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Prof. Dr Snežana Šerbula

18-21 June 2019, Hotel Jezero, Bor Lake, Serbia

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ECOLOGICAL TRUTH AND ENVIRONMENTAL RESEARCH – EcoTER'19

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PREFACE

Today's growing environmental and ecological imbalances require a multidisciplinary approach in finding adequate sustainable solutions. That is why environmental and ecological issues are at the focus of the 27th International Conference Ecological Truth and Environmental Research 2019 (EcoTER'19), which will be held at Bor Lake, Serbia, 18-21 June 2019. On behalf of the Organizing Committee, it is a great honor and pleasure to wish all the participants a warm welcome to the Conference.

The EcoTER'19 is organized by the University of Belgrade, Technical faculty in Bor, and co-organized by the University of Banja Luka, Faculty of Technology, University of Montenegro, Faculty of Metallurgy and Technology – Podgorica, University of Zagreb, Faculty of Metallurgy – Sisak, University of Pristina, Faculty of Technical Sciences – Kosovska Mitrovica and the Association of Young Researchers, Bor.

The primary goal of EcoTER'19 is to bring together academics, researchers, and industry engineers to exchange their experiences, expertise and ideas, and also to consider possibilities for collaborative research.

These proceedings include 105 papers from authors coming from universities, research institutes and industries in 15 countries: Russia, Belarus, Turkey, Kazakhstan, Czech Republic, Portugal, Sweden, Switzerland, Slovenia, Bulgaria, Croatia, Bosnia and Herzegovina, North Macedonia, Montenegro, and Serbia.

The support of the donor and their willingness and ability to cooperate has been of great importance for the success of EcoTER'19. The Organizing Committee would like to extend their appreciation and gratitude to the donor of the Conference for their donation and support.

We would like to thank all the authors who have contributed to these proceedings, and also to the members of the scientific and organizing committees, reviewers, speakers, chairpersons and all the Conference participants for their support to EcoTER'19. Sincere thanks to all the people who have contributed to the successful organization of EcoTER'19.

On behalf of the 27th EcoTER Organizing Committee,

Snežana Šerbula, PhD Full Professor

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EVALUATION OF URBAN BIOTOPES – TOOL FOR BIODIVERSITY PROTECTION AND SUSTAINABLE DEVELOPMENT OF CITIES

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Abstract

Cities have a large heterogeneity of habitats (i.e. biotopes) in a relatively small area. Urban biotope mapping is a procedure for determining and describing the size and distribution of different habitats (natural and man-made) in the entire urban area with the intention of creating a biotope (habitat) network. Mapping of urban biotopes and their value assessment is therefore considered to be a valuable tool for the purpose of sustainable development and preservation of biodiversity in cities. Methodology for mapping and evaluation of urban biotopes in Serbia was developed for Belgrade city. Typology for biotope mapping as well as criteria for biotope value assessment were also created. Actual value of selected i.e. representative biotopes, as well as the potential value of all mapped biotopes were assessed. Results of biotope mapping are possible to transform for practical use, and certain recommendations and measures are defined for application in the planning process. So far, only limited use of these information was noticed for the purpose of urban planning, nature protection and scientific researches. Thus, there is a need to promote the projects regarding mapping and evaluation of urban biotopes as a useful tool for biodiversity protection and sustainable development of cities in Serbia.

Keywords: biotope mapping, biotope value assessment, GIS, urban planning

INTRODUCTION

Urbanization is one of the leading demographic trends, thanks to which more than half of the world's population today lives in cities. The percentage of Europe's urban population in 2018 was 74% (Statistic 2018). It is expected that within next 20 years the city population reaches up to 5 billion people, making the planet become global megalopolis. Urbanization always had a strong impact on biodiversity and landscape modification. Due to this, cities and nature have long been regarded as the opposites. As cities became "more urban", they had less natural places in them and vice versa. However, nowadays opinion that the city and nature do not necessarily imply the opposite prevails. Cities can play an important role for nature, in case they do not present a barrier but linkage to the regional biotope system [1–3]. Therefore, the map of urban and suburban biotopes represent a key part of the information system in many cities of the world, and the mapping and evaluation of urban biotopes is a tool for the integration of biodiversity protection into the urban planning process.

Protection and preservation of the urban biodiversity has not been given special attention in the past. This is due to the fact that the green areas in the city suffer from unique and powerful influences such as: the high density of buildings and infrastructure, small size of the isolated (fragmented) nature within the city, intense pressure of visitors etc. However, in spite of this, recent researches show that there are numerous ecologically and economically valuable biological resources in the cities [4–8]. According to Mansuroglu *et al*. [9], besides urban and suburban biotopes being a home for plant and animal species, they are also of importance for environmental design and aesthetics, natural and cultural history, protection of species, environment and landscape elements (water, air, soil, etc.), as well as for ecological research, education, recreation opportunities for urban dwellers and playgrounds for children.

Large influx of population in cities and unplanned urbanization pose a threat to the preservation of total biodiversity on planet Earth. Urban planners in many countries have failed to include environmental concerns in the planning of urban areas [10]. Incorrect urban management is not an inevitable fate of cities. Urbanization and ecology can co-exist, but only with active participation of involved stakeholders, including the private and public sector, as well as the citizens [11]. According to Teofilović *et al*. [10], some scientists in the field of biodiversity protection predict that cities will determine the fate of remaining biodiversity on our planet in the future. Namely, the struggle for life will be acquired or lost in the cities [12]. In this respect, this overview paper aims to affirm the importance and need of a systematic approach to mapping and evaluating urban and suburban biotopes as a useful tool for biodiversity protection and sustainable development of cities in Serbia.

City as ecosystem

Cities represent complex unities with different habitats and ecosystems that are inhabited by man and by other living species. Therefore, modern urban planning should also be an instrument that ensures the functioning and preservation of other living beings, since the existence of the man depends on them.

The city is a typical example of an non-autonomous ecological system, since it does not have its own autonomy. Unlike natural (autonomous) ecosystems, such as forests, meadows, pastures, lakes, rivers or seas, the city is unable to sustain and renew itself. Namely, green plants, as primary food producers, are insufficiently represented in the urban ecosystem. On the other hand, consumers, dominantly man, are present in abundance, while decomposers, as well as the producers, have disproportionately low presence. In such circumstances, in order to maintain itself, the urban ecosystem must take organic matter, water and energy from the surrounding natural and semi-natural ecosystems to meet the needs of many consumers. Because of this, the city is often figuratively described as "parasitic ecosystem" or "biospheric parasite" [1]. Man is the dominant ecological factor that even contributes to modification of climate in urban ecosystems. It is known that the climate of the city significantly differs from the surrounding environment. According to Kuttler [13], the most important features of urban climate include higher air and surface temperatures (urban heat island effect), changes in radiation balances, lower humidity, and restricted atmospheric exchange that causes accumulations of pollutants from a variety of sources.

Cities are typically warmer, drier, nutrient–laden, and floristically enriched by human activity [14]. The diverse vegetation types occurred in certain parts of many cities, and natural and man-made waterways attracts different birds, mammals, insects, and fish. But nutrient enrichment and extensive land conversion often allow a few tolerant species to attain extremely dense populations. The importance of plant diversity to birds, bats, and insects is consistent and suggests that if people allow structurally complex vegetation to occur, invertebrate and vertebrate diversity will prosper. At the same time, human facilitation of invasions by exotic species is general characteristic and probably the greatest concern for biological diversity of cities [14]. During the last two decades the number of studies focused on urban ecosystems in Europe increased, especially related to biological invasions and global environmental changes [4,15–19].

Legislative framework for mapping and evaluate of urban biotopes

Conservation of habitats is generally defined by a number of international documents. The most famous are the Convention on Biological Diversity (CBD - UNCED, Rio de Janeiro, 1992), Berne Convention (Council of Europe, Berne, 1972), and Habitat Directive (EU Habitats Directive 92/43 / EEC). All of them have been ratified by Serbian parliament. Also, development strategies and national legislation require the conservation of flora and fauna as well as their habitats, providing a starting point and obligation for sustainable urban planning.

The most direct approach to this issue is the Curitiba Declaration on Cities and Biodiversity (Curitiba city, Brazil, 2007). This declaration confirms global commitment to integrate biodiversity issues into urban planning and development in order to improve the lives of urban inhabitants and to ensure a sustainable basis for cities.

Mapping of urban and sub-urban biotopes

Compared to the natural areas, cities have a large heterogeneity of habitats (i.e. biotopes) in a relatively small area, which are additionally exposed to direct or indirect human influence. Although urban habitats are often considered to be abound with non-native or alien plants and animals [20,21], flora and vegetation of towns and cities harbour a significant number of specialized species, including rare and threatened ones [22]. Most of them are sensitive to habitat and management changes and thus represent good indicators of the environmental conditions and the socio-economic status of a city [23]. In recent urban management practice, biotope areas are often considered as passive green spaces which resulted in no specific protection measures taken, leaving them open to every kind of human disturbance [9].

Biotope is the basic topographical unit in ecology [24] representing an area of uniform environmental conditions that provides a living place for a specific assemblage of plants and animals. Biotope is sometimes used as synonym with the term habitat, though the first is more accurately delineated to describing an area with boundaries within which plants and animals can live [25]. In terms of mapping, biotope represents a clearly edged surface with a relatively unique structure of vegetation and land use. Urban biotope mapping is a procedure for determining and describing the size and distribution of different habitats (natural and man-made) in the entire urban area with the intention of creating a biotope (habitat) network. The mapping area of urban biotopes includes even empty parcels, abandoned blocks, steep slopes, transport corridors, abandoned agricultural land, etc. [10].

According to Jarvis & Young [26] and Mansuroglu *et al*. [9], two methods of mapping of biotopes in urban environments are commonly used: a) Selective Biotope Mapping, where only the biotopes worth of protection are mapped, and b) Comprehensive Biotope Mapping, where all existing biotopes are mapped. Germany is leading country in the terms of usage of biotope maps in spatial planning studies. Biotope mapping in Germany was started in the 1970s both at provincial and urban levels [27]. Today, biotope maps of 160 cities in Germany (that additionally include detailed information about the geology, water, climate, land use, traffic/noise and energy, etc.) have been prepared and are widely used as fundamental references in urban planning and management. In the later period, the biotope mapping method has been utilised in an increasing number of countries including the UK, Sweden, Turkey, Japan, South Korea, New Zealand and China [28]. Development of remote-sensing technologies and geographical information systems (GIS) offers new possibilities for very accurate and quick mapping of biotopes. The data obtained are more reliable and easy to update [9].

So far, Belgrade is the only city in Serbia that established a system of mapping and evaluation of urban biotopes. Establishment of Belgrade GIS on biotopes is the result of a two-year work on the project "Mapping and evaluation of the biotope of Belgrade" (third phase of the "Green Regulation of Belgrade" project, 2008). Methodology of mapping the urban and suburban biotopes involves several successive phases: a) divisioning the city territory to typical biotopes, b) graphic and cartographic representation of their size and distribution, c) inventorization of contents and specificities of the abiotic and biotic environment, and d) evaluation of the biotopes [3,11]. Previously, it was necessary to define the typology for biotope mapping [29]. The key for biotope mapping is organized in 9 main groups. Within each main group, further biotope classification was carried out at three to four hierarchical levels of resolution (type, subtype, variation, and specific plant community). Using the method of photointerpretation of aerial photographs, the mapping of the territory of Belgrade was carried out at the third hierarchical level of typology, i.e. at the level of the biotope subtype. In the subject area of 77,460 ha, 161,484 individual biotopes were isolated.

The next phase involves the selection of representative biotopes in selected areas of the city, presenting most subtypes previously defined by typology. For representative selected biotopes (504 entities at the level of subtype), detailed field survey of flora, fauna, vegetation, level of disturbance and other parameters defined for evaluation purposes has been done. Each biotope is geometrically defined as a closed polygon having a unique code (IDBiotop) with a certain set of data [10].

EVALUATION OF URBAN BIOTOPES

Evaluation criteria – example of Belgrade city

The criteria and their scale of values which were applied for urban and suburban biotopes assessment in Belgrade were partially defined according to model from Germany [30] but improved, modified and adjusted to Belgrade conditions as follows [10]:

1. Basic biotope importance (Cr 1)
    - (5) Priority for conservation habitats (Habitats of special conservation interests on international lists)
    - (4) Habitats included in international lists of important habitats
    - (3) Habitats with endemic, relict or rare species as edificators
    - (0) Other habitats
2. Degree of typicality for natural environment (Cr 2)
    - (5) Biotopes with climatogenic (primary) and well-preserved forms of vegetation
    - (4) Biotopes with climatogenic (primary) and relatively well-preserved forms of vegetation
    - (3) Biotopes with a higher stage of succession - vegetation progradation typical for the natural environment
    - (2) Biotopes with a lower stage of succession - vegetation progradation typical for the natural environment
    - (1) Initial and pioneer stages of natural succession of vegetation typical for the natural environment
    - (0) Biotypes of no significance and connection with natural succession of vegetation typical for the natural environment
3. Age and biotope regeneration ability (Cr 3)
    - (5) Very old (about 250-1000 years old) and non-renewable biotopes
    - (4) Old (about 75-250 years old) and very hard-to-renew biotopes
    - (3) Medium-age biotopes (approximately 20-75 years old) and possibly renewable at least within the similar time period
    - (2) Relatively young biotopes (about 5-20 years old), slightly dependent on age and easily renewable without additional care
    - (1) Very young biotopes (approximately 0-5 years old), independent of age and very easily renewable almost everywhere
    - (0) Biotypes with no possibility and need for estimation of age and regeneration ability
4. Biodiversity richness (Cr 4)
    - (5) Biotopes rich in primarily indigenous species of flora and fauna with a significant share of characteristic species
    - (4) Biotopes relatively rich in indigenous species of flora and fauna with lower participation of characteristic species
    - (3) Biotypes medium rich with species of autochthonous flora and fauna and with the participation of allochthonous species
    - (2) Biotope poor in species of autochthonous and allochthonous flora and fauna
    - (1) Biotypes extremely poor with species of autochthonous and allochthonous flora and fauna, or without representatives of groups selected for the biotope evaluation

5. Specificity of biotope (endemism - relic - rarity) (Cr 5)

    (5) Biotopes with more than 10 endemic, relict or rare species of flora or fauna

    (4) Biotopes with 8 to 10 endemic, relict or rare species of flora or fauna

    (3) Biotopes with 5 to 7 endemic, relict or rare species of flora or fauna

    (2) Biotopes with 2 to 4 endemic, relict or rare species of flora or fauna

    (1) Biotopes with at least one endemic, or relict, or rare species of flora or fauna

    (0) Biotopes without endemic, relict and rare species of flora and fauna

6. Significance as a habitat for endangered species (Cr 6)

    (5) Biotopes with one or more critically endangered (CR) or endangered (EN) species of flora or fauna

    (4) Biotopes with one or more vulnerable (VU) species of flora or fauna

    (3) Biotypes with one or more flora or fauna species on the lists of internationally and/or nationally protected species (e.g. species found in different annexes of conventions such as Bern, Bonn, Habitat Directive, Bird Directive, etc. that do not have a defined status of vulnerability, but are treated as species of significance for protection)

    (2) Biotopes with one or more species of flora or fauna of lower categories of vulnerability (LR, DD)

    (1) Biotopes with one or more species of flora or fauna that are under the control of collection and traffic

    (0) Biotopes without endangered or protected species of flora or fauna

7. Level of disturbance (Cr 7)

    (5) Biotopes without or with very low level of disturbance

    (4) Biotopes with low level of disturbance

    (3) Biotopes with medium level of disturbance

    (2) Biotopes with a high degree of disturbance

    (1) Biotopes with a very large (extreme) degree of disturbance

8. Size, isolation and fragmentation of biotope (Cr 8)

    (5) Extremely large compact biotopes (more than 2 minimal surfaces for stable functioning)

    (4) Large compact biotopes (between 1 and 2 minimum surfaces for stable functioning)

    (3) Isolated biotope belonging to a large mosaic of fragmentally distributed biotopes within the same category

    (2) Isolated biotope belonging to a small mosaic of fragmentally distributed biotopes within the same category

    (1) Small fully isolated biotope

In addition to the actual (real) value, which represents the current state of each specific biotope, defined primarily by the influence of anthropogenic factor, a potential value category is defined, which reflects the ability of the biotope to reach a certain value in natural conditions without direct and indirect anthropogenic impacts.

Potential value is estimation of the development state that a particular biotope can achieve if anthropogenic impact is excluded. For this type of evaluation, criterion 3 (age and biotope regeneration ability) and criterion 7 (level of disturbance) has not been taken into account, with a certain modification of the criteria related to the degree of typicality for natural environment (Cr 2) and the specificity of biotope in terms endemic, relict and rare species of flora and fauna presence (Cr 5) as key criteria for this assessment [8,10].

Criteria for determining the potential value of biotope are definitely new in this area and provide the possibility that, regardless of the quality and quantity of data collected during the field work on the research of representative biotopes, a general map of the potential biotope value of Belgrade can be made. The set of criteria for determining the potential value of Belgrade biotope is defined primarily on the basis of the generalization of data from the database "Habitats of Serbia" and "Phytocoenosis of Serbia", as well as on the basis of published data on wider analyzes related to problems of diversity, endemism and vulnerability of vascular flora of Serbia [31–33].

Potential biotope value is a special quality assessment that was developed for application in the biotope evaluation of Belgrade (Figure 1). In this way, in practical terms, a preliminary assessment of the value of concrete biotopes is enabled, for example if certain parts of city should be exempted from interventions in the area, or be treated with special care, before detailed data are collected for the estimation of real values.

Urban biotopes value estimation

For the assessment of the real and potential value of biotope in the area of Belgrade, a seventh-grade scale was established, according to [10]:

Grade 1 - Extremely poor biotopes, often resulting in heavy burdened surrounding living spaces. Primarily built areas, such as areas under buildings, roads and covered areas, biotopes extremely poor with species, large areas without vegetation e.g. illegal parking lots, commercial and industrial areas, areas treated with herbicides, etc.

Grade 2 - Extremely impoverished biotopes with limited ability to develop, without refugial function, with intensive use and low diversity of species. Biotopes that can be quickly compensated everywhere. They always burden the adjacent valuable living spaces (eg. sports areas, settlement areas with cultivated ornamental gardens and isolation greenery poor in species, young fallows rich in nutrients, arable land, etc.);

Grade 3 - Depleted biotopes, but capable for development. If necessary with a small refugial function, useful areas with low diversity of species (eg. intense grassland and pastures, intensively used young fallows rich in nutrients, etc.);

Grade 4 - Still valuable biotopes with good development abilities, extensively used in the past and sufficiently structured. The habitat of the medium diversity, in the built areas and in areas with intensive agriculture, with the existing refugial function, as well as the slightly disturbed fallows of old family houses and old garden colonies with high participation of utilitarian species and woody fruit trees, cemeteries, damaged hedges, etc;

Grade 5 – Valuable biotopes, extensively used and with a rich structure. Habitat of a large number of species and with the important function of refugium or buffer in build areas, or in areas with intensive agriculture (e.g. location with ruderal vegetation of older succession stages, extensive grasslands and pastures, hedges, etc.);

Grade 6 - Highly-valued, close-to-nature biotopes with high refugial function, worth of protection, slightly disturbed remains of the former natural areas. They are not used extensively anymore; habitats of endangered species (for example, the old forests close to nature, slightly degraded wetlands and wet meadows, close to nature streams, old hedges/meadows, old grasslands, etc.); and

Grade 7 - Natural or biotopes very close to nature, with prominent values for biotope and species protection, worth of nature protection with international and national significance, remains of the former natural areas or older cultivated ecosystems. They are not used extensively anymore. They represent the habitat of many endangered species (eg. wetlands, peat bogs, natural climatogenic forests, natural meadows or primary scrub, left to nature streams and lakes with a pronounced sediment accumulation zone).

Figure 1 *Map of potential biotope values (grade 5-7) of Belgrade city (extracted from Teofilović 2013)*

On the basis of the defined criteria and grade scale, in the area of Belgrade (within the boundaries of the General Urbanistic Plan), the evaluation of representative biotopes was carried out. Firstly, an estimate of the real value for the 504 representative selected biotopes has been completed based on filed data related to flora, vegetation and fauna (insects, amphibians, reptiles, birds, mammals, fish and zooplankton), as well as the air quality and climate (air temperature and precipitation). In addition to the evaluation of representative biotopes, estimation and potential biotope values estimation were carried out for the entire area of Belgrade [10]. By analyzing the data of the formed GIS database, information that can further serve the city administration, local governments, institutions dealing with urban planning, management and protection of natural resources, education and scientific institutions, as well as citizens, has been improved and moved towards more sustainable development.

Summarization of biotope mapping and evaluation in Belgrade

Some of the important data that came out from this database are: a) the most represented type of biotopes are agricultural fields and vegetable gardens, however, about 7,400 ha of abandoned agricultural lands are recorded in the area; b) about 5000 ha of biotopes belong to a group of fallows, representing the areas covered by ruderal vegetation of different succession stages; c) illegal landfills were recorded on a number of locations on about 400 ha; d) biotopes which are estimated as potentially highly valuable with prominent values for the protection of habitats and species and worth of nature protection of international and national importance were recorded on an area of over 10,700 ha; e) real high-value biotopes and biotopes with prominent values (grades 6 and 7) are located at the following areas: Veliko blato, forland zones of the Danube and Sava, Reva swamp, Zvezdara forest, Big War island, Ada Ciganlija, Makiško polje, Košutnjak, Topčider park, Manastirska forest, Stepin grove, Avala, part of Velikoselski rit, etc. [8,10].

Considering that the mapping and evaluation of the biotope of Belgrade lasted only two years (2005-2007), there was not enough time for the complete this information system. Therefore, it is necessary to permanently update the existing database of representative biotopes, as well as the other mapped biotopes with new data [34].

Transformation of the results for the use in planning

In the period following the formation of the GIS database, the data were used for different purposes and at different levels of detail. Namely, more than 100 excerpts of the database have been prepared, most often for the needs of the urbanistic plans preparation, but also for the needs of environmental impact assessment studies, the project for the protection of natural assets, as well as expert and scientific papers. The level of detail and the way data is displayed in the prepared excerpts depends on the detail of the database for the subject area. Based on this, certain recommendations are defined as well as general and detailed measures to be taken into account during the planning process. Detailed measures depend on the specific space, types of biotope, their values and the immediate environment, while recommendations can be generalized in the following way, according to [8] and [10]:

<u>Biotopes with value 6 or 7</u> - In the planning process the sites of high-value biotypes and biotope with prominent values should be absolutely preserved, and adequate measures for the maintenance of such biotopes should be recommended. Also, in the case of highly valuable

biotopes, it is necessary to carefully plan the areas in immediate surrounding, as inadequate uses would potentially endanger biotopes worth of protection.

Biotopes with values 4 and 5 - In the planning process valuable and still valuable biotopes should be preserved as much as possible, with recommendations for their improvement in order to preserve biodiversity. These biotopes should be considered as habitats of a large number of species with important function of refugium or buffer in built areas (eg. non-built living quarters, etc.).

Biotopes of value 3 - Degraded biotopes that do not set specific requirements in terms of planning, but represent potential as biotopes capable for development. On the premises of these biotopes, if possible, open and green areas should be considered in the context of the green area system planning, thus encouraging their ability to develop.

Biotopes of values 1 and 2 - Extremely poor and highly degraded biotopes with a limited ability to develop, which do not set specific requirements in relation to making planned solutions.

Practical significance and application

The mapping and evaluation of urban biotopes has practical application in number of cases, such as: a) development of environmental impact assessments of various facilities; b) development of landscape plans and plans of the greenery system in cities, c) delineation of protected areas and development of programs of their management d) scientific work in the field of urban ecology [3,11]. The initial step and prerequisite for the successful realization of each of these activities is the biotope map, that is, the information system with relevant data on their biogenic and abiogenic characteristics and estimated values.

Planning of green infrastructure in order to preserve biodiversity has become a practice in the European countries, but also a worldwide. According to Benedict and McMahon [35], green infrastructure is a link between the environment inside and between the cities, settlements and villages. It is a network of open spaces, waterways, gardens, forests, green corridors and alleys, which brings many social, economic and environmental benefits to the local population. Careful planning of green infrastructure can reduce the impact of various stressors such as: urban development, watercourse modification, drainage of swamps, introduction of foreign (allochthonous) species, removal of indigenous species, global climate change, etc. Many species can find suitable habitats in urban and suburban environments, provided people recognize their needs and integrate them into urban development. In order to promote and implement sustainable urban development and protection of biodiversity, the local population and city administration must first identify local wildlife and their habitats, and then provide and support basic needs for their survival (plant cover, food, water, space for living and reproduction).

CONCLUDING REMARKS

The system of urban biotope mapping as well as assessment of biotope value is an inter-discipline comprehensive work which provides a useful database for sustainable planning and urban landscape management. Rapid growth of areas and population in cities is causing inevitable changes (such as concentration of people and infrastructure, microclimate change and degradation of natural values), so mapping of urban biotopes and creation of databases for the purpose of sustainable development of cities and preservation of biodiversity in cities is gaining importance. The number of cities developing informational systems on urban biotopes is growing. Methodology of biotope mapping follows certain general rules, but should be modified for each city and geographical region, especially in regard of biotope typology. Results of biotope mapping are transformed for practical use in planning, and certain recommendations as well as general and detailed measures to be taken into account are defined for application in the planning process. Although ecosystem services are of high policy interest in EU countries and increasingly incorporated in urban planning, “urban green space” generally does not consider the diversity of the spontaneous urban flora, vegetation and fauna as well as their relationships to different or changing environments.

Mapping of urban biotopes in Serbia was conducted in Belgrade in 2008. In addition to the evaluation of representative biotopes, an estimate of the potential value of biotope in the entire area of Belgrade was made as a base for further use in urban planning and management. So far, only limited use of these information and maps was noticed for the purpose of urban planning, nature protection and scientific researches. There is a need to promote ecological planning approach in the Belgrade urban planning process as important factor leading to the preservation and development of urban biotopes.

Considering the size of city and the diversity of its biotopes, the typology of urban and suburban biotopes, as well as the mapping and evaluation methodology used for Belgrade can be adapted to other major Serbian cities such as Novi Sad, Nis, Kragujevac, Kraljevo and others. Smaller cities, however, would require certain adjustment of this methodology and biotope typology to their size and the local characteristics. However, it is necessary for local governments to make strategic decisions about the future planning and development of their cities on the principles of sustainability, especially with regard to the green area system, which presents kind of `green capital` in the terms of an elevated quality of urban life for people and other biota.

ACKNOWLEDGEMENTS

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TOWARDS DEVELOPING GREEN CHEMISTRY METHOD FOR SYNTHESIS OF NEW SUCCINIMIDES AS THE ANTIMICROBIAL AGENTS

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Abstract

In the present study, three new series of N-(3- and 4-substituted phenyl)-2,2-disubstituted succinimides, as potentially new heterocyclic compounds with promising bioactivity, were synthesized via green synthetic route. A conventional synthesis of N-arylsuccinimides requires tedious multistep transformations. It involves reaction of substituted aniline with succinic anhydride, in apropriate solvent, to give matching succinimic acid, which cyclizes to succinimide derivative in refluxing acetyl chloride or acetic anhydride/sodium acetate. In the final step of applied synthetic route, condensation between 2,2-dialkyl or 2-alkyl-2-aryl succinic acid and substituted anilines has been improved utilizing the microwave irradiation under solvent-free conditions. Some noteworthy features of our eco-friendly method are its cleanliness, short reaction time and moderate to high conversion rate, and the reaction proceeds by a simple procedure. The certain newly synthesized succinimides were screened for antimicrobial activity. Generally speaking, tested compounds showed higher antifungal potential than antibacterial effect. The preliminary biological results indicated that N-(4-bromophenyl)-2-ethyl-2-methylsuccinimide (4h) exhibited significant in vitro antifungal activities against different species of fungus, and could be observed as a novel potential fungicide.

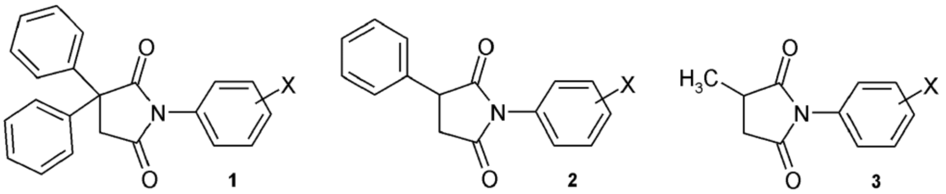
Keywords: succinimide, green synthesis, antimicrobial activity, antifungal activity

INTRODUCTION

Succinimides are cyclic imides that are privileged pharmacophores and important building blocks for the synthesis of bioactive compounds [1]. A variety of biological activities and pharmaceutical uses have been attributed to them. To name a few, succinimide is a part of many bioactive molecules [2] possessing activities such as anti-convulsant [3,4], antimicrobial [5–8], analgesic [9,10], antitumor [11,12], nerve conduction blocking [13], anti-tubercular [14] etc. [1]. Additionally, certain *N*-phenyl succinimide derivatives showed the seedling growth stimulator activities against wheat and radish [15], as well as strong activity against fungal diseases of plants like rice blast, rice brown spot and kidney bean stem rots [16].

As a result of the numerous side effects produced by antimicrobial drugs, there is a growing need for both drug improvement and search for novel agents. Moreover, the overcoming drug resistance has become an important issue in medicinal chemistry due to the increased consumption and misapplication of antimicrobial drugs [17]. Besides, the hazardous impacts of agricultural fungicides in human and animal health and environment are well known and with their excess applications pest resistance may exist [18]. Therefore, design and synthesis of modern specific-target new bioactive compounds with eco-friendly properties has become an urgent need in the future.

The focus of our former study [19–21] was on investigation of three series of succinimide derivatives containing a substituted phenyl moiety on the nitrogen atom of the succinimide ring (Figure 1).



Comp.	X
1a	4-OH
1b	3-OH
1c	4-OCH$_3$
1d	4-CH$_3$
1e	H
1f	4-F
1g	4-Cl
1h	3-Cl
1i	4-Br
1j	4-I
1k	4-COCH$_3$
1l	4-CN
1m	3-CN
1n	4-NO$_2$

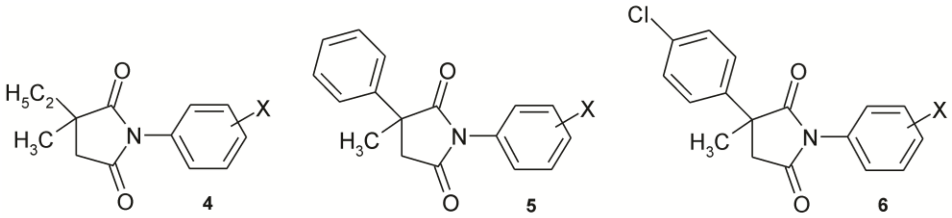
Comp.	X
2a	4-OH
2b	4-OCH$_3$
2c	4-CH$_3$
2d	3-CH$_3$
2e	H
2f	4-Cl
2g	3-Cl
2h	4-Br
2i	3-Br
2j	4-COCH$_3$
2k	4-NO$_2$

Comp.	X
3a	4-OH
3b	4-OCH$_3$
3c	4-CH$_3$
3d	H
3e	4-Cl
3f	3-Cl
3g	4-Br
3h	3-Br
3i	4-I
3j	4-COCH$_3$
3k	4-CO$_2$H
3l	4-NO$_2$

***Figure 1** Chemical structure of previously investigated N-arylsuccinimides: N-(3- and 4-substituted phenyl)-2,2-diphenylsuccinimides (Series **1**, labeled as **1a**–**1n**), N-(3- and 4-substituted phenyl)-2-phenylsuccinimides (Series **2**, labeled as **2a**–**1k**) and N-(3- and 4-substituted phenyl)-2-methylsuccinimides (Series **3**, labeled as **3a**–**3l**)*

In our previous research, we have established relationships between their chromatographic data and the selected structural features which are related to the ADMET (absorption, distribution, metabolism, excretion, and toxicity in pharmacokinetics) parameters, obtained by the established computational medicinal chemistry methods. In view of the results of this study the investigated succinimides meet the pharmacokinetic criteria of preselection of drug candidates and hence qualify for the pharmacodynamic phase of drug development. In addition, compounds with halogen substituents in *meta* and *para* position in benzene ring of *N*-aryl-2-phenylsuccinimides **2f**–**2i** are proposed as the best drug candidates for the further research and development studies.

Our recent studies have demonstrated three new series of *N*-(3- and 4-substituted phenyl)-2,2-disubstituted succinimides **4a**–**4l**, **5a**–**5l**, and **6a**–**6j**, as potentially biologically active compounds (Figure 2).



Comp.	X
4a	4-OH
4b	4-OCH$_3$
4c	4-CH$_3$
4d	H
4e	4-F
4f	4-Cl
4g	3-Cl
4h	4-Br
4i	3-Br
4j	4-CO$_2$H
4k	4-CN
4l	4-NO$_2$

Comp.	X
5a	4-OH
5b	4-OCH$_3$
5c	4-CH$_3$
5d	H
5e	4-F
5f	4-Cl
5g	3-Cl
5h	4-Br
5i	3-Br
5j	4-CO$_2$H
5k	4-CN
5l	4-NO$_2$

Comp.	X
6a	4-OCH$_3$
6b	4-CH$_3$
6c	H
6d	4-F
6e	4-Cl
6f	3-Cl
6g	4-Br
6h	3-Br
6i	3-I
6j	4-NO$_2$

***Figure 2** Chemical structure of novel three series of investigated N-arylsuccinimides: N-(3- and 4-substituted phenyl)-2-ethyl-2-methylsuccinimides (Series **4**, labeled as **4a**–**4l**), N-(3- and 4-substituted phenyl)-2-phenyl-2-methylsuccinimides (Series **5**, labeled as **5a**–**5l**) and N-(3- and 4-substituted phenyl)-2-(4-chlorophenyl)-2-methylsuccinimides (Series **6**, labeled as **6a**–**6l**)*

In the present work, these three series of succinimides **4a**–**4l**, **5a**–**5l**, and **6a**–**6j**, were synthesized via green synthetic route. Additionally, antimicrobial activities of the certain succinimide derivatives were assessed.

MATERIALS AND METHODS

Experimental

Chemistry

The chemicals used in the synthesis were purchased from Sigma-Aldrich Chemicals (Sigma-Aldrich Corp., St. Louis, MO, USA) or Merck Chemicals (Merck KGaA, Darmstadt, Germany). Microwave synthesis was performed in Anton Paar Monowave 300 (Ashland, VA, USA) microwave reactor. The FT-IR spectra of the compounds (in KBr pellets) were determined using an ABB Bomem MB Series 100 Fourier transform infrared (FT-IR) (Quebec City, Canada) spectrophotometer. The NMR spectral measurements have been performed on a Bruker AC 250 spectrometer at 200 MHz for the ^{1}H NMR and 50 MHz for the ^{13}C NMR spectra or on a Bruker 300 spectrometer at 400 MHz for the ^{1}H NMR and 100 MHz for the ^{13}C NMR spectra. The spectra were recorded at room temperature in deuterated dimethyl sulfoxide (DMSO-d6) with TMS as the internal reference signal. All melting points were uncorrected. The elemental analyses of the investigated compounds were carried out by standard analytical micromethods using an Elemental Vario EL III microanalyzer.

Antimicrobial activity

Human pathogens

The newly synthesized succinimides were evaluated for antibacterial activity against eight bacterial species: *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (clinical isolate), *Micrococcus flavus* (ATCC 10240), *Listeria monocytogenes* (NCTC 7973) (gram positive bacteria), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter cloacae* (clinical isolate), *Salmonella typhimurium* (ATCC 13311), *Escherichia coli* (ATCC 35210) (gram negative bacteria).

Antifungal activity was evaluated against eight fungal species: *Aspergillus flavus* (ATCC 9643), *Aspergillus ochraceus* (ATCC 12066), *Aspergillus versicolor* (ATCC 11730), *Aspergillus niger* (ATCC 6257), *Penicillium ochrochloron* (ATCC 9112), *Penicillium funiculosum* (ATCC 36839), *Penicillium verrucosum var.cycopium* (food isolate), *Trichoderma viride* (IAM 5061).

Broth microdilution method

The antimicrobial activity was performed by using microdilution method [22–28]. The minimal inhibitory (MICs), minimal fungicidal (MFCs) and minimal bactericidal concentrations (MBCs) of the synthesized succinimides for each species of studied microorganisms were determined.

MIC determination

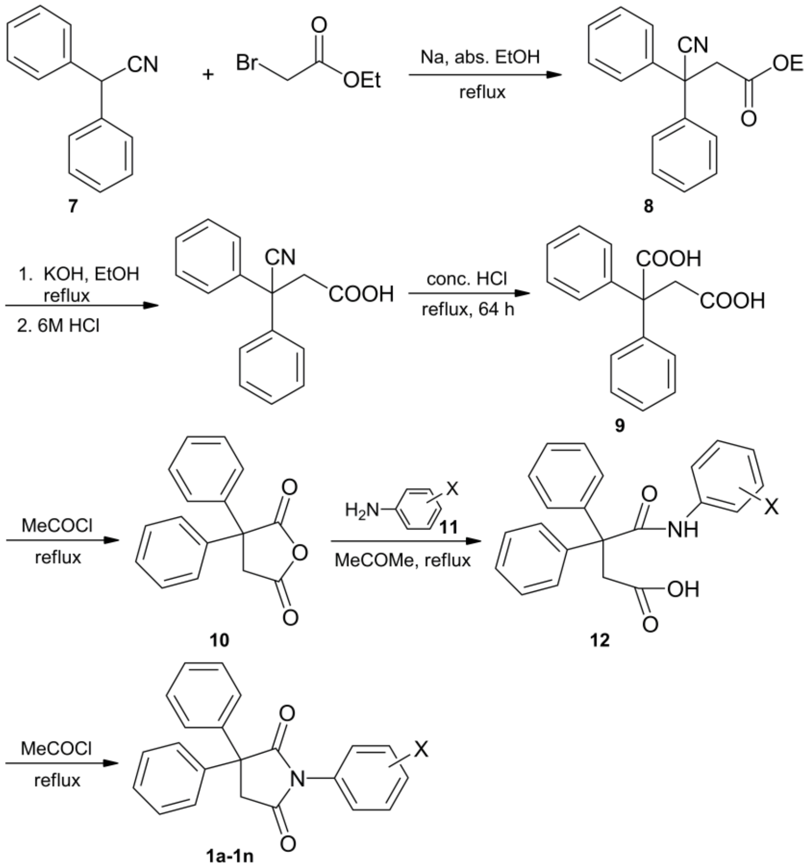
The minimum inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent that completely inhibits bacterial and fungal without visible growth, observed under a binocular microscope [29–31]. The MIC values were determined by counting cell numbers by spectrophotometric methods [25,26].

RESULTS AND DISCUSSION

This review provides an overview on the synthesis and antimicrobial activity of certain succinimide derivatives. In the first part we intent to outline the general conventional method by which substituted succinimides are proposed. In the second part we described the synthesis of succinimide under microwave irradiation. Finally, we reported antimicrobial activity of investigated succinimides.

The following reactions (Scheme 1) illustrate the conventional synthetic method generally employed for the preparation of *N*-aryl-2,2-diphenylsuccinimides of series **1**. The alkylation of diphenylacetonitrile (**7**) with ethylbromo acetate gave cyanoester **8**. Subsequent conversion of **8** to 2,2-diphenylsuccinic acid (**9**) was achieved through hydrolysis. 2,2-Diphenylsuccinic acid (**9**) was cyclized to anhydride **10** in refluxing acetyl chloride. The subsequent reaction of **10** with substituted anilines **11** gave the corresponding succinimic acid **12**, which were further cyclized to *N*-(3- and 4-substituted phenyl)-2,2-diphenylsuccinimides (Series **1**) in refluxing acetyl chloride. This synthetic route is a modified literature procedure [32–34].

All the *N*-arylsuccinimides of series **1** were synthesized from 2,2-diphenylsuccinic anhydride (**10**) and the corresponding aniline **11** (Scheme 1) [35]. Anhydride **10** was previously synthesized by the method of Miller *et al.* [33].

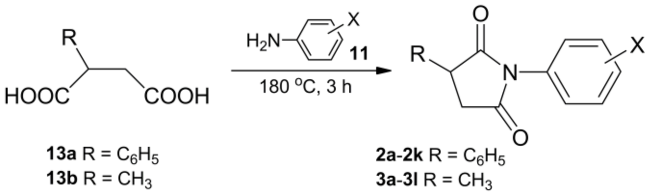


***Scheme 1** Conventional synthetic route of N-(3- and 4-substituted phenyl)-2,2-diphenylsuccinimides (Series **1**)*

In accordance with the reaction workup, the crude *N*-arylsuccinimides **1a**–**1n** was filtered off and recrystallized from acetone. The yield of crystalline product was from 23 % to 37%.

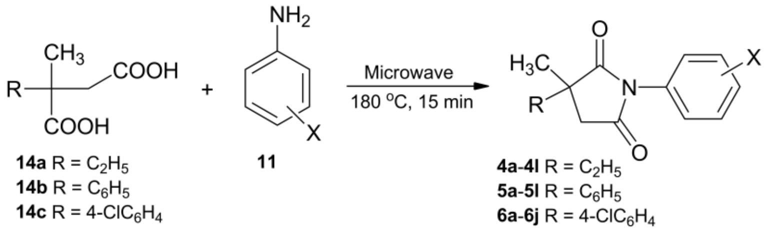
A conventional synthesis of *N*-aryl-2,2-diphenylsuccinimides **1a**–**1n** from diphenylacetonitrile (**7**) (Scheme 1) consists of six steps sequence and has its own synthetic limitations when applied to a range of derivatives, such as: use of hazardous reagents, poor yield and tedious workup and purification process.

N-aryl-2-substituted succinimides **2a**–**2k** and **3a**–**3l**, were prepared from appropriate 2-substituted succinic acids **13a** and **13b**, and the corresponding anilines **11** under free-solvent conditions (Scheme 2), using a modified conventional procedure from the literature [36]. Poor to fair yields of crystalline products were obtained.



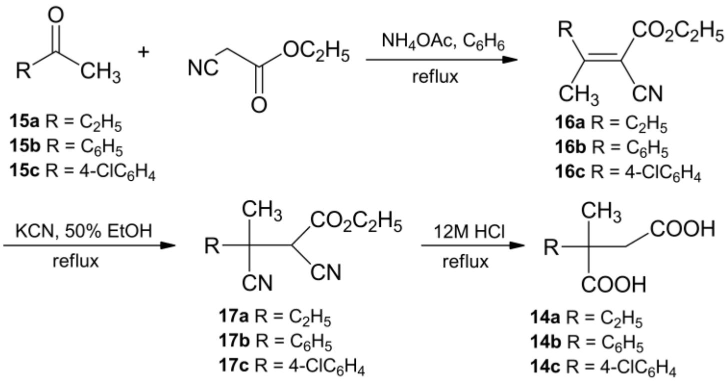
***Scheme 2** Sunyhesis of N-(3- and 4-substituted phenyl)-2-fenil- and N-(3- and 4-substituted phenyl)-2-methylsuccinimides (Series **2** and **3**)*

Microwave-assisted preparation of investigated *N*-aryl-2-disubstituted succinimides **4a**–**4l** [37], **5a**–**5l** [38] and **6a–6j**, were performed by reacting 2,2-disubstituted succinic acids **13a**–**13c** and corresponding anilines **11** under solvent-free conditions (Scheme 3). This eco-friendly approach is superior to conventional synthetic approach in both required reaction time (15 min *vs.* 3 h of conventional heating) and isolated yield of the product (very good and fair *vs.* fair and poor of conventional heating).



Scheme 3** Green synthetic route of novel three series of investigated N-arylsuccinimides **4–6

2,2-Disubstituted succinic acids **14a**–**14c** were prepared according to modified conventional synthetic procedure [32–34] presented in Scheme 4. The condensation of ketones **15a**–**15c** with cyanoacetate gave α,β-unsaturated cyanoesters **16a**–**16c** which were further treated with KCN and the intermediate dicyanoesters **17a**–**17c** obtained were subjected to acid hydrolysis and decarboxylation. 2,2-Disubstituted succinic acids **14a**–**14c** were obtained in fair yields.



Scheme 4** Conventional synthetic route of 2,2-disupstituted succinic acids **14a**–**14c

In the present study, some synthesized succinimides of series **4**, **5** and **6**, have been performed against eight bacterial strains (the standard used is Streptomycin and Ampicillin) and eight fungal strains (the standard used is Ketoconazole and Bifonazole) [37].

The phenyl substitution of 4-OCH_3, 4-CH_3 and 4-COOH group on *N*-aryl-2-ethyl-2-methylsuccinimides (**4b**, **4c** and **4j**, respectively), has shown good antibacterial activity on tested bacteria, in some cases even better than streptomycin and ampicillin (Table 1). Succinimide **4b** has better MIC/MBC activity than ampicillin, except in the case of *Escherichia coli*, where it has no activity. The compound **4c** has no activity to *Escherichia coli* and *Salmonella typhimurium*, but its activity (MIC/MBC) is better for other bacteria then ampicillin. The succinimide **4j** shows activity to four bacteria: *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Enterobacter cloacae* and *Salmonella typhimurium*. Other compounds from this series did not show any antibacterial activity.

***Table 1** MIC/MBC activity of certain N-aryl-2-ethyl-2-methylsuccinimides **4b**, **4cb**, and **4j** (Values expressed in mg/mL (10% DMSO))*

Comp.	*Staphyl. aureus*	*Bacillus cereus*	*Micrococcus flavus*	*Listeria monocytogenes*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Salmonella typhimurium*	*Escherichia coli*
4b	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	n.a.
4c	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.125/0.5	0.125/0.25	n.a.	n.a.
4j	n.a.	n.a.	n.a.	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	n.a.
Streptomycin	0.05/0.1	0.1/0.2	0.2/0.3	0.2/0.3	0.2/0.3	0.3/0.5	0.2/0.3	0.2/0.3
Ampicillin	0.3/0.4	0.3/0.4	0.3/0.4	0.4/0.5	0.8/1.25	0.4/0.8	0.3/0.5	0.3/0.5

*n.a. no activity

The succinimides of series **4** showed great activity against different species of fungi (Table 2). *N*-(4-bromophenyl) derivative **4h** which should be particularly highlighted, having higher antifungal potential even then a standard ketoconazole and bifonazole. All compounds of series **4**, except **4k** that has no activity, have much better MIC/MFC activity to *Penicillium ochrochloron* than commercially available drug ketoconazole (Figure 3). The same trend is observed in the case of MIC/MFC activity to *Trichoderma viride* with exception of succinimide **4j** that has no antifungal potential.

***Table 2** MIC/MFC activity of N-aryl-2-ethyl-2-methylsuccinimides **4a–4l** (Values expressed in mg/mL (10% DMSO))*

Comp.	*Aspergillus flavus*	*Aspergillus ochraceus*	*Aspergillus versicolor*	*Aspergillus niger*	*Penicillium ochrochloron*	*Penicillium funiculosum*	*Penicillium verr.var.cycl.*	*Trichoderma viride*
4a	0.063/0.125	0.063/0.125	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	n.a.	0.25/0.5
4b	0.125/0.25	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.125/0.25	0.125/0.25
4c	0.125/0.25	0.25/0.5	0.25/0.5	n.a.	0.25/0.5	n.a.	n.a.	0.25/0.5
4d	0.25/0.5	0.25/0.5	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5
4e	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.125/0.25	0.25/0.5
4f	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.25/0.5	0.5/0.5	0.25/0.5
4g	n.a.	0.25/0.5	0.25/0.5	0.125/0.25	0.063/0.125	n.a.	n.a.	0.25/0.5
4h	**0.125/0.25**	**0.125/0.25**	**0.125/0.25**	**0.125/0.25**	**0.063/0.125**	**0.25/0.5**	**0.063/0.125**	**0.032/0.063**
4i	0.25/0.5	0.25/0.5	0.125/0.25	0.125/0.25	0.125/0.25	0.25/0.5	0.25/0.5	0.25/0.5
4j	0.063/0.125	0.125/0.25	0.063/0.125	0.25/0.5	0.25/0.5	n.a.	n.a.	n.a.
4k	0.25/0.5	0.25/0.5	0.25/0.5	0.5/0.5	n.a.	0.25/0.5	0.25/0.5	0.25/0.5
4l	0.25/0.5	0.25/0.5	0.125/0.25	0.25/0.5	0.25/0.5	n.a.	0.25/0.5	0.25/0.5
Ketoconazole	0.20/0.50	0.15/0.20	0.20/0.50	0.20/0.50	1.00/1.50	0.20/0.50	0.20/0.30	1.00/1.50
Bifonazole	0.15/0.20	0.15/0.20	0.10/0.20	0.15/0.20	0.20/0.25	0.20/0.25	0.10/0.20	0.15/0.20

*n.a. no activity

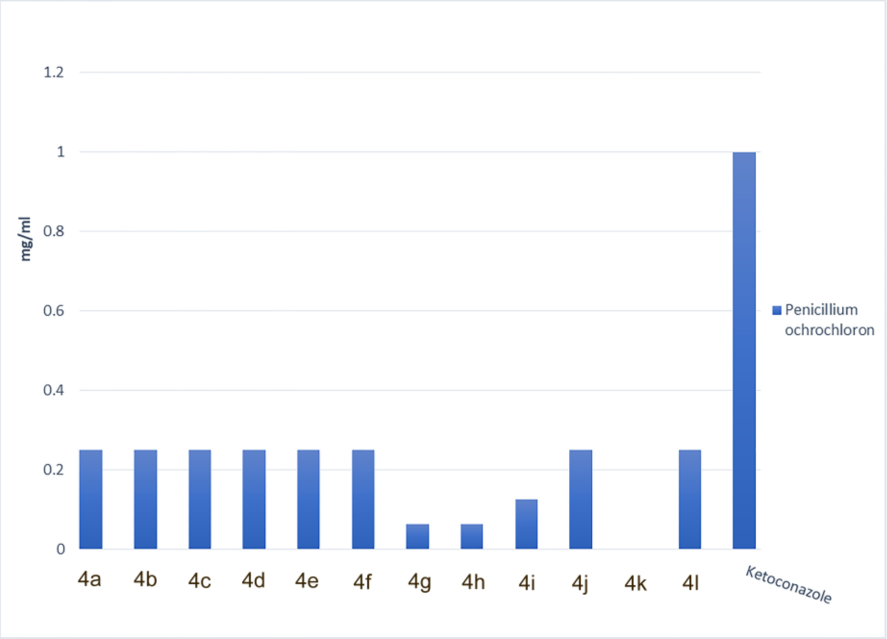


Figure 3 *Antifungal activity of N-aryl-2-ethyl-2-methylsuccinimides **4a–4l** and ketoconazole to Penicillium ochrochloron*

The results indicated that tested compounds showed higher antifungal effect than antibacterial potential and are effective against almost all fungal species, with exception toward *Penicillium* species. These particular antimicrobial results candidate the investigated succinimides of series **4** as promising antifungal agents.

Table 3 *MIC/MFC activity of certain N-aryl-2-methyl-2-phenylsuccinimides **5f–5i** and N-aryl-2-(4-chlorophenyl)-2-methylsuccinimides **6e–6h** (Values expressed in mg/mL (10% DMSO))*

Comp.	*Aspergillus fumigatus*	*Aspergillus ochraceus*	*Aspergillus versicolor*	*Aspergillus niger*	*Penicillium ochrochloron*	*Penicillium funiculosum*	*Penicillium verr.var.cycl.*	*Trichoderma viride*
5f	4.00/>4.00	4.00/>4.00	4.00/>4.00	n.a.	4.00/>4.00	4.00/>4.00	2.00/4.00	2.00/4.00
5g	2.00/4.00	2.00/4.00	4.00/>4.00	n.a.	4.00/>4.00	4.00/>4.00	2.00/4.00	4.00/>4.00
5h	1.90/3.80	1.90/3.80	3.80/>3.80	n.a.	n.a.	3.80/>3.80	3.80/>3.80	3.80/>3.80
5i	3.80/>3.80	3.80/>3.80	3.80/>3.80	n.a.	3.80/>3.80	3.80/>3.80	1.90/3.80	3.80/>3.80
6e	2.00/4.00	4.00/>4.00	4.00/>4.00	n.a.	4.00/>4.00	4.00/>4.00	2.00/4.00	2.00/4.00
6f	4.00/>4.00	4.00/>4.00	4.00/>4.00	n.a.	4.00/>4.00	4.00/>4.00	2.00/4.00	4.00/>4.00
6g	1.00/2.00	2.00/4.00	4.00/>4.00	n.a.	4.00/>4.00	4.00/>4.00	2.00/4.00	4.00/>4.00
6h	3.80/>3.80	3.80/>3.80	3.80/>3.80	n.a.	3.80/>3.80	3.80/>3.80	1.90/3.80	3.80/>3.80
Ketoconazole	0.20/0.50	0.15/0.20	0.20/0.50	0.20/0.50	1.00/1.50	0.20/0.50	0.20/0.30	1.00/1.50
Bifonazole	0.15/0.20	0.15/0.20	0.10/0.20	0.15/0.20	0.20/0.25	0.20/0.25	0.10/0.20	0.15/0.20

*n.a. no activity

Considering the significant antifungal potential of halogen succinimide derivatives **4e–4i**, in the pursuit of our study, halogen derivatives of series **5** and **6** (**5f–5i** and **6e–6h**, respectively) were screened for antifungal activity, and MIC/MFC values are given in Table 3.

Selected halogen succinimide derivatives: *N*-(4- and 3-chloro-, 4- and 3-bromophenyl)-2-methyl-2-phenylsuccinimides (**5f**–**5i**) and *N*-(4- and 3-chloro-, 4- and 3-bromophenyl)-2-(4-chlorophenyl)-2-methylsuccinimides (**6e**–**6h**), did not show desired antifungal potential. As it can be seen from Tables 2 and 3 and Figure 4, succinimides **4e**–**4i** have much greater antifungal potential than succinimides **5f**–**5i** and **6e**–**6h**.

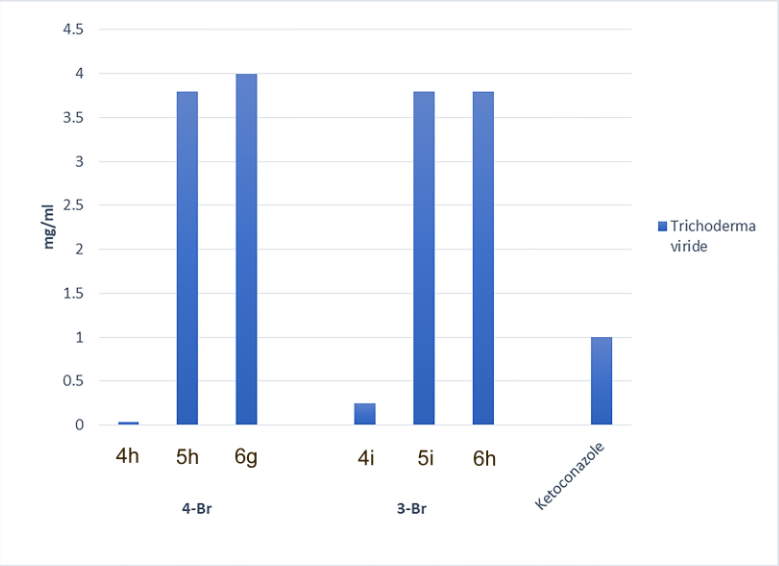


Figure 4 *Antifungal activity of bromo sucinimide derivatives fo series **4**–**6** and ketoconazole to Trichoderma viride*

Very likely, a presence of phenyl group in position 2 of succinimide nuclei is an important cause for poor antifungal activity of chloro and bromo succinimide derivatives **5f**–**5i**, and **6e**–**6h** in comparison to **4e**–**4i**. Phenyl group instead of ethyl, induces higher molar refractivity (parameter that reflects molar volume and easiness in polarization of molecule) of the molecule of series **5** and **6**. The phenyl group increases the molar volume of the molecule by its steric effect, and with its electronic effect increases the delocalization of the electrons through the entire succinimide molecule, which leads to a reduction in the antifungal activity of the succinimides **5f**–**5i** and **6e**–**6h**.

CONCLUSION

In summary, microwave-assisted condensation procedure for closure of the succinimide ring is performed in the absence of solvent, which reduces waste, it is energy efficient, therefore, it represents a greener preparation than the conventional synthesis of succinimide derivatives. Additionally, this approach is superior to conventional synthetic approach in both isolated yields of the product and required reaction time. The certain newly synthesized succinimides were tested for antimicrobial activity and showed higher antifungal potential than antibacterial effect. Antimicrobial activity screening of *N*-aryl-2,2-disubstituted succinimides **4a**–**4l** displayed the potency of bromo derivative **4h** as a new potential fungicide.

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Conference Papers

ACTIVITY LEVELS OF ^{137}Cs AND ^{40}K IN MOSS COLLECTED IN 2018 FROM THE NP ĐERDAP

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Abstract

Moss samples (33 samples, 10 species) were collected in June 2018 on the territory of NP Đerdap from 14 management units and two regions: Dobra (17 samples, 7 species) and Đerdap (16 samples, 8 species). ^{137}Cs and ^{40}K were present in all samples. Activity levels of ^{137}Cs and ^{40}K in investigated samples were from 14.9 to 251 Bq/kg and from 127 to 721 Bq/kg, respectively. The average activity level of ^{137}Cs in moss samples was 82.7 Bq/kg and for ^{40}K it was 297 Bq/kg. Research in 2018 shows that activity levels of ^{137}Cs and ^{40}K in moss from the Đerdap region were higher compared to the levels in moss from the Dobra region. Activity levels of ^{137}Cs in moss collected in 2018: Hypnum cupressiforme Hedw. (Dobra, Đerdap), Polytrichum formosum Hedw. (Dobra, Đerdap) and Isothecium alopecuroides (Dubois) Isov. (Dobra) were lower when compared to values obtained in previous years. The ratio between average values of ^{137}Cs and ^{40}K is from 0.049 to 0.786. The Pearson correlation coefficient between ^{137}Cs and ^{40}K is positive (0.265) leading to the conclusion that there is no linear correlation between these two radionuclides.

Keywords: National Park Đerdap, mosses, radioactivity, ^{137}Cs, ^{40}K

INTRODUCTION

The National park (NP) Đerdap, is one of the parks in Serbia. It is situated in the Carpathian mountain region in Northeastern Serbia close to the boundary with Romania. Its area is 63608 ha, about 100 km long along the left bank of the Danube. It encompasses a narrow woodland mountain area about 3-9 km wide along the Danube, with a height of about 50-900 m altitude. It was founded in 1974 [1].

Development of ecology as a science and better understanding of the food chain and biochemical cycles indicated the necessity of viewing short and long-term consequences of the influence of radionuclides on individuals, populations, communities and ecosystems. The behavior of radionuclides in the environment has been the subject of scientific research based on radiation biology and ecology. Radionuclides enter the soil and water through migration and accumulation processes. This way they can enter into a plant and by its consumption into an animal or human body [2].

In nature, radionuclides are found in the air, water and soil and are part of rocks, seas and oceans. Over 1500 radionuclides are known and they can be grouped into two categories: natural and anthropogenic (produced). The origin of natural radionuclides can be primordial (initial - before the formation of Earth) and cosmogenic (formed as the result of interaction between cosmic rays). ^{40}K is a primordial radionuclide that is present in 0.0117% in natural potassium [3]. ^{40}K is one of the main radionuclides in magma, an unavoidable component in the food chain (soil-plant-human). Humans ingest about 44000 Bq yearly of this radionuclide with food. The physical half-life of ^{40}K is $T_{1/2}=1.28\cdot10^9$ years. Since the sixties of the last century produced (artificial) sources of ionization radiation represent a significant problem besides natural sources of ionization radiation. According to the UNSCEAR data large amounts of ^{137}Cs entered into the environment after nuclear tests (1945-1980) [4] that is most dangerous and significant for the living world. The physical half-life of ^{137}Cs is 30.2 years and it is a chemical analogue of potassium and follows its metabolitic paths in an organism. In April 1986, the accident in the Chernobyl (present Ukraine) nuclear power plant took place. $3.8\cdot10^{16}$ Bq ^{137}Cs was emitted into the environment. Different parts of Europe were contaminated differently. It was estimated that during 1986 about 10% of the total emitted ^{137}Cs was deposited on the territory of Yugoslavia [5]. Research by our scientists have shown that the accident in the Fukushima nuclear power plant (2011, Japan) did not have an influence on ^{137}Cs deposition in Serbia [6].

Mosses are suitable bioindicators for research and tracking of the deposition of radionuclides in from the air. They do not have a developed root system, body, leaf and cuticle. The absence or strong reduction of cell membrane and their thin leaves enable easy adoption of water and nutrients from the atmosphere. They can absorb radionuclides, heavy metals, pesticides and other pollutants in measurable concentrations. About 661 species of moss grow in Serbia and 229 moss species grow on the territory of the NP Đerdap [7]. Mosses as indicators of environment pollution were first used in Sweden.

MATERIALS AND METHODS

Research that has been conducted on the territory of the NP Đerdap in the last few years has shown that the content of ^{137}Cs and ^{40}K in moss samples is not homogeneous. Moss samples (33, 10 species) were collected in June 2018 on the territory of NP Đerdap from 14 management units (MU), from two regions (number of samples): Dobra (17) and Đerdap (16). The following mosses were collected (number of samples): **1.** *Hypnum cupressiforme* Hedw. (13), **2.** *Brachythecium salebrosum* (F. Weber and D. Mohr Schimp) (4), **3.** *Atrichum undulatum* (Hedw.) P. Beauv (3), **4.** *Polytrichum formosum* Hedw. (4), **5.** *Brachythecium rutabulum* (Hedw.) Schimp (3), **6.** *Dicranum scoparium* Hedw. (2), **7.** *Isothecium* *alopecuroides* (Dubois) Isov. (1), **8.** *Anomodon viticulosus* (Hedw.) Hook and Tayl. (1), **9.** *Anomodon attenuatus* (Hedw.) Hueb (1), **10.** *Plagiothecium denticulatum* (Hedw.) Schimp (1). After sample transport to the laboratory, they were cleaned from noticeable dirt (earth, grass, pine needles), dried and homogenized and packed into Marinelli vessels with a volume of 1.0 L. The sample mass was up to 150 g.

An ORTEC - AMETEK, USA semiconducting germanium high purity detector with 8192 channels, resolution of 1.65 keV and relative efficiency of 34% at 1.33 MeV for ^{60}Co was used to determine the radionuclide content. Calibration of the detector efficiency and energy was performed by the Department of Physics, Faculty of Natural Science University of Novi Sad. All samples were measured for 60000 s. Spectra analyses was performed using the Gamma Vision 32 software [8]. The ^{40}K content was determined using the gamma line at 1460 keV, while the ^{137}Cs content was determined using the line at 661.6 keV.

In order to secure checking of the measuring device for potential pollution periodic measurements of the detector system background was performed. Background measurement was performed prior to sample measurements. The relative measurement instability of all results was up to 10% of activity levels in analyzed samples. Quality control of gamma spectrometric measurements was performed using a calibration standard and reference materials and by regular yearly participation in comparisons between laboratories organized by the International Agency for Atomic Energy.

RESULTS AND DISCUSSION

Table 1 shows the moss species (number of samples), management unit, activity level (Bq/kg) of ^{137}Cs and ^{40}K, their ratio in moss collected in 2018 from the Dobra region. Activity levels of ^{137}Cs in investigated moss samples were from 14.9 Bq/kg (moss 7, MU Čezava) to 128 Bq/kg (moss 1, MU Leva reka). Activity levels of ^{40}K in investigated moss samples were from 127 Bq/kg (moss 4, MU Boljetinska reka) to 470 Bq/kg (moss 5, MU Kožica). Research shows that the average activity levels and standard deviation for ^{137}Cs and ^{40}K in moss from the Dobra region were 60.1±43.0 Bq/kg and 278±116 Bq/kg, respectively.

Table 2 shows the moss species (number of samples), management unit, activity level (Bq/kg) of ^{137}Cs and ^{40}K, their ratio in moss collected in 2018 from the Đerdap region. Activity levels of ^{137}Cs in investigated moss samples were from 36.5 Bq/kg (moss 1, MU Tekija) to 251 Bq/kg (moss 3, MU Crni vrh). Activity levels of ^{40}K in investigated moss samples were from 145 Bq/kg (moss 9, MU Tekija, Kosovica) to 721 Bq/kg (moss 2, MU Brzujka). Research shows that the average activity levels and standard deviation for ^{137}Cs and ^{40}K in moss from the Đerdap region were 107±62.7 Bq/kg and 318±133 Bq/kg, respectively.

From Tables 1 and 2 follows that ^{137}Cs and ^{40}K are present in all investigated samples, mosses are radionuclide bioaccumulators, and activity levels of ^{137}Cs and ^{40}K in moss from the Đerdap region are higher in relation to the values in moss from the Dobra region.

The average activity levels and the standard deviation for ^{137}Cs and ^{40}K in moss collected in 2018 on the territory of NP Đerdap, in the Dobra and Đerdap regions, were 82.7±57.7 Bq/kg (min 14.9 Bq/kg (moss 7, MU Čezava), max 251 Bq/kg (moss 3, MU Crni vrh)) and 297±124 Bq/kg (min 127 Bq/kg (moss 4, MU Boljetinska reka), max 721 Bq/kg (moss 2, MU

Brzujka)), respectively. The average activity levels for ^{137}Cs are lower compared to the values obtained in samples collected in the previous years that leads to the conclusion that there were no new pollutions with this radionuclide on the territory of the NP Đerdap [9–11].

Table 1 *Moss species (number of samples), management unit, activity levels (Bq/kg) of ^{137}Cs and ^{40}K, their ratio in moss collected in June 2018 in the Dobra region*

Moss (number of samples)	**Management unit**	^{137}**Cs**	^{40}**K**	^{137}**Cs/**^{40}**K**
		(Bq/Kg)		
1 (7)	Čezava	25.2	203	0.124
		17.4	221	0.079
	Leva reka	128	360	0.356
	Desna reka	32	157	0.204
	Boljetinka	116	435	0.267
	Pecka bara	126	335	0.376
		114	419	0.272
2 (3)	Desna reka	15.5	319	0.049
	Kožica	70.4	408	0.173
		101	157	0.643
3 (1)	Leva reka	35.2	348	0.101
4 (2)	Boljetinska reka	67.9	127	0.535
	Boljetinka	81	131	0.618
5 (1)	Kožica	36.7	470	0.078
6 (2)	Čezava	19.1	224	0.085
		20.6	257	0.080
7 (1)	Čezava	14.9	157	0.095

Table 2 *Moss species (number of samples), management unit, activity levels (Bq/kg) of ^{137}Cs and ^{40}K, their ratio in moss collected in June 2018 in the Đerdap region*

Moss (number of samples)	**Management unit**	^{137}**Cs**	^{40}**K**	^{137}**Cs/**^{40}**K**
		(Bq/Kg)		
1 (6)	Crni vrh	138	263	0.525
		102	269	0.379
	Tekija, Dafin	120	334	0.359
	Tekija	36.5	269	0.136
		48	296	0.162
	Manastirički gaj	79	192	0.411
2 (1)	Brzujka	72.6	721	0.101
3 (1)	Crni vrh	251	417	0.602
		141	246	0.573
4 (2)	Crni vrh	53.9	413	0.131
	Faca Tekija	93.7	250	0.375
5 (2)	Crni vrh	65	276	0.236
	Tekija, Kosovica	111	356	0.312
8 (1)	Crni vrh	45.3	226	0.175
9 (1)	Tekija, Kosovica	114	145	0.786
10 (1)	Prapezešće	237	407	0.582

The ratio between average activity levels of ^{137}Cs and ^{40}K was from 0.049 (moss 2, Dobra region, MU Desna reka) to 0.786 (moss 9, Đerdap region, MU Tekija, Kosovica). The

Pearson correlation coefficient between ^{137}Cs and ^{40}K is positive (0.265) and its value leads to the conclusion that there is no linear correlation between these two radionuclides.

CONCLUSION

Radionuclides ^{137}Cs and ^{40}K were present in all investigated moss samples collected in 2018 from the NP Đerdap. Mosses are good bioindicators of radionuclide pollution. Activity levels of ^{137}Cs in investigated moss samples from the Dobra region were 14.9-128 Bq/kg and for ^{40}K they were 127-470 Bq/kg. The average activity level in moss from the Dobra region for ^{137}Cs was 60.1 Bq/kg and for ^{40}K it was 278 Bq/kg. Activity levels of ^{137}Cs in investigated samples from the Đerdap region were 36.5-251 Bq/kg and for ^{40}K they were 145-721 Bq/kg. The average activity level in moss from the Đerdap region for ^{137}Cs was 107 Bq/kg and for ^{40}K it was 318 Bq/kg. The activity levels of ^{137}Cs and ^{40}K in moss from the Đerdap region were higher compared to the levels obtained in moss from the Dobra region.

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ESTIMATION OF HAZARDOUS MINING AND QUARRYING WASTE QUANTITY IN THE REPUBLIC OF SERBIA

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Abstract

The aim of this paper is estimation of generated hazardous waste amount in the Republic of Serbia (RS) from mining and quarrying during the period between 2014 and 2018 due to exemption of this kind of hazardous waste from the scope of the Waste Framework Directive by The Mining Waste Directive after the reference 2015 year, with prognosis for the period 2020 – 2024. The greatest quantities of hazardous waste have been reported in mining sector in RS. The share of mining and quarrying waste in total amount of generated waste was around 80% during period 2014 – 2018, and one-third of this waste, or more, represents the hazardous waste. Based on available data, amount of at least 35,000 tons per year of mining hazardous wastes for physical, chemical or thermal treatment can be estimated – the minimum 18,000 tons in the period between 2020 and 2024. Furthermore, waste highly influenced by mineral residues deriving from mining activities disposed to landfills are in the range 30,000 to 40,000 tons per year, with prognosis that it can be expected much more, approximately 60,000 tons. Based on simplified calculations quarrying hazardous waste would maintain its quantity in the range 11.0 – 17.0 million tons.

Keywords: mining, quarrying, waste, hazardous, generation

INTRODUCTION

Hazardous waste is defined by the Waste Framework Directive (WFD) 2008/98/EC as "waste which displays one or more of the hazardous properties listed in Annex III of WFD" 0. The hazardous waste represents the "waste that according to its origins, composition or concentration of hazardous substances may cause danger to the environment or human health and has at least one of dangerous characteristics determined by special regulation", in compliance with the Waste Management Law of the Republic of Serbia 0.

The share of hazardous wastes in total amount and generation of waste is relatively low. However, the generation of hazardous waste requires a special attention due to its potential negative affects to human health and the environment 0. Hazardous waste is generated almost in all economic activities. The main sources of hazardous waste represent the sectors of industry, mining and quarrying, construction, services and commerce, waste treatment and health, households etc. The waste generated in mining and quarrying sector might be identified as rocks (stone pieces) and mill tailings (residues obtained after separation minerals from the ore) 0, which after treatment might contain hazardous substances.

The largest quantities of hazardous waste reported in the Republic of Serbia (RS) come from mining and quarrying, i.e. mining and stone exploitation sector and the manufacturing industry 0. The mining sector is an important part of the Serbian economy. It was estimated that there are approximately 200 active mines in RS, according to preliminary projections by the Ministry of Mining and Energy, which primarily produce copper, iron, coal, and significant production of gold, lead, salt and selenium 0. However, there are also around 250 mining waste sites which have been abandoned, inactive or their precisely location is unknown 0. Systematized and complete information about these mining waste sites does not exist, especially data regarding type, characteristics and quantity of mining waste, i.e. data about residual waste and pollution. Likewise, precise informations of generated amount of hazardous waste from mining and quarrying in RS are not exactly defined.

The aim of this paper is to estimate the amount of generated hazardous waste in RS from mining and quarrying sector during the period 2014 – 2018 due to exemption of this kind of hazardous waste from the scope of the WFD by the Mining Waste Directive after the reference 2015 year 0 with prognosis for future period 2020 – 2024.

MINING AND QUARRYING WASTE AND LEGISLATION

Mining and quarrying waste represents the waste generated during the geological exploration, exploitation, preparation and storage of mineral resources and working of quarries. As mentioned, the mining waste is excluded from the scope of the WFD and the same approach has also been transposed into the Waste Management Law of RS 0.

Mining Waste Directive (2006/21/EC), i.e. Directive on mining waste management imposes reduction of treatment and disposal of extractive waste impact on the environment and human health, but not under the waste legislation. This directive was transposed by the Law on Mining and Geological Exploration in RS legislation in 2015 0. This particular extractive waste must be managed in specialized facilities in compliance with specific rules. From reference year the Law on Mining and Geological Exploration sets up the framework for proper management of wastes resulting from extractive industries in RS along with the Mining Waste Directive. In 2017 RS has established the Regulation on the Conditions and Procedures for Issuing Permits for Waste Management, as well as the Criteria, Characterisation, Classification and Reporting on Mining Waste 0. Based on the Waste Management Law the preparation of a cadastre of Mining Waste is underway, aiming to develop and improve the mining waste management system in the state 0.

Identification of mining wastes sites, environmental assessment, and data base establishing due to management, remediation and re-cultivation represent the crucial issues. The provisions of the Mining Waste Directive and the Law on Mining and Geological Exploration do not apply to waste streams which, although generated during mineral extraction, treatment operations or transport to other location, are not directly linked to the extraction or treatment process, e.g. waste oils from maintenance, end-of-life vehicles, spent accumulators etc. These wastes are regulated by the Waste Management Law and its by-laws.

The European waste list is structured on the first level by origin of wastes. Since hazardous waste may arise in all economic activities, the List of waste contains entries for all wastes types, i.e. chapters (categories) of waste. Chapters from 02 to 16 are attributed to wastes from agriculture and forestry, and to industrial and commercial wastes. Separate chapters exist for waste from the construction and demolition sector, medical sector, treatment of waste and wastewater, and sector of mining and quarrying 0. The wastes from mining and quarrying are covered by chapter 01. This chapter is named “Wastes resulting from exploration, mining, quarrying, physical and chemical treatment of minerals” 0. The waste quality from exploitation of mineral raw materials ranges from inert residues (e.g. 01 04 08), to potentially leachable waste (e.g. 01 03 04*) or to highly toxic chemicals and extraction residues (e.g. 01 04 07*) 0. In accordance with data from Statistical Office of the Republic of Serbia (SORS) for a period from 2014 to 2017 a share of 32.4 – 44.3% of mining and quarrying waste is characterized as hazardous waste (data for 2018 have not been yet processed) 0.

MINING AND QUARRYING HAZARDOUS WASTES QUANTITY

The two main data sources for generation of hazardous waste in RS are available 0: Statistical Office of the Republic of Serbia (SORS) and the Serbian Environmental Protection Agency (SEPA). However, they are based on different legal reporting obligations, i.e. SEPA data are based on WFD, while SORS data are unifies all collected data 0.

The main results from both of these data sources are presented in the following description of generation and treatment of hazardous wastes in RS 0. Due to implementation of the Mining Waste Directive in legislation, SEPA and SORS data published in April 2017 in the Serbian Integrated Hazardous Waste Management Plan, contains only amounts of mining and quarrying wastes until 2015 as the reference year 0. According to SEPA data, the total generation of industrial and commercial waste in RS in 2014 was approximately 5.9 million tons, of which 209,877 tons was classified as hazardous waste 0. These data include 155,044 tons of wastes from mining and quarrying, which from the 2015 reference year are not included in SEPA statistics on hazardous waste generation. It should be noted that share of hazardous wastes from mining and quarrying of the total generated hazardous wastes quantity from industrial and commercial sectors in 2014 was even about 73.9% 0.

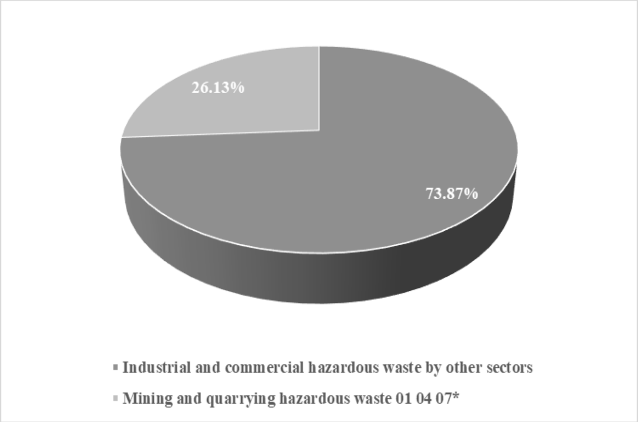

Figure 1 *The share of hazardous wastes from mining and quarrying of the total quantity of hazardous wastes generated by industrial and commercial operators in 2014 0*

This waste belongs to 01 04 07* category, i.e. "wastes containing hazardous substances from physical and chemical processing of non-metalliferous minerals" 0. Its amount includes hazardous wastes resulting from exploration, mining, quarrying or from physical and chemical treatment of minerals. The data on the generation of hazardous waste covers quantities reported by companies which submitted to SEPA annual reports on the types and quantities of waste generated in the course of their business activities 0.

According to SORS Statistical Release ZS60 data the total amount of waste generated and treated during period 2014 – 2017 has been almost eight times higher then SEPA statistics for 2014 (Table 1) 0. Differences between amounts of SORS and SEPA came from statistics of SORS which includes wastes from mining and quarrying, which are not covered by the statistic of SEPA from 2014, since these wastes are not covered by the WFD anymore 0. The quantities of generated waste for 2018 is yet not available, as mentioned. Also, SORS Statistical Release ZS60 for 2017 represent the preliminary results, but this document shows that generated waste quantities recorded decrease in majority of economic activities, i.e. mining and quarrying sector have shown decrease of 0.8%.

Table 1 *Generated waste in RS according to SORS (total and mining and quarrying amount by year)*

Year	Total (million tons)	Mining and quarrying (million tons)	Mining and quarrying hazardous waste (%)
2014	47.5	41.5	28.3
2015	49.3	41.4	N/A
2016	47.2	38.7	44.3
2017	48.9	N/A	N/A

According to SORS the share of generated waste in RS by mining and quarrying sector in generated waste total amount is around 80% during period 2014 – 2018, and one-third of this waste or more represents the hazardous waste 0.

MINING AND QUARRYING HAZARDOUS WASTES QUANTITY PROGNOSIS

The objectives of this study as well were forecasting of mining hazardous waste quantities. The prognosis of waste quantities depends on several factors, e.g. demographic, economic and social. The developments in the waste management sector, e.g. the improvements in the separate collection of waste types, also have a high influence on the future waste quantities and waste composition. The forecast presented here is based, inter alia, on data of current waste quantities, assumptions on developments of separate waste collection, prognosis of economy and population development, and in some cases, on data of selected other countries with similar generation of specific hazardous waste streams. The forecasts presented below are based on population and migration 0, as well as industrial production and hazardous wastes stream from mining and quarrying in past five years according to SORS and SEPA data. Industrial production taken into account was focused on mining (without stone exploitation), processing industry, metallurgical and chemical production.

According to available data, the annual volume of mining waste generated in RS is estimated to be around 150 million m^3 of solid mining waste (not subject to the Waste Management Law), around 15.5 million m^3 of waste from flotation, and 35,000 m^3 of oil mud (at least partly covered by the Waste Management Law) 0. Improved management of mining waste after the 2015 under the control of the Law on Mining and Geological Exploration is likely to increase the amount of hazardous wastes from mining operation for treating in dedicated waste management facilities (mainly various types of sludge). This investigation has shown that the amount of at least 35,000 tons per year of wastes for Physical/Chemical (P/C) or thermal treatment can be estimated. Although wastes from mining and quarrying sector were excluded from the statistics of SEPA, according to some preliminary data, in 2016 about 18,000 tons of hazardous wastes from mining still were treated in facilities which require permitting in accordance with the WFD. Therefore, it can be assumed that similar minimum quantities would be treated also in near future, i.e. period 2020 – 2024. Estimated quantities based on prognostic numbers of mining hazardous waste generation, i.e. waste highly influenced by mineral residues deriving from mining activities disposed to landfills are in the range 30,000 to 40,000 tons per year. Experience from other EU members shows that expected quantity of mineral hazardous residues generated by multiple industrial and commercial activities could be even higher, approximately up to 60,000 tons 0.

Although quarrying hazardous waste was excluded from above prognosis, since its large share in mining and quarrying waste it might be concluded that this kind of waste would not change its quantity in the next years radically. Therefore, based on forecast for mining hazardous waste amount and mining and quarrying waste SORS data for period 2014 – 2018, it can be calculated that this hazardous waste would maintain the quantity in the range between 11.0 to 17.0 million tons.

The best practice and solution for this amount of dangerous waste types would be reuse (i.e. renew) after process of waste inertisation, i.e. solidification and stabilisation, as raw materials in civil engineering whenever possible 0.

CONCLUSION

The largest quantities of hazardous waste reported in RS come from mining and quarrying. The aim of this paper was to estimate the amount of generated hazardous waste from this sector during 2014 – 2018 due to exemption of this kind of hazardous waste from the scope of the WFD by The Mining Waste Directive after the reference 2015 year with future prognosis for period 2020 – 2024. According to SEPA, the total generation of industrial and commercial waste in RS in 2014 was approximately 5.9 million tons, of which 155,044 tons were mining hazardous waste. According to SORS, the total waste generated amount during 2014 – 2017 was eight times larger per year. Differences came from different statistics, i.e. corresponding directives/laws. The SORS data have shown that a share of 32.4 – 44.3% of mining waste was characterized as hazardous waste (data for 2018 have not been yet processed).

This study has shown that at least 35,000 tons per year of mining wastes for P/C or thermal treatment can be estimated (18,000 tons of hazardous wastes from mining need to be treated in facilities which require permitting in accordance with the WFD) in period 2020 – 2024.

Estimated quantities for hazardous mining waste disposed to landfills are in the range 30,000 to 40,000 tons per year. The quantity prognosis for quarrying hazardous waste was calculated to be in the range 11.0 to 17.0 million tons for the same period.

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APPLICATION OF STANDARD ISO 14001 IN THE ENVIRONMENTAL PROTECTION SYSTEM

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Abstract

Implementation of ISO 14001 standards - The environmental management system, following the ISO 9001 standard, is becoming increasingly common in our organizations. This is particularly true for construction companies that are significant waste generators and environmental pollutants. On this, they are bound by increasingly stringent legislation, on the one hand, and a more favorable position in public vacancies if they have introduced a standard, on the other. Bearing in mind the experience of the author in the implementation of the ISO 14001 standard, the paper will present the basic procedures for the efficient functioning of the environmental management system.

Keywords: standard, management, environment, aspects and impacts, waste

INTRODUCTION

In order to ensure sustainable development, it is essential to strike a balance between the environment, society and the economy. Social expectations for sustainable development, transparency and accountability have evolved with increasingly stringent legislation, growing pressures on the environment from pollution, inefficient use of resources, improper waste management, climate change, degradation of ecosystems and loss of biodiversity [1].

This has led organizations to adopt a systematic approach to environmental management using the environmental management system according to the ISO 14001 model.

The purpose of this international standard is to provide organizations with a framework to protect the environment and respond to changing environmental conditions in balance with socio-economic needs. It specifies requirements that enable an organization to achieve the intended outcomes it sets for its environmental management system. A systematic approach to environmental management can provide top management with information to build success over the long term and create options for contributing to sustainable development by [2]:

- protecting environmental by preventing or mitigation adverse environmental impacts;
- mitigation the potential adverse effect of environmental conditions on the organization;
- assisting the organization in the fulfillment of compliance obligations;
- enhancing environmental performance;
- controlling or influencing the way the organization’s products and managing and services are designed, manufactured, distributed, consumed and disposed by using a

life cycle perspective that can prevent environmental impacts from being unintentional shifted elsewhere within the life cycle;

- achieving financial and operational benefits that can result from implementing environmentally sound alternatives that strengthen the organization's market position;
- communicating environmental information to relevant interested parties.

The following text will describe aspects of the environment, environmental impact assessment, and the waste management process for a company engaged in the design and execution of construction works.

ASPECTS OF ENVIRONMENT AND IMPACTS

Definitions

Environmental definitions are given in the Law on Environmental Protection [3], and for the purpose of this work only some of the following will be listed:

- Environment - the environment in which a particular organization operates, including air, water, land, natural resources, flora, fauna, people and their mutual relationships. In this context, the environment extends from the one within the organization to the global system.
- Environmental aspect - an element of an activity, product or service of a given organization, which can be in a mutual relationship with the environment.
- Environmental Impact - Any change in the environment, deterioration or improvement, which is completely or partially the result of the environmental aspects of the organization.
- Evaluation of the significance of environmental aspects - determining the significance of environmental aspects based on legal regulations, frequency, severity, location and level of control.

Identification of aspects

Identification of aspects is done in such a way that for every business process of the enterprise all activities with environmental aspects are listed. For the analysis of the aspects, technical documentation is used, as well as other documentation of the management system (QMS, EMS, OHSAS).

Impacts that are of importance in the construction company are:

- Emissions of gases into the air (transport means and working machines on the construction site),
- Discharge of technical, sanitary and atmospheric waters into the wastewater system,
- Wastes (solid and liquid) that arise as a result of activities in the realization of the service of export of construction works,
- Pollution of soil and groundwater,
- Use of natural resources

Determining the importance of the environmental impact

The intensity of the environmental impact is based on the following five criteria [4]:

The frequency of the occurrence of the impact is calculated in relation to the periodicity of the occurrence of the impact. Depending on the frequency, grades 1 to 4 are given, where 1 is the smallest and 4 with the greatest influence.

- U = 1 - rarely (once a year and less),
- U = 2 - 1-2 times a year,
- U = 3 - 1 times a week to 1 time a month,
- U = 4 - every day.

The severity of the environmental impact. Depending on the severity, estimates are given from 1 to 4, where 1 assesses a situation without a serious environmental impact, and grade 4 is given for major environmental impacts.

- O = 1 - without affecting the LC,
- O = 2 - small seriousness, doesn’t require remedy,
- O = 3 - medium seriousness, greenhouse effect, natural resources,
- O= 4 - great influence, flora and fauna disorder, expensive remediation.

The location where it occurs, the impact is estimated from the point of view of the geographical spread of the impact on the ZS. The smallest rating is given for the impacts that affect the place where the facility/equipment is used/built, and the largest estimate for the effects that are spreading beyond the location, the location of the construction/use of the facility.

- L = 1 - at the place of construction/use,
- L = 2 - in the circle of the location,
- L = 3 - immediate environment,
- L = 4 - wider surroundings.

The pressure of the stakeholders (lawmaker, buyer, etc.), is the criterion where the legislator or other interested party exerts pressure on the company. The smallest rating is given when the activity being monitored has no correlation with legal requirements, and the largest when there is a violation of the law, and no measures are taken.

- Z = 1 - without legal and other requirements/compliance with legal and other requirements,
- Z = 2 - requirements are not respected but are planned, or are in progress, an annual measure, in accordance with the law.
- Z = 3 - requests are not respected despite the measures taken,
- Z = 4 - requests have not been complied with and no measures have been taken.

Control of aspects and impacts is the criterion of evaluating aspects in relation to the control of operations occurring in the enterprise. Aspects that are not controlled, and they do not happen often, must be addressed through reaction in emergency situations.

- K = 1 - the aspect is completely under control;
- K = 2 - rare corrections, corrective / preventive measures;
- K = 3 - frequent corrections, corrective / preventive measures;

- K = 4 - the aspect is not under control

The overall significance is the product of all five criteria, and the significance of which is the value of ≥ 48. An example of an analysis of aspects and impacts, for a construction company is given in Table 1.

Table 1 *Analysis of aspects and impacts [4]*

Process	Activity	Aspect	Influence	Eligibility criteria					Rank of charac-ter
				U	O	L	Z	K	
Waiting and contracting	Telephone and e-mail communication, mail, reception of parties, presentations	Power consumption	Use of natural resources	4	1	1	1	1	4
		Consuming paper for printing	Use of natural resources	4	2	1	1	1	8
	Tour of potential buyers and construction sites	Emission of exhaust gases from the vehicle	Air pollution	4	2	2	1	1	16
Designing	Telephone and e-mail communication, generic documentation	Electricity consumption	Use of natural resources	4	1	1	1	1	4
		Consuming paper for printing	Use of natural resources	4	2	1	1	1	8
		Printing (toners)	Pollution of the environment	4	2	1	1	1	8
Procurement	Procurement Administration	Electricity consumption	Use of natural resources	4	1	1	1	1	4
		Consuming paper for printing	Use of natural resources	4	2	1	1	1	8
	Transport material to site	Emission of exhaust gases	Air pollution	4	2	3	1	1	24
Construction work	Craftsmanship in construction (mason, carpenter, armor, locksmith, auxiliary worker)	Electricity consumption	Use of natural resources	4	1	1	1	1	4
		Emission of exhaust gases	Air pollution	4	2	3	1	1	24
		Fuel consumption	Use of natural resources	4	2	1	1	1	8
		Creating dust	Air pollution	3	2	3	1	1	18
		Construction of construction waste (shot, brick, concrete, stone, wire,...)	Pollution of the environment	4	2	2	1	2	32

Great significance	$R > 48$
Monitoring and control	$12<R<48$
Tolerant significance	$R< 12$

WASTE MANAGMENT

Definitions

Definitions used in the field of waste management are given in the law [5] and subordinate acts (ordinances). For this occasion we will mention only some of the most important in our opinion:

- Waste - any matter or item contained in the list of waste categories that the owner discards, intends or must discard, in accordance with the law.

- Waste management - implementation of prescribed waste management measures in the context of collection, transport, storage, treatment and disposal of waste, including monitoring of these activities.
- Characterization of waste - a test procedure that determines the physico-chemical and biological properties and composition of waste and determines whether the waste contains one or more hazardous characteristics.
- Waste Generator - enterprise, other legal or natural person or entrepreneur, registered for the performance of a waste-generating activity, which owns waste, or in whose territory the waste is located.

Waste management activities

Waste management is carried out in accordance with the Law on Waste Management and must define the following:

- Waste disposal sites at the headquarters and at the site,
- The dynamics of waste disposal,
- Different containers for certain types of waste,
- Agreement with the communal institution on the takeover of municipal waste,
- Agreement with the company for the purchase of secondary raw materials.

Selection of waste - Waste arises as a result of the development of the work process at the headquarters of the company, as a result of the performance of services (execution of works) on the site itself. The following types of waste have been identified:

- Construction waste,
- Waste,
- Other waste

All employees in the company are responsible for the selection of waste. The responsible person for the collection and proper disposal of certain types of waste in his organizational unit is the manager of this organizational unit. The site manager is responsible for the proper collection and disposal of waste at the site.

Waste storage - Temporary disposal of collected, classified waste is carried out in accordance with the Rulebook on conditions and manner of classification, packaging and storage of secondary raw materials [6]. The selection of waste sorting is done at the place of origin. All waste shall be disposed of in places for the temporary disposal of waste to the appropriate amount of generated waste for the period until the next disposal by the authorized institution in the following manner:

- *Construction waste* - Construction waste and demolition wastes, including excavated land from contaminated sites (index 17 from the Catalog of Waste) is created on the site and collected and deposited in a separated and marked place. The removal of this waste from the casing is regulated between the construction company and the authorized company (possessing a permit) for the removal and storage of this type of waste.
- *Municipal waste* - Municipal waste generated at the company's headquarters is collected and deposited in polyethylene bag baskets, which are located in each office.Waste from the basket is discharged at the end of each shift by placing bags in

municipal waste containers outside the company's scope and within the jurisdiction of the Public Utility Company. The removal of municipal waste from the container is regulated between the construction company and the Public Utility Company.

- *Other waste* - Expired parts of equipment, old furniture and the like, it is deposited in a designated place in a warehouse, from where it is controlled remotely outside of the company. The disposal of this waste is regulated between the construction company and organizations for the purchase of secondary raw materials.

Shipments of waste - The commercial function organizes waste dispatch and the necessary documentation.

CONCLUSION

The implemented ISO 14001 standard in organizations is the tool of management for efficient waste management that the organization generates, resulting in a reduction in environmental pollution. The paper presents, for a construction company, aspects of the environment and their environmental impacts, as well as types of waste and method of waste management. In response to emergency situations, which are not considered due to space constraints, these are the key requirements of ISO 14001 standards.

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ECO-INDUSTRIAL PARKS

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Abstract

The goal of Eco-industrial parks is to activate unused development potentials as preconditions for an integrated, balanced, stable and self-sustaining economic and social development in a given area. The Eco-industrial park would enable companies to reduce production costs by increasing energy efficiency and material consumption efficiency, increasing waste recycling levels, and reducing the adverse impacts on the environment. Eco-industrial parks also allow better coordination between the private and public sectors, and create preconditions for a better business environment and job creation.

Keywords: industrial zone, eco-industrial park, circural economy

INTRODUCTION

In the modern world, in which industrialization and globalization are two basic tendencies of economic and social development, a special significance is attributed to a sustainable, environmentally conscious industrial production. Industrial parks around the world, and especially in developed countries, are extremely economically important for society, but at the same time they are the main polluters of the environment. In order to optimize the impact and results of industrial parks, modern industrial production is focused on the development of production systems that follow the principles of cleaner production, industrial ecology, and circular economy, in order to increase the both, and economic and environmental efficiency. In the other words, throughout the world, construction designing and networked industrial plants planning in purpose to improve the economic performance of associated companies, are in progress. At the same time environmental problems caused by rapid economic growth might be avoided [1]. Such closed systems, in which the relationship of industrial symbiosis is established between the participating companies belonging to different branches of industry [2], and material flows circulate and produce no waste [1], are called eco-industrial parks.

INDUSTRIAL PARK

Industrial park (industrial land, area, and zone economic and business zone, industrial estate, high tech and biotechnology park are some of the synonymous terms that circulate in practice and literature [1,3]) represents different branches of industry in a limited area, i.e. land that (the most often municipal) authorities devoted to industrial development [2,3].

Industrial parks are located today on all continents, and are the most numerous in the developed west countries. Industrial parks are built in the wide or narrow areas of a large and medium-sized urban settlements, in a location that is suitable for transport, has water in abundance and is equipped with energy, communal and transportation infrastructure.

By size, industrial parks are divided into: micro-parks (cover up to 10 ha), small parks (10 -50 ha), medium-sized parks (50-100 ha), and large parks (over 100 ha) [1]. According to diversity of cross-industries industrial parks can be complex [composite], auxiliary [ancillary], and the parks of a particular sector [single-trade] [2]. In complex parks (development zones), companies from different industrial branches are integrated. In subsidiary companies there is one main company or parent company integrated with a several smaller companies from other sectors (usually the procurement or service sector), while in single-purpose parks all participating firms belong to the same branch of industry, e.g. chemical, pharmacological or metal industry [2,3]. The most common industrial parks are parks of composite type [2].

The industrial parks could be also possible to divide according to type of investment, i.e. the initial readiness for investment [1]. Two types of the investments also differ, the so-called greenfield investments (investments in green areas) and brownfield investments (investment in brownfield areas). Greenfield investments involve the construction of a "zero-point" park at new locations outside the city center, while brownfield investments involve investments in locations where there is already a certain production or business activity, the most often in the narrow area of the urban settlement. The development of industrial parks encourages local economic development and attracts foreign investment.

However, industrial parks at the same time could have a detrimental impact on the quality of the environment. Their massive construction increases the level of air, water and soil pollution, leads to impoverishment and loss of arable soil, uncontrolled forest cutting, causing water shortages and endangering biodiversity. As a solution for improving the ecological efficiency of industrial parks, it is proposed to build Eco-industrial parks that would bring multiple benefits to the city, region and country in which they are located, as well as the world, to the environment, economy and society [2].

ECO-INDUSTRIAL PARK

The Eco-industrial park is a model of modern resource management through public-private partnership, whose task is to support the environment by supporting economically viable business [1].

Unlike traditional industrial parks based on one-way production [4] which inevitably leads to resource exhaustion and accumulation of waste, eco-industrial parks are based on the circular economy model.

In Eco-park waste, by-products, water and energy are reused, resulting in the conservation of natural resources and the reduction of production costs, industrial processing, and waste reduction [3].

As in the natural ecosystems of the interconnected, durable and complex communities of plants, animals, fungi and microorganisms, in symbiosis to survive and efficiently utilize the available resources, in the eco-park the company participants work together and strive for collective benefit, which is greater than benefits for each company individually [1–3]. Natural ecosystems function according to principles of the optimum production, unlike traditional industrial systems that strive for maximum production. Regarding this, the Eco-industrial park is the part of biosphere, and it can be considered as an attempt to establish a similar balance in the technosphere [1]. In Table 1, the general metaphors of natural and technical systems are presented.

Table 1 *General metaphors of natural and technical systems [1]*

Biosphere	**Tehnosphere**
Environment	Market
Organism	Company
Natural product	Industrial product
Natural selection	Competition
Ecosystem	Eco-industrial Park
Ecological niche	Market niche
Anabolism / Catabolism	Production / Waste Management
Mutations and selection	Ecological design
Heritage	Economic growth
Customization	Innovation
Food chain	Product lifetime

Leading industrial parks around the world are working to improve their reputation and become more attractive to investors, and therefore compete for the status of the eco-industrial park [2]. The conditions that some industrial park must officially fulfill to acquire the marking as Eco-Industrial park will be presented by the case of industrial policy in the People's Republic of China.

In the 1990s, certain Chinese industrial parks adopted strategies to improve their ecological status, with the help of the United Nations Environment Program [2]. This project involved waste production and environmental pollution control, ecological infrastructure modernizing, environmental management offices establishing, extending the industrial symbiosis network, etc. In Figure 1, Zhounggancun Technology Park in Beijing is displayed.

***Figure 1** Zhounggancun Technology Park, Beijing [5]*

In 2003, the Chinese (SEPA) State Environmental Protection Administration, established a new national standard for Eco-industrial parks [2] and issued official guidelines for state-level eco-park planning. The SEPA issues comprehensive guidelines that define the concept of eco-park, the criteria that need to be met, as well as indicators of the success and efficiency of such plants. This document is also the first national standard of this type in the world [2].

The 2006 Standard sets out the following criteria that the industrial park must fulfill when applying for the status of an eco-industrial park [2]:

a) The park must respect and apply all state regulations and regional regulations concerning the protection of the environment.

b) During the last three years there were no ecological catastrophes or accidents within the park that led to environmental pollution.

c) The environment in the local park surroundings must meet the standards established at the state level.

d) The degree of pollution and emissions of harmful substances must be controlled.

e) The plan for the construction of an Eco-industrial park must be evaluated and approved by the SEPA, and adopted by the local government.

One of the general conditions for establishing a modern and environmentally sustainable industrial park, and therefore an eco-park, is adequate equipment for energy, communal and transport infrastructure. Also, the existence of green areas within the park is of paramount importance.

By planting plant species that are highly resistant to gases, smoke and dust, a sanitary protection zone is created that protects against harmful emissions and noise. With this standard SEPA also established indicators of the performance of industrial parks and defined precise formulas for the budget [2].

Eco-industrial parks provide long-term economic, environmental and social benefits.

From the financial point of view, eco-parks are considerably more profitable than traditional industrial parks. First of all, it saves on insurance costs and protection against environmental disasters and no penalties for disrespecting environmental standards are paid.

Contaminants are removed, and hazardous and toxic substances are replaced by less harmful materials. Thanks to the principles of eco-design, products are produced with as high a percentage of biodegradable content or potential for recycling and reuse [1]. Finally, well-being is achieved in the wider social community. Public health and safety are promoted, new jobs are opened and better cooperation is established between the participating companies and the administration of the park. The cooperation between the private and public sectors is enhanced, and municipalities and cities are gaining a significant role in the economic development process [1].

In practice, the construction of eco-industrial parks is an expensive, time-consuming and demanding process. The construction of such park in the Republic of Serbia and the region would be impossible without foreign investments. The Republic of Serbia should have to solve the inadequate planning of land use, poor cross-sectoral co-ordination, unclear property rights, inefficient economic policy, insufficiently educated staff, lack of public and political awareness and insufficient participation of the public, and the education system before serious thoughts on the construction of an Eco-industrial park.

On the other hand, countries that have much more experience with the establishment of Eco-industrial parks, such as China, face practical problems that accompany projects for the construction of eco-parks.

GUIDELINES FOR OVERCOMING THE CHALLENGE OF BUILDING ECO INDUSTRIAL PARK

The first step in improving the ecological and economic efficiency of industrial plants is to raise awareness of the importance of building eco-parks, which can be achieved by informing the public through promotional programs and activities, training workshops, public debates, media content, etc. [2]. Countries must first of all devote themselves to meeting the conditions of the circular economy, not just the construction of Eco-industrial parks [1]. Government authorities must support the establishment of industrial symbiosis among different companies, as well as cooperation between industrial parks and the wider environment, because only in this way can true genuine development be discussed [2].

CONCLUSION

The aim of this paper is to represent the potential achieving way for sustainable resource management and protection of the environment within the industrial system. One way to achieve this goal is to establish the Eco-industrial parks.

Eco-industrial park depends on the local community, its material and human resources.

Companies within the park can share some common business services, such as waste management, shopping, training, information on environmental protection systems, thus achieving greater economic efficiency. From this paper it can be concluded that with the help of Eco-industrial parks, the numerous pollution and waste sources or excessive use of natural resources decreases, new jobs and economic growth of the city will be strengthened.

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GC-MS ANALYSIS OF BOSCALID IN APPLE ORCHARD SAMPLES FROM THE REGION OF JABLANICA - VUČJE

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Abstract

Commercial production of apples involves the use of pesticides for the control of diseases, not only during the growth stages, but also during the post-harvest ripening in the storage, which can leave pesticide residuals in fresh and even in processed fruit. This study was conducted to determine the residue levels of boscalid, pesticide from the class of fungicides, in apples, after five months of field treatment with commercial pesticide formulation. Samples were analyzed by a novel, few-steps only method with suitable performance criteria, according to SANTE guidelines. GC-MS analysis of samples from orchard showed that amount of boscalid was below MRL value i.e. it was 0.1932, 0.3090 and 0.7014 mg kg^{-1} in Golden Delicious, Granny Smith and Idared variety samples, respectively.

Keywords: apples, GC-MS analysis, boscalid

INTRODUCTION

Apples represent a rich source of minerals, vitamins, dietary fibers, and phytochemical antioxidants such as polyphenols [1]. The controlled atmosphere storage of 25% of this crop has allowed a fresh market on the annual basis where the greater part, about 56.5%, is consumed in the fresh state and the rest is processed into dried, canned or frozen products [2]. In European Union in the first half of 2018 the fresh apple production reached 10 million tons, of which 6.4 million tons was freshly consumed, and 3.3 million tons was processed [3].

A regular part of the commercial production of apples is a practice termed integrated pest management and includes pesticides application either during the growth stages or prior to the cold storage. The fact that apples belong to the group of climacteric fruits, enables the fruit to be stored for months *i.e.* provides the availability on the market through the whole year. However, post-harvest fruit ripening usually accompanies increased susceptibility to post-harvest diseases, which are controlled with chemicals from the class of fungicides. This practice can leave residuals in fresh and even in processed fruit in much higher quantities, leading to a harmful effect on consumers health. Thus, the monitoring of the pesticide residue levels in apples is of a great significance.

Boscalid (2-chloro-N-(4'-chlorobiphenyl-2-yl) nicotinamide) is one of the fungicides from the anilid class used in the protection of apple crops, developed by BASF. In EU was approved in 2008 [4]. Several studies revealed the boscalid degradation after the fruit treatment procedures, with the following residue levels significantly below Maximum Residue Level (MRL) [4–6].

This study was conducted to assess the boscalid residue levels in orchard samples of *Granny Smith, Golden Delicious* and *Idared* variety after five months of pesticide application (the analysis was conducted in January 2019). Determination of boscalid was performed by GC-MS as one of the most common techniques for pesticides residues level determination.

MATERIALS AND METHODS

Chemicals

High purity pesticide standard of boscalid was purchased from AccuStandard® (New Haven, CT, USA). HPLC grade ethanol, HPLC grade hexane and HPLC grade methanol were purchased from Fisher Scientific (USA).

Standard preparation

Primary stock solution of 1000.00 µg mL^{-1} was made of high purity pesticide standard in ethanol. Secondary stock solutions of 100.00 and 10.00 µg mL^{-1} were prepared in ethanol by diluting the primary stock solutions. All solutions were stored in freezer at –15 °C.

Samples

For validation procedures, apples of the *Granny Smith, Idared* and *Golden Delicious* variety were randomly chosen at the open-air market (JP "Tržnica", city of Niš, Serbia). Apple fruits were free of visible damages and pests. Screening analysis did show the presence of boscalid in all samples.

For real sample analysis apples of the *Granny Smith, Idared* and *Golden Delicious* variety were harvested form an apple orchard located in the region of Jablanica - Vučje, Serbia. Apples were treated with commercial formulation of boscalid according to the pre-defined treatment plan.

One kilogram of each apple variety was collected, chopped into smaller pieces and homogenized by chopping in food processor. The homogenized portions were transferred to a glass container and stored in the refrigerator until analysis.

Sample preparation procedure

Ten grams (10 ± 0.1 g) of the sample portion was added 20 mL of water and blended. The sample was quantitatively transferred to a glass container with the additional 5 mL of water. Afterwards, solvent extraction was performed, successively with two 10 mL-portions of hexane. The organic phase was separated from the solids-water dispersion and after centrifugation for 5 minutes at 3500 rpm, was subjected to the sample clean-up procedure. Namely, the extract was subjected to a procedure comprising three steps: sample concentration (SC), solvent exchange (SE) and low-temperature precipitation (LTP). Ten milliliters of the extract were evaporated to dryness under a stream of nitrogen, in water bath set at 40 °C. The solid residue was added 1 mL of methanol and left for 10 minutes.

Afterwards, it was thoroughly mixed on a vortex for 1 minute and stored in refrigerator at -15 °C for 30 minutes. The obtained solution was then filtered through a filter of 0.45 µm porosity and analyzed.

Sample preparation for the method limit of detection ($_m$LOD), method limit of quantification ($_m$LOQ) and recovery evaluation procedure

Ten grams (10 ± 0.1 g) of the sample portion was added 20 mL of water and blended. The sample was quantitatively transferred to a glass container with the additional 5 mL of water and fortified. The fortified mixture was manually shaken, then thoroughly mixed on a vortex for 1 minute and left to equilibrate for 30 minutes. Afterwards, solvent extraction was performed, successively with two 10 mL-portions of hexane. The organic phase was separated from the solids-water dispersion and after centrifugation for 5 minutes at 3500 rpm, was subjected to the SC-SE-LTP procedure and analyzed.

Instruments

In sample preparation procedure, following appliances were used: balance KERN KB 2000-2N, Germany (acc. ± 0.01 g); food processor TEFAL 0.9L BL142A, centrifuge Thermo Scientific Jouan C4i and TurboVap® LV evaporator, Zymark USA.

GC-MS analysis

The gas chromatographic analysis was performed on Agilent 6890 gas chromatograph equipped with 5973 Mass Selective Detector (MSD) and 7683 autosampler and SGE 25QC2/BPX5 0.25 capillary column (25 m×0.22 mm×0.25 µm, non-polar). The mass spectra were recorded under an electron ionization (EI) voltage of 70 eV. The gas chromatograph was operated in the splitless injection mode. The oven temperature was programmed from 90°C (hold time 0 min) to 280°C (4 min) at 20°C min^{-1} rate; post run: 300°C (2 min). Helium was the carrier gas with constant flow rate of 1.0 mL min^{-1}. The target compound was identified and confirmed at scan mode (m/z 50–400) and analyzed at selected ion monitoring (SIM) mode with confirmation ions as given in Table 1. Both data acquisition and processing were accomplished by Agilent MSD ChemStation® D.02.00.275 software.

Table 1 *Properties and qualification parameters of boscalid; quantifier ion is given in bold*

Pesticide	Class	MRL, mg kg^{-1}	Structure	Molecular mass	Log K_{ow}	Retention time, min	Target ions, m/z
Boscalid	Fungicide	2	Pyridine-carboxamide	343.207	2.96	12.34	140,342,112

Validation procedures

Recovery evaluation procedure

Mean recovery – as a measure of trueness – was evaluated on 5 replicates, at 0.10, 1.00 and 5.00 mg kg^{-1} fortification level. Quantification of non-fortified and fortified samples and recovery calculation was performed by using the standard addition to extract aliquots procedure, according to the EURL-SRM workflow [7].

$_m$LOD and $_m$LOQ evaluation procedure

The limit of detection and the limit of quantification of the method were evaluated by using the equation 1:

$$_m\mathrm{LOD}\ (\text{or } _m\mathrm{LOQ}) = k \times S_{dr}/s \qquad (1)$$

Where, k equals 3.3 and 10 in $_mLOD$ and $_mLOQ$ calculation formula, respectively. S_{dr} stands for the standard deviation of the residuals and s for the slope of the calibration function. The calibration function is the function of the matrix-matched calibration curve, developed with the samples fortified prior to extraction, at the following levels: 0.01, 0.025 and 0.05 mg kg^{-1}.

Instrumental LOD and LOQ evaluation procedure

Limit of detection and limit of quantification of the instrument were evaluated by using the equation 1. The calibration function is the function of the solvent-based calibration curve, developed with the pesticide solutions at the following concentration levels: 0.005, 0.010 and 0.025 µg mL^{-1}.

Real sample analysis

The pesticide in the apple orchard samples was quantified by using the standard addition to sample portion procedure according to the EURL-SRM workflow [7].

RESULTS AND DISCUSSION

The instrument limit of detection and limit of quantification were 0.0017 and 0.0053 µg mL^{-1}, respectively. The lowest method limit of detection and therefore the lowest method limit of quantification of boscalid were evaluated for the *Idared* matrix (Table 2).

Table 2 *Method validation parameters and detected amounts of boscalid in orchard samples (mean ± SD, n=3)*

Sample	Fortification level, mg kg^{-1}	Trueness, %	Precision, %	$_m$LOD, mg kg^{-1}	$_m$LOQ, mg kg^{-1}	Found in orchard samples, mg kg^{-1}
Golden Delicious	0.1	78.45	12.34	0.0308	0.0934	0.1932±0.004
	1	90.81	17.24			
	5	97.40	7.58			
Idared	0.1	73.68	17.65	0.0050	0.0152	0.7014±0.014
	1	83.79	15.06			
	5	96.80	12.52			
Granny Smith	0.1	64.76	15.99	0.0092	0.0278	0.3090±0.009
	1	32.48	17.86			
	5	35.86	7.12			

The trueness ranged from about 78 to 97 % for *Golden Delicious* and from about 73 to 96 % for *Idared* variety, depending on the fortification level, with precision in terms of repeatability below 20 %, thus meeting SANTE/11813/2017 guidelines criteria [8]. For

Granny Smith the trueness was lower, especially at higher fortification levels i.e. ranged from about 32 to 64 %. However, since the precision was below 20 %, recovery rates outside the range of 70-120 % are acceptable [8].

Apple samples were collected from an orchard located in the region of Jablanica, aimed for the commercial apple production. Apples were treated with the commercial formulation of boscalid (BOSCO®, Agromarket) in the mid-August (22.08.2018) against *Carpocapsa pomonella* and *Monilinia sp*., 15 days before picking, with 1 kg ha^{-1} formulation dose, as part of the integrated pest management plan. Boscalid residue levels in orchard samples were below MRL; the highest amount was determined in the samples of *Idared* variety (Table 2).

CONCLUSION

Apples of *Granny Smith*, *Golden Delicious* and *Idared* variety from orchard located in the region of Jablanica were analyzed after five months of commercial boscalid field application. The novel, few-steps only method, matched all needed performance criteria, according to SANTE guidelines. GC-MS analysis of samples from orchard showed that amount of boscalid was below MRL value *i.e.* it was 0.1932, 0.3090 and 0.7014 mg kg^{-1} in *Golden Delicious*, *Granny Smith* and *Idared* variety samples, respectively.

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SCREENING OF SEVEN ANIONS IN SOIL AND WATER SAMPLES FROM THE LALINAC SALT MARSH

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Abstract

Saline soils represent a soil type abundant in soluble salts, originating from the high soil evaporation potential or high saline water table. Assessment of the water-soluble inorganic ion content in saline soils and waters is important for many scientific and practical fields. The focus of this study was to evaluate seven anions content by ion chromatography i.e. *the content of* F^-, Cl^-, NO_2^-, Br^-, NO_3^-, PO_4^{3-} *and* SO_4^{2-} *in soil and water sampled from several sites within the Lalinac salt marsh, a protected area in the south-eastern Serbia. The analysis of water and water extracts of soil showed that sulphate and chloride ions were representatives in all samples, with the highest determined amounts of 991.40 and 18.63 mg* $100g^{-1}$, *respectively, while other five anions were present in amounts dozens of times lower. Sampling sites were more uniform in the amount of sulphates, bromides, chlorides and less uniform in the amount of other four ions. Nitrates and phosphates were not found in one soil sampling site, while several water sampling sites did not contain nitrites and phosphates. The highest total amount of anions determined within a soil and water sampling site was 1,025.54 mg* $100g^{-1}$ *and 1,631.94 mg* L^{-1}, *respectively.*

Keywords: anions, ion chromatography, salt marsh, soil, water

INTRODUCTION

Soil is usually defined as superficial layer of the earth's crust made of decayed and partly decayed parent rock material with associated organic matter in various stages of decomposition. Its chemical composition is determined by the nature of the starting materials from which was formed and by the processes that it has suffered over time. A type of soil, expressively different from other in chemical properties, is salt-affected soil which can be classified as saline, sodic, or saline-sodic.

Salinity can be defined as “the concentration of dissolved mineral salts presents in waters and soils on a unit volume or weight basis” [1]. In other words, saline soils are abundant in soluble salts. The main causes of soil salinity could be the lack of water, which especially occurs in arid and semiarid climates, that would leach soluble salts from the soil, high evapotranspiration potential of soil, which increases the concentration of salts in both soils and surface waters or poor drainage of soils due to a high-water table or to low soil permeability caused by high sodium content of water [1].

The major cations and anions in saline soils and waters are Na^+, Ca^{2+}, Mg^{2+}, K^+, Cl^-, HCO_3^-, CO_3^{2-}, and NO_3^-, SO_4^{2-}. Anions as soil constituents and their amounts are significant for agriculture as components in plant nutrition and vegetable production, especially in the case of increased amounts. Thus, the analysis of the water-soluble inorganic ion concentrations in soils is important for many scientific and practical fields, such as environmental management and protection, water-use management or agricultural crop improvement *i.e.* assessment of the suitability of areas for various agricultural activities.

The leading instrumental technique for the ion analysis in environmental samples is ion chromatography [2–4]. It offers a non-destructive separation, a multipurpose, selective and sensitive analysis with minimal sample preparation, and relatively low costs.

Lalinac salt marsh (Serbian: Lalinačka slatina) is one of the largest salt marshes at the foothill of Mali Jastrebac mountain, near a settlement called “Lalinske pojate”. This area includes Oblačina and Lepaja salt marshes as well. It is formed from water with high content of mineral salts, primarily of sodium chloride, which in many sites springs from the depths and floods the surrounding land. This study was focused on evaluation of anion levels in soil and water samples from different sites within the Lalinac salt marsh.

MATERIALS AND METHODS

Chemicals

The seven-anion stock standard solution containing fluorides (20 mg L^{-1}), chlorides (30 mg L^{-1}), bromides (100 mg L^{-1}), nitrites (100 mg L^{-1}), nitrates (100 mg L^{-1}) as the sodium salt, phosphates (150 mg L^{-1}) as the monobasic potassium salt and sulphates (150 mg L^{-1}) as the dibasic sodium salt, and Dionex AS22 Eluent concentrate (1.4 mM of Sodium Bicarbonate and 4.5 mM of Sodium Carbonate, after 100-fold dilution) were purchased from Thermo Fisher Scientific.

Preparation of the calibration-plot solutions

An appropriate volume of the seven-anion stock solution was added to a 10-mL volumetric flask and filed up with deionized water. The resulting calibration range for anions is given in Table 1.

Samples

Soil and water samples were collected at six sites, about hundred meters distant from each other, in the part marked as II (Figure 1) of the protected area “Lalinačka slatina” in May 2018. The soil sampling was performed by digging small pits of 15 cm depth. Soil and water were preserved in plastic bags and plastic tubes, respectively, until transportation to the laboratory.

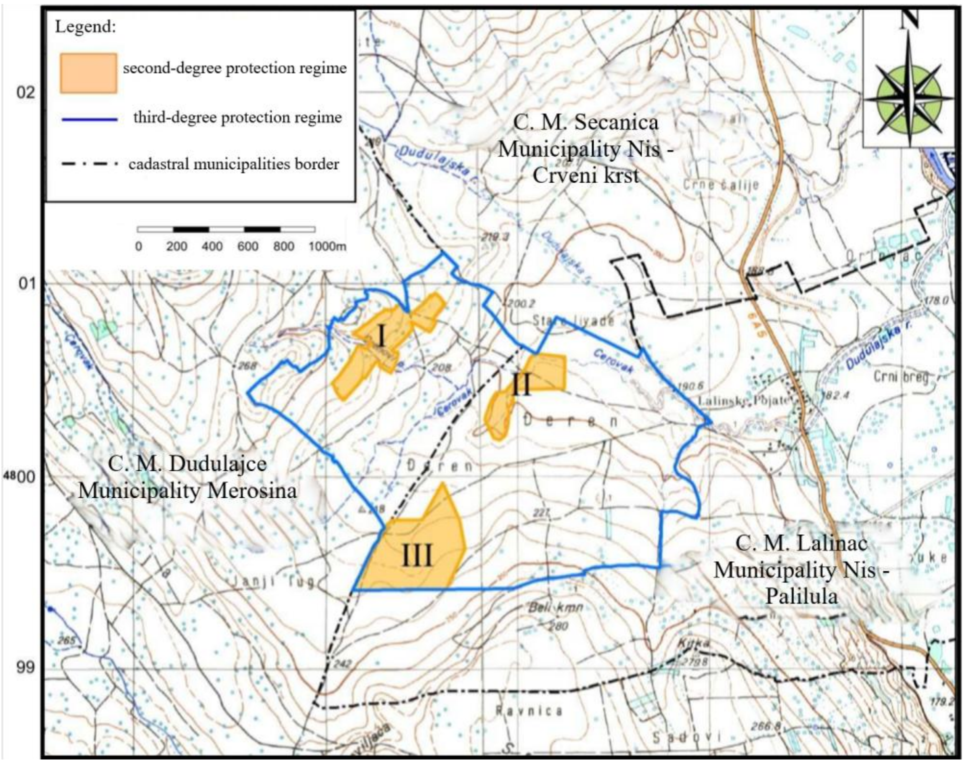

Figure 1 *Location of the Lalinac salt marsh, (source: Institute for nature conservation of Serbia)*

Sample preparation

The soil was air-dried by placing in a shallow tray in a well-ventilated area. Afterwards, it was crushed so that the gravel, roots and large organic residues become separated. The smaller residues were further separated by sieving the soil through a 2 mm sieve. Finally, the soil was ground in a mortar reaching the powder form. The powdered soil samples were preserved in separate, labelled plastic tubes.

Five grams of each of the soil sample was weighed and transferred to a 250 ml conical flask. Fifty millilitres of deionized water were added, and the mixture was shaken for 30 minutes with short breaks. The supernatant was separated from the solid part by centrifugation at 3800 rpm for 5 minutes and further filtered with a 0.45 μm syringe filter. One millilitre of the sample was transferred to a 5-mL volumetric flask and diluted to the final volume with deionized water, prior to analysis. The procedure was conducted in triplicates.

Prior to analysis water samples were filtered with a 0.45 μm syringe filter and, in order to fit the calibration range, diluted according to each ion sample concentration, previously estimated.

Instruments

A Thermo Scientific™ Dionex™ Aquion™ Ion Chromatography (IC) system with electrolytic suppression, an AS Autosampler, and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software were used for the analyses. The chromatography conditions were as follows: column – Dionex IonPac™ AS22 (4×250 mm), temperature 30 °C; eluent - 4.5 mM sodium-carbonate, 1.4 mM sodium-bicarbonate - 1.2 mL min^{-1} flow rate; detection - suppressed conductivity (AERS Carbonate 4mm, 41 mA), cell temperature 35 °C; data acquisition duration - 15 min.

RESULTS AND DISCUSSION

The calibration range included low concentration levels for fluoride and chloride ions and high concentration levels for the rest of tested anions. Good linearity was achieved through a wide concentration range for all anions, with coefficients of determination ranging from 0.9996 to 0.9998 (Table 1).

***Table 1** Retention time of tested ions and the single ion concentration ranges with associated parameters*

	Retention time, min	Concentration range, mg L^{-1}	Coefficient of determination	Calibration function
F^-	3.221	0.050–2.00	0.9998	y=0.2339x+0.0042
Cl^-	4.697	0.075–3.00	0.9998	y=0.1569x+0.0244
NO_2^-	5.681	0.250–10.00	0.9997	y=1038x-0.0078
Br^-	6.771	0.250–10.00	0.9997	y=0.0687x-0.003
NO_3^-	7.647	0.250–10.00	0.9998	y=0.086x+0.0015
PO_4^{3-}	10.651	0.375–15.00	0.9996	y=0.0461x-0.0035
SO_4^{2-}	12.377	0.375–15.00	0.9998	y=0.1198x+0.0172

Among anions, in both soil and water samples, the most represented were sulfates followed by chlorides. The amount of sulphates ranged from 50.32 mg $100g^{-1}$ at site 7 to 991.40 mg $100g^{-1}$ at site 4 in soil and from 260.70 mg L^{-1} at site 5 to 1,602.70 mg L^{-1} at site 2 in water (Figure 2). The amount of chlorides ranged from 5.50 mg $100g^{-1}$ at site 5 to 18.63 mg $100g^{-1}$ at site 4 in soil and from 11.46 mg L^{-1} at site 5 to 73.36 mg L^{-1} at site 3 in water samples (Figure 3).

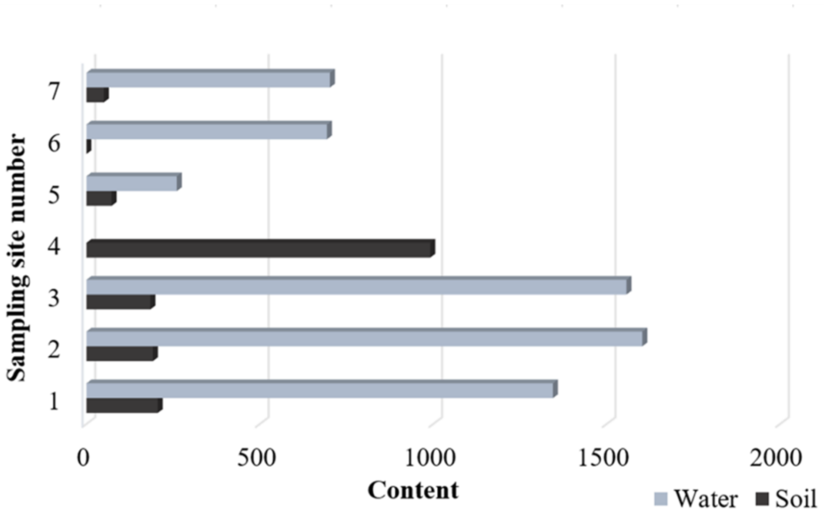

***Figure 2** Content of sulphates in soil (mg $100g^{-1}$) and water samples (mg L^{-1}); at site 4 only soil was collected; at site 6 only water was collected*

When compared to the amount of chlorides, the amount of fluorides and bromides was many times lower in both sample types. The highest determined amount of fluorides was 1.39 mg $100g^{-1}$ in soil from the site 2 and 0.86 mg L^{-1} in water from the site 1. The highest amount of bromides was 0.38 mg 100g^{-1} in soil from the site 2 and 0.57 mg L^{-1} in water from the site 3.

Regarding soil samples, notable amounts of nitrites, nitrates and phosphates were present in the samples collected at 4th and 5th sampling site (Figure 3) *i.e.* the highest amount of phosphates and nitrites and nitrates was 0.62 mg 100g^{-1} at site 4 and 0.34 and 0.70 mg 100g^{-1} at site 5, respectively. Nitrates and phosphates were not detected in samples from the site 7. Regarding water samples nitrites and phosphates were not detected in samples from sites 1, 6, 7 and 2, 3, 6, respectively (Figure 3).

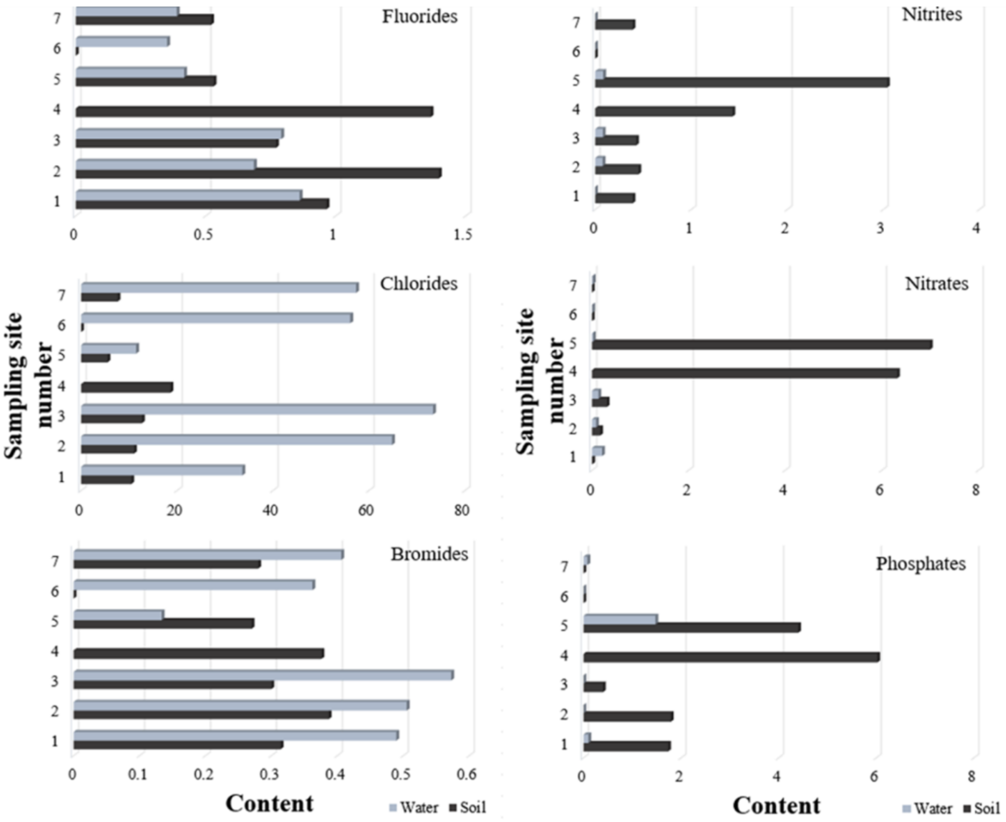

Figure 3 *Content of five anions (F^-, Cl^-, Br^-, NO_2^-, NO_3^-, PO_4^{3-}) in soil (mg 100g^{-1}) and water samples (mg L^{-1}); at site 4 only soil was collected; at site 6 only water was collected*

Water and soil sampling sites were more uniform in the amount of sulphates, bromides, chlorides and fluorides and less uniform in the quantity of nitrites, nitrates and phosphates. Soil sample from the site 4 could be distinguished from other sampling sites since it contains the highest total amount of tested anions *i.e.* 34.14 mg 100g^{-1} (excluding sulphates) or 1,025.54 mg 100g^{-1} (including sulphates).

CONCLUSION

Anion content in soil and water samples from several sites within Lalinac salt marsh was evaluated. Sulphate and chloride ions were representatives in all samples. The amount of sulphates ranged from 50.32 to 991.40 mg 100g^{-1} in soil and from 260.70 to 1,602.70 mg L^{-1}

in water samples. The amount of chlorides ranged from 5.50 to 18.63 mg $100g^{-1}$ in soil and from 11.46 to 73.36 mg L^{-1} in water samples.

Compared to the amounts of sulphates and chlorides, other five anions were present in significantly lower amounts. Soil sample from the site 7 did not contain nitrates and phosphates. Water samples from sites 1, 6, 7 and 2, 3, 6, did not contain nitrites and phosphates, respectively.

Both water and soil samples were more uniform in the amount of sulphates, bromides, chlorides and more variable in the amount of other four ions. One of six soil sampling sites could be distinguished from others due to a highest total amount of tested anions *i.e.* the soil sample from the site No. 4 (1,025 mg $100g^{-1}$). Regarding water samples, the sample from the site No. 3 contained the highest total amount of tested anions *i.e.* 1,632 mg L^{-1}, however it was free of phosphates.

ACKNOWLEDGEMENT

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OIL SLUDGE OF MANGYSTAU REGION OF THE REPUBLIC OF KAZAKHSTAN

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Abstract

The article discusses the oil sludge of the Mangystau region of the Republic of Kazakhstan for the possibility of their processing into target petroleum products. Based on the study of the elemental composition and physicochemical characteristics of oil and oil sludge, the possibility of their use for the production of paraffins and petroleum coke is proposed.

Keywords: oilsludge, chemical composition, fractional composition, paraffin, petroleum, coke

INTRODUCTION

In the context of market relations, many states and large multinational oil and gas companies pay serious attention to the real needs of the world economy in energy resources, especially in hydrocarbon resources, to develop key guidelines for their future development. At the same time, oil refining and petrochemistry is an attractive sector of the manufacturing industry, taking into account market prospects, economic effect and competitiveness of the Republic of Kazakhstan. Priority commodity groups are determined based on the volume of markets in Kazakhstan and the macroregion, technological "complexity" of commodity groups, as well as the possibility of their production, taking into account the requirement to reduce environmental emissions [1]. In this regard, environmental safety is one of the main strategic components of the national security of the Republic of Kazakhstan and the most important aspect of state priorities. As many domestic and foreign researchers reasonably believe, environmental pollution has become a serious obstacle to the socio-economic development of the country [2]. On the territory of the Republic there are about 2.5 thousand enterprises belonging to the 1-2 highest hazard classes among the 5 existing classes [3]. According to the Environmental code of the Republic of Kazakhstan dated January 9, 2007, the requirements for environmental standards and increasing the responsibility of industrial enterprises for the state of the environment are tightened. This is also relevant because the Republic, implementing the "Strategy 2030", "the Concept of transition to sustainable development for 2007-2024" aims at a long-term environmental strategy - the harmonization of the interaction of society and the environment, as well as the creation of environmentally safe, favorable habitat. Implementing the strategic objectives of the Concept, Kazakhstan, increasing the efficiency of resource use, increasing life expectancy, providing an increase in the index of environmental sustainability, will create an opportunity to be on the level of quality of life among the most competitive and developed countries of the world [4].

The growth of oil production in Kazakhstan, the volume of its processing and transportation is accompanied by an increase in the volume of oil pollution and other toxic waste. Every year in our country during the processing or transportation of oil, as a result of natural spills and accidents, about 400 thousand tons of oil waste are formed, and the resources located in earthen barns are estimated at 4.5 million tons [5]. This problem requires an immediate solution, since the accumulation of oily waste affects not only the ecological state of the environment, but also the sanitary well-being of the inhabitants of the region. During oil production, there is a strong contamination of soil and water with drilling slime containing hydrocarbons, and oil spill during its production is associated with a number of negative phenomena that lead to "oil contamination" and soil degradation of huge territories. It is natural that after such influence useful properties of the earth are broken and demand restoration, i.e. reclamation. Reclamation is a set of measures aimed at restoring the former fertile qualities of the land, its biological and economic value, as well as improving the environmental conditions. The creation of high-performance technology of processing of contaminated soil will solve the problem of their complete elimination with the return of land to the user, reduce environmental pollution and enable the rational use of organic raw materials from waste. Accordingly, to carry out measures for the reclamation and restoration of land, it is necessary, initially, to collect and dispose of oily waste. It should be understood that the presence of oil sludge collection in the barns increases the risk of death of animals, pollution of groundwater, air. To improve the ecological status and return to the economic circulation of land contaminated with oil sludge, various environmental technologies for processing and disposal are proposed [6–10].

EXPERIMENTAL PART

Physical and chemical characteristics of oil and oil sludge were carried out according to the method [11,12]. Determination of the density of oil and oil sludge was carried out by the well-known method of pycnometric method at a temperature of 20°C. First of all, the "water number" of the pycnometer was determined. To do this, an empty pycnometer was kept in a thermostat with a temperature of 20°C for 30-40min. The pycnometer is filled with distilled water at a temperature of 20°C and placed in a thermostat for 30 minutes. Determine the "water number" of the pycnometer by the formula:

$$q = m_2 - m_1 \tag{1}$$

where m_1 is the mass of the pycnometer with air, m_2 is the mass of the pycnometer with water.

After that, water is poured from the pycnometer, dried and filled with oil sludge tightly to the mark. Pycnometer with oil sludge is placed in a thermostat with a temperature of 20°C and kept 40min. After that, it is removed from the thermostat, wipe dry and weighed. This process is carried out 3-4 times for statistical processing of the results.

The density is determined by the formula:

$$\rho_{20}^{20} = \frac{m_3 - m_1}{q} \quad (2)$$

where: m_3 is the weight of the pycnometer with the oil sludge at 20°C

When calculating the density to an accuracy of 0.0001, it is necessary to make an adjustment for the loss of mass in the air, since the mass of air in the volume of the pycnometerwas not taken into account when determining the mass of water. Then the calculation should use the formula:

$$\rho_4^{20} = \rho_{20}^{20} \cdot (0.9982 - 0.0012) + 0.0012 \quad (3)$$

where: 0.0012 is the density of the 1 cm^3 of air at a pressure of 760 mm of mercury. art. and a temperature of 20°C.

The coking ability of petroleum products is calculated by the formula, %:

$$X = \frac{m}{m_1} 100 \quad (4)$$

where m is the mass of coke residue, g; m_1 is the mass of the tested oil product, g.

Analysis of hydrocarbons released up to 3000C was performed on GC DANI Master GC (capillary column of molten quartz Varian capillary Column CP-PoraPlot Q; 27.5 m, 0.32 mm, 10µm). The temperature of the evaporator is 350°C, and the columns are 200°C. Detection – ionization-flame). Fraction 160-3200C of sludge NSH-1 was also analysed on GC-MS spectrometer.

IR spectra were recorded on the X-29 spectrometer (4000-400 cm^{-1}) in potassium bromide and vaseline oil. Distillation of crude oil and sludge was performed using the ARN-3 according to GOST 11011-85. Hot water (80-90°C) was used to separate mechanical impurities from oil sludge (oily soil). Asphaltenes were isolated using solvent - n-heptane, paraffins – alcohol.

Elemental analysis for carbon and hydrogen is based on the non-sufficient combustion of the organic mass of oil in the oxygen current to carbon dioxide and water. The latter catch and calculate the content of these elements by their number. It is necessary that the combustion is complete (the co FORMED is oxidized to CO_2), and the combustion products are purified from sulfur oxides, Halogens and other impurities.

Determination of sulfur can be carried out by various methods. For medium and heavy oil products suitable method of flushing condensate by burning the sample in a calorimetric bomb. The combustion method in the tube is fundamentally no different from the tube method, only the sulfur dioxide formed during combustion is oxidized by hydrogen peroxide to sulfur trioxide; further determination is carried out as in the previous method. The principle of the bomb flushing method is to burn oil in a calorimetric bomb, which is pre-filled with 10 cm^3 of distilled water. After burning, the water from the bomb and wash it off the walls and other parts are transferred to the flask, acidified, boiled to remove CO_2, then barium chloride is added. The precipitated barium sulfate precipitate is isolated, dried and the sulfur content is calculated by its mass.

The nitrogen content is determined by method for determination of total nitrogen. The percentage of oxygen was determined by the difference between 100 and the total content of all other elements in percent. This is an inaccurate method, as its results are affected by errors in the determination of all other elements. For reliability, the determination of oxygen by the gravimetric method of pyrolysis of oil sludge in an inert gas current in the presence of platinum graphite and copper oxide was carried out in parallel. The oxygen content was determined by the mass of the released CO_2.

RESULTS AND DISCUSSION

Determination of the elemental composition of oil and oil sludge are summarized in Table 1. According to the sulfur content, oil belongs to the second class. According to the elemental composition of oil and oil sludge differ in the content of the main elements. In oil sludge oxygen content increases from 1.6 to 6.0-6.5% by weight, nitrogen from 0.4 to 2.1-2.6% by weight. The carbon in oil sludge is reduced from 84.4 to 74.4-76.6 wt.%

Table 1 *Elemental composition of oil and oil sludge*

Name	Content of elements,%					Other,%
	C	H	N	S	O	
Oil № 1	84.4	11.4	0.4	1.2	1.6	**1.0**
Oil sludge1	74.4	9.8	2.6	2.0	6.2	**5.0**
Oil sludge 2	76.6	8.0	2.1	1.9	6.4	**5.0**
Oil sludge 3	75.4	9.6	2.5	2.0	6.5	**4.0**
Oil sludge 4	75.6	9.4	2.4	1.6	6.0	**5.0**

To separate the organic part of the smeared soil, an aqueous solution with a surfactant content of 0.5% by weight was used. After purifying oil sludge the content of organic compounds in the soil is 0.05%. The physical and chemical characteristics of oil and oil sludge are represented in Table 2. There are not hydrocarbons with boiling point below 300°C in oil sludge. It shows that oil sludge couldn’t be used for producing gasoline and dyzel.

The content of asphaltenes, paraffins and resinous substances in the organic part of oil sludge was determined using appropriate selective solvents. Paraffin- naphthenes in oil 21.0% and in oil sludge it changes from 23.5 to 26.0%. In oil sludge the percentage of resin increase to 26.0 – 28.0 from 6.0 in oil, and also increase asphaltenes up 11.0 from 2.4.

Table 2 *Physical and chemical characteristics of oil and oil sludge*

Name	**Oil sludge-1**	**Oilsludge-2**	**Oil**
Color	Dark brown	Dark	Dark brown
Dencity at 20°C, g/cm^3	**0.916**	**0.944**	**0.864**
Freezing point,°C	**18-20**	**22-24**	**10-11**
Flash point in closed Cup,°C	**260**	**300**	**60**
Coking,%	**10.0**	**14.0**	**4.0**
Boilingpoint(BP), °C	**280**	***2.0% Water is released at 96°C**	**54**
* Fractional composition, %mass. **BP – 160°C**	**-**	**-**	**9.5**

***Table 2** continued*

160-180	**-**	**-**	**6.0**
180-240	**-**	**-**	**8.0**
240-280	**-**	**-**	**7.0**
280-320	**2.0**	**-**	**10.5**
Residue, incl.:	**98.0**	**97.0**	**56.4**
Paraffin - naphthenes	**26.0**	**23.5**	**21.0**
Arenas	**36.0**	**34.5**	**24.0**
Resins	**28.0**	**26.0**	**6.0**
Asphaltenes	**4.0**	**11.0**	**2.4**
Other	**6.0**	**5.0**	**3.0**

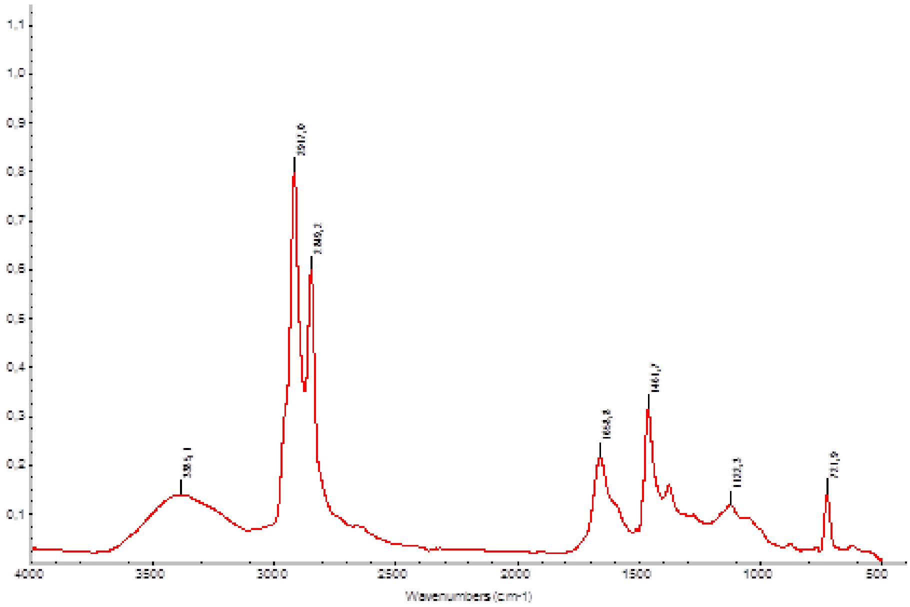

***Figure 1** IR spectra of the organic part of oil sludge*

From Figure 1 it can be seen that IR spectra of oil sludge have characteristic absorption frequencies of hydrocarbon and functional groups. The frequencies of absorption bands in the IR spectra with wave numbers 2920-2950 cm^{-1} and 2800-2910 cm^{-1} belong to the methyl and methylene groups, respectively. Absorption bands 2851-2918 cm^{-1} hartery naphthenic hydrocarbons, especially decalin and its derivatives. For aromatic structures C=C characteristic absorption bands 1635 cm^{-1}, and there is a set of bands 722-806 cm^{-1}, which belong to the CH aromatic groups. Absorption bands 1700-1760 cm^{-1} and 1680-1700 cm^{-1} are characteristic of aliphatic and aromatic carboxylic acids.

The frequency of the absorption band 3410, 3415 cm^{-1}, characteristic of OH - groups. The presence of the absorption band of oxygen-containing groups in the samples of oil sludge in comparison with the initial oil means the interaction of hydrocarbons of oil sludge with air oxygen. Absorption bands 3380-3540 cm^{-1} to be related to amino groups is complicated by overlap of hydroxyl groups. The absence of amino groups with an absorption frequency of 3380-3540 cm^{-1} in oils and their appearance in oil slime suggests the probable binding of atmospheric nitrogen to the hydrocarbon part of the oil sludge. As oil and sludge phenolic hydroxyl is detected at values of 1140-1230 cm^{-1}.

CONCLUSION

On the basis of the obtained experimental results and the separation of oil sludge components may be used to produce paraffins and petroleum coke. In [11–13] some characteristics of bitumen production are considered, on which the quality of road and construction bitumen, anticorrosion, waterproofing coatings and other properties of these materials depend. According to the regulations, the role of bitumen is to ensure the adhesive and cohesive strength of the coating, the deformation ability when exposed to mechanical loads. In this respect, oil sludge the content of long paraffin side chains in asphalt - resinous substances makes it difficult to use them as oil bitumen.

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FLY ASH DISPERSION MODELING IN EXTREME WEATHER CONDITIONS FROM LANDFILL IN OBILIĆ

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Abstract

The paper presents the use of an environmental software application in the modeling and simulation of air and soil contamination from the landfill fly ash generated by the combustion of lignite in the Kosovo A and B thermal power plants in Obilić, near Priština. Deposits of fly ash, together with pit and lagoon, make up an area of about 234 ha and are located on the banks of the Sitnica river. In order to assess the environmental risk, the characterization of the emitted particles was carried out and the movement of particulate pollutants in different climatic conditions was investigated. Using scanning electron microscopy and X-ray diffraction analysis, the characterization of fly ash in terms of concentration, morphology, particle size distribution (TSP) and elemental composition was performed. The concentrations of ash particles in the air were measured and compared to the concentration range calculated using the SCREEN view. The simulation is done for different climate conditions typical for the region: humidity ranges from 20%, wind speed of 2-7 m/s and temperature range from -10 to 36^{0}C. The SCREEN view software showed that in these conditions the pollution range exceeding the maximum limits covers an area greater than 20 km in diameter, endangering a large populated area.

Keywords: Environment, fly ash, SCREENVIEW software, modelling and simulation

INTRODUCTION

Air pollution with fine solid particles from mining landfills has been observed in all regions with developed mining. Creation of dust and its dispersion are a major problem in mining areas, primarily contributing to particulate matter - PM, especially PM_{10} [1]. These particles represent one of the most challenging problems both for air quality and for climate change policy [2]. In addition to air pollution, which is characteristic of mine locations, pollution of land and water, as well as disruption of the health of the local population are another source of concern [3].

Regional geology of Kosovo and Metohija is rich in deposits of ore and lignite [4]. The city of Obilić is located in the central part of Kosovo and Metohija, not far from Priština, famous for its abundant reserves of lignite in the Kosovo basin. Coal exploitation is one of the key industries in Obilić and is an important factor in the economic development of this region. The industrial complex has developed surface pit, Badovac and Dobro Selo, as well as a powerful thermal power system "Kosovo A" and "Kosovo B". The first lignite plant, Kosovo A1, started operating in 1962, and in the period from 1962 to 1984, other units were built.

Mineral coal is transported by conveyor belts to the separator (dry process) where it is crumbled and scattered. The fine assortment is used for the needs of the power plants, that is, the lignite is milled before it is inserted into the furnace and it turns into coal that mixes with air and thus burns easily [5].

During these activities, the main waste and emissions include emissions of gases, discharge of wastewater and waste by-products of slag and ash from the production current. Since the first operation of the power stations in 1962 until today, the ash has been collected and stored as waste without any exploitation, which poses a great danger as an environmental pollutant [6]. Also, there was no significant rehabilitation since the construction of thermal power units. Moreover, the technology of energy production in existing power stations does not meet the ecological criteria defined by EU legislation [7]. The thermal power "Kosovo A" has five blocks and belongs to the old type of thermal power station. In coal combustion, it emits over 15% of the produced ash in the atmosphere, and produces about 1,200,000 t of ash per year. The second power station "Kosovo B" is composed of two energy blocks and belongs to the modern type of thermal power stations. The annual quantity of ash produced in this thermal power station is around 1,000,000 t. All facilities and landfills of the thermal power Kosovo A and B are near the river Sitnica, the main water recipient in this region (Figure 1).

Figure 1 *Industrial landfill of fly ash - Obilić*

The asphalt ash dumpsite in Obilić is located within the industrial circle, close to residential buildings, with a lot of attention to its impact on the environment. Published studies on this landfill [5,8–12] analyze the impact of ash on the environment, as well as the possibility of using ash for specific purposes [6,13,14]. In order to assess the environmental impact, it is necessary to determine the emission rate of pollutant solid particles and the quantification of concentrations of pollutants. Depending on the meteorological conditions, the particles can reach quite a considerable distance, so that wind power, atmospheric stability, and particle size distribution are the three main factors that influence the concentration of pollutants [15]. For these reasons, in the changing climatic conditions, the characterization of the materials and the specific domain of air dusting was carried out, based on which the modeling and obtaining of the pollution map will be carried out.

MATERIALS AND METHODS

In order to determine the environmental impact, fly ash samples were characterized by chemical, physical and mineralogical investigations. The dusting factor was determined in a laboratory and then the climate factor was taken into consideration.

Microscopy and mineralogy

SEM investigation was carried out on Scanning Electron Microscopy instrument from JEOL (JSM6460), with Energy Dispersive Spectrometer, EDS by Oxford Instruments. XRD (X-Ray Diffractometry) analysis was used for mineralogical investigation. Samples were investigated using diffractometer Philips PW 1710 under following conditions: radiation from copper anticatode with $CuK\alpha = 1.54178$ Å and graphite monochromator, working voltage U=40 kV, current strength I=30 mA. Samples were investigated in the range of 5 – 70º 2θ (with step of 0.02° and time 0.5 s).

Dusting experiment

A fan type ABVE-3,5 apparatus was employed for measuring dust loading (mg/m^2) in the laboratory, using a flow of 3600 m^3/h and a vacuum of 200 Pa for airflow simulation, with a gravimetric sampler of the respiratory dust. The sample was set in a shallow metal plate, along with the measuring scale for the residual solid particles on the filter paper and a digital anemometer (DA-4000). The measurements were performed with the material set in the airflow direction from the fan and before the apparatus for polluted air vacuuming. The wind velocity was changed by the distance between the fan and the metal plate for each sample. The measurements were performed in wind velocities of 3, 4 and 5 m/s. A wind velocity larger than 10 m/s is outside the interval of wind velocities occurring in this region. The humidity in the laboratory was within the interval of 20–53 % and the dust concentration was 0 %.

Modeling and simulation

The meteorological data for the wind speed, wind directions and frequency distribution were taken from the nearest meteorological station, 4 km from the site. The data was analyzed using the program Wind Rose Plot for Meteorological Data, Version 7.0. The obtained results are imported into the SCREEN view together with the PM_{10} properties, terrain data, receptors grid and impact area for modeling and simulation.

RESULTS AND DISCUSSION

The basic aim of the chemical analysis was to determine the presence of toxic elements as well as the elements present in the ash. The average chemical composition of the ash is given in Table 1.

***Table 1** Chemical composition of examined sample given in mass%*

Element	O_2	Na	Mg	Al	Si	P	S	K	Ca	Mn	Fe	Cu
Sample 1	55.08	0.51	2.48	1.68	8.14	0	6.18	0.14	22.36	0.17	2.88	0.37
Sample 2	53.02	0.39	2.50	2.12	6.69	0	6.21	0.19	25.36	0.19	3.33	0
Sample 3	52.16	0.38	2.30	1.79	6.13	0.18	6.63	0.15	26.58	0	3.70	0

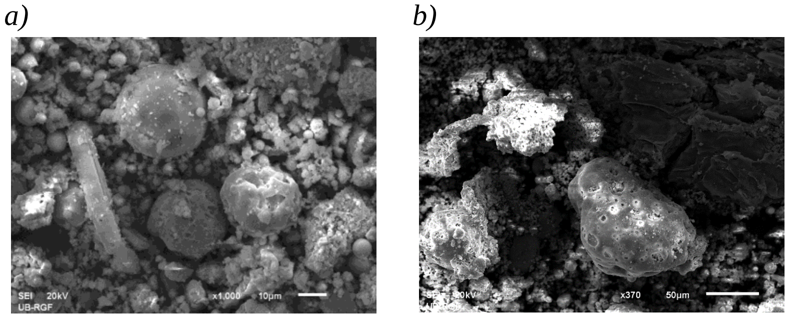

Figure 2 *SEM microstructure for fly ash*

Based on chemical analysis, all detected elements react with O_2 to form oxides (SiO_2, CaO, MgO, Fe_2O_3). In addition to O_2 in almost all compounds, Ca is also detected, so most of the compounds besides O_2 have Ca. It mostly reacts with O_2 and thus builds SiO_2. In addition to O_2, Si is the only one with Al and Ca, and thus build compounds $Ca_{0.92}$ $(Al_{1.8}Si_{2.2}O_8)$ $(H_2O)_{4.32}$ and $Ca_2Al_2SiO_7$. Mg reacts with O_2 and builds MgO. Ca reacts with almost all the elements, most of Ca is the only Si and O_2, thus forming the compounds CaO, $Ca(OH)_2$ and $CaCO_3$, and with S, causing $Ca(SO)_4$. A small amount of Fe, which reacts with O_2, has been determined to build Fe_2O_3.

The mineralization analysis was carried out on a composite ash sample using X-ray diffractometry, and the obtained results are shown in Figure 3. The fly ash is characterized by a high content of calcium oxide and other oxides which give the ash base the basic character and, by mineral composition, bind into a group of silicate-calcium ash.

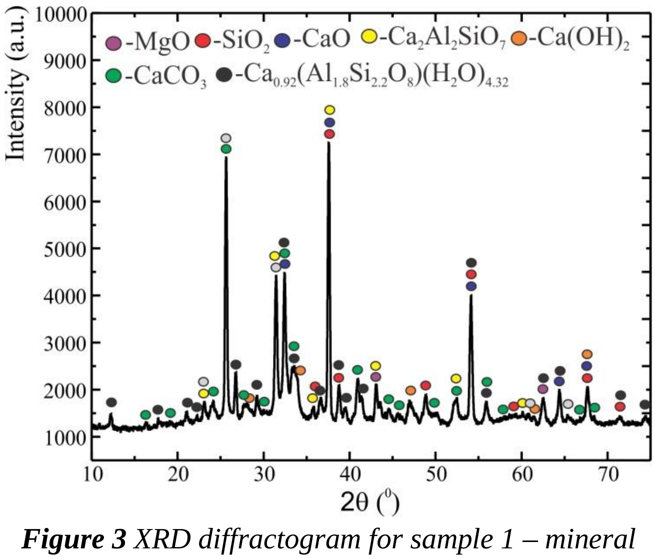

Figure 3 *XRD diffractogram for sample 1 – mineral*

The XRD diffractogram indicates that the most important phases that are indentified in the ash are quartz (SiO_2), gehlenite ($Ca_2Al_2SiO_7$), calcite ($CaCO_3$), perclasses (MgO), monoxide calcium (CaO) and portlandite ($Ca(OH)_2$).

Meteorological data

As the measurements were performed at a relatively small distance and under the actual climate conditions for the defined period, it was necessary to define the approximate dusting under different climatic conditions. The initial conditions such as dust under laboratory conditions, waste characteristics, deposit dimensions and surrounding relief were inputted into the software SCREEN view and the obtained diagram showed reasonable agreement with the measured values. The calculated results are presented in Figure 4.

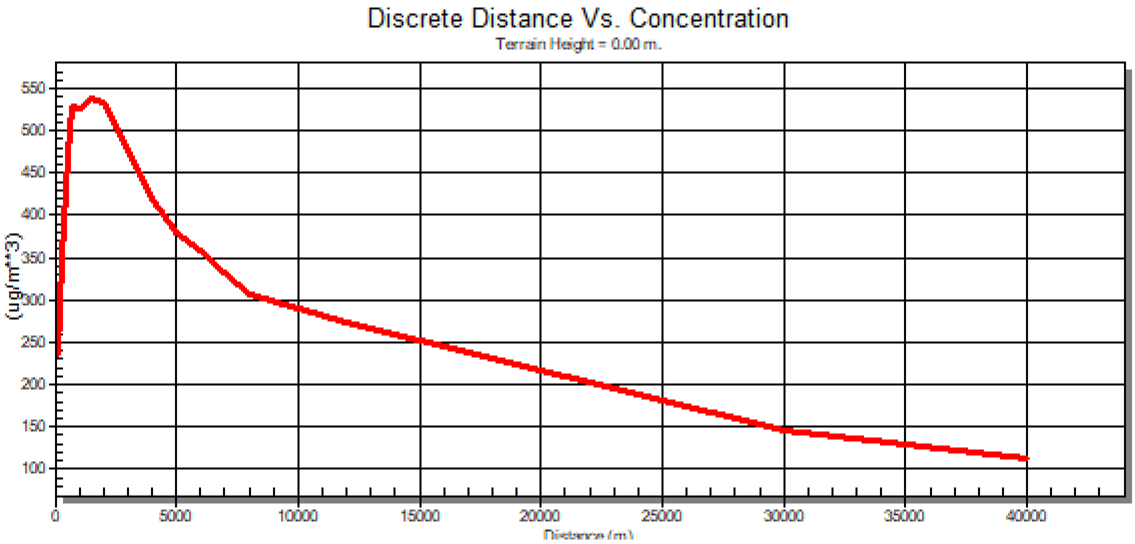

Figure 4 *Calculated concentration of fly ash particles in the relative humidity of 20% and wind speed of 3 m/s*

Observing the climatic conditions at the location of the landfill in the vicinity of Obilić during the period when the measurements were made and the data showing the trend of decreasing the minimum relative humidity, as shown in Figure 5 and the maximum values of wind speed for the winter period of 5.6 m/s there was a need to simulate dusting for all possible climatic conditions on the ground.

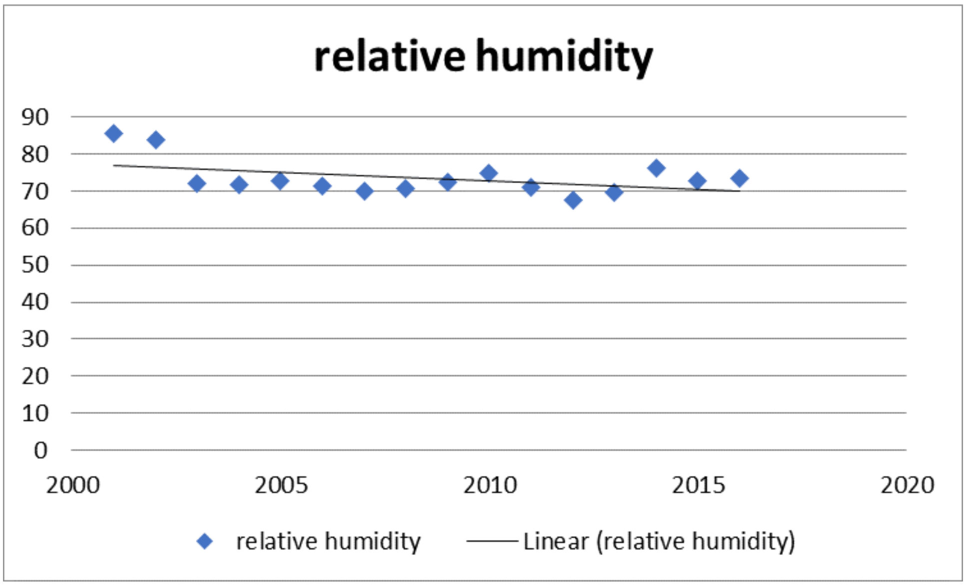

***Figure 5** Minimum relative humidity recorded at the meteorological station in Priština*

Taking into consideration all the wind classes, the results of airborne dusting (mg/m^2/s) in the laboratory, as initial data, for a relative humidity of 20 %, are presented in Figure 6.

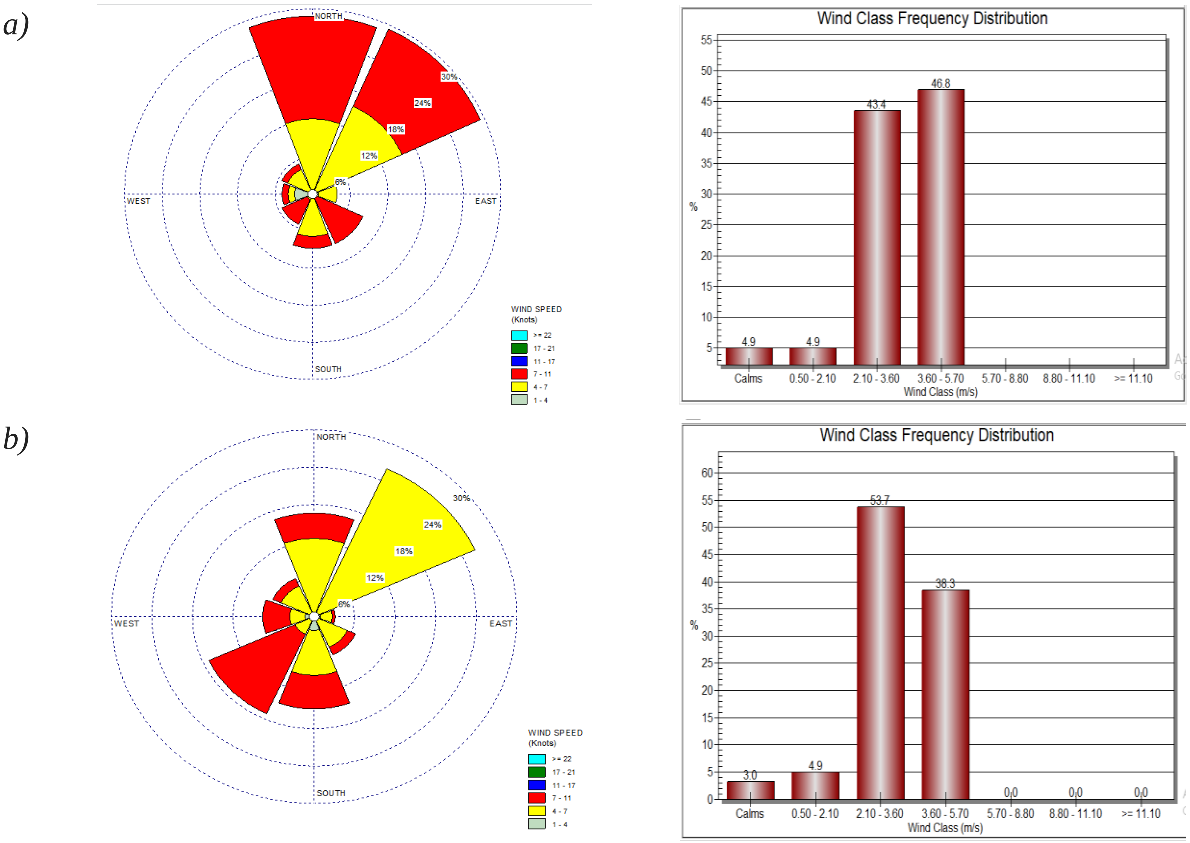

***Figure 6** Frequency of wind classes at Priština meteorological station a) Wind rose for the winter and b) Wind rose for the summer*

CONCLUSION

Air pollution is affected by climatic conditions, because the domain of fly ash particles carried by wind is higher in dry and windy weather. Simulation made using a software application can be useful in environmental impact assessment, because ash dumps are located near the city of Obilić, and 10 km from Priština. Simulation of air pollution domains with ash particles showed that the emission values of total solid particles exceeded the maximum permissible concentrations up to 30 km from the landfill. The maximum dusting factor was 570 mg/m^3, which is three times higher than the maximum permissible. Given the global trend of changing climatic conditions, this problem can only be increased.

ACKNOWLEDGEMENT

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ORGANIC DYE REMOVAL FROM AQUEOUS SOLUTIONS BY ULTRASOUND SYNTHESIZED LAYERED Mg/Co/Al DOUBLE HYDROXIDE

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Abstract

A novel layered double hydroxide sorbent (Mg/Co/Al-LDH) was synthesized by a co-precipitation method for Reactive Blue 19 (RB19) removal from aqueous solutions. The results indicated that the sorbent exhibited a high sorption capacity when it has been tested using various concentrations of RB19 ranging from 10 to 100 mg dm^{-3}*. A dosage of 0.5 g dm*$^{-3}$ *of Mg/Co/Al-LDH was found to be effective for maximum uptake of RB19. The maximum sorption capacity of RB19 for Mg/Co/Al-LDH are 152.54 mg g*$^{-1}$ *at 20°C and pH 4. The sorption process is very fast and reaching an equilibrium after 5 minutes. The sorption kinetics followed pseudo-second order model.*

Keywords: Reactive Blue 19, layered double hydroxides, sorption, kinetics

INTRODUCTION

Dyes are widely used in various industries. However, presence of hazardous dyes in wastewater is one of the serious global environmental concern [1–3]. Reactive dyes are highly soluble in water, chemically and photolytically stable and originally produced to be resistant to the weather, light, water, and detergents. These dyes present a serious threat to human health and the ecosystem because they are highly toxic, carcinogenic and mutagenic and caused serious consequences to human health and marine system. Reactive Blue 19 (RB19) or disodium salt of 1-amino-2-sulfo-4-(3-sulfoxy-ethyl-sulfophenyl-1-ylamino)-5,10-anthraquinone is one of the very stable and resistant anionic dye. RB19 it may be mutagenic and toxic because of the presence of electrophilic vinylsulfone groups [4]. Therefore, it is extremely important to remove organic dyes from industrial effluents before its discharge to aqueous environment. To date, biodegradation [5], ozonation [6], photocatalytic degradation [7], biological oxidation [8], coagulation [9], filtration [10] and sorption [11] have been applied to eliminate and removal dye effluents from water. Many types of sorbents have been tested in attempts to reduce dye concentrations from aqueous solutions, such as modified bentonite [12], hydrolytic and aerobic microorganisms [13], hexagonal MgO [14], L-arginine-functionalized Fe_3O_4 [15], silica [16], chitosan [17] and biosorbents [18]. Layered double hydroxides (LDH) are used in recent years as potential sorbent materials for wastewater treatment because of high sorption capacity (the large surface area), layered structure and interlayer ion exchange, non-toxicity, economically and feasible. The general formula of the layered double hydroxides can be represented as $\left[M_{1-x}^{2+}M_{x}^{3+}(OH)_2\right]^{x+}\times\left[A_{x/n}^{n-}\times mH_2O\right]$, where

M^{2+} and M^{3+} are divalent and trivalent metal cations, respectively, x is the molar ratio of trivalent cations and A – anions, e.g., Cl^{-}, NO_3^{-}, CO_2^{2-}, SO_4^{2-} etc.).

In this study, triple-metal Mg/Co/Al-LDH was designed and prepared by using simple co-precipitation method as novel sustainable, environmental friendly and effective sorbent for water treatment. The effect of sorbent dose and initial dye concentration was evaluated. The experimental results of RB19 sorption on sorbent Mg/Co/Al-LDH were analyzed using pseudo-first and pseudo-second kinetics model.

MATERIALS AND METHODS

Reagents

All chemicals were of reagent grade and used without further refinement. HNO_3, NaOH, and Na_2CO_3, $Mg(NO_3)_2 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Al(NO_3)_3 \cdot 9H_2O$, RB19, were purchased from Merck (Darmstadt, Germany). All solutions were prepared with deionized water (18 MΩ).

Preparation of the sorbent

Mg/Co/Al-LDH were synthesized by a co-precipitation method by mixing the aqueous solution containing $Mg(NO_3)_2 \cdot 6H_2O$, $Co(NO_3)_2 \cdot 6H_2O$ and $Al(NO_3)_3 \cdot 9H_2O$ with atomic ratio of 1:2:1. The solution was heated to 80°C with a reflux condenser and mixed by agitating and ultrasonication with the power of ultrasound of 100 W dm^{-3} and agitation speed 400 rpm, in period of 1 h. Then, it was dropwise added 1M Na_2CO_3 and 2M NaOH to a vigorously stirred and ultrasonication solution with refluxing at 80°C to pH 11. After complete precipitation with 1M Na_2CO_3 and 2M NaOH the suspension was stirred for 8 h at 80°C. Following this, the mixture was allowed to settle for 24h without stirring, ultrasonication and heating to improve their crystallinity. The resultant suspension was centrifuged and the collected precipitate was washed for several times with deionized water to remove any unreacted component. Finally, the products were dried at 80°C overnight.

Batch sorption experiments

The sorption study was carried out by using RB19 concentration from 10 to 100 mg dm^{-3} and at the temperature of 20°C. A 0.075 g amount Mg/Co/Al-LDH was added into 150 cm^3 of RB19 solution with the certain initial concentration at the pH 4. The effects of Mg/Co/Al-LDH concentration on the removal of RB19 were studied by varying the dose of Mg/Co/Al-LDH from 0.25 to 1.5 g dm^{-3} on 150 mg dm^{-3} of the RB19 concentration, at pH 4 and the temperature of 20°C. The pH of the solution was adjusted to designated value by adding HNO_3 or NaOH (0.1 M and 1 M) with a pH meter (H260G, HACH, USA). RB19 concentration in the samples was determined by using the UV–vis technique by the spectrophotometer Shimadzu UV–vis 1650 PC (Shimadzu, Japan), after filtration through a 0.45 µm membrane filter (Agilent Technologies, Germany).

The sorption capacity q_t (mg/g) was determined by using the equations 1:

$$q_t = \frac{(c_0 - c_t) \times V}{m} \tag{1}$$

Data analyses

In order to investigate the sorption mechanism of RB19 on triple-metal sorbent Mg/Co/Al-LDH, confirm the efficiency of the sorption process and predict the rate, a kinetics investigation was conducted. The kinetics results were examined by using two reaction-based kinetics models: pseudo-first-order (Eq. 2) [19] and pseudo-second-order (Eq. 3) [20].

$$q_{\mathrm{t}} = q_{\mathrm{e,cal}}(1 - e^{-k_1 t}) \tag{2}$$

$$q_{\mathrm{t}} = \frac{k_2 q_{\mathrm{e,cal}}^2 t}{1 + k_2 q_{\mathrm{e,cal}} t} \tag{3}$$

where q_{t} (mg/g) is sorption capacity in time (t), $q_{\mathrm{e,cal}}$ (mg g^{-1}) is equilibrium sorption capacity of the sorbent, and k_1 (min^{-1}) is the pseudo-first order reaction rate equilibrium constant, k_2 (g mg^{-1} min^{-1}) is the rate constant of second order sorption.

RESULTS AND DISCUSSION

Effect of sorbent dose

The effect of Mg/Co/Al-LDH doses on the RB19 removal was investigated by calculating the sorption capacity after contacting various doses with RB19 solution (150 mg dm^{-3}) after 60, 120 and 180 minutes.

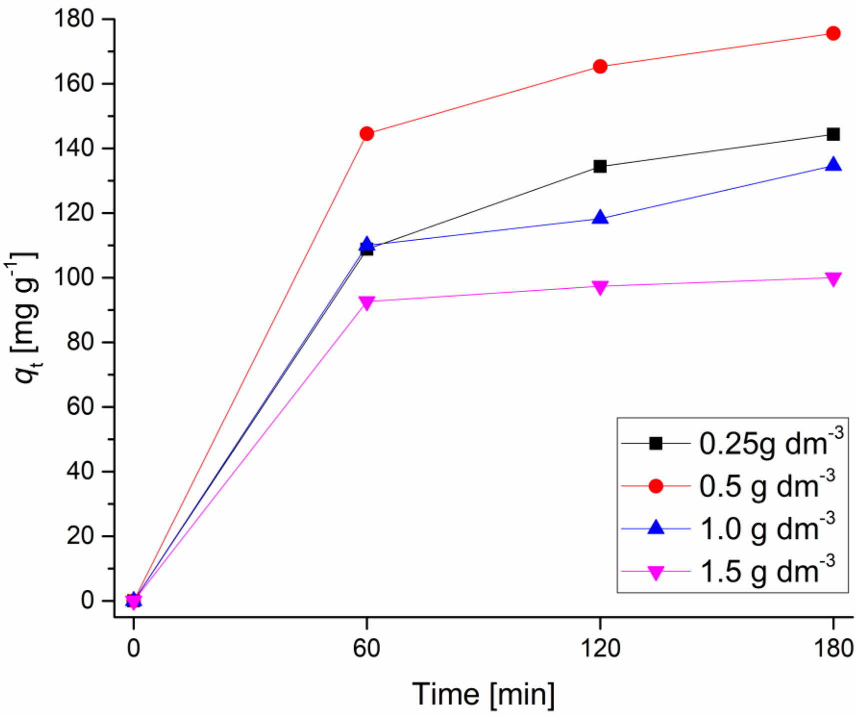

Figure 1 *Results of the effect of sorbent dose on RB19 removal using of Mg/Co/Al-LDH*

From Figure 1 it can be seen that the sorption capacity of RB19 increases with increase of sorbent dose from 0.25 to 0.5 g dm^{-3}, and after that decreases with an increase in the sorbent dose from 0.5 to 1.5. The initial increase in the sorption capacity can be attributed to increased sorbent surface area and the availability of more sorption sites. The decrease in the sorption capacity is due to a decrease in the concentration gradient and the complete removal of RB19.

Kinetics study

The dependency of RB19 sorption on time was studied by varying sorption time from 0 to 60 min, while keeping other parameters such as pH, sorbent dose and initial RB19 concentration constant at 4, 0.5 g dm^{-3}, 100 mg dm^{-3}, respectively. The results (Figure 2) indicate that the sorption was fast and reached an equilibrium value in approximately 5 min for Mg/Co/Al-LDH. The determination coefficient values for sorption RB19 on sorbent Mg/Co/Al-LDH show the greater value for pseudo-second order (Table 1). Also, calculated $q_{e,cal}$ values are very close to experimental value $q_{e,exp}$ values from pseudo-second order. Thus, it can be concluded that the pseudo-second order model better describes kinetics sorption for RB19 onto Mg/Co/Al-LDH, what further implies that the rate-controlling step in the sorption process is the chemical interaction between functional groups of Mg/Co/Al-LDH and RB19 in the solution.

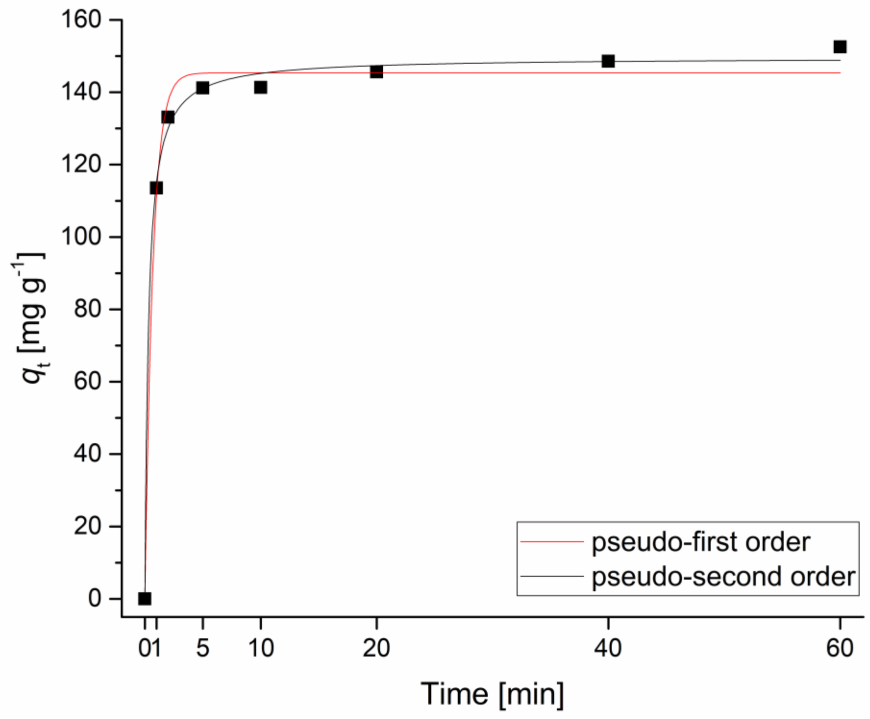

***Figure 2** Pseudo-first and pseudo- second order plot for sorption of Rb19 onto Mg/Co/Al-LDH*

Table 1 *Parameters of kinetics modelling for sorption of RB19 onto Mg/Co/Al-LDH*

Kinetic model	Parameter	Value
	$q_{e,exp}$	152.54
Pseudo-first-order	k_1	1.455
	r^2	0.992
	$q_{e,cal}$	145.37
Pseudo-second-order	k_2	0.022
	r^2	0.998
	$q_{e,cal}$	149.60

CONCLUSIONS

The Mg/Co/Al-LDH has been synthesized by using co-precipitation method at elevated temperature in presence ultrasound. The optimum sorbent dose for the removal of RB19 was 0.5 g dm^{-3}. The equilibrium sorption time was 5 min and the pseudo-second order kinetic model accurately described the RB 19 sorption kinetics by Mg/Co/Al-LDH. The maximum sorption capacity of 152.54 mg g^{-1} for RB19 at temperature of 20 °C and pH 4. The Mg/Co/Al-LDH can be used as potential and effective sorbent for the removal of RB19 from water.

ACKNOWLEDGEMENT

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CuO INCORPORATED $Bi_6O_6(OH)_3(NO_3)_3 \cdot 1.5\ H_2O$ WITH SUPERIOR PHOTOCATALYTIC ACTIVITY FOR DECOLORIZATION OF DYE

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Abstract

CuO incorporated $Bi_6O_6(OH)_3(NO_3)_3 \cdot 1.5\ H_2O$ *as novel photocatalyst was successfully synthesized via a facile hydrothermal method and utilized in aqueous solution for the photocatalytic decolorization of textile dye. The photocatalyst was characterized by scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDS). The optimal photocatalyst dose was 500 mg* dm^{-3}*. The photocatalytic activity was evaluated by removal of Reactive Orange 4 (RO 4) dye, where total decolorization was achieved in less than 15 minutes.*

Keywords: photocatalytic process, Reactive Orange 4, decolorization, SEM, EDX

INTRODUCTION

Wastewaters from dye production and application industries presents a serious threat to the environment [1]. In the past 20 years, many treatments have been developed for water purification. Besides standard technologies, several new technologies, such as advanced oxidation processes (AOPs), have been developed [2]. Nowadays, heterogeneous photocatalysis has become one of the most promising technologies for environment remediation because it is environmentally friendly, capable of performing at room temperature and it can treat organic contaminants at very low concentration [3,4]. The main advantage of heterogeneous photocatalysis is ability of the total mineralization of organic dyes, resulting in formation of CO_2, H_2O, and the corresponding mineral acids, leaving no waste for second disposal [5,6]. Other advantages of heterogeneous photocatalysis are that it does not require using of any other chemicals or soluble catalysts, except insoluble photocatalyst, which can be recovered and re-used, so that secondary pollution is not formed. The effective photocatalytic treatment of all kinds of organic contaminants requires that the photocatalyst should have high efficiency and long life. However, some photocatalysts show low activity, while others lack the catalytic stability, or require rigorous synthetic conditions. Developing a new efficient photocatalyst is still a great challenge in the photocatalysis field so far.

In this manuscript, we synthesized one of the bismuth basic photocatalyst CuO incorporated $Bi_6O_6(OH)_3(NO_3)_3 \cdot 1.5\ H_2O$ via hydrothermal process. The photocatalytic performance for the degradation of a typical anionic dye Reactive Orange 4 (RO 4) over the CuO incorporated $Bi_6O_6(OH)_3(NO_3)_3 \cdot 1.5\ H_2O$ sample is investigated. Furthermore, the explanation for the enhanced photocatalytic performance of CuO incorporated $Bi_6O_6(OH)_3(NO_3)_3 \cdot 1.5\ H_2O$ sample is provided based on the results of SEM and EDX.

MATERIALS AND METHODS

Materials and reagents

The azo reactive dye C.I. Reactive Orange 4 (RO 4) (Mw = 781.74 g mol^{-1}) was obtained from Sigma-Aldrich (USA) and used without any purification. $Bi(NO_3)_3$ x $5H_2O$, $Cu(CH_3COO)_2$, ethanol, HNO_3 and NaOH were of analytical grade and purchased from Merck (Germany). All solutions were prepared with deionized water (18 MΩ).

Characterization of photocatalyst

The surface morphology of the C-BONH surface was analyzed by SEM (Hitachi SU8030). EDS analysis (Thermo Scientific NORAN System 7, USA) provides elemental information via the analysis of X-ray emissions from the catalyst surface.

Experimental set-up and procedure

A stock solution of RO 4 was made by dissolving 1.0 g dye in 1000.0 cm^{-3} of deionized water. Working solutions were prepared freshly, before irradiation, by diluting the stock to the desired concentration with deionized water. The pH of solutions was adjusted by addition of NaOH or HNO_3 (0.1/0.01 mol dm^{-3}). The suspensions of dye and C-BONH were magnetically stirred in dark for 30 min to attain adsorption-desorption equilibrium between dye and photocatalyst, and then the dye solutions are treated in the UV reactor.

Photochemical experiments were carried out in a batch photoreactor handmade in our laboratory (Fig. S1 in Supplementary material section). The UV lamps were turned on 10 min before performing each experiment. The intensity of UV radiation was measured by UV radiometer Solarmeter model 8.0 UVC (Solartech, USA). The total UV intensity was controlled by turning on different numbers of UV lamps and the maximum intensity was 1950 $\mu W\ cm^{-2}$ (with all ten UV lamps on) at the distance of 220 mm, from working solution surface.

The suspensions of dye and photocatalyst were magnetically stirred in dark for 30 min to attain adsorption-desorption equilibrium between dye and photocatalyst, and then the dye solutions are treated in the UV reactor. During irradiation, the solution was magnetically stirred (Are, Velp Scientifica, Italy) in a constant rate and temperature was maintained at $25 \pm 0.5^{o}C$ by thermostating. At required time intervals, 4.0 cm^{-3} of samples were withdrawn, centrifuged (3000 rpm, 5 min) and filtered through a 0.20 μm regenerated cellulose membrane filter (Agilent Technologies, Germany) to separate the catalyst. Absorbance was measured using a UV-vis spectrophotometer Shimadzu UV-1800 PC (Shimadzu, Japan) Shimadzu, Japan), after filtration through a 0.20 μm membrane filter (Agilent Technologies, Germany), to determine the degree of decolorization of the solution. A pH/ISE meter (Orion Star A214, Thermo scientific, USA) was used for pH measurements.

RESULTS AND DISCUSSION

Structure and physicochemical properties of photocatalyst

The morphology and size of sample were studied by SEM. It could be seen that photocatalyst is composed of two and three-dimensional sheet-like nanostructures with uniform thickness of about 200 nm and relatively smooth surface (Figure 1). This implies that the photocatalyst has a relatively large surface area important characteristic for the photocatalytic activity. In addition, the component of photocatalyst was further investigated by EDS. The EDS spectrum and the quantitative elemental composition were show in Figure 2. It was concluded that photocatalyst was composed with Bi, Cu, O and N elements.

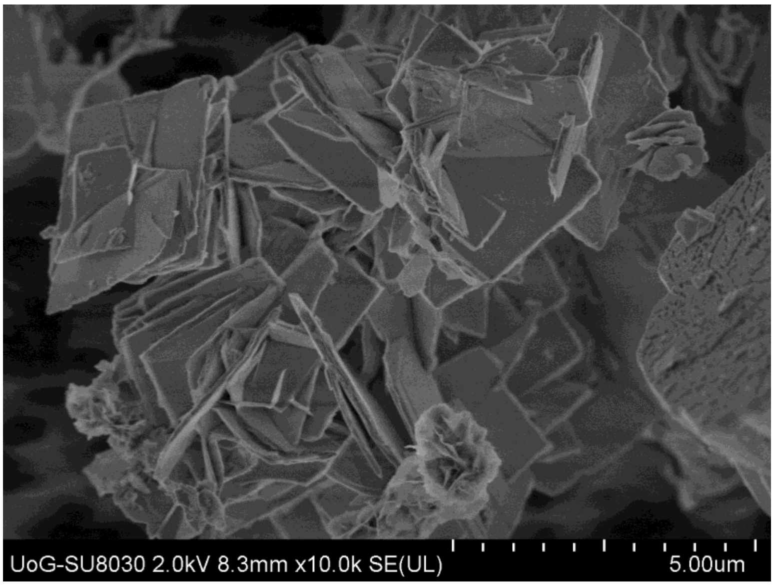
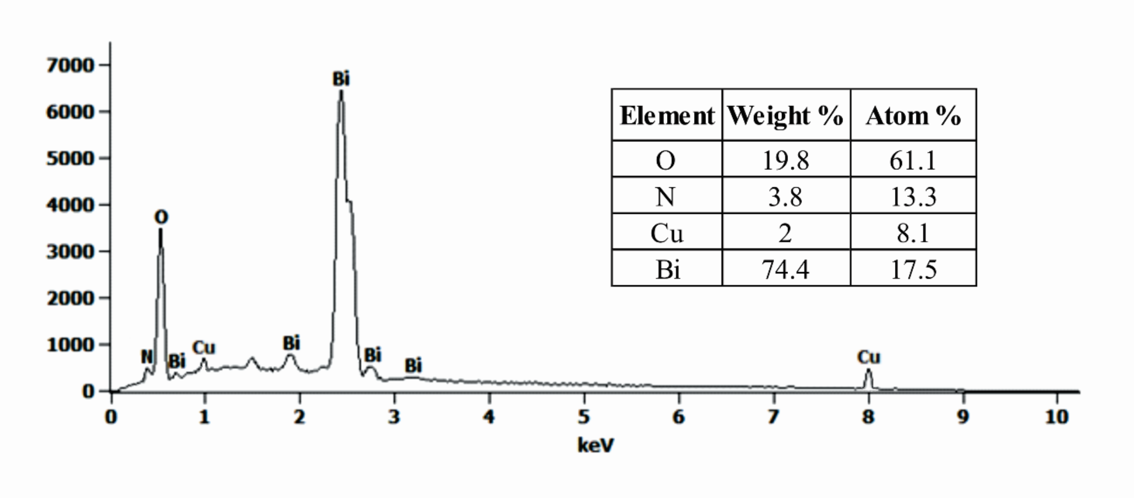


Figure 1 *SEM images of photocatalyst*



Element	Weight %	Atom %
O	19.8	61.1
N	3.8	13.3
Cu	2	8.1
Bi	74.4	17.5

Figure 2 *EDS spectrum of photocatalyst*

Photocatalytic studies

Effect of photocatalyst dose on the photocatalytic performance

To determine the optimal photocatalyst dose, a series of experiments was conducted by varying the initial photocatalyst dose (20, 100, 500, 1000, and 2000 mg dm^{-3}) at native pH and fixed initial dye concentration. The results are shown in Figure 3. An increase in photocatalyst dose from 20 to 2000 mg dm^{-3} increases the decolorization rate rapidly. The total active surface area increases with the increasing of photocatalyst dosage, hence decolorization is faster [7]. Due to the large increase in the catalyst concentration, there is no consequential change in removal efficiency. Thus, it can be concluded that higher photocatalyst dose may not be useful. Hence, the optimal photocatalyst concentration is 500 mg dm^{-3}.

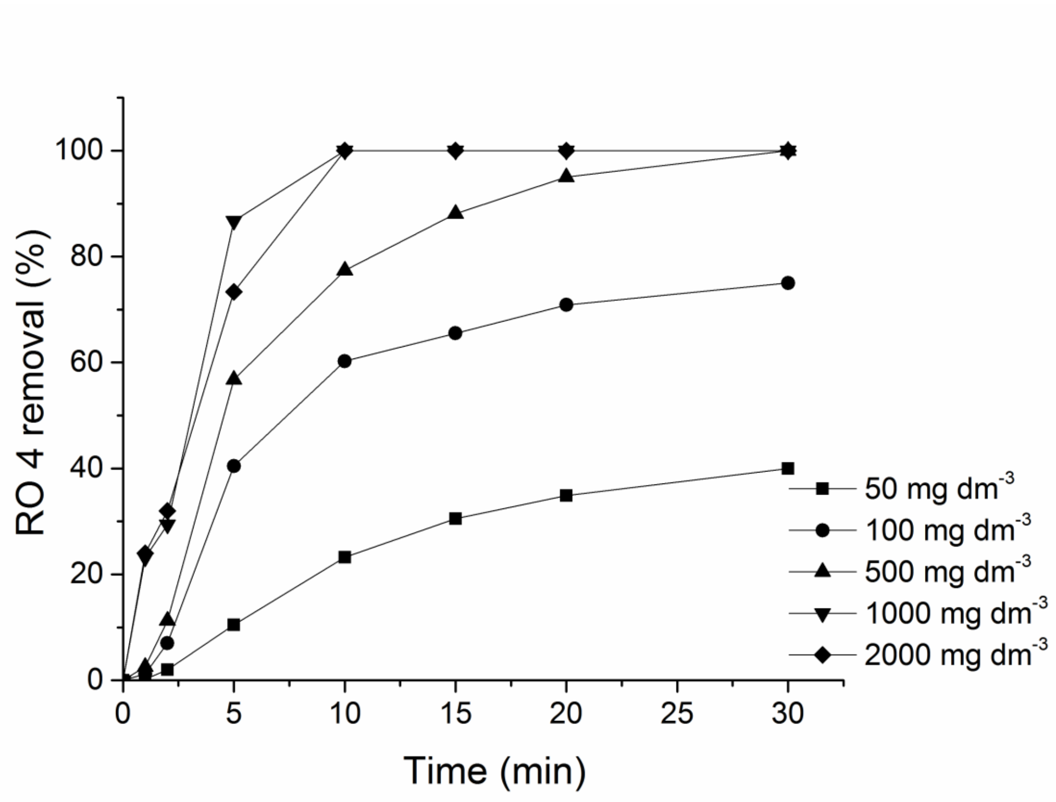


Figure 3 *The effect of photocatalyst dose (c_{RB19} 25 mg dm^{-3}). UV light intensity 1950 μW cm^{-2}, temperature 25 ± 0.5ºC*

CONCLUSION

A new photocatalyst based on bismuth oxo citrate was successfully synthesized by the facile precipitation method. The photocatalyst was characterized by SEM and EDS methods and results showed that obtained material has two and three-dimensional sheet-like nanostructures. The decolorization of RB19 is directly related to the dose of photocatalyst in the suspension, and the optimal photocatalyst dose is 500 mg dm^{-3}. High efficiency, good reusability, facile synthesis, low cost and eco-friendly starting materials makes the new photocatalyst a promising means for the removal of reactive dyes from water.

ACKNOWLEDGEMENT

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FLUORESCENCE SPECTROSCOPY AND PRINCIPAL COMPONENT ANALYSIS IN THE HONEY SAMPLES CLASSIFICATION

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Abstract

Steady state fluorescence spectroscopy in combination with Principal Component Analysis (PCA) for spectral analysis was used to differentiate multifloral honeys from different parts of Serbia. The emission spectra were recorded in the wavelength range 280 – 550 nm, after excitation in the 270 – 370 nm range. After normalization of the spectra, chemometric evaluation of the spectral data was carried out using principal component analysis (PCA). This study indicates that front-face fluorescence spectroscopy is a promising technique for the authentication of geographical origin of honey and may also be useful for determination of the botanical origin within the same unifloral honey type.

Keywords: honey, spectrofluorometry, Principal Component Analysis

INTRODUCTION

Honey is a pure natural food produced by bees from the nectar of flowers. Honey can be considered as sugar syrup, mainly composed of fructose and glucose, along with some proteins, free amino acids, enzymes, vitamins, polyphenols and minerals.

Honey has intrinsic emission properties, which are reportedly attributed to a mixture of fluorophores, like amino acids, vitamins and polyphenols. The positions of emission maxima of the phenolic components vary for various honey samples, but they are in the same emission range 415–450 nm. Component related to the proteins and syringic acid emit at 340 nm and 370 nm, respectively [1–3].

Spectroscopic techniques are fast, relatively low–cost, and provide considerable information about the sample with only one test. They are considered as sensitive, non–destructive, rapid, environmentally friendly, and non–invasive.

The fluorescence spectra, in combination with appropriate statistical methods, may provide useful fingerprints in food analysis [4]. Steady state fluorescence spectroscopy in combination with Principal Component Analysis (PCA) for spectral analysis has been applied to differentiate samples of honey. We have chosen the samples of multifloral honey from different parts of Serbia.

MATERIALS AND METHODS

In this pilot study, eight honey samples were analyzed. All samples are multifloral honeys, collected in 2013, and were obtained from 8 beekeepers from different parts of Serbia. Samples were stored at room temperature in the dark before analysis.

Fluorescence spectroscopy

The fluorescence spectra of the honey samples were recorded using a Fl3–221 P spectrofluorimeter (JobinYvon, Horiba, France), equipped with a 450W Xe lamp and a photomultiplier tube. The sample was placed in solid sample holder, in front-face configuration. The illumination's incident angle was set to 35°C, to minimize light reflections, scattered radiation and depolarization phenomena. The Reyleigh masking was applied in order to reduce Rayleigh scattering from the solid sample which limits the sensitivity and accuracy of the measurement. The fluorescence emission spectra in range from 280 to 550 nm, were recorded with excitation wavelengths of 270 to 370 nm. The integration time was 0.1 s, and the wavelength increment in excitation measurements was 5 nm, and emission increment was 1 nm. A spectral band width of 2 nm was employed for both the excitation and emission slits.

Statistical analysis

The PCA was used to classify honey samples according to the differences in characteristic emission spectra. Principal Component Analysis (PCA) is an application of chemometrics used as a tool in exploratory analysis, which applies algorithms and being designed to reduce large complex data sets, or rearranges the data to exploit linear structure. PCA is a technique using mathematical procedures, such as orthogonal linear transformation from original data. The transformation of new data must have correlation between the new variable, called Principal Components (PCs). PCA was performed by using Unscrambler software (X10). For each sample the average of the 18 emission spectra recorded for various excitation wavelengths was used as the input value in PCA, in order to take into account contribution of all fluorophores present in the sample.

RESULTS AND DISCUSSION

The excitation-emission spectral series were recorded for various excitation wavelengths for each sample. Figure 1a shows excitation–emission spectral series for one of the honey samples. The averaged normalized emission spectra for different samples (Figure 1b), enabled the study of the main emitting compounds in honey, which are the base for estimation of differences between the samples. The spectral shapes, number, and positions of the emission maxima differed among the samples.

All honey samples have maximum in the range 400–450 nm. As phenolic and polyphenolic compounds have been described as reliable indicators of botanical and geographical origin of honeys [5–8] the fluorescence properties of these intrinsic and unique fluorophores may inform identification of floral source reliably.

The PCA was used to classify honey samples according to the differences in characteristic emission spectrum. The dependent variables were honey samples of different geographic origin. The independent variables were the recorded fluorescence emission spectra.

Principal component analysis of honey samples suggested that a two–component model explains 98% of total variance (PC1 accounted for 86% and PC2 for 12%). The scores plot is shown in Figure 2. The PCA scores plot discriminated four groups of samples, which correspond to their geographic origin. This grouping is based on the similarity of the spectra of the samples in corresponding groups. Zlatar, Zlatibor and Tara (Western Serbia) samples have emission maxima at 430nm; Avala and Šumadija (Central Serbia) have emission maxima at 438nm; Deliblatska peščara and Fruška gora (Vojvodina) have emission maxima at 425nm and sample from Kučevo (Eastern Serbia) has emission maxima at 420nm. Since all honey samples are multifloral, similarities of the spectra within the groups on PCA plot indicate differences in plant species at particular geographic regions.

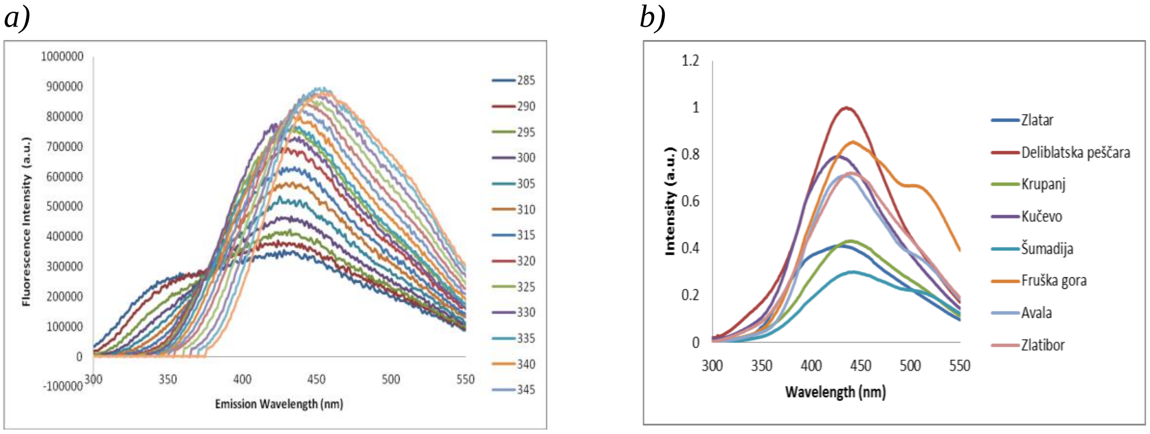

***Figure 1** a) Excitation-emission matrix for the raw spectra b) The normalized emission spectrum of each honey sample is an average of the 18 spectra recorded for various excitation wavelengths*

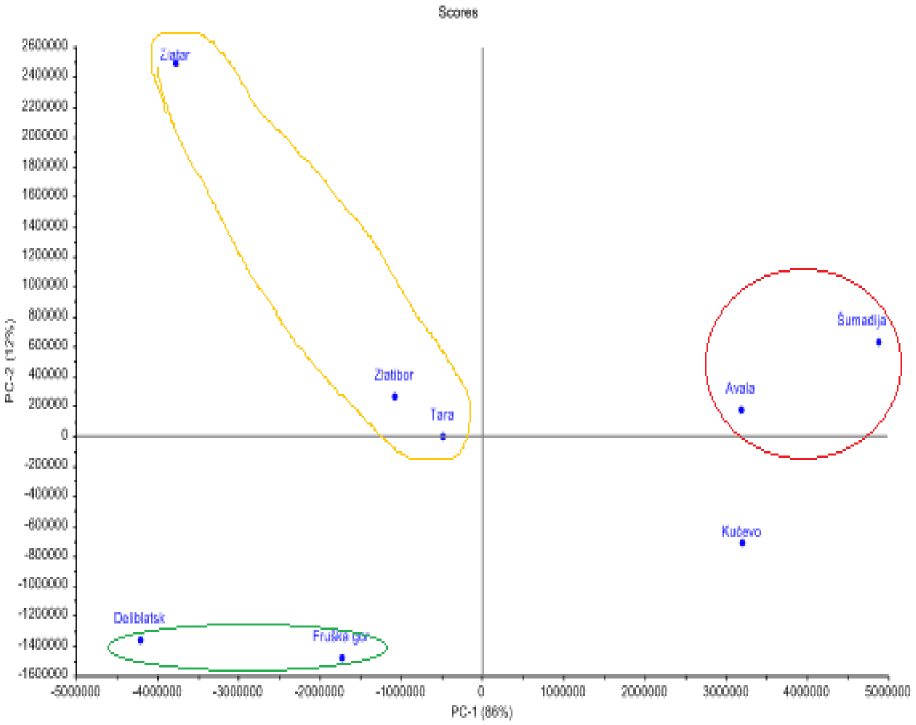

***Figure 2** Principal component analysis of the fluorescence emission spectra*

CONCLUSION

This preliminary study shows that front–face fluorescence spectroscopy combined with chemometrics offers a promising approach for the authentication of the geographical origin of honey. The technique is non-destructive, rapid, easy to use, and not expensive. It does not need any particular sample preparation. The measurements that we performed focused on the fluorescence of a small set of pure honey samples. However these preliminary findings should be confirmed with a larger set of samples and additional honey types.

ACKNOWLEDGEMENT

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DETERMINATION OF PHTHALATES IN PVC BY FTIR AND A PRECIPITATION METHOD

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Abstract

Phthalates are widely used as plasticizers in plastic materials and given their physicochemical properties, they can easily migrate from plastics and thus pollute the environment. In this investigation medical equipment made from PVC was used. In order to isolate phthalates from PVC and determinate then precipitation method and Fourier Transform Infrared Spectroscopy (FTIR) was used. The obtained FTIR spectra show the presence of phthalates in the investigated plastic samples. The characteristic FTIR spectra absorbance band at 743 cm^{-1}*and doublet bands at 1579 cm*$^{-1}$*and 1599 cm*$^{-1}$ *prove the presence of phthalates in PVC samples. Based on the absorption intensity, it can be determined which plastic sample containes the most phthalates. The absence of given absorbance bands in FTIR spectra of plastic samples that was released from phthalates by the precipitation method shows the efficiency of the precipitation method. The FTIR method ensures rapid, reliable and not expensive qualitative determination of phthalates.*

Keywords: phthalates, PVC, FTIR, precipitation

INTRODUCTION

Phthalates are a class of organic compounds produced by esterification of phthalic acid with different aliphatic alcohols. The chemical structures of phthalates are similar, the difference is in the ester groups attached to the benzene ring in an ortho position. The basic chemical structure of phthalates is given on Figure 1.

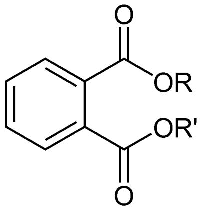

Figure 1 *The basic chemical structure of phthalate (R, R`=*C_nH2_{n+1}*, n=1-15)*

The physicochemical properties of phthalates depend on molecular mass of phthalates. The common feature of all phthalates is colourless, odourless, volatility, high density, solubility in non-polar solvents [1]. With an increase of length of hydrocarbon chain and thus, increase in molecular mass, their insolubility in polar solvents increases.

Phthalates are commonly used as plasticizers in the production of plastics. The most commonly used phthalate is di-*n*-ethyl hexyl phthalate (DEHP). 15-30% phthalates are added to polymers, such as PVC, in order to transform the glassy unmodified PVC into a soft and flexible rubbery material, and in this way make it easier to process these plastic materials [2]. Since phthalates are physically bonded to the plastic, they can easily leach out to their environment, including beverages, food products, air and water [3]. In this way, humans are exposed to phthalates in several ways: through ingestion, inhalation and dermal contact throughout their lifetimes. Literature data indicate the effect of phthalates on human health and phthalates are classified as endocrine disruptors and affect reproduction [4–6].

Instrumental techniques for the separation and detection of phthalates are different and depend on the physicochemical properties of phthalates and the matrices being examined. The most commonly used techniques are liquid (LC) and gas (GC) techniques coupled to mass spectrometry (MS detection systems). The Fourier Transform Infrared Spectroscopy (FTIR) has the significance in the pre-screening for phthalates in plastic materials, because this technique is reliable, non-destructive, does not require special preparation of the samples, thus it is more rapid and far less costly than the GC-MS method.

The aim of this paper is the analysis of presence of phthalates in medical equipment, which is a reliable source of phthalates, because it is made of PVC. First, a qualitative analysis will be performed using FTIR technique, after which the phthalate will be removed from the PVC by precipitation method, which will also be verified using the FTIR technique.

MATERIALS AND METHODS

Chemicals and reagents

The tetrahydrofuran (HPLC grade) was purchased from Fischer scientific (USA). The *n*-hexane (HPLC grade) was purchased from Carlo Erba (France).

Medical equipment made of PVC was taken from local Clinical Center Niš, Serbia. Transfusion set with transfer bag, quadrupole blood bag, Sag-M bag and transfusion tubing (TIANHE Pharmaceutical, Chine) was used for analysis.

FTIR technique

FTIR spectra were recorded on a laser Fourier Transform Infrared Spectroscop (BOMEM Hartman & Braun — Michelson MB series 100).

The manual hydraulic press (Graseby Specac) was used. This hydraulic press is designed fundamentally to create pellet samples which can then be processed using analytical equipment such as FTIR and provide high optical throughput for precise and efficient analysis.

Amalgamator (Wig-L-Bug) was used for mixing a variety of materials including precipitates from this analysis.

Gravimetric Techniques

n-Hexane was used to produce the PVC precipitate from tetrahydrofuran solution. The centrifuge (Jouan C4I Benchtop, Termo Fisher) was used to separate precipitates from the aliquot. Mass of PVC precipitate after drying was measured on the analytical balance with accuracy of ±0.00001 g.

PVC sample dissolve in THF

The noted medical equipment (plasticized PVC) was used as sample for this investigation. 0.01 g of sample was weighted and transferred into 8 mL vial. After that, 4 mL of tetrahydrofuran was added in vial. In order to quickly dissolve the plastic into tetrahydrofuran the vial was placed on the hot plate and obtained covered with glass to prevent evaporation and loss of tetrahydrofuran. After dissolving PVC, the obtained solution was poured into evaporating dish. After evaporation of the tetrahydrofuran for 30-40 minutes, a polymer film was formed. The polymer film was created on the evaporating dish and was mounted on an IR cell holder on FTIR instrument and FTIR spectrum was scanned. Each analysis is repeated three times.

PVC sample dissolve in THF and precipitation by *n*-hexane

The mass of the sample of 0.01 g was dissolved in 4 ml of tetrahydrofuran. After dissolution, 10 ml of *n*-hexane was added to the obtained solution. The PVC precipitate without phthalates was obtained, while dissolved phthalates were found in the aliquot above the precipitate.

The PVC precipitate was separated from the aliquot by centrifugation (3500 rpm). In the oven at 80°C the PVC precipitate was dried. After that, PVC precipitate was mixed in amalgamator with KBr and pellets were formed by manual hydraulic press. FTIR spectrum was recorded to confirm completely release of phthalates from PVC sample. In order to confirm the complete removal of phthalates from PVC sample by n-hexane precipitation, the following was done. Phthalates-free PVC precipitate was dissolved in tetrahydrofuran, after which the obtained solution was poured into evaporating dish, left for tetrahydrofuran evaporation and obtained polymer film that did not contain phthalates was scanned on FTIR instrument.

The aliquot with dissolved phthalate was placed into evaporating dish and after evaporation obtained disk was gravimetrically measured. These was the plasticizers from the PVC sample. The weight of the film represented mass of the phthalates in PVC, so the mass percentage of phthalates in PVC samples was determinate.

Safety precautions and reduction of sample contamination by phthalates

Used solvents are volatile and flammable, therefore, care must be taken. Tetrahydrofuran is toxic by inhalation and ingestion and the evaporation must be done in the digester. In order to reduce laboratory phthalates contamination, and since tetrahydrofuran dissolves PVC, only glassware equipment was used. All glassware was washed with soap, tap water and ultrapure water, then washed with acetone and n-hexane and dried at 200 °C in the oven for 4 hours [7].

RESULTS AND DISCUSSION

Characterization of IR spectra of PVC sample dissolved in tetrahydrofuran

The strong absorbance band at 743 cm^{-1} in the infrared spectrum identifies the phthalates, due to their ortho substituted aromatic ring. Other useful bands for infrared analysis of phthalates are the doublet bands at 1599 cm^{-1} and 1579 cm^{-1}. These bands appear as a result of the aromatic ring quadrant stretching vibration, and their frequency and intensity depends on the aromatic ring substitution. Also, the characteristic FTIR spectra absorption bands for PVC were: 2960 cm^{-1} (for -C-H bond), 1718 cm^{-1} (for the phthalate ester), 1450 cm^{-1} (for $-CH_2-$ and $-CH_3$), 1278 cm^{-1} (for $-CH_2-$), 1073 cm^{-1} (for $-CH_3$). In the figure 2 IR absorption spectrum of PVC sample is given.

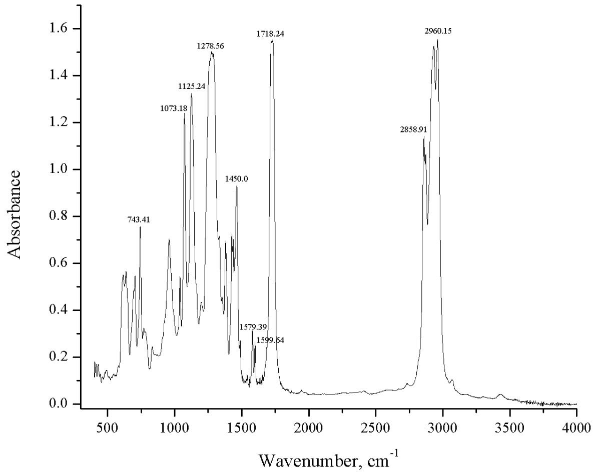

***Figure 2** The FTIR spectrum of transfusion tubing, with qualitative bands identified*

In the Figure 3 two regions of the IR spectra of PVC samples were given. Based on the given spectra it can be concluded which PVC sample contains the most phthalates. The tubing for transfusion contains the most phthalates, followed by quadrupole blood bag, transfer bag and Sag-M bag.

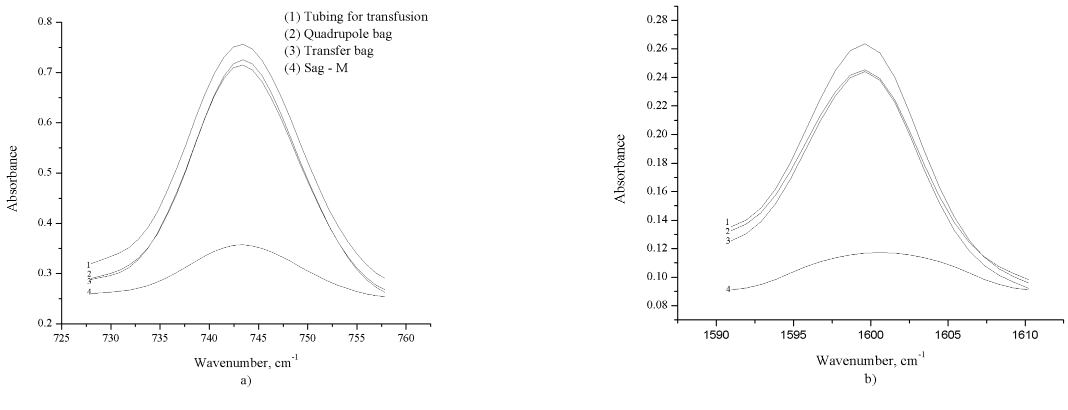

***Figure 3** The FTIR spectrum of PVC samples: a) region 730-760 cm^{-1} b) region 1590-1610 cm^{-1}*

Characterization of IR spectra of PVC sample dissolved in tetrahydrofuran and precipitated by *n*-hexane

Obtained KBr pellets were recorded by FTIR instrument and given spectra show absence of characteristic absorbance bands (743 cm^{-1}, 1579 cm^{-1}, 1599 cm^{-1}) for phthalates. In Figure 4 FTIR spectrum of one KBr pellet is given. These data indicate that the precipitation method of PVC by *n*-hexane completely releases phthalate from PVC samples. Identical FTIR spectra was obtained by recording a plastic film obtained by evaporation of the dissolved precipitate in tetrahydrofuran.

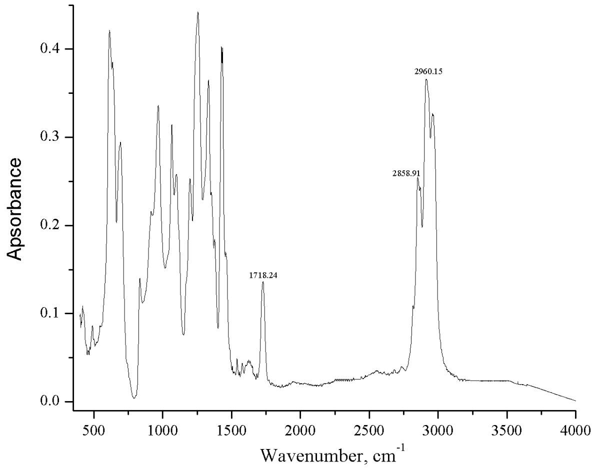

Figure 4 *The FTIR spectrum of transfer bag without phthalates*

Analysis of aliquots above precipitates, with dissolved phthalates, give the following results. The weight of polymer film after evaporation of *n*-hexane-tetrahydrofuran give the total mass of phthalates in the sample. Obtained calculated mass percentage of phthalates in PVC samples is given in Table 1.

Table 1 *Mass % of phthalates of PVC sample*

Sample	Mass % of phthalates
Tubing for transfusion	29.25±0.31
Quadrupole bag	25.51±0.06
Transfer bag	25.11±0.10
Sag-M	5.50±0.09

During this analysis, two polymer films were obtained. The first polymer film was obtained by evaporation of solvent from the solution of dissolving PVC with phthalates in tetrahydrofuran was elastic and highly flexible, which is related to the effect of phthalates. The second polymer film was obtained by evaporation of solvents from the *n*-hexane-tetrahydrofuran solution of the PVC precipitates without phthalates was non-flexible, which showed that the phthalates are removed from the PVC sample.

CONCLUSION

Bearing in mind structure of phthalates and their ortho substituted aromatic ring, expected characteristic absorbance bands in FTIR spectra occurred. In this way pre-screening of phthalate content in PVC samples can be done, because this method is a rapid and efficient, after which the GC-MS analysis can be performed. For the phthalate analysis the precipitation technique and evaporation method of the aliquot above precipitate can also be used. A more efficient method for recording FTIR spectra of PVC-free phthalates, give the same results, is the method of making KBr pellets, because it is rapidly than the method of re-dissolving the precipitates in tetrahydrofuran.

ACKNOWLEDGEMENT

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STUDY OF DI-*N*-ETHYL HEXYL PHTHALATE MIGRATION FROM PLASTIC MATERIALS

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Abstract

The aim of this paper was to examine the migration of the most frequently used phthalate, di-n-ethyl hexyl phthalate (DEHP), from the most commonly used plastics: polyethylene terephthalate (PET), high-density polyethylene (HDPE), polyvinyl chloride (PVC), low-density polyethylene (LDPE), polypropylene (PP), polystyrene (PS) and polycarbonate (PC). The investigation was performed through leaching of phthalates from plastic into n-hexane for three different leaching duration periods (6, 15 and 30 days). DEHP determination was carried out by gas chromatography coupled with mass spectrometry (GC/MS). The results showed that PVC samples contain DEHP in signaficient amount, from 5.19 to 28.76% by weight of samples after 30 days of leaching, which is related to the fact that phthalates are always added to PVC to be come softer, more elastic, with a longer lifetime. Bearing in mind results and that tested PVC is medical equipment, that is used in important medical procedures (peritoneal dialysis, transfusion and infusion), presence of DEHP in investigated samples is a potential risk to human health. The GC/MS analysis found that other types of plastics do not contain DEHP and that food and beverages packaging are safe to use.

Keywords: DEHP, plastic materials, migration, GC-MS

INTRODUCTION

Plastic materials that characterize suitable properties and relatively low cost are more commonly used as compared to other materials in the production of food packaging, cutlery, children toys, cosmetic packaging. Some plastics from an environmental aspect are appropriate and safe to use, while other plastics contain harmful chemicals and pollute the environment during production and usage. There are recyclable codes that are stamped on the underside of plastic products to indicate to consumers which plastic is safer for use. The most usually used plastics are marked with numbers from 1 to 7. In Table 1 the names of plastics, their abbreviations and recyclable codes are noted. Harmless plastics are marked with numbers 1, 2, 4 and 5. Plastic marked with numbers 3, 6 and 7 could be potentially harmfull because it contains toxic chemicals which affect human health. PVC releases chemicals known as phthalates which inhibit hormone activity in the human body. In spite of this, PVC is still the most commonly used bottle plastic.

***Table 1** The most commonly used plastics*

Recyclable codes	Name of plastic	Abbreviations
1	Polyethylene terephthalate	PET
2	High-density polyethylene	HDPE
3	Polyvinyl chloride	PVC
4	Low-density polyethylene	LDPE
5	Polypropylene	PP
6	Polystyrene	PS
7	Polycarbonate	PC

Esters of phthalic acid, known as phthalates, are added in the PVC during production process, in order to achieve its flexibility, softness and transparency. Given that phthalates are not bound by chemical bonds to plastics, they can be easily migrated from PVC under the influence of external factors, making the plastic inelastic and breakable. The most commonly used plasticizer is di-*n*-ethyl hexyl phthalate (DEHP) and plastics may content up to 40 % (w/w) of DEHP [1]. Annual production of DEHP is about 2 million tons worldwide [2]. DEHP is used in many products, such as medical equipment (peritoneal dialysis bag with tubing, transfusion set with blood bag and tubing, infusion tubing, *etc*.), consumer products (food packaging, beverages packaging, *etc*.), toys, cosmetics [3-4]. Considering the already mentioned nature of the bonding between phthalate and plastics, DEHP can be easily migrated from plastics in the environment, especially in fatty foods, because of its physicochemical properties (DEHP is soluble in non-polar solvents and poorly soluble in polar solvents). In this way, DEHP can come into human by ingestion, inhalation and through the skin. Scientific studies have shown harmful effect of DEHP on human health, making it one of endocrine disruptors [5]. In Europe tolerable daily intake (TDI) value for DEHP is 50 μg/kg bodyweight [6]. Bearing in mind impact of DEHP on human health, it is important to determine phthalates in different plastics.

There are different techniques for determining DEHP in plastics, but the most common technique is gas chromatography mass spectrometry analysis (GC/MS). GC/MS analysis is rapid and reliable and requires appropriate preparation of the samples.

The aim of this work is determination of DEHP in samples of plastics, marked with number 1 to 7, which is used for food and beverages containers and medical equipment, by GC/MS analysis. DEHP determination is carried out by leaching of DEHP in a non-polar *n*-hexane which is related to its lipophilic character. Leaching of DEHP was monitored during 6, 15 and 30 days. In this way, the potential pollution of food and beverage samples by DEHP is demonstrated.

MATERIALS AND METHODS

Reagents and materials

Chemical reagents

HPLC grade *n*-hexane was purchased from Carlo Erba (France). Di-*n*-ethyl hexyl phthalate (DEHP) was purchased, in the highest available purity, from Sigma–Aldrich (USA). Dibutyl adipate (DBA) was purchased from Fluka (Switzerland) and used as an internal standard.

Investigated samples

Samples used for this analysis was plastics used for food containers marked with number 1 to 7. All plastic samples were purchased at local store at Niš (Serbia), except medical equipment made of PVC which were taken from local Clinical Center Niš (Serbia).

Preparation of calibration standards

All stock, intermediate and working solutions were prepared in *n*-hexane. The stock solutions of DEHP and DBA were prepared at a concentration of 1000 µg mL^{-1} of each. The stock standard was diluted stepwise with *n*-hexane to prepare working solutions of DEHP. The calibration standard series is obtained with DEHP concentration range 0.25, 0.50, 1.00, 1.50 and 2.50 µg mL^{-1} with DBA at a concentration of 1 µg mL^{-1}.

GC-MS technique

Gas chromatographic analysis was performed by gas chromatograph 6890 (Hewlett-Packard, USA) equipped with a mass selective detector (MSD) 5973 (Hewlett-Packard, USA) and Autosampler 7683 (Agilent, USA) and SGE 25QC2/BPX5 0.25 capillary column (25 m×0.22 mm×0.25 µm, non-polar). The gas chromatograph was operated in the split less injection mode. The oven temperature was programmed from initial temperature 90 °C (hold time 0 min) to 280 °C at a rate of 20 °C min^{-1} with hold time of 4 min, and post run 300 °C (2 min). Helium was the carrier gas (flow rate of 1.0 mL min^{-1}). The operating temperature of the MSD was 280 °C with the electron impact ionization (EI) voltage of 70 eV. The dwell time was 100 ms. The MSD was used in the single ion-monitoring mode (SIM), the quantification ion is *m/z* 149 for DEHP and ion *m/z* 185 was chosen as representative ion of DBA. Analyte response was normalized to DBA as internal standard. The identification and quantification of target compound was based on the relative retention time, the presence of target ions and its relative abundance. Both data acquisition and processing were accomplished by Agilent MSD ChemStation® D.02.00.275 software.

Sample preparation

In this study a total of 44 samples of plastics marked with number from 1 to 7 were analysed. Seven different types of medical equipment made of PVC was examined: dialysis bag with coupled tubing which constitute a set for peritoneal dialysis (Baxter, USA), three bags (quadruple blood bag, SAG-M transfer bag and transfer bag) and coupled tubing (TIANHE Pharmaceutical, China) and tubing for infusion set (Mediaset, Spain). Plastic packaging used for dairy products and water, made of PET and PP, was investigated on content of DEHP. Also, these include 28 different samples: one milk bottle, two plastic bags for milk, eight yoghurt bottles, three fruit yogurt bottles, seven yogurt cups, cap for sour milk, one bottle for non-carbonated mineral water, one bottle for carbonated mineral water, one disposable plastic foods packaging, one cellophane, one freezer bag and one cutting board. An egg box made from PS and disposable platter of the same content were examined. One bag from HDPE plastic and HDPE granulate from which further packaging are produced for different uses were analysed. Bottle for infusion (Hemofarm, Serbia), bottle with Ringer's solution for parenteral nutrition (Zdravlje, Serbia), granulate and zip bag for food made of LDPE was examined. One packaging bag made of PC was examined. At the end, dialysis bag (Biofine, Germany) made of a substance from the group of polyolefins were analysed.

On the analytical balance with accuracy of ±0.00001 g, was measured about 0.02 g of each plastic sample (each plastic sample was cut into pieces with area about 0.5 cm^2). *n*-Hexane (5 ml) was added in each plastic sample in glass vial. DEHP migration into *n*-hexane was monitored within 6, 15 and 30 days. The analysis of each sample was done three times, to obtain reliable data covered by standard deviation (SD). After 6, 15, and 30 days, a certain amount of *n*-hexane (900 µL) was removed from *n*-hexane solution, DBA (100 µL from solution of DBA 10 µg mL^{-1}) was added and recording was performed by GC/MS.

In order to avoid laboratory phthalates contamination, only glassware was used. The glassware was prepared in the manner described in the reference [8].

RESULTS AND DISCUSSION

GC-MS analysis

The chromatogram of a standard solution of DEHP and DBA, is given in Figure 1. The given chromatogram shows good separation of DEHP and DBA occurred within a running time of 13.5 min with retention times for DBA and DEHP 6.413 and 10.078 min, respectively.

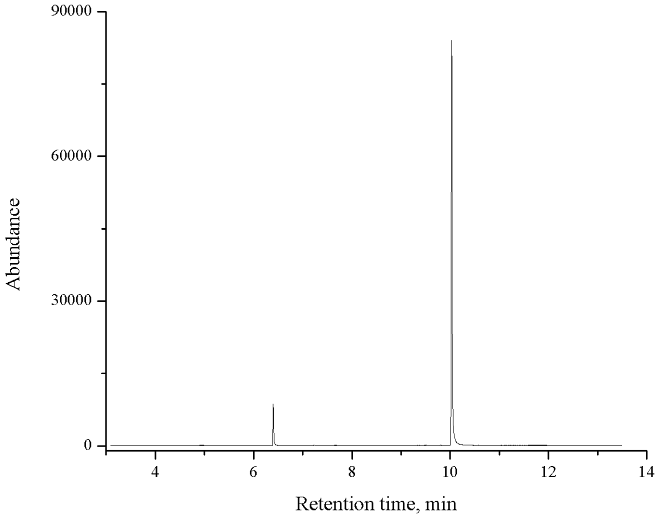

***Figure 1** GC/MS chromatogram of a standard solution of DEHP and DBA in a concentration of 2.5 µg mL^{-1} and 1.00 µg mL^{-1}, respectively*

The calibration curve was linear in the range from 0.25 to 2.5 µg mL^{-1} with correlation coefficient of $R^2 = 0.99$ and linear equation $y = (2488.2 \pm 17947.8)\ x + (358701.3 \pm 14027.4)$.

The limit of determination (LOD) and limit of quantification (LOQ) were calculated from the signal/noise ratios which were multiplied with the factor 3 and 10 respectively [9]. The LOD and the LOQ values for DEHP are 0.04 µg mL^{-1} and 0.12 µg mL^{-1} with relative standard deviation (RSD) value 3.6 % ($n = 3$).

Leaching of DEHP

In order to determine the amount of leached DEHP in samples, the GC/MS analysis was performed after 6, 15 and 30 days of leaching. Chromatograms were recorded after addition of internal standard. On the basis of the performed calibration, the quantification of the samples was carried out. The amount of leached DEHP in the investigated PVC samples is given in Table 2. DEHP was found in extracts from all PVC samples (medical equipment) in concentration levels of 5.19-28.76% by weight of PVC samples. These are the expected results since it is known that phthalates are added to the PVC to soften it.

***Table 2** Concentration of leached DEHP (mg g^{-1}) from PVC material determined for different extraction times (6, 15 and 30 days) SD – standard deviation (n = 3)*

	Concentration of leached DEHP (mg g^{-1})		
PVC samples	6 days	15 days	30 days
Transfusion tubing	139.40±1.27^{a}	177.80±3.44^{b}	287.58±7.67^{c}
Tubing for infusion set	127.35±1.20^{a}	161.43±1.76^{b}	263.60±6.36^{c}
Quadruple blood bag	119.45±1.48^{a}	152.60±2.21^{b}	257.21±5.95^{c}
Transfer bag	120.20±0.57^{a}	154.62±7.35^{b}	251.68±3.79^{c}
Dialysis tubing	101.45±1.34^{a}	109.91±4.41^{a}	205.71±7.23^{b}
Dialysis bag	91.50±2.97^{a}	100.33±4.98^{a}	188.14±6.87^{b}
SAG-M transfer bag	23.60±0.71^{a}	26.37±2.16^{a}	51.90±0.14^{b}

$^{a–c}$ values with the same letter within a row are not statistically significant different at the $p < 0.05$ level (Tukey's HSD test)

Difference between the leached concentration of DEHP obtained after different extraction times (6, 15 and 30 days) was determinated using the post-hoc test, Tukey's test. In order to determinate significant of difference, difference was compared to a critical value. The adjustment multiple testing was carried out with the aim of comparing difference between each pair of mean values. The critical value (q) is the point when a mean difference becomes honestly significantly different and it was obtained from table values. The critical value for the examined samples was 4.34 [9]. Values of HSD (honest significant difference) for each pair were computed by Origin© program. Comparing was performed in case $p < 0.05$.

Obtained results using Tukey's post-hoc test indicated significant difference between the obtained amounts of leached DEHP for 6 days extraction period and 30 days extraction period for all samples. There are significant diiferences between the obtained results for 6 days extraction period and 15 days extraction period for transfusion tubing, tubing for infusion set, quadrupole blood bag and transfer bag. For the remaining three samples, results showed no significant difference between these periods of leaching. Obtained amount of leached DEHP showed significant difference between the obtained amounts of leached DEHP for 15 days extraction period and 30 days extraction period for all samples.

Migration of DEHP from other plastic samples (PET, LDPE, HDPE, PP, PS and PC) did not occur even after 30 days, as determined by GC/MS analysis of *n*-hexane extracts of these plastic samples. From this it can be concluded that these types of plastics do not contain phthalates. Bearing in mind these results, the use of these investigated food and beverages packaging and mediqal equipment made from LDPE, can not negatively affect human health, as they don't contain DEHP. Otherwise, DEHP from the samples would tend to migrate to fatty foods and milk, which would endanger the human health.

In this work, the peritoneal dialysis bag made of Biofine materials, which does not contain phthalates, was also examined. Analyisis confirmed this fact. In view of this, these results should be kept in mind because using this bag does not cause harmful effects of DEHP on human health.

CONCLUSION

Considering the technical and economic superiority of a variety of plastic materials, they have been used in the production of consumer products and in practically all areas of the technique. Given the nature of the bound between phthalate and plastics, DEHP migration from plastics under certain conditions was tested. In this study was found that PVC plastic contains DEHP in significant amount which may endanger the environment. In view of these results, it can be concluded that the tested samples for food and beverage packaging are safe to use.

ACKNOWLEDGEMENT

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INVESTIGATION OF AMMONIUM HYDROXIDE EFFECT ON DnBP EXTRACTION FROM MILK SAMPLES

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Abstract

In this study, influence of ammonium hydroxide addition on di-n-buthyl phthalate (DnBP) extraction from milk samples was investigated. Ammonium hydroxide addition was done before extraction step. Effects of ammonium hydroxide were studied using spiked milk samples with known amount of DnBP standard solution in concentration range 0.25 to 1.25 mg L^{-1}*. DnBP determination was carried out by gas chromatography – mass spectrometry (GC-MS). Obtained results indicate that extraction method which included addition of ammonium hydroxide higher mean recovery for all spiked concentrations of DnBP (51.91%) than extraction without addition of ammonium hydroxide (29.90%). The recoveries were 25.54% to 35.27% at five spiked levels for samples treated by extraction procedure without addition ammonium hydroxide, and in range from 47.88% to 61.50% for samples treated by extraction procedure with addition of ammonium hydroxide.*

Keywords: phthalate, milk, extraction, ammonium hydroxide

INTRODUCTION

Phthalates are used as plasticizers in producing of polymeric materials in order to increase their physical properties such as flexibility, transparency and softness. Millions of tons of phthalates are produced over the world annually and the most popular plasticizer is di-2-ethylhexyl phthalate (DEHP), followed by di-*n*-buthyl phthalate (D*n*BP), di-iso-decyl phthalate (D*i*DP) and di-iso-nonyl phthalate (D*i*NP) [1]. Phthalates are not physically bonded to plastics and can migrate into air, groundwater, soil, food and then come into food chain [2]. A lot of packaging materials are made of polymeric material that could contain phthalates. The most used phthalates in polymers of food packaging materials are DEHP and DBP [3-5]. These phthalates could metabolize to bioactive phthalate monoesters and they are listed as endocrine disruptors [6].

Due to fact that phthalates are not physically bonded to plastic material, in case of contact with fat-containing food they tend to migrate in food, due to their lipophilic structure [7]. Milk as a complex matrix, can contains proteins and different amounts of fat, and it causes that different phthalates can be bound to this matrix constituents with different affinity, depending of their structure and length of chain. Bearing in mind that milk is a major source of nutrition for infants and children, determination of D*n*BP and DEHP presence in milk is of increasing interest [8].

Techniques such as solvent extraction and solid phase extraction are used to clean up and concentrate milk samples before analysis. It is very important to find appropriate solvent or combination of few solvents to obtain high recovery [9]. The main objective of this work was to investigate if addition of ammonium hydroxide can improve extraction of D*n*BP from milk samples based on fact that addition of ammonium hydroxide causes disruption of milk fat globule and forms free fatty acids which then remain in the aqueous phase and are not extracted.

MATERIALS AND METHODS

Reagents and equipment

D*n*BP standard, dibuthyl adipate (DBA) and hexane were purchased from Sigma Aldrich, USA. Ethanol was purchased from Baker, USA. All used solvents were HPLC grade and screened to determine the phthalate background.

All sample and standard manipulation was done avoiding any contact with plastic equipment. Amounts of DnBP and DBA standard were accurately weighted out by analytical balance with precision at ±0.0001 g (Kern, Germany) and stock solutions were prepared by hexane diluting. Stock and working standards were stored in fridge.

Sample preparation

Milk samples with 2.8% milk fat content were purchased from local supermarket (Niš, Serbia). Effects of solvent extraction were studied by spiking known amount of D*n*BP standard solution in concentration range from 0.25 to 1.25 mg L^{-1} in samples before extraction steps. A volume of 10 mL milk sample was put in a glass centrifuge tube and 5 mL of ethanol was added. Mixture was treated under ultrasonic conditions for 30 min and centrifuged for 10 min at 4000 rpm. After this step, 10 mL of supernatant was transferred into clean polypropylene tube, 5 mL of hexane was added, and mixture was shaken and centrifuged once more by the same conditions. Hexane layer was analysed by GC-MS. All spiked samples and blank sample were analysed in three replicates. This procedure was repeated for the same milk samples with additional step. Before addition of ethanol, 1 mL of ammonium hydroxide was added to each analysed sample.

GC-MS analysis

Analysis was carried out by gas chromatograph coupled to mass spectrometer (Hewlett Packard 6890 series GC System with autosampler connected with Agilent 5973 Mass Selective Detector (Electron Ionization MSD-EI, single quadrupole)). The separation was achieved with 30 m × 0.25 mm × 0.25 µm a non-polar AGILENT DB-5MS column coated with 5% phenyl, 95% dimethylpolysiloxane. The MSD was used in the single ion-monitoring (SIM) mode. The identification of target compounds was based on the relative retention time, the presence of target ions and their relative abundance. The most abundant ion *m/z* 149 was chosen for quantification of D*n*BP. Ion *m/z* 185 was chosen as representative ion of DBA internal standard. The dwell time was 100 ms.

RESULTS AND DISCUSSION

The chromatogram in Figure 1 shows that the separation of D*n*BP and DBA, as internal standard, occurred within a running time of 15 min. Retention times for DBA and DEHP were 9.940 and 11.557 min, respectively.

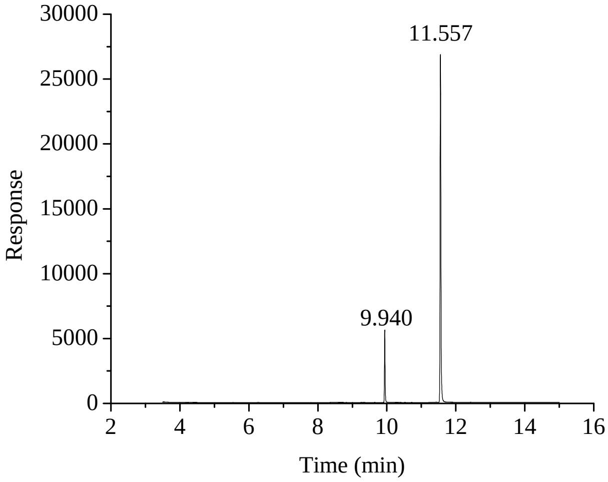

***Figure 1** Chromatogram of a standard solution containing DnBP in concentration 0.25 µg mL^{-1} and DBA in concentration 1 µg mL^{-1}*

All quantifications were performed by an external calibration method based on response ratios between internal standard and D*n*BP. The analytical curve obtained for D*n*BP in concentration range 0.25–10.0 µg mL^{-1} was linear for the given range with coefficient of determination, R^2, 0.99922 (Figure 2).

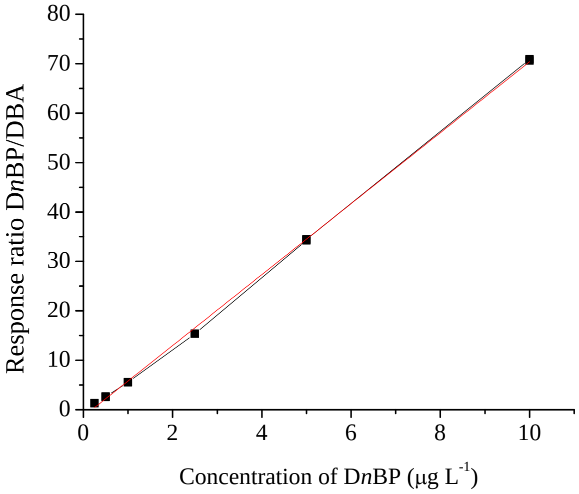

***Figure 2** Analytical curve for DnBP for concentration range 0.25–10.0 µg cm^{-3}*

Recovery values were calculated for all spiked milk samples. Mean values of recovery obtained by using two different extraction procedures for all investigated spiked samples are given in Table 1.

Table 1 *Recovery values obtained by extraction without and with addition of NH_4OH*

Spiked concentration of D*n*BP (mg L^{-1})	Recovery (%)	
	Without NH_4OH	With NH_4OH
0.25	29.73	61.50
0.50	25.54	52.25
0.75	27.00	49.83
1.00	31.96	47.88
1.25	35.27	48.10
Mean value of recovery (%)	29.90	51.91

D*n*BP concentration correlation between hexane extract from both procedures and milk matrix are showed in Figure 3.

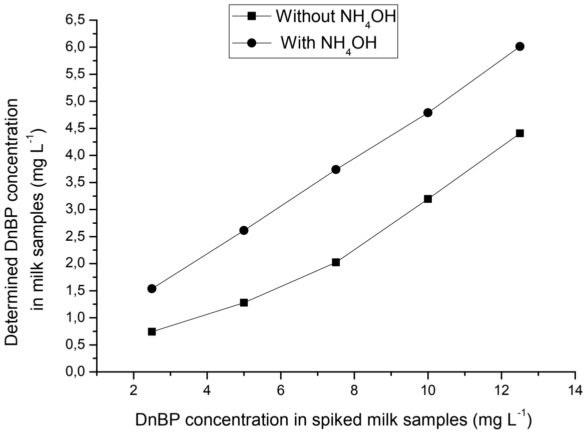

Figure 3 *Correlation between spiked and determined DnBP concentration in milk samples (mg L^{-1})*

Obtained results indicate that extraction method which included addition of ammonium hydroxide showed higher recovery for all spiked concentrations of DnBP. These results could be explained by fact that ammonium hydroxide causes disruption of milk fat globule. This process allows better extraction of DnBP from samples that contains free fatty acids in the aqueous phase.

CONCLUSION

The effectiveness of ammonium hydroxide addition on D*n*BP extraction from milk samples was investigated. Results showed that addition of ammonium hydroxide causes more efficient extraction with mean recovery value of 51.91% compare to mean recovery value obtained by extraction without addition of ammonium hydroxide (29.90%). The highest value of recovery was obtained from milk samples with the lowest concentration of D*n*BP (61.50%) by procedure with addition of ammonium hydroxide.

ACKNOWLEDGEMENT

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CHARACTERIZATION OF ELECTRIC ARC FURANCE SLAG BY XRD, SEM/EDS AND THERMAL ANALYSIS

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Abstract

SEM/EDS, XRD, TGA/DTA and dilatometric analysi of electric arc furnace slag (EAFS) was carried out. Ca, Fe, Si, Al and Mg were found as a main constituent of EAFS. Larnite, wüstite and gehlenit were found as major crystal phases. Mass increase and shrinkage of EAFS upon the heating was confirmed by TGA/DTA and dilatometric analysis.

Keywords: Slag, characterisation, XRD, SEM/EDS, TGA/DTA

INTRODUCTION

Electric arc furnace slag (EAFS) is a by-product of iron and steel scrap re-melting in electric arc furnaces during the steel production. It is mainly comprising of CaO, FeO, SiO_2, Al_2O_3, MgO, MnO, while the main mineral phases are dicalcium and tricalcium silicates, dicalcium ferrite and wüstite [1]. This slag is mainly used in a civil engineering [2–4] but potential application as neutralising agent in bioleaching [5] and for phosphorous and heavy metals removal from water was also considering [6–8].

In recent decades, great attention has been paid to the use of alkali activated slag (AAS) [9,10], as a possible replacement for ordinary Portland cement (OPC) binder due to the enhanced durability of alkali activated slag in comparison to the cement binder [11,12].

The variety of possible slag application impose the necessity to carried out the characterization of EAFS to access the slag potentials.

The slag was characterized using the next methods: X-ray powder diffraction (XRPD), scanning electron microscopy (SEM) coupled with the energy dispersive spectrometer (EDS) thermal (TGA/DTA) and dilatometric analysis.

MATERIALS AND METHODS

The EAFS used in this investigation was obtained from the Steel Mill Nikšić in Montenegro and its chemical composition is presented in Table 1. Prior to analysis, EAFS was grinded and the mean particle size of the grinded slag was d_{50}= 24μm, measured on a MALVERN 2000Laser Particle Size Analyzer (Figure 1).

Table 1 *Chemical composition of EAFS*

Component	CaO	FeO	SiO_2	Fe_2O_3	MgO	MnO	Cr_2O_3	Al_2O_3	TiO_2
%	46.5	23.5	12.2	0.9	6.5	1.3	0.8	7.2	1.06

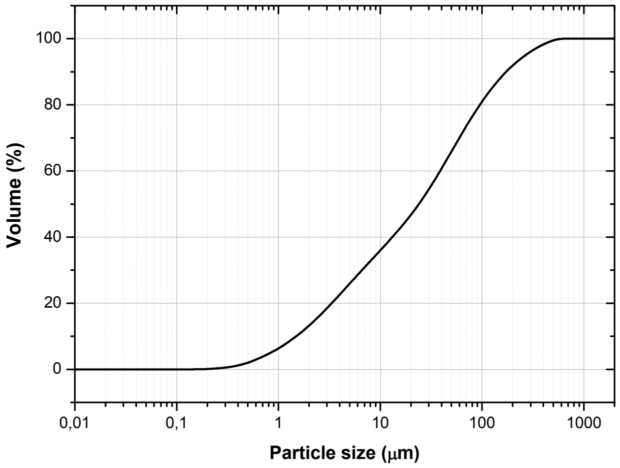

Figure 1 *Cumulative particle size distribution of EAFS*

Microstructural investigations were carried out using the FEI 235 DB focussed ion beam system, equipped with the EDAX energy dispersive spectrometer (EDS). The SEM images were recorded with various electron detectors, including secondary electron detector (SED).

The qualitative analysis of EAFS phase composition was performed by X-ray powder diffraction (XRPD) technique. The XRPD data was collected on a Rigaku RINT-TTRIII diffractometer, with Cu-Kα radiation of λ=1.5406 Å at room temperature in the 2θ range of 10–70° with a scanning step of 0.02° and scan speed of 5 s per step.

TGA/DTA measurements were performed using the TA-SDT 2060 instrument. Approximately 20 mg of the sample was crushed and placed in a platinum crucible and heated to 1200°C at a constant heating rate of 15 °C/min.

Dilatometry measurements of EAFS was performed using a sensitive Thermomechanical analyzer SETSYS Evolution TMA (Setaram Instrumentation, Caluire, France). Heating was carried out in air atmosphere with a heating rate of 10°C/min, from room temperature to 1200°C.

RESULTS AND DISCUSSION

SEM microphotographs of EAFS the results of EDS analysis are given in Fig. 2 and Table 2, respectively. The results obtained indicated that EAFS comprises of calcium, iron, silicon, aluminum and magnesium as the most abundant elements, which is in agreement with the chemical composition of EAFS given in the Table 1.

The results of XRPD analysis (Fig. 3) has shown that EAFS is poorly crystalline. The main crystalline phase is larnite, β-Ca_2SiO_4 followed by wüstite a complex nonstoichiometric oxide of iron with the general formula Fe1-xO. Moreover, the presence of small quantity of gehlenite, $Ca_2Al(AlSiO_7)$ is observed.

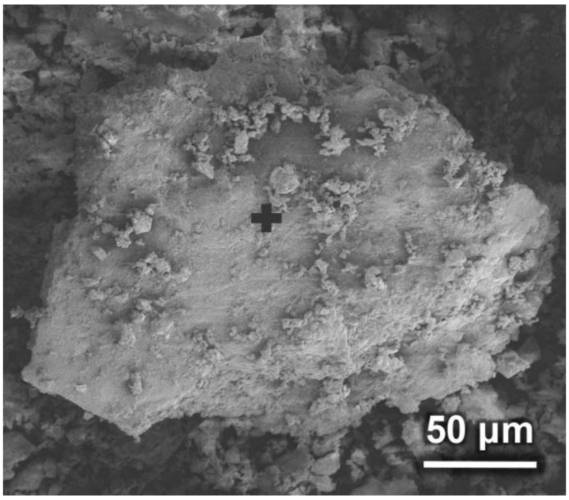

***Figure 2** SEM of electric arc furnace slag*

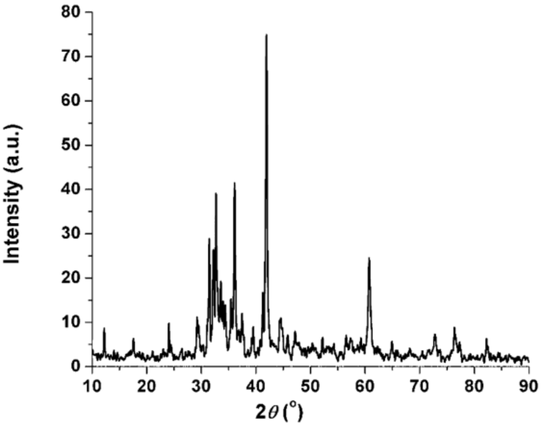

***Figure 3** XRD of electric arc furnace slag*

***Table 2** The content (wt.%) of most abundant element in EAFS obtained by EDS*

Component	O	Na	Mg	Al	Si	S	Ca	Cr	Mn	Fe
%	28.6	1.0	2.2	5.41	9.51	0.3	39.6	5.6	2.2	20.56

Thermal behaviour of EAFS was evaluated using the TG/DTA and dilatometric analysis. The TGA curve (Fig. 4) exhibited the small mass loss up to approximately 250 ^oC due to the loss of adsorbed water. This is accompanied with the endothermic peak at DTA curve (Fig. 5). Further, heating up to 600 ^oC lead to the increase of mass (0.3 %). The major mass change EAFS undergoes above 600 ^oC. Additional mass increase of 1.5 % occurs in the range between 600 and 1000 ^oC due to the mineralogical transformation of wüstite into magnetite [13]. This transformation is accompanied by the appearance of small endothermic peak at the DTA curve. Above 1000 ^oC the sample displayed the mass loss of 0.5 %.

The dilatometric curve (Fig. 6) of EAFS indicate the expansion of sample with maximum of 5.5 % at 1000 ^oC due to the mineralogical transformation of wüstite into magnetite. Above 1000 ^oC the sample displayed the mass loss of 0.5 % and shrinkage of 0.8. The subsequent cooling is accompanied with the shrinkage of sample. After the one cycles of heating and cooling the final expansion of EAFS 3.5 % were observed.

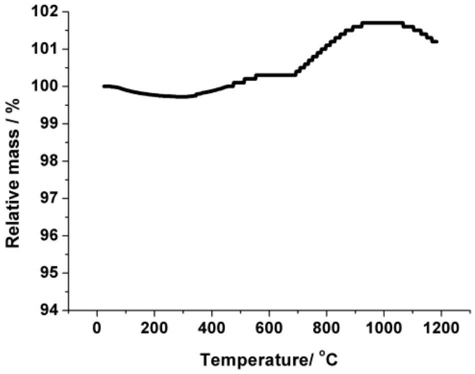

Figure 4 *TGA of electric arc furnace slag*

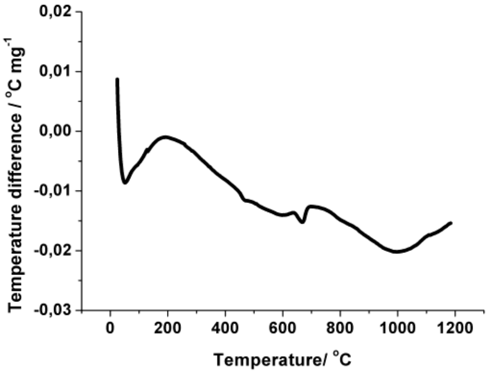

Figure 5 *DTA of electric arc furnace slag*

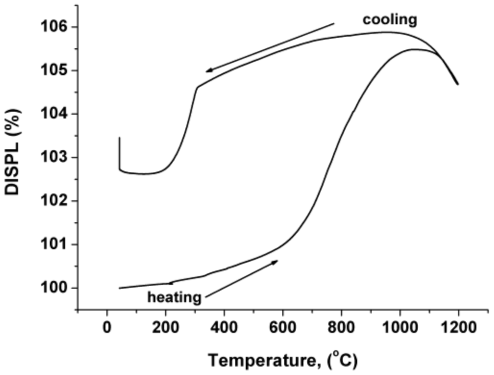

Figure 6 *Dilatometric analysis of electric arc furnace slag*

CONCLUSION

The results of SEM/EDS analysis has confirmed the presence of Ca, Fe, Si, Al and Mg as the most abudant elements in EAFS. X-ray diffraction study shows the presence of larnite, wüstite and gehlenite as major phases. TGA/DTA analysis indicated the mass increase of EAFS during the heating which is atributed to the expansion of EAFS sample as confirmed by dilatometric analysis.

ACKNOWLEDGEMENT

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COMPARISON OF DIFFERENT RISK ASSESSMENT METHODS FOR EVALUATION OF GROUNDWATER IN LANDFILL AREAS

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Abstract

The aim of this study was to examine the use of different risk assessment methods for evaluation of environmental state of groundwater at six landfills with different characteristics. Risk assessment was performed using variety of sub-indices, including pollution indices (LWPI and Nemerow index) and descriptive quantification of groundwater state. Different methodologies yielded different final scores, particularly in the case of landfills with no pollution control measures. On the basis of obtained values of groundwater risk assessment methodologies (eV_{gw}) *it is recommended to include sampling and analysis of groundwater samples for development of environmental impact assessment model for the prioritization of landfills for closure and remediation. However, large amount of resources is needed to construct piezometers/boreholes on territory of 3500 identified unregulated landfills. Therefore, risk quantification using descriptive methods (e.g. mean annual precipitation) should only be used for landfills without preinstalled piezometers/boreholes, small landfills that don't represent great environmental threat and landfills that have pollution control measures, taking into account other environmental factors like landfill age, soil quality, air quality, waste composition and etc.*

Keywords: Landfill pollution, groundwater, multi-parameter indices, risk assessment

INTRODUCTION

Waste disposal is important element of integrated waste management, and the most used method for municipal solid waste treatment. In Serbia, due to an undeveloped solid waste management system, there are approximately 3500 identified municipal solid waste landfills (MSWLF) without proper pollution control. Such landfills represent great environmental threat [1,2] releasing numerous pollutants through landfill leachate and gas, making inadequate waste management one of the major environmental problems in Serbia.

The main action for solving this environmental problem in Serbia is construction of regional sanitary landfills. However, no real action program has been established to deal with the serious environmental problems that unregulated landfills present. The only measures taken thus far have been to identify landfills according to national regulation [3]. Not surprisingly, no methods have been implemented as decision making tools for evaluation or cleaning up landfill sites. In order to perform required actions for closure or remediation of critical group of landfills, priority order list must be made based on credible environmental impact assessment model for the prioritization of landfills for closure and remediation.

Along with air and soil, groundwater is under the greatest negative affect of the landfill. Therefore, quality of groundwater must be part of overall environmental risk assessment of landfills. General recommendations for groundwater quality assessment at the territory of the landfill are instalment of two investigatory and one control piezometers or boreholes. However, this requires significant amount of resources, as almost none of identified 3500 landfills have piezometers/boreholes. Therefore, other methods for groundwater quality assessment must be investigated. Appropriate groundwater risk assessment could also be useful for risk assessment of other environmental elements (e.g. surface water, air, soil).

Aim of this study was to investigate different risk assessment methodologies for groundwater under the influence of MSWLFs with different characteristics. These way available resources can be distributed for solving the most critical problems landfills currently present.

MATERIALS AND METHODS

Investigated sites

Groundwater at six different landfills was subject of this research. Characteristics of these landfills are given in Table 1. Quality of groundwater at these landfills was assessed by continuous monitoring program, including analyses of wide range of pollutants. Detailed results of groundwater quality assessment can be found in our previous studies [1,4–6]. All of these landfills have preinstalled piezometers/boreholes for sampling of groundwater, and therefore are applicable for investigation of different groundwater risk assessment methodologies.

Table 1 *Characteristics of investigated landfills*

Landfill	Surface (ha)	Population that uses landfill	Yearly disposed waste (metric tons)	Established control measures	Investigative piezometers or boreholes	Control piezometer or borehole	References
G	1.2	2 300	480	-	1	No	[1,4–6]
M	1.6	2 700	570	-	1	No	
Z	7.2	9 000	5 800	-	3	No	
SM	5.8	118 000	50 000	(1) (2)	2	Yes	
S	12.9	100 000	48 000	-	3	No	
NS	22.1	340 000	126 000	(1) (2) (3)	5	Yes	

(1) Leachate collection and treatment system
(2) Landfill gas extraction
(3) Waste separation

Risk assessment methodologies

To preform groundwater risk assessment, first it is necessary to evaluate state of groundwater under the influence of landfill. According to our previous studies [1,4-6] and relevant literature [7,8], the best methods for overall evaluation of groundwater quality are: LWPI and Nemerow index. LWPI is more complex method, because it considers background quality of groundwater that is in the close area of landfill, but is not under the influence of landfill itself. Original equation was used for calculation of LWPI [7], with additional 15 parameters (variables) [1]. On the other hand, Nemerow index method includes use of only two sets of data: concentrations of specific parameters in groundwater samples, and standard values of the same parameters. Improved equation was used for determination of Nemerow index. Grading of final LWPI and Nemerow index scores were adopted from original sources [7,8].

In order to generate priority list for closure and remediation of MSWLFs, overall risk assessment of groundwater must be quantified with sufficient degree of certainty. The environmental impact assessment model for the prioritization of landfills for closure and remediation must include use of impact indicators for environmental components (eV), one of which is groundwater (eV_{gw}). Generally, two approaches can be used for final quantification of groundwater sensitivity: a) pollution index score (e.g. LWPI) calculated based on sample analysis; b) quantification of descriptive characteristics (e.g. use of groundwater). Therefore, several different sub-indices (A) can be used for calculation of final eV_{gw} value: calculation and classification of LWPI ($A1_a$); calculation and classification of Nemerow index ($A1_b$); use of groundwater (A2); mean annual precipitation (A3). Use of groundwater is not indicator of groundwater quality, but has important role for overall risk assessment [9]. These sub-indices can be used individually or in combination, in order to obtain sufficient degree of certainty for decision making process. Additionally, not all of these sub-indices are applicable for every landfill. For example, landfill G doesn't have installed control piezometer, so LWPI cannot be calculated and sub-index $A1_a$ is not usable. For better understanding, different methods for quantification of groundwater status at the landfill are shown on Figure 1.

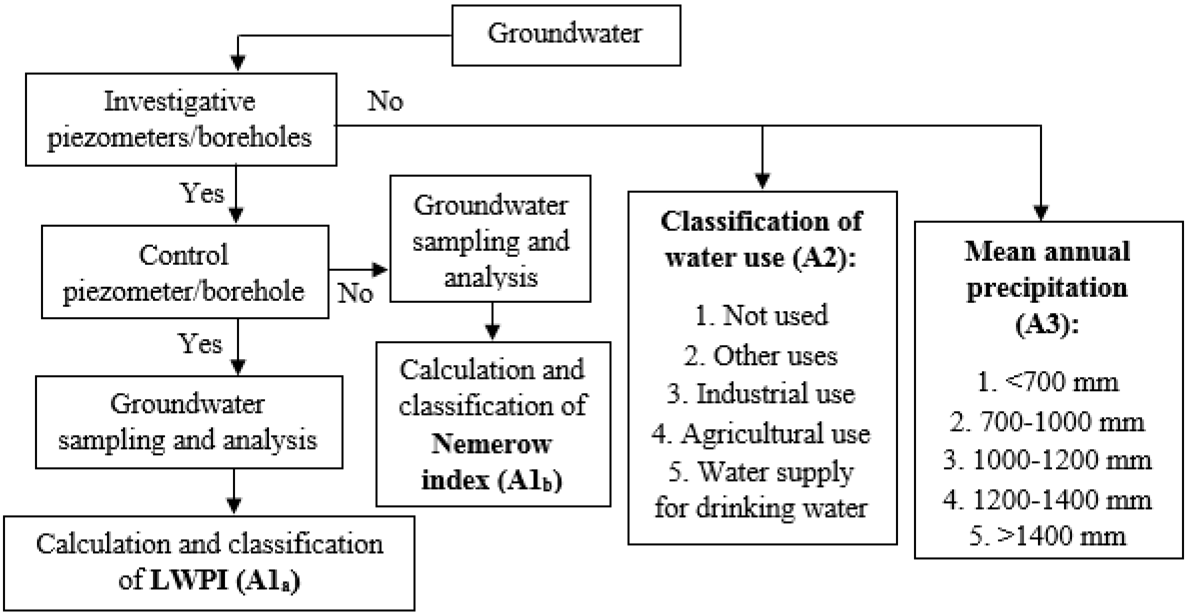

Figure 1 *Connection between different methods for landfill groundwater risk assessment*

All of sub-indices range from 1 to 5, so they can be compared. LWPI classification originally ranges from 1 to 5 [7]. LWPI values above 5 are normalized to 5, and all values below 1 are normalized as 1. Originally, Nemerow index (PI) classification goes from 0.7 to 3.0 [8], but in case of classification of sub-index $A1_b$, classification is modified to fit 1-5 range. Classification of other sub-indices is presented in Figure 1.

Advantages of groundwater risk assessment quantification using sub-indices A2 and A3 are much less need for recourses and it is relatively easy implementation for large number of landfills, but these sub-indices are based on assumption, while sub-indices A1a and A1b are based on real quality monitoring data. Clearly, water use only is not enough for risk assessment of groundwater. Same goes for mean annual precipitation. Therefore, investigation of different combination of sub-indices for quantification of groundwater risk assessment was performed and presented in Table 2.

RESULTS AND DISCUSSION

LWPI and Nemerow index values obtained by our previous studies [1,4–6], are shown in Figure 2, along with normalized values for calculation of overall groundwater risk assessment (eV_{gw}) (Table 2). Other sub-indices values for investigated landfills are given in Table 2. As it is show in Figure 2 and Table 2, normalized values of LWPI and Nemerow index does not significantly differ. This means that either LWPI or Nemerow index can be used for quantification of groundwater quality influenced by landfill.

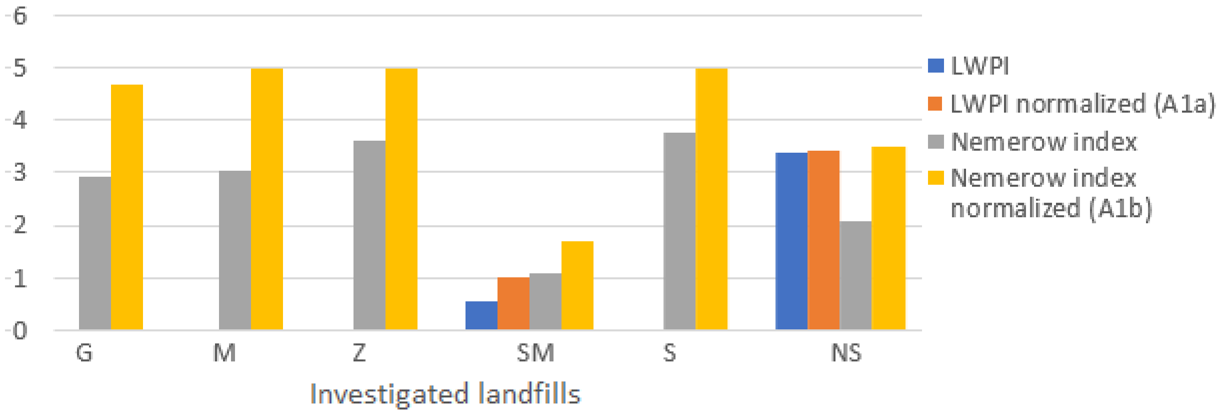

***Figure 2** LWPI and Nemerow index values for groundwater of investigated landfills*

Results of different sub-index combinations for calculation of groundwater risk assessment are also presented in Table 2. Significant variation can be observed in some of the final scores for the same landfill, which can also be seen in Box and Whisker plot shown in Figure 3. The most significant variations are detected for landfills G and Z. According to $eV_{gw}1$ (only Nemerow index) value, groundwater at the G landfill is characterized as highly polluted ($eV_{gw}1$=4.7) with intensive negative influence of landfill. On the other hand, according to $eV_{gw}4$ values (combining water use and annual precipitation for quantification) the same landfill is characterized as only moderately polluted with moderate to low landfill influence ($eV_{gw}4$=1.5). Similar results were obtained for landfill Z (Table 2). These variations are great indicators that combinations for calculation of $eV_{gw}1$ and $eV_{gw}4$ are not sufficient to be solely used as markers for groundwater risk assessment.

It has to be noted that only in the case of landfills G and M boreholes are installed for monitoring of groundwater quality, and only one is installed. It could be possible that if two or three piezometers were installed at the territory of landfills G and M, instead of one borehole per landfill, different $eV_{gw}1$ values would be obtained. This shows the need for further investigations regarding difference in groundwater quality from shallow and deep aquifers.

Table 2 *Obtained values of individual sub-indices and different methods for quantification of groundwater risk assessment*

Sub-index	**G**	**M**	**Z**	**SM**	**S**	**NS**
$A1_a$	-	-	-	1.0	-	3.4
$A1_b$	4.7	5.0	5.0	1.7	5.0	3.5
A2	1.0	4.0	1.0	1.0	4.0	2.0
A3	2.0	2.0	2.0	1.0	2.0	2.0
Groundwater risk assessment quantification (1)						
$eV_{gw}1=A1_b$	4.7	5.0	5.0	1.7	5.0	3.5
$eV_{gw}2=(A1b+A2)/2$	2.9	4.5	3.0	1.4	4.5	2.8
$eV_{gw}3=(A1b+A3)/2$	3.4	3.5	3.5	1.4	3.5	2.8
$eV_{gw}4=(A2+A3)/2$	1.5	3.0	1.5	1.0	3.0	2.0
$eV_{gw}5=(A1b+A2+A3)/3$	2.6	3.7	2.7	1.2	3.7	2.5

(1) Classification of final eV_{gw} values: $eV_{gw} \leq 1$: no or very low influence of landfill (very low priority); $1 < eV_{gw} \leq 2$: low landfill impact (low priority); $2 < eV_{gw} \leq 3$: moderate negative effect of landfill (medium priority); $3 < eV_{gw} \leq 4$: significant influence of landfill (medium-high priority); $4 < eV_{gw} \leq 5$: high negative impact of landfill (high priority); $5 \leq eV_{gw}$: very high influence of landfill (very high priority).

As shown on Figure 3, the least variation between final scores of different groundwater risk assessment methods were obtained for landfills SM and NS, which both have implemented pollution control measures (Table 1). Groundwater at the territory of landfill SM is characterized with very low influence from landfill using all of investigate methods for risk assessment (eV_{gw}=1.0-1.7). According to obtained eV_{gw} values groundwater under the influence of landfill NS is under moderate-high impact from landfill (eV_{gw}=2.0-3.5). This in fact shows that under appropriate conditions, all of the investigated combination of sub-indices can be effectively used for risk assessment of landfill groundwater.

Sub-indices values are the same for groundwater from landfills M and S, regardless of different landfill characteristics (Table 1), meaning that other factors must be taken into account for final decision-making regarding priority actions. According to obtained eV_{gw} values (Table 2), groundwater from both of these landfills is under high to very high negative influence from landfill (eV_{gw}=3.0-5.0).

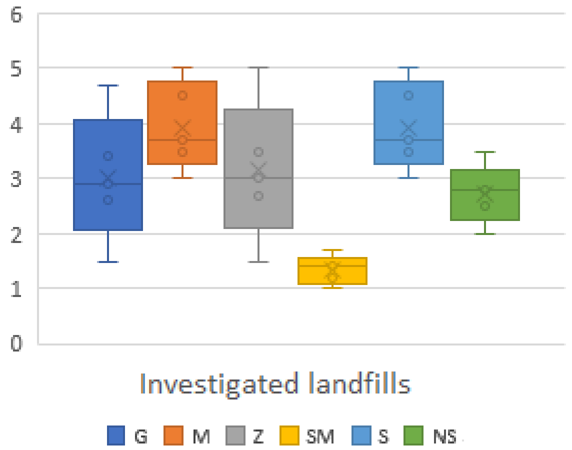

Figure 3 *Box and Whisker plot for results of groundwater risk assessment quantification*

The lowest eV_{gw} values were obtained by combination of following sub-indices: water use (A2) and mean annual precipitation (A3) which are both descriptive in nature. As was already mentioned, water use does not give notion of water quality or even landfill influence, but is very important factor for calculation of overall risk assessment, especially if these values are used for development of model for prioritization of landfills for closure or remediation.

CONCLUSION

Different risk assessment methods were performed on groundwater from landfills with dissimilar characteristics. The methods described in this paper have been shown to be valid for analysis of landfill sites. These methods generate indices that give information regarding state of groundwater under the influence of landfill. Based on obtained eV_{gw} values it can be concluded that equations for calculation of $eV_{gw}2$, $eV_{gw}3$ and $eV_{gw}5$ gives similar results, and all of these equations can be used for groundwater risk assessment quantification.

This study shows that this instrument has the potential to assist planners, decision-makers and other agents involved in development of credible environmental impact assessment model for the prioritization of landfills for closure and remediation. This “model” could only work using trustworthy input data obtained by continuous monitoring and adoption of pollution indices and risk assessment methodologies, which were demonstrated in this study. Therefore, further Application of such a robust model and use of adequate in-situ and/or ex-situ remediation techniques will largely contribute to solving the problem of unregulated landfills in the region.

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PREDICTION OF OZONE CONCENTRATION IN BELGRADE URBAN AREA USING ANNs APPROACH

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Abstract

This paper presents the results of the statistical modeling of the ground-level ozone concentration in the air in the Belgrade urban area (Serbia). Artificial neural networks (ANNs) were used as the tools for the mathematical analysis of dependence of ozone concentration on the following predictors: NO_2, *NO, NOx, Toluene and m,p-Xylene concentration in the air, as well as on the meteorological parameters (the wind speed and air temperature). The results have shown that ANNs provide good estimates of ozone concentration on the monitoring site.*

Keywords: ozone, artificial neural networks, pollutants, air pollution

INTRODUCTION

Air pollution in urban areas directly affects public health, therefore it is very important to predict the concentration of air pollutants. Air quality is a complex function of emissions, meteorology and topography, and ANNs provide a framework for linking all these variables [1,2]. The most important issue of the environment is the deterioration of the quality of urban air. It has been scientifically proven that exposure to contaminated air causes serious health consequences. For these reasons, it is extremely important to monitor the quality of air in urban environments in order to quantify current and future health risks [3].

Tropospheric ozone (O_3) is a secondary air pollutant formed in the troposphere from the photochemical oxidation of methane (CH_4), carbon monoxide (CO), and non-methane volatile organic components (NMVOC) in presence of nitrogen oxides (NOx) and sunlight [4]. Ozone is a very important greenhouse effect gas, which directly contributes to climate changes [5]. The exposure of the urban population to tropospheric ozone is a serious health problem in Europe [6]. Ozone levels are the subject of many global research studies, precisely because of its negative impact on human health [7] and animals and plants [8,9]. In order to control and reduce the negative impact of tropospheric ozone, the European Commission has established regulations to promote clean air throughout Europe. Accordingly, Fontes and associates [10] engaged in the development of tools for identifying sources of ozone emissions.

The surface concentrations of ozone are directly affected by climate changes [11,12], bearing in mind that chemical production, dilution and precipitation of ozone is regulated by temperature, cloudiness, humidity, wind and precipitation [13]. Ozone is a reactive gas that negatively affects human health [14,15]. Exposure to ozone in outdoor spaces accounts for 43-76% of total exposure to ozone [16], because people usually spend almost 90% of their lives indoors [17–20]. Prediction of ozone concentration in the outdoor and indoor environment is very useful in order to reduce the level of ozone-related health risks. For this purpose, artificial neural networks (ANNs) are the most commonly used technique [21].

Statistical models are very popular solutions for different types of predictions, due to their ability to establish a link between predictors and output variables, without considering all physical parameters of transmutation and dispersion of pollutants. The most commonly used statistical methods in the prediction are ANNs. ANNs represent a new branch of intelligent science that is applied in order to understand complex systems [22] and mathematical models that can imitate the processes of the biological brain learning and which are capable for modeling of complex and nonlinear relations between input and output variables [23].

ANN consists of a number of interconnected nodes that simulate individual neurons, similar to the biological neurological system. It can be used to classify, identify, and forecast problems in complex process situations [24]. However, ANNs are unable to describe in detail the basic physical and chemical processes of air pollutants, so moderators are left with a dilemma how to select significant prediction variables, relying on a large set of available data [25].

Abdul-Wahab and Al-Alawi [26] dealt with the modeling of ozone in a lower atmosphere by using neural networks, in order to predict the concentration of tropospheric ozone, depending on meteorological conditions and different air quality parameters.

Kumar *et al.* [27] have studied seasonal variations of ozone, together with NO_2 and meteorological parameters. The monitoring of ozone concentrations during the whole year showed a trend of increase during the summer and a declining trend during the winter.

MATERIALS AND METHODS

Study area

Belgrade is capital of Serbia (latitude 44°27′44″N, longitude 20°27′44″E) and has about 2 millions inhabitants and it is located at 116.75 m a.s.l., at the confluence of the Sava and Danube. Climate, typical for this area is moderate continental with 4 seasons, while the average annual temperature is 11.7°C. The main sources of pollution are anthropogenic in origin. Usually in such areas, industry and traffic present the main sources of pollution. For the purpose of this research, data collected at automatic measuring station Pancevo Bridge in Belgrade, in 2010 (Table 1), were used. The data were collected from official website (www.sepa.gov.rs) during January-Jun 2010, in the time periods in which all pollutants and meteorological parameters were measured simultaneously. This way, representative data base for credible statistical analysis was generated.

***Table 1** Characteristics of measuring station*

Name	Longitude	Latitude	Altitude (m)	Operated since	Pollutants and meteorological parameters	Type of station
Pancevo bridge	20°49 E	44°81N	105	2007	SO_2, O_3, NOx, NO, NO_2, xylene, toluene, PM10, PM2.5, PM1, T, WS, RH, BPR, WD	Urban traffic

The aim of this study was to develop an accurate ozone prediction model using the Multilayer Perceptron (MLP) network. The MLP is a common mathematical model and a type of FFNN (feed forward neural network). MLP has been successfully applied in a number of applications, including regression problems, classification problems, or time series prediction using simple autoregressive models [28, 29]. The effectiveness of the MLP for prediction, its ability to generalize and applicability in non-linear problems were main reasons for choosing this method for ozone prediction in Belgrade urban area. The basic component of MLP is 'neuron' which is a simple processing element. Neurons are arranged in layers and each layer is fully connected with the next one by means of weights.

The values of the measured input parameters and output parameter in the form of descriptive statistics results are presented in Table 2.

***Table 2** Descriptive Statistics for pollutants' and meteorological parameters' measured values*

	Range	Minimum	Maximum	Mean	Std. Deviation
SO_2	22.9	32.0	54.9	40.640	5.1253
NO_X	127.1	8.9	136.0	45.384	26.0420
NO	127	3	130	33.80	24.691
NO_2	48.9	10.5	59.4	31.835	12.4408
O_3	51.9	8.5	60.4	28.651	12.9656
Toluen	1.3	.1	1.4	.584	.3352
Xylene	.7	.0	.7	.286	.1971
PM1	152.4	10.6	163.0	48.330	31.7761
PM2.5	156.2	13.8	170.0	51.426	31.9111
PM10	145	27	172	62.02	31.488
WS	4.1	.8	4.9	1.853	.9064
T	28.1	-6.6	21.5	5.430	7.5016
RH	48.0	47.0	95.0	72.653	11.9553
BPR	42	993	1035	1013.28	9.597
WD	283.8	39.5	323.3	159.051	81.9442

For the purpose of defining the correlation dependence in the form: the output of the model as the function of the input of the model, a bivariate correlation analysis was performed. As the result of this analysis, the Pearson correlation (PC) coefficients with responding statistical significance were calculated (Table 3).

Table 3 *Correlation matrix*

	SO_2	NO_X	NO	NO_2	O_3	Toluen	Xylene	PM1	PM2.5	PM10	WS	T	RH	Air press.	Wind direc.
SO_2	1.000	.733**	.750**	.614**	-.321**	.371*	.385*	.647**	.640**	.650**	-.212	-.461**	.228	-.132	-.141
NO_X		1.000	.989**	.925**	-.716**	.652**	.636**	.658**	.670**	.723**	-.645**	-.035	.238	.007	-.055
NO			1.000	.864**	-.682**	.594**	.570**	.629**	.640**	.685**	-.586**	-.029	.263	-.034	-.040
NO_2				1.000	-.718**	.722**	.707**	.678**	.691**	.753**	-.742**	-.064	.164	.131	-.131
O_3					1.000	-.577**	-.679**	-.399**	-.417**	-.347**	.751**	.652	-.290	-.218	-.121
Toluen						1.000	.869**	.366*	.385*	.496**	-.533**	.147	-.090	.112	.064
Xylene							1.000	.369*	.388*	.556**	-.645**	.126	-.044	.152	.063
PM1								1.000	.998**	.934**	-.357*	-.595**	.469**	-.132	-.132
PM2.5									1.000	.943**	-.378*	-.579**	.459**	-.125	-.128
PM10										1.000	-.511**	-.471**	.313*	.072	-.168
WS											1.000	-.210	-.112	-.335*	.058
T												1.000	-.444**	.092	.090
RH													1.000	-.366**	.293
Air pressure														1.000	-.327*
Wind direc.															1.000

**. Correlation is significant at the 0.01 level (2-tailed).
*. Correlation is significant at the 0.05 level (2-tailed).

In order to define the dependence of the output parameter as the function of the input parameters by applying MLP with an acceptable level of fitting (a strong correlation), it is necessary that the value of the PC should be near ± 0.5, with the statistical significance of ($p \leq 0.05$). A correlation with the probability (p) above 0.05 has no statistical importance. The obtained results (Table 3) reveals that, in the following cases, there is no statistical significance for the correlation between the dependent variable (ozone) and the predictors (pollutants and meteorological parameters): SO_2, PM_{10}, $PM_{2.5}$, PM_1, RH, BPR, WD. Due to that, remained indicators (NOx, NO, NO_2, m,p - Xylene, toluene, temperature, wind speed) were used for model building phase (Figure 1).

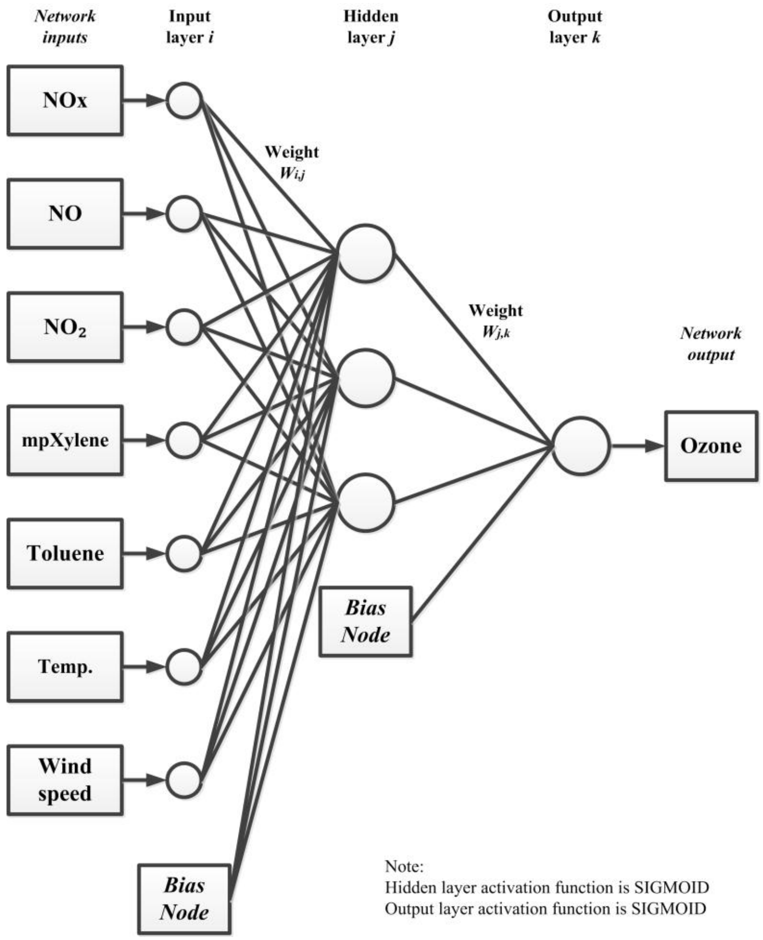

Figure 1 *ANN architecture*

The ANN presented in Figure 1 consists of the following three layers: the input, output, and hidden layers. The neurons comprising the input layer represent the information on the input process parameters (the independent variables), whereas the only neuron in the output layer generating the output information is the process quality indicator (the dependent variable). The appropriate number of the neurons in the hidden layer was determined by training and testing several networks. The best results were obtained for the network architecture with three neurons in hidden layer.

For such a network, the obtained coefficient of determination is 0.873 for the training phase. During the ANN testing phase, the calculated coefficient of determination (R^2) is slightly lower, equaling 0.775. Figure 2 illustrates a comparative presentation of the measured values and the values calculated by applying the ANN approach for the investigated occurrence.

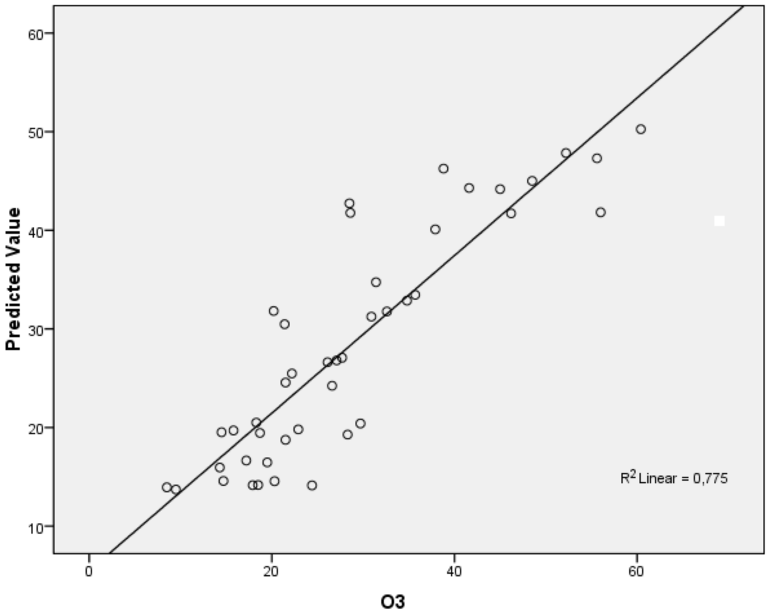

***Figure 2** Comparison of measured and calculated values using ANN to predict the ozone concentration in the air*

CONCLUSION

In this paper, the ANNs approach was used to explore the complex relationship between ozone and the other variables based on the data collected at the measuring station in Belgrade urban area, Serbia. This work has shown a good potential of the Artificial Neural Networks method for the prediction of ozone concentrations in ambient air. Before the model building phase, the initial data set was reduced by eliminating those polluters and meteorological parameters which didn’t have coefficient of correlation in relation to ozone concentration near ± 0.5. In that way only remained variables were included in network architecture. The obtained results indicate that the data collected in this study can be used to predict ozone concentration in the air.

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INTEGRATION OF ARTIFICIAL NEURAL NETWORKS INTO MODELLING MORAVA RIVER SYSTEM WATER QUALITY

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Abstract

For the effective natural resources management, adequate monitoring system, and prediction of development scenarios are required. Increasing development of mathematical and statistical tools accelerated the creation of numerous numerical models successfully used in solving environmental problems. However, it is difficult to select a model which is suitable for the real and practical needs. This causes constant efforts of scientists to improve and upgrade current models by a combination of various techniques. The aim of this article is to examine the possibility of Artificial Neural Networks technique to predict quantitative water quality parameters. The study was conducted on the example of Morava river system in Serbia, by forecasting following water quality parameters: electrical conductivity, pH value, and potassium. These parameters were extracted as a discriminant for spatial pattern determined in previous research. Satisfactory values of determination coefficients prove that the water quality model, based on the recognition of spatial pattern using Cluster Analysis and Discriminant Analysis, can be improved by integration of ANN's technique.

Keywords: water quality, Artificial Neural Networks, prediction, spatial pattern, Morava river system

INTRODUCTION

Water resources pollution occurred as a result of agricultural and urban runoff and wastewater discharges from industrial and municipal activities, is considered as one of the main contemporary problems. In order to protect and improve the state of the quality of water resources, especially surface waters, it is necessary to establish a reliable monitoring system, within which regular measurements of physical, chemical and biological parameters, on as many stations as possible, are carried out. Well designed monitoring network should enable data that can generate information used to undertake preventive measures and timely reaction. However, modern studies highlight that most of the countries from all over the world are not enough dedicated to the creation of an adequate monitoring system [1,2,3].

In the environmental management, there are more and more advanced mathematical and statistical methods, used for data set simplification and determination of patterns through the construction of the models valuable in the simulation of variations and future scenarios. The prediction accuracy notably depends on open boundary conditions that serve as input variables of the model and on the adopted numerical scheme [4]. Multivariate statistical techniques are considered as a tool which is very useful in water resources management, because of its' possibility to reduce complex and extensive data sets resulting from long-term monitoring of numerous water quality parameters [5–7]. Also, these techniques could be used for identifying pollution sources and their origin and for determination of spatial and temporal patterns of water quality variations, as well as the prediction [8,9].

One of the techniques which are increasingly present in modeling in the field of environmental and water resources management are Artificial Neural Networks. During the last 20 years, the usage of this technique directed towards prediction of variations of water quality parameters has been studied and confirmed. It is intensively applying for prediction and forecasting of numerous variables like precipitation rate, flow rate, surface level, and water quality parameters [10,11]. According to a detailed literature review and critical overlook on studies from this field, the authors Maier et al. [12] and Wu et al. [13] have developed the procedure aimed to apply Artificial Neural Networks (ANN’s) in water resources evaluation, with an explanation of every single phase.

The aim of this research is, on the example of the Morava river system in Serbia, to prove the reliability of ANN’s method in modeling and prediction of water quality variations. This river system has the biggest potential for Serbian water industry growth, so its' monitoring, evaluation, and efficient management are the most prominent issues, especially if we consider numerous industry centers located in river valleys, developed agriculture, but also inadequate or missing wastewater treatment. Also, the aim is to present the ANN’s technique as an addition to one of the models created to evaluate variations of spatial water quality patterns. The obtained results should help to evaluate the possible development scenarios and create starting point for future directions, in terms of minimizing the negative effects of human activities on water quality of this river system.

MATERIALS AND METHODS

Artificial Neural Networks (ANN’s)

Considering that water quality is influenced by numerous factors with non-linear interrelations, there was a need to replace the traditional linear statistical methods aimed to predict the normal distribution of variables in water quality modelling with advance, non-linear methods like ANN is. ANN is a complex, non-linear function in which numerous parameters are adjusted (calibrated) to make the function looks like the measures are known data set. Neural network operate by learning the relationship between the input parameters and the controlled and uncontrolled variables by studying previously recorded data [14]. In this process, problem-solving is inspired by the neural structure of the human brain and its’ functioning during the development of the strategy of data treatment. Neural networks can be trained to solve problems that are difficult for conventional computer programs or human beings.

Study area

The Morava River Basin lies approximately between 42° 28′ and 44° 43′ of the North latitude, and 19° 33′ and 23° 00′ of the East longitude. In this study, the investigation of the water quality of the Morava River system included 14 measuring stations situated on the main courses—the South Morava, the West Morava, and the Great Morava. This region characterizes humid continental climate. Various relief forms beginning from the plain at the mouth of the Great Morava in the Danube, the hillsides to the south, as well as the mountainous regions on the western, eastern and southern sides are present. Given that it covers 42.38% of total area of Serbia, this is its' the most widespread river system.

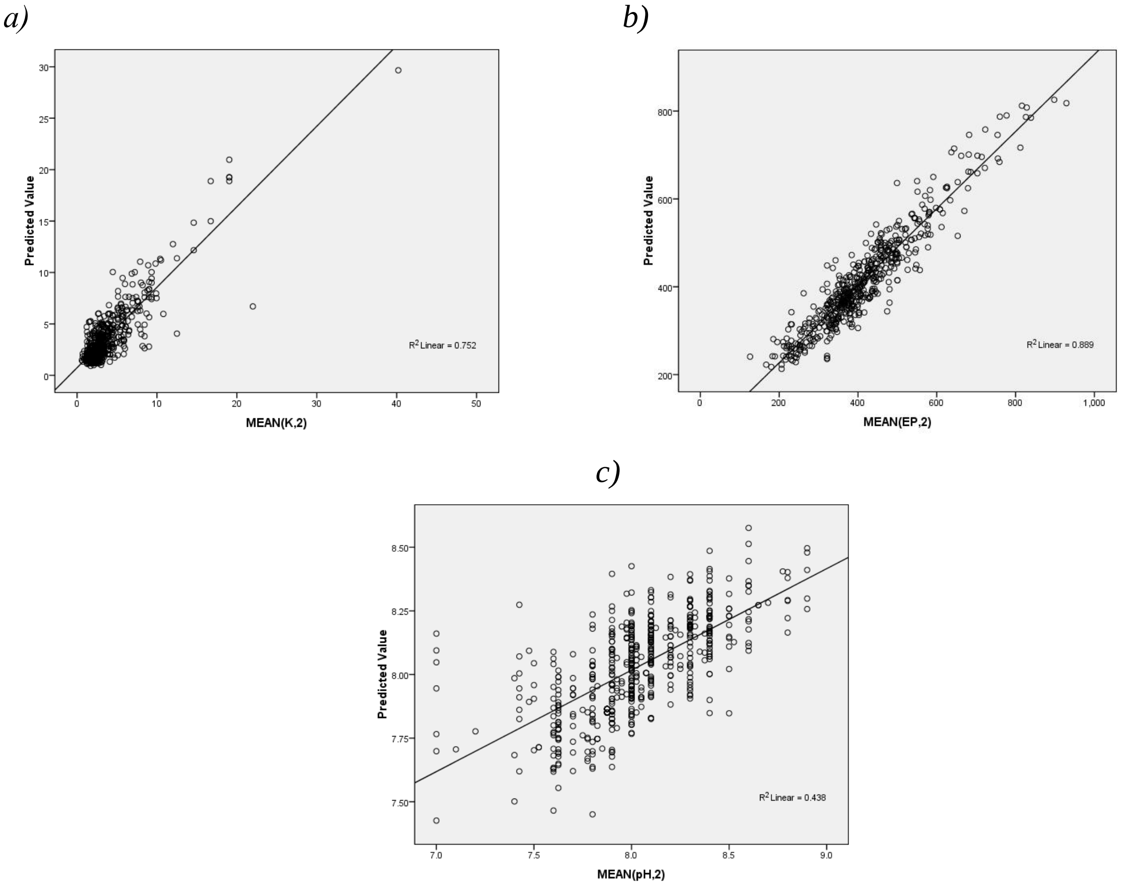
Data set

Data set analysed in this study consists 18 water quality parameters (water temperature, pH value, dissolved oxygen, 5-day biochemical oxygen demand, suspended solids, dissolved oxygen, total hardness, electrical conductivity, phosphates, nitrates, silicates, total nitrogen oxides, sodium, potassium, magnesium, calcium and chloride), The measuring of the observed parameters was generally performed once a month, during a 10-year period (from January 2005 to December 2012). These data are part of the public database created by the Republic Hydro-Meteorological Service of Serbia.

RESULTS AND DISCUSSION

According to the results obtained in the study that was conducted by the authors Voza et al. [15], directed towards the implementation of Cluster Analysis on the same data set, 14 measuring stations located on the main watercourses of Morava river system were divided into three groups. The conclusion of this study was that similar water quality characteristics present the basis for the cluster formation. Also, the Discriminant Analysis used in this study extracted three discriminant (pH value, electrical conductivity, postassium) from twelve parameters. It was concluded that the differences between the groups of sampling stations are mostly reflected in these parameters. So, these parameters were considered as responsible for the spatial pattern definition. Considering that the discrimination factors were defined, it can be concluded that it will be necessary to strive to the increasing the accuracy of measuring these parameters in the future, so that their changes can be clearly predicted [16]. With this aim, in this paper was tested the possibility of predicting the variations of these parameters based on existing measurements. For this purpose, ANN method with Multilayer procedure (MLP) was applied. Analyzed water quality parameters presented independent variables, while the discrimination factor was a dependent variable. Also, the original data set was limited to the observed spatial cluster.

The results of ANN's that indicate the possibility of discriminating parameters (pH, EP, K), limited on the first spatial cluster, are presented in Figure 1. During the testing phase, coefficients (R^2) for potassium, electrical conductivity and pH were obtained, and their values accounted for 0.752, 0.889 and 0.438, respectively. These values indicate a high level of possibility for K and EP prediction. On the other hand, coefficient R^2 obtained in the case of pH indicates that ANN's technique cannot help in predicting this parameter's value.

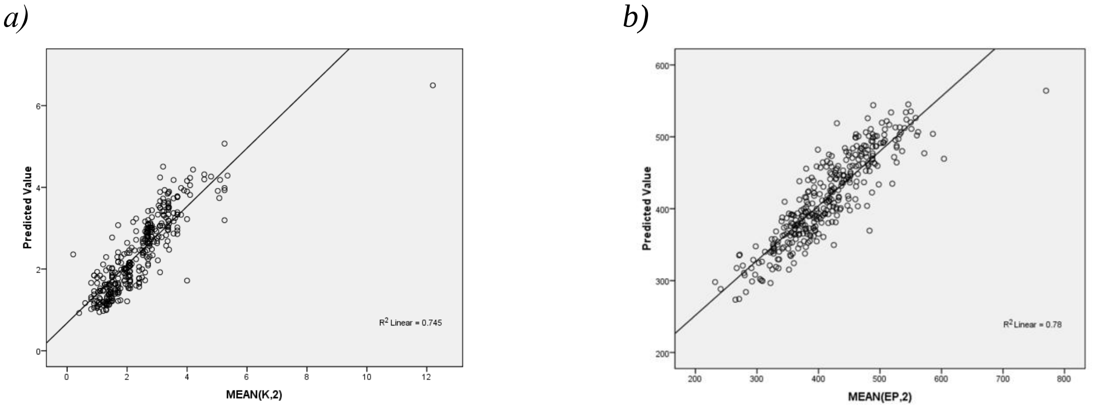

a)

b)

c)

Figure 1 *Scatter plots of actual versus predicted values of discriminant parameters for the first cluster*

The examination of possibility for predicting variations of parameter K in the second spatial cluster, the coefficient value R^2 =0.745 was calculated (Figure 2a). Regarding the parameter EP, the coefficient amounts 0.78 (Figure 2b). The analysis indicated a slightly lower R^2 value (0.62) for parameter pH (Figure 2c).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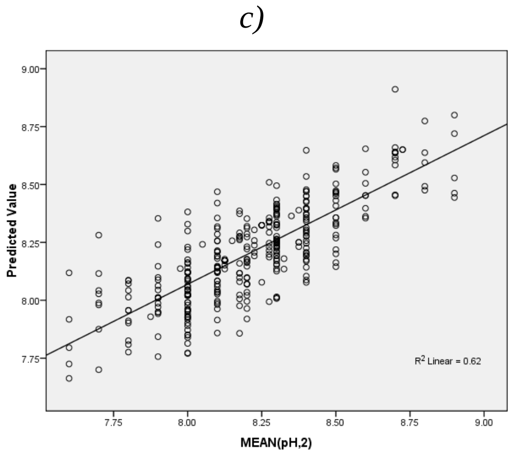

***Figure 2** Scatter plots of actual versus predicted values of discriminant parameters for the second cluster*

Calculation of the determination coefficients for the third cluster resulted in the high value (0.927, 0.816 and 0.924) of every discriminant parameter (Figure 3).

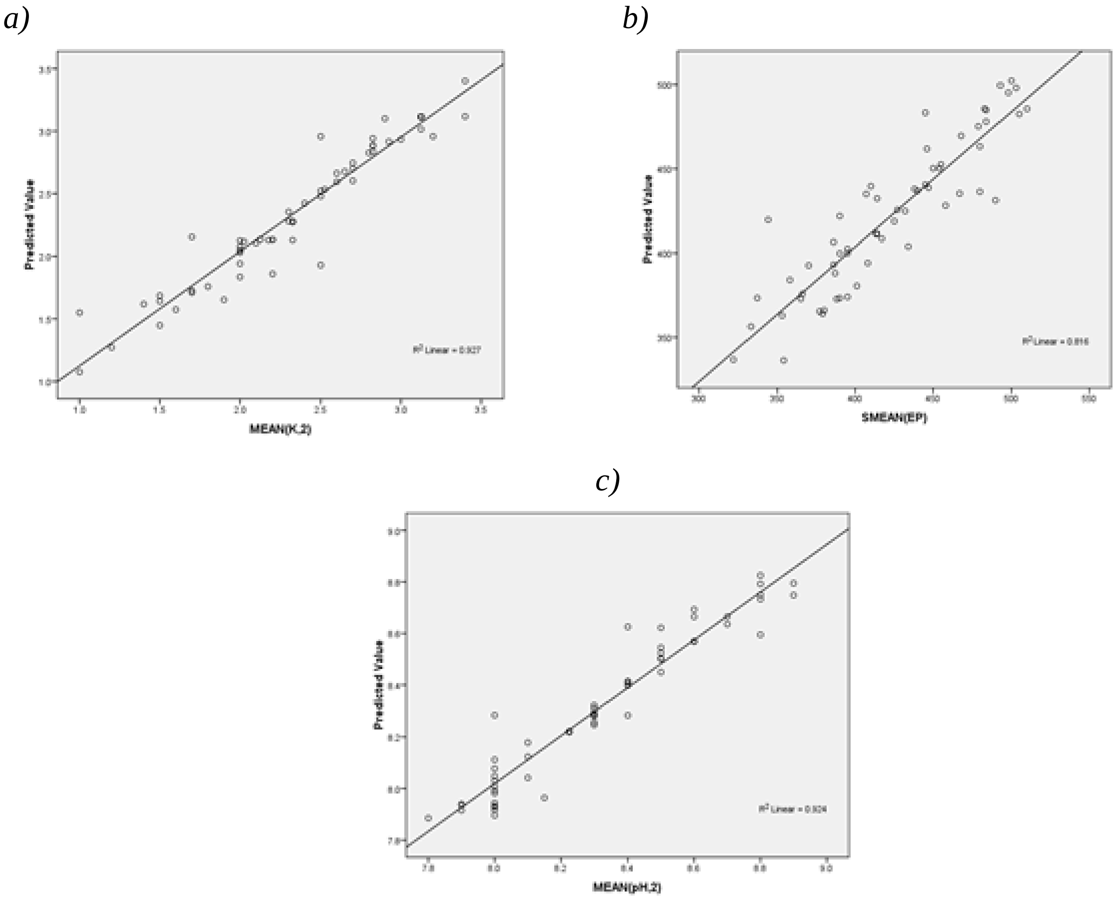

***Figure 3** Scatter plots of actual versus predicted values of discriminant parameters for the third cluster*

High values of determination coefficients confirmed the utility of ANN’s method in predicting variations of defined spatial clustering.

CONCLUSION

Monitoring of discriminant parameters’ values contributes simpler and easier analysis of water quality from various spatial clusters, while prediction enabled by ANNs’ technique can accelerate reactions aimed to prevent unwanted changes. The presented results confirm that the ANN’s technique is useful in determination of the differences between the groups of monitoring stations located along the analysed course and provides reliable prediction of discriminant water quality parameters (EP, pH, and K). For discriminant parameters in every spatial cluster, high values of determination coefficients were obtained. This allows the decision-maker to timely react by undertaking activities aimed to prevent the disturbance of river ecosystems. Also, it enables the basis for creating effective and efficient monitoring system. Finally, it can be concluded that this technique is suitable for integration with other multivariate statistical techniques in the process of water quality modelling.

ACKNOWLEDGEMENT

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ANALYSIS OF CO EMISSIONS IN BOR AND ZAJEČAR

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Abstract

Air pollution in cities due to the presence of carbon monoxide (CO) is most often due to the operation of engines with internal combustion and combustion of solid fuel materials such as coal and wood. The paper presents a comparative analysis of the measured concentrations of carbon monoxide in the two cities of Timočka krajna. The paper analyzes the influence parameters on the concentration of CO in these two structurally different cities. Bor is a well-known mining and metallurgical-industrial center, with very poor air quality, but in this city central district heating covering 80% of housing and space, and Zajecar as a city where the industry is not developed, but on the other hand there is a large number of individual boiler rooms for heating of the living space. The analysis shows drastically unfavorable CO concentrations in Zajecar in the winter period. The main reason of this is the operation of individual boiler rooms. But even in the period when heating is not active, the concentration of CO in the air of Zajecar is higher than in Bor. The conducted analysis was based on statistical data processing.

Keywords: CO, air pollution, SEPA

INTRODUCTION

Air pollution is a global environmental problem facing increasingly developed countries in the world today. Air pollution is also a serious problem for every individual, because it affects its health irreversibly, in addition to the fact that air pollution encourages the development of chronic diseases, it is estimated that 2 million people are affected by air pollution annually [1]. The term air pollution means the emission of pollutants into the surrounding atmosphere. City environments, especially in large industrial centers, are the area with the highest degree of air pollution. In the vicinity of cities, due to high energy consumption, densely populated areas, a large number of cars and the work of industry, there is increased emissions of smoke, dust and other pollutants. It is thought that in urban areas as much as 60% of the total amount of all pollutants from air comes from engines with internal combustion, [2]. Internal combustion engines are a priority source of air pollution with carbon monoxide. In addition to the engines with internal combustion, the increased concentration of CO is also influenced by energy plants that burn fossil fuels. Carbon monoxide occurs due to incomplete combustion of solid fuels, [3]. The usual CO concentration in the atmosphere is 0.1 ppm, but in the environment of polluted cities, this concentration can be even higher. The maximum allowed carbon monoxide (MDC) content in the working environment for 8 hours of working time is limited to 50 ppm, [4].

Having this in mind, this paper analyses the impact of individual heating boilers and internal combustion engines on the degree of CO emissions in the urban environment. The analysis monitors the change in the concentration of CO in the cities of Bor and Zajecar, because they have different ways of heating of residential and commercial space. In Bor, 80% of housing space is warmed up by the central heating system, while in Zajecar only 20% of housing space is warmed on this way.

The work is based on a comparative statistical analysis.

MATERIALS AND METHODS

Carbon monoxide

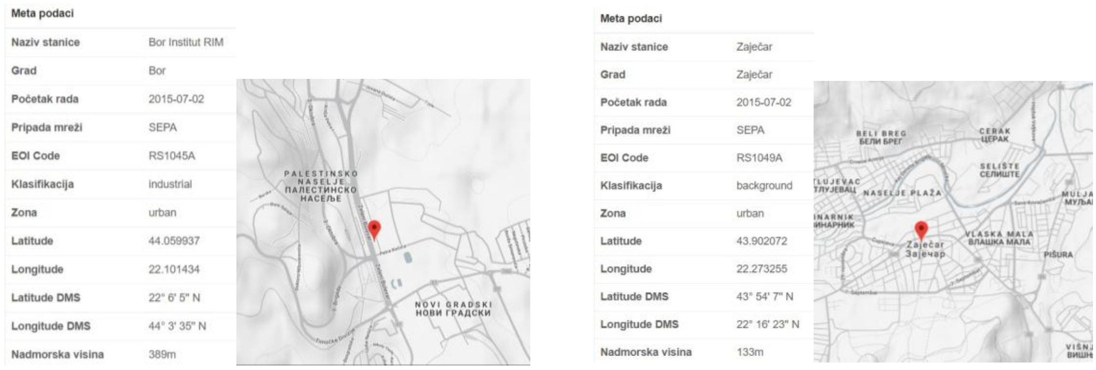
Carbon monoxide (carbon (II) oxide) is a gas composed of carbon atoms and oxygen atoms. CO is a gas without color, flavor and smell, so it is impossible for people to register their presence in this manner with their own senses. Its essential characteristic is that it is slightly lighter than air. CO belongs to the so-called. neutral oxides, i.e. do not react with water, acids and bases. It is highly poisonous and belongs to the group of chemical contaminants and the biggest air pollutants. It does not support combustion, but it burns with a blue, flickering flame, with the creation of carbon dioxide, [5]. CO occurs as a result of incomplete oxidation of organic matter. Motor vehicles are the largest pollutants of the atmosphere with this gas (1 to 14 %). Followed by gases produced during the production of iron as well as gases by combustion of coal in thermal power plants, and in the process of production in oil refineries and the chemical industry. Carbon monoxide is a highly poisonous gas for living beings, introduced into the human organism, binds to hemoglobin, which has a higher affinity for it than to oxygen, and causes general hypoxia of the organism. Its toxic effect occurs very quickly even at very low concentrations. The death toll for humans is from 1,000 ppm to 2 000 ppm when the gas is injected for 30 minutes. At high concentrations of carbon monoxide in the inhaled air, death can occur from 1 minute to 2 minutes, [6].

Monitoring CO

Environmental monitoring is an information system for tracking, assessing and forecasting changes in the state of the environment. The establishment of an air monitoring system arises from the need for air quality management, which is only possible through day-to-day gathering of information. Such monitoring allows insight into the state of air quality, undertaking certain measures for improving the quality of air, as well as checking the efficiency of the measures taken, [7].

Environmental monitoring of air in Serbia was established as a continuous monitoring of air quality to a total of 45 measuring stations. In Serbia, monitoring is carried out within IPA 2012 "Establishment of an integrated environmental monitoring system for air and water quality" part of "Supply of ICT equipment and software for Air Quality Monitoring System". The main objective of the project is to enable the competent national institution (Environmental Protection Agency) to collect, update and process the results of automatic monitoring of air quality from the network of automatic measuring stations for air quality for the purpose of reporting at national and European level. The project enables the public to instantly monitor real-time monitoring of all measuring stations in 38 cities in Serbia. The

Environmental Protection Agency has been designated as the responsible implementer of the establishment and operation of the system for automatic air quality monitoring in the Republic of Serbia [8].



a)

Meta podaci	
Naziv stanice	Bor Institut RIM
Grad	Bor
Početak rada	2015-07-02
Pripada mreži	SEPA
EOI Code	RS1045A
Klasifikacija	industrial
Zona	urban
Latitude	44.059937
Longitude	22.101434
Latitude DMS	22° 6' 5" N
Longitude DMS	44° 3' 35" N
Nadmorska visina	389m

b)

Meta podaci	
Naziv stanice	Zaječar
Grad	Zaječar
Početak rada	2015-07-02
Pripada mreži	SEPA
EOI Code	RS1049A
Klasifikacija	background
Zona	urban
Latitude	43.902072
Longitude	22.273255
Latitude DMS	43° 54' 7" N
Longitude DMS	22° 16' 23" N
Nadmorska visina	133m

***Figure 1** Locaton of a) measuring station Bor Institut; b) measuring station Zajecar, [8]*

The data presented in this paper can be found on the website of the Environmental Protection Agency (SEPA), whose appearance is shown in Figure 1.

RESULTS AND DISCUSSION

CO concentration data obtained from the SEPA Agency for the period from 01.01.2018. to 28.02.2019. are used in this paper. The annual limit value of carbon monoxide concentrations is 3 mg / m^3 and it is not exceeded at any measuring point in the observed period. The limit value of the maximum daily eight hours concentration of carbon monoxide, which is 10 mg / m^3, is also not exceeded, but approximate values of 9, 6 mg/m^3 were recorded at the station in Zaječar.

By observing possible sources of carbon monoxide pollution in Zajecar and Bor, it has been concluded that there are two primary sources:-in the winter period, the boiler rooms play a key role in air polluting. In summer period pollution is present due to internal combustion engines. This trend is clearly evident, figure 2, the CO concentration significantly lower in the April-September (summer period), than in the winter period. Because of that, these two periods are analyzed separately.

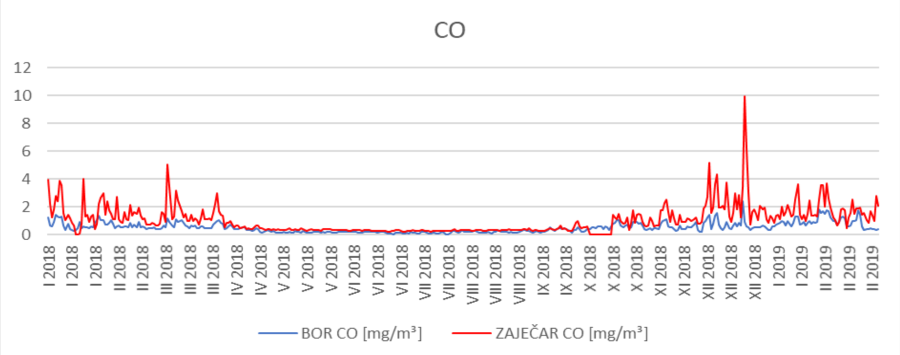


***Figure 2** Comparative display of CO emissions for cities: Bor and Zajecar, [5]*

The period April-September is analyzed in relation to the number of motor vehicles present in each of the cities. As seen in Table 1, a large number of vehicles have been registered in Zajecar. This ratio is not negligible and the difference in the number of registered vehicles in 2017 is 27% higher in Zajecar.

***Table 1** Number of registered motor vehicles in 2017., [9]*

City	Moped	Motorcycles	Passenger cars	Buses	Trucks	Total vehicles	Total vehicles	%
Zaječar	301	394	13136	21	1027	32	3212	100
Bor	73	204	12653	99	891	31	2018	63

Based on the previously stated conclusion that CO_2 emissions are directly proportional to the number of vehicles, the assumption is that pollution in Bor will be 30% lower than in Zajecar. In this analysis it was not possible to take into account the frequency of using the vehicle, so it is assumed that both people of Zajecar and people of Bor equally use their vehicles. Figure 3 shows that these trends are "monitored", but the Bor trend line in relation to the Zajecar trend line is lower for a value that is proportional to the number of cars.

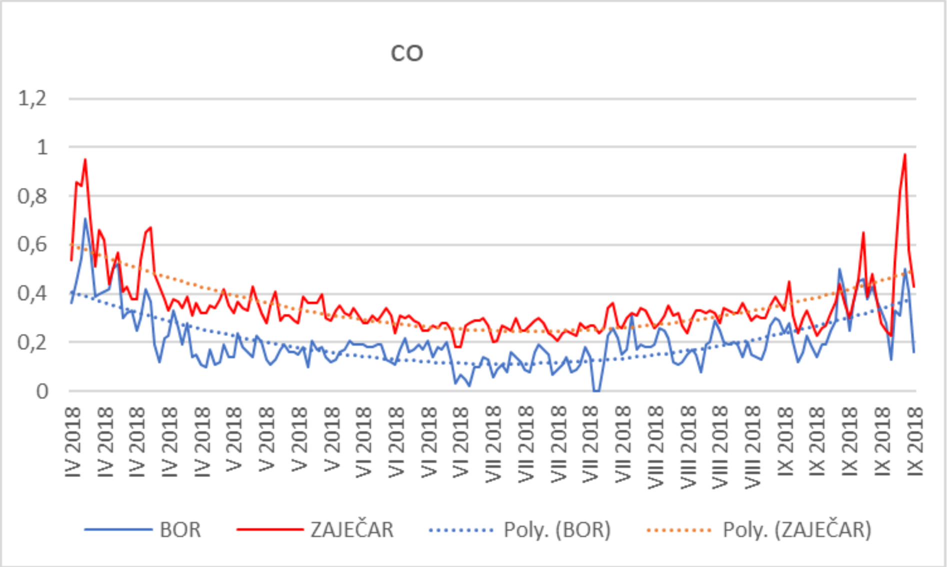


***Figure 3** Comparative display of CO emissions for cities: Bor and Zajecar, for the period: April-September 2018., [5]*

Correlation of the number of cars and CO emissions is clearly evident. Is established relation for the average daily measured CO (mg/ m^3/ day) in the period April-Septemer and the number of registered motor vehicles - the average daily CO emission per registered vehicle. The average daily CO emission per registered vehicleit amounts for Zaječar 0.000102764 (mg/ m^3/ day/ reg.mot.v.), and for Bor 0.000108864 (mg/ m^3/ day/ reg.mot.v.).

Another important CO emission parameter is incomplete combustion of solid fuels. Therefore, the dependence between temperature oscillations in both cities, or the temperature level of the outside temperature that heating intensity depends on outside temperature the heating intensity, was analyzed, but the relevant correlation was not established. The analysis showed very small differences between the mean daily temperature in Bor and Zaječar Figure 4), so this parameter is not significant for the big difference in the CO gas emission, Figure 5.

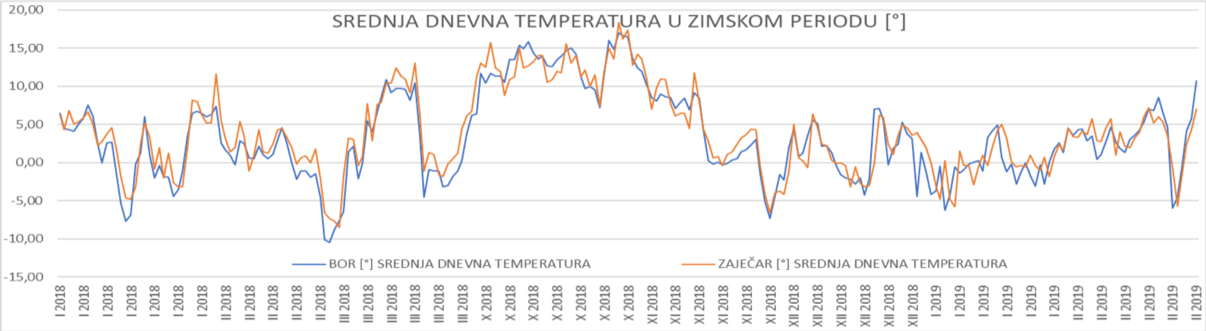


Figure 4 *Comparison graph of the average daily temperature for the cities: Bor and Zajecar in the winter period, [10]*

In relation to the type of heating, i.e. there are individual boiler rooms or heating plants in the city, an appropriate dependency has been established. In Bor, the City Heating Plant meets the needs of 80% of the household, and only 20% of them use individual boiler rooms, while in Zaječar there are 80% of individual boiler rooms, while only 20% use district heating services, i.e. City heating plants.

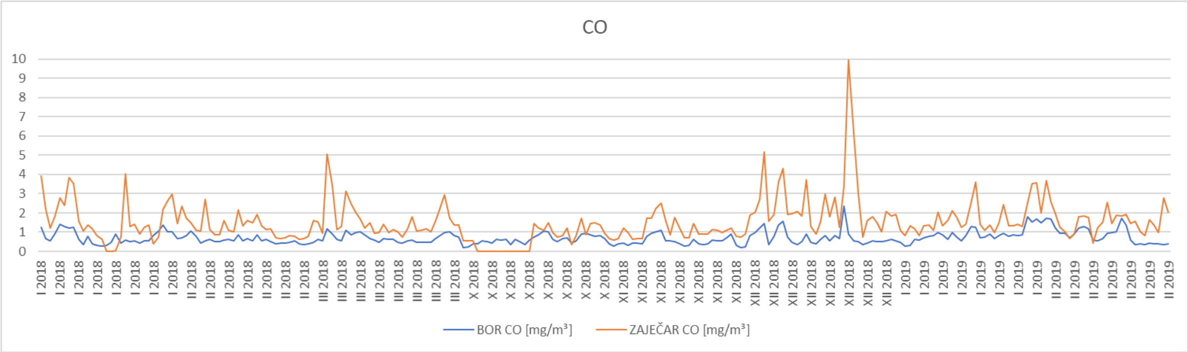


Figure 5 *Comparative graph of CO emissions for cities: Bor and Zajecar for the winter period, [5]*

Figure 5 shows a comparative CO emission graph in Bor and Zaječar during the winter period. Based on the available data, indicators such as the average daily CO emissions in the winter period were calculated.

The CO emission in Zaječar is higher than in Bor exclusively, because in this town a large number of households are heat by using their own boiler rooms predominantly on solid fuels such as coal and wood, these data are presented in Table 2.

The average daily CO emissions for the cities of Bor and Zajecar are shown in Table 2, which shows that CO emissions can be displayed in relation to the number of registered vehicles and that these indicators are approximately equal in both annexed city. In column 4, which shows the average daily CO emissions for the winter period, higher emissions are recorded in Zajecar, and if these values are shown in relation to the percentage of individual boiler rooms present in both cities, the dependence is easily visible.

Table 2 *Average daily emissions of CO for Bor and Zajecar*

City	Average daily CO emissions for the summer period (01.04. to 31.09.2018) (mg/m^3/day)	Average daily CO emissions for the summer period by number of registered vehicles (mg/m^3/day/reg.mot.v.)	Average daily CO emission for the winter period (01.01.-31.03. and 01.10. 2018 to 28.02.2019) (mg/m^3/day)	Average daily CO emissions for the winter period minus the average daily CO emissions for the summer period (mg/m^3/day)	Percentage participation of average daily CO emissions for the winter period, %
Bor	0.207377049	0.000102764	0.522907935	**0.315530886**	25
Zaječar	0.349672131	0.000108864	1.356385000	**1.006712869**	80

CONCLUSION

Based on the analyzed data, it is concluded that throughout the year there is a pollution of air by gas CO due to the operation of internal combustion engines and it amounts to approximately 0.000105 mg/ m^3/ day/ reg.mot.v. In the winter period, additional CO pollution is present, which is proportional to the volume of active boiler plants in the analyzed cities.

The results of this paper suggest that the reduction of air pollution by CO gas in Zaječar could be effectively addressed by using district heating on a larger scale than the present one.

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ANALYSIS OF ENVIRONMENTAL POLLUTION WITH DUST FROM NON-METALLIC OPEN PITS

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Abstract

Surface mining is source of the environmental pollution in the working and living area in the vicinity of the open pit. In the surface mining of non-metallic deposits, dust is the dominant pollutant. It occurs in all parts of the technological process of obtaining non-metallic minerals and represents practical and legal problem in exploitation. In order to solve this problem, the method of mineral dust formation, critical points with increased dust emissions, measures for reducing dust emissions and measures for discharge reduction into the environment are analyzed in this paper. The problem is considered in the case of the exploitation of sandstone deposit „Deo“ near the village Donja Bela Reka.

Keywords: open pit, mineral dust, pollution

INTRODUCTION

Surface mining affects the pollution and change of the immediate environment. These changes can be manifested in the immediate working and living mine area, but also in the wider area. Changes in the natural environment can be characterized as permanent and temporary. Permanent changes are most expressive in the aspect of the land degradation due to excavation of mineral raw materials. The excavation leads to the creation of new artificial relief structures and irreversible changes of the existing terrain, [1]. Another permanent form of degradation of the environment is the creation of a waste dumps, which irreversibly destroys large ground surfaces. Permanent changes contribute to changes in the water regime, microclimate conditions in the wider area, changes in flora and fauna. Also, mining processes, but also waste dumps are often the cause of contamination of soil with heavy metals, [2,3]. Impact on the environment depends primarily on the size of the open pits and waste dumps, i.e., surface directly affected by mining operations. In addition to the size of the open pit, there are other factors that significantly influence the increase in environmental pollution, such as: precipitation, watercourses, groundwater regime, types of pollutants, their chemical composition, etc. It is believed that the winds are most responsible for the rapid transport of contaminants through the immediate environment, with particular emphasis on atmospheric aerosols as substances responsible for various respiratory organs diseases, [4].

The manifestation of temporary changes is reflected in pollutants that are not permanent, i.e. their effects disappear after the end of the exploitation, specifically two years after the end of exploitation, [5]. Temporary pollutants are active during the exploitation and they are closely related to the technological processes that take place at the mine. Most often lead to the creation of increased emissions of mineral dust, excessive noise, vibration, rupture hazards, accumulation of mine waste, etc.

The concept of air pollution involves the emission of pollutants into the surrounding atmosphere, carried by wind can endanger human health, inflict damage on animals, plants and other natural and labor-created values. Chemical harmful substances can be stimulated from the rock mass, from mechanization in the form of exhaust gases or from materials used in the exploitation process, such as explosives for blasting, [6].

Dust is the accompanying phenomenon for all open pits, occurs in all technological phases, especially during drilling and blasting, loading, hauling and crushing mineral materials. The presence of dust is particularly expressed in the exploitation of stone aggregates. Pollution due to dust emissions depends on the characteristics of the soil, relief and landscape, the area climate, the roses of the wind and the characteristics of the non metallic minerals that are exploited. The effect of dust is more expressed during dry summer periods with a small number of rainy days. Dust by definition has the same chemical composition as the exploited mineral material. In this paper, the degree of pollution by emission of dust, sources of pollution, and the efficiency of the applied measures in the vicinity of the open pit „Deo“ Donja Bela Reka, are analyzed, [6].

MATERIALS AND METHODS

Open pit *„Deo“ Donja Bela Reka*

The open pit „Deo“ is placed in mountainous area with an altitude of 310 – 447 m. Exploitation of quartz sandstone in the part of the „Deo“ deposit is done for many years, which results in the mining works being affected and degraded by the larger area of the surrounding land. Further development of the open pit implies the exploitation of Deo Sever deposit and the expansion of degraded areas towards the north side. In the vicinity of the open pit there are forests, but also cultivated areas such as fields and orchards. Figure 1 present relation between open pit and waste dump affected areas as well as the nearness of the Donja Bela Reka village [6].

Dust on the open pit is result of preparatory and auxiliary works, drilling and blasting, loading and haulage, crushing and screening. On the quartz sandstone open pit the discontinuous method of exploitation of this non-metallic mineral resource is applied. Mineral dust is generated in following processes of exploitation:

- drilling,
- blasting,
- loading of blasted material into the trucks,
- transport of blasted material to the mineral processing (crushing plant) plant and waste dump,
- crushing and screening.

Dust is created also during the loading of final products from depot in Crushing plant, as well as transporting final products to the customers with trucks.

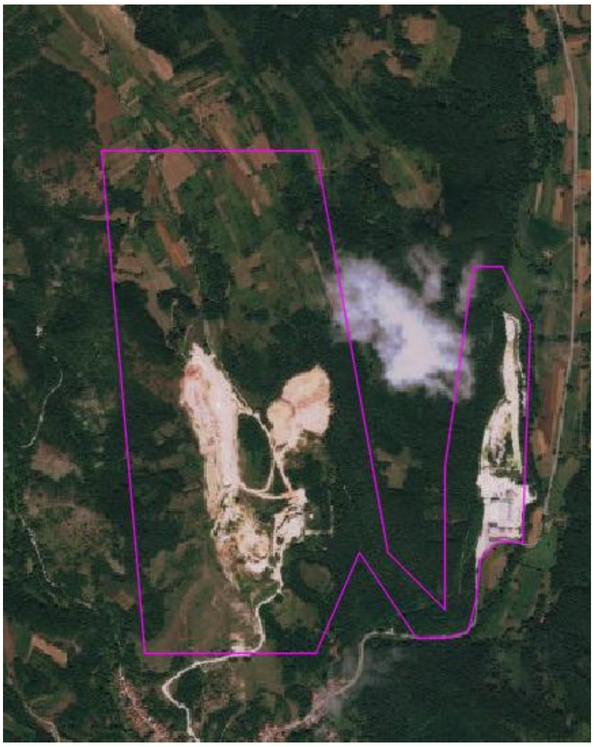

Figure 1 *Surface affected by open pit Deo Donja Bela Reka*

Also the eolic erosion of the open bench surfaces on the open pit and waste dump is present. The effect of wind in dry periods over already dry surfaces is a significant source of dust, which is particularly expressed on roads inside the open pit and waste dump. Also the surface sources of dust are depots of materials.

RESULTS AND DISCUSSION

Except for eolic erosion and haulage by trucks defined as the linear type of air pollution with mineral dust, all other phases (drilling, blasting, loading, crushing and screening) represent point sources of air pollution. In addition to eolic erosion that has the character of general pollution and haulage which effect is characterized by local and general pollution, other sources of pollution have the character of local pollution, except in the conditions of strong winds, when they may have an environmental impact, [6].

Linear air pollutants on the open pit

The haulage with trucks is the biggest pollutant of the living and working environment, of all haulage methods. Its contribution in the total pollution of the environment in terms of dust generation amounts to 60 ÷ 70%, [6]. For the haulage of material on the open pit „Deo“, three trucks MAN TGS 33.400 are used. The best way for determination of dust emission generated by the operation of these transporters is measuring dust emissions in the field and it depends on the truck speed, the wind speed and direction, and the period of year (dry or rainy). Based on the measurements, dust emissions of 7500 mg/s for all three trucks were determined, [6].

Figure 2 *Haulage by the trucks on the open pit*

Point air pollutants on the open pit

Drilling is the biggest source of fine respiratory dust on open pits. On the open pit „Deo“ the drill type DTH-KG910B with the air compressor Kaishan LGCZ-12/10 is used for drilling. This drilling equipment doesn’t have installed dust protection equipment. Dust emission at drilling can be determinate by the empirical formula, [7]:

$$E = i \cdot V_0 \cdot d^2 \cdot \rho \cdot f \cdot K_1 \cdot K_2 \cdot K_3 = 155 \cdot 10^{-8} \cdot 4{,}44 \cdot 89 \cdot 2{,}7 \cdot 5{,}5 \cdot 1{,}7 \cdot 0{,}7 \cdot 1 = 0{,}01082376 mg/s$$

where:

E- dust emission [mg/s],

i – coefficient dependable of the drilling method and cleaning of the drillhole ($i=155\cdot10^{-8}$),

V_0- drilling velocity (V_0= 4,44 mm/s),

d- drillhole diameter (d= 89 mm),

ρ- rock density (2,7 mg/mm^3),

f- rock strenght coefficient by Protodiakonov (f= 5,5),

K_1- coefficient of simultaneous operation of multiple drill hammers (K_1= 1, for one drill hammer),

K_2- coefficient of drillhole angle inclination (K_2= 0,7 for sidelong and vertical drillholes drilled downward),

K_3- coefficient depended of the impact of drilling machine type (K_3= 1 for the drill hammer on the rafter).

Blasting represents a surface source of dust pollution. This phase is performed periodically and is limited by the time duration and zone of distribution within the working environment. The total amount of dust rising with the cloud of gaseous detonation products can be calculated according to the formula, [7]:

$$I = 0{,}149 a^2 V_b / t_f = 0{,}149 \cdot 0{,}76 \cdot 1500 / 30 = 4{,}30312 kg/s$$

where:

I- total amount of dust that is raised during blasting [mg/s],

a- specific consumption of explosives (a= 0,76 kg/m^3),

V_b- volume of the block for blasting (V_b= 1500 m^3),

t_f- blasting duration time (t_f= 30 s).

Also, loading machines are point sources of dust. For the loading of quartz sandstone and waste into trucks on the open pit „Deo“, the excavator Doosan 225 DX and the wheel loader Liebherr L550 XPower are used. By measuring the dust emission a quantity of 1500 mg/s was established.

Primary and secondary crusher, and two-stage vibration screen are located in closed buildings at Crushing Plant. Since they are not in the open space and the dust extraction system is installed in the Crushing Plant, these dust sources have no impact on the environment and they are not considered.

Table 1 *Sources of pollution and their character and participation in total pollution*

Source of pollution/process	Participation in open pit pollution (%)	Character of pollution	Harmfulness
Drilling	5	Local	Dust
Blasting	20-25	Local and general	Gasses and dust
Mining mechanization	5-15	Local	Gasses and dust
Haulage	60-70	Local and general	Gasses and dust
„Eolic erosion”	20-30	Local and general	Dust

Improvement measures overview

The measures applied to reduce the emission of mineral dust had a positive effect in the Crushing Plant. In order to reduce the total air pollution in the vicinity of the open pit „Deo“, it is necessary to apply measures of their suppression.

Drilling represents point source of pollution with local character. Treatment of dust release during drilling can best be solved by applying a wet drilling or using a dust extractor that can be an integral part of the drill. Since the existing drill is not equipped with any of these two systems, it is easiest to install a dust extracting system consisting of a cyclone and filters.

The most important source of dust is haulage by trucks. The quality of roads has a major impact on the reduction of dust emissions during haulage. On a properly made road, the surface of the road will slowly degrade, resulting in a reduction in the dust emission potential. Although the proper construction of the haulage routes is important for reducing the emissions of dust due to the movement of haulage equipment, there is still a need to implement measures for the suppression and reduction of dust on the transport routes. The most commonly used method for dust removal on roads is the wetness of roads with water. The application of this method is the simplest and easiest and is achieved by using a tank with water spray nozzles. Also, reducing the haulage speed can be an effective method for reducing dust emissions, provided that this does not jeopardize production capacity.

The effect of eolic erosion on air pollution depends on the wind velocity and the period of the year (dry or rainy). Sprinkling with water is obviously recommended for reducing dust emissions at waste dumps and open spaces at the open pits. However, although sprinkling is a good measure for reducing dust emissions, in order to prevent erosion, the humidity of the material in the waste dump must be constantly monitored and maintained in an appropriate amount of moisture in the material. The final surfaces (bench levels and slopes) on the open pit and waste dump will be subjected to technical and biological recultivation according to the established dynamics, after their formation, which will significantly influence to the reduction of dust from these surfaces by the wind.

CONCLUSION

In the vicinity of the open pit „Deo“ is the village of Donja Bela Reka, the water supply Surdup and the river Ravna reka that can be endangered by mining operations during the exploitation. Therefore, it is necessary to conduct an environmental impact assessment of the environment in the vicinity of the open pit to prevent possible negative environmental impacts. In this paper an analysis of pollution of the environment from dust from the open pit was conducted and the proposal of measures of protection was given. By analyzing the technological phases of exploitation, it is concluded that the greatest amount of dust is generated by blasting, but this process is periodical, since the blasting is carried out only 3-4 times in a month and lasts briefly. The largest dust emission from the open pit is generated during the haulage of materials by trucks, and this technological phase is time-consuming, while the eolic erosion affects only at wind velocity greater than 2 m/s. By appropriate suppression measures for dust generated during the exploitation on the open pit „Deo“, the environmental impact of dust can be reduced and environmental protection can be improven.

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EVALUATION OF SOIL POLLUTION IN THE BOR AREA

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Abstract

Concentrations of 10 elements (As, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn) in the samples of hazel root zone soil, from the Bor area, were used for the calculation of four soil pollution indexes: Contamination Factor (CF), Pollution Load Index (PLI), Geo–accumulation Index (I_{geo}) and Enrichment Factor (EF). According to the obtained values of the indexes, the evaluation of the soil pollution at 12 sampling sites was performed. The results showed that soil in the study area was mostly heavily to extremely polluted with Cu and As, less polluted with Pb and Zn, while pollution of soil with Co, Cr, Fe, Mn, Mo and Ni was in the range of none to moderate, depending on the pollution index and its classification. According to PLI, three sites were in overall classified as heavy polluted (R1, R2 and T2), while all the other sites were moderately polluted in the study area.

Keywords: soil pollution, hazel, Bor area, soil indexes

INTRODUCTION

Soil is substantive component of the biosphere, which is exposed to excess of various pollutants [1]. Smelting of nonferrous metals is one of the most significant anthropogenic pollution sources of the environment. In the steps of refining and processing of mineral concentrates significant quantities of atmospheric deposition, containing heavy metals, are discharged [2]. Unlike organic contaminants, metals are generally non degradable and persistent in various media, such as soil and water [3,4]. Kang *et al.* [2] suggested that considerable inputs of metals, thought emission and deposition of dust from Zn–smelter, were responsible for polluting the nearby agricultural soils. According to Yang *et al.* [4], Pb–Zn ore exploitation, in duration of more than 50 years, reduced the quality of the agricultural soil, in which the primary metal pollutants were Pb, Cd, Zn and As. Akopyan *et al.* [5] showed that the amount of As and Pb in the residential soil was associated with proximity of Cu–smelter. Pattnaik and Equeenuddin [6] suggested that mining and processing of chromite ore resulted in degradation of soil quality, whereas the soil was severely contaminated by Cr, Ni, Co, Mn, Cu, Zn and Pb. As indicated in the study by Cai *et al.* [3], elevated concentrations of heavy metals (Cd, As and Pb) in soil, crop, well water and fish, were found due to emissions from the nearby Cu–smelter.

Due to long–term mining and metallurgy activities, pollution inputs in the study area of Bor and its surroundings were high. The aim of the paper was to evaluate the contamination of the root zone soil of hazel (*Corylus* spp.) by 10 selected elements (As, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn) using soil pollution indexes.

MATERIALS AND METHODS

Study area

The study area was the city of Bor and its surroundings. Mining–metallurgical operations associated with copper production are the dominant pollution sources in the study area (Figure 1a). During 2013, in the time of soil sampling, the „old“ copper smelter was operational. Hazardous substances, such as SO_2 and particulate matter enriched with Cu and accompanying elements from processed ores, were emitted into the atmosphere and distributed by winds on the surrounding area. The secondary sources of pollution were associated with the city heating plant and traffic [7].

Concentrations of As, Co, Cr, Cu, Fe, Mn, Mo, Ni, Pb and Zn were obtained from the hazel root zone soil (*Corylus* spp.) at 12 sampling sites, including background (Figure 1b). The sampling and analysis of soil were performed according to the standard procedure, as previously explained in Radojevic *et al.* [8].

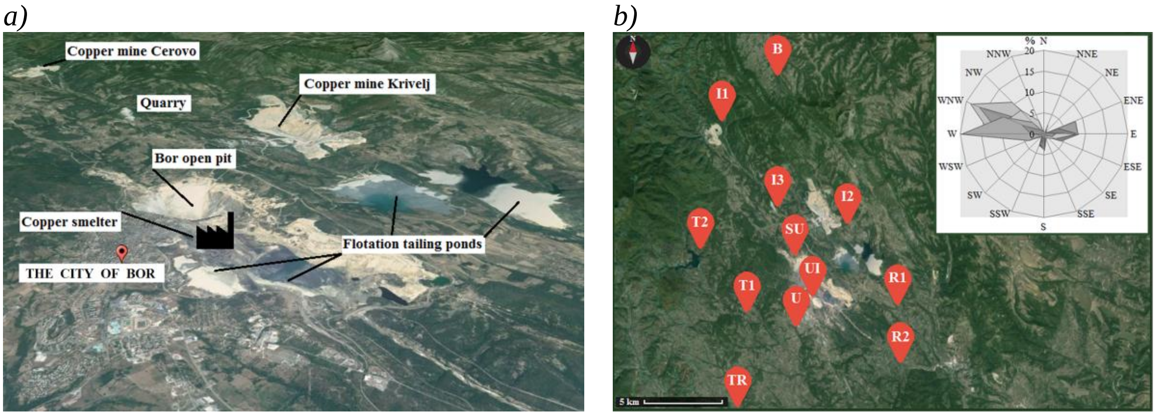

Figure 1 *a) The map of the study area with major pollution sources; b) location of the sampling sites of the hazel root zone soil and wind rose diagram for 2013 [8]*

The sampling sites urban (U), urban–industrial (UI) and suburban (SU) were located in the city of Bor; rural settlements Oštrelj (R1) and Slatina (R2) were sites located on the prevailing wind directions; the sites associated with mining operations were the mine Cerovo (I1), ore waste heap of the Veliki Krivelj mine and the quarry (I3); the sites T1 and T2 were located in the touristic area of Bor, i.e. Brestovac spa and Bor Lake, respectively; the site TR was the site associated only with traffic pollution, since it was dislocated from mining–metallurgical operations. The rural settlement Gornjane was selected as the background, due to its position behind the mountain range of Veliki and Mali Krš, which provided natural shield from the pollution [8].

Data analysis

The obtained element concentrations in the soil (mg/kg of dry weight), were used for the calculation of four soil pollution indexes, which are explained below.

Contamination Factor (CF) could be calculated for each element at each sampling site, in order to evaluate the soil contamination level for the particular site [1,4,9] using equation 1:

$$\mathrm{CF} = \frac{C_{\mathrm{sample}}}{C_{\mathrm{control}}} \tag{1}$$

where:

C_{sample} – element concentration in the soil sample (mg/kg),

C_{control} – element concentration in the soil sample from control/unpolluted area (mg/kg).

Based on the CF values, the soil contamination could be ranged from 1 to 6, and in the terms of a pollution, the ranks represent following pollution levels: 0 – none, 1 – none to moderate, 2 – moderate, 3 – moderate to strong, 4 – strongly polluted, 5 – strongly to very strong, and 6 – very strong pollution of soil.

After the calculation of CFs for each element, the overall level of soil pollution at particular sampling site can be evaluated by Pollution Load Index (PLI) [4,10] as:

$$\mathrm{PLI} = \left(\mathrm{CF}_1 \times \mathrm{CF}_2 \times \mathrm{CF}_3 \times \ldots \times \mathrm{CF}_n\right)^{1/n} \tag{2}$$

where:

CF_n – Contamination Factor calculated for n elements at each sampling site.

According to the PLI values, following soil pollution levels could be distinguished: PLI < 1 represent no pollution, 1 < PLI < 2 are related to moderate pollution, 2 < PLI < 3 are related to heavy pollution, and the values of PLI > 3 represent extremely heavy pollution of the soil at particular sampling site.

Geo–accumulation Index (I_{geo}) is used to assess the soil contamination by comparing the element concentration in the soil with the concentration of the corresponding element in uncontaminated soil [2], according to the equation 3:

$$I_{geo} = log_2 \frac{C_n}{1.5 \times B_n} \tag{3}$$

where:

C_n – concentration of element in the soil sample (mg/kg),

B_n – geochemical background concentration of the element (mg/kg),

1.5 – background matrix correction factor used due to the lithological variability.

The values of I_{geo} are divided in seven classes: Class 0 is uncontaminated soil ($I_{geo} \leq 0$), Class 1 is uncontaminated to moderately contaminated ($0 < I_{geo} \leq 1$), Class 2 is moderately contaminated ($1 < I_{geo} \leq 2$), Class 3 is moderately to heavily contaminated ($2 < I_{geo} \leq 3$), Class 4 is heavily contaminated ($3 < I_{geo} \leq 4$), Class 5 is heavily to extremely contaminated ($4 < I_{geo} \leq 5$), and Class 6 is extremely contaminated soil ($I_{geo} > 5$). The element concentration (C_n) in the Class 6 can be hundredfold greater than the geochemical background value (B_n) [2]. For the geochemical background concentrations of the elements (B_n), the concentrations of analysed elements from the background site B were used.

The most often used soil contamination index is Enrichment Factor (EF) [6,10]. The preferred way of evaluating soil contamination is by normalization with the referent element (usually Al or Fe), according to the equation 4. This normalization compensates the natural variability caused by influence of grain size and mineral composition of soil on elemental concentration [6]. In this study, normalization was done by Al, not Fe, due to anthropogenic input of Fe, since the processed ores contain Fe in some degree [8]. In the terms of referent concentration of elements, different approaches have been used thought the literature. For the calculation of EF in this paper, the concentrations of elements in upper crust were used [11].

$$\mathrm{EF} = \frac{\left(\frac{C_n}{C_{\mathrm{Al}}}\right)_{\mathrm{sample}}}{\left(\frac{C_n}{C_{\mathrm{Al}}}\right)_{\mathrm{referent}}} \tag{4}$$

where:

$\left(\frac{C_n}{C_{\mathrm{Al}}}\right)_{\mathrm{sample}}$	– element concentrations (C_n) and Al concentration in soil sample (mg/kg),
$\left(\frac{C_n}{C_{\mathrm{Al}}}\right)_{\mathrm{referent}}$	– element concentrations (C_n) and Al concentration in the referent soil sample (mg/kg).

According to the EF values, there are five levels of soil enrichment by elements: EF < 2 corresponds to depletion or minimal enrichment (i.e. minimal pollution), 2 < EF < 5 refers to moderate enrichment, 5 < EF < 20 refers to significant enrichment, 20 < EF < 40 refers to very high enrichment, and values of EF > 40 correspond to extremely enriched soil.

RESULTS AND DISCUSSION

Soil contamination indexes were calculated according to the equations 1–4, and the results of the contamination of the hazel root zone soil are presented in Tables 1–3.

According to CFs (Table 1), analysed soil was not polluted with Cr (CFs < 1), while CFs of Co, Mn, Fe and Ni were < 2 indicating none to moderate pollution. Contamination with Mo, Zn and Pb varied up to: moderate to strong (Mo; CFs ranged 0.80–3.14), strong (Zn; 1.24–4.74) and very strong (Pb; 1.60–6.49) pollution of the soil. The CF values of As were in the range of none to moderate pollution (at the TR site) to very strong pollution (R2 and T2). In the case of Cu contamination, the contamination of soil was mostly in the range of very strong pollution (at 8 out of 11 sampling sites). The highest CFs of As and Cu in the study area were obtained at the R2 site, amounting 12.12 and 20.12, respectively. The evaluation of sampling sites contamination was done using calculated CFs. The PLI values (Table 1) indicated that, in overall three sites were heavy polluted (R1, R2 and T2), while all the other sites in the study area were moderately polluted. However, the PLI values of the sites UI and U were >1.90, which is close to the classification of heavy polluted soil (2 < PLI < 3). The lowest PLI values were obtained of the sites I1, I2 and TR, indicating lower pollution load at these sampling sites in the study area.

According to I_{geo}, the analysed soil was not contaminated with Cr, and none to moderate contamination was noted for Co, Fe, Mn, Mo and Ni (Table 2). The results of I_{geo} were very similar to the results of CFs. The soil was generally less contaminated with Pb than with Zn, mainly in the range of moderate contamination, except at the site T2 where soil was moderately to heavily contaminated with Pb. Based on the I_{geo} values, As and Cu were also elements of the highest concern, particularly Cu. The highest values were obtained for As at the T2 site (Class 4), and for Cu at the T2, R2 and UI sites (Class 4). However, the soil pollution Classes 5 and 6 (“heavily to extremely” and “extremely pollution”, respectively), were not remarked in the study area.

***Table 1** Contamination Factor (CF) and Pollution Load Index (PLI) for the elements in the root zone soil of hazel*

Site	**CF**										**PLI**
	As	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Zn**	
UI	4.07 [e]	1.06 [b]	0.37 [a]	14.40 [f]	1.42 [b]	1.34 [b]	3.14 [d]	0.63 [a]	2.45 [c]	3.02 [d]	1.91 [*]
U	2.61 [c]	1.29 [b]	0.49 [a]	11.23 [f]	1.63 [b]	1.43 [b]	2.14 [c]	0.83 [a]	2.70 [c]	3.44 [d]	1.93 [*]
SU	4.83 [e]	0.99 [a]	0.49 [a]	10.77 [f]	1.30 [b]	1.27 [b]	1.71 [b]	0.57 [a]	2.56 [c]	2.80 [c]	1.77 [*]
I1	2.03 [c]	1.08 [b]	0.38 [a]	3.11 [d]	1.31 [b]	1.11 [b]	0.94 [a]	0.56 [a]	1.65 [b]	1.67 [b]	1.18 [*]
I2	3.44 [d]	0.76 [a]	0.94 [a]	1.49 [b]	1.10 [b]	0.76 [a]	0.85 [a]	1.25 [b]	1.60 [b]	1.24 [b]	1.20 [*]
I3	4.41 [e]	0.76 [a]	0.35 [a]	9.06 [f]	1.12 [b]	1.05 [b]	2.03 [c]	0.54 [a]	2.39 [c]	2.29 [c]	1.54 [*]
R1	3.23 [d]	0.94 [a]	0.67 [a]	9.77 [f]	1.15 [b]	1.29 [b]	2.02 [c]	1.61 [b]	2.91 [c]	4.74 [e]	2.05 [**]
R2	6.54 [f]	1.06 [b]	0.49 [a]	18.41 [f]	1.46 [b]	1.38 [b]	2.19 [c]	0.77 [a]	3.67 [d]	3.78 [d]	2.22 [**]
T1	3.11 [d]	1.56 [b]	0.32 [a]	8.32 [f]	1.94 [b]	1.57 [b]	0.89 [a]	0.58 [a]	2.25 [c]	2.55 [c]	1.61 [*]
T2	12.12 [f]	1.04 [b]	0.42 [a]	20.12 [f]	1.31 [b]	1.27 [b]	2.61 [c]	0.60 [a]	6.49 [f]	3.71 [d]	2.42 [**]
TR	1.56 [b]	1.20 [b]	0.85 [a]	1.98 [b]	1.48 [b]	1.31 [b]	0.80 [a]	1.23 [b]	1.68 [b]	1.64 [b]	1.33 [*]

[a] none pollution; [b] none to moderate pollution; [c] moderate pollution; [d] moderate to strong pollution; [e] strongly polluted; [f] very strong pollution; [*] moderate pollution; [**] heavy pollution.

***Table 2** Geo–accumulation Index (I_{geo}) for the elements in the root zone soil of hazel*

Site	I_{geo}									
	As	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Zn**
UI	1.44 [c]	-0.49 [a]	-2.01 [a]	3.26 [e]	-0.08 [a]	-0.17 [a]	1.07 [c]	-1.26 [a]	0.71 [b]	1.01 [c]
U	0.80 [b]	-0.22 [a]	-1.61 [a]	2.90 [d]	0.12 [b]	-0.07 [a]	0.51 [b]	-0.85 [a]	0.85 [b]	1.20 [c]
SU	1.69 [c]	-0.59 [a]	-1.60 [a]	2.84 [d]	-0.20 [a]	-0.24 [a]	0.19 [b]	-1.39 [a]	0.77 [b]	0.90 [b]
I1	0.44 [b]	-0.48 [a]	-2.00 [a]	1.05 [c]	-0.19 [a]	-0.43 [a]	-0.68 [a]	-1.43 [a]	0.14 [b]	0.15 [b]
I2	1.20 [c]	-0.90 [a]	-0.67 [a]	-0.01 [a]	-0.45 [a]	-0.98 [a]	-0.82 [a]	-0.27 [a]	0.10 [b]	-0.27 [a]
I3	1.56 [c]	-0.97 [a]	-2.10 [a]	2.60 [d]	-0.42 [a]	-0.51 [a]	0.44 [b]	-1.48 [a]	0.67 [b]	0.61 [b]
R1	1.11 [c]	-0.67 [a]	-1.16 [a]	2.70 [d]	-0.38 [a]	-0.21 [a]	0.43 [b]	0.11 [b]	0.96 [b]	1.66 [c]
R2	2.12 [d]	-0.50 [a]	-1.62 [a]	3.62 [e]	-0.03 [a]	-0.12 [a]	0.55 [b]	-0.96 [a]	1.29 [c]	1.33 [c]
T1	1.05 [c]	0.06 [b]	-2.22 [a]	2.47 [d]	0.37 [b]	0.06 [b]	-0.76 [a]	-1.36 [a]	0.58 [b]	0.77 [b]
T2	3.01 [e]	-0.53 [a]	-1.83 [a]	3.75 [e]	-0.19 [a]	-0.24 [a]	0.80 [b]	-1.31 [a]	2.11 [d]	1.31 [c]
TR	0.06 [b]	-0.32 [a]	-0.82 [a]	0.40 [b]	-0.02 [a]	-0.20 [a]	-0.90 [a]	-0.29 [a]	0.16 [b]	0.13 [b]

[a] uncontaminated; [b] uncontaminated to moderately contaminated; [c] moderately contaminated; [d] moderately to heavily contaminated; [e] heavily contaminated.

***Table 3** Enrichment Factor (EF) for the elements in the root zone soil of hazel*

Site	**EF**									
	As	**Co**	**Cr**	**Cu**	**Fe**	**Mn**	**Mo**	**Ni**	**Pb**	**Zn**
UI	21.09 [d]	1.82 [a]	0.87 [a]	57.28 [e]	1.66 [a]	2.47 [b]	2.69 [b]	0.92 [a]	3.31 [b]	4.18 [b]
U	13.35 [c]	2.19 [b]	1.13 [a]	44.11 [e]	1.88 [a]	2.60 [b]	1.81 [a]	1.20 [a]	3.61 [b]	4.70 [b]
SU	36.88 [d]	2.51 [b]	1.70 [a]	63.20 [e]	2.25 [b]	3.47 [b]	2.16 [b]	1.23 [a]	5.10 [c]	5.73 [b]
I1	14.74 [c]	2.58 [b]	1.22 [a]	17.32 [c]	2.14 [b]	2.87 [b]	1.12 [a]	1.14 [a]	3.13 [b]	3.24 [b]
I2	22.48 [d]	1.64 [a]	2.76 [b]	7.48 [c]	1.62 [a]	1.77 [a]	0.92 [a]	2.30 [b]	2.74 [b]	2.17 [b]
I3	40.79 [e]	2.33 [b]	1.46 [a]	64.34 [e]	2.33 [b]	3.47 [b]	3.11 [b]	1.40 [a]	5.76 [c]	5.67 [b]
R1	25.78 [d]	2.48 [b]	2.40 [b]	59.84 [e]	2.08 [b]	3.68 [b]	2.67 [b]	3.63 [b]	6.06 [c]	10.10 [c]
R2	46.63 [e]	2.51 [b]	1.57 [a]	100.88 [e]	2.36 [b]	3.50 [b]	2.58 [b]	1.55 [a]	6.84 [c]	7.21 [c]
T1	16.25 [c]	2.70 [b]	0.75 [a]	33.43 [d]	2.30 [b]	2.93 [b]	0.77 [a]	0.86 [a]	3.07 [b]	3.57 [b]
T2	83.17 [e]	2.36 [b]	1.30 [a]	106.09 [e]	2.03 [b]	3.11 [b]	2.97 [b]	1.17 [a]	11.64 [c]	6.81 [c]
TR	6.86 [c]	1.75 [a]	1.68 [a]	6.69 [c]	1.47 [a]	2.05 [b]	0.59 [a]	1.52 [a]	1.93 [a]	1.93 [a]
B	9.16 [c]	3.03 [b]	4.12 [b]	7.04 [c]	2.07 [b]	3.27 [b]	1.52 [a]	2.58 [b]	2.39 [b]	2.45 [b]

[a] minimal enrichment; [b] moderate enrichment; [c] significant enrichment; [d] very high enrichment; [e] extremely enriched soil.

Depletion or minimal to moderate soil pollution in the study area were observed for Co, Cr, Fe, Mn, Mo and Ni according to EFs (Table 3). Somewhat different results were noted for Pb and Zn, which showed mostly moderate to significant enrichment of the soil, compared to the results of the other indexes. Regarding the enrichment with As and Cu, the analysed soil was in the range of significant to extremely enriched, particularly with Cu (EFs > 40 at seven sites).

CONCLUSION

Improving the characterization of soil is becoming an important component of risk assessment and environmental policies, particularly where unsustainable intensive industrial development is in progress. Regarding the Bor area, the highest pollution of soil was noted for As and particularly Cu, less pronounced pollution existed with Pb and Zn, while pollution with Co, Cr, Fe, Mn, Mo and Ni was in the range of none to moderate, depending on the pollution index and classification. The sites that were characterised with higher pollution load, compared to the others, were the sites located on the prevailing W and E wind directions, such as R2 (Slatina), R1 (Oštrelj) and T2 (Bor Lake), as well as the sites in the close vicinity of the copper smelter (UI, U and SU). Based on the values of four soil pollution indexes, it can be concluded that long–term pollution from the mining–metallurgy activities of copper production, have left severe consequences' on the soil quality of Bor and its surroundings.

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INDICATION OF THE POLLUTION EMITTED FROM THE QUARRY

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Abstract

Polution assessment of the environment around the quarry using soil as well as branches and leaves of linden and elder was presented in this study. Concentrations and values of the Enrichment factors (EFs) of Cu, Zn and Cd in the soil, sampled around linden roots from the vicinity of the quarry (Q), indicated soil pollution with these elements. Soil was potentialy enriched with Pb, Ni and As, according to the values of EFs, although contents of these elements were below the Limit values (LV) defined in the Serbian Regulation. Branches of both plant species from the Q sampling site stood out with the lowest, and unwashed leaves with the highest concentrations of almost all the studied elements. Branches, unwashed and washed leaves of linden and elder indicated environmental enrichment with Cu and Pb, while enrichment with As was indicated only by leaves. The amounts of elements remowed by washing of linden and elder leaves from the Q and control (C) sampling site indicated the increased concentrations of some metals and metalloids in the air around the quarry compared to the C sampling site.

Keywords: quarry, linden, elder, biomonitoring

INTRODUCTION

The city of Bor and the surroundings (Eastern Serbia) are well known for mining metallurgical copper production more than 100 years. Due to outdated smelting technology which was replaced at the year of 2016, the environment of the urban–industrial and rural zones of the Bor municipality was considered to be extremely polluted [1]. In the vicinity of the city of Bor there is the limestone quarry. Quarrying is a form of land use where nonmetallic rocks and aggregates are extracted. Dimension and crushed–stones are the final output of such industry in which these products are used for different purposes. Mineral exploration, mining and processing have resulted in environmental damages including ecological disturbance, destruction of natural flora, pollution of the air, land and water, instability of soil and rock masses, landscape degradation and radiation hazards [2,3]. One possibility for the monitoring of the environmental pollution is biomonitoring using soil and plants. A lot of biomonitoring studies of the pollution from the mining metallurgical facilities have been conducted in the Bor area [4–6]. It was concluded that some of the used plant species were suitable for detection and monitoring of the extreme soil and air pollution. According to those results, linden and elder were used for the examination of environmental pollution in the vicinity of the limestone quarry which was presented in this study.

MATERIALS AND METHODS

Soil and plant samples were collected from the area around the quarry (Q) which was affected by dust emissions from exploiting and crushing. The control (C) sampling site represented the surroundings of the village Gornjane, which was without the impact of the pollution from the quarry and mining and metallurgical facilities for the copper production (Figure 1).

Figure 1 *Location of the sampling sites*

Sampling was conducted from September to mid October (just before shedding, to assure maximum metal accumulation). Particulate composite samples of soil, leaves and branches of each plant species per sampling site were formed. Soil was sampled around roots of plants at 10–20 cm depth. The samples of branches (2 cm thickness) and leaves were collected at 1.5 to 2 m height above the ground from outer branches of the canopy. Branches (B) were not washed. One half of the foliar samples were retained as an unwashed sample (UL), while the second half was thoroughly washed (WL) with running distilled water for about 1 min at the room temperature, to remove the particle fraction deposited on the surfaces of leaves. After air drying at the room temperature, all the samples were ground to a fine powder and stored at the room temperature until analysis. Digestion [6] and chemical analysis (ICP–AES) of the soil and plant samples were conducted at the Institute of Mining and Metallurgy Bor (Serbia). The values of the Enrichment factor (EF) were calculated by dividing the concentration of a certain element in the sample from the polluted zone by the concentration of the corresponding element in the sample from the control zone. The values of EF>2 indicated environmental pollution.

RESULTS AND DISCUSSION

Assessment of soil pollution

Al and Fe soil contents were in the typical range given by the US EPA [8,9] (Table 1). Concentrations of Fe in linden and elder soil were slightly above the average soil content of 3.5% given by Kabata−Pendias [10]. Linden soil indicated the enrichment with Fe.

Table 1 *Concentrations of Al, Fe, Cu, Zn, Pb, Ni, As and Cd in soil samples (µg/g)*

Sampling site	**Plant species**	**Al**[*]	**Fe**[*]	**Cu**	**Zn**	**Pb**	**Ni**	**As**	**Cd**
Quarry	**linden**	4.22	3.48	391.78	167.14	52.94	17.34	27.88	1.60
	elder	5.41	4.00	116.27	93.93	23.96	17.78	14.47	<0.3[a]
Control	**linden**	2.59	1.25	22.74	40.80	22.50	8.62	5.10	<0.3[a]
	elder	3.39	2.44	39.42	84.22	22.70	15.59	7.39	<0.3[a]
LV[b]		n.d.[c]	n.d.[c]	36	140	85	35	29	0.8
EF	**linden**	1.63	**2.78**	**17.23**	**4.10**	**2.35**	**2.01**	**5.47**	**>5.33**[d]
	elder	1.60	1.64	**2.95**	1.12	1.06	1.14	1.96	/[e]

[*]Concentrations were expressed in %; [a]<concentrations were below the lower limit of determination; [b]Limit value [7]; [c]not defined; [d]>values were obtained by dividing the element concentration in soil from the Q sampling site with the lower limit of determination of the corresponding element in the sample from the C sampling site; [e]/ cannot be calculated because both element concentrations were below the lower limit of determination; the values of EFs >2 were given in bold.

Soil Cu contents were above the LV in all the samples, except in the linden soil from the C sampling site. The enrichment of soil of the both plant species from the Q sampling site with Cu was indicated, especially of the elder soil. The LV for Zn was exceeded only in the linden soil from the vicinity of the quarry. This sample also indicated the enrichment with Zn. Concentrations of Pb, Ni and As were below the LVs, but the linden soil showed the enrichment with these elements, especially with As. Only Cd content in the linden soil from the Q sampling site was above the LV, and this sample indicated the soil pollution with Cd.

Assessment of air pollution using aboveground plant parts

Linden branches stood out with the lowest Al, Fe, Cu, Zn and As concentrations compared to other parts of the examined plant species from the Q sampling site (Table 2). The lowest Pb and Ni contents were determined in the washed linden leaves. The elder branches also contained lower concentrations of the studied elements compared to the WL and UL, except of Ni. Concentrations of Al, Fe, Cu, Zn, Pb and As were the highest in the unwashed linden and elder leaves, compared to the washed leaves and branches from the Q sampling site. The highest Ni contents were determined in the branches of the both plant species from the vicinity of the quarry. The examined elements in the plant parts from the C sampling site showed some different regularities depending on the element. Linden and elder branches contained the lowest Al, Fe, Cu and Ni concentrations compared to other plant parts, with exception of elder where the lowest Cu content was determined in the unwashed leaves. The plant part with the highest contents of the elements depended of the particular element. The highest Al, Fe and Pb concentrations were determined in the linden unwashed leaves. The washed linden leaves contained the highest Cu and Ni concentrations, while the linden branches contained the highest Zn concentrations. The highest Al, Fe, Zn and Pb concentrations were obtained in the unwashed elder leaves, Ni in the elder washed leaves and Cu in the branches. Cd concentrations in the linden and elder aboveground parts were below the lower limit of determination (<0.2 μg/g).

Table 2 *Concentrations (μg/g) of elements in leaves and branches of linden and elder*

Sampling site	Plant species	Plant part	Al	Fe	Cu	Zn	Pb	Ni	As
Quarry	**linden**	**WL**	118.82	146.64	23.96	11.57	4.79	0.35	2.07
		UL	190.97	204.57	36.05	14.09	5.44	0.43	3.30
		B	26.34	39.39	14.83	7.98	5.23	0.48	<1.0[a]
	elder	**WL**	70.30	125.74	20.93	17.94	3.52	0.44	1.86
		UL	133.38	343.38	41.40	22.95	7.09	0.41	3.53
		B	36.13	45.05	13.47	8.14	2.26	0.45	<1.0[a]
Control	**linden**	**WL**	63.01	90.26	7.88	9.20	1.01	0.99	<1.0[a]
		UL	81.28	92.14	7.06	8.35	1.15	0.95	<1.0[a]
		B	19.78	31.66	4.27	9.92	1.06	0.63	<1.0[a]
	elder	**WL**	139.54	162.28	6.60	21.75	0.95	2.17	<1.0[a]
		UL	214.01	193.49	6.46	22.36	1.05	0.96	<1.0[a]
		B	34.18	42.85	6.74	8.42	<1.0[a]	0.27	<1.0[a]

[a] concentrations were below the lower limit of determination.

The EF values for Al and Fe were >2 only for unwashed leaves of linden (Table 3). All the examined parts of linden and elder indicated the enrichment of the environment with Cu. According to the values of EFs, there was no enrichment with Zn and Ni. The enrichment with Pb was somewhat higher than with Cu.

Table 3 *The values of Enrichment factors of Al, Fe, Cu, Zn, Pb, Ni and As in leaves and branches*

Plant species	Plant part	Al	Fe	Cu	Zn	Pb	Ni	As
linden	**WL**	1.89	1.62	**3.04**	1.26	**4.72**	0.35	**>2.07**[a]
	UL	**2.35**	**2.22**	**5.10**	1.69	**4.75**	0.45	**>3.30**[a]
	B	1.33	1.24	**3.47**	0.80	**4.96**	0.75	/[b]
elder	**WL**	0.50	0.77	**3.17**	0.83	**3.71**	0.20	>1.86[a]
	UL	0.62	1.77	**6.41**	1.03	**6.78**	0.43	**>3.53**[a]
	B	1.06	1.05	**2.00**	0.97	**>2.26**[a]	1.66	/[b]

[a]>values were obtained by dividing the element concentration in plant part from the Q sampling site with the lower limit of determination of the same element in the sample from the C sampling site; [b]/concentrations in plant part from the both sampling sites were below the lower limit of determination; the values of EFs>2 were given in bold.

The values of EF for As in the washed and unwashed linden leaves as well as unwashed elder leaves were higher than 2 (Table 3). The highest EF values for Cu, Pb and As were calculated for the unwashed leaves of elder. These results are consistent with those obtained in the previous study by Kalinovic *et al.* [6], where elder leaves were better for detection of enrichment with the studied metals and metalloids in the atmospheric deposition and for biomonitoring. Comparing the obtained values of EFs for Cu in the unwashed and washed leaves of linden and elder from the Q sampling site with the EF values for leaves from the UI zone (UL–linden EF≈34, UL–elder EF≈74; WL–linden EF≈18, WL–elder EF≈30) in the study by Kalinovic *et al.* [11], it was concluded that enrichment of the air with Cu in the vicinity of the quarry persisted, but it was far lower.

In Table 4 were given differences between the unwashed and washed leaves of linden and elder for the particular element, at the three sampling sites. Except the Q and C, results were given for the UI sampling site [6,11] that was indicated as the most polluted area in the Bor and surroundings. The determined differences for all the examined elements (except Cd) in the linden leaves were the lowest for the leaves sampled at the B, and the highest for the leaves from the UI sampling site. The same regularity was observed for the elder leaves, with the exception of Al, where differences were higher for the samples from the C than for the Q sampling site. These regularities indicated that the air in the vicinity of the quarry contained higher amounts of the examined elements in the atmospheric deposition than the air from the C sampling site. However, the air of the urban–industrial zone of Bor stayed the most polluted compared with the air in the vicinity of the quarry.

Table 4 *Differences (μg/g) between element contents in the unwashed and washed leaves of linden and elder*

Plant species	**Sampling site**	**Al**	**Fe**	**Cu**	**Zn**	**Pb**	**Ni**	**As**	**Cd**
linden	**UI**[a,b]	115.17	233.99	98.57	19.52	11.33	0.87	6.65	0.19
	Q	72.15	57.93	12.09	2.52	0.65	0.08	1.24	/[c]
	C	18.27	1.89	-0.82	-0.85	0.13	-0.04	-0.28	/[c]
elder	**UI**[a,b]	247.26	721.01	287.20	52.24	32.02	0.37	11.56	0.38
	Q	63.08	217.63	20.47	5.01	3.57	-0.03	1.68	/[c]
	C	74.47	31.21	-0.14	0.61	0.10	-1.20	/[c]	/[c]

[a,b] [6,11]; [c]concentrations of elements in the unwashed and washed leaves were below the lower limit of determination.

CONCLUSION

Soil pollution with Cu in the vicinity of the quarry was indicated by the Cu contents which were above the LV and the values of the EFs>2. Zn and Cd concentrations as well as the EFs for the linden soil from the Q sampling site point out pollution with these elements. The LVs for Pb, Ni and As were not exceeded, but the EFs still indicated the enrichment of the linden soil. According to the obtained results, the soil sampled around the root of linden was more efficient for indication of the soil pollution with the examined elements. However, it should not be ignored the potential plant effect on the concentrations of elements in the soil around roots. Linden and elder branches from the Q sampling site stood out with the lowest contents of almost all the studied elements, while the highest concentrations were determined in the unwashed leaves of both plant species. The values of EFs for the aboveground parts of linden and elder confirmed the environmental enrichment with Cu and Pb, but there was no enrichment with Zn and Ni. Enrichment with As confirmed EFs for the washed and unwashed linden leaves as well as the unwashed elder leaves. The unwashed leaves of elder were better for indication of the environmental pollution with Cu, Pb and As due to the highest calculated EF values. Amounts of elements removed after a washing treatment of the linden and elder leaves showed that levels of the studied elements were higher in the atmospheric depositions from the vicinity of the quarry than in the air of the control sampling site (except of Cd in the linden leaves and Al in the elder leaves). Such results indicated the moderate environmental pollution near the quarry.

ACKNOWLEDGEMENT

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ASSESSMENT OF SOIL CONTAMINATION WITH HEAVY METALS BY SOIL POLLUTION INDICATORS

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Abstract

Although naturally present in the environment, the relative abundance of heavy metals can be seriously impaired due to different anthropogenic activities, among which mining and smelting have been describes as main pollution sources. Soil acts as a sink of the emitted heavy metals, posing a serious threat to all living organisms due to their toxicity, persistence and bioaccumulation, which is particularly noticeable in the urban soils. Thus, the adequate soil pollution assessment should be performed in order to obtain soil contamination levels. Different soil pollution indicators, which can be applied in the soil contamination degree assessment, have been described, including single pollution indicators (Contamination Factor, Pollution Index, Enrichment Factor, Geoaccumulation index, potential ecological risk factor) and integrated pollution indexes (Pollution Load Index, Contamination degree, modified Contamination degree, Integrated Pollution Index, Nemerow Integrated Pollution Index, Improved Nemerow index and potential ecological risk index).

Keywords: heavy metals, soil pollution, soil pollution indicators

INTRODUCTION

Anthropogenic activities have influenced the mobilization of heavy metals into the biosphere, thus impairing their geochemical cycling. This is particularly noted in the urban areas in which both stationary and mobile sources have led to the releasing of heavy metals into the environment in quantities which highly exceed their natural emission rates [1]. Heavy metals in the urban areas can originate from the industrial activities, traffic emissions, municipal waste and domestic activities [2]. Mining activities and metal smelting have been described as one of the main sources of heavy metal pollution [3]. By these activities, heavy metals are released into the atmosphere, and subsequently deposited on the soil [1]. Soil represents an important biogeochemical sink for heavy metals, which can remain for a long time. Heavy metals in the soil pose a potential threat to human health and ecological systems, due to their toxicity, persistence and bioaccumulation [2,3].

In order to assess the degree of soil contamination with heavy metals different pollution indexes have been described in the literature [1–16]. Calculated soil pollution indexes can be used to provide a relative ranking of soil contamination levels [13]. Single pollution indexes, as well as the integrated pollution indexes, which can be subsequently calculated, are presented in this paper.

ASSESSMENT OF SOIL POLLUTION

Simple and integrated pollution indexes

Contamination Factor (CF) is frequently used to assess soil contamination status [3–6]. CF was originally introduced by Hakanson [7], who defined it as the ratio between the mean content of the substance from at least 5 sampling sites and the corresponding standard preindustrial reference level. In recent literature, CF is usually defined as the ratio obtained by dividing the concentration of each metal in the soil by the baseline or background value (concentration in unpolluted soil) [3–6]:

$$\mathrm{CF} = \frac{C_{\mathrm{metal}}}{C_{\mathrm{background}}} \tag{1}$$

In order to evaluate the contamination level of the selected sampling sites, CF should be calculated for each heavy metal at each site [4]. The contamination level may be classified on the scale ranging from 1 to 6 [3–5]: 0 (none); 1 (none to moderate pollution); 2 (moderate pollution); 3 (moderate to strong pollution); 4 (strong pollution); 5 (strong to very strong pollution); 6 (very strong pollution). CF can be classified in different ranges [6]. Other authors have described this factor as Concentration factor [8] or Pollution factor [9].

CF can be used to calculate Contamination degree (Cd) which represents the sum of the CFs for each sample. The values Cd<6 indicate a low degree of contamination; 6<Cd<12 represents a moderate degree of contamination; 12<Cd<24 is a considerable degree of contamination; and Cd>24 is a high degree of contamination (serious anthropogenic pollution) [6].

Modified Contamination degree (mCd) can be used in the estimation of the overall degree of contamination at the given site [6]:

$$\mathrm{mCd} = \frac{\sum_{i=1}^{n} \mathrm{CF}}{n} \tag{2}$$

where n represents number of analyzed elements, *i* is i^{th} element and CF is contamination factor. For the classification and description of the modified degree of contamination (mCd) in sediment, the following gradation are proposed [6]: mCd<1.5 (none to a very low degree of contamination); 1.5≤mCd<2 (low degree of contamination); 2≤mCd<4 (moderate degree of contamination); 4≤mCd<8 (high degree of contamination); 8≤mCd<16 (very high degree of contamination); 16≤mCd<32 (extremely high degree of contamination) and mCd≥32 (ultra high degree of contamination).

Pollution Load Index (PLI) can be calculated from CFs. PLI is regarded as a simple, useful and comparative means in the assessment the level of heavy metal pollution [3,5,8]. For the overall level of soil pollution across the sampling sites, PLI can be calculated as [5]:

$$\mathrm{PLI} = \left(\mathrm{CF}_1 \times \mathrm{CF}_2 \times \mathrm{CF}_3 \times \ldots \times \mathrm{CF}_n\right)^{1/n} \tag{3}$$

where CF is the metal contamination factor and n is the number of samples analyzed. Four pollution levels for each sampling site can be defined [3,4]: no pollution (PLI<1), moderate pollution (1<PLI<2), heavy pollution (2<PLI<3) and extremely heavy pollution (PLI>3).

According to Cabrera *et al.* [8], PLI=1 indicate heavy metal loads close to the background level, while PLI>1 indicate pollution.

Chen *et al.* [1] expressed Pollution Index (PI) as the ratio of the measured heavy metal concentration in the soil to the geochemical mean of the background concentration of the corresponding metal. The PI value for each metal can be classified as low ($PI \leq 1.0$), moderate ($1.0 < PI \leq 3.0$) or high ($PI > 3.0$). Another commonly used criterion to evaluate the soil heavy metal pollution is Integrated Pollution Index (IPI), which is defined as the mean values for all the PIs of all considered metals. According to IPI, there are several classes of soil contamination: low ($IPI \leq 1.0$), middle ($1.0 < IPI \leq 2.0$) and high ($IPI > 2.0$).

PI can also be described as the ratio between concentration of the given heavy metal in soil samples and the corresponding target concentration in guidelines [2] or the corresponding target concentration from the circular on target values and the intervention values for the established soil remediation [10]. Based on the obtained PI, Nemerow Integrated Pollution Index ($IPI_{Nemerow}$) can be calculated as [10]:

$$IPI_{Nemerow} = \left[\left(IPI_{ave}^{2} + PI_{max}^{2}\right)/2\right]^{1/2} \qquad (4)$$

where IPI_{ave} is the mean value of all PI_i of the analyzed metals, and PI_{max} is the maximum value. The $IPI_{Nemerow}$ is divided into five levels, while the relationship between $IPI_{Nemerow}$ and the metal pollution level are: $IPI_{Nemerow} \leq 0.7$ (safe); $0.7 < IPI_{Nemerow} \leq 1.0$ (precaution); $1.0 < PI_{Nemerow} \leq 2.0$ (slight pollution); $2.0 < IPI_{Nemerow} \leq 3.0$ (moderate pollution); and $IPI_{Nemerow} \geq 3.0$ (heavy pollution).

Enrichment Factor

The contribution of the anthropogenic inputs of metals in soil can be estimated from the enrichment relative to unpolluted reference soil, or widely accepted background (pre–industrial) levels, such as the average values for continental shale or crustal abundances [6]. Enrichment Factor (EF) is applied in order to differentiate between metals originating from the anthropogenic activities, and those from natural provenance, as well as to assess the degree of anthropogenic inputs. EF can be calculated using the following expression [12]:

$$EF = \frac{\left(C_n/C_{ref}\right)_{sample}}{\left(C_n/C_{ref}\right)_{background}} \qquad (5)$$

where C_n is the concentration of heavy metal in soil, and C_{ref} is the concentration of the reference element.

Generally, normalization of metal concentration with conservative elements like Al has been used in calculation of EF to study the anthropogenic impact on soil. In order to compensate this normalization is introduced. The soil enrichment can be: minimal ($EF<2$), moderate ($2<EF<5$), significant ($5<EF<20$), very high ($20<EF<40$) and extreme ($EF>40$) [11]. Other authors [5] use the concentration of Fe as the normalizing element. The EF values close to unity indicate crusted origin, those <1.0 suggest a possible mobilization or depletion of metals, whereas EF>1.0 indicates that the element is of anthropogenic origin, and EF>10 is considered to be non–crusted source. On the other hand, Luo *et al.* [2] describe EF as the ratio between the concentration of the examined metal in soil and the baseline concentration of the corresponding metal in soils of the study area, where EF>1 indicates anthropogenic inputs to soils.

Geoaccumulation index

In order to identify a single heavy metal contaminant or the co–contamination of multiple metals, the comparation of metal concentration with the established guidelines or quantification of geoaccumulation index based on background values is perfomed [10]. By calculating Geoaccumulation index (I_{geo}), the effects of parent rocks and prominent artificial effects on the soil heavy metal content can be reduced, therefore it is suitable for the evaluation of soil heavy metal contamination in the areas affected by mining activities [13]. I_{geo} is determined by Muller *et al.* [14]:

$$I_{geo} = \log_2 \frac{C_n}{1.5B_n} \tag{6}$$

where C_n is the concentration of metals examined in soil samples and B_n is the geochemical background concentration of the metal (*n*). Factor 1.5 represents the background matrix correlation factor due to lithospheric effects. The Geoaccumulation index consists of seven grades or classes [5,13]: Class 0 (practically uncontaminated): $I_{geo} \leq 0$; Class 1 (uncontaminated to moderately contaminated): $0 < I_{geo} < 1$; Class 2 (moderately contaminated): $1 < I_{geo} < 2$; Class 3 (moderately to heavily contaminated): $2 < I_{geo} < 3$; Class 4 (heavily contaminated): $3 < I_{geo} < 4$; Class 5 (heavily to extremely contaminated): $4 < I_{geo} < 5$; Class 6 (extremely contaminated): $I_{geo} > 5$.

However, the evaluation of I_{geo} is only done for a single heavy metal contaminant, thus this index cannot provide a comprehensive description of the contamination status of the study area. Thus, an evaluation based on the comprehensive index method is necessary. Qin *et al.* [13] have described Improved Nemerow index (I_N) by the following equation:

$$I_N = \left[(I_{geomax}^2 + I_{geoave}^2)/2 \right]^{1/2} \tag{7}$$

where I_N represents the comprehensive contamination index of a sample; I_{geomax} is the maximum I_{geo} value of such sample; and I_{geoave} is the arithmetic mean value of I_{geo}. Based on I_N, the soil can be described as [13]: $0 < I_N \leq 0.5$, uncontaminated (Class 0); $0.5 < I_N \leq 1$, uncontaminated to moderately contaminated (Class 1); $1 < I_N \leq 2$, moderately contaminated (Class 2); $2 < I_N \leq 3$, moderately–heavily contaminated (Class 3); $3 < I_N \leq 4$, heavily contaminated (Class 4); $4 < I_N \leq 5$, heavily to extremely contaminated (Class 5); $I_N > 5$, extremely contaminated (Class 6).

Potential ecological risk index

Heavy metals present in the soil can enter the human body through various exposure approaches, and subsequently cause serious health risks. In areas impaired by mining activities, which have a high degree of heavy metal exposure, the assessment of potential ecological risks is necessary. To quantify the potential hazard from soil contamination with heavy metals, the potential ecological risk was introduced [13]. The quantitative expression of the potential ecological risk index (RI) was originally established by Hakanson [7]. This index should indicate the contamination agents, and where contamination studies should be prioritized [6]. RI based on the heavy metal abundance and release capacity, is used in the assessment of the degree of heavy metal pollution in soils, according to the toxicity of heavy metals and the response of the environment. RI is calculated as [9]:

$$C_f^i = \frac{C^i}{C_n^i} \tag{8}$$

$$E_r^i = T_r^i \times C_f^i \tag{9}$$

$$RI = \sum E_r^i \tag{10}$$

where C_f^i represents the pollution factor of heavy metal *i*; C^i is the measured concentration of heavy metal *i* in the sample; C_n^i is the corresponding background value; E_r^i is the monomial potential ecological risk factor of each heavy metal; T_r^i is the toxic–response factor of each metal. The terminology used to describe E_r^i and RI is [7]: $E_r^i<40$ (low potential ecological risk); $40<E_r^i<80$ (moderate ecological risk); $80<E_r^i<160$ (considerable ecological risk); $160<E_r^i<320$ (high ecological risk) and $E_r^i>320$ (very high ecological risk); $RI<150$ (low ecological risk); $150\leq RI<300$ (moderate ecological risk); $300\leq RI<600$ (high ecological risk); and $RI\geq 600$ (serious ecological risk). Different classifications of the ecological risks are given by other authors [6,15,16].

CONCLUSION

Heavy metals as soil contaminants have been characterized as one of the most important worldwide environmental problem due to their toxicity, persistence and bioaccumulation. Among different anthropogenic activities, mining and smelting have been described as one of the most important sources of heavy metals. Different indexes have been proposed in the assessment of soil contamination with heavy metals, including single pollution and integrated pollution indicators.

Concentration of heavy metals in soil can be used to calculate indexes such as Contamination Factor, Pollution Index, Enrichment Factor, Geoaccumulation index. The reference values for calculating single indexes include either background level or target values established for soil remediation. By calculating single pollution indexes, integrated indexes can be computed. These include: modified Contamination degree, Pollution Load Index, Integrated Pollution Index, Nemerow Integrated Pollution Index, Improved Nemerow index. With these indexes, soil can be classified into the corresponding classes. One of the indexes, which is particularly important in the areas with high degree of heavy metals exposure, is potential ecological risk index, since it is based on the toxicity of heavy metals and the response of the environment.

Since soil is regarded as one of the most important ecosystem component representing important geochemical sink, the assessment of soil contamination with heavy metals is necessary, especially in the urban soils.

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ENDANGERING GROUND BY SURFACE MINING WORKS ON THE AREA OF THE MUNICIPALITY BANOVIĆI

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Abstract

The exploitation of coal in Banovići mine causes the development of degraded earthen surfaces. In the course of the process, and especially after the completion of the exploitation, the obligation of the Mine and the local community is to undertake planning procedures that will remediate these surfaces and bring the purpose. This paper presents the scale of the negative impact of mining operations coal exploitation on natural resources and the environment, as well as the actions taken by the Mine and the local community in order to remedy the consequences of carrying out mining works.

Keywords: degraded land, surface works, recultivation

INTRODUCTION

The area of Banovići municipality occupies a total area of about 182 km^2 which of the urban area is about 11 km^2. The town is set in a beautiful natural environment, surrounded by dense forests which are lying about 100 km^2 on this area-(they occupies about 55% of the total space of the municipality). Endangering the land with mining works in Banovići municipality dates from the time of the first excavations that were done here at the beginning of the 20th century. Surface exploitation was carried out mainly in the southern parts of Banovići on open pits: "Odžak, Delnice, Bukve, Mušići, Podgorje, Ravne i Čubrić". Today the exploitation is carried on two open pits: Turija and Grivice whose work areas occupy the space of cca 1000 ha [1].

ENDANGERING AREA OF THE MUNICIPALITY BANOVIĆI BY MINING WORKS

The elementary economic activity which the municipality relies on is mining. Mining activities are polluting water, air and create large degraded areas. By surface mode of coal exploitation creates large mining surfaces (landfills). Landfills are made from marls oligocene pharmaceutics. By chemical composition, the basic pH is 8-8.5 contains low macro and micro nutrient content and no humus component. Such composition imposes the need to refinement said substrate with technical and agrotechnical measures, all in order to restore the land for a specific purpose [2]. Table 1 shows the chemical composition of the soil.

On new landfills from one year to two, there is almost no vegetation. The basic starting color to poor, phytocenologic landscape gives the coltsfoot. Later, there are showing a burdock and a horsetail plant.

After 7-8 years the coltsfoot was slowly disappearing and leaving an improved substrate. In this way, conditions are creating for clover appearing. Today in the area of the municipality have endangered 2410 ha ground. Just 72.8 ha is biologically recultivated, 450 ha is technically recultivated and ready for construction, 609 ha are landfills which are in greater extent technically recultivated. Most of the endangered area generate of the working area open pits and active landfills which occupy area of 1277 ha.

Table 1 *The chemical composition of the materials from which the landfills are formed*

Participation %	Average sample	Gray marl	White limestone marl
moisture	4.050	5.87	1.60
insoluble	0.911	-	-
Loss (annealing)	21.009	27.11	37.32
S,O_2	28.024	23.29	8.46
Fe_2O_3	5.138	3.44	3.12
$Al_2O_3{}^5$	12.78	9.22	2.56
MgO	1.597	2.78	2.25
SO_3	1.29	1.06	0.90
CaO	24.955	28.48	43.24
$CaCO_3 = C_aO$ x1.786	44.57	50.87	72.23
Marl 40-75% $CaCO_3$		Limestone marl 70-90% $CaCO_3$	

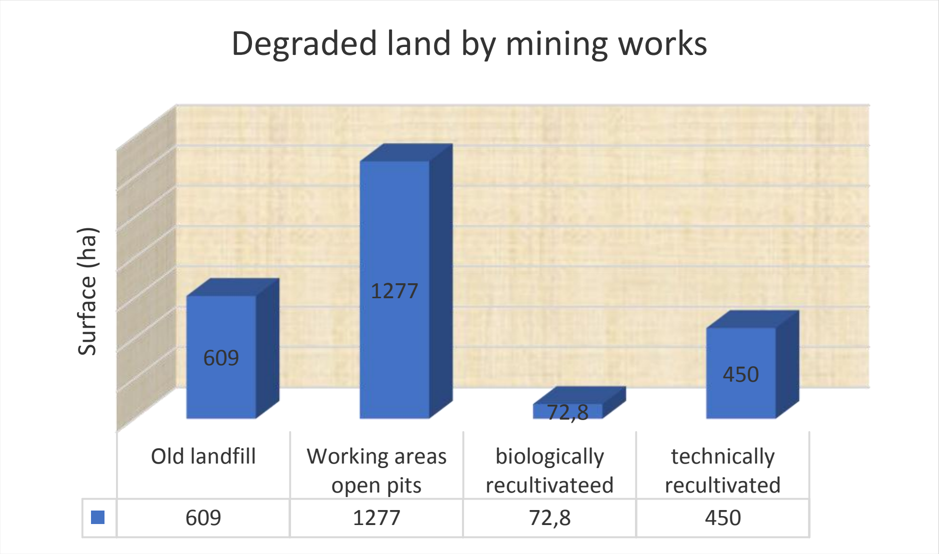

Figure 1 *Degraded land by mining works*

RECULTIVATION OF LAND THREATED BY MINING WORKS

The technology of performing recultivation endangered parts of surface terrain isn't universal not even, and it can't be prescribed for all surface open pits. This means the recultivation depends from a number of factors that are characteristic for a specific locality. All present factors need to be analytically analyzed so that one can be opt for one of the aspects of recultivation, make choices of culture, technology, technical and biological recultivation.

Today, recultivation is only considered as measures which are involving phases in its performance.

Recultivation phases can be:

- ✓ technical
- ✓ agrotechnical
- ✓ biological (forestry or agricultural)

Technical recultivation

Technical recultivation is a set of engineering-technical measures whose task is to biologically recultivate it and adapt the degraded space to the surrounding terrain. So, technical recultivation covers the total treatment of the damaged area almost until the moments of sowing or planting of planned crops [3].

This treatment encompasses:

- ✓ determination of the type of the overburden, humus and other substrates, its addition and cover over the overburden
- ✓ technical soil shaping,
- ✓ agro and forest - technical melioration.

Until now the recultivation has been carried out in the sense of retention of existing landfills for construction residential objekts, So that settlements were built in the former landfills. They are: "Selo II", "Bagremik", "Stražbenica", "Kasumovići", "Čubrić" and "Mrdići", which have lasting character. All constructed buildings have construction consent from the mining organization. Other areas that are technically recultivated are more or less naturally covered with petty vegetation (grass, burdock, petty overgrowth).

Biological recultivation

Biological recultivation consists of:

- ➢ Vegetables production
- ➢ Sowing with the clover-grass mixture,
- ➢ Afforestation,
- ➢ Planting orchards, and
- ➢ Restocking and landscaping.

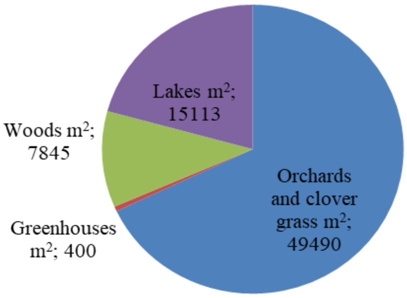

***Figure 2** Biologically recultivated areas*

Vegetable production

Vegetable production takes place in four greenhouses per 100 m^2. Vegetables are sown in every greenhouse separatly: onion, salad, tomato and pepper. Figure 3 shows the greenhouses that are already used on recultivated soil.

***Figure 3** The soil of clover-grass,greenhouses and vegetable farming*

Clover and grass

Clover is sown in one direction and grass in the other direction (crosswise). After sowing, rolling of sown soil carries out. The fostering of clover-grass area includes the following measures: harrowing, fertilization, weed control and irrigation.

In the first year do not use phosphorus and potassium fertilizers for nutrition, because with meliorative fertilization before sowing is secured enough reserve for use in the first year of sowing. On recultivated areas in Banovići's mine, seedlings of clover and grass are most commonly combined with fruit seedlings (between the lines), very little as an independent agro culture.

Forest recultivation

In the structure of forest cultures the advantage is given to the coniferous species, because of their bigger economic benefits and environmental reasons. On the slopes it is foreseen contour planting in trench-terraces so the plants can have the necessary moisture in soil, which such planting allows [4].

On the slopes, practically it is not possible to mix evenly the fertilizer with soil, so fertilization will be placed only on planting sites and on space around them. Two-year seedlings are recommended for planting [4]. Experiences from forest recultivation have shown that spring planting gives better effects.

Figure 4 *Pine seedlings in Podgorje with low overgrowth between*

Recultivation of fruit

Estimating the suitability of land for fruit growing has particular significance, as fruits are long-lasting cultures and remain in the same place for several years. Of course, fertility of fruits and health, as well as the quality of the fruit and the duration of fertility depend to a great extent on the suitability of soil for some cultures. There are many types of fruits that are sown on the recultivated surface: plum, pear, apple, cherry, walnut, hazel and the like [2].

Figure 5 *The young orchard on recultivated soil with the clover between trees*

Recultivation of water accumulation

With mining works on the area of PK Turija was created Ramičko lake which is located on the periphery of the landfill. The lake is located 10 km from Banovići and it’s one o of the most beautiful and cleanest lakes in the municipality Banovići. It has a well-decorated beach and access to the lake is accessible and well-maintained. It is also suitable for fishing because it is rich with: carp, pike and roach. Every year the lake is enriched with other kinds of fish.

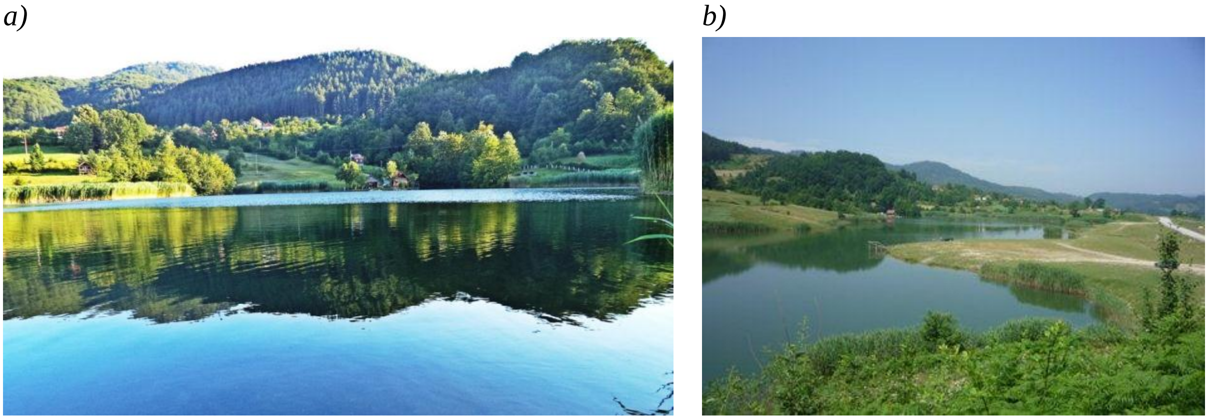

Figure 6 *Ramičko lake*

CONCLUSION

On Banovići Municipality's surface mining works, some 2410 ha of land were degraded, and about 20% of that land was converted for other purposes. Rocks that are deposited in their composition have a high content $CaCO_3$ and as such are suitable for cultivating forest and agro crops.

In addition to forest cultures, clover cultivation, various fruit crops (plums, apples, pears, walnuts, etc.), agricultural crops are also grown (peppers, tomatoes, cucumbers, potatoes, etc.).

After the completion of the technical recultivation and consolidation of the deposided material on this area can be started with the construction of urban settlements.

However, the investments so far are insufficient, and the need for remediation of damaged land in the forthcoming period will require more material resources and more efforts to study the reclamation procedures of damaged land and the application of complex methodology in the stages of investigative works of recultivation.

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ACTIVITY OF GLUTATHIONE S-TRANSFERASE IN *Cepaea vindobonensis* (GASTROPODA: HELICIDAE) FROM POLLUTED AREA

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Abstract

This study investigates the activity of snail glutathione-S-transferase (GST) and metal bioaccumulation for assessing ecotoxicological effects of urban metal pollution in Belgrade city, Serbia. This investigation was performed in the viscera of roadside Cepaea vindobonensis snails collected from two different sites in the city. The results showed that mean concentrations of the measured metals, cadmium (Cd) and lead (Pb) were higher in site next to the major federal highway (HS) when compared to the background levels of the reference site (RS) 1.5 km away from the highway. The pattern of metal accumulation at both sites was Pb > Cd. At the HS site, snails displayed higher mean of GST activity compared to snails from the RS. The activity of GST enzyme was positively related to metal concentrations. The tested antioxidant enzyme is sensitive parameter that could be used as biomarker in snails exposed to the actual metals in the environment. The overall results of this study showed the importance of C. vindobonensis as a sentinel organism for biomonitoring the biologic impact of atmospheric pollution in urban areas.

Keywords: *Cepaea vindobonensis*, cadmium, lead, glutathione-S-transferase

INTRODUCTION

The release of inorganic contaminants such metals in natural ecosystems can consequently pose environmental and human health risks. In the case of urban areas affected by high traffic density, most of the metal pollution primarily comes from atmospheric depositions. Heavy metals like cadmium (Cd) and lead (Pb) can contaminate soils from atmospheric deposits, continuous application of large amounts of fertilizer, disposal of industrial waste sludge and vehicle emissions [1]. The presence of heavy metals in terrestrial ecosystems is of special concern for their high toxicity and ability to be biomagnified through the food web [2,3].

Metal pollution causes oxidative stress which can induce the activity of antioxidants in exposed organisms. Chronic exposures to metals lead to excessive production of reactive oxygen species (ROS) such as superoxide anion radical ($O_2^{\cdot-}$), hydroxyl radical (•OH) and hydrogen peroxide (H_2O_2). This can result in a shift in the redox status of the cell, thereby causing damage to biomolecules such as lipids, nucleic acids and proteins and consequently to oxidative stress in the organisms [4].

Aerobic organisms have a variety of enzymatic and nonenzymatic antioxidant defenses that maintain endogenous reactive oxygen species (ROS) at relatively low levels and attenuate the damage related to their high reactivity. Glutathione-S-transferase (GST) is a detoxifying and antioxidant enzyme that protects cells from oxidative stress. This enzyme catalyzes the conjugation of glutathione (GSH) with a variety of electrophilic metabolites and heavy metal ions resulting from detoxification of both reactive intermediates and oxygen radicals [5].

Soil organisms, like terrestrial snails as an important part of the invertebrate biomass, are continuously exposed to soil inorganic contaminants [1]. Snails accumulate substantial quantities of pollutants in their bodies, often without lethal consequences [6,7]. Due to their capacity to accumulate heavy metals in their tissues and limited ability to excrete them, terrestrial or land snails have been extensively studied as bioindicator organisms of environmental contamination by metals [7].

Previous studies have shown that land snail can be good biomonitoring organisms and hyper-accumulators of heavy metals pollution [7,8]. The land snails are involved in transfer of trace elements in food webs, because of their position in trophic webs - detrivorous and herbivorous species, being dietary item of numerous invertebrate and vertebrate predators [9].

The objective of this study was to investigate the impact of vehicle traffic on soil contamination using the urban land snail *Cepaea vindobonensis* (C. Pfeiffer, 1828) which is very abundant and widespread gastropod species in Balkan Peninsula [10]. Concentrations of cadmium (Cd) and lead (Pb) were determined in soil and snail samples. In order to measure the effects produced by high environmental heavy metal concentrations, the oxidative biomarker GST in snail was assessed.

MATERIALS AND METHODS

Sampling sites

Soil samples and snails were collected from two sites in Belgrade city, Serbia, during the April of 2017. The first site (HS, Lat. 44°48'43.65"N, Long. 20°25'17.12"E) was chosen due to its close proximity to the major federal highway - a permanent source of soil pollution via heavy metal atmospheric deposition. The second site, designated as referent site (RS, Lat. 44°49'08.81"N, Long. 20°26'27.06"E) was 1.5 km away from the highway and placed in the city park “Ušće”. Three subsamples of soil (~100 g) were randomly taken at a depth of 10 cm from each site using a plastic grab. After sampling, soils were packed in polyethylene bags, transported to the laboratory and stored at 4 °C until further treatment. The adult snails (around 60 individuals per site) were collected from sampling sites by hand, washed with distilled water and transferred into clean plastic bags. Upon arrival at the laboratory, the samples were stored at –80 °C until further analysis.

Heavy metal extraction and analysis

Adult snails belonging to the *C. vindobonensis* were classified according to the presence of a clear white or brown-black lip at the mouth of their shell [11]. Before performing the metal analyses, the soft bodies of the snails were separated from the shell and dried in an oven (60°C) to a constant dry mass. The dried samples were ground into a fine powder by mortar and pestle. Snails were generally analyzed individually, but when their dry mass was lower than 0.1g, three to five individuals were pooled to obtain a sufficient biomass for metal analysis.

Soil and tissue digestion was conducted according to the procedure of Thevenon and Poté [12]. Analysis in the digests of soils and soft tissues was performed by inductively-coupled plasma optical spectrometry (ICP/OES) and comprised assessment of the concentrations of Cd and Pb. The values are expressed by the mean ± standard deviation (SD) in the analysis of three subsamples for each soil and tissue sample. Metals were analyzed in triplicate and concentration was expressed in mg kg^{-1} of dry mass.

Determination of enzyme activity

The whole soft tissue (n = 10) was minced and homogenized in 5 volumes of 25 mmol l$^{–1}$ sucrose containing 10 mmol l$^{–1}$ Tris–HCl, pH 7.5 using a Janke & Kunkel (Staufen, Germany) IKA-Werk Ultra-Turrax homogenizer [13] and sonicated for 15 s at 10 kHz on ice [14]. Sonicates were centrifuged at 100,000 × *g* for 90 min at 4°C. Protein content and enzyme assay were performed with the supernatant fraction.

Protein content was determined by the Folin–Phenol reaction as described by Lowry *et al.* [15] using bovine serum albumin as a standard. The activity of GST toward 1-chloro-2,4-dinitrobenzene as a substrate was determined according to Habig *et al.* [16]. The reaction rate was recorded at 340 nm and expressed as nmol of glutathione (GSH) min$^{–1}$ mg protein$^{–1}$.

Statistical analyses

With the aim of detecting normality of data and homogeneity of variances the Kolmogorov–Smirnov test and Levene’s test were used, respectively. Significant differences were analyzed using one-way ANOVA and post-hoc Tukey honest significant difference (HSD) multiple-comparison test. $P < 0.05$ was considered significant. All analyses were performed using SAS 9.1.3 software (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

In order to determine the level of *C. vindobonensis* biomarker response and heavy metal contamination of soil, the concentrations of the most toxic heavy metals Cd and Pb were measured in soil and snails’ samples at two sites with different pollution pressure (Table 1). Notably, HS site which showed increased Cd and Pb levels during sampling is located near major road network that is abundant source of Cd and Pb pollution exposure from highway traffic. At both sites, higher values of Pb were measured in soil and snails’ samples compared to Cd concentration. Lower heavy metal concentrations were registered in the RS site, which validates its selection as a reference site.

It can be seen from Table 1 that accumulation in the organism is higher than that of the medium from which the metal was taken and the organism can be considered as concentrator as proposed by Dallinger [17]. Only soil concentrations of Cd exceeded maximum allowed concentration (MAC) values at site HS according to Serbian legal regulations, Official Gazette of FRY, No.23/94 (Table 1), thereby placing it in the range of chemical concentration that is occasionally associated with adverse biological effects. In addition, the adverse biological effects of Pb cannot be ruled out, even though its concentration did not exceed MAC value (Table 1).

The detrimental effect of Cd takes several forms, such as blocking of signalling receptors, interactions with some enzymes, induction of oxidative stress, genotoxic and necrotic effects

[18]. Cadmium presents a higher ecological risk than Pb and any other metal, which is due to the high toxicity coefficient of Cd despite its lower concentration (Table 1). Therefore, Cd can be regarded as one of the most probable stressors affecting GST activity. Consequently, GST activity showed positive relation with concentrations of Cd and Pb.

Table 1 *Cadmium (Cd) and lead (Pb) content (mg kg^{-1} dry weight) in the soil and snail Cepaea vindobonensis collected from referent site (RS) and polluted site (HS). Metal concentrations are presented as average concentration levels measured in these specimens (for soil n = 3 per site; for snails n = 10 per site). Significant differences in metal concentrations between referent and polluted samples were calculated by one-way ANOVA. The level of statistical significance of differences between the sites was defined as p < 0.05. Statistical significance is marked with an asterisk: ** p < 0.01 and *** p < 0.001.*

Metal concentration (mg kg^{-1} dry weight), mean ± SD	**Cd**		**Pb**	
Locality	RS	HS	RS	HS
Soil	1.75 ± 0.09	3.46 ± 0.1**	15.88 ± 1.77	46.49 ± 2.63**
Snail	2.65 ± 0.11	6.19 ± 0.56***	11.25 ± 0.84	36.73 ± 2.08**
MAC[a]	3		100	

[a]MAC(s) - maximum allowed concentrations of potentially toxic elements in the soil as prescribed by Serbian legal regulations, Official Gazette of FRY, No.23/94.

Data on GST activity in snails collected from two studied sites are presented in Figure 1. At the site located in the close proximity to pollution source (HS), snails showed higher GST activity compared to snails from the site RS with significant statistical difference (p < 0.01).

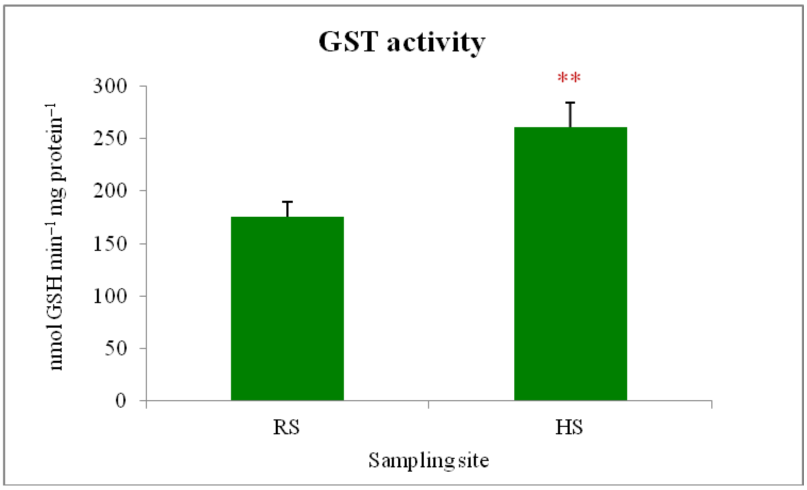

Figure 1 *Activity of glutathione S-transferase (GST) in viscera of Cepaea vindobonenesis sampled from referent (RS) and polluted (HS) site during April 2017. Data are presented as mean + SD (n = 10 for each site). Significant difference between referent and polluted samples were calculated by one-way ANOVA and marked with an asterisks, ** p < 0.01*

GST activity was significantly elevated in polluted snails, indicating that Cd and Pb can induce oxidative stress by producing ROS, and GST may be involved in cellular protection against metal-induced toxicity. Pollution of the soil with heavy metals is often less visible and direct than other types of land pollution, but its effects on terrestrial ecosystems and biota are long-lasting and severe. Different types of interactions, like that synergic and antagonic relationships found between heavy metal ions may increase their toxic potential. These findings are very important, because in the environment heavy metals usually co-occur in complex mixtures [19]. It is already shown that GST enzyme in land snail *Cantareus apertus* is responsive to Cd and Pb and their combination [20]. The induction of GST activity indicates an adaptation of the organism to enhanced pollution stress like is previously demonstrated [21].

CONCLUSION

The results in this study confirm that snail *C. vindobonensis* is able to accumulate Cd and Pb in concentrations higher than that in surrounding soil accompanied by significant difference in the GST values in the field. Increased GST activity suggests that the detoxification process against pro-oxidation forces, which are mediated by this enzyme, is induced. Therefore, this study suggests *C. vindobonensis* is a suitable sentinel organism and GST as suitable biomarker for terrestrial heavy metal biomonitoring.

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Lagerstroemia indica L. IN CLIMATE CHANGE CONDITIONS IN BELGRADE

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Abstract

This paper presents Lagerstroemia indica L., recorded and researched at Banovo Brdo area in Belgrade, which complements the floristic review of allochthonous dendrophlora adapted to local climatic conditions. Because of its ornamental values - habit, flowers, bark, and autumn colours - it is planted more frequently in Belgrade than before. A statistical analysis of vitality and decorative value, of the 12 noted Crepe Myrtle in five different locations, confirmed that the taxa of the Crepe Myrtle were very well adapted to local environmental conditions. So this ornamental woody plant could have multiple practical applications in landscape architecture, as well as in cultivation and production..

Keywords: Crepe Myrtle, allochthonous species, acclimatization, introduction

INTRODUCTION

By introducing woody plants into areas beyond their natural habitat, a local dendrofond is enriched. A large number of allochthonous species and intraspecies taxa have been recorded and researched in Serbia, especially in Belgrade area [1–13]. Due to global warming and climate change, many foreign species have shown remarkable adaptability to the new environmental conditions in Serbia. The greatest number of exotic species is introduced by the anthropogenic factor. The reasons for this are different - exploitation, agriculture, horticulture, use in landscape architecture, aesthetics, etc. Due to its ornamental values: decorative flowers, long flowering period, unusual bark and autumn colours, the *Lagerstroemia indica* L. species has been introduced and used more often. It is often planted in the Mediterranean region - France, Italy, Greece and others, but in recent years it can also be found in Serbia. It is also more frequent in Belgrade, as it tolerates temperature to -17°C [3,14,15]. Research and recording of this species was carried out in Belgrade in the municipality of Čukarica in the Banovo Brdo area. Twelve specimens in 5 locations were recorded.

The paper analyses the vitality and decorative value of the Crepe Myrtle in the conditions of the changed temperate continental climate. Because it is used frequently in the Mediterranean region, its recording in Belgrade is of particular importance. The monitoring confirmed that this ornamental woody plant is very well adapted to the changed climatic conditions in our region.

MATERIALS AND METHODS

The specimens of Crepe Myrtle were observed in five locations (Figure 1), in private yards, on Banovo Brdo in Belgrade. The specimens 1, 2 and 3 were noted in Bogoljub Čukić Street 21 (Location 1), the specimen number 4 in Bogoljub Čukić Street 25 (Location 2), the specimen 5 in Straško Pindžur Street 7 (Location 3), the specimens 6, 7, 8, 9, 10 and 11 in Petar Martinović Street 26 (Location 4) and the specimen 12 in Praška Street no. 20 (Location 5). All specimens are located in the full sun on sloping terrain in the south-west exposition.

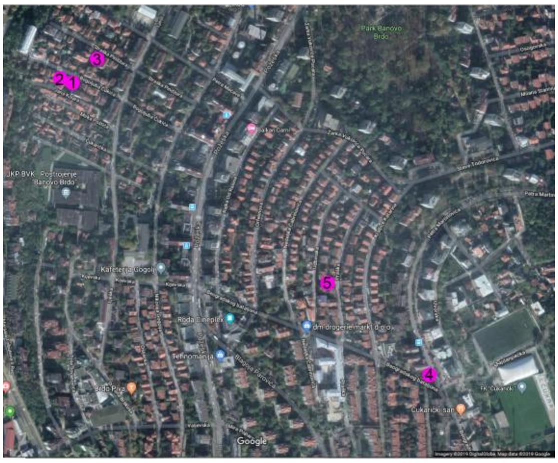

Figure 1 *Locations on Banovo Brdo in Belgrade where specimens of Lagerstroemia indica L. are analysed*

The field research included the recording and mapping of *Lagerstroemia indica* L. specimens on green spaces on Banovo Brdo. The height of the specimen (measured by the altimeter Haglof Vertex IV) was analysed, while decorative value and vitality were rated on a scale ranging from 1 to 5; where 1 is a specimen with no aesthetic values, low vitality, and 5 – a specimen with outstanding esthetical qualities and excellent vitality.

RESULTS AND DISCUSSION

Family Lythraceae J. St.-Hil., nom. cons., which is in the order of Myrtales L., contains 62 genera [16], among which is the genus Lagerstroemia L., which is named after Swede Magnus von Lagerstroem, who was Carl Linne's friend and associate. In the genus Lagerstroemia L. there are about 80 deciduous and evergreen species of trees and shrubs, which are naturally distributed in Indonesia, East Asia and Australia. Within this genus is a species of *Lagerstroemia indica* L. This species was introduced into Europe in 1759. Although scientific and one of the local names (Indian lilac) plants refer to India, the species of these species are China, Korea and Japan [15,17].

The analysed specimens *Lagerstroemia indica* L. grow on Banovo Brdo. The geological background of this locality consists of limestone, clay and sandy clay. The type of soil on which the noted specimens grow is degraded chernozem soil type. According to literature, a Crepe Myrtle thrives in nutrient rich, fresh and well drained soils. However, it can grow on nutrient deficient, sandy and clay soil. However, a Crepe Myrtle grows poorly on soil saturated with water [3,14,15], which is not a problem in the researched locations where the water easily flows through the soil surface due to the slope.

In the investigated area 12 specimens of the *Lagerstroemia indica* L. taxa were recorded. The average height of the analysed specimens on all five locations is 1.9 m. The smallest achieved height is 1.5 m, and the maximum is 3 m. Crepe Myrtle, which is growing more abundant not only at the site, but also in Belgrade, failed to reach a height of 8 m, which is stated by literature [14]. Crepe Myrtle thrives in full sun [17], as are the locations where the observed specimens are.

The specimens 1, 2, and 3 are at 99 m altitude. The tree height of all three specimens is 3 m. Specimen number 4 is located at 98 m altitude. The height of this specimen is also 3 m. Specimen number 5 is located at 100 m altitude. The height of the specimen is 2 m. Specimens number 6 (Figure 2a), 7 (Figure 2b), 8, 9, 10 (Figure 3) and 11 are located at 177 m altitude. The tree height of all six specimens is 1.5 m. Specimen number 12 is located at 158 m altitude. The height of the specimen is 1.5 m. The recorded heights indicate that no specimen of the Crepe Myrtle on Banovo Brdo can reach the height indicated for the species in the literature. However, specimens still have not reached full maturity, so it is necessary to continue the research in the following period in order to determine the growth rate and long-term resistance.

Crepe Myrtle is drought and polluted air tolerant. It is a very adaptive species [3,14,15], which is confirmed by the conducted field research showing that the Crepe Myrtle has adapted well to the local environment. Average ratings of vitality and decorative value are identical: 4.9 and 4.9. For both parameters, there was no lower score than 4 recorded for the all specimens.

By a comparative analysis of vitality and decorative value, for twelve specimens and both parameters, the highest grades from 4 to 5 were determined, indicating good adaptability of the species in the investigated environmental conditions. Based on presented data, the similarity of the analysed parameters of all 12 specimens was demonstrated, which showed successful acclimatization to the Belgrade modified temperate continental climate.

Figure 2 *Habit of specimen a) number 6 and b) number 7 of Lagerstroemia indica L., in Belgrade*

***Figure 3** Flowers of specimen number 10 of Lagerstroemia indica L., in Belgrade*

***Table 1** Recorded parameters of the analysed specimens of Lagerstroemia indica L.*

Taxa		Location	Number of specimen	Hight (m)	Vitality (1-5)	Decorativity (1-5)
Lagerstroemia indica L.	1	Bogoljuba Čukića 21	3	3	5	5
	2	Bogoljuba Čukića 25	1	3	5	5
	3	Straška Pindžura 7	1	2	4	4
	4	Petra Martinovića 26	6	1,5	5	5
	5	Praška 20	1	1,5	5	5

CONCLUSION

Acclimatization and the use of introduced allochthonous plants are largely dependent on environmental conditions. Thus, along with the physiology of species, climatic factors have the greatest effect on successful adaptability.

Lagerstroemia indica L. is a allochthonous ornamental species that is becoming more frequent in Serbia. By analysing the green areas on Banovo Brdo in Belgrade, twelve specimens of the Crepe Myrtle were recorded on 5 locations. The results of the research show that the specimens are very well adapted in the conditions of changed moderate-continental climate of Belgrade, which is confirmed by high ratings of vitality and decorative value. The mean value for both vitality and decorative value is 4.9. However, even though the noted specimens have high estimates of adaptability, the tree height of the observed specimens has not reached the maximum specified for the species in the literature. It is therefore recommended that the research continues in the following period for the selected specimens (until they reach full maturity), and also that the research should be extended to the territory of other Belgrade municipalities.

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MEDITERRANEAN HERBACEOUS PLANTS IN CLIMATE CHANGE CONDITIONS IN BELGRADE GREEN AREAS

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Abstract

Climate changes are happening they have a great impact on living beings, which makes it necessary to prepare for the upcoming periods. Problem of space greening is facing with great challenge. A number of plants of Mediterranean and submediterranean origin have been used for a decade for the purpose of greening the area. Some plants represent potential material, but there is also a large group of plants that have an invasive, unfavorable character that appear and become dominant. The task of profession and science field is to find the best way to explain this process, point out the dangers that climate changes carry out and provide solutions that optimize the sustainability of the self-generated and planned green fund. One of the mechanisms is the greater use of plants from the Mediterranean floral element.

Keywords: Mediterranean floral element, climate change, herbaceous plant, landscape architecture, green areas

INTRODUCTION

Climate change is not just a term about what is possible one day, they are a reality. They are much more pronounced in the northern Earth's hemisphere than in the conditions of the moderate-continental climate, and the trend of strengthening the pronouns of change is rising. Climate change has been acknowledged as a key driver of change affecting the phenology, invasiveness and range of a wide variety of species [1]. However, recent data showed that besides climate, change human activities are very important determinate of distribution boundaries for many terrestrial species, especially for those inhabiting regions such as Mediterranean landscapes [2,3]. Although the Mediterranean ecoregion has been recognized as one of the world's most vulnerable [4], the flora of the Mediterranean remains insufficiently studied [5]. Therefore, it is important improving the knowledge on plant species that are most vital in the conditions of the Mediterranean climate, as well as monitoring and modified adopted patterns on the geographical distribution of species [6].

Climate change and preparations for the future of green areas in the Scandinavian countries are active through plans for plant selection and application, soil preparation, plans for accumulation and regulation of rain water, etc. Following mentioned patterns and plans to introduce Scientifics and professionals with Mediterranean floral elements, is extremely important especially with the species present in our temperate continental climate zone and with the possibilities of their application, as a possible mechanism of action in accordance with the climate change.

PAPER GOALS AND METHODS

Today in the flora of some parts of Europe there is a large number of plants that historically were not present in this area [7]. There are different conditions that affecting biodiversity of the urban environment such as different historical periods, wars, anthropogenic influences, climatic factors, homogeneity of habitats, etc [8]. According to these characteristics, urban diversity of flora is often different from the flora that is biogeographically defined. On the other hand, green areas play a fundamental role in preserving biodiversity in cities. Sukopp and Werner [9] emphasize the importance of parks, gardens and other green areas in urban environment and their role as biofilter of contaminated urban environments [10].

Recording herbaceous plants that are fitogeographically belonging to the Mediterranean floral elements and have been located on public green areas in Belgrade is the main goal of the work. Available literature sources as well as existing floristic databases are examined. Also, an assortment of plant species in horticultural nurseries in Belgrade has been explored. Plant species that are widely used and those for which there is a possibility of greater application in the future are determined.

The paper emphasizes importance of field research monitoring and analysis, as well as the use of modern equipment and technology, assessment of condition and vitality, assessment of adaptability and endangering plants and biodiversity assessment. Otherwise, the aim of the work included the expert and scientific public preparation on the inevitability of climate-related changes.

RESULTS AND DISCUSSION

Term of Mediterranean floral elements

Mediterranean region is one of the most diverse in the world in terms of flora. More than 6,000 plant species have been recorded in the Mediterranean region in Greece, while in the UK there are four times more plant species belonging to Mediterranean floral elements [11].

Mediterranean plants make the basis of the Balkan flora. The largest number of these plants originates from the tertiary, from the Mediterranean islands and mountains and represents the core of the ancient Balkan flora. All these species were widespread on the Balkan Peninsula by the end of the tertiary period and represent the main part of flora and vegetation [12].

Vegetation spreading along the coast of the Mediterranean Sea is characterized by a Mediterranean climate. It is distinguished by evergreen plant species and forest degradation of varying degrees, a large number of plant species are endemics and /or tertiary relicts [11].

Endangering and reducing biodiversity is a global process that gets worrying. Since the mid-twentieth century Mediterranean vegetation has been confronted with disappearance, mostly due to the rise of tourism and climate change [13]. As a result of climate change new types of plants find easier way to our market and wider usage in urban green areas, among which the plants of the Mediterranean floral elements stand out.

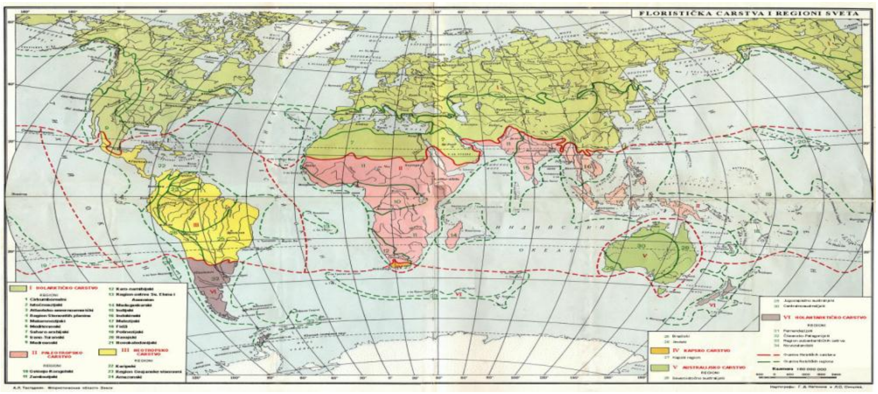

Figure 1 *Phytogeographic division according to Tahtadžjanu [14]*

Representatives of herbaceous plant species of the Mediterranean floral element on the Belgrade green areas

Many plant species are used outside the Mediterranean region as exotic and decorative due to aesthetic values, frequent use is related to the soil stabilization, sandstones greening or as food for livestock [7]. In recent years, they are used in Serbia on public green areas, but also in private gardens. Due to high demand, their production in nurseries is growing.

The research results showed that the recorded herbaceous plants on the Belgrade green areas which are most resistant in the conditions of the Mediterranean climate can be divided into two categories:

1. plants present with a large participation in green areas
2. species distinguished by the potential for greater usage in the future.

The first group contains herbaceous plants that are part of the regular assortment of nurseries in Belgrade (recorded in more than a half of the researched nurseries), and this can be considered as one of the reasons for a greater presence on green areas. It is noticed that these species except on public green area also make a significant part of special-purpose green areas. One part of the introduced Mediterranean flora exists in cities has been entered by conscious man activity and has been cultivated as decorative [7] on special purposes green areas such as private gardens, botanical gardens, arboretums, etc. On the other hand some of Mediterranean herbaceous species have expanded considerably more, in parks and other general green areas [7]. Among the species that are intentionally planted and have usage in the Belgrade conditions next can be distinguished: lavender (*Lavandula officinalis* Chaix.), sage (*Salvia officinalis L.*), cotton lavender (*Santolina chamaecyparissus L.*), thyme (*Thymus longicaulis L.*) and wild sage (*Lantana camara L.*).

Dobrić *et al*. [15], made the inventory of fruit plant species and ornamental dendroflora in private gardens in the area of Knin town (Croatia) and recorded 54 typical Mediterranean plant. Among recorded plants, species *Lavanda officinalis* Mill. and Rosemary (*Rosmarinus officinalis* L.) from the fam. Lamiaceae can be distinguished.

In the second group are plant species according to next criteria: recorded with a low percent on the Belgrade green areas, hasn't been a part of the nurseries offer, present in less than a half of the investigated plant production and marketing facilities. These plant species spontaneously inhabited green areas and their occurrence is a consequence of the compliance of climatic and microclimate factors with the bioecological characteristics of the species. In addition, their occurrence may also be the result of an unintentional anthropogenic impact. Among the plant taxa in this group there are: yellow bugle (*Ajuga chamaepitys* (L.) Schreb.), grape hyachinth (*Muscari neglectum* Guss. ex Ten.), lemon balm (*Melissa officinalis L.*), *Cephalaria transsylvanica (L.)* Roemer & Schultes, Arabian star flower (*Ornithogalum gussonei* Ten.).

In some long lasting research in the Belgrade area [16,17] some of the herbaceous plant species of the Mediterranean flora as spontaneous vegetation have been recorded: (*Ajuga chamaepitys* (L.) Schreb, *Ornithogalum gussonei* Ten., *Melissa officinalis* L., *Cephalaria transsylvanica* (L.) Roemer & Schultes, *Muscari neglectum* Guss. ex Ten.). Their appearance is equally present in urban, suburban and rural areas of the city of Belgrade. The usage of some of them, for example *Ajuga chamaepitys*, *Muscari neglectum* and *Melissa officinalis*, is possible in one-year flowerpots. For the use of a large number of other taxa additional studies with more aspects (invasive character, adaptability, reproduction, etc.) must be carried out.

In Belgrade on public green areas, in private gardens, garden communities, terraces and balconies, there are very often some of the herbaceous plants of the Mediterranean region (*Lavandula officinalis* Chaix, *Salvia officinalis* L., *Santolina chamaecyparissus* L., *Lantana camara* L., *Thymus longicaulis L.*). Their role on these areas is primarily aesthetic, but often the main reason for their planned usage is spice and medicinal plants purpose [18]. Also, these plant species are suitable for the alpine garden design [19].

CONCLUSION

Introduction scientific, professional and general public with climate change and consequences is extremely important, because they represent reality and it is necessary to be preparing for them. Many of the plant species listed in this paper have wide usage in the territory of Serbia, some are offered by nurseries, while some plant taxa are individual specimens spreading as a part of existing spontaneously vegetation on urban green areas. In urban gardens requires new standards of low intensity maintenance (LEED standards), herbaceous species belonging to the Mediterranean floral element will find much more application in the future.

Compared to woody plant species from the Mediterranean floral element, herbaceous plant species are significantly more sensitive to climatic extremes, especially to frost and drought, and their usage in conditions of moderate-continental climate is questionable and correspond to greater risk in terms of the coverage of the green areas.

Monitoring of changes on green areas is important for the purpose of revaluation, assessment of changes and multi-purpose use of plant material for the greening of urban areas. The determination and a state assessment of the diversity of Mediterranean plants on green areas of Belgrade is a long-term process that is important for the preservation of these types of plants. It is necessary to establish a database and carry out inventorying at the national level as well as beyond. Formation of a single database can be the basis for protecting and conserving species in order to facilitate the adjustment of green areas to climate change.

ACKNOWLEDGEMENT

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MODERN MANIFESTATIONS OF A KARST IN THE TERRITORY OF THE REPUBLIC OF BASHKORTOSTAN

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Abstract

Information about the modern manifestations of karst, represented by karst failures is collected and systematized. The analysis of their formation is made over the past five years (2014-2018) depending on the types of karst on the composition of karst rocks, the degree of their nudity and the time of karst dips to the relief elements. It is established that, most often they occur in the southern Urals in the karst country of the East European plain and are associated with the development of sulfate karst in gypsum Kungur layer of lower Perm in the areas of development of overlapped karst.

Keywords: karst, failure, funnels, sulphatic karst, water tightness

INTRODUCTION

The Republic of Bashkortostan is a classic region of sulphate and carbonate karst development, which covers the Ural and East European karst countries. Within the first, also sulfide karst locally developed (in the plains of the Urals on Cisilo-ARTOZYMA and Sakmara-Canalaska plains), and within the second (southern Urals) – customart (Pribelskaya and Priiskovy plains) and the karst calcareous tuffs (Bulinska-Belebeevskaya upland) [1].

In the region, karst rocks are distributed on ~50% of the territory, and the manifestations of karst are represented by all known forms – both surface (funnels, wells, basins, logs, etc.) and underground (grottoes, caves, mines, etc.). Every year the formation of new karst dips are recorded on its territory, once every 5-6 years there are abnormally large dips with a diameter of more than 15 m and a depth of >10 m [2].

The most remarkable modern form of manifestation of a karst are karst failures which arise suddenly, often are followed by shudder of soil and sound effects, cause sometimes even earthquakes measuring up to 3 points. The largest of them seldom remain unnoticed by local population and is fixed by it, as in the territory of RB, so around the world [3,5].

Karst downfall is a sharply limited, closed, usually rounded in plan, a pronounced decrease in the earth's surface, formed as a result of the collapse of rocks over the cavities in the thickness of karst rocks. Despite the annual formation of karst downfall on the territory of the Republic of Bashkortostan (RB) information on them is not systematized today. Synthesis of data on them for the last five years is made for the first time.

PURPOSE OF RESEARCH

Analysis of karst manifestations in the territory of Republic of Bashkortostan over the past five years (2014-2018), represented by karst downfalls.

INITIAL DATA

The initial data for the study were the information about the modern manifestations of karst which contained in the acts and conclusions of their operative examination: OOO "Geostroyispytaniya", OAO "Bashkirgeologiya", the monitoring Department at the RB branch of "Volga Regional center of SMMR", JSC "Arkhstroyisyskaniya", Institute of Geology APNIC RAS (Figs. 1-4), as well as data of the State reports of the Ministry of ecology of the Republic of Bashkortostan on the state of natural resources and environmental protection [4].

Figure 1 *Karst failure in the village Vavilovo. 04/26/2016. Photo by V.G. Kamalova*

Figure 2 *Karst failure in s. Novokulevo. 12.03.2017. Photo O.V. Sadykova*

Figure 3 *Karst failure in Blagoveshchensk, st. Sedov, 62. 05/31/2018 Photo by O. Sadykova*

Figure 4 *Karst failure in Ufa, st. Bolotnaya, d. 7. 19.11.2018. Photo A.I. Smirnova*

RESULTS AND DISCUSSION

The collected material on modern manifestations of karst in the territory of Bashkortostan in 2014-2018 with a fixed time of formation is given in the table below. It shows the following. Modern manifestations of karst on the surface are represented by dips with steep sides usually well-shaped.

Table 1 *The list of karst manifestations on the territory of the Republic of Bashkortostan, formed in 2014-2018*

№ pp.	Date (Time) of education	Location	Karst type	Position in relief	Brief description of failure (shape, size)
1	2	3	4	5	6
1	28/04/2014	Ufa district, D. Zinino, SNT "Dawn", the site No. 62, 4-5 m from the house	Sulphate (P1 k), overlapped (subluxual or Kama)	The rear part of the floodplain terrace at the junction of the left slope of the r. Ufa and the right slope of the valley r. White	Well dip diameter 12 m depth 8 m
2	13/11/2014	Kirovsky district Ufa, st. Aviatorskaya, 33 (under the apartment building)	Sulphate (P1 k), closed (Russian)	The watershed area at the edge of the right steep slope of the valley. White	Well-shaped dip in plan size 4 X 5 m depth 4.4 m
3	Spring 2014	Ufa district. Between md Dema Ufa and D. Myrmyleva	Sulphate (P1 k), closed (Russian)	Lower flat part of the right slope of the valley r. White	Cone-shaped dip with a diameter of 18-20 m deep ~ 8 m
4	23/06/2015	Mishkinsky District. North-North-Western outskirts with. Mishkino	Sulphate (P1 k), closed (Russian)	Near-valley part of the watershed on the right bank of the r. Inyak	Boiler dip diameter ~ 5 m depth ~ 3 m. Filled with water
5	The end of the summer of 2016	Birsky district. with. Staroburnovo	Sulphate (P1 k), closed (Russian)	Lower flat part of the right slope of the valley r. Bir	Cup-shaped dip diameter ~ 50 m deep 15 m
6	End of November 2016	Blagoveshchensky district. SNT "Energy", on the southeast coast oz Abyzovo	Sulphate (P1 k), overlapped (subluxual or Kama)	Foot of the right valley slope r. White.	A bowl-shaped dip with a diameter of ~ 20 m and a depth of up to 8 m. At the bottom there is a ponor that absorbs the waters of the lake. Abyzovo
7	26/04/2016	Ufa district. D. Vavilovo	Sulphate (P1 k), overlapped (subluxual or Kama)	Rear end II floodplain valley terraces r. White on its left bank	The cauldron-shaped dip in the plan size is 30 X 20 m and 7 m deep. Filled with water, the water level at a depth of 1.8 m from the surface.
8	05/02/2016	Ufa district with. Milovka, SNT "Spring"	Sulphate (P1 k), overlapped (subluxual or Kama)	The rear part II of the floodplain terraces of the valley r. White on its left bank	The cauldron-shaped dip in plan size is 20 X 10 m deep - 4 m, filled with water

***Table 1** continued*

9	25/11/2016	Kalininsky district Ufa, st. International, d. 193/2 from NE side 9-storey residential building	Sulphate (P1 k), covered (sub-eluvial or Eastern European)	The lower part of the gentle slope of the valley r. Ufa (completed by urban development)	Boiler dip 3x4 m deep 6-8 m. The failure completely swallowed the car "Lada Kalina", which could not be raised. In September 1984, a karst dip in the shape of a well with a diameter of about 4.5 m and a depth of 12 m was formed at 7 m to the NE of the corner of the neighboring village of 193
10	12/03/2017	Nurimanovsky district, with. Novokulevo, south-western outskirts	Sulphate (P1 k), overlapped (subluxual or Kama)	Rear end II floodplain valley terraces R. Ufa on its left bank	Bowl-shaped dip with steep sides in the upper and middle parts with a diameter of ~ 40 m and a depth of 11.1 m. Half filled with water
11	31/05/2018	Blagoveshchensk, st. Sedova, 62	Sulphate (P1 k), closed (Russian)	Near-valley part of the watershed on the right bank of the r. White	Well dip diameter 9-12 m depth ~ 6 m
12	Weights 2018	Ufa, Demsky district. Chebaksarsky lane between houses №№ 15 and 20	Sulphate (P1 k), overlapped (subluxual or Kama)	Rear end II floodplain valley terraces R. White on its left bank	Repeat failure at the failure site of 2000 (05/09/2000), which took about 4 thousand m3 of clay soil
13	19/11/2018	Ufa, Leninsky district, st. Bolotnaya, d. 7, 3 m to the west of a residential private house, grabbed the lawn and the whole roadway	Sulphate (P1 k), overlapped (subluxual or Kama)	High right-bank floodplain of the valley. White	A bowl-shaped dip with a plan size of 10.5 x 9.10 m and a depth of 2.20 m. Through the entire contour of the dip, cracks were fixed, taking into account that the overall dimensions were 14.4 x 12.5 m

All of them are located in the southern Urals in the karst side of the East European plain in horizontally and flat rocks and are associated with the development of sulfate karst in gypsum of the Kungur tier of the lower Permian (P1 k) [5].

Most often (7 pieces) karst downfalls occurred under conditions of blocked (padillawalker or Kama) type of the karst, where the gypsum Kungur occur below the alluvial Pleistocene formations of permeable pores. Somewhat less often (5 PCs.) they were formed in a closed (Russian) type of karst, where karst gypsum are under the rock and semi-rocks of the Ufa tier with fractured water permeability, and in the areas of gypsum coating loam-clay neogene-quaternary weakly permeable sediments only one downfall formed over the past five years. That is, the frequency of formation of modern karst downfall depends on the composition of the sediments covering the karst rocks and the nature of their water permeability: it is maximum in the areas of development of the overlapped type of karst, minimum – covered.

At the level of relief elements in the southern Urals, the highest frequency of formation of karst dips in the considered period was observed in river valleys, mainly in the rear parts of the floodplain terraces of river valleys and the foothills of their slopes. Extremely rare (2) they arose in parts of the near-valley hill on the watershed spaces, which is caused established in 1962 by D. S. Sokolov in [6,7] the overall pattern of more intensive valleys in comparison with the "core" of the watershed spaces.

The maximum amount of formation, karst downfalls (5 pic.) was recorded in 2016, which in a number of observations (2014-2018) at the weather station "Ufa" differed and the maximum amount of precipitation (604 mm, with an average value for 5 years – 560 mm), which is consistent with the established overall relationship between the activity of karst development and the degree of humidification of the territory [8].

CONCLUSION

The presented karst downfalls are not limited to all modern surface manifestations of karst for the specified period. Only the large ones are reliably recorded and described, but small ones left, apparently not being documented, particularly in considerable removal from settlements.

Meanwhile, the collected data on the formation of karst downfalls allow us to assess the current course of the karst process in the territory of Bashkortostan. They show the greatest activity of its development in the areas of distribution of sulfate karst in the southern Urals, which is the most economically, developed part of the territory of the Republic of Bashkortostan.

Further studies of karst downfalls should be aimed at their study over a longer period of time with the analysis of a wider range of factors of their formation, especially man-made.

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DICLOFENAC, IBUPROFEN AND KETOPROFEN PHOTOCATALITIC DECOMPOSITION BY TiO_2 NANO POWDER

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Abstract

The TiO_2 photocatalytic degradation of diclofenac, ibuprofen and ketoprofen been studied in a laboratory-scale experiment using a medium pressure mercury lamp. UV/TiO_2 photocatalys is proved to be efficient in the elimination of investigated pharmaceuticals from aqueous solution in batch mode. The photodegradation rates after 90 minutes were 49.97%, 94.69%, 90.88% for ibuprofen, diclofenac and ketoprofen, respectively.

Keywords: photocatlysis, TiO_2, diclofenac, ibuprofen, ketoprofen

INTRODUCTION

Among water treatment technologies employed thus far [1], advanced oxidation processes (AOPs) represent a good option for treating a wide range of emerging pollutants such as pharmaceuticals in municipal effluent [2–4], bearing in mind that pharmaceutical pollutants are persistent contaminants in aquatic media and that conventional treatment plants are inefficient for their removal [5–7].

Diclofenac (2-(2,6- dichlorophenylamino)phenylacetic acid, DCF), ibuprofen **(**α-Methyl-4-(isobutyl)phenylacetic acid, (±)-2-(4-Isobutylphenyl)propanoic acid) and ketoprofen (2-(3-Benzoylphenyl)propionic acid) are some of the commonly used NSAIDs (non-steroidal anti-inflammatory drugs) and some of the most frequently detected emerging pollutants (EP) in water matrix.

Semiconductor photocatalysis based on titania and induced by UV/Vis illumination comprises a promising technology for water purification with many investigations over the past several years [4], although it has not been extensively employed for NSAIDs degradation in practice. Nanostructured TiO_2 is being the most explored material up until now [8–10], due to its high efficiency in photo degradation and TiO_2 its stability in acid environments.

MATERIALS AND METHODS

Nano powder used as photocatalysts in present study was a commercial TiO_2, Sigma Aldrich, purity 99.9%.

A HPLC (high performance liquid chromatography) with diode array detector (Agilent 1260 series) was used for the measurement of diclofenac, ibuprofen and ketoprofen concentrations after photocatalytic degradation. Chromatography separation was performed at reverse, stationary phase Eclipse XDB-C18 (150 x 4.6, particle size 5µm) at flow rate of 0.8 mL min^{-1} and injection volume of 10 µL at room temperature. Mobile phases consisted of: A (50%) – 0.1 formic acid and B – acetonitrile in 7 minute initial conditions. The stationary phase temperature is 25 °C. Mass of TiO_2 used for each batch was 40 mg.

RESULTS AND DISCUSSION

As we have recently published in Štrbac *et al*. [11], the commercial TiO_2 (Sigma Aldrich, purity 99.9%, particle size <1µm), also used for present study, comprise particles which are characterized by globular shape with average particle size of ~100 nm (Figure 1), the crystallite size 84(5) nm and the specific surface area of 9.3(1) m^2/g.

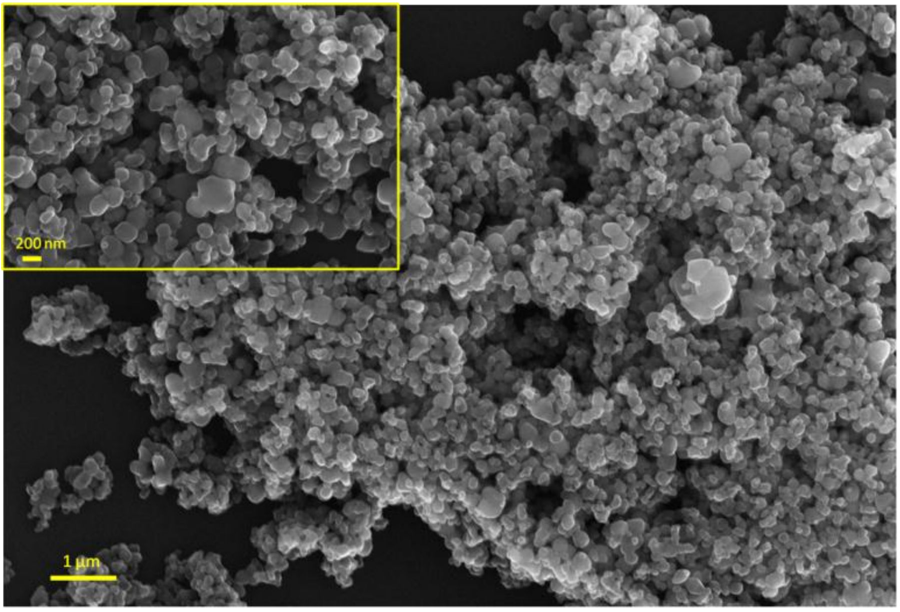

Figure 1 *Representative FE-SEM image of the* TiO_2 *powder [11]*

The value of TiO_2 powder optical band gap (3.45 eV [11]), indicated that photocatalytic decomposition can be carried out under the UV irradiation. The optical band gap value was estimated from the measured diffuse reflectance data [11] applying the analysis of the Kubelka–Munk theory.

The PC activity of TiO_2 was evaluated by the photodegradation of diclofenac, ibuprofen and ketoprofen in distilled water under UV irradiation. The photocatalytic degradation rates as a function of time for the investigated pharmaceuticals are shown in Figure 2. The efficiencies of photodegradation (Table 1) after 90 minutes were 49.97%, 94.69%, 90.88% for ibuprofen, diclofenac and ketoprofen, respectively.

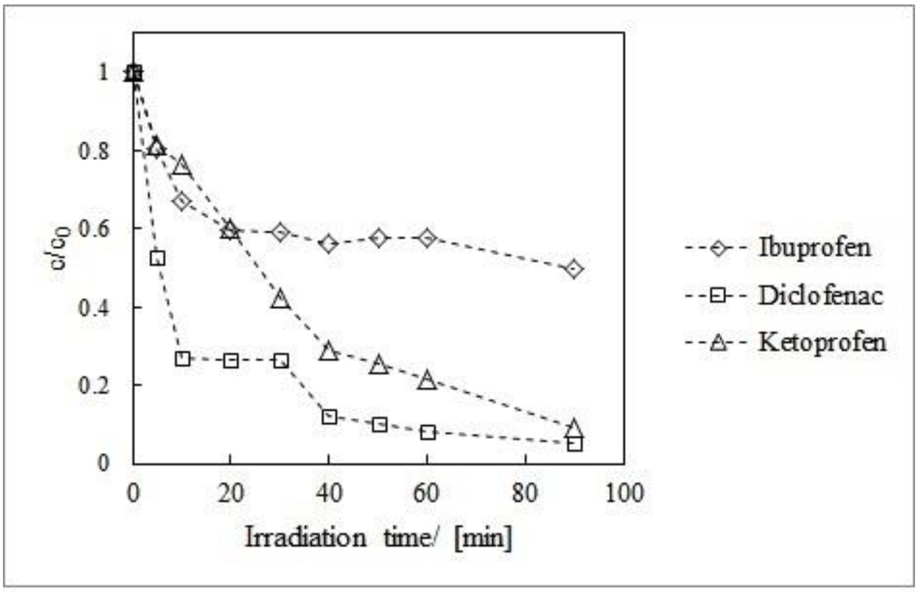

Figure 2 *Photocatalytic degradation efficiency of ibuprofen, diclofenac and ketoprofen by* TiO_2 *(the line is used as guide to the eye)*

Table 1 *Photocatalytic degradation efficiency of ibuprofen, diclofenac and ketoprofen by* TiO_2

	ibuprofen		**diclofenac**		**ketoprofen**	
Time (min)	**Concentration (mg/L)**	**Efficiency (%)**	**Concentration (mg/L)**	**Efficiency (%)**	**Concentration (mg/L)**	**Efficiency (%)**
5	4.03	19.46	2.64	47.23	4.08	20.54
10	3.35	32.93	1.34	73.11	3.82	25.45
20	2.98	40.40	1.33	73.46	3.00	41.44
30	2.96	40.79	1.32	73.63	2.11	58.94
40	2.81	43.72	0.62	87.70	1.46	71.56
50	2.89	42.27	0.52	89.59	1.27	75.18
60	2.88	42.34	0.42	91.58	1.09	78.68
90	2.50	49.97	0.27	94.69	0.47	90.88

It is important to emphasise here that a more comprehensive understanding of the environmental fate and biological effect of nanoparticles in general in environmental systems are required. Although titanium dioxide nanoparticles are already widely used in commercial products such as sunscreens and toothpastes, paints, lacquers and paper, novel studies strongly indicate that TiO_2 nanoparticles strongly impact soil microbial function [12].

CONCLUSION

Results of the conducted investigation indicated that the application of UV/TiO_2 photocatalysis to diclofenac, ibuprofen and ketoprofen contaminated water matrices is a promising technique, due to the exhibited high degradation efficiency. The efficiency of the photodegradation is determined by the chemical nature of the pharmaceutical. Clearly, process performance is affected by several factors, such as photocatalyst type, catalyst loading, pharmaceutical initial concentration, irradiation, presence of ions, other pharmaceuticals or pollutants of other type. This indicates that further investigations still need to be done until the final confirmation of feasibility of using photodegradation for NSAIDs removal from wastewaters.

ACKNOWLEDGEMENT

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UPTAKE OF COPPER BY WATER LETTUCE IN MULTIPLY METAL-CONTAMINATED WATER

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Abstract

Anthropogenic factor mainly contributes to the heavy metal water contamination. The problem key nowadays is mostly focused on the phytoremediation practice involving hyperaccumulating plants. This study was conducted to determine the copper uptake behavior of Pistia stratiotes, *macrophyte from the family* Araceae, *in multiply metal-contaminated water. The amount of copper was compared among plant samples grown for 28 days in nutrient-copper water solution (control solution) and in nutrient-copper water solutions containing different amounts of nickel, cadmium, lead and zinc. Results of atomic absorption spectroscopy analysis showed 32.53, 76.02, 9.50 and 54.71 % higher amount of copper, expressed as* Cu^{2+} *ion, in plants from reservoirs enriched with nickel, cadmium, lead and zinc, respectively. The presence of the selected metals altered the copper uptake in terms of enhancement. Since the alteration is assumed to be the consequence of many direct or indirect effects displayed in multi-contaminated systems, the copper or any other metal uptake behavior in even slightly different medium from the tested one could be distinct, thus, the investigation of the targeted metal behavior uptake in a specific environment prior to phytoremediation is required.*

Keywords: water lettuce, bioremediation, copper

INTRODUCTION

Many processes of anthropogenic nature, such as industrial, domestic or agricultural, significantly contribute to the release of metal species into the environment. Typical for such source of contamination is the presence of a metal in a form more available to biological systems than a natural form found in soils and sediments, which leads to a greater susceptibility of living organisms to the contamination. Another side of the problem is the metals persistence and their subsequent accumulation in the environment.

The main disadvantages of common soil and water metal remediation technologies, arising from the principles of metal isolation or immobilization in contaminated area [1,2], such as the failure of metal removal from the polluted site and the failure of remediation of trace levels of metal ions, are overcome with the use of phytoremediation. Phytoremediation is defined as a cost-effective eco-friendly technology based on the use of specially selected metal-accumulating plants for heavy metal removal. In comparison to non-accumulator plants, hyper-accumulating plant species can concentrate heavy metals such as Cu, Zn, Co, Mn, Ni, Pb from a hundred to a thousand times [3]. In addition, for a plant to be classified as a hyperaccumulator of a given metal, it must be able to tolerate concentrations above a set threshold for that metal, for example 100 $\mu g\ g^{-1}$ for Cd, Se and Tl, 300 $\mu g\ g^{-1}$ for Cu, Co and Cr, 1000 $\mu g\ g^{-1}$ for Ni, As and Pb, 3000 $\mu g\ g^{-1}$ for Zn and 10000 $\mu g\ g^{-1}$ for Mn [4].

Many aquatic plants are currently under investigation to determine their phytoremediation potential, particularly high-growth rate plants *i.e.* macrophytes. *P. stratiotes* or water lettuce is a free-floating macrophyte from the family *Araceae*. The key properties of its potential phytoremediation effectiveness are high growth rate, high coverage of water surface and easy harvestability. Several studies showed excellent phytoremediation potential of the plant towards different metals such as Cr, Cu, Fe, Mn, Ni, Pb, and Zn [5–9].

Metal-contaminated environments usually contain a combination of toxic metals and other contaminants instead of one metal only, from which may arise many synergistic or antagonistic effects displayed between essential and non-essential metals. This is an important factor when evaluating the phytoremediation efficiency of plant species. Thus, the aim of this study was to evaluate the uptake efficiency of water lettuce towards Cu^{2+}, an essential metal ion, from the water enriched with cadmium, nickel, zinc and lead. Water lettuce was selected for the experiment because it is an endemic species naturally found near Niš, Serbia.

MATERIALS AND METHODS

Chemicals

Water solutions of the following salts were used as metal sources - $ZnSO_4 \times 7H_2O$ (Zdravlje, Leskovac), $Pb(NO_3)_2$ (Merck, Germany), $NiCl_2 \times 6H_2O$ (Kemika, Zagreb), $CuCl_2 \times 2H_2O$ (Zdravlje, Leskovac), $CdCl_2 \times H_2O$ (Zorka, Šabac) at following concentrations – 16.26, 17.07, 10.78, 15.61 and 10.33 mg mL^{-1}, respectively. As source of nutrients for plant growth, the solutions of potassium-di-hydrogen-phosphate (KH_2PO_4) and ammonium-nitrate (NH_4NO_3) were used at 13.61 and 60.91 mg mL^{-1}, respectively.

Biosorbent

The samples of aquatic macrophyte *P. stratiotes* were sampled from the natural freshwater habitat near city of Niš (Serbia), in mid-September 2016.

Experiment setup

Collected plant samples were washed with tap water to eliminate the remains of pond sediments and particulate matter, placed in five different 35 L plastic reservoirs (labelled from 1 to 5) and filled with tap water. Each reservoir contained 15 specimens of *P. stratiotes*, in total 850 grams of plant material. Reservoirs were equal on the amount of nutrients and copper ion but contained different amount of other metal ions (Table 1) *i.e.* the reservoir No. 1 was the copper-only metal solution, while other reservoirs were the metal-mixture solutions. In order to simulate the natural growth conditions, the plastic reservoirs were placed outdoor in a sunny place for 28 days.

Table 1 *Chemical composition of water from experimental reservoirs*

Component	**Reservoir 1**		**Reservoir 2**		**Reservoir 3**		**Reservoir 4**		**Reservoir 5**	
Substance	**μmol L^{-1}**	**μg mL^{-1}**	**μmol L^{-1}**	**μg mL^{-1}**	**μmol L^{-1}**	**μg mL^{-1}**	**μmol L^{-1}**	**μg mL^{-1}**	**μmol L^{-1}**	**μg mL^{-1}**
Copper (II)-chloride	50	6.72	50	6.72	50	6.72	50	6.72	50	6.72
Nickel (II)-chloride	0	0	50	6.48	10	1.29	10	1.29	10	1.29
Cadmium (II)-chloride	0	0	10	1.83	50	9.16	10	1.83	10	1.83
Lead (II)-nitrate	0	0	10	3.31	10	3.31	50	16.56	10	3.31
Zinc (II)-sulphate	0	0	10	1.61	10	1.61	10	1.61	50	8.07
Potassium-dihydrogen-phosphate	30	4.08	30	4.08	30	4.08	30	4.08	30	4.08
Ammonium-nitrate	120	9.60	120	9.60	120	9.60	120	9.60	120	9.60
Correspondent ions										
Cu^{2+}	50	3.17	50	3.17	50	3.17	50	3.17	50	3.17
Ni^{2+}	0	0	50	2.93	10	0.58	10	0.58	10	0.58
Cd^{2+}	0	0	10	1.12	50	5.62	10	1.12	10	1.12
Pb^{2+}	0	0	10	2.07	10	2.07	50	10.36	10	2.07
Zn^{2+}	0	0	10	0.65	10	0.65	10	0.65	50	3.27
K^+	30	1.17	30	1.17	30	1.17	30	1.17	30	1.17
$H_2PO_4^-$	30	2.90	30	2.90	30	2.90	30	2.90	30	2.90
NH_4^+	120	2.16	120	2.16	120	2.16	120	2.16	120	2.16
NO_3^-	120	7.44	120	7.44	120	7.44	120	7.44	120	7.44
SO_4^{2-}	0	0	10	0.96	10	0.96	10	0.96	50	4.80
Cl^-, total	100	3.54	240	8.50	240	8.50	240	8.50	240	8.50

Preparation of plant material for analysis

After 28 days in plastic reservoirs, samples of *P. stratiotes* were washed out of excessive substrate solution with fresh water and left to dry indoor at ambient temperature until complete dryness. Afterwards, in order to remove possible residual humidity, plants were additionally dried in a laboratory oven, at 100 °C for 30 minutes, prior to weighing. Weight measurement showed that the total initial plant mass of 850 g after drying reduced to 22.45, 21.98, 18.40, 20.73 and 24.42 g in 1st, 2nd, 3rd, 4th and 5th reservoir, respectively. Each of 45 (3×15) plant specimen (including leaves and root), was cut in small pieces and pulverized. A weight of each plant sample was then digested with 15 mL of a mixture (v/v 3:1) of concentrated 63 % nitric acid and 30 % hydrogen peroxide and the solution was evaporated to dryness. The solid residue was re-dissolved in 13 mL of 0.1 mol dm^{-3} nitric acid, filtered through filter paper, then through micro-filter (0.45 μm), after which the mineralized plant material was ready for the instrumental analysis. Instrumental analysis: in order to determine the metal content in plant material, the obtained extracts were analysed by atomic absorption spectroscopy (AAS) using the Perkin Elmer AAS instrument (software AAnalyst 300) with acetylene/air flame.

Chemical speciation of the tank water solutions

In order to verify if the chemical species due to the interactions among each other express any influence on the amount of free metal ions, the speciation of all components was done with the software package MINTEQA2. The reservoirs were considered as systems in contact with the atmospheric CO_2 (partial press. 0.00038 atm). The sulphates amount in tap water (70 μmol L^{-1}), determined additionally by ionic chromatography, was summed up with added amount and counted in the MINTEQA2 speciation.

Statistical analysis

Statistical analysis was performed with software package OriginPro 8.5©. The means calculation was based on 13 samples. For the means comparison one-way ANOVA was used. For the identification of the statistically different means the Dunnett's test was used.

RESULTS AND DISCUSSION

The measured water temperature in all reservoirs was 24.3 °C. The measured water pH values were: 6.57 (reservoir 1), 6.52 (reservoir 2), 6.45 (reservoir 3), 6.46 (reservoir 4) and 6.42 (reservoir 5), after the addition of chemicals.

Zinc, nickel and cadmium were mostly in the form of free ion, with the percentages of total concentration ranging from 94.89 to 98.43 among reservoirs (Table 2). Below 90 % of lead was in the form of free ion, while the percentage of Cu^{2+} ranged from 83.11 to 86.23. Other copper species with notable percentage of total concentration were also dissolved species.

Table 2 *Components speciation in water from each reservoir*

		Reservoir 1	Reservoir 2	Reservoir 3	Reservoir 4	Reservoir 5
Component	**Species name**	**% of total concentration**				
Zn^{2+}	Zn^{2+}	n.a.	97.64	97.80	97.78	97.85
Pb^{2+}	Pb^{2+}	n.a.	87.86	89.14	88.97	89.63
Cu^{2+}	Cu^{2+}	80.93	83.11	85.41	85.05	86.23
	$CuOH^+$	8.61	7.76	6.79	6.92	6.39
	$CuHPO_{4(aq)}$	4.87	4.39	3.91	4.04	3.79
K^+	K^+	99.93	99.93	99.93	99.93	99.93
PO_4^{3-}	HPO_4^{2-}	18.50	16.92	14.68	15.19	14.09
	$H_2PO_4^-$	73.35	74.07	75.52	76.38	77.67
	$CuHPO_{4\,(aq)}$	8.13	7.32	6.53	6.73	6.32
NH_4^+	NH_4^+	99.65	99.68	99.71	99.71	99.73
NO_3^-	NO_3^-	99.98	99.95	99.95	99.92	99.95
Cl^-	Cl^-	99.99	99.87	99.55	99.76	99.86
SO_4^{2-}	SO_4^{2-}	99.11	97.71	97.60	96.96	97.68
Ni^{2+}	Ni^{2+}	n.a.	98.32	98.40	98.39	98.43
Cd^{2+}	Cd^{2+}	n.a.	94.89	95.15	95.10	95.22
CO_3^{2-}	HCO_3^-	61.16	58.25	54.79	54.18	53.20
	$H_2CO_{3\,(aq)}$	36.46	38.76	42.83	41.39	44.57

The amount of copper (Cu^{2+}) in plant is presented in Figure 1. Since copper is an essential element, it is expected to be transported under metabolic control, reaching a constant concentration in plant despite the variations in the growth medium. However, in plants from reservoirs representing the metal-mixture solutions (No. 2, 3, 4 and 5) compared to the plants from the reservoir representing the copper-only solution (No. 1), the copper sorption enhancement could be observed, assuming the consistent pre-contained quantity of copper in plants among reservoirs. The amount of copper in plants from reservoirs enriched with nickel, cadmium, lead or zinc was 32.53, 76.02, 9.50 and 54.71 % higher, respectively, than the amount in plants from copper-only reservoir. In addition, the differences were statistically significant (Figure 1), (one-way ANOVA (F (4,60) = 7.399, p = 0.05)).

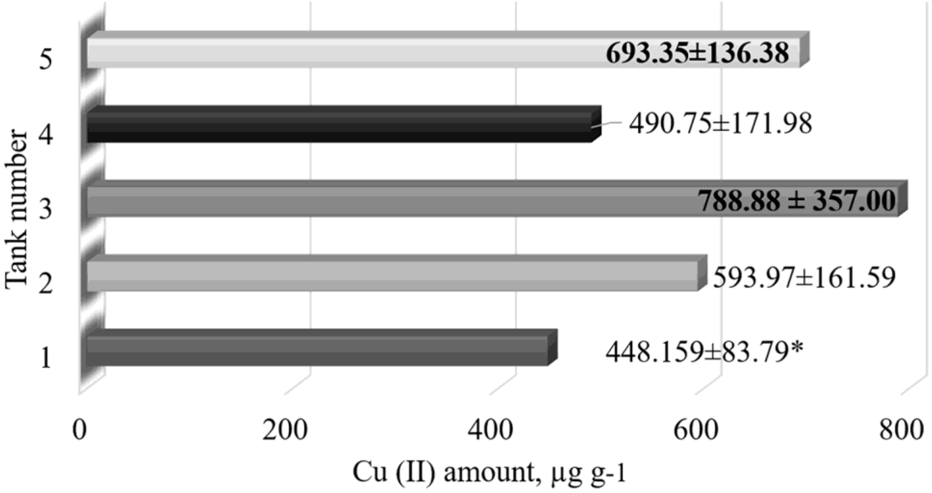

***Figure 1** Amount of copper, as Cu^{2+} ion, in plant material (dry weight) expressed as mean ± STD (n=13). Bolded values are significantly different from the control value (*) at $p<0.05$ level (Dunnett's test)*

The Dunnett's test showed that statistically significant sorption enhancement of copper was present in reservoirs enriched with cadmium and zinc. It is known that the mechanisms of uptake and transport of non-essential metals typically use pathways normally involved in the uptake of essential metals, thus the variation, in terms of suppression, in the copper uptake could be expected. However, beside direct effects, such as competition over specific binding sites or co-uptake by transport proteins, more indirect mechanisms exist, in which the presence of one metal activates defensive processes that protect the plant from other metals or on the contrary, trigger the enhanced uptake of another metal [4]. The last-mentioned effect could be an explanation for the enhanced copper uptake from the medium enriched with zinc and toxic cadmium.

CONCLUSION

Water lettuce represents an excellent choice for water phytoremediation practice and many studies confirmed great phytoremediation potential of the plant towards many metals. An important factor when evaluating the phytoremediation efficiency of plant toward a specific metal is a fact that metal-contaminated environments are not one-metal systems but a combination of toxic metals and other contaminants, where different synergistic or antagonistic effects regarding biosorption could be displayed. The study evaluated the copper uptake by water lettuce from water enriched with several heavy metals *i.e.* nickel, zinc and two non-essential metals - cadmium and lead. After 28 days in contaminated water, in plants from the metal-mixture contaminated water compared to the plants from the copper-only contaminated water, the copper sorption enhancement was observed. The enhancement was statistically significant in reservoirs with water enriched with cadmium and zinc - the amount of copper in plants, expressed as Cu^{2+} was 76.02, and 54.71 % higher, respectively. A range of effects can be expressed among metals when found together at contaminated sites, affecting the metal uptake or transport mechanisms and hence biosorption capacity of a plant. Thus, the investigation of the targeted metal uptake behaviour in a specific environment prior to phytoremediation is desirable.

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ELECTROCHEMICAL OXIDATIVE DEGRADATION OF TWO SYNTHETIC DYES IN WATER BY ELECTROSYNTHESIZED Ti/Bi_2O_3 ANODE

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Abstract

Ti/Bi_2O_3 anode was synthesized by potentiostatic electrodeposition from acidic Bi^{3+} solution on Ti substrate, followed by thermal treatment at 600°C. Characterization by SEM, EDX and XRD showed that the anode surface was fully covered with monophase α-Bi_2O_3, which formed inhomogeneous coat. Two dyes with different chromophores, Reactive Red 2 and Methylene Blue were degraded by electrochemical oxidation at the synthesized anode in the presence of hydrogen peroxide. Decolorization followed the pseudo-first order kinetics model for both of the dyes. Optimal degradation current density was 40 mA cm^{-1} and 50 mA cm^{-1} for Reactive Red 2 and Methylene Blue, respectively.

Keywords: anode, synthesis, dyes, degradation, electrochemical

INTRODUCTION

Synthetic dyes are very stable and resistant to most of the common wastewater treatments. Removal of dyes from water medium by electrochemical oxidation is possible via anodically generated hydroxyl radical •OH, a very strong oxidant that ensures fast reaction with most organics. Anode material is a very important factor in the processes of electrochemical decolorization. Anodes based on certain metal oxides have high surface area and excellent mechanical and chemical resistance even at high current densities [1,2]. Microcrystalline Bi_2O_3 can offer large surface area, electrochemical stability and photo- and electrocatalytic behaviour which makes it an interesting material for electrochemical oxidation of various organic pollutants [3,4]. In this work, Ti/Bi_2O_3 electrode was prepared by potentiostatic electrodeposition from acidic Bi^{3+} solution, followed by thermal treatment and used as the anode for electrochemical degradation of Reactive Red 2 (RR 2) and Methylene Blue (MB).

MATERIALS AND METHODS

All electrochemical experiments were carried out using Amel 510 DC potentiostat (Materials Mates, Italy) furnished with VoltaScope software package. All reagents were of analytical grade and used without further purification. Electrodeposition was performed in the three electrode cell with a Ti substrate (cathode) a Pt auxillary electrode and saturated calomel electrode as a reference electrode, at constant potential of 0.4 V during 2 minutes, from 0.1 M Bi^{3+} in 1M HNO_3. After the deposition, the substrate covered with deposited film was heated at 600°C for 90 minutes in air. Anode was characterized by SEM, EDX (Hitachi SU8030) and XRD (Siemens D500). Dye degradation was done under galvanostatic conditions in one compartment two-electrode cell, with synthesized anode and Pt sheet as the cathode, at constant temperature of 20 ± 0.5°C. The working dye solutions were prepared by diluting the stock solutions, which were prepared by dissolving the required amount of powdered dye in deionized water. The required amount of H_2O_2 and Na_2SO_4 were added to the working dye solutions. Dye concentrations were determined spectrophotometrically at λ_{max} = 538 nm and 668 nm for RR 2 and MB, respectively.

RESULTS AND DISCUSSION

Figure 1 shows surface morphology of the synthesized anode. Ti surface is fully covered with mechanically stable, non-uniform and relatively porous, foam-like bismuth oxide coat.

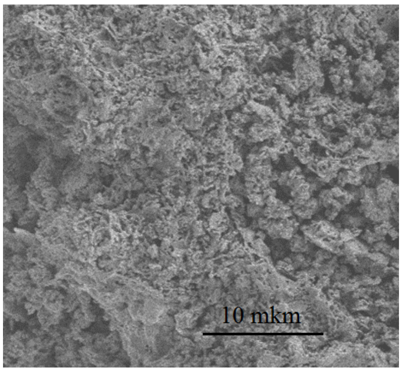

***Figure 1** Surface morphology of the synthesized Ti/Bi_2O_3 anode*

EDX showed that the weight ratios of Bi and O on the surface were 90.25% and 9.75%, respectively, which are close enough to their theoretical weight ratios in Bi_2O_3 of 89.70% and 10.30%, respectively, indicating the formation of Bi_2O_3 coat on the Ti surface and that the formed Bi_2O_3 is somewhat oxygen-deficient. XRD analysis revealed the presence of monophase, highly crystalline monoclinic (α) Bi_2O_3 on the Ti surface (Figure 2). Narrow diffraction peaks with characteristic 2θ values at: 27.4°, 33.3° and 46.3°, corresponding to (120), (200) and (041) reflection, respectively, are attributed to α-Bi_2O_3.

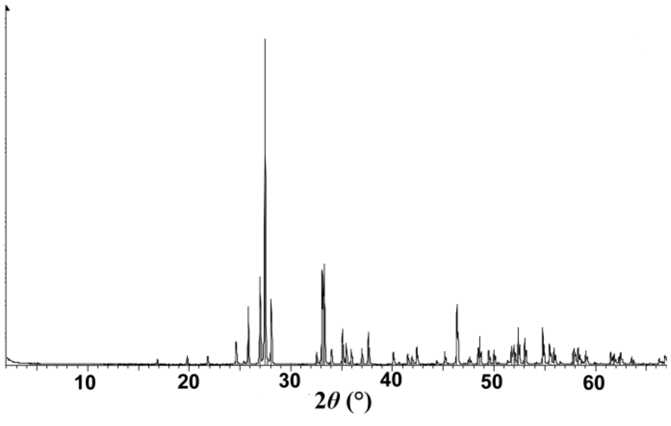

***Figure 2** XRD pattern of the synthesized Ti/Bi_2O_3 anode*

RR 2 and MB are very stable in the presence of H_2O_2 and their initial concentration of 50 mg dm^{-3} did not appreciably change in the 10 mmol dm^{-3} aqueous H_2O_2 solution during the time period of 24 hours. Electrolysis of 50 mg dm^{-3} RR 2 and MB in 10 mmol dm^{-3} Na_2SO_4 water solution in the absence of H_2O_2 at the constant current density (j) of 40 mA cm^{-2} also gave poor results: about 10% of RR 2 and 5% of MB was removed after two hours. However, in the presence of 10 mmol dm^{-3} H_2O_2 solution, RR 2 and MB were degraded in about 35 and 180 minutes, respectively under the same electrolysis reaction conditions.

As shown in Figure 3, the electrolysis current density has a significant effect on the decolorization rate. Complete decolorization can be achieved at any current density in the investigated range for both of the dyes, but at different rates. Decolorization rate of RR 2 is higher than that of MB, i.e., higher current density must be applied to degrade MB. At a current density of 10 mA cm^{-2}, the decolorization rate of RR 2 is considerably low and it takes more than 60 for RR 2 to completely decolorize the dye solution. With increasing current density, the dye decolorization rate also increases, that is the decolorization time decreases. The effect is more significant at lower current densities and the greatest difference in rate of decolorization is observed between 10 and 20 mA cm^{-2}. When the current density reaches 40 mA cm^{-2}, the dye solution is decolorized in less than 40 minutes and any further increase in current density does not bring a significant increase in the decolorization rate, so, it is assumed that the optimal current density for the process is 40 mA cm^{-2} for RR 2. MB shows similar behaviour, although it takers significantly more time to decolorize it at the same current densities; the optimal current density for degradation of MB, at which the dye is completely decolorized in about 2 hours, is 50 mA cm^{-2}. Further increase of current density does not bring significant increase of degradation rate. Different degradation rates of two dyes at the same current density are caused by the differences in their molecules chemical stabilities.

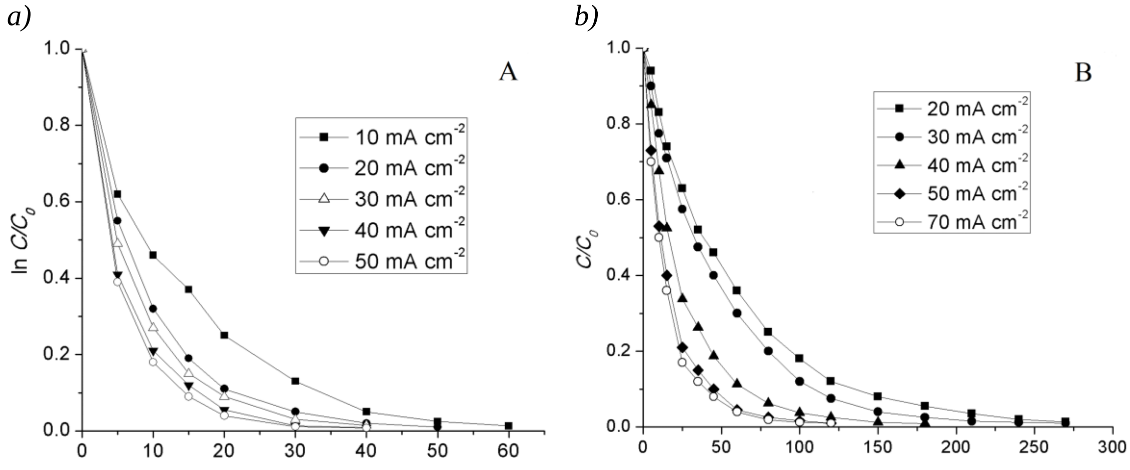

Figure 3 *Effect of current density on the degradation of a) RR 2 and b) MB at Ti-Bi$_2$O$_3$ anode (C_0 (RR 2) = C_0 (MB) = 50 mg dm^{-3}, C_0 (H_2O_2) = 10 mmol dm^{-3}, C_0 (Na_2SO_4) = 10 mmol dm^{-3})*

Dye degradation reactions follow the pseudo-first-order kinetics for both RR 2 and MB:

$$\ln(C/C_0) = -kt \qquad (1)$$

where C_0 is initial dye concentration (mg dm^{-3}), C is dye concentration after reaction time t (minute), and k is the reaction rate constant (min^{-1}). The plot of ln (C/C_0) against time scale for electrochemical decolorization of RR 2 and MB at the Ti/Bi_2O_3 anode for a series of solutions containing an initial dye concentration ranging from 20 to 200 mg dm^{-3} is presented in Figure 4. For all solutions, ln (C/C_0) shows a good functional linear decrease with increasing reaction time. The corresponding correlation coefficient squared (R^2) values for the initial dye concentrations of: 20, 30, 50, 100 and 200 mg dm^{-3} are high: 0.995, 0.998, 0.998, 0.985 and 0.987 respectively for RR 2 and 0.995, 0.994, 0.994, 0.984 and 0.982, respectively for MB, confirming the validity of treating the process as a pseudo-first order kinetic reaction. This finding is in accordance with those reported in literature [5,6] for the advanced oxidation processes of organic molecules, in which those molecules are oxidized by •OH radicals, strongly indicating that •OH radicals is the main oxidizing reagent, which is electrogenerated at Ti/Bi_2O_3 anode in the presence of H_2O_2. The initial dye concentration has a significant impact on dye decolorization rate. The corresponding reaction rate constants are: 0.191, 0.165, 0.146, 0.083 and 0.066 min^{-1}, respectively for 20, 30, 50, 100 and 200 mg dm^{-3} of RR 2, and 0.053, 0.047, 0.046, 0.028 and 0.024 for the same C_0 values of MB. MB shows lower degradation rates than RR 2 for all the examined C_0 values, which is a consequence of the difference in the two dyes molecular structure, and therefore, their stability. A significant decrease in the decolorization rate for the higher initial dye concentrations probably occurred because the ratio between the dye and •OH radical concentration increases, decreasing the amount of •OH available for the reaction with the dye molecules, thus prolonging the decolorization reaction time.

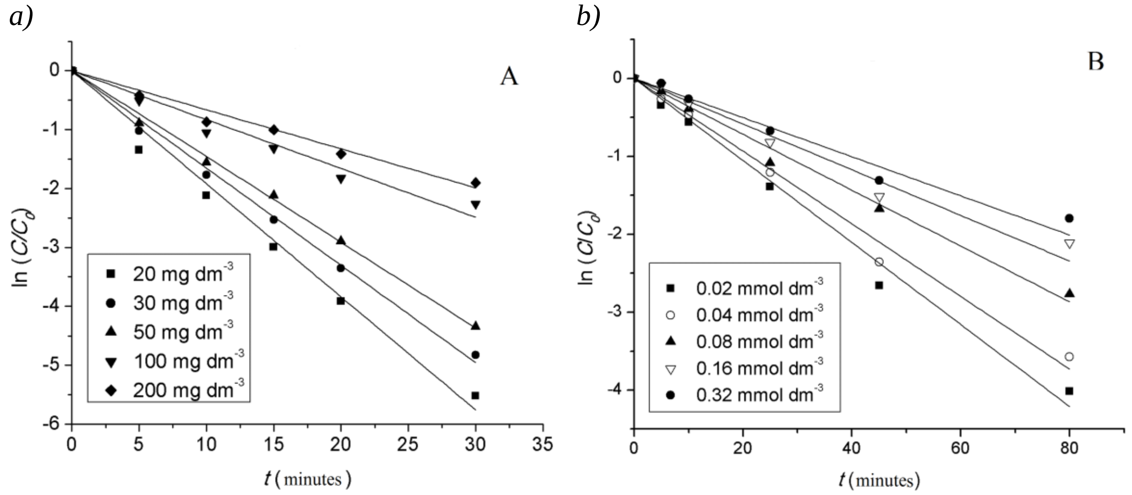

***Figure 4** Pseudo-first order kinetics of degradation of a) RR 2 and b) MB at Ti-Bi_2O_3 anode (C_0 (RR 2) = C_0 (MB) = 50 mg dm^{-3}, C_0 (H_2O_2) = 10 mmol dm^{-3}, C_0 (Na_2SO_4) = 10 mmol dm^{-3})*

CONCLUSION

Ti/Bi_2O_3 anode was synthesized by electrodeposition from acidic Bi^{3+} solution on Ti substrate, followed by thermal treatment at 600°C. Anode surface is entirely covered by relatively porous, non-uniform coat of monophase α-Bi_2O_3. Reactive Red 2 and Methylene Blue were completely degraded by electrochemical oxidation at the anode in the presence of H_2O_2. Increase of current density increased dye degradation rate, but only up to a certain value: 40 mA cm^{-2} for Reactive Red 2 and 50 mA cm^{-2} for Methylene Blue, which is taken as their optimal degradation current density. Degradation reactions proceed via oxidation by •OH radicals, electrogenerated at the synthesized anode, following the pseudo-first order kinetics. Degradation rate decreases with the increase of initial dyes concentration. Reactive Red 2 shows higher degradation rate constants than Methylene Blue under the same reaction conditions. The difference in degradation rate constants are caused by the difference in dyes chemical stability.

ACKNOWLEDGEMENT

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PAULOWNIA LEAVES AND THEIR HYDROCHAR FOR Pb^{2+} IONS REMOVAL FROM AQUEOUS SOLUTION

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Abstract

Adsorbents prepared from agricultural and industrial biowaste are being investigated as efficient material for removal of various pollutants from wastewater. In this study, Paulownia leaves powder and its hydrochar prepared from hydrothermal carbonization at 200°C was investigated as potential adsorbents for Pb^{2+} *ions from aqueous solution. Paulownia leaves and hydrochar at 200°C were characterized by FTIR spectroscopy before and after adsorption of* Pb^{2+} *ions. The experimental results showed that Paulownia leaves had better adsorption capacity (q=34.53 mg/g) than hydrochar produced at 200°C (q=11.26 mg/g) and could be considered as effective biosorbent for* Pb^{2+} *removal from wastewaters.*

Keywords: Paulownia leaves, hydrothermal carbonization, biosorption, Pb^{2+}, FTIR

INTRODUCTION

Industrial wastewater is a type of environmental pollution and in the 21st century is one of major problem for humanity and nature. Containing various organic and inorganic pollutants, water represents source of toxically matters which negatively affect human health and environment [1,2]. Lead (Pb^{2+} ions) is one of the most toxic metals which often could be found in industrial effluents. It is well known that in humans lead may cause headaches, visual disturbance, anaemia, mental and liver damage, and also can be deadly for animals and plants [2–4].

Through the influence of industrial production and wastewaters, non-biodegradable, heavy metal ions are easily available and could be harmful for living organisms and their removal from aqueous media, before discharge into watercourses, are of the great importance [4]. Various methods have been reported for purifying wastewaters such as coagulation, ion exchange, membrane separation, oxidation, flocculation but most of them are costly or create undesirable byproducts [1,2]. The biosorption has represented powerful tool for the removal of pollutants from wastewaters. The adsorption of lead ions using waste biomass has been confirmed as a simple, inexpensive and efficient method for treatment of polluted waters [2,3,5]. During past years, there has been increasing in the use of plant waste and their products for heavy metals removal. Some alternative biosorbents have the advantage due low-cost, ease of operation, greater efficiency and profitability [5]. However, the most biosorbents have limited biosorption capacities and new economical acceptable biomasses are always in demand.

The Hydrothermal Carbonization (HTC) is a promising process used to convert wet biomass into functional materials (called hydrochars) [6]. In HTC process added water reacts with fibrous component of biomass and cause reconstruction and degradation (hydrolysis, dehydration, decarboxylation, condensation, polymerization and aromatization) [6,7]. Hydrochar is a homogeneous and hydrophobic material which contains micro- to nano-sized carbon spheres with a porous structure and oxygen-containing functional groups. Due to its physicochemical features, hydrochars could be considered for numerous practical applications [6,8]. So far this material has been tested for use as solid fuel, soil supplement, as feedstock for pellets. By this technology, various types of biomass, municipal waste, wood and sewage sludge, can be quickly transformed into the solid carbonaceous products which characteristics strongly dependent on reaction conditions and feedstock type [8].

Paulownia is a genus of about 20 species of fast growing trees native to China. Good Paulownia wood characteristics, such as easily adaptable, fast growth, high biomass production, are reason that this plant is very popular and commonly used in industry [9–11]. Beside industry, Paulownia is grown like a decorative tree in parks thanks to the beautiful flowers and leaves. However, paulownia leaves represent biowaste during wood processing and in parks at the end of the season when it falls of trees [1].

In order to investigate the ability and efficiency of Paulownia leaves (PL) and Paulownia leaves hydrochar (200-PL) to remove lead ions from aqueous solution, preliminary adsorption test was performed. Additionally, for characterization of materials FTIR analysis was used.

MATERIALS AND METHODS

Materials preparation

The PL was collected from park in Belgrade, wash with distilled water, air-dried for two weeks and extensively grinded in order to obtain homogenous samples. Sieved fraction of 0.5 mm was used in HTC and biosorption experiments.

The HTC process was carried out in laboratory autoclave (Carl Roth, Model II). For production of hydrochar 10 g of PL powder was stirred with 150 ml of ultrapure water on reaction temperature at 200ºC constant for 1 h. After that period, the hydrochar was separated from liquid by filtration, rinsed three times with distilled water and dried at 105°C for 24 h.

Preliminary adsorption test

For preliminary adsorption test we used: adsorbent (PL and 200-PL) mass 0.08 g, lead solution with initial concentration 100 mg/L in volume 0.05 L, contact time 2 hours on pH = 5 value and at room temperature (25°C). Solution was separated from adsorbent by filtration and Pb^{2+} concentrations before and after adsorption were determined using atomic adsorption spectrophotometer (AAS, Perkin Elmer AAnalyst 300).

FTIR Analysis

Fourier transform infrared (FTIR) spectroscopic analysis was used to determine the presence of functional groups in surface of PL and 200-PL responsible for Pb^{2+} binding. The

FTIR of samples before and after metal sorption were recorded on Thermo Fisher Scientific Nicolet IS-50 spectrophotometer, in the spectral range of 400 - 4000 cm^{-1}. The FTIR spectra of the samples were converted into pellet form, using KBr as the reference material.

RESULTS AND DISCUSSION

Preliminary adsorption test

The amount of Pb^{2+} ion adsorbed on PL and 200-PL surface was calculated using equation:

$$q = ((C_0 - C_e) / m) \times V \tag{1}$$

Where, q - adsorbent capacity [mg/g]; C_0 - initial concentration of Pb^{2+} [mg/L]; C_e - metal concentration after adsorption [mg/L]; m - amount of adsorbent [g]; V - volume of the Pb^{2+} solution [L].

The percentage of lead ions removal (R) was calculated based on the difference in lead concentration in initial solution (C_0) and in solution after adsorption (C_e):

$$R[\%] = ((C_0 - C_e) / C_0) \times 100 \tag{2}$$

The preliminary adsorption test showed that leaves without structural changes had better adsorption capacity which was q=34.53 mg/g than hydrochar produced at 200°C, q=11.26 mg/g (Figure 1). Additionally, percentage of Pb^{2+} removal confirm that PL has higher efficiency as adsorbent (60.87%) in comparison with 200-PL (19.57%).

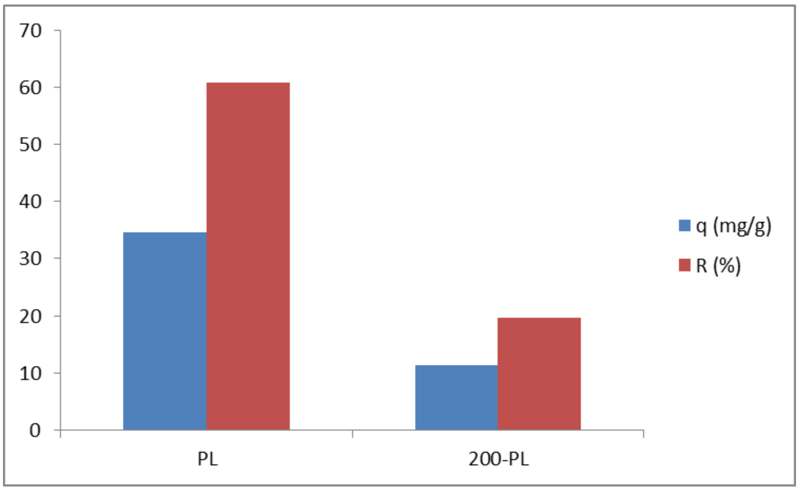

Figure 1 *Adsorption capacity (q) and Pb^{2+} removal (R) using PL and 200-PL*

FTIR Analysis

Selected FTIR spectrums of PL and 200-PL were shown in Figure 2 and the oxygen functional groups such as hydroxyl and carboxylic groups are crucial for adsorption of lead ions. Also, it can be seen that hydrothermal carbonization leads to a decrease in the intensity of the peaks in FTIR spectra. Unlike the PL and 200-PL, adsorbents with Pb^{2+} ions had a lower intensity of IR peaks between 1000 and 1400 cm^{-1}, peak at about 1700 cm^{-1} and peak at about 3300 cm^{-1}. The IR peaks between 1000 and 1400 cm^{-1} indicated C-O and O-H groups from alcohols, phenols, carboxylic acids [6,8], peak at about 1700 cm^{-1} can be attributed to the carbonyl group (C=O) from fibrous structure [6,7]. The wide and intense band at about

3300 cm^{-1} belongs to the vibration of the –O-H group [7,8]. Since the intensities of these peaks are reduced after the adsorption of Pb^{2+} ions, it could be concluded that those groups were involved in a metal adsorption on the surface of adsorbents.

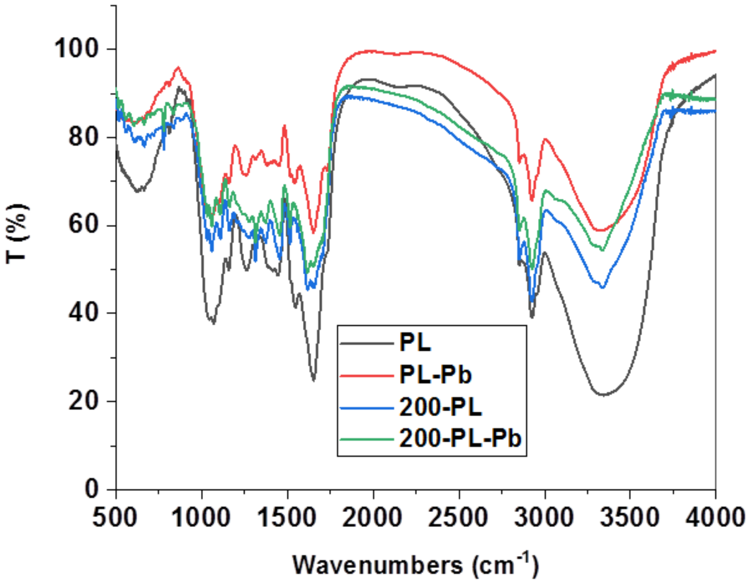

Figure 2 *FTIR spectra of* ***PL*** *and* ***200-PL*** *before and after* Pb^{2+} *adsorption*

CONCLUSION

The adsorption preliminary test showed that PL had better adsorption characteristic for lead ions removal. Adsorption capacity of PL was 34.53 mg/g and adsorption capacity of 200-PL was 11.26 mg/g. Also, adsorption efficiency was 60.87% for PL and 19.57% for 200-PL. With FTIR analysis it was observed that oxygen functional groups may have been the most important for adsorption of Pb^{2+} ions and hydrothermal carbonization process reduced their number on the surface of the sample. This study suggests that waste Paulownia leaves without any structural changes could be considered as more effective adsorbent for Pb^{2+} removal than their hydrochar at 200°C.

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REMOVAL OF Cd (II) USING HYDROCHARS PREPARED FROM SUBSTRATE FOR CULTIVATING MUSHROOMS

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Abstract

In this study, the sorption capacity of Cd (II) removal from aqueous solution was investigated using hydrochar obtained from substrate for cultivating mushrooms. In order to increase the adsorption capacity, hydrochar was previously modified with 2M KOH. The obtained results showed that alkally modification increased the sorption capacity from 41.5 mg/g to 53.5 mg/g. Structural characterization of alkally modified and unmodified hydrochars were performed by Fourier transform infrared spectroscopy (FTIR). According to obtained results of FTIR analysis, the increased of the oxygen functional groups (OFG) in alkally modified hydrochars was observed, which is contributed to the increase in the adsorption capacity of this hydrochar. Results indicated that substrate for cultivating mushrooms can be converted into hydrochar as a sorbent for sorption of Cd (II), and the presence of OFGs play important role in the hydrochar's high adsorption capacity.

Keywords: substrate for cultivating mushrooms hydrochars, Cd(II) removal, alkally modification

INTRODUCTION

Excessive increased of population, industrialization and agricultural activities have caused contamination of water and soil with heavy metals [1]. The presence of these pollutants in the waters can have negative effects on the environment, because heavy metals are highly toxic and not degradable. Elements such as Cd, Hg, Ag, Se and Pb can be extremely toxic, while Cu, Zn, Mn, Fe, Ni and Co are considered essential for the functioning of living organisms and they are toxic in higher concentrations [2]. Cadmium is one of the most toxic heavy metal. Phosphoric fertilizers, wastewaters from industry, batteries, and dyes are often responsible for bringing Cd (II) into the aquatic environment [3]. Constant exposure to Cd (II) can cause various diseases in human such as lung cancer, prostate proliferative lesions, bone fractures, kidney dysfunction, and hypertension [2]. In order to avoid mentioning negative health effects, industrial wastewater should be purified and recycled using appropriate methods.

In recent years, various conventional wastewater treatments methods have been used, such as: precipitation, ion exchange, adsorption, electrochemical processes and membrane processes [3]. However, adsorption relative to other technology is the most commonly used method because it is efficient, environmentally friendly and economical [4]. A large number of different sorbents have been investigated for water purification, especially waste biomass. Biosorbents are by-products of agriculture that are easily accessible, high efficiency, and ability to remove organic and inorganic compounds from water [5]. Research on biosorbents was mainly focused on application of biochar from the pyrolysis process [2]. However, lately a greater emphasis is placed on the using of hydrochar, which is obtained during hydrothermal process. Kambo and Dutta [6] states that hydrochar has substantially greater adsorption capacity than biochar, considering that it is richer in functional groups on its surfaces and also it has lower surface area and porosity.

Hydrothermal carbonization (HTC) is thermochemical technology that can treat various types of organic waste materials into functional products with minimal environmental pollution [7]. HTC is operated in water as a reaction medium, moderate temperatures (180–260°C) and autogenous pressure [8]. The products obtained from HTC of feedstock are a carbon-rich solid product (hydrochar), process water and small amount of gas. The process parameters such as pressure, temperature, reaction time and biomass and water ratio has a significant impact on degree of carbonization of waste organic matter [9]. In contrast to feedstocks, the hydrochar is characterized by higher mass and energy density, better dewaterability, and improved combustion performance as a solid fuel [10]. HTC has been considered for various purposes, such as catalysis, adsorption, fuel, electrochemistry, template synthesis, nanostructured materials [11]. A number of studies have been performed on hydrothermal carbonization of a wide range of waste organic matter: food waste, animal manure, glucose etc. [9].

Petrovic *et al*. [12] examined the adsorption of Pb^{2+} from aqueous solution with alkally modified hydrochar and found that the hydrochar showed the highest Pb^{2+} sorption capacity of 137 mg/g. Elaigwu *et al*. [13] compared the adsorption capacities of the hydrochars and biochars, and found that hydrochars were able to adsorb the Pb (II) and Cd (II) more efficiently in contrast to biochars.

The aims of this study were to investigate the efficiency of substrate for cultivating mushrooms hydrochar as a sorbent for the removal of Cd (II) from wastewaters. Besides, the sorption capacity between alkali-modified and unmodified hydrochars will be compared, as well as their structural changes.

MATERIALS AND METHODS

Substrate for mushroom cultivation (SM) is obtained from the local mushroom industry located in the vicinity of Belgrade. The compost used is from the following components: wheat straw, urea, gypsum and horse manure.

HTC hydrochar preparation

The HTC experiments were carried out in 250 ml autoclave reactor (Carl Roth, Model II). The HTC reactor was equipped with a cooling system and controller for pressure and temperature. To analyse the effect of the HTC temperature on 200°C were adopted under the same reaction time of 1 h. For each test, 10 g of SM feedstock was mixed with 150 ml of deionized water into autoclave. The autoclave was held at final temperature at 60 min and the reactor was cooled down to the room temperature. The hydrochar was washed with deionized water and then dried at 105°C for 24 h.

Alkaline modification

Alkaline hydrochar modification was carried out according to the procedure shown in the paper Petrovic *et al*. [12].

Adsorption studies and characterization of hydrochar

A stock solution of 100 mg L^{-1} Cd^{2+} was prepared by dissolving Cd $(NO_3)_2{\cdot}4H_2O$. For adsorption experiment, 0.025 g of one of hydrochars (unmodified (SM-200) or alkali-modified (SM-KOH-200)) was mixed with 25 ml of Cd^{2+} solutions. The flasks were capped and shaken at room temperature for 120 min at 250 rpm. After that, the mixtures were filtered and the concentrations of Cd^{2+} were determined by atomic absorption spectroscopy (Perkin Elmer, AAS Analyst 300). The solids remaining on the filter were collected and dried at 105°C for Fourier transform infrared spectroscopy (FTIR; Thermo Scientific Nicolet iS50 FT-IR spectrometer in transmission mode) analysis. Materials were recorded in the spectral range 4000–400 cm^{-1}.

Adsorption capacities qe (mg/g) were calculated as follows:

$$qe = (C_0 - C_e) \times V/m$$

where V, is the volume of the Cd^{2+} solution (L), m is the amount of adsorbent (SM-200 or SM-KOH-200) (g); C_0 and C_e are the initial and equilibrium concentrations of the Cd^{2+} ions (mg L^{-1}), respectively.

RESULTS AND DISCUSSION

The FTIR analysis was carried out to explain the internal chemical structural changes of SM-200 and SM-KOH-200 after adsorption of Cd (II). The FTIR spectrums are presented in Fig. 1. The intensity of the peak 3280 cm^{-1} was associated to -O-H stretching vibration from hydroxyl and carboxyl groups in the SM-200 and SM-KOH-200 [2,12]. The two bonds at around 2920 and 2850 cm^{-1} probably originated from vibration of aliphatic C–H stretching in cellulose from wheat straw [14].

The bonds at 1620 cm^{-1} could be attributed to C=O vibrations [15]. The band at 1521 cm^{-1} are the results of the of N-O nitro groups, which can originated from protein and uric acid in horse manure [16]. The bonds at 1420 cm^{-1} were associated with aromatic C=C stretching, indicating the presence of lignin in the hydrochars [17]. The stretching -COR aliphatic ethers and –COH primary alcohols were attributed to the bonds at 1030 cm^{-1} [11]. The absorbance peaks at around 779 and 470 cm^{-1}, presented to the aromatic C-H bending vibration and aliphatic C-H stretching vibrations [11].

Based on research Han *et al*. [2], it can be noticed that there are a large number of active functional groups (-COOH, -CO, -OH) that can form complexes with Cd (II) on the surface of the hydrochars.

Similar observation was also noticed in this paper. The Figure 1 showed that SM-KOH-200 had a significantly higher intensity of peak at 3280, 1620 and 1030 cm^{-1} compared to SM-200. This confirmed that SM-KOH-200 had more OFG in contrast to SM-200, which SM-

KOH-200 improved ability to adsorb Cd(II). Petrovic *et al*. [12] and Sun *et al*. [18], also found that alkaline modification of the hydrochars increased the number of OFGs, which had an impact on the improvement of the metal adsorption capacity to the hydrochars.

The results from FTIR analysis was confirmed by determining the preliminary adsorption capacity of the applied hydrochars. The adsorption capacity of the SM-KOH-200 was qe=53.5 mg/g, while the SM-KOH showed smaller capacity (qe=41.5 mg/g). It can be shown that alkally modified hydrochar had more OFGs on their surface, and thus more electron donating sites for adsorption of Cd (II) ions.

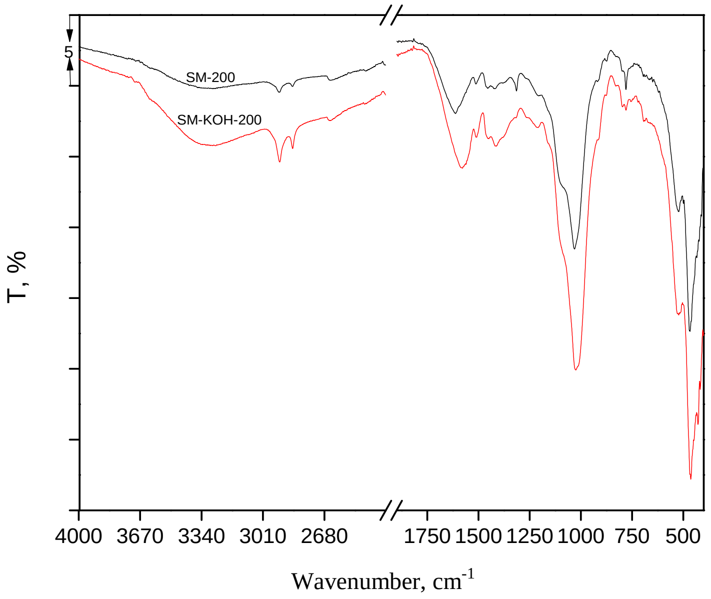

Figure 1 *FT-IR spectra of SM-200 and SM-KOH-200 after Cd (II) sorption*

CONCLUSION

Based on this paper, we can conclude that alkaline modification contributed to the increase of OFG in hydrochars, which increased the adsorption capacity from 41.5 mg/g for SM-200 to 53.5 mg/g for SM-KOH-200. It showed that these materials could be used as low cost adsorbents in wastewater treatment and that HTC is sustainable method for conversion of this type of biomass.

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OPTIMIZATION OF HORSERADISH PEROXIDASE ENCAPSULATION WITHIN TYRAMINE-ALGINATE FOR PHENOL REMOVAL

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Abstract

Phenolic compounds are one of the most common pollutants in aqueous systems, so their removal from water is of major interest. Among biocatalysts used for phenol removal, horseradish peroxidase is the most investigated for this purpose. Enzyme inactivation is a major problem which could be successfully overcome by immobilization of the enzyme onto different polymers. Tyramine-alginate micro-beads were tested for the immobilization of horseradish peroxidase. Different concentrations of tyramine-alginate were used and their influence on specific activity of the enzyme was tested. Increasing concentration of oxidized alginate results in increase of specific activity. Immobilized HRP was tested for phenol removal in a batch reactor. Presented results were obtained with HRP immobilized within 10 mol% tyramine-alginate micro-beads. These biocatalysts can be used up to three cycles.

Keywords: horseradish peroxidase, immobilization, phenol removal, tyramine, alginate

INTRODUCTION

On a daily basis, large amounts of pollutants reach water courses leaving the consequences to the entire ecosystem. Phenol is one of the most common water pollutants, because of its toxicity even at low concentrations [1]. Phenols and phenolic compounds, commonly found in wastewaters, are derived from a number of industrial processes, including coal conversion, mining, petroleum refining, plastics and textiles. Methods used for the removal of phenolic compounds from waste effluents include many physical, chemical and biological processes [2,3].

Different enzymatic approaches have been used for the removal of phenolic compounds. Peroxidases are able to oxidize phenol like compounds in the presence of hydrogen peroxide, forming water-insoluble polymers that could be easily removed from aqueous phase using filtration or sedimentation. Among investigated peroxidases, horseradish peroxidase (HRP) was successfully used for the purpose of phenol removal from wastewaters. Inactivation of the enzyme is a major problem in the phenolic compound treatment. Immobilization on different materials was tested in order to overcome this difficulty and to increase operational stability of the enzyme [4].

In this work, horseradish peroxidase was encapsulated within tyramine-alginate hydrogels that we have previously developed and tested for the phenol removal in a batch reactor.

MATERIALS AND METHODS

Materials

Sodium alginate, horseradish peroxidase, phenol, hydrogen peroxide and other chemicals used for this research were purchased from Sigma-Aldrich, USA.

Synthesis of tyramine-alginates

Alginate was modified with sodium metaperiodate and tyramine hydrochloride as was previously described Prodanovic et al. [5]. Briefly, appropriate amount of sodium alginate (0.28 g) was dissolved in water to a final concentration of 1% (w/v). Sodium metaperiodate was added to this solution to a final concentration of 2.5 mM and 5 mM so that molarity ratio of periodate to C6 glycoside units in alginate was set to 5 mol% and 10 mol%. Reaction was stopped by adding glycerol at 50 mM concentration and oxidized alginate was precipitated by adding NaCl at 1% (w/v) and 2 volumes of 96% ethanol (v/v). The precipitate was separated, dried and dissolved in 0.1 M sodium-phosphate buffer pH 6. Modification of oxidized alginate was carried out by adding solid tyramine hydrochloride and sodium cyanoborohydride to this solution. Modified alginate was precipitated by adding NaCl at 1% final concentration and two volumes of 96% ethanol.

Immobilization of horseradish peroxidase

Tyramine-alginates were dissolved in 50 mM Tris HCl buffer pH 7.0 at final concentration of up to 15%. Horseradish peroxidase, glucose oxidase and glucose were added in tyramine-alginate solution. Micro-beads were formed by pouring this mixture into 600 µL of light mineral oil containing 3% Span 80 detergent. Reaction was stopped by adding 1 mL of 0.5% Triton X-100 in 5% calcium chloride solution.

Measurement of enzyme activity

Peroxidase activity was measured using pyrogallol and H_2O_2 as substrates. For immobilized enzyme 130 µL of suspension and 30 µL of 0.97 M H_2O_2 were poured into 3 mL of 13 mM pyrogallol solution. Reaction was performed under constant stirring for 7 min. Every 120 s aliquots were taken and filtrated and absorbance at 420 nm was measured. 1 U of enzyme activity was defined as the amount of enzyme that produces 1 mg of purpurogallin in 20 s.

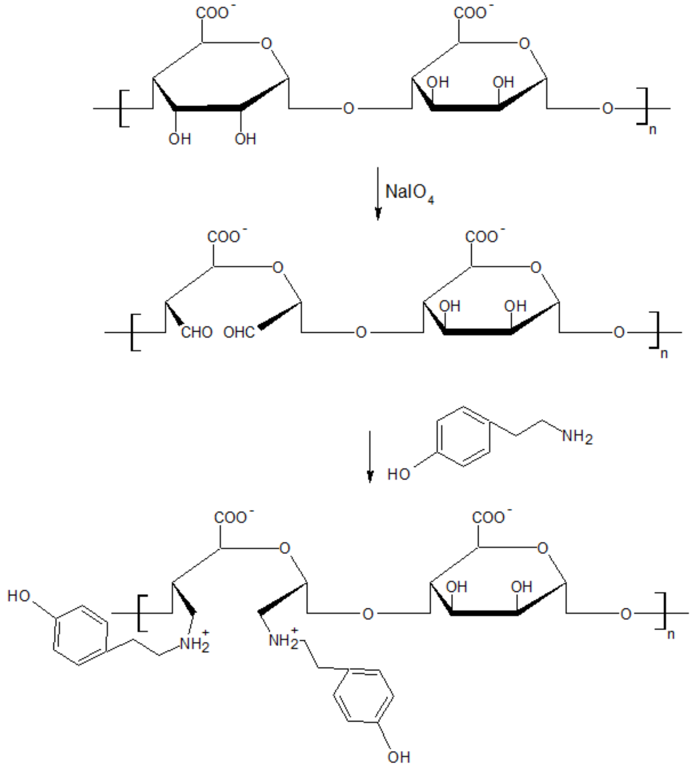
Phenol removal

Phenol removal was carried out in a batch reactor by adding alginate-tyramine-beads into 3 mL of 2 mM phenol solution in 50 mM Tris HCl buffer. For the delivery of hydrogen peroxide we tested system composed of glucose oxidase and glucose. Reaction mixture composed of phenol solution, glucose oxidase and glucose, stirred for 6h using magnetic stirrer. Aliquots were taken every 30 minutes. Phenol concentration was determined using a colorimetric assay in which phenol reacts with 4-aminoantipyrene (AAP) in the presence of potassium ferrocyanide ($K_3Fe(CN)_6$). Absorbance was measured at 510 nm after 10 min of color development.

Concentration of removed phenol was measured using calibration curve. Removal of phenol was followed for three days within 120 minutes, until decrease of the immobilized enzyme was noticed.

RESULTS AND DISCUSSION

Alginate was oxidized with various molar ratios of sodium metaperiodate and modified further with tyramine as it is shown on Scheme 1.



Scheme 1 *Tyramine-alginate synthesis: reaction of periodate oxidation and reductive amination with tyramine*

Influence of tyramine-alginate concentration (w/v) on specific activity of immobilized HRP was tested. The results presented in Table 1 show that specific activity of the enzyme is increasing with increase of tyramine-alginate concentration.

Table 1 *Influence of tyramine-alginate concentration* (w/v) *on specific activity of immobilized peroxidase*

Tyramine-alginate concentration (w/v)	5%	10%
Specific activity (U/mL)	0.66	2.04

Obtained micro-beads with encapsulated HRP were used for the removal of phenol in a batch reactor. The reusability studies showed that after three times of repeated use, these biocatalysts show decrease in their catalytic performance (Figure 1).

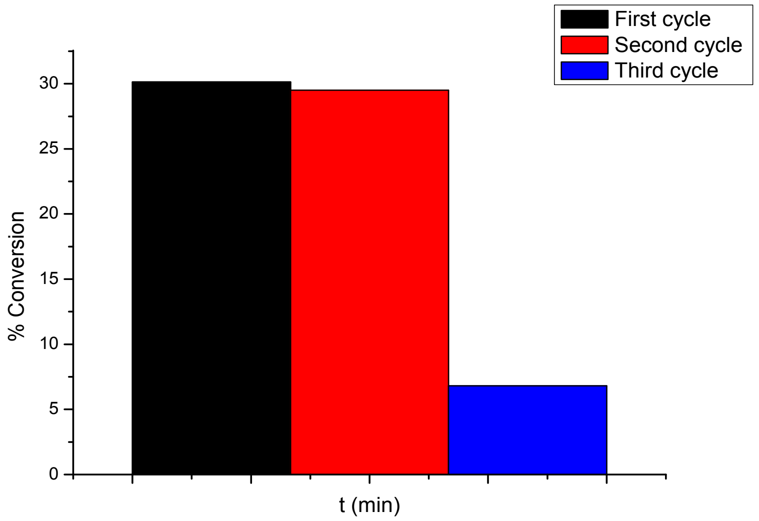


Figure 1 *Reusability studies of micro-beads, 10 mol% tyramine-alginate*

CONCLUSION

The preparation and application of immobilized horseradish peroxidase within tyramine-alginate micro-beads was investigated. Tyramine-alginates were used for the encapsulation of horseradish peroxidase within hydrogel micro-beads in a coupled emulsion polymerization reaction. Application of immobilized peroxidase for phenol removal was tested. Obtained experimental results revealed the effectiveness of the peroxidase encapsulation in the removal of phenol.

ACKNOWLEDGEMENT

This work was supported by Ministry of Education and Science of Republic Serbia (Project OI 173017, OI 172049, III 41007).

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IMMOBILIZATION OF TYRAMINE-HRP ONTO TYRAMIDE-CARBOXYMETHYL CELLULOSE MATRIX FOR WASTEWATER TREATMENT

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Abstract

Horseradish peroxidase (HRP, E.C. 1.11.1.7) catalyzes oxidation of aqueous aromatic compounds using hydrogen peroxide. Enzymatic treatment methods for phenol removal from wastewaters has become an efficient and environmentally friendly alternative for the traditional methods. Carboxymethyl cellulose (CMC) derivative with tyramine attached via amide bond to carboxyl groups has been chosen as carrier for immobilization. In effort to overcome the main disadvantage of entrapment immobilization method, loss of enzyme activity due to washing out from the carrier, HRP was modified in a reductive amination reaction and tyramine was bound to the enzyme. Immobilization of tyramine-HRP onto tyramide-carboxymethyl cellulose carrier was carried in an emulsion polymerization reaction that produced carboxymethyl cellulose microbeads. The highest specific activity of the obtained biocatalyst was 0.227 U/ml and after 48h of storage 0.197 U/ml. Immobilized tyramine-HRP retained 87% of activity after 48 h. Immobilized HRP is a suitable candidate for wastewater treatment.

Keywords: immobilization, HRP, carboxymethyl cellulose, tyramine, phenol removal

INTRODUCTION

Aromatic compounds that are found in wastewaters, like phenols and its derivatives, are dangerous for the environment due to their toxicity and suspected carcinogenicity [1]. Methods for their removal such as microbial degradation, chemical oxidation, activated carbon adsorption and solvent extraction, have been thoroughly investigated. Some disadvantages of the traditional methods like low efficiency, extreme reaction conditions, high cost or generation of even more toxic products could be overcome using enzymatic treatment [2]. Peroxidases, like horseradish peroxidase (HRP) or soybean peroxidase (SBP), have been successfully used for enzymatic removal of phenolic compounds from aqueous solutions [3]. Advantages of enzyme-based treatment methods are high specificity and low energy requirements. Enzymes operate over wide range of conditions and have minimal environmental impact [4].

HRP catalyzes oxidation of aromatic compounds in the presence of hydrogen peroxide producing free radicals that polymerize to produce high molecular weight polymers with low solubility and easy to separate from the solution [4]. Although enzymes proved to be efficient biocatalysts, their wider application is limited due to high cost of their purification and inability to retrieve them from the solution after treatment. Enzyme immobilization onto solid carriers like cellulose provides some improved characteristics like stability, reusability, improved performance in organic solvents, pH tolerance, heat stability and selectivity [5]. Cellulose, as constituent of the primary plant cell wall, is a common biopolymer and an excellent source of renewable polymeric material. Like all natural polymers it is nontoxic, biocompatible and biodegradable, therefore an ideal carrier for enzyme immobilization [6,7]. A major drawback to entrapment method of enzyme immobilization is a so called "leaking" (or washing out) of the enzyme from the carrier followed by reduced specific activity [8]. Carboxymethyl cellulose (CMC), a low cost, water-soluble cellulose derivative, has been conjugated with tyramine using carbodiimide activation reaction with 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC) in the presence of N-Hydroxysulfosuccinimide (NHS) [9]. Obtained tyramide-CMC was suitable material for hydrogel formation through enzyme catalyzed covalent crosslinking and an ideal carrier for HRP immobilization.

In our previous work we immobilized peroxidase onto tyramine modified natural polysaccharides like alginate and pectin. In this work our goal was to reduce enzyme leakage from the carrier so we used a similar procedure to modify the enzyme and immobilized tyramine modified HRP onto tyramide-carboxymethyl cellulose polymer synthesized as previously described by Ogushi *et al.* [9]. HRP was first oxidized with sodium periodate and tyramine was covalently bound to a glycosidic side chain. Specific activity was measured 1 h, 24 h and 48 h after immobilization, in order to determine the stability and enzyme leakage from the matrix.

MATERIALS AND METHODS

Material

Horseradih peroxidase, carboxymethyl cellulose, glucose oxidase (160 U/mg), triton X-100 (t-octylphenoxypolyethoxyethanol), tyramine hydrochloride, N-hydroxysulfosuccinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDAC), mineral oil and span 80 (sorbitan monooleate) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium cyanoborohydride was obtaineed from Fluka (Buchs, Switzerland). TRIS was obtained from SERVA Electrophoresis GmbH (Heidelberg, Germany). Glucose was purchased from Zorka Pharma (Šabac, Serbia).

Modification of HRP

Horseradish peroxidase was dissolved in 50 mM sodium carbonate buffer pH 8.0 to a final concentration of 0.5 mg/ml. Two hundred microlitres of 50 mM sodium periodate was added into 2 ml of buffered HRP solution and incubated in the dark for 6 h at 4 °C with occasional shaking. Reaction was stopped by adding 20 μl of 99% glycerol, left for another 30 min at 4 °C and afterwards dialyzed against 50 mM sodium carbonate buffer pH 8.0. After dialysis, obtained HRP sample volume was measured and tyramine hydrochloride in the same buffer was added to final concentration of 50 mM and incubated for 2 h at 25 °C. Sodium cyanoborohydride was added to final concentration of 1 mg/ml and left for 1 h at +4 °C in the dark. Solution was dialyzed against 100 mM sodium phosphate buffer pH 7.0 with minimum 3 solution changes. Finally, solution volume was measured to calculate modified enzyme concentration and absorbance of native and modified enzyme was recorded at 260 nm and 280 nm on UV-VIS spectrophotometer (Shimadzu Corporation UV-2501PC, Japan).

Immobilization of tyramine-HRP

Tyramide-carboxymethyl cellulose solution in 0.1 M Tris HCl buffer pH 7.0 was mixed with 0.1 U of glucose oxidase, 0.5 U of tyramine-HRP and 0.13 M glucose. Polymerization reaction started as soon as glucose was added so a total of 300 μl containing 2 % (w/v) tyramide-CMC was quickly poured into 600 μl of light mineral oil with 3 % Span 80 and stirred for 10 minutes on magnetic stirrer. Reaction was stoped using 1 ml of 0.5 % Triton X-100 in 5 % $CaCl_2$ solution and stirred for another 5 minutes. Immobilized tyramine-HRP onto CMC microbeads was rinsed 3 times with Triton X-100 and 3 times with $CaCl_2$ in 10 mM Tris HCl buffer pH 7.0 and stored at 4 °C.

Enzyme activity assay

Specific activity of tyramine-HRP immobilized inside tyramide-CMC microbeads was measured by adding 150 μl of 60 % (v/v) of immobilized enzyme suspension into 3 ml of 13 mM pyrogalol containing 0.1 U glucose oxidase and 0.1 M glucose. Solution was constantly stirred during reaction and absorbance was measured after 10 min at 420 nm.

RESULTS AND DISCUSSION

In effort to minimize leaking of the enzyme from the carrier after immobilization, HRP was modified to introduce tyramine functional group into glycosidic side chain. HRP is folded as a single polypeptide chain, that is glycosylated at eight specific sites [7]. Peroxidase was oxidized with sodium periodate and subsequently modified with tyramine in a reductive amination reaction. Absorbances at 260 nm and 280 nm were recorded to confirm the modification (Table 1).

***Table 1** Absorbance of 0.33 mg/ml HRP and tyramine-HRP at 260 nm and 280 nm*

Wavelenghts	$A_{260\text{ nm}}$	$A_{280\text{ nm}}$
HRP	0.264	0.278
Tyramine-HRP	0.247	0.292

Increased absorbance at 280 nm of tyramine-HRP compared to the native enzyme originates from tyramine since its absorbance maximum is at 275 nm [9].

Tyramine-HRP immobilization was carried out in emulsion polymerization reaction resulting in carboxymethyl cellulose microbeads formation. Tyramide-carboxymethyl cellulose was enzymatically crosslinked and HRP was covalently immobilized onto hydrogel microbeads through phenol moieties. Instead of using hydrogen peroxide, it was gradually generated in glucose oxidase reaction with glucose as substrate. Obtained specific activity of immobilized tyramine-HRP after 1, 24 and 48 h is presented in Figure 1.

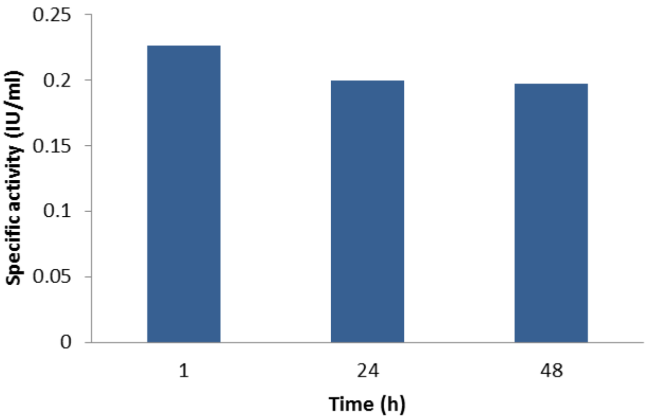

Figure 1 *Specific activity (U/ml) of immobilized tyramine-HRP versus time (h)*

Specific activity of the obtained immobilized enzyme was 0.227 U/ml immediately after immobilization. Recorded activity 24 and 48 h after immobilization, 0.200 U/ml and 0.197 U/ml respectively, showed that enzyme leakage from the carrier was reduced to 13 %.

CONCLUSION

This study showed that modified tyramine-HRP was successfully immobilized onto carboxymethyl cellulose carrier. With introduction of tyramine on the glycosidic side chain of HRP the enzyme was attached covalently to the matrix in a peroxidase catalyzed oxidation of phenolic groups in the presence of internally delivered hydrogen peroxide. Retained specific activity after 48h of storage was 87 % of the initial 0.227 U/ml. Therefore, leaking of the enzyme from the matrix has been significantly reduced due to stronger, covalent attachment of HRP inside crosslinked hydrogel polymeric network. Obtained biocatalyst has great potential for applications in wastewater treatment and phenol removal from polluted waters.

ACKNOWLEDGEMENT

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ADSORPTION OF METHYLENE BLUE FROM AQUEOUS SOLUTION USING ACTIVATED CARBON NORIT HYDRODARCO C

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Abstract

The discharge of colored wastewater from different industries into natural streams has caused many significant problems such as increasing the toxicity and chemical oxygen demand (COD). This work describes the adsorption process of one of the most dominant dye in textile and paper industry, methylene blue (MB) from aqueous solutions with commercially available activated carbon (AC). The adsorption process is generally known as effective and complementary to conventional treatments in the removal of dyes compounds. Adsorption studies were performed on powdered activated carbon Norit Hydrodarco C, in ambient temperature, on pH value between 2 – 10, with different masses of adsorbent (20 – 200 mg), The results show that commercial activated carbon Norit Hydrodarco C can be applied in the process of adsorption for the purpose of removing MB from aqueous solution.

Keywords: methylene blue, adsorption, activated carbon, pH value

INTRODUCTION

Every day growth of industrial, agricultural and domestic activities is resulted in the production of large amounts of wastewater containing a number of toxic materials which are continuously polluting the available fresh water [1]. Many industries such as textile, chemicals, refineries, leather, plastic, and paper, use different kinds of dye stuffs in various processing steps and these dyes are chemically and photolytically stable with different complex of aromatic structures [2]. Most commercial dyes due to their chemical stability and difficulty in decomposition cause serious environmental and health problems such as mutagenic and carcinogenic effects [3]. Such colored effluents reduce the sunlight penetration to the aquatic environment and significantly hinder photosynthetic processes. On the other hand, dyes by reducing oxygen levels in water lead to suffocation of aquatic flora and fauna [4].

Among various developed technologies for water purification, sorption is proven as a superior one because of its cost-effectiveness, simplicity of operation, insensitivity to toxic pollutants, etc. [5]. Due to their wide availability and environmental safety, materials such as commercial activated carbon are the most commonly used sorbents for the removal of pollutants from wastewater.

The adsorption process is preferred as it is environmentally friendly with high treatment efficiency and where the selection of adsorbent plays important role in determining its cost effectiveness [6].

Methylene blue (MB) (Figure 1) (3,7-bis(Dimethylamino)-phenothiazin- 5-iumchloride) as the selected model compound (thiazine cationic dye) is commonly used for coloring paper, temporary hair colorant, dyeing cottons, wools, etc. Although MB is not considered to be a very toxic dye, it can reveal very harmful effects on living things such as difficulties in breathing, vomiting, diarrhea and nausea [7].

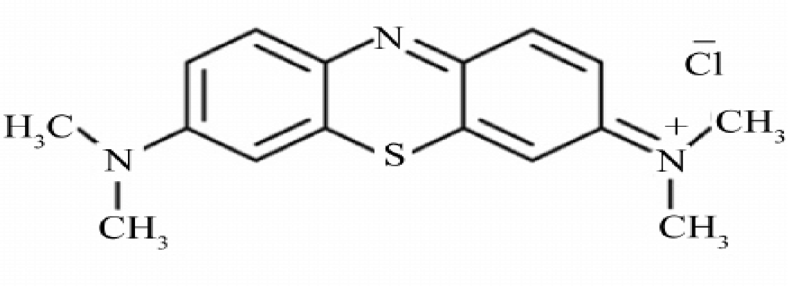

***Figure 1** Chemical structure of Methylene blue [8]*

The aim of this study was to investigate the adsorption of methylene blue onto commercial activated carbon Norit Hydrodarco C. The effects of contact time and pH on methylene blue adsorption were evaluated.

MATERIALS AND METHODS

In this study commercial activated carbon Hydrodarco C by Norit, Belgium, was used. Activated carbon is used directly before any kind of treatment such as washing and drying. Methylene blue was chosen for this study because of its known strong adsorption onto solids. Methylene blue (CI = 52015; chemical formula: $C_{16}H_{18}ClN_3S$; molecular weight = 319.86 g/mol; maximum wavelength = 662 nm) supplied by Merck was used as adsorbate and was not purified prior to use.

An accurately weighted amount of MB was dissolved in deionized water to prepare 1000 mg/L as stock solution, while the working solution was prepared by diluting this solution to the required initial concentrations.

For adsorption experiments, 50 mL of dye solution of known initial concentration, in Erlenmeyer flasks was agitated on stirrer (model Heidolph Unimax 1010) with a certain amount of adsorbent at desired pH at room temperature, with speed of 140 RPM for 20 minutes. pH value of MB solution was 6.0 and during experiments it was adjusted with 0.1 N HCl or 0.1 N NH_3OH and measured by using Multi 3320 pH-meter with a combined pH electrode. The pH-meter was standardized with NBS buffers before every set of measurement.

The MB concentration evaluation was carried out using UV–visible spectrophotometer (model HACH Lange) at a wavelength of 668 nm. The removal percentage of MB was calculated using the following relationship.

$$\%MB\ removal=\left(\frac{C_0\text{-}C_t}{C_0}\right)\times 100 \tag{1}$$

where C_0 (mg/L) and C_t (mg/L) are the dye concentration at initial and after time t respectively. The entire experiment was conducted in Accredited Laboratory for the monitoring of landfill, wastewater and air, at Department of Environmental Engineering and Occupational Health and Safety, Faculty of Technical Sciences, Novi Sad, Serbia.

RESULTS AND DISCUSSION

In order to determine the effects of the adsorbent dosage on MB adsorption efficiency on commercial activated carbon, different amounts (20, 50, 70, 100, 150 and 200) mg of Norit Hydrodarco C were mixed with 50 mL of MB solution, initial concentration of 85 mg/L.

By increasing the amount of adsorbent, adsorption capacity significantly increased. The removal percentage increased rapidly and further addition has not significantly affected the MB removal percentage. Therefore, 50 mg of adsorbent was selected for subsequent work (Figure 2).

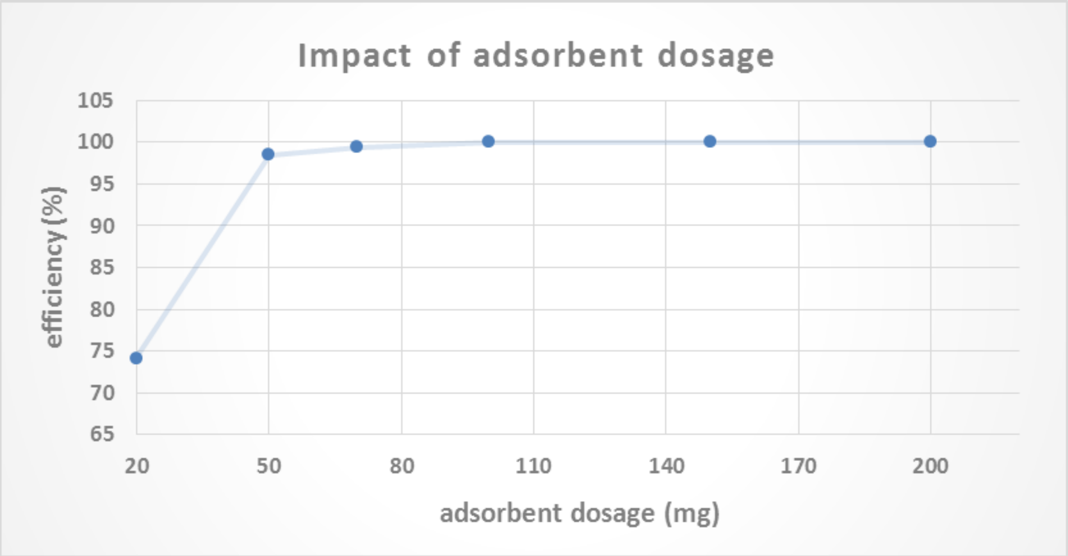

Figure 2 *Mass odsorbent effect on the adsorption of MB onto Norit Hydrodarco C (T = 25°C, C_0 = 85 mg/L, V = 50 mL, contact time = 20 min, pH value >9)*

Figure 2 shows that on the smallest mass of adsorbents, 20 mg, the efficiency of MB removal is about 75%, with a mass of 50 mg efficiency is higher than 98%. With higher dosage of adsorbent, efficiency in the removal of MB is 100%.

After determination of the adsorbent dosage effects on MB adsorption efficiency on commercial activated carbon Norit Hydrodarco C, using different amounts of adsorbent, it was found that 50 mg is ideal mass for this study and this amount was applied in order to determine a favourable pH value.

As it is known, the solution pH has a dominant impact on ions sorption. Figure 3 shows the effect of the solution pH on the adsorption of MB on the AC Norit Hydrodarco C.

The initial MB solution with a concentration of 85 mg/L has the pH value 6.0 and after adding 50 mg of adsorbent in volume sample of 50 mL, pH value was 9.5. The influence of pH values was analysed at different pH points (2, 3, 5, 7, 8, 10) using 0.1 N HCl or 0.1 N NH_3OH. On lower pH values and acidic environment, efficiency of MB removal is slightly lower than in alkaline environment.

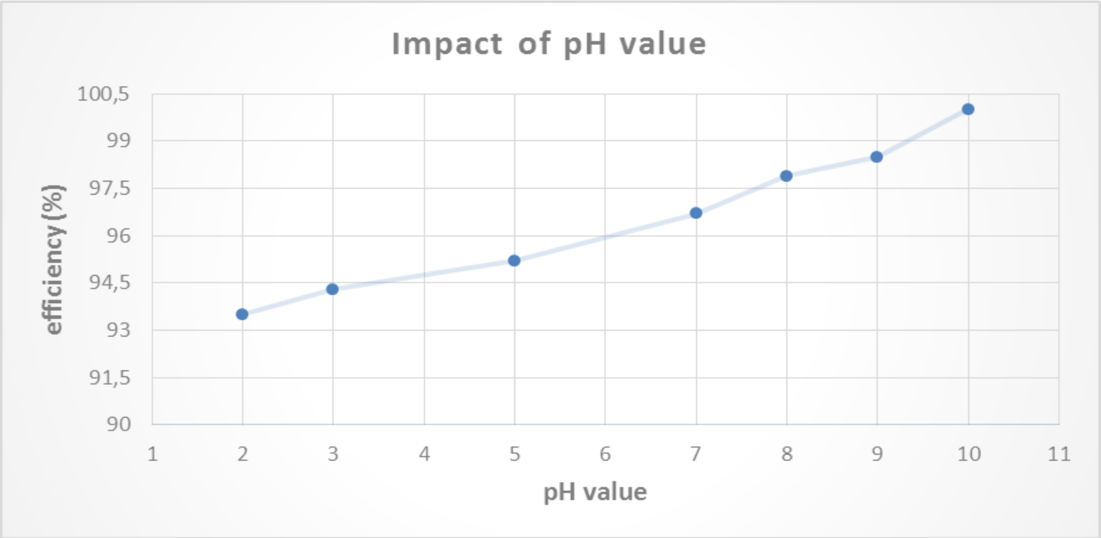

Figure 3 *pH value effect on the adsorption of MB onto Norit Hydrodarco C (T = 25°C,* C_0 *= 85 mg/L, V = 50 mL, contact time = 20 min)*

Figure 3 shows small deviations in efficiency of removal MB by AC Norit Hydrodarco C, on lower pH values. The small reduction of the MB adsorption in the acidic solutions could be attributed to the neutralization of free OH groups on the surface of AC Norit Hydrodarco C by bonding with H^+ from HCl used for pH adjustment. Accordingly, since it was found that the highest sorption efficiency was obtained at pH 9.5 further experiments should be prepared under the same pH condition.

CONCLUSION

The conducted study and presented results demonstrate that it is possible to use Norit Hydrodarco C as a highly efficient adsorbent for the removal of pollutants from water solutions. The obtained results show that the efficiency of Norit Hydrodarco C, for the removal of methylene blue, a non-degradable cationic dye, from aqueous solutions, depends more on the adsorbent amount and not so much of pH value of the dye solution. AC shows excellent abilities in the adsorption process at all pH values and the most important data is that, free OH groups on the surface of AC Norit Hydrodarco C results of no use of an acid in experiments and possibility of next research under the same pH condition.

ACKNOWLEDGEMENT

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CHARACTERIZATION OF CHROMATE RESISTANT AND REDUCING BACTERIAL STRAINS

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Abstract

Microorganisms can be used in bioremediation of chromium contaminated sites as they can biosorb chromium and/or reduce highly toxic Cr(VI) to less toxic Cr(III). We investigated 13 environmental Bacillus cereus group strains with high chromate resistance and bioreduction ability, by determining their biofilm formation potential (BFP) in different conditions and tolerance to other heavy metals. Biofilm formation potential was lower on nutrient rich LB medium compared to minimal M9 medium. Concentration of 0.1 mM Cr(VI) did not influence BFP, while 1 mM caused a decrease in bacterial growth accompanied with decreased BFP in most strains. Very adherent or moderately adherent strains have the potential to be used in immobilized form in chromate bioremoval. Remaining strains with the low BFP could be used in the processes with free suspended cells. All tested strains had almost identical heavy metal MIC values, indicating their metal homeostasis is based on conserved mechanisms present in the B. cereus group. Wolfram and molybdenum were the least toxic heavy metals among those tested (MIC >30 mM), while silver was the most toxic (MIC 0.5 mM for all the strains). To conclude, strains possess multiple metal tolerance and varying BFP, indicating a potential for bioremediation application.

Keywords: hexavalent chromium, heavy metal pollution, bioremediation, biofilm

INTRODUCTION

Chromium (Cr) is a heavy metal used in an array of industrial applications and, as a result, is a common pollutant of soil, sediment, surface and ground water. In the environment, Cr is mostly present in the two stable oxidation states: hexavalent [Cr(VI)] and trivalent [Cr(III)]. Naturally occurring geogenic Cr is almost exclusively found in trivalent form, while anthropogenic pollution releases hexavalent Cr compounds. Trivalent Cr has very low bioavailability in the environment and shows little effect on living organisms [1]. Contrary, hexavalent Cr is highly water soluble and bioavailable, with pronounced toxic, carcinogenic, mutagenic and teratogenic effects [2].

Certain microorganisms have the ability not only to grow in the presence of high chromium concentrations, but also to reduce highly toxic hexavalent to the more innocuous trivalent form. Microorganisms can also be used in chromium biosorption from aqueous media and wastewater. Therefore, they can be employed in bioremediation of contaminated environments in an environmentally friendly and cost-effective way. For the successful use of microorganisms in bioremediation, a detailed knowledge of their biology, physiology and metal tolerance mechanisms is crucial.

Ability to grow in the form of a biofilm could be a useful trait for practical use of microorganisms in bioremediation [3]. Biofilms are microbial communities consisting of microbial cells which are attached to surfaces and encased within EPS (extracellular polymeric substances) matrix. EPS can contribute to higher metal resistance and metal biosorption [4]. Increased resistance to heavy metals in biofilm form facilitated by different mechanisms has been proven in many studies as reviewed by Harrison et al. [3]. Finally, secondary waste water treatment is often based on the activity of a biofilm formed on various types of carriers (e.g. trickling sand filters, rotating biological contactors, sequencing batch reactors) [5]. Taken together, strong biofilm formation potential could be considered as an advantage for strain's prospective bioremediation use.

Waste materials rarely contain only one or few pollutants. In majority of cases, waste contains complex mixture of polluting substances belonging to various chemical types. Consequently, it would be beneficial if microorganisms employed in Cr bioremoval are tolerant to a wider range of pollutants.

Having this in mind, we tested a number of bacterial chromate reducing strains for their biofilm formation potential and heavy metal tolerance.

MATERIALS AND METHODS

Chromate resistant and reducing bacterial strains

Strains examined in the present study were isolated from a range of environmental samples including both Cr contaminated and uncontaminated samples, as described previously [6]. Four strains (LCr6, LCr7, LCr8 and LCr9a) were isolated from soil and water samples obtained from the unpolluted salt marshes. Six strains (NCr1a, NCr1b, NCr1c, NCr2, NCr3 and NCr4) originated from serpentine soils with elevated concentrations of chromium of natural geological origin. Finally, three strains (PCr1, PCr2a and PCr12) were isolated from locations impacted by industrial Cr pollution.

All strains were identified as members of the *Bacillus cereus* group. Strains are highly resistant to hexavalent chromium (able to tolerate at least 2 mM Cr(VI) in the form of chromate anion in minimal media). To that, strains have a notable ability to reduce Cr(VI) to less toxic Cr(III) (some of them reduce up to 98.5 % of the starting 0.2 mM Cr(VI) after 72h).

Biofilm formation assay

Biofilm formation potential in media with different concentrations of Cr(VI) was tested by the standard microplate procedure [7]. Plates were filled with 100 μl of medium (LB or M9) with varying concentrations of Cr(VI). In LB medium Cr(VI) concentrations tested were 0, 0.1, 1 and 16 mM Cr(VI), while 0, 0.1 and 1 mM were tested in M9 medium, each in triplicate. Biofilm formation was assessed after 24 h or 7 days of incubation at 28°C by staining with crystal violet and spectrophotometrical measurement of absorbance at 550 nm as described previously [7]. Polystirene flat bottom plates (Biolite, Thermo Scientific) were used in all the assays. In addition to environmental isolates, reference strains *B. cereus* ATCC

14579, *B. pseudomycoides* DSM 12442, *B. subtilis* PY79 and *Pseudomonas aeruginosa* ATCC 15692 were also tested.

Heavy metal tolerance determination

Overnight culture of the strain on LB agar was used to make a suspension of 2 McFarland units in sterile saline solution. Suspension was spot inoculated on M9 agar plates with various concentrations of heavy metals (10 µl per spot). M9 agar plates with various concentrations of heavy metals were prepared by adding filter-sterilized stock solutions of heavy metal to an autoclaved medium cooled to 45°C. Each strain was tested on three separate plates. After 7 days of incubation, absence/presence of growth was detected by visual inspection. The lowest metal concentration on which growth was completely inhibited was recorded as MIC. In addition to environmental isolates, reference strains *Bacillus cereus* ATCC 14579, *B. subtilis* PY79, *B. pseudomycoides* DSM 12442 and *Escherichia coli* ATCC 25922 were also tested.

RESULTS AND DISCUSSION

Influence of Cr(VI) on biofilm formation potential

Living in the form of a biofilm provides microorganisms with increased protection from many environmental factors, including heavy metal toxicity. Also, microorganisms with the ability to form biofilm can easily be immobilized on different types of carrier materials and subsequently used in waste treatment. Therefore, biofilm formation could be a useful trait for practical use of microorganisms in bioremediation.

For that reason, we tested the ability of the 13 chromate tolerant *Bacillus cereus* group isolates to form biofilm in two media (nutrient rich LB and minimal M9 medium) after 1 and 7 days of incubation. Influence of different Cr(VI) concentrations on biofilm formation potential was also examined. Biofilm formation potential was categorized in 4 groups: 0 - unadherent; 1 - weakly adherent; 2 - moderately adherent; 3 - very adherent [8]. Results are presented in Table 1.

Biofilm formation potential was lower on nutrient rich LB medium compared to minimal M9 medium. Longer incubation did not promote biofilm formation. In LB medium strains were generally unadherent or weakly adherent, except for the reference strains B. subtilis PY79 and P. aeruginosa ATCC 15692, which were more adherent in LB compared to M9 medium.

Biofilm formation potential was the highest in M9 medium without Cr(VI) after 1 day of incubation. In stated experimental conditions, strains NCr4, PCr12, LCr7, LCr8 and LCr9a were moderately adherent, while LCr6 was very adherent. Concentration of 0.1 mM Cr(VI) did not influence biofilm formation, while 1 mM concentration caused decrease in bacterial growth accompanied with decreased biofilm formation potential in most strains. In some instances, biofilm formation potential was higher in the presence of Cr(VI) compared to no Cr(VI) medium.

Reference strains of B. cereus group (B. cereus ATCC 14579 and B. pseudomycoides DSM 12442) showed similar biofilm formation potential as environmental isolates. They were unadherent to moderately adherent in most of the experimental conditions. Reference strain B. subtilis PY79 exhibited higher biofilm formation, especially in LB medium after 24h, when it was very adherent.

***Table 1** Biofilm formation potential of chromate tolerant Bacillus cereus group strains in various concentrations of Cr(VI) (mM) in LB and M9 medium*

Strain	Incubation duration, medium and Cr(VI) concentration (mM)													
	1 day							7 days						
	LB				M9			LB				M9		
	0	0.1	1	16	0	0.1	1	0	0.1	1	16	0	0.1	1
NCr1a	0	0	0	0	0	1	1	0	0	0	0	1	1	1
NCr1b	0	0	0	0	1	1	1	1	0	0	0	0	1	1
NCr1c	0	0	0	1	1	1	1	1	0	0	0	1	1	1
NCr2	0	0	0	0	1	1	1	1	0	0	0	0	1	1
NCr3	0	0	0	0	0	1	1	0	0	0	0	1	1	1
NCr4	0	0	1	1	2	2	1	1	0	0	0	0	1	1
PCr1	0	1	1	1	1	1	1	1	1	0	0	1	1	1
PCr2a	1	1	1	1	1	1	0	0	0	0	0	0	1	1
PCr12	0	0	1	1	2	2	1	0	0	0	0	0	0	1
LCr6	0	0	0	1	3	3	0	0	0	0	0	0	1	1
LCr7	0	0	0	0	2	2	0	0	0	0	0	0	0	1
LCr8	0	0	0	1	2	2	0	0	0	0	0	1	1	1
LCr9a	0	0	0	1	2	1	1	0	0	0	0	0	1	1
B. cereus ATCC 11775	0	0	1	1	2	1	1	0	0	0	0	1	1	1
B. pseudomycoides DSZM 12442	0	0	0	1	1	1	1	0	0	0	0	1	1	1
B. subtilis PY79	1	2	1	0	1	1	1	3	3	1	0	1	1	1
P. aeruginosa ATCC 15692	3	3	3	2	1	1	1	2	2	2	2	1	1	1

0 (red) - unadherent; 1 (yellow) - weakly adherent; 2 (green) - moderately adherent; 3 (blue) - very adherent

Treatment of Cr contaminated wastewater has been conducted by biofilms on different types of carrier materials. For instance, glass beads, pebbles, coarse sand [9], activated carbon granules [10,11], alginate [12] or granular biofilms [13] have been employed. Biofilm-based reduction of Cr(VI) has certain advantages: *(i)* the cells in biofilm form can tolerate higher Cr(VI) concentrations compared to planktonic cells, and *(ii)* separation of the treated liquid from the biomass is comparably easier [13].

Strain which proved to be very adherent (LCr6) or moderately adherent (NCr4, PCr12, LCr7, LCr8 and LCr9a) have the potential to be used in immobilized form for chromate bioremoval. Parameters of the process (carrier material choice, temperature, medium composition, pH, etc.) could be optimized in order to further improve their biofilm formation potential. Unfortunately, other strains isolated in the present study did not exhibit particularly high biofilm formation potential. However, their high chromate tolerance even in the planktonic form offers the opportunity of their application in waste treatments with free suspended cells, such as activated sludge (especially true for strain NCr1a which forms highly flocculent growth in liquid culture).

Heavy metal tolerance

As pollution is rarely comprised of only one particular pollutant, multiple heavy metal tolerance would be an advantage for a strain in prospective bioremediation application. Thus, we tested heavy metal tolerance of chromate tolerant *Bacillus cereus* group isolates (Table 2). Reference *Bacillus* sp. and *E. coli* strains were also included in the measurements.

MIC values of Hg, Cd and Zn were out of the scope of the testing and were reported as higher than the highest tested concentration. Thus, their MIC could possibly be much higher. For that reason, their toxicity was not discussed in detail.

Majority of strains exhibited similar pattern of metal tolerance, while some strains had different response. Wolfram (W) and molybdenum (Mo) were the least toxic heavy metals among those tested, with MIC values of >30 mM for all the tested strains. The most toxic metal was silver (Ag) with MIC value of 0.5 mM for all the strains (the lowest precisely determined MIC among the metals tested). Strains NCr1a, *B. subtilis* PY79 and *B. pseudomycoides* DSM 12442 were more sensitive to cadmium (Cd) than remaining strains – their MIC was 0.5 mM compared to 4mM for the rest of the strains. Generally, *B. subtilis* PY79 was the most sensitive strain, with comparably lower tolerance to Hg, Cd, Cu, Co, Ni and Zn. *E. coli* ATCC 25922 was more sensitive to Cr(VI), compared to other strains with MIC of 2 mM.

Based on our results, general order of toxicity of heavy metals to *Bacillus cereus* group chromate resistant isolates, in terms of MIC values could be (most toxic to least toxic from left to right):

$$Ag^{+} > Cu^{2+}, Cr^{6+} > Co^{2+}, Ni^{2+} > Cr^{3+} > W^{6+}, Mo^{6+}$$

***Table 2** Heavy metal resistance of chromate tolerant Bacillus cereus group isolates*

Strain	MIC (mM)									
	Hg^{2+}	Ag^{+}	Cd^{2+}	Cu^{2+}	Co^{2+}	Ni^{2+}	Zn^{2+}	Cr^{6+}	Cr^{3+}	W^{6+}	Mo^{6+}
NCr1a	>0.1	0.5	0.5	10	10	10	>10	10	30	>30	>30
NCr1b	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
NCr1c	>0.1	0.5	>4	10	>10	>10	>10	10	30	>30	>30
NCr2	>0.1	0.5	>4	10	>10	10	>10	10	30	>30	>30
NCr3	>0.1	0.5	>4	10	10	>10	>10	10	30	>30	>30
NCr4	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
PCr1	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
PCr2a	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
PCr12	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
LCr6	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
LCr7	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
LCr8	>0.1	0.5	>4	10	10	10	>10	10	30	>30	>30
LCr9a	>0.1	0.5	>4	10	>10	10	>10	10	30	>30	>30
BC	>0.1	0.5	>4	10	>10	10	>10	10	30	>30	>30
BS	0.1	0.5	0.5	<0.1	0.5	1	10	10	30	>30	>30
BP	>0.1	0.5	0.5	10	>10	10	>10	10	30	>30	>30
EC	>0.1	0.5	>4	>10	>10	10	>10	2	30	>30	>30

BC – *Bacillus cereus* ATCC 14579; BS – *B. subtilis* PY79; BP – *B. pseudomycoides* DSM 12442; EC - *Escherichia coli* ATCC 25922; green color indicates the highest and orange color the lowest metal tolerance among the tested strains.

It should be noted that only a limited range of concentrations with large gaps in between was tested. Therefore, the results obtained give only a rough preliminary assessment of heavy metal tolerance.

As expected, trivalent chromium was less toxic to strains than hexavalent form (MIC of Cr(VI) was 10 mM, while MIC of Cr(III) was 30 mM). Although it is uniformly regarded that Cr(VI) is far more toxic than Cr(III) [2,14], some studies found significant negative effects of trivalent chromium as well. For instance, among bacteria isolated from treated tannery effluents and agricultural soil irrigated with these effluents, more were resistant to Cr(VI) than to Cr(III) [15].

In general, chromate tolerant isolates as well as reference *B. cereus* group strains were comparably more metal tolerant than *B. subtilis* PY79. Specifically, *B. cereus* group strains were more resistant to Hg, Cd, Cu, Co, Ni and Zn. All tested *B. cereus* group strains had almost identical MIC values, indicating their metal homeostasis is based on conserved mechanisms innately and constitutively present in all the strains of the group. This multiple metal tolerance could be advantageous for their application in bioremediation.

CONCLUSION

A number of strains reported in this study have the ability to resist high Cr(VI) concentrations and to reduce it to less toxic Cr(III). Moreover, certain strains can be employed in the form of immobilized biomass on carrier materials as they possess moderate to high ability to form biofilm. Nonadherent strains could be used in the form of suspended planktonic cells. Strains exhibited tolerance to other heavy metals, which is also a useful trait for bioremediation application as pollution is rarely present in the form of single pollutant. Taking all in account, strains possess high potential for further bioremediation investigation.

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HEXAVALENT CHROMIUM REDUCTION OF INDIVIDUAL BACTERIAL STRAINS AND CONSORTIA

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Abstract

Hexavalent chromium is a serious environmental pollutant due to its high toxicity, bioavailability and solubility. A variety of microorganisms with the capability to reduce hexavalent form of chromium to its less toxic trivalent form have been identified. In this study, we investigated the ability of 21 bacterial consortia, each consisting of two environmental bacterial strains, to grow in the presence of Cr(VI) and to reduce it to Cr(III). Reduction of Cr(VI) was tested on a minimal medium (M9) containing 0.5 mM Cr(VI). The growth in the presence of 0.5 mM Cr(VI) was also measured and compared to the growth in medium without Cr(VI). The results indicate that the highest percentage of chromium reduction after 48h of incubation is shown by consortia containing strains Arthrobacter ilicis LCr10 or Cellulosimicrobium cellulans PCr3b, in combination with various strains from Bacillus cereus group. Our results also indicate that the consortia exhibit significantly higher efficacy in chromium reduction than any individual strain tested.

Keywords: chromate reduction, bioremediation, bioreduction, bacteria

INTRODUCTION

Chromium is a common contaminant of aquatic and terrestrial ecosystems, due to its wide use in industrial processes [1]. Chromium has several oxidation states, but in nature it is present mostly in the state of stable hexavalent or trivalent form. Trivalent form is biologically inert, with little or no effect on cells and it can be naturally found in the environment or released by pollution [2]. In contrast, hexavalent form has toxic, mutagenic and carcinogenic effects on cells, because of its high water solubility, bioavailability and transport across the cell membrane [3]. Hexavalent chromium is released in the environment by anthropogenic pollution.

Many bacterial strains resistant to chromium are already isolated and investigated. Mechanisms of resistance to chromium include reduced uptake of chromium ions, active efflux of Cr(VI) from the cytoplasm, intracellular/extracellular reduction, DNA reparation and protection against oxidative stress. These mechanisms may be encoded by chromosomal genes or by plasmids [4]. The most studied and the most common mechanisms of resistance to chromium compounds are chromium reduction and biosorption, due to their applicability in removal of chromium pollution [5].

Conventional physicochemical methods for remediation of contaminated sites are numerous and include precipitation, adsorption, ion exchange, adding chemical reducing agents and others. However, these methods are expensive, environmentally damaging and require a lot of infrastructure and maintenance. In addition, the use of these techniques can lead to the creation of highly toxic secondary contaminants [6,7]. In contrast, bioremediation through bioaugmentation and biostimulation processes is cost-effective and environmentally friendly solution for the treatment of polluted areas.

There are many experiments indicating that the significant number of bacterial strains is capable of reducing hexavalent chromium under the laboratory conditions, such as *Escherichia coli* [8], *Pseudomonas putida* [9], *Streptomyces sp.* [10], *Cellulosimicrobium sp.* [11], *Arthrobacter sp.* [12], *Ochrobactrum tritici* [13], *Bacillus cereus* [14] and many more. A significantly smaller number of studies is conducted in order to test the ability of bacterial consortia to reduce chromium [15–18]. Some of these results demonstrate that the utilization of microbial consortia in chromium reduction is much more effective than the use of pure bacterial cultures, indicating that, in the future, microbial consortia could be powerful tool for bioremediation of chromium, and possibly other heavy metals.

Having this in mind, we tested chromium reduction ability of a number of different bacterial consortia consisted of two bacterial strains, and compared their activity to the activity of a single bacterial strains.

MATERIALS AND METHODS

Description of bacterial strains and consortia

Bacterial strains used in this study were previously isolated and their resistance on hexavalent chromium was tested by Tamindžija *et al.* [14]. Out of all tested strains (total of 37), those with the highest percentage of chromium reduction (Table 1) were selected for further examination and were used to create 21 different bacterial consortia, consisting of two strains each.

Table 1 *Individual strains used to form consortia*

Strain	Strain mark	Sample type
Arthrobacter ilicis	LCr10	Low Cr
Bacillus cereus	NCr2	High Cr of natural origin
Bacillus cereus	PCr2a	Low Cr
Cellulosimicrobium cellulans	PCr3b	High Cr caused by pollution
Microbacterium marytipicum	PCr11	High Cr caused by pollution
Ochrobactrum grignonense	PCr2b	Low Cr
Bacillus cereus	NCr1a	High Cr of natural origin

Reduction experiments

Reduction experiments were conducted on modified minimal medium M9 containing the following per litre of distilled water: Na_2HPO_4, 6 g; KH_2PO_4, 3 g; NH_4Cl, 1 g; NaCl, 0.5 g. This basal medium was autoclaved and then supplemented with the following solutions:

glucose (3 mM), 4 ml; $MgSO_4$ (1 mM), 1 ml; thiamine (3 mM), 1 ml; $CaCl_2$ (0.1 mM), 1 ml; yeast extract (0.5 g L^{-1}), 5 ml.

Strains were grown in M9 medium for 24h at 26°C, washed three times and resuspended in saline solution. Suspension was used for inoculation of two variations of M9 medium – one without Cr(VI) and the other with the 0.5 mM Cr(VI) added in the form of a K_2CrO_4 stock solution. Each strain in consortium was added to an optical density of 0.25 MF units. This resulted in a starting optical density in all test tubes of 0.5 MF units, which is approximately $1.5x10^8$ CFU/ml (DEN-1 McFarland densitometer). In the case of individual strains starting optical density was also set to 0.5 MF units.

Inoculated test tubes were incubated aerobically for 48h, at 150 rpm (IKA KS 4000i control), at 26°C, in dark.

Evaluation of chromium reduction and bacterial growth

Residual Cr(VI) concentration was determined through reaction with diphenylcarbazide (DPC). Aliquots (300 µl) of incubated cultures were taken at 0, 24 and 48h in order to conduct DPC test. The reaction mixture contained 225 µl H_2SO_4, 50 µl sample and 125 µl DPC (2.5 mg ml^{-1}). Absorbance was determined spectrophotometrically at 540 nm (Thermo Scientific Multiskan GO). Residual Cr(VI) concentration was calculated from absorbance using the calibration curve made from a series of standard chromium solutions. Concentration was used to calculate the percentage of chromium reduction during 24h and 48h period of incubation.

The optical density of incubated cultures was measured at 24 and 48h spectrophotometrically at 600 nm. It was further used to calculate the percentage of bacterial growth on medium with 0.5 mM Cr(VI), compared to the growth in medium without Cr(VI).

RESULTS AND DISCUSSION

In this study, total of 21 consortia and 7 individual bacterial strains were analyzed. Percentage of reduction for all of the 7 individual strains was above 30% (Table 2). As can be seen in Table 2, the most effective strains were *Cellulosimicrobium cellulans* PCr3b, *Bacillus cereus* NCr2 and NCr1a. All of the strains showed high percentage of growth (>68%), after the incubation period of 48h.

Table 2 *Percentage of Cr(VI) reduction and growth by individual strains after incubation period of 48h*

Strain	Strain mark	Reduction 24h±stdev	Reduction 48h ±stdev	Growth 24h±stdev	Growth 48h±stdev
Arthrobacter ilicis	LCr10	33.4±3.6	34.5±3.2	94.1±13.1	83.1±5.5
Bacillus cereus	NCr2	39.0±0.8	62.5±6.6	76.9±14.2	83.6±9.8
Bacillus cereus	PCr2a	32.0±2.4	48.0±3.0	81.9±13.9	68.9±8.9
Cellulosimicrobium cellulans	PCr3b	61.7±0.2	68.4±1.3	116.7±10.2	98.4±0.2
Microbacterium marytipicum	PCr11	16.7±2.5	33.1±3.8	88.4±14.0	95.1±7.0
Ochrobactrum grignonense	PCr2b	33.6±12.1	46.8±1.6	79.6±3.2	86.4±1.9
Bacillus cereus	NCr1a	39.6±2.5	68.9±2.6	98.7±11.0	79.1±3.3

Some of the previous studies show that the bacterial species belonging to genera *Arthrobacter* [19], *Bacillus* [14] and *Cellulosimicrobium* [11] are exhibiting high capability of chromium reduction.

Most of the consortia show reduction percentage of over 50% after the incubation period of 48h (Table 3). Consortia consisting of either *Arthrobacter ilicis* or *Cellulosimicrobium cellulans*, in combinations with various strains of *Bacillus cereus* group, exhibited the highest percentage of reduction, with values of 100% or nearly 100%. All consortia exhibited high percentages of growth after incubation period of 24h (>60%) and 48h (>50%). The results suggest that the growth of some consortia declined slightly after 48h, while the percentage of growth of other consortia increased, compared to the growth after 24h.

***Table 3** Percentage of Cr(VI) reduction and growth of consortia after incubation period of 24 and 48h*

	Strains in consortium	Strain mark	Reduction 24h ± stdev	Reduction 48h ± stdev	Growth 24h ± stdev	Growth 48h ± stdev
1	*Arthrobacter ilicis* *Bacillus cereus*	LCr10 NCr2	53.3±11.1	100.0±0.0	82.9±5.5	85.3±14.6
2	*Arthrobacter ilicis* *Bacillus cereus*	LCr10 PCr2a	43.4±6.6	99.2±1.1	72.6±16.2	87.7±2.0
3	*Arthrobacter ilicis* *Cellulosimicrobium cellulans*	LCr10 PCr3b	58.6±2.0	73.2±2.2	114.1±15.1	104.3±7.2
4	*Arthrobacter ilicis* *Microbacterium marytipicum*	LCr10 PCr11	43.0±1.4	66.3±5.6	96.5±7.6	85.5±11.2
5	*Arthrobacter ilicis* *Ochrobactrum grignonense*	LCr10 PCr2b	39.5±6.8	62.4±8.6	85.0±7.2	78.2±1.0
6	*Arthrobacter ilicis* *Bacillus cereus*	LCr10 NCr1a	43.7±1.0	95.9±5.8	77.5±22.6	65.6±6.7
7	*Bacillus cereus* *Bacillus cereus*	NCr2 PCr2a	35.1±11.2	67.3±5.4	81.2±20.7	71.0±10.2
8	*Bacillus cereus* *Cellulosimicrobium cellulans*	NCr2 PCr3b	90.4±8.6	100.0±0.0	114.7±6.1	97.9±17.4
9	*Bacillus cereus* *Microbacterium marytipicum*	NCr2 PCr11	33.8±11.3	56.7±8.9	83.0±0.9	69.3±9.7
10	*Bacillus cereus* *Ochrobactrum grignonense*	NCr2 PCr2b	38.9±1.6	51.8±0.2	77.8±26.6	96.4±8.8
11	*Bacillus cereus* *Bacillus cereus*	NCr2 NCr1a	39.0±8.3	64.2±16.8	80.5±9.1	76.9±2.2
12	*Bacillus cereus* *Cellulosimicrobium cellulans*	PCr2a PCr3b	81.4±24.1	100.0±0.0	126.9±6.5	91.5±12.1
13	*Bacillus cereus* *Microbacterium marytipicum*	PCr2a PCr11	37.7±1.4	55.4±2.6	75.5±8.7	59.5±9.8

***Table 3** continued*

14	*Bacillus cereus* *Ochrobactrum grignonense*	PCr2a PCr2b	45.6±8.7	63.1±1.3	69.5±5.8	86.0±36.7
15	*Bacillus cereus* *Bacillus cereus*	PCr2a NCr1a	46.7±10.8	70.8±5.2	71.9±21.5	57.4±10.2
16	*Cellulosimicrobium cellulans* *Microbacterium marytipicum*	PCr3b PCr11	65.6±0.9	90.7±8.7	108.1±3.7	88.6±14.2
17	*Cellulosimicrobium cellulans* *Ochrobactrum grignonense*	PCr3b PCr2b	71.9±6.0	100.0±0.0	99.2±0.2	97.6±7.9
18	*Cellulosimicrobium cellulans* *Bacillus cereus*	PCr3b NCr1a	71.4±17.3	100.0±0.0	86.2±24.4	70.4±21.1
19	*Microbacterium marytipicum* *Ochrobactrum grignonense*	PCr11 PCr2b	26.7±8.3	39.8±10.4	83.3±8.5	88.4±5.7
20	*Microbacterium marytipicum* *Bacillus cereus*	PCr11 NCr1a	33.0±6.9	57.0±12.7	98.7±8.9	72.8±44.0
21	*Ochrobactrum grignonense* *Bacillus cereus*	PCr2b NCr1a	42.5±3.0	57.9±0.2	63.1±1.3	69.6±12.8

Percentage of reduction in almost every consortium significantly increased after the incubation period of 48h, compared to the values after 24h (Figure 1). Consortium number 8 from Table 3 showed very high percentage of reduction even after 24h, reducing more than 90% of Cr(VI). The lowest rate of reduction (≈40%) was shown by consortium number 19.

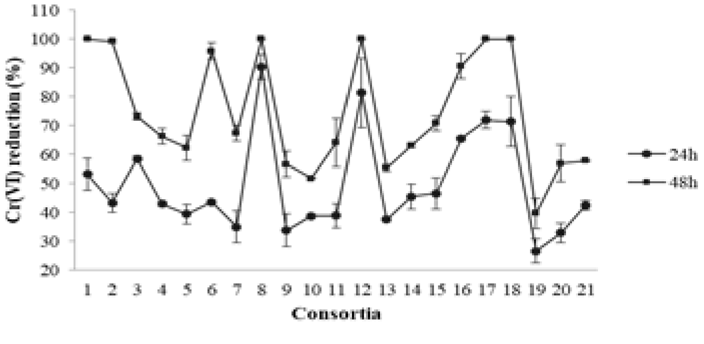

***Figure 1** Comparison of chromium reduction by consortia after incubation periods of 24 and 48h*

Comparison of reduction percentages of individual bacterial strains and consortia indicates that most of the strains are more effective in consortia than individually (Table 4). Table 4 shows by how much reduction improved, calculated by dividing percentage of reduction of consortium with the percentage of reduction of individual strain. Therefore, all of the strains with values over 1.00 are exhibiting higher reduction percentage in combination with some other strain, than individually. The results indicate that the strain *Arthrobacter ilicis* LCr10 shows the greatest improvement in reduction in combination with *Bacillus cereus* strains NCr2, PCr2a and NCr1a. Specifically, reduction improved nearly threefold in consortia compared to individual strains (2.90, 2.87 and 2.78-fold, respectively).

Table 4 *Comparison of reduction by individual bacterial strains and by consortia (reduction of consortium divided by the reduction of individual strain)*

Strain	LCr10	NCr2	PCr2a	PCr3b	PCr11	PCr2b	NCr1a
LCr10	-	2.90	2.87	2.12	1.92	1.81	2.78
NCr2	1.60	-	1.08	1.60	0.91	0.83	1.03
PCr2a	2.07	1.40	-	2.08	1.18	1.08	1.34
PCr3b	1.07	1.46	1.46	-	1.33	1.46	1.46
PCr11	2.00	1.71	1.67	2.74	-	1.20	1.72
PCr2b	1.33	1.11	1.35	2.14	0.85	-	1.24
NCr1a	1.39	0.93	1.03	1.45	0.83	0.84	-

Some of the previous experiments indicate that the utilization of consortia in bioremediation have the advantage over the use of individual strains, due to the synergy of their metabolic capacities [16]. Our results are in compliance with these earlier findings.

CONCLUSION

This study is pointing out the advantage of the utilization of microbial consortia in bioremediation, over the use of pure cultures. Our results indicate that the consortia exhibit significantly higher efficacy in chromium reduction than any individual strain tested. Significant differences between the growth percentages of consortia and individual strains were not shown, with strains exhibiting high growth rate either individually or in consortia.

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THE QUALITY OF IRRIGATION WATER AND ASSESMENT OF ITS USE IN TOPLICA DISTRICT

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Abstract

In the area of Toplica district in 2018, samples of irrigation water were sampled at 34 locations. Water quality assessment for irrigation was carried out using the traditional classifications by Stebler, Nejgebauer and the classification of the US Laboratory for saline soils and by the more recent FAO and RSC classifications. Following parameters were assessed: pH value - potentiometric; EC - electroconductivity - electrochemical; dry residue - thermogravimetric; ionic balance: CO_3^{2-}; HCO_3^-; Cl^- -volumetric. The acid-available fraction of heavy metals and other microelements (As, B, Cd, Cr, Cu, Fe, Ni, Pb, Zn) and SO_4^{2-}; Ca^{2+}; Mg^{2+} preparation and reading on the ICP-OES method EPA 200.7; Content K^+, Na^+ - plamenephotometric; SAR (Sodium Adsorpcio Ratio) - using calculation. In all tested water samples, the contents of the tested microelements and heavy metals were below the maximum permissible concentrations, except in one sample, number 29 from location no. 21 where an increased concentration of boron (B) content was determined. The reason for this phenomenon was probably related to the contamination of the accumulation due to the spillage of the fertilizers with high concentration of this element, were stored directly beside the water intake.

Keywords: classifications, quality, water, irrigation, contamination

INTRODUCTION

Irrigation is an agro-technical measure that allows stable yields of cultivated plants to be achieved and in this way satisfies the ever-growing needs for food due to population growth. Therefore, in many areas of the world without irrigation, agricultural production would not be possible and it is necessary to pay special attention to available water resources, both in quantity and quality [1]. Inadequate quality of irrigation water can significantly reduce the expected economic yield of agricultural production [2,3]. Water quality is a term used to describe the physical, chemical and biological parameters of water characteristics and defines the suitability for a specific purpose [4]. The application of water of inadequate quality can result in decay of soil structure, slower plant growth, deformation of the fruit, and in some cases the complete absence of plant growth. In order to overcome this, specific indicators for assessing the quality of water for irrigation have been adopted. Those indicators are a set of parameters that are widely accepted and used in the decision-making process [5].

Irrigation means the use of water from natural and artificial sources. Natural sources are from the waters and lakes, and artificial can be wells and artificial lakes. In addition, the source of water for irrigation can be wastewater from the settlement, from production and industrial capacities [6]. Water of inadequate quality can affect the salinity, alkalization and deterioration of water-physical properties of the soil [7,8].

It is very important to assess the risk of salinization of irrigation water source of any agricultural land in order to achieve maximum yields of cultivated crops [9]. For the assessment of the quality of water for irrigation, there are traditional and modern methods and classifications. None of them can be considered absolutely applicable to all conditions in plant production [10]. They are mainly based on the estimation of the total amounts of salt in water, relative to the concentration of Na^+ ion on the ion content Ca^{2+} and Mg^{2+}, the presence of the Cl and B salts and the electrical conductivity.

MATERIALS AND METHODS

Description of the area of research and methodology of sampling

Toplica district is located in the basins of the river Toplica and Kosanica, in the south of the Republic of Serbia in the central Balkan region (Figure 1). The research area in which research has been conducted extends from 42°52'-43°24' north latitude and from 20°55'to 21°49' east longitude and covers an area of approximately 2. 231 km^2.

The irrigation water sampling site coordinates are presented together with results of the analysis in Table 2. At the sites where research was conducted, the drip irrigation system is applied at nine locations; in eight places plants are irrigated by artificial rain, and within the three parcels it is planned to use some of the irrigation methods in the following period. 34 samples of irrigation water were sampled, 24 from wells, three from streams, four from artificial reservoirs, and three from lake.

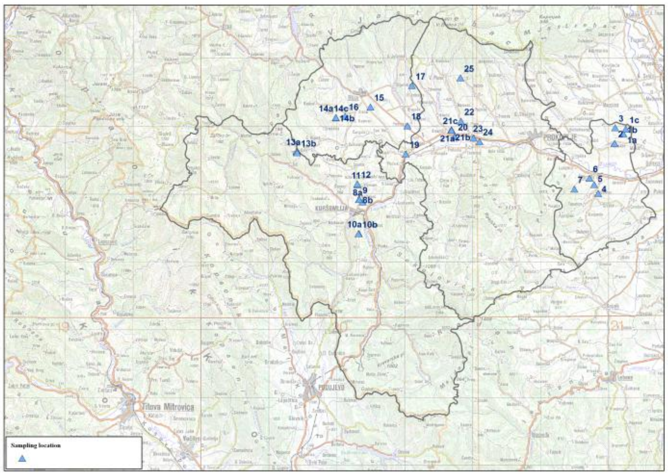

***Figure 1** Location map with selected sample sites*

Research methods

In the irrigation water samples, the following parameters were determined: pH - potentiometric (SRPS H.Z1.111: 1987) [11], electrical conductivity (EC) - (SRPS EN 27888: 1993) - electrometric [12]; the total dissolved solids content (TDS) - gravimetric [13]; CO_3^{2-}; HCO_3^-; Cl-volumetric, K^+; Na^+ - plamenfotometric (APHA) [14]. The content of heavy metals and other toxic elements (As, B, Cd, Cr, Cu, Fe, Ni, Pb, Zn) and SO_4^{2-}; Ca^{2+}; Mg^{2+}

were determined by EPA method 200.7, [15] on the ICAP 6300 ICP optical emission spectrometer (ICP-OES); (SAR) - by calculating [16].

RESULTS AND DISCUSSION

In relation to the Stebler classification, based on the estimation of the irrigation coefficient determined by the content of Na^+, Cl^-, SO_4^{2-}, all the tested irrigation water samples are of good quality, which means that they can be used without special measures to prevent the accumulation of harmful salts in the soil. 88.23% of the samples were with good water quality, 8.82% of satisfactory quality and 2.95% water were of unsatisfactory quality.

Based on the Nejgebauer classification [17], which takes into account the total amount of salt in irrigation water in interaction with the concentration of Na^+ with Ca^{2+} and Mg^{2+}, of the tested samples 55.9% belonged to the Ia class, in which the dry residue is below 700 mg l^{-1}, and the ratio (Ca+Mg) : (Na+K) is > 3, and 32.4% was in Ib class, where the dry residue is less than 700 mg l^{-1}, and the ratio (Ca+Mg): >3. These are impeccable water with ameliorative characteristics of flushing salt marsh. IIa (2.9%) class, where the dry residue is less than 700 mg l^{-1}, and the ratio (Ca+Mg): Na> 1 and IIIb (8.8%) class, where the dry residue is less than 700-3000 mg l^{-1}, and the ratio (Ca+Mg): Na> 1.

Experts from the University of Riverside, USA [18] made the largest contribution to the study of irrigation water quality and its classification as regards the benefits of irrigation of agricultural crops, and it is applied worldwide. The basis for assessing the method is EC and SAR. In the tested water samples for irrigation, the C1-S1 class belongs to 11.7% of the tested samples with characteristic that EC≤0.250 dS m^{-1}; SAR 0-10. These are waters where there is a small risk of dredging / alkalization, or water suitable for irrigation. 35.4% of the tested samples belong to the class C2-S1 class of water, in which the EC values range from 0.250 to 0.750 dS m^{-1} and can be used for irrigation of plants with a mean salt tolerance. The remaining 50.0% of the tested samples belonged to the class C3-S1, in which the EC values range from 0.750 to 2.250 dS m^{-1}, and their use requires the application of special measures in the prevention of soil depletion. 2.9 % belonged to the class C4-S1 (S4-highly salinated - high danger of salinization of soil (EC from 2.25 to 4 dSm^{-1}); S1-(SAR 0-10) - water with low content of Na, with low risk of salinization.

Modified FAO classification [19], analyzes in detail the influence of dissolved salt in irrigation water and its impact on the water-physical properties of the soil, primarily on infiltration. It takes into account the risk of sedimentation, based on the amount of electrical conductivity (EC) and salt concentration in the test sample (TDS).

Table 1 shows the values of the parameters on the basis of which the irrigation water samples were estimated in relation to the above classification. It was found that 35.3% of the samples belonged to the class of drinking water and irrigation (EC <0.7dS m^{-1}, TDS <500 mg l^{-1}), 61.8% of the samples belonged to the class for irrigation (EC: 0.7-2 dS m^{-1}; TDS 500-1500 mg l^{-1}), and 2.9 % - to the class for primarily drainage and ground water (EC: 2-10 dS m^{-1}; TDS 1500-7000 mg l^{-1}).

An additional estimate using the possible influence of some elements dissolved in irrigation water, analyzing Na^+ effects through different relationships with other tested substances (Na_2CO_3) was determined on the basis of the RSC-Residual Sodium Carbonate classification [10]. Based on this classification, 91.2% of the tested irrigation water samples belonged to the class of good waters (RSC <1.25) and 2.9% water - to the class with usability limit (RSC = 1.25-2.50); 5.9 % of the water samples were with poor quality (RSC>2.50).

The obtained values of the content of the studied microelements and heavy metals are shown in Table 1, and the interpretation was carried out on the basis of the limit values in the Ordinance on the permitted quantities of hazardous and harmful substances in soil and irrigation water [20] and the literature data [18] (*).

***Table 1** Maximum permitted levels (MAC) of hazardous and harmful substances in irrigation water*

Element	As	B	Cd	Cr	Cu	Fe*	Ni	Pb	Zn	Hg
	(mg l^{-1})									
MAC	to 0.05	to 1.0	to 0.01	to 0.5	to 0.1	to 5	to 0.1	to 0.1	to 1.0	to 0.001

In all tested water samples, the content of the tested microelements and heavy metals were below the maximum permissible values, except in one sample, number 29 from location No. 21 where an increased concentration of boron (B) content was determined. The reason for this phenomenon was probably related to the contamination of the accumulation due to the spillage of the fertilizers with high concentration of this element, were stored directly beside the water intake.

***Table 2** Chemical and physical properties of water samples for irrigation*

N°	Coordinate X	Y	pH	EC (dSm^{-1})	TDS (mg l^{-1})	SAR (mg l^{-1})	As (mg l^{-1})	B (mg l^{-1})	Cd (mg l^{-1})	Cr (mg l^{-1})	Cu (mg l^{-1})	Fe (mg l^{-1})	Ni (mg l^{-1})	Pb (mg l^{-1})	Zn (mg l^{-1})
1	562174	4787965	7.1	0.76	380	0.31	bdl	0.0203	bdl	bdl	0.0297	bdl	bdl	bdl	0.2228
2	562178	4787952	7.1	0.919	459	0.49	bdl	0.0183	bdl	bdl	bdl	bdl	bdl	bdl	bdl
3	562535	4788642	7.4	0.856	428	0.74	bdl	0.0786	bdl	0.0161	bdl	bdl	bdl	bdl	bdl
4	560901	4786541	7.4	0.835	418	1.03	bdl	0.0631	bdl	bdl	bdl	bdl	bdl	bdl	bdl
5	560946	4788855	7.2	0.532	268	0.63	bdl	0.0321	bdl	bdl	bdl	bdl	bdl	bdl	0.0094
6	558452	4779163—	7.4	0.688	344	0.91	bdl	0.0073	bdl	bdl	bdl	bdl	bdl	bdl	bdl
7	557868	4780465	7	0.751	375	0.77	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl
8	557171	4781405	7	0.229	115	0.47	bdl	bdl	bdl	bdl	bdl	0.0827	bdl	bdl	0.0136
9	554978	4779802	7.3	0.913	457	0.93	bdl	0.0119	bdl	bdl	bdl	bdl	bdl	bdl	bdl
10	523836	4777919	7.3	0.916	458	0.58	bdl	0.0855	bdl	bdl	bdl	bdl	bdl	bdl	0.3722
11	523303	4778327	7.6	0.866	433	0.61	bdl	0.0994	bdl	bdl	bdl	bdl	bdl	bdl	bdl
12	523303	4778294	7.8	0.488	244	0.13	bdl	0.0647	bdl	bdl	bdl	bdl	bdl	bdl	bdl
13	523275	4773241	8.1	0.124	62	0.15	bdl	bdl	bdl	bdl	bdl	0.0214	bdl	bdl	bdl
14	523216	4773229	7.8	0.38	190	0.24	bdl	0.0053	bdl	bdl	bdl	bdl	bdl	bdl	bdl
15	523068	4780447	7.5	1.12	581	1.32	bdl	0.0166	bdl	bdl	bdl	bdl	bdl	bdl	0.0124
16	523034	4780570	7.9	0.989	494	1.08	bdl	0.1244	bdl	bdl	bdl	0.088	bdl	bdl	bdl
17	514124	4785377	7.6	0.714	357	0.01	bdl	bdl	bdl	bdl	bdl	0.011	bdl	bdl	0.0083
18	514223	4785177	7.9	0.623	311	0.05	bdl	bdl	bdl	bdl	bdl	0.0157	bdl	bdl	bdl
19	519905	4790324	8.7	0.501	250	0.05	0.0082	0.0225	bdl	bdl	bdl	0.1167	bdl	bdl	bdl
20	519888	4790318	7.6	0.855	427	1.04	bdl	bdl	bdl	bdl	bdl	bdl	bdl	bdl	0.0742
21	519897	4790339	7.6	0.878	439	0.43	bdl	0.028	bdl	bdl	bdl	bdl	bdl	bdl	0.0389
22	524991	4791914	7.4	0.952	476	0.72	bdl	0.0413	bdl	bdl	bdl	bdl	bdl	bdl	0.0098
23	521234	4790539	7.3	0.41	205	0.84	bdl	0.0244	bdl	bdl	bdl	bdl	bdl	bdl	0.0156
24	531080	4795050	7.5	0.626	312	0.09	bdl	bdl	bdl	bdl	0.0128	bdl	bdl	bdl	0.0547
25	530412	4789145	8.3	1.06	531	0.76	0.0164	0.2643	bdl	bdl	bdl	0.0245	bdl	bdl	bdl
26	530190	4784978	8.9	0.513	257	0.52	bdl	0.036	bdl	bdl	bdl	bdl	bdl	bdl	bdl
27	537328	4787548	7.4	0.74	370	1.05	bdl	0.1614	bdl	bdl	bdl	0.0169	bdl	bdl	0.0151
28	536939	4788514	7.3	2.26	1130	2.51	bdl	0.1216	bdl	bdl	bdl	bdl	bdl	bdl	0.0089
29	536909	4788548	8.3	1.73	869	6.89	bdl	1.683	bdl	bdl	bdl	0.064	bdl	bdl	bdl
30	536879	4788485	7.6	1.2	580	1.58	0.0076	0.0889	bdl	0.0095	bdl	bdl	bdl	bdl	0.0403
31	538266	4789799	7.8	0.54	272	0.36	bdl	0.0355	bdl	bdl	bdl	bdl	bdl	bdl	bdl
32	540137	4787341	7.5	1.44	722	2.22	bdl	0.2407	bdl	bdl	bdl	0.0595	bdl	bdl	0.0281
33	540997	4786817	7.7	0.616	308	0.46	bdl	0.0457	bdl	bdl	bdl	bdl	bdl	bdl	0.1645
34	538212	4796190	7.8	0.482	241	0.13	bdl	bdl	bdl	bdl	0.0252	bdl	bdl	bdl	0.1235

bdl - below detection limit

CONCLUSION

Based on the obtained and analyzed results of the quality study of irrigation water, it can be concluded that water from sampling sites can be used mostly without restrictions to irrigate cultivated crops and there is no risk to have a negative impact on the structure of the soil on which it is applied. Special attention should be paid at locations where irrigation water didn't meet the criteria for applicability, the irrigation measures could be applied with restriction because the use of contaminated water can damage the structure of the agricultural soil.

At the location where increased content of (B) boron was determined, it is necessary to implement measures for cleaning and washing water intake, and if the results after implementation of those measures do not give satisfactory results, it is necessary to relocate the water intake location.

Nevertheless, the irrigation water and soil tests should be carried out periodically in order to prevent the creation of a rupture and breakdown of the structure.

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ELECTROCOAGULATION OF TEXTILE DYEING WASTEWATER CONTAINING AN AZO DYE

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Abstract

This research focused on electrochemical treatment (electrocoagulation) of synthetic textile industry wastewater containing azo dye (BEMACID RED E-TL). It is well know that many of azo dyes are toxic, cause skin and eye irritation, and are extremely dangerous to aquatic organisms. The study was conducted on a prepared synthetic wastewater in a batch laboratory electrochemical reactor. As electrode material it was used steel and stainless steel. The concentration of dye before and after the treatment was measured spectrophotometrically and the results are shown trough dye removal efficiency. It was examined the impact of current density (2.5; 5; 10 mA/cm^2) and initial dye concentration (100; 200; 500 mg/L). It was found that the process of electrocoagulation can very effectively carry out almost complete removal of dye by using steel anode. For 30 minutes of treatment it was achieved 86.2% removal efficiency at lowest examined current density (2.5 mA/cm^2) and highest initial dye concentrtation (500 mg/L).

Keywords: electrocochemical treatment, steel anode, azo dye, removal efficiency.

INTRODUCTION

The wastewater of textile dyeing and finishing factories causes various environmental problems not only because of its huge quantity of poisonous organic pollutants, but also due to its color and the high water consumption in this industry [1]. General characteristics textile dyeing wastewaters are, above all, a high content of organic matter, heavy metals and high coloration. The dye pollutant in water causes dangerous problems because those are products that are toxic, mutagenic, and carcinogenic to the life forms.

Azo dyes are the largest group of dyes used in textile industry. Nearly two-thirds (60% - 70%) of all synthetic dyes are azo dyes [2]. Azo dyes have one or more azo groups as a chromophore, with each group attached to two radicals of which at least one is an aromatic ring containing functional groups such as OH and SO_3H. The complex aromatic conjugated structure is responsible for the intense color, high water solubility, high stability against oxidants, light, and biological degradation [3].

An extensive literature reporting the characteristics and applications of most important conventional technologies developed for this purpose including physico-chemical and chemical methods, advanced oxidation processes (AOPs), adsorption, microbiological treatments, enzymatic decomposition, electrochemical technologies has been published [4–10].

Bassyouni *et al.* [1] examined the use of electrooxidation (EO) and electrocoagulation in the treatment of wastewater containing dye Acid Brown 14, which showed that EC was more efficient compared with EO, under the same operating conditions [1].

Removal of Acidic Red 18 by electrocoagulation with an aluminum anode was investigated by Khosravi *et al.* [10]. The effects of different parameters including initial pH, current density, reaction time, initial dye concentration, distances between electrodes, and type of the electrolyte solution on the efficiency of EC process were investigated. Optimum conditions were obtained at pH 4. Removal percentage of the dye was significantly increased with increasing current density until reaching to critical point of 26 mA/cm^2 [10].

In our previous study, it was investigated the possibility of treatment textile dyeng wastewater containing a mixture of azo dyes (Acid Black 194, Acid Black 107 and Acid Yellow 116) by EC process with iron anode. It was concluded that process is mostly influded by concentration of supporting electrolyte, dye concentration, current density and reaction time. The highest achieved removal efficiency was 80.6% for an initial dye concentration of 200 mg/L (γ_{NaCl}=1 g/L, j=10 mA/cm^2) [9].

This paper describes an increasingly present electrocoagulation (EC) treatment for removing dye from wastewater. EC implies the formation of coagulants *in situ* by electrolytic dissolution of the electrode made of aluminium or iron. During the EC process, the anode leads to the formation of metal ions, and at the cathode evolution of hydrogen gas exiting the system and carries the flocculated particles on the surface of the water.

Usage of high amount of chemical coagulants can be avoided, due to direct generation of coagulation by electrooxidation of an anode in EC process. EC equipment is simple and easy to operate. Relatively low reaction time and small quantities of sludge production are other advantages of the process [10].

MATERIALS AND METHODS

Experimental part of the research is contained by the application of EC for removing dye from simulated textile industry wastewater. Electrochemical batch reactor (Fig. 1) is made of polypropylene of capacity 250 cm^3 with possibility of constant mixing (200 rpm/min), which contains two electrodes of the same dimensions (area), P=44 cm^2, and distance between electrodes, d=20 mm. Electrodes were connected to digital power source (Atten, APS3005SI; 30V, 5A).

Used electrode materials are made out of metals known compositions, and comply with prescribed standards, respectfully, steel (EN10130-91; max. 0,08% C, max. 0.12% Cr, max. 0.45% Mn, max. 0.60% Si) and stainless steel (EN 1.4301/AISI 304; max. 0.07% C, 18.1% Cr, 8.2% Ni). Steel was used as anode and stainless steel as cathode.

For the experimental purpose it was used commercially available 99,5% sodium chloride, NaCl, 35% hydrochloric acid, HCl, acetone, $(CH_3)_2CO$ (Lachner, Czech) and “BEMACID RED E-TL” dye (CHT GmbH, Germany). Mentioned dye is harmful to aquatic life with long lasting effects, causes serious eye irritation [11].

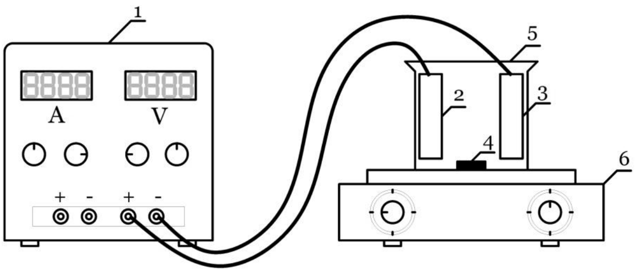
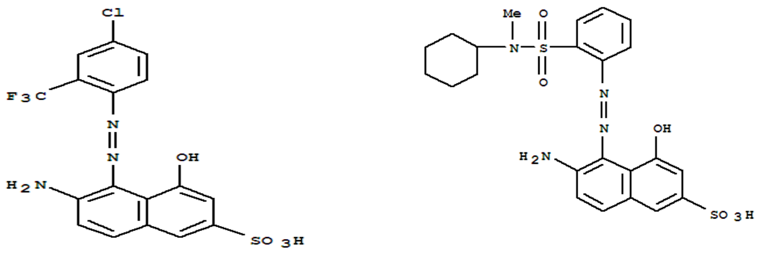


Figure 1 *Schematic view of electrochemical reactor: 1 – source of electric power; 2 – anode; 3 – cathode; 4 – magnetic stir bar; 5 – electrochemical cell; 6 – magnetic stirrer)*

All the experiments were performed at an ambient temperature and with synthetic wastewater volume of 250 cm^3. Initial wastewater concentration was 100 mg/L, 200 mg/L and 500 mg/L of azo dye and it was added 1 g/L sodium chloride as supporting electrolyte. Before each treatment, current density was set at desirable value (2,5; 5 and 10 mA/cm^2) and electrodes were mechanically cleaned and washed with detergent and acetone in order to remove surface grease. Electrode surfaces were cleaned by emerging in diluted (1:2) solution of HCl before each treatment.



(a) *(b)*

Figure 2 *Chemical compositon of used azo dye: (a) - sodium 6-amino-5-[[4-chloro-2-(trifluormethyl)phenyl]azo]-4-hydroxynaphthalene-2-sulphonate; (b) - sodium 6-amino-5-[[2-[cyclohexylmethylamino)sulphonyl]phenyl]azo]-4-hydroxynaphthalene-2-sulphonate [11]*

The dye concentration before and after treatment was determined spectrophotometrically (λ_{max} = 510 nm) on UV-VIS spectrophotometer (Perkin Elmer, Lambda 25) according to standard methods [12].

RESULTS AND DISCUSSION

Results of the electrochemical dye removal are showed through mass concentration, γ (mg/L), and dye removal efficiency, *Eu* (%), whose ratio could be described by following equation:

$$E_U = \frac{\gamma_i - \gamma_f}{\gamma_i} \cdot 100[\%] \qquad (1)$$

where is γ_i and γ_f initial and final dye concentration (mg/L).

Figure 3 shows dye removal efficiency at different current densities (2.5; 5; 10 mA/cm^2) and different reaction time, at an initial dye concentration of γ_i=100 mg/L and supporting electrolyte concentration γ_{NaCl}=1 g/L. For 20 minutes of treatment it was achieved almost same removal efficiency at all examined current densities, *Eu*=95.2% (*j*=2.5 mA/cm^2), *Eu*=92.2% (*j*=5 mA/cm^2) and *Eu*=95.5% (*j*=10 mA/cm^2), respectively.

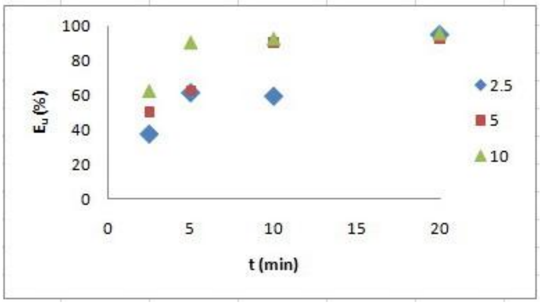


Figure 3 *Effect of current density and electrolysis duration on dye removal efficiency at initial dye concentration of γ_i=100 mg/L*

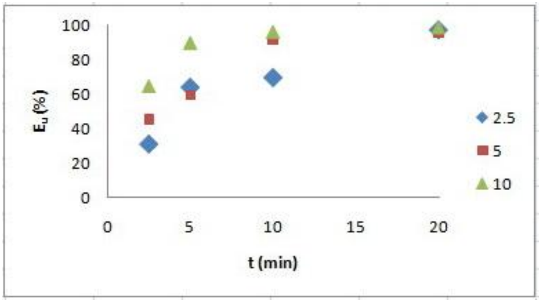


Figure 4 *Effect of current density and electrolysis duration dye removal efficiency at initial dye concentration of γ_i=200 mg/L*

High removal efficiencies are also achieved at higher initial dye concentration (γ_i=200 mg/L) and with same concentration of supporting electrolyte, which is showed in Figure 4. For 20 minutes of treatment it was achieved almost complete removal of dye at all examined current densities, *Eu*=97.6% (*j*=2.5 mA/cm^2), *Eu*=95.9% (*j*=5 mA/cm^2) and *Eu*=99.1% (*j*=10 mA/cm^2) respectively.

Since there is no significant difference in the efficiencies achieved with the examined current densities, in the further study a current density of 2.5 mA/cm^2 was used to reduce the specific energy consumption in the treatment.

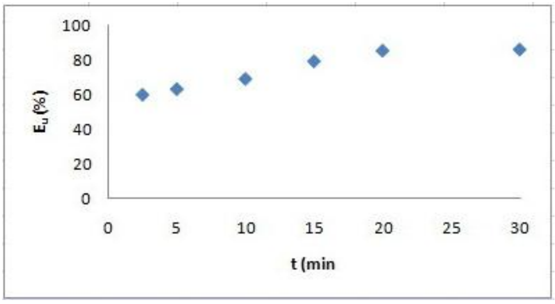


Figure 5 *Effect of electrolysis time on dye removal efficiency at current density 2.5 mA/cm^2 and initial dye concentration of γ_i*=500 mg/L

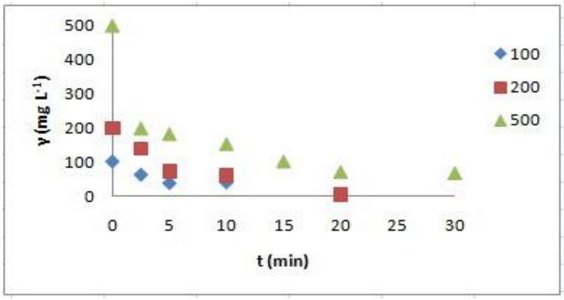


Figure 6 *Effect of electrolysis duration on different initial dye concentration at current density of 2.5 mA/cm^2*

Figure 5 shows effect of electrolysis duration on dye removal efficiency at current density of 2.5 mA/cm^2 and initial dye concentration of γ_i=500 mg/L. It can be noticed that for a very short time, high removal efficiency is achieved. For 2.5 minutes of treatment removal efficiency of *Eu*=60.0% was achieved, while in the 30 minutes removal efficiency was *Eu*=86.2%.

The effect of treatment time on decrease of dye concentration at different initial dye concentrations (j=2.5 mA/cm^2, γ_{NaCl}=1 g/L) is shown in Figure 6. It can be seen that at the beginning of the treatment high removal efficiency was achieved, so in the very begin of treatment the reaction was not diffused controlled. This is most noticeable at the highest examined concentration. By prolongation of treatment, removal efficiency is increasing, but significantly slower than in the beginning of the treatment.

CONCLUSION

This study shows that EC with iron anode is efficient process for removing of this azo dye in which most effect has dye concentration and reaction time. The treatment characteristic is high efficiency at the very beginning of the treatment. Also, the process is very efficient at a low current density, which is desirable to reduce the specific energy consumption.

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ARSENIC REMOVAL FROM GROUNDWATER BY *IN LINE* COAGULATION

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Abstract

In this work we investigate ways to improve arsenic removal from groundwater by in line coagulation (with $FeCl_3$*) using different pretreatment techniques. Pretreatment involved pH correction (to pH=7) and preoxidation of groundwater with chlorine (0.5 mg* Cl_2/l*). We show that successful oxidation of As(III) to As(V) is critical to improve arsenic removal by in line coagulation, with the arsenic speciation results confirming that the in line coagulation process is significantly less effective for As(III). Better arsenic removal was thus achieved by in line coagulation with the pre-treatment, which was able to convert all the As(III) to As(V), with the coagulation process also proving more effective at neutral pH. The combined approach, with pH correction, preoxidation and in-line coagulation, is a good solution for arsenic removal from contaminated groundwater in small water utilities.*

***Keywords**: arsenic, drinking water, *in line* coagulation

INTRODUCTION

Arsenic is considered to be one of the most hazardous elements for living organisms. Its presence in natural waters used as drinking water resources thus presents a serious issue in many parts of the world, including Serbia [1,2]. It is well know that long term consumption of arsenic contaminated drinking water may cause different health problems, with carcinoma as the most serious consequence of chronic arsenic exposure [3,4]. In order to minimize the possible health risks and protect public health, the World Health Organization (WHO) recommend a maximum allowable concentration (MAC) for arsenic in drinking water of 10 μg/l [5].

In water resources, arsenic usually exists in inorganic forms such as arsenite (As(III)) and arsenate (As(V)), although in groundwaters, As(III) is the predominant form of arsenic [6]. In comparison with As(V), As(III) is more toxic because of its high affinity for thiol groups and enzyme inhibiting action [7]. Additionally, at the pH typical for groundwater (pH=6.5-8.5), As(III) exists in the neutral form H_3AsO_3, which make its removal much harder than anionic As(V) ($H_2AsO_4^-/HAsO_4^{2-}$). Therefore, preoxidation is usually required to convert As(III) to As(V) to achieve high removal efficiencies and reduce toxicity [8].

Different techniques for arsenic removal have been developed and applied, including coagulation, adsorption, ion exchange and membrane processes [9]. Among them, coagulation is considered the most suitable conventional technique due to its high efficiency and relatively low cost [10-12]. However, this technique is not recommended for point of entry systems due to the necessary technical skills and high maintenance costs. In addition, the sludge produced in this process is difficult to separate from the treated water and due to the high concentration of arsenic, it must be managed in an adequate manner. Disposal and treatment of waste requires high material costs, which further restricts the application of this process in small water supply systems [9].

Compared to classical coagulation and flocculation, *in line* coagulation does not require large infrastructure investments and the dosage of applied coagulant is usually significantly lower, such that as well as having lower operation costs, the problems and other shortcomings of classical coagulation can also be avoided with respect to disposal of excess sludge.

As part of efforts to find simple, economically and technologically viable water treatment solutions for small water supply systems with arsenic contamination problems, the aim of this study was thus to investigate the efficiency of *in line* coagulation with and without pretreatment (preoxidation with Cl_2 and pH correction) for arsenic removal from groundwater.

MATERIALS AND METHODS

In line coagulation

Coagulation experiments were carried out in Jar tests using a FC6S Velp Scientific apparatus, with 0.5 l groundwater samples in 0.8 l beakers at room temperature (22-25ºC). Iron(III)-chloride was used as coagulant and applied at a dose of 7.30 $FeCl_3$/l. Before coagulation, the pretreatment consisted of adjusting the groundwater pH to 7 with 0.1 M HCl, and adding 0.5 mg Cl_2/l, in order to oxidise As(III) to As(V). Coagulation was carried out with rapid stirring at 120 rpm/min for 2 min, after which the coagulated water was filtered through a glass column filled with quartz sand (particle size 0.5-1.5 mm). Arsenic concentrations in the thus filtered samples were determined by inductively coupled plasma mass spectrometry (ICP/MS) analysis. The main characteristics of the packed column are given in Table 1.

Table 1 *Characteristics of filtration unit*

Parameter	Value
Column length (cm)	80
Internal diameter (cm)	1.7
Bed depth (cm)	30
Flow rate (ml/min)	12
EBCT (min)	5.7

Analytical methods

DOC concentrations were analysed after filtration through a 0.45 μm membrane filter on an Elementar LiquiTOCII, using Pt-catalysed combustion at 850°C to oxidize the carbon, in accordance with standard method SPRS ISO 8245:2007 [13]. The practical quantitation level (PQL) of the method was 0.5 mg C/l. pH and conductivity were measured using InoLab pH/ION 735 and WTW Cond 3210 portable instruments. Total arsenic concentrations (EPA method 7010) and arsenic speciation analyses were carried out by ICP/MS coupled with high performance liquid chromatography (HPLC) [14,15]. Water alkalinity (p- and m-alkalinity) were measured according to Standard Methods [16]. The content of orthophosphate was determined according to method SRPS EN ISO 6878: 2008 [17]. Concentration of chloride and ammonium was determinate according to methods SRPS ISO 9297/1:2007 and SRPS ISO 5664:1992, respectively [18,19].

RESULTS AND DISCUSSION

The characteristics of the investigated groundwater are presented in Table 2. The arsenic concentration in the groundwater was 126±8.97 μg/l, about 13 times greater than the maximum allowed concentration of 10 μg/l [5]. According to inorganic content, water belongs to the Ca-Mg-Na-carbonate type. Investigated groundwater has relatively low content of natural organic matter (Table 2).

***Table 2** Characteristics of the investigated groundwater*

Parameter	Value
pH	8.05±0.15
Conductivity (μs/cm)	669±14.3
Alkalinity (mmol/l)	6.93±0.37
Hardness (mg $CaCO_3$/l)	133±48.3
Arsenic (μg/l)	126±8.97
Fe (μg/l)	20.2±25.8
TOC (mgC/l)	2.40±0.71
DOC (mgC/l)	2.12±0.51
Phosphate (mgPO_4/l)	1.33±0.05
Chloride (mgCl/l)	17.7±1.36
Ammonium (mgN/l)	0.15±0.10

Preliminary tests of arsenic specification show that the dominant form of As in the investigated groundwater is As(III) (80%) (Figure 1).

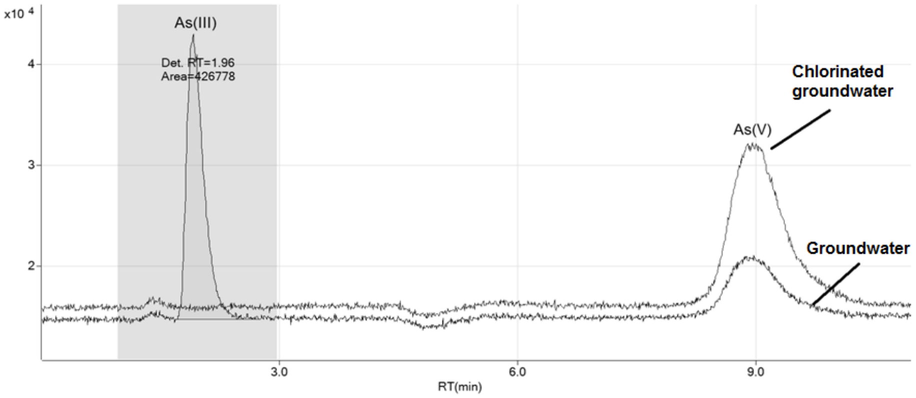

***Figure 1** HPLC/ICP/MS chromatogram of arsenic in groundwater before and after oxidation with 0.5 mg Cl_2/l and a contact time of 1 min*

As mentioned above, As(III) is much more mobile, more toxic and harder to remove from water than As(V), due to its neutral form [20]. 0.5 mg Cl_2/l was thus added to the groundwater to oxidise the As(III) and improve overall arsenic removal. Laboratory testing showed that a chlorine dose of 0.5 mg/l, applied for 1 min, was enough to completely convert As(III) to As(V) (Figure 1). This result was in accordance with USEPA recommendations [21].

The arsenic removals from groundwater achieved by *in line* coagulation and filtration through quartz sand, with and without pretreatment (preoxidation 0.5 mg Cl_2/l and/or pH correction to pH=7) are shown in Figure 2.

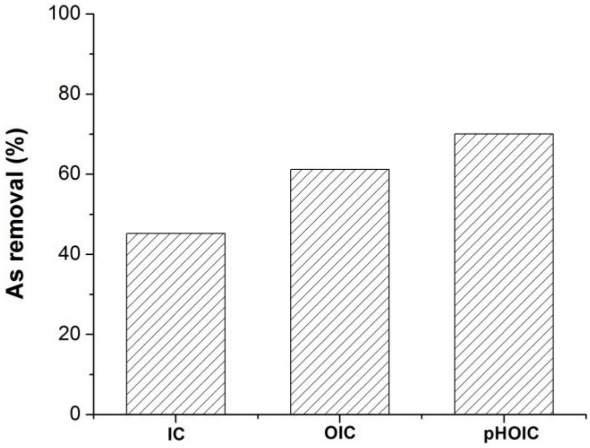

Figure 2 *Percentage of total As removal by in line coagulation with and without pretreatment (IC: in line coagulation; OIC: oxidation and in line coagulation; pHOIC: pH correction, oxidation and in line coagulation)*

Without the pretreatment step, only about 36% of the arsenic was removed by *in line* coagulation. Chlorination of water prior to *in line* coagulation increased arsenic removal up to 55% However, the best total As removals of 70% were achieved when both pretreatment steps, (pH correction to pH=7 and chlorine oxidation) were applied, resulting in an almost two-fold improvement in arsenic removal by the *in line* coagulation process (Figure 2).

This can be explained by the presence of different arsenic species in the groundwater (Figure 3) and the effect pH on efficiency of *in line* coagulation. As can be seen in Figure 3, the addition of chlorine provides complete oxidation of As(III) to the more readily removed As(V). Furthermore, application of pH correction created optimal conditions for arsenic removal by coagulation, since iron salts are most effective at neutral pH [22]. Thus, oxidation of As(III) to As(V) and subsequent As(V) removal by coprecipitation and adsorption on the flocs formed, significantly improved total arsenic removal from groundwater.

Similar observations were made by Jiang [23], who found that arsenic concentrations can be reduced from 10-500 μg/l to 5-10 μg/l by oxidation, coagulation and adsorption.

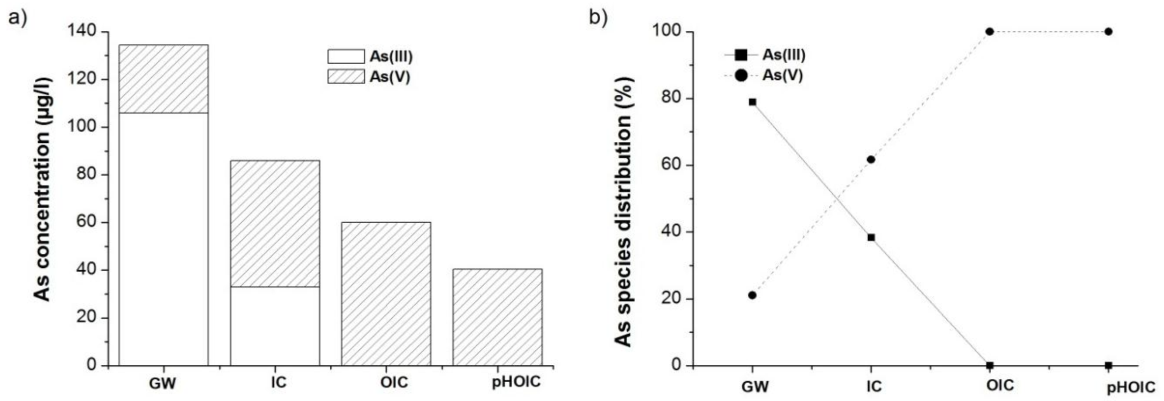

***Figure 3** a) Arsenic concentrations and b) distribution of As species in the groundwater before and after in line coagulation*
(GW: raw groundwater; IC: in line coagulation; OIC: oxidation and in line coagulation; pHOIC: pH correction, oxidation and in line coagulation)

The same author found that the most effective treatment, in which 100% removal of arsenic was achieved, involved oxidation by chlorination, coagulation with $FeCl_3$, sedimentation and sand filtration, which is accordance with the results obtained in this study.

CONCLUSION

This work investigates the effect of pretreatment (pH correction and oxidation with Cl_2) on the efficiency of *in line* coagulation for arsenic removal from groundwater. It was shown that introducing the pretreatment steps significantly improves arsenic removal. Preoxidation of the groundwater with 0.5 mg Cl_2/l results in complete oxidation of As(III) to As(V). pH correction of the groundwater also increased the effect of *in line* coagulation. The combined approach, with pH correction, oxidation with Cl_2, and subsequent *in line* coagulation, resulted in significantly improved arsenic removal from groundwater, and represents a technologically and economically feasible treatment solution for small water systems.

ACKNOWLEDGEMENT

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NEWFOUND RARE SPECIES OF COCCINELLID, *Henosepilachna argus*, IN SPECIAL NATURE RESERVE "ZASAVICA"

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Abstract

While revising old insect collections and samples, a rarely occurring Coccinellide species for Serbia was found, Henosepilachna argus. It was collected on 08 May 2007, at Široka Bara, Ravnje, a humid locality that's covered by water during the summer by up to 0.5 m in depth. Until now it was only documented on five different occasions according to the electronic database of biodiversity Alciphron. Documenting this species in Special Nature Reserve Zasavica is the first of its kind in Northern Mačva and Posavina, and is forwarding the complete understanding of its distribution in Serbia. It's also expected that finding this species in SNR "Zasavica" furthers its importance as a protected nature area.

Keywords: *Henosepilachna argus*, rare species, Zasavica

INTRODUCTION

Even besides a lenghty period of research of Coccinellidae fauna in Serbia, with many notable researchers (Apfelbek, Košanin, Matić, Horvat, Raheb, etc.), there are a select few published papers of their abundance in protected areas. Special Nature Reserve "Zasavica" along with National park „Đerdap" [1] and Fruška Gora [2,3] is one of the rare localities with well known coccinellid fauna [4].

Zasavica represents a fossil riverbed of Sava and Drina, wich formed during the holocene period. Its curved stream was built on large deposits of river aluvium. Today it's a river, 33.1 km in lenght, about 20 – 80 m in girth, with a depth of about 3m, which was declared a Special Nature Reserve of the first category in 1997., representing a locality of great interest. It has a surface area of 1876 ha, of which 671 ha comprises the I`st category of protection [5]. Out of the terrestrial habitats there are two types of forests and meadows. The meadows are wet and semiaquiatic, mostly of steppe characters. The forrests are mainly composed of ash, while poplar, willow and alder are much less in abundance [6]. The total forrested area in the Reseve, which includes willow and northamerican poplar, is 16.67% on about 110 ha [7]. The diverse and numerous types of habitats in the Zasavica river valley are home to a large number of insect species, of which some are Natura recognized, as well as several rarities and endemites [8–10]. What sets apart Zasavica from other protected nature areas in Serbia, is the fact that the entomofauna is well studied, whilst the most representative groups, comprising of butterflies [11–14], odonates [15,16], orthopterans [17], certain coleopteran families [18–23] and hymenopterans [24,25] are extremely well documented.

Across the world there are 6000 known species of coccinellids and of that 207 are occurring in Europe [26]. In Serbia, according to Živojinović [27] there are 26 species and 16 intraspecific forms. But according to yet unpublished records there are around 60 species. There are 10 species in 9 genuses of coccinellids documented in SNR [4]. While going through and revising old collections and samples, the rare species for Serbia, *Henosepilachna argus,* was found.

Species description

Species from the genus *Henosepilachna* are found in Europe, Asia and Australia, while one species, *H. vigintioctopuncata*, was accidentally introduced in South America (Brasil and Argentina). Until now there are 17 known species from the genus *Henosepilachna*, and the species *H. argus* was first described by the French doctor Etienne Louis Geoffroi in 1762. It has a body length of around 5-7 mm. Color of the elytra can range from orange to dark brown and have 11 dark spots on them. The pronotum and extremities are orange. This species is occurring in urban localities, such as parks and parking areas, but is much more occurring in natural habitats. It feeds mostly on leaves from plants in the *Cucurbitaceae* family. According to Fauna Europaea [28], *Henosepilachna argus* is documented in England, Austria, Belgium, Bulgaria, Croatia, Czech Republic, France, Germany, Hungary, Italy, Poland, Portugal, Romania, Sardinia, Sicily, Sweden, Netherlands and Yugoslavia (Figure 1).

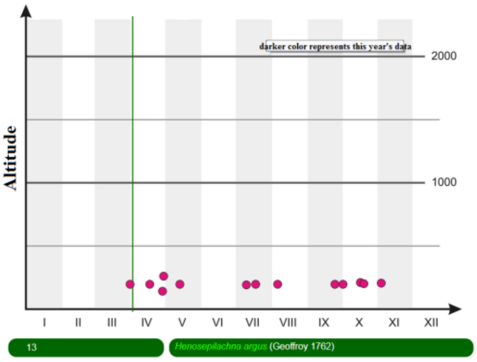

Figure 1 *Diagram of annual occurrence of the species*

MATERIALS AND METHODS

Coccinellidae are a very diverse group, morphologically and ecologically. With that in mind, the methods that are used in their collection are also diverse. The most common method is using a sweep net in dense vegetation. It was this specific way that the sample used in this paper was collected. After collecting, the sample is then stored in containers, after which it's taxidermy. The species was identified according to Christoph [26].

Locality

Široka Bara [44º54'55.14"N; 19º24'53.62"E] is located near Ravnje and is a naturally formed denudational plane that is hidrologically active until the end of May, sometimes even until the beginning of June, often resembling a pond. After the water recedes, mostly wet grasslands are left behind, which can dry in extremely arid years. These are also the most endangered localities, because after the water has dried up, higher terrain becomes accessible to farming which is connected to habitat destruction. The terrain has plenty of depressions and microdepressions on one side and aluvial beams on the other. On this location we have an occurrence that a part of the grasslands, underbrush or forrests in the depression has a totally different floral elements then the one on the alluvial beam. The sporadic inudational depressin that comprieses Široka bara is covered by the select vegetation elements *Genisto elatace-Quercetum Horv, 1938. subass. Leucoio-Fraxinetum angustifolia Glav, 1959.* or *Brachypodio silvaticae palustris-Quercetum Erdeši, 1955*. The geographical location is shown on the map below (Figure 2).

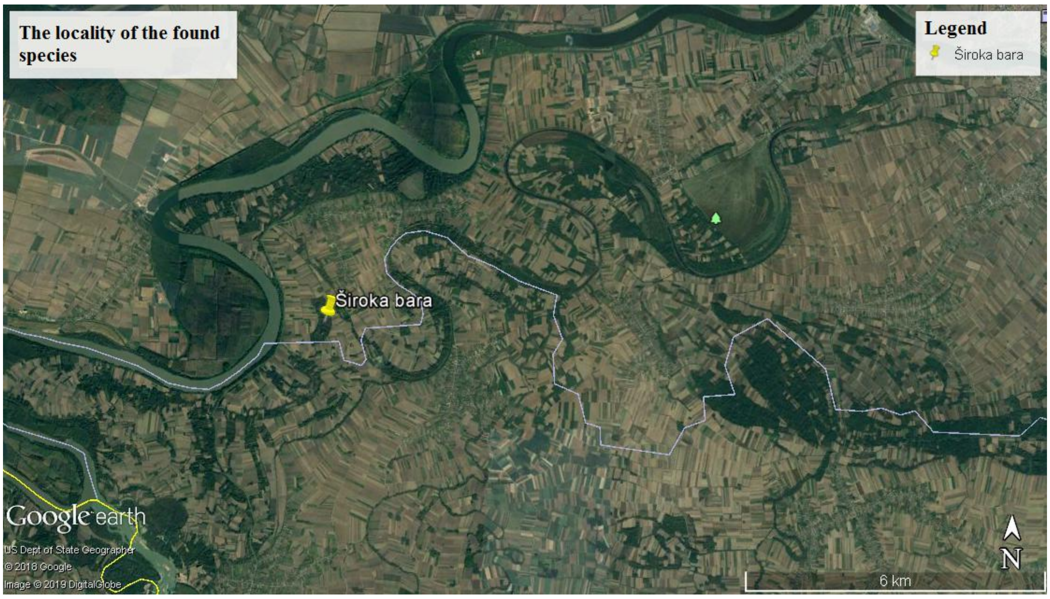

Figure 2 *Position of Široka Bara, Ravnje*

RESULTS AND DISCUSSION

As it was mentioned above, while going through and revising old samples, one sample from Široka Bara, Ravnje had the above mentioned *Henosepilachna argus*. It fit the description of the species and the habitat on which it was found had two species of host plant from the Cucurbitaceae family, *Echinocystislobata.*

Until now, apart from the Reserve, according to the online database Alciphrone, *H. argus* was documented in Vojvodina, Šumadija, Eastern Serbia and twice in Southeastern Serbia (Figure 3). Online database BioRas however showed no documentation of this species at all. This coccinellids is also present in neighboring countries, Hungary, Bulgaria and Romania, and in Croatia besides *H. argus* there is also another species present, *H. eleferi* [29].

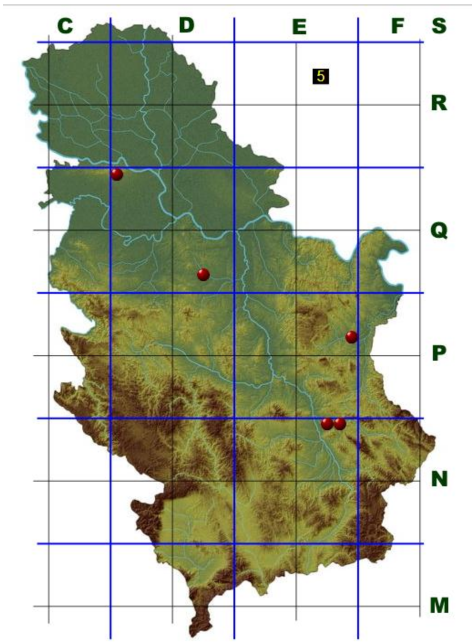

Figure 3 *Documented findings of Henosepilachna argus in Serbia*

CONCLUSION

This finding in Special Nature Reserve “Zasavica” is the first of its kind in Mačva and Posavina, and brings forth better understanding of its distribution in Serbia. The very low number of documented sightings may indicate the rarity of this species or simply not adequate research. Without a doubt it raises the importance of this protected area and further efforts will be put into detailed research of similar habitats where this species might be found again.

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OVERVIEW OF NECESSARY TYPES FOR NATURE PROTECTION OF EUROPE IN A SPECIAL NATURE RESERVE ZASAVICA

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Abstract

Species of importance for the protection of Europe's predecessors in the Zasavica reserve are: Vascular plants: Aldrovanda vesiculosa, Galanthus nivalis, Lindernia procumbens, Ruscus aculeatus; Invertebrata: Coleoptera: Carabus variolosus, Cerambyx cerdo, Morimus asper funereus, Pilemia tigrina, Stenopterus similatus, Prionus coriarius, Saperda punctata,Cucujus cinnaberinus, Graphoderus bilineatus, Lucanus cervus, Dorcus parallelopipedus, Osmoderma eremite; Lepidoptera: Lycaena dispar, Zerynthya polyxena; Odonata: Gomphus flavipes, Cordulegaster bidentata; Orthoptera: Pachytrachis gracilis, Poecilimon fussii, Phoenixoptera frivaldskyi, Zeuneriana amplipennis, Leptophyes boscii, L. discoidalis; Vertebrata: PISCES: (Barbus peloponesius, Cobitis taenia, Misgurnus fossilis, Rhodeus sericeus amarus, Silurus glanis, Umbra krameri). AMPHIBIA: Bombina bombina, Bufo bufo, B.viridis, Hyla arborea, Pelobates fuscus, Rana kl.esculenta, R. lessonae, R. ridibunda, R. dalmatina, R.temporaria, Salamandra salamandra, Triturus vulgaris, Triturus dobrogicus; REPTILIA: Emys orbicularis, Testudo hermanii, Anguis fragilis, Lacerta viridis, Podarcis muralis, Coronella austriaca, Natrix natrix, N. tesselata, Vipera berus, Zamenis longissimus; AVES: Of the 216 species of birds in the reserve, 196 species are significant for the protection of nature in Europe. MAMMALIA: Crocidura suaveolens, C.leucodon, Sorex araneus, S.minutus, Neomys anomalus, Barbastella barbastellus, Miniopterus schreibersii, Myotis bechsteinii, M. daubentonii, M. emarginatus, M. myotis, M. mystacinus, M. nattereri, Nyctalus leisleri, N. noctula, Pipistrellus kuhlii, P. nathusii, P. pipistrellus, Plecotus auritus, P. austriacus, Rhinolophus ferrumequinum, Vespertilio murinus, Castor fiber, Cricetus cricetus, Glis glis, Sciurus vulgaris, Muscardinus avellanarius, Canis aureus, Felis silvestris, Lutra lutra, Martes foina, M. martes, Meles meles, Mustela erminea, M. nivalis, M. putoris, Capreolus capreolus.

Keywords: flora, fauna, Zasavica, Europe

INTRODUCTION

Nature protection in the European Union is regulated by two directives: the Birds Directive and the Habitats Directive. According to the Habitats Directive, a European network of protected areas was established. Natura 2000, consisting of the Special Protected Areas under the Birds Directive and Special Protection Areas under the Habitats Directive in order to conserve other plant and animal species and habitats. Zasavica Reserve has been protecting and preserving flora, fauna and habitat for 20 years. There are a large number of endemic, relict, critically endangered, endangered and vulnerable species in the area of the reserve, as well as species on the border of their areas and newly discovered species for Serbia and the Balkans. The aim of the paper is to present the representatives of the flora and fauna that are located in the area of the Zasavica Nature Reserve and are on the list of species important for nature protection in Europe.

MATERIALS AND METHODS

The methodology for writing this work involved collecting all the so far published data on the special diversity of the Nature Reserve of Zasavica. In addition to published publications, the data from field diaries or from the reserve natural collection have not been published yet.

RESULTS AND DISCUSSION

Europe is distinguished by the abundance of wild plants and animals, and many of them are unique to this continent, but unfortunately many are at risk of extinction [1]. The SRP Zasavica has a large number of plant and animal species that are important for nature protection in Europe. There are about 680 species of vascular plants and mosses in the reserve, and for 657 species, data have been published so far [2]. Out of the total number of species, 45 species are distinguished as the most important representatives of the flora according to national and international criteria. In the flora of Zasavica from plants important for nature protection in Europe according to Dožai [3] there are: *Aldrovanda vesiculosa,Galanthus nivalis, Lindernia procumbens, Ruscus aculeatus*. Of these four species, *Aldrovanda vesiculosa* is the most significant, which was supposed to have disappeared from Serbia [4]. During 2005 and 2006, the presence of this critically endangered marshland flotation-submerged species was detected at 7 km of plantation plant along the valley to the forest beetle, in the contact zone between the cane and rush and free water belt within the vegetation of the *Lemneta minoris* W.Koch & tx.1955 and *Utricularietea intermedio-minoris* Den Hartog & Segal, 1964 em. Pietsch, 1965. The units from Zasavica had a length of 5-13 cm (max.24 cm) and, bearing in mind that its annual increment of about 1 cm indicates that the aldrovide is present here for at least a decade [2], and the species *Lindernia procumbens*, which is very rare in Serbia [5], and was found in 2005 in a wetland habitat in the community of the *Isoëto-Nanojuncete* class. An overview of the insects important for nature protection in Europe and recorded in the SRP Zasavica are given in order: From the order of Coleopter as an insect species important for the protection of nature in Europe and recorded in SRP Zasavica belong to the following families: Carabidae, Cerambycidae, Cucujiidae, Dytiscidae, Lucanidae and Scarabaeidae [1]. Fam.Carabidae in the reserve has been seized with 72 species, which is 12,08% of the total number of species of shrimps in Serbia [6]. From the species Fam.Carabidae, the species important for the protection of nature in Europe is *Carabus variolosus*, which was once widespread in Central and Southern Europe, but today the population of this trunk is more rare [7]. Variety was recorded in the reserve according to Ćurčić and Stanković [8] *C. variolosus variolosus*, which has been recorded so far only in the territory of Vojvodina [6]. There is one finding of this species in the reserve in 2010, and this is the first data for northwestern Serbia [8]. Fam. Cerambycidae: In the Zasavica Reserve, there are about 30 species of sheepsub [9], and as species of importance for nature protection in Europe are: *Cerambyx cerdo, Morimus asper funereus, Pilemia tigrina*. Here, the species of *Pilemia tigrina* with the larvae is hosted by the plant from the genus *Anchus sp.*, While adults stay in plants where they are well camouflaged [10]. In the Zasavica flora there is one species of the genus *Anchusa* (*Anchusa officinalis*), which is cosmopolitan in the wild, and yet there is one finding of this 2001 syringe, and this is the first data for northwestern Serbia. The remaining two species are inhabited by forest communities *Genisto elate-quercetum* Horv.1938 *subass Leucoio-fraxinetum* Gloves. 1959; *Genisto elate-quercetum* Horv.1938 *subass Carpinetosum betuli* Vuk.1959; *Rusco aculeti-Tilio-Quercetum* Erdeši.1955; [11]. Fam. Cucujiidae: According to Živojinović [12], the Cucujidae family in Serbia is represented by six species, among which the presence of the genus Cucujus is not mentioned. According to the BIORAS database, two species of this species are living in Serbia: *C. cinnaberinus* and *C. haematoes* [13]. *Cucujus cinnaberinus* from the Cucujiidae family, as a species important for nature protection in Europe. In 2017, a specimen of the species *Cucujus cinnaberinus*, found on a broken willow tree (*Salix alba*), was found beneath the bark. Finding this potentially endangered species (Nt) in Serbia is significant because it is the first to the south of the Sava and the Danube. Familia Dytiscidae: According to Mesaros and Stanković [14] 47 species from the Adephag suborder were recorded in the reserve, of which 34 species belong to Fam. Dytiscidae, including the genus *Graphoderus*. From the Dytiscidae family, as a species important for the protection of nature in Europe, the *Graphoderus bilineatus*. The species in the reserve was not found at Šumareva ćuprija, 2011. So far, in Serbia, the species was known on the basis of literature data, with no closer location. The species is in most parts of Europe before the spraying, is found on all European nature protection lists and significant efforts are being made in some European countries to preserve its habitat [14]. Fam. Scarabaeidae: In the entomofauns reserve, about ten species of the Scarabaeidae family were found. From the Scarabaeidae family as a species important for nature protection in Europe, the *Osmoderma eremita*, and in the reserve there is one finding of this species from 1998. [15]. Fam. Lucanidae: Two species from the Lucanidae family are registered in the reservation: *Lucanus cervus* L. and *Dorcus parallelopipedus* (L.) [15,16], and as a species of importance for the protection of nature in Europe, The *Lucanus cervus*, whose survival, both as a larvae and as an adult insect, is directly dependent on old trees. Red Lepidoptera: The reserve contains 68 species of butterflies [17], and as species of importance for nature protection in Europe, two species are present in the reserve: *Lycaena dispar* and *Zerynthya polyxena* [18]. Both species are on the European Red List (1999) and have the status of vulnerable species (VU) in Serbia, with the species *L.dispard* included in the World Red List (1996) [19]. Red Odonata: From the Odonata order, there are 43 species in total, of which 42 data were previously published [20–22] as a species important for nature protection in Europe and present in the Zasavica Reserve is *Gomphus flavipes* [23]. For the protection of nature in Europe from the SRP Zasavica area, there are also other types of insects from the European red list, as well as endemic and relict species. From the European aspect of nature conservation, the following species from the European red list are also important: in the fauna of the Orthoptera with the status of critically endangered species (CR) (*Pachytrachis gracilis, Poecilimon fussii*) and endangered species (EN) (*Phoenixoptera frivaldskyi*) [24]. In addition to the European red list in Serbia, the Red Book of fauna of Serbia IV-Orthoptera has been made, where three species from Zasavica and those with endangered species (EN) *Zeuneriana amplipennis*, with the status of worryingly endangered *Leptophyes boscii* and with the status of almost worrying threat *L. discoidalis* [25]. In the fauna of Coleopter there is a significant presence and species dependent on protection (Lrnt) at the European level (*Prionus coriarius, Saperda punctata, Protaetia cuprina, Valgus hemipterus, Dorcus parallelopipedus, Bostrichus capucinus*). From the European aspect of nature protection in the reserve fauna, five endemic and relict species of invertebrates have been recorded. A variety of endemic benthic broom *Chirocephalus brevipalpis* and relict living fossil *Lepidurus apus* [26] were found, as well as three endemic species, the endemic Europe-*Cordulegaster bidentata* [27], Balcans-*Stenopterus similatus* [9] and Danube-*Zeuneriana amplipennis* [28]. PISCES: According to Bajic and Stankovic [29] in SRP Zasavica, 23 species of fish were registered, and Zasavica is today only one of the remaining three fish habitats in *Umbra krameri* in Serbia. From species of fish significant for nature protection in Europe and recorded in SRP Zasavica according to Bajić [30] are: *Barbus peloponesius, Cobitis taenia, Misgurnus fossilis, Rhodeus sericeus amarus, Silurus glanis, Umbra krameri*. AMPHIBIA: Batrahofaun Reserve Zasavica has 13 species of which 3 species are from the Ordo Caudata, and 10 species are from the Ordo Anura [31]. An overview of the species of Amphibians important for nature protection in Europe recorded in the SRP Zasavica according to Mesaroš [32] are: from Anura: *Bombina bombina, Bufo bufo, B. viridis, Hyla arborea, Pelobates fuscus, Rana kl. esculenta, R. lessonae, R. ridibunda, R. dalmatina, R. temporaria;* from Caudata: *Salamandra salamandra, Triturus vulgaris, Triturus dobrogicus.* Many of the amphibians are cosmopolitan species and the populations in Europe and Serbia are stable and have a vulnerability category -Lc [33]. In the reserve are from the list and three species from the Serbian Red Book of Fauna, and these are (*T. dobrogicus, P. fuscus* and *R. lessonae*), of which *T. dobrogicus* is an endemic species of the Balkans. REPTILIA: According to Stankovic [31], 14 species (11 + 3 ssp) reptiles live in the reserve. Of the 14 species of the two species belong to the ordo of Testudines, 5 species (+ 1 ssp) belong to the ordo of the Saurida and 7 species (+ 2 ssp) belong to the ordo Serpentes. An overview of the species of reptiles significant for nature protection in Europe recorded in SNR Zasavica according to Mesaroš [32] are: Testudines: *Emys orbicularis, Testudo hermanii*; Saurida: *Anguis fragilis, Lacerta viridis, Podarcis muralis,* Serpentes: *Coronella austriaca, Natrix natrix, N.tesselata, Vipera berus, Zamenis longissimus*. Many of the reptiles are cosmopolitan species whose populations in Europe and Serbia are stable so as to have a category of vulnerability -Lc [34]. In the reserve, there are three species found in the list of reptiles that are important for nature protection in Europe, which are in the Red Book of Serbian Fauna II, which are from the species Testudines (*Emys orbicularis* and *Testudo hermanni*) and from the Serpentes (*Viper berus*) line. AVES: The IBA area of Zasavica includes SNR Zasavica. In the year 2018. there are 216 species of birds, 214 of which are previously published data [35,36]. Of the species recorded, 110 species are nesting in the reserve, which is 46% of the total diversity of the breeding gardens in Serbia [35]. Of the 216 species of birds in the reserve, 196 species are significant for the protection of nature in Europe [37] and these are: *Accipiter gentilis, A. nisus, Acrocephalus arudinaceus, A. palustris, A. schoenobaenus, A. melanopogon, A. scirpaceus, Aegithalos caudatus, Alauda arvensis, Alcedo atthis, Anas acuta, A. clypeata, A. crecca, A. penelope, A. plathyrhynchos, A. querquedula, A. strepera, Anser albifrons, Anthus campestris, A. cervinus, A. spinoleta, A. trivalis, Apus apus, Aquila clanga, A. heliaca, A. pomarina, Ardea cinerea, A. purpurea, Ardeola ralloides, Asio flammeus, Aythya farina, A. fuligula, A. nyroca, Bombycilla garrulous, Butaurus stellaris, Bubulcus ibis, Buteo buteo, B. lagopus, B. rufinus, Calidris alpina, C.*

minuta, C. temminckii, Caprimulgus europaeus, Cardu-elis cannabina, C. carduelis, C. flavirostris, C. spinus, Egrettaalbus, Certhia brachydactyla, C. familiaris, Charadrius dubius, Chlidonias hybridus, Ch. leucopterus, Ch. niger, Ciconia nigra, C.ciconia, Cricaetus gallicus, Circus aeruginosus, C. cyeneus, C. macrourus, C. pygargus, Coccothraustes coccothraustes, Columba livia, C.oenas, C. palumbus, Coracias garrulus, Corvus corax, C. cornix, C. frugilegus, C. monedula, Coturnix coturnix, Cuculus canorus, Cygnus cygnus, C. olor, Delichon urbica, Dendrocopus major, D. medius, D.minor, D. syriacus, Dryocopus martius, Egretta garzzeta, Emberiza citrinella, E. hortulana, E. schoeniculus, Erithacus rubecula, Falco cherrug, F. columbarius, F. peregrinus, F. subbuteo, F. tinnunculus, F. vespertinus, Ficedula albicollis, F. hypoleuca, Fringilla coelebs, F. montifringilla, Fulica atra, Galerida cristata, Gallinago gallinago, G.media, Galinula chloropus, Garrulus glandarius, Gavia stellata, Grus grus, Gyps fulvus, Haliaeetus albicilla, Himantopus himantopus, Hippolias icterina,Hirundo daurica, H. rustica, Ixobrichus minutus, Jynx torquilla, Lanius collurio, L. excubitor, Larus cachinnans, L. canus, L. melanocephalus, L. ridibundus, Limosa limosa, Locustella fluviatilis, L.luscinioides, Loxia curvirostra, Lullula arborea, Luscinia liscinia, L. megarhynchos, L.svecica, Mergus merganser, Merops apiaster, Milvus migrans, Motacilla alba, M. cinerea, M. flava, Muscicapa striata, Numenius arquata, N. phaeopus, Nycticorax nycticorax, Oenanthe oenanthe, Oriolus oriolus, Pandion haliaetus, Panurus biarmicus, Parus ater, P. caeruleus, P. major, P. palustris, Passer montanus, Perdix perdix, Pernis apivorus, Phalacrocorax carbo, Ph. pygmeus, Phasianus colchicus, Philomachus pugnax, Phoenicurus ochropus, Ph. phoenicurus, Phylloscopus colybita, Ph. sibilatrix, Ph. trochilus, Pica pica, Picus canus, Platalea leucorodia, Plegadis falcinellus, Porzana parva, P. porzana, Prunella modularis, Pyrrhula pyrrhula, Rallus aquaticus, Recurvirostra avosetta,Regulus ignicapillus, R. regulus, Remiz pendulinus, Riparia riparia, Saxicola rubetra, Scolopax rusticola, Serinus serinus, Sitta europaea, Sterna hirundo, Streptopelia decaocto, S. turtur, Sturnus vulgaris, Sylvia atricapilla, S.borin, S. communis, S. curruca, Tringia erythropus, T. glareola, T. nebularia, T. ochropus, T. stagnatilis, T. totanus, Troglodytes troglodytes, Turdus iliacus, T. merula, T. philomelos, T. pilaris, T. torquatus, T. viscivorus, Upupa epops, Vanellus vanellus. Of the 196 species of birds, 45 species were confirmed breeding gardens, 66 species were non-gardens, 14 species were nesting in the nest. Zasavica is the only nesting site of the globally endangered species (SPEC 1) *Phalacrocorax pygmaeus* in Western Serbia and the most important location for nesting another globally endangered species of *Aythya nyroca* with 5% of the national population. In Zasavica, there are over 50% of the breeding population of Western Serbia and 1% of the national population of the globally endangered species *Haliaeetus albicilla* as well as 50% of the breeding population of Western Serbia and 2% of the national population of the endangered species *Anas querquedula*. the recorded species of *Phylloscopus trochilus* is a new breeding node for Serbia Šćiban, M., (2017). Out of a total of 196 species of birds important for the protection of the nature of Europe in the reserve, 7 species are vulnerable (VU) and 1 is endangered (EN). It is worth mentioning that after more than a century, in 2016, two birds of the species *Gyps fulvus* were observed in the thermal, which according to the data of the brothers Dombrovski (1895) and nesting in Macva near Šabac. MAMMALIA: Of the 65 species of mammals in the reserve [39], 37 species are significant for nature protection in Europe. As species of mammals important for the protection of nature in Europe and recorded in SRP Zasavica towards [40] are given in the systematic order: Ordo Eulipotyphla: In the Zasavica region, the presence of 7 species from the line of the insectivora [39], of which 5 species (*Crocidura suaveolens, Crocidura leucodon, Sorex araneus, S. minutus, Neomys anomalus*), are significant for nature protection in Europe. Ordo Chiroptera: So far, in the area of SRP Zasavica, total of 24 species have been confirmed, and 16 species of bats have been proven. Of the 24 species, 17 species (*Barbastella barbastellus, Miniopterus schreibersii, Myotis bechsteinii, M. daubentonii, M. emarginatus, M. myotis, M. mystacinus, M. nattereri, Nyctalus leisleri, N. noctula, Pipistrellus kuhlii, P. nathusii, P. pipistrellus, Plecotus auritus, P.austriacus, Rhinolophus ferrumequinum, Vespertilio murinus*) in the reserve is significant for the protection of nature in Europe. Row. Rodentia: In the SNR Zasavica area there are 18 species of rodents, of which 5 species (*Castor fiber, Cricetus cricetus, Glis glis, Sciurus vulgaris, Muscardinus avellanarius*) are significant for nature protection in Europe. Ordo Carnivora: In SRP Zasavica, 12 species of beasts have been recorded, of which 9 species are important for the protection of nature in Europe (*Canis aureus, Felis silvestris, Lutra lutra, Martes foina, M. martes, Meles meles, Mustela erminea, M.nivalis, M. putoris*). According to the type of the stanza of 9 species, one is a semi-aquatic (*Lutra lutra*), 6 species are silvicols, which beside the forest inhabit both the scissors, scabs, reeds and the like. The wildest species in the wildlife reserve is *Felis slivestris*, which has only a few finds in the reserve for 20 years. Ordo Cetartiodactyla: According to the data of the Serbian Hunting Federation, two species of this species are present in the SRP area of Zasavica, and a species of significant nature protection in Europe is *Capreolus capreolus* and this is a very rare species because there are up to five data for the past 20 years.

CONCLUSION

With the placement of Zasavica and its surroundings under the protection of the state in 1997 as the Special Nature Reserve of Zasavica I category as an area of international, national or extraordinary importance of the IVth IUCN category, conditions have been created for preserving the flora and fauna of the area. Conservation of nature is also given by more intensive research of the area whose results are included in the management plans. From all of the exhibited, we recognize the importance of the Zasavica Reserve in preserving both national and European natural, and a confirmation of this is the proclamation reserve for: the Ramsar area since 2008; The IBA area was proclaimed in 2000 and a revision of the area was made in 2009; The IRA area listed since 2005 in the international botanically important areas of Central and Eastern Europe. One of the 40 selected areas for daily butterflies in Serbia (PBA - Prime Butterfly Areas). Due to all of Zasavica's all values, it has been designated as the Areas of Special Conservation Interest (ASCI) under the code RS0000014.

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CLIMATE AS A FACTOR OF WATER DEFICIT AND REAL EVAPOTRANSPIRATION IN THE SOIL WATER BALANCE OF THE PART OF CENTRAL SERBIA

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Abstract

Through the calculation of the water soil balance of the part of Central Serbia for Kraljevo based on climatic elements, air temperature and precipitations over a 3-year period under the conditions favouring the potential evapotranspiration (ETP) during the vegetation, it was found to amount to 604 mm on average and to 782 mm on a year basis, denoting to the water deficit recorded in July averaging 53 mm, to that in August of 71 mm and to that in September of 18 mm. The total average deficit of soil water over the vegetation as well as the annual one amounted to 142 mm being the norm of irrigation conditioned by climate. Thus, for the needs of plant production based on climate, the real water consumption that it ETR (real evapotranspiration) was reported to amount to 462 mm at the level of vcegetation period and to 640 mm on a year basis.

Keywords: climate, evapotranspiration, water balance, soil, water deficit

INTRODUCTION

Due to an increasing need of the world population for food, it seems necessary to increase its production by increasing yield and/or sowing areas. Thus, to provide necessary water amounts for plants seems to be of a prior importance. Water deficit has resulted from its higher consumption, surface, underground and atmospheric waters contamination as well as from the issues of the global changes in climate.

Furthermore, within the technical-technological and agronomical-biological process of agricultural production, the use of irrigation is of paramount importance. Under the conditions of irrigation, the optimal soil humidity, varying due to a precipitation regime, farming culture etc., should be kept [1].

For the needs of plants for water (potential evapotranspiration, i.e. ETP) and towards their normal growth and development, it is necessary to analyse the natural conditions of the local area, primarily the environmental, soil conditions as well as the natural water inflow from the rainfalls. Rainfall and evapotranspiration are the two major climatic factors affecting agricultural production. While rainfall can be directly measured, evapotranspiration is estimated from weather data [2]. ETP is a critical component of water balance at plot, field, farm, catchment, basin or global level. From an agricultural point of view, ETP determines the amount of water to be applied through artificial means (irrigation) [3].

For the ETP calculation, numerous methods, which, at best, approximate the average values of the potential evapotranspiration (ETP) are used. These are mainly empirical methods based on the regional relationship between the evapotranspiration and climatic conditions [4]. The precipitation analysis over the summer months denoted to regular droughts appearing in Serbia [5]. The precipitation deficiency is considered to be the basic factor of the commencement of dry weather [6].

The values of ETP and precipitation amounts of the area concerned make up the basis of water balance in the soil, from which the water deficiencies in it as well as the real evapotranspiration (ETR) may be defined. The calculated water deficit is, therefore, the basis for irrigation while ETP denotes to the real water consumption of the soil belonging to the area underway, being the objective of this paper.

MATERIALS AND METHODS

The data of the mean air monthly temperatures for the area of Kraljevo [7] were used to calculate the mean air temperatures over the vegetation period and the mean annual air temperatures for the period 2015-2017. The sums of the monthly precipitations made at the station (MS) of Kraljevo [7] for 2015, 2016 and 2017, make up the basis of precipitations on a vegetation and a year basis.

The water balance of Kraljevo, which, after being calculated, denoted to the water shortages in the soil as well as to the value of the real evapotranspiration (ETR) was aided by the average monthly values of the evapotranspiration (ETP) [8], calculated according to the Penman-Monteih method for the long-term period (1971-2000), individually, for each year separately. The water balance of the soil of Kraljevo was made following Thorntwaite method [9] for the 3-year period of studies (2015-2017) separately for each year.

Having resulted from the water balance, the water deficit in the soil of Kraljevo was established through calculation for each of all the three study years. Along with calculation within the balancing procedure, the average values of water deficit for the period underway, were recorded.

Throughout the course of water balance calculation, all the soils of the area studied, both those of light and those of heavy mechanical composition were assumed to consist of the reserve of the readily available water (RRAW) of 100mm in the rhizosphere zone. In addition, evapotranspiration is asssumed to be fairly stable up to this water reserve. Unless there is no water in the soil up to the limit of the reserve of the readily available water, then it is prevalently deficient, meaning its deficit when the irrigation is approached. The differences in water-physical traits of the different soil types seemed to be almost insignificant when the water deficit was determined [10].

RESULTS AND DISCUSSION

Geographically, Kraljevo offers fairly suitable conditions for plant production. The variety of natural resources is also favoured by various soils existing on the area of Kraljevo. The soils of weak productional ability due to an unsettled water-air regime appear most.

The area of Kraljevo is characterised by a moderate continental climate being affected by peak moment [11].

The air temperatures were found to affect the water consumption by plants, i.e. the evapotranspiration (ETP) value resulting from them.

The mean air temperature of the area of meteorological station of Kraljevo for 2015 was recorded to be 19.5°C and the mean annual one 12.5°C (Table 1). As regards 2016, that ratio was 18.7°C i.e., 12.2°C and regarding 2017, it was 19.3°C i.e. 12.4°C . Throughout the 3-year period, the average air temperature over the vegetation period amounted to 19.1°C and the mean annual air temperature to 12.3°C. On average, the lowest mean monthly air temperature was reported in January (-0.9°C) and the highest one in July (23.9°C) succeeded by those reported in August (22.9°C) and in June (21.1°C).

***Table 1** Mean monthly, growing season and mean annual air temperatures (°C), Kraljevo (2015-2017)*

Years	Months												Growing season	Mean annual
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2015.	2.3	2.9	6.3	11.6	17.6	19.7	24.6	23.9	19.5	11.6	7.3	2.3	19.5	12.5
2016.	-0.1	8.8	7.8	14.1	15.5	21.3	23.2	20.6	17.6	10.6	6.8	0.0	18.7	12.2
2017.	-5.0	4.5	10.3	11.3	16.2	22.4	24.0	24.2	17.7	11.9	6.8	4.0	19.3	12.4
Average	-0.9	5.4	8.1	12.3	16.4	21.1	23.9	22.9	18.3	11.4	7.0	2.1	19.1	12.3

The vegetation sum of precipitations of the area of Meteorological station of Kraljevo over the 3-year analysis appeared to be similar to the one already presented, being 346 mm (2017), 367 mm (2016) and 375 mm (2015) (Table 2). The annual precipitation sum was reported to be the lowest in 2017 (682 mm), more than 777 mm in 2015 and 835 mm in 2016. Therefore, over the vegetation, 362 mm of precipitations fell on average and of up to 763 mm fell on a year basis. The analysis made revealed that March was the rainiest month (126 mm), followed by May (109 mm) and October (91mm).

***Table 2** Monthly, growing season and annual precipitations (mm), Kraljevo (2015- 2017)*

Years	Months												Growing season	Annual
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
2015.	43	66	163	64	91	90	9	37	84	57	64	9	375	777
2016.	86	53	158	40	136	49	29	63	50	84	78	9	367	835
2017.	22	35	58	82	100	56	35	29	44	133	32	56	346	682
Average	50	51	126	62	109	65	24	43	59	91	58	25	362	763

The value of the potential evapotranspiration, being its mean values for the area of Kraljevo proved to be the highest over the summer months, being in July (127 mm), succeeded by those recorded in August of up to 114 mm, in June (113 mm) and in May (100 mm) (Table 3). Thus, the average ETP for the vegetation period amounted to 604 mm and its mean annual value to 782 mm. The lowest values of ETP were reported in January (15 mm) and in December (16 mm).

Table 3 *Mean monthly, growing season and annual potential evapotranspiration (ETP) (mm), Kraljevo (1971-2000)*

Parameter	**Months**												**Mean growing season**	**Mean annual**
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**		
ETP, mm	15	24	50	73	100	113	127	114	77	48	25	16	604	782

The results of the soil water balance of the area of Kraljevo are the water deficits (Tables 4 and 5) and real evapotranspiration (ETR) (Table 6).

Table 4 *Water deficit in the soil (mm), Kraljevo (2015- 2017)*

Years	**Months**												**Annual**
	I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	
	Water deficit, mm												
2015.							59	77					136
2016.							62	51	27				140
2017.							49	85	33				167

Thus, the water deficits (normal irrigation) in the soil of the area of Kraljevo were reported from July to September over all the three research years (Table 4). During 2015, the total water deficit of up 136 mm was recorded, with 59 mm recorded as deficient in July and 77 mm in September. Further, the total water deficit of 140 mm was recorded during 2016 with 62 mm reported in July, 51 mm in August and 27 mm in September. As for 2017, the total of 167 mm of water was recorded as deficient, with 49 mm evidenced in July, 85 mm in August and 33 mm in September.

The water balance calculated based on the average input values (ETP and precipitations) for the 3-year period, denoted to the average values of water deficit for the aerial soils amounting to: 53 mm in July, 71 mm in August and 18 mm in September averaging the entire vegetation and the annual water deficit of up to 142 mm (Table 5).

Table 5 *Average water deficit in soil (mm), Kraljevo (2015-2017)*

Months												**Annual**
I	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**	**X**	**XI**	**XII**	
Water deficit, mm												
						53	71	18				142

Real evapotranspiration (ETR) denoted to the real state of the water existing in the soil without irrigation. Thus, 462 mm of water was reported in the soils of Kraljevo during the vegetation period and 640 mm annually (Table 6).

Table 6 *Average monthly, growing season and annual real evapotranspiration (ETR) (mm), Kraljevo (2015-2017)*

Parameter	Months												Mean growing season	Mean annual
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII		
ETR, mm	15	24	50	73	100	113	74	43	59	48	25	16	462	640

CONCLUSION

The value of the potential evapotranspiration of the area of MS Kraljevo, on average amounted to 604 mm and to 782 mm annually. Based on the average climatic parameters for the area of Kraljevo for the 3-year period, it may be inferred that soil water balance was evidently deficient averaging 142 mm over the vegetation period and annually. Real avapotranspiration averagely amounted to 462 mm over the vegetation period and to 640 mm on a year basis.

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ENVIRONMENTAL EVALUATION OF GROUNDWATER QUALITY IN THE PROTECTED AREA OF LAKE ZOBNATICA

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Abstract

The uncontrolled and irresponsible use of water for long period of time can lead to environmental pollution and water quality degradation, particularly in sensitive protected water bodies as lakes. The Lake Zobnatica, is characterized as protected natural area, and is surrounded by agricultural land. The key physicochemical parameters that were analyzed are pH, electroconductivity, dissolved oxygen, chemical oxygen demand (COD), biological oxygen demand (BOD_5), total organic carbon (TOC), anions - nitrites ($NO_2^-{}_{(aq)}$) and nitrates ($NO_3^-{}_{(aq)}$), orthophosphates ($PO_4^{3-}{}_{(aq)}$) and ammonium nitrogen cation (NH_4^+-N $_{(aq)}$), total nitrogen and phosphorus, sulphates ($SO_4^{2-}{}_{(aq)}$), chlorides ($Cl^-{}_{(aq)}$), fluorides ($F^-{}_{(aq)}$), total chlorine and cations of metals (nickel ($Ni^{2+}{}_{(aq)}$), iron ($Fe^{2/3+}{}_{(aq)}$), zinc ($Zn^{2+}{}_{(aq)}$), chromium ($Cr^{6+}{}_{(aq)}$), copper ($Cu^{2+}{}_{(aq)}$). The high measured values of orthophosphates with maximum concentrations of 2.459 mg/L for ground water indicate high agricultural activities. Results showed the alkaline ground water polluted by nutrients, and lower values of dissolved oxygen than 5 mg/L. Research study of ground water quality has been conducted from 2018 to 2019 on the protected Lake Zobnatica for the first time within the Interreg IPA CBC Croatia-Serbia Project AF_HR-RS135_SeNs_Wetlands.

Keywords: protected area, key physicochemical parameters, ground water, monitoring of water quality

INTRODUCTION

Lakes as natural water resources are linked by horizontal and vertical transport of nutrients, cations of metals and dissolved oxygen to wastewater as a result of anthropogenic activities, and can be under vast pressure due to pollution, dumping of waste and urbanisation [1,2]. The contamination properties and sources of ground water pollution by agricultural and domestic sources are poorly researched with scarce results, particularly in Serbia. The Lake Zobnatica is surrounded by agricultural land and is a countryside touristic destination, with a vast number of leisure activities. The Lake Zobnatica is protected area formed in 1976 in the valley of a small river, Krivaja, with an area of 226 ha and length of about 5 km in the western part of Vojvodina (Bačka), Serbia (Figure 1). Krivaja is the right tributary of the Danube-Tisa-Danube canal and is the longest river that flows through Vojvodina. The area of the basin is 956 km² and belongs to the Black Sea basin. The primary purpose of the Lake is the irrigation of agricultural areas, but it has become a tourist attraction. Urbanized area of the Lake has a beach, sports facilities, restaurants and bars that are available for the tourists. In the close vicinity of the Lake is the rich agricultural area, which in some parts comes close to the Lake water, to beach and rural settlements [3,4]. Therefore, groundwater quality from nine piezometers was evaluated within research study of the Project.

Figure 1 *Geo-location of the Lake Zobnatica*

In the paper, environmental evaluation of ground water was investigated as good indicator of ecological status of Lake Zobnatica. The analyses of key physicochemical parameters: pH, electroconductivity, dissolved oxygen, COD, BOD_5, TOC, anions - $NO_2^-{}_{(aq)}$ and $NO_3^-{}_{(aq)}$, $PO_4^{3-}{}_{(aq)}$ and NH_4^+-N $_{(aq)}$, total nitrogen and phosphorus, $SO_4^{2-}{}_{(aq)}$, $Cl^-{}_{(aq)}$, $F^-{}_{(aq)}$, total chlorine, and cations of metals ($Ni^{2+}{}_{(aq)}$, $Fe^{2/3+}{}_{(aq)}$, $Zn^{2+}{}_{(aq)}$, $Cr^{6+}{}_{(aq)}$, $Cu^{2+}{}_{(aq)}$) were performed in Accredited Laboratory for monitoring of landfills, wastewater and air, Department of Environmental Engineering and Occupational Safety and Health, Faculty of Technical Sciences, University of Novi Sad. This type of research has been performed for the first time in cooperation of Serbia and Croatia within the Interreg IPA CBC Croatia-Serbia Project „Active SEnsor monitoring Network and environmental evaluation for protection and wiSe use of WETLANDS and other surface waters".

MATERIALS AND METHODS

Physicochemical parameters were analysed in Accredited Laboratory for monitoring of landfills, wastewater and air, Department of Environmental Engineering and Occupational Safety and Health, Faculty of Technical Sciences, University of Novi Sad. The standard EPA and HACH methods were used for all Laboratory measurements - EPA 170.1, EPA 150.1, EPA 120.1, EPA 360.1, EPA 365.3, HACH 8507, HACH 8192, HACH 8155, HACH 8021, HACH 8113, HACH 8023, HACH 8167, HACH 8023, EN ISO 11905-1, Method 8047, Method 8150, Method 8146, Method 8009, Method 8143 and ISO 15705. The sampling procedure was conducted according the Standards SRPS EN ISO 5667-1:2008 (Guidelines for development of sampling programs). SRPS EN ISO 5667-3:2007 (Guidelines for protection and handling of the sample). SRPS EN ISO 5667-6:1997 (Guidelines for abstraction of samples from rivers and streams). The classification of surface water quality prescribed by Regulation on emission limit values of polluting substances in surface and groundwater and deadlines for their achievement (Official Gazette of the RS 50/2012) is shown in Table 1 and Table 2 [5]. The lakes are natural reservoirs of dynamic and static water body types, and the sensitivity of this type of eco-system is higher and more fragile. Henceforth, the maximum allowable values of physicochemical parameters in lakes are more restrictive than in rivers.

Table 1 *The maximum allowable values of physicochemical parameters for class I and II quality of water for lakes within the 200 m of sea level [5]*

Parameter	Class I	Class II
pH	**6.5-8.5**	**6.5-8.5**
Dissolved oxygen	**8.52**	**7**
TOC	**2**	**6**
BPK5	**2**	**5**
NH_4^+-N	**0.1**	**0.3**
NO_3^-	**1**	**3**
PO_4^{3-}	**0.02**	**0.1**
Ptot	**0.05**	**0.2**
Cl^-	**50**	**100**

pH has no unit, and other parameters are expressed in mg/L

Table 2 *The limit values of physico-chemical parameters for classification of water quality [5]*

Parameter	Class I	Class II	Class III	Class IV	Class V
pH	6.5-8.5	6.5-8.5	6.5-8.5	6.5-8.5	<6.5 or >8.5
Conductivity	<1000 / BLQ	1000	1500	3000	>3000
Dissolved oxygen	- / BLQ	- / BLQ	5	4	<4
PO_4^{3-}	- / BLQ	-	0.2	0.5	>0.5
NO_2^-	0.01 / BLQ	0.03	0.12	0.3	0.3
NO_3^-	- / BLQ	-	6	15	>15
NH_4^+-N	- / BLQ	-	0.6	1.5	>1.5
$SO_4^{2-}{}_{(aq)}$	50 / BLQ	100	200	300	>300
$Cl^-{}_{(aq)}$	50 / BLQ	-	150	250	>250
Total Cl	0.005	0.005	-	-	-
TOC	- / BLQ	-	15	50	>50
COD	10 / BLQ	15	30	125	>125
BOD_5	- / BLQ	-	7	25	>25
Cr (total)	25 / BLQ	50	100	250	>250
$Cu^{2+}{}_{(aq)}$	5/22/40/112*	5/22/40/112*	500	1000	>1000
$Zn^{2+}{}_{(aq)}$	30/200/300/500*	300/700/1000/2000*	2000	5000	>5000
$Fe^{2/3+}{}_{(aq)}$	200	500	1000	2000	>2000
Fenols	<1	1	20	50	>50

*BLQ – base-line quality; * - depending on the water hardness 10, 50, 100 and 500 mgCaCO$_3$/L, pH has no unit, conductivity is expressed in µS/cm, TOC, COD, BOD in mgO$_2$/L and other parameters in mg/L*

RESULTS AND DISCUSSION

The results of key physicochemical parameters of ground water of the Lake Zobnatica are shown in Table 3. According to the results and maximal allowable values Zobnatica Lake can be classified as class IV at best. According to the national Law the lakes within the 200 m of sea level should have water quality of class I or II. Some parameters, pH, dissolved oxygen,

NO_2^-, total chlorine, COD, conductivity, $PO_4^{3-}{}_{(aq)}$ and NH_4^+-N exceed even the values set for class V quality of water.

Table 3 *Results of physicochemical parameters of ground water in the vicinity of the Lake Zobnatica*

02.07.2018.									
Parameter	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
pH	7.81	7.82	**9.07**	7.88	7.76	**8.92**	**9.00**	**9.09**	**9.14**
Conductivity	509	620	598	516	**1217**	794	478	**1002**	910
Dissolved oxygen	**4.80**	**4.63**	**5.40**	**5.58**	**5.45**	**0.86**	**1.38**	**3.94**	**4.42**
NO_3^-	0.80	0.45	0.02	0.02	0.35	0.02	0.02	0.34	0.42
NO_2^-	**0.501**	**0.525**	0.008	0.014	**0.165**	0.013	0.008	**0.225**	0.024
NH_4^+-N	0.29	0.15	0.03	0.02	<0.01	0.02	0.3	0.18	<0.01
Total nitrogen	2.03	3.84	4.42	1.17	**11.8**	0.782	1.12	**15.9**	**20.1**
$PO_4^{3-}{}_{(aq)}$	**0.310**	**0.484**	**0.308**	**0.379**	**0.407**	**0.489**	**0.350**	**0.309**	**0.346**
Total chlorine	**0.02**	**0.04**	**0.03**	**0.04**	**0.16**	**0.07**	**0.01**	**0.03**	**0.02**
$Cl^-{}_{(aq)}$	<0.01	2.2	<0.01	<0.01	1.8	0.9	<0.01	1.1	2.7
$F^-{}_{(aq)}$	0.19	0.65	0.94	2.56	0.73	1.04	0.14	0.34	0.41
Cr^{6+}	0.015	0.012	0.014	0.013	0.039	0.022	0.015	0.009	0.008
$SO_4^{2-}{}_{(aq)}$	0	11	0	1	61	42	1	80	70
TOC	5.438	**46.44**	3.38	**9.07**	**15.91**	**34.51**	**7.295**	**29.97**	**13.13**
COD	**24.9**	**155**	9.07	**346**	**44.8**	**91.4**	**26.5**	**100**	**96.8**
30.08.2018									
Parameter	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
pH	7.07	7.06	7.17	7.22	7.18	7.29	7.33	7.46	7.07
Conductivity	477	718	599	519	**1360**	477	965	866	477
Dissolved oxygen	**4.10**	**0.80**	**6.77**	**4.21**	**6.77**	**5.70**	**5.80**	7.53	**4.10**
NO_3^-	0.007	0.585	0.066	0.010	0.078	0.004	0.048	0.048	0.007
NO_2^-	0.01	**1.50**	**1.07**	**0.04**	**1.5**	0.03	**0.92**	**1.6**	0.01
NH_4^+-N	0.01	0.03	0.03	0.01	0.01	0.02	0.01	0.01	0.01
Total nitrogen	1.34	2.15	2.96	0.803	**26.5**	0.817	1.98	2.55	1.34
$PO_4^{3-}{}_{(aq)}$	**0.420**	**0.621**	**1.798**	**1.325**	**1.182**	**1.835**	**2.429**	**0.956**	**0.420**
Total chlorine	**0.01**	**0.09**	**0.02**	**0.01**	**0.02**	**0.01**	**0.01**	**0.01**	**0.01**
$Cl^-{}_{(aq)}$	12.1	48.6	17.6	10.9	35.4	5.5	5.8	94.1	12.1
$F^-{}_{(aq)}$	0.18	0.03	0.22	0.10	0.18	<0.02	0.04	0.48	0.18
Cr^{6+}	0.001	0.036	0.004	0.009	0.011	0.01	0.010	0.007	0.001
$SO_4^{2-}{}_{(aq)}$	0	31	11	1	71	0	79	66	0
COD	**80.9**	**98.2**	8.73	**50.9**	10.4	**38.2**	**28.7**	**57.3**	**68.2**
BOD_5	0.30	1.3	1.1	0.44	0.52	1.90	2.1	3.26	0.36

***Table 3** continued*

20.02.2019.								
Parameter	**B1**	**B2**	**B3**	**B4**	**B5**	**B7**	**B8**	**B9**
pH	7.20	7.26	7.48	7.47	7.25	7.34	7.52	6.95
Conductivity	832	**1222**	959	900	**1446**	**1055**	990	705
Dissolved oxygen	**5.51**	**5.80**	**6.26**	**5.41**	**6.02**	**5.43**	**5.24**	**2.63**
NO_3^-	0.026	0.028	0.050	0.005	0.068	0.009	0.001	0.013
NO_2^-	2.55	2.31	2.8	0.02	3.42	0.06	0.01	0.04
NH_4^+-N	0.01	0.01	0.02	0.01	0.06	0.05	0.08	0.03
Total nitrogen	**18.3**	**24.1**	9.5	5.2	**25.9**	1.24	3.84	**16.7**
$PO_4^{3-}{}_{(aq)}$	<0.01							**4.075**
$Cl^-_{(aq)}$	26	44.6	41.3	34.6	**100.2**	32.8	18.3	26
$F^-_{(aq)}$	0.23	0.22	0.23	0.11	0.12	0.17	0.42	0.21
Cr^{6+}	0.004	0.004	0.007	0.002	0.002	0.002	0.004	0.002
$SO_4^{2-}{}_{(aq)}$	75	72	65	43	77	42	83	10
COD	6.11	8.99	3.21	2.21	1.01	1.38	2.38	3.52

16.04.2019.									
Parameter	**B1**	**B2**	**B3**	**B4**	**B5**	**B6**	**B7**	**B8**	**B9**
pH	8.182	8.089	8.213	8.043	7.520	7.925	7.689	8.073	7.91
Conductivity	1000	**1428**	**1112**	**1094**	**1670**	**1616**	**1256**	**1141**	817
Dissolved oxygen	**6.47**	**5.69**	**6.73**	**5.23**	**5.73**	**6.74**	**4.86**	**6.47**	**1.54**
NO_3^-	0.001	0.001	0.001	0.002	0.008	0.019	0.002	0.007	0.003
NO_2^-	**1.72**	**1.81**	**1.56**	**1.68**	**2.01**	**0.66**	0.03	**2.37**	**0.06**
NH_4^+-N	<0.01	<0.01	0.01	<0.01	0.15	**0.52**	0.06	<0.01	**2.5**
Total nitrogen	**76.23**	**46.01**	**21.59**	**23.05**	**46.38**	**12.77**	<1	**25.47**	**31.74**
$PO_4^{3-}{}_{(aq)}$	<0.01	<0.01	<0.01	<0.01	<0.01	**8.042**	<0.01	<0.01	**3.678**
$Cl^-_{(aq)}$	23.5	24.1	27.7	47.3	**144.1**	25.2	23.8	11	18.1
$F^-_{(aq)}$	0.36	0.45	0.31	0.28	0.30	1.69	0.19	0.44	0.16
Cr^{6+}	<0.01	<0.01	<0.01	<0.01	0.012	0.012	<0.01	0.021	0.01
$SO_4^{2-}{}_{(aq)}$	32	58	61	21	80	94	28	85	17
TOC	**21.91**	**11.51**	**11.41**	**13.55**	**9.25**	**20.73**	**22.35**	**14.31**	**45.19**
COD	11.2	2.07	5.63	3.87	**21.5**	**30.7**	3.5	5.82	**96.9**

The values of parameters in the Table 3 that exceed the values set for class V water according to the limiting values of polluting substances in surface and groundwater and deadlines for their achievement are marked grey and bold, and the values that exceed the values set as the maximum allowable values of physicochemical parameters for class I and II quality of water for lakes below 200 m of sea level are bold.

All the metal cations, BOD, phenols and NH_4^+-N (except one measurement on location B6) measured were within the ranges set for class I quality of ground water. According to the Urban Waste Water Directive (91/271/ EEC) discharge from sewage treatment plants to surface water cannot exceed 10 mg/L of total nitrogen [6]. In the case of Lake Zobnatica this value has been exceeded mostly during the winter and spring, and it is shown in the range of 11.08 to 76.23 mg/L. The pH value indicates whether the solution is acidic, neutral or alkaline. In natural unpolluted waters the pH value depends on free CO_2, carbonate and bicarbonate. Natural waters have a pH value of 6.5 to 8.5. pH values in samples B3 and B6-B9 of ground water were higher than 8.5, which point out pollution of ground water. Higher pH value indicates alkaline ground water in the summer months. The parameter conductivity suggests that water quality is in flux between class II and III. The most interesting parameters for evaluation and discussion are dissolved oxygen and orthophosphates. The dissolved oxygen in limnology (the study of lakes), is an essential factor [7]. A dissolved oxygen level that is too high or too low can harm aquatic life and affect water quality. In the ground water of the Lake Zobnatica dissolved oxygen was measured in range from 0.8 to 7.53 mg/L. Values of orthophosphates in concentrations <0.01 to 8.04 mg/L suggests mostly pollution from agricultural activities.

CONCLUSIONS

Results of the research study within the Project SeNs Wetlands showed the pollution of ground water with nutrients during summer months, alkaline water with pH value higher than 8.5 during winter and low values of dissolved oxygen in the most of the samples are reason for concern. High values of orthophosphates and total nitrogen suggest the mostly pollution from high agricultural activities. Concentration levels of nutrients, cations of metals and dissolved oxygen could serve as good indicator of ecological status of Lake Zobnatica.

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RAPID ALERT SYSTEM FOR FOOD AND FEED - RASFF AND REPUBLIC OF SERBIA

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Abstract

A key tool to ensure the flow of information to enabling reaction when risks to public health are detected in the food chain is RASFF - the Rapid Alert System for Food and Feed. The aim was to present notification results of unsafe food concerning Republic of Serbia. From January 2018 until May 2019, 25 notifications were found when Serbia is mention as a country where food was delivered or a country of origin of food. In 10 of 25 cases Serbia was country of origin for notified food. In all cases risk decisions were serious except dietetic foods where risk decision was undecided. In 15 notifications, Serbia was country where unsafe food is distributed. Almost in all cases risk decisions were serious. The establishment of the Rapid Alert System in the territory of the Republic of Serbia, leads to the highest level of human health protection and unappropriate economic damage.

Keywords: food, notification, unsafe, RASFF

INTRODUCTION

The EU has one of the highest food safety standards in the world - largely thanks to the solid set of EU legislation in place, which ensures that food is safe for consumers. A key tool to ensure the flow of information to enabling swift reaction when risks to public health are detected in the food chain is RASFF - the Rapid Alert System for Food and Feed.

Created in 1979, RASFF enables information to be shared efficiently between its members (EU Member State national food safety authorities, Commission, EFSA, ESA, Norway, Liechtenstein, Iceland and Switzerland) and provides a round-the-clock service to ensure that urgent notifications are sent, received and responded to collectively and efficiently. Thanks to RASFF, many food safety risks had been averted before they could have been harmful to European consumers [1].

Vital information exchanged through RASFF can lead to products being recalled from the market. A robust system, which has matured over the years, RASFF continues to show its value to ensure food safety in the EU and beyond.

The RASFF portal features an interactive searchable online database. It gives public access to summary information about the most recently transmitted RASFF notifications as well as the ability to search for information on any notification issued in the past.

The RASFF consumers' portal has been available for consumers to use since June 2014. The portal provides the latest information on food recalls and public health warnings in all EU countries.

The European Commission created the RASFF database to keep its information as transparent as possible to the consumers, business operators and authorities around the world. In doing so, however, it needs to strike a balance between openness and protection of information that could lead to disproportionate economic damage [1].

The effectiveness of RASFF is ensured by keeping its structure simple: it consists of clearly identified contact points in the Commission (DG Health and Food Safety), EFSA, EFTA surveillance authority and at national level in member countries [2].

EFSA is a European agency funded by the European Union that operates independently of the European legislative and executive institutions (Commission, Council, Parliament) and EU Member States.

It was set up in 2002 following a series of food crises in the late 1990s to be a source of scientific advice and communication on risks associated with the food chain.

The General Food Law created a European food safety system in which responsibility for risk assessment (science) and for risk management (policy) are kept separate. EFSA is responsible for the former area, and also has a duty to communicate its scientific findings to the public.

Thanks to this system, European consumers are among the best protected and best informed in the world as regards risks in the food chain.

As the risk assessor, EFSA produces scientific opinions and advice that form the basis for European policies and legislation. Our remit covers: food and feed safety, nutrition, animal, health and welfare, plant protection and plant health.

EFSA also consider, through environmental risk assessments, the possible impact of the food chain on the biodiversity of plant and animal habitats. Since it was set up, EFSA has delivered scientific advice on a wide range of issues such as Bovine Spongiform Encephalopathy (BSE), Salmonella, food additives such as aspartame, allergenic food ingredients, genetically modified organisms, pesticides, and animal health issues such as avian influenza. EFSa has an important role in collecting and analysing data to ensure that European risk assessment is supported by the most comprehensive scientific information available. It does this in cooperation with EU Member States.

Communicating on risks associated with the food chain is another key part of EFSA’s mandate. This means providing appropriate, accurate and timely information on food safety issues to raise awareness and explain the implications of our scientific work. Scientific results cannot always be easily converted into simple guidelines and advice that non-scientists can understand. One of EFSA’s tasks, therefore, is to communicate clearly not only to its principal partners and stakeholders but also to the public at large, to help bridge the gap between science and the consumer [3].

The Commission carries out, every week, a screening of all notifications reported in the Administrative Assistance and Cooperation (AAC) system and in the Rapid Alert System for Food and Feed (RASFF).

The objective is to identify a potential intentional violation of the EU agri-food chain legislation which may have remained undetected by Member States and potential violations of the EU agri-food chain which might need an adequate coordination and follow up at EU level.

These notifications are identified in RASFF to the attention of the EU countries for further reaction or transferred directly to the EU Food Fraud Network for further treatment and action. Stakeholders are also informed when new or unexpected fraudulent schemes are detected.

The Commission services - at the request of one or more EU countries or by its own initiative - can coordinate activities at EU level. This happens when operators in several EU countries are involved in a possible fraudulent scheme, when operators in non-EU countries might be involved and when the suspicion presents either a health risk or a significant socio-economic risk.

Decision criteria for such coordination at EU level take into account the seriousness of those risks, the reliability of the information available and its similarity to previous occurrences.

When the suspicion is related to imported products, the Commission works with the concerned non-EU countries and asks for targeted information and investigations.

Very concrete results have been achieved in the fight against food fraud thanks to the cooperation between EU countries in the framework of the AAC and between EU countries and non-EU countries. And also thanks to consumer awareness and industry vigilance, with the scientific support from the Knowledge Centre for Food Fraud and Quality - Joint Research Centre and the European Food Safety Authority (EFSA) [4].

Republic of Serbia is not yet member of RASFF network, but Serbia sets regulation for establishing and organisation rapid alert system for food and feed. The responsibility for this matter is on Ministry of agriculture, forestry and water management [4].

MATERIALS AND METHODS

The RASFF portal was searched under following criteria: notified from 01/01/2018 - notified till 15/05/2019, product type: food and country Serbia (RS).

Under product type food, following product categories were searched: cereals and bakery products, cocoa and cocoa preparations, coffee and tea, dietetic foods, food supplements, fortified foods, fruits and vegetables, herbs and spices, milk and milk products, non-alcoholic beverages, nuts, nut products and seeds and poultry meat and poultry meat products.

RESULTS AND DISCUSSION

During period from 01.01.2018 until 15.05.2019. it was 25 notifications when Serbia is mention as a country where food was delivered or as a country of origin of food.

In 10 of 25 cases Serbia was country of origin for notified food. Number of notifications per product category is presented in Table 1.

Table 1 *Number of notifications according to product category*

Product category	Number of notifications
cereals and bakery products	4
cocoa and cocoa preparations	1
dietetic foods, food supplements, fortified foods	1
fruits and vegetables	4

Reasons (subjects) for notification according product category and notification basis were:

- cereals and bakery products - atropine (4.5 µg/kg - ppb) and scopolamine (4.3 µg/kg - ppb) in corn grits from Serbia (official control on the market) and high content of acrylamide (950 µg/kg - ppb) in biscuits from Serbia (official control on the market) and deoxynivalenol (DON) (970 µg/kg - ppb) and fumonisins (2480 µg/kg - ppb) in corn flour from Serbia (official control on the market) and ochratoxin A (25.8 µg/kg - ppb) in bread crumbs from Serbia (border control - consignment under customs)
- cocoa and cocoa preparations, coffee and tea - atropine (200.5 µg/kg - ppb) and scopolamine (488.7 µg/kg - ppb) in peppermint from Serbia (company's own check)
- dietetic foods, food supplements, fortified foods - unauthorised substance magnesium in metal form (particles) in food supplement from Serbia (official control on the market)
- fruits and vegetables - norovirus (GII /25g) in frozen raspberries from Serbia (border control), aflatoxins (Tot. = 24.39 µg/kg - ppb) in dried figs from Serbia, with raw material from Turkey (border control), glass fragments in frozen blackberries from Serbia, via Switzerland (consumer complaint) and oxamyl (0.042 mg/kg - ppm) in cucumbers from Serbia (border control)

In all cases risk decisions were serious except dietetic foods where risk decision was undecided.

In 4 cases (1 cereals and bakery products and 3 fruits and vegetables) the notification type was border rejection. In other 6 cases, the notification type was information for attention and alert.

In 15 notifications, Serbia was country where unsafe food is distributed. Product categories, number of notifications and subjects for notifications are presented in Table 2.

Table 2 *Notifications of unsafe food distributed in Serbia*

Product category	Number of notifications	Notification basis	Notification type	Subjects for notification	Risk decision
cereals and bakery products	2	company's own check and official control on the market	alert	metal strips in chilled puff pastry from Austria, with raw material from Italy and atropine (6.60 µg/kg - ppb) and scopolamine (1.77 µg/kg - ppb) in popcorn from France	serious

***Table 2** continued*

cocoa and cocoa preparations, coffee and tea	1	official control on the market	alert	high content of aluminium (1910; 1782 mg/kg - ppm) in matcha powder (dried green tea) from Germany, with raw material from Japan	serious
dietetic foods, food supplements, fortified foods	5	company's own check (3) and official control on the market (2)	Alert (4) and information for follow-up	sulphite (40 mg/kg - ppm) undeclared in organic chlorella and spirulina powder from China, via the United Kingdom, sulphite undeclared in organic chlorella powder and tablets from China, via the United Kingdom traces of lactoprotein (23.2 mg/kg - ppm) in vegan protein from Belgium, unauthorised novel food ingredient Salvia hispanica in food supplement from the United States, via the Netherlands, aflatoxins (Tot. = 0.582 / Tot. = 0.545 / Tot. = 0.489 / Tot. = 0.670 / Tot. = 0.503 µg/kg - ppb) in fruit-based infant purees from Germany, manufactured in Italy	Serious (4) and undecided for the last subject
fruits and vegetables	1	company's own check	alert	methomyl (0.087 mg/kg - ppm) in table grapes from Peru	serious
herbs and spices	1	official control on the market	alert	benzo(a)pyrene (16.1 µg/kg - ppb) and polycyclic aromatic hydrocarbons (sum of PAH4: 59.8 µg/kg - ppb) in ground black pepper from Austria	serious
milk and milk products	1	consumer complaint	alert	plastic fragments in yogurt from Poland, with raw material from Morocco, via the Netherlands	serious
non-alcoholic beverages	1	company's own check	alert	risk of physical injury due to explosion of overpressurised (yeast fermentation) bottles containing non-alcoholic carbonated grape flavoured drink for children from Hungary	serious
nuts, nut products and seeds	2	official control on the market and company's own check	information for attention and alert	atropine (16177.6 µg/kg - ppb) and scopolamine (4658.3 µg/kg - ppb) in whole cumin seeds from Hungary sulphite (22 mg/kg - ppm) undeclared on grated coco from Indonesia	serious
poultry meat and poultry meat products	1	official control on the market	alert	Salmonella enterica ser. Typhimurium (presence /25g) in frozen quails from Italy	serious

CONCLUSION

The establishment of the Rapid Alert System in the territory of the Republic of Serbia, as well as the inclusion of that system in the system of rapid notification for food or feed safety of the European Union or at the international level in accordance with the international agreement and the law regulating food safety has the aim to ensures that food is safe for consumers. The RASFF database is available for consumers and keeps its information as transparent as possible to the consumers, business operators and authorities around the world.

In doing so, it leads to the highest level of human health protection and unappropriate economic damage.

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SENS WETLANDS PROJECT: OVERVIEW OF THE INTERREG IPA PROJECT BETWEEN CROATIA AND SERBIA

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Abstract

Wetlands are areas of diverse biodiversity that are becoming more sensitive and vulnerable by modernization and exploitation, mainly due to exponential growth of agricultural activities. The Lake Zobnatica, Serbia and the Wetlands of Tompojevci, Croatia are characterized as protected natural areas and are surrounded by agricultural land, and thus selected for the research area of Project. The key goal of the Project is local support, education on purpose of vegetation in the wetland ecosystem and positive effects on the sustainable development of the area, community and public via remote-sensing and analytical monitoring of key physicochemical parameters. Pollution analysis will provide bases for development of guidelines specified for the growth and maintenance of a protective green belt, proven to minimize or eliminate contaminants. This research is performed in cooperation of Faculty of technical sciences, University of Novi Sad, Institute for Nature Conservation of Vojvodina Province, Serbia, Faculty of Electrical Engineering, Computer Science and Information Technology, University of Osijek, and Public Institution for Management of Protected Natural Values of Vukovar-Srijem County, Croatia within the Interreg IPA CBC Croatia-Serbia Project „Active SEnsor monitoring Network and environmental evaluation for protection and wiSe use of WETLANDS and other surface waters" SeNs Wetlands financed by EU realized through 5 work packages.

Keywords: SeNs Wetlands Project, key physicochemical parameters, sensors, protective green belt, wetlands

INTRODUCTION

The main objective of the SeNs Wetlands Project is to enforce integrated cross-border monitoring for key parameters and existing risks, as well as environmental and biodiversity protection [1]. It will be carried out by implementation of integrated continuous monitoring system for the purpose of cross-border data collection and evaluation. The expected changes are improvement of the knowledge level and existing data, development of guidelines for surface and groundwater and biodiversity protection, use of modern sensor methods. The results will be the Guidebook, for the establishment and maintaining vegetated strips along the banks of surface waters in agriculture landscapes of Pannonia Region. The optimal structure of multifunctional bank vegetation belt can be defined by the analysis of merged data. The main target groups are Municipalities and Local Authority, Local Community, Public Utilities, Private and Governmental Institutions, Environmental and civil NGOs and University and Research Institutes.

The Project sites are within hydrological systems of watercourses recognized as ecological corridors, as the wetland in Croatia has a direct influence on the water of the Natura 2000 site, and the site in Serbia is proposed for the Nature Park. Water pollution from agricultural sources is a common problem of both Serbia and Croatia. The water quality is one of the drivers of biodiversity loss in wetlands. Buffer/filter function of the bankside vegetation have not been recognized widely, field data from the Pannonian region are scarce and mostly gained by indirect methods. The continuous monitoring by sensors located in measurement wells will produce data about the quantity and seasonal dynamics of the most important pollutants responsible for the eutrophication. Direct beneficiaries of the Project will be the local municipalities and/or water authorities. Disimination of results will provide data to research facilities or water authorities. Indirect benefits will be accessible data for all stakeholders. The Project SeNs Wetlands emerged from the necessity of applying an extra effort in the process of protection of wetlands and habitats which represent the most sensitive and endangered ecological systems. Wetlands are areas of diverse biodiversity that are becoming more sensitive and vulnerable by modernization and exploitation, mainly due to exponential growth of agricultural activities.

Impaired and degraded ecosystems have reduced tolerance and adaptability to environmental condition variations. The optimal revitalisation of ecosystem require high inter and transdisciplinary, cross-border activities. The project addresses one of the major cross-border challenges of the Pannonia Region and the bordering agricultural landscapes characterized by the high percent of arable land and increased pressures on the natural systems. Water pollution from agricultural sources is a common cross-border problem, caused by cultivation reaching the edge of water. The poor water quality is not only causing the loss of biodiversity in wetlands, but often makes the surface waters inadequate for irrigation. Although the natural habitats are heavily fragmented, a significant number of existing and planned Natura 2000 sites are in the region. Project sites are within hydrological systems of watercourses (Bosut in Croatia, Krivaja in Serbia) recognised as ecological corridors. The wetland in Croatia have a direct effect on the water quality of the Natura 2000 site located downstream, and the project site in Serbia is proposed for the protection as Nature Park. The fragmentation/isolation problems, eutrophication and pollution of wetlands in these agricultural areas have to be solved for the purpose of biodiversity conservation. Predicted climate changes, in North Serbia and eastern part of Croatia, raise the needs for the extension of irrigation capacities and for the improvement of landscape resilience by habitat revitalisation and establishment of green infrastructure. Filter and buffer function of the bank vegetation have no wider recognition, data from the region are scarce and gained by indirect methods. The frequent summer droughts considerably increase the costs of the afforestation and hedge planting. There is a need to find the optimal vegetation structure, adapted for local climatic conditions and effective in pollution reduction.

The key goal of the SeNs Wetlands Project is monitoring of water quality, nurturing and maintaining flora (green belt) and fauna, education on purpose of vegetation in the wetland ecosystem and positive effects on the sustainable development of the area, local community and the general public. The pollution analysis will provide bases for development of guidelines and the Guidebook specified for the growth and maintenance of a protective green belt that is proven to minimize or eliminate contaminants.

All the key physicochemical parameters (in Serbia and Croatia) will be the result of collaboration of the Project Partners. The analytical determination of key physicochemical parameters is performed in Accredited Laboratory for monitoring of landfills, wastewater and air, Department of Environmental Engineering and Occupational Safety, Faculty of Technical sciences, University of Novi Sad. Monitoring by fibre optical system (FOS) will be set *in-situ*. These types of research have been performed for the first time in Serbia and Croatia within the Interreg IPA CBC Croatia-Serbia Project „Active SEnsor monitoring Network and environmental evaluation for protection and wiSe use of WETLANDS and other surface waters“ AF_HR-RS135_SeNs_Wetlands financed by European Union.

MATERIALS AND METHODS

The Project addresses the major cross-border challenge of the Pannonia Region and the bordering agricultural land characterized by the increased pressures on the natural systems. Water pollution from agricultural sources is a common cross-border problem. The poor water quality is causing the loss of biodiversity and often makes surface waters inadequate for irrigation. Surface and ground water pollution from agricultural sources is a pressing cross-border problem.

The seasonal sampling for quantitative and qualitative analysis of the selected physicochemical parameters of water by analytical laboratory methods and fiber optic sensors (FOS) will be conducted. The key physicochemical parameters pH, electroconductivity, dissolved oxygen, COD, BOD_5, TOC, anions - $NO_2^-{}_{(aq)}$ and $NO_3^-{}_{(aq)}$, $PO_4^{3-}{}_{(aq)}$ and NH_4^+-N $_{(aq)}$, total nitrogen and phosphorus, $SO_4^{2-}{}_{(aq)}$, $Cl^-{}_{(aq)}$, $F^-{}_{(aq)}$, Cl_2, and cations of metals ($Ni^{2+}{}_{(aq)}$, $Fe^{2/3+}{}_{(aq)}$, $Zn^{2+}{}_{(aq)}$, $Cr^{6+}{}_{(aq)}$, $Cu^{2+}{}_{(aq)}$) are performed in Accredited Laboratory. Laboratory analysis of physicochemical parameters of run-off, groundwater wells and surface water samples from 18 locations in Serbia, and 14 in Croatia are performed. Key physicochemical parameters will be selected for determination by FOS device. Laboratory equipment based on FOS will be developed and calibrated to monitor the quality of the studied water bodies. Efficiency of the device will be tested. Field FOS device based on laboratory prototype will be developed. Analysed physicochemical parameters in water by fibre optic sensors device are: NO_2^- and NO_3^-, NH_4-N, Cl_2, PO_4^-, SO_4^{2-}, $Cl^-{}_{(aq)}$, $F^-{}_{(aq)}$, Ni^{2+}, Fe^{3+}, Zn^{2+}, Cr^{6+}, Cu^{2+}.

The planned biodiversity survey enables the comparison of the buffer capacity and habitat quality of the bank vegetation.

The contamination properties and sources of wetlands with the reference to the industrial, agricultural and domestic sources are poorly researched and still highly unknown, particularly in Western Balkan countries. Sensitive water bodies, the Lake Zobnatica and the Wetlands of Tompojevci, are surrounded by agricultural land, and Lake Zobnatica, as a countryside touristic destination, has a vast number of leisure activities (Figure 1).

Figure 1 *The wetlands of Tompojevci and the Lake of Zobnatica*

RESULTS AND DISCUSSION

According to the results and maximal allowable values Wetlands of Tompojevci belongs to the class V water quality (orthophosphates and dissolved oxygen), with high trophic state and bad eco-status. The results of preliminary screening analyses in Wetlands of Tompojevci indicated pollution and need for detailed monitoring of surface water as well as ground water and run-off water. The preliminary results and maximal allowable values show that the Lake Zobnatica can be classified as class IV at best, as poor ecological status and utilization for irrigation and industrial use (process and cooling water) [2,3].

Following the development and progress of the Project the parameters shown of most importance to the analytical study of the wetlands in Tompojevci and Lake Zobnatica are pH, dissolved oxygen, conductivity, ortophosphates, nitrates, nitrites and total nitrogen. The sensors of FOS system are in the process of development expected to be set on locations.

All obtained results of sampled ground and surface water, indicate significant pollution, leading to the conclusion that the surrounding agricultural land has high impact onto the sensitive water bodies Wetlands of Tompojevci, Croatia and the Lake Zobnatica, Serbia.

CONCLUSIONS

The SeNS Wetlands Project main objective is development of integrated cross-border monitoring for protection of environment and biodiversity, which will provide new cross-border data correlation and evaluation. Specific objectives of the Project are development and implementation of active sensor monitoring system which will provide cross-border database; analysis of physicochemical parameters in water by standard analytical and new fiber optic sensor methods; definition of guidelines for enhancing the ecosystem services of bankside vegetation in the agricultural landscapes in Pannonia region and adjacent areas; collection of data about the interests of local communities and other stakeholders in the sustainable use of the multifunctional vegetation strips. The SeNS Wetlands Project main outputs are Development of wireless water quality measurement stations and monitoring network system for Data Acquisition, Processing and Presentation (DAPP); New fiber optic sensor (FOS) for monitoring of environmental water quality; Examination of environmental water quality inside the structural elements of the ecological network and measurement wells; Guidelines for the establishment of multifunctional bankside vegetation strips and plan for the establishment of multifunctional vegetation strip.

Highly inter and multidisciplinary area of environmental protection has a significant segment of transdisciplinarity, cross-border and inter regional character. The significance of the obtained results is also the possibility to disseminate knowledge, experience to wider areas of similar geological, climatic and physical-chemical characteristics.

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VIABILITY ASSESSMENT OF MAIZE (*Zea mays* L.) SEEDS CONTAMINATED WITH AFLATOXIN USING FLUORESCENCE SPECTROSCOPY

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Abstract

Maize seeds are a primary source of nourishment and their viability is a critical consideration to ensure a reasonably high harvest and seed quality. In this work, we study the effect of aflatoxin induced stress on the seed viability concerning the germination, as well as the estimation on the impact on the viability using fluorescence spectroscopy. Our results showed that the contaminated seeds exhibit significant decreases in the percent of germination, even at low levels, if compared to the uncontaminated seeds (P < 0.05). Furthermore, the fluorescence ratio of 453/680 and 680/751, could be used for the rapid screening of the viability of the maize seeds.

Keywords: maize (*Zea mays* L.), Seed viability, aflatoxin stress, fluorescence ratios

INTRODUCTION

Aflatoxins are toxic secondary metabolites produced by the fungi, such as Aspergillus flavus. They contaminate a variety of food commodities, including wheat, maize, rice, dried fruits, and nuts [1]. Maize is one of the main energy source for animal feed amd hence, from an economical point of view, it is one of the most important crops. Maize seeds can be infested with toxigenic fungal species in the field or during storage, which can lead ultimately to a contaminating of the food, as well as the feed with mycotoxins, showing toxic effects on animals and humans [2].

The phytotoxic effect of aflatoxin is observed on the basis of the remarkable inhibitory effect on chlorophyll and carotenoid synthesis and the reduction of seed germination and seedling growth of lettuce mung, mustard, cowpea and sesame [3].

In this work we use fluorescence spectroscopy to investigate the phytotoxic effects of aflatoxin in low-stressed maize seeds, in terms of the seeds' viability.

MATERIALS AND METHODS

Plant Materials

The maize (*Zea mays* L.) seeds, both uncontaminated, as well as contaminated with aflatoxin B1(AFB1), were used in this study. The AFB1 concentration in the maize samples was 33 ppb. A total of 80 seeds were divided into two groups, with four replicates per group.

The seeds were immersed in a 0.2% sodium hypochlorite solution, after that the seeds were washed with plenty of water. The seeds are placed between the two paper filters in the Petri dishes, and 10 ml of distilled water is poured onto the upper filter paper. Incubation was performed in the laboratory, at a temperature of approximately 25° C in dark, sealed in aluminium foil bags for four days. The number of germinated seeds was noted every day (Germination was considered as the detection of the radicle breaking through the seed coat). For the preparation of the maize extracts, the seeds were homogenized with 80% cold methanol. The homogenates were kept in the shaker for one hour and then centrifuged at 10 000 x g for 5 min.

Fluorescence spectroscopy

The fluorescence spectra were recorded for both the contaminated and un-contaminated maize seeds before germination (BG), and after the fourth day of germination (compare nonviable (G0) and viable seeds (G1)). The samples' fluorescence spectra were recorded using an Fl3-221 P spectrofluorimeter (JobinYvon, Horiba, France), equipped with a 450 W Xe lamp and a photomultiplier tube. The emission spectra, ranging from 400 to 800 nm, were recorded with an excitation wavelength of 375 nm. A spectral bandwidth of 2 nm was set for both the excitation and emission slits. The fluorescence ratios 453/680 nm and 680/751 were calculated.

RESULTS AND DISCUSSION

Figure 1 shows that the aflatoxin-contaminated seeds exhibited significant decreases in the percentage of germination, compared to the uncontaminated seeds ($p<0.05$). Our results suggest that the presence of aflatoxin leads to changes in the seed metabolism, leading to a possible delay of the germination process.

The fluorescence spectra of the methanolic extracts from both contaminated and uncontaminated maize seeds, before germination (BG), as well as after the fourth day of germination, are shown for the non-viable seeds in Figure 2 a), and for viable seeds in Figure 2 b). As shown in the Figure 2, three different maxima were observed at 453 nm, 680 nm and 751 nm in all analyzed samples in the emission range between 400 nm to 800 nm. Both types of the non-viable seeds (G0) have a more pronounced fluorescence peak near 680 nm. The analyzed fluorescence peaks arising from chlorophyll fluorescence [4].

The changes in the fluorescence ratios 453/680 nm and 680/751 nm are shown in figure 3. The fluorescence in the blue and green regions is emitted by secondary metabolites (mainly plant phenols) related to plant defence [4,5]. We observed a lower ratio at 453/680 nm for the nonviable seeds that are contaminated with AFs compared to the non-contaminated maize seeds (G0X and G0Y). The opposite effects were observed for viable seeds. That could imply a difference in the seed's metabolic state under the influence of aflatoxin and that could be used as an indicator of aflatoxin stress.

On the other side, the ratio 680/751 nm, an indicator of the Chlorophyll content, was higher in the aflatoxin-stressed seed than in the non-contaminated nonviable maize seeds. This observation is in agreement with early works [6], which showed that seeds with a high chlorophyll fluorescence signal were of lower quality (viability).

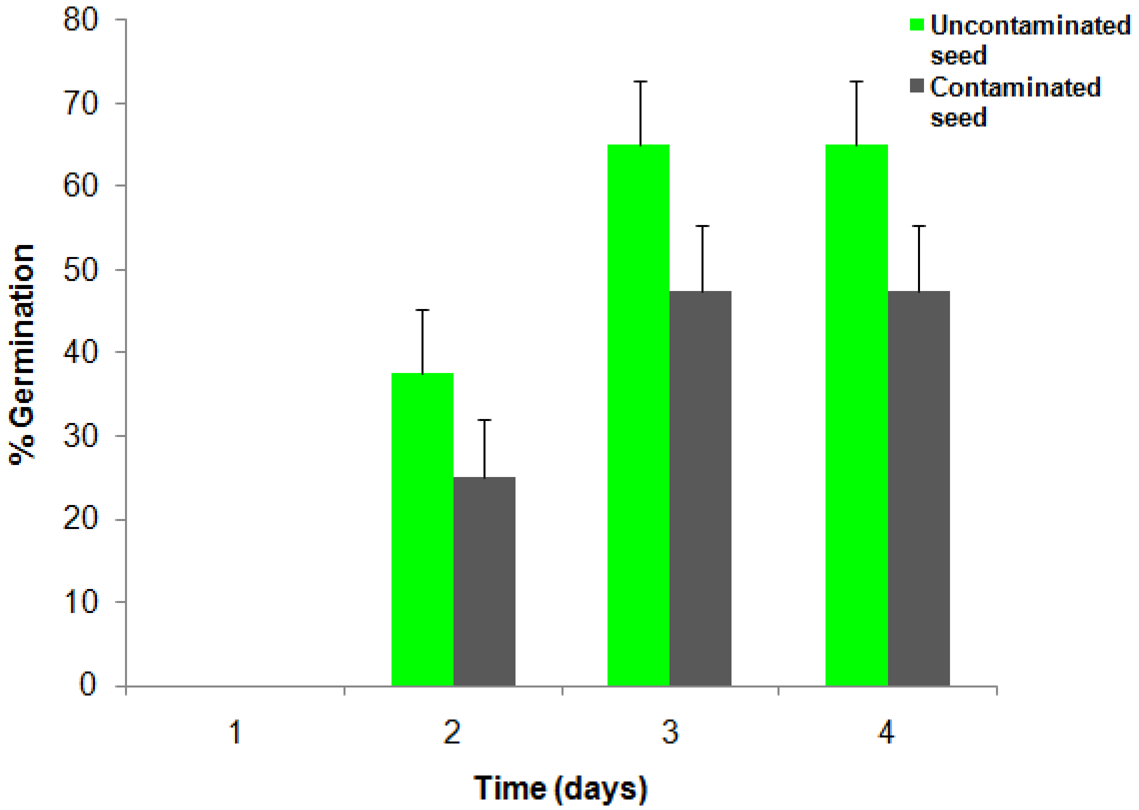

Figure 1 *Variations in germination dynamics of uncontaminated and aflatoxin-contaminated maize seeds after 1, 2, 3 and 4 days*

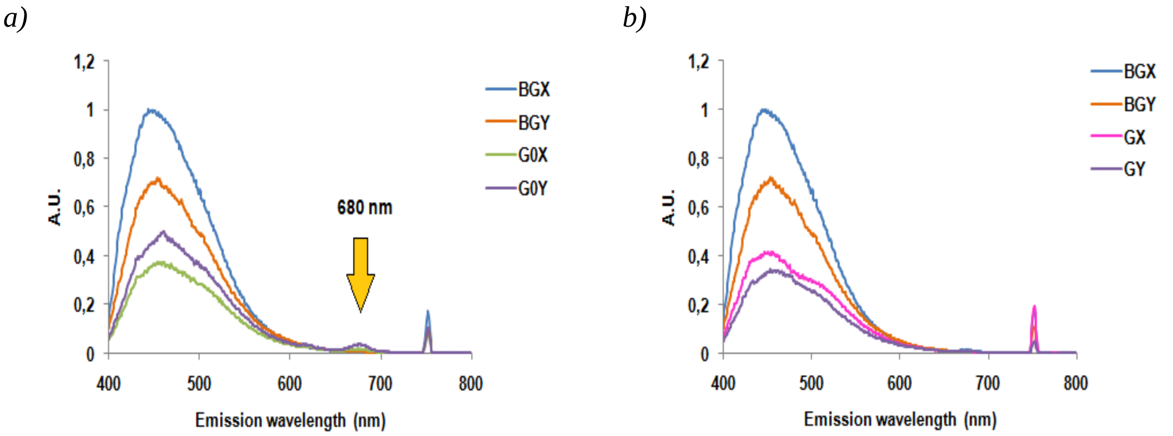

Figure 2 *Comparison fluorescence spectra of the methanolic extracts for the contaminated (Y) and uncontaminated (X) seeds before germination (BG) with a) nonviable (G0) b) viable (G1) seeds*

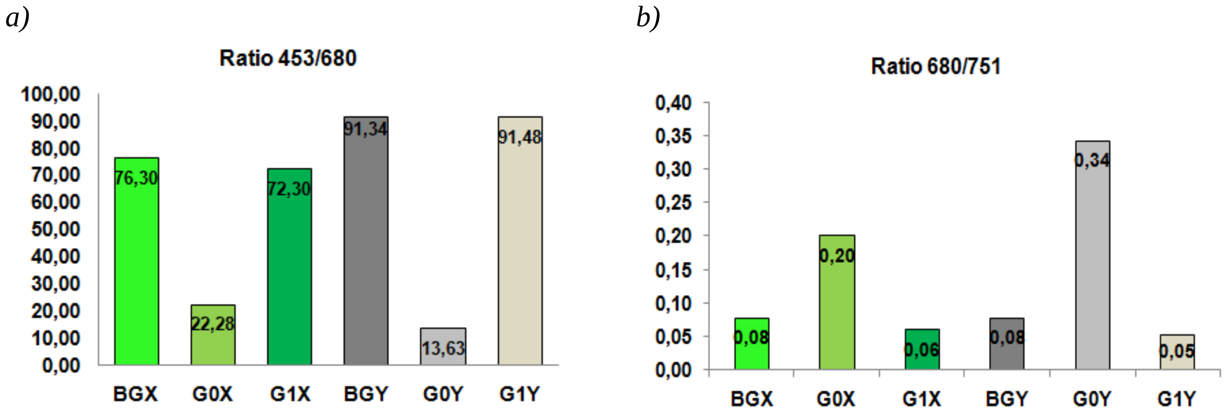

***Figure 3** Changes in the fluorescence ratios a) 453/680 nm and b) 680/751 of analyzed maize seeds*

CONCLUSION

This study shows that fluorescence spectroscopy can be a valuable tool to assess the viability of maize seeds due to aflatoxin stress, even at a low level of contamination. The observed differences in the fluorescence ratios imply that aflatoxin stress induces damage, or compromises, in the chlorophyll system of maize seeds.

ACKNOWLEDGEMENT

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THE EXAMINATION OF ECOTOXIC EFFECT OF FOLIC ACID BASED CARBON DOTS ON MAIZE

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Abstract

Carbon dots (CDs) are popular or emerging nanomaterial which found application in many fields such as drug delivery, optoelectronic, and imaging due to their high solubility, low cost and easiness of their functionalization. Their effect on plants is not sufficiently investigated, so it is necessary to investigate their ecotoxicity. In this research, CDs prepared from folic acid was used for the treatment of maize plants at two different concentrations. The treatment was performed during the plant growth in hydroponics. ICP method was used for the analysis of macronutrients (Ca, K, Mg, P, S) uptake in plants from the hydroponic medium, which was used for the plant growth. The obtained TPC results demonstrated low oxidative stress proportional to the used concentration, which was not significant. The similar trend was observed in TAA where the only significant increase was in plant shoots after the treatment at 500 µg L^{-1}.

Keywords: carbon dots, maize, phenolics, antioxidant activity, ecotoxicity

INTRODUCTION

Carbon dots (CDs) are metal free nanomaterial with an inner graphitic core [1]. High solubility, chemical stability, stable luminescence, photo-bleaching resistance, low cost and easiness of their functionalization are advantages of these nanoparticles in comparison with conventional heavy metals based quantum dots and organic dyes [2]. Their applications have been tested in printing inks [3], drug delivery [4], imaging [5], photocatalysts [6], fingerprint [7] and optoelectronics [8]. Their elemental content (in carbon, oxygen, and heteroatoms) and graphitization degree significantly depends on the type of starting materials and the synthetic route, giving an expanding gallery of photoactive materials [9]. The literature data about the effect of CDs on plants are scarce, so there is a need to examine their ecotoxic effect.

In this study, CDs were synthesized from folic acid (FA) as starting material and their ecotoxic effect was examined on maize at 167 and 500 µg L^{-1} during 7-day exposure in hydroponics. The main objective of this study is to examine if CDs have an impact on macronutrient concentration, total phenolic content (TPC) and total antioxidative activity (TAA), as indicators of plant response to the nanoparticles.

MATERIALS AND METHODS

Folic acid (≥ 97%,), H_3PO_4 (85% wt%, 99.99%), NaClO, $Ca(NO_3)_2·4H_2O$, KNO_3, $MgSO_4·7H_2O$, KH_2PO_4, HNO_3, H_2O_2, K_2HPO_4, KH_2PO_4, Folin-Ciocalteu reagent (2N), CH_3OH, Na_2CO_3, gallic acid, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid (ABTS), horseradish peroxidase (HRP) type II (150-250 units per mg solid) and cellulose membrane for dialysis were supplied from Sigma-Aldrich Química S.A. (Spain) were used. All reagents were obtained from Sigma Aldrich (St. Luis, USA). Ultrapure Millipore water and further reagents of analysis quality were used throughout all experiments.

Synthesis of CDs from FA

CDs were obtained by adding 10 mg of FA to 5 mL phosphoric acid solution (7.31 M) and heated under reflux at 100ºC for 1 h. Then, 500 µL of the previous solution was diluted with deionized water and was dialyzed *vs* water for 1 h [1].

Experimental design for plant treatments

Maize (*Zea Mays*, L.) seeds (cv. VA35; Maize Research Institute "Zemun Polje", Serbia) were surface-sterilized with 4% NaClO for 2 min and rinsed with distilled water (2-3 times for 1 min each).

Four replicates of 20 maize seeds were germinated in a filter paper moistened with 10 mL of distilled water. After germination, 20 seedlings per each treatment (control, 167 µg L^{-1} and 500 µg L^{-1}) were transferred to the 17.5 cm height-plastic vessels containing 2.5 L half-strength nutrient solution in the presence of CDs nanoparticle. The seedlings were grown for the next 7 days under 16h/8h photoperiod and the nutrient solution was aerated by bubbling. After 7 days, the shoots and roots of 10 plants (2 plants per replicate) were collected, frozen in liquid nitrogen and kept at -80˚C until the determination of TPC and TAA. Concentrations of macronutrients (K, Ca, Mg, P and S) were determined in the remaining 10 plants.

Determination of micronutrients

Dry pulverized plant materials were digested with conc. HNO_3 and 30% H_2O_2 (1:4) in the Tecator digestion system [10]. After cooling to the room temperature, the solutions were filtered using Whatman filter paper and volume was adjusted with MilliQ water to 25 mL. The concentrations of macronutrients (excluding N) in the plant samples and nutrient solutions were determined by inductively coupled plasma optical emission spectrometry (ICP-OES; SpectroGenesis EOP II, Spectro Analytical Instruments GmbH, Kleve, Germany).

Extraction of phenolics and determination of TPC

In order to obtain phenolic extracts, roots and shoots of ten plants (2 per sample, in 5 replicates) were separately homogenized in a mortar with liquid nitrogen. Then, homogenates were resuspended in 80% methanol in the 1:10 (m:V) ratio and stirred for 60 minutes at room temperature. The extracts were centrifuged at 10000 rpm for 5 minutes, and extracted phenolics were obtained in the supernatant.

For determination of TPC in the samples, Folin-Ciocalteu's spectrophotometric procedure [11] was used. Phenolic extracts were mixed with Folin-Ciocalteu reagent in 1 mL of total volume. After 3 min sodium carbonate solution was added and the mixture was incubated for 60 min at 25°C. Gallic acid was used for the construction of the standard curve (0.1 - 2.0 mM). Absorbance was read at 724 nm (2501 PC spectrophotometer, "Shimadzu", Japan) and the results were expressed as micromoles of gallic acid equivalents per gram of fresh weight.

Determination of TAA

ABTS/HRP endpoint method was used for measuring of TAA in the samples, according to the modified procedure of Cano et al. [12]. In brief, the reaction mixture contained 2 mM ABTS, 15 µM H_2O_2, 0.25 µM horseradish peroxidase (HRP) type II and 20 µL of 80% methanol extract of the samples in 50 mM potassium-phosphate buffer, pH 7.5, in 1 mL of total volume. The assay was performed at the temperature 25°C, in 5 replicates per treatment. The reaction was monitored at 730 nm (2501 PC spectrophotometer "Shimadzu", Japan) until a stable absorbance, due to ABTS radical ($ABTS^{\cdot+}$) formation in the reaction with HRP. After adding methanol extracts of plant, the decrease of absorbance due to $ABTS^{\cdot+}$ depletion was used for calculation of TAA from the standard curve obtained with ascorbic acid (0.1 - 1 mM) as a universal antioxidant. The TAA was expressed as micromoles of ascorbic acid equivalents per gram of fresh weight.

Statistical analysis

The raw data (macronutrients' concentration, dry biomass, TPC and TAA in root and shoot parts of maize plants treated with different concentrations of CDs) were used as input variables. Exploratory and data analysis were performed using the IBM SPSS Statistics 20 software (IBM, USA). A non-parametric Kruskal-Wallis test for independent samples was used to test the differences in TPC and TAA (n=5), as well as in concentrations of macronutrients (n=3) measured in root and shoot parts in plants under the different treatments. Post hoc inter-group comparisons of variables (between different treatments and control) were performed by the non-parametric Mann-Whitney test at the level of the significance $p<0.05$.

RESULTS AND DISCUSSION

Uptake of macronutrients

The treatment of plants with CDs did not lead to any visible signs of tissue damage such as chlorosis or necrosis. Interestingly, CDs even increased shoot dry biomass in comparison with the control (Table 1).

***Table 1** Dry biomass of shoot and root per individual plant*

Treatment	Dry biomass of shoot per individual plant	Dry biomass of root per individual plant
Control	0.0215 ± 0.0021	0.0092 ± 0.0019
167 CDs	0.0414 ± 0.0039	0.0196 ± 0.0024
500 CDs	0.0351 ± 0.0041	0.0085 ± 0.0026

Figure 1 shows the concentration of macronutrients in maize roots and shoots after the treatment with CDs. K showed the highest uptake, while the lowest uptake had Mg. According to the concentration of macronutrients in roots and shoots, it can be concluded that root-to-shoot translocation is similar for all macronutrients.

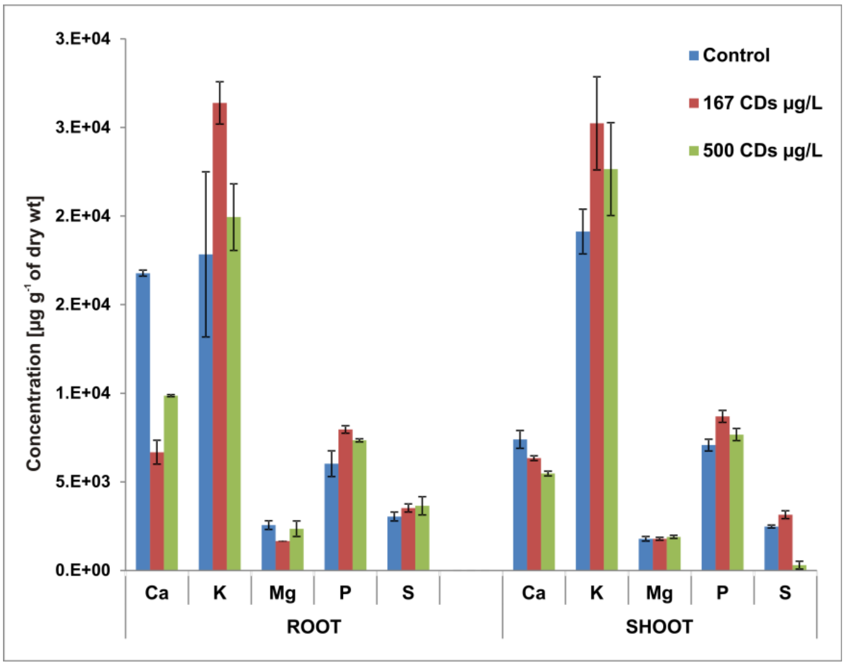

Figure 1 *Concentration of macronutrients in maize after the treatment with different CDs concentrations*

Effect of CDs on TPC

In order to investigate the potential for oxidative stress due to CDs treatment of plants, we measured phenolic content, as an indicator of the plant defense capacity. The effect of CDs treatments on TPC in maize roots and shoots is presented in Figure 2.

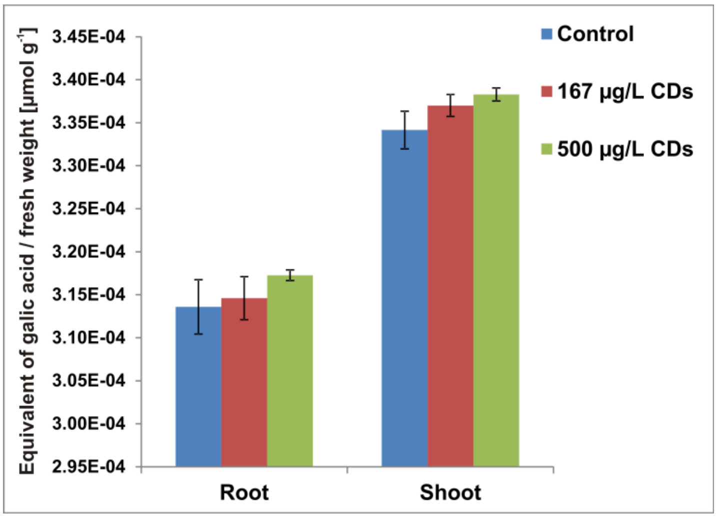

Figure 2 *Effect of CDs on TPC in roots and shoots in maize. Values are shown as mean ± SE; * indicates statistically significant differences in comparison with the corresponding control, p<0.05*

Although TPC was increased in both parts of plants after treatment with CDs at both concentrations, this effect is negligible because it is not significant. It can be concluded that CDs does not cause significant oxidative stress in treated plants.

Effect of CDs on TAA

Since TAA may be an indicator of metabolic disorder in plants, it was analyzed in phenolic extracts of maize shoots and roots after treatment with CDs (Figure 3). Results suggested that TAA was significantly increased compared to the control only after the treatment with 500 µg L^{-1} CDs. This can be explained with the ability of CDs to induce excessive production of ROS [13], which activates plant defense system leading to the increase of TAA in plants.

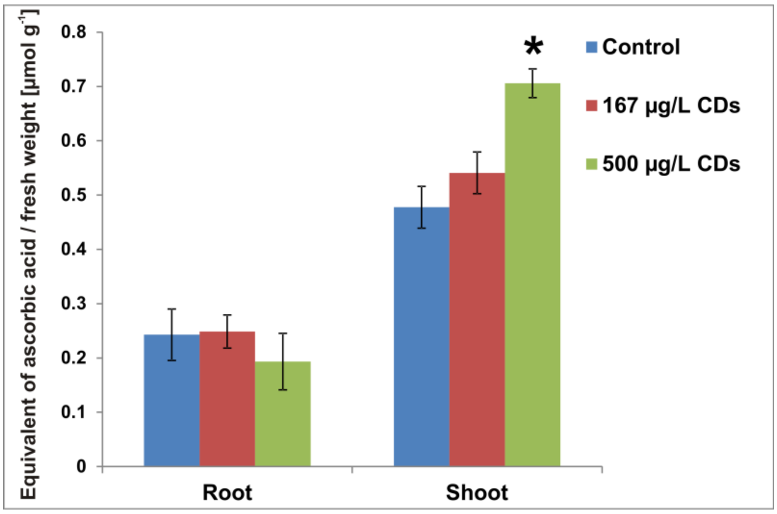

Figure 3 *Effect of CDs on TAA in roots and shoots in maize. Values are shown as mean ± SE; * indicates statistically significant differences in comparison with the corresponding control, p<0.05*

CONCLUSION

The treatment of maize plants with CDs did not any phytotoxic symptoms at both tested concentrations. CDs at both concentrations significantly decreased leaf Ca accumulation while 500 µg L^{-1} significantly increased TAA. This may indicate the phytotoxic effect of CDs at higher concentration, which remains to be examined in future research.

ACKNOWLEDGMENT

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MERCURY CONTAMINATION OF SEAWATER, SEDIMENT AND FISH FROM BAR MUNICIPALITY

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Abstract

Once released into the environment either from natural or anthropogenic sources, mercury undergoes thorugh a series of complex transformations and cycles among pedosphere, hydrosphere and atmosphere. The most common mercury form in the food chains is methyl mercury. Fish and fish products are the dominant source of methyl mercury in food. This chemical form of mercury could be rapidly accumulated by the most aquatic organisms where maintaines the highest concentrations in fish which are usually at the top of the aquatic food chains. The aim of this research was to obtain data on mercury contamination in seawater, sediment and fish samples from the Bar region. All collected fish had mercury contamination in muscle tissue near or above the proposed levels by Commission Regulation (EC) No 1881/2006.

Keywords: mercury, contamination, biota, water, sediment

INTRODUCTION

The Adriatic Sea is a large bay of the Mediterranean Sea central part, placed between the Balkan and Apennine Peninsulas. The mainland Adriatic coastline in Montenegro is 249 km long, with the harbour Bar as the most important Montenegrin port. It is located in a natural bay between the old city of Bar and Ulcinj, sheltered from the south by the hill Volujica, with a wide opening to the west. The total length of the port operational shore is 3 km, can host 20 ships at once, with the closed storage area of more than 100.000 m^2 [1]. The harbour Bar is capable for all types of goods transhipment, especially, ores, concentrates, metallurgy products, oil and its derivatives, grain and its products, fertilizers, pesticides, cement and wood.

The extremely strong anthropogenic influence in this region has caused significant changes in both, marine and terrestrial ecosystems. Considering these numerous harmful effects on the marine ecosystem and the vigorous antagonism between ecology and economy, awareness of the necessity for continuous monitoring sediment - water - organism interactions has been increased, with the aim to protect and preserve marine environment [1]. The oceans and seas are ecosystems with natural mechanisms to store, and in many cases, decompose pollutants and contaminants released by the industry or agriculture in limited extent. Nevertheless, this ability varies depending on numerous factors, such as size, currents, circulation speed, tides, temperatures, depth, freshwater flow and even marine wildlife density and diversity [1].

Regarding specific diet as consumers in food chains, fish can represent suitable and accurate bioindicators and biomonitors for the marine water pollution and contamination analyses. They are accurate and economically cost-effective natural bioindicators that could in appropriate time warn of the presence of the harmful substances in the water. The pollutants and contaminants are accumulated in different fish tissues, usually in the larger quantities than those found in the water column. Metals, such as mercury, lead, cadmium and arsenic, accumulate in fish from water, food, sediment and some suspended particulate materials [2]. Fish are frequent in human nutrition, especially in the coastal regions and consequently they could directly affect human health. According to [3], marine and farmed fish and shellfish are significant contributors to the consumers' intake of some contaminants, due to their presence in the aquatic environment and their accumulation in the fish and shellfish tissues.

Mercury is a non-essential metal which is released into the environment from natural and anthropogenic sources. Once released, mercury undergoes through a series of complex transformations and cycles between atmosphere, hydrosphere and pedosphere. The most common mercury form in the food chain is methyl mercury. Fish meat is the dominate contributor to human methyl mercury dietary exposure, followed by the fish products and shellfish. Methyl mercury is rapidly accumulated by the most aquatic biota and the highest concentrations are maintained in fish which are at the top of the aquatic food chain [4]. Fish accumulate methyl mercury in their tissues, where it becomes strongly bound and cannot be removed from fish tissue by any practical cooking method [5]. According to WHO (World Health Organization), the mercury contents in these commodities widely vary among different fish species, but generally is higher in predatory fish. Methyl mercury contamination negatively impacts human and animal health. The response of fish methyl mercury concentrations to changes in mercury deposition has been difficult to establish, according to [6], as sediment contain large pools of historical contamination.

The aim of this research was to obtain data on mercury contamination in water, sediment and fish from Bar coastal region.

MATERIALS AND METHODS

The water, sediment and fish were sampled at three localities in Bar region: Utjeha, Bar harbour and Sutomore. The water samples were collected with a Friedinger sampler (1 dm^3) from two depths of the water column (0.5 and 3 m) and two points regarding the coast distance. The water was stored in the polyethylene bottles and preserved with nitric acid (1:1).

The sediment samplings were collected using an Ekman Grab and stored in the zip-loc plastic bags. The fish were collected from the daily catch of the local fisherman by the random principal choice. The water, sediment and fish samples were prepared for mercury testing according to [7]. The mercury contamination was tested using Atomic Absorption Spectroscopy (AAS) by the Thermo electron S2 AA System. The standard metal solution (stock solution, 1000 mg/L) was made by dissolving 1 g of metal or its salt (calculated on 1 g of metal) in hydrochloric acid (1:1). Diluting the stock solution (with water), a series of lower concentration of metal were prepared.

RESULTS AND DISCUSSION

The water and sediment mercury contamination is presented in Table 1. During research period, eight fish species were collected: *Mullus surmuletus* Linnaeus, 1758 (surmullet or striped red mullet); *Oblada melanura* (Linnaeus, 1758) (saddled seabream) and *Lophius budegassa* Spinola, 1807 (blackbellied angler) from Utjeha; *Solea solea* (Linnaeus, 1758) (common sole); *Mugil cephalus* Linnaeus, 1758 (flathead grey mullet) and *Raja miraletus* Linnaeus, 1758 (brown ray) from Bar harbour; *Pagellus erythrinus* (Linnaeus, 1758) (common pandora); *Trachurus mediterraneus* (Steindachner, 1868) (Mediterranean horse mackerel) and *R. miraletus* from Sutomore. The mercury contamination of muscle tissue is shown in Table 2.

Table 1 *Mercury contamination of water and sediment*

	Depth	Water samples (μg/dm^3)		Sediment samples (mg/kg)	
	(m)	Coastal area	Open sea	Coastal area	Open sea
Utjeha	0.5	0.00	0.00	0.10	0.05
	3.0	0.00	0.00		
Bar harbor	0.5	0.00	0.00	0.21	0.14
	3.0	0.02	0.00		
Sutomore	0.5	0.00	0.00	0.13	0.07
	3.0	0.00	0.00		

The highest mercury concentrations in water and sediment are obtained in samples collected at Bar harbour, which is correlated to the ports' capacity, the number of hosted ships and operational importance in state economy, transport and industry. Temporal studies conducted in Lavaca Bay on the southeastern Texas coast indicated that bay sediments have a strong seasonal methylation cycle, with a short, active period in spring, followed by a slow year-long decrease [8]. According to [9] it is difficult to observe trends or seasonality in the data obtained in Bar harbour, since processes in the sea are complicated and easily influenced by numerous unknown factors, such as winds, underwater currents, sediment characteristics and previous pollutions. The further studies are more than necessary as mercury bioavailability and speciation have been revealed to occur on a seasonal basis and with correlation to the other chemicals, like the increase of sulfide levels [10] and Fe redox cycle [8].

Table 2 *Mercury contamination of fish muscle tissue*

	Species	Average concentration of detected Hg (mg/kg)	Diet**	Feeding habit**
Utjeha	*Mullus surmuletus*	0.285	zoobenthos, detritus, zooplankton	demersal: hunting macrofauna (predator)
	Oblada melanura	0.162	zoobenthos	benthopelagic: hunting macrofauna (predator)
	Lophius budegassa	**0.640***	nekton	bathydemersal: hunting macrofauna (predator)
Bar harbour	*Solea solea*	0.009	zoobenthos	demersal: hunting macrofauna (predator)
	Mugil cephalus	0.047	zooplankton, zoobenthos, detritus, plants	benthopelagic: variable
	Raja miraletus	**0.850***	zoobenthos	demersal: hunting macrofauna (predator)
Sutomore	*Pagellus erythrinus*	0.250	zoobenthos, zooplankton, nekton	benthopelagic: hunting macrofauna (predator)
	Trachurus mediterraneus	0.190	zooplankton, nekton	pelagic-oceanic: hunting macrofauna (predator)
	Raja miraletus	**0.050***	zoobenthos	demersal: hunting macrofauna (predator)

*results above the maximum permissible concentrations (MPC) according to Commission Regulation (EC) No 1881/2006

** according to www.fishbase.in

According to [11], 90 to 99% of the total Hg in the environment is associated with the sedimnet, and only <1% accumulates in the biota. Furthermore, the same authors reported that only 1 to 10% of methyl mercury is associated with the sediment, while 90-99% accumulates in the biota. The reason for this is the lipophilic nature of methyl mercury, which enhances its ability to be bioaccumulated comparing to inorganic Hg and results in enhanced biomagnification of methyl mercury in the marine food chains.

The highest concentrations of mercury are detected in *L. budegassa* and *R. miraletus*, both bottom-dwellers that typically feed on molluscs, crustaceans and fish, which make them the secondary consumers in the food chains. The mercury level in pelagic samples can be considered low as the habitat of these species is near to the water surface [2].

CONCLUSION

Fish meat has become a frequent dish included on the list of many healthy diets, due to their protein composition and high concentration of essential fatty acids and docosahexaenoic acid, recommended by nutritionists as a mean to prevent cardiovascular diseases and obesity. However, fish also may contain toxic metals that cause serious disorders in human metabolism and disease occurrence. Mercury is one of the most toxic elements and exposure to high levels of this heavy metal could permanently damage the developing foetuses, adult brains and kidneys. The continuous monitoring of mercury and other heavy metals in marine water, sediment and fish is more than necessary in order to prevent human and animal diseases and environment pollution.

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OCCURRENCE OF CYANOBACTERIAL TOXINS IN LUDAŠ LAKE

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Abstract

Nutrient rich, eutrophic, warm and low turbulent conditions in freshwater bodies typically promote the dominance of cyanobacteria within phytoplankton communities. The excessive proliferation of cyanobacteria leads to blooms that disrupt ecosystems, adversely affect the taste and odor of water. There have been 234 microcystins (MCs) identified so far. Owing to the high surface water temperature during August and September 2017, cyanobacterial water blooms have occurred in Ludaš lake. Ludaš lake is a shallow lake in the Province of Vojvodina in the northern part of Serbia. It is a special natural preserve designated as a swamp area of international significance by the Ramsar Convention. The water blooms of cyanobacteria and their cyanotoxins are common in the majority of surface waters in Serbia so the determination of microcystin-LR, WR, LA, LY, LW and LF in the two months period by the LC-ESI-MS/MS method in the multiple reaction monitoring mode (MRM) for the determination of MCs was done. Only MC-LR was detected in 66.67% of the analyzed samples. The concentrations were in the range 0.005 - 0.045 µg/L.

Keywords: microcystins, Ludaš lake, LC-ESI-MS/MS

INTRODUCTION

In freshwater areas, toxic cyanobacterial blooms due to eutrophication have been reported all over the world. Many of the common cyanobacterial species (called "blue-green algae") [1], are unicellular organisms often growing in colonies or filaments [2]. Bloom-forming cyanobacteria share the ability to produce a wide variety of bioactive compounds including neurotoxins, hepatotoxins, cytotoxins, and lipopolysaccharide endotoxins, which it creates a significant water quality problem. Among cyanotoxins, microcystins (MCs) are considered to be the most common and one of the most dangerous groups [3].

MCs are a group of hepatotoxic cyclic peptides consisting of five common amino acids and two variable L-amino acids. MCs are produced by several genera of freshwater cyanobacteria, including Microcystis, Nostoc, Dolichospermum (Anabaena), Planktothrix, Hapalosiphon, and Anabaenopsis [4].

MCs are a large group of cyclic heptapeptides, with more than 100 structural variants [5]. The most common and toxic among them is microcystin-LR, in which the two variable amino acids are leucine - L and arginine - R [6]. MCs are chemically stable compounds, resistant to heat and chemical hydrolysis at near neutral pH.

Eutrophication in lakes, reservoirs, and recreational water with frequent occurrence of cyanobacterial blooms has been recognized as a worldwide problem. Exposure to MCs represents a health risk to aquatic organisms, wild life, domestic animals, and humans upon [7, 8].

Ludaš lake is a shallow Pannonia lake in the northwest of Bačka, Vojvodina, Serbia with the average depth of 0.9 m. Taking into consideration the specific morphology, all shallow stagnant or flow running waters and particularly shallow lakes are especially sensitive [7]. As regards shallow lakes the euphotic zone extends to the bottom together with the light thus making the photosynthesis throughout the entire depth possible. The major objective of this study was to examine the distribution of six microcystins (MC-LR, WR, LA, LY, LW and LF) in the lake biomass in the two months period by the liquid chromatography tandem mass spectrometry (LC-ESI-MS/MS) method from Ludaš lake.

MATERIALS AND METHODS

Water sample collection and preservation

Six water samples from Ludaš lake were collected on 01/08 and 09/09/2017. The water sample was taken from the boat, from the three localities (north (N), middle (M) and south (S) part of the lake) (Figure 1). The water was collected in amber glass bottles (1 L) by plunging at a depth of 50 cm and closing with the lid under the water surface. The sample was transported to the laboratory and immediately analysed.

Figure 1 *Sampling points*

Analytical standards

The MCs and nodularin (internal standard) were purchased from Sigma (St. Louis, MO, USA) in the concentration of 10 µg/mL in methanol. Stock standard solutions of MCs were prepared in methanol, and stored at -20 ºC. In the same conditions the nodularin in the concentration of 0.05 µg/mL, was stored.

Instrumentation and chromatographic conditions for LC-MS/MS

LC was performed with an Agilent 1200 HPLC system equipped with a G1379B degasser, a G1312B binary pump, a G1367D autosampler and a G1316B column oven. Chromatography separation was achieved by Zorbax Ecllipse XDB C18 column (50 x 4.6 mm, 1.8 µm) maintained at 30 °C. The analytical separation was performed using methanol as mobile phase A, and as water mobile phase B, both containing 0.1% formic acid with gradient mode (0 min: 60% B, 10 min: 5% B, 15min: 5% B, stop time: 17 min, post time: 5 min. The flow rate was maintained at 0.5 mL/min. The mass analysis was carried out with an Agilent 6410B Triple Quadrupole mass spectrometer equipped with multi-mode ion source in +ESI. The data acquisition and quantification was conducted using MassHunter Workstation software B.04.01.

Validation parameters

The method was validated according to SANTE/11945/2015 [9].

The limit of the detection (LOD) was determined as the lowest concentration giving a response of three times the average baseline. The ratio signal/noise in the obtained chromatograms for the LOD was calculated by MassHunter Qualitative Software. The linearity was checked at the concentrations of 0.01, 0.025, 0.05, 0.1 and 0.2 µg/mL. The recovery was checked, by enriching of a blank sample (tap water) with the working standard of MCs, to get the final mass concentration of 0.02, 0.04 and 0.1 µg/L (in five replicates). The precision of the method in terms of repeatability (% RSD_r) (intra-day precision) and reproducibility (% RSD_R) (inter-day precision) was investigated in the spiked samples analyzed in five replicates on the same and different days.

MCs extraction

After addition of nodularin (IS), the water samples (0.5 L) were passed through the filter paper. The filter paper extraction was done in 50 mL PP tubes with 3x3 mL of methanol, during 10 minutes. All the methanol extracts (from the same sample) were collected, centrifugated at 4000 rpm for 5 minutes. Aliquot was evaporated to dryness and reconstituted in 0.5 mL of mobile phase. After that the extract was ready for the LC-MS/MS analyses [10].

RESULTS AND DISCUSSION

For the quantification the ion with the best signal sensitivity (Q) was preferred and for the confirmation the second transition (q) and the ratio of abundances between both ion transitions (Q/q) was used. The cone voltages were selected according to the sensitivity of the precursor ions and the collision energies were chosen to give the maximum intensity of the fragment ions obtained. The product-ion spectra obtained on triple quadrupole instrument generally provide fragments which are of diagnostic value for structural elucidation and confirmation.

The basic validated parameters were obtained for all investigated MCs: good linearity with $R^2>0.99$ within the calibration ranged from 0.01-0.2 µg/mL; the average recoveries (0.02, 0.04 and 0.1 µg/kg) were in the range of 80.4 to 93.7% with the RSD<20%. The good accuracy and precision results were also obtained in intra-day and inter-day analysis. The LOQs were set at 0.1 µg/L value, while the LODs were mathematically calculated.

The MRM transitions (*m/z*) for all MCs and nodularin were given in the Table 1.

Table 1 *Acquisition data*

Compound Name	Precursor Ion		Product Ion	Dwell	Frag (V)	CE (V)	Polarity
MC-LR	996.2	→	213.2	100	135	70	Positive
MC-LR	996.2	→	135.2	100	170	90	Positive
MC-WR	1068.6	→	135.2	100	120	40	Positive
MC-WR	1068.6	→	159.1	100	90	40	Positive
MC-LA	910.5	→	135.3	100	120	40	Positive
MC-LA	910.5	→	213.2	100	90	40	Positive
MC-LY	1002.5	→	135.3	100	120	40	Positive
MC-LY	1002.5	→	868.7	100	120	40	Positive
MC-LW	1025.5	→	135.3	100	120	40	Positive
MC-LW	1025.5	→	213.0	100	120	40	Positive
MC-LF	986.5	→	135.4	100	120	40	Positive
MC-LF	986.5	→	852.8	100	120	40	Positive
Nodularin	825.5	→	135.0	100	100	70	Positive
Nodularin	825.5	→	103.1	100	80	100	Positive

The LC-MS/MS chromatogram of the sample collected on 09/09/2017 from the south part of Ludaš lake was shown in figure 2.

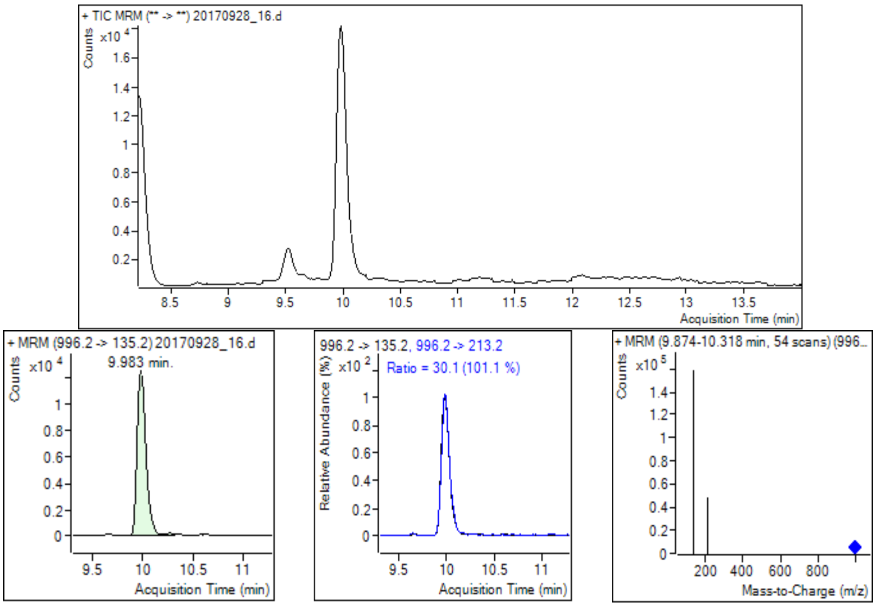

Figure 2 *TIC and MRM chromatogram of water sample wit detected MC-LR*

From the obtained data, in all the analysed samples, only MC-LR was detected (Table 2).

Microcystin-LR is one of the most prevalent and potent microcystins [8]. It is designated as possibly carcinogenic to humans by the International Agency for Research on Cancer (IARC). The variable residues of MC-LR are L-arganine and L-leucine. The World Health Organization [11] guideline recommended the limitation of MC-LR on 1 μg/L in drinking water.

Table 2 *MC-LR detections (μg/L)*

	01/08/2017			09/09/2017		
Sample	N	M	S	N	M	S
MC-LR	-	0.006	-	0.005	0.009	**0.045**

CONCLUSION

The shallowness of the Ludaš lake is a crucial factor why it is chosen for the determination of six microcystins (MC-LR, WR, LA, LY, LW and LF) in it. The samples were taken from three sites (north (N), middle (M) and south (S)).

After the filtration of water samples, in the obtained biomass using LC-MS/MS with the addition of nodularin as internal standard, the content of microcystins was determined.

From the obtained data, in all the analysed samples, only MC-LR was detected in the concentration range from 0.005 to 0.045 μg/L. Microcystin-LR is one of the most prevalent and potent microcystins, designated as possibly carcinogenic to humans. MC-LR have negative influence on aquatic organisms as well.

ACKNOWLEDGEMENT

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STUDY OF OBSERVING ADSORPTION OF HEXAVALENT CHROMIUM FROM AQUEOUS ENVIRONMENT ON MORDENITE

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Abstract

Since the compounds of hexavalent chromium are very toxic and have a harmful effect on humans and human environment, it is necessary to continuously control the quantity of chromium in all its spheres, and particularly in the surface and subterranean waters which the industrial wastewater gets emitted into. This study deals with the observation of adsorption of anion species of hexavalent chromium from the aqueous environment in the temperature interval from 283 K to 303 K on the original sample of synthetic mordenite (MOR), a product of the Zeolyst International company, USA (mark: CBV10A), for the purpose of examining of its adsorption capacity and potential application for removal of chromium from the wastewater. Concentration of chromium was determined spectrophotometrically with 1.5-diphenylcarbazide at the wave length of 545 nm. Characterization of adsorption systems was described by the Freundlich model. Results showed that active centers on the surface of mordenite had affinity to chromium adsorption, and adsorption parameters indicated that it was physisorption.

Keywords: adsorption, hexavalent chromium, mordenite, waste water

INTRODUCTION

Chromium gets in the composition of a big number of chemical compounds, out of which the most spread and most famous are the compounds of trivalent and hexavalent chromium. Sources of chromium can be of natural and anthropogenic origin. It is widely used in the industry of heavy metals and galvanization, wood and textile industry, as well as in chemical production of pigments, and may be contained in the wastewater in a high concentration. Chromium (VI) ions act as a strong oxidation agent and they are very toxic, potentially carcinogenic and mutagenic for all human beings. The most significant compounds of hexavalent chromium are chromates and bichromates [1–3]. It is very important to control the quantity of hexavalent chromium and to remove the surplus from the wastewater before being emitted in the recipient. There are several methods for removal of heavy metals from the aqueous environment, such as chemical precipitation, membrane filtration, ion exchange, biosorption by the means of different biomaterials. Adsorption as a method is a good choice due to the simplicity of performing it and its efficiency. It is known that zeolite materials are characterized by an exceptional adsorption, catalytic and ion-exchange characteristics. An exceptionally developed internal network of delicately distributed channels and cavities makes them very porous materials [4–8]. In this study, the selected adsorbent was synthetic mordenite (MOR) at which adsorption was observed of hexavalent chromium from the aqueous solutions (simulated wastewater) of different concentrations. Concentrations of chromium solutions, before and after adsorption, were determined spectrophotometrically. Quantity of adsorbed chromium was calculated from the difference. Obtained experimental data and adsorption parameters determined based on the Freundlich isotherm indicate that mordenite possesses active centers suitable for this adsorbate and that the tie between adsorbent and adsorbate was established mainly by dispersed forces.

MATERIALS AND METHODS

Adsorption of hexavalent chromium was observed on the original sample of synthetic mordenite (MOR) as adsorbent, which is a product of the American company Zeolyst International. Before the use, zeolite was thermally processed at 378 K up to the constant mass. Basic characteristics of this sample are presented in Table 1.

***Table 1** Basic characteristics of MOR zeolite*

Adsorbent	Nominal cation form	Chemical formula	Na_2O (weight %)	SiO_2/Al_2O_3 (mole ratio)	Surface area (m^2g^{-1})
MOR CBV 10A	Sodium	$Na_x \cdot Al_x \cdot Si_{(48-x)} \cdot O_{96}$	6.6	13.0	425

Potassium-bichromate, $K_2Cr_2O_7$, p.a., product of Merck, Germany, was used to prepare the adsorbate solution. Aqueous solutions of different concentrations of hexavalent chromium were prepared by diluting of the basic solution (0.01 mol/L). Concentrations of adsorbate solutions were within the range from $8 \cdot 10^{-4}$ to $2.9 \cdot 10^{-3}$ mol/L (pH= 5-6). Temperatures at which adsorption was observed are: 283, 293 and 303 K. Mass of adsorbent in all experiments was cca 0.25 g, and volume of adsorbate was 50.00 mL. Reaction system for the time of adsorption observance (three hours) was always thermostated (Thermostat: WiseCircu WCR, model WCR-P22, Witeg, Wertheim, Germany). Concentrations of chromium solutions before and after adsorption were determined spectrophotometrically (λ_{max} = 545 nm) on the spectrophotometer: Perkin Elmer UV/VIS Spectrometer Lambda 25. Quantity of adsorbed chromium was calculated from the difference (x) and by the mass unit of adsorbent (x/m). Obtained experimental results were described by the Freundlich model. Degree of removed chromium expressed in percentages (S/%) was calculated according to the equation (1):

$$S/\% = \frac{\gamma_o - \gamma_e}{\gamma_o} \cdot 100 \tag{1}$$

where: γ_o equals original concentration of chromium in mg/L; and γ_e equals balance concentration of chromium in mg/L.

RESULTS AND DISCUSSION

For spectrophotometric determination of Cr(VI)-ion there was a calibration curve constructed (Figure 1).

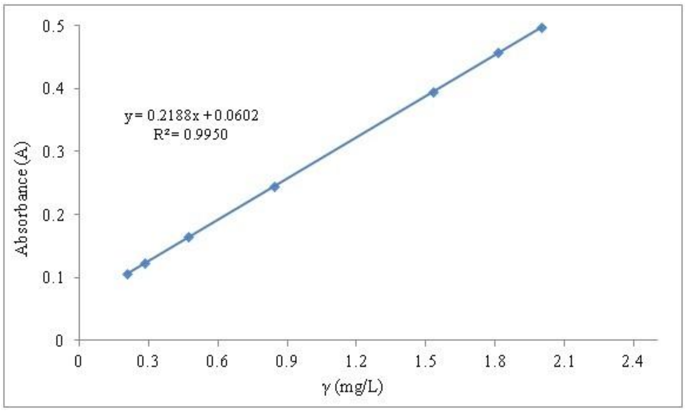

Figure 1 *Calibration curve was constructed for determination of Cr(VI)-ion concentration*

Dependence of adsorbed quantity of hexavalent chromium in the solutions of different concentrations was presented by the Freundlich adsorption isotherm, and results were presented in tables from 2 to 4 and in Figure 2.

Table 2 *Adsorption of Cr(VI)-ion on MOR (T=283 K)*

$c_0\cdot10^3$ [mol/L]	$c_{balan.}\cdot10^3$ [mol/L]	$x\cdot10^4$ [mol]	$m_{adsorb.}$ [g]	$\frac{x}{m}\cdot10^4$ [mol/g]	$\ln\frac{x}{m}$	$\ln c_{balan.}$
0.8366	0.3346	0.2510	0.2517	0.9972	-9.2131	-8.0026
1.1270	0.5135	0.3068	0.2506	1.2243	-9.0080	-7.5743
1.3886	1.1116	0.1385	0.2512	0.5514	-9.8056	-6.8020
2.1386	1.6059	0.2664	0.2523	1.0559	-9.1559	-6.4341
2.5790	1.6213	0.4788	0.2512	1.9061	-8.5653	-6.4245
2.8848	1.9249	0.4800	0.2526	1.9002	-8.5684	-6.2529

Table 3 *Adsorption of Cr(VI)-ion on MOR (T=293 K)*

$c_0\cdot10^3$ [mol/L]	$c_{balan.}\cdot10^3$ [mol/L]	$x\cdot10^4$ [mol]	$m_{adsorb.}$ [g]	$\frac{x}{m}\cdot10^4$ [mol/g]	$\ln\frac{x}{m}$	$\ln c_{balan.}$
0.8366	0.3404	0.2481	0.2506	0.9900	-9.2204	-7.9854
1.1270	0.5462	0.2904	0.2509	1.1574	-9.0642	-7.5125
1.3886	1.1653	0.1116	0.2510	0.4446	-10.021	-6.7548
2.1386	1.4809	0.3288	0.2522	1.3038	-8.9451	-6.5151
2.5790	1.7021	0.4384	0.2507	1.7487	-8.6515	-6.3759
2.8848	2.1540	0.3654	0.2514	1.4535	-8.8364	-6.1404

Table 4 *Adsorption of Cr(VI)-ion on MOR (T=303 K)*

$c_0\cdot10^3$ [mol/L]	$c_{balan.}\cdot10^3$ [mol/L]	$x\cdot10^4$ [mol]	$m_{adsorb.}$ [g]	$\frac{x}{m}\cdot10^4$ [mol/g]	$\ln\frac{x}{m}$	$\ln c_{balan.}$
0.8366	0.2212	0.3077	0.2512	1.2249	-9.0075	-8.4164
1.1270	0.5924	0.2673	0.2519	1.0611	-9.1510	-7.4313
1.3886	1.1982	0.0952	0.2504	0.3802	-10.177	-6.7269
2.1386	1.7097	0.2144	0.2520	0.8508	-9.3719	-6.3714
2.5790	2.1213	0.2288	0.2518	0.9087	-9.3061	-6.1557
2.8848	2.4040	0.2404	0.2503	0.9604	-9.2507	-6.0306

Figure 2 presents Freundlich adsorption isotherms for the systems Cr(VI)-MOR at 283, 293 and 303 K.

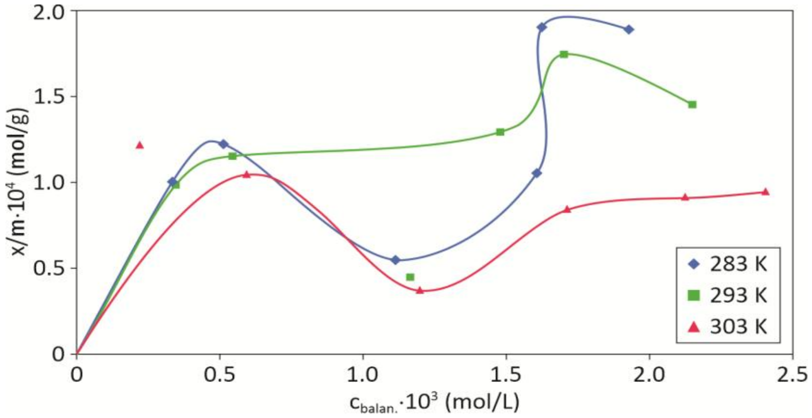

***Figure 2** Freundlich adsorption isotherm for the systems Cr(VI)-MOR*

Adsorption isotherms according to their form belong to the group S4 according to the classification of Giles and associates [9]. Several plateaus get registered, and that suggests that it is a multi-layer adsorption and that most probably each plateau represents a mono-layer. Convex beginning of dependence x/m = f(c) could mean that there is a distinct affinity between adsorbent and adsorbate.

Figures from 3 to 5 present the degree of removal of chromium from the aqueous environment by means of zeolite MOR depending on the concentration and temperature. It can be noticed that the degree of removal of chromium gets decreased by the increase of temperature and concentration.

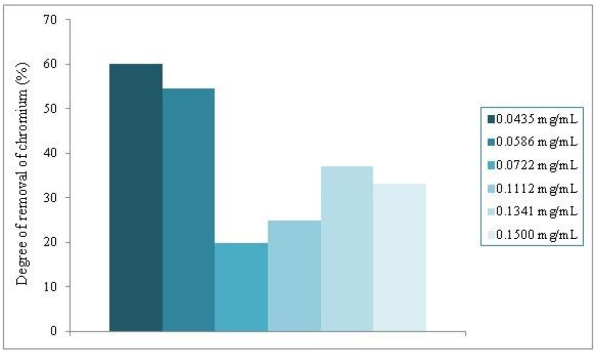

***Figure 3** Degree of removal of chromium (283 K)*

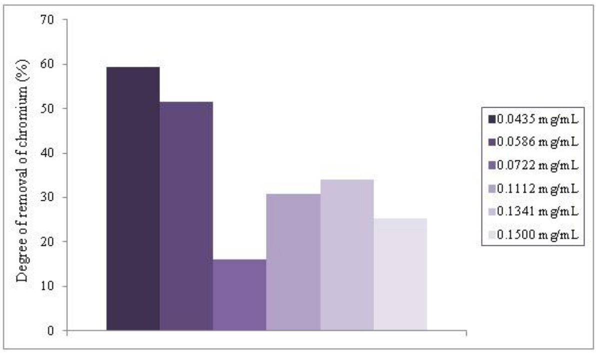

***Figure 4** Degree of removal of chromium (293K)*

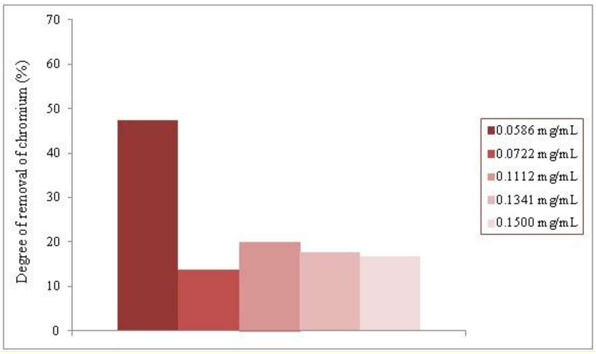

***Figure 5** Degree of removal of chromium (303K)*

Table 5 presents a summary review of obtained results of observance of adsorption of hexavalent chromium from the aqueous environment on the zeolite MOR as adsorbent. Table contains values *x/m* (quantity of adsorbed chromium by the unit of adsorbent mass), then values of adsorption constants *k* and *n*, determined graphically based on functional dependence of *lnx/m* on $c_{balan.}$, as well as the values of adsorption heat.

Table 5 *Summary reviews of parameters for adsorption system Cr(VI)-MOR*

Adsorbent	T [K]	$x/m \cdot 10^4$ [mol/g]	$k \cdot 10^4$	n	$\Delta_{ads}H_m$ [kJ/mol]
MOR	283	I =1.20 II =1.90	5.52	4.46	-10.48
	293	I =1.20 II =1.65	6.77	4.20	-10.22
	303	I =1.05 II = 0.90	0.96	32.15	-81.00

Based on the presented data in Table 5 it can be concluded that the increase of adsorption temperature makes the quantity of adsorbed chromium fall (at II plateau at 303 K less by 47 % compared with the same plateau at 283 K), which indicates that the adsorption took place as a multi-layer physical one. Obtained values $\Delta_{ads}H_m$ confirm that. Based on the values of constants n and k it can be concluded that there is a relatively strong interaction between the active centers of adsorbents and adsorbates and that it is biggest at the temperature of 303K, where the release of the most of energy was registered. As it is known, hexavalent chromium in the aqueous environment may exist in several anion forms, and which species will be dominant depends on pH value and concentration. In case pH solution is below 1.0, the dominant species is H_2CrO_4, for pH between 1.0 and 6.5, the dominant species is $HCrO_4^-$, and if pH is above 6.5, the dominant species is CrO_4^{2-}. With the increase of acidity of solution and with the high concentrations of chromium, the dominant species is $Cr_2O_7^{2-}$. Chromate ions are weak bases and they are probably tied to the mordenite surface through the protons from hydroxide groups, i.e. to Brönsted acid centers that are located on the outer surface of this zeolite. Due to the specific structure of the inner surface of mordenite, which are made up of long, relatively narrow channels, of 0.581x0.695 nm in dimensions [10,11], it can be assumed that the active centers on the inner surface were not accessible for the chromate species, because of which the degree of removal is lower in higher concentrations.

CONCLUSION

Compounds of hexavalent chromium are widely spread as polluters of life environment in which they most often get through unpurified industrial wastewater. Keeping in mind that these compounds are very toxic for the living world, it is necessary to remove them from the wastewater before its emission into the water flows. Adsorption methods are a good choice for that. This study observed adsorption of chromium from the aqueous environment on the zeolite MOR as adsorbent in the temperature interval of 283-303 K. Concentrations of chromium before and after adsorption were determined spectrophotometrically with 1.5-

diphenylcarbazide. Obtained results showed that there is a satisfactory interaction between the species of chromium and surface of adsorbent. Adsorption takes place in many layers and has the character of physisorption, which is confirmed by the obtained values of adsorption heat.

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REGISTRATION OF UNSAFE TOYS ON THE RAPID ALERT SYSTEM FOR NON-FOOD CONSUMER PRODUCTS (RAPEX)

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Abstract

Products (excluding food) that could present health and safe risk for consumers would appear at Safety Gate rapid alert system (RAPEX). This study would show the reasons why some of the toys have been reported as unsafe during the period from 2016 till 2018. The number of unsafe reported toys was 682. Bigger amount of products was reported unsafe during increased level of phthalates. Phthalates are generally in use as plasticizers. The compliance control was conducted at the border-line and at the market. Measurements that had been taken in large number of cases were: Cancelation the products and withdrawing the products from markets. The announcements on RAPEX have good impact on health protection, while it does not affect the fast course of articles and services principle.

Keywords: toys, safety, risk, chemicals, phthalates, RAPEX

INTRODUCTION

The aim of this research is to show the fast unsafe products reporting system (food excluded) at RAPEX site of European commission [1].

At site of Ministry of trade of Serbia you may find NEPRO where is the list of reported products in our country. After joining the European Union would accompany EU reporting system.

At RAPEX site the products are reporting by category, brand, the country where the product has been produced. They are announcing the types of risks as well as the types of warning. Also, they are giving the information about which country has reported and the measurements that are about to be taken. The warnings are split by dangerous risk, second degree risk and others.

MATERIAL AND METHODES

In this paper has been showed the analysis of unsafe products registration- toys up from 2016-2018 at European Commission site- RAPEX. The assessment of product safeness has been done due Toy Safety Directive, relevant European standard and REACH Regulation (for chemical risks) [2–4].

The reporting was conducted by EU countries and the results were given by organ for compliance assessment. All the data relevant for products identification could be found within report (Figure 1). Furthermore the report involve information about control location (border-line or market) along with the measurements that are about to be taken.

Alert number:
Category: Toys
Product:

Brand:
Name:
Type / number of model:
Batch number / Barcode:
Risk type: Dangerous
Chemical , Microbiological,Choking….

Measures taken by economic operators:
Measures ordered by public authorities(to:Manufacturer):

Figure 1 *Displaying the application on RAPEX site*

RESULTS

During the period from 2016 to 2018 the number of products reported as unsafe for children health is 682. The chemicals were declared as the type of risk for all toys while 6% of them also listed suffocation as addition risk. The increased level of phthalates had 79% of toys, 9% had increased metal migration, 8% had mechanical defect and 9% had increased migration of other chemicals (nitrosamines, formaldehydes, citric acid).

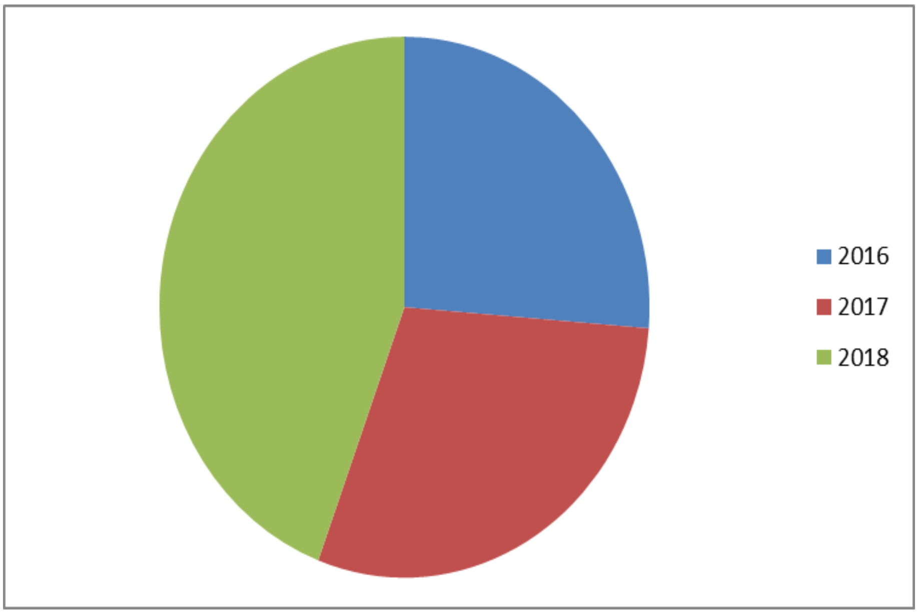

Figure 2 *Incorrectness causes in period between 2016-2018*

In 2016 the number of reported toys was 179 and from that sum 92% had increased level of phthalates, 4% had increased metal migration, 2% had increased level of nitrosamines and 2% had other defects. In 2017 the increased level of phthalates had 72% of toys, 13% had mechanical defects and 2% increased migration of other chemicals (nitrosamines, formaldehydes). In 2018 the increased level of phthalates had 76% of toys, 9% had increased metal migration, 9% had mechanical defect and 6% had increased migration of nitrosamines.

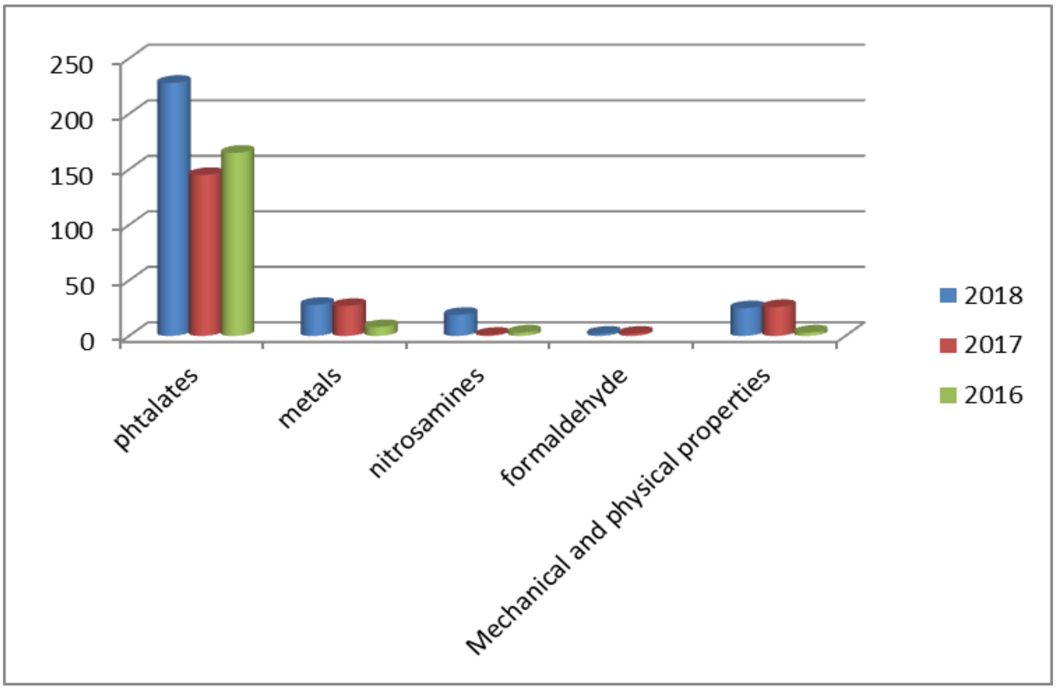

Figure 3 *Incorrectness causes by years*

The type of warnings for 99% of toys was: “Products that pose a serious health risk”. From the list of all reported toys 164 of them were not allowed to pass the border-line (20%) while others were taken to analysis during the market control. The measurements that had been taken in 72% of cases were: Cancellation of products and withdrawing the products from markets, in 24% of cases: Import denied, 3% of toys were destroyed and 1% of them were withdrawn from Ali Express market [1].

CONCLUSION

Based on results showed we could conclude that the biggest health risk for the most sensitive population (children) are chemicals, phthalates the most. Phthalates being in use for plastic emollient is the reason why it is presented in all soft toys made of polymer mass. They present the serious risk for reproductive children organs. After products reported as unsafe analysis we are making a conclusion that the monitoring system is very important.

The fast reporting system could help the consumers to have the right insight in products found on markets in their country as well as markets in other countries. On time information is the only help in consumer’s protection.

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REMOVAL OF COMMONLY USED ANTIBIOTICS FROM HOSPITAL WASTEWATER

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Abstract

Feasibility for removal of two commonly used antibiotics (amoxicillin and ciprofloxacin) from hospital wastewater was experimentally investigated using sub- and super-critical water oxidation. Effect of temperature and flow rate on the concentration of antibiotics was observed in the temperature range from 473 K to 773 K and at flow rates of 3 and 5 mL/min. Samples were analysed in terms of their total organic compound (TOC) and chemical oxygen demand (COD). Highest COD reduction was achieved at the highest temperature of 773 K, where it was reduced for 76 %. Furthermore, the highest decrease in TOC was 63 % at 773 K. Using liquid chromatography tandem-mass spectrometry (LC-MS/MS) amoxicillin and ciprofloxacin were identified and the structure of products was assigned by MS fragmentation patterns.

Keywords: antibiotics, ciprofloxacin, amoxicillin, supercritical water, hospital waste water

INTRODUCTION

Antibiotics are a class of so-called ‘emerging’ contaminants that are receiving considerable attention owing to their potential to negatively impact the environment and to pose risks to human health. They are extensively used in human and veterinary medicine to prevent or to treat microbial infections. Antibiotics could not be completely removed using the wastewater treatment. Most of antibiotics are usually water soluble and poorly degradable [1].

Amoxicillin is one of the most widely used antibiotics for middle ear infection, strep throat, pneumonia, skin infections, and urinary tract infections among others. It belongs to the group of β-lactam antibiotics, which belongs to the group of penicillins. It is semi-synthetically produced and works bactericidally on several gram-positive and gram-negative microorganisms [3]. Due to its chemical composition, level of consumption, solubility, pharmacological characteristics and environmental toxicity, it is known as one of the main pollutants to be persistent in the aquatic environment. Compared toward other pharmaceuticals, amoxicillin has a very low metabolic rate in humans, since 80-90 % of amoxicillin is released to the environment unchanged [4,5].

Ciprofloxacin is an antibiotic used to treat a number of bacterial infections such as bone and joint infections, intra abdominal infections, certain type of infectious diarrhea, respiratory tract infections and skin infections [2]. It is known as a powerful antimicrobial agent used in human and veterinary medicine, primarily they active against gram-negative bacteria like *Pseudomonas aeruginosa* [5].

Traces of antibiotics and their metabolites have been found in all environmental compartments (soil and water). Indeed, biological sewage treatment plants are in most cases not effective in removing residues of antibiotics. Furthermore, antibiotics inhibit microorganisms as working agents. A lot of scientist reported about purification of antibiotics by adsorption on activated carbon [6]. General problem arises in separating the activated carbon from the water [7]. Sub- and supercritical water has unique properties. Besides, water is known as the cheapest solvent that changes its physicochemical properties dramatically from a solvent for ionic species to a solvent for non-ionic species. Critical temperature and pressure of water are 647 K and 22.1 MPa, respectively. At temperatures above the critical, diffusivity of water increases considerably and water also shows more acidic properties. Working with water at high temperatures increases reaction rate, which in consequence increases the stirring speed, leading to better dissolving of organic matters, and undergoing of a fast reaction with oxygen. Due to its high diffusion ability and low viscosity water in supercritical state shows excellent transport properties [8]. A great feature of supercritical water oxidation (SCWO) is its low environmental impact. The technology can be classified as green chemistry, according to the sustainable development [9]. Unless catered for by careful engineering design, the high-temperature environment within SCWO reactors and processing systems can present significant reliability and performance problems. Traditional SCWO processing systems are designed to operate at pressures in excess of the critical pressure of water ($P>220.55$ bar) [10]. Traversing a supercritical isotherm from, say, 250 bar to lower pressure, the corresponding changes in physical properties of the reactants and byproducts are only gradual in nature.

In the study two antibiotics, from two major antibiotic group – β-lactams (amoxicillin) and fluoroquinolones (ciprofloxacin) were used. The selection was based on various factors; intensity of the use in Slovenian hospitals, environmental stability; and concentration reported in waste water. The present work represents the development of a novel method for treatment of wastewater containing amoxicillin and ciprofloxacin by applying sub- and super-critical water oxidation. To our knowledge, this is the only study which has attempted to reduce amoxicillin and ciprofloxacin from synthetic hospital wastewater, using sub- and supercritical water oxidation.

MATERIALS AND METHODS

Chemicals

Buffered peptone water, obtain from Biolife (Italy) was used for the preparation of synthetic wastewater. Ciprofloxacin (99.5% purity), ciprofloxacine-D8 hydrochloride hydrate (99% purity) and amoxicillin trihydrate (85.7% purity) were purchased from Sigma-Aldrich, Fluka (Germany). Amoxicillin: $3H_2O$ Phenyl-13C6 (≥ 95% purity) was purchased from

Cambridge Isotope Laboratories (USA). All reagents were HPLC grade obtained from Sigma-Aldrich, Fluka (Germany) or Merck (Germany).

Synthetic hospital wastewater and sample preparation

The composition of synthetic hospital wastewater is presented in Table 1.

Table 1 *Typical composition of buffered peptone water*

Compound	Concentration (mg/L)
Peptone	250
Sodium chloride	125
Disodium hydrogen Phosphate anhydrous	87.5
Monopotassium Phosphate	37.5

Sub- and supercritical water oxidation

Experiments were performed at a high temperature in a high pressure reactor system. Operating temperatures varied between 373 K up to 773 K. Pressure was maintained between 20 and 30 MPa in order to keep the water in the liquid state. The flow rates of the reaction mixtures were 3 and 5 mL/min.

Analysis of extracts

Total organic compound (TOC) and chemical oxygen demand (COD)

The concentrations of TOC and COD were determined using Hach cuvette tests LCK 381 and LCK 514, and DR 2800 spectrophotometer (Hach Co., Loveland, USA). Samples were heated in HT 200 S high temperature thermostat (Hach).

LC/MS-MS analysis

The antibiotics added to the synthetic wastewater were identified and quantified using LC-MS/MS. Agilent 1260 Infinity HPLC system, coupled with the API 2000 triple-quadrupole mass spectrometer was used.

RESULTS AND DISCUSSION

In this work, investigation has been focused on amoxicillin (β-lactam group) and ciprofloxacin (fluoroquinolone group) because of their wide consumption and the consequence presence in the aquatic environment.

To study the effect of temperature on the amount of ciprofloxacin and amoxicillin, experiments were performed at four different oxidation temperatures, namely 473 K, 573 K, 673 K and 773 K. It can be observed that temperature has significant impact to the final concentration of amoxicillin and ciprofloxacin (Table 2).

The influence of time and temperature on TOC removal (mg/L) is given in Fig. 1. The results clearly demonstrate that reaction temperature had a significant effect on TOC removal, which was increased at higher reaction temperature. At lowest operating temperature of 473 K very low decrease was observed, while decrease in TOC for 63 % was measured at 773 K. The removal of TOC partially reflects the mineralization degree of organic compound. The higher removal efficiency of TOC implies higher decomposition rate of antibiotics to CO_2 and H_2O.

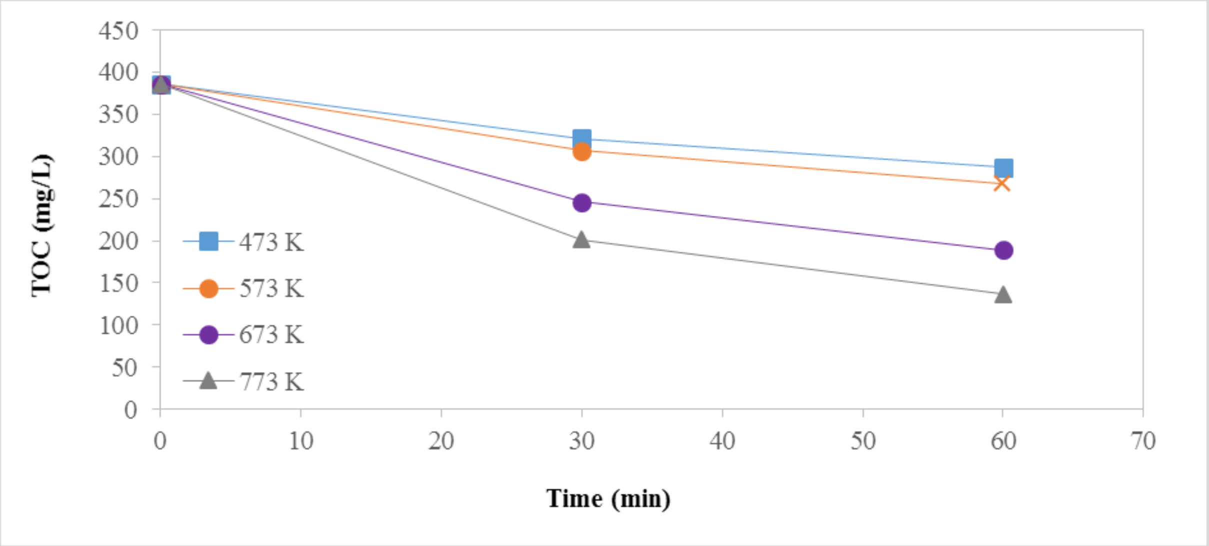

Figure 1 *Changes of TOC of synthetical wastewater sample with reaction time at different temperatures*

When comparing degradation efficiency of both antibiotics, amoxicillin degraded faster than ciprofloxacin. Ciprofloxacin showed a high thermal stability up to 673 K, while amoxicillin was almost fully decomposed at 473 K. The effect of flow rate has also been considered. According to the results presented in Table 2; flow rate does not have much impact on the concentration at the temperatures, lower than 673 K. After exceeding the critical temperature at 673 K, the decrease in concentration became much more visible. In the temperature range from 673 K to 773 K, the higher flow rate (5 mL/min) improved the solubilisation of both contaminants for almost two times compared to the lover flow rate (3 mL/min).

Table 2 *Process parameters and results of concentration of amoxicillin and ciprofloxacin after 2 h*

Temperature (K)	Pressure (MPa)	Flow rate (mL/min)	Concentration of ciprofloxacin (ng/mL)	Concentration of amoxicillin (ng/mL)
473	25	3	815	41
473	25	5	813	34
573	27	3	675	21
573	27	5	605	< 20
673	29	3	171	< 20
673	29	5	86	< 20
773	30	3	45	< 20
773	30	5	28	< 20

Apparently the higher temperature and the flow rate contribute to improve the solubilisation of amoxicillin and ciprofloxacin, so all the compounds are removed at a faster rate.

CONCLUSION

In this study, synthetic hospital wastewater containing amoxicillin and ciprofloxacin was subjected to the process of SCWO. Amoxicillin and ciprofloxacin removal rate has been evaluated as a function of operating pressure, temperature and flow rate. Experiments have been conducted at four different oxidation temperatures of 473, 573, 673 and 773 K and in the pressure range from 25 MPa up to 30 MPa.

High reduction/removal rate of amoxicillin and ciprofloxacin from hospital wastewater has been attained. Thus sub- and supercritical water oxidation have been proved to be efficient methods not only to reduce, but also to completely remove both antibiotics from synthetic hospital wastewater. It has been demonstrated that impact of temperature on amoxicillin and ciprofloxacin concentration is high. After exceeding the critical temperature at 673 K, the removal rate was greater; the concentration of both antibiotics decreased below 20 ng/mL. Comparatively, amoxicillin degraded faster than ciprofloxacin.

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MICROSTRUCTURAL PROPERTIES AND DYNAMIC-MECHANICAL BEHAVIOUR OF CuAlMn SHAPE MEMORY ALLOY

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Abstract

Shape memory alloys (SMAs) are a relatively new advanced functional metallic materials used in different industrial fields. Among them CuAlMn alloys can be used due to their ductility. In this work the results of microstructural and dynamic-mechanical properties of Cu-8.3Al-9.4Mn (wt. %) alloy after casting and plastic deformation are presented. After cold rolling of strip the solution annealing at 900 °C holdnig for 30 minutes following water quenching was carried out. Metallographic analysis of the alloy was carried out by an optical microscopy (OM) and scanning electron microscopy (SEM) equipped by device for energy dispersive spectroscopy (EDS). Damping properties of the alloy were carried out by dynamic-mechanical analyzer (DMA) method. It was found that after casting and hot+cold rolling two-phase ($\alpha+\beta$) microstructure existed, while after quenching of the strip only martensite microstructure occurred. Dynamic-mechanical analysis results confirmed large energy loss in the investigated samples, as a result of movement of interfaces of martesite structure.

Keywords: CuAlMn, shape memory alloy, microstructure, dynamic-mechanical analysis

INTRODUCTION

Shape memory alloys (SMAs) are metallic materials which are able to memorize and recover its original shape. The shape memory effect (SME) is the remembrance of the previously introduced material shape. Today the three most popular polycristalline shape memory alloys are: Ni-Ti (nitinol), Cu-based (CuZnAl, CuAlNi, CuAlMn etc.) and ferrous alloys (FePt, FeMnSi, etc.) [1-5]. The main advantage of Cu-based SMAs are their low price compared to Ni-Ti alloy. Among Cu-based SMAs the CuAlMn alloys can be competitive due to their high ductility. These alloys have high potential for practical applications [6-8]. The addition of manganese to binary Cu-Al alloys leads to better ductility of the alloy. The CuAlMn alloys with higher Mn content (above 8 at. %) and lower Al content (below 18 at. %) have excellent cold-workability without decreasing of shape memory effect. Investigation on the CuAlMn alloys with 10-14.5 wt. % aluminium and 0-10 wt. % manganese were shown that lower aluminium and higher manganese contents improving superelasticity [9]. By quenching of the alloys martensite transformations occurs. Depending on composition of the CuAlMn alloys three types of martensite microstructures could be presented (α', β_1' and γ_1'). In the different industrial fields (energy-storage technological applications) increasing need for vibration and noise reduction. SMAs are relatively high damping materials because of a high damping capacity and good ductility [10]. The goal of this work was to test the microstructure of the CuAlMn strip after hot and cold rolling including heat treatment (quenching) with special regard to the damping properties.

MATERIALS AND METHODS

The shape memory alloy (Cu-8.3Al-9.4Mn, wt. %) was produced by melting pure elements in vacuum induction furnace and casted by vertical continuous casting procedure. A bar of 8 mm in diameter solidifies in the crystallizer and comes out passing between two rolls which rotate in opposite directions. After the casting alloy was submitted to plastic deformation (hot rolling and cold rolling). The rod from 8 mm was inserted in the furnace at 900 °C and heated for 5 minutes. Number of hot rolling passes was seven, while number of cold rolling passes was ten. Thickness of strip after cold rolling was 1.02 mm, while strip width after cold rolling was 13.9 mm. After cold rolling of strip the solution annealing at 900 °C for 30 minutes followed by water quenching was carried out. Samples for microstructural analysis were prepared by mechanical grinding with paper No. 240-1000 and polishing with 0.3 μm Al_2O_3. After that the samples are etched in mixing solution (2.5 g $FeCl_3$, 48 ml methanol and 10 ml HCl) for 12 s. Metallographic analysis of the alloy was carried out by an optical microscopy (OM) and scanning electron microscopy (SEM) equipped by device for energy dispersive spectroscopy (EDS). Damping properties of investigated alloy were carried out by dynamic-mechanical analyzer (DMA) 983, TA Instruments. Measurements were performed under fixed frequency mode in nitrogen atmosphere, in the temperature range from -50 °C till 250 °C, with heating rate of 10 °C/min. Modulus were detected at frequency 1 Hz, oscillation amplitude 0.1 mm and shear distortion 1.500. Specimen dimensions were: 20.1400 x 3.9580 x 0.2380 mm.

RESULTS AND DISCUSSION

Figures 1-3 show results of metallographic analysis by OM, SEM and EDS methods. As can be seen the CuAlMn alloy after casting and hot+cold rolling has two-phase (α+β) microstructure (Figures 1a and 2a). After quenching the only martensite microstructure exists (Figures 1b, 2b and 3a). At high temperature in the CuAlMn alloy β-phase with bcc structure is present. During quenching of hot+cold rolled strip in the α-phase is not present solid-state transformation, but the β-phase undergoes to order-disorder transformation: $\beta(A2)\rightarrow\beta_2(B2)\rightarrow\beta_1(L2_1)$. By rapid cooling at M_s temperature the thermoelastic martensite transformation ocurrs: $\beta_1(L2_1)\rightarrow\beta'_1(18R)$.

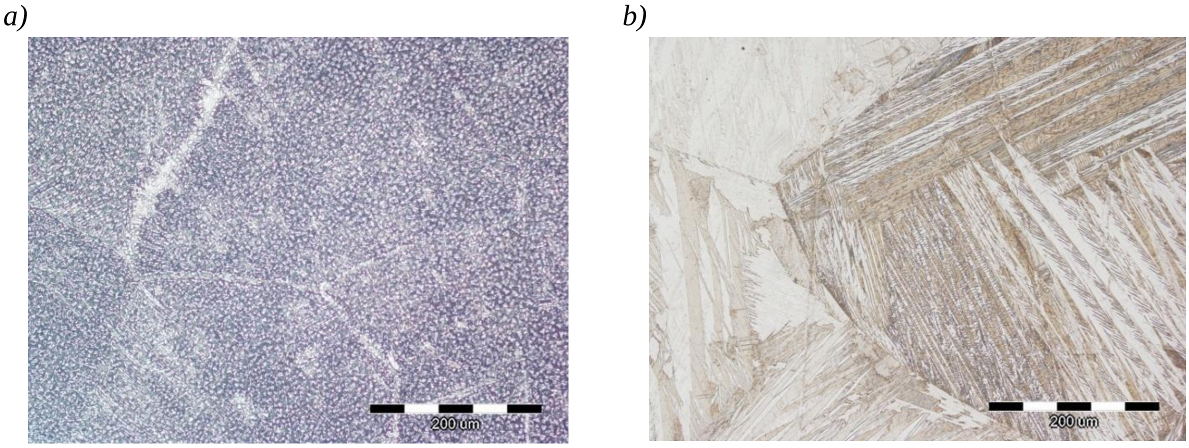

Figure 1 *Optical micrographs of CuAlMn cold rolled strip in as-cast state (a) and after quenching at 900 °C/30'/H_2O (b)*

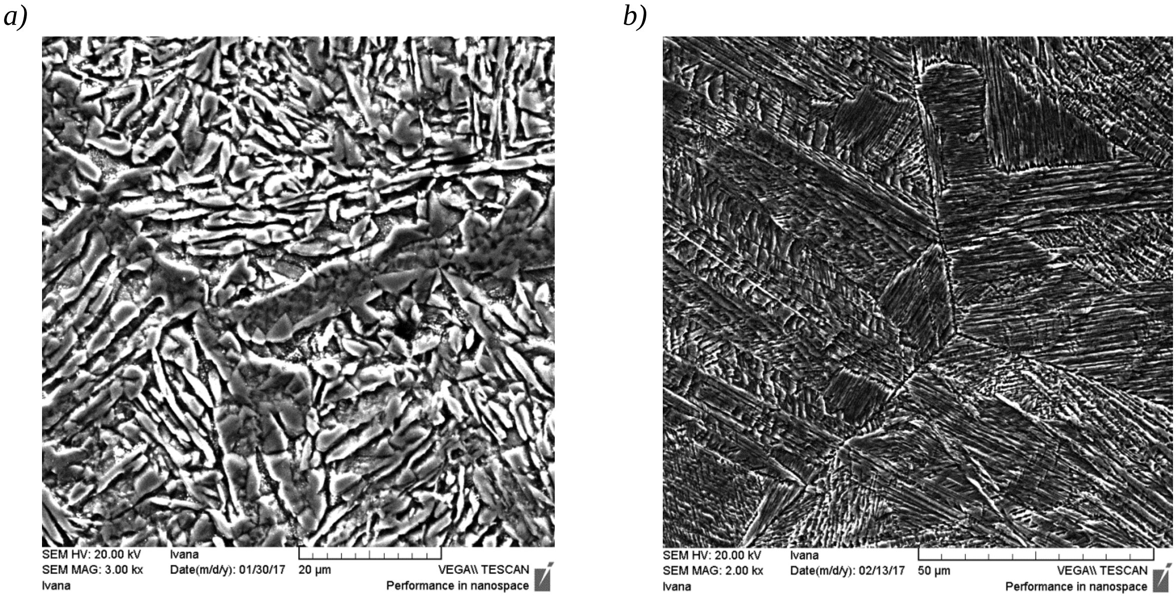

Figure 2 *SEM micrographs of CuAlMn cold rolled strip in as-cast state (a) and after quenching at 900 °C/30'/H_2O (b)*

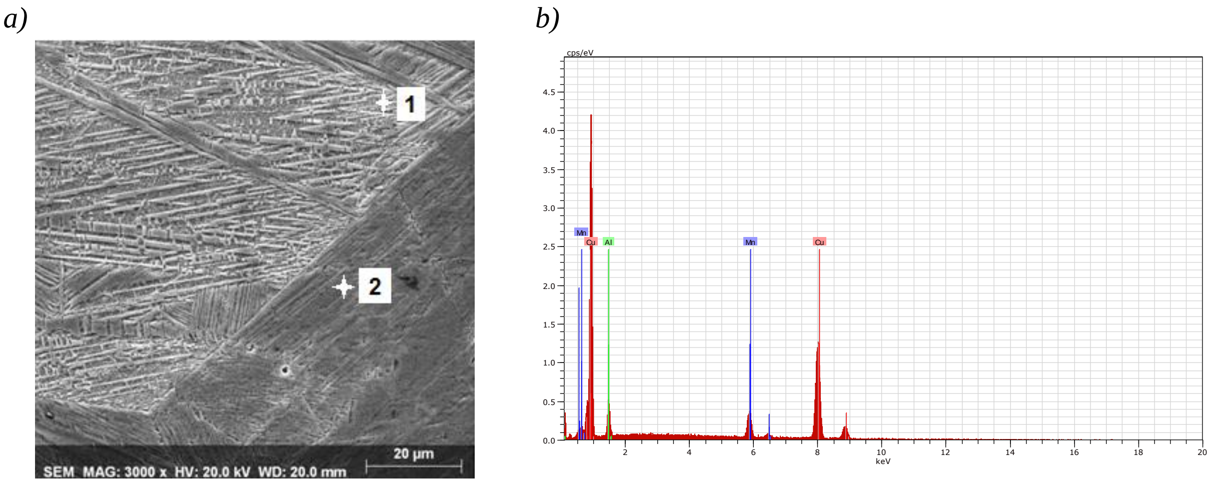

Figure 3 *SEM micrographs (a) and EDS spectrum (b) of CuAlMn cold rolled strip after quenching at 900 °C/30'/H_2O (b)*

Table 1 *Chemical composition of positions marked at Figure 3, wt. %*

Position	Cu	Al	Mn
1	84.00	6.70	9.30
2	84.50	6.81	9.50

Figures 4 and 5 present results of dynamic-mechanical analysis of Cu-8.3Al-9.4Mn alloy in the as-cast and quenched state. Changes in the storage modulus as a function of temperature for both samples indicate austenitic transformation only after quenching at 900 °C (Figures 4, 5a). Transition range starts at -20°C with maximum intensity of martensite+parent two phase state at 31 °C (Figure 5a). Loss tangent (tg δ) curve for quenched of Cu-8.3Al-9.4Mn alloy shows higher value of tgδ in the martensite single-phase in relation to parent single phase (Figure 5b). Level of tgδ in the martensite phase region is 0.03, in the austenitic phase 0.01, while the maximum of loss tangent, tgδ, is in the two-phase region 0.052 (Figure 5b). Obtained loss tangent values correspond with results of previous investigations of Cu-based shape memory alloys [11,12]. In the transition region Cu-8.3Al-9.4Mn alloy presents maximum damping limit, due to dissipated energy during martensite/austenite transformation [13]. Generally a high damping capacity of the CuAlMn alloys is due to revesibile martensite phase transformation, microstructural control (such as grain size and texture control), unrealistic strain of interfaces including twin boundaries, thermoelastic martensite variant interfaces etc. [6,10]. Dynamic-mechanical analysis results confirmed large energy loss in the investigated sample, as a result of movement of two interfaces of martesite structure. Martensite variant interface showed lower mobility and consequently lower damping ability in regards to two-phase habit planes [14].

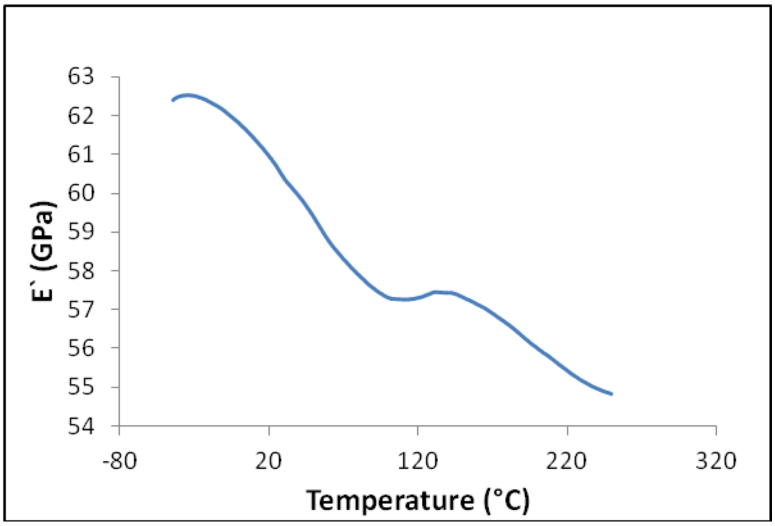

Figure 4 *Storage modulus (E`) of Cu-8.3Al-9.4Mn alloy in the as-cast state*

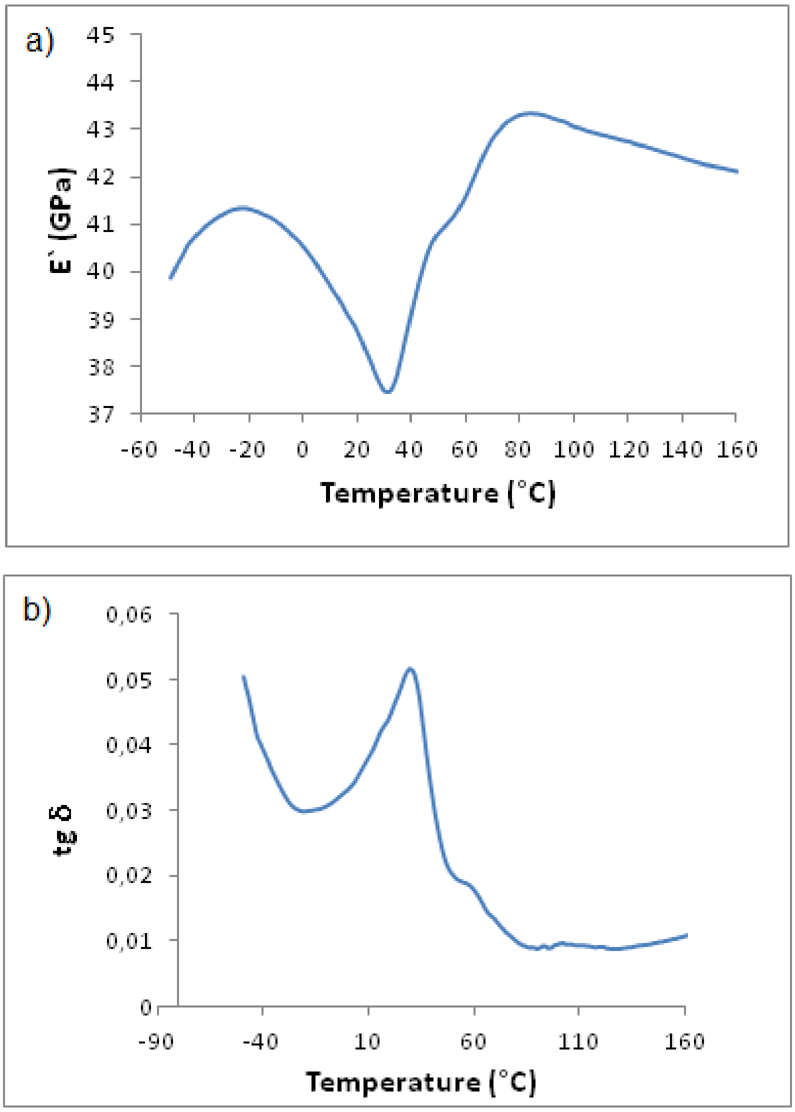

Figure 5 *Storage modulus (E`) of quenched Cu-8.3Al-9.4Mn alloy (a) and loss tangent (tgδ) of quenched Cu-8.3Al-9.4Mn alloy (b)*

CONCLUSION

Results of metallographic analysis of CuAlMn shape memory alloy by OM and SEM methods shows that after casting and hot+cold rolling has two-phase (α+β) microstructure, while after quenching the martensite microstructure occurred. Results of dynamic-mechanical properties indicate good damping properties. Dynamic-mechanical analysis results confirmed large energy loss in the investigated samples, as a result of movement of interfaces of martesite structure.

ACKNOWLEDGEMENT

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SUPERCRITICAL FLUIDS AS GREEN SOLVENTS

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Abstract

Supercritical fluids (SCFs) offer the possibility for obtaining and designing of new environmentally friendly and sustainable products with special characteristics. Recently, SCFs have been applied for polymer processing, polymer extraction and purification, preparation of optical materials, supercritical blending of additives into polymers, as a foaming agent for microcellular materials, impregnation, fractionation, purification and formation of powdered polymers. This review is focused on some applications of supercritical fluids with special emphasis on their properties of supercritical fluids in water and carbon-dioxide green industrial chemical processes.

Keywords: supercritical fluids, carbon dioxide, water, technology, process

INTRODUCTION

The wide industrial application of different organic solvents represents a serious threat to the environment. However, modern industrial processes are focused on development of organic solvents free processes, such as supercritical fluids (SCFs), which are based on the high-pressure theory and green chemistry principles [1,2]. SCFs present an adequate substitute for processes, which use toxic chlorinated, aromatic, and chlorofluorocarbon solvents. In the supercritical state, there is no phase boundary between the gas phase and the liquid phase. The properties of SCFs are in between that of gas and liquid. SCFs are highly compressible, particularly near the critical point, and their density and thus the solvation power can be carefully controlled by small changes in temperature and/or pressure [3,4]. The special combination of gas–like viscosity and liquid–like density and solvating properties of a SCF makes it an excellent solvent for various application. The environmental benefits of using SCFs in industrial processes, such as low energy consumption during operation, show their potential of replacing the far more environmentally damaging convential organic solvents [5–7]. Therefore SCFs are sometimes called green solvents for the future. Water and carbon dioxide in their supercritical states are primary candidates as solvent for green chemical processes, since they are compatible with the environment and have enhanced transport properties for reaction and separations. Gases such as nitrous oxide or ethane have low critical values, but can generate explosive flammable mixtures. Although the properties of SCFs are well known, they are as yet not fully exploited for industrial application [8].

APPLICATION OF SUPERCRITICAL FLUIDS

Supercritical fluid extraction (SCFE) has been successfully introduced for a range of industrial processes, examples include: coffee decaffeination, isolation of some flavoring components from hops, fatty acid refining and production of herbal products. There is still much activity in the food, chemical and pharmaceutical fields in processing and product development with SCFs. SCFs fluids applied to food products have met with commercial acceptance as noted in the chemical engineering literature [9–11]. Recent research on the application of SCFs showed that they could be used as new reaction media for chemical and biochemical reactions, for synthesis of new materials and new catalyst supports such as aerogels, for special separation techniques such as chromatography using SCFs and extraction processes, and for particle formation and product formulation. There is a great variety of potential applications of SCFs in the industrial processing of fats, oils and their derivatives. Several industrial plants, which use different gases for isolation/ fractionation of components, are in operation for extraction of oils from seeds, fruits, leaves and flowers, which are further applied in food, pharmaceutical and cosmetic industry. The application of SFE in vegetable oil industry has been extensively investigated as an alternative to conventional refining, separation and fractionation processes. Several industrial plants are in operation also for extraction of spices for food industry and natural substances for use in cosmetics. In pharmaceuticals and biomedical areas, however, although there are commercial developments that use SCFs, industrial acceptance of SCFs produced products including particle technology has lagged, which is probably due to the necessity of clinical studies that are required. Extractions with SCFs will continue to play an important role in separation science and engineering. The promising new areas of SCFs application are functional foods from algae and microalgae, micronization and high-liquid free-flowing powders for foods, bioactive compounds and lipids. Much activity exists in the areas of energy conversion, biomass gasification, biofuels, biodiesel, wet algae conversion, and petroleum upgrading. The use of SCFs for heat transfer is a relatively new field, which has been recently intensively studied; however, the data about the efficiency and applicability of these features in practice is still scarce. The heat transfer and refrigeration properties of SCFs found also other applications, such as conductor cooling to obtain superconductivity effects, cooling of rockets and military aircrafts, of turbine blades, supercomputer elements, magnets and power transmission cables. SCFs have been also applied for sustainable biodiesel production. Biodiesel has become attractive recently due to its carbon-neutral effect and environmental benefits. Recently, SCFs have been applied for polymerization, swelling, impregnation, fractionation, purification and formation of powdered polymers and polymer processing with supercritical fluids. Frequently, it is necessary to extract small molecules (plasticizers, antioxidants, solvent and monomer residues, oligomers, stabilizers) from polymer matrices. SCFs can interact not only with polymers at temperatures higher than the softening point but also with polymers in the glassy state [12]. In the dyeing processes of the textile industry, the use of SCFs an alternative solvent instead of water based processes has been gaining much interest for environmental reasons. The environmentally friendly SCFs dyeing process does not require any water, dispersing agents or surfactants and also does not involve any drying stage after dyeing.

SUPERCRITICAL CARBON DIOXIDE AND WATER

The best known and widely employed SCF is carbon dioxide (CO_2) used industrially on a large scale for the decoffeination of coffee. CO_2 is environmentally friendly. Its contributions to the greenhouse gas inventory is negligible compared to that resulting from the burning of fossil fuels. It is nonflammable, nontoxic (at low concentration), and noncorrosive to common structural materials and is cheap. Supercritical CO_2 is becoming an important solvent due to its role in chemical extraction. The relatively low temperature of the process and the stability of CO_2 also allow most compounds to be extracted with little damage or denaturing. $SCCO_2$ has been used in polymer processing as solvent, anti-solvent or plasticizer for microcellular foaming, particle production, polymer blending, obtaining polymer composites, impregnation of polymers and solvent extraction. In the field of polymeric foams, $scCO_2$ was used as blowing agent (Figure 1).

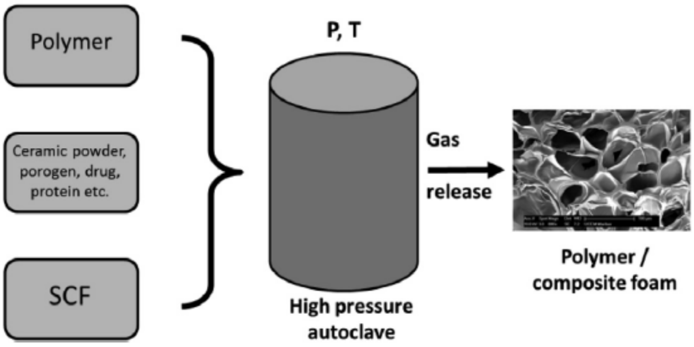

Figure 1 *The preparation of polymer and composite foams using supercritical fluid technologies*

In heating and ventilating systems, CO_2 is seen as a competitive fluid for heat pumps. The commercial EcoCute transcritical CO_2 water heater developed by Japanese manufacturers reached installation of more than 2 million units as of October, 2009 and has a remarkably high COP (coefficient of performance) of 3.5 or more than for heating hot water for domestic and commercial-scale. $SCCO_2$ is a superior mass-separation agent compared with liquid solvents because it can easily be recovered and recycled. The use of CO_2 as a mass separation agent not only makes the separation efficient, but more importantly, it protects worker health and thus is the beginning of a green chemical process. $SCCO_2$ can be used to reduce the viscosity of an ionic liquid mixture and this viscosity reduction can also allow reactions to occur. Some examples of classical organic synthetic procedures that have been successfully performed in $SCCO_2$ include: Friedel Crafts, Diels-Alder, Aldol, Claisen rearrangement, Michael addition and Kolbe-Schmitt reaction in $scCO_2$ media [13-15]. $SCCO_2$ has provided a successful reaction medium for the production of some polymers including: amorphous fluoropolymers, polysiloxanes, some hydrocarbon polymers, polybutylacrylate and polystyrene [16]. The use of $scCO_2$ to produce polymers provides polymers free of solvents, in many cases also water, that are required for many applications. CO_2 is a good solvent for many non-polar and some polar molecules with low molecular weights but it is a very poor solvent for most macromolecules at normal operating conditions. Although the solubility of most polymers in CO_2 is extremely low its, solubility in many polymers is substantial. This can lead to the enormous decrease of the polymer glass transition temperature at modest pressures. The plastification of polymer materials using CO_2 plays a key role for processing, purifying, extracting or foaming of thermally sensitive materials. Particle design using $scCO_2$ is demonstrating significant potential in applications involving the pharmaceutical, cosmetic and specialty chemical industries. The dyeing of fabrics using $scCO_2$ as a dye solvent has been the focus of increasing research. $scCO_2$ emulsion extraction of the organic phase has been successfully applied to the production of microparticles and microcapsules for controlled drug delivery. The general idea of electrospinning in $scCO_2$ is to produce the fiber in a high pressure vessel filled with $scCO_2$, enhancing solvent extraction and obtaining also porous fibers. Levit at al. [17] demonstrated the feasibility of this process using two electrodes immersed in $scCO_2$ located at a distance of 3 mm. In presence of $scCO_2$, a polymer can show a reduced viscosity (and surface tension) due to the capacity of CO_2 of penetrating inside the polymeric structure; at these conditions. Polydimethylsiloxane (PDMS) and PLA fibers were electrostatically produced without the use of a liquid solvent, processing the polymers at 40°C and 14 MPa. Supercritical water (SCW) is also used as solvent, especially in last year. It has properties differing considerely from those of $scCO_2$. Its critical temperature is twice and its critical pressure almost three times higher. Its ranges of application are about 400 – 600ºC and 30 – 100 MPa. Water at ambient conditions is a polar solvent of polar molecules. It is a good solvent for polar compounds and salts. At supercritical conditions at e.g. 550ºC and 20 MPa, water behaves as a nonpolar organic solvent like pentane with good solubility for organic components and gases and low solubility for salts. Generation of hydrogen and other compounds that may be used as fuel is one of the most studied applications of SCW. Recently, hydrogen production from wet biomass and organic compounds in sub and supercritical water has gained significant attention. The advantages of SCW gasification over conventional biomass gasification (such as steam reforming) refers to the possibility of processing feedstock with high water content and the generation of pressurized gases, which simplifies their transportation and further use. However, there are a number of limitations to the SCW gasification process, such as the formation and deposition of carbon, which may cause plugging of the reactor or deactivation of the catalyst, and the specific requirements for reactors and other high pressure equipment. Supercritical water oxidation (SCWO) initially was seen as the best method to destroy toxic and dangerous compounds and to clean liquids and solids. Process development and various applications soon showed that several problems are connected with SCWO including salt precipitation, plugging, and severe corrosion. Although the technology has matured, it still has challenges related to corrosion and solids handling although some of these issues are being addressed through reactor design [17]. SCW can be used to develop material formation processes that operate at low temperatures (ca. 400ºC) as opposed to present industrial processes that operate at high temperatures (ca.

1200ºC). Supercritical water gasification (SCWG) can convert organic waste streams from industrial activities into energy. SCW gasification technology can potentially gain energy in the form of combustible gases from organic waste streams. Along with energy gain via gas production, it also minimizes pollution from waste stream and as a result reduces its potential noxious and dangerous effects. Thus, SCWG of waste stream is a future perspective /potential solution to solve two problems at once. SCWG is a thermochemical conversion process, which includes also pyrolysis, liquefaction, dry gasification and combustion. Among them, gasification is one of the most favorable processes since the products can be used for different markets such as heat, electricity and transportation. During SCWG, the organic matter decomposes into char, tar, gas, or other intermediate compounds. These products are subsequently reformed into gases such as CO, CO_2, CH_4 and H_2. Gasification in SCW involves mainly thermal decomposition and reforming reactions. The main disadvantages of the process are high capital and operating costs. The required extreme temperatures and pressures cause higher costs in the system leading to the need for special materials and operating equipment. In addition to water and CO_2, compounds such as ethane, propane, methanol, ethanol are also used in their supercritical phase for certain applications (extraction, chemical reactions).

CONCLUSION

SCFs are already applied in several processes developed to commercial scale in industries. CO_2 and water have been the most used SCFs. Many natural compounds, such as vitamins, aromas, natural pigments or essential oils, are extracted with SCFs, thus avoiding the use of organic solvents and of high temperatures. SCFs were proposed for different applications in the energy field. The practical application of SCFs requires the design of technical components and plants for production. Compared with other technical systems, SCFs production plants are relatively simple from the mechanical and control points of view. SCFs processes are also energy efficient, particularly when they can replace organic solvent based processes that at some stages require removal of organic solvents by energy consuming procedures. The development of sustainable processes is one of the aims of the SCFs technologies.

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ECOLOGICAL ADVANTAGES OF ORGANIC GROWING COTTON

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Abstract

There are a number of important differences between conventionally and organically grown cotton, which related to different aspects of cotton growing, such as soil tilling, seed selection, resources consumption, primarily water and chemicals, management of various pests, engagement of labor, etc. Moreover, organic cotton exhibits significant environmental benefits. In this paper, different cotton growing practices are compared.

Keywords: conventional cotton, organic cotton, ecological benefits

INTRODUCTION

Cotton growing agricultural practises differ in the world, even in certain areas, from farm to farm and at various geographical locations. Conventional methods of cotton growing are standard practices that are widely applied. It is estimated that 90% of the total cotton produced is grown in a conventional way. Due to the large ecological problems caused by conventional growing of cotton, methods of organic cotton growing and so-called *Responsible Livelihood Enhanced Environment* (REEL) are increasingly applied [1,2].

AGRICULTURAL PRACTICES IN COTTON GROWING

The main factors for the cotton growing methods selection are the type of cotton and soil and climate local conditions. In addition to the cottonseed itself, key elements for successful cotton growing are: temperature, sunlight, precipitation (rainwater) / water for irrigation and soil fertility / supplementary nutrients (fertilizers) [3].

Cotton requires a large amount of water – rainwater or irrigation during the growth season. About 73% of globally produced cotton comes from irrigated soils. Cotton consumption is responsible for about 2.6% of total water consumption in the world. This causes soil salinization, especially in dry areas and hence the degradation of soil fertility. The amount of land used to grow cotton hasn’t changed since 1930s, but yields have been increased three times through hybridization, intensive land management and use of chemical pesticides and fertilizers [4].

Cotton is still the fourth-largest consumer of agricultural chemicals. According to various sources, cotton production globally use over 25% of all pesticides (insecticides, fungicides and herbicides), and takes place on about 2.5% of arable land in the world. Often, pests develop resistance to pesticides that have been used for a long time. What's more, chemicals remove not only pests but also their enemies, which significantly reduces biodiversity and can result in pests that initially were not significant (secondary pests) become the main problem. Artificial fertilizers are used for growing cotton in almost all countries. From a life-cycle perspective, energy consumption for the production of cotton fibers is not significant [2,5–7].

Protection of cotton crops from harmful impacts – After planting, cotton plants must be protected from impacts which can damage crops, in particular from: insects, nematodes, infectious diseases and weed. A large number of insecticides are used against cotton parasites. Most diseases are controlled by seed treatment with appropriate chemicals. The most important method for weed control is chemical – herbicides application [8].

Impacts on the environment and human health – Cotton production contributes to climate change. Industrial fertilizers are produced using considerable quantities of finite energy sources (1.5% of the world's energy consumption), releasing large amounts of carbon dioxide. Furthermore, the excessive application of nitrates to agricultural land leads to their transformation into nitrous oxide (N_2O – "laughing gas"), a greenhouse gas that is 300 times more destructive than CO_2 in terms of global warming. Finally, soils are important carbon sinks. Soil degradation seriously reduces their carbon sequestration capacity, thereby contributing to the greenhouse effect [2]. The main impacts of cotton growing on the environment are the contamination by the use of chemical fertilizers, pesticides and chemicals that help reading (desiccant, defoliants), water consumption and land degradation, as well as the use of land and the disposal of wild habitats. The use of synthetic fertilizers can cause contamination of groundwater, nutrient enrichment, excess vegetation (eutrophication) and pollution of drinking water by nitrates, the occurrence of problems with insects and diseases. Pesticides are often non-selective and kill natural enemies of parasitic species, in addition to the parasites themselves. Secondary parasites can begin to compete to major parasites. Also, insects can become resistant to pesticides. Some pesticides get into water systems, where plants and animals are destroyed or poisoned. Spraying pesticides on cotton fields also pollutes the air outside boundaries of the cotton field, which affects people and nature in the environment [6,9,10]. The World Health Organization estimates that there are 20000 deaths annually from pesticide use, mostly in contact in the application [11]. The danger in cotton fiber production is also plant fiber dust, which leads to respiratory illnesses. Research has shown that cotton dust contains up to 26 inorganic elements (bromine, nickel, cadmium, barium, silicon, cobalt, zirconium, manganese, arsenic, etc.) in different relationships.

The most widely used practices in cotton growing

Various agricultural practices in cotton growing are applied in the world, which depends on the ecological load and cotton fibers properties [12]. The most widely used practices are:

- **conventional cotton growing** (traditionally, usually), which is by far the most appropriate and least restrictive in the use of chemicals and seeds; mainly uses synthetic agrochemicals (pesticedes and fertilizers);
- **hybrid cotton growing** – Responsible Livelihood Enhanced Environment (REEL), by which farmers train for the use of agrochemicals in a much more careful and effective way than the conventional;

- **organic cotton growing**, using techniques such as crop rotation, compost and biological pest control, which leads to improved ecosystem and soil condition, and prohibits the use of synthetic chemicals.

The main differences between conventional, REEL and organic cotton growing relate to chemical inputs [1]. It is also important to mention Integrated Pest Management (IPM) practice and Genetically Modified (GM) pest resistant plant cotton (insect-resistant crops (Bt cotton), Herbicide-tolerant (HT) crops).

Conventional cotton growing

The conventional method of cotton growing is a standard practice, widely used with the combination of mainly synthetic agrochemicals for pest control and fertilizers and is least restrictive in the application of chemicals. Conventional cotton production has many harmful effects on the environment and human health – it has a series of social and economic risks, especially for small farmers in developing countries, which fall ill or die due to a lack of adequate equipment and knowledge about how to handle pesticides properly. Besides, the income from such cotton harvest is often lower than the costs of the inputs due to low crop yields and market prices, which is a major problem for farmers, especially in regions with unstable climatic conditions. Cotton producers are highly dependent on the unstable world market [2].

Hybrid cotton growing (REEL)

A great step forward from conventional cotton farming is REEL, which is more stringent in the use of synthetic chemicals than conventional growing and offers a number of other benefits – promoting the practice of sustainable cotton production, which also includes the education of farmers [1]. It is a plantation capacity building program that provides training during the cotton season. It concerns the management of land, waters and pests, as well as decent working conditions, focusing on: **ecological sustainability** (reducing toxic chemicals, increasing water efficiency, improving soil condition and biodiversity, planting in traps and using natural / organic fertilizers and pesticides) and **socio-economic sustainability** (increasing productivity of farmers by reducing input costs and increasing yields, hence improving profitability and life expectancy; care of decent working conditions, including health and safety, the importance of education and the elimination of child labor (as defined in ILO Conventions 138 and 182). The program also offers farmers training in: financial and business management, gender equality, supply chain mapping, supply chain conventions, etc.

Organic cotton growing

Organic cotton production is steadily growing, but it still accounts for less than 1% of total cotton production in the world. In 2017 organic production of cotton reached 117,525 metric tons of fibers, which represents an increase of 10% compared to the previous year, and it took place on about 473000 ha of certified areas, or on over 220,000 farms in 18 countries. About 89% production comes from five countries (India 51%, China 19%, Kyrgyzstan 7%, Turkey 7% and Tajikistan 5%). An additional 215,000 ha is in the process of obtaining certificates for organic cotton growing, of which over 80% are in India [6,13,14]. The price of organically grown cotton is about 20-30% higher than conventional cotton [9].

Organic cotton growing applies methods that are environmentally, economically and socially sustainable. It does not permit the use of chemical fertilizers, pesticides, desiccants, defoliants and all other harmful substances, prohibited by organizations for organically grown cotton registration. Organically grown cotton does not use genetically modified seeds [15]. Up to 80% of organic cotton production uses rainwater rather than irrigation systems [16,17].

Reduction in yield can be prevented by the use of alternative methods, other types of unconventional parasite management, using techniques such as appropriate seedling, crop rotation, pre-irrigation and programmed weed treatment, time-harvesting / hand-picking, compost and manure utilization, and biological control of pests, which leads to an improved ecosystem and the soil, water and air conditions – prohibits the use of synthetic inputs, and aims to make organic inputs more assimilated by natural ecosystems. Organic fertilizers should provide intensive growth, as well as to improve soil structure, biological activity, mineralization process and maintenance of water capacity in the soil [18]. Farms must provide set standards three years prior to obtaining the organic status and must maintain them in order to comply with the certificate issued by accredited, third-party organizations [4]. In addition, later in the life cycle, when cotton is processed into textile materials, there will be no residues of pesticides and harmful chemicals on cotton fibers in organically grown cotton. Hence there will be no contamination risk to the environment with these substances, nor threatening human health [1,9].

Relevant EU regulations and GOTS (Global Organic Textile Standard) regulate the rules and principles of organic production of textile products. Agricultural production, processing, packaging and coding are covered by inspection procedures. All fields of organically grown cotton, including the transient phase, buffer zones and reading equipment are controlled, as well as manufacturers and operators. Organic grown cotton and cotton in the transition phase are isolated from contact with other cotton and many chemicals used in the finishing are avoided.

ORGANIC COTTON ADVANTEGES

By comparing the practices of conventional and organic cotton production, numerous ecological advantages of organic cotton can be identified [9,15,19,20]: reduced global warming potential, less acidification potential, reduced eutrophication (soil erosion) potential, reduced blue water consumption, reduced primary energy demand. REEL practice is somewhere between and, depending on the applied methods and chemicals, is closer to one or the other option. The Table 1 outlines some of the major differences at each stage of the production process [12].

There are also contrary opinions that the choice of the best ecological option is not always clear and that an organic cotton product can consume more resources than the same product from conventional cotton and can have a higher total environmental impact [21,22].

Table 1 *Organic cotton vs. conventional cotton [12]*

Topic	Organic	Conventional
Seeds	GMO-free; untreated	Treated with fungicides, insecticides, possible genetic modification
Soil	Crop rotation; cover cropping, better retention of moisture; higher concentrations of organic matter, animal manure additions	Synthetic fertilizers, loss of soil due to mono crop culture, intensive irrigation
Weed & insect control	Beneficial insects and 'trap crops' used; flame weeding and cultivation; border management of vegetation (to increase population of native beneficial);use of certain bio-pesticides such as bacteria, virus and fungal insect pathogens	Aerial spraying of insecticides used & pesticides; FYI 9 of the most commonly pesticides are known cancer-causing agents
Harvesting	Natural defoliation from freezing temperatures or through the use of water management	Defoliation induced with toxic chemicals
Whitening	Safe peroxide is used	Chlorine bleaching is used; toxic by product produced and released into environment in process
Finishing	Soft scour in warm water with soda ash for pH 7.5-7.8	Hot water, synthetic surfactants, additional chemicals (sometimes formaldehyde)
Dyeing	Low impact or natural dyes used with low metal or sulfur content	High temperature with heavy metals and sulfur content (can leach into skin as well as ecosystem)
Printing	Low impact, water based inks and/or pigments with no heavy metals	Pigments may be petroleum based and contain heavy metals. Run off easily spills into water ways and ecosystem
Fair trade	Organic certification includes requirements for fair wages and treatment of workers in the supply chain Criteria are in place to ensure safe, healthy and non-abusive, non-discriminatory compensation for workers	No fair trade or living wage requirements in place Widespread evidence of child labor, forced labor, slave labor/wages and work condition in countries where cotton growing exists

CONCLUSIONS

Organic cotton represents a return to safe and sustainable practices. It is an eco-friendly processing that does not compromise workers' health and helps reduce water and electric use and toxic runoff, e.g. non-chlorine bleach, silicon-free softeners and low impact, azo-free dyes, etc. It is grown with natural fertilizers and is free from toxic chemicals. Manual farming and organic practices have a lower carbon footprint as the entire process consumes less fuel and energy and emits fewer green house gases.

The rapid increase in the share of organic cotton on the market in recent years indicates that organic cotton growing is possible in large proportions. In cotton textile fabrics, organic growing cotton can therefore be recommended as a superior alternative to the environment, compared to the conventional cotton.

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OPTIMIZATION OF FLAVONOID EXTRACTION USING MICROWAVE-ASSISTED EXTRACTION AS ECO-FRIENDLY TECHNIQUE

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Abstract

Microwave-assisted extraction (MAE), as efficient and eco-friendly technique for extraction of valuable bioactive compounds, was optimized using response surface methodology (RSM). The effect of three independent variables such as microwave power (300, 450, 600 W), ethanol concentration (25, 50, 75%) and extraction time (5, 10, 15 min) on the flavonoid extraction efficacy was investigated. Experimental maximum of total flavonoid content (TFC) of 23.2 mg rutine (RE)/g dry extract were obtained at 600 W, 50%, 15 min, while the TFC value predicted by proposed quadratic model was 22.09 mg RE/g dry extract and obtained at proposed optimal conditions: 556.5 W, 50.84%, 15 min. RSM suitability in optimizing MAE of chokeberry was confirmed within a 88.2% confidence interval. The set of economically most accepted process condition in terms of reducing production costs was 417.15 W, 43.03%, 11.24 min which resulted with TFC of 20.18 mg RE/g dry extract. Such TFC value presents decrease of only 16.5% but its production needs less power, ethanol consumption and extraction time for 30.4%, 13.5%, 25%, respectively. In advantages such as fast heating, shorter extraction time and lower solvent consumption, this technique is also classified as environmentally friendly so-called green methods.

Keywords: chokeberry, microwave-assisted extraction, flavonoid, response surface methodology, optimization

INTRODUCTION

Chokeberry (*Aronia melanocarpa*), a plant belonging to the Rosaceae family, is a bush that is being widely cultivated in Europe but originates in North America [1]. Chemical composition investigations of fresh fruit or products derived from these berries indicate the significant content of phenolic compounds related to their positive health effects such as: antioxidant, antiradical, antimutagenic and antiinflammatory activity [1–3]. This fruit also provide cardioprotective effects by reducing blood pressure, total cholesterol, LDL cholesterol and trigylceride level, exhibits antitumor activity as well as hepatoprotective and gastroprotective effect [1,4].

MAE presents efficient eco-friendly and so called “green” extraction technique of bioactive compounds, by increasing the yield and quality of extracts (avoiding chemical degradation and modification of bioactive compounds), along with reducing of extraction time, energy and solvent consumption [2,5,6]. High extraction efficiency of MAE has been confirmed along with enhanced contents of antioxidants in obtained extracts at many investigations [2,6–8]. Microwave power, solvent type and concentration and extraction time are the factors which individual and/or combined mostly influence the MAE extraction efficiency. RSM presents combined statistical and mathematical technique, which overcome impossibility of classical single variables optimization (“one-variable-at-a-time”) to analyse both individual and combined interaction of extraction factors (variables) and it is used to evaluate response function and these variables relationship as well as to estimate the optimal extraction conditions [3,9]. Three-dimensional graphic is designed as surface which presents extracted bioactive substance when plotted against different pairs of variables and maximum point on the surface defines optimal extraction [3,5,10,11].

The aim of present study was to evaluate the potential of using MAE as a useful eco-friendly method for extraction of flavonoid compounds from chokeberry, as well as to determine the individual and combined effects of extraction parameters (ethanol concentration, microwave power and extraction time) and propose the optimal and economically most acceptable extraction conditions which results in a maximum TFC.

MATERIALS AND METHODS

The plant material (frozen berries) originating in Poland was purchased at “Fungo-jug”, Leskovac, Serbia. Modified microwave oven (“SAMSUNG”, Type M1712N, Malaysia) [9] was used for MAE. Aqueous ethanol solutions (250 cm^3, 25, 50 and 75%) and grinded plant material (50 g) were subjected under different microwave power (300, 450 and 600 W) for 5, 10 and 15 min. Separation of liquid extracts from the exhausted plant material was realized under vacuum on the Büchner funnel through Whatman filter paper (No. 2). Liquid extract was evaporated to a dryness by rotary vacuum evaporator (800 rpm) at 40^oC.

Total flavonoid content (TFC) was determined by spectrophotometric method based on aluminium-chloride use [12].

Experimental design

Process parameter influence on TFC (depended parameter, Y) was designed using 3^3 full factorial experiment design. Range of independent parameters (variables): microwave power (300, 450, 600 W, X_1), ethanol concentration (25, 50, 75%, X_2), and extraction time (5, 10, 15 min, X_3) were defined by application of preliminary experiments data. Each of the experiments was performed in triplicate.

Response surface methodology

Influence of variables (process parameters, varied on tree levels) on TFC (depended parameter, Y) and determination of their optimal combination was performed by RSM.

Obtained mean values of determined TFC were used for the regression analysis. Experimental data analyze and prediction of the optimal conditions was accomplish using Design Expert software and fitted to a second-order polynomial model given in following equation:

$$Y = \beta_0 + \sum \beta_i X_i + \sum \beta_{ii} X_i^2 + \sum \beta_{ij} X_i X_j \qquad (1)$$

where: Y presents the predicted response (TFC mg RE/g dry extract), β_o, β_i, β_{ii}, and β_{ij} are regression coefficients for intercept, linear, quadratic and interaction terms, respectively and X_i and X_j are the actual levels of the independent variables.

Also, experimental design, regression coefficient calculations and graphical analysis of the experimental data (response surfaces and contour plots) were realized using the statistical software Design Expert (Trial version 7.0.0, STAT-EASE Inc.). Confirming the adequacy and statistical significance of the fitted model as well as regression coefficients and individual independent parameters and their interactions was performed using Analysis of variance (ANOVA).

RESULTS AND DISCUSSION

RSM modelling and optimization

Multiple non-linear regression analysis of experimental data obtained with 33 design experiments (TFC was in the range of 12.9 to 23.2 mg RE/g dry extract) was used for determination of a quadratic polynomial equation which describes the influence of independent variables (MAE process parameters) on TFC (dependent variable-response):

$$Y_{TFC} = 20.13 + 1.26X_2 + 2.05X_3 - 1.03X_1^2 \qquad (2)$$

The value of the coefficient of determination R^2=0.8821 indicates that the model is adequate and that 88.21% of the variation in the response values could be explained by the model. Value of R^2_{adj}=0.8045 is greater than 0.75 suggesting that the values of the fitted model are reliable and confirm the statistical significance of the model. The fit model is reliable and reproducible to optimize the MAE of the chokeberry in order to obtain maximal TFC, according to relatively small (less than 10%) coefficient of variation CV=5.73%.

Geometric interpretation of regression equation is the response surface graphically represented by a three-dimensional response surface plots and as a two-dimensional contour surface plots. Visual presentation of the relations between the response and each independent variable or the interaction of the two process parameters at the same time when the third parameter is fixed was derived. In this way determination of optimum values of independently variable, on which the dependent variable will have a maximum response is possible [13]. The influence of extraction parameters (concentration of ethanol, microwave power and extraction time) on TFC in chokeberry extracts is shown in Figure 1. It could be concluded that regardless of the microwave power and extraction time, when the ethanol concentration increases from 25% to 50%, the TFC value increases and reaches the maximum using 50%. This is also confirmed by the statistically significant (F=10.43, *p*=0.0040) quadratic factor of ethanol concentration (X_1^2). However, the coefficient of this polynomial member has a negative sign, which means that after reached its maximal value the TFC decreases.

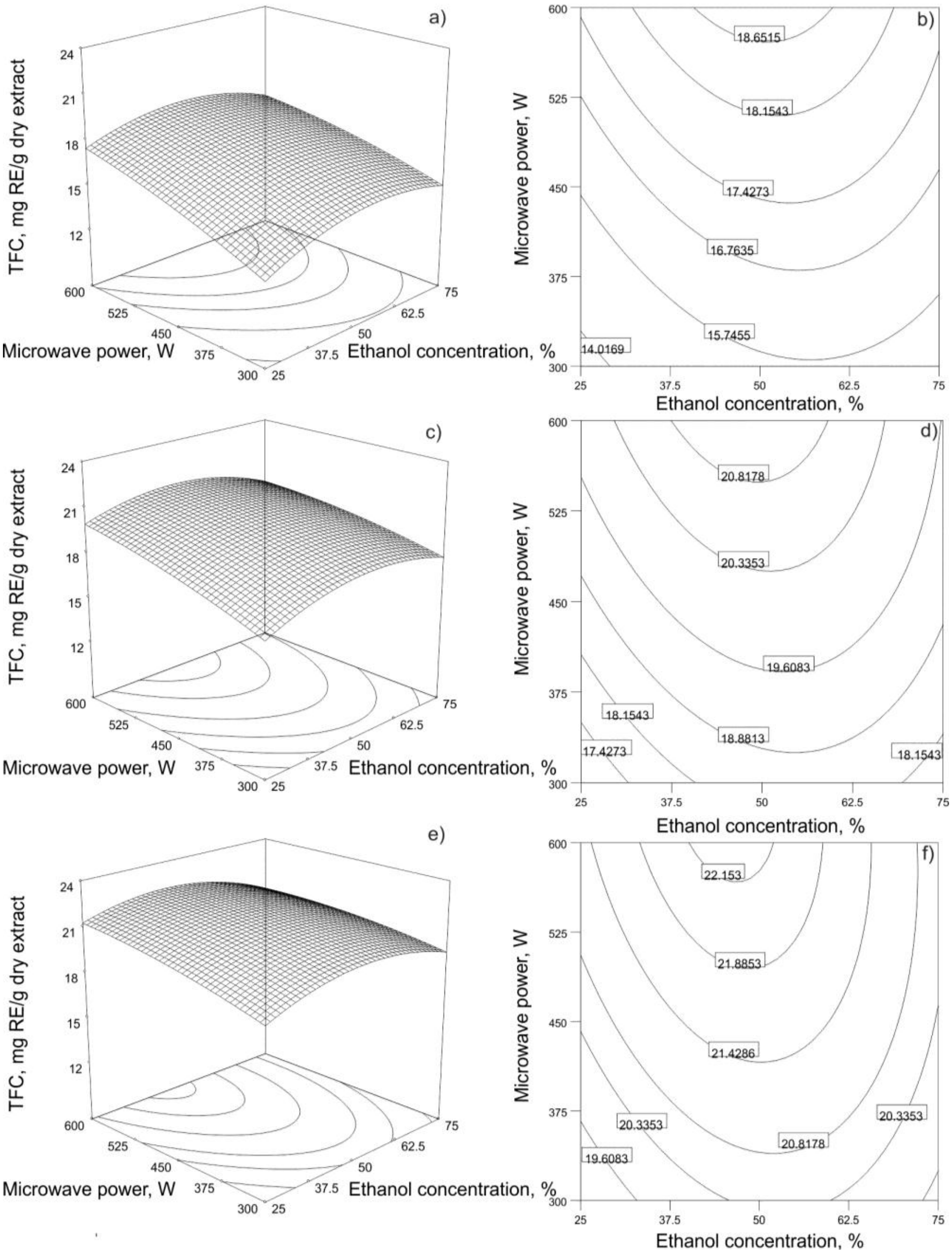

Figure 1 *3D response surface plots and two-dimensional plots for TFC as a function of microwave power, ethanol concentration and extraction time of 5 min (a, d), 10 min (b, e) and 15 min (c, f)*

Extraction time (X_3) is the most influential factor on the TFC (F=64.40 and *p*<0.0001), which agrees with the value of the coefficient for X_3=2.05 in the polynomial regression equation. The increases in the microwave power and extraction time values positively influence the TFC (most pronounced at moderate concentrations of ethanol-50%), so it reaches a maximum value at 600 W and 15 min, independently of the third factor. According to the proposed model the maximum TFC of 22.09 mg RE/g dry extract could be obtained at the microwave power at 525 W, the ethanol concentration at 62.5% for 15 min. Experimental design matrix and results are presented in Table 1.

***Table 1** Experimental design matrix and results*

Run	Design matrix						Response	
	Coded factors			Uncoded factors			TFC mg RE/g dry extract	
	Factor A (X_1)	Factor B (X_2)	Factor C (X_3)	Ethanol concentration, % (X_1)	Microwave power, W (X_2)	Time, min (X_3)	Experimental	Predicted
1	0	0	0	50	450	10	20.25	20.13
2	0	-1	0	50	300	10	18.53	18.87
3	0	0	-1	50	450	5	18.01	18.08
4	1	0	0	75	450	10	19.94	18.83
5	-1	-1	-1	25	300	5	12.85	15.52
6	0	1	1	50	600	15	23.25	23.24
7	-1	0	1	25	450	15	20.11	20.88
8	1	-1	1	75	300	15	19.56	19.62
9	0	0	0	50	450	10	17.67	20.13
10	1	1	-1	75	600	5	15.61	18.04
11	-1	1	0	25	600	10	18.67	20.09
12	0	0	0	50	450	10	19.78	20.13
13	0	-1	1	50	300	15	19.12	20.92
14	1	1	0	75	600	10	19.81	20.09
15	-1	0	-1	25	450	5	15.67	16.78
16	-1	-1	0	25	300	10	17.89	17.57
17	1	0	1	75	450	15	20.31	20.88
18	-1	1	1	25	600	15	21.09	22.14
19	0	0	0	50	450	10	20.09	20.13
20	0	1	-1	50	600	5	19.75	19.34
21	0	0	0	50	450	10	20.57	20.13
22	1	-1	-1	75	300	5	15.37	15.52
23	1	-1	0	75	300	10	17.50	17.57
24	1	1	1	75	600	15	20.17	22.14
25	-1	1	-1	25	600	5	18.30	18.04
26	1	0	-1	75	450	5	17.50	16.78
27	0	-1	-1	50	300	5	14.98	16.82
28	0	0	1	50	450	15	21.46	22.18
29	0	0	0	50	450	10	22.03	20.13
30	-1	-1	1	25	300	15	19.92	19.62
31	0	1	0	50	600	10	21.84	21.39
32	0	0	0	50	450	10	19.38	20.13
33	-1	0	0	25	450	10	18.17	18.83

According to the proposed model the maximum TFC of 22.09 mg RE/g dry extract (which is slightly lower than the maximum experimentally obtained) could be obtained at: 525 W, 62.5%, 15 min. Modified criteria which require optimization of the extraction process based on the cost-effectiveness and less environmental impact was performed. New optimal economic conditions reduce the extraction time, energy and solvent consumption providing a relatively high level of TFC under 43.03% ethanol concentration, 417.5 W microwave power and extraction time of 11.24 min. Decreasing of TFC for just 8.6%, the extraction could be realized with 30.4% less power, approximately 13.5% less ethanol consumption and 25% faster compared to experimental conditions need to obtain the maximum TFC.

CONCLUSION

Using a multiple nonlinear regression analysis of experimental data obtained by 3^3 factorial design, a quadratic polynomial equation was created describing the influence of independently variable on the TFC. The proposed model is statistically significant with the values of statistical parameters that indicate its adequacy and relatively good fit with the experimental data. Extraction time represents the factor with the highest significance for the analyzed response. In order to rationalize and reduce the costs of the chokeberry MAE process, simultaneous multicriterial numerical optimization was performed, and two sets of conditions were proposed: optimal (50.84%, 556.5 W, 15 min) and economically most acceptable (43.03%, 417.15 W, 11.24 min). This would provide a reduction in the cost of the final product (extract), which still coincides with the relatively high amount of targeted bioactive compounds along with the less impact on environment (by reducing the consumption of energy and solvents).

ACKNOWLEDGEMENT

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LASER CLEANING OF CORROSION, EFFICIENT AND ENVIRONMENTALLY FRIENDLY METHOD

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Abstract

In recent years, lasers have been used more and more in different areas of human life, science, industry, medicine, military, agriculture, and show business, protection of environmental and cultural-heritage objects, in the arts and so on. Laser technology has some advantages in many areas over classical methods. This paper presents the results of laser application for cleaning corrosion of metal objects, cooper and brass. The first sample was oxidised cooper plate and the second ashtray made of brass. Nd: YAG laser was used as a source of radiation to clean surface corrosion deposits. Removal of layers from the surface occurs through the process of laser ablation. Three wavelengths were used with different fluences. The cleaning results, i.e. the morphological and chemical changes were investigated by optical microscopy, XRF analysis and colorimetry. The obtained results show that lasers are efficient corrosion cleaning and environmentally friendly method.

Keywords: laser cleaning, metal, corrosion, Nd:YAG laser, cultural heritage

INTRODUCTION

Corrosion is a problem for all metallic objects. Corrosion is the destruction of metals and alloys due to chemical or electrochemical reaction with the surrounding environment. Electrochemical corrosion is a consequence of electrochemical reactions and is subject to the laws of electrochemical kinetics. The important condition for its appearance is the contact of the metal with the second phase, which has the properties of electrolytes, whereby the metal creates a thin layer with the properties of the electrochemical double layer. This includes all cases of corrosion in a humid atmosphere, as well as corrosion of metals in electrolyte solutions [1].

Pollution of the environment is a major problem facing the modern world that leads to the destruction of eco system, the achievements of civilization and cultural heritage. Interest in the use of laser technologies in this area is caused by the catastrophic degradation of the monuments in recent decades, which is the consequence of the increasing effect of anthropogenic factors. The distressing state of many architectural monuments, sculptures, historical buildings, and museum artefacts and the acceleration of their breakdown processes request the use of new and effective technologies for restoring and preserving such objects. The study and conservation of museum collections request the application of scientific methodology in the examination, analysis and dating of objects. In all these methods, lasers play an important role [1].

The conventional techniques of metal surface cleaning are based on mechanical or chemical methods which can lead to the substrate being damaged or polluted. There is numerous literatures [1–11] that describes the classic and laser methods and problems accompanying their implementation in cleaning and protection of metals. It can be conclude that lasers can to change some classical, pollutant method with ecological friendly ones in different applications. Lasers cannot prevent pollution or reduce the concentration of harmful substances, but can detect pollutants and determine in which amount are present.

Laser cleaning is of interest due to its great potential for removing contamination or films from different substrates [3]. Lasers have many advantages over conventional techniques. They can be used for contactless and fast surface cleaning and processing (drilling, marking, grooving, cutting and welding) with precisely controlling energy deposition in the material.

Precisely controlled laser cleaning features a unique feature: removes impurities and corrosive products from the surface without damaging the base surface. Laser systems are very efficient, economical, and environmentally friendly compared to existing conventional methods. They do not require chemicals and abrasive materials, or storage for waste disposal. Laser, mobile systems, equipped with different types of lasers can be impulsive or continuous, with high mean power. These can be equipped with an optical cable up to 50 m long, making them suitable for applications such as historical monuments, high voltage pylon cleaning, metal contaminated parts of nuclear plants or facades of large cultural heritage sites. Laser cleaning is applicable in the automotive industry. Lasers can be used for welding, removal of oils and lubricants, cleaning of electronic components and cleaning of tools. Lasers can also be used in the food industry. In the airline industry, laser cleaning is most used in the removal of colours and other coatings from the surface of the aircraft. Lasers can also be used effectively in the selective sintering of different materials.

Many studies [4,5,11] confirm that Nd:YAG lasers (l = 1.06 mm), with ns pulses, are highly effective in removing inorganic encrustations from artifacts; however, the absorption of laser radiation by bulk material can raise the surface temperature and can cause some changes. Because of that, every investigation of Nd:YAG laser ablation is of great interest for laser application in the cleaning process.

MATERIALS AND METHODS

In this paper are presented the results of cooper and brass laser cleaning. The first sample was oxidised cooper plate and the second ashtray made of brass [11].

All experiments were performed in laboratory and normal atmospheric conditions. Nd:YAG laser, Thunder Art Laser, produced by Quanta System (with wavelengths $\lambda = 1064$ or $\lambda = 532$ nm, optical pulse duration < 8 ns, and output pulse energy up to 1000 mJ) was used in the presented experiments. The repetition rate is up to 20 Hz, with a beam diameter of 10 mm and 70 % fit to Gaussian energy distribution. The lens with focal length of 150 mm is used to focus laser beam.

Copper is a metal that has been used for millennia for a wide variety of purposes, and today it is irreplaceable in electronics, computer industry, energy, construction and medicine. Numerous objects of cultural heritage are made of copper or its alloys, bronze and brass. Laser cleaning of copper surfaces is very important with several aspects [1,7].

The experimental parameters are provided in Table 1. The laser cleaned objects are presented in Figure 1. The dimensions of corroded cooper and brass plates are 50x30x1 mm and 60x30x2 mm, consequently. The relief ashtray belongs to a private collection, dated to the beginning of the twentieth century.

The results of laser beam-material of the samples were recorded with the optical digital USB microscope with different magnification. Chemical composition was determined by XRF analysis and the colour changes by colorimeter [11].

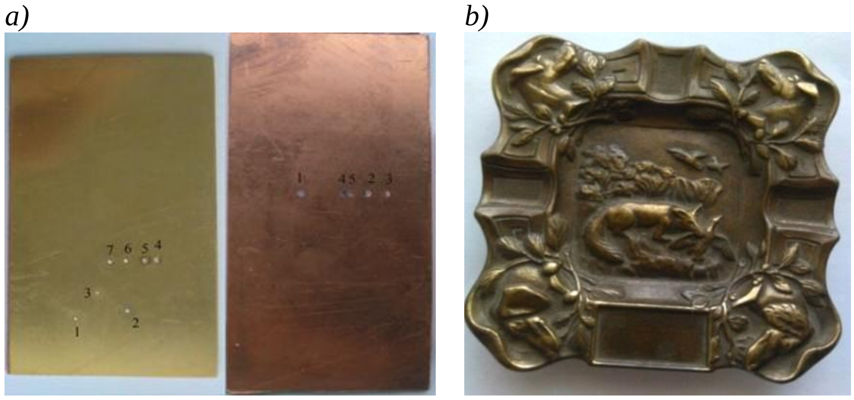


***Figure 1** a) Oxided cooper and brass plates with laser cleaned zones, b) ashtray*

***Table 1** The experimental conditions during laser cleaning of some zones on cooper and brass plates and ashtray*

Oxide cooper plate					Ashtray				
Zone number	f, Hz	t, s	E (mJ)	λ, nm	Zone number	λ, nm	E, mJ	f, Hz	t, s
		cooper			1	1064	250	20	5
1	20	5.5	500	1064	2	1064	350	20	5
3	20	4	500	1064	3	1064	450	20	5
5	20	5	200	532	4	1064	600	20	5
		brass			5	1064	750		5
2	10	3	500	1064	6	532	375	20	5
5	20	5	500	1064	7	532	375	20	240
7	20	4	200	532	9	1064	600	20	240
					10	355	250	20	240
					11	Mechanically cleaned			

RESULTS AND DISCUSSION

Surface cleaning, based on laser ablation, is a delicate and irreversible process, followed by many potential complications. It is very important to choose the most suitable laser cleaning methodology and laser parameters in accordance with the material properties. Laser ablation occurs when the laser fluence (pulse energy per unit area) overcomes a critical threshold, which is an intrinsic property of the material structures under irradiation.

The application of laser in cleaning the corrosion layers on metallic threads is a complex phenomenon, depending on characteristics of laser lights and materials. It is based on several processes: absorption of laser energy within a very short period of time (several ns), melting of material in the heating layer depending on the applied energy, and ablation or evaporation of material. Depending on the fluence and quantity of absorbed energy, mechanical, expansion waves can be formed, consisting of evaporated material and ambient gas, which also rips off parts of the surface layers and discards them from the irradiated zone. The strength of the expansion wave increases if the sample is damp, and thereby creates conditions for more efficient cleaning of corrosion products. A laser re-melting process is the fundamental process, which occurs in parallel with the cooling of the melted layer.

Microscopic tests, conducted by optical microscopy, allow the study of the threshold of ablation fluence and efficiency of cleaning process. Figure 2 shows some zones on cooper and brass plates cleaning with parameters presented in Table 1. The analysis of images show that the corrosion is removed, but some colour changes occur in and around irradiated zones (in HAZ). It can conclude that laser radiation (1064 nm, 8 ns) at fluence levels above 0.6 J/cm^2 causes some melting to copper and brass. Brass appeared to be the most sensitive of the metals tested.

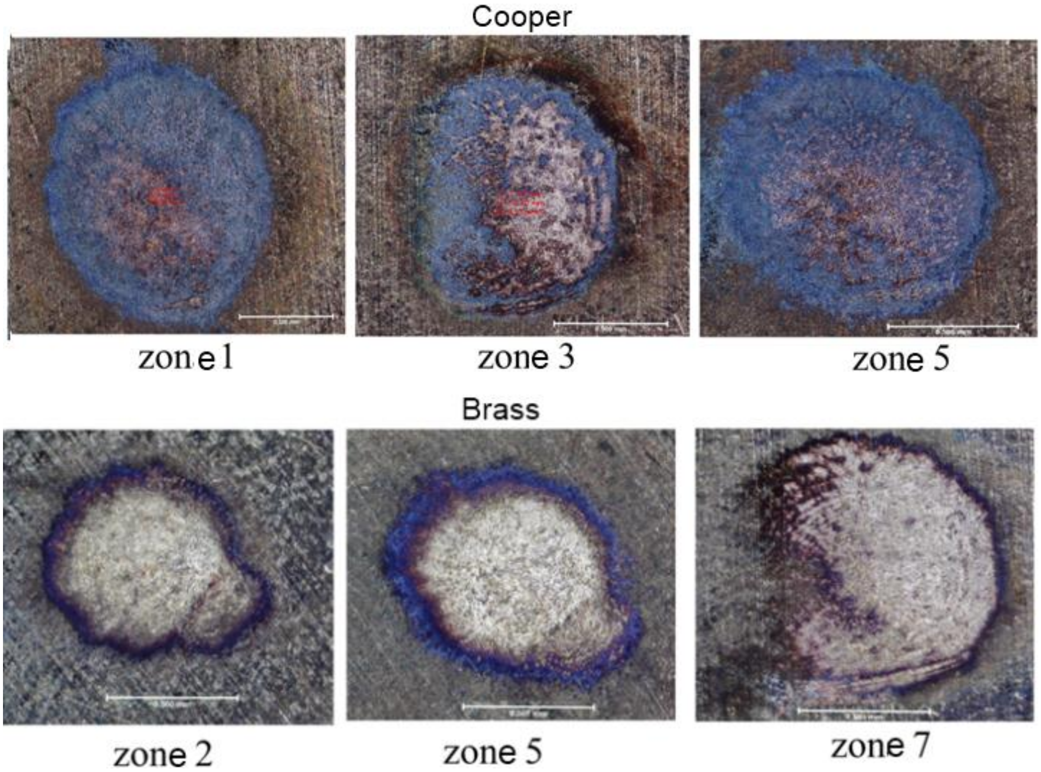


***Figure 2** OM of laser cleaning zones on brass and copper plate*

Figure 3 presents the appearance of the ashtray after partial laser cleaning (rear side) with laser treated zones. Experimental conditions during laser cleaning are given in Table 1. In these studies, the laser parameters have been selected very carefully and they are below the material damage threshold.

Chemical composition determined by XRF analysis on laser treated and untreated zones, (table 2) points to the fact that there is no coating, there is a thin layer on the surface of the sample that is corroded and which protects the surface against further corrosion. Since the chemical analysis is carried out by XRF method, which cannot detect light elements, it is very likely that on the surface of the ashtray, besides the oxide layer, there is a thin layer of organic materials, which during the laser irradiation leaves dark clues.

***Table 2** XRF analysis of ashtray [11]*

Zone	Concentration % $\cdot 10^{-3}$													
Elements	Sb	Sn	Ag	Mo	Nb	Pb	Zn	Cu	Ni	Fe	Mn	Cr	V	Ti
Cleaned	17	845	159	5	6	2564	35488	59043	755	580	150	140	159	46
Uncleaned	17	870	159	5	7	2562	33383	59089	754	599	140	164	170	44

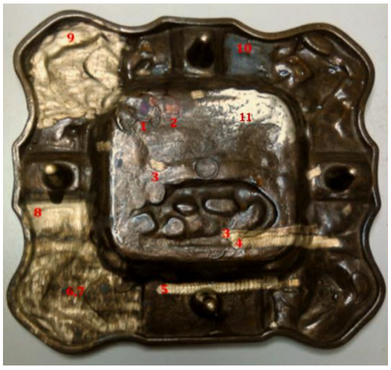
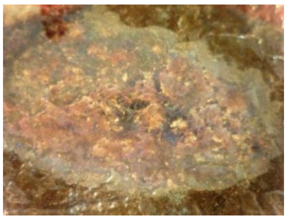


***Figure 3** Laser cleaning of ashtray rear side*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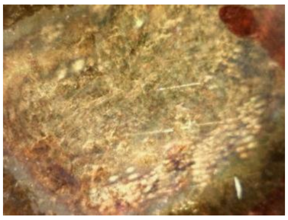
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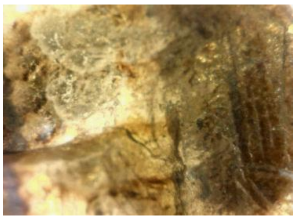
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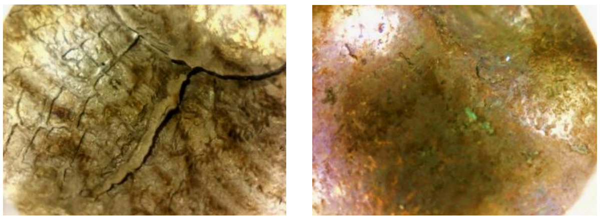

Figure 4 *OM analysis of laser cleaned zones*

The images in Figure 4 are made with a USB optical microscope, with a magnification of 40x. An analysis of the images confirmed the inhomogeneity of surfaces that were cleaned by the laser. Around the zone 1-3 appears dark ring, while in the zone cleaned by the scanning laser beam with constant velocity, periodic segments of curved shape are formed.

The results of colorimetric tests for uncleaned surface and for zone 9 are presented in Figure 5. The analysis of the surface colour was based on the L∗a∗b parameters.

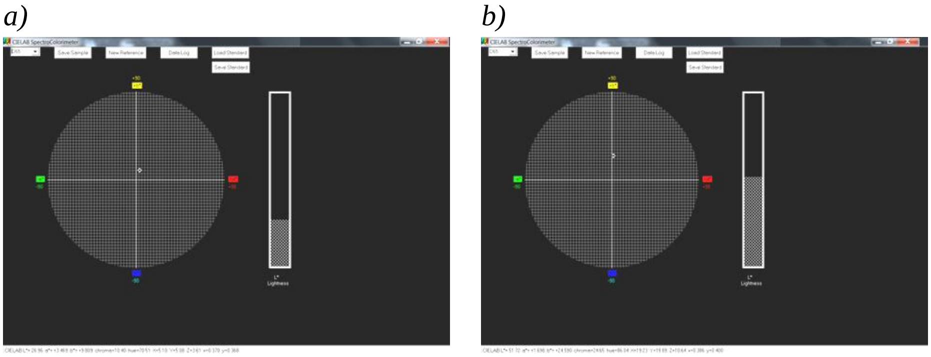

Figure 5 *Colorimetry of: a) uncleaned zone, b) zone 9*

CONCLUSION

From the results of the conducted tests, it can be concluded that the use of a Q-switched Nd:YAG laser at 1064 nm and 532 nm is suitable method of cleaning thin corroded layer on copper and brass objects. According the obtained results the ablation threshold fluence for oxidized layers from cupper surface is estimated below 0.5 mJ/cm^2. Localised surface melting was found to occur at this fluence. The ablated layers evaporate and it is only one by-product of cleaning. No other pollutant in laser ablation. Because of that, laser technology is environmentally friendly and is becoming more and more applicable.

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RECYCLING VALUABLE AND HAZARDOUS METALS FROM WEEE AND GREEN TECHNOLOGIES

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Abstract

Waste electrical and electronic equipment (WEEE) or electronic waste (e-waste) is one of the fastest growing waste streams in the urban environment worldwide. A concentration of metals in discarded E-waste, or materials intended for recycling, is much higher than the minimal concentration of these metals in profitable ores. Conventional approaches to the exploitation and extraction of metals from the ore and recovery of metals from e-waste, are rapid and efficient, but cause secondary pollution and economically unviable. The present research suggests that involving microbiological organism in leaching of metals presents innovative methods of extraction which is eco-friendly manner. The application of the integrated modified technological processes combined with bioleaching technique that involving microbiological leaching of metals both from ore, and from e-waste, optimization of certain factors such as microbial species, (Chromobacterium violaceum, Pseudomonads spp., and mixed culture) pH, temperature, nutrients, and aeration rate affect the bioleaching process and can lead to profitable recovery of metals. The development of metabolic engineering in process mining and recycling of WEEE are very important from the aspect of efficient, economical and environmentally friendly manner exploitation and recovering valuable metals from WEEE.

Keywords: modern mining, e-waste, integrated process, bioleaching, environmental

INTRODUCTION

Very rapid technological development creates the need for constant upgrading of information and technical systems in order to achieve the appropriate technology requirements, while outdated devices are being discarded [1,2]. The main component of e-waste is printed circuit boards (PCBs), which contain substantial quantities of precious metals in concentrations significantly higher than those typically found in corresponding ores [3]. Urban mining is related to e-waste recycling and developing a 21st century, high-quality recovery process for the valuable materials in electronic waste is very important [4]. Management of electronic waste is one of the major problems of today. Printed circuit boards are found in almost all electronic devices, and consist mainly of polymers, ceramics and metals. At such a small place there are 40 and more different metals, which make up only 3-6% of the total weight of electronic waste [5,6]. Recycling is a very important segment in the hierarchical approach to waste management and can contribute to many benefits: recovery of metal from rejected electrical and electronic devices, which reduces the amount of waste destined for landfilling, removes heavy metals from the environment, preserves natural resources, reduces emissions of gases that produce an effect greenhouse gases and pollutants that degrade the environment, open new jobs and, finally, but no less important, realizes economic gain. Conventional pyrometallurgical and hydrometallurgical processes for recycling and processing of WEEE, in particular PCBs of electronic devices, cause discomfort to human health and the environment due to: various hazardous combustion products [7], harmful chemical reagents, large quantities of by-products [8]. The processes of recycling require major investment in highly efficient and environmentally friendly technologies [9]. It has been observed that the presence of various microorganisms/fungi in the natural environment catalyzes the processes of degradation of various metals and metalloids. This ability of microorganisms has contributed to the development of a new approach to recovering valuable and precious metals from WEEE, as well as dangerous and harmful ones, whose presence in the environment would contribute to further degradation and pollution [10]. The bioleaching process is now emerging as commercial exploitable technology applicable for metal extraction from electronic waste and low grade ores. The wide range of waste treated by bioleaching is a clear indication of the ease of application of the process [11]. Bioleaching process is offer many benefits over the traditional metallurgical techniques including its relative simplicity, lower energy requirements and operational cost, reduced need for skilled labor, highly efficient environmental detoxification, lesser secondary pollution, and is eco-friendly [12,13]. Bioleaching process involves conversion of metallic compounds into their water-soluble states by employing diverse range of microorganisms, mainly chemolithoautotrophs, which uses CO as carbon source and inorganic compounds (Fe^{2+}, reduced S) as an energy source [14]. Microorganisms can catalyses bioleaching through oxidative and reductive dissolution, mediated by the oxidation and reduction of ferrous and ferric iron, respectively. Moreover, bioleaching can be achieved through the production of biogenic acids, alkali and ligands, such as cyanide, thiosulfate, organic acids and iodide [10]. Bacteria such as *Thiobacillus ferrooxidans* and *T. thiooxidans* and fungi such as *Aspergillus niger* and *Penicillium simplicissimum* are able to mobilize high percentages of Cu, Sn, Al, Ni, Pb and Zn from WEEE [15]. *Ch. violaceum* may solubilize Au and Cu as dicyanoaurate and dicyanocuprate ($[Au(CN)_2]$ and $[Cu(CN)]$), respectively [16]. *Ch. violaceum* is a Gram-negative bacterium (facultative anaerobe), that is considered to be nonpathogenic for humans and found in tropical and subtropical areas of several continents. However, in some cases it can act as an opportunist pathogen for animals and humans and cause fatal septicemia. Many reviews were published related to this pathogenesis, biological activities and its possibilities in many applications [17,18]. In any case, by applied biotechnological approaches of recycling could not only reduce the toxicity of various waste streams, such as mining wastes, e-wastes and even municipal waste sludge, already and also unearth the potential of those wastes as secondary raw materials for precious metal recovery [19–21]. Also, treating of the sludge, which is residue after the extraction of gold and silver, using the microorganisms of the genus *A. thiooxidans*, can be achieved mobilization some rare metals, such as: Ce, Eu and Nd at high percentages (>99%), whereas for La and Y can be reached extraction yield of 80% [22].

BIOLEACHING METALS FROM E-WASTE

Bioleaching is widely used in mineral processing. It is an environment-friendly and low-cost method [23]. Biotechnological approaches to extraction and recover metals are based on the application of microbial catalyzed processes for the extraction of metals from different raw materials. Bioleaching has also some potential for metal recovery and detoxification of industrial waste products, sewage sludge and soil contaminated with heavy metals. Biotechnological approaches cover all cutting-edge areas of biohydrometallurgy, which include bioleaching, bioprecipitation, bioflotation, bioflocculation, biooxidation, biosorption, bioreduction, bioaccumulation and the application of biosensors in analytics [24]. The first studies on microbiological leaching of metals from electronic waste they were conducted authors who noticed toxic influence of waste on microorganisms. In an external environment the bioleaching process of metals was slowed down. In nature, there are many plants, bacteria, algae and fungi which are able to produce cyanide. In spite of cyanide toxicity, there are organisms able to survive in its presence and some of them are able to use it as a nitrogen source [25]. Gradual adaptation of bacteria to the environment conditioned the introduction of the bioleaching process under controlled conditions. Interactions of bacteria with solid matrix or substrate and development of biofilm are of utmost importance in critically analyzing the bioleaching process [26].

MATERIALS AND METHODS

From electronic waste, specially are isolated parts of printed circuit boards, rich in precious metals. (GFMPCs, contacts, composites, PCI slots, IDE, connectors, processors, chips) and were used for hydrometallurgical treatment. The rest parts of the printed circuit boards are crushed and treated in bioreactors by the action of microorganisms. The residual sludge from hydrometallurgical process was treated in separately in a special bioreactor. SEM-EDS were used for the analysis of separated, melted parts of computers and mobile phones. For characterization and control quality of residual sludge from hydrometallurgical treatment of electronic waste was used X-ray diffraction (XRD). Prior to the analysis, the samples of the sludge were washed with distilled water, filtered through filter paper, and dried in a drying oven at 105 °C for 2h. The microbiological cultures which were used in the process of bioleaching processes (*Acidithiobacillus ferrooxidans, Chromobacterium violaceum, Pseudomonas aeruginosa* and *Pseudomonas fluorescents*) are been grown in laboratory, in Petri's cups and in Erlenmeyer flasks on appropriate substrates/mediums according to the instructions of producer and in appropriate conditions.

RESULTS AND DISCUSSION

Procedure for the extraction of valuable metals from electronic waste and efficiency of the bioleaching process

The process of metal extracting is more take place under controlled conditions (mixing, aeration, the appropriate temperature and occasional addition of solvents) in a closed device with digester, neutralizer and waste gas treatment, shown in Figure 1. In the same device, it's possible to use various solvents such as aqua regia, thiosulphate, thiourea or nitric acid (in this process, as a solvent was used diluted nitric acid).

The achieved results of bioleaching metals from PCBs computers and mobile phones, as well as residual sludge from hydrometallurgical treatment are shown in Table 1.

A. ferrooxidans strain was used to remove any residual copper after electromagnetic separation. When copper is present in the sample, it prevents the effective chelating of Au from the sample, therefore it is necessary to remove it prior to the bioleaching of gold. For the efficient bioleaching of Cu from crushed samples of PCBs size 0.5-1mm, a concentration of Fe^{3+} ions of 6.8 g/dm^3 was needed, which was achieved, for 85 h with a Cu degree of bioleaching rate 97.7% and 83.1% for Zn. The degree of bioleaching of Cu and Zn from residual sludge the hydro-metallurgical process was realized with an efficiency of 78.2% and 54.5% respectively.

With *Ch. violaceum*, the efficiency of Cu, Au, Zn, and Ag from PCBs is achieved (from shredded, and separated samples) of 79.5%, 61.9%, 45.2% and 5.25% respectively, while 43.3%, 21.4% 25.3% and 3.9%, respectively were allocated from the residual sludge from hydrometallurgical treatment under the same conditions. The parameters and conditions for bioleaching were given in Table 4. The dried inert biomass of the nutrient agar at 35-37 °C proved to be the most effective substrate for the growth of *P.aeruginosa* and selective separation: in addition to 76.1% Cu, 45.8% Zn, 58.3% Au and 4.5% Ag, then, from the sludge of hydrometallurgical treatment 39.8% Cu, 20.1% Zn, 21.1% Au and 2.2% Ag, was achieved bioleaching Gd, Eu, La, Yb (from speakers and microphones) with efficiencies of 87%, 20%, 15%, 15% respectively, which is of special importance from the aspect of bioleaching rare metals from WEEE. Gold bioleaching from hydrometallurgical sludge has been achieved, of 21.1%, which at first glance seems to be low, but if it is known that the percentage of gold remaining the 7.5 ppm per kg of sludge (heterogeneous composition - small % share of a large number of metals), then this percentage is promising. The approximate bioleaching of 86% Gd, 18% Eu, 13% La and 12% Yb was also shown on the sample from the hydrometallurgical precipitates, which can be explained by the affinity of the *P. aeruginosa* metabolite according to these rare metals, especially according to Gd, to build cheated complexes and the production of organic acids as byproducts that facilitate bioleaching.

In all variants, the flushing of silver was very low. The most important bioleaching of gold and copper was accomplished by the mixed cultures of *Ch. violaceum* and *P. aeruginosa*, followed by *Ch. violaceum*, as well as with the mixture *Ch. violaceum* and *P. fluorescents*. The affinity of the bacteria used for bioleaching, according to metals from the hydrometallurgical sludge, was similar, but with considerably lower level of leaching (Table 1), which could be explained by the fact that the presence of different metals in the precipitate at the same time is inhibitory to the process bioleaching.

Table 1 *The bioleaching of some metals from PCBs parts of computers and mobile phones and from residual hydrometallurgical sludge with some mesophilic microorganisms in controlled condition*

Microorganism	**Leaching mechanism**	**Pulp density of PCBs waste (w/v)**	**Particle size of PCBs (µm)**	**Temperature of bioleaching**	**pH**	**Aeration rate (rpm)**	**Bioleaching metals from PCBs parts (%)**				**Bioleaching metals from residual hydrometallurgical sludge (%)**			
							Cu	**Zn**	**Au**	**Ag**	**Cu**	**Zn**	**Au**	**Ag**
Acidithiobacillus ferrooxidan (A.f.)	Acidolysis (SO_4^{2-}), redoxolysis (Fe^{3+})	0.1	500	Mesophilic (25-37 °C)	2.5	150	97.7	83.1			78.2	54.2		
Chromobacterium violaceum (C.v.)	Complexolysis (CN^-)	0.5	350-500	Mesophilic (25-37 °C)	7.0-9.6	150	79.5	45.2	61.9	5.25	43.3	21.4	25.3	3.9
Pseudomonas fluorescens (P.f)	Complexolysis (CN^-)	0.5	350-500	Mesophilic (25-37 °C)	7.0-7.8	150	16.7	42.5	25.2	4.2	15.3	24.8	14.9	2.5
C.v. and P.a	Complexolysis (CN^-)	0.5	350-500	Mesophilic (25-37 °C)	7.2-9.2	150	80.5	46.1	71.9	5.5	41.7	24.4	35.6	2.7
C.v. and P.f.	Complexolysis (CN^-)	0.5	350-500	Mesophilic (25-37 °C)	7.2-9.2	150	75.3	42.7	61.4	4.5	33.1	19.7	29.8	2.6
P.a. and P.f.	Complexolysis (CN^-)	0.5	350-500	Mesophilic (25-37 °C)	7.0-9.3	150	52.9	15.7	42.3	4.9	29.7	22.4	17.6	2.5
Pseudomonas aeruginosa (P.a.)	Complexolysis (CN^-)	0.01	150-180	Mesophilic (25-37 °C)	5.6-6.5	150	76.1	45.8	58.3	4.5	39.8	20.1	21.1	2.2
							Gd	Eu	La	Yb	Gd	Eu	La	Yb
							87	20	15	15	86	18	13	12

In Figure 1, is presented, innovative, recycling process whose provides an environmentally friendly recycling and the recovery of valuable, precious metals with the application a hybridized modified technological process and bio-extraction of the remaining metals from residual, precipitated sludge and shredded, solders metal parts of the printed circuit boards of computers and mobile phones. The process of bioleaching in the bioreactor, takes place under controlled conditions: adequate pH, temperature, nutrients, aeration.

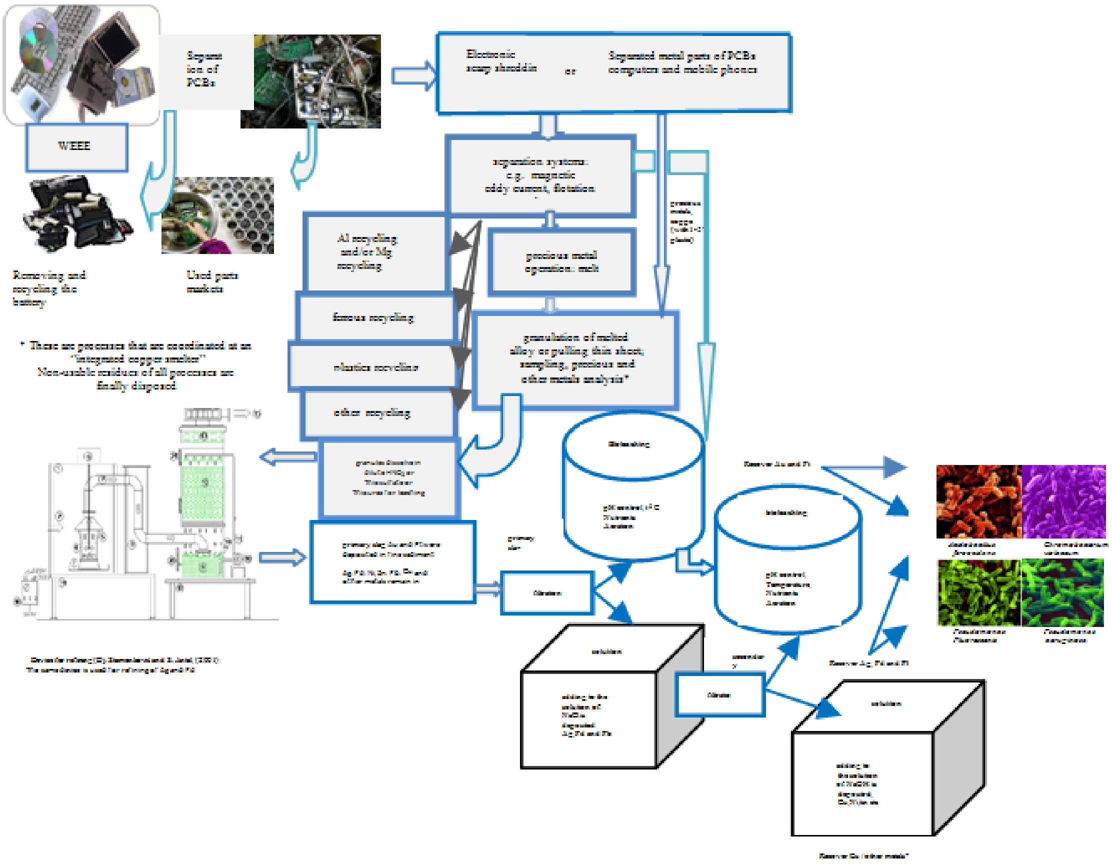

Figure 1 The integrated modified technological processes recycling metals from electronically waste combined with bioleaching technique that involving microbiological leaching

CONCLUSION

For the purpose of more efficient recovering of metal from electronic waste and environmental protection, the proposed process of recycling includes bioleaching processes in a bioreactor; by treating the residual sludge from the hydro-leaching process, as well as parts by crushed PCBs of computers and mobile phones.

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INFLUENCE OF MODIFICATION OF ACTIVE CARBON MATERIAL SURFACE ON ITS ANTIMICROBIAL PROPERTIES

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Abstract

Active carbon materials were obtained by hydrothermal carbonization process. Fructose and plane tree seeds were used as raw materials for the production of active carbon material. Diluted phosphoric acid was used as an activating agent. Sodium hydroxide was used for modification of active carbon material surface. The obtained active carbon materials were analyzed in detail, by determining: the specific surface area according to the Brunauer Emmett Teller (BET) method, micro and meso, as well as the total area according to the αs method, the volume of micro pores according both to the Gurwitch rule, as well as with αs method, the size of the micro and meso pores according to Horvath-Kawazoe (H-K) method and Barrett, Joyner, and Halenda (BJH) method. The complete calculation was carried out on the experimentally obtained adsorption / desorption isotherms of N_2 *at 77 K on these materials. A standard agar dilution test has been used for the in vitro testing antimicrobial activity of the obtained materials. The anti-microbial effect of the material before and after modification was examined on the microbes that are located on the surface of the skin. It has been noted that the modification of the material can improve its antimicrobial effect.*

Keywords: active carbon material, adsorption characteristics, surface modification, agar test, antimicrobial activity

INTRODUCTION

Carbon materials have always played important roles for human beings; for example, charcoals as a heat source and adsorbent since prehistoric times, flaky natural graphite powder as pencil lead and soot in black ink in the development of communication techniques, etc., etc. They are widely used from the home to the industrial setting. The earliest documented carbon material is the bamboo-char filament made by Thomas Edison for use in incandescent light bulb in 1880. After a successful explanation of the graphite structure (Bernal, 1924), it became clear that there are chemical bonds in the graphite which, by their strength, differ drastically between one another - one among the strongest, and the other among the weakest in nature. This indicated the possibility of obtaining different carbon materials. With time, carbon materials have repeatedly proven to be an inexhaustible source for scientific research and technological development [1–6].

Classification of carbon materials can be done on various different bases; for example, the chemical nature of the carbon-carbon bonds (structures), the production procedure, properties, structural change at high temperatures, nanotexture, and time of appearance etc. Structurally, they can be divided into diamond, graphite, pyrocarbon, glass-like carbons, fullerenes, carbynes, etc., and by production procedure into artificial graphite, intercalation compounds, activated carbons, carbon/carbon composites, etc. [1–6]. One of the most important, most famous and most used types of carbon materials are definitely active carbon materials. Prior to the use of what we call today activated carbon, either wood char, or coal char or simply a partially devolatilized carbonaceous material was employed as an adsorbent since ancient times. Powder and granular activated carbons are typical carbon adsorbents. These are non-graphitic, non-graphitizable carbons with a highly disordered microstructure. Other forms of carbon materials are also used as adsorbents such as activated carbon fibers, fabrics and felts [5–7].

Carbon surfaces can form chemical or physical bonds with a wider spectrum of substances than any other material. Even very small contents of heteroatoms can significantly influence on the chemical properties of active carbon material surface. Oxygen is invariably the most common heteroatom found on carbon material surface. Both acidic and basic functional groups can exist on the activated carbon surface indicating the amphoteric nature of these materials. The presence of various functional groups on the active carbon material surface depends on the raw material nature and on the activation technique employed in the manufacturing process. Furthermore some post treatments of a material can modify the surface chemistry, resulting in new useful properties [3–7].

Considering the energy crisis, and the environmental protection, hydrothermal carbonization, denoted from here on as HTC, becomes very popular process for carbon material production. It is facile, low-cost, environmentally friendly, and nontoxic routes to produce novel valuable carbon materials. In HTC, carbohydrates and raw lignocellulosic biomasses are converted into valuable carbon materials using mostly water as a carbonization medium at mild temperatures under self-generated pressures [8–14].

Medical applications of activated carbons are based on their powerful adsorption properties. They can remove even harmful toxins from the human body. Due to the bactericidal and bacteriostatic activity of silver ion, it is commonly usually used as an antimicrobial agent in the form of nitrate, acetate or organic compound silver-sulfadiazine [15–18].

In this study fructose and plane tree seed were used as cheap and suitable raw material for preparation of activated carbonaceous materials by the HTC process. Materials were post treatment modify. The antimicrobial potency of obtained materials was examined.

MATERIALS AND METHODS

Materials

Two types of raw materials were used for samples preparation: plane tree seed and fructose (F). The plane tree seed were collected from Platanus orientalis growing in Belgrade (Serbia) parks. Collected plane tree fruits were cut open to take the inside achenes. Fructose was purchased from "Centrohem", Serbia.

Methods

Sample preparation

All samples have been prepared by hydrothermal carbonization of those two precursors at the 260°C for 20 hours. The precipitate was collected by filtration and then washed repeatedly with distilled water and ethanol. Finally, the resulting product was dried in a dryer oven. These samples were denoted as PTS, when plane tree seed was used as a raw material and F, when fructose was used as a raw material.

Plane tree seed balls were cut open and washed with distilled water to remove dust and dirty. After that the samples were dried in dry oven. The samples were ground in a coffee mill for easier handling during the experiment. Plane tree seed (2.5 g) was put in the vessel with 40 ml solution of phosphoric acid (pH=6.4) and the mixture was homogenized by ultrasonic homogenizer for 30 minutes. After that vessel was puts in an autoclave for further hydrothermal carbonization.

2M solution of fructose in 60 ml of phosphoric acid diluted to the pH=4.5 was homogenized by magnetic stirrer for 15 minutes. Than the solution was puts in an autoclave for further hydrothermal carbonization as was mentioned before.

The modification of the samples was performed by immersing the samples in a 0.1 M solution of NaOH. These samples were denoted as MPTS (sample PTS modified by NaOH) and MF (sample F modified by NaOH).

Sample characterization

The textures of these materials were examined with scanning electron microscopy (SEM). Adsorption and desorption isotherms of nitrogen were measured on the activated carbon samples at 77K applying the gravimetric method by McBain balance. The specific surface area, S_{BET}, was calculated using Brunauer–Emmett–Teller (BET) theory [19]. Total pore volume, $V_{TOT,}$ was calculated using Gurwitch rule [19]. Pore size distribution was estimated by applying Horwath Kawazoe (HK) method, for micropores and Barrett, Joyner, and Halenda (BJH) method for mesopores to the desorption branch of the isotherms [20,21]. The High-resolution α_s plot method, proposed by Kaneko and co-workers, was used to estimate external surface area, S_{EXT}, total surface area, S_{TOT}, and micropore volume, V_{mic}, of the samples [20,21]. Micropore surface area can be calculated by subtracting S_{EXT} from S_{TOT}.

Samples of skin swab were collected and pre-cultivated in the sterile LB medium. Before and after pre-cultivation with samples MPTF and MF, swabs were vigorously mixed, and seeded on LB-agar Petri dishes. After incubation in microbiological incubator, the number of CFUs (colony forming units) was manually counted. Results were expressed as percentage of decreased viable CFU on LB-agar plates with addition of specific carbon material.

RESULTS AND DISCUSSION

Differences in texture of PTS and F samples are best observed by SEM analysis. After HTC process texture of both samples were changed according to the severe experimental conditions. Influence of acid, temperature and pressure changed texture that became relief, but very similar see Figure 1 a) and b).

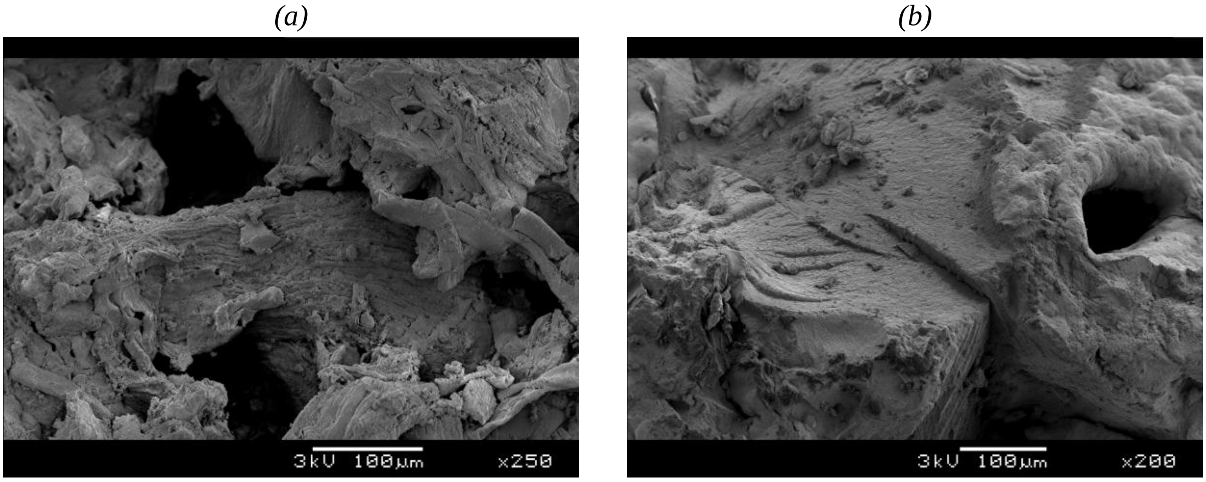
(a)
(b)

***Figure 1** SEM micrographs of active carbon material obtained from different raw material: a) plane tree seed - PTS and b) fructose - F*

Active carbon materials with developed microporosity and with presence of mesoporosity were obtained by hydrothermal carbonization process. This is confirmed by results obtained with detailed analysis of the experimentally obtained data for adsorption/desorption of N_2 at 77K on these materials, Table 1 and Figure 2. Adsorption characteristics such as specific surface area (S_{BET} and S_{TOT}), the micro and meso surface area (S_{mic}, S_{ext}, respectively) volume of pores (total and micropores), are presented in Table 1, while the size of the micro and meso pores in form of pore size distribution in Fig 2. PTS sample has a wider range of pore sizes, in both the micro and meso pores, Figure 2.

***Table 1** Adsorption characteristics of active carbon materials PTS and F*

Sample	S_{BET} (m^2g^{-1})	V_{TOT} (cm^3g^{-1})	$V_{\alpha s}$ (cm^3g^{-1})	S_{TOT} (m^2g^{-1})	S_{mic} (m^2g^{-1})	S_{ext} (m^2g^{-1})
PTS	544	0.324	0.214	525	408	117
F	664	0.259	0.236	643	531	112

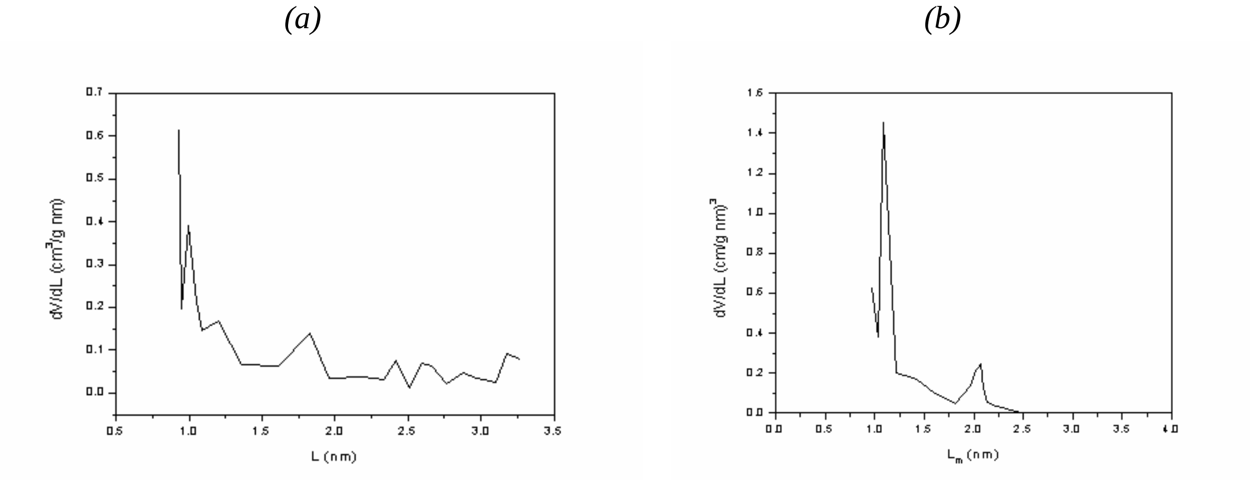
(a)
(b)

***Figure 2** Pore size distribution of active carbon material obtained from different raw material: a) plane tree seed - PTS and b) fructose - F*

Modification of material by sodium hydroxide can change the surface of the material, by removing acide oxide groups such as carboxylic, phenolic, lactonic and lactolic [19].

The results of microbial activity of these samples together with probe sample, which is in fact active carbon, very similar to the sample F, but without surface modification, are presented in Table 2. It is clear that the modification of the surface of the material leads to the reduction in microbial development.

Table 2 *Decrease in the number of colony forming units (d CFU) compared to the control plate, for both concentrations*

Sample	d CFU (%) 1 mg ml^{-1}	d CFU (%) 3 mg ml^{-1}
Probe	52	61
MPTS	91	92
MF	77	92

Both samples, after hydrothermal carbonization process under given conditions, showed significant antimicrobial effects.

CONCLUSION

The results obtained suggest that the material surface modification can improve the properties of the material, in this case its antimicrobial effect. Increasing of antimicrobial agent concentration increases antimicrobial effect.

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ISOTHERMAL TITRATION CALORIMETRY STUDY OF Cu BINDING TO HUMIC ACIDS FROM TECHNOSOLS ON RECLAIMED Cu POST FLOTATION TAILINGS (BOR, SERBIA)

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Abstract

Part of Cu post flotation tailings of Copper Mine Bor were reclaimed by reconstructing the topsoil with arable soils and revegetation in 1991. Humic acids were isolated from these Technosols and binding of copper present at high concentration, to selected humic acid was studied by Isothermal Titration Calorimetry (ITC). Same binding experiment was performed with the standard IHSS soil humic acid and results obtained were compared. According to Isothermal Titration Calorimetry results revealing negative sign of heat effects, binding of Cu^{2+} *to both humic acids studied is endothermic process. Comparing thermograms obtained, heats evolved are slightly higher for* Cu^{2+} *binding for Elliot Soil than for technosol humic acid. Since in our previous investigations most of these soils were characterized by degraded structure, low humus content, predominantly low pH, high As and Cu concentrations, and low soil microbial activity, stronger metal binding of Elliot Soil humic acid is expected. This emprical fact will be quantitavely confirmed by determination of thermodynamic parameters associated to the metal-humic acid complexation process.*

Keywords: Cu post flotation tailings; technosol humic acids; Cu binding; isothermal titration microcalorimertry

INTRODUCTION

One of the largest mines in Serbia is the Copper Mining and Smelting Combine Bor. Beside other mine wastes, post-flotation tailings (PFT) dumps as a result of copper ore processing were formed. The PFT dump "Polje 2" was definitively abandoned in 1987. In addition to degradation of large land areas, these mine wastes are sources of pollution to the surrounding environment. In order to restore the function of a portion of PFT dumps, reclamation measures were implemented. In 1991, an area of about 16 ha was reclaimed by reconstructing the topsoil with natural arable soil, an average depth of 40 cm. The soil was taken from the southern part of the town of Bor where the residential area expanded. One part of the reclaimed area was planted with grass and the other with trees. Previous investigations of reclaimed PFT 20 years after remediation [1] have shown that A horizon (about 45 cm deep) was formed on the soil surface by reclamation process, mixing of natural arable soils with tailings. Most of these soils are characterized by degraded structure, low humus content, predominantly low pH, high As and Cu concentrations, and low soil microbial activity. According to WRB 2007 [2], these soils are classified as RSG Technosols.

Humic substances (HSs) are the most important organic components present in water, soil, and sediments and have significant environmental functions. They are recognized for their role in controlling both the fate of environmental pollutants and the biogeochemistry of organic C in the global ecosystem [3]. Humic substances affect the soil and water properties through their participation in dynamic processes where their constituent molecules interact with other molecules or ions (complexation/ decomplexation), solid surfaces (adsorption/desorption), and among themselves (aggregation/deaggregation) [4]. Their roles in removal of heavy metals from soil and water, inhibition of free radicals formation by metal catalysis, reduction, and stabilization of metal nanoparticles are among the most important [5].

To get quantitative insight into interaction energies between Cu^{2+} ion and humic acids and to determine thermodynamic parameters associated to the metal-humic acid complexation process, Isothermal Titration Calorimetry technique was used [6,7].

MATERIALS AND METHODS

Soil samples and analyses

Humic acids were isolated from soil samples collected from the A horizon of Technosols (T) on reclaimed Cu PFT "Polje 2", Bor - Serbia (44º05’N, 22º06’E). Selected T1 sample, classified as a silt loam, was taken at a 0-25 cm depth [8,9]. HA sample was isolated from T using a modified IHSS method (HA gel was dried at 35°C, powdered, and sieved using a 0.05-mm sieve) [10]. Results obtained for Technosol humic acid (T1 HA) were compared to well characterized standard IHSS humic acid isolated from Elliott Soil (ES HA), which is silt loam, silty clay loam or loam, moderately acid to neutral [11].

Isothermal Titration Calorimetry

Termal events were monitored by the Thermal Activity Monitor (227, LKB Bromma, Sweden) using perfussion cell as measuring device. Titrations were performed using Radiometer automatic titrator (ABU 80, Denmark). Prior to titration experiments, loaded measuring cell of the LKB perfussion system (227-401/402, Sweden) was thermally equilibrated and calibrated (10 µW range) using both static and dynamic calibration procedure. 17 sequential doses of 30 µl of 5 mmol/l $Cu(NO_3)_2$ was added into 2.5 ml of 0.8 g HA/l 0.1 M KNO_3. Time interval between two additions was 10 minutes. Initial pH of the HA sol was 5.0. For each titration, the output signal, power (µW) versus time (min), was recorded by the TAM assistant software and thermograms were obtained.

RESULTS AND DISCUSSION

ITC thermograms representing heat changes upon Cu^{2+} complexation with ES and TC HA are given in Figures 1 and 2.

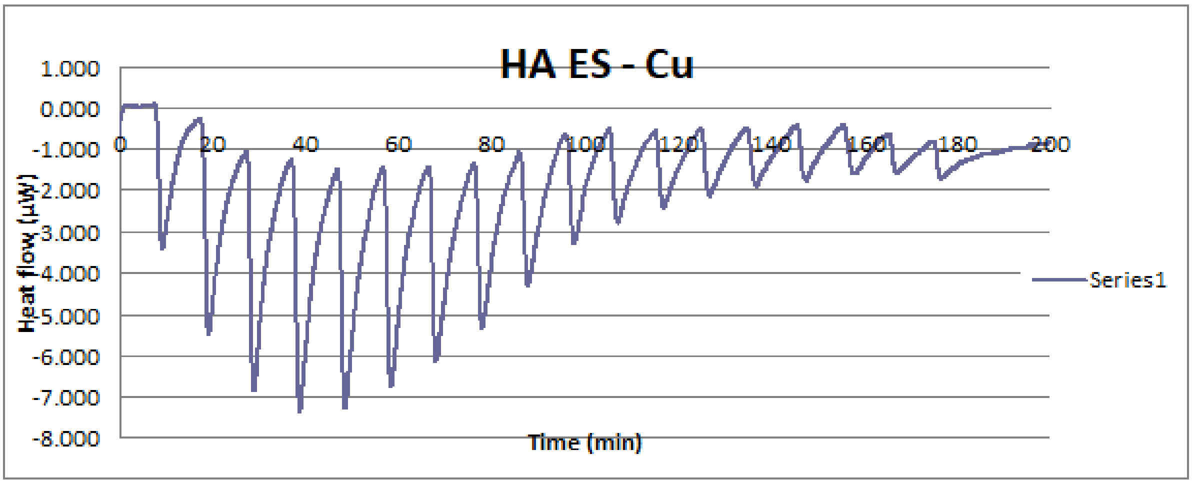

Figure 1 *Termogram representing heat flow (μW) as a function of time (min) for complexation of* Cu^{2+} *with Eliot Soil Humic Acid (10 μW,* $pH_{initial}=5.0$*)*

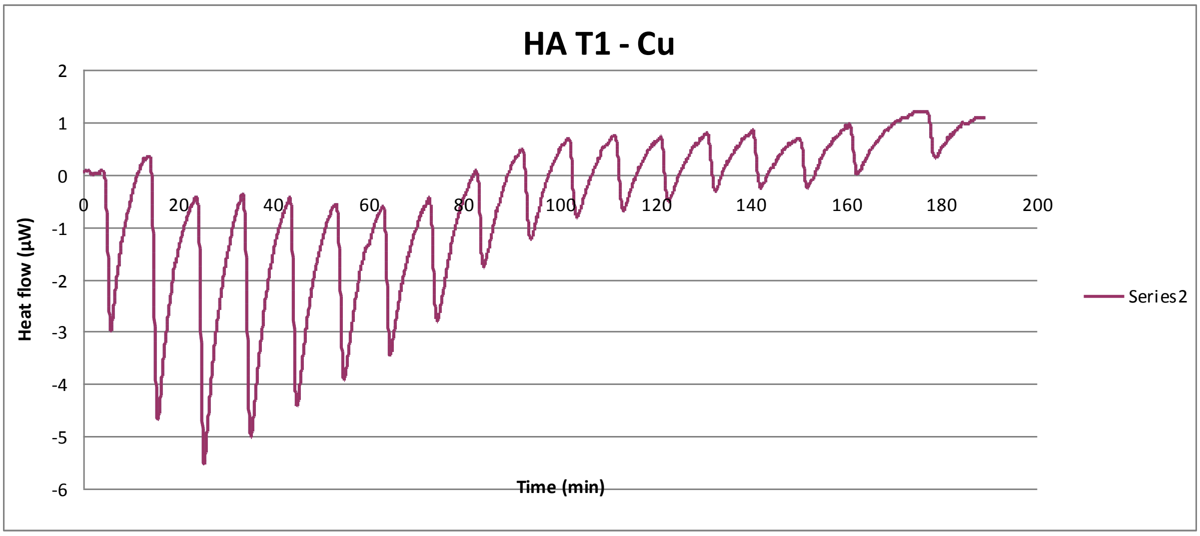

Figure 2 *Termogram representing heat flow (μW) as a function of time (min) for complexation of* Cu^{2+} *with Technosol Humic Acid (10 μW,* $pH_{initial}=5.0$*)*

From results presented in Figs. 1 and 2 revealing negative sign of complexation reaction heat effects, it is obvious that binding of Cu^{2+} to both humic acids studied is endothermic process. Qualitative comparison of thermograms shows that heats evolved are slightly higher for Cu^{2+} binding to ES HAl than to T1 HA. Since in our previous investigations [1] most of these soils were characterized by degraded structure, low humus content, predominantly low pH, high As and Cu concentrations, and low soil microbial activity, stronger metal binding of ES humic acid is expected. Quantitative confirmation of metal binding intensity will be done by determining thermodynamic parameters of metal-humic acid complexation process. Thermodynamic treatment of ITC data obtained will be performed two theoretical models relevant for the process investigated [6,7].

CONCLUSION

From the results presented it can be concluded that ITC technique can be successfuly used to get quantitative insight into interaction energies between metal ions and soil humic acids. In addition, row ITC results can be used to determine thermodynamic parameters associated to the metal-humic acid complexation process applying relevant theoretical models. Theoretical treatment could even distinguish various humic acid reactive groups.

As at the location studied arsenic as very toxic metal is present at extremely high concentrations, future investigations will be focused to investigation of As binding to humic acids.

ACKNOWLEDGEMENT

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THE EFFECTS OF DIFFERENT AGING TREATMENTS ON THE MICROHARDNESS AND THERMAL DIFFUSIVITY OF THE EN AW-6060 AND EN AW-6082 ALUMINUM ALLOYS FROM 6000 SERIES

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Abstract

In this paper an investigation was performed on two aluminum alloys from 6000 series, EN AW-6060 and EN AW-6082. The goal was to investigate the influence of natural aging on mechanical and thermal properties. In the first set of experiments the effect of natural aging on microhardness was investigated. Afterwards, in the second set of experiments, the effect of natural aging (room temperature pre-aging) on artificial aging was studied. From the first set of experiments the obtained results showed the hardening effect of natural aging in both alloys. During natural aging, the microhardness gradually increased up to around 17% for the EN AW-6060 alloy and 19% for EN AW-6082 alloy in comparison to the quenched state. The second set of experiments showed some interesting results. For the EN AW-6060 alloy room temperature pre-aging showed a positive effect in general. Sample that was pre-aged for 70 days before artificial aging had around a 14 % increase in microhardness values compared to the sample that was directly artificially aged. Thermal diffusivity was higher in the samples that were pre-aged for 3 and 10 days. In the other case, for the EN AW-6082 alloy, room temperature pre-aging showed a negative effect in general. Samples that were pre-aged all had lower microhardness values when compared to the samples that were directly artificially aged. Thermal diffusivity was only higher in the samples that were pre-aged for 40 days or more.

Keywords: aluminum alloys, natural aging, pre-aging, microhardness, thermal diffusivity

INTRODUCTION

Aluminum alloys from 6XXX series (Al-Mg-Si) are often chosen in automotive and other industries because they have very good formability and strength followed by high resistance to corrosion. These alloys contain the Mg and Si as two primary elements for alloying. [1–7]. The main characteristic of these alloys is that they can be age hardened. The age hardening process involves solution heat treatment, quenching and aging, which can be either natural (performed at room temperature) or artificial (performed at higher temperatures) [1,6]. During the aging process very fine precipitates are formed. Through the Orowan mechanism these precipitates hinder the movement of dislocations thus strengthening the alloy [1]. Usually, investigation of these alloys starts with DSC analysis which gives the precipitation sequence in the function of temperature. The precipitation sequence has been very well studied over years of research and it is given as: α_{ssss} → Mg:Si clusters→ G.P. zones→ β’’→ β’→ Si (if excess silicon is present)→ β [4,8–13]. Precipitates given in this sequence appear at different temperature ranges and influence (change) the structure in specific way. Natural aging can only produce clusters of magnesium and silicon due to very low temperature, in this case room temperature. Many researchers devoted their attention to the investigation of cluster formation due to natural aging and their influence on the subsequent artificial aging [1–3,6,7]. The research shows that the natural aging can either have a positive or a negative influence on the subsequent artificial aging resulting in better or worse mechanical properties. The positive effect of natural aging (room temperature pre-aging) means higher peak-aged microhardness values after the subsequent artificial aging [14,15]. The negative effect is reversely proportional to the positive one, giving lower microhardness values of the peak-aged samples if they are stored at room temperature before artificial aging [1,2,6,16]. The positive response of pre-aging is often detected in the so-called lean alloys (alloys with small amount of alloying elements), while the negative effect is present in high solute alloys (alloys with larger amount of alloying elements).

In this paper two alloys were chosen for investigation. EN AW-6060 which belongs to the lean alloy category and EN AW-6082 which is a high solute alloy. Regardless of many investigations on the subject of natural aging and its influence on alloys and their properties, this subject is still interesting to the scientific community. The aim was to better understand the influence of natural aging on microhardness and thermal properties of the aluminum alloys. Also, the investigation of positive or negative effects of natural aging (room temperature pre-aging) on subsequent artificial aging was performed.

MATERIALS AND METHODS

Two aluminum alloys, EN AW-6060 and EN AW-6082 were used for investigation. The alloys were delivered from “AlCu metali d.o.o.” company, in peak aged condition (T6 temper) in the form of extruded rectangular bars. The Tables 1 and 2 represent the chemical compositions of investigated alloys determined by the optical emission spectrometer “Belec Compact Port”. In order to remove peak aged condition and obtain the O-temper for all of the samples, annealing at 550 °C for 6 hours in the electric resistance furnace Heraeus K-1150/2 was performed. After that, a super saturated solid solution (α_{ssss}) was obtained by solution heat treatment at 550 °C for 1 hour followed by quenching in water with ice. After obtaining the super saturated solid solution, the samples were subjected to natural aging for a different time from 3 up to 70 days (referenced as NA samples). One sample was directly aged without any room temperature delay in order to achieve peak microhardness state (presented on graph as directly aged or AA sample). In order to investigate the influence of natural aging on artificial aging (AA), samples that were naturally aged for 3, 10, 40 and 70 days were chosen and artificially aged in the same manner as the directly aged sample (those samples are referenced as NA+AA samples). All the NA samples were compared to the quenched sample (quenched state on the presented graphs), while all the NA+AA samples were compared to only the artificially aged samples (AA samples).

During the aging treatment, all samples were subjected to various characterization methods. Firstly, microhardness values were measured using a PMT-3 Vickers microhardness tester using 100 gf loads with load duration of 15 s. The ASTM E384 standard was followed during the microhardness measurements [17]. Additionally, thermal diffusivity was measured on the DXF 500 thermal analyzer manufactured by TA Instruments by using a Flash method.

Thermal diffusivity was measured on NA+AA samples and AA samples, but not on NA samples.

Table 1 *Chemical composition of the EN AW-6060 (mass. %)*

Si	Fe	Cu	Mn	Mg	Cr	Ni	Zn
0.49	0.182	0.012	0.006	0.594	<0.003	0.028	0.01
Ti	**Pb**	**V**	**Co**	**Sn**	**Zr**	**Al**	
0.005	<0.003	0.014	<0.003	<0.003	<0.003	98.62	

Table 2 *Chemical composition of the EN AW-6082 (mass%)*

Si	Fe	Cu	Mn	Mg	Cr	Ni	Zn
0.807	0.354	0.042	0.453	0.696	<0.012	0.012	0.115
Ti	**Pb**	**V**	**Co**	**Sn**	**Zr**	**Al**	
0.025	0.01	<0.003	0.006	<0.003	<0.003	97.45	

RESULTS AND DISCUSSION

Analysis of the graph presented in Figure 1 shows that natural aging caused a hardening effect in both alloys. All of the naturally aged samples had higher microhardness values in comparison to the quenched state.

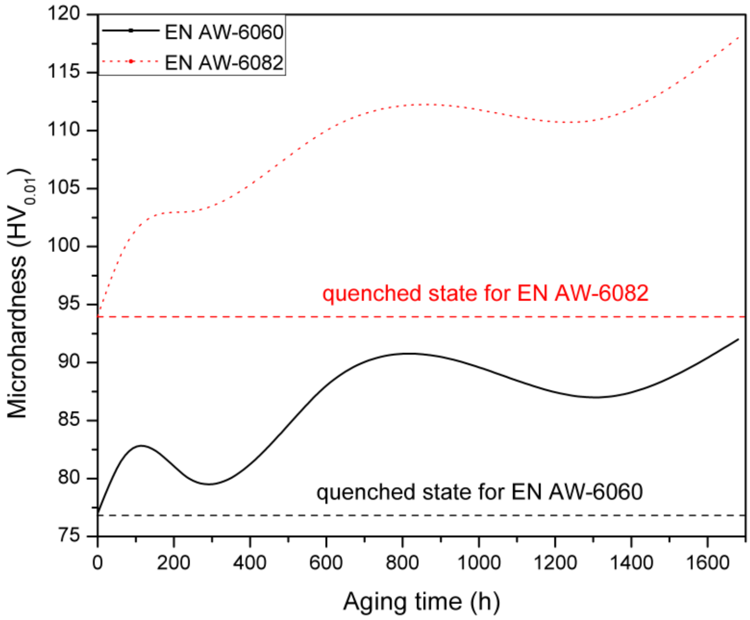

Figure 1 *Change in microhardness during natural aging*

Microhardness increases gradually until it reaches microhardness saturation after which the curve shows the plateau state where microhardness remains almost the same up to 70 days. Saturation is achieved after 40 days of aging. This value is around 17 % higher than the value for the quenched sample for the EN AW-6060 and around 19 % for the EN AW-6082 alloy. Magnesium and silicon atoms that originally dissolved into the Al matrix during solution heat treatment and remained in the matrix after quenching now precipitate in the form of clusters and co-clusters due to the natural aging. This formation of clusters is causing the restriction of dislocation motion and increasing the microhardness of the alloy [1, 2, 6, 7, 16]. Cao *et al*. using 3D atom probe technique discovered that clustering between Mg and Si atoms occurs after several hours of natural aging. In this way it is explained the steepness of the curve presented in Figure 1 [18].

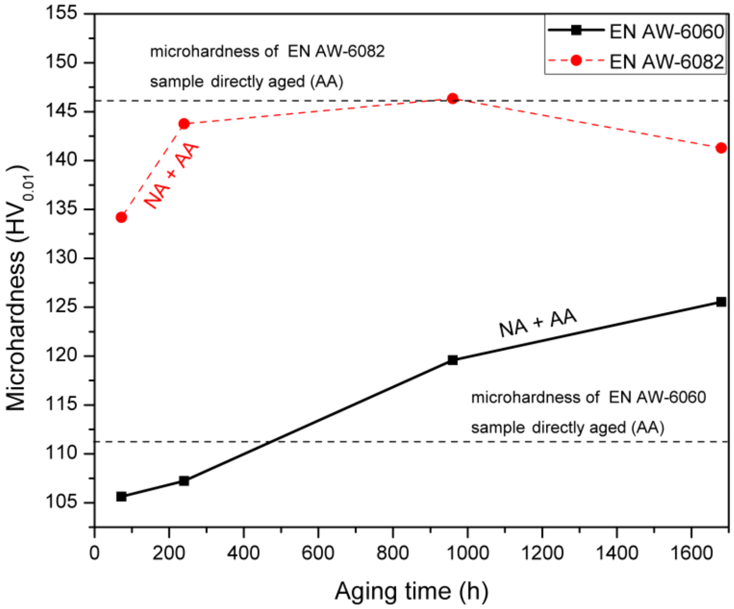

Figure 2 *Vickers microhardness as a function of pre-aging time*

In Figure 2 the influence of natural aging (room temperature pre-aging) on the artificial aging has been presented. In order to obtain the highest microhardness values the samples need to be peak aged. The highest values of microhardness in peak aged samples are achieved after the precipitation of β’’ phase. Any stimulation of the precipitation of this phase results in higher microhardness values. In the case of lean Al alloys, clusters and co-clusters formed during the pre-aging help the nucleation of the β’’ phase in later precipitation during artificial aging [14, 19]. For the high solute alloy, those clusters are very stable so they inhibit the formation of β’’ phase causing the microhardness to decrease in NA+AA samples [1, 2]. This statement was proven in the analysis given in Figure 2. In the case of EN AW-6060 pre-aging had a positive effect on subsequent artificial aging. Samples that were pre-aged for 40 and 70 days before artificial aging had higher values of microhardness compared to the samples that were directly artificially aged. For the EN AW-6082 alloy, room temperature pre-aging showed a negative effect in general. Samples that were pre-aged all had lower microhardness values when compared to the samples that were directly artificially aged.

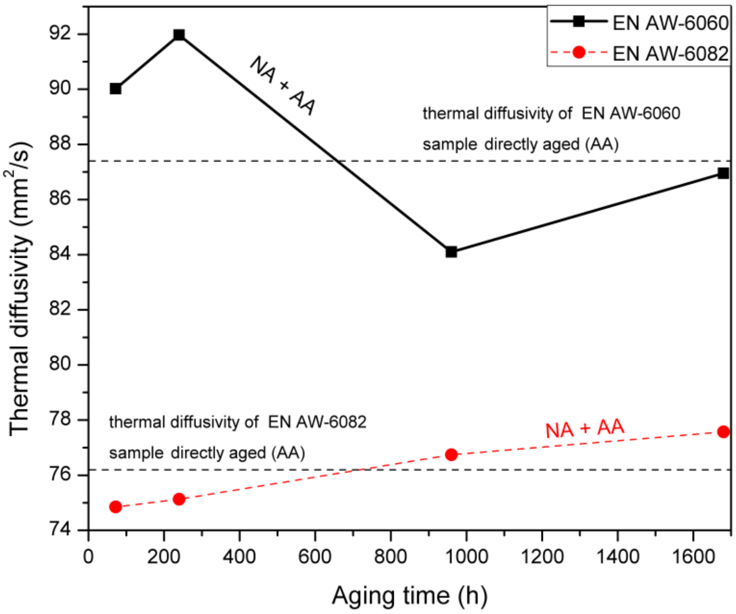

Figure 3 *Thermal diffusivity as a function of pre-aging time*

Thermal diffusivity was inversely proportional to microhardness for the samples of EN AW-6060 alloy. Thermal diffusivity was higher for the samples that were pre-aged 3 and 10 days, and in the case of EN AW-6082 alloy higher values of thermal diffusivity were obtained for the samples that were pre-aged for 40 and 70 days, as can be seen on Figure 3. The largest increment for the lean alloy was around 5% (after 10 days of pre-aging), and for the high solute alloy it was around 2% (after 70 days of pre-aging).

CONCLUSION

The main focus of this research was the influence of natural aging on microhardness and thermal diffusivity of the aluminum alloys with small and larger amount of alloying elements. The key conclusions can be outlined as:

- The microhardness of the samples naturally aged is higher than those in the quenched state for both alloys. Maximal microhardness is achieved after the 40 days of natural aging after which the microhardness goes into the plateau state and microhardness remains more or less the same until 70 days of natural aging.
- Natural aging (room temperature pre-aging) showed positive or negative effect on artificial aging. The positive effect was expressed through higher microhardness values for the EN AW-6060 alloy with small amount of alloying elements. Samples that were pre-aged for 40 and 70 days before artificial aging had higher values of microhardness compared to the samples that were directly artificially aged. A 14% increase in microhardness value compared to the sample that was directly artificially aged was detected in the sample pre-aged for 70 days. For the EN AW-6082 alloy with larger amount of alloying elements, room temperature pre-aging showed a negative effect, as all of the samples that were pre-aged had lower values of microhardness when compared to the directly aged samples.

- Thermal diffusivity was not as much affected by the pre-aging process. The largest increment for the lean alloy was around 5% (after 10 days of pre-aging), and for the high solute alloy was around 2% (after 70 days of pre-aging).

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ELECTROCHEMICAL BEHAVIOR OF STAINLESS STEEL 316L IN RINGER'S SOLUTION IN THE PRESENCE OF L-TRYPTOPHAN

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Abstract

The corrosion behavior of stainless steel 316L in Ringer's solution in the presence of L-tryptophan was investigated. For this purpose electrochemical methods such as open circuit potential measurements and potentiodynamic polarization measurement were used. The results obtained by potentiodynamic polarization indicated that investigated amino acid behaves as mixed type inhibitor with more pronounced influence on cathodic corrosion processes. The inhibition efficiency of L-tryptophan increases with increasing its concentration. According to results obtained by electrochemical measurements, in the presence of L-tryptophan, protective film is formed on the steel surface.

Keywords: steel, Ringers solution, amino acid, corrosion inhibition

INTRODUCTION

Stainless steel 316L is one of the most used material for orthopedic implants in medicine [1,2]. Stainless steel possesses good properties which allow it to be used in such aggressive media like human body. However, in aggressive solutions stainless steel undergoes corrosion and the pitting corrosion occurs as the most commonly type of corrosion. The mechanism of pitting corrosion is however, a complex one, and the nucleation and propagation of pits occur in several steps [3]. An important method of protection of metals against deterioration from corrosion is by using organic inhibitors [4–6]. Unfortunately, many common corrosion inhibitors are health hazards and to overcome this, new environmental friendly and non-toxic inhibitors were investigated in few past years [7–9]. It is very important to find health-safe inhibitor that can be used in aggressive environments such as the human body. Amino acids are nontoxic, relatively cheap and easy to produce with purities greater than 99%.

The aim of this paper is to examine inhibition efficiency of different concentration of L-tryptophan (Figure 1) on stainless steel corrosion in artificial physiological solution.

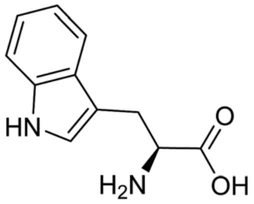

***Figure 1** Molecular structure of L-tryptophan*

MATERIALS AND METHODS

In this investigation a three-electrode cell system was used. The stainless steel 316L electrode was used as working electrode, standard calomel electrode as the reference and platinum as the auxiliary electrode. Electrochemical measurements were performed by potentiostat (IVIUM XRE, IVIUM Technologies) with appropriate software. The working electrode was polished with SiC paper, washed with distilled water and dried before each measurement. Open circuit potential (OCP) measurements, potentiodynamic polarization and cyclic voltammetry (CV) measurements were applied in this study. Potentiodynamic polarization curves was recorded from the open circuit potential to 0.5 V (vs. SCE) in anodic direction and from the open circuit potential to −0.6 V (vs. SCE) in the cathodic direction, while the cyclic voltammetry was recorded in a wider potential range from −1.0 V (vs. SCE) to 1.0 V (vs. SCE). Scan rates were 1 mV/s and 10 mV/s for potentiodynamic polarization and cyclic voltammetry measurements, respectively. Electrochemical behavior of stainless steel 316L was investigated in Ringer's solution with and without addition of L-tryptophan ($1\cdot10^{-2}$, $5\cdot10^{-3}$, $1\cdot10^{-3}$, $1\cdot10^{-4}$ M). Chemical composition of Ringer's solution is shown in Table 1.

***Table 1** Chemical composition of Ringer's solution*

Compounds	Concentration (g/dm^3)
NaCl	8.60
KCl	0.30
$CaCl_2$	0.33

RESULTS AND DISCUSSION

Open circuit potential

Figure 2 showed the open circuit potential (OCP) curves for 316L steel in Ringer's solution in the absence and presence of various concentrations of L-tryptophan.

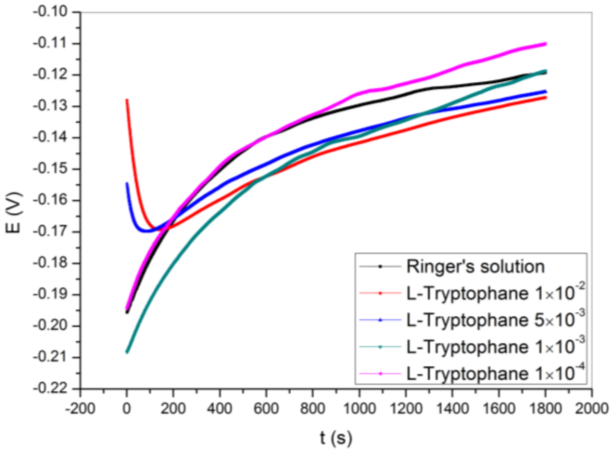

***Figure 2** The open circuit potential of steel electrode in Ringer's solution without and with the addition of different concentrations of L-tryptophan*

Decrease of OCP values in solutions with higher concentration of amino acid ($1\cdot10^{-2}$ and $5\cdot10^{-3}$M) indicates dissolution of oxide film on steel surface as a result of adsorption of chloride ions [10, 11]. Further increase of OCP values reveals that comes to the passivation of electrode surface due to formation of chromium and iron oxides on electrode surface. Nonetheless, in Ringer's solution with addition of amino acid, adsorption of L-tryptophan molecules on stainless steel surface contributions to the better protective effect of formed layer [12].

The cyclic voltammetry measurements

In order to obtain more information about corrosion behavior of stainless steel 316L in artificial body solution in the presence of amino acid, the cyclic voltammetry is applied in a wide range of potential, from −1.0 V vs SCE to 1.0 V vs SCE. The obtained curves are showed in Figure 3.

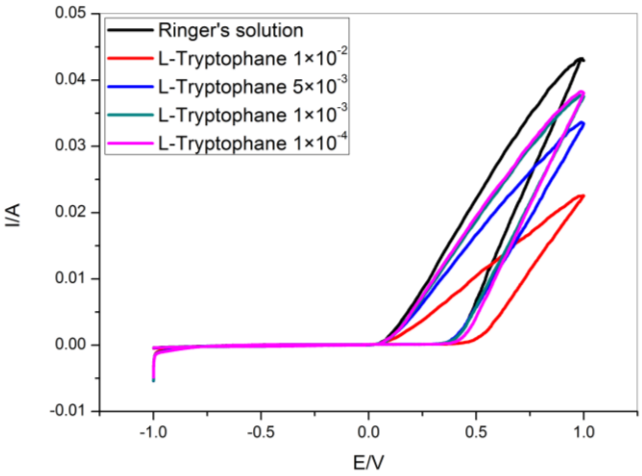

Figure 3 *The cyclic voltammetry curves for steel in Ringer's solution without and with the addition of different concentration of L-tryptophan*

Cyclic voltammetry curves obtained in Ringer's solution with and without addition of L-tryptophan are similar and reveal that no modification in corrosion processes in solution with addition of amino acid. Shapes of CV curves indicate that on steel surface exists thin layer of Cr_2O_3 which was formed in contact with the air. With further increment of potential, passivity of surface increase due to formation of iron oxides [13]. At the beginning, the oxidation of Fe to Fe^{2+} occurs according to the equation [14]:

$Fe = Fe^{2+} + 2e^-$

After that comes to the formation of Fe_3O_4 [14]:

$3Fe^{2+} + 4H_2O = Fe_3O_4 + 8H^+ + 2e^-$

Further polarization leads to the formation of Fe_2O_3 [15]:

$2Fe_3O_4 + H_2O = 3Fe_2O_3 + 2H^+ + 2e^-$

Beside that, nickel oxide and molybdenum oxide also contribute to the passivity of formed layer. Nickel and molybdenum oxides were formed according to the equations [14]:

$Ni + H_2O = NiO + 2H^+ + 2e^-$

$Mo + 2H_2O = MoO_2 + 4H^+ + 4e^-$

However, in artificial physiological solution with addition of L-tryptophan adsorption of amino acid molecules occurs onto steel surface, which leads to the formation of stronger barrier and prevents the contact of aggressive ions with the steel surface.

Potentiodynamic polarization measurements

After the determination of OCP values, the potentiodynamic polarization measurements were performed. The obtained curves for stainless steel in artificial physiological solution without and with different concentrations of L-tryptophan are presented in Figure 4.

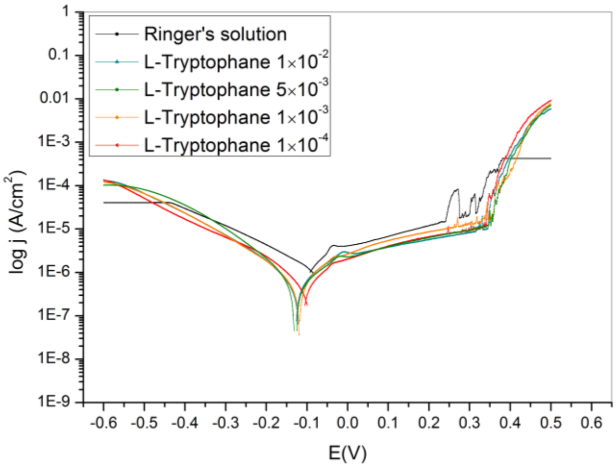

Figure 4 *Potentiodynamic polarization curves of steel in Ringer's solution without and with the addition of different concentrations of L-tryptophan*

Potentiodynamic polarization curves obtained in Ringer's solution with amino acid point out that decrease of anodic and cathodic current density occurred in the presence of different concentrations of L-tryptophan. This suggests that comes to the decrease of hydrogen evolution and anodic dissolution of steel and implies inhibitive effect of L-tryptophan. Also, the effect is enhanced with the increase in concentration of inhibitor. Thus, it can be assumed that inhibitor is adsorbed on the steel surface and hinders both anodic and cathodic reactions.

Kinetic corrosion parameters including corrosion potential (E_{corr}), corrosion current density (j_{corr}), anodic (ba) and cathodic (bc) Tafel slopes and inhibition efficiency (IE) are calculated from potentiodynamic polarization curves and shown in Table 2. The inhibition efficiency (IE) is calculated according to equation:

$IE = [(j_{corr} – j_{corr(inh)}) / j_{corr}] \cdot 100\%$

where j_{corr} and $j_{corr(inh)}$ are the corrosion current densities without and with the addition of L-tryptophan.

Table 2 *Electrochemical parameters and inhibition efficiency of steel corrosion in Ringer's solution without and with the addition of different concentrations of L-tryptophan*

Solution	E_{corr} (V)	j_{corr} (A/cm^2)	b_c (mV/dec)	b_a (mV/dec)	IE (%)
Ringer's solution	-0.092	$1.24 \cdot 10^{-6}$	-0.019	0.027	/
L-tryptophan $1 \cdot 10^{-2}$	-0.124	$2.76 \cdot 10^{-7}$	-0.12	0.13	77.2
L-tryptophan $5 \cdot 10^{-3}$	-0.121	$2.87 \cdot 10^{-7}$	-0.13	0.14	76.8
L-tryptophan $1 \cdot 10^{-3}$	-0.118	$2.98 \cdot 10^{-7}$	-0.11	0.13	75.9
L-tryptophan $1 \cdot 10^{-4}$	-0.103	$4.37 \cdot 10^{-7}$	-0.12	0.11	64.7

By analyzing these results, it should be noted that the values of E_{corr} are shifted toward negative values with the increase in L-tryptophan concentration. Since the displacement of E_{corr} value is lower than 85 mV, L-tryptophan can be classified as mixed type inhibitor. Results reflected in Table 1 indicate that both anodic and cathodic Tafel slopes are changed in the presence of inhibitor. This behavior may be explained by the formation of protective film on the steel surface [16].

CONCLUSION

L-tryptophan was studied as potential inhibitor of stainless steel 316L corrosion in artificial body solution. Results of the electrochemical measurements indicate that L-tryptophan inhibits corrosion in satisfactory levels. Cyclic voltammetry measurements and potentiodynamic polarization measurements show that in the presence of amino acid comes to the formation of protective layer on stainless steel surface. Also, electrochemical tests indicate that L-tryptophan acts as mixed-type inhibitor with stronger influence on cathodic corrosion processes.

ACKNOWLEDGEMENT

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THE INHIBITORY EFFECT OF N-ACETYL-L-LEUCINE ON CORROSION OF BRASS IN SYNTHETIC ACIDIC RAIN SOLUTION

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Abstract

The effect of N-Acetyl-L-Leucine on brass in artificial acid rain solution was investigated. Electrochemical methods were used to show behavior of brass electrode. The effect of the addition of potassium sorbate to the investigated system was also studied. Obtained results showed that N-Acetyl-L-Leucine and potassium sorbate have good inhibitory characteristics. Increase of concentration of N-Acetyl-L-Leucine lead to current density decrease. Cyclic voltammograms confirmed inhibitory properties of used inhibitor. N-Acetyl-L-Leucine obeys Langmuir adsorption isotherm.

Keywords: amino acid, acid rain, brass, corrosion inhibition

INTRODUCTION

Corrosion has an impact everywhere and on all aspects of our existence [1]. The impact of the corrosion effect is reflected on economy, safety and environment [2]. Too much money is spent to prevent the effect of a seemingly simple corrosion process. The proposed solution for this problem is the formation of a thin layer of organic film on the surface of the metal that will prevent further corrosion. It is well known that corrosion never stops but the corrosion rate can be reduced [1].

Brass is one of the most famous copper alloys due to good thermal conductivity and mechanical workability [3]. Copper-based alloys usually enter the construction of roofs and facades and are also used as coatings due to good physical and mechanical properties, as well as positive corrosion properties [4]. Brass is widely used for casting decorative items, which are mostly used for outdoor use [5]. Brass is also used as a material for the production of pipes, heat exchangers, propellers, forage, valves and pump parts [6].

In copper alloys, the susceptibility to the corrosion process increases with the increase in the percentage content of Zn [6]. Brass is subject to atmospheric corrosion (dezincification) when it is exposed to aggressive anions such as halogen ions and SO_4^{2-} ions that are the result of the pollutant in the atmosphere [7]. Due to the increase of pollution of air and acid rain, brass constructions in the urban environment accelerate the process of corrosion and changes in their surfaces [5].

In order to protect metals against corrosion, organic substances are added in very small amounts that we call inhibitors, but many proved good inhibitors are toxic that was the reason to examine eco-friendly corrosion inhibitors.

The purpose of this work is to examine corrosion inhibition efficiency of environmentally safe inhibitor N-Acetyl-L-Leucine (NAL).

MATERIALS AND METHODS

For electrochemical tests, potentiostat (IVIUMSTAT XRe, IVIUM Technologies) was used with the appropriate software. The methods applied during the test are: measurement of open circuit potential (OCP), linear voltammetry (LV) and cyclic voltammetry. All measurements were made at room temperature.

The open circuit potential was determined for 30 minutes. The rate of potential change in LV was 1 mV / s. The final values of the potential of the OCP measurement were the baseline values of the potential of the LV measurement. The measurement in the anode direction for the LV was carried out up to 0.5 V, while in the cathode direction the measurement was carried out to -0.5 V. The potential values for cyclic voltammetric measurements were from -1.0 V to 1.0 V. The rate of potential change was 10 mV / s.

A solution of acid rain was used as a basic solution. Acid rain solution with a pH value of 2.42 was prepared by dissolving certain amounts of the components listed in Table 1 by distilled water. The role of the inhibitor had N-Acetyl-L-Leucine in acidic rain solution at concentrations of $1 \cdot 10^{-2}$ M, $5 \cdot 10^{-3}$ M, $1 \cdot 10^{-3}$ M and $1 \cdot 10^{-4}$ M. Various inhibitor concentrations were obtained by dilution of the N-Acetyl-L-Leucine solution of concentration $1 \cdot 10^{-2}$ M. For the examination of synergism, 1% potassium sorbate dissolved in N-Acetyl-L-Leucine concentration was $1 \cdot 10^{-2}$ M. pH value of basic solution was achieved by adding the H_2SO_4.

Table 1 *Chemical composition of acid rain solution*

Compounds	Concentration (g/dm^3)
Na_2SO_4	0.2
$NaHCO_3$	0.2
$NaNO_3$	0.2

RESULTS AND DISCUSSION

Open circuit potential

Measured values of open circuit potential in acid rain solution and in the presence of different inhibitor concentrations are presented in Figure 1.

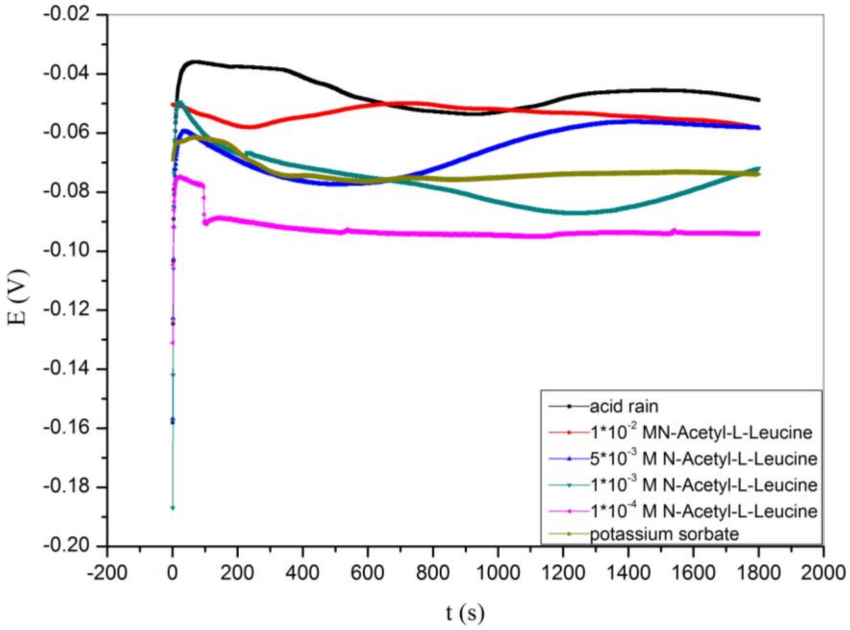

Figure 1 *The open circuit potential of brass electrode in acid rain solution without and with the addition of different concentrations of N-Acetyl-L-Leucine*

Moving the OCP into a more negative area can be attributed to the adsorption of the inhibitor on metal surface [8] or the formation of a complex. The constant value of the OCP indicates the creation of a protective film that further prevents the dissolution of the brass.

The cyclic voltammetry measurements

To test the behaviour of brass in solution of acid rain with and without the presence of N-Acetyl-L-Leucin and with the addition of potassium sorbate, voltammograms were recorded.

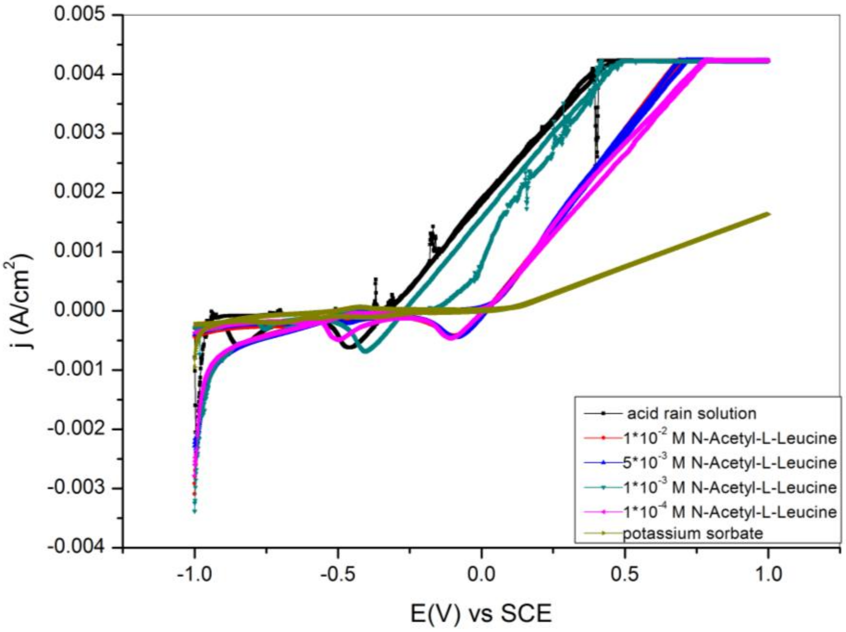

Figure 2 *The cyclic voltammetry curves for brass in acid rain solution without and with the addition of different concentration of N-Acetyl-L-Leucine*

Based on cyclic voltammograms it could be concluded that brass undergoes oxidation and current density increasing with potential increasing. In reverse scan two peaks were observed which means that the reduction of the dissolved species proceeds in two steps corresponding to the reduction of Cu^{2+} to Cu^{+} as the first one and Cu^{+} to Cu as the second one [9].

Potentiodynamic polarization measurements

Figure 3 present the potentiodynamic polarization curves obtained after the determination of OCP. Potentiodynamic polarization curves for brass were recorded in blank acid rain solution and in presence of N-Acetyl-L-Leucine as corrosion inhibitor.

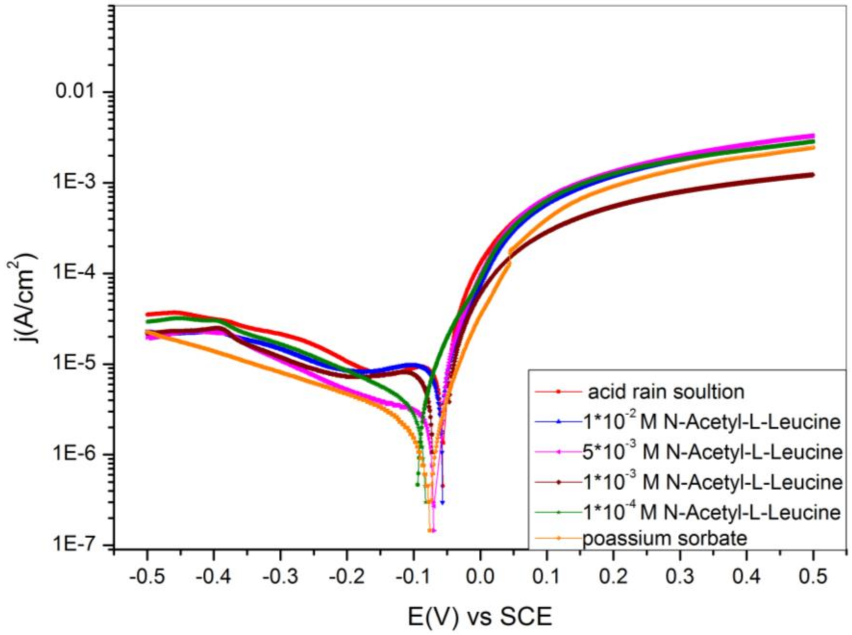

Figure 3 *Potentiodynamic polarization curves of brass in acid rain solution without and with the addition of different concentrations of N-Acetyl-L-Leucine*

The obtained results suggest that presence of different concentration of N-Acetyl-L-Leucine diminish the value of current density in cathodic and anodic direction. Also, presence of potassium sorbate additionally decreases anodic and cathodic current density. Presented results imply inhibitive effect of inhibitor and potassium sorbate. The addition of N-Acetyl-L-Leucine and potassium sorbate do not shift the corrosion potential value more than 85mV and studied compounds can be considered mixed-type inhibitors [10].

The anode reaction of the brass electrode immersed in the acidic solution is:

-Oxidation of zinc [11]:

$$Zn \rightarrow Zn^{2+} + 2e \tag{1}$$

-Copper oxidation [12]

$$Cu = Cu^{+} + e^{-} \tag{2}$$

$$Cu^{+} = Cu^{2+} + e^{-} \tag{3}$$

The cathode reaction of brass electrode is reduction of oxygen [11]:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^- \qquad (4)$$

Voltamogram curves in the presence of inhibitor show the formation of two peaks in the negative part of the voltamogram as a result [13]:

- the reduction of Cu (II) (formed during the oxidation process) in Cu (I):

$$Cu^{2+} + e^- = Cu^+ \qquad (5)$$

The reduction of Cu(I) in Cu:

$$Cu^+ + e^- = Cu \qquad (6)$$

Inhibition efficiency was calculated from the linear voltammograms according to the following equation:

$$IE = 100 \cdot (j - j_{corr}) / j (\%) \qquad (7)$$

Where j and j_{corr} are the corrosion current densities in the test solution without and with inhibitor. Calculated values for inhibition efficiency, anodic and cathodic Tafel slopes, corrosion current densities and corrosion potential are presented in Table 2. According the data presented in Table 2 using the potassium sorbate with inhibitor at the same time, lead to better inhibition efficiency.

***Table 1** Electrochemical parameters and inhibition efficiency of brass corrosion in acid rain solution without and with the addition of different concentrations of N-Acetyl-L-Leucine*

Concentration (M)	E_{corr} (V)	$I_{corr} \cdot 10^6$(A)	-$ß_c$ (V)	$ß_a$ (V)	EI(%)
AR	-0.055	6.987	0.059	0.035	/
NAL **$1\cdot10^{-2}$**	-0.059	2.902	0.087	0.041	58.47
NAL **$5\cdot10^{-3}$**	-0.077	3.699	0.114	0.049	47.06
NAL **$1\cdot10^{-3}$**	-0.060	4.350	0.116	0.052	37.74
NAL **$1\cdot10^{-4}$**	-0.092	4.509	0,150	0.069	35.46
Potassium sorbate +**$1\cdot10^{-2}$**	-0.077	1.934	0.115	0.026	72.32

CONCLUSION

Obtained results led to following conclusions:

- N-Acetyl-L-Leucine is environmentally safe corrosion inhibitor.
- In synthetic acid rain solution the inhibition efficiency of N-Acetyl-L-Leucine toward brass corrosion is evident and maximum of inhibitor efficiency was observed at $1\cdot10^{-2}$ M.
- The addition of potassium sorbate increasing the inhibition efficiency.

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PROTECTION OF THE PERSONNEL FROM IRRADIATION DURING PYRO-METALLURGICAL PROCESSING OF METALLIC RADIOACTIVE WASTE ON THE BASE OF MODEL CALCULATIONS

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Abstract

The theoretical grounds of radioactive loading of the organism of work staff received during utilization of metallic radioactive waste from nuclear power plants are considered. Methods for protection of the personnel and the environment from radioactive emissions are indicated. Based on model calculations practical measures are prescribed for protection of the personnel in the metallurgical deactivation installation from radioactive emissions.

Keywords: radioactivity, radioactive waste, installation for radioactive waste remelting

INTRODUCTION

The operation of nuclear power plants (NPP) “produces” from 100.0 to 300.0 t of metallic radioactive waste annually depending on their type and capacity. The operation of an installation with a nuclear thermal cycle (NTC) can yield from 700.0 to 7,000.0 t of metallic radioactive waste per year. During decommissioning of such facilities the amount of metallic radioactive waste increases significantly and can reach 15,000 t, and during decommissioning of the most powerful NTC (gas-diffusion plants for uranium enrichment) – up to 230,000 t. According to existing data about 1 million tons of metallic radioactive waste has been accumulated only in Russia. About 340,000 t of them are high alloyed steels and non-ferrous metals and alloys. The main sources of metallic waste are: nuclear power plants, radiochemical enterprises, military and civilian fleets, ship-repair industry, oil-gas complex industry, etc.

The annual dynamics of metallic radioactive waste accumulation is about 10,000-20,000 t [1,2]. According to estimates made in the USA as a result of the operation and decommissioning of all facilities and installations working as of the present moment, the generated metallic radioactive waste, amount to about 12,000,000 t [3,4]. The metallic radioactive waste from the Kozloduy NPP is also included here.

The aim of this work is, on the basis of model calculations, to prescribe measures for protection of the personnel engaged in the metallurgical deactivation of metallic radioactive waste.

THEORY

Essence of the process

It is known that the atoms of all elements consist of a nucleus with a positive electric charge with a certain number of electrically negative electrons, circulating around it. The atomic nucleus consists of protons and neutrons. The latter are electrically neutral. The number of the positive and negative charges is equal and hence the atom is electrically neutral in normal state. The total number of protons and neutrons is called nucleons. They give so called mass number of the nucleus. For example, the nucleus of helium has two protons and two neutrons and its mass is four times the mass of the nucleus of hydrogen.

The atoms that differ in their nucleus composition are called nuclides, i.e. they have different number of nucleons in their nucleus or different number of protons and neutrons for the same number of nucleons.

In 1927 E. Schrodinger found that the electrons can occupy only certain energy levels, i.e. they can have only exactly specified energy. Being at a specified state, the electrons do not absorb and emit energy. This happens only when they are transferred from one energy state to another. If the atom is not excited the electrons occupy the energy levels most close to the nucleus with the lowest energy. The electron is transferred to a higher energy level only if it receives external energy. This occurs when the atoms collide or when radiation with appropriate energy is absorbed. The electron can return back to a lower energy level. Then it emits electromagnetic (photon) irradiation with the respective frequency [5].

Radioactivity, or else radioactive decay (from Latin “radio” – emit, and “activus” – active), is called the ability of atomic nuclei of unstable isotopes of chemical elements to be transformed spontaneously into nuclei of other chemical elements, thereby emitting energy in the form of electromagnetic radiation or particles, for example alpha-particles, beta-particles or gamma-rays.

The measuring unit of radioactivity in the International System of Units (SI) is becquerel in the honor of Henry Becquerel, symbolized as Bq and with dimension 1/s.

There are two types of radioactivity:

- Natural – from interaction of cosmic rays with the Earth’s atmosphere, or simply arbitrary decay of nuclei of elements, existing in nature (the most common being Rn^{222}, obtained from the decay of Ra^{226}).
- Artificial radioactivity – as a result of human activity, of nuclear reactions in particular.

Types of radioactivity according to the emitted particles

Alpha-decay

This is a spontaneous decay of the atomic nucleus, resulting in a sub-nucleus and a α-particle (He^4 nucleus). The alpha-particles consist of two protons and two neutrons. The alpha-rays penetrate only a few thousandths of a centimeter in an aluminum foil and hardly penetrate through a sheet of paper.

Beta-decay

The beta-rays are accelerated streams of high energy electrons or positrons. They are accompanied by neutrino (antineutrino) emission, which explains the continuous energy spectrum of this type of decay. Beta-rays are with greater penetration capacity and in contrast to alpha-rays they represent a hazard in external irradiation.

Gamma-radiation

The gamma-rays are a stream of high energy electromagnetic waves with a very small wavelength. Because of their lack of mass, their penetration capacity is greater than that of the alpha and beta radiation. This makes them extremely dangerous both in internal and external radiation. Shielding materials of heavy metals (mainly lead) combined with thick concrete barriers can play a relatively good protective role against gamma radiation [5].

PROCESSING OF METALLIC RADIOACTIVE WASTE

One of the basic methods for utilizing metallic radioactive waste from decommissioned nuclear facilities or closed NPP units is the remelting of radioactive waste in melting furnaces of various types. Thus several important goals are achieved. On the one hand the volume of metallic radioactive waste is reduced (sometimes up to 50 times). On the other hand, after processing the liquid melt with special fluxes, metal is obtained that is suitable for further limited or unrestricted use.

Both the pre-treatment of metallic radioactive waste (cutting, preliminary deactivation, etc.) and the melting process itself take place in a radioactive environment, which requires the protection of the work personnel in this respect. Sources of radioactive radiation are the melting equipment and the material obtained, as well as the secondary radioactive waste – filter powder, slag, refractories from furnace repair, etc.

Input control of metallic radioactive waste, introduced for remelting

The input radiation control consists in measuring the gamma-ray dose power at a specified distance, measuring the beta-particle stream from the elements subjected to remelting and gamma spectrometric measurement to determine the radioisotope composition of the contamination.

The radioactive scrap for remelting introduced in the melting installation in special containers should meet the following requirements:

- the presence of scrap, contaminated by organic and inorganic materials, as well as the presence of these individual materials in the containers is inadmissible;
- the presence of waste, representing closed containers and vessels under pressure, as well as vessels, containing substances that release great amount of gases, is inadmissible;
- the scrap dimensions should ensure full use of the furnace volume.

Output radiation control of secondary radioactive waste

The output radiation control includes measuring the gamma-ray radiation dose power at a specified distance from the radiation sources, measuring the beta-particle stream from the castings and obtaining gamma spectrometric information about the radioisotope composition of the residual contamination.

The aim of the output radiation control is to verify the compliance of residual radioactivity of the castings with the norms for release of materials outside the Installation and their secondary use with or without restrictions.

As a result of the output control the castings are sorted as:

- unconditionally released, suited for release for unrestricted use;
- conditionally released, which are slightly contaminated, for storage on the territory of NPP as radioactive metals;
- heavily contaminated for “disposal”, for storage on the territory of NPP as radioactive waste in repositories.

Criteria for releasing the metallic scrap

We present the articles and paragraphs with criteria for releasing the metallic scrap according to the currently applicable regulation Ordinance on Radiation Protection (ONRZ-2012) [6]:

‘Article 11 (3)’ In accordance with the criteria under ‘paragraph 2’ a radioactive material from regulated activities can be released unconditionally from regulation if the following is provided:

- the specific activity of an individual radionuclide of technogenic origin does not exceed the level for release from regulation, indicated in ‘Table 2’;
- in case of a radioactive material, containing more than one technogenic radionuclide, the sum of the ratios of the specific activity of each of the present radionuclides to the specific activity of the respective radionuclide, indicated in ‘Table 2’, does not exceed unity;

‘Article 12 (2)’ Metals can be exempt from regulation conditionally for subsequent recycling if the sum of the specific activity ratios of each of the present radionuclides to the specific activity of the respective radionuclide, indicated in ‘Table 4’, does not exceed unity. For each particular case, the applicant also justifies admissible levels of surface radioactive contamination.

An excerpt from ‘Tables 2 and 4’ (ONRZ-2012) for the radionuclides present in metallic radioactive waste, subject to deactivation, is shown below (Table 1).

Key easily measurable nuclides are Co^{60} and Cs^{137}, whose specific activity is between 75 and 95% of the sum of the specific activities of all nuclides, included in the nuclide vector. Conservatively maximum values can be assumed for the sum of the specific activities of Co^{60} and Cs^{137} – 0.75 Bq/g for the conditional release of metals subject to recycling.

***Table 1** Excerpt from 'Table 2 and Table 4' (ONRZ-2012)*

Radionuclide	***Specific activity for unconditional release (Bq/g)***	***Specific activity for release from regulation of metals, subject to recycling (Bq/g)***
Co^{60}	0.1	1.0
Cs^{137}	0.1	1.0
Am^{241}	0.1	1.0
Pu^{241}	10	10
Sr^{90}	1.0	10

EXPERIMENT

Model calculations were carried out under conditions of premises, similar to those in closed NPP units of the PWR 400 type with an Installation for metallurgical deactivation under the following conditions:

- a furnace with capacity of 2 t, 2 melting processes per shift, 250 workdays per year;
- maximum specific activity of 1,000 Bq/g for β/γ emitters.

The results of the modeling prove that the worker, removing manually the slag from the molten metal surface, receives the highest dose. The total estimated annual dose is 8.53 mSv, 6.5 mSv of which being from external irradiation. This value does not exceed the normatively defined limit of the effective dose for professional irradiation – 20 mSv per year.

The data for the secondary radioactive waste are:

- Slag – 1–4%/t; powder – 0,01%/t;
- Operational lifespan of the furnace lining – 55–110 melting processes depending on the composition and radioactive contamination of the scrap and the temperature regime of melting.

The expected annual amounts of secondary radioactive waste for the above model are:

- Slag – 15 t; Powder – 0.1 t; Refractories – 10 t.

Obtained results and analysis

In most cases the protection of the personnel from external α-irradiation is not a serious problem. The protection can be accomplished using protective clothing and gloves [6]. Light screens of aluminum, glass and plexiglass can be used against the β-radiating radionuclides.

One of the materials most commonly used for protection screens against γ-radiation is lead [6]. Because of economic reasons, in some cases lead is replaced by other materials. In terms of minimum screen weight, it is expedient to choose materials with higher density and higher atomic weight.

From a technological viewpoint there are two types of materials for protection against γ-radiation: containing lead (lead, lead rubber, lead glass) and lead-free (tungsten, reinforced concrete, bricks, water). Table 2 presents some of the characteristics of the cited protective materials.

Table 2 *Characteristics of protective materials*

Material	*Atomic weight*	*Density, g/cm^3*	*Scope of application*
Lead	207	17.3	Universal material. For transport and technological containers. Protection of measuring devices.
Lead rubber		3.3–5.8	Protection against X-ray radiation.
Lead glass		3.4–4.6	Protection against X-ray radiation. Hot chambers.
Tungsten	148	17–19	Transport and technological containers.
Depleted uranium	238	18.9	Containers.
Iron	56	7.5–10	Containers, screens, reactors.
Barite		2.0–2.7	Building structures and plasters.
Concrete		2.1–2.4	Building structures. Reactors.
Bricks		1.4–1.9	Building structures.
Water		1.0	Reactors.

Practical measures for protection of the personnel in the Installation for metallurgical deactivation

According to the requirements of the “Ordinance for Ensuring the Safety of Nuclear Power Plants”, to ensure the radiation protection of the personnel, all real and potential sources of ionizing radiation should be identified and measures should be envisaged to ensure the necessary technical and administrative control in their use.

The metallic scrap subject to remelting is the main source of ionizing radiation. In the course of the technological process the melting furnace, over which radioactive aerosols are released, becomes the main source of ionizing radiation.

Practical measures for protection of the personnel

After analysis of the available documentation and reference literature and based on the experience in this area accumulated so far, the following practical measures are defined for the effective protection of the work staff, servicing the Installation for metallurgical deactivation of metallic radioactive waste from radioactive exposure:

- an area for scrap storage, an area for ready castings and an area for storage of secondary radioactive waste have to be distinguished on the installation site. The duration of the stay of the personnel in these areas is restricted depending on the gamma-radiation dose power;
- the number of the people working simultaneously in the premises is restricted – up to 5 workers permanent staff in a shift and up to 4 people temporary staff for current facility maintenance;
- the time of sojourn of the permanent and temporary staff at the site of the Installation is restricted to the limits concerning only the specific activities to be carried out according to the regulations;

- the use of personal protective equipment according to a prescribed procedure is obligatory for the permanent and temporary staff;
- medical examinations shall be performed periodically for the workers at the Installation for metallurgical deactivation.

The radiation protection of the personnel requires that all sections of the Installation, conveyor belts and especially the control panel should be shielded. Therefore the following requirements should be also met in the design and equipment of the Installation for metallurgical deactivation:

- the premises and the components that might be contaminated with radioactive substances are designed in such a manner that they could be easily deactivated by chemical or mechanical means;
- the ventilation cleaning system should ensure: evacuation of the air from the potentially contaminated zones close to the pollution source; remote distance between the areas of discharge and influx of air and higher pressure in the zones with lower pollution than in the zones with higher pollution levels. The filtering components of the purification system should be sufficiently reliable to perform their functions with the necessary purification coefficient under all operation modes;
- in compliance with the requirements it is obligatory to ensure shielding of the section for temporary storage of the finished blocks and especially of the section for temporary storage of the secondary radioactive waste – slag, refractories, filter powder, etc. The latter should be stored in special containers of steel or concrete;
- in order to protect the work staff, continuous air control should be ensured at the site of the Installation for metallurgical deactivation for the presence of radioactive contaminants.

CONCLUSIONS

1. The theoretical grounds of radioactive contamination, generated in the process of utilization by melting of metallic radioactive waste from nuclear power plants, have been considered. Methods for protection of the personnel and the environment from radioactive emissions are indicated.

2. Model calculations have been performed under conditions of premises, analogous to those in closed NPP units of the PWR 400 type with equipment for metallurgical deactivation. The quantities of released secondary radioactive waste and the annual radiation exposure doses of the personnel are specified.

3. Practical measures are prescribed for protection of the personnel in the Installation for metallurgical deactivation from radioactive emissions.

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ASSESSMENT OF THE ENVIRONMENTAL SITUATION IN EUROPEAN COUNTRIES USING NEUTRON ACTIVATON ANALYSIS

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Abstract

The purpose of this work is a comprehensive study of the use of Neutron Activation Analysis to assess the environmental situation in European countries, and in particular in Belarus, Serbia, Greece and Norway. A total of 68 soil samples were collected in Belarus for ^{137}*Cs and* ^{129}*I studies. A total of 30 samples of moss Sphagnum girgensohnii from Serbia were examined for the presence of trace elements such as Al, V, Cr, Mn, Fe, Ni, Cu, Zn, As, and Cd. 95 moss samples of the Hypnum cupressiforme were collected in the Northern region of Greece to determine* ^{7}*Be,* ^{210}*Pb,* ^{137}*Cs and* ^{40}*K. In Norway, since 1977, atmospheric deposition of metals has been studied every 5 years using samples of moss Hylocomium splendens. Then all samples were analyzed using neutron activation analysis and other analytical technologies.*

Keywords: ecology, environmental monitoring, radionuclides, Neutron Activation Analysis

INTRODUCTION

The end of the 20th - the beginning of the 21st century is characterized by a strong anthropogenic impact on the ecosystem of various sources of radioactive and technogenic pollution. A wide range of sources of various pollution requires the development of methods for reducing the anthropogenic load on the ecosystem and the development of high-precision environmental monitoring methods.

One of the methods of environmental monitoring is Neutron Activation Analysis (NAA). NAA is actively used to detect in the soil, water, air and biological material of radioactive and unnatural for this sample elements or elements found in high or low quantities. NAA has become popular due to its accuracy in determining the elements in the sample under study. It has a high sensitivity, selectivity, does not destroy the sample under study, but requires subsequent disposal of the sample as it remains radioactive after irradiation. NAA allows the simultaneous determination of many elements in one sample and does not require complex chemical separation methods [1].

The action of the NAA is based on the neutron bombardment of the analyzed sample with a duration of several nanoseconds to hours. When exposed to neutrons, isotopes with a certain half-life and emission of gamma rays with different wavelengths that pass through a gamma spectrometer begin to form from the sample atoms, which allows determining the content of any atoms in the sample under study. Thus, thanks to this information, it is possible to determine the presence of certain elements in the sample and their concentration [2,3].

DISCUSSION

The constant increase in the concentration of artificial radionuclides and abnormal quantities of unusual elements in the ecosystem causes a deterioration of the ecological background of the planet, which further affects people's health.

The accident at the Chernobyl Nuclear Power Plant in 1986 led to irreversible contamination of the vast territories of Belarus with various radionuclides for millions of years. Over the past 33 years after the catastrophe, an enormous amount of research has been conducted using various methods to study the effects of radionuclides on organisms and its distribution in the ecosystem. Among such methods, NAA can be distinguished.

For example, study has been conducted on the distribution of ^{137}Cs and ^{129}I in the Republic of Belarus 15 years after the accident at the Chernobyl Nuclear Power Plant by Mironov *et al*. [4]. Samples of soil from territories that were not studied before were used as an object of study, and NAA was the method of determining the presence and concentration of elements in the object. Soil samples were collected from the south-eastern part of the country near the Chernobyl Nuclear Power Plant (Figure 1).

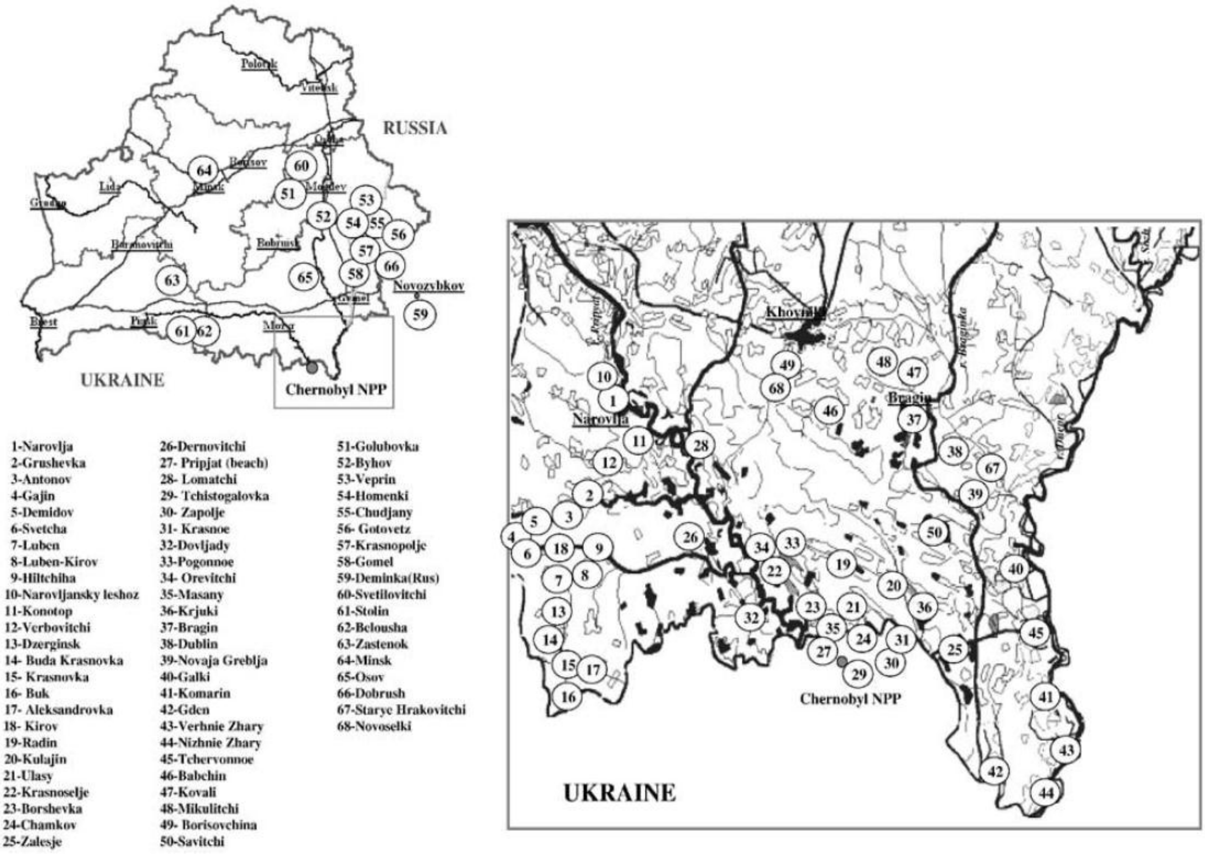

***Figure 1** Map showing the locations of the soil sampling sites from the study of Mironov et al. [4]*

Based on the results of research obtained through the use of NAA, table 1 presents the corresponding values for ^{137}Cs and ^{129}I [4].

Table 1 *The relation between concentrations of* ^{129}*I and* ^{137}*Cs in soils in different regions of Belarus according to Mironov et al. [4]*

Map number	Sample site	^{129}I (*10^{10} at/kg)	^{137}Cs (kBq/kg)	Fractionation factor
19	Radin	510 ± 130	35 ± 4	0.73 ± 0.20
36	Krjuki	1100 ± 280	45 ± 5	1.2 ± 0.3
46	Babchin	57 ± 14	3.1 ± 0.3	0.94 ± 0.25
49	Borisovchina	12 ± 3	0.82 ± 0.08	0.75 ± 0.20
55	Chudjany	260 ± 70	30 ± 3	0.44 ± 0.12
61	Stolin	50 ± 14	1.7 ± 0.2	1.5 ± 0.4
66	Dobrush	140 ± 40	4.7 ± 0.5	1.5 ± 0.4

Thus, the use of NAA gives researchers and scientists in the field of ecology and other scientific disciplines a fairly accurate determination of the presence of elements in objects of animate and inanimate nature. This information is very important in assessing the migration of radionuclides in the atmosphere and the future determination of the effectiveness of various measures to eliminate the negative effects of not only the Chernobyl Nuclear Power Plant, but also other technological accidents and disasters of various sizes [3].

Another example of the use of NAA in ecology is the study of atmospheric deposition of trace elements in urban areas of Belgrade, Serbia, from the study of Aničić *et al*. [5]. The urban atmosphere is exposed to a large anthropogenic impact of pollutants from stationary (industry, CHP) and mobile (motor) sources. Trace elements are widely distributed in the environment, and their interaction with various components of nature leads to a toxic effect on the biosphere (Figure 2) [5].

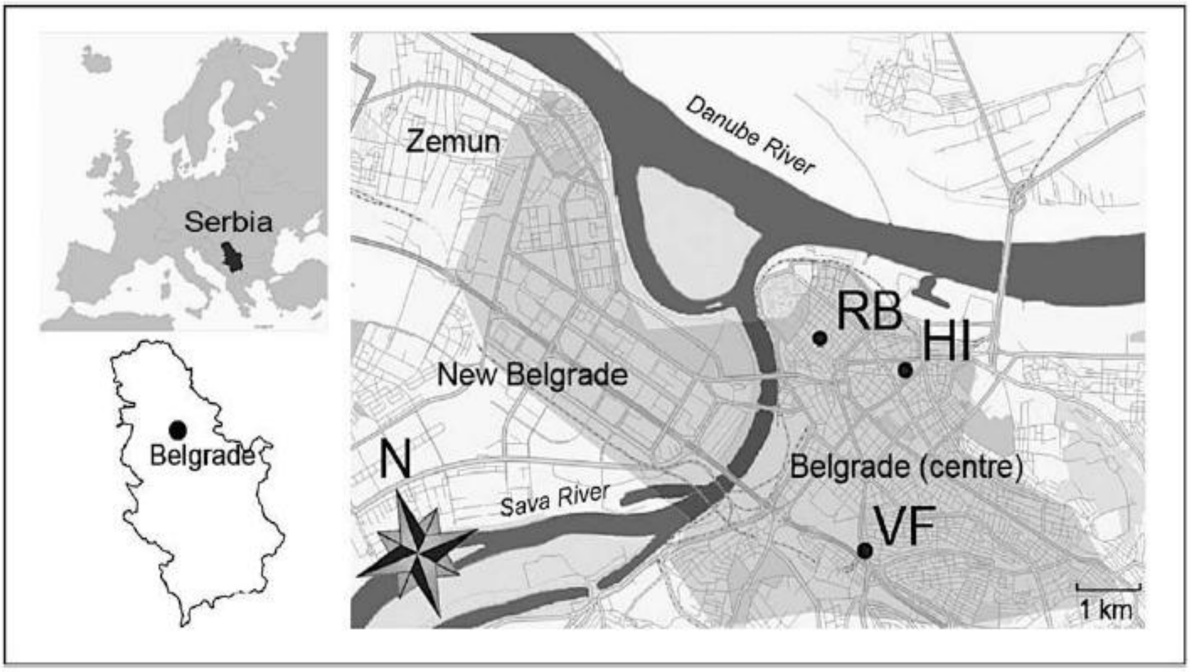

Figure 2 *Location of the sampling sites in Belgrade urban area: Rector's Office building (RB); Faculty of Veterinary Medicine (VF); Public Health Institute (HI), according to Aničić et al. [5]*

The object of the study was the moss-biomonitor *Sphagnum girgensohnii*. The moss was packed in special bags installed in three typical urban areas for three three-month periods (July 2015 - October 2016) (Figure 2) [5].

The research method was NAA, which determined the concentration (mg g^{-1} dry weight) Al (1043), V (2.1), Cr (1.7), Mn (173), Fe (864), Ni (1.4), Cu (7.6), Zn (36), As (0.67), and Cd (0.3) [5].

In the Northern region of Greece in 2016, Betsou *et al*. [6] conducted studies of atmospheric deposition of heavy metals and radionuclides released into the ecosystem due to various technogenic impacts, and in particular the accident at the Chernobyl Nuclear Power Plant in 1986. A total of 95 samples of mosses from *Hypnum cupressiforme* were selected, according to whose coordinates a map of the ^{137}Cs radionuclide contamination level of the Northern regions of Greece was constructed (Figure 3).

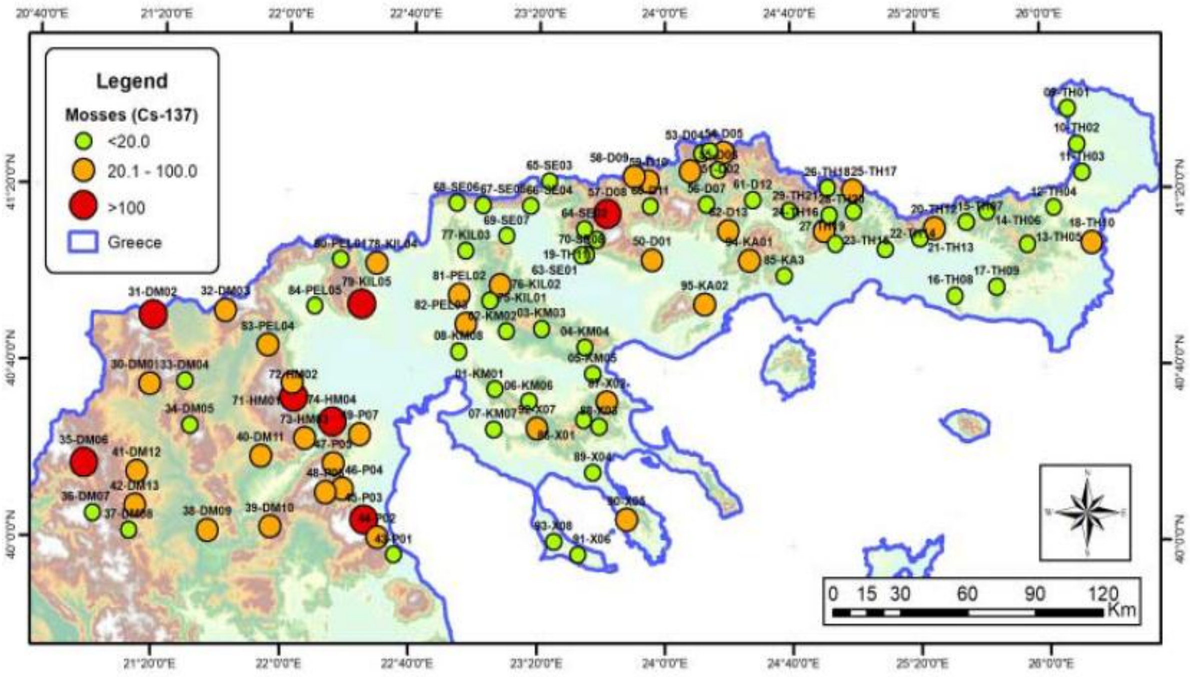

***Figure 3** The activity concentrations of ^{137}Cs in moss collected in the Northern Greece from the study of Betsou et al. [6]*

The results of contamination of the territory of the Northern region of Greece with heavy metals were also obtained using the Instrumental NAA (Table 2) [6].

***Table 2** Heavy metal concentrations (μg g^{-1}) in moss H. cupressiforme in Greece in during summer 2016, according to Betsou et al. [6]*

	Al	As	Cr	Fe	Ni	V	Zn
Min	1350	0.52	2.04	1010	1.72	2.61	14.60
Max	46.10	17.90	222.00	28700	138.00	33.40	282.00
Median	6160	1.62	14.70	4630	10.00	8.66	38.30
Stand. Err.	648	0.39	4.47	502	2.57	0.67	4.91

Monitoring of atmospheric fallout of heavy metals in Norway using moss *Hylocomium splendens* as a biomonitor began in 1977 and was repeated every 5 years. NAA (until 1985) and atomic absorption spectrometry were used as a research method. The results of the study for 1977 and 2005 for Zn and As are presented below (Figure 4) [7].

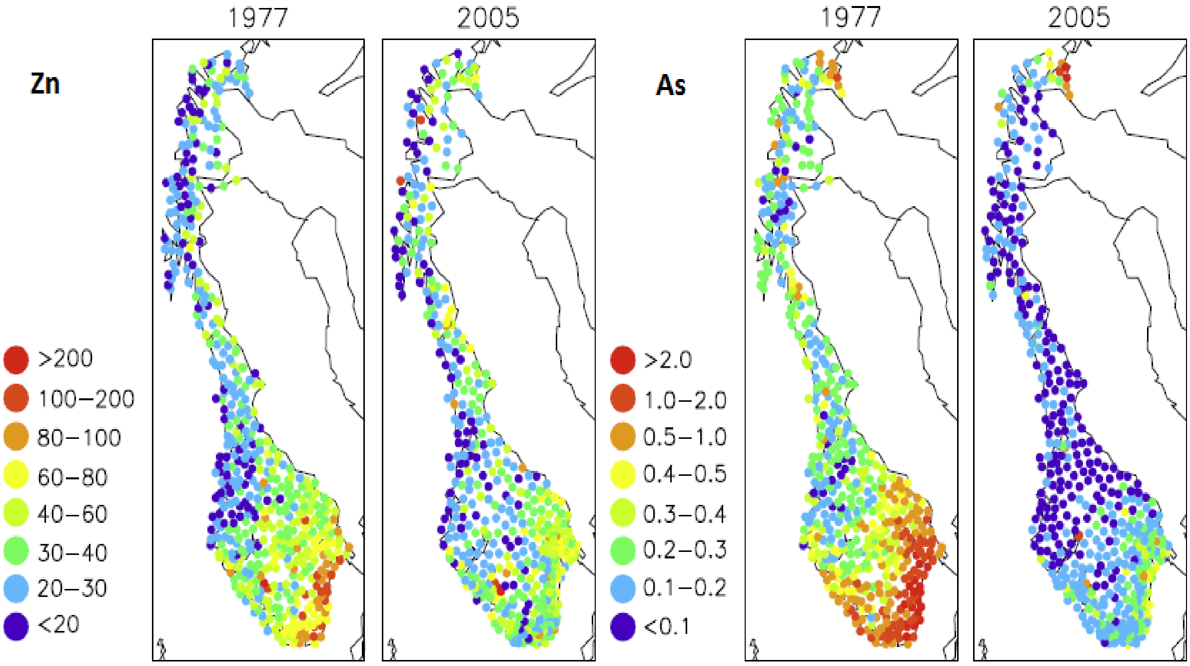

Figure 4 *Zn and As concentrations in moss samples in Norway 1977 and 2005 (mg kg^{-1}) from the study of Steinnes et al. [7]*

CONCLUSION

NAA is currently one of the most reliable methods for determining the set of elements in the sample under study, although it is gradually being superseded by more modern technologies. Despite its economic value and demands in specially trained personnel, this method is still actively used to assess the state of the environment and in many other areas of scientific research [2].

In the rapidly developing modern world, continuous improvement of the reliability and operability of sources of radiation and technogenic pollution of the environment is required to avoid the risk of man-made disasters. Also, a large role is assigned to regular monitoring of the state of the environment in order to predict the environmental situation and warn the population about the danger, and one of the roles in this area belongs to the NAA [3].

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ASSESSMENT OF ANNUAL EFFECTIVE DOSE DUE TO INGESTION OF ^{137}Cs, ^{40}K AND ^{210}Pb IN MEDICINAL HERBS FROM SERBIA AND FROM MONTENEGRO

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Abstract

Three selected medicinal herbs (Hypericum perforatum L., St. John's wort, kantarion, Rosa canina L., Dog rose, šipurak, and Artemisia absinthium L., Wormwood, pelin) from the regions of Serbia and of Montenegro have been analyzed from the radiobiological point of view. Specific activities of radionuclides ^{137}*Cs,* ^{40}*K, and* ^{210}*Pb in samples of these medicinal herbs were measured using semiconductor HPGe spectrometer system. Individual annual effective dose of ingestion of the radionuclide based on measured activity concentrations are: less than 7.2 nSv for* ^{137}*Cs, in the range (1.4 ÷ 3.4) μSv for* ^{40}*K, and in the range (1.0 ÷ 6.6) μSv for* ^{210}*Pb. Thus, the values obtained for effective dose, presented in this investigation, are less than 100 μSv, which means that the daily use of 200 ml herbal infusion from the region of Mt. Kopaonik, Sokobanja, Mt. Zlatar, Majdanpek (Serbia) and Mt. Durmitor (Montenegro) does not represent a significant radiological risk for human health. Nevertheless, more attention should be paid to the investigation concerning individual annual effective dose of ingestion of radionuclides in medicinal herbs, especially for radionuclide* ^{210}*Pb since it is not sufficiently investigated worldwide.*

Keywords: medicinal herbs, radioactivity, annual effective dose

INTRODUCTION

Natural radionuclides are present in everything that surrounds us and even in ourselves, but in very small quantities. The main sources of natural radioactivity in the terrestrial environment are ^{40}K and the radionuclides of the uranium and thorium series [1]. On the other hand, contamination of the environment with anthropogenic radionuclides appeared as a result of different application of radionuclides in every day's life, particularly after the nuclear accident in Chernobyl in 1986.

There are some areas on Earth where the concentration of natural radionuclides in soil is much higher than elsewhere, and plants that grow on such soil have higher uptake of radionuclides. The same statement is valid for contamination with artificial radionuclide, especially for ^{137}Cs as the main source of plant contamination, because ^{137}Cs deposited into the soil can be accumulated into the herbs by their root system [2]. Thus, the concentration of radionuclides may have different levels in various plants and their parts, among others in herbal medicines.

In recent decades, medicinal herbs have increasing use in modern therapies, and for preparation of herbal infusions as refreshing drinks. Due to increase use of herbal infusions it is important that their use be safe concerning human health which requires monitoring as well as the assessment of radiological risk. Thus, it is essential to have in mind that it is recommended to use herbal infusions during a longer period of time for the purpose of medicinal treatment, and it can lead to the accumulation of harmful substances in the organism, and consequently to the chronicle radiation exposure. They tend to accumulate in various tissues of the human body; lead in skeleton and hair [3] and caesium and potassium in muscles [4].

St John's wort belongs to the Clusiaceae family and originates from Minor Asia, Greece and Siberia. St. John's wort is an herbaceous perennial plant which blooms from May till September. It grows on grass meadows, along the edge of forests and roads mostly in moderate and subtropical climate. St. John's Wort is collecting at the time of flowering in July and used as an antidepressant, sedative, antibiotic, and can be used externally and internally [5].

Dog rose belongs to the rose family (Rosaceae). It is widespread in Europe, most of Asia and North Africa, and grows in the shrubs area, forest glades, meadows, burned areas and roadsides. Dog rose is a deciduous shrub, which blooms from May till July and fruit ripens in September and October. The unripe fruits are collecting at the end of the summer or at beginning of the autumn, has many uses, e.g. preparation of refreshment drink, which has a beneficial effect on the heart and kidneys [5].

Wormwood (Asteraceae family) is native species to temperate regions of Eurasia and Northern Africa, while, in Canada and the northern United States, is widely naturalized. It grows on uncultivated arid ground, on rocky slopes, and at the edge of footpaths and fields. Wormwood is an herbaceous perennial plant flowering from early summer to early autumn. As medicine, the apical herbaceous parts at the time of flowering (July to September) are collecting and are used for dyspepsia and for various infectious diseases [5].

As it is already mentioned St John's wort, Dog rose and Wormwood are suitable for preparation of herbal infusion, but literature data on the recommended amount of dry medicinal herb for preparation of infusion are different from source to source. In Table 1, the list of selected medicinal herbs analyzed in this study, used part of the herbs, as well as the averaged values of the recommended amount of dry medicinal herb for preparation of 200 ml infusion is presented [2,6-8].

The objective of the present work is to determine the activity concentrations of ^{137}Cs, ^{40}K and ^{210}Pb in three different medicinal herbs: St John's wort, Dog rose and Wormwood (kantarion, šipurak, pelin, respectively names on native languages), and to calculate the individual annual effective dose due to ingestion of ^{137}Cs, ^{40}K and ^{210}Pb present in herbal infusions.

Table 1 *Recommended amount of dry medicinal herb for preparation of 200 ml herbal infusion*

Common English and *native languages names	Scientific name	Family affiliation	Part of the plant	Recommended amount [g]
St John's wort, *kantarion	*Hypericum perforatum* L.	Clusiaceae	Leaf and flower	2.5
Dog rose, *šipurak	*Rosa canina* L	Rosaceae	Fruit	3.0
Wormwood, *pelin	*Artemisia absinthium* L.	Asteracea	Leaf	2.0

MATERIALS AND METHODS

Samples of three different medicinal herbs, St John's wort, Dog rose and Wormwood, originating from the regions of Mt. Kopaonik, Sokobanja, Mt. Zlatar, and Majdanpek (Serbia) and Mt. Durmitor (Montenegro) were purchased on green markets during the spring and summer of 2018. Already dried samples were prepared in a laboratory blender to fine powder, and stored in a suitable standard cylindrical PVC container of 125 ml volume. The mass of each individual sample was approximately 30 g, adequate to working standard which contains dried grass as a matrix, with similar biological composition. The matrix of dried grass was spiked with radioactive solution with common mixture of gamma ray emitters (^{241}Am, ^{109}Cd, ^{139}Ce, ^{57}Co, ^{60}Co, ^{137}Cs, ^{113}Sn, ^{85}Sr, and ^{88}Y) purchased from the CMI (Czech Metrology Institute) [9]. The working standard was prepared in Laboratory for nuclear and plasma physics, University of Belgrade - Institute of Nuclear Sciences “Vinča” [10,11]. All samples were measured in close to detector geometry by coaxial HPGe spectrometer: Canberra GX5019, with 55 % relative efficiency and 1.9 keV resolution for ^{60}Co at the 1332.5 keV. Spectra of medicinal herbs samples were recorded and analyzed using Canberra’s Genie2000 software. In order to achieve acceptable statistics, the samples were measured up to 585 000 s.

The activity concentrations of radionuclides ^{137}Cs, ^{40}K, and ^{210}Pb in medicinal herbs were determined directly by analyzing full-energy peaks at 661.66 keV, 1460.82 keV, and 46.54 keV, respectively, taking into account corrections for background. It should be noted that the net peak area at 1460.82 keV was corrected for the contribution of the ^{228}Ac peak at 1459.13 keV energy. The value of uncertainty of measurement was determined by applying the general law of propagation of uncertainty. The largest contribution to the total uncertainty was due to the statistical uncertainty (in some samples more than 50 % for ^{137}Cs) and efficiency calibration (5 %), whereas uncertainty due to measured mass of the sample could be neglected.

The average annual effective dose that individual adult receives due to ingestion by daily consumption of 200 ml herbal infusion is calculated according to the following formula [2,7,12]:

$$E_{\mathrm{ing}} = A_{\mathrm{s}}\, H\, C_{\mathrm{d}}\, DF_{\mathrm{ing}}$$

where E_{ing} is the individual annual effective dose due to ingestion of specific radionuclide in the herbal infusion [Sv], A_s is the activity concentration of specific radionuclide in medicinal herb sample [Bq/kg], H is the annual amount of consumed medicinal herb [kg], C_d is the dissolution coefficient for specific radionuclide in herbal infusion, and DF_{ing} is the effective dose coefficient for ingestion of specific radionuclide [Sv/Bq].

The values of dissolution coefficient for specific radionuclide in herbal infusion are 0.6 for ^{137}Cs [12, 13], 0.9 for ^{40}K [14] and 0.5 for ^{210}Pb [15]. The effective dose coefficient for ingestion of specific radionuclide: 1.3 10^{-8} Sv/Bq for ^{137}Cs, 6.2 10^{-9} Sv/Bq for ^{40}K and 6.8 10^{-7} Sv/Bq for ^{210}Pb [16,17].

RESULTS AND DISCUSSION

The calculated values of the individual annual effective dose due to ingestion of specific radionuclide in the herbal infusion are shown in Table 2.

Table 2 *The calculated values of the individual annual effective dose due to ingestion of specific radionuclide in the herbal infusions with uncertainties of measurement, at the confidence level 1 σ*

Radionuclide	Herb	Kopaonik	Sokobanja	Zlatar	Durmitor	Majdanpek
^{137}Cs [nSv]	St John's wort	< 2.1	< 2.1	3.8 ± 1.6	< 2.1	< 2.1
	Dog rose	6.4 ± 2.1	< 1.7	< 2.6	< 2.6	< 2.6
	Wormwood	< 1.1	3.1 ± 1.5	< 1.7	7.2 ± 1.9	7.1 ± 1.6
^{40}K [μSv]	St John's wort	1.6 ± 0.1	1.7 ± 0.1	2.0 ± 0.1	1.4 ± 0.1	1.6 ± 0.1
	Dog rose	3.4 ± 0.2	1.6 ± 0.2	1.8 ± 0.1	2.2 ± 0.1	2.1 ± 0.1
	Wormwood	1.6 ± 0.1	3.1 ± 0.2	2.0 ± 0.1	1.7 ± 0.1	2.2 ± 0.1
^{210}Pb [μSv]	St John's wort	4.3 ± 0.6	3.6 ± 0.3	6.6 ± 0.6	3.6 ± 0.7	2.9 ± 0.4
	Dog rose	1.4 ± 0.2	2.1 ± 0.4	1.0 ± 0.2	2.6 ± 0.4	1.5 ± 0.2
	Wormwood	2.9 ± 0.2	4.0 ± 0.6	3.8 ± 0.4	4.6 ± 0.5	4.4 ± 0.5

The values of individual annual effective dose due to ingestion of ^{137}Cs, ^{40}K and of ^{210}Pb in the infusion prepared from medicinal herbs collected in investigated regions are less than 7.2 nSv for ^{137}Cs, in the range (1.4 ÷ 3.4) μSv for ^{40}K, and in the range (1.0 ÷ 6.6) μSv for ^{210}Pb and are lower than 100 μSv which represents the recommended limit for individual annual effective dose due to ingestion [18]. The results obtained for ^{137}Cs and ^{40}K are in accordance with data given in available literature [2, 6, 19, 20]. On the other hand, in the available literature there are no data for ^{210}Pb.

CONCLUSION

The values of individual annual effective dose due to ingestion of ^{137}Cs, ^{40}K and ^{210}Pb in the infusion prepared from medicinal herbs analyzed in this study are less than 7.2 nSv, 3.4 μSv and 6.6 μSv, respectively, and are lower than 100 μSv which represents the recommended limit for individual annual effective dose due to ingestion [18]. Having in mind that obtained results showed that daily use of herbal infusion prepared of selected medicinal herbs from the regions of Mt. Kopaonik, Sokobanja, Mt. Zlatar, and Majdanpek (Serbia) and

Mt. Durmitor (Montenegro) are below recommended limit it can be concluded that there is no significant or enhanced radiological risk for consumers.

However, it should be noted that the content of radionuclides ^{210}Pb in medicinal herbs in general is not sufficiently investigated. Therefore, since the individual effective annual dose due to ingestion of ^{210}Pb is slightly higher than that of ^{40}K and significantly higher than that of ^{137}Cs it should pay more attention to this topic.

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INTEGRATED PEST MANAGEMENT IN COTTON GROWING

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Abstract

The problem of all cotton producers is possible harmful impacts on the plant, which can reduce the yield and quality of cotton. In order to reduce these impacts, different pest control techniques are applied. One of the measures to increase sustainability of pest management in cotton production, including the reduction of pesticides use, is the application of Integrated Pest Management (IPM). In this paper, integrated pest management in the cotton growing is discussed.

Keywords: cotton, pests of cotton, Integrated Pest Management

INTRODUCTION

Cotton is one of the most important natural fiber and causes very polluting loads, especially wastewater, which comes not only from the raw materials and auxiliary materials used in the processes of pre-preparation, dyeing, printing and finishing, but also from the fibers themselves (various types of protection and stimulus in growing) and auxiliary agents used for spinning and winding. Also, different cotton growing practices have different ecological loads [1,2]. Efforts to increase sustainability of pest management in cotton production, including the reduction of pesticide use, are largely based on the application of some form of Integrated Pest Management (IPM) [3]. Food and Agriculture Organization of the United Nations (FAO) defines it as: "IPM means the careful consideration of all available pest control techniques and subsequent integration of appropriate measures that discourage the development of pest populations and keep pesticides and other interventions to levels that are economically justified and reduce or minimise risks to human health and the environment. IPM emphasises the growth of a healthy crop with the least possible disruption to agro-ecosystems and encourages natural pest control mechanisms" [4].

COTTON PROTECTION FROM HARMFUL IMPACTS

Cotton fibers are obtained from seeds of cotton plants, which include 40 species in the genus *Gossypium* (Malvaceae family), some of which are used for commercial purposes. These fibers account for about 30% of the world's fiber production. Cotton is growing in over 100 countries. It is estimated that cotton is growing on 34 million hectares of plantations annually [4] and that as many as 100 million households are directly involved in cotton production. It is also estimated that around 300 million people work in the cotton sector.

According to the data for 2018, the largest cotton producers in the world are India, China and the United States, followed by Brazil, Pakistan, Australia, Turkey, etc.

The problem of all cotton producers is possible harmful impacts on the plant, which can reduce the yield and quality of cotton. Namely, after planting, cotton plants must be protected from impacts that can damage the crop, in particular: insects, nematodes, infectious diseases and weeds [1]. There are different types of pests, and some of them are shown in the Table 1, along with symptoms of damage to cotton plants [5].

***Table 1** Different types of pests and damage symptoms to cotton plants [5]*

Phase development in cotton / Name of insect / Scientific name	Phase development in cotton / Name of insect / Scientific name
	
Seedlings/ Trips / Thrips tabaci (Thripidae: Thysanoptera)	*Seedlings / Cotton Aphid / Aphis gossypii (Aphididae: Homoptera)*
	
Seedling / Cricket / Gryllotalpa gryllotalpa (Gryllotalpidae: Orthoptera)	*Seedling / Worm / Agrotis ipsilon (Noctuidae: Lepidoptera)*
	
Growing/ Worm / Spodoptera exigua (Noctuidae: Lepidoptera)	*Growing / Cotton leaf worm / Spodoptera littoralis (Noctuidae: Lepidoptera)*
	
Growing / Ehite fly / Bemisia tabaci Or. Hemiptera	*Growing / Jassid / Empoasca lybica (Cicadellidae: Homoptera)*
	
Fruiting/ Worm / Heliothis zea (Noctuidae: Lepidoptera)	*Fruiting / American bollworm / Heliothis armigera (Noctuidae: Lepidoptera)*
	
Fruiting / Pink bollworm / Pectinophora gossypiella (Gelechiidae: Lepidoptera)	*Fruiting / Worm / Earias insulana (Noctuidae: Lepidoptera)*

In the conventional growing of cotton as a protection against these pests, various pesticides are generally used. There are three main primary pesticide groups: insecticides (targeting insect and other arthropod pests); fungicides (substances that target fungal diseases of crop plants) and herbicides (targeting unwanted plants, i.e. weeds). Ideally, a pesticide would act only on the specific target, unwanted organism, without risk or harm to non-target animals (including humans), microbes or plants. Most pesticides, however, can affect a fairly broad range of organisms, i.e. they are non-selective in their action. Most of the older generation insecticides can kill or harm a broad spectrum of insects and arthropods, including beneficial ones such as honey bees. Organophosphate and carbamate insecticides are very broad-spectrum. The most used active ingredients are mainly insecticides, and cover several pesticides groups (Table 2) [4].

Table 2 *Pesticides that are widely used in cotton growing*

Insecticides				Herbicides
Organophosphate	Pyrethroids	Neonicotinoids	Organochlorine	
Acephate	Cypermethrin	Imidacloprid	Not widely, but still used	Glyphosate
Chlorpyrifos	Deltamethrin	Thiamethoxam		**2,4 D**[**]
Monocrotophos	λ-Cyhalothrin	**Acetamiprid**[*]	Endosulfan	
Profenofos				

[*] The pesticides in bold are not included in the PAN (Pesticide Action Network) list of Highly Hazardous Pesticides (HHPs)

[**] 2,4 D (2,4-dichlorophenoxyacetic acid)

Insect control – Many countries subsidize pesticides use, which can lead to excessive pesticide use, as well as the insect resistance to specific pesticides. There are significant indirect losses as a result of the destruction of useful insects and the development of resistance to insecticide [6].

Nematodes control – Chemical control of nematodes is the most widely used method for reducing losses caused by plant parasites. These methods use the chemical control agents to protect the early season, to reduce the initial population of plant parasites [7].

Diseases control – There are many causes of the disease of cotton plants around the world (bacteria, fungi). Most diseases are controlled by seed treatment. Fungicidal chemicals for the protection of seeds and seedlings can be used [8].

Weed control – The most important method for weed control is chemical – herbicide application. Types of herbicides vary depending on the type of weeds, local conditions and application. There are potentially harmful effects of pesticides and herbicides on humans and nature [9].

Integrated Pest Management in cotton growing

Over the last few decades, over-dependence on chemical control of cotton pests (including insects, pathogens, weeds, etc.) has led to many adverse effects, notably changes in pest and insect status, an increase in sticky cotton, development of resistance, environmental pollution and health problems [4,10]. These problems have led to a greater awareness of the need for integrated pest management (IPM) or, in more practical terms, integrated crop management.

Integrated pest management can be defined as the practice of preventing or suppressing damaging populations of insect pests by application of the comprehensive and coordinated integration of multiple control tactics. Tactics are the various control methodologies, e.g., cultural, biological, and chemical. Strategies are the planned manipulations undertaken to optimize the dynamic integration of control methodologies in the context of their economic, environmental and social consequences. Ideally, control methods cause minimal environmental damage and pose little or no risk to human health. Components of IPM are: crop rotation, time of planting, closed season, cultural control, quarantine, host plant resistant, behavioral control, biological control, resistance inducers, chemical control and resistance management, into a single pest control efforts [5,11].

Crop rotation is important to control pest and nematodes. In many cotton-producing areas practical rotation crops are limited by environmental or economic factors. Care must be exercised in selecting appropriate rotation crops; they must be poor or non hosts for the nematode, yet economically feasible to. Also, rotations, tillage, and fertilizer treatments can affect yield, costs, and profitability, depending on their effects on pests. Rotation or planting different crops reduces soil erosion and pests that build up when a field is planted to the same crop each year. Minimum tillage reduces the number of trips over a field, lessening soil compaction and reducing costs [5].

Adjustment planting time to escape pest damage is the most important means of keeping pest damage bellow economic levels, and can help the crop to avoid the infestation of some pest.

Closed season is legally required in most cotton growing countries, but is difficult to implement fully where growers retain the stalks as a fuel. Better removal of unharvested bolls reduces the risk of pink bollworm survival with stalks. Shredding and ploughing in crop residues is the best policy, as this improves the organic matter content in the soil. This legislation stresses that all cotton plants should be uprooted and destroyed (or burned) by a certain date and quite clearly no seed would be planted until the following rain arrives. This approach to pest control is applicable to the tropics, while in temperate regions there is already stablished very firmly a close season for virtually all crops, namely winter.

Cultural control and the influence of production practices such as cultivation, irrigation, fertilization, cultivar selection, weed control and planting date on the crop's susceptibility to damage and suitability for insect infestations remain an extremely important aspect of effective pest management. For example, stalk destruction, field sanitation, efficient harvest, tillage and winter irrigation can effectively control or reduce populations of some pests. Early planting and early crop termination are long-standing principles of cultural control and pest avoidance that are still relevant for many pest species, and in some regions help to avoid fall and winter rains and resulting in important economic advantages. Delayed planting also may contribute to the reduction of some pests population. The application of colonization of crops of some cultures (eg alfalfa) through the trap of cotton crops has been restored, but it is rarely applied due to the wide applicability of effective chemical control options [7].

By forming **quarantine zones** that prohibit the planting of cotton to prevent the spread of certain pests, the threat of the spread of pests and diseases is reduced as a result of moving cotton seeds from infected to non-infected areas.

Host plant resistance can be broadly categorized as antibiosis (reduced fitness or pest status), antixenosis (avoidance or behavioral factors) and tolerance (ability of plant to compensate for damage). Genetically controlled traits useful in cotton resistance to insects include: crop earliness, a range of plant morphological and varying concentrations of plant secondary compounds. The tools of biotechnology have provided new opportunities to enhance traditional approaches to host plant resistance.

Behavioral control of some insects leads to population suppression or even elimination. First of all, it refers to the use of pheromones and sterile insects. A pheromone is a chemical that mediates behavioral interactions between members of the same species. The most common examples are sex pheromones which are involved in mating, but aggregation and alarm pheromones are also known from cotton pest species. The main applications of pheromones are monitoring, mating disruption and mass trapping (lure and killing). One of the techniques of sterile insects is the production of sterile insects by exposing the sublethal dose of gamma radiation, and another technique is the introduction of deadly mutations in the insect genome.

Biological control includes three major approaches: classical biological control, where exotic agents are introduced for permanent establishment against exotic and native pests, augmentation biological control, which involves the rearing and periodic release of natural enemies, and conservation biological control, which attempts to protect, manipulate and enhance existing natural enemies for improved control.

Some acids (jasmonic, salicylic), present in many plants, are considered as **inductors of resistance**, involved in regulating different plant functions, such as plant resistance and aging.

Chemical control implies the use of insecticides, which, in addition to their broad activity against pests, pose hazards to the environment and human health. The use of chemical pesticides should remain as the latest choice in IPM and be used in minimum quantities.

Resistance management – The development of resistance in pests population to insecticides is a continual threat to successful implementation of chemical control. The mitigation of resistance is based on insecticide type, which means: limiting insecticide use through adherence to economic thresholds, diversifying modes of action through rotations, mixtures and use of synergist, partitioning of insecticide use in space and time by adoption of seasonal stages or crop-specific usage [7].

Implementation of IPM

There are a lot of information required to be able to implement the IPM on a crop or an area. These include the following information: correct pest identification; understanding of pest and crop dynamics; planning preventive strategies; monitoring; decision making; selection of optimal pest control tactics to manage the problem while minimizing economic, health and environmental risks; implementation; evaluation.

CONCLUSIONS

The methods used in cotton pests control have passed through several stages during the last two decades, varying from manual control to extensive use of chemical control to the encouragement of using the biological control in the frame of the integrated pest management under the wide strategy of integrated crop management. The current situation of the cotton insect pests control tends to extend the use of the environmental friendly methods.

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DEPOSITING CADMIUM AND THE THERAPEUTIC EFFECT OF α-LIPOIC ACID IN THE TISSUE OF LIVER AND KIDNEY RATS

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Abstract

Pollution of air, soil and water by cadmium is a great ecotoxicological problem that greatly negatively affects the health of people and all other living beings. Professional exposure to the action of cadmium will manifest itself in a series of disorders and diseases, especially in the organs where this metal is deposited. In this regard, the toxicological aspect and content of the mentioned metal as well as the physiological role of α-lipoic acid in the homogenates of the liver and kidney tissue in the albino rats of Wistar soy was examined. The content of toxic metals in tissue homogenates was determined by the method of potentiometric striping analysis. The measured content of metal in the liver and kidney was higher in animals that received the supplement on the day after poisoning, which by binding to the metal contributes to its retention in the tissue. According to the structure, the α-lipoic acid exhibits an affinity for lead binding, resulting in covalent modification and the change in the pathophysiological properties of the toxic metal itself.

Keywords: cadmium, α-lipoic acid, depositing, liver, kidney

INTRODUCTION

Since ancient times was known toxic effects of heavy metals on the body of living creatures, including humans, so that the heavy metals are among the oldest poisons. For the absorption of heavy metals from the most significant air intake through the respiratory tract and by inhalation in the form of an aerosol or a vapour into the lungs where are absorbed, and then delivered via the bloodstream to the target organs. Via contaminated foods heavy metals due to the intestinal tract, and then enters the absorption of blood, and in certain organs which are being deposited. Mainly the heavy metals are deposited in the target organ, such as bone, kidney, liver or brain. The most significant amounts of heavy metals are excreted via the urine and the digestive tract [1].

Cadmium (Cd) as a metal was discovered in 1817. The use of cadmium is large, for example in the production of nickel-cadmium batteries, ceramics, paints, textiles and plastics. Mining and smelting zinc and lead cadmium is released into the environment [2,3]. Inhaling through the respiratory system cadmium from the environment enters the lungs. Through cigarette 0.1-0.2 μg of this element enters the human body. Inhalation of smoke with high cadmium content may cause acute pneumonitis and pulmonary edema. Also, through contaminated food or beverages infused much of the cadmium in the human body. Acute cadmium intoxication leads to nausea, vomiting, abdominal pain. In blood, cadmium binds to red blood cells and blood proteins such as albumin or metallothionein. About 50-70% of the total content of cadmium is deposited in the liver and kidneys [4]. The effects of chronic exposure to low concentrations of cadmium in the working environment are manifested as chronic obstructive pulmonary disease, emphysema, lung fibrosis and chronic renal failure [5].

It has been observed that cadmium affects the metabolism of calcium in the body, so in people with nephropathy, osteomalacia, osteoporosis and deformation of the bone system are also observed. Of great importance for the organism's transport and distribution of cadmium, which is bound to metallothionein [6]. Cadmium bound to metallothionein is accumulated in the liver, kidneys, salivary glands, pancreas, and in minor amounts can also be found in other tissues, in particular of glandular (thyroid and adrenal glands). After 24 hours the cadmium concentration in most tissues remains constant, except for the kidneys which is gradually increasing. Cumulating in the renal cortex in this way is explained by the long half-cadmium (about 30 years). Part of cadmium is excreted via the respiratory tract, salivary gland, a part with skin, sweat and sebaceous glands, a certain percentage of cadmium is excreted via urine [7].

Nutritional supplements such as melatonin (N-acetyl-5-methoxy-tryptamine), N-acetylcysteine (NAC), thiamine, and methionine were found to be potent antioxidants which enhance the effectiveness of the chelating effect of the treatment with cadmium intoxication. It is known that chelators and antioxidants protect against potential adverse effects of reactive oxygen species and of lipid peroxides [8].

α-lipoic acid (α-LA) was the cyclic disulfide and the same is over the carboxyl group linked to the protein portion of an enzyme as an amide. In the chemical view, 6,8-dithioctanoic acid is present. Belongs to the group of fatty acids, and it is very important for the performance of the proper metabolism. Alpha lipoic acid is the most efficient known antioxidants for their lipo and hydrosolubility which allows the easy penetration into the cytoplasm to participate in the protection against free radicals, reactive, energy metabolism, regulation of gene expression, and so on [9,10].

In animal models is in the course of subacute intoxication investigated the effect of cadmium and studied its consequence a high concentration in the target organs. It is also accompanied by the effect of supplement of added α-lipoic acid and accumulation of cadmium in the target organs.

MATERIALS AND METHODS

In vivo tests

The experimental model to study the toxic effects of cadmium and protective roles α-lipoic acid was studies on albino Wistar rats, females of 2-3 months, the average weight of about

250-300 grams. Animals were kept in metal cages and acclimated to the laboratory conditions in the vivarium Scientific Research Center for Biomedicine Faculty of Medicine. Experimental animals were grown in the laboratory, where they feed and water were available ad libitum. The rats were divided into 2 groups. The first group of rats was administered cadmium (II) chloride [11]. The second group of rats, in addition to cadmium (II) -chloride received α-lipoic acid [12]. All chemicals were of analytical grade purity, manufactured by Merck. After the extraction is carried out anesthesia organs, the liver and kidneys in order to determine the concentration of metal, ie. cadmium in the tissue homogenates. In experimental work is fully respected the Code of Ethics of scientific research Faculty of Medicine in Nis.

Determination of the metal concentration in the tissue homogenates of liver and kidneys

Toxic metal content in the tissue homogenates was determined by potentiometric stripping analysis (PSA). The mineralization of the tissue homogenate was performed nitric acid ($C = 6$ ml/dm^3), followed by treatment with hydrochloric acid and finally by dissolving in deionized water twice. Heavy metal content was measured by the potentiometric stripping analyser (Universal Leskovac-TMF Novi Sad) [13].

STATISTICAL ANALYSIS

All measurement results are shown as the mean-AVERAGE. Statistical analysis of the results was done by Student's t-test for independent samples (Microsoft Office Excel). Results of these analyses are presented in tables.

RESULTS AND DISCUSSION

The content of heavy metals in the investigated organs

Effect of exposure to cadmium may be monitored by various techniques and various methods for the analysis of biological and biochemical samples. These can be monitored by measuring the metal content in biological fluids (plasma, saliva, urine, cerebrospinal fluid, gastric fluid, sweat, tears, amniotic and synovial fluid) and tissues of some organs (liver, kidneys, brain, pancreas, and spleen). These measurements are carried out by atomic absorption analysis (AAS), the emission spectrography (ICPOES) and potentiometric stripping analysis (PSA) [14].

Table 1 shows the measured mean value of the content of toxic metal cadmium in the tissue homogenates of liver and kidneys in terms of heavy metal intoxication by this. The measurement of the metal content in the liver was higher in animals that are received one day after poisoning supplement that binding with the metal contributes to its retention in a tissue.

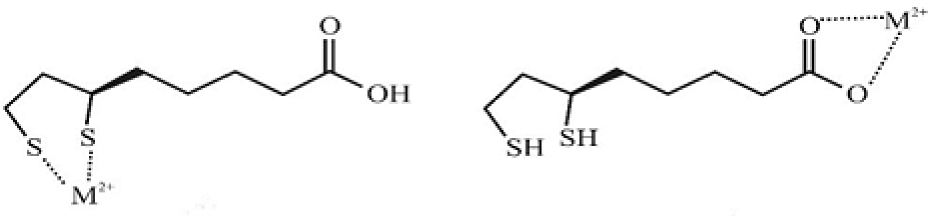

Figure 1 *Possible modes of interaction of metal ions (M^{2+}) and construction of appropriate complex associations with α-lipoic acid*

Figure 1 shows the possible products of the interaction of metal ions (M^{2+}) Cd with α-lipoic acid is likely type of covalent coordinated particles. According to the structure of α-liponic acid, it has the ability to interact with M^{2+} ions and, therefore, it can effectively reduce the toxic effect of metals. According to the results of these tests α-lipoic acid effectively reduces or blocks the toxic effects of Cd^{2+} ions in the tissue homogenates of liver and kidney.

Table 1 *Values of concentrations of metals in the tissue homogenates (liver and kidney) in the experimental group of animals in conditions of chronic cadmium intoxication and with the addition of an appropriate supplement*

Experimental groups	**The metal concentration (mg/kg)**	
	Liver	Kidney
Cd	0.1	0.06
Cd + α-LA	0.125	0.06

CONCLUSION

Based on measuring the amount of heavy metals (Cd) in the tissue homogenates of liver and kidneys can be concluded that the toxic effect of the heavy metal may be reduced by the addition of supplement (α-lipoic acid). It is believed that through the donor atom -SH groups supplement achieves relatively strong co-ordination with said metal and thereby block the possibility for the expression of the negative effects of heavy metals.

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DISPOSAL OF LEAD AND PHYSIOLOGICAL EFFECT OF GLUTATHIONE IN THE LIVER AND KIDNEY TISSUE IN RATS

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Abstract

Many industrial plants and traffic allow the emission of harmful substances, particularly of lead in the environment. This leads to pollution of the working and living environment. Necessary is a continuous emissions monitoring of certain metals, particularly lead. Continuous and long-term analysis and monitoring as well as the introduction of adequate measures for protection systems with the aim of reducing emissions of toxic substances is a good step to protect the health of individuals and entire populations. The setting of this animal study, albino Wistar rats, is to understand the effect of the toxic effects of lead and physiological role of glutathione when depositing the same in the target organs. Analysis of the measurements and content the lead and the supplement in the tissue homogenates of liver and kidneys was accomplished by the potentiometric stripping analysis. Glutathione as the peptide of the present thiol group (-SH group) capable of binding a building with its lead complexes of the type of associations. In this way, facilitates deposition of the metal and also the limit stops and a number of chain reactions which cause structural and functional changes at the level of cells, tissues and organs within many diseases.

Keywords: lead, glutathione, deposition, liver, kidney

INTRODUCTION

Environmental polluters, which include a variety of chemicals used in industry and household pesticides, fertilizers, heavy metals and ionizing radiation, are a serious threat to human health. Poisoning by heavy metals, especially lead, cadmium, arsenic and mercury are key factors responsible for the occurrence of oxidative stress [1,2].

Lead (Pb) is a widely-used heavy metal on the environment with extremely toxic effects on biological systems. The use of lead and its compounds is large in the household, workshops and in particular in the industry (for the production of lead color, production of various alloys, the battery industry, the discharge plate, where the network is processed by the lead and collect the lead preparations) [3]. The most common method of human exposure is through food, drinks through the digestive tract, then the smoke and dust in industrial environments over respiratory system and the combustion gas near the roads in big cities. After resorption by said system, entering the blood lead where more than 90% in the blood is related to the erythrocyte hemoglobin. In the body, lead accumulates in bone, central nervous system, liver, kidneys and other organs [4]. Lead disrupts numerous metabolic processes that take place in a living organism. Important dosage, exposure time, and exposure to lead in order to fully comprehend and evaluate the mechanisms toxicity and disorders that occur due to excessive input to the generation of the deposited metals into the human body.

The tripeptide γ-l-glutamyl-l-cysteinyl-glycine, also known as glutathione (GSH) is a thiol compound of low molecular weights, which makes 90% of the total non-protein sulfhydryl compounds cells [5]. Glutathione plays a role in many metabolic processes in mammalian organisms. It is known as an antioxidant protecting cells from free radicals and oxidative damage, as well known to him, and many other functions: detoxification of xenobiotics, regulation of synthesis and degradation of the protein, maintenance of protein structure and function, metabolism of leukotrienes and prostaglandins, regulation of the cell cycle, as well as gene expression [6]. GSH degradation takes place by catalytic action of γ-glutamyl transpeptidase, γ-glutamyl cyclotransferase, 5-oksoprolinase and intracellular protease. Thanks to the sulfhydryl-SH group in the glutathione molecule, its most important function is provided, which is to prevent cell apoptosis. This is achieved by removing reactive oxygen species, lipid peroxides and many xenobiotics, as well as direct repair of oxidative damage on the DNA molecule itself and proteins [7].

AIM OF EXPERIMENTAL

Animal models of Wistar rats, female sexes that imitate human conditions of exposure to lead and glutathione, were used. The aim of this study is to monitor the depositing of the mentioned metal and compare the concentrations of metals and glutathione metals at the target site, i.e. in the liver and kidneys.

MATERIALS AND METHODS

The testing was carried out on female albino Wistar rats, age of 2 months, body weight about 250 g. During working with animals, ethical principles of labor in laboratory animals are respected. Animals were kept in a vivarium Scientific Research Center of Biomedicine the Faculty of Medicine of the metal cages, in the air conditioning controlled conditions (temperature of 22 ± 2°C, relative humidity of 50 ± 5%, with the cycle of the light/dark 12h/12 h). All animals are living during the duration of the experiment under the same conditions with free access to food and water. All experimental procedures on animals were carried out between 9 and 11 o'clock.

The rats were divided into two groups. The first group of rats was administered lead(II)acetate $Pb(CH_3COO)_2$ [8,9]. The second group of rats, in addition to the application of lead(II)acetate obtained the glutathione. All chemicals were of analytical grade purity, manufactured by Merck. After the extraction is carried out anesthesia organs, the liver and kidneys in order to determine the concentration of metal, i.e. lead in tissue homogenates.

Euthanasia occurs bleeding. In experimental work is fully respected the Code of Ethics of scientific research Faculty of Medicine.

Determination of the metal concentration in the tissue homogenates of liver and kidneys

Content of toxic metal in the tissue homogenates was determined by potentiometric stripping analysis (PSA). The mineralization of the tissue homogenate was performed nitric acid (C = 6 ml/dm^3), followed by treatment with hydrochloric acid and finally by dissolving in deionized water twice. Heavy metal content was measured by the potentiometric stripping analyzer (Universal Leskovac-TMF Novi Sad).

STATISTICAL ANALYSIS

All the measurement results are shown as the mean value. Statistical analysis of the results was done by Student's t-test for independent samples (Microsoft Office Excel). Results of these analyzes are presented in tables.

RESULTS AND DISCUSSION

In physiological conditions, there is a balance between the production and the elimination of free radicals, that maintains homeostasis of the body, preserves the integrity of the cell and allows the normal functioning of the structure in the cell (DNA, proteins). During the evolutionary development of the organism has been established a specific defense system of the potential harmful effects of free radicals, which is characterized as the antioxidant system. This system is aimed at preventing the formation of free radicals, their removal or stop chain reactions. Oxidative stress is caused by an imbalance created between free radicals and antioxidant protection of the organism. Due to the increased production of reactive oxygen species (Parkinson's and Alzheimer's disease) or a reduced ability of cells that neutralize them, through its internal antioxidants leads to the formation of oxidative stress [10,11].

Direct prooxidant effect manifested metals with variable valencies (Cu, Fe, Mn and V) in such a way that generate free radicals through a Fenton reaction, favor the autooxidation parts of a biomolecule with the ortho-hydroxyl groups in the structure (the catecholamines and related structures) and contribute to the induction and propagation process of lipid peroxidation. Indirect effects occur prooxidant metals with a stable valence (Cd and Pb), and metals with variable valencies (Cu, Fe, Hg and Cr) in such a manner as to interact with the bioelement, i.e. react with the metals in the active site of the enzymes, interfere with the same ionic channels and disrupt the homeostasis of the intracellular ions [12]. The connection with the parts of biomolecules is realized through -O, -N or -S donor atoms of these bioligands, and thus can be lowered to their effective concentration and availability for interaction under physiological conditions of functioning of organisms.

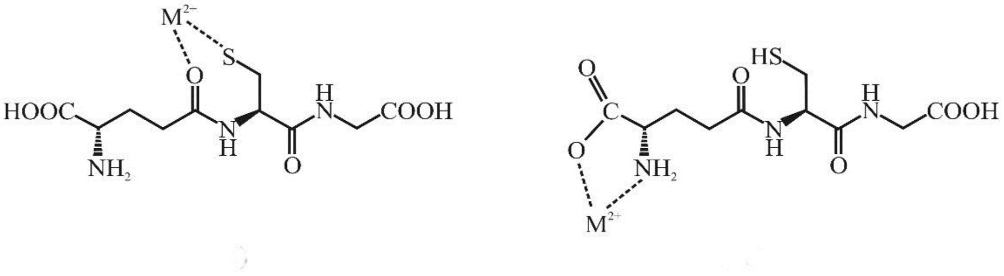

***Figure 1** Possible modes of interaction metal ion (M^{2+}) and the corresponding construction of associations with glutathione*

According to the nature and method of action, antioxidants can be divided into: enzymatic (superoxidismutase, catalase, glutathione reductase, glutathione-S-transferase, etc.) and non-enzymatic (glutathione, vitamins A, C and E, albumin, ceruloplazmin, transferrin, bilirubin, acid, etc.). Glutathione is one of the most important ligands that bind to metals, so that it has an important role in the transport, metabolism and the deposit metal. Sulfhydryl groups of cysteine in GSH molecule has a high affinity for metals and formed with a mercaptide of several endogenous metals such as cadmium, lead, copper, selenium, zinc and mercury [13]. Glutathione exists in two forms, reduced thiol GSH form and oxidized disulfide GSSG form. The key functional element of the glutathione molecule is the amino acid of cysteine, which has a reactive thiol group (-SH). In normal physiological conditions, more than 98% of intracellular glutathione is in reduced state, while the rest is present in the cell as a disulfide form of GSSG, mixed disulfides (mainly GSS protein) and thioesters.

Lead is considered to be an enzyme poison because it binds to sulfhydryl groups of proteins. In high concentrations, lead can change the structure of intracellular proteins, leading to their denaturation, cell death, and tissue inflammation. However, in conditions of chronic lead intoxication and supplements with some of the antioxidants, for example, GSH results in the possible interaction of metals with said supplements and the formation of associations of the type of complex that reduces the toxic effect of lead and at the same time leads to a deposit of metals in individual organs, e.g. liver and kidney (Table 1).

***Table 1** Values of lead concentration in tissue homogenates (liver and kidney) in experimental groups of animals under conditions of chronic lead intoxication and with the addition of appropriate supplements*

Experimental groups	**Metal concentration (mg/kg)**	
	Liver	**Kidney**
Pb	0.217	0.14
Pb + GSH	0.19	0.12

CONCLUSION

Based on measuring the amount of a heavy metal (Pb), the individual and pooled exposure with supplements glutathione in the tissue homogenates of liver and kidneys can be concluded that the lead could be toxic metal forms complexes with the sulfhydryl groups of said supplements (e.g. glutathione). In this way reduces the level of its deposit in the liver and kidney and consequently multiple toxic effects of lead in the target organs.

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PATHOPHYSIOLOGICAL EFFECTS OF COPPER AND PHYSIOLOGICAL ROLE OF α-LIPOIC ACID THROUGH THE ACTIVITY OF ENDONUCLEASES IN RATS

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Abstact

Due to the continuous development of the industry and the accelerated urbanization process, there is a significant increase in the emission of copper and air, water and soil pollution. Additional efforts should be made to protect the environment from anthropogenic activities. Also, preventive measures and compliance with legal and by-laws and acts of working and environmental protection, or ecosystems as a whole, should be taken. This study presents the toxicological and pathophysiological aspects of copper activity, as well as the physiological and biochemical role of α-lipoic acid through the activity of alkaline and acidic DNase in the homogenate of the pancreatic tissue. Model systems were experimental animals albino Wistar rats were female. Analysis of the determination of acid and alkaline DNase activity was performed on the Beckman DU 530 spectrophotometer. On the basis of increasing levels of alkaline and acidic DNase was observed that the copper induces autocatalytic chain reactions leading to the cell to apoptosis. It also explains the protective effects of α-lipoic acid which is linked to the toxic metal and is intended to prevent, limit, and stops the process of apoptosis.

Keywords: copper, α-lipoic acid, endonucleases, pancreas

INTRODUCTION

In the group of essential metals are copper, zinc, iron, cobalt, magnesium, selenium, manganese and molybdenum. These metals, taken in low concentrations, are part of the important substances in the human organism, for example, proteins, enzymes, vitamins and other biologically active compounds [1]. In this way they indirectly participate in important metabolic processes of the functioning of the organism and maintenance of homeostasis. However, higher concentrations of these metals disturb the major processes of the organism, such as growth and development. Also, there are literature data indicating the potential deposit of essential metals in certain organs and the consequent potential toxic effects of these metals if they are taken in higher concentrations [2].

Application of copper in copper products industry is extremely high. Due to a wide use of copper salts and copper daily, in workers occupationally exposed to the effect of copper, introduce minor amounts of this metal in the organism of people, which is roughly equivalent, 0.02 g of copper [3]. Copper a component of enzymes that are involved in oxidation-reduction reactions in the body, for example: cytochrome oxidase, superoxide dismutase, peptidylglicine monooxygenases, monoamine oxidase, tyrosinase and other. Its presence is also important due to iron because it is part of ceruloplasmin. Normal copper concentrations in human serum are about 120-145 μg/L. In the serum binds to albumin or ceruloplasmin. Copper is deposited in the liver and bone marrow, where it can bind to metallothionein. Eliminates the bile. Copper deficiency can cause anemia due to the occurrence of the disorder in the synthesis of hemoglobin [4].

Acute copper poisoning can occur due to oral ingestion of large amounts of copper salts, such as e.g. copper sulfate, which has been applied as a fungicide. Symptoms accompanying acute intoxication are vomiting, hypotension, hemolytic anemia, liver damage, coma and death. Symptoms accompanying acute intoxication are vomiting, hypotension, hemolytic anemia, liver damage, coma and death. As a result of chronic intoxication with copper, there is a disorder of copper metabolism, and indirectly the expression of its toxic effects. Increased copper accumulation in the liver, kidneys, brain and eye is characterized by the formation of Wilson's disease. Menke's disease, characterized by severe mental retardation, neurological damage, is also due to disturbed metabolism of the copper, that’s it degeneration of the cerebral cortex and white substance. In these patients, the content of copper in the liver and brain is low, while in other tissues it is relatively high.

α-lipoic acid (α-LA) is a disulfide compound. In terms of the chemical 6,8-ditiooktanska acid. Belongs to the group of fatty acids, and it is very important for the performance of the proper metabolism. α-lipoic acid is present in the matrix of the mitochondria where energy is being produced in the form of ATP in the cell. α-lipoic acid in the metabolic process, glucose improves the functioning of the pyruvate dehydrogenase so that it is necessary for energy production in the cell. At the cellular level, it promotes the growth of muscle cells [5], inhibits adipocyte differentiation and has a preventative effect on weight gain. Useful in the treatment of many diseases (e.g., diabetic polyneuropathy, diabetic angiopathies, cardiovascular, neurodegenerative diseases and intoxication metals) [6,7].

The enzymes responsible for the internucleosomal cleavage of DNA or RNA molecules in apoptosis are the endonuclease [8]. The endonuclease group includes DNase I or alkaline DNase and DNase II or acid DNase. DNase I is a glycosylated polypeptide which hydrolyze one or both strands of the DNA to form 5'-phospho-tri/tetra-oligonucleotides [9]. The deoxyribonuclease II in mammals is endonuclease, which functions in the optimum acidic environment and with the absence of divalent cations.

AIM OF STUDY

The aim of this study is an attempt to understand the effect of copper, and the effect of α-lipoic acid alone and in parallel with the exposition of the copper which could be administered to humans and in chronic i.e. professional exposition with copper.

MATERIAL AND METHODS

Tests were conducted on albino Wistar rats, body weight 220-250 g, grown in a vivarium Scientific Research Center of Biomedicine Faculty of Medicine in Nis. During working with animals, ethical principles of labor in laboratory animals are respected. All animals lived under the same conditions during the duration of the experiment with free access to food and water.

Animals were randomly divided into the control group, the group of animals that received copper(II)sulfate, following the group which received α-lipoic acid and a group of animals that had received the combined administration of the copper and the α-lipoic acid.

Preparation of the homogenate for measuring the activity of the enzyme, DNase

After sacrificing animals, after the medial laparatomy, extraction of the pancreas was carried out, which, after washing in physiological saline, was decapulated and freezed. After incomplete defrosting and preparation of pancreatic tissue tissue for analysis, homogenization of the sample on ice was performed with Teflon beater K Ultra Turrax IKA® T18 basic homogenizers in physiological saline as a medium [10]. The resulting supernatant is analyzed immediately to determine the activity of acidic and alkaline DNase spectrophotometer (Beckman DU 530 spectrophotometer) according to the method Bartholeyns *et al*. [11].

STATISTICAL ANALYSIS

For statistical analysis of data was used Student's t test. The values are presented as the mean and standard deviation, i.e., AVERAGE ± SD. The differences were considered statistically significant for $p < 0.05$.

RESULTS AND DISCUSSION

From these results (Table 1), it can be seen that copper significantly increases the level of alkaline DNase in the tissue of the pancreas (5.76 ± 1.70 to 11.48 ± 0.75). Acid DNase values after chronic copper intoxication were increased in pancreas (5.54 ± 0.81 to 9.94 ± 0.92) compared to the control group of animals that were in normal diet and life without heavy metal inoxing and without adding supplement. The excess Cu in rats increases the oxidative damage of lipid membranes and DNA in the pancreas, which increases enzymatic activity [12]. According to the literature, at higher doses, of 10^{-6} mol/dm^3, Cu inhibits certain enzymes (acid phosphatase), prevents binding of other essential trace element, or binds to some co-factors, such as glutathione. Cu, at high concentrations, participate in the formation of free oxygen radicals [13,14].

Table 1 *Activity a) alkaline DNase and b) acid DNase in experimental groups in conditions of chronic intoxication with copper and with the addition of suitable supplement*

Experimental Groups	Pancreas	
	Alkaline DNase [IJ⁄mg]	Acid DNase [IJ⁄mg]
Control	5.76 ± 1.70	5.54 ± 0.81
Cu	11.48 ± 0.75	9.94 ± 0.92
Cu + α-LA	8.71 ± 1.00	8.56 ± 1.11
α-LA	4.66 ± 1.05	4.28 ± 0.70

Copper also participates in the production of reactive species oxygen that lead to oxidative stress, which later leads to the oxidative modification of DNA molecules. α-LA supplementation day after intoxication with copper, reduces the level of alkaline DNase I (11.48 ± 0.75 to 8.71 ± 1.00) in the pancreas compared to the experimental group intoxicated with copper. The value of the acid DNase in the α-LA supplementation was also reduced on the day after copper injection into the pancreas (9.94 ± 0.92 to 8.56 ± 1.11) in relation to the group of animals receiving copper.

CONCLUSION

Albino Wistar rats, there was an increase in the activity of endonucleases of alkaline and acid of the DNase in the pancreatic tissue. This can be considered as biochemical marker of oxidative damage individual cells in the tissue homogenates of the pancreas caused by intoxication with copper. Application of α-lipoic acid leads to a decrease in the activity of DNase, alkaline and acidic in the tissue of the pancreas. It is believed that α -lipoic acid is a substance with antioxidant action because it initiates the mechanism of antioxidant protection.

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HERBICIDE EFFICACY IN CONTROLLING WEEDS IN ALFALFA

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Abstract

Alfalfa is one of the economically most important perennial forage crops. Alfalfa provides first class fodder that is suitable for feeding of all live stock, especially ruminants. Grass and broadleaf weeds are a serious threat to the quality and viability of a alfalfa stand. Weeds can reduce the nutritional value of the crop and cause significant reduction in yield as they compete aggressively for water, nutrients, sunlight, and space. In our research we used herbicides for controlling weeds in alfalfa (Butoxsone, Agil, Basagran, Corum, Treflan, Fusilade forte, Pulsar and Focus ultra). On the tested surfaces we have observed the presence of annual and perennial weeds, typical of alfalfa plantations: Capsella bursa pastoris, Stelaria media, Lamium amplexicaule, Viola arvensis, Amaranthus retroflexus, Setaria spp., Sorghum halepense, Cirsium arvense, Plantago lanceolate, Rumex crispus and others. According to our research, the most effective herbicides are Basagran, Butoxsone and Corum, which are intended to suppress broadleaf weeds. While herbicides Fusilade forte and Pulsar proved to be very effective in controlling grass weeds. A very good reaction was also demonstrated by the combinations of herbicides Pulsar + Fusilade forte and Pulsar + Basagrane, as this has expanded the spectrum of activity to the grass and broadleaf weeds. The aim of this paper was to determine which herbicide is most effective in controlling weeds in alfalfa.

Key words: alfalfa, herbicides, weeds

INTRODUCTION

Alfalfa (*Medicago sativa*) is an important perennial forage crop used around the world. Alfalfa is the fourth largest crop, in terms of acreage, grown in the world behind corn, soybeans, and wheat [1]. Unlike annual cropping systems, alfalfa management differs greatly due to its perennial habit of growth. The alfalfa crop will remain in the field for several growing seasons and will be harvested several times each season. Therefore, with alfalfa, management practices will not only affect the first harvest of the crop, but will also affect subsequent harvests within the life of the alfalfa stand [2,3].

Weeds are serious economic pests of alfalfa. A variety of different weed species, including annuals and perennials, warm and cool season grasses and broadleaf plants, and parasitic and poisonous plants, infest alfalfa hay grown throughout the arid alfalfa production regions of the West. Weeds in alfalfa directly compete for the same resources required for alfalfa growth and development: water, nutrients, light, and space. If weeds are left uncontrolled, they will reduce alfalfa yield and weaken or even destroy the stand, particularly if left unchecked during the seeding period [3]. Weeds have a large effect on forage quality. Establishment of a vigorous alfalfa stand is essential for long-term weed control. In older alfalfa fields, weeds are quick to fill in open spaces when stands decline [4]. It is nearly impossible to control weeds in a thin or weak alfalfa stand. Weeds affect alfalfa during two distinct periods: stand establishment and in established fields. Yield is sometimes reduced, but more often yield is the same or actually higher when weeds are not controlled [5]. However, the feeding value of the hay is usually drastically reduced. Weeds affect quality because most weeds are less palatable and less nutritious than alfalfa. Although some weeds make high quality forage, they are too mature at the time alfalfa is harvested. The loss of feeding value from weed infestation can be due to physical, chemical, or toxic factors. Many weeds are much lower in protein and higher in fiber than alfalfa [2]. Herbicide requires a wide range of effects on weeds, long persistence and the preservation of the human environment. The most important weed species at this stage are: *Capsella bursa pastoris*, *Stelaria media*, *Senecio vulgaris*, *Lamium amplexicaule*, *Veronica hederifolia*, *Viola arvensis*, *Taraxacum oficinalle*. After the first rolled and later predominantly late-spring weeds - *Amaranthus retroflexus*, *Chenopodium album*, *Sorghum halepense*, *Setaria* spp., *Panicum Crus-galli* and other [5,6].

The aim of this paper was to determine which herbicide is most effective in controlling weeds in alfalfa for the more economical and efficient use of herbicides in order to preserve the environment.

MATERIAL AND METHODS

The experiment was set in the spring of 2014 in the experimental field of the Institute for rorage crops Kruševac in Globoder. The alfalfa variety K-28 was planted on the experimental field on parcels measuring 15 meters long and 1 meter wide. Herbicides registered for alfalfa and soy in Serbia were included in the study of Petrović and Sekulić [7]. Before sowing, on 8 April 2014, three plots were treated with Treflane herbicide using different concentrations (Table 1). The herbicide was incorporated in the soil to a depth of 5 cm using rakes. After five days, the sowing of alfalfa seed was carried out on 14 April 2014, when 40 grams of seeds of alfalfa seed were planted per plot.

Fourteen treatments were done with different herbicides (Table 1) from the Serbian market, which have been registered for the treatment of alfalfa and soy [7]. Alfalfa plants treated with water were used as the control. The alfalfa plants were treated with foliar herbicides at an incremental stage from 10 cm to 15 cm, when the weeds were in phases of 2 to 6 with permanent leaves. Treatment was carried out by hand-sprayer for vegetable crops of

5 liters, using standard methods. Weed flora on treated areas, as well as in control, was determined. Analysis of the results was done after the first release. Cutting was done on 21 July 2014, as it was delayed due to weather conditions. The flora was analyzed in squares of 1 m x 1 m and the total green mass was measured. Grass weeds were separated from broadleaf weeds, the total green mass of grass and broadleaf weeds were measured.

Table 1 *Types of herbicides and treatments*

Herbicides	**Treatments, date of the treatment**
Butoxsone (2.4-DB sodium salt**)**	3 l/ha (3 ml/2l water), 20 May
Agil (propakvizafopa)	1 l/ha (1 ml/2l water), 20 May
Corum (bentazon + imazamox**)**	0.9 l/ha+0.9 l/ha (0.9 ml +0.9 ml/2l water), 20 May
Basagran (bentazon)	3 l/ha (3 ml/2l water), 20 May
Pulsar - one time (imazamoks)	1.2 l/ha (1.2 ml/2l water), 20 May
Pulsar - double (imazamoks)	0.6 l/ha (0.6 ml/2l water), 20 May 0.6 l/ha (0.6 ml/2l water), 20 May
Agil + Butoxsone (propakvizafopa + 2.4-DB sodium salt)	0.5 l/ha + 1 l/ha (0.5 ml +1.5 ml/2l water), 20 May 0.5 l/ha +1 l/ha (0.5 ml +1.5 ml/2l water), 27 May
Pulsar + Focus ultra (imazamoks + cikloksidim)	0.5 l/ha + 1 l/ha (0.5 ml +1.5 ml/2l water), 20 May 0.5 l /ha +1 l/ha (0.5 ml +1.5 ml/2l water), 27 May
Pulsar + Fusilade forte (imazamoks + fluazifop-p-butil)	0.5 l/ha + 1 l/ha (0.5 ml +0.6 ml/2l water), 20 May 0.5 l/ha +1 l/ha (0.5 ml +0.6 ml/2l water), 27 May
Pulsar + Butoxsone (imazamoks + 2.4-DB sodium salt)	0.5 l/ha + 1 l/ha (0.5 ml +1.5 ml/2l water), 20 May 0.5 l/ha +1 l /ha (0.5 ml +1.5 ml/2l water), 27 May
Pulsar + Basagrane (imazamoks + bentazon)	0.5 l/ha + 1 l/ha (0.5 ml +1 ml/2l water), 20 May 0.5 l/ha +1 l/ha (0.5 ml +1 ml/2l water), 27 May
Treflan 1 L/ha (trifluralin)	1 l/ha (1 ml/2l water), 8 April
Treflan 1.5 L/ha (trifluralin)	1.5 l/ha (1.5 ml/2l water), 8 April
Treflan 2 L/ha (trifluralin)	2 l/ha (2 ml/2l water), 8 April
Control	-

DB – 2.4-DB or 4-(2.4-dichlorophenoxy)butyric acid

RESULTS AND DISCUSSION

The applied herbicides showed different effects on different types of weeds in alfalfa, which can be seen from the yield of alfalfa's green mass, as well as the total weight of broad-leaved and grass weeds (Table 2).

On the tested areas, the presence of annual and perennial weeds was observed, which are typical for alfalfa plantations: *Capsella bursa pastoris*, *Stelaria media*, *Senecio vulgaris*, *Lamium amplexicaule*, *Veronica hederifolia*, *Viola arvensis*, *Taraxacum oficinalle*, *Amaranthus retroflexus*, *Chenopodium album*, *Setaria* spp., *Panicum crusgali*, *Sorghum halepense*, *Cirsium arvense*, *Plantago lanceolata*, *Taraxacum oficinalle*, *Rumex crispus* and others, which is in the agreement with the results [4–10].

Table 2 *Efficiency of herbicides on weeds in alfalfa*

Treatments	Grass weeds	Grass weeds mass	Broadleaf weeds	Broadleaf weeds mass	Alfalfa mass
Butoxsone	*Agropyrum repens, Sorghum halepense, Setaria* spp.	2 kg	-	-	7.0 kg
Agil	*Agropyrum repens*	1.6 kg	*Convolvulus arvensis, Taraxacum oficinalle, Senecio vulgaris*	5.8 kg	4.0 kg
Corum	*Agropyrum repens, Sorghum halepense*	2.7 kg	*Rumex acetosa, Cirsium arvense*	1.5 kg	6.2 kg
Basagran	*Sorghum halepense, Agropyrum repens, Panicum Crus-galli, Setaria* spp.	1.9 kg	*Rumex acetosa, Convolvulus arvensis, Taraxacum oficinalle*	2.0 kg	6.5 kg
Pulsar - one time	*Agropyrum repens*	0.8 kg	*Cirsium arvense, Rumex acetosa, Convolvulus arvensis, Taraxacum oficinalle*	1.2 kg	2.9 kg
Pulsar - double	-	-	*Rumex acetosa, Taraxacum oficinalle*	4.2 kg	8.0 kg
Agil + Butoxsone	*Agropyrum repens*	0.8 kg	*Taraxacum oficinalle, Rumex acetosa*	1.8 kg	3.0 kg
Pulsar + Focus ultra	-	-	*Convolvulus arvensis, Cirsium arvense, Polygonum aviculare, Rumex acetosa, Capsella bursa pastoris*	2.8 kg	3.0 kg
Pulsar + Fusilade forte	-	-	*Convolvulus arvensis, Cirsium arvense, Polygonum aviculare, Rumex acetosa, Capsella bursa pastoris*	2.4 kg	4.0 kg
Pulsar + Butoxsone	*Agropyrum repens*	0.9 kg	-	-	5.5 kg
Pulsar + Basagrane	*Agropyrum repens*	0.8 kg	*Rumex acetosa, Cirsium arvense, Senecio vulgaris*	1.0 kg	5.9 kg
Treflan 1.0 l/ha	*Sorghum halepense, Agropyrum repens*	1.6 kg	*Rumex acetosa, Senecio vulgaris, Cirsium arvense*	2.8 kg	2.4 kg
Treflan 1.5 l/ha	*Sorghum halepense, Agropyrum repens*	1.4 kg	*Rumex acetosa, Senecio vulgaris, Cirsium arvense*	3.0 kg	2.4 kg
Treflan 2.0 l/ha	*Sorghum halepense, Agropyrum repens*	1.5 kg	*Rumex acetosa, Senecio vulgaris, Cirsium arvense*	5.5 kg	0.3 kg
Control	*Sorghum halepense, Agropyrum repens, Panicum Crus-galli, Setaria* spp.	5.2 kg	*Convolvulus arvensis, Cirsium arvense, Polygonum aviculare, Rumex acetosa, Capsella bursa pastoris, Symphytum officinale, Taraxacum oficinalle, Lamium purpureum, Senecio vulgaris, Amaranthus retroflexus, Chenopodium album*	5.8 kg	4.5 kg

According to our research, the most effective herbicides are Basagran, Butoxsone and Corum, which are intended to suppress broadleaf weeds (*Taraxacum oficinalle*, *Amaranthus retroflexus*, *Chenopodium album* and etc.), similarly noted by Konstantinović *et al*. [5].

While the herbicides Fusilade forte and Pulsar proved to be very effective in controlling grass weeds such as *Sorghum halepense*, *Setaria* spp. and *Panicum crusgali* [7,9], very good reaction was also demonstrated by the combinations of Pulsar + Fusilade forte, Pulsar + Basagrane and Pulsar + Butoxsone, because this expanded the spectrum of action on the grass and broadleaf weeds, which is in the line with notes of Petrović and Sekulić [7] and Janjić [9].

CONCLUSION

The chemical control of weeds in alfalfa does not differ in any way considering the application of herbicides in other forage crops. The task and goal are the same, to eliminate the undesirable weed species at the right time, and that herbicides exhibit satisfactory selectivity to the crop and not contaminate the environment, regardless of whether they are residues or metabolites in biomass, hay, plant residues and soil.

To remove weeds from alfalfa fields, numerous herbicides are used after sowing, after emergence and during the resting phase of vegetation. The herbicides are required to have a wide range of effects on weeds, long persistence and the preservation of the human environment.

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CHARACTERIZATION OF *Saccharomyces cerevisiae* YEASTS BY MATRIX ASSISTED LASER DESORPTION/IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY

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Abstract

Yeasts are a large part of environment they have important role in the ecosystem and food industry. Matrix assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometric characterization of yeast strains Saccharomyces cerevisiae was done in this work. This yeast strains were grown in the two different media, three different methods of sample preparation and three different matrices were applied for MALDI-TOF MS characterization. Results from MALDI-TOF MS show that SMA and DRBC have almost the same peeks, according to m/z values, but with different intensities; CHCA as a matrix gives clearer spectrum with higher intensity than other MS spectra; direct transferring method and drying of colonies on the plate after that adding matrix solution and drying, is better than other methods.

Keywords: yeast, *Saccharomyces cerevisiae*, MALDI-TOF MS.

INTRODUCTION

A large numbers of yeast species can occur in soil, different types of water, they are normal inhabitants on plants and animals. Yeasts are very useful in food and beverage industry [1] and play an important role in the ecosystem. One of ecological problem is that estimates indicate that only 1% of yeast species that exist in nature have been described. Yeast ecology, therefore, is at a stage of discovery [2]. Yeasts can be defined as a unicellular eukaryotic fungus reproducing by budding or fission [3], also can be defined as ascomycete and bazidiomycetic fungus that are also reproduced by vegetative infusion or fission, with or without the formation of hifa or pseudohifa and those having a sexual form that is not in the fetal body. Some yeasts can be reproduced sexually, which leads to a change in the generation with characteristic cells in which meiosis takes place [4,5].

The definition of a yeast species is a summary made by systematicians and is based on the observation of taxonomically informative characteristics of one or several strains, thought to belong to the same biological and phylogenetic entity. These entities can be large, like *Saccharomyces*, or small when a species is known from a single strain only [1]. *S. cerevisiae* is one of the best model systems used for understanding microbial ecology and evolutionary genetics [6]. Yeasts of this genus are probably the oldest commercially used microorganisms. The most famous yeast *S. cerevisiae* strains are used today in the production of wine, beer, bread and strong alcoholic beverages. Its characteristic of high fermentation activity and good tolerance of various extreme environmental conditions present in industrial plants has led to the selection of several hundred strains of this yeast with known characteristics [7]. The correct identification and characterization of yeast species in the ecosystem and ecology is major factor of studies [1]. Given that traditional techniques, such as phenotypic, morphological, immunological and genotypic, common methods for identification yeasts, they can have a high percentage of inaccurate identification in addition are time-consuming and not easy to use as routine analyses [8].

In recent times, method of MALDI-TOF MS is applicable for the analysis of macromolecules (proteins, DNA, peptides, carbohydrates) [9], and natural or fragmented biomolecules (viruses, bacteria, molds and yeasts) [10]. MALDI-TOF MS in the identification of yeast strains using protein analysis has become a popular method, as an alternative to chromatographic, and even methods of analyzing the characterization and systematization of DNA. The technique is based on the detection of microbial protein in the mass range of 2-20 kDa, and time of flight (TOF) mass ions through the analyzer, in which the speed of the ions depends of the mass to charge ratio [11]. This MALDI-TOF MS technique analyses the protein content from treated or intact cells of microorganisms under the form of a spectrum that is considered as a protein fingerprint specific of a microorganism [12].

MATERIALS AND METHODS

Yeast strain and medium

Yeast strain of the genera *S. cerevisia* ATCC 9763 was used for testing. Prior to scanning, strain was cultivated on two solid media during 48 h at 25 °C in triplicate [13]: Sabouraud Maltose Agar (SMA, Lab a Neogen Company, UK) with the addition of chloramphenicol 50 mg/L (HiMedia, India) and gentamycin 50 mg/L (Galenika AD, Serbia) and on Dichloran Rose Bengal Chloramphenicol agar (DRBC, HiMedia, India).

Matrix preparation

Matrices prepared by dissolving 10 mg of α-cyano-4-hydroxycinnamic acid (CHCA, Sigma-Aldrich, Switzerland), 15 mg of 2,5-dihydroxybenzoic acid (DHB, Acros Organics, China), 20 mg of sinapinic acid (SA, Sigma-Aldrich AG, Switzerland) in the solution containing 500 μL acetonitrile (ACN, J.T.Baker, Avantor, Netherlands), 480 μL deionized H_2O and 20 μL trifluoroacetic acid (TFA, Sigma-Aldrich, USA). After vortex-mixing 3 minutes, centrifugation was performed at 13,000 rpm.

Sample preparation

The first one method consisted of the fast formic acid extraction. A thin layer of a colony was transferred directly on the MTP 384-well polished steel target plate (Bruker Daltonics, Germany) in two replicates and allowed to air-dry. Then, 1 µL of 70% formic acid was applied to the sample spot and after a short drying period, the spot was covered by 1 µL of a daily prepared matrices solution.

The second one method of sample preparation was extraction of yeast strain. Yeast colonies were picked and suspended in 300 µl of sterile distilled water and vortex-mixed thoroughly than added 900 µl of absolute ethanol. The mixture was centrifuged at 10,000 × g for 2 min. After the supernatant was discarded, the pellet was centrifuged again. Residual ethanol was removed by pipetting and the pellet was allowed to dry at room temperature. Subsequently 30 µl of formic acid (70%) was added and vortex-mixed with the pellet. Next, 30 µl of acetonitrile was added and vortex-mixed thoroughly. The solution was centrifuged at maximum speed for 2 min again and 1.5 µl of the supernatant was spotted on the target plate in two replicates. Immediately after drying 1.5 µl of the matrix solution was added to each spot and allowed to air dry.

The third one method was dried-droplet. More precisely, 2 µl of above mentioned supernatant were mixed with 2 µl of the matrix solution and vortex-mixing 3 minutes.

MALDI-TOF MS

MALDI-TOF MS experiments were performed on an Autoflex speed instrument (Bruker Daltonics, Germany) operating in linear positive mode in the *m/z* range of 2–20 kDa. A solid-state, Nd:YAG 355 nm SmartBeam laser, with a frequency of 1000 Hz was used for ionization. Typically, spectra were collected by accumulating 3000 laser shots from 6 different spots. The spectra were acquired using the FlexControl software (Bruker Daltonics, Germany).

RESULTS AND DISCUSSION

The MALDI-TOF mass spectra of *S. cerevisiae* grown on SMA medium (a), and DRBC medium (b) obtained by using CHCA as a matrix, are shown in Figure 1.

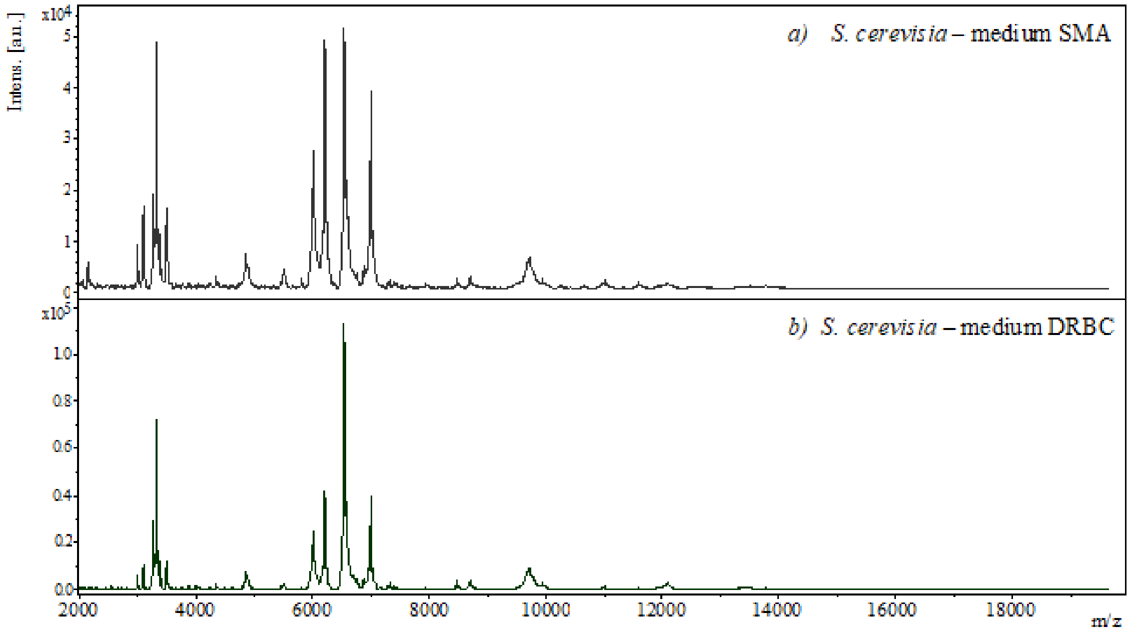

Figure 1 *The MALDI-TOF mass spectra of yeast Saccharomyces cerevisiae ATCC 9763 grown on two different mediums: a) sabouraud maltose agar (SMA) and b) dichloran rose bengal chloramphenicol agar (DRBC) with matrix: α-cyano-4-hydroxycinnamic acid (CHCA)*

The MALDI-TOF mass spectra on Figure 1 show that yeast grown on SMA and DRBC mediums have almost the same peeks, according to *m/z* values, but with different intensities.

The MALDI-TOF mass spectra of *S. cerevisiae* grown on SMA medium with three different matrices: a) CHCA, b) DHB and c) SA are shown in Figure 2.

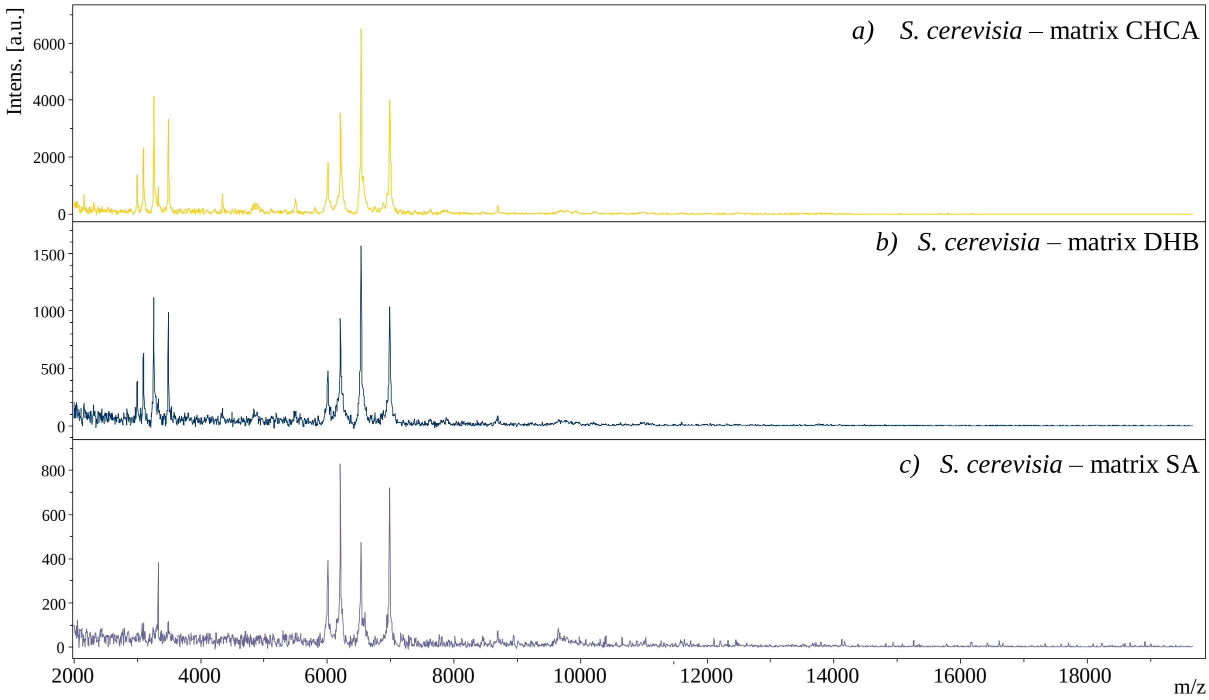

***Figure 2** The MALDI-TOF mass spectra of yeast Saccharomyces cerevisiae ATCC 9763 grown on sabouraud maltose agar (SMA) with three different matrices: a) α-cyano-4-hydroxycinnamic acid (CHCA), b) 2,5-dihydroxybenzoic acid (DHB), c) sinapic acid (SA)*

The MALDI-TOF mass spectra on Figure 2, where three different matrices (CHCA, DHB and SA) were used, CHCA as a matrix gives clearer spectrum with higher intensity than other MS spectra. On the MALDI-TOF mass spectrum, where SA was used as a matrix, there are no clear and intensive peaks in the area between 3000 and 4000 m/z values.

The MALDI-TOF mass spectra of S. cerevisiae grown on SMA medium with two different matrices CHCA and DHB with three different methods for sample preparation: direct transferring and drying of colonies on the plate after that adding matrix solution and drying, extracting cells, transferring and drying the supernatant on the plate after that adding matrix solution and drying, drying-droplet ie. mixing supernatant with matrix solution, transferring on the plate and drying are shown in Figure 3.

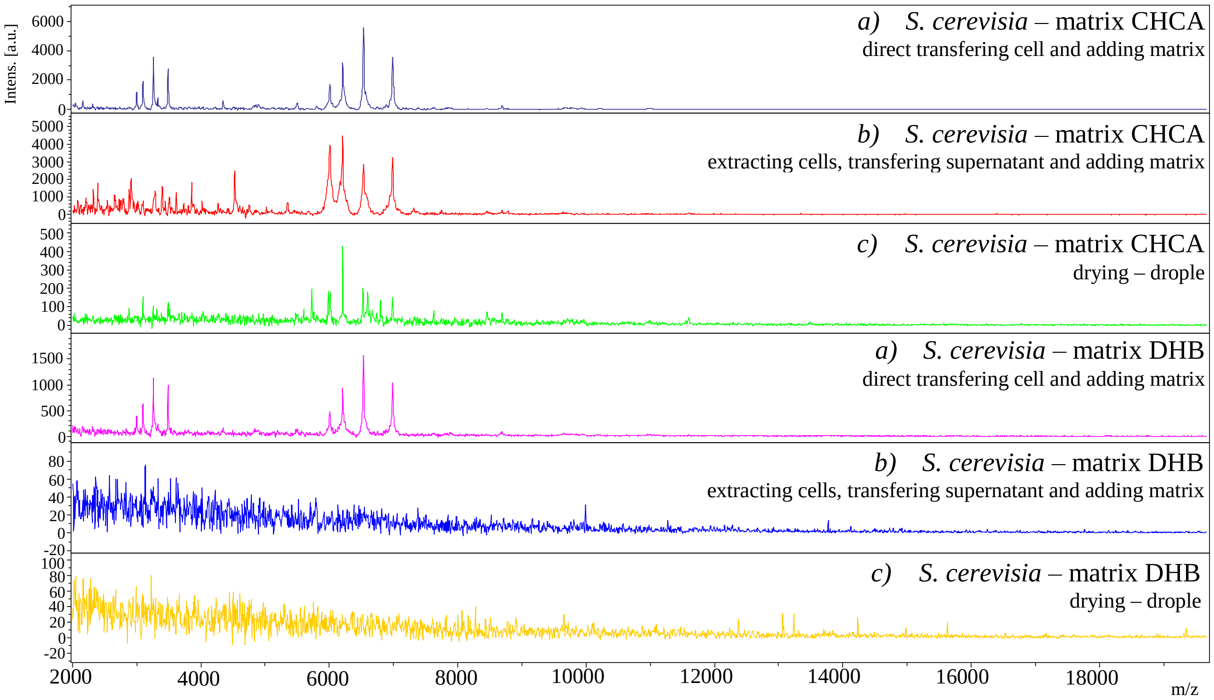

***Figure 3** The MALDI-TOF mass spectra of yeast Saccharomyces cerevisiae ATCC 9763 grown on sabouraud maltose agar (SMA) with two different matrices: α-cyano-4-hydroxycinnamic acid (CHCA) and 2,5-dihydroxybenzoic acid (DHB) with three different methods for sample preparation: a) direct transferring and drying of colonies on the plate after that adding matrix solution and drying, b) extracting cells, transferring and drying the supernatant on the plate after that adding matrix solution and drying, c) drying-droplet ie. mixing supernatant with matrix solution, transferring on the plate and drying*

The MALDI-TOF mass spectra on Figure 3, where three different methods of sample preparation were applied, give information that direct transferring method and drying of colonies on the plate after that adding matrix solution and drying, is the better than other methods.

CONCLUSION

According to ISO 21527 standard for microbiology of food and animal feeding stuffs the basic medium for growing yeast and mould is DRBC agar, but useful medium also is SMA. The MALDI-TOF mass spectra give information that yeast grown on SMA and DRBC mediums have almost the same peeks, according to m/z values, but with different intensities.

Comparison of the MALDI-TOF mass spectra for three different matrices CHCA, DHB and SA, MS with CHCA as a matrix gives clearer spectrum with higher intensity than other MS spectra. MALDI-TOF mass spectrum where SA was used as a matrix, shows that no clear and intensive peaks in the area between 3000 and 4000 m/z values.

Comparison of the MALDI-TOF mass spectra for three different methods of sample preparation gives information that direct transferring method and drying of colonies on the plate after that adding matrix solution and drying, is better than other methods.

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SOIL CONTAMINATION TESTING AT THE AGRICULTURAL AREAS OF THE TERRITORY OF BELGRADE

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Abstract

The purpose of the paper is to present the results of soil contamination testing at the agricultural areas of the territory of Belgrade Laboratory. Testing results of soil quality that are shown in this paper were collected in the period 2011-2018, during the implementation of the Program of soil contamination testing on the territory of Belgrade. The obtained results show higher concentration levels of one or more pollutants, mostly heavy metals and organic pollutants, at depths of 10cm and 50cm at most of the locations. Pollution of soil with dangerous substances in the territory of Belgrade may endanger the crops that are used for human consumption.

Keywords: soil, contamination, agricultural soil, heavy metals

INTRODUCTION

The soil is one of the most important natural resources under the constant "pressure" of anthropogenic impact. The soil contamination monitoring program on the territory of Belgrade is performed in a collaboration of the Secretariat for Environmental Protection of the City of Belgrade and the Institute of Public Health of Belgrade. The objectives of the Program are to monitor indicators of the state of the environment, through the soil pollution monitoring, and, at the same time, to protect the health of the population of the City of Belgrade.

In the period from 2011 to 2018 a total number of 458 soil samples from 6 types of the City zones (such as rural, green, traffic, water supply protected areas, etc.) were analyzed, out of which 39 samples were sampled on agricultural areas from the depths of 10 and 50cm.

MATERIALS AND METHODS

The number of samples, the extent and the dynamics of examination are determined by the soil contamination monitoring program, and sampling locations are determined afterwards, according to needs of the Secretariat for Environmental Protection of the City of Belgrade. At each location composite samples are taken. Sampling and laboratory testing are performed by accredited analytical methods in accordance with SCS ISO/IEC 17025:2006. The interpretation of the results for the examination period was carried out in accordance with the regulation referring to soil quality: „Official Gazette”, No 30/2018.

Table 1 *Parameters and methods of laboratory soil testing*

PARAMETER	METHOD
Moisture %	SRPS ISO 11465:2002
pH in H_2O	SRPS ISO 10390:2007
Organic(550°C)%	VDM 0081
Clay %	ISO 11277:2009
Pb	VDM 0131
Cd	VDM 0131
Cu	VDM 0131
Zn	VDM 0131
Cr	VDM 0131
Ni	VDM 0131
As	VDM 0131
Hg	VDM 0131
Pesticides	ISO 10382:2002
PAU - Polycyclic aromatic hydrocarbons	ISO 18287:2006
PCB - Polychlorinated biphenyls	ISO 10382:2002
Total petroleum hydrocarbonsC10-C40	ISO 16703:2004 ISO 16703:2013 *since 2016
Total petroleumhydrocarbonsC10-C28	ISO 16703:2004

RESULTS AND DISCUSSION

The results of the laboratory examinations showed that the pollutants were present in the surface soil layer (up to 50 cm) at the most sampling locations. In 2 soil samples the content of nickel exceeded the upper limit according to the regulation, as well as the remediation value. The increased content of nickel is related to the specific geochemical structure of the surface layers of soil in this area and, in the most cases, it is not dominantly caused by anthropogenic impact. The content of the DDT residues (8 samples) is not highly important in terms of its present concentrations, but it indicates the need for further soil monitoring, having in mind significant eco toxicological characteristics of this incesticide.

CONCLUSION

The results of performed analysis show that in the most of tested agricultural soil samples from the depth up to 50cm some of tested parameters, mostly heavy metals, do not comply with the regulation. For Nickel, this is due to geological characteristic of the soil, but for the others heavy metals, this is due to use of agro technical measures and the other potentially harmful influences in the previous period.

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SWOT ANALYSIS OF POTENTIAL PROJECTS OF RENEWABLE ENERGY SOURCES IN THE MUNICIPALITY OF STRPCE

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Abstract

Štrpce is a municipality in the south of Kosovo and Metohija. One of the problems this municipality faces for years is the unstable supply of electricity. On the other hand, there are certain potentials for renewable energy sources (RES) utilization. However, each of the RES projects is characterized by certain strengths and weaknesses and faces various threats and opportunities. In this paper SWOT analyzes are presented for several different RES projects (biomass, wind, hydro and solar sources) for the municipality of Štrpce. In order to conduct SWOT analysis 5 local experts from various fields (energy, mechanical engineering, management, law and electrical engineering) are involved in the research.

Keywords: SWOT analysis, Štrpce, renewable energy sources

INTRODUCTION

The municipality of Štrpce is located in the south of Kosovo and Metohija and covers area of 247.36 km². One of the problems Štrpce faces since 1999 and to this day is the unstable supply of electricity. However, it can be said that the municipality of Štrpce has certain potentials of biomass, solar energy, wind energy and hydro energy that can be used for energy purposes. Nevertheless, the RES potentials of Štrpce are not currently used enough.

The most important research related to this territory was carried out by the Serbian Academy of Sciences and Arts (SANU) in the early 1990s. The research of the SANU was published in three editions: I Natural characteristics [1], II Demographic Development and Particularities of the Social Space [2], III Socio-Economic Development [3]. Also, some of the recent works devoted to this topic and the territory relate to the research of energy security of Štrpce [4], consideration of the RES potential of Štrpce [5], determination of current energy situation and development of strategies to improve energy security of Štrpce [6].

In the literature, SWOT analysis is used standalone or in combination with other methods to solve various energy problems. In [7] SWOT analysis is used for regional energy planning. [8] discusses the energy mix at the Association of Southeast Asian Nations. [9] used the combined SWOT and Fuzzy AHP (FAHP) to analyze the status of the biofuel industry in China. [10] use the hybrid SWOT-AHP method for the selection and prioritization of RES projects. [11] use SWOT and modified DELFI technique for strategic planning of oil and gas pipelines in the Caspian Sea.

Each RES project is accompanied by certain strengths and weaknesses, but also the opportunities that shall be used and the threats that need to be neutralized or mitigated. In this sense, the aim of research is to conduct SWOT analysis for potential RES projects that can be realized in Štrpce. The research involved 5 local experts from various fields (energy, mechanical engineering, management, law and electrical engineering). The paper presents only final SWOT analysis for each of RES projects. The research consists of several steps: first, defining a group of experts which participate in the research; second, collecting relevant literature; thirdly, defining RES projects that will be the subject of research; fourth, a SWOT questionnaire is delivered to each expert with request to fill in a questionnaire based on the collected literature, local conditions and personal knowledge and experiences of experts; fifth, results (SWOT analysis factors) for each RES project obtained from all 5 experts are grouped into a global SWOT matrix; sixth, the factors of the global SWOT matrix are evaluated by experts in a scale of 0-1 with an incremental increase of 0.1 in order to select the most important factors for each RES project; seventh, in the final SWOT matrix, only the factors whose significance is over 0.7 are included.

SWOT ANALYSIS – BIOMASS

Agricultural production in municipality of Štrpce is small and there are no significant potentials for using agricultural residues for energy purposes. On the other hand, there is a large forest fund that can be a huge potential in terms of using biomass for energy purposes.

Table 1 *SWOT analysis of biomass projects*

Strengths	Weaknesses
✓ Electricity can be produced 24 hours a day (if raw materials are provided and if maintenance time is excluded) ✓ Independence of weather conditions ✓ Various types of bio-waste can be used ✓ Contribution to energy security of Štrpce	✓ Increase in the amount of harmful gases ✓ Waste disposal requirements ✓ Cost of raw material collection ✓ Incomplete research on RES potentials in the territory of the municipality of Štrpce ✓ Significant land requirements

Opportunities	Threats
✓ Starting of local wood processing industry ✓ Large quantities of waste from the Sharr Mountain National Park ✓ Use of municipal solid waste from the municipality ✓ Donor support ✓ Political and financial support of the Republic of Serbia ✓ Contributions to the local economy	✓ Insufficient agricultural raw materials (residues) to cover the annual needs of the biomass power plant ✓ Possible abuses in NP Šar planina ✓ Prohibition of residues collecting from the NP Šar planina by the administration or authorities ✓ Prohibition of licensing and networking by various Kosovo institutions

SWOT ANALYSIS – HYDRO PROJECTS

Natural conditions for the exploitation of the hydro potentials of the Lepenac river and its tributaries in the area of Štrpce municipality can be classified into the most productive basins in Serbia [3]. The hydropower potential of the municipality of Štrpce was used in the 1950s until the electrification of the municipality. A mini-hydroelectric power plant of 18 kW supplied Brezovica. Another mini hydro power plant (50 kW) located on a sawmill in Berevce, supplied villages Štrpce and Berevce. The largest hydro power plant (175 kW) supplied a large number of municipal settlements until electrification.

According to Nikolic [3], it is possible to build around 27 derivative flow hydroelectric power plants in the territory of the municipality of Štrpce with the installed capacity of about 20 MW.

Table 2 *SWOT analysis of hydro projects*

Strengths	Weaknesses
✓ Does not produce waste and do not pollute the air ✓ Contribute to strengthening the local economy through employment, but also creating conditions for tourism development ✓ Well-known technology ✓ Contribution to energy security of Štrpce	✓ Threats to water quality and water flow ✓ Endangering the fish fund ✓ Reduction or cessation of electricity in case of drought ✓ Incomplete research on RES potentials in the territory of the municipality of Štrpce ✓ Endangering the natural habitat of people, flora and fauna in the case of dam construction
Opportunities	**Threats**
✓ Lepenac River - one of the most productive river basins in Serbia to use for energy purposes ✓ Defined locations for construction of small hydro power plants ✓ Donor support ✓ Political and financial support of the Republic of Serbia	✓ Possible opposition from local farmers and individuals ✓ Possible ban on the issuance of permits and connection to the network by various Kosovo institutions ✓ Unreliable / outdated data on hydro potentials

SWOT ANALYSIS – SOLAR PROJECTS

Based on research conducted 1984-1988 its concluded that Sirinićka župa with an average annual sunshine duration of over 1,938 hours is extraordinary sunny [1]. Especially, the sum of solar radiation is greater on the higher slopes of Šar mountain, which is in the winter months regularly over the layers of low clouds [1]. This feature is very important from the aspect of the possibility of using solar energy for energy purposes during the winter. It is because Ski resort Brezovica consumes the most electricity and the municipality of Štrpce in this period is very often exposed to restrictions [5].

In Figure 1 it is noticeable that the municipality of Štrpce belongs to the part of the country where the average daily energy of global radiation on the horizontal surface in January is the highest and amounts to > 1.6 kWh / m^2, while in July 6.4 - 6.5 kWh / m^2.

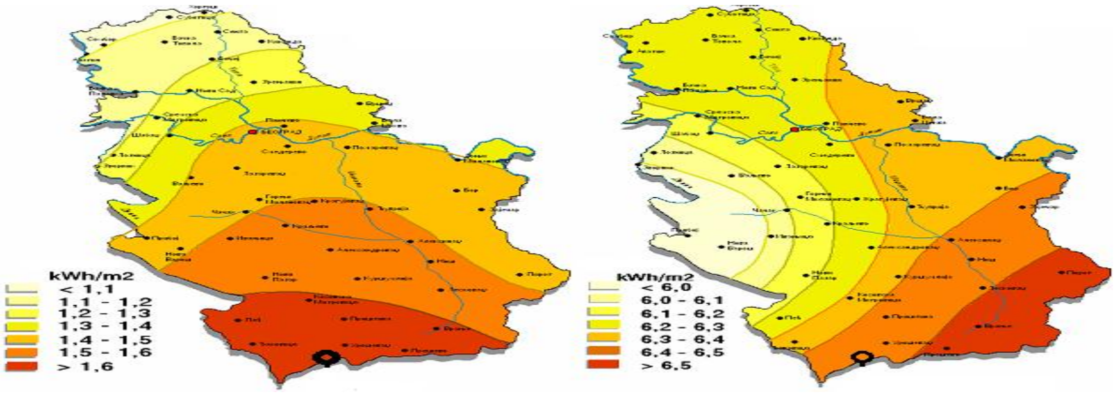

Figure 1 *The average daily energy of global radiation on the horizontal surface in January (left) and July (right) (kWh / m*^{2}*)*

Table 3 *SWOT analysis of solar projects*

Strengths	**Weaknesses**
✓ Well-known technology ✓ Easy installation of production capacities ✓ Solar farm does not produce noise, waste or other types of pollution ✓ Significantly lower maintenance costs than other RES technologies ✓ Long lifetime of technology ✓ Solar panels can also be used by households ✓ Contribution to energy security of Štrpce	✓ Required significant land areas ✓ No electricity production at night ✓ Low efficiency (14% - 25%) ✓ Significant variations in the production of electricity during the day and the year ✓ Incomplete research on RES potentials in the territory of the municipality of Štrpce
Opportunities	**Threats**
✓ Financial support of donors for the realization of RES projects ✓ Political and financial support of the Republic of Serbia ✓ Significant land areas that are not currently in use and could be used to install a solar farm ✓ Constant reduction in the price of solar panels ✓ Contributes to the local economy	✓ Potential shrinkage of the forests of the Šar mountain National Park for the needs of a solar farm. ✓ Possible opposition from local associations and individuals ✓ Permits issues caused by local authorities ✓ Possible ban on the issuance of permits and connection to the network by various Kosovo institutions

SWOT ANALYSIS – WIND PROJECTS

The five-year observations of wind (1984-1988) in Brezovica show that the frequency of silence (388 ‰) is significantly higher than in Prizren (301 ‰), especially in Dragash (181 ‰) and Urosevac (271 ‰) [1]. If the frequency of winds is observed in seasons, then it is the highest in the spring and summer, and the smallest during autumn and winter. The most frequent wind is from the east (199 ‰), then from the southwest (136 ‰), while the winds from the southeast (108%) and west (106 ‰) have almost the same frequency [1]. The rose of the wind for the territory of the municipality of Štrpce is shown on Figure 2.

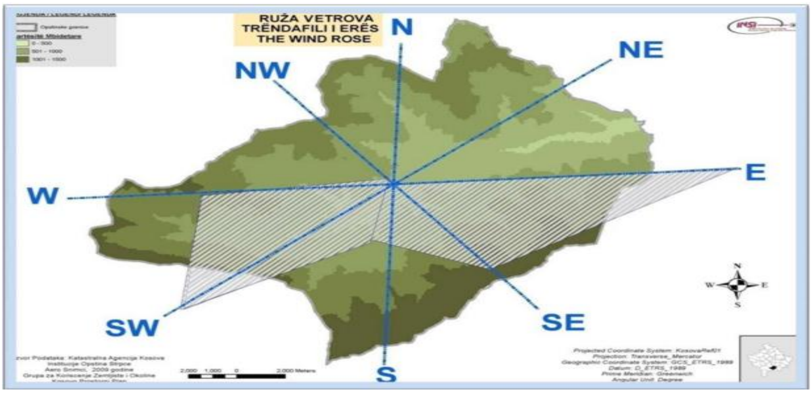

Figure 2 *Rose of winds in the municipality of Štrpce [12]*

Table 4 *SWOT analysis of wind projects*

Strength	**Weaknesses**
✓ A wide range of available turbines. Depending on the local possibilities and needs, adequate turbines can be selected. ✓ Does not produce waste, harmful gases and does not use water during exploitation ✓ It can be easily combined with solar energy ✓ It can significantly increase energy security in remote and severed mountain areas of Štrpce	✓ High investment costs ✓ Variable wind power and, therefore, a variable amount of energy produced ✓ Increasing maintenance costs in the event that the wind farm is away from the main roads and distribution networks ✓ Possible negative impact on the fauna of Shar Mountain National Park (birds, bats and butterflies) ✓ Noise ✓ Narrowing the natural appearance of the Sharr Mountain National Park ✓ Incomplete research on RES potentials in the territory of the municipality of Štrpce
Opportunities	**Threats**
✓ Significant potential of the municipality of Štrpce ✓ Donor support ✓ Political and financial support of the Republic of Serbia ✓ Contributes to the local economy ✓ Interested in distant and cut-off settlements for installing wind farms	✓ Possible diversions and terrorist attacks due to distance from settlements ✓ Possible opposition from local associations and individuals ✓ Prohibition of licensing and networking by various Kosovo institutions

CONCLUSION

In this paper 5 experts participated to analyse 4 different RES from perspective of their strengths, weaknesses, opportunities and threats. Results of research point to the need for more detailed research in the field of RES. However, the results of research can be a good basis for evaluating and selecting RES projects in future. Also, results could help decision makers at local level in strategic RES planning.

As the main conclusions we can state the following:

- ✓ RES potentials in the territory of the Municipality of Štrpce have not been sufficiently explored;
- ✓ The realization of each of the RES projects would contribute to the improvement of the energy security of Štrpce;
- ✓ The potential problem of (non) issuing necessary permits by the Kosovo institutions is noticeable;
- ✓ Each of the RES projects would contribute to a certain extent to the local economy through employment, taxes and engagement of local companies.

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FOREST-BASED BIOMASS AS ALTERNATIVE ENERGY SOURCE IN SERBIA

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Abstract

Biomass is the most significant renewable energy source, due to abundance of forests and agricultural areas. Despite its great potential, the use of wood biomass in Serbia has not been efficient. Significant amounts of wood residue produced in the course of tree felling and timber assortment production are left unused in forests. Intensive use of forest-based biomass requires the establishment of system measures for control and supervision in the chain of use as well as the adaptation of the planning and forest management pattern. On this moment in Serbia legislative and regulatory frameworks and support mechanisms aimed to increase the usage of biomass and other renewable energy sources are developing. Investors interested in biomass utilization in district heating (DH) systems and combined heat and power (CHP) plants are also present. However, despite all potential and advantages of biomass and the existence of potential investors, we cannot be satisfied with the level and modes of utilization for energy production in Serbia. It is necessary that wood and wood biomass be given the right importance, not only in energy balances and official consumption analyses in Serbia but also in the application of methods for estimating its consumption.

Keywords: biomass, forests, renewable energy source, potential, Serbia

INTRODUCTION

The possibility and rationality of the use of certain forest functions, as well as the rate of attainment of objectives related to forest management, depend on the already existing conditions, especially in terms of functional optimism determined for some primary purposes.

By the middle of the last century, forests were treated as natural raw material resources for the production of wood intended for mechanical and chemical processing, for construction and thermal energy. In the last decade, the interest of the professional and general social community in assessing forest-based biomass as an alternative energy source in Serbia is growing.

Biomass is the only form of renewable energy source that can be used to produce liquid fuel whose consumption exceeds other energy sources, with projections that it won`t change after 2030 [1].

Forest-based biomass includes wood for heating, plant masses of rapidly growing plants, branches and wood waste from wood, sawdust, bark and wood residue from the wood processing industry. Forest based biomass has a dominant share of about 60% of total energy produced from renewable sources in Serbia [2].

The paper analyses the resources and makes recommendations for increasing the utilization of forest biomass as an alternative energy source in Serbia in order to improve the use of forest biomass and wood processing industry.

METHODOLOGY

The research was based on the methods of analysis and synthesis of data related to the currently available resources of forest-based biomass to determine the significance and potential from forestry for alternative energy in Serbia. Also, this paper gives the recommendations and proposals of measures for more efficient use of wood biomass for energy purposes in Serbia.

Data on the forest-based biomass in Serbia were obtained from the data analyzed in relevant scientific papers, studies, projects and monographs dealing with this issue, as well as legal regulations and data obtained from the Institute of Statistics of the Republic of Serbia, SE "Srbijašume", SE "Vojvodinašume" and other sources.

RESULTS AND DISCUSSION

In accordance with the Kyoto Protocol, the Paris Agreement and the EU Directives, and based on the obligation to increase the share of renewable energy sources in the total energy consumption, a lot of EU countries are encouraging the use of biomass as fuel. In the last few years significance of biomass started to grow again, due to exhaustion of fossil fuel reserves and their negative impact on climate and environment. Compared to oil, coal and natural gas, the share of renewable energy sources in the global energy consumption is only 2.8% in the world today [3] (Figure 1).

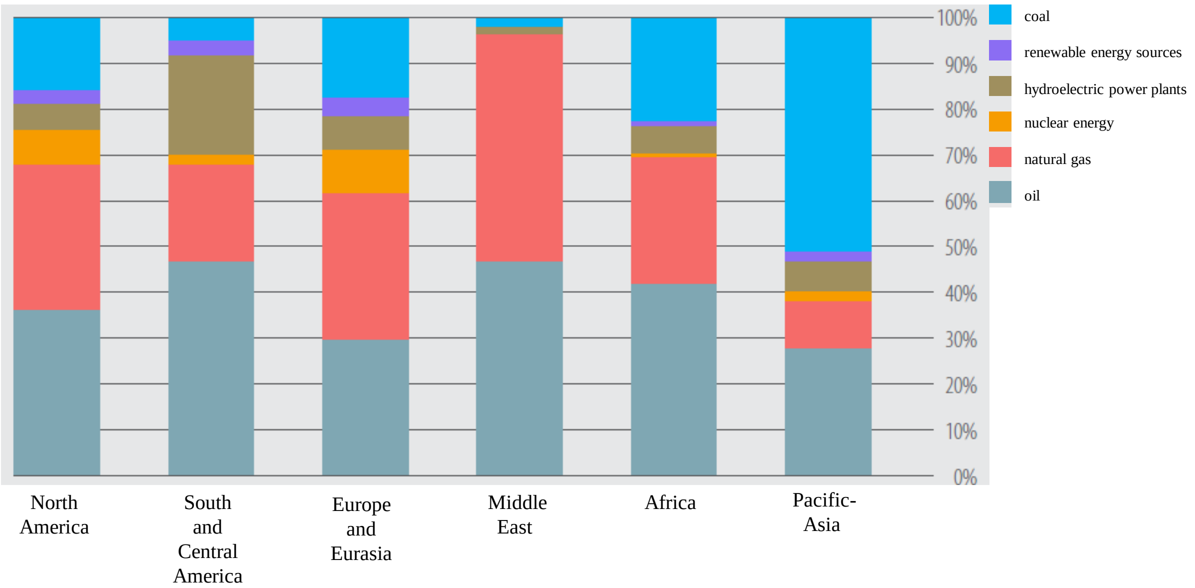

***Figure 1** Energy consumption from different sources in the world (Source: [4], modified)*

Serbia is one of the countries of Southeastern Europe with significant potentials for using forest-based biomass and wood processing industry in the form of wood residues for the production of modern fuels. This type of biomass has not been used efficiently.

Forests in Serbia account for 29.1% of the total area (7.1% in Vojvodina, 37.6% in Central Serbia). The other forest land, which by the international definition also includes bushes and scrubs, accounts for 4.9% of the territory, which in total accounts for 34.0% or 36.3% of the productive soil of Serbia [5].

By Bankovic *et al*. [5], 40 broadleaf and 9 conifer tree species were reported, as well as the uneven presence of the reported tree species in the total growing stock.

The beech is dominant, since it accounts for 29.0% of the total area, for 40.5% of the total volume, and it accounts for 30.6% of the total volume increment, the second dominant species is Turkey oak, which accounts for 15.0% of the total area, for 13.0% of the total volume, and for 11.4% of the volume increment.

The beech, as the most dominant broadleaf species in Serbia, is a mesothermal species, well-adapted to the moderate temperatures, quantities of moisture, and shady conditions (sciophytes). In contrast to the beech, most oaks in Serbia are xerothermic (they grow at dry and warm sites) and light-loving species (heliophytes). Regarding conifers, pines are light-loving species, adaptable to the ecological condition of temperature, moisture and soil. The spruce and fir are sciophilic, i.e. shade-loving species.

One of the reasons is the traditional use of firewood for domestic heating needs despite its lower energy efficiency compared to pellets and briquettes (Figure 2).

Figure 2 *Pellets and briquettes (Source: [4], modified)*

According to Glavonjić *et al*. [6], the estimated energy potential of the available biomass derived from forests and wood processing industry in Serbia amounts to 1.53 Mtoe/year. According to the Energy Sector Development Strategy of the Republic of Serbia for the period by 2025 with projections by 2030 [7], together with relevant Decisions on the energy balance determination, the degree of the use of potentially available forest-based biomass and wood processing industry is around 1.02 Mtoe/year (66.7%). The remaining unused potential of this biomass is estimated at around 0.51 Mtoe/year. Production levels in the forestry sector depend on a series of market and institutional factors (Table 1).

Table 1 *Potential and actual wood biomass from forests and wood processing industry: energy potential [7,8]*

Biomass from forests and wood processing industry	**Energy potential (GJ)**	**Energy potential (Mtoe)**
Potentially available wood biomass	63.960.822	1.53
Degree of potentially available wood biomass utilization	42.747.228	1.02

In the structure of the final consumption of biomass from forests and wood processing industry in Serbia, the industry accounts for 13%, households for 84% (use of firewood for heating purposes), and other sectors for 3%, with extremely small amount spent in heating plants [2].

According to Brasanac-Bosanac [9], there are numerous reasons for such a structure of consumption: rural areas and peripheral parts of suburban areas, where households mostly use firewood for heating purposes, gravitate towards the areas with high production of timber or they are far from other supply sources, while households` low purchasing power makes firewood the most affordable heating alternative; insufficient incentive measures of the state in the field of biomass and its use for energy purposes (construction of biomass power plants); limited economic power of potential investors interested in investing money in the biomass sector (construction of power plants, cogeneration plants); unfavourable commercial loans and loan terms and conditions; lack of financial resources of households, small businesses and institutions to change the heating mode (to buy pellet boilers), etc.

Although firewood has lower energy efficiency than pellets and briquettes, it will be still used by households in the future due to the low purchasing power of the population, high prices of conventional fuels (fuel oil, liquefied gas, coal), slow development of gas distribution network and costly gas installation (without subsidies and favourable loan conditions), inability of households to afford the purchase of boilers and pellet heating stoves.

The use of forest-based biomass in Serbia will not be satisfactory enough until investments are encouraged and stimulated, especially for the establishment of a district pellet heating system. Also, the establishment of intensively managed forest plantations of fast-growing tree species in Serbia should be considered as a possible solution for meeting the ever-growing energy needs by using renewable energy sources, which would further reduce the pressure on forests (unproductive areas of agricultural and forest land would be given purpose and the engagement of local workforce and professional support would provide additional funding sources). Potential growers of energy crops are not sufficiently familiar with the comparative advantages of energy crops of the second generation biofuels, nor with the technology of their cultivation, which makes education in this area very important in the coming period.

CONCLUSION

Although forest resources in Serbia represent an important resource for economic growth, primarily in rural areas, the traditional approach in their management and use is still dominant. This approach is characterized by being based on wood as the primary forestry product, while other forest products are mainly neglected and their economic evaluation is often absent. Besides, a certain amount of wood biomass is left in the forest although it could be economically evaluated, or offered to the market with minimum investment in infrastructure and technological solutions which would enable its more effective exploitation.

It must not be allowed for the increase of woody biomass demand to lead to the increased pressure on forests and exceeding of allowed cuts. In that case, positive effects of biomass use on one side could lead to the degradation of forests on the other. In order to improving the use of forest-based biomass for energy purposes in Serbia it is necessary to adopt relevant measures existing in other European countries and the regulations harmonized among decision makers in the fields of agriculture, forestry, energy and environmental protection.

High fossil fuel prices and political decisions directed toward increasing energy security and climate change mitigation provide a strong stimulus to the development of renewable energy sources, especially the wood-based energy. In order to improve the production process and enable a more effective use of forest resource potentials, additional funds should be invested into the accompanying infrastructure, primarily into the opening of the forest complexes.

As the biomass from forests is increasingly becoming the interest of national and international market, a detailed research on overall potential of woody supply from Serbian forests is required.

ACKNOWLEDGEMENT

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ACTUAL STATE OF APPLICATION OF MOTOR VEHICLE AGGREGATES

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Abstract

The paper reviews alternative technologies for propulsion of motor vehicles. The alternative fuels and technologies applied in the cities of some of the European countries are reviewed including the examples of some of the solutions of motor vehicles realized by applying alternative propulsion systems. The paper presents actual situation of European regulations in the field of motor vehicle exhaust emissions.

Keywords: motor vehicles, alternative technologies, IC engine, regulation, exhaust emission

INTRODUCTION

Today, a lot of effort is being invested in introducing environmentally cleaner and removing ecologically "dirty" vehicles from the road. Clean air represents the basis of a healthy life and well-being of all living beings. One of the major causes of air pollution is traffic. At present, there are about seven billion people living on Earth, and the number of registered vehicles is about one billion and six million. In France, Japan and Germany there are about 600 vehicles per 1000 inhabitants, while in the US there are 800 vehicles per 1000 inhabitants. In Serbia, the number of registered cars is around 1,500,000 vehicles. The number of cars in the world is constantly rising. For this reason, it seeks to ensure that the motor vehicle is in accordance with nature and its laws. This means that the harmful influence of motor vehicles on the environment is reduced to the lowest possible extent. This is achieved, inter alia, by the introduction of legislation on the issue of exhaust emissions. Alternative fuels, which are intended for use in motor vehicles, cause less air pollution, compared to petrol and diesel fuel. Today, or are planning to apply in the vehicles the following alternative fuels: bio fuels (biodiesel, ethanol and biogas), methanol, natural gas, liquefied petroleum gas, hydrogen, fuel cells and electricity. Too much of the price of these fuels nowadays, to a greater degree, prevents them from massive use in vehicles. For this reason, the use of internal combustion engines is still dominant for the propulsion of motor vehicles. The development of alternative fuels is normally followed by the development of appropriate technological solutions for the propulsion systems of motor vehicles. These are: drive to compressed natural gas, hydrogen, fuel cells, hybrid and electric drives.

THE STATE AND THE DEVELOPMENT OF NATURAL CONCEPTIONS OF THE VEHICLE DRIVE

As a driving unit for motor vehicles today, the most commonly used internal combustion engine, in which the chemical energy of fuel is converted into mechanical work. The advantages of using an internal combustion engine are: Relatively good fuel economy; Good construction compatibility; Quick readiness for work; They only burn fuel when they work; They use fuel with high energy compression. The disadvantages of piston engines are: Bad ecological characteristics; High dependence on fuel quality; Unwavering start of work; Significant nonsense; Complicated construction; Inability to overload the engine; Requires expert handling and maintenance; High fuel prices; High production cost.

Adverse effects during combustion in the engines are [1,2,3]: The occurrence of dissociation (diminishing efficiency); Occurrences of uncontrolled ignition and detonation (endangering the construction); Occurrence of toxic components in the exhaust emission (endangering the environment). Incorrect events during combustion in diesel engines are: Combustion; Smoke in exhaust; Exhaust emission toxicity. The development of alternative fuels for the propulsion of motor vehicles has led to the development of appropriate technical and technological solutions for their subsystems, or technologies in order to meet the ever-increasing environmental regulations and improve energy efficiency. The impact of vehicles on the environment, from the aspect of environmental indicators, can be improved through the application of modern technical and technological solutions for their operation. Alternative, or "clean" fuels, which are intended for use in motor vehicles, can greatly improve air quality, thanks to their characteristics that make them cleaner than gasoline and diesel. Generally speaking, these fumes emit, with combustion, less hydrocarbons that are less reactive and less toxic, and CO_2 emissions are also reduced, which contributes to the reduction of global pollution.

The most common alternative fuels include: bio fuels (biodiesel, ethanol and biogas); Methanol; Natural gas; Liquefied petroleum gas; Hydrogen; Fuel cells; Electricity. Due to its high price, alternative fuels have not yet found wider application, although they have more environmental characteristics than petrol and diesel. The development of alternative fuels was accompanied by the development of appropriate technological solutions for the propulsion systems of vehicles, the most famous of which are: Compressed natural gas; Hydrogen; Fuel cells; Hybrid and electric drive. Based on the research of the International Public Transport Organization (UITP), the International Association of Public Transport (UITP) concluded that about 90% of the city's fleet is with diesel engines, and the rest belongs to alternative fuels: Compressed natural gas (about 41% Liquid petroleum gas (about 1.7%), Biodiesel (2.8%), Biogas (0.3%). The total share of other fuels, such as [4]: ethanol, various diesel and biodiesel blends and fuel cells is less than 0.5%. About 68% of diesel-powered buses use diesel fuel with low content. The water and diesel emulsion is used on 6% of buses in France, about 5% in Italy, while in other countries it is almost out of use.

CNG buses are represented with around 20% in Athens and Helsinki fleets. LPG is equipped with 100% bus in Vienna and around 14% of buses in Koperhagen. Biodiesel is used around 29% in the Luxembourg fleet, 18% in Austria and 6% in Spain. The use of biogas is negligible, except in Sweden. The use of electrically powered buses in Italy is five times higher than the average application in all other countries. Hybrid buses, mostly with diesel-electric drives, are represented with around 0.25% of the total fleet analyzed in 90 countries. In the use of hybrid drives, Luxembourg (from 8%), followed by Italy (with 1%). The largest number of buses is with Euro2 and Euro3 engines (which make up about 2/3). In most countries most buses are below the Euro standards.

Compressive natural gas is the most commonly used alternative fuel in motor vehicles. Today, there are over 9600,000 vehicles in the world that are fuelled by this fuel. Leading countries, according to the number of CNG vehicles, are: Pakistan, Argentina, Brazil, Iran and India. In Europe, more than 1100,000 vehicles are on CNG (78% passenger cars, 5.6% buses, 8.2% commercial vehicles). Italy ranks first in Europe by number of CNG vehicles. MAN, Mercedes, Solaris and Volvo have in the production of CNG buses. Hydrogen, as an alternative fuel, has the smallest detrimental effect on the environment as well as the highest energy density. In combustion, water is released and a little NO_x, which can be controlled using catalytic converters.

Hydrogen, in motor engine, is experimentally applied for some twenty years.

In the application of hydrogen, Germany is leading.

An electrochemical process, from hydrogen-rich fuels, usually natural gas or methanol, separates hydrogen from hydrogen fuel cells, which in combination with oxygen produces electricity and water.

The fuel is converted into electric energy by an efficient electrochemical process.

From the point of view of ecology, this is a particularly good drive for motor vehicles.

Hybrid drive is a combination of multiple drives (usually electric motors and conventional fuelled engines). A regular or parallel connection between these drives is in use. Diesel hybrid buses can operate, under urban traffic conditions, with zero exhaust emissions. Vehicles with hybrid drive are located between conventional fuel vehicles and fuel cell vehicles.

The use of hybrid drives on vehicles significantly reduces exhaust emissions compared to diesel engines. Fuel consumption is lower by about 15-30%, as well as total CO_2 emissions. Some European manufacturers (MAN, Mercedes, Solaris) have developed their models of road vehicles on hybrid drives. The drive system of road vehicles, with pure electric drive, consists of an electric motor, a battery pack that serves for energy storage and control systems. Today, mainly lead and nickel-cadmium batteries are in use. It is intensively used to increase the use of nickel-metal-hybrid, lithium-ion and sodium-nickel-chloride batteries. The main advantage of the electrically driven vehicle is the absence of exhaust emissions.

STATE AND TRENDS OF THE EXAMINATION EMISSION OF MOTOR VEHICLES

In California, 1966, first legislation on limitation of exhaust emissions of passenger vehicles was passed. In Europe in 1970, the first directive on the limitation of exhaust emissions of passenger vehicles (Directive 70/220 / EC) was adopted. Directive 98/69 / EC prescribes limit values for the following exhaust and diesel engine components [5]: Carbon monoxide (CO); Non-Hazardous Hydrocarbons (HC); Nitrogen oxides (NO_x); Solid particles.

From the Euro 4 regulations, all parts of vehicles that affect the exhaust emissions must prove a 100,000 km duration, and from the Euro 5 regulations, the lifetime is extended to 160,000 km. In 1990, the California Environmental Protection Agency (CARB - California Air Resource Board) adopted a program called Low Emissions Vehicle Regulations (LEV). This program has demanded from the automotive industry the continuous introduction into the market of an increasing number of "clean vehicles", with a permanent On Board Diagnose (OBD) control. Four categories of vehicle legislation have been introduced, which were continually in line with the increasingly stringent legislation: TLEV - Transient Low Emissions Vehicles (since 1995), LEV - Low Emissions Vehicles (since 1988), ULEV - Ultra Low Emissions Vehicles (from 1998), ZEV - Zero Emissions Vehicles (2% since 1998, 10% since 2010).

Since the request for ZEV to date could not be fulfilled, in 1998 the legislator passed the LEV II regulation, which introduced two other categories: SULEV - Super Ultra Low Emissions Vehicles, PZEV - Partial Zero Emissions Vehicles.

In 2012, a new LEV III regulation was adopted, which tightens the limit values for HC, NO_x and PM and applies to all vehicles since 2020.

In almost all countries of the world, exhaust emission limitation regulations apply, which rely on the regulations of the European Union, the US, or Japan.

Together for the regulations of all countries, tests are carried out at the test bench and have their own specific driving schedule. Differences in test methods in the EU, USA, Japan, ... significantly increase the costs of development and homologation.

Currently, 125 ECE Regulations are in force, of which 15 are in the field of exhaust emissions and energy of motor vehicles, namely: ECE15 - passenger vehicle emissions (no longer relevant); ECE24 - smoke emission of heavy engines and vehicles; ECE40 - motorcycle emission; ECE47 - moped emission; ECE49 - Emission of gases and particles of heavy engines and vehicles; ECE67 - LPG fuelled vehicle equipment; ECE83 - Emissions of passenger and light goods vehicles; ECE84 - Measurement of fuel consumption; ECE85 - engine power measurement; ECE96 - emissions of tractor diesel engines; ECE101 - carbon dioxide emission and fuel consumption of passenger vehicles; ECE103 - replacement of catalytic converters; ECE110 - specific equipment for compressed priming gas (CNG); ECE115 - Subsequent installation of LPG and CNG devices and ECE120 - Measurement of power and fuel consumption of tractors.

Global Technical Regulation (GTR) includes: Worldwide Heavy-Duty Certification Procedure (WHDC) - GTR No.4; Worldwide Motorcycle Emission Test Cycle (WMTC) - GTR No. 2; OBD System for Heavy Duty Vehicles (WWH-OBD) - GTR No. 5; Heavy Duty Off-Cycle Emissions (OCE) - GTR No. 10; Particulate Measurement Program (PMP); Non-Road Mobile Machinery (NRMM); Worldwide Harmonization of Light-Duty Test Procedure (WLTP); Hydrogen and Fuel Cell Vehicles (HFCV).

In order to improve exhaust emissions of gasoline engines, the application of new technologies is required (in order to meet the requirements of Euro 5 and 6), which can be expressed as follows [6]: Engine improvement (electronic injection, turbo charging, variable distribution scheme, EGR); Subsequent treatment (TWC, catalyst heating, SCR); OBD;

Direct injection (reduction of emissions and consumption, higher price, particulate emissions); Alternative fuels (bio-ethanol, hydrogen); Hybrid drive (parallel, serial, parallel-serial, mild, plug-in: good emissions and consumption, higher price and weight of the vehicle); Fuel cells (perspective solution but far from application); High quality fuel (benzene and lead). The diesel engine is a big pollutant in big cities and is therefore significantly more than an octagonal engine at the stroke of the law. In order to meet ever more stringent regulations, in terms of exhaust emissions of motor vehicles, it is necessary to develop new modern technologies. Introducing new, alternative drives to serial production require comprehensive research, as well as finding solutions that will meet not only the criteria for exhaust emissions, but also in terms of performance and vehicle characteristics. In doing so, it is also necessary to provide and lower the costs of production and exploitation of vehicles with such facilities. In parallel with the measurement of exhaust emissions, fuel consumption is measured.

Automotive producers, as well as fuel producers, must be involved in solving the "Vehicle Influence on the Environment" problem. The fact that "clean" motors also require "clean" fuels is known. In this context, cooperation between the oil and automobile industry is necessary. New concepts of engines and new technologies for subsequent exhaust gas engine exhaustion require the optimization of the complete system. In addition to the engine and exhaust system, fuel, as an important part of the engine, must be involved in the process of optimization. The world's largest vehicle manufacturers have set requirements that fuels have to meet to be successfully applied in their SU engines. After eliminating lead, reducing the amount of aromatic, especially benzene, attention has been paid to reducing and completely removing. And the smallest amount in the fuel has a negative effect on the operation of the exhaust gas purification system. In the EU, fuel consumption is not directly limited, but since 1978 manufacturers are obliged to provide information on the amount of consumption. Indirectly, fuel consumption is limited by CO_2 emissions. CO_2 emissions and consumption of fossil fuels (B) have the following dependence: CO_2 = 27 x B [l / 100 km] for diesel engine, CO_2 = 24 x B [l / 100 km] for vehicles with an octagon.

The introduction of the regulation 443/2009 EC should contribute to reducing the CO_2 emissions of one producer from 2012 to 2015 to 120 gCO_2 / km (about 5 l / 100 km). Until 2021, this value is expected to decrease to 95 gCO_2 / km (≈3.5 - 4.0 l / 100km). In the US, fuel consumption, through the so-called. CAFE standard (Corporate Average Fuel Economy) has been limited since 1978. In 2010, new rules for fuel consumption and CO_2 emissions were adopted in the United States. The limit values for fuel consumption and CO_2 emissions depend on the geometric surface of the vehicle. In order to reduce the emissions of non-combustible hydrocarbons from the vehicle, it is also necessary to take into account the evaporation of fuel, rubber, plastics and various adhesives. In the EU since 1983, and in the United States since 1993, the tests for the evaporation of hydrocarbons of the complete vehicle at rest, which are carried out in special chambers - so-called SHED-test (Sealed Housing for Evaporate Emission Determination).

Despite the efforts made to find another (alternative) power train for motor vehicles, the S SU engine has remained an unmodified power train generator of motor vehicles. Almost 140 years of diesel and octagonal motors show that the best technical solutions of the power train aggregates of the fuel-efficient vehicle contained in fossil fuels. It is believed that both engine variants will retain their significance as driving aggregates for motor vehicles and the future, and that the development regarding the reduction of fuel consumption and the harmful effects of exhaust emissions on the environment will continue to be intensively continued. By 1975 only the theoretical comparative cycles of the oto and diesel engines were analyzed, which only counts on the economy of the engines. In 1975, a new theoretical cycle was patented, which also takes into account the reduction of exhaust emissions (HC and NO_x), so-called thermodynamic cycle with isothermal expansion. Otto and diesel engines with multiple, direct spraying, which are now produced and applied, work according to this cycle.

Since the introduction of the first legislation on the limitation of exhaust emissions, in the early 1970s, motorcycles have been facing ever more stringent requirements regarding the satisfaction of requirements related to the composition of the exhaust emission. The development of the Otto and Diesel engines enabled them to meet ever more stringent requirements, which enabled them to be the most applicable motor drive units today. Measures taken to reduce exhaust emissions and reduce fuel consumption (and thus CO_2 reduction) include optimizing a large number of parameters and systems of these engines. All vehicles, which are produced today, must have an activated carbon reservoir, to reduce vehicle evaporation in peace, as well as a complete exhaust emission control system (OBD). Today's results of the analysis of the impact of CO_2 emissions on possible climate change have intensified their efforts in the development of engines in the field of reducing fuel consumption. Opening constantly new roads to reduce fuel consumption. The development of new materials, the application of new technologies of production, control and process control in the engine, contribute to the improvement of the characteristics of the engine sus, both from the aspect of reducing fuel consumption and from the aspect of reducing the harmful impact on the environment.

CONCLUSION

The intensive development of traffic, which is a logical consequence of the progress of society, has adverse effects on the environment: Increase in the number of traffic accidents; Pollution of the environment by exhaust emission of the engine; Noise; Non-ferrous materials of vehicle parts; Converting natural surfaces into asphalt-concrete surfaces for roads and parking spaces. All parameters indicate today that the car will retain its primacy as a means of transport in the foreseeable future. Replacement for it has not yet been found. Of course, it will still have to meet all the demands placed before it by increasing traffic density, especially in big cities, with all the problems related to energy consumption and environmental damage. The question of the drive unit for vehicles will find a response primarily in the supply of energy on the market. Forecasts about the amount of existing oil reserves are constantly changing for decades. Even today, as it was half a century ago, it is claimed that they will last another 30 to 40 years. But regardless of the amount of reserves, it is known that they are limited and that is why looking for other fuels is a constant companion for development in the automotive industry. For more than 100 years, the intensive search for alternative automotive driving systems has shown that none of the proposed systems, in the sum of their features, could compete with a four-stroke engine. At the beginning of the 21st century, the engine is thought to be the main driver of the car in the next 20 to 30 years. The only alternative that does not replace, but is complemented by the engine is the so-called hybrid drive, combining a motor drive with an additional electric drive. Cars with pure electric drive or drive through the so-called fuel cells will not be used in mass traffic for a long time. But no matter which drive unit drives cars, environmental requirements will be tougher. Future propulsion engines, new materials, new fuels will also have to comply with all environmental protection regulations at all stages of its lifetime, from raw materials to recycling. Because one will not change, for the survival of mankind, four elements on which the ancient world rested: air, water, earth and fire remain of great importance.

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THE POSSIBILITY OF IMPROVING THE SECURITY OF TRANSPORT OF DANGEROUS CARGO FROM THE ASPECT OF A VEHICLE

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Abstract

All those substances due to improper and / or negligent work may lead to harmful consequences for humans, flora and fauna, as well as material goods are classified as dangerous goods. In order to transport hazardous materials industry took place with maximum security, it is necessary to provide the necessary and sufficient conditions. The paper provides an analysis of the possibilities of improving the security of transport of dangerous goods by road, particularly in terms of the vehicle.

Keywords: dangerous goods, road safety, vehicle

INTRODUCTION

All those substances that, due to incompetent and / or insecure work, can lead to harmful effects on humans, plant and animal life, as well as material goods, are classified as dangerous substances.

All hazardous substances, according to the European Agreement on the International Carriage of Dangerous Goods in Road Traffic - ADR [1], are classified in several so-called the class. The suitability of the appropriate class depends primarily on the aggregate state and the hazard characteristics. In order to ensure that the transport activity of hazardous substances takes place with maximum safety, it is necessary to provide the necessary and sufficient conditions prescribed primarily by the relevant legislation.

An analysis of the possibilities for improving the safety of traffic in the transport of dangerous goods can be done from the following aspects: Existing legislation; Transport operations; Transport means; Traffic and traffic infrastructure; People participating in the transport process; Ambient and road conditions; Transport organizations; The work of the competent institutions in the field of preventive action; The work of the competent institutions in the field of eliminating or mitigating the consequences of the negative effects of dangerous substances on humans, the flora and fauna and material assets.

A special attention is paid to the analysis of the possibility of improving the transport of dangerous goods, primarily from the aspect of the vehicle.

VEHICLES FOR TRANSPORT OF HAZARDOUS MATERIALS

The Law on Transport of Dangerous Goods stipulates that means of transport for transporting dangerous goods must be technically correct, manufactured, equipped and marked in accordance with special conditions. Vehicles must be specially designed and must comply with the conditions prescribed by the Law on the Transport of Dangerous Goods by the European Agreement on the International Carriage of Dangerous Goods in Road Traffic - ADR. According to the ADR, depending on the characteristics of the dangerous substance that is being transported, vehicles of different characteristics are used. The following classification of these vehicles has been introduced to facilitate the definition of the requirement [1]:

"FL vehicles" of vehicles for the transport of liquids with a ignition point of not more than 61ºC, (with the exception of diesel fuel, gas and crude oil mixture and light oil for heating, UN No. 1202, even if their ignition temperature is greater than 61ºC) or for flammable gases in tank containers, portable tanks or multi-element gas container (MEGC) with a volume greater than 3000 l or in tightly coupled tanks or demountable tanks and battery-vehicles with a capacity greater than 1000 l for transport for flammable gases.

"OX vehicles" of hydrogen peroxide vehicles, aqueous solution stabilized, with more than 60% hydrogen peroxide (class 5.1, UN number 2015) in a container of tanks in portable tanks with a volume exceeding 3000 l or in tightly coupled tanks or demountable tanks, a volume greater than 1000 l.

"AT vehicles" of vehicles that do not belong to FL or OX types, which are intended for the transport of dangerous goods in tank containers, portable gas tank containers for multi-element gas (MEGC) with a capacity of more than 3000l in tightly connected tanks or demountable tanks for batteries of more than 1000l of non-FL types.

In the case of a code, in accordance with the provisions of the ADR, it is necessary to use a FL vehicle for the transport of dangerous goods, then only the vehicle that has the FL can be used; OX, then only the OX vehicle can be used; AT, then a vehicle with AT, FL and OX can be used. In addition to the aforementioned types of vehicles used for the transport of dangerous goods, for the transport of Class 1 dangerous goods, the types of vehicles EX / II or EX / III and MEMU are used. Vehicle type must be indicated in the vehicle certificate, which directly affects the amount of dangerous goods that can be transported in one transport unit. In the case of vehicles for the transport of dangerous goods, special attention must be paid to the existence and the correctness of the parts listed in the ADR.

Vehicles for the transport of dangerous goods should also satisfy certain specific requirements, in accordance with the existing legislation, regarding the implementation of braking systems, electrical equipment, fire protection, speed limiter and coupling devices for towing and connecting vehicles, sidewalls protection devices and from behind, marking, necessary equipment and devices. Some of the regulations and norms relating to vehicles for the transport of dangerous goods, according to international regulations, are [2,3]: EEC94 / 63 - way of filling tank, EEC 97/27 - general conditions, ECE R48 - electrical installation, ECE 105 - conditions for chassis, ECE 111 - lateral stability of vehicles tank, ECE R70 - vehicle marking plates, EN12972 - safety and testing, EN13094 and EN 14025 - test pressure, EN 13082 - exhaust valve, EN 14595 and EN 14596 - valves and safety valves, ...

Required documentation in the vehicle for the transport of dangerous goods Before the start of the process of transporting dangerous goods and dangerous goods, it is necessary to provide the necessary documentation that is issued for the needs of transport by the competent institutions. This documentation must be valid at the time the transport is performed, and its validity is checked by controls. In the cabin of a vehicle transporting dangerous goods, the following documents must be: Certificate of safety of vehicles for the transport of dangerous goods, or matter, of a particular class, which is carried; Certificate of driver training; drivers must attend and place special training for drivers who manage transport units for the transport of dangerous goods; Factory leaves for all dangerous goods being transported; Written instructions in the event of an accident, for each dangerous substance being transported; Permits granted to this transport.

Certificate of vehicle safety In accordance with existing regulations, it is mandatory to carry out testing of motor vehicles and trailers for the transport of dangerous goods in order to issue Certificates and Certificates, which confirms the correctness and equipment of vehicles for the transport of dangerous goods. Motor vehicles and trailers intended for the carriage of dangerous goods, according to the existing national and international regulations, must be subject to a special inspection regarding the fulfilment of conditions provided for by the Law and the ADR for the transport of a specific hazardous substance. Examination of vehicles, designed and constructed for the transport of dangerous goods, includes the determination of reliability and safety from the aspect of exploitation conditions in road traffic. Examination of vehicles for the transport of dangerous goods basically encompasses performance vehicles, determination of functional characteristics, from the aspect of special requirements for the transport of certain dangerous substance, that is, determining the constructive characteristics of the equipment and equipment, as well as the constructive implementation of the vehicle as a whole, from the aspect of the specific requirements defined in transport of dangerous goods.

The methodology for testing vehicles for the transport of dangerous goods is primarily based on the characteristics of dangerous goods being transported, as well as the current legislation. It is especially important to emphasize that when testing vehicles for the transport of dangerous goods it is necessary to respect all applicable regulations (in particular all recommendations and requirements of the European Agreement - according to ADR).

In order to create the conditions for the safe transport of dangerous goods, it is necessary to carry out proper control in accordance with the current legislation. Control can be successfully performed by persons who have undergone appropriate training. Supervision is carried out to gain insight into the execution of tasks, instructions, decisions, verification of the legality of work and taking appropriate measures. Vehicles transporting dangerous goods must be subject to an annual check in the country where they are registered to determine whether they meet general and special requirements that are imposed prior to the performance of vehicles used by hazardous substances.

Pay particular attention to the control of requirements relating to: Electrical installations; Appropriate battery circuit breaker; Speed limiter; ABS braking system and appropriate system for permanent braking; Marking. If vehicles are made as trailers and semi-trailers, i.e. connecting vehicles that connect to the towing vehicle, then the towing vehicle is subject to the same technical inspection. If a vehicle is required to be equipped with a durable retarder, the first review for the issuance of an ADR certificate requires a manufacturer's declaration of compliance with the requirements imposed on permanent retarders. The competent institution in the country of registration of the vehicle shall issue a certificate of compliance with the requirements prior to the performance of the vehicle, for each vehicle. The certificate shall be issued in the language, or languages of the issuing country, as well as in English, French or German, unless otherwise provided by the contract between the countries between which the transport is performed. A certificate issued by the competent institution of one of the contracting parties, for a vehicle registered in the territory of that Contracting Party, must be respected within the time limit of its validity by the competent institution of the other Contracting Party. The validity of the vehicle's validity certificate can not last longer than one year, counting from the date of issuance of the certificate immediately after the inspection. Also, the date of the last examination must be indicated on the certificate.

When forming a complete record, on the basis of which the Certificate for a specific vehicle according to the ADR is issued, it is necessary to use the prescribed procedure in its entirety. When inspecting the vehicle, for the purpose of issuing the Certificate for the Transport of Dangerous Goods, it is necessary to determine the existence and the correctness of specific details in the construction of the vehicle and to record it explicitly. Dangerous substances, classified in different classes, also condition the differences in the equipping of vehicles for the carriage of these materials. For each class of dangerous goods, depending on their categorization and legal regulations (Laws, Regulations, Procedures, etc.), on the one hand, and the construction of vehicles depending on the manufacturer and vehicle type, on the other hand, it is necessary to draw up a each vehicle examined, which will be in the file of the specified subject - Certificate. At the request of the manufacturer or his legally accredited representative, the basic vehicles of new motor and trailer vehicles subject to type approval may be type-approved by the competent institution. This type-approval must be accepted as ensuring the conformity of the base vehicle in the event of the approval of a complete vehicle, provided that no modification of the base vehicle endangers its importance.

For each vehicle that demonstrates satisfactory results at the technical control, an authorized laboratory from the country where the vehicle is registered and issued by the ADR - a certificate of vehicle safety. This certificate must be completed in the official language or in one of the official languages of the issuing country, as well as in English, French or German, provided that that language is not one of these three, except insofar as the international agreement of the interested countries otherwise regulated. The ADR certificate must be a safer model that is shown in the ADR.

A special certificate of the ADR on the safety of vehicles issued by the authorized institution of the Contracting Party in the Agreement on the territory of which the vehicle is a vehicle shall be accepted by the authorized institutions of the other Contracting Parties until the expiration of its validity.

A special ADR certificate shall cease to be valid no later than one year from the date of the technical control that preceded its issuance. The next term is related to the last date of the official expiration date, insofar as the technical control is executed and the time interval of one month before or after this date. In the case of tanks subject to mandatory periodic control, this regulation does not imply that the testing of sealing, hydraulic pressure test or tank interior inspection must be performed at time intervals shorter than prescribed by ADR.

The task of the authorized institutions for checking the conformity of vehicles for the carriage of dangerous goods with ADR regulations, and the existing legislation, is that, based on the results of the inspection and testing, they issue the appropriate approval and certification for a particular vehicle intended for transport of dangerous goods, which confirms that it meets the prescribed requirements.

The overall content of this paper is an effort to show the key aspects of the necessity of providing the necessary conditions that the vehicle must satisfy to be able to be used for the transport of dangerous goods according to the ADR.

SPECIFICATIONS OF THE CONTROL OF A VEHICLE FOR TRANSPORTING HAZARDOUS MATERIALS

Vehicles for the transport of dangerous goods, whether newly manufactured or repaired for this purpose, are subject to mandatory control of technical safety, in accordance with the legal regulations on the transport of dangerous goods in road traffic.

Vehicles for the carriage of dangerous goods must satisfy all the conditions prescribed for a particular class and category of vehicles, but also additional conditions that are directly related to the dangerous goods carried by that vehicle, which are precisely given under the ADR. It is absolutely necessary to strictly observe all the provisions of the existing legislation, which apply to these vehicles, but also all provisions of the ADR. When checking the technical correctness of these vehicles, pay special attention to the following [2,4]:

- Does the motor vehicle have a valid certificate of the safety of vehicles for transport of dangerous goods, as well as appropriate certificates on the correctness of certain components and parts of these vehicles, according to ADR and certain legal regulations (for example: On the testing of the tightness of courts for the storage of dangerous goods; On testing the ability to withstand certain pressures; On testing of safety components and assemblies of these vehicles, ...);
- Whether the vehicle is equipped and marked with basic equipment and devices, according to ADR and certain legal regulations;
- Whether vehicles, depending on the hazard of transported substance, are equipped with additional equipment and devices, according to the ADR and certain legal regulations.

Vehicles for the transport of dangerous goods must have: basic and additional equipment.

It is mandatory to have the following basic equipment: Driving tool and vehicle jack; At least two fire extinguishers, one for fire fighting on the engine and one for fire extinguishing at the vehicle's cargo and for such a charge, which, in terms of quantity and other properties of dangerous substance, enables efficient fire extinguishing; Two hand-held electric flashlights with a flashing or continuous light color, visible from a distance of at least 150m; Two signs indicating a motor vehicle stopped on a cart track; Two flags marking vehicles transporting hazardous substances; Two shovels and one beverage; A portable lamp that can be plugged into the vehicle battery and which is designed to not cause an explosion or fire. In addition to this basic equipment that every vehicle that transports dangerous goods must have, vehicles for the transport of radioactive materials must still have: Radiation control device and means for protection and marking of the terrain; Two flags for marking vehicles transporting radioactive substances. It is also necessary to have the following additional equipment: identification boards, labels and inscriptions, according to the provisions of the ADR.

CONCLUSION

Traffic accidents, when it comes to hazardous matter, can lead to catastrophic consequences for humans, plant and animal life, but also for material goods. In order to raise the safety of the transport of dangerous goods to the highest possible level, appropriate conditions are necessary. In this context, the creation of the necessary conditions for the safe realization of the transport of dangerous goods plays an important role: Legislator; The authorized state institutions that regulate the subject matter and supervise the implementation of legal regulations (Ministry of Internal Affairs - Traffic Police Directorate, Ministry of Transport, Construction and Telecommunications - Agency for Traffic Safety, Directorate for Transport of Dangerous Goods); Designated bodies for the control of vehicle safety; Designated bodies for vehicle testing; Designated bodies for testing vessels and vehicle equipment; Designated bodies for education of people participating in the transport of dangerous goods; Traffic control services; Participants in the transport of dangerous goods. In order to realize the transport of dangerous goods in road traffic in a satisfactory way, primarily from the aspect of safety, it is necessary:

- Define adequate national legislation, which is harmonized with international legal regulations in this area; - Compliance with all provisions of the European Convention on the Transport of Dangerous Goods in Road Traffic - ADR, as well as all provisions of national legislation in the subject area; - The existence of a properly trained person who carries out tasks related to the transport of dangerous goods in road traffic; - Existence of the appropriate equipment necessary for the control of the technical correctness of the vehicle, servicing of vehicles, regulation and traffic control; - Existence of adequate traffic infrastructure with adequate traffic regulation system; - Existence of an adequate organization and methodology for testing and controlling the technical safety of vehicles; - The existence of adequate vehicles that comply with all regulations so that they can be used for the transport of dangerous goods; - Existence of an adequate organization in monitoring and control of dangerous cargo; - Existence of an adequate organization, training and equipment of people within the intervention teams that should act in conditions of traffic accidents when it comes to the transport of dangerous goods. There is much work to improve the safety of dangerous goods transport in our country, and today great efforts are being made in that direction.

In the future, in the future, on the basis of realized activities undertaken to improve the transport of dangerous goods, the level of safety of dangerous goods transport will continue to grow. In this context, as far as vehicles intended for the transport of dangerous goods are concerned, special attention should be paid to the selection of adequate vehicles, their proper testing and control of their technical safety, as well as their proper maintenance and exploitation.

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PUBLIC WATER SUPPLY AS AN ALTERNATIVE ENERGY

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Abstract

Using the potential energy of water in the water supply system is one of the ways to obtain alternative energy. The paper presents the basic criteria and characteristics for the selection of the equipment for small hydroelectric power plant as well as the production of electricity for the past 6 years. Overview of the limitations during the production is given and the way that required quantities of water are provided.

Keywords: energy, water, unit, SHHP

INTRODUCTION

Most of the greenhouse gases are generated in the production of electricity from non-renewable energy sources. Large investments are made in European countries to increase the quantity of renewable energy sources in total electricity production. The electricity production using renewable sources is important because it is a safer and more reliable way of obtaining electricity and does not affect environmental pollution. Water is the most important renewable energy source in Macedonia.

SMALL HYDRO POWER PLANT (SHPP)

Hydro power plants use water and gravity (a fully carbon free and non-exhaustive source) to drive turbo generators that generate electricity. Unlike power plants using fossil fuels, hydro-capabilities do not generate any gases or ash. In comparison with nuclear power plants, there is no radioactive waste in the hydroelectric power plant, which is a special problem, nor does it waste any resources because the water can't be lost or polluted as a result of the process of electricity generation from hydro potential.

Macedonia started to develop the concept of small hydropower plants in 2007 through the Ministry of Economy, i.e. calls for participation in a tender concession with applications for over 140 locations for SHPP with a capacity of 35 to 2,500 kW. The projections in that period were that in the near future SHPP will reach much larger number near 400. Today, according to the registry of privileged producers from SHPP released on the site of the Energy and water services regulatory commission of the Republic of North Macedonia there are a total of 79 SHPPs [1].

SHPP STARA FILTERNICA BITOLA

The small hydro power plant “Stara filternica” is located next to the reservoir near the facility named Stara Filtrnica at locality Dovlezhik in Bitola. It is planned to utilize the potential energy of water that is coming gravitatively into the existing reservoir-knot No. 5.

The required water is provided through three intakes of the watercourses of Mountain Baba, including the catchments of the rivers Sapuncica, Lak Potok and Crvena, as well as from the accumulation of the artificial lake Strezevo in the case of a shortage of water from the intakes, i.e. the water from the accumulation is pumped to the reservoirs at the purification plant located in the village of Dihovo.

In the purification station so called Nova Filternica raw water from the intakes is being process and as a chemical and biologically clean drinking water is sent to the consumer via a pipeline with a length of about 1850 m and a diameter of 812 mm to the intermittent chamber (knot 5) filter plate. The pipeline is underground (covered) and protected by cathode protection.

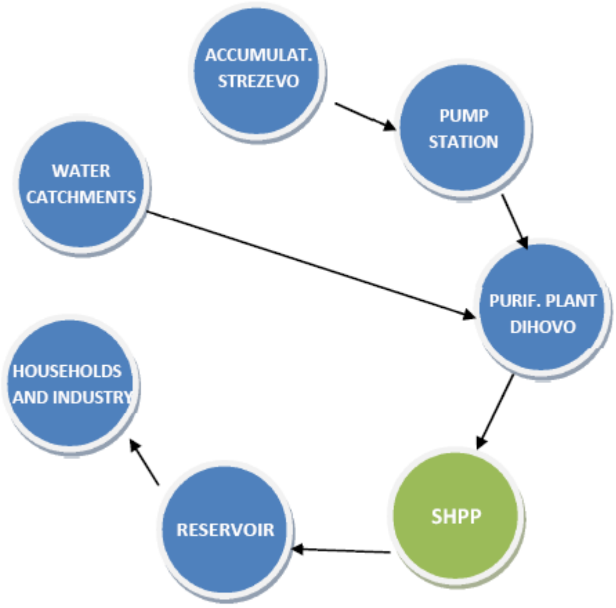

Figure 1 *Disposition of the SHPP in the water circle*

Drained water from the sedation chamber of the SHPP is directed directly into the reservoir or in the pipeline for a lower zone.

Already constructed facilities owned by JKP Vodovod Bitola were highly important for the relatively short and successful realization of this project. These facilities include: purification plant-Nova Filternica, intermittent chamber (PK-8) built right below the purification plant where the supply pipeline starts, a pipeline with a length of 1850 m and a diameter of 812 mm, built an intermittent chamber at the Stara Filternica facility and a reservoir with a size of 600 m^3 [2].

From another side, the concerns pointed by the environmentalists' that include negative influence of the construction of small hydro power plants to the environment including river beds destruction, eco systems disturbance, reduced water level and fish stock, destruction of soil and water with hydraulic oils and cement, are unsustainable in our case because all of these phases were previously implemented in the 60th years when the intakes and network for the water supply system for the city of Bitola were made.

GENERAL TECHNICAL DATA

Technical solution of the object

The technical solution of the facility and the selected equipment is based on the existing technical documentation for the constructed facilities, the topographic conditions and the disposition of the facilities, as well as the technical conditions for the selection of the equipment. The main power equipment consists of a single unit with a horizontal axis (Francis turbine directly coupled to a synchronous generator). The hydropower plant is equipped with control, signalling and protection equipment, which enables a fully automated drive.

The SHPP Stara Filternica facility is made up of the following composite units:

- Water catchment upstream of the existing pipeline at the site planned for connection to the projected reservoir
- Connected supply pipeline to the hydro power plant
- Hydro power plant building
- Drainage water with a calming chamber

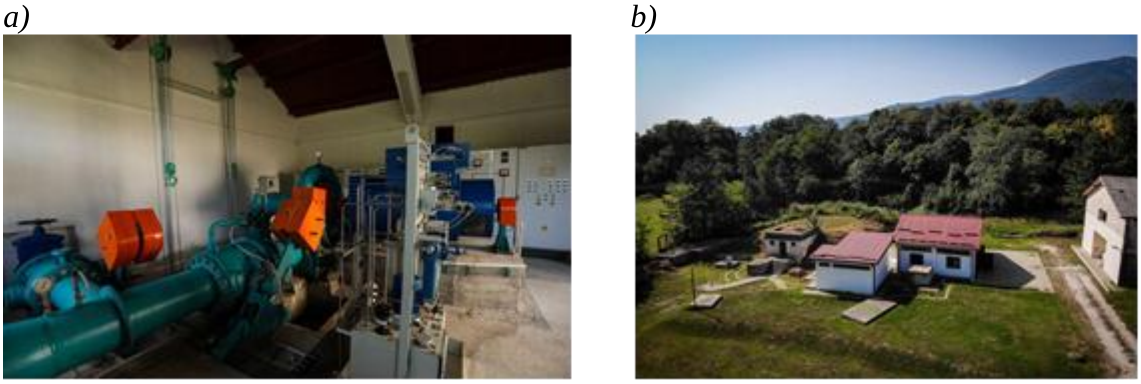

Figure 2 *SHPP, a) hydromechanical and electrical equipment; b) plant building*

Technical parameters of the hydro power plant

In order to accurately determine the energy losses, and considering the relatively large length of the pipeline, as well as the time life that the pipeline is in operation, hydraulic measurements were made to determine the available energy depending on the flow of the pipeline [2].

Taking into account the performed hydraulic measurements, the hydrological data from the multi-annual exploitation period and the fact that the water supply needs of the system are constantly increasing the minimum (500 l/s) and the maximum daily flow (720 l/s) are determined.

Based on the conditions for selection of the equipment, the following main technical parameters of the unit are determined:

- Type of turbine: Francis with horizontal shaft
- Net drop: 60 m
- Nominal flow: 0.7 m^3/s
- Rotation speed: 1000 min^{-1}
- Runaway turbine speed: 1800 min^{-1}
- Power: 388 kW
- Generator type: synchronous
- Nominal voltage: 0.4 kV
- Apparent power: 440 kVA
- Power factor: 0.8

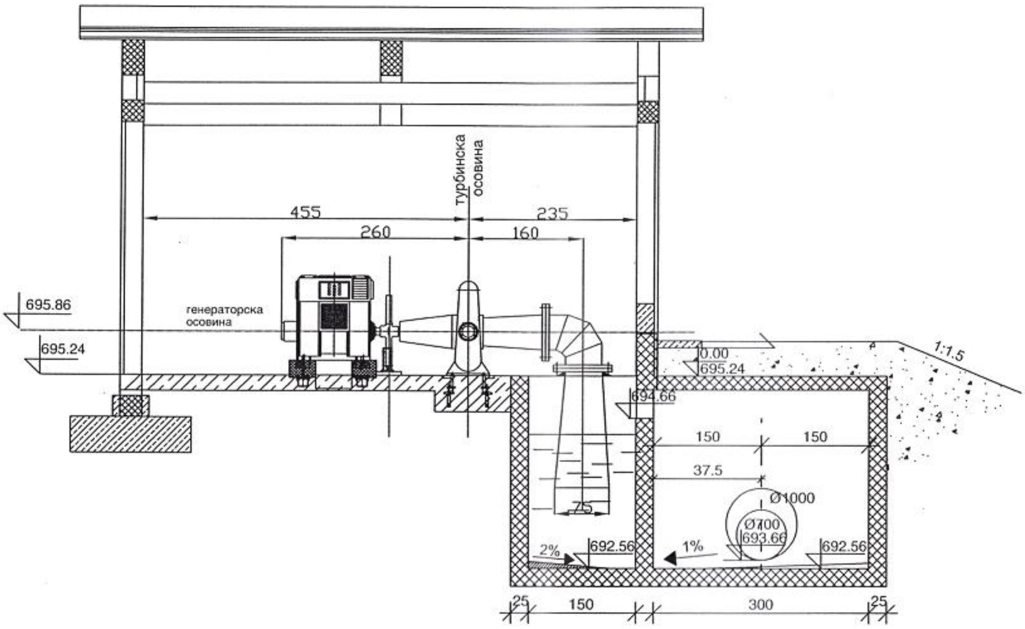

Figure 3 Unit cross section

PROJECTED ELECTRICITY PRODUCTION

The possible production of energy is calculated depending on :

• The available flow from the curve of duration as the average monthly values,

• Operating time of the unit (adopted-taking into account the following interruptions: regular annual overhaul of 10 days and unplanned outages from a total of 9 days).

With the above adopted values, the calculation of energy production as average monthly average production was carried out. In the calculation, a 5% reserve availability or 95% availability of the forecasted water quantities was adopted. Based on this, the total projected average annual production of SHPP is:

$E_{yr} = 2.41$ GWh

REALLY PRODUCED QUANTITY OF ELECTRICITY

The quantity of electricity produced in SHPP Stara Filtrantica for the period 2013-2018 is shown in Table 1 [3].

Table 1 *Electricity production by month from 2013 to 2018 year [kWh]*

Month	2013	2014	2015	2016	2017	2018
January	142,755	139,379	157,278	166,050	134,167	115,583
February	122,050	104,473	148,250	172,766	112,543	133,642
March	207,758	135,562	172,434	191,781	165,699	178,549
April	222,330	183,045	188,238	185,486	173,822	180,126
May	229,370	200,357	215,379	187,332	173,070	188,709
June	218,108	187,791	175,798	169,821	161,382	176,366
July	120,376	124,527	133,007	139,138	134,504	154,622
August	123,224	124,088	121,052	122,723	139,150	145,635
September	106,795	157,877	110,397	141,848	124,105	125,679
October	103,617	165,806	160,658	156,725	118,334	121,647
November	133,273	196,581	145,877	169,852	127,266	115,216
December	134,769	189,870	122,411	130,337	162,014	129,637
Total	**1,864,425**	**1,909,356**	**1,850,779**	**1,933,859**	**1,726,056**	**1,765,411**

If we compare the total projected (theoretical) average annual production of the SHPP and the real one realized in the given period, we will see that there is a deviation from 19.76% to 29.38%.

In the work of the SHPP we can recognize two periods:

A period of favorable climatic conditions, when the required raw water is provided in full from the three intakes of the Baba Mountains, and

Period of unfavorable climatic conditions, when the natural springs of the Baba Mountains have reduced yield and when with the pumping station the necessary quantities are supplemented from the accumulation of the artificial lake Strezevo.

In the first case, in addition to the quantities for the needs of the households and the industry, a part of the overflow of the reservoirs can be allowed in order to increase the electricity production in the SHPP, which is not allowed in the second case when there is a strictly controlled level of the reservoirs.

Regarding the planned annual overhaul of 10 days and unforeseen 9 day outages, it can be said that in reality over the past 8 years, the planned repair time is only 3 days, and the unplanned outages are from 3-4 days per year.

The official release date of the SHPP exploitation was 23 of March 2011 and already in the beginning of 2014, we gained return of the investment. Monthly costs included salaries for 4 operators who serve 24/7 during the whole calendar year and costs for repairs, protection examination, as well as unforeseen deferrals, which amount to not more than 1% of the investment costs (equipment and construction part total 536.000 EUR).

The short investment return period is also a result of previously constructed facilities owned by JKP Vodovod Bitola mentioned before in this paper and privileged tariffs for energy production given by Energy Regulatory Commission of the Republic of North Macedonia.

CONCLUSION

Advantages of Small Hydro Power Plants, but also hydroelectric plants in general, are unambiguous. They do not generate greenhouse gases emissions, they contribute to sustainable energy development by being economically viable by respecting the environment and allowing decentralized production of electricity (social benefits for distant places). Small Hydro Power Plants provide a clean energy (no waste is generated in the water or in the air) and they represent alternative renewable energy source.

Small hydropower plants are an efficient, reliable, clean, renewable and sustainable resource and they are especially feasible for the construction from the techno-economical aspect and from the aspect of environmental protection if they can be build and exploit on the existing pipelines which are already build and used for the water supply needs for the inhabited areas and industry, as in the case of the Small hydro power plant "Stara Filternica".

Small investments are required in maintaining the equipment and have a good investment profitability index (PI) and low discounted payback period (DPP).

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CLIMATE ASSESSMENT ACCORDING TO THE DROUGHT INDEX OF THE PART OF CENTRAL SERBIA

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Abstract

The research conducted on climate humidity and drought onset throughout the long-term series of data obtained on temperature and precipitation sum of the hydrological years (1996/97-2016/17) for Kraljevo (central Serbia), was conducted. The drought index (I) according to De Martonn was used to identify drought with the categories of semiarid (moderately dry) climate throughout the vegetation period 2010/11 and 2011/12. On average, the vegetation period is featured by moderately humid (semihumid) climate with the drought index of 30. On a year basis, the climate is humid which is indicated by the average drought index value for that period of 34. The average monthly level of drought index denoted to prevalently semihumid climatic conditions over July, August and September and to the humid ones over the remaining months of the hydrological year.

Keywords: climate, drought index, climate humidity, precipitations, air temperature

INTRODUCTION

The effect of climatic changes has been evidenced at the global level. Thus, the result of climatic changes and that of global heating are considered to be two extreme natural hydrological phenomena, droughts and floods as entirely unpredictable beforehand. Definitions of drought should reflect a regional proclivity since water supply is largely a function of climatic regime [1]. Drought is a slow-onset natural hazard that is often referred to as a creeping phenomenon. It is often difficult to know when a drought begins. Drought is a recurring climatic phenomenon over the land resulting from below-normal precipitation and/or high temperatures over the period of months to years [2]. Precipitation deficits generally appear initially as a deficiency in soil water; therefore, agriculture is often the first sector to be affected [3]. In this regard, in Serbia, the areas of eastern Serbia and Vojvodina seem to be highly affected.

The air temperature rise leading to a higher evapotranspiration (ETP) and drought are limiting agricultural production as the most sensitive sector of Serbian economy. The usual weather conditions may not occur due to climatic change. The snow cover and duration of winter period visibly decrease, affecting the winter soil water reserves. The onset of drought and floods (2005, 2006, 2007, 2009, 2010, 2013, 2014) in Serbia appears to be more frequent with consequences being more costly and forest fires being rather serious. The onset of landslide has become more intensive. The soil has been degraded, the fields flooded, buildings damaged, roads, energy sector and, therefore, tourism seriously impaired to the extent of catastrophes even with human lives being lost. The droughts and floods are said to have been caused by natural and antropogeneous phenomena. The role of a man is to manage such risks by preventing them in the first place and by enhancing overall resistance to their manifested consequences.

The analysis made and the data collected about climate categorisation into the levels (zones) of an area humidity, droughts and high precipitation amounts, provided significant information which might be used for improving water management strategies, agricultural production planning and environmental concerns, which altogether contributes to a better economic growth of a particular area.

Different methods are used to identify drought and how it is manifested, at which drought indices are often used when analysing it. The range of drought and its intensity determination ought to be clearly presented and linked with the concrete consequences that might arise subsequently [4].

The analysis made on the climate elements, air temperature and precipitations, drought constituents in agriculture and its categorisation on the area of central Serbia will, beside drought control enabled by protection measures, help the man gain better knowledge about the detrimental effects and adapt to the new conditions using new technologies within soil amendment, which is the major goal of this paper.

MATERIALS AND METHODS

The longterm series of 20 hydrological years (1996/97-2016/17) with the data about the monthly air temperatures of the area of Kraljevo [5] and monthly precipitation sum [6] were used to calculate the mean monthly air temperatures, the mean air temperatures over the vegetation period, the mean annual air temperatures, total and mean precipitation sums based on month, vegetation and on year, too.

Draught indices (I), denoting to its identification on the area studied, were calculated according to De Martonne [7]. The calculation included the air temperature parameters (T, ºC) and precipitation sum (P, mm) for the period of studies, monthly, vegetation and annual level.

The character of climatic conditions ascertained for drought assessment, i.e. climate humidity and its categorisation (dry, humid and moderate areas), was determined on the basis of marginal values of drought index primarily for agricultural purposes from the aspect of soil amendment.

RESULTS AND DISCUSSION

Kraljevo stretches on the area encircling the middle course of the Zapadna Morava and the lower course of the river Ibar. It is situated at 43°43’N and 20°41’E, at the altitude of 206 m. Seven separate relief entireties may be singled out on the territory of Kraljevo, being four mountains and three valleys, lying among these mountains. Farming, field crops, livestock and vegetable production are mostly suited by the flat ground and by the lowest parts of valleys. The soils appear in a larger range from those of lighter to those of heavier composition which may equally provide conditions for agricultural production.

The climate is moderately continental in the lower parts of Kraljevo municipality with mountain parts having mountainous climate.

Based on the analysis, the mean annual air temperature of the area of Kraljevo for the period of 20 hydrological years (1996/97-2016/2017) was found to amount to 12.0ºC and over the vegetation to 18.7ºC (Table 1), with the warmest months being July (22.7ºC) and August (22.4ºC) and the lowest air temperatures registered in January and February (0.7ºC and 1.7ºC, respectively).

Table 1 *Monthly, vegetation and annual air temperature means (ºC), Kraljevo (1996/97-2016/17)*

Years	**Months**												**Vegetation**	**Annual**
	X	**XI**	**XII**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**		
1996/97-2016/17	11.8	7.0	1.7	0.7	3.0	7.5	12.4	16.8	20.7	22.7	22.4	17.2	18.7	12.0

The average annual precipitations for Kraljevo amount to 755 mm, and that over vegetation period to 430 mm (Table 2). On average, annually and over vegetation, the rainiest month was May (87 mm) and June (85 mm). The lowest precipitations fell in January (46 mm) and in February (47 mm) while 59 mm of precipitations fell over the vegetation period in August.

Table 2 *Monthly, vegetation and annual precipitations (mm), Kraljevo (1996/97-2016/17)*

Years	**Months**												**Vegetation**	**Annual**
	X	**XI**	**XII**	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**	**VIII**	**IX**		
1996/97-2016/17	68	50	53	46	47	61	67	87	85	66	59	66	430	755

Further, the lowest precipitation amount of 528 mm was recorded in 2011/12 accounting for 69.9% of the annual precipitations and 246 mm accounting for 57.2% of the annual vegetation ones. Speaking of 2002/03, the annual precipitations amounted to 602 mm and those during vegetation to 336 mm. In 2010/11, the precipitations amounted to 609 mm and over the vegetation period to 280 mm.

The highest annual precipitation sum of 1079 mm recorded in 2013/14 was by 26% higher than the average annual ones. In the same year, 856 mm precipitation fell over the vegetation exceeding the average annual precipitation sum by 101 mm and also being 1.99 the precipitation average evidenced over the vegetation. Also, 921 mm fell in 1998/99, 571 mm over vegetation, 910 mm, i.e. 456 mm in 2009/10.

Based on the drought indices (I_y) per years of the area of Kraljevo recorded throughout a 20-year-period of analysis, 6 years had mildly humid climate, i.e. semihumid (SH) (30%), meaning that such a climate recurs every 3.3 years (Figure 1). Humid (wet) climate (H) appeared more frequently accounting for 70%, i.e. 14/20 implying (I_y) to recur every 1.4 year.

Such a finding seems to be similar to the conclusion made on the analysis that the climate of the northern and central parts of Vojvodina region becomes wetter in terms of precipitation value and frequency, reflecting the characteristic of the central European regime, whereas the southernmost part of the region is drier, reflecting the characteristic of the Mediterranean regime [8]. Taken from the average annual drought index average, the estimated mean value I_y amounting to 34, denoted that the area of Kraljevo had characteristics of humid (H) climate.

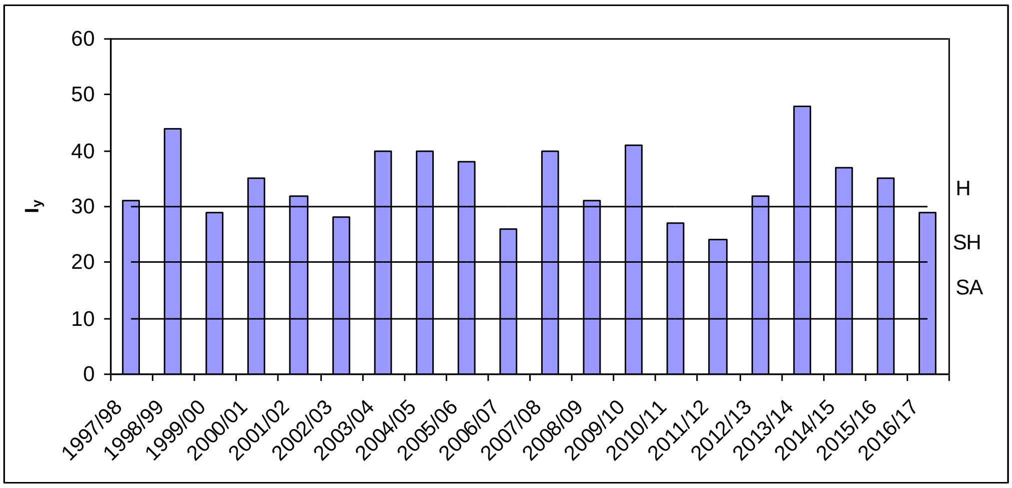

Figure 1 *Drought indices (I_y) per years (1997/98-2016/17) for Kraljevo region*

Drought index based on vegetation (I_y) denoted to the climate of Kraljevo over vegetation to be mildly dry, i.e. semiarid (SA), of 2/20 frequency, i.e. manifested every tenth year (2010/11 and 2011/12) (Figure 2) accounting for 10%. The climate signified as SH and H also characterises the vegetation period, appearing as frequently as 9/20 i.e. 45% may be expected over vegetation every 1.4 year in Kraljevo. The estimated average value I_y amounting to 30, suggests semihumid (SH) climatic features for the Kraljevo region if taken from the average annual drought index.

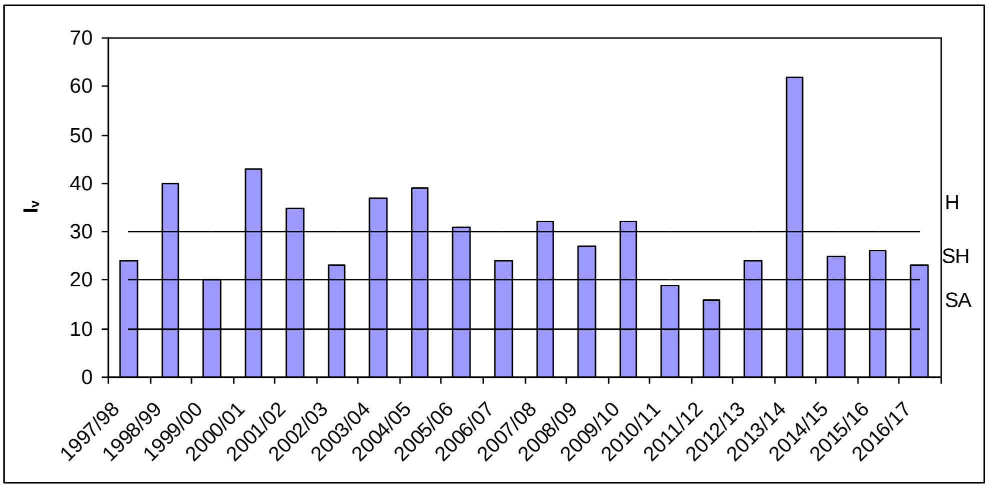

Figure 2 *Drought indices (I_y) for the vegetation period (1997/98-2016/2017) for Kraljevo region*

The average monthly drought indices (I_m) evidently denote to the features of semihumid (SH) and humid (H) climate (Figure 3). Based on the monthly averages, the climate of Kraljevo may be considered to be semihumid (SH) for July, August and September, with index values of 24, 22 and 29 along with remaining months of having prevalently humid climate (H). The relevance of obtained data comprises not only clearly defined climatic features, but also more clearly defined elements of the hydro melioration systems [9].

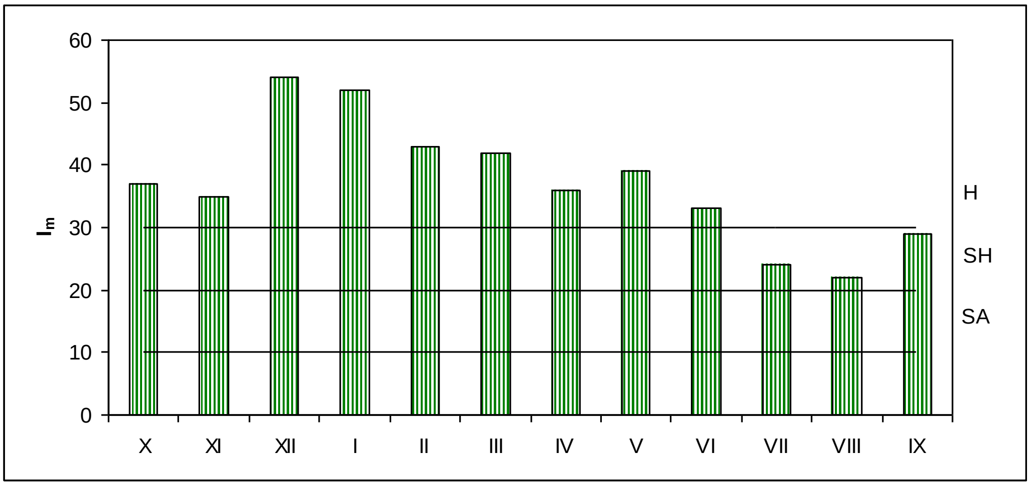

Figure 3 *Average monthly drought indices (I_m) (1997/98-2016/2017) for Kraljevo region*

CONCLUSION

The climate of Kraljevo may be inferred to have the features of all the three categories of climate, being humid, moderately humid and moderately dry (irrigation needed) with an exception to arid or dry climate. Such a determination of climate is corroborated by the analysis of climatic elements or drought indices. Humid climate proved to appear most frequently, denoting to the climate studied to have become more humid regarding precipitation amounts due to which irrigation of the soils of heavier mechanical composition should be used. 2013/14, 1998/99 and 2009/10 may be considered as extremely rainy years.

ACKNOWLEDGEMENT

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DEGRADATION OF THE MAKIŠ MARINA GREEN AREA DUE TO ANTHROPOGENIC IMPACTS

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Abstract

The paper presents the impact the anthropogenic factor on the degradation of to a close-to-nature landscape, the green space of Makiš Marina. The explored location is located on the right bank of the Sava River, in the suburban part of the city of Belgrade. It was investigated in which ways man influences the marina area, and the ways in which that affects the soil, water, flora and fauna. The anthropogenic impact and changes in the study area were analyzed on the basis of the natural characteristics of the area, environmental conditions, urban planning characteristics of the study area and current state of the green spaces. A detailed analysis of the bio-ecological properties of the dendroflora of Makiš Marina enabled us to determine the vitality and decorative properties of the local trees and shrubs. Lawns in the investigated area were evaluated on the basis of the main visual characteristics. Construction-architectural elements were categorized, described and evaluated. Human impacts on the fauna, changes and pollution of soils and underground and surface waters were also analyzed and shown.

Keywords: Makiš Marina, landscape degradation, human impact, landscape architecture, biodiversity preservation

INTRODUCTION

The degradation of the natural environment that occurs as a result of human activity happens everywhere around us (pollution of watercourses, soil, air, forest destruction, etc). Through these activities, man changes the biodiversity of a certain area and promotes the occurrence and spread of invasive plant species that endanger the survival of autochthonous vegetation, promotes the appearance of harmful biotic factors (pathogenic fungi, harmful insects, etc.) and affects the number of plant and animal species in a given area and causes their extinction.

The destruction of autochthonous vegetation started some 100 to 150 years ago, but by its intensity it overcomes all other disorders of the natural environment. A more significant deterioration of the natural quality of the landscape was created due to the construction of industrial complexes in places not intended for that purpose, unplanned construction of weekend settlements, as well as the illegal construction of facilities [1].

The main goal of this paper is to examine the ways in which the anthropogenic factor influences the area of Makiš Marina. Anthropogenic impacts were analyzed by determining the state of the greenery (its vitality and decorative properties), the presence of invasive plant species, the fauna of the investigated area and the ways in which humans use space. Changes in the space over time were considered, and whether these changes and manner of use are in accordance with the applicable laws: the Water Law [2], Ordinance on Minimum Technical Conditions for the Construction, Arrangement and Equipping of Nautical Facilities and Categorization Standards for Marinas [3] and the 2016 General Urban Plan [4].

MATERIALS AND METHODS

Data on the research area (Figure 1) were collected from printed and electronic sources, as well as in direct contact with professionals from various institutions involved in the process of managing the Makiš Marina area (PE Srbija šume, PE Ada Ciganlija, Nature Conservation Institute of Serbia). Literature data on the habitats and ecological conditions of the investigated area were collected, i.e. data on climatic, geological, pedological and vegetational characteristics of the area. The fauna of the investigated area was analyzed through literature data related to the fauna of “Ada Ciganlija” and “Obrenovački Zabran” Nature Monument, which are located in the close proximity to the investigated site. The data were collected from the applicable legal acts [2,3,4], and satellite images of the terrain [5]. Field research included the determination of plant material, measurement of dendrometric parameters, determination of the health status of dendroflora, presentation, description and evaluation of objects and elements of the spaces of anthropogenic origin. The results were used to provide guidelines and recommendations for preventing or reducing further degradation and devastation of the Makiš Marina area.

Figure 1 *Orthophoto image of the investigated area [5]*

RESULTS AND DISCUSSION

The investigated area is located in the territory of the city of Belgrade, in the municipality of Čukarica. It is separated from the urban part of the city by the river Sava, Ada Ciganlija and a wetland forest. According to Jovanović *et al.* [6], microclimatic conditions in the area of Makiš are significantly different from the climate of Belgrade. Forest massifs on Ada and in Makiš, the aquatoriums of the Sava River and the Sava Lake mitigate extreme summer temperatures. The main aquifer is in river-lake sediments and its waters are used for the water supply of Belgrade [1]. In Makiš, the natural-potential vegetation is made up of the community of white willow (*Salicetum albae*) [6].

According to the GUP (2016) [4], the existing purpose of the investigated area of Makiš Marina is a green space where agriculture, municipal activities, housing, and commercial activities are not allowed. The area of the Great War Island, Ada Ciganlija and the area of Makiš are areas with built Renney wellsand tubular wells in the riparian area of the Sava and Danube rivers, for which retention of purpose as green spaces is intended [4].

An increase in population, intensive urbanization and industrialization have lead to a change in the land use of large areas and loss of their original character. Soil pollution caused by the anthropogenic factor is considered much more dangerous than air and water pollution, since the consequences are more permanent and more difficult to remove [7]. In the area of Makiš Marine, the soil has been changed under the influence of man. The entire space is filled with a large amount of demolition waste (Figure 2) and the anthropogenic soil of different granulations is created (stones and other materials, i.e. pieces of concrete, bricks, and various objects and waste). The right bank of the Sava River was raised by backfilling with demolition waste, which changed the surface layer of the soil as well as its air-water regime.

Natural-potential vegetation was removed to build facilities such as restaurants, stilt houses, housing and parking space sat the investigated location. The construction of facilities (Figures 3 and 4) required introduction of additional infrastructure (access roads, paths, elements of energy infrastructure (lamp posts, power lines, etc.), toilets, drinking water fountains, animal areas, fountains etc.), thus introducing new elements into the landscape. Types of construction materials used for paths, plateaus, rest areas and other paved areas within restaurants and other facilities (concrete, asphalt and concrete elements) reduce the porosity of a green area Makiš Marina.

Figure 2 *Backfilled demolition waste*

Figure 3 *Housing facility*

Figure 4 *Stilt house*

A large number of objects and riverhouses in the immediate proximity (Figure 5) are located next to the very source of water supply of the city of Belgrade with drinking water. Such a location is not suitable and negatively affects the quality of drinking water, since waste water from these facilities is discharged, and in the marina and on the Sava River there is also gas leakage from water vehicles. The presence of riverhouses also implies a large number of barrels that retain a significant amount of sludge and plant material in the riparian zone. The barrels are made from sheet metal prone to corrosion, which results in further contamination of the water in the river. Over the years, a large amount of sludge is accumulated, and the river bed is narrowed, resulting in flooding.

Figure 5 *Riverhouses on the right bank of the Sava River*

Water in the river in the area of Makiš Marina is polluted with waste produced and left behind by the users of the marina, restaurant guests, owners of riverhouses, restaurants and stilt houses. The waste remains on the riverbanks after the withdrawal of water, which takes place during the summer months, and large quantities of sludge, mud, empty bottles, bags and other waste were recorded in the area.

The natural-potential vegetation of the study area is considerably changed due to the introduction of plant species, which are not typical for this habitat type (flood prone area). The presence of a large number of plant species whose bioecological characteristics are not in accordance with the conditions prevailing in the area of Makiš Marina resulted from the activities of users who applied plant material in this area at their own convenience.

Invasive species are a major issue and an obstacle to biodiversity conservation in many different habitats. The introduction of invasive species poses a threat to the structure and composition of communities, as well as to ecosystem processes [8,9,10]. The sensitivity of plant communities to the invasion by new species depends on the level of anthropogenic disruption [11,12]. Invasive species appear in large numbers in the investigated area, due to the cutting down of autochthonous vegetation, a change in the surface soil layer and the regime of surface and underground waters. In the research area, man accelerated the spread of invasive species such as: *Acer negundo* L., *Ailanthus altissima* (Mill.) Swingle and *Amorpha fruticosa* L.

The research results have shown that ground vegetation is also disturbed. The use of the area by man leads to the deterioration of the grass cover and creates bare surfaces. The reduction of areas covered by ground vegetation is pronounced, provoked by the anthropogenic factor, after the creation of covered areas especially around restaurants where sitting areas (restaurant gardens) are formed aroud the facilities. The lawns are also destroyed by the construction of parking lots and in fenced areas for animals which walk on lawns anduse them for food. Likewise, bad state of the lawns is also the consequence of the use of spaces by visitors. In the investigated area, roe deer and pheasant appear, and in the spring months also swan (the genus *Cignus*) was spotted. The explored area, along with Ada Ciganlija, located in its close proximity, is a habitat to a large number of birds, autochthonous species of small mammals, different insect species and fungi species [13].

The area is rich in fauna, with the most numerous populations of bird communities. It is a habitat to the endangered species of Pygmy Cormorant (*Microcarbo pygmeus*), mallard (*Anas platyrhynchos*) and gull (fam. *Laridae*), that gather on the lake. In addition, this area is inhabited by singing birds such as: thrush (fam. *Turdidae*), woodpecker (fam. *Picidae*), etc. [14]. Although it is a small area with disturbed natural characteristics and pronounced human impacts, Makiš Marina has significant preserved ornitofauna. The conservation of habitats such as this one creates a connection among the ecosystems in the Sava valley, which is important for the global preservation of ornitological values. The most common are species that are otherwise characteristic of the nearby forest area [15]. With regard to the quality of the fish stock in the Sava River, fish from 6 families were found in the area [15]. The endangering factor for the local fauna is also the way man uses electric power. In order to provide a more comfortable living, people made connections for electric power, but not in an adequate way - cables, electric meters and connections are not protected and can cause the mortality of fish that is in contact with water. Likewise, they can directly affect birds that can be hurt, when landing on trees and lamp posts with inadequately secured cables.

The results of the study show that, due to the construction of Makiš Marina and cutting down of the natural-potential vegetation, the ecosystem was disturbed as well as its connections. The areas of “Obrenovac Zabran” and “Ada Ciganlija” are located in the coastal zone of the Sava near the Makiš Marina and are very important from the point of view of biodiversity conservation and improvement of the environmental quality [16].

In order to avoid further disturbance of biodiversity, destruction of autochthonous vegetation and fauna, as well as pollution of the water supply sources in the Makiš Marina area, a more elaborate work of inspection services is needed. Inspection supervision activities are carried out by different departments in the inspection Secretariat [17]. The departments should co-ordinate each other’s activities and invest joint efforts to prevent further degradation and devastation of the nature of nearby spaces. In addition, experts in the field of landscape architecture, forestry, biology, ecology and environmental protection should be engaged during the reconstruction of the investigated area. The team work of these professionals can lead to the best results when returning the area to the conditions that are close to its original state.

CONCLUSION

Anthropogenic impacts in the investigated area are high and in order to prevent further impacts and return the area to its almost original condition, the existing facilities (restaurants, bars and riverhouses) and the marina must be removed. It is necessary to comply with the provisions of the Water Law and the General Urban Plan. Without the protection of the area there is no preservation of its biodiversity, and it is necessary to protect the area in accordance with the Law on Environmental Protection and the Law on Nature Protection. It is necessary to conduct reforestation of the area and a proper selection of plant species is crucial in the process of greening, which should be conducted by landscape architects.

Marinas, as nautical objects, will be built and developed. Their impact on nature and landscape cannot be stopped, but it can be reduced. This is achieved by a careful selection of the marina construction site. It is necessary to make an assessment of the impact of marina construction on the environment, i.e. the analysis of the quality of environmental factors and the prediction of direct and indirect harmful effects on the entire area.

In order to point out the importance of preserving close-to-nature areas, the significance of aquatic ecosystems, the importance of preserving autochthonous plant species and animals and prevention of their disappearance, it is necessary to conduct permanent education of people. It is necessary to introduce the general public to the importance of preserving close-to-nature spaces and raise awareness of the importance of using their natural benefits.

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NEGATIVE EFFECTS OF ANTHROPOGENIC IMPACTS ON THE HEALTH STATUS OF TREES: A CASE STUDY OF MAKIŠ MARINA IN BELGRADE

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Abstract

The investigated area (Makiš Marina) is located on the right bank of the Sava River, which is characterized by the presence of numerous plant and animal species typical of humid flood prone areas, as well as a large number of different fungi species. Due to the intense logging of autochthonous vegetation and urbanization of the area, there is a disruption of biodiversity and habitats of the local flora and fauna. A total of 105 specimens of trees were recorded in the investigated area. The largest number of trees belongs to the families of willows and poplars on which the presence fungi species was recorded. Those fungi are Spongipellis spumeus, Pholiota destruens,Ganoderma resinaceum, Lentinus tigrinus, Fomes fomentarius, Laetiporus sulphureus, Phellinus igniarius and Ganoderma applanatum, which have a negative impact on the health status of the trees.

Keywords: Sava River, Makiš Marina, trees health status, human impact, green area degradation

INTRODUCTION

Special-purpose green areas above all have a functional character. Special purpose areas include protective zones around industrial objects, protective shelterbelts (against wind, snow, etc.), protected areas, roadside plantations, etc. [1]. Green spaces around marinas also belong to the category of special-purpose green areas. According to the *Law on Navigation* and Ports on Inland Waters [2], a marina is defined as a special port intended for the reception, keeping of vessels and equipment for recreation, sport and entertainment. The development of marinas resulted from the need of people to recreate and rest on their own vessels (boats and yachts), but also on the coast. For these reasons, there was a need for an area where their vessels could be parked, tied, serviced, etc., but also where their owners could take a break. According to the classification of the green spaces, marinas of every city belong to the class of public use areas [3].

Makiš Marina is located in the city of Belgrade, in the municipality of Čukarica and belongs to the suburban part of the city (Figure 1). Makiš is separated from the city center by floodplain forests, the Sava River, the Sava Lake and Ada Ciganlija.

The climate in Makiš is wetter than the city climate due to the proximity of the Sava River, which increases relative humidity of the air. During summer months the air temperature is lower than usual and in winter it gets higher. Vegetation affects the daily and annual temperature changes making them milder and increases relative humidity, keeps the snow, while soils under forest vegetation retain more moisture.

Figure 1 *The position of the Makiš marina in relation to Belgrade city center*

The marina area extends in the alluvial plain of the Sava River. The community which is widespread in this area is *Salicetum albae* (white willow) forest, spreading in a narrow strip along the Sava River [4].

Changes in natural areas last for centuries as a result of natural disasters and climatic factors, but the most pronounced consequences are caused by the anthropogenic factor, i.e. man. The removal of vegetation in the flood prone areas by the Sava River, concreting and "regulation" of the coast threatens the survival of species (plants, animals) in terrestrial aquatic habitats. Possible discharge of hazardous liquid chemicals from ships and other vessels and coastal belt pollution along the Sava River can have negative consequences for the biota, due to the relatively narrow flood prone zone between the river flow and the constructed embankment [5]. Deforestation in the investigated area disrupted the protective autoregulation mechanisms of the forest and enabled the impact of various negative environmental factors. The remaining parts of the forest represent disturbed forest biocenoses. Parasitic fungi that cause plant diseases are among the harmful biotic factors that endanger forest ecosystems [6]. A large number of fungi species have been discovered in various ecosystems, where they play different roles [7].

The aim of the paper is to examine the health status of individual tree specimens in the area of Makiš Marina and to identify negative causing agents affecting the health status of those specimens.

METODOLOGY

The recording and identification of the tree specimens was performed by Vukićević [8], within the boundaries of the investigated area and their health status was analyzed (Figures 2 and 3), while fungi perceived on the stems of the tree specimens were also determined.

The analysis of the health status of the specimens involved recording of the changes on the leaves, bark, trunks and root collar of trees caused by the activity of parasitic and saprophytic fungi. The fungi that occur on the trunks of specimens in the investigated area were recorded and described after Karadžić *et al*. [9]. The determination of fungi, collected in the field, was performed in the phytopathological laboratory of the University of Belgrade, Faculty of Forestry.

Figure 2 *Orthophoto image of the researched area*

Figure 3 *Makiš marina image from the air*

RESULTS AND DISCUSSION

A total of 105 tree specimens were recorded in the investigated area. The number of specimens with damage caused by parasitic and saprophytic fungi in the investigated area is eleven, of which four specimens had damage on the foliage, and seven specimens were observed to have damage on the trunk. Taxa whose observed damage was located on the foliage were *Malus sylvestris* L., *Platanus orientalis* L. and *Tilia cordata* Mill., while wood rotting fungi were recorded on the trunks of the species *Populus nigra* L., *Salix alba* L. and *Salix babylonica* L. A total of eight different wood rotting fungi species were detected. Two fungi were detected on poplar trees: *Spongipellis spumeus* and *Pholiota destruens*, while six of them were found on willow stems: *Ganoderma resinaceum*, *Lentinus tigrinus*, *Fomes fomentarius*, *Laetiporus sulphureus*, *Phellinus igniarius* and *Ganoderma applanatum*.

The results of the study show that species: *Acer negundo* L., *Betula alba* L., *Fraxinus angustifolia* Vahl., *Fraxinus ornus* L., *Fraxinus pensilvanica* Marsh., *Juglans regia* L., *Morus alba* L., *Pinus sylvestris* L., *Prunus cerasus* L., *Prunus domestica* L., *Tamarix sp.* L., *Tilia tomentosa* Mnch., *Thuja occidentalis* L., *Ulmus effusa* Willd., *Ulmus montana* With., demonstrated tolerance to pathogens at the investigated site.

Pathogens appearing on poplars species:

- *Spongipellis spumeus* (Sow.: Fr.) Pat., Fam: Polyporaceae, Genus: *Spongipellis* Pat. *Spongipellis spumeus* (Fig. 4) develops as a parasite on broadleaved species such as

Populus, *Ulmus*, *Fraxinus* and *Juglans*. Infections occur through the wound scars from where the fungi spreads into the heartwood and causes rot in that part of the stem [9].

- Pholiota destruens (Brond.) Gill. (Syn. Pholiota populnea /Pers.: Fr. / Kuyp. & Tjall.), Fam: Strophariaceae, Genus: Pholiota (Fr.) Kummer.

Pholiota destruens (Figure 5) usually appears in a group (and rarely also individually) on stumps, trunks or in the damaged areas of standing trees of *Populus* species. This fungus causes white heartwood in poplar trees, which already appears on standing trees [9].

Figure 4 *Spongipellis spumeus*

Figure 5 *Pholiota destruens*

Pathogens appearing on willow species:

- *Ganoderma resinaceum* (Boud in Pat.), Fam: Ganodermataceae, Genus: *Ganoderma* P. Karst.

This species is very rare in central Europe and resembles *Ganoderma pfeifferi*, which has a reddish-brown stem and a mesh-cracked cap surface. Specimens of *Ganoderma resinaceum* (Figure 6) with a stem can be mixed up with *G. lucidum*. However, *G. lucidum* has a smaller cap and longer stem [9].

- *Lentinus tigrinus* (Bull.; Fr.) Sing., Fam : Polyporaceae, Genus: *Lentinus* Fr.

Lentinus tigrinus (Figure 7) most commonly occurs in flood prone forests, along rivers and river embankments. It mainly appears on trunks and broken branches of *Salix*, *Populus* and other broadleaved species, and sometimes on certain fruit trees. This fungus causes white rot. At first, it causes dark changes followed by white rot with dark lines in the rotten mass [9].

- *Fomes fomentarius* (L.: Fr.) Fr. (Syn.: *Ungulina fomentaria*/Linn./Pat.), Fam: Polyporaceae, Genus: *Fomes* (Fr.) Fr.

Fomes fomentarius (Figure 8) develops as a parasite or saprophyte on beech (genus *Fagus*), birch (genus *Betula*), alders (*Alnus glutinosa*, *A. incana*), oaks (*Quercus robur*, *Q. petraea*, *Q. cerris*) and poplar (*Populus spp.*) and on other broadleaved species. In the early stage of development, the infected tree gets a subdued color. In the further course of disease development, the tree becomes pale yellow with many brown or black curved lines. The rot caused by this fungus is known as the "white mottled rot" [9].

Figure 6 *Ganoderma resinaceum*

Figure 7 *Lentinus tigrinus*

Figure 8 *Fomes fomentarius*

- *Laetiporus sulphureus* (Fr.) Murrill, Fam: Fomitopsidaceae, Genus: *Laetiporus* Murrill.

Laetiporus sulphureus (Figure 9) develops as a parasite or saprophyte more often on the deciduous trees, and very rarely on conifers. It is especially common in species of the genera *Quercus*, *Salix*, *Alnus*, *Prunus*, *Fagus*, *Juglans, Pyrus, Robinia u Populus* [9].

- *Phellinus igniarius*, Fam: Fomitopsidaceae, Genus: *Phellinus*

Phellinus igniarius (Figure 10) is a parasite of various broadleaved tree species. The early stage of rotting is characterized by the appearance of yellowish-white stains in the heartwood, streaked with a strip of yellow-green to dark-red color. In the end, white layered rot appear, which is localized in the heartwood, and rarely spreads into the sapwood [9]. Infections take place through the rest of the branches and various injuries. The presence of only a few fruiting bodies on the stem indicates widespread rot inside the stem. This fungus is called "false tinder fungus" [10].

Figure 9 *Laetiporus sulphureus*

Figure 10 *Phellinus igniarius*

Figure 11 *Ganoderma applanatum*

- *Ganoderma applanatum*, Fam.: Ganodermataceae, Genus: *Ganoderma*

Fungi of this genus evolve as parasites, facultative *parasites* or saprophytes on broadleaved species [11]. Fruiting bodies (carpophores) have a hoove-like or shell-like shape and sometimes irregular form. They are very hard and woody. *G. applanatum* (Fig.11) causes white rot on the foliage. Carpophores are formed at the stem base [12]. The rot can continue its activity even after tree logging on logs that remain in the woods for a long time, in favorable conditions of humidity and temperature [13]. *Ganoderma* spp. can cause canker wounds on the buttress [14].

According to literary sources, Karadzic *et al*. [6], Karadžić *et al*. [9], Karadzic *et al*. [12], Karadžić *et al*. [13], Lazarev [10], Keča [11], there are many types of mushrooms that can be recorded on poplar and willow trees, but at this site, due to the specific conditions of the environment, number of mushrooms is much lower.

CONCLUSION

Anthropogenic impacts disrupted the biodiversity of the Makiš Marina area. The natural-potential vegetation was changed as a result of logging of the autochthonous species. The canopy opening of high vegetation, disturbed habitat conditions, heterogenization of habitats etc. have led to the physiological weakening of the autochthonous vegetation.

In addition, logging and the inadequate attitude of man towards the existing vegetation, above all damage to the stems, reduced the vitality of the plants. The plants became more sensitive and susceptible to the penetration of pathogens that caused disease. Forest logging in the investigated site also disrupted forest autoregulatory protection mechanisms and enabled the operation of various negative environmental factors. The remaining parts of the forest represent disturbed forest biocenoses.

In order to reduce the occurrence of parasitic and saprophytic fungi, it is necessary to plant specimens which have shown adaptability to the existing conditions and tolerance to the present pathogens, while belonging to the natural potential vegetation of the investigated area. In addition, it is important to apply tending measures to the specimens in the investigated area at the right moment and in the right way.

If the uncontrolled anthropogenic impacts in the study area persist because of the process of urbanization which is unstoppable, biodiversity degradation and physiological weakening of the vegetation will also continue. Therefore, it is necessary to comply with the laws and applicable regulations in order to protect the area of Makiš Marina. In addition to that, all planning documents such as the plans of water supply, nature protection, development, etc. must not be conflicted, which is the only way to make anthropogenic impacts conducive to sustainable development of this area without its further devastation.

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LANDSCAPE EVALUATION FOR THE PURPOSES OF ECOTOURISM – CASE STUDY OF BURGENLAND

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Abstract

The objective of this paper is the landscape evaluation of Burgenland (federal state of Austria) for the purposes of ecotourism, based on the Quantitative method of diversity (V-Wert method), where the natural elements of landscape (forests, water surfaces, relief, climate) and land use of considered area are taken as the main criteria for the evaluation. The research was carried out using modern mapping methods and GIS software. By applying this method, more or less favorable surfaces are determinated for the given purposes, with the aim of identifying landscape units which have a high degree of convenience for the development of different types of sustainable and ecotourism, where the main motives of tourist activity are natural heritage and natural touristic values, which are making that landscape more exclusive and more attractive among tourists and visitors. The results of geospatial analysis and landscape evaluation of considered spatial entity in this paper could greatly contribute to an additional tourist valorization of Burgenland.

Keywords: quantitative method of diversity, landscape evaluation, ecotourism, GIS, Burgenland

INTRODUCTION

In recent decades, global trends in tourism have undergone a gradual transformation. Modern society becomes aware of the risks posed by the ecological crisis, and relies more on individual and collective responsibility to protect the environment. In many social activities, the environmental aspect strengthens, leaving a deep trace in the tourist activity itself, and the need arises to create an entirely new alternative concept with the expressed ecological dimension of "ecotourism" in contrast to traditional, massive forms of tourism (one of the most valuable manifestations of a sustainable tourism) [1,2]. Tourism based on ecologically responsible behavior, where the main motive is natural and cultural heritage, in many European countries such as Austria, Germany, the Netherlands, France and the United Kingdom is an effective instrument for protecting the natural environment, establishing sustainability and improving the health of the human population as well as local and regional economic development [3,4]. Burgenland is characterized by very rich and diverse landscapes, which due to their importance and specificity are often found under protection, such as nature reserves, nature parks (6), the famous National Park Neusiedler See, numerous areas within the European Natura 2000 network, etc. Preserved nature, diversity of biodiversity, protected areas and regional specificities are important resources on which the prosperity of this province rests. High quality and authentic landscape features except for the development of tourism and agriculture, contribute to education in the field of environmental protection, energy production from renewable sources and overall regional development. Tourism is slowly becoming one of the key pillars of the regional economy of Burgenland and has been intensely developing over the last two decades. The promotion of tourism contributes to linking different types of tourist offer and introducing new tourist facilities that basically rely on the natural and cultural elements of the region: wellness, spa and health tourism, birdwatching, sports and recreational tourism (hiking, alpinism, cycling, wind and kitesurfing), camping, hunting and fishing, various water activities, educational, wine and gastronomic tourism, agritourism etc. Only in the period 2001-2015 the number of total overnight stays increased from 1 to 3 million (in 2015 it was 2 914 753). According to the official statistics, 5.9 million tourists visited Burgenland in 2015, with total tourist costs amounting to 1040 million euros. The highest growth rates are recorded in the area of sports and recreational, excursion, cultural and manifestation, educational, wellness and spa tourism [5]. However, not all the parts of the province were equally rapidly developing in tourism. Tourism has traditionally represented in northern Burgenland, where there is a largest number of tourists and visitors annually. Central and Southern parts experience their more intensive development in recent years, although growth rates are still lower than the northern zone. The main objectives of the future development of tourism in Burgenland are extending the duration of stay of tourists and visitors (currently is 3 days on average), expansion of accommodation facilities, development of daily and weekend tourism, as well as the organization of specific forms of sustainable and ecotourism for additional diversification of tourist offer [6]. Examples of strong growth are well-known spa centers – cities Lutzmannsburg, Bad Tatzmannsdorf (East and West part of Burgenland) and St. Martin (Neusiedler See), but lately more and more cities Gussing (southeast part) and Stadtschlaining (Central part) who managed to significantly increase tourist frequency in a relatively short time due to new and interesting ecotourism facilities. An interesting example of the new type of tourist offer is "energy tourism" where tourism activities are related to renewable energy sources (the most famous example is the European Center for Renewable Energy – EEE Gussing and wind, solar and biomass power plants). This type of tourism is in the initial stage of development, it is promising and provides numerous opportunities, so can easily be combined with educational tourism and tourism trips whose main motives are science and technological achievements in the field of sustainable development. Evaluation of natural and cultural areas and their most important elements, as well as additional valorization of tourist sites and zones, will enable the existing potentials to be used in an even more efficient and sustainable way, which will contribute to the conservation of landscapes and natural ecosystems on the one hand, and regional development of Burgenland on the other [7].

MATERIALS AND METHODS

Main goal of this paper is geoecological evaluation of landscapes of Burgenland for the needs of ecotourism. The evaluation was performed using the Quantitative method of diversity (V-Wert method). The criteria used in the geospatial analysis, on which the mentioned model is based, are: forests and forest land (W), water surfaces (G), relief (R), land use (N) and climate factor (K). Using these criteria authors will determined more or less favorable areas for the given purposes. More or less suitable surfaces for the development of tourism are determined by the following formula [8,9]:

$$V = \frac{W + G \cdot 3 + R + N}{1000} K \tag{1}$$

By this model, for the criteria of relief energy and the land use, the values of weight factors are defined (Table 1) by means of which the final value of the mentioned evaluation criteria are determined.

Table 1 *Scale of relief values and Weight factors for each category of land use [10]*

Altitude difference (m)	**Values of the relief**
10-20	220
20-30	300
30-60	400
60-100	590
100-250	860
250-500	1200
Land use	**Weight factors**
Agricultural areas (arable land)	6
Orchards and vineyards	8
Wetlands	10
Meadows and pastures	15
Forests	19
Artificial surfaces	21
Water bodies	50

At the beginning of the research, a raster network was formed with dimensions of 2000 x 2000 m (2 x 2 km) which covers the whole area of the Federal state of Burgenland, so that for each spatial unit - GRID cell (in total 1173) it would be possible to determine the category of suitability in relation to each evaluation criterion. After that, on the basis of the collected data, several thematic maps were created, and at the end by overlapping of these layers, as a final result was obtained a Map of Suitability of Burgenland for tourism purposes, which was the main goal of this research. For the needs of landscape evaluation, geospatial analysis and cartographic presentation of the results, GIS software based on ESRI technology (ArcGIS 10.2) was used.

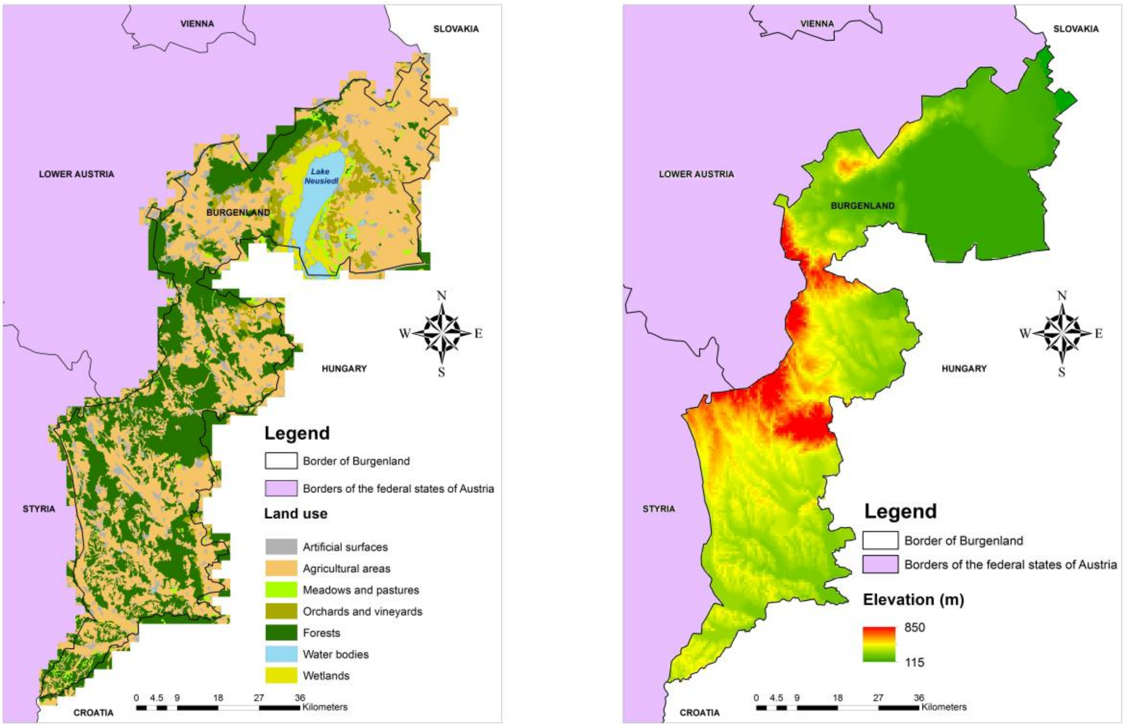
Evaluation criteria

Forest edges and water edges - Forest edges (W) are the bearers of contrast and change in the space that have an impact on the observer's senses and represent the typical elements of the natural and cultural landscape [11,12]. Forest complexes represent the ideal spatial units intended for carrying out a number of tourist activities, but apart from tourism, they also have a great ecological significance as they contribute to the preservation of biological diversity.

The forests on the territory of Burgenland occupy about 130 000 ha, or 32.81% of the total area. The share of monoculture decreases (mostly coniferous), while the percentage of resistant and mixed forests is growing. Among the deciduous species dominate the oak (especially important is European oak-*Quercus robur*), linden, chestnut, hornbeam and black locust (*False acacia*), and among the conifers are fir tree (*abies*), pine and spruce. Forests are predominantly concentrated in the central, western and southern parts of the country. The northern parts are distinguished by vegetation predominantly composed of agricultural crops, wetland vegetation and steppe areas with spacious meadows and pastures [13]. Forests are represented to a lesser extent and as the dominant stand the temperate deciduous forests [14], [15]. Water edges (G) increase the tourist value of the area, make it more attractive and, from a recreational aspect, more inviting because they are suitable for the development of a number of recreational and tourist activities [12]. The most important, most famous and the largest hydrographic object of Burgenland is Lake Neusiedler See - a national park located on the territory of two European countries, an important element of cross-border cooperation, and since 2001 it has been on the UNESCO World Heritage List as Fertö/Neusiedlersee Cultural Landscape. The World Heritage Site corresponds to the Austrian National Park Neusiedler See and the Hungarian park Fertő-Hanság Nemzeti Park. The area is recognizable by the wide tourist offer: hunting and fishing, sports and recreational tourism, gastronomic and wine tourism, cultural, manifestation, educational, adventurous, wellness, spa and health tourism etc. The lake is a characteristic element of the landscape of northern Burgenland and it is a factor of recognizing the region in a wider environment. Lake surfing, wind and kite surfing are the most popular sporting activities that attract around 95 000 visitors annually. The National Park is a significant destination in the field of cultural and manifestation tourism, where the most famous is The Seefestspiele Mörbisch - the world's largest open-air opera and concert festival, which annually visits up to 220 000 visitors. The distinctive steppe landscapes harmoniously fit into the backdrop of the world's largest open-air stage. The area of National Parke also features a unique flora - the most typical representative is the reed (the reed belt around Neusiedler See is the second largest belt in the world) [16] and fauna, especially orinthofauna, so it is also an attraction for birds lovers (birdwatching). The area offers rich facilities for both athletes and recreationalists: arranged bicycle trails around the lake, hiking trails that match the wine routes (Gols town), as well as more demanding hiking trails on Leitha Mountains. The Neuseidler See region is known for its high level of integration of tourism and viticulture. Wine manifestations, numerous wine trails and tours (e.g. Jois a small town in the district of Neusiedl am See) complete the tourist offer of the region [7,14,17]. The roles of the forest and water edges in the geospatial analysis and geoecological evaluation of the landscapes, both natural and cultural, are very similar, with the fact that waters additionally enriching the area, the lengths of all water shores are multiplied by a value of 3 [12]. In order to obtain data on the lengths of the water and forest edges, a digital database on the status and changes of the land cover and the way of land use throughout Europe CORINE Land Cover (2012) was used [18]. The database contains 44 categories of land use, and on the basis of available data for the state of Burgenland, three types of forest are distinguished: deciduous, coniferous and mixed forests. The analysis also includes transitional woodland shrub (forest-bush ecosystems). Then, the lengths of forest and water edges were determined in meters, i.e. km^2 for each individual GRID cell, for the entire surface of the analyzed area. The types of vegetation covered by the research, as well as the position of hydrographic objects are shown on Figure 1.

Relief energy - Relief (R) is one of the most important natural components that can be analyzed and evaluated [12]. The relief energy represents the height difference between the highest and the lowest point in the corresponding square of the raster, expressed in meters. To determine this evaluation criterion, it was necessary to use the data obtained from a digital elevation model (DEM) [19]. The digital elevation model is shown on the Relief map of Burgenland (Figure 2). Each GRID cell is assigned a value that represents the difference between the highest and the lowest point of altitude. According to this difference, the relief values are assigned [10], (Table 1). Burgenland is mostly part of the Pannonian Basin, which is morphologically different from the rest of Austria. The relief is predominantly flat, with altitudes between 120 and 250 m. On the territory of this province is the lowest point of Austria (115m). A smaller part, in the western parts has characteristics of mountainous terrain, altitudes up to 850m and belongs to the first, lower mountains of the Alps.

Land use - Land use (N) is an inevitable criteria in this analysis in order to determine the possibilities of different elements of landscape for the needs of ecotourism and recreation. In order to ascertain the values of this evaluation criterion, it is necessary to calculate the percentage of participation of different types of land use in the appropriate square of the raster, which is then multiplied by the corresponding weight factor for this criterion (Table 1) (Figure 1). By summing up the partial values of each type of land use, the final value for every GRID cell is obtained. For the purposes of determining this criterion, the data from the digital database CORINE Land Cover (2012) were used.



Figures 1 and 2 *Land use of the territory of Burgenland (left), Relief map of Burgenland (right)*

Climate factor - The climate factor (K) is calculated based on the annual values of the air temperature and the amount of precipitation, altitude and type of landscape. The value of the climate factor is taken as equal to the entire considered area, ie. all raster units carry the same value of the mentioned factor. Burgenland is characterized by a warm and humid continental climate witch suits to the development of various tourist activities.

RESULTS AND DISCUSSION

Landscape evaluation was conducted in a wider area compared to administrative boundaries of Burgenland, so instead of 3 962 km^2 (the surface of this federal state of Austria), the analysed area amounted to 4 692 km^2. The reason for this was the consideration of each raster unit within which there is even the smallest part of the territory of Burgenland. The degree of suitability of the various parts of considered area for ecotourism purposes are shown in Figure 3, according to the categories of diversity presented in the Table 2.

Table 2 *Categories of diversity (V-Wert method) [9]*

Categories	Classes	Span
I	Unfavorable	$V < 3.72$
II	Conditionally favorable	$3.72 < V < 7.44$
III	Favorable	$7.44 < V < 11.16$
IV	Very favorable	$V > 11.16$

All spatial units (GRID cells) are classified into one of four categories according to the degree of suitability: unfavorable areas of 1632 km^2 (34.78%), conditionally favorable areas 1200 km^2 (25.58%), favorable areas 932 km^2 (19.86%) and very favorable areas 928 km^2 (19.78%). Based on the results obtained, it can be easily concluded that a large part of Burgenland territory has the basic amenities for ecotourism. Very favorable and favorable areas account for almost 40% of the total considered area. They are predominantly concentrated in the southern and western parts and coincide with the zones of distribution of forest complexes, while in the central part they coincide with the mountainous area that has significant tourist potential (e.g. Rosalia Mountain). In the northern part of Burgenland, the area around the Neusiedler See is recognized as dominantly favorable - which stands out as the most attractive ecotourism destination of this federal state. A large part of northern Burgenland lies between the Danube and Lake Neusiedler See in the so-called "Green center", a space that is located between three major European cities: Vienna, Bratislava and Gyor. In the last decade, the North Burgenland region has experienced dynamic development, not only due to its natural values, but also due to its favorable geographical location and proximity to the international tourist market. What is interesting about this region is that, according to the results of the conducted evaluation, it has been singled out as an area with the largest share of unfavorable areas, but the reason for this should be found in the land use of this area, dominated by arable agricultural land and numerous vineyards – the most famous feature of Burgenland as a wine region. One of the main principles of the State is the permanent protection of fertile land intended for sustainable agriculture - the basis of the rural development of Burgenland. At the same time, the importance of the intensive cooperation of tourism and agriculture, harmonization of the development objectives of these two fields, the placement of joint brands (e.g. wine tourism, rural and agritourism - Austria, along with Italy, a pioneer of agritourism) are emphasized, so the "unfavorable" should be understood only conditionally.

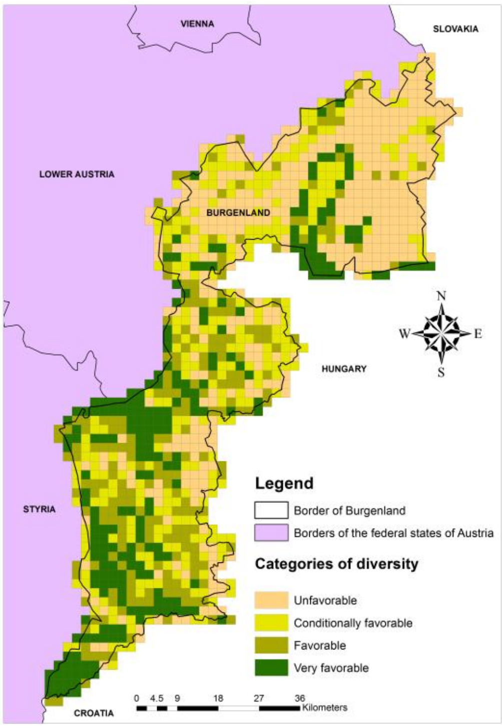


Figure 3 *Suitability of Burgenland for ecotourism purposes*

CONCLUSION

Tourism is an important economic support of Burgenland, especially in the domain of innovation and creation of new tourism products and services, primarily in the field of ecotourism. Especially important is the cooperation and networking of tourism activities with other sectors such as agriculture, forestry, nature protection, energy production from RES and culture. The preserved environment is an important resource that can be successfully used for the promotion of Burgenland as an attractive ecotourism destination at the international level. Advantages of the Quantitative method of diversity in evaluating the natural elements of landscapes for planning of tourist and recreational zones are confirmed once again by this paper. By applying this model, on the territory of Burgenland an area of 1860 km^2, or 39.64% of the total area, was assessed as particularly suitable for the given purposes. Significant potentials have also those parts which are designated as conditionally favorable (25.58%), which means that more than 2/3 of the area of this federal state has the appropriate conditions of its natural components that have an important, often a decisive role in the development of ecotourism.

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BACKCASTING AND TNA (Technology Needs Assessment) METHODS FOR SELECTION OF TECHNOLOGIES FOR REDUCTION OF GHG EMISSIONS IN TRANSPORT SECTOR IN BOSNIA AND HERZEGOVINA

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Abstract

In Bosnia and Herzegovina, climate change is increasingly considered as a matter of key strategic importance, especially by local authorities and the academic community. The State development vision is that by 2025 Bosnia and Herzegovina will be a sustainable and progressive 'green economy'. Mitigation of climate change is focused on the sectors where the greatest potential for reducing emissions of GHG gases is identified: energy production, district heating, buildings, transport, waste, agriculture and forestry. The use of renewable energy sources and the implementation of energy efficiency measures will lead to a reduction in the state's energy dependence and improvements in the quality of the environment, as well as increasing the competitiveness of B&H's economy. Backcasting approach, having in mind its descriptive character and the characteristic of problem solving at the very beginning, is much more convenient for solving long-term problems and offering long-term sustainable solutions. The aim of this paper is identification of the optimal technologies in the transport sector using backcasting approach, which can be applied in B&H and their analysis through the TNAssess process to evaluate their acceptability and the possibilities of their use in B&H.

Key words: backcasting, transport sector, technology assessment

INTRODUCTION

In Bosnia and Herzegovina, climate change is increasingly considered as a matter of key strategic importance, especially by local authorities and the academic community. The state development vision is that by 2025 Bosnia and Herzegovina will be a sustainable and progressive “'green economy” [1,2]. When Bosnia and Herzegovina joins the European Union (EU), it will have low emissions, high quality of life for all, preserved natural ecosystems, sustainable management of natural resources and a high level of resistance to climate change as a member state. Increased levels of energy efficiency, increased use of renewable energy and improved energy and transport infrastructure and services will lead to attracting international investment, job creation and business entrepreneurship in an economy based on the efficient use of resources. Negative impacts of climate change will be minimized by reducing the level of sensitivity and exploiting climate change opportunities [1,2]. Thus, although Bosnia and Herzegovina is among the countries with the lowest greenhouse gas emissions per capita in Europe (five tons of equivalent carbon dioxide per capita per year, about half of the EU average value), climate change has already been observed [2]. Bosnia and Herzegovina is particularly vulnerable to climate change due to its geographical position, the economic importance of the agriculture and forestry sector, as well as its limited capacity to adapt to climate change. Implementation of measures for reduction of emission greenhouse gases (GHG) is really optimal opportunity and chance to start, with international professional and financial help, technological transition. However, the problem is many barriers: from ignorance and distrust, to inadequate legal regulations. It is therefore appropriate and necessary to demonstrate technology transfer in B&H, with all their aspects: technical, economic, environmental, market, legal and social. It is very important that after beginning of implementation of certain technology to establish monitoring, to track results and removes all difficulties with introducing of new projects [1,2]. The mitigation of climate change is focused on the sectors where the greatest potential for reducing emissions of GHG gases is identified [2]: energy production, district heating, buildings, transport, waste, agriculture and forestry.

Backcasting approach started in the early 1970s and was originally developed as an alternative to traditional forecasting and planning methods [3]. The focus was on the analysis of energy planning policies and later on for examining sustainable solutions in the future, while stakeholder involvement and achievement goals through the implementation of action plans became a significant part of this approach at the beginning of the 21st century. This enables such approach to be used at the level of organizations, local communities, the region, the industry and other spheres of social development, as well as at the state level and globally [4]. However, backcasting approach is not the only approach that uses descriptive or desirable visions of the future, but it is also possible to recognize it in other approaches that combine descriptive scenarios with the stakeholder involvement [5]. In this way, backcasting approach can be viewed as a philosophical concept, both as a study, both as an approach, both as a methodology, and as an interactive process between participants, as an analysis, and as a specific retrograde step (from the desired future) in the whole planning process [4]. Also, although this approach is described as a phase (step by step) and in some way linear it certainly is not. It is possible to repeat individual cycles, and there is a mutual influence of the two phases (steps) that accompany each other [5]. Furthermore, the backcasting process has a dynamic nature, which means that at one time there may be a change in the main actors. Backcasting is normative in nature and problem-oriented, multidisciplinary and involves the participation of all stakeholders, which makes it even trans disciplinary. Interested stakeholders are very important, not only because of their specific content-related knowledge, but also obtaining consent for the results obtained and the implementation of the proposed activities [6]. Backcasting approach, having in mind its descriptive character and the problem solving at the very beginning, is much more convenient for solving long-term problems and offering long-term sustainable solutions. Therefore, it is better to consider backcasting as an approach rather than a method. Furthermore, backcasting studies should provide decision-makers and the public in an entirely acceptable and interesting picture of the future of the whole society on which an opinion should be formed for quality decision making [7]. Therefore, the scenarios of the project (in our case, transport scenarios) using the backcasting approach should provide a wide description of the solutions that should be considered for the adoption of final options of different future [5]. It has been confirmed that the backcasting approach is particularly promising in cases of complex problems, the need for radical changes, in cases where dominant trends are part of the problem and external influences that cannot be sufficiently addressed in the current market. Sustainable development issues clearly combine all the above mentioned features, and then it is clear why the application of backcasting approaches from the initial energy sector has spread to all sectors of sustainable development [8]. What we refer to as backcasting in the modern world is the so-called participatory backcasting approach. It requires the participation of all stakeholders in the planning process, as already stated at the very beginning of this paper.

Although most literary approaches show certain deviations in the applied methods, the method of involving stakeholders and the number of steps, it is possible to generalize and group them into a single methodological framework for participatory backcasting approach consisting of five stages (steps) [9]:

1. Orientation towards the strategic problem;
2. Developing sustainable vision of the future or scenarios;
3. Backcasting;
4. Development, analysis and defining of all activities with the development of an action plan;
5. Include results and generate later activities and implementations.

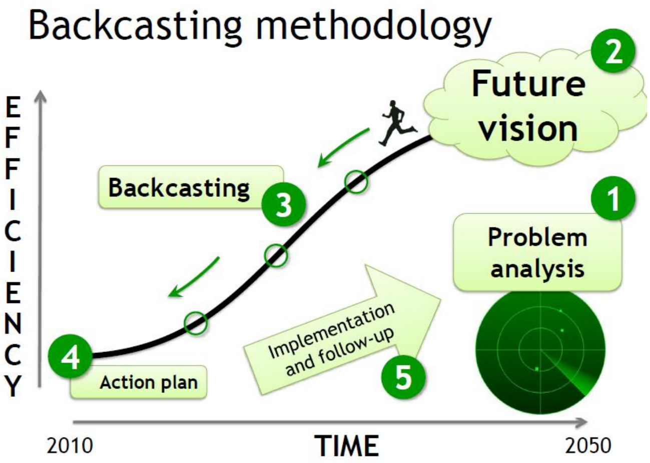


Figure 1 *Steps in backcasting*

A wide range of methods and tools is necessary when using a participatory backcasting approach and can be divided into four groups of actors, which together constitute the basic backbone of the necessary tools in the process. Participatory tools and methods make the first group [10]. The second group consists of the tools and methods needed for creation. This is not just about creating scenarios, but also about developing and embedding details of all system elements and creating process tools. The third group consists of analytical methods and tools. This does not only apply to the assessment of scenarios and studies, such as economic analysis or environmental impact analysis, but includes methods for process analysis and assessment, identification of actors and their analysis and analysis of their impact. Since the backcasting approach also requires effective management, coordination in communication, these methods, tools and skills are the fourth group. This includes the methods and skills necessary for effective communication, networking among actors and management of that network. It must be emphasized that each stage of the backcasting approach generally requires some of the methods and tools listed, while some specific methods and tools are used in some specific phases of the process [11].

The TNA process can very well fit into the context of Bosnia and Herzegovina as it can play a significant role in the process of harmonizing domestic legislation with EU legislation and practice in the field of climate change and contributing to the fulfillment of obligations under the UNFCCC. The timing and future implementation of this document coincides with the growing importance at global level of transfer of technologies and the growth of global funds for the issue of climate change, an opportunity that Bosnia and Herzegovina should not miss. In addition, the results of this process can also serve as input data for the development of other strategic development documents.

The aim of this paper is to identify the optimal technologies in the transportsector using backcasting approach that can be applied in B&H and their analysis through the TNAssess process evaluates their acceptability and the possibilities of their use in B&H.

WORKING METHODS

The TNA Handbook [12] foresees that the process takes place in five basic steps (shown in Figure 2), which are realized in a participatory way, through workshops involving all relevant stakeholders. Based on these recommendations, when preparing the assessment of technological development needs for mitigation of climate change and adaptation to climate change in B&H, all planned steps were implemented through a series of activities of the broader and wider TNA team, engagement of technical experts for technology identification, cost estimation and emission reduction, and through organization of workshops and several consultative and working meetings during the implementation of technology assessment.

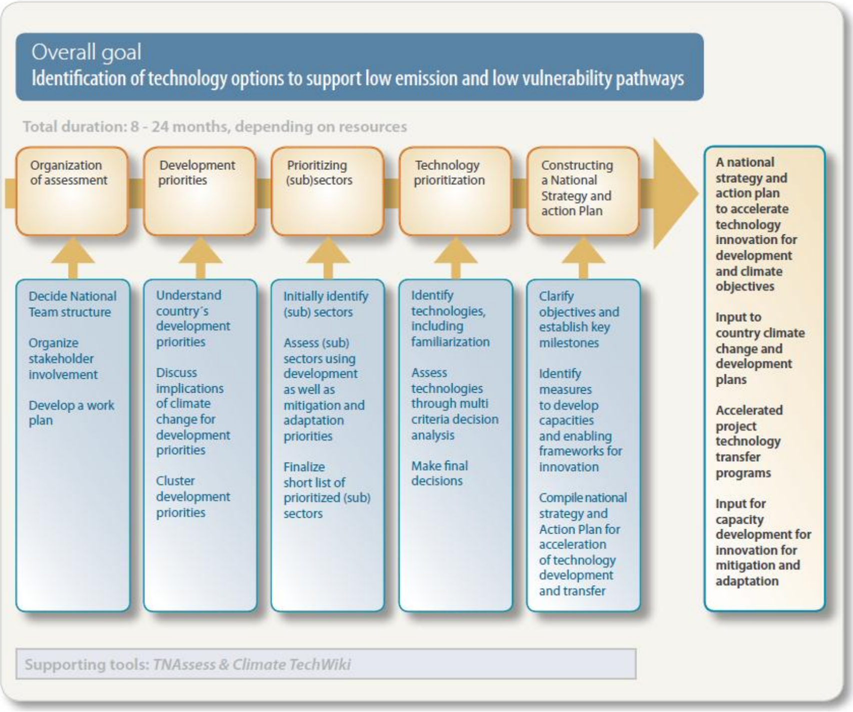


***Figure 2** Steps in conducting Technology Needs Assessment*
Source: [12]

Collected information about individual technological options from shorter lists are presented on the workshops for introduction with technologies with aim to provide to the participants of backcasting process on the TNA workshop, active participation and all collected information include in the prioritization . Workshops are also were an opportunity to verify technology list with participants, that were in position to suggest complementation and/or abbreviation of the proposed list, ie corrections offered information. After finishing the identification process, information about the selected technologies were entered into the multi-criteria TNAssess software in the appropriate categories. Thus, these processed and categorized data on technologies served as base for next step in the process - determining priority option that will contribute the mostly to the achievement of climate and development goals in the country.

RESULTS AND DISCUSSION

Collected information about individual technological options from shorter lists are presented to the participants on the workshops for introduction with technologies aiming to provide active participation and all collected information include in the prioritization. Workshops are also were an opportunity to verify technology list with participants. After finishing the identification process, information about the selected technologies were entered into the multi-criteria TNAssess software in the appropriate categories. Thus, these processed and categorized data on technologies served as base for next step in the process - determining priority option that will contribute the mostly to the achievement of climate and development goals in the country. Technologies for emissions reduction and Technologies/measures for climate change adaptation for transport sector (vehicle and fuel technologies) are given in following table.

Table 1 *Selected technologies for emissions reduction and adaptation in transport sector*

Technologies for emissions reduction and Technologies/measures for climate change adaptation	
1. Electric vehicle	6. Compressed natural gas (CNG) in transport
2. Efficient diesel	7. Hydrogen technologies
3. Hybrid vehicle	8. LPG in transport
4. Plug in hybrid	9. Bioethanol technologies
5. Biodiesel	10. Biomethane technologies

Points should be assigned for every of the selected criteria on the scale from 0 to 100, in a way that score of 0 points does not meant that the application of these technologies would not be achieved any benefits but to make a contribution of the concrete technology was the lowest in the relationship with the others options in the group (rating of 100 points would mean the biggest contribution).

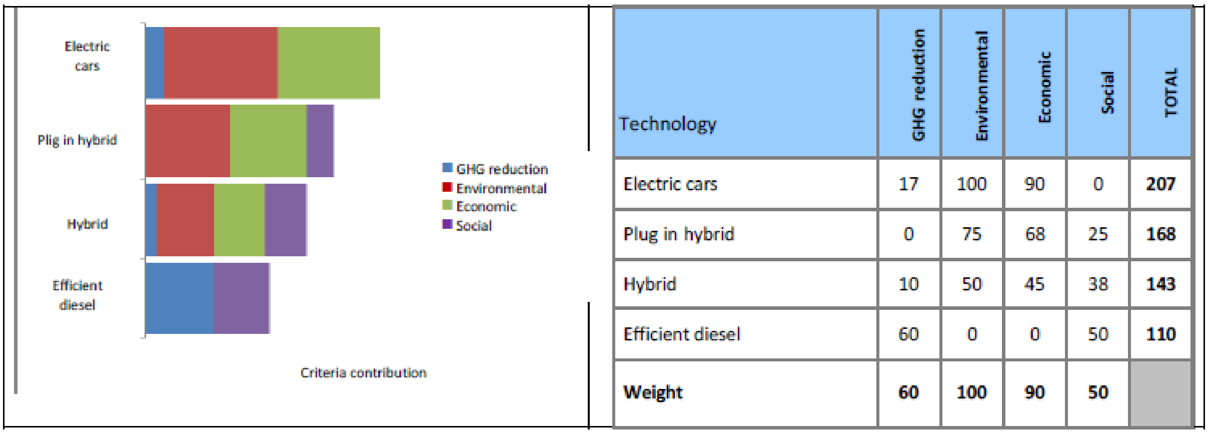
Table 2 *Results for sector: transport sector (vehicle and fuel technologies)*

No.	**Technology**	**Contribution to reduction of GHG / Contribution to the reduction in sensitivity**	**Contribution to environmental protection**	**Contribution to economic development**	**Contribution to social development**
1.	Hybrid vehicle	10	50	45	38
2.	Plug in hybrid	0	75	68	25
3.	Efficient diesel	60	0	0	50
4.	Electric cars	17	100	90	0

Table 3 *Results of giving weight to the criteria*

	Weight to improvement		
Criteria	**The least desirable option**	**The most desirable option**	**Weight**
GHG reduction/Sensitivity	2	3	60
Environmental	3	4	100
Economic	3	4	90
Social	4	3	50

Based on these points and weight assigned, the average grade for each criterion is calculated and such results are included in the software. The software generates total benefits from the application of technology and allows their graphical and tabular overview.



Technology	GHG reduction	Environmental	Economic	Social	TOTAL
Electric cars	17	100	90	0	**207**
Plug in hybrid	0	75	68	25	**168**
Hybrid	10	50	45	38	**143**
Efficient diesel	60	0	0	50	**110**
Weight	**60**	**100**	**90**	**50**	

Figure 3 *Chart with contribution to the overall benefits of prioritized technologies and table with weighted points*

INTERPRETATION: Chart on left side of Figure 3. shows cumulative development and climate benefits for each prioritized technology in this sector, based on assigned points and weights. Table on chart above shows same results as number values. Different colors on bars indicate how we achieved total results, ie how each criteria: GHG reduction, environmental, economic and social benefits contributes to total benefits. For example, total benegits for electric cars mostly origin from environmental and economic benefits that could be achieved by application of this technology. Low values of assigned points for electric cars for GHG reduction and social benefits are result of low estimation of contribution to GHG reduction due the small scope of use (3% out of total car number over 25-year period) and low social acceptability due the high price of electric cars.

Sensitivity analysis

Chart with sensitivity analysis illustrates consistency (robustness) of the most desirable rating technological option in relationship on the changes in weight criteria. At the top is stated name of the most desirable options from prioritization. Thus are listed all of the criteria and colors (or empty fields) that highlighted how robust rating on the most desirable option in relationship on the increase (to right pages) or on the decrease (at left pages) in the assigned weight. Tags have following meanings:

- **Empty field**: None changes in the cumulative weight would not change most desirable option.
- **Red color**: Cumulative weight should be changed for 5 percent points or less to change most desirable option.
- **Yellow color**: Cumulative weight should be changed for 5 to 15 points to change most desirable option.
- **Green color** : Cumulative weight should be changed for more than 15 points to be change most desirable option

In the case that an increase or decrease of criteria weight led to a change of the most desirable options, the new most desirable the option is displayed next to colored fields . Charts with green or empty fields show that the results of concrete ratings are robust since even not significant changes in points or the weights would not change most desirable option in relevant category.

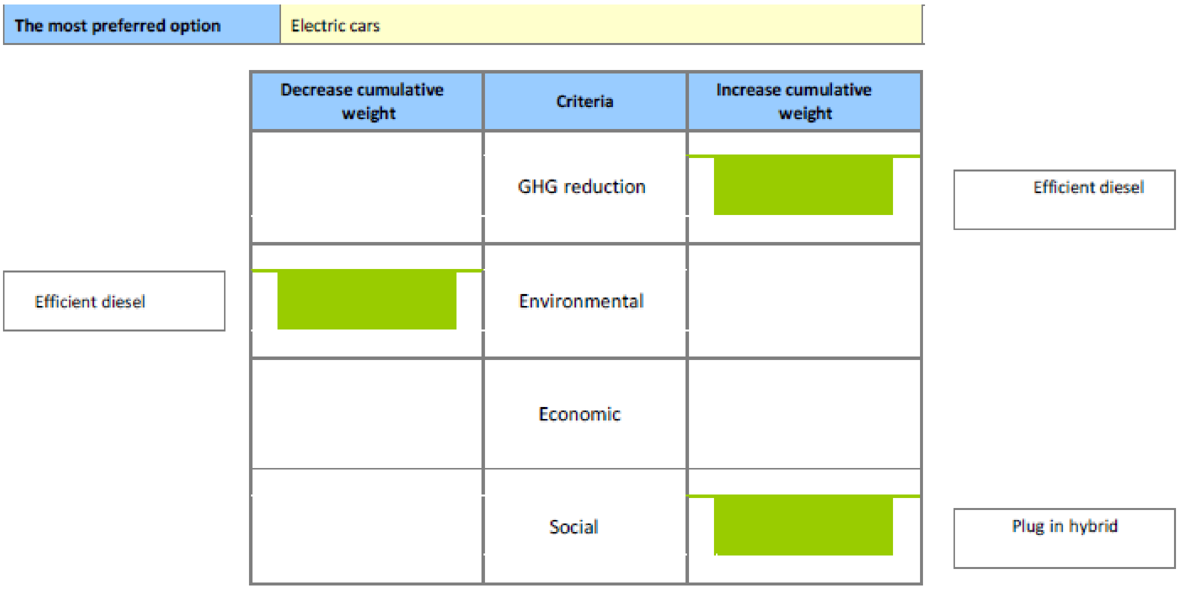
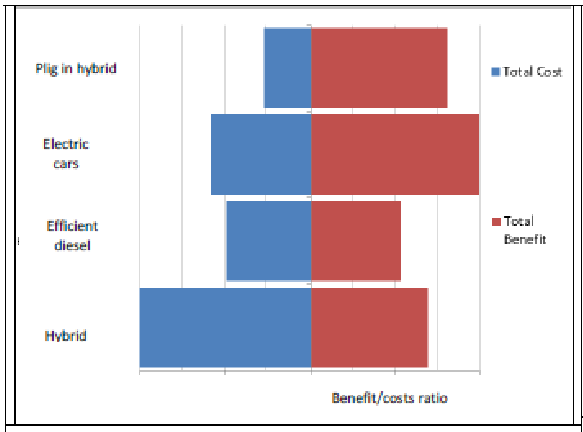


__Figure 4__ Chart with sensitivity analysis

INTERPRETATION: From chart above can be seen that with weight increase for 15 points for sensitivity criteria, efficient diesel engines will become the most preferred option. Also, efficient diesel engines will become the most preferred option with weight decrease for 15 points for environmental criteria. Plug in hybrid will become the most preferred option with weight increase for 15 points for social criteria. According to TNA assess MCDA (Multi-criteria decision analyses) guidelines, decision that electric cars are the most preferred option can be considered as robust, having in mind that even significant decrease/increase in assigned weight would not change results of prioritization. This is illustrated with green color or empty fields for each criteria on chart above.



Technology	Total costs	Total benefit	Benefit per unit costs
Plug in hybrid	180.000.000	168	9,3 E-07
Electric cars	393.300.000	207	5,2 E-07
Efficient diesel	330.000.000	110	3,3 E-07
Hybrid	681.000.000	143	2,1 E-07

__Figure 5__ Chart of the benefit ratio (red bar) and total costs (blue bar) and table with estimated costs and estimated benefits

INTERPRETATION: The chart on the left side compares for each technology option and uses the estimated costs associated with the application of the given option. Costs are as investment, operational and other costs, assuming a realistic possible impact of technology within the boundaries of technical potential over a period of 25 years. The amount of costs is shown in the table on the right. Benefit graphs correspond with those from the previously displayed graph with benefits (without showing the contribution of various criteria or without color). Hybrid have got lowest ratio of benefits/costs (this means that for them high investment are necessary in relationship what are benefits from their application as this was rated by participants in the TNA process). Plug in hybrid have the most favorable benefits/costs ratio.

CONCLUSIONS

Experiences with applying backcasting in the process of technology selection in transport sector using the TNA Handbook and other available tools can be summarized in the following way:

- Backcasting and TNA methodology are a solid framework for systematic implementation of processes with a clear link between different steps in the process;
- Regarding contribution to the overall benefits of prioritized technologies, use of electric cars have been calculated as the optimal technology aiming to achieve sustainable development;
- Having in mind benefits/costs ratio, most favorable technology is use of plug in hybrids;
- The introduction of multi-criteria decision analysis (MCDA) in the process of evaluation of technologies gave new quality to the overall process and offered a possible model for further consideration of the country's sustainable development options;
- It is proven that backcasting and TNA methodology can be efficiently used for selection of technologies in energy production sector aiming to provide adequate information and analyses to decision makers;
- Realization of development, climate and EU integration goals is possible if politics that favorites emission and energy intensive projects and solutions will be modified and support redirects on the new technologies that are contributing to achieve objectives of sustainable development and enhance total benefits.

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IMPROVING FUEL UTILISATION EFFICIENCY BY OXYGEN-ENRICHED AIR COMBUSTION

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Abstract

Industrial production has been driven by the requirements of maintaining or improving product quality and minimization of production costs. The improving fuel utilisation efficiency is the key factor in achieving these goals. This paper identifies cost-effective energy savings that can be achieved through performance improvement of combustion process in the industrial furnaces. The energy efficiency technologies and measures to reduce the specific fuel consumption and the pollutant production of burners and combustion chambers for the existing furnaces were described, giving the focus on the effect of oxygen-enriched air combustion on the combustion efficiency.

Keywords: industrial furnaces, energy efficiency, oxygen-enriched air combustion

INTRODUCTION

In practice, natural gas is often the default choice as a fuel in industrial furnaces, although due to its low carbon/hydrogen ratio results in low emissivity combustion products and relatively poor radiant heat transfer. There are many options for increasing the energy efficiency as well as improving performance of the industrial furnaces. To overmatch the challenges of rising energy cost and environmental regulations, the furnace user is necessary to choose the best possible options with respect to: performance, energy efficiency, low emissions and low maintenance, taking into consideration the investment costs.

To meet the higher requirements, it is necessary to improve existing furnace by optimising the efficiency and by reducing the pollutant production of burners and combustion chambers. Likewise, the choice of combustion technology for industrial furnaces is one of the key factors in minimization of energy consumption per unit of product. In general, the cost-effective energy savings at a furnace in the metallurgical industry can be achieved through performance improvement of combustion process and heat transfer.

Actual industrial furnace operational issues are listed as follows:

- Energy efficiency and operating cost;
- GHG emissions;
- Combustion efficiency;
- Emissions control;
- Product quality;
- Integrity/life and reliability parts of plants;
- Maintenance costs;

- Safety.

Within this requirement the energy efficiency plays important role. First of all, solutions to optimize the furnace energy efficiency with substantially reduced pollutant emissions should be sought in improving the governing processes in open-flame furnaces:

- Fuels and combustion;
- Burner and furnace aerodynamics;
- Heat transfer.

In this connection, it is especially important to pay attention to the operating conditions such as:

- Excess Air;
- Pressure Control;
- Oxygen Enrichment;
- Combustion Air Preheat;
- Load Temperature (at inlet and discharge);
- Flame Temperature and Emissivity;
- Wall Emissivity.

It is equally important to examine the possibility of replacement of the existing conventional combustion technology by the so-called ’advanced’ commercially available combustion techniques, for example High Temperature Air Combustion (HiTAC) or Oxy-Fuel combustion [1,2], under which the furnace should be operated in order to maximize the efficiency and minimize the pollutant emissions. In the process of choosing a certain technology it is essential to take into account the cost effectiveness of the investment i.e. the payback period.

But, in any case, reducing energy consumption through the application of appropriate working conditions in practice, as well as investment in advanced technologies, can enhance competitiveness in metallurgical production.

In this paper the effect of oxygen-enriched air combustion on the combustion efficiency was analysed.

EFFECTS OF OXYGEN ON THE COMBUSTION PROCESS

Effects of oxygen on the combustion process are manifested through:

- Increased flame temperature and consequent more intense heat transfer by radiation;
- Increased furnace production;
- Improved energy efficiency and reduced specific fuel combustion;
- Improved product quality;
- Increased burning velocity;
- Decreased ignition temperature,

but also has an impact on the composition and quantity of flue gas, as can be seen from the following stoichiometric equations of methane combustion at the excess air $n = 1$:

- Stoichiometric combustion of methane with air:

$1\ CH_4 + 2\ O_2 + 2\cdot3.76\ N_2 \rightarrow 1\ CO_2 + 2\ H_2O + 2\cdot3.76\ N_2$

The composition of the flue gases: 9.5% CO_2, 19% H_2O, 71% N_2

The amount of flue gas: 10.52 m^3 flue gases/m^3 CH_4

- Stoichiometric combustion of methane with oxygen:

  $1\ CH_4 + 2\ O_2 \rightarrow 1\ CO_2 + 2\ H_2O$

  The composition of the flue gases: 33.3% CO_2, 66.7% H_2O

  The amount of flue gas: 3 m^3 flue gases/m^3 CH_4

According to the above equations, the influence of oxygen on the combustion process is as follows:

- Increased CO_2 and H_2O concentrations which result in the higher flame emissivity and consequently more intense heat transfer by radiation;
- Reduced exhaust mass flow rate, for 3 and a half times, and consequent improvement in thermal efficiency.

CALCULATIONS OF THE EFFECT OF EXCESS AIR AT COMBUSTION OF NATURAL GAS

Calculations are based on combustion of natural gas of the following composition:

97% CH_4, 2.06% C_2H_6, 0.80% N_2, 0.14% CO_2, the net heat value of H_i=34.18 MJ/Nm^3 and at the excess air $n = 1$.

The *combustion efficiency* (η_f) is defined as [3]:

$$\mu_f = \frac{h_{fuel(NHV)} + h_{air} + h_{fluegas(inlet)}}{h_{fuel(NHV)}} \tag{1}$$

Where $h_{fuel(NHV)}$ is the net heat value of the fuel, h_{air} is the enthalpy of the preheated combustion air, and $h_{flue\ gas(inlet)}$ is the enthalpy of the gas at the inlet of the recuperator. Its physical meaning is identical to the available heat, i.e. to the percentage of the gross energy input which is available to heat the load and energy oriented to the furnace superstructure.

As can be seen from the expression (1) the enthalpy, i.e. the mass flow rate of flue gases leaving the furnace has the greatest impact on reducing the combustion efficiency, which is highlighted above.

From Figure 1 it can be seen that the available heat for the oxy-fuel technology is higher than that for air-fuel technology for the same temperature of exhaust, for example, at the temperature of exhaust flue gases of 1000°C the available heat for air-fuel combustion (21% O_2) has the value of 55%, and for oxygen-fuel combustion 84%. The difference in available heat increases as the exhaust temperature increases. The values of the available heat for oxygen enhanced combustion are in between these two technologies.

METHODS OF APPLICATION OF THE OXYGEN-ENRICHED AIR COMBUSTION

Many conventional air/fuel burners can be adapted for air/fuel process in which the air is enriched with O_2. The oxygen may be injected into the incoming combustion air supply (Figure 2). This is usually an inexpensive retrofit method, which will shorten and intensify the flame. However, the flame shape may become unacceptably short and the higher flame temperature may damage the burner or burner block. Because of this, the oxygen concentration in the air is limited to the interval of 23-35%, and usually at conventional burners is 25%.

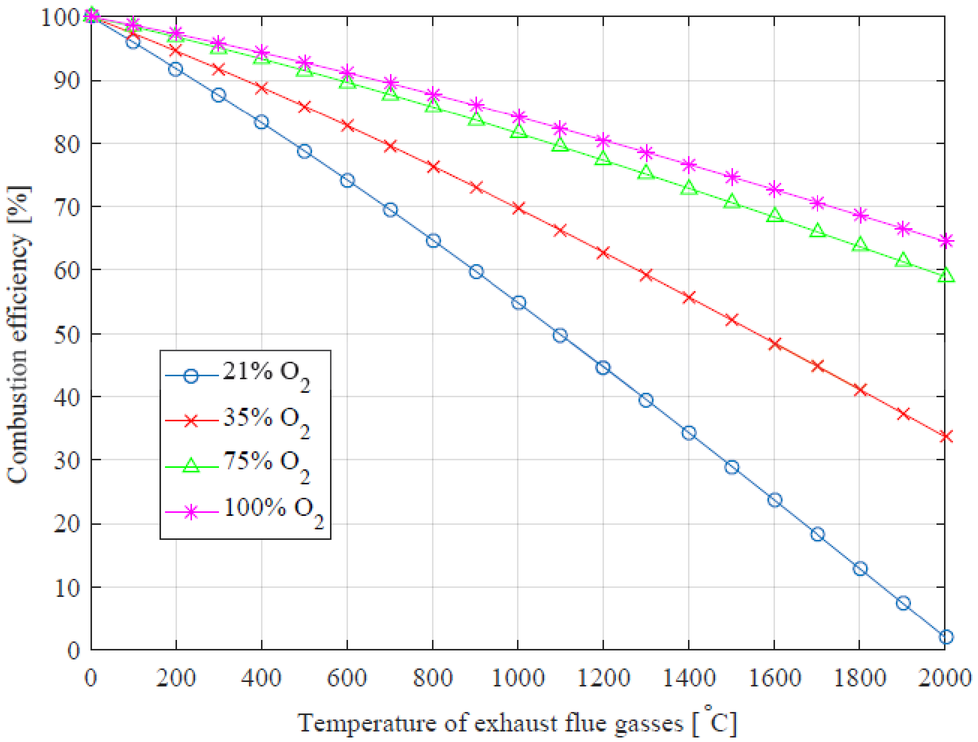

Figure 1 *Combustion efficiency vs. flue gas temperature for different oxygen contents in air*

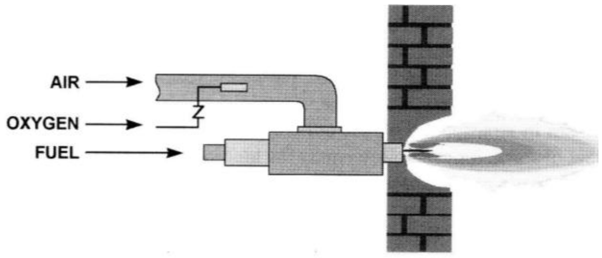

Figure 2 *Air-oxy/fuel burner where oxygen is injected into the air stream [4]*

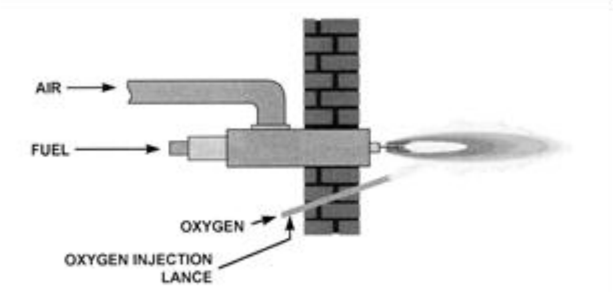

Figure 3 *Underflame oxygen enrichment [4]*

The second method is introduction of oxygen through the lance under flame (Figure 3). This is a direct technique where there is no need to modify an existing burner, and it is possible to regulate the length of the flame. The disadvantage is also limited oxygen concentration up to 30%.

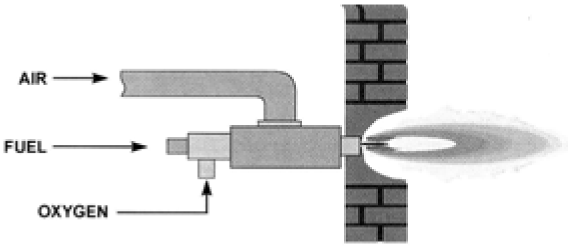

Figure 4 *Air-oxy/fuel burner where oxygen is injected into the gas stream [4]*

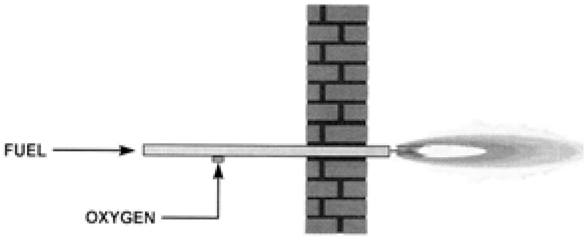

Figure 5 *Oxy/fuel combustion [4]*

In a burner where oxygen is introduced into the gas stream (Figure 4), a higher oxygen level (O_2> 90%) may be applied in relation to the first two techniques.

In the case of an oxy/fuel burner (Figure 5) the high-purity O_2 (>90% O_2 by volume) is used to combust the fuel. Because of the extremely high reactivity of pure O_2, there is the potential for an explosion if the gases are premixed. For safety reasons, it can only be used for combustion in a nozzle-mix burner, which produces a diffusion flame.

In previous cases, the higher flame temperature causes, except for the above-mentioned problems, and higher levels of NO_x. The High Temperature Air Combustion (HiTAC) (Figure 6) and Oxy-Fuel combustion technologies (Figure 7) exhibit superior combustion characteristic for heating applications in direct fuel fired industrial furnaces in terms of high energy efficiency, uniform heat flux distribution and low NO_x emissions.

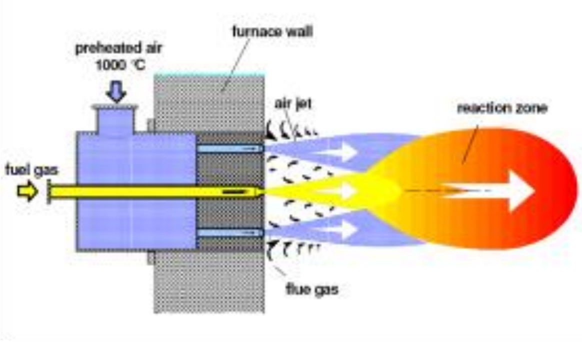

Figure 6 *Flameless Oxidation Burner [5]*

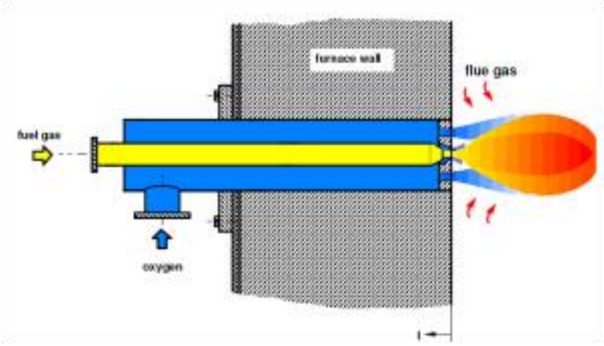

Figure 7 *Low NOx Oxy Fuel Burner with Staged Combustion [5]*

Because of the lower temperature of exhaust gases (approximately 200°C at the flameless oxidation regenerative burners compared to approximately 1000°C at the oxy-fuel burners) the available heat is equal or almost greater at the regenerative burners in relation to the oxy-fuel burners due to the effect achieved by the high temperature air preheating. Consequently, applying HiTAC technology, there is no justification to apply oxy-fuel combustion, because the oxygen is additional cost.

Also, there is no need for oxygen enriched air at the regenerative burners. As it can be seen from Figure 1, the available heat for HiTAC burner without oxygen enrichment is about 92%, and with oxygen enrichment to 35% (vol) is 95% at the same exhaust gas temperature of 200°C and 80% extracted flue gases through the burner regenerator. It is evident that the slight difference in the available heats cannot justify the cost of oxygen. Application of oxygen would be justified only if it would be necessary to implement a reduction in CO_2 emission.

The others limits of oxy-combustion are: maintenance of combustion system is delicate (must be executed by specialized technicians) and with very short steps, due to really high flame temperature reached, refractory life is shorter than at air combustion, temperature uniformity on reheating load is not improved, production can be increased to increase set-point temperatures but it is not necessary in the case of the furnace under consideration.

CONCLUSION

One of the main reasons for this poorer energy utilization is inadequately organized combustion process. With regard to this fact, the furnaces in the industry operate mainly with low energy efficiency. This paper points to the ways of improving the energy utilization in open-flame furnaces considering how various operating factors and combustion technologies, affect the thermal performance of industrial furnaces, particularly analysing the effect of oxygen-enriched air combustion on the combustion efficiency.

The oxy/fuel combustion has the greatest potential for improving a process, but it also may have the highest operating cost. In addition, the oxy-fuel combustion is less efficient in regards to HiTAC technology considering the available heats and non-economic costs of oxygen producing. Accordingly, in the absence of any need to reduce CO_2 emissions the oxy-combustion is not competitive.

However, oxy-fuel is a viable alternative for removing CO_2 from the flue gas in a conventional air-fired fossil fuel plant. Namely, the big advantage of oxy-combustion is reduction of the mass and volume of the flue gases for approximately 75%.

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ANALYZING ENERGY SECURITY FROM AVAILABILITY PERSPECTIVE – ENERGY POLICY DEVELOPMENT IN THE REPUBLIC OF SERBIA

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Abstract

Energy security has received a plethora of attention in recent years. There is a difference between "classic" and "contemporary" energy security studies. Within contemporary" energy security studies, it is important to consider dimensions and parameters of energy security and their relationships. A variety of dimensions and parameters are found in the literature. This study identifies the following dimensions of energy security: availability, diversity, cost, technology and efficiency, location, timeframe, resilience, environment, health, culture, literacy, employment, policy, military, and cyber security, if energy security is to be dealt with adequately. Even though some of the dimensions might be interconnected, it is beyond the scope of this study to analyze each of them separately. This study analyzes the availability dimension of energy security. The approach to the analysis has three parts: analyzing the availability dimensions and its parameters, revealing the relationship between the parameter and energy security, and addressing the threats to availability dimension. The questionnaire illustrates that the influence of availability dimension on energy security is apparent. As shown, there are comprehensive risks and threats associated with the availability.

Keywords: energy security, energy, energy availability

INTRODUCTION

Energy security has received a plethora of attention and has been the subject of studies within different fields of science [1], international relations [2], politics [3], policymaking [4], national energy policies [5], and as a national security issue [6]. The necessity to deal with energy security is due to its ability to influence policies and countries behaviour [7]. All actions should therefore be taken to provide energy security, and to fulfil the objective of making societies lives better.

There is a difference between "classic" and "contemporary" energy security studies. For instance, during the 1970s and 1980s, energy security meant stable supply of cheap oil under threats of embargoes and price manipulations by exporters. On the other hand, contemporary energy security issues go further than oil supplies. They and include a wider range of topics, e.g. mitigating climate change and providing equitable access to modern energy.

Furthermore, it is important to consider dimensions and parameters of energy security and their relationships. A variety of dimensions and parameters are found in the literature. This study identifies the following dimensions of energy security: availability, diversity, cost, technology and efficiency, location, timeframe, resilience, environment, health, culture, literacy, employment, policy, military, and cyber security, if energy security is to be dealt with adequately. Even though some of the dimensions might be interconnected, it is beyond the scope of this study to analyze each of them separately. This study analyzes the availability dimension of energy security. The approach to the analysis has three parts: analyzing the availability dimensions and its parameters, revealing the relationship between the parameter and energy security, and addressing the threats to availability dimension.

LITERATURE

Definition

Since energy security is a concept rather than a policy, a clear definition of energy security is necessary. In a generic manner, energy security can be defined as "the feature (measure, situation, or a status) in which a related system functions optimally and sustainably in all its dimensions, freely from any threats" [8].

Availability dimension of energy security

Availability dimension is a critical dimension for energy security. In literature, availability is mostly discussed in relation to access to services [4] to sufficient supplies [9] or to the availability of consumers [10]. Availability thus has three parameters: availability of energy resources, e.g., oil or gas, availability of means to transform resources into services, e.g., energy infrastructure or pipelines, and the availability of energy consumer, e.g., public use of energy or industrial use.

The principle meaning of availability is existence. If energy resources do not exist, there is no notion of energy system. Nowadays, the current energy system (coal, oil, gas) has no problem with energy resources (see [11]). The global coal reserves are $1 \cdot 10^7$ TWh, the global oil reserves are $2.5 \cdot 10^6$ TWh, and the global natural gas reserves are $2 \cdot 10^6$ TWh [6]. Based on current global energy consumption, the world's proven reserves of oil are estimated to last for more than 55 years [11], proven reserves of coal are estimated to last for 122 years [12], and proven reserves of natural gas are estimated to last for 61 years [12]. In addition, there is ambient/renewable energy (e.g., wind power, solar energy, flow-of-the-river, and marine energy), which can be used to cover the demand (see [13]). There are no energy security issues with reference to availability of the energy sources on a global level [14,15]. However, these resources (mainly fossil fuels) and the demand are not distributed evenly around globe [16].

The second parameter of the availability dimension is the consumers or the demand perspective. The demand parameter is as legitimate concern as it is the resource parameter for countries where their revenues are from energy exports [17]. Nowadays, the discussion about the demand side is as part of energy security known as "security of demand" [18]. From energy producers' perspective, energy security is a quest for a market for their energy exports which correlates to increased (government) revenues [2]. Today's population is around 7.35 billion and is expected to increase to 11.21 billion in 2100 [19]. Therefore, people will continue to demand energy for various needs and, as long as they live, there will be available consumers for the available resources. Furthermore, the need for energy in different sectors of an industrialized society is high. So similarly, as long as the civilization continues, the energy demand will continue.

The third parameter of the availability dimension is access to these energy resources, transferring them to energy services for consumers. Having access to the energy services was a concern through the history [20], and individuals, societies, and countries pay a huge cost to obtain such access. Full access is considered to enhance energy security and drives the development path of a society [21]. On the other hand, less access reduces energy security. The access importance relates to infrastructure. Secure infrastructure and transport routes are important for energy security [17]. Developing infrastructure is thus needed to enhance energy security [22].

Research questions

This paper analyzes energy policy development in the Republic of Serbia. It focuses on availability dimension as one of the key aspects of energy security. Serbia, with a population of around 7.1 million, fulfills its electricity demand mostly from domestic production. Electricity production in Serbia is based around 70% on coal, while the remaining 30% is generated in hydropower plants. The electricity market in Serbia is dominated by the national power utility EPS (Elektroprivreda Srbije – Power Industry of Serbia), which owns and manages all large generation capacities and supplies most consumers. This paper represents systematic empirical research organized around the following question:

- What is the influence of different parameters of the availability dimension on energy policy development in the Republic of Serbia?
- What other dimensions can be identified with reference to potential influence on energy policy development the Republic of Serbia?

METHODOLOGY

Development of research hypothesis

A hypothesis is a “proposition that can be tested for association or causality against empirical evidence” [23]. Present research obtained research hypothesis based on the literature review. The main research hypothesis thus might be summarized as follows:

H1: Ceteris paribus, the greater is the availability of energy resources, the more secure the system is.

H2: Ceteris paribus, the greater is the availability of means to transform resources into services, the more secure the system is.

H3: Ceteris paribus, the greater is the availability of energy consumer, the more secure the system is.

Research design

Research can be understood as a process of finding solutions to a problem after a thorough study and analysis of the various factors. Business research can be understood as a methodical and organized effort to examine a particular problem encountered in the work setting that requires a solution. Present research is an exploratory research. There are no earlier studies to which we can refer for information about the availability aspect of energy security in the Republic of Serbia. It is a qualitative research carried out to deal with the research questions and design a study that involves collecting qualitative data and analyzing them using interpretative methods. Qualitative data collection instruments are researcher itself and a questionnaire. A sample of present dissertation is a joint stock company EPS. Data collection methods include e-post, group distribution and individual distribution.

RESULTS AND DISCUSSION

The questionnaire illustrates that the influence of availability dimension on energy security is apparent. Hypothesis 1 shows that 92% of respondents strongly agree that an increase in the availability of energy resources increase the security of the energy system. Similarly, hypothesis 2 shows that with the appropriate energy infrastructure the more secure the energy system is (89% of the respondents). Finally, in case of hypothesis 3, increase in public or industrial use of energy correlates to the more secure energy system (93% of the respondents).

CONCLUSION

The questionnaire illustrates that the influence of availability dimension on energy security is apparent. As shown, there are comprehensive risks and threats associated with the availability.

Such an overview of energy security with all the issues included in discussion hopefully provides recommendations for policymakers, governments, and researchers. However, to come up with inclusive and comprehensive agenda for energy security, all identified dimensions and their parameters should be taken into consideration. The exclusion of any of these dimensions and their parameters might result in an imbalanced outcome and decisions with reference to energy security since all the dimensions are interdependent and can affect each other.

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RENEWABLE ENERGY POLICY DRIVERS – ESTIMATING ECONOMIC IMPACTS OF DEPLOYING RENEWABLES

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Abstract

Climate change mitigation has been the primary rationale behind policies to support renewable energy (RE) development. The renewable energy technologies have developed sufficiently and are becoming cost-competitive with fossil fuels and nuclear sources. An increase in cost-competitiveness eases other drivers of RE as well. Investments in RE increase income, improve trade balances, contribute to industrial development and create jobs. Three main drivers for using RE can be identified: (1) energy security, (2) economic impacts, and (3) CO_2 emission reductions. Even though some of the drivers might be interconnected, it is beyond the scope of this study to analyze each of them separately. Instead, this study analyzes the economic impacts of deploying renewables. It shows that the influence of renewable energy technologies on sustainable economic growth is apparent. Technological capabilities and innovation success in renewables result from a broad range of beneficial factors influencing the innovation chain. In addition, in isolated rural areas with underdeveloped access to electricity, off-grid renewable technologies provide a sustainable and cost-effective alternative to the diesel generators.

Keywords: renewable energy, policy drivers, economic impacts, climate change

INTRODUCTION

Climate change mitigation has been the primary rationale behind policies to support renewable energy (RE) development. The renewable energy technologies (RET) have developed sufficiently and are becoming cost-competitive with fossil fuels and nuclear sources. An increase in cost-competitiveness eases other drivers of RE as well. Investments in RE increase income, improve trade balances, contribute to industrial development and create jobs. Three main drivers for using RE can be identified: (1) energy security, (2) economic impacts, and (3) CO_2 emission reductions [1]. Even though some of the drivers might be interconnected, it is beyond the scope of this study to analyze each of them separately. Instead, this study analyzes the economic impacts of deploying renewables.

LITERATURE

Definitions

According to the Cambridge Dictionary [2], the term “renewable energy” is defined in the following way: “energy that is produced using the sun, wind, etc. from crops, rather than using fuels such as oil or coal”. Similarly, Lund [3] defined the term “renewable energy” as “energy that is produced by natural resources—such as sunlight, wind, rain, waves, tides, and geothermal heat– that are naturally replenished within a time span of a few years”. RE includes the technologies that convert natural resources into useful energy services: “(1) wind, wave, tidal, and hydropower (including micro- and river-off hydropower); (2) solar power (including photovoltaic), solar, thermal, and geothermal; (3) biomass and bio-fuel technologies (including biogas); and (4) renewable fraction of waste (households and industrial waste)”.

Estimating economic impacts of deploying renewables

As written, three main drivers for using RE can be identified: (1) energy security, (2) economic impacts, and (3) CO_2 emission reductions [1].

The usage of RET is often given high priority within a strategy towards more sustainable economic growth, and is referred to as “green growth” [1]. RET are able to contribute to sustainable development by allowing exploitation of natural but replenishing resources. The most important aspect of a green growth framework is recognition of “natural capital” as a factor of production and its role in improving societal well-being which refers to factors entering the production process that are provided by nature itself, in particular fossil energy resources. The IEA [1] states that “existing production technology and consumer behavior can be expected to produce positive outcomes only up to a point; beyond that point, depleting natural capital has negative consequences for overall growth”. To determine accurately where this frontier lies is unknown in all cases. Also, the ability of reproducible capital to substitute for natural capital is limited in the absence of innovation. Economic policy decisions therefore need to incorporate a longer time horizon. In reference to green growth, policies that support renewable electricity (RES-E) production have two major objectives: “first, they aim to create new markets that recognize the importance of natural capital and of reconciling limited natural resources with economic growth; second, they provide an exit strategy from the fossil energy-based development path to which the global economy is currently committed” [1].

Several established RE market leaders (e.g. Germany, Denmark, Japan, etc.) have long placed industrial and economic development objectives at the centre of their support for RE technologies [1]. They stimulated the creation of industrial clusters and developed domestic markets by setting up stable, enabling policy frameworks. They also specialized at an early stage in providing RET which were characterized by high knowledge intensity and learning potential, and thus became front-runners in terms of innovation. For example, Walz *et al.* [4] illustrate factors that improve a country’s ability to benefit from a first-mover advantage in external trade: (1) positive market conditions in the country; (2) innovation-friendly regulation in the country; (3) technological capability of the country; (4) the competitiveness of related industry clusters in the country; and (5) technology characteristics that form obstacles to international relocation. An important indicator of a country’s level of specialization in certain technologies and a measure of future potential for market share growth is a patent activity. In fact, patent shares “reflect the success of government policies to stimulate innovation in the field of renewable energies” [1]. Technological capabilities and innovation success in renewables therefore result from a broad range of factors influencing the innovation chain. The challenge is whether these established RE market leaders can maintain their first-mover advantage in spite of growing competition from emerging economies with lower production costs, i.e. so-called “fast followers” [1].

In addition, in rural areas with poorly developed access to electricity, grid extensions are often cost ineffective [1]. Off-grid RETs provide a sustainable and cost-effective alternative to the diesel generators kerosene lamps and conventional biomass that would be typically used in such areas. Strengthening the economy in rural areas is another rationale for using RE in developed countries [1], i.e. the agricultural sector can diversify its activities and open access to new markets that are economically viable in the long term by introducing support policies for the production and consumption of biofuels.

Research questions

This paper analyzes policy drivers for deploying renewables. It focuses on economic impacts of using RE. This paper thus represents systematic empirical research organized around the following question:

- What are the economic impacts of deploying renewable energy technologies?

METHODOLOGY

Development of research hypothesis

A hypothesis is a “proposition that can be tested for association or causality against empirical evidence” [5]. Present research obtained research hypothesis based on the literature review. The main research hypothesis thus might be summarized as follows:

H1: Ceteris paribus, the higher is the deployment of renewable energy technologies, the more sustainable economic growth is.

H2: Ceteris paribus, the higher is the patent share reflected as a deployment of renewable energy technologies, the more sustainable economic growth is.

H3: Ceteris paribus, the higher is the deployment of off-grid renewable energy technologies in rural areas, the more sustainable economic growth is.

Research design

Research can be viewed as a process of finding solutions to a problem after a thorough study and analysis of the various factors. Similarly, business research can be seen as a methodical and organized effort to examine a particular problem in the work setting that requires a solution.

Research is classified in accordance with the “(1) purpose of the research (the reason why the research is conducted); (2) process of the research (the way in which the data were collected and analyzed); (3) logic of the research (the course of research from the broad to the specific or vice versa); and (4) outcome of research (the solution to a specific problem or a broad contribution to knowledge)” [5].

Present research is an exploratory research. It will evaluate which existing theories and concepts can be applied to the problem or whether new ones should be developed. Present research is also a qualitative research carried out to deal with the research questions and design a study that involves collecting qualitative data and analyzing them using interpretative methods. Qualitative data collection instruments are researcher itself and a questionnaire. A sample of present research is venture capital and private equity funds in European Union (EU). 150 – 200 questionnaires will be sent to the employees of such funds. Data collection methods include e-post, group distribution and individual distribution. Furthermore, present research is applied research designed to apply it findings to solving a specific, existing problem. It is the application of existing knowledge to improve management practices and policies. Finally, present research is a deductive research in which a conceptual and theoretical structure is developed which is then tested by empirical observation. Particular instances are, thus, deducted from general inferences.

RESULTS AND DISCUSSION

The questionnaire shows that the influence of RET on sustainable economic growth is apparent. Hypothesis 1 illustrates that 77% of respondents strongly agree that the deployment of RET lead to more sustainable economic growth. Hypothesis 2 shows that 79% of respondents strongly agree that the patent share reflects a deployment of renewable energy technologies and the sustainability in economic growth. Similarly, in a hypothesis 2, 72% of the respondents strongly agree that the deployment of off-grid RET in rural areas will inevitably lead to the more sustainable economic growth.

CONCLUSION

RET contribute to sustainable economic development by exploiting natural but replenishing resources, providing new sources of natural capital. The most important feature is recognition of “natural capital” as a factor of production and its role in enhancing societal well-being.

Established RE market leaders have placed industrial and economic development objectives at the centre of their support for RET by putting in place stable, enabling policy frameworks along the innovation chain, along with favorable investment conditions for innovative RET, including solar PV and wind. They specialized in the supply of RET characterized by high knowledge intensity and learning potential, and therefore became front-runners in terms of innovation. Technological capabilities and innovation success in renewables result from a broad range of beneficial factors influencing the innovation chain. In the same manner, patent activity is an important indicator of a country’s level of specialization in certain technologies and a measure of future potential for market share growth.

In isolated rural areas with underdeveloped access to electricity, off-grid renewable technologies provide a sustainable and cost-effective alternative to the diesel generators.

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THE EVOLVING CONCEPT OF ENERGY SECURITY – WHETHER CULTURE PLAYS A ROLE IN SECURING SUFFICIENT ENERGY SUPPLIES?

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Abstract

Energy security has received a lot of interest in recent years. It is necessary to consider dimensions and parameters of energy security and their relationships. A variety of dimensions and parameters are found in the literature: availability, diversity, cost, technology and efficiency, location, timeframe, resilience, environment, health, culture, literacy, employment, policy, military, and cyber security, if energy security is to be dealt with adequately. This study analyzes the cultural dimension of energy security. The approach to the analysis has three parts: analyzing the cultural dimensions and its parameters, revealing the relationship between the parameter and energy security, and addressing the threats to cultural dimension. Culture is an important aspect of energy security. It affects how people shape, react, or deal with specific issues. Two parameters how culture affect energy security are found: first during the three stages of energy systems (production, transfer, and consumption), and second how energy conditions shape or modify cultural behavior. This study confirms that the influence of cultural dimension on energy security is apparent and hopefully provides recommendations for policymakers, governments, and researchers.

Keywords: energy security, culture, production, transfer, consumption

INTRODUCTION

Energy security has received a plethora of attention and has been the subject of studies within different fields of science [1], international relations [2], politics [3], policymaking [4], national energy policies [5], and as a national security issue [6]. The necessity to deal with energy security is due to its ability to influence policies and countries behavior [7]. All actions should therefore be taken to provide energy security, and to fulfil the objective of making societies lives better. Although the notion of energy security is quite old, analysis as a part of scientific research started around 1975 [8] and due to following reasons: climate change [9], political conflict [3], complex global markets [10], social development [8], threats to the energy system [11], energy demand and competition [12], the global energy supply crisis [13], major disruptions in oil markets [1] related to military upheavals [14], increasing energy prices [12], the perception that views energy security in relation to national security [15], and the increasing dependence of industrialized economies on energy [16] as a condition for economic growth [17] and a driver for civilization [18] and society [19].

There is a difference between “classic” and “contemporary” energy security studies. For instance, during the 1970s and 1980s, energy security meant stable supply of cheap oil under threats of embargoes and price manipulations by exporters. On the other hand, contemporary energy security issues go further than oil supplies. They and include a wider range of topics, e.g. mitigating climate change and providing equitable access to modern energy.

It is further important to consider dimensions and parameters of energy security and their relationships. A variety of dimensions and parameters are found in the literature. This study identifies the following dimensions of energy security: availability, diversity, cost, technology and efficiency, location, timeframe, resilience, environment, health, culture, literacy, employment, policy, military, and cyber security, if energy security is to be dealt with adequately. Even though some of the dimensions might be interconnected, it is beyond the scope of this study to analyze each of them separately. This study analyzes the cultural dimension of energy security. The approach to the analysis has three parts: analyzing the cultural dimensions and its parameters, revealing the relationship between the parameter and energy security, and addressing the threats to cultural dimension.

LITERATURE

Definition

Since energy security is a concept rather than a policy, a clear definition of energy security is necessary. A variety of different definitions of energy security can be found in the literature. For instance, the Cambridge Dictionary [20] defines the term “energy” in the following way: “the power from something such as electricity or oil that can do work, such as providing light and heat”. Energy is thus the power or the strength that can result in work. The Cambridge Dictionary [20] also defines the term “security”: “freedom from risk and the threat of change for the worse”. Energy security can be viewed as “the provision of sufficient and reliable energy supplies to satisfy demand at all times and at affordable prices, while also avoiding environmental impacts” [21]. Similarly, but in a more generic manner, energy security can be defined as “the feature (measure, situation, or a status) in which a related system functions optimally and sustainably in all its dimensions, freely from any threats” [22].

Cultural dimension of energy security

Culture is an important aspect of energy security [23]. Cultures affect how people shape, react, or deal with specific issues. It is referred to as social acceptability [24] or public participation [23]. On the other hand, events and conditions can change some parts of the culture [25], and energy systems and energy security are not exceptions.

The first parameter is how cultures affect energy security. Humans’ effect is in the three stages of energy systems (production, transfer, and consumption). In the production stage, energy poverty will encourage societies to produce energy no matter how to cover their demand even if such energy resources cause a lot of harm on the environment or the health which is reducing energy security [7]. The way how energy is transferred is affected by both public and private suppliers. If the culture is more materialistic, suppliers are inclined to concentrate on fulfilling the energy demand from industrial consumers at the cost of the poor with low demand [26], which obstructs energy security of the poorest. Referring to the consumption side, liberal individuals are more likely to engage in a responsible consumption behavior to achieve energy security [27]. Social justice promotes secure energy systems that benefit the masses rather than the elite [28]. From demographical stance, age and gender also play a role. For instance, for youngest groups energy security is their top priority compared to elderly [27].

The second parameter is how energy conditions shape or modify cultural behavior. From the historical stance, it is shown how the discovery of resources changed civilizations and cultures [29]. Disruption of energy production that leaves societies without energy can cause social conflicts [30]. Wealthy and powerful societies achieve energy security easier compared to the poor ones [31]. Such financial consideration change consumers' purchasing behaviour [32].

Research questions

This paper analyzes cultural dimension of energy security based on systematic empirical research organized around the following questions:

- What is the influence of different parameters of the cultural dimension on energy policy development?
- What other dimensions can be identified with reference to potential influence on energy policy development?

METHODOLOGY

Development of research hypothesis

A hypothesis is a "proposition that can be tested for association or causality against empirical evidence" [33]. Present research obtained research hypothesis based on the literature review. The main research hypothesis thus might be summarized as follows:

- H1: Ceteris paribus, the greater is the uncoordinated production of energy by societies to cover their demand, the less secure the energy system is.
- H2: Ceteris paribus, the more frequent is the disruption of energy production that leaves societies without needed energy, the less secure the energy system is.

Research design

Research can be understood as a process of finding solutions to a problem after a thorough study and analysis of the various factors. Business research can be understood as a methodical and organized effort to examine a particular problem encountered in the work setting that requires a solution. Present research is an exploratory research as there are no earlier studies to which we can refer for information about the cultural aspect of energy security. It is a qualitative research carried out to deal with the research questions and design a study that involves collecting qualitative data and analyzing them using interpretative methods. Qualitative data collection instruments are researcher itself and a questionnaire. A sample of present research is companies engaged with venture capital, private equity, public capital markets and investment banking. Data collection methods include e-post, group distribution and individual distribution.

RESULTS AND DISCUSSION

The questionnaire illustrates that the influence of cultural dimension on energy security is apparent. Hypothesis 1 shows that 69% of respondents strongly agree that uncoordinated production of energy by societies will lead to less secure energy system. In a similar manner, in a hypothesis 2 77% of the respondents strongly agree that any disruption of energy production will lead to less secure energy system.

CONCLUSION

The questionnaire illustrates that the influence of cultural dimension on energy security is apparent. The concept of energy security is evolving and there are comprehensive risks and threats associated with its cultural aspect. In addition to the described influences, the threat to this dimension is also the psychological effect [34]. Societies and individuals are manipulated by media, which is a distorted reality, misleading conclusions, and wrong facts.

An overview of energy security from a cultural stance hopefully provides recommendations for policymakers, governments, and researchers. However, to reach inclusive and comprehensive agenda for energy security, all identified dimensions and their parameters should be taken into consideration. The exclusion of any of these dimensions and their parameters might result in an imbalanced outcome and decisions with reference to energy security since all the dimensions are interdependent and can affect each other.

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STRUCTURAL CHARACTERISATION AND ORIENTATION OF CELL WALL POLYMERS IN MAIZE LEAVES

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Abstract

Cell wall can be considered as a nano-composite in which cellulose, lignin and hemicelluloses are interconnected in a specific manner. Mechanical and physical propreties of plant fibres are dependent on the orientation of constituent polymers (cellulose, hemicellulose, lignin). Fourier transform infrared (FTIR) microscopy was used to examine the orientation of the main plant polymers in transversal and longitudinal direction of the isolated cell wall of the maize leaves. Polarised FTIR measurements indicated an anisotropy, i.e. orientation of the cellulose microfibrils that was more or less parallel to the longitudinal axis of the cell wall. Xylan has parallel orientation with regard to the orientation of cellulose, as well as lignin.

Keywords: cell wall, cellulose, xylan, lignin, anisotropy

INTRODUCTION

Plant cell walls (CW) are the most abundant, renewable and biodegradable composite on Earth. The specific form and function of the cell walls and interaction with the environment are based on variation in its chemical composition and connections between the building macromolecules. Cell wall can also be considered as a nano-composite in which cellulose, lignin and hemicelluloses are interconnected in a specific manner. Biopolymers such as cellulose, hemicellulose and lignin, have wide applications in different industries, especially for biofuels and biomaterials [1,2].

Understanding structural organisation of the cell wall and related polymers is important for understanding mechanical properties of a plant, which has implications in plant response to stress, but also in possible applications of corn as a source of new biomaterials. By using imaging FT-IR microscopy, run in transmission mode and at different polarisation modes (from 0° to 90°), it is possible to follow chemical variability and orientation of cell wall polymers [3]. The orientation of cellulose, xylan and lignin, as essential components of plants, were analysed by iFTIR with regard to the sample axis.

MATERIALS AND METHODS

Cell wall isolation

To obtain extractive free cell walls, 1 g of maize leaves were homogenized in 10 mL of 80% methanol in 50 mL Big Clean tubes filled with a stainless steel matrix for 45 s at a speed of 4.5 m/s, using a FastPrep-24 apparatus (MP Biomedicals, Santa Ana, CA, USA). After stirring for 5 min at room temperature, the sample was again subjected to FastPrep homogenization at the same speed, which is a preferred technique in the case of plant material (Melton and Smith 2001). The homogenate was stirred at room temperature for 1 h and centrifuged at 1500 x g for 5 min. The pellet obtained was re-extracted twice, using 10 mL of 80% methanol. In order to remove the extractives, the pellet that had been obtained was then subjected to washing steps: 1M sodium chloride, 1% Triton X-100, distilled water, methanol, acetone [4].

FTIR microscopy

FTIR microscopy measurements were carried out using a Spectrum Spotlight 400 FTIR Imaging System (Perkin Elmer Inc, Shelton, CT, USA). The area of interest was first displayed, using a visible CCD camera to locate the cell wall area, which was then irradiated using mid-IR light. The scanning was carried out in imaging mode using an array detector, providing a pixel resolution of 6.25 μm x 6.25 μm, a spectral resolution of 4 cm^{-1} and a spectral range from 1,800 to 720 cm^{-1}. Polarisation: the incident IR radiation was polarised by a gold wire grid polariser from 0° to 90° polarisation in relation to the fibre orientation with intervals of 5°. The sample was mounted on the sample stage as parallel as possible to the orientation of the 0° polarisation. The IR spectra were processed by the software Spotlight 1.5.1, HyperView 3.2 and Spectrum 6.2.0 (Perkin Elmer Inc., Shelton, CT, USA) [3].

RESULTS AND DISCUSSION

From the in-depth study of polymer orientation, three areas from the sample (corn leaf) were selected. The transmission spectra were recorded from 0° to 90° polarisation modes. Figure 1 shows FTIR spectum of cell walls of maize leaves in the region 800–1800 cm^{-1}. Spectral signals related to absorptions from cellulose, xylan and lignin can be identified.

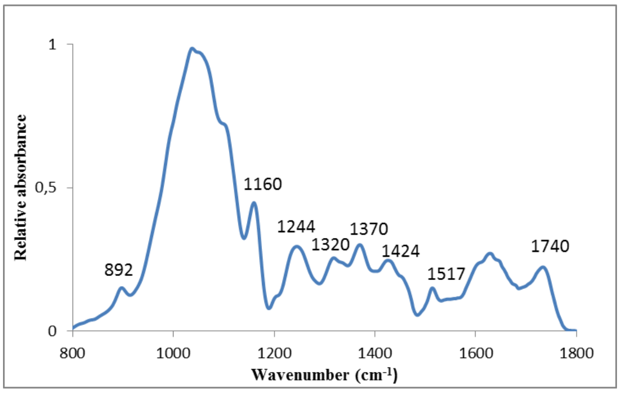

Figure 1 *Average absorbance spectrum of maize leaves cell wall*

Four distinct vibration bands that were related to cellulose, viz. the antisymmetric C–O–C bridge stretching vibration at 1160 cm^{-1}, CH_2 wagging vibration at 1320 cm^{-1}, the C–H bending vibration at 1370 cm^{-1} and the C–OH bending vibration of the CH_2–OH group at 1424 cm^{-1} were found [5–7]. The bands at 1244 cm^{-1} (the C–O stretching in the O=C–O group) and 1740 cm^{-1} (carbonyl group vibration, the C=O stretching vibrations in the O=C–OH group of the glucuronic acid units) are characteristic for xylan [5–7]. The band at 1517 cm^{-1} (aromatic skeletal vibrations) is characteristic for lignin [8,9].

The relative absorbance spectra are presented (Figure 2) as specific absorption peaks ($RA = (I_p - I_{min}) / (I_{max} - I_{min})$) where RA is relative absorbance, I_p is intensity of the absorbed IR radiation at a given angle of the polarisation, I_{max} is maximal intensity observed for a given vibration and I_{min} is minimal intensity observed for a given vibration. These relative absorbance values were presented in relation to the angle of the incident IR polarisation (from 0° to 90°).

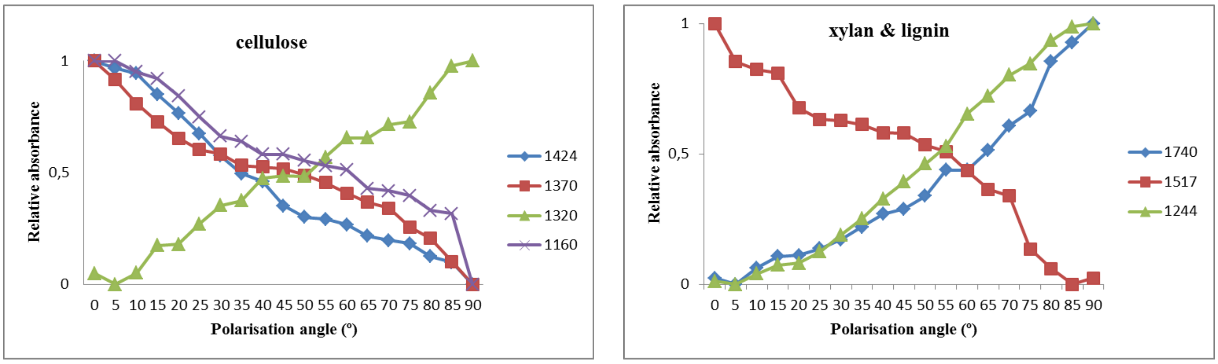

***Figure 2** The relative absorbance of IR specific absorption wavenumbers plotted against the polarisation angle for the different polymers for maize leaves*

It is evident (Figure 2 left) that the three cellulose peaks (1160 cm^{-1},1370 cm^{-1} and 1424 cm^{-1}) [3–5] had high absorption levels at low polarisation angles, which is a consequence of a more parallel orientation of the corresponding groups to the CW longitudinal axis. The fourth cellulose peak (the perpendicular signal at 1320 cm^{-1}) had the greatest intensity at a high polarisation angle, due to the perpendicular orientation of the corresponding group (Figure 2 left). For the xylan, the characteristic band signals (1244 cm^{-1}, 1740 cm^{-1}) [5–7] increased with an increase in the polarisation angle. Due to the parallel orientation of these side groups in xylan, an orientation parallel to the longitudinal CW axis is indicated (Figure 2 right). For the lignin, the characteristic band signal (1517 cm^{-1}) [8,9] decreased with an increase in the polarisation angle (Figure 2 right), indicating that lignin is organised in parallel with the longitudinal CW axis.

CONCLUSION

It has been shown that xylan is oriented in parallel to the cellulose and more or less parallel to the axis of a cell wall, in isolated CW fragments from maize leaves. There was also a clear indication of lignin orientation parallel to the longitudinal CW axis. This means that all of these components show strong anisotropic behaviour and organisation.

ACKNOWLEDGEMENT

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WIDESPREAD OF ASIAN FLY (*Drosophila suzukii*, MATSUMURA) IN DIFFERENT PLANTATIONS

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Abstract

The fruit fly Drosophila suzukii is a fruit crop pest and is a serious economic threat to soft summer fruit. As a polyphagous pest it infests a wide range of fruit crops, including grape, as well as an increasing number of wild fruits. In Serbia, since its first occurrence in 2014 D. suzukii has been causing damage in different crops. The damage caused by D. suzukii larvae makes the fruit unmarketable. This work presents monitoring of D. suzukii in 2018 and it shows the widespread of this pest in the area of regional centre Sremska Mitrovica. The presence of pests in traps was registered on 7 localities. The increased number of individuals compared to 2017, their widespread and favorable natural conditions for development of D. suzukii on the territory of Srem indicate risks for production of fruit and grape.

Keywords: pest, *Drosophila suzukii*, fruit

INTRODUCTION

In june, 1916, larvae were found in pre-harvest cherries (*Prunus avium*) in Japan. The invaded fruits were collected and the adult flies that appeared were confirmed as a species of *Drosophila*. Later, in 1931, Dr. Shounen Matsumura described the species as *Drosophila suzukii*. On new territories the species had spread by international trade. In Europe was recognized for the first time in 2008 and in Serbia 2014 [1]. Its adaptability, large number of generations and wide number of hosts enabled their survival and spreading on new territories. Unlike other Drosophila, *D. suzukii* can cause damage on fruit in ripening phase. *Drosophila suzukii* has two imago forms: summer and winter form. Male summer forms are brown-yellow colored with black stripes on the abdomen and have red eyes. They are 2-3 mm in length. The main characteristics are a black-grey spot in the corner of the wings and short hairs on the front feet. Females don't have a spot on the wings, but have a large serrated ovipositor. Summer forms are light colored and have black stripes on their back. The antennas are short and covered with feather-like hairs. The oral apparatus is specialized for sucking liquids. Compared to the body, the oral apparatus is long and has inoculum of microorganisms for fruit degradation. Females lay eggs by ovipositor. Larvae feeding in fruits can cause their damage. The development of an egg lasts 1-3 days, of a larva 3-13 days and of an adult 4-16 days [2]. Adults live 3-9 weeks, and favorable natural conditions can cause the development of up to 13 generations per year. During one oviposition term, a female lays maximum 3 eggs. Oviposition takes place in the phase when the fruit changes its color, when the fruit skin is softened and the sugar level is higher. The female prefers juicy fruits with thin skin. Damage appears as an effect of feeding on fruits, the fruit becomes soft, changes color and the places of stab can be susceptible for secondary infections. Accommodation of *D. suzukii* for surviving winter takes place in fall when its feeding is reduced and then transit to reproductive diapause. If the larvae were feeding themselves and suddenly low temperatures appear, they die because of the „nucleus“ in their intestines. Imagoes search for a place to survive winter. Unlike other potentially invasive pests, *Drosophila suzukii* is not an object of regulation in USA and Europe, for now it’s just monitoring and it is on EPPO A2 quarantine list [3]. The purpose of monitoring is to determine the widespread of Asian fly in different plantations in Srem and to determine flying dinamic during vegetation season by the help of traps with apple vinegar basis.

MATERIALS AND METHODS

Monitoring of widespread of *Drosophila suzukii* is implemented during 2018 in 5 vineyards, 3 raspberry, 2 peach and 2 blackberry plantations. Climate conditions and air temperature are very important, so the data were taken from the Republic hidrometeorological institute for the region of Sremska Mitrovica and because of this research the data was upgraded. The monitoring was performed by the help of traps for which were used apple vinegar and wine solutions (40:60), because it is more attractive than yeast. This solution was poured in plastic bottles which had a small hole 3 cm under the bottle cap, so the adults could go in it. A few drops of detergent were added in apple cider vinegar (ACV), so the flies could fall to the bottom. The traps were set up: 1 trap per plot bigger than 5 ha and 2 traps per plot smaller than 5 ha. In vineyards and peach plantations the traps were set up for the first time on April, and in raspberry and blackberry plantations in July. Samples were collected once in a week until October 2018. Vinegar was changed twice a month. Most of the traps were set up in shadowy places. During the inspection of the catch, in order to reduce the damage to the flies, vinegar solution was filtered through funnel or soft fabric, and then distilled water was added to remove the excess. In this way refined catch is put on white paper to decant liquid, and then transferred into another bowl. Before recording, water was added to separate the flies from each other. The number of pests was monitored in collaboration with PIS Vojvodina.

RESULTS AND DISCUSSION

The presence of *Drosophila suzukii* was registered for the first time in July in grape plantations in all 5 localities. The total number of caught adults was 2200 (Figure 1).

In raspberry plantations the first individuals were also registered in July in 3 locations with total number of 869. In peach plantations the first catch was at the end of July, the number of adults were 748, while the first catch in blackberry plantations was registered at the begining of August, with the number of 378 adults (Figure 2).

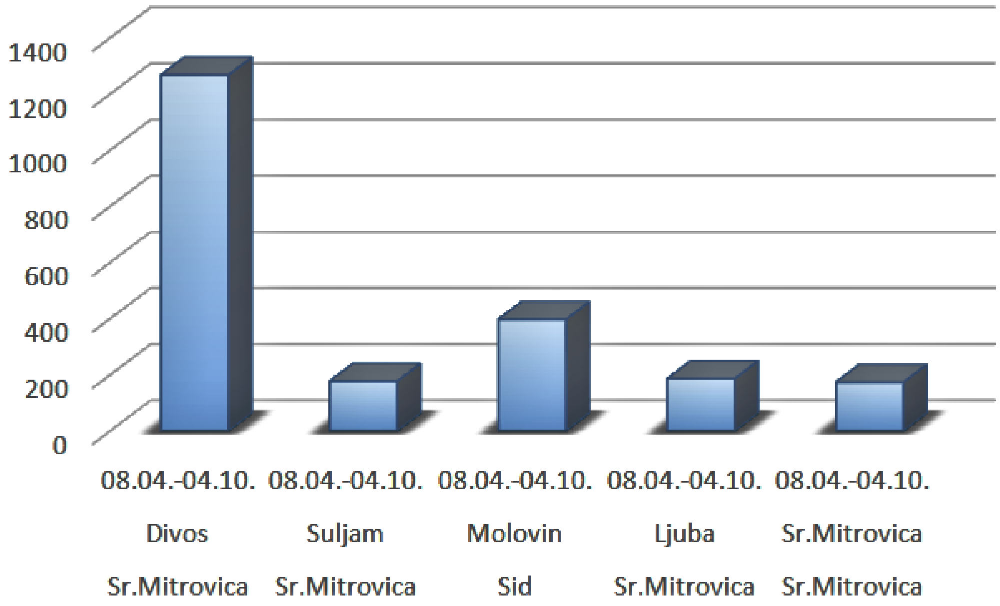

Figure 1 *The number of Drosophila suzukii adults in vineyards*

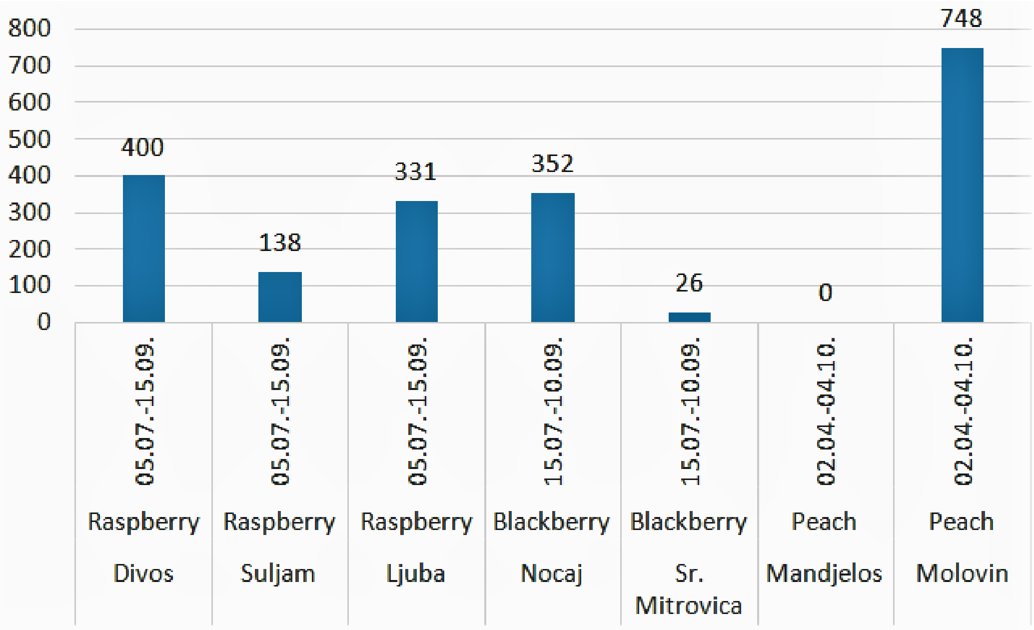

Figure 2 *The number of D. suzukii adults in plantations of blackberry, raspberry and peach*

Considering these results it can be assumed that in plantations on the territory of Srem, the number of pests was sporadical, increase was in ripening phase, and the further survival of the population was enabled by the fruit they were in. According to research [4], medium annual temperatures of 5°C to 20°C increase the survival of *D. suzukii*. Medium annual temperature in Sremska Mitrovica is 13.3°C, which means that the terms were in favor of the development of *D. suzukii*. It is induced that the optimal temperature needed for activity of *Drosophila suzukii* is 10-20°C, and the minimal needed for flying is 10°C [5]. According to Figure 3, it is obviously that the average monthly temperatures from March to November were in the optimal range for activity. The minimum temperature for the flight was present during all days in the months from July to November when it was 7°C. The largest number of captured individuals was registered in the vineyard in the Divos 1267, followed by the peach orchard in Molovin with 748 adults caught, while in raspberry plantations the largest number was registered in Divos 400, and in the blackberry plantation at the locality of Nocaj 352 individuals (Figure 4).

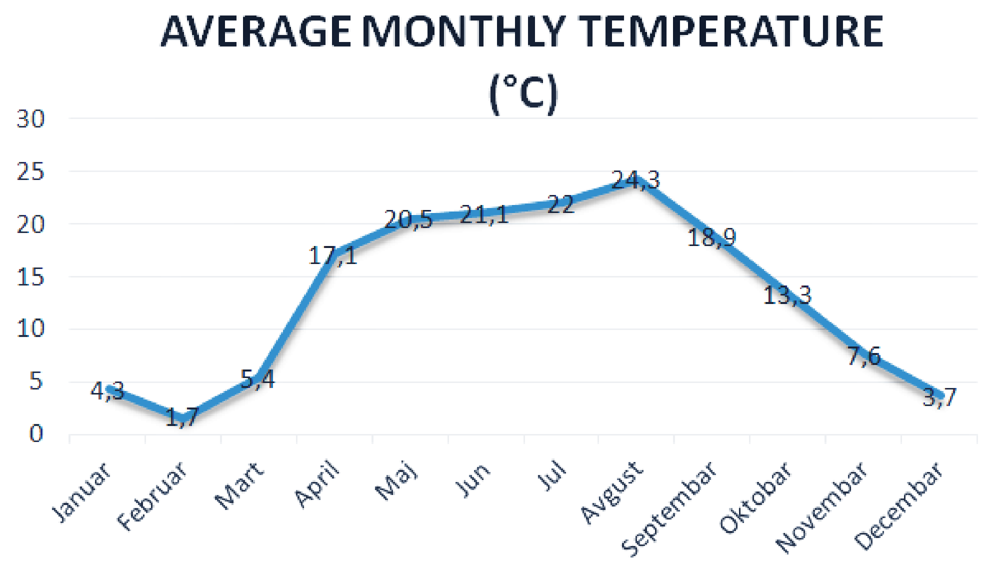

Figure 3 *Average monthly temperature in Srem*

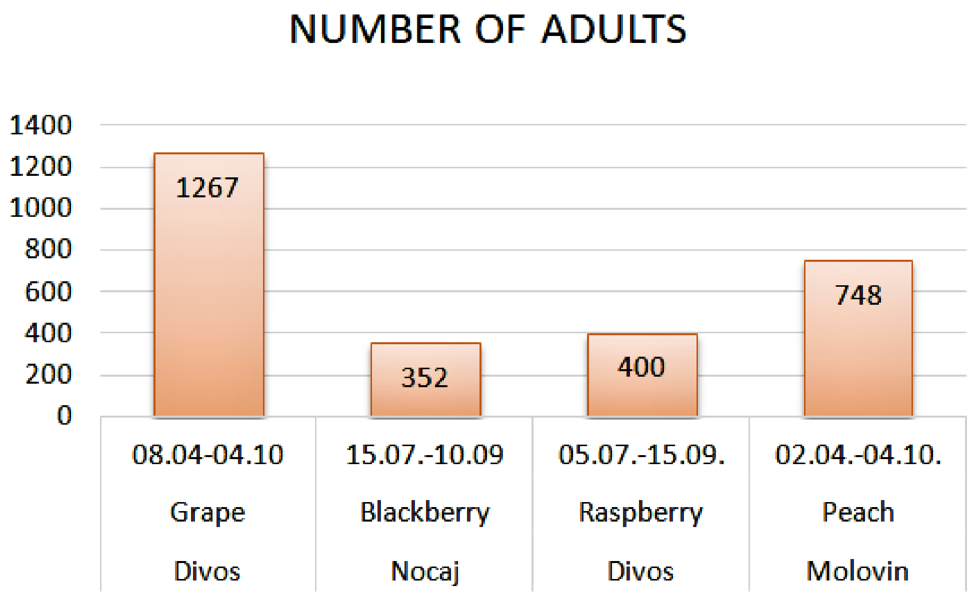

Figure 4 *The largest number of D. suzukii*

If the number and the presence of *Drosophila suzukii* in Srem (44° 58'N 19° 36'E) are compared with data from Zagreb (45°48'N 15°58'E), one can notice a large deviation in number in the same plantations of raspberries, peach and blackberries. During the research period, 847 adults were captured in Zagreb, while 1545 individuals were caught in Srem at 3 locations, which indicates that the climatic conditions in Srem, selected variety, the surrounding vegetation contributed to the greater settlement of Asian fly in this territory.

CONCLUSION

According to these results, based on monitoring of *Drosophila suzukii* in 12 plantations on the territory of Srem in 2018, it can be concluded as follows:

- *Drosophila suzukii* was present in all 12 plantations
- Total number of caught adults in period of monitoring was 4195
- Climate conditions on the territory of Srem were in favor for the development of the pest, medium annual temperature was 13.3°C.
- Compared to other monitorings on the territory of Srem it developed a large number of generations thanks to favorable climate conditions, domestic and wild vegetation in the surrounding area.
- The program of protection in Serbia should include all producers of fruits and grapes, otherwise their plantations present potential danger for other plantations and localities in which *Drosophila suzukii* is not present or it is present in a small number.

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THE IMPACT OF ALIEN SPECIES ON MACROZOOBENTHOS COMMUNITIES AND ASSESSMENT OF WATER QUALITY BY BIOTIC INDICES

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Abstract

Invasion of alien species can effects ecological state of water bodies through changes of composition and structure of macrozoobentos communities. We assessed bio-contamination (SBCI index) and water quality by biotic indices at 12 sites along the Danube River in Serbia and at 17 sites including the Neman, Narew and Western Bug river basins in Belarus for different years. The correlation analyses show that SBCI was weakly correlated with biotic indices in Danube River. Also, during the time Corophium sp. disappeared from macrozoobenthos community in the presence of Dikerogammarus villosus what could be a possible reason for the increase in the number of species and of the values of biotic indices. SBCI and densities of Orconectes limosus at sampling sites at Neman River are negatively correlated with biotic indices. At other sites in Belarus, SBCI metrics showed positive correlation with biotic indices. Differences in the impact of alien species on the assessment of water quality may be related to the intensity of biological pollution and the duration of the invasion.

Keywords: macroinvertabrates, alien species, biocontamination, water quality assessment

INTRODUCTION

Great number of alien species, especially macroinvertabrates, invaded the major European river basins due to human activities and related impacts [1]. Besides, domination of non-native species along the numerous European large rivers is confirmed [2,3]. Invasion of alien species can effects native communities due to direct predation and competition [4, 5]. Benthic macroinvertebrates are one of five biological quality elements of WFD used to assess of ecological status of European aquatic ecosystems. Changes in macroinvertebrate community structure due to pressures of invasive species may influence assessment of water quality [3,6].

To establish possible impact of aliens on native macroinvertabrates and assessment of water quality by biotic indices we conducted analysis of data from Serbian part of Danube River and Belorussian part of Neman River and its tributaries and rivers of Narew and Western Bug basins for different years. The Danube River is a part of Southern Invasive Corridor of Europe [7] and the Neman River is the main pathway for invaders from the Baltic Sea regions. The other rivers are part of the Ponto-Caspian Basin.

MATERIALS AND METHODS

Sampling of aquatic macroinvertebrates in the Danube River was carried out at 12 sites in 2014, 2015 and 2018. Sampling in Belarus was cried out at 17 sites in 2007, 2015 and 2018. Macroinvertebrates were collected using benthic hand nets with mesh size 500 µm, using Kick and Sweep multihabitat method in proportion to the evaluated habitat percentage at the sampling section. Material was preserved using 95% ethanol.

To assess biocontamination by alien species we used the site-specific biocontamination index (SBCI) [2]. It was calculated from two metrics: an abundance contamination index (ACI) and a richness contamination index (RCI). The sampling site classifies by obtained SBCI into one of four classes ranging from 0 (no contamination) to 4 (severe contamination).

Water quality was assessed using Biological Monitoring Working Party Index (BMWP) [8] and Altered Indice Biotico Esteso (IBE AQEM) [9]. To calculate biotic indices and other metrics we used Asterics Software Version 4.0.4.

Pearson correlation coefficient was used to test relationships between biocontamination indices and biological metrics.

RESULTS AND DISCUSSION

The 12 alien species were observed at sites along the Danube: *Hypania invalida* (Grube, 1860), *Dikerogammarus villosus* (Sowinsky, 1894), *Dikerogammarus bispinosus* Martynov, 1925, *Echinogammarus ischnus* (Stebbing, 1899), *Obesogammarus obesus* (Sars, 1894), *Limnomysis benedeni* Czerniavsky, 1882, *Dreissena bugensis* Andrusov, 1897, *Dreissena polymorpha* (Pallas, 1771), *Paramysis lacustris* (Czerniavsky, 1882), *Corbicula fluminea* (Müller, 1774), *Physella acuta* (Draparnaud, 1805) and *Orconectes limosus* (Rafinesque, 1817). The most abundant among them were *D. villosus* and *L. benedeni*.

Table 1 *Correlation coefficients between biocontamination and biotic indices and abundances of Dikerogammarus vilosus at sites of Danube River*

Index	ACI	RCI	SBCI	*D. villosus*
BMWP	-0.112	-0.119	0.061	**0.424**
IBE AQEM	0.085	-0.070	0.212	**0.510**

The most sites of Danube River had a high biocontamination level, which is in concordance with previous investigations [2,3]. The correlation analyses showed that SBCI metrics were weakly correlated with biotic indices (Table 1). The lasts were positively related with numbers of species in macrozoobentos communities (r = 0.744 for BMWP and r = 0.697 for IBE AQEM, P<0.05) and densities of *D. villosus* (r = 0.570, P<0.05).

Regarding certain amphipods species detected in the Danube River, we can see that during the time corophiid species were disappeared in the presence of *D. villosus* (Figure 1).

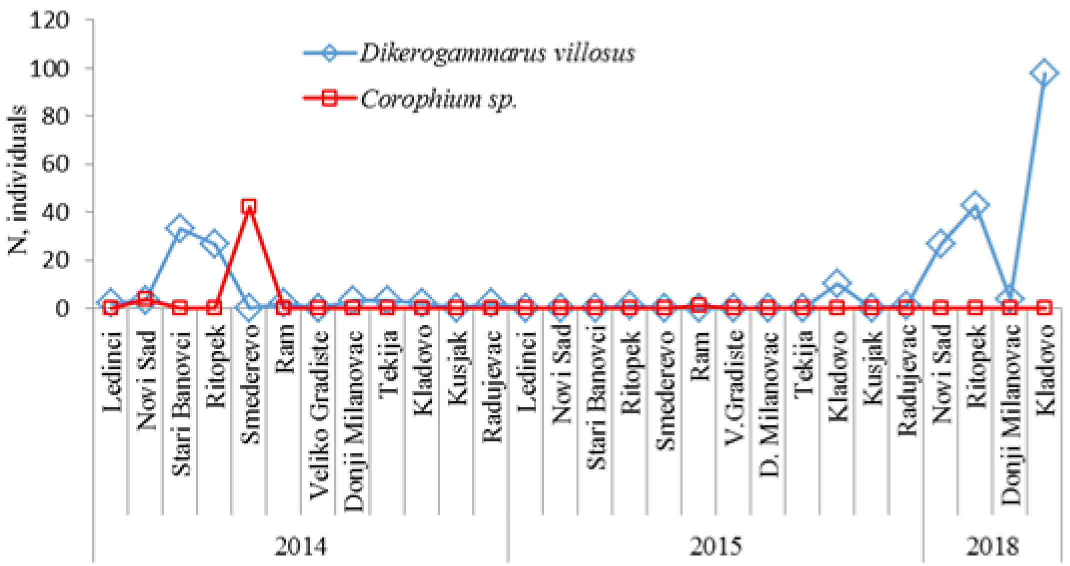

Figure 1 *The densities of Dikerogammarus vilosus and Corophium sp. at different sites of Danube River*

Decrease of *Corophium* sp. densities could be a reason for the increase in the number of species and values of biotic indices. Same situation was observed in Rhine River when *D. villosus* decreased abundance of *Corophium curvispinum*, resulting in a reduction of mud on the stone substrate, leading to an increased diversity of other macroinvertebrates [10].

Only 3 alien species were found at Belorussian river sites: Neman - *Lithoglyphus naticoides* (Pfeiffer, 1828), *Dreissena polymorpha* (Pallas, 1771) and *Orconectes limosus* (Rafinesque, 1817). Different metrics of biocontamination index and densities of *O. limosus* show negative correlation with biotic indices (Table 2). Another analyses showed that biotic indices were positively correlated with the proportions (%) of EPT taxa (Table 3).

Table 2 *Correlation coefficients between biocontamination and biotic indices and abundances of Orconectes limosus at sites of Neman River*

Index	ACI	RCI	SBCI	*O.limosus*
BMWP	-0.064	**-0.605**	-0.415	**-0.562**
IBE AQEM	-0.177	**-0.555**	-0.455	**-0.552**

Table 3 *Correlation coefficients of different groups of macroinvertebrates with biotic indices and the numbers of Orconectes limosus for study sites of Neman River*

taxa	BMWP	ASPT	BBI	IBE Aqem	N *O.limosus*
Gastropoda	-0.147	0.074	-0.315	-0.325	0.405
Oligochaeta	-0.231	-0.021	-0.370	-0.364	0.351
Hirudinea	0.006	-0.403	-0.132	-0.135	0.122
Crustacea	0.023	-0.166	0.046	0.049	-0.022
Ephemeroptera	0.072	-0.128	0.173	0.228	-0.285
Odonata	-0.298	**-0.663**	**-0.504**	-0.341	0.453
Plecoptera	**0.558**	0.446	**0.527**	**0.514**	-0.316
Heteroptera	0.459	0.365	0.459	0.374	-0.342

***Table 3** continued*

Trichoptera	0.337	**0.492**	**0.581**	0.364	**-0.549**
Coleoptera	0.083	-0.355	0.001	0.239	0.172
Diptera	0.174	0.363	0.358	0.266	-0.298
Hydrachnidia	0.466	**0.578**	**0.575**	0.449	-0.389
EPT/OL	-0.334	**-0.504**	-0.270	-0.145	0.266
- EPT (abundances)	**0.549**	**0.782**	**0.813**	**0.572**	**-0.799**

In contrast, biocontamination index positively correlated with biotic indices at sites along Narew and Western Bug basins rivers and Neman tributaries (Table 4). Biotic indices are negatively correlated with proportion of Oligochaeta, but have positive correlation with EPT taxa. Density of *Orconectes limosus* tends negative relation with proportion of Oligochaeta and are positively correlated with Ephemeroptera (Table 5).

***Table 4** Correlation coefficients between biocontamination and biotic indices and abundances of Orconectes limosus at sites of Neman tributaries and Narew and Western Bug basins rivers*

Index	ACI	RCI	SBCI	*O. limosus*
BMWP	0.418	**0.855**	**0.825**	**0.797**
IBE AQEM	0.326	**0.707**	**0.686**	**0.680**

***Table 5** Correlation coefficients the proportions (%) of different groups of macroinvertebrates with biotic indices and the numbers of Orconectes limosus for study sites of different rivers of Narew and Western Bug basins and Neman tributaries*

taxa	BMWP	ASPT	BBI	IBE AQEM	N *O. limosus*
Oligochaeta	-0.594	**-0.729**	**-0.836**	**-0.695**	-0.406
Hirudinea	-0.194	-0.399	-0.518	-0.196	-0.385
Crustacea	0.274	0.452	0.271	0.187	0.546
Araneae	**0.681**	0.584	0.459	0.612	**0.700**
Ephemeroptera	0.520	**0.708**	**0.694**	0.635	**0.695**
Odonata	0.337	0.551	0.407	0.263	0.560
Plecoptera	-0.036	0.231	0.408	0.198	0.060
Heteroptera	0.486	0.535	0.347	0.439	**0.713**
Trichoptera	-0.049	0.132	0.173	0.240	-0.217
Coleoptera	-0.061	0.093	0.419	0.252	-0.164
Diptera	0.170	-0.130	-0.069	0.109	-0.185
Hydrachnidia	0.032	0.061	0.056	0.018	-0.001
EPT/OL	0.528	**0.729**	0.603	0.531	**0.853**
- EPT (abundances)	-0.380	-0.360	-0.327	-0.529	-0.322

CONCLUSION

Differences in the impact of alien species on the assessment of water quality may be related to the intensity of biological pollution and the duration of the invasion. The study sites of Danube River have high level of biocontamination and predation of *D. villosus* on *Corophium* sp. possibly promotes increase of the number of species and values of biotic indices. Data from Belorussian river sites presuppose that *O. limosus* can influence macroinvertebrate communities through decreasing of EPT and Oligochaeta taxa, which has already been proven in our experiments [6]. From one hand, a decrease of Oligochaeta abundance increases values of biotic indices, but decrease of EPT taxes tends to decrease biotic indices and in that way, *O. limosus* can influence an assessment of water quality. *O. limosus* established its populations in Neman River at the end of 1990 and in the other rivers this species appeared later [11]. It is possible, that differences in *O. limosus* impact were due to the differences in the time of invasion and in community composition of macroinvertebrates. Thus, predatory invasive invertebrates, can affect the composition of macroinvertabrate communities and an assessment of water quality by biotic indices.

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GROWTH OF TURKISH HAZEL (*Corylus colurna* L.) IN THE BEECH-TURKISH HAZEL FOREST IN THE AREA OF "DJERDAP" NATIONAL PARK

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Abstract

This paper presents the growth characteristics of Turkish hazel in the area of "Djerdap" National Park in north-eastern Serbia. Dominant trees were felled in a mixed stand of beech and Turkish hazel. The site is classified as Corylo colurnae-Fagetum moesiace. At the age of 90, Turkish hazel trees at the study locality (340 m. a.s.l.) reached the height (H) of 27 m, diameter (DBH) over 42 cm and volume (V) of up to 1.80 m^3*. The defined models of the H, DBH and V growth and increment of dominant trees indicate that Turkish hazel can be classified into the category of fast-growing tree species, with early increment culminations, especially in the current annual height increment (between the age of 9 and 20), and a relatively rapid decline after the peak. The culmination of the current annual volume increment occurred much later (at the age of 76), while the mean annual volume increment did not culminate till the studied age. It can be concluded that Turkish hazel can grow well in relatively poor site conditions, on a very shallow soil on the skeletal limestone* colluvium, *with clear outcrops in some places.*

Keywords: Turkish hazel, tree growth and increment, NP Djerdap, Serbia

INTRODUCTION

Among numerous and diversified natural resources and outstanding beauty of the NP "Djerdap", mixed, even-aged and uneven-aged stands of beech and valuable broadleaves attract special expert and scientific attention [1, 2]. Bearing in mind a number of very positive characteristics of valuable broadleaves (beauty of the wood, early attainment of substantial wood volume and desired dimensions, high revenue obtained from the sale of extremely valuable wood assortments, aesthetic properties), Assmann [3] notes that the "enrichment" of pure beech stands with valuable broadleaves is a very effective and efficient means of increasing their economic value. From the scientific, professional, ecological and historical point of view, the relict impoverished community of beech and Turkish hazel (*Corylo colurnae-Fagetum moesiace B. Jov. 1979)* is one of the particularly interesting communities of beech and valuable broadleaves.

Turkish hazel (*Corylus colurna* L.) is one of the valuable broadleaved species of Southeastern Europe and the Orient distinguished by its highly-prized wood and successful growth on unfavourable terrains, with insufficient soil moisture and air humidity [4]. The results obtained by Mišić [5] show that this species is very tolerant of different substrates: it grows in deep, medium-deep and shallow rocky substrates, on screes, at the bottom of sinkholes, in karst valleys, etc. Stojkovic [6] was one of the first to say that “*Turkish hazel is one of the best forest tree species growing in our region. It has considerable hardness, particularly beautiful colour and it does not require any particular skill to enhance it; it can be easily worked to make it nice and smooth* ...“Pointing to the high quality of its wood, Stojković [6] further states that *“... Turkish hazel can replace the expensive mahogany wood in many ways ...* “ and therefore “... *special attention should be given to the cultivation of Turkish hazel in the areas where it grows* ... “.Similarly, Yaltirik and Efe [7] pointed out that the fine wood of Turkish hazel has a pinkish-brown colour and excellent finishing characteristics [8]. Hazelnut wood is under-utilized, although it has limited use in the manufacture of veneers, furniture, decorative inlays, and novelty items [8].

The growth of Turkish hazel in Serbia has been almost completely unexplored and unfamiliar. Therefore, the aim of this paper was to determine the characteristics of height, diameter and volume growth of Turkish hazel trees in a mixed stand of beech and valuable broadleaved tree species in the relict community of *Corylo colurnae-Fagetum moesiacae* in the NP “Djerdap“ area in north-eastern Serbia.

MATERIALS AND METHODS

The research of the tree growth of Turkish hazel in a mixed stand of beech and valuable broadleaved tree species was carried out in the “Djerdap“ National Park (44°26'36" N, 22°09'45"). Djerdap has a highly modified humid-continental (typical of the greater part of Serbia) and continental climate of the Vlaška and Bulgarian lowlands [5].

The growth of Turkish hazel trees was studied in a mixed stand of beech and valuable broadleaved tree species on the site belonging to the syntaxonomic units of *Corylo colurnae-Fagetum*, sub-associations *aceretosum*. The stand has the average number of trees per ha and the average basal area per ha of 401 and 34.9m^2, respectively, while the average volume per ha amounts to 431 m^3. Visual assessment of the tree size structure and stand physical appearance reveals that the stand structure is closest to even-aged.

Because of the high ecological value of the trees and the fact that they grow in the forest under the highest level of protection, only three dominant trees were used to investigate the diameter (DBH), height (H) and volume (V) growth of Turkish hazel. These trees were felled and cross-sectional discs were taken at every 1.0 m from the base to the top of the stems. To estimate the height from the stem analysis data, using Carmean’s procedure [9] annual rings on each disc were precisely measured and counted.

For the presentation of the age (T) - growth (Y) relationships, the Chapman-Richards function (eq. 1) was applied:

$$Y = a \cdot \left(1 - e^{-b \cdot T}\right)^{c} \quad (1)$$

where: *a*, *b* and *c* are the parameters to be estimated.

The current annual increment (CAI) was obtained as the first derivative of the growth function (eq. 2):

$$CAI = f(T) = F'(T) = \frac{dY}{dT} \tag{2}$$

The mean annual increment (MAI) was obtained using the following formula (eq. 3):

$$\text{MAI} = \frac{Y}{T} = \frac{\int_0^T f(T) \cdot dT}{T} = \frac{F(T)}{T} \tag{3}$$

or as the quotient of the attained tree dimensionY (DBH, H and V) and age (T).

RESULTS

The defined DBH, H and V growth models of dominant Turkish hazel trees for the studied site conditions, as well as the graphic illustrations of the growth (Y), CAI and MAI values are presented in Figure 1.

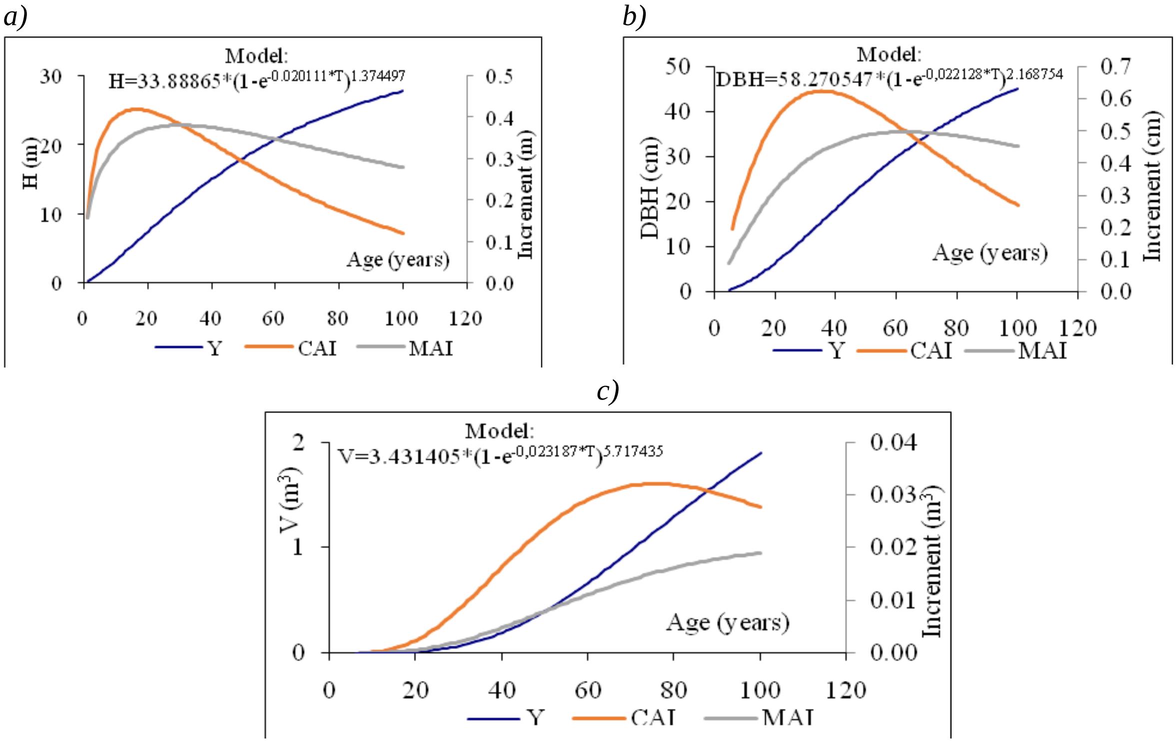

***Figure 1** Height (a), diameter (b) and volume (c) growth and increment curves of dominant Turkish hazel trees*

At the highest common age (90 years), Turkish hazel trees attained a height of 26.5 m, 27.2 m and 26.5 m. According to the defined height growth model, dominant Turkish hazel trees should have the H of 27.8 m at the age of 100 (Figure 1). The CAI_H and MAI_H of the trees culminated at the age of 9-20 and 16-36, respectively. According to the adopted height growth model, the CAI_H and MAI_H culmination was predicted to be at the age of 16 and 30, respectively. The values of CAI_H and MAI_H at the culmination age amounted to 0.42 m and 0.38 m, respectively.

At the age of 90, the DBH amounted to 42.2 cm, 44.5 cm and 40.5 cm. According to the defined diameter growth model, dominant Turkish hazel trees should have a DBH of 42.4 cm at the age of 90, and 45.3 cm at the age of 100 (Figure 1). The CAI_{DBH} and MAI_{DBH} of the trees culminated at the age of 30-38 and 54-70, respectively, with the values of 0.67-0.75 cm and 0.49-0.51 cm, respectively. According to the applied growth model, the CAI_{DBH} and MAI_{DBH} culminated at the age of 32 (0.70.cm) and 63 (0.50 cm), respectively.

The volume growth pattern of the three analyzed trees was quite similar before the age of 60-70, after which the growth differences became increasingly notable. At the age of 90, the stem volume ranged from 1.42 m^3 to 1.80 m^3. CAI_V of the trees culminated at the age of 64-95, and its values ranged from 0.0270 m^3 to 0.0385 m^3, while MAI_V did not culminate before the studied age. According to the defined volume growth model, the CAI_V culmination of 0.0321 m^3 was predicted for the age of 76.

DISCUSSION

Turkish hazel is a widely widespread species in the area of the “Djerdap“ National Park - from the sites near the banks of the Danube River to the highest ridges and peaks, such as Veliki Štrbac and Šomrda, over 800 meters above sea level [10]. In general, it is fairly resistant to windthrows and can grow at sites which are specific in terms of the aspect, inclination, soil, and other ecological factors [2].

As already indicated, the characteristics of the Turkish hazel growth in Serbia have been scarcely explored and studied. There have been only some general information regarding the possibility of Turkish hazel trees to reach a height of up to 23 m [4] or up to 30 m [11], and diameters of over 1 m. Stojanović and Jovanović [12] studied the growth of this species in a mixed stand of beech and fir with valuable tree species in western Serbia. The first extensive study on the characteristics of the Turkish hazel growth was the result of Stajić's research [13].

The NP ‘’Đerdap’’ area is one of the most important sites of Turkish hazel, with a variety of different communities in which this tree species grows. According to Stajic and Vilotic [2], at some sites and parts of sites in the area of the NP ‘‘Đerdap’’, for instance in the areas with plenty of air humidity and sheltered from the effects of extreme climate, such as deep sinkholes and depressions, in the community with beech as the main species, it is distinguished by considerably high values of the basic growth elements (diameter, height, wood volume, crown diameter). It achieves slower growth rates and smaller increment values in oak forest stands with different variants of the southern aspect and in shallow sinkholes, while the slowest growth and increment values are attained in the communities with lilac, with lilac, Montpellier maple and Oriental hornbeam at highly exposed sites with very shallow soil [2].

The obtained results indicate that the lines of the height and diameter growth of Turkish hazel resemble the Latin letter *S*, which in terms of growth means that the growth was first slowed in the earliest youth, then it was intense and again slowed down at the old age. The described characteristics of the growth characteristics are most typical of the so-called classic type of growth [14]. The line of the volume growth has a similar form, but due to the much slower volume growth compared to the height and diameter growth and the fact that the trees have not reached the maturity yet, one part of the right half of the typical S-curve growth is “missing“. However, the typical *S*-form of the height and diameter growth curves is not so pronounced, especially in the early youth, which suggests that the growth of this tree species is more intense than the growth, for example of oak as a typical representative of the S-type growth. Additionally, the results showed that the growth of DBH and V is not as intense as the growth of H, bearing in mind that the Turkish hazel trees grew up in an abandoned forest stand, which was excluded from the regular forest management (protected forest) for several decades. So, the obtained results indicated that the dominant Turkish hazel trees had a diameter of ≈45 cm and a volume smaller than 2 m^3 at the age of 100.

The fact that Turkish hazel can be categorized as a fast-growing tree species was shown by the early culmination of the CAI_{DBH} and, especially the CAI_H (between the age of 9 and 20), and their relatively rapid decline after the peak. Similar results were obtained by Stojanović and Jovanović [12], who noted that the CAI_H and CAI_{DBH} of Turkish hazel trees culminated at the age of 9-20 and 20, respectively. As expected, the culmination of the CAI_V occurred much later, while the MAI_V did not culminate till the studied age of 90.

In order to get a deeper insight into the pattern of the Turkish hazel growth, the obtained results were compared with the growth dynamics of beech, lime and Norway maple from the same stand, as presented by Stajić [13]. In the same conditions, the most intense height growth at youth was attained by lime, followed by Norway maple and Turkish hazel, and finally, by beech trees. This period in the life of trees when the height growth of beech trees lagged significantly behind Turkish hazel and other valuable broadleaved tree species lasted for about 50-55 years, after which the beech height dominance started. For that reason, at the age of 90 and 100, beech attained a greater height compared to the analysed valuable broadleaved tree species.

Regarding the increment values and dynamics, the empirical (9-20 years) and the estimated (16 years) age of the CAI_H culmination is very similar to the age of the CAI_H culmination of the valuable broadleaved tree species. Namely, according to the research by Stajić [13], if site conditions are the or same similar, Norway maple attains the CAI_H culmination at the age of 9, lime at the age ranging from 8 to 13, sycamore from 7 to 10, and in the case of European ash between age of 11 and 12. The same conclusions can be derived if the CAI_{DBH} data of Turkish hazel are compared to the CAI_{DBH} data of these valuable broadleaved tree species. The estimated age (32 years) of the CAI_{DBH} culmination is close to the estimated age when the CAI_{DBH} culmination of the above-mentioned trees occurred [13]: Norway maple – 34 years, lime – 27 years, sycamore – 25 years and European ash – 29 years. The same relationships between Turkish hazel and other valuable broadleaved tree species mentioned here could be also applied to the dynamics of the MAI_H and MAI_{DBH} as well as the CAI_V. Namely, the obtained results showed that the predicted age of the CAI_V culmination for Turkish hazel was 76, which is a quite similar to the estimated age when the CAI_V culminated for the mentioned tree species from Stajić’s [13] study. This author found that Norway maple attained the culmination of the CAI_V at the age of 74, lime at the age of 58, sycamore maple at the age of 82 and European ash at 91.

CONCLUSION

In order to preserve and protect certain tree species, some useful and necessary measures of forest management (tending, protection cuttings, restoration, etc.) have been limited or completely forbidden in some stands, parts and even entire forests and these forests are left to spontaneous growth and development. This ‘‘no management concept’’ and their further spontaneous development is explained by the need for the ‘‘natural development” of forests, but it is often overlooked that this kind of protection also includes forests, stands and certain tree species which are already in a state of “disturbed balance”, with a reduced vitality and a regressive trend of their development [15]. In some forests, these protection measures have ‘‘unintentionally’’ catalyzed the negative processes, enabled the further progression of the degradation processes of these forests so that they are increasingly distant from the appropriate natural site structure and composition. In this way, the “natural spontaneous development” that has arisen as a result of declaring different types of protected forests and the prohibition of cutting as a measure of their tending and regeneration, can lead to a significant disturbance of their stability and vitality and disappearance of some competitively weaker tree species.

One of the less competitive tree species in the mixed forests of beech and oak is Turkish hazel, which has been widespread in Serbia since ancient times. Today, Turkish hazel belongs to the category of rare and endangered tree species, to whom it is necessary to pay special attention in the planning of management objectives in the forests in which they are represented. It is precisely the study of the growth of these species and the effects of numerous impact factors on these growth processes in mixed stands that can greatly contribute to the acquisition of important scientific and practical knowledge on the conflicts in growth, competitiveness and vitality of the mixed tree species which enable these tree species (Turkish hazel too) and their stands to be better protected and improved. In that sense, the here obtained results point to the conclusion that Turkish hazel can be categorized as a fast-growing tree species, with the early culmination of increments and their relatively rapid decline after the peak.

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ECONOMIC AND ENVIRONMENTAL EFFECTS OF COMPOSTING TECHNOLOGY WITHIN THE REGIONAL WASTE MANAGEMENT CENTRE IN LESKOVAC, SERBIA

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Abstract

PWW Company, the operator of the regional waste management centre in Leskovac, was founded on August 24, 2005. The main activity of the company is waste management. In the Republic of Serbia, PWW is a strategic partner of cities and municipalities in the field of complete waste management, based on a partnership model between the public and the private sector including the following services: collection, transport, treatment and disposal of waste. This partnership model includes: the city of Leskovac and the municipalities of Lebane, Medveđa, Bojnik, Vlasotince, Crna Trava, Vladicin Han, Žitorađa and Prokuplje. Environmentally aware parts of the world have established an organized approach and have achieved significant results in waste recycling such as the application of composting as biodegradable waste recycling technology. In this paper, description of the composting plant location, planning, selected composting technology description, cost structure and the conclusion that includes: economic and ecological effects of the selected composting technology, are presented.

Keywords: waste management, recycling, composting technology

INTRODUCTION

Composting technology is one of the most acceptable ways to handle biodegradable waste and can be placed in many environments from a simple open pit to compost in sophisticated closed vessels with temperature, pH value, flow and humidity control. Reliable financial planning is an important step in the successful development of the composting program.

Capital and operating costs are particularly important as well as market value and quality of materials received, guaranteed generated quantities of biodegradable waste and the need for disposal, must be considered. Due to the complexity of the integrated waste management system, not all concepts are sustainable, so, in order to achieve a long-term optimal and sustainable solution, adequate and timely initiatives and financial support are needed [1].

MATERIALS AND METHODS (TECHNOLOGIES)

Materials suitable for composting include various compostable materials of bio-organic origin [1] that are classified into "green" and "brown". Green materials are rich in nitrogen and proteins, and help faster growth of useful microorganisms. Green compostable materials [1] among others are: mowed grass, coffee plant, tea bag filter, fruit and vegetable residues, all kinds of animal manure, etc. Brown materials are rich in carbon and carbohydrates and serve as a source of food for microorganisms. Brown compostable materials [1] among others are: opalescent leaves, pine needles, bark trees, granaries, straw, hay, sawdust, chips, paper, fabrics made of natural materials (cotton, hemp), etc.

The way of oxygen delivery to microorganisms classifies methods or technologies of composting. In Figure 1, classification of composting methods, is given [1].

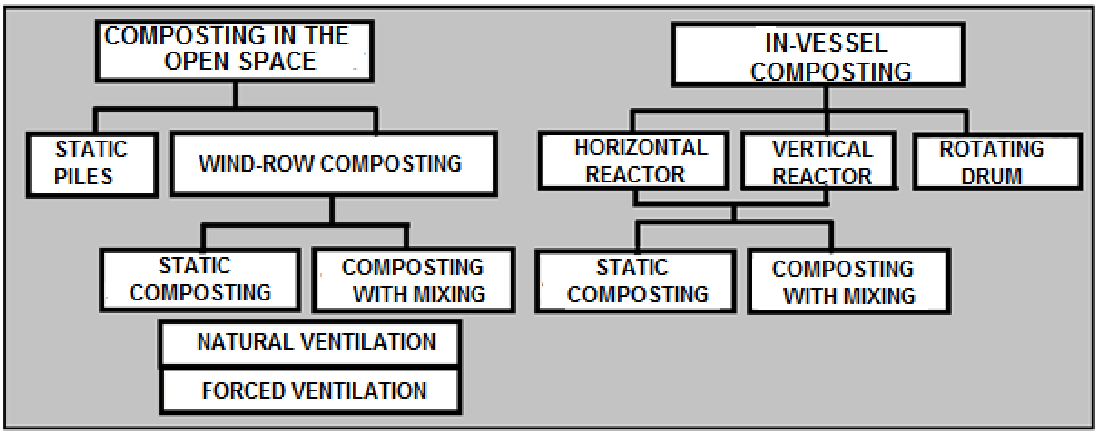

Figure 1 *Classification of composting methods*

Table 1 shows an overview of existing composting systems that are grouped according to the degree of technology to: systems with low, medium and high composting technology [1].

Table 1 *An overview of existing composting systems that are grouped according to the degree of technology*

LOW DEGREE OF COMPOSTING TECHNOLOGY	MEDIUM DEGREE OF COMPOSTING TECHNOLOGY	HIGH DEGREE OF COMPOSTING TECHNOLOGY
Static piles	• Forced aerated wind-rows • Forced aerated bunkers	• Rotating drums • Closed tunnels • Silos

Table 2 shows the mass capacities of some existing composting technology systems [2].

***Table 2** Capacities of some existing composting technology systems*

COMPOSTING TECHNOLOGIES	WASTE MASS SUITABLE FOR COMPOSTING
Static pile	50÷1.500 t/a
Aerated wind-row	Up to 25,000 t/a
Simpler channels and tunnel systems	Up to 100,000 t/a
Complex channels and in-vessels	Up to 250,000 t/a

Table 3 shows the amounts of capital investments in individual composting systems [2].

***Table 3** Capital investments in individual composting systems calculated for at least of 50,000 t/a of raw material*

COMPOSTING TECHNOLOGIES	CAPITAL INVESTMENTS (€/ton)
Static piles	30÷40
In-vessel wind-rows	70÷100
In-vessel	200÷350

The lower the technology, the smaller the capital investment, the higher operating costs and vice versa for higher technology are higher capital investments and lower operating costs.

RESULTS AND DISCUSSION

The initial projected capacity of the composting plant is around 11,520 tons per year. After market research in respect of the amount of waste considered, the existing composting technology, the existing solutions of the composting plant in the world as well as their characteristics, composting plant with static piles technology with natural material ventilation and continuous process monitoring, was chosen. The composting process would last about 45 days [3]. In Figure 2, selected composting plant using static piles technology is shown [4].

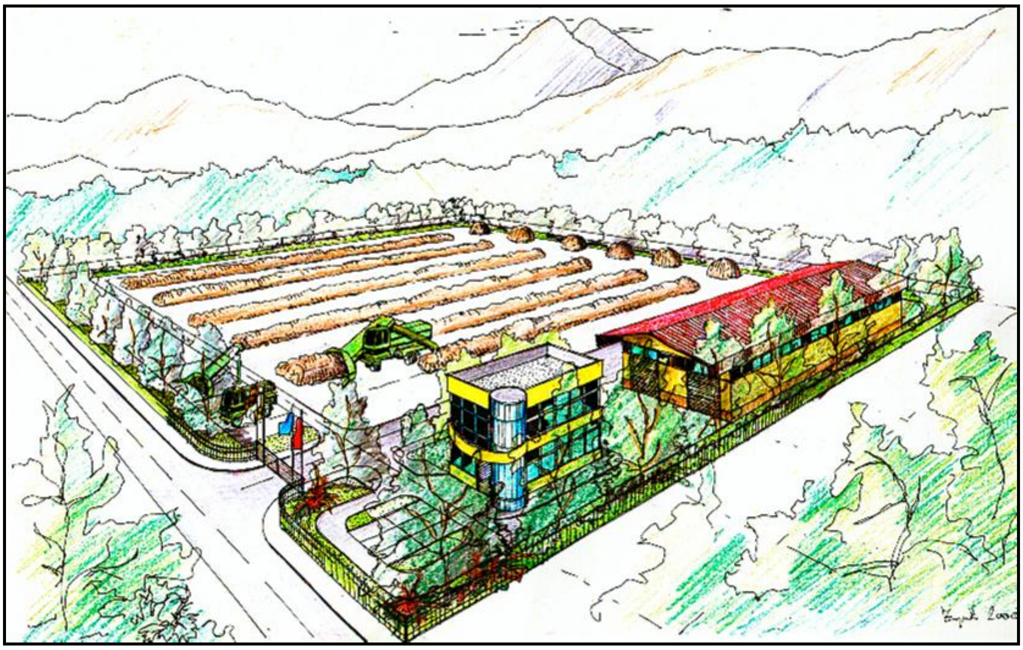

***Figure 2** Composting plant using static piles technology*

The total costs of the plant, which normally include investment and operating costs, amount to around € 1,000,000 and the construction and commissioning period is around 3 months. Investment costs are around € 800,000 and operating costs for a period of one year amount to about € 200,000. The planned annual production capacity of the plant would be about 5,760 tons of compost [3]. Table 4, summarizes the total cost of the composting plant, which includes investment and operating costs.

Table 4 *Summarized total cost of the composting plant [3]*

INVESTMENT COSTS	**€**
Mechanical equipment and parts	36,500
Mobile equipment	328,000
Electrical equipment and works	118,000
Construction facilities and works	311,850
TOTAL	**794,350**
OPERATIONAL COSTS	**€/god**
Electricity	415
Fuel	13,474
Maintenance	17,472
Labor, general, administrative and other costs	61,432
Financing costs	106,354
TOTAL	**199,147**

In Table 5, the operational costs of the composting plant with regard to the input material to be composted and to the output material which represents the final product of the composting technology.

Table 5 *Operational costs of the composting plant with regard to the input material and output material [3]*

OPERATIONAL COSTS OF THE COMPOSTING PLANT WITH REGARD TO THE MATERIAL TYPE	**€/t**
Input material (various compostable materials of the bio-organic origin)	34.57
Output material (compost produced as the final product)	17.29

Figure 3 shows the final product of the composting technology - compost ready to use.

Figure 3 *The final product of the composting technology - compost ready to use [4]*

Economic effects

The contribution of composting technology on the economic plan is multiple because it includes not only the profit from the sale of compost, but also the economic effects of savings achieved by composting technology [3].

In the concrete case, the annual projected amount of biodegradable waste for composting amounts to 11,520 tons, and the quantity of compost produced is 5,760 tons. Selling this amount of ready-made compost at a price of € 110 per ton [5] generates a gross profit of € 633,600. After deducting the annual operating costs per ton of output material or compost in the amount of 17.29 €/t, the net amount of the profit is € 534,010. Based on this, it can be concluded that the time period of the return on investment costs (€ 794,350) is about a year and a half or about 18 months, making the selected technology extremely profitable [3].

Also, by composting the projected amount of biodegradable waste of 11,520 tons or 23,040 cubic meters, valuable landfill space is saved. Costs for 1 square meter of landfill space are about 60 €, so, it is possible to save about € 1,382,400 (23,040 X 60) [3]. Additionally, it also saves the costs of landfilling, which amount to 22.11 € per ton of waste. In this case, taking into account the total amount of biodegradable waste for composting expressed in tons, the savings amount to € 254,707 (11,520 X 22.11) [6]. This means that the total economic effect is the sum of all the above mentioned effects, ie in total it amounts to € 2,171,117 (534,010 + 1,382,400 + 254,707) [3].

Environmental effects

The environmental effects or effects of composting technology in the environmental protection plan are multiple [4]:

- Prevention of negative effects of biodegradable waste on landfills to ecosystem, because in the uncontrolled decomposition of organic matter, very strong toxins are formed - dioxins, either as a product of the work of anaerobic microorganisms or by chemical means, and their environmental and health influence is very negative. In the uncontrolled decomposition of organic matter, substances in liquid and gaseous aggregate state are formed. One part of the gaseous substances goes into the atmosphere and creates compounds that, later in the form of precipitation, cause negative consequences on the surface of the earth (smog, acid rains, etc.). The second part of the gaseous substances that are lighter than air goes to the ionosphere where they directly affect the reduction of the ozone layer. A part of the liquid substances sinks through the soil and walls and contaminates the groundwater that feeds the watercourses used as the cleanest water and drinking water. The second part is directly washed through atmospheric precipitation in the watercourses.

- Composting creates materials needed for plant and soil ecosystems, carbon dioxide, water, plant growth stimulators, minerals, stable organic matters, in such a state that plants can use them as well as compost that increases the number of microorganisms living in symbiosis with plants.
- The use of compost eliminates the need for artificial fertilizers, which is a direct contribution to the production of health-safe food, because compost is the famous organic substrate for its production;
- By using compost, healthy plants are produced and there is no need to be treated with various chemicals because they return natural immunity, increase fertility, produce more oxygen, producing conditions for a healthier life of people and other living beings on the planet;
- Reducing the amount of organic waste, creating new knowledge about compost and transferring this knowledge as well as reducing their impact on the ecosystem;
- Compost is an excellent soil conditioner because it improves the structure, nutritional value, looseness, and moisture retention capacity. It reduces: the pH value of the soil, erosion, use of artificial fertilizers and pesticides as it dampens certain plant diseases and parasites.

CONCLUSION

Taking into account the current situation and practice in waste management at the national level, it can be concluded that compost technology is a project with the greatest potential, especially because this technology is foreseen in all three sectors of the clean development mechanism: waste management, agriculture and forestry.

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MATERIAL FLOW MANAGEMENT: ZERO EMISSION CONCEPT AND CIRCULAR ECONOMY

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Abstract

Material flow management is a method of efficiently managing materials in the field of sustainable development. Zero Emission concept represents a responsible approach to the environmental, social and economic orientation, and it contributes to creation of efficient technology, new production process, conservation and recycling of natural resources (waste into energy). Circular Economy provides a new vision on the treatment of resources, energy and, primarily, presents a new way of value creation, keeping the benefit in products as long as possible, simultaneously eliminating the waste.

Keywords: material flow management, Zero Emission, Circular Economy, environment

INTRODUCTION

The reduction of energy consumption, environmental protection and waste management become imperative to every technological process. Thus, it is important to create eco- friendly materials using new methods of combining raw materials and waste products, or by the evolution of traditional technologies, in order to meet different range of environmental performances and appearance requirements [1,2]. The Zero Emission (ZE) presents the ideal of zero environmental stress by suppressing resource consumption and minimizing waste. Using ZE concept, it is possible to predict the circulation of material flows and reduce the emission of material and energy to a minimum, ideally zero [3]. Based on the concern for economic growth and environmental protection in the developing countries, different emission reduction methods were developed in the 1980s such as "source reduction", "waste reduction", "low-waste" technology, "design-for-environment", "industrial ecology" and "eco-efficiency" [4,5]. There are no significant differences among mentioned approaches. Waste reduction approach emphasizes energy conservation and materials utilization efficiency. The aim of clean technology is to ensure needed technical changes that reduce emissions at the source; design-for-environment underlines the possibility of reducing wastes by making products more recyclable or remanufacturable [6]. The idea of industrial ecology is based on the potential for recycling waste from one industry as feedstocks to another, as biological organisms recycle nutrients [7]. The Circular Economy, as the logical descendant of the linear economy that dominated since the start of the industrial revolution, is an economy that enables producers to show the value and quality of the performance of their products to the customer [8]. Transition to a more circular economy requires changes throughout value chains, from product design to new business and market models, from new ways of turning waste into a resource to new modes of consumer behavior which implies full systemic change, and innovation not only in technologies, but also in organization, society, finance methods and policies. Products are designed for performance and re-use of all materials in different phases [7].

THE ZERO EMISSION OR ZERO DISCHARGE CONCEPT

Three most important concepts used for resolving the problem of waste control and reduction are: “End-of-Pipe” pollution control technologies, Cleaner Production concept and finally, taking a historical perspective, the Zero Emission ZE concept, which represents the next phase in the evolution in the control and reduction of emissions from industrial pollution sources [9]. It offers the optimum solution to waste control of air, water and land, removing pollutants from the waste streams and converting them into products or feeds for other process. Such model, expressed regarding no solid waste, no wastewater, no gases to contribute greenhouse gasses, no energy losses, predicts the circulation of material flows and consequently reduces the emission of material and energy to a minimum, ideally to zero. Achieving the ZE goals is not always possible according to physical laws of nature [10,11]. For instance, chemical reactions do not reach exactly 100% yield and waste heat emissions are inevitable. In this case, ZE concept does not assert that all emissions of a set of industrial processes can reach zero. ZE offers the best available industrial process and technologies that threat waste streams and is using advanced tools and techniques, such as life cycle assessment, for analyzing and designing environmentally friendly material and less energy consuming process (Figure 1). Applying ZE concept, the amount of generated waste is minimal, due to all used flows, enabling also the economic benefits. Zero emission economy is defined as an economy, in which all materials must be recovered, repaired, reused, remanufactured or, recycled [3].

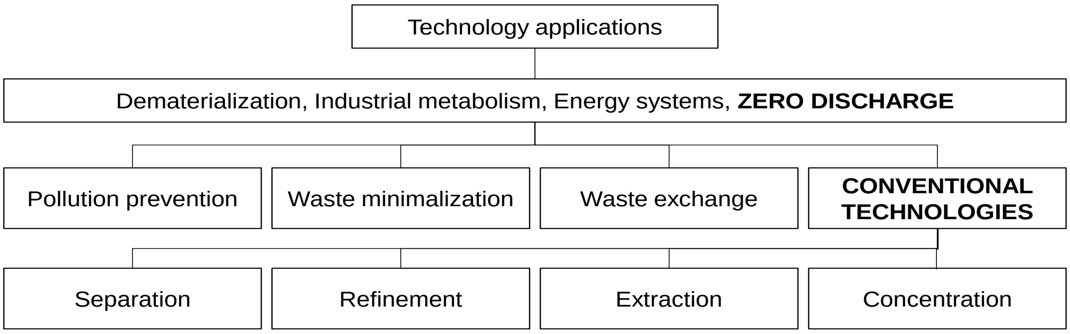

Figure 1 *The classification of Zero Emission techniques and methodologies*

Long term sustainability is dependent on massive reductions in the generation of waste and pollution which implies a comparable reduction in the extraction and processing of virgin raw materials, and a gradual closure of the materials cycle. It is crucial to insure technical opportunities for increasing the lifetime value provided by durable goods, through redesign for increased operating efficiency and recovery of asset value by reuse, reconditioning and remanufacturing [4]. From an engineering point of view, there are three important types of technologies to reduce waste and emissions: energy and materials conservation; product life extension: re-use, repair, renovation, re-manufacturing, recycling; waste mining: conversion of waste into useful products [12]. Rational energy use and recovery of waste heat from the process are the two most important ways of reducing costs. Utilization of waste streams and renewable sources, based on the comprehensive nature of zero emissions systems (Figure 2), are two main tools to reduce costs and to preserve the environment and reduce waste. The first step towards reducing the energy consumption is detailed analysis for which is necessary to have a good knowledge about material and energy balances based on all inputs and outputs (Figure 3).

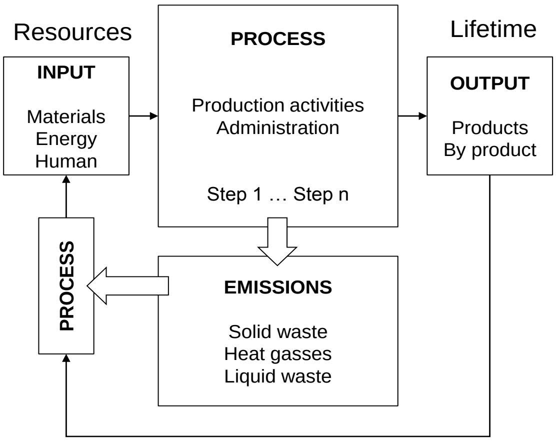

Figure 2 *Flow chart considered for zero emissions systems approach*

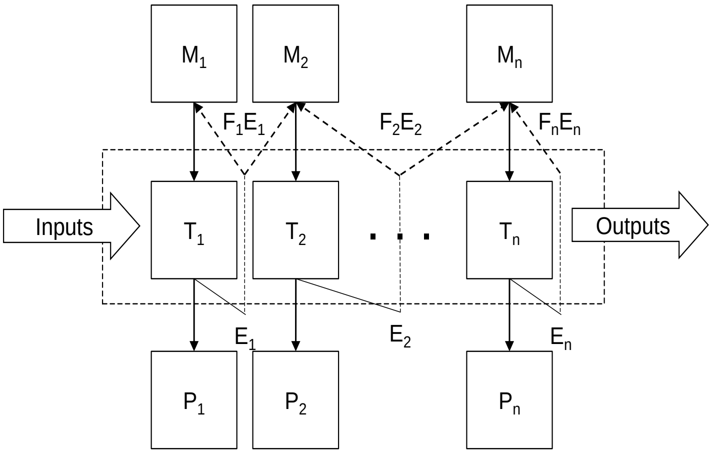

Figure 3 *The scheme of material flows in process approaching zero emissions*

Basic principles and benefits of circular economy

The Circular Economy is an industrial system that is restorative or regenerative by intention and design, and it is focused at the combination of products and services incorporated into a business model that facilitates a company's ambition to gain value. Substantial principles of the Circular Economy [13] are:

- Design of waste.
- Understand that everything within the economy has value.
- Design with disassembly and reuse in mind, with minimal changes required to reuse components of a product.
- Differentiate between consumable and durable components. Biological materials go back into nature; durable, or technological, materials stay in use for as long as possible.
- Find ways to reuse materials across the value chain (for instance, Nike uses recycled plastic bottles in its polyester products).
- Eliminate toxic chemicals, making it easier to reuse components without risk of contamination.
- Fuel the system with renewable energy.
- Build resilience through diversity.
- Adjust process to reflect the truce cost of the effort required to produce a product
- Think in systems, taking into account how one action will impact the whole.

There are four principles that define the circular economy: minimization of the use of inputs and eliminate waste and pollution, maximization of the value created at each stage, management of flows of bio-based resources from and back into the biosphere, and recover and retain flows of non-renewable resources in closed loops [14]. Three major trends support the shift towards adopting a circular economy: a new era of resource scarcity, a new age of innovation as seen by clean technology advancements in the energy sector, and the emergence of a bio-age to replace the oil age [15].

Designing and innovations for a circular economy

Circular economy approaches 'design out' waste and typically involves innovation throughout the value chain, rather than relying solely on solutions at the end of life of a product [16]. An important starting-point is the design of production processes, products and services. Products can be redesigned to be used longer, repaired, upgraded, remanufactured or eventually recycled, instead of being thrown away. Production processes can be based more on the reusability of products and raw materials, and the restorative capacity of natural resources, while innovative business models can create a new relationship between companies and consumers [17]. The conceptual diagram is given in Figure 4. illustrates in a simplified way the main phases of a circular economy model, with each of them presenting opportunities in terms of reducing costs and dependence on natural resources, boosting growth and jobs, as well as limiting waste and harmful emissions to the environment.

The phases are interlinked, as materials can be used in a cascading way, for instance; industry exchanges by-products, products are refurbished or remanufactured or consumers choose product-service systems. The aim is to minimize the resources escaping from the circle so that the system functions in an optimal way.

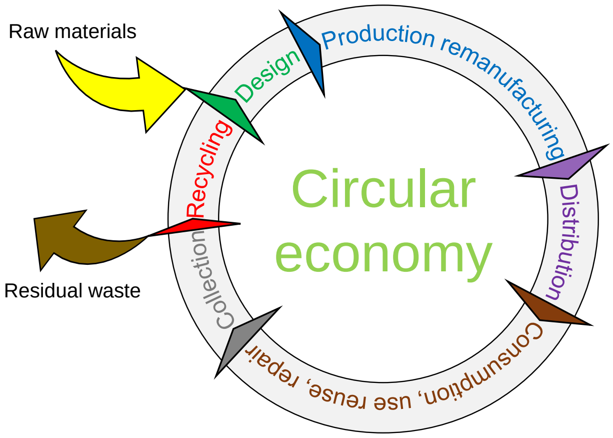

Figure 4 *Circular Economy Conceptual Diagram*

CONCLUSION

In order to be able to precisely and completely define industrial material flow, it is necessary to understand and analyze the technological process in detail. It is clear that this topic is very wide, and it requires engineering knowledge about the main principles of technological processes and equipment, regarding environmental protection and sustainability. Applying zero emissions concept, the amount of generated waste is minimal, due to all used flows, enabling also the economic benefits. The industry with zero emission, industrial clusters, eco-industrial parks and circular economy is given as the practical impacts/benefits of the concept which is the key to sustainable development and industrial ecology. In this way, waste is used as process input materials in a closed-loop of the production and emissions are considered as an unexpected part of production processes. Circular Economy, as trending topic in business and policies, promotes a systemic change that creates continuing loops of materials: resources are only temporary part of a product and at the end of life or end of use (of the product), the resources will be available again for re-use in new products. Using this approach, the materials can be safely returned into the biosphere, thus generating more raw materials for the future.

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SYNTHESIS OF BIOBASED THERMOPLASTIC POLYURETHANE ELASTOMERS

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Abstract

The fabrication of polymeric materials from renewable resources is very important task for scientists, because it is known that the fossil sources will be depleted in the future. Due to this fact, the interest for the novel polyurethanes based on renewable resources increases. This work analyzed the properties of thermoplastic polyurethane elastomeric (TPU) materials obtained from the renewable resources. Superhydrophobic polyurethane elastomers were prepared by a two stage process in solution (first the synthesis of the pre-polymer and second synthesis of the polyurethanes). Two types of isocyanates (isophorone diisocyanate and hexamethylene diisocyanate), polyols (polydimethylsiloxane and polycaprolactone) and chain extender isosorbide were used as reactive component. FTIR spectroscopy was used to determined molecular structures of obtained TPU. It was assessed that used silicone polyol has a significant influence on the thermal properties of prepared PU elastomeric materials, due to significant interphase separation of formed soft and hard segments of polyurethane materials.

Keywords: biobased polymers, biopolyurethanes, polydimethylsiloxane, polycaprolactone

INTRODUCTION

The use of the monomers from renewable resources can help in solving the environmental and economical issues related to large amount fossil fuels consumption [1]. Therefore, the main efforts in the science and industry are aimed to develop novel polymer from renewable raw materials. Therefore, it is necessary to design new materials with the properties that will be able to compare to the traditional plastics. Currently, most polyols for the polyurethane industry are derived from petrochemical resources, but due to the reduction of these resources, the materials with a biological base gain importance with the rise of the oil crisis and the danger of global warming [2]. Polyurethanes are polymeric materials that differ from other polymers, among other things, because there is no urethane monomer. The first polyurethane was synthesized by Otto Bayer in 1937, in the reaction of 1,6-diisocyanatohexane and 1,4-

butanediol. The physical and chemical properties of polyurethane depend primarily on their structure. Due to the fact that their properties can be changed in a wide range by selecting the appropriate raw material, catalyst and auxiliary compounds PU founds the broad range of applications. Depending on the type of components and their functionality, thermoplastic and thermosetting polyurethanes can be obtained in polymerisation reaction. Polyurethanes with permanent crosslinking are characterized by higher thermal stability, but also with weaker mechanical properties compared to linear polyurethanes. Crosslinking is also possible to establish by physical interaction (temporary crosslinking) between polymer chains in the liner polyurethane. Linear thermoplastic polyurethane elastomers are typically block copolymers of different structures, consisting of an elastomeric, soft phase as a matrix and a discontinuous solid phase. They can appear as diblock and triblock copolymers, linear and star structures. The result is their specific microphase structure, consist rigid hard segments and flexible chains of soft segments. Polyurethanes have good elasticity with relatively high mechanical strength and are resistant to abrasion and also have controlled hardness. Thermoplastic elastomers are multiphase polymeric materials, with unique combinations of mechanical and process properties. They are obtained without vulcanization but retain flexibility and elasticity. They are specific in that they behave as elastomers at room temperature, while they behave as thermoplast at elevated temperatures. Due to the above mentioned characteristics thermoplastic elastomers have great advantages over traditional thermosetting elastomers. Termoplastic polyurethane elastomers (TPU) can be synthesized in reaction between aromatic or aliphatic diisocyanate, polymer diol (so-called macrodiol) and a low molecular weight chain extender, which is by chemical composition diol or diamine. Their basic structure consists of randomly produced soft and hard segments, which are very different in terms of chemical and physical properties. The hard segment consists of diisocyanate and chain extender, while the soft segment consists of long chain polyols. The thermodynamic incompatibility, or the non mixability of the two types of segments, leads to phase segregation in the material and the creation of a two-phase microstructure, where the soft segments form an amorphous matrix, while the hard segments are interconnected by secondary bonds and represent a physical networking. The structure and mass fraction, molar mass, as well as the conditions of synthesis, affect morphological factors such as the degree of macro- and microphase separation of soft and hard segments, crystallinity and domain size. Many polyols are used in the TPU formulation such as polyester, polyethers, polysiloxanes, i.e. compounds with –OH groups. Poly(dimethylsiloxane)s (PDMSs) were used as soft segments in TPUs because of their extraordinary properties (such as good flexibility, high thermal oxidative stability, biocompatibility, low surface tension) [3]. Unlike organic polymers, whose structure consists of carbon atom chains, the main raw material from which siloxane is made is silicon (Si), which is the second most common element in the Earth's crust, after oxygen. The siloxane chain consists of alternately connected atoms of silicon and oxygen, for which various organic side groups are attached, such as methyl (CH_3-), phenyl (C_6H_5-), vinyl (CH_2CH-), ethyl (C_2H_5-), propyl (C_3H_7-), which also affects the properties of silicone itself. Their higher thermal and chemical stability differ silicones from other materials. Due to their outstanding properties they have wide application. One of the most famous silicone oils is polydimethylsiloxane, (Figure 1), which is used as an elastomer, as a component of the shampoo, of silicone emulsions, and is also used as a lubricant. The basic PDMS chain consists of [$(CH_3)_2SiO)_n$] units.

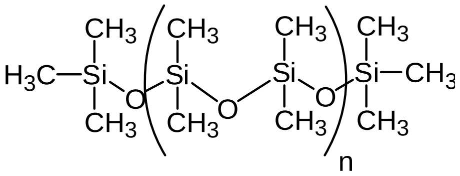

Figure 1 *The structure of linear polydimethylsiloxane (PDMS)*

Due to their extraordinary properties many polyurethanes were used as medical devices, but some researcher groups recognized the possible advantages of combining silicone and polyurethane in a single biomaterial [4,5]. In these new copolymers, the silicone may be present along the polymer backbone and/or in the form of covalently bonded end groups or grafts [6]. With very low silicone content in such hybrid polyurethanes it is relatively easy to obtain silicone-like surface properties due to exceptional silicon surface activity [7]. Due to the exceptional properties of polyurethane as well as the possibilities of improving the properties of PU (biocompatibility and elasticity) the aim of this work is the development of biodegradable polyurethanes based on silicone polyols. Also, the petrochemical chain extender is replaced by a biodegradable isosorbide, so that a part of the hard segment is made of renewable resources.

MATERIALS AND METHODS

Materials

For the synthesis of superhydrophobic polyurethane elastomeric materials, two types of diisocyanates were used: hexamethylenediisocyanate (HDI) and isopropoxydiisocyanate (IPDI). As a polyols, silicone polyol polydimethylsiloxane molecular weight of 2000 g/mol (bishidroksi αω HS, 2311 Evonic) and polycaprolactone, molecular weight of 1000 g/mol, manufactured by Sigma Aldrich, were used. As chain extender, isosorbide (1,4:3,6-Dianhydro D-glucitol, Sigma Aldrich) was used. The solvent was tetrahydrofuran (THF) having a density of 0.899 g cm^{-3} at 25°C. As the catalyst, dibutyl-tile-dilaurate (DBTDL) was used.

Sample preparation

Polyurethane elastomers were synthesized by a two-step process in solution. Synthesis was performed in vials in which a certain amount of diisocyanate and polyol components was measured depending on the desired portion of the soft segment, then 10 ml of solvent THF and 0.5% by weight of the DBTDL catalyst were added, Table 1. NCO/OH ratio was 1:1.15. The synthesis of prepolymer was performed for 1 h at 50°C with continuous stirring on the magnetic stirrer, and then chain extender was added and the temperature was increased to 120°C for about 30 min, in order to complete the polymerisation. After that the samples were poured into the petri dish to obtain films after evaporation of the solvents in a vacuum oven at 50°C.

Table 1 *Composition of thermoplastic polyurethane samples with different content of soft segments (SSC)*

Sample	IPDI, g	HDI, g	PDMS, g	Polycaprolactone, g	Isosorbide, g	SSC, %
SI-HDI-30 %	-	0.494	0.145	0.145	0.294	30
SI-HDI-50 %	-	0.288	0.194	0.194	0.160	50
SI-HDI-70 %	-	0.172	0.227	0.227	0.055	70
SI-IPDI-30 %	0.557	-	0.156	0.156	0.205	30
SI-IPDI-50 %	0.329	-	0.200	0.200	0.131	50
SI-IPDI-70 %	0.170	-	0.235	0.235	0.065	70

Sample characterisation

FTIR spectra were recorded in the wavelength range from 400 to 4000 cm^{-1}. The DSC method was used to assess the thermal properties of synthesized PU samples based on two types of isocyanates and with different content ration of soft segments. Measurement was carried out in the temperature range from -20 to 180°C, at a heating rate of 10°C min^{-1}, and in a cooling regime from 180 to -90°C at a heating rate of 10°C min^{-1}.

RESULTS AND DISCUSSION

The results of the FTIR analysis are one of the best indicators of whether synthesis of polyurethane with a different proportion of soft and hard segments based on bio polyols occurs. Figure 2 shows absorption peaks at wavelengths of about 3300 cm^{-1} that are characteristic of the presence of the N-H group from urethane bond, which confirms that the all components have been fully reacted and that samples have been successfully synthesized. In addition, the absorption peak at 1700 cm^{-1} identifies the presence of the C=O group as well as the -NH group at 1500 cm^{-1} from the synthesized PU, while the silicone groups are identified by peaks at 1250-1300 ($Si-CH_3$) and 1000-1100 cm^{-1} (Si-OR), the peaks are more pronounced with an increase in the proportion of the silicon from the soft segment.

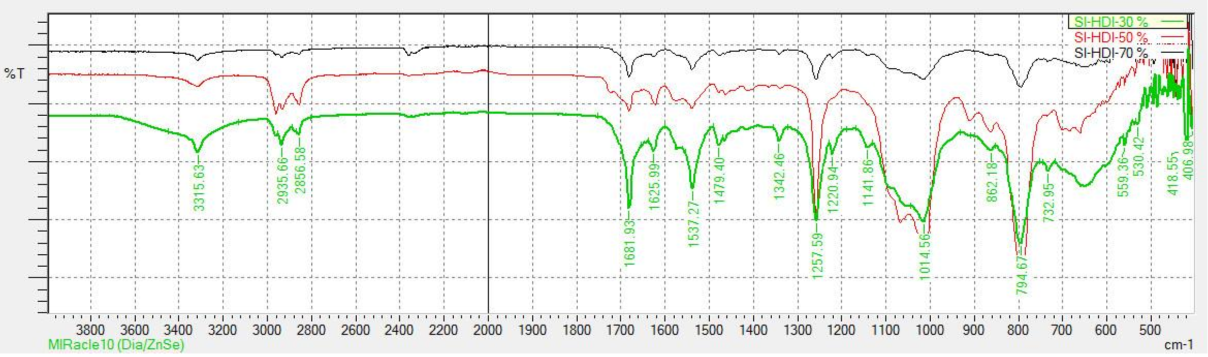

Figure 2 *FTIR spectra of PU samples based on HDI containing 30%, 50% and 70% of soft segments*

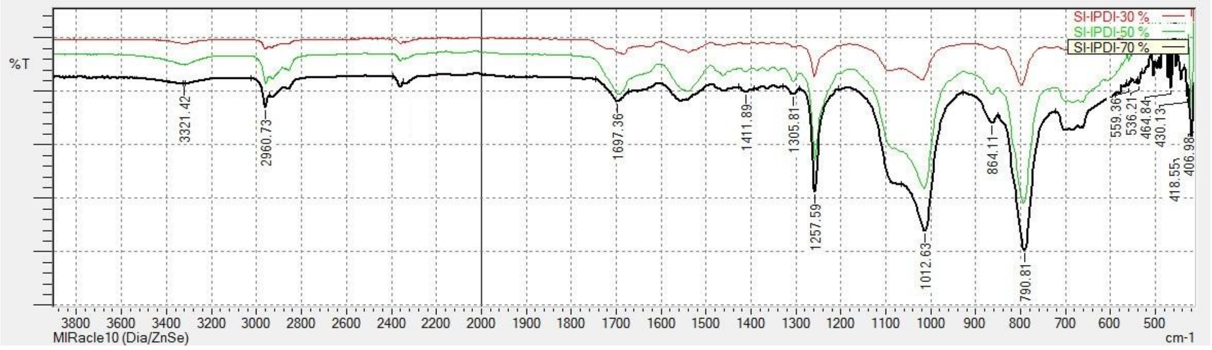

***Figure 3** FTIR spectra of PU samples based on IPDI containing 30%, 50% and 70% of soft segments*

In Figure 3, the FTIR spectra obtained by analyzing the IPDI-based PU sample containing 30%, 50% and 70% soft segments, are observed peaks as in previous HDI-based samples, thereby concluding that the components are in this case were completely reacted and that the synthesis of samples based on IPDI as an isocyanate component was done. The absence of a band of about 2200 cm^{-1}, derived from free NCO groups, confirms the quantitative reaction of isocyanate groups, that is, the choice of the ratio of the reaction components is optimal.

Based on the results shown in Table 2 it is noted that increasing the ratio of soft segments leads to an increased Tg value, which is the aim of this paper, which confirms that the desired microphase separation of soft and hard segments occurred in TPU. Crystallization is more common in samples with a higher ratio of hard segments, which is expected.

***Table 2** Thermal properties of thermoplastic polyurethanes*

Sample	**1st Heating**			**Cooling**	
	Tg (°C)	**Tm (°C)**	**ΔHm (J/g)**	**Tc (°C)**	**ΔHc (J/g)**
SI-HDI-30%	48.23	113.9	5.06	85	14.89
SI-HDI-50%	52.78	145.4	17.75	96.9	4.14
SI-HDI-70%	92.05	/	/	70.3	1.38
SI-IPDI-30%	33.12	132.2	2.97	64.1	3.61
SI-IPDI-50%	44.98	145.5	8.36	41.3	0.18
SI-IPDI-70%	89.88	/	/	/	/

In the case of IPDI based TPU, the increase of Tg with increase of the ration of soft segments is also observed due to the good microphase separation of the segments. In sample SI-IPDI-30% crystallization is most pronounced, while in sample SI-IPDI-70% there is no crystallization phenomenon, which is normal as soft segments prevail in the sample. The degree of crystallization of the analyzed samples is also affected by the structure of the used IPDI and HDI, which also influences the mechanical properties of the samples.

CONCLUSION

In this paper, the process of the synthesis of hydrophobic thermoplastic polyurethane elastic materials has been successfully developed. TPU was obtained from two types of isocyanates, different polyols siloxane and polycaprolactone and isosorbide as chain extenders. The FTIR analysis has confirmed that the use of the components in the given ratio leads to a quantitative reaction to the formation of polyurethane materials. The obtained silicone polyurethane elastomers show a higher range of viscoelasticity, as confirmed by DSC results, where a higher Tg value was obtained with an increase in the soft segments ratio. Silicone polyol contributes to the expansion of the temperature range of the possible application of this material.

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SETTING THE FRAMEWORK FOR DEVELOPMENT OF SUSTAINABLE DESIGN CURRICULUM

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Abstract

In next decades demographical, economic and environmental changes, have to cope with limited natural resources. The need for fresh solutions for obtaining secondary resources is the challenge of nowadays. Introducing the sustainable design in the field of education is a step toward obtaining sustainable production and consumption of materials and products. While recycling takes waste products and breaks them down in order to remade their base materials into a new consumer product, upcycling is design practice that has a long-term and positive impact on the environment by creating something new of waste products and materials of same quality as starting material. The paper presents the results of the conducted survey of the attitudes of Belgrade Polytechnic' students toward the introduction of a new study program in the field of sustainable design, as well as the results of the survey conducted for companies in order to define their needs for employees in the field of design with better knowledge of production methods and material properties.

Keywords: sustainable design, survey, students, organizations

INTRODUCTION

In the coming decades important demographic and environmental changes will take place in the urban environment. Those changes have to be followed by developing of integrated sustainable solutions for environment problems as well as economic and social needs of urban areas. Natural resources are limited and cannot be changed. Resources of second order are the answer of society as a helping tool to overcome the unequal distribution of natural resources: substitution of critical or scarce raw materials, technology for improved efficiency of use, as well as recycling [1].

Within the EU, the Circular Economy Package presented in May of 2018. covers the whole cycle from production of materials and products to waste management, and promote design as important part for implementing new concept [2]. Promoting better connections between technology and design fields, could be good start for development and uptake of viable solutions for sustainable development. Strengthening co-operation between higher institutions and local stakeholders (production sectors), providing experts in sustainable design for the enduring and new challenges and demands in the field of sustainable production and consumption in contemporary society, by tackling skills gaps and mismatches reinforcing links between education, economy and innovation, is in line with the challenges identified in the Renewed EU Agenda for higher education [3].

College of Vocational Studies - Belgrade Polytechnic, has two main areas of education: Technology and Design. Connecting these areas can be a good start for development of sustainable design course, harmonized with sustainable development goals, that would contribute to students transferable, entrepreneurial skills.

MATERIALS AND METHODS

In order to set the framework for development of new sustainable design curricula (the structure and content of a short multidisciplinary course), in line with the market needs and requirements of future students, two separate surveys were realized in March of 2019.

One questionnaire was formed for Belgrade Polytechnic` students of design with aim to obtain their attitudes as potential future users of the new development of course and

Questionnaire for organizations (stakeholders). For all surveyed organizations product design or development is in the core of their business activities. This survey for organizations was conducted in order to perceive perceptions of the position of sustainable design from the perspective of the business goals in accordance with the type of organization (start up, small business, company, ect), as well as the readiness and needs of the organization for employees with a specific kind of knowledge from the field of sustainable design, potentially obtained within newly developed course.

Below are the most interesting research results from both areas. The collected answers are shown in a separate sections.

RESULTS AND DISCUSSION

Student survey

Undergraduate students from all departments of design of Belgrade Polytechnic were interviewed, and 131 responses were obtained (from Graphic design department 48.9 % of all interviewed students, from Industrial design: 27.5%, from Fashion design of leather products 13.7% and the rest is from Concept art and character design department).

For continuing education after completing bachelor degree are interested 73.3% of interviewed students (while 21.4% do not know yet).

Grate number of students, 61.8% support the idea of collaboration of the study programs of design and technology departments through interdisciplinary courses, while 28.2% have not developed opinion toward this question.

This is in relation with obtained data that only 5.3% of students see design as related only to the aesthetic component of the products (Figure 1), while 79.4 % of them responded that it is necessary for designer to understand material properties and the basics of the production processes.

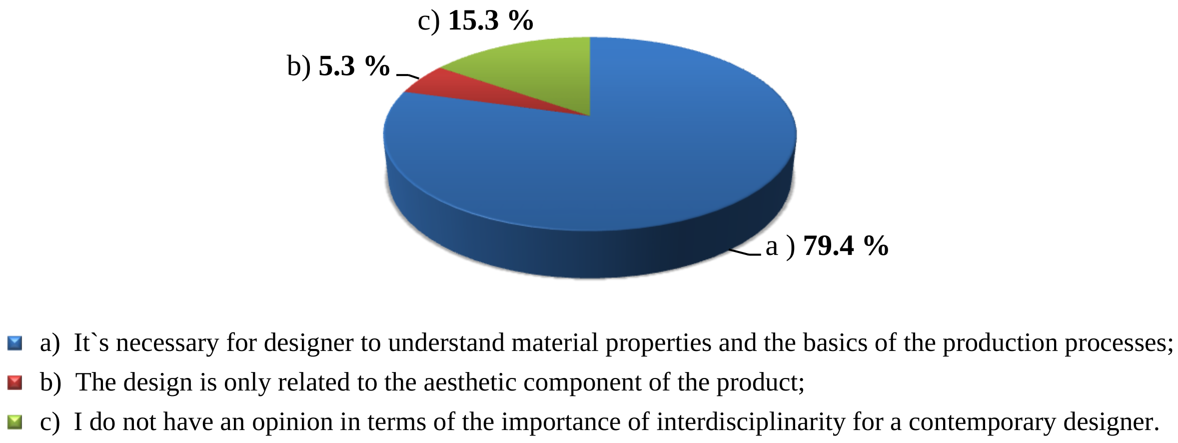


Figure 1 *Responds on the survey question: "Do you think that interdisciplinarity is an important part of the education of a contemporary designer?"*

On question *Are you interested in enrolling specialized design courses if they are in your area of interest?* (Figure 2) students have divided in answers (while 23.7 % of them are for one semester course, 29.8% are interested in one year long course)

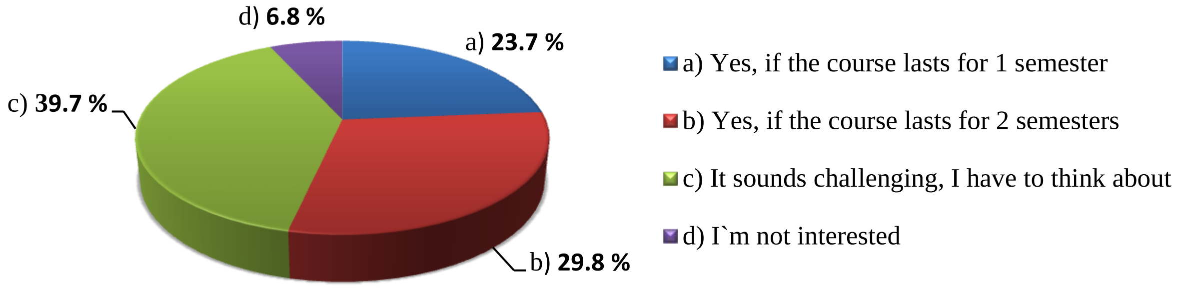


Figure 2 *Responds on the survey question: "Are you interested in enrolling specialized design courses if they are in your area of interest?"*

Students care of environment and of preservation of resources - as support of this claim is 95.4 % of respondents that agreed that within the designing of products it is important to take care of its impact on the environment and the preservation of resources. All the rest of interviewed students (4.6 %) didn't think about it.

More than half of surveyed students (53.4 % of them) are interested in to acquire a deeper knowledge in areas of sustainable design (eco design, design of accessories, or, unique design).

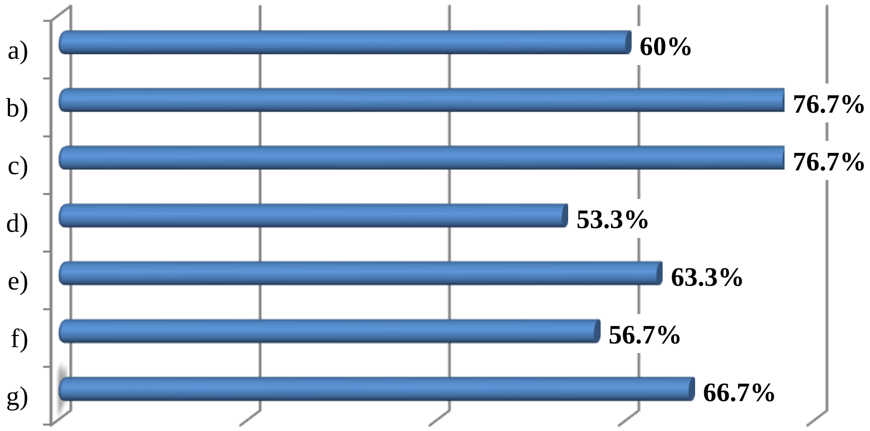
Results within the Student Survey confirms impressive number of undergraduates are eager to continue with their formal education, recognize the need for environment protection and are very curious and interested in short courses.

Companies survey

The questionnaire for organizations (stakeholders) obtained 30 responds, most of which were design studios (53.3 %) and the rest were almost equally represented bureaus, independent crafts workshops, start-ups, small firms and companies.

Having in mind that the questionnaire were designed for design oriented organizations, it is no wonder that most of respondent organizations were with maximum of five employees (70 %), while 20 % have between five and twenty employees, and the rest are with over 100 employees.

Figure 3 shows knowledge and skills detected as important within design oriented surveyed organizations (each given criteria should be treated separately from others).



a) Following of innovations and contemporary trends in the field of eco-design and introducing them into the production process in accordance with the organization's policy.
b) Acquainting with the properties of materials that are the subject of manufacturing process.
c) Designing and projecting the products according to the design requirements (aesthetic, ergonomic, functional and technical-technological requirements).
d) Actively participating in the marketing activities of bureaus, studios, companies, etc.
e) Proposing new solutions in accordance with good practice of using recycled materials, economics in terms of raw material utilization, environmental care and product quality.
f) To be familiar with the world's best design practices in the field of sustainable, eco-friendly or green design.
g) Good theoretical and practical knowledge and skills and providing critical opinion.

***Figure 3** Knowledge and skills detected as important within design oriented surveyed organizations*

Results within the Companies Survey confirms high interest for employees that are acquainted with the properties of materials that are the subject of manufacturing process, that follow innovations and contemporary trends in the field of sustainable design and can propose new solutions in accordance with good practice of using recycled materials, economics in terms of raw material utilization, environmental care and product quality, as well as are familiar with the world's best design practices in the field of sustainable, eco-friendly or green design (Figure 3).

Almost all of respondents consider recyclable materials or materials that can be reused significant for economy of country (93.3 %), and consider importance of understanding of reusing of materials potential by designers, in order of applying that knowledge within design or production processes (96.7 %).

CONCLUSION

For now no short cycle programmes have yet been accredited in either academic or professional studies in the education system of Serbia. However, there is a growing need for this, as confirmed by Student Survey results. Students are highly interested in continuation of their education and are eager to try innovative, blending courses, particularly if they are also recognized by the companies/studios (confirmed by Companies Survey).

The Student Survey confirms impressive number of undergraduates recognize the need for environment protection and are very curious and interested in short courses.

Students attitudes, expressed through the responses obtained through the survey, can be a solid basis for detecting gaps in their previous education, and at same time basis for developing more contemporary educational programs, in correlation with the sustainable development goals in order to achieve a better and more sustainable future for all.

The stakeholders` attitudes expressed through the responses obtained through the Companies Survey, can be a solid basis for finding adequate ways to establish a sustainable business concepts compatible with the principles of circular economy.

Sustainable design course in the future could support, promote and apply interdisciplinary cooperation between technology, engineering and arts. Overall, are aiming at higher quality of education and support permeability between the different education and training pathways, allowing our graduates to fulfill their ambition for continuous education (confirmed in Student Survey), while offering them course recognized and valued by professionals and companies from Serbia and region.

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SUSTAINABLE FASHION: A PUSH TOWARD CIRCULAR FASHION

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Abstract

Fashion has never been about the clothes, but about objects of desire and status since the dawn of time. It went through many changes during time but today is a huge global industry with a large income and even larger polluter. Fashion is a massive industry worldwide that employs 60 million people globally has a revenue of incredible $2.5 annually with prediction of doubling in the decade to come. That comes with a great price in loss of money but even more importantly resources. Extensive usage of water, chemical-treatment pollution and creating enormous amounts of waste from unused and unsold clothes are the main reasons why fashion industry is one of the major polluters in the world with 10% of carbon footprint and 1.2 billion tones green gas and is the second largest polluter of fresh water. Even though many steps and ideas are subjected to suppress the problem – the real push forward would be to change the global outlook on the matter and make sustainable and circular clothes fashionable and objects of desire through media, as well as finding the appropriate way to deal with already generated amounts of end of life products.

Keywords: fashion design, circular design, waste

FASHION: HISTORICAL AND SOCIAL OUTLOOK

Fashion is the style and custom that prevails at a given moment. In the broadest sense, "fashion" means popular style and the term is usually applied to clothing, hairstyle, jewellery, ways of public behavior, entertainment, etc. The beginning of today's fashion perception in the Renaissance courts, and the main driving force is a constant desire of newness and individualization. It is believed that from the transition from the Middle Ages and domination of the church, in to Renaissance Europe, the body, spirit, and thinking have been liberated, which lead to slowly going through secularization, humanization and aesthetics, and is a transition from a religious domination in Europe. That all came along with the idea of progress, great social and spiritual changes, as well as great discoveries in various sciences.

Sociology considers that taste is socially determined and that it is not a matter of a universal feeling for a good one, but that different socio-economic groups have different aesthetics and tastes, and therefore the taste is a socio-empirical category. It has long been considered that the taste of the ruling class is the only valid and accepted taste, while the lower classes mimic the aesthetics of the higher, with the constant imposition of new standards and trends again. How many times have we heard the say to „dress for the job we want and not for the one we currently have“? Vance Packard called it "Status Hunters", where in aesthetics and manners he always tends to represent a higher layer than he realistically belongs, and that hobbies and manners that require more time and are more complex on a scale of luxury. At first it was considered that the original idea of clothing was to protect the human body from external influences, but archaeologists and anthropologists, interpreting images in caves, came to the conclusion that natural shelters and fire were originally the protection of the body, and that the original clothes were made deer and other animal skins - actually served for magical and ritual purposes. Also, in a number of primitive and original communities, people lived naked in communities, which additionally confirms the theory that clothes were always a notion for society, not mere physical need.

The focus is on the desire, and not so much on having the object of the desire, although fashion is often repeated and visually recycled. Thus, in the eighteenth century, in newspaper fashion advertisements and posters the emphasis is on tags such as "improved", "old-fashioned", "newest fashion", "new trends", etc. Hegel claimed that there are three elements that combine: spirit, body, and clothes.

German philosopher Walter Benjamin (1892-1940) considered various aspects of society and culture in his works. Since he lived in a period when mass culture and cultural industries were only in the beginning - he is considered one of the first theoreticians of media studies and mass culture. His work “Artwork in the Age of Mechanical Reproduction" from 1935 takes the decisive work in this field, where he primarily deals with changes in art and its perception as a consequence of industrialization and changes in the way in which the work of art is made and the manner of its reproduction. He considered that the work was losing its authenticity, which he had designated as the author, unless he was in contact with the author, that is, if the author does not "enter" in the material when it is created. In his never-ended "Arcading Project", which does not have a heterogeneous form, but makes notes and reflections on various topics, Benjamin mentions fashion more often, above all as a socio-political factor of society, as an economic power and visual signifier.

She calls fashion a "seller's art" and thinks that it "Fashion prescribed the ritual by which the fetish Commodity wished to be worshiped". According to Benjamin, fetishism reaches its climax at the World Exhibitions which is called "pilgrims of goods like fetishes". He considered that on these occasions, capitalist phantasmagoria, or obsessive, was experiencing its climax and that Paris then confirmed itself as the center of luxury and fashion.

Also, one of the more interesting aspects of fashion that he dealt with in the text "Paris, the capital of the twentieth century" is the notion of a tiger jump (ger/Tigersprung) in a fashion that denotes the combination of past, costume and modernity in an eclectic circle of various elements.

SPEED OF FASHION

Post-Second World War is known as the Golden Age of Capitalism. A deeply divided and wounded world became the global village as the Marshall Mcluhan called it in his book “Understanding Media” from 1964. Fashion was a big factor in the fast growing global economy flying on the wings of glossy magazine and ever-growing number of electronic media such as TV and radio leading to internet and social media today. The old say “Clothes to not make the man” was rewritten by Mark Twain into “Clothes do make the man. Naked people have little or no influence on society" stressing the importance of fashion in everyday life.

With the acceleration of everyday life, fashion became faster while designers and industry had to produce two collections annually and more recently even faster. Global multinational companies dictate very fast changing trends on a global scale causing massive production of clothes - the using about 79 billion cubic meters of textiles annually [1]. USA, largest importer of fashion in the world, has tripled the number of clothing items bought by the average citizen since the 1960s. This causes the need for more resources, and therefore the necessity for a faster production that leads to a greater pollution creating a vicious circle. Every year our global fashionable village produces about 80 billion items of clothing [2]. Having that number in mind, some researches show that only 30% of fashion items is sold at the retail price, while same amount gets sold on sale and the remaining 40% stays unsold or doesn't even reach the shops [3]. This unwanted fashion items end up being thrown in land field or incinerated, creating massive loss of energy and materials while only 20% of that pile of clothes ends up recycled or reused.

According to Ellen MacArthur Foundation report on new textiles about $500 billion [4] is "lost every year due to clothing underutilization and the lack of recycling". Some researches show that, for example around 350,000 tons of clothing ends up as landfill every year in UK and that average American throws away 70 pounds of clothes annually [5] making the textile waste about 5% of all the municipality litter in US. This causes extensive usage of water, chemical-treatment pollution as well as creating enormous amounts of waste from unused and unsold clothes. For all of those reasons, fashion industry is one of the major polluters in the world. It creates 10% of carbon footprint, is responsible for total greenhouse at 1.2 billion tones (more than international flights and maritime shipping combined) and is the second largest polluter of fresh water with an incredible numbers such as that to create a single simple cotton t-shirt a staggering 1,083 gallons of water is consumed.

Fashion is a massive industry worldwide with a revenue of incredible $2.5 annually and with prediction of doubling in the decade to come. The industry employs 60 million people globally. In terms of socio-economy, the fashion rise started in France and Italy but due to high production costs, strict rules and speed of mass production in Europe, it moved to third world countries. Low pay and long hours put in by men, women and especially children in inhumane condition were brought to light by a 1999 book "No logo" by Naomi Klein. It created waves globally by calling out big multinational brands such as Nike or The Gap. Even so, little has changed since. Today clothing is huge export of low GDP countries such as Haiti (88%), Bangladesh (79%), Lesotho (59%), Cambodia (52%), Sri Lanka (43%) just to name a few [5].

Another interesting aspect of today's fashion industry is gender. Even thought that vast majority of fashion consumers are women (85%) and majority of its employees on a global scale (68%) – they seldom make it to top management. From 50 major fashion brands – only 7 are run by women [5]. Also they make only a quarter of board members of publicly-traded fashion companies.

CONSCIENCE OF FASHION

First ideas about stopping or rethinking the wheel of fashion came up in the 1960s in the book Silent Spring by American biologist Rachel Carson. Second big milestone was in late 1980 with the coining of the term sustainable development that lead to various derivate including sustainable design and sustainable fashion.

Nowadays fast fashion cycles are followed with depletion of non-renewable resources, massive energy and water demands, emissions of GHG, human toxicity, and severe environmental pollution, vast amounts of textile waste at the end of life and huge waste generation, as well the problems with limited landfill space. The main challenge of contemporary fashion industry is finding the way to design out waste and pollution and keep materials in use rather than sending to landfills.

The paths of unwonted textile productscan be:

1. Landfilling - dumping into landfills;
2. Incineration - burning the textile waste with or without energy recovery option;
3. Recycling - processing and converting it to a new productfor the same or for different purpose;
4. Upcycling- converting textile old/discarded materials into something useful
5. Remanufacturing – adding value to the products (restoring used products to a “new-like” condition)
6. Resale of the productsat secondary products’ market (without any processing - in present condition) or internal reuse of products.

As the fashion industry relies on a high volume of sales and a steadily rising rate of growth, that resulting in an upsurge of textile waste, the one of the most environmentally beneficial ways would be to reutilize clothes is reusing garments. But, even thou reuse and recycling of waste products and materials of fast fashion sphere, became necessity for now only a small part of used clothing is recycled into fibers and ultimately made into new clothes.

According some analysis, extending a garment’s life by just three months can lower its water, carbon and waste footprint by 5-10%, however, currently only 8% of garments are reused [6].

Although contemporary waste management is mainly limited to improving the technology of its processing, it is expected that the introduction of new, circular, economic business models in the future will enable a complete and irreversible redefinition of the term waste, to his final elimination - at least in the way we see it today [7]. Fast fashion industry slowly became an important part of these economic processes (an example of *Circular textiles value chain* is shown on Figure 1).

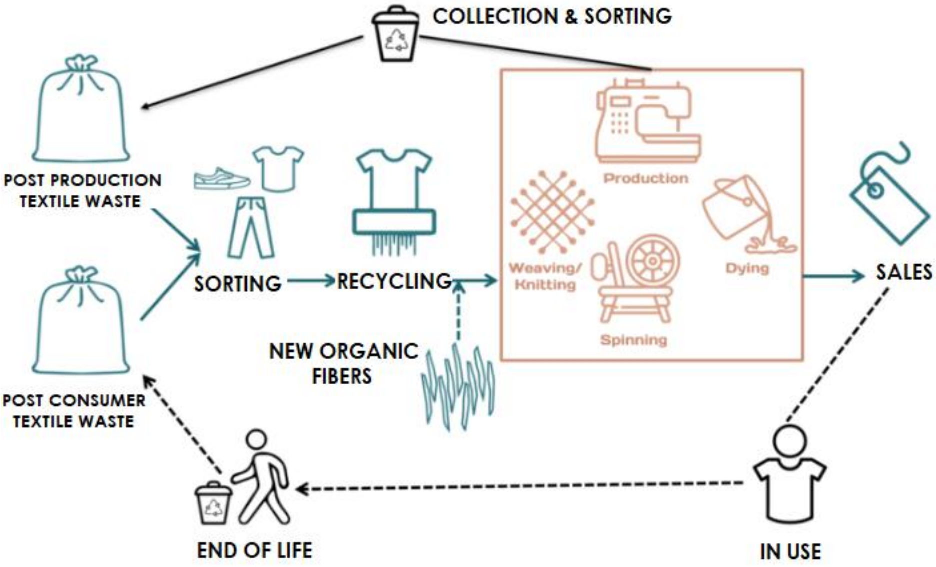

Figure 1 *Circular textiles value chain [8]*

The important question is how to build a sustainable fast fashion business. There are several approaches for retailers to develop sustainable fast fashion, and all of them includes circular economy principles. It is important for materials to remain in the loop as long as it is possible, and with focus sat on [7]:

- design for reuse and recycle,
- managing a sustainable supply chain,
- increase industry collaboration,
- drive awareness among businesses and consumers.

As waste-free fashion business is nearly impossible to achieve with today’s business models and technologies it is a moment to make a shift in approach to production and consumption of garments, and at that way rearrange relations within fashion industry approach.

CONCLUSION

Fashion is a massive industry worldwide, and its production comes with a grate environmental cost (extensive usage of water, chemical-treatment pollution, and by creating enormous amounts of waste from unused and unsold clothes). Generation of new amounts of textile waste can upend the entire fast fashion industry with catastrophic consequences to the environment, economy and society.

In the search for more productivity, efficiency and lesser environmental impacts of textile products, the design process should integrate requirements from all fields affecting the industry.The challenge is to develop new, more circular model for fashion business, to help this industry in transition to production without waste. True sustainability can be tackled only if this sector takes proactive approach towards production and promotions of products.

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ECOLOGICAL FOOTPRINT OF TEXTILE FIBERS

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Abstract

Ecological impacts occur in all phases of the textile products life cycle. Increased interest in sustainable textiles has contributed to the development of tools for assessing and comparing the environmental impact of textiles products with respect to sustainability. One of the most comprehensive indicators of ecological sustainability is Ecological Footprint. In this paper, ecological footprint of textile fibers is discussed.

Keywords: textile fibers, cotton, Ecological Footprint

INTRODUCTION

The textile industry is one of the biggest causes of environment pollution. Fiber production has grown to over 130 million tons a year, and it is estimated that clothing accounts for about 3-6.7% of global CO_2 emission. Textile production and consumption have high water, chemicals and energy consumption and occupy land suitable for food production. Textile industry affects biodiversity and water flows. Ecological impacts occur in all phases of the textile products life cycle. Wet treatment of textiles like desizing, prewashing, mercerizing, dyeing, printing etc. includes a lot of chemical applications on the fibers and fabrics. Some fibres need to be bleached with chlorine before dyeing. This causes organochlorine compounds to be released, which are very dangerous to the environment. From dyes to transfer agents, around 2000 different varieties of chemicals are used. During the process of wet treatment, huge quantity of fossil fuels is consumed which have carbon content and react with oxygen to form carbon dioxide. This results in acidification, fossil fuel depletion and ultimately global warming. Fabrics take a lot of energy to produce textiles. The clothing use phase includes dry cleaning, washing and drying, which contribute to the high consumption of water, energy and chemicals. The life cycle of the textile product ends with one of the options for waste management – return, reuse, recycling, incineration or disposal at the landfill, which affects its ecological load. In response to the ever-growing demands for sustainable textile production, there is a need to assess the environmental impact of certain types of fibers on the environment [1–3].

ECOLOGICAL IMPACTS OF DIFFERENT ORIGIN FIBERS

In the production of textile products, a large number of different fibers are used, mostly: cotton, wool, viscose, acrylic, polyester, polyamide, etc. Acrylic, polyester and polyamide fibers come from non-renewable sources - petroleum. Viscose, cotton and wool fibers are from natural, renewable sources. However, these fibers come from different sources and origin can not be treated in a similar way. Fiber from renewable sources is also different related to the location of the textile producing plant. Wood is a renewable source, but only in the long time, if afforestation is managed on sustainable principles [4]. Similarly, the ecological impacts of textiles in all life cycle phases can be considered. Thereby, there are different tools / methodologies for assessing the environmental impact of textile products made from different fibers, which allow their comparison according to their ecological loads.

TOOLS FOR THE ASSESSMENT OF TEXTILE ECOLOGICAL IMPACTS

Increased interest in sustainable textiles has contributed to the development of tools for assessing and comparing the environmental impact of textiles and textile products to sustainability, an alternative approach to eco-labeling of products as "green". Since fiber content is one of the few characteristics available on all clothing items, it is also the most common basis for comparison. The most widely used fiber-based comparison tools are the *Higg Materials Sustainability Index* (MSI) and the *MADE-BY Fiber Benchmark*. They assess the environmental impact taking into account the entire life cycle of the product (LCA) [3]. Most often, the environmental impact is assessed according to the MIPS methodology (Material Input Per unit Service or output) [5]. The schematic calculation of MIPS is shown in Figure 1. One of the most comprehensive indicators of ecological sustainability is Ecological Footprint [6].

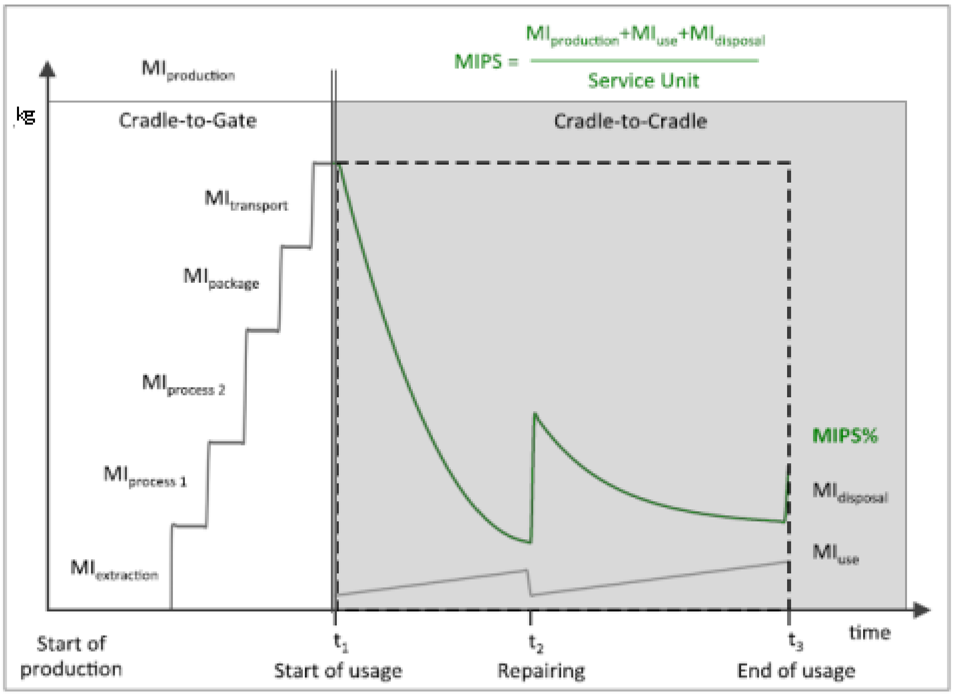

Figure 1 *Schematic calculation of MIPS*

Ecological Footprint

Ecological Footprint (EF) accounting measures the demand on and supply of nature. On the demand side, the EF measures the ecological assets that a given population requires to produce the natural resources it consumes (including fiber products) and to absorb its waste, especially carbon emissions. On the supply side, a biocapacity is important, which represents the productivity of the ecological assets. Both the EF and biocapacity are expressed in global hectares (gha) – globally comparable, standardized hectares with world average productivity [7,8]. The EF essentially accounts for the use of the planet's renewable resources. Nonrenewable resources are accounted for only by their impact on, or use of, renewable, bioproductive capacity. The EF deals only with demands placed on the environment. It does not attempt to include the social or economic dimensions of sustainability [9]. EF indicator is a means to evaluate the sustainability and environmental performance of textile processes and products [10]. The total EF estimation should consider all resources (materials, water, energy, waste) that are part of a process or an organization – EF methodology for the evaluation of products, is based on the EF component method [11].

Thus, environmental footprints measure the areas of the Earth's surface needed to maintain consumption patterns and assimilate generated waste. The assessment of ecological footprint for any type of fiber is carried out according to the same methodology. For example, in the case of cotton, the ecological footprint takes into account where cotton is produced, how much water and energy is needed, chemical inputs, transport and waste disposal. Usually the following categories of prints are evaluated [1,8]:

- **Material footprint**, which relates to abiotic and biotic raw materials that are input in the production of certain quantities of cotton.
- **Land footprint** is the extent of land needed to produce a certain amount of cotton, considering the differences in productivity of land in different regions and the long-term impacts on land resources of various methods of cotton growing.
- **Water footprint** measures the amount of water for irrigation of a certain area under the crop, which is required for the cultivation of a certain quantity of cotton and the amount of water needed to dilute toxic chemicals to a safe level.
- **Carbon footprint** measures the area needed to isolate carbon emissions related to planting, maintenance and reading, irrigation, chemical input production and waste disposal. The carbon footprint reveals how much CO_2 in total is emitted along the value chain of a product. It is the total set of greenhouse gas (GHG) emissions caused by an organization, event or product. It is calculated for the time period of a year and expressed in terms of the amount of carbon dioxide, or its equivalent of other GHGs emitted.

When a consumer buys a textile product, he also buys an invisible backpack – environmental impacts generated during its production, use and textile waste disposal (Figure 2) [6,10,12,13]. For example, the load of ordinary T-shirt (171 g) in environmental impacts, calculated according to the MIPS methodology, is given in Tab. 1 [5].

The comparison result of such estimation of EF cotton fibers grown in an organic and conventional manner in California is shown in Figure 3 [8].

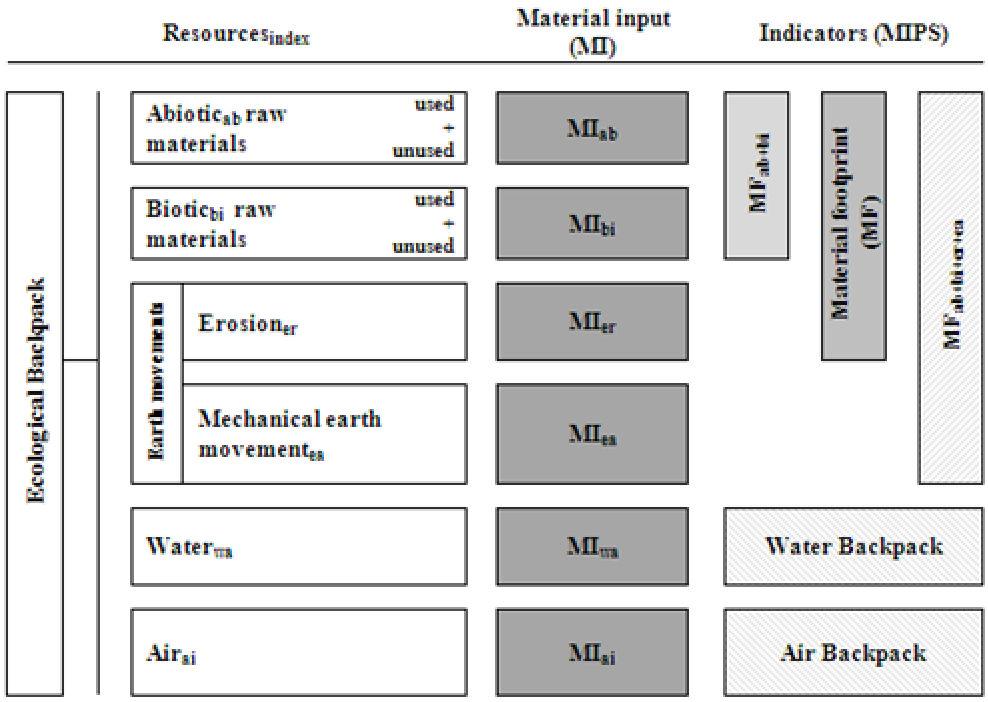

Figure 2 *Ecological backpack of product*

Table 1 *Results of the analysis – material intensities (MI) and material input per unit service or output (MIPS) of a cotton T-shirt*

MI	Abiotic material, kg	Biotic material, kg	Erosion earth-work, kg	Water, kg	Air, kg
Production	2.00	1.20	233.00	1480.00	12.50
Use	117.35	0.00	Not specified	2719.60	27.44
Disposal	0.15	0,00	Not specified	0.40	0.06
Total	119.50	1.20	233.00	4200.00	40.00

Note: Resource consuption of a black T-shirt by *Hess Natur* weighing 171 grams (service unit “worn 100 times”: manufacture + 100 × drying + 100 × ironing)

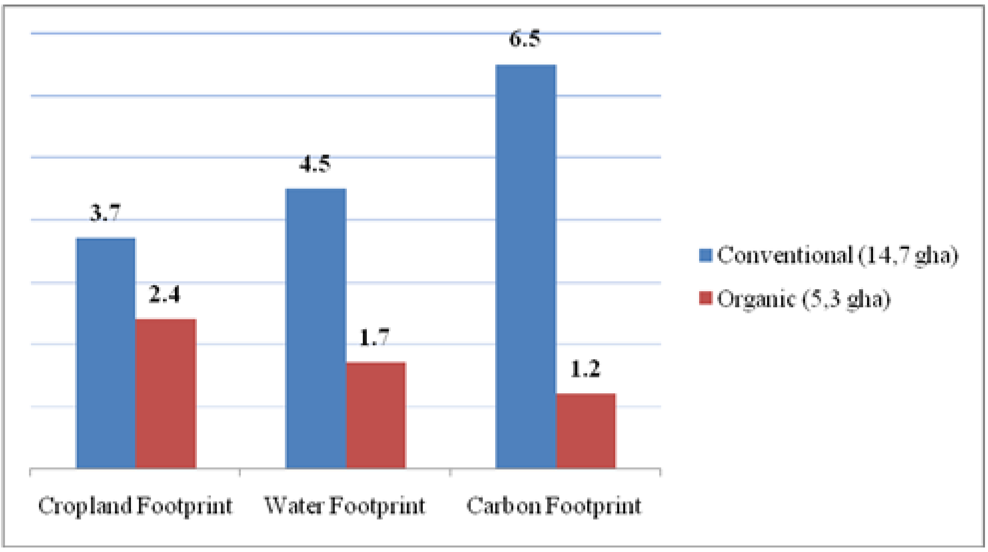

Figure 3 *Estimation comparison of the ecological footprint of cotton fibers growing in an organic and a conventional way*

Water footprint

In the life cycle of textile products, the influence on watercourses through water consumption and generated wastewater is particularly important. In order to understand the impact of textiles on global water resources, a water footprint is measured in the supply chain. The global water footprint of cotton products is estimated at 233 billion cubic meters of water annually [14].

Water footprint is an indicator of the use of fresh water, in a direct and indirect manner, for all production activities. It includes water consumption and pollution at all production process phases and value chain and includes three components [15]:

1. **Blue water footprint** – represents the amount of fresh surface or groundwater used to crops growing or goods or services produce. This is the quantity of evaporated water incorporated in the product or returned to another place or at different times in relation to the time it is withdrawn; the blue water print occurs when irrigation is applied;
2. **Green water footprint** – total amount of precipitation or moisture from the soil used for growing plants; this is relevant for products that include agricultural crops and wood and other forest inputs;
3. **Gray water footprint** – is a pollution measure; it is expressed as the volume of water required to assimilate pollutant load to meet the requirements of the standards for the quality of the aquatic environment; a pollutant that requires a higher assimilation volume is regarded as a critical contaminant and is used to calculate the gray water footprint; if there is pollution of both surface and groundwater, the gray water footprint is calculated for each pollution separately; in the case of cotton, this footprint includes nitrogen and phosphorus fertilizers and active ingredients of pesticides.

The assessment of the water footprint is a process that answers questions such as: what is the water footprint and what is the relationship between the green, blue and gray water footprint, whether the water footprint is sustainable or not, which strategies can improve its sustainability, etc. This assessment includes four phases:

1. setting goals and scope – identification of objectives and scope of assessment, including geographical / time limits and process / supply chain limits;
2. water footprint calculation – calculation of the operational (direct) water footprint and the water footprint of supply chain (indirect), by quantity and quality;
3. assessment of the water footprint sustainability – assessment of the water footprint in relation to the environment, social and economic criteria and
4. formulation of the response to the water footprint – the choice of strategic activities to reduce the water footprint or improve its sustainability.

CONCLUSIONS

The textile industry is one of the biggest causes of environment pollution. Eliminating as much as possible harmful processes and inputs in textile production provides a safer and sustainable environment. Thereby, it is important to evaluate the harmful environmental impacts of textile processes and products throughout the life cycle, and this can be achieved using the EF estimation methodology, which is very useful as an environmental indicator.

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THE VULCANIZATION PROPERTIES OF HYBRID ELASTOMERIC MATERIALS BASED ON WASTE RUBBER POWDER

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Abstract

Elastomeric materials have viscoelastic properties and can be designed for numerous applications, tires production, vibration isolation and damping. These properties can be tailored for a wide temperature range and in ozone-rich atmospheres. Processing capacity of a rubber compound can be predicted by their vulcanization properties. In goals of raising environmental demands rubber manufacturers are now focusing on the uses of recycled product. Re-use, recycle, pyrolysis and recovery are the common methods in sustainable management of waste elastomeric products. Waste rubber can be used in civil engineering, for composite material preparation, as energy source, for sport devices, tires fabrication. The goal of this applicative work was to estimate vulcanization behavior of rubber compounds based on recycled rubber powder and triple rubber matrix NR/BR/SBR which is used for tire tread manufacturing. It was estimated that the values of optimum vulcanization time decrease with the increase of recycled rubber powder amount.

Keywords: rubber blend, elastomers, waste rubber, recycling, composites

INTRODUCTION

Crosslinking of rubbers as network precursors causes substantial changes at the rubber macromolecules. The long rubber macromolecules (molecular weight usually between 100,000 and 500,000 daltons) become linked together with junctures (crosslinks) spaced along the chains with the average distance between junctures corresponding to a molecular weight between crosslink points of about 4,000 and 10,000 daltons. Because of network formation, the material becomes essentially insoluble, and cannot be processed by methods that requires it to flow (processing in a mixer or extruder; on a mill or calendar; or during forming, or molding. It is substantial that crosslinking occur only after the rubber product is in its final geometric shape. Effects of vulcanization on elastomeric material final properties are given in the Figure 1. The static modulus increases with crosslinking to a greater level than does the dynamic modulus. The dynamic modulus is a composite of viscous and elastic behavior, whereas static modulus is largely a measure of only the elastic component of rheological behavior. Dynamic modulus is determined by the imposition of a sinusoidal small strain in the frequency range from 1 to 100 Hz. Crosslink point formation reduces hysteresis. Hysteresis is the ratio of the rate dependent or viscous component to the elastic component of deformation resistance. It is also a measure of not stored deformation energy, but is converted to heat. Vulcanization then causes a trade-off of elasticity for viscous or plastic behavior. Toughness and tear strength are related to the breaking energy. Values for these properties enlarge with small amounts of crosslink points but they are diminished by further crosslink point formation [1].

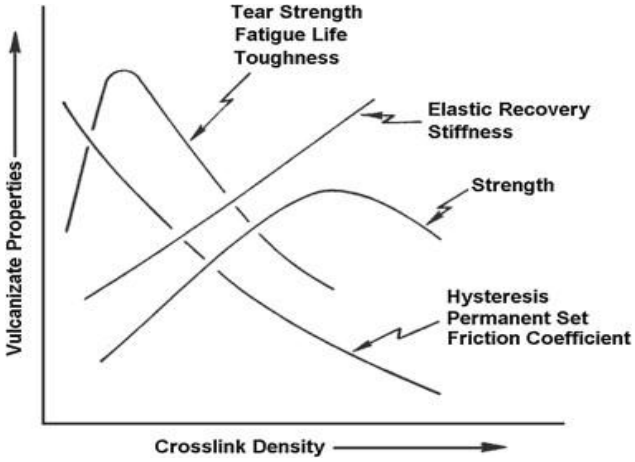

Figure 1 *Typical changes of elastomeric material properties as a function of the obtained crosslinking density (vulcanization level)*

The majority of rubber blend have been found to be multiphase either completely immiscible or multiphase with limitations. This is due to the small mixing combinatorial entropy of the dissimilar macromolecules. The remaining portions are single phase, melt-miscible mixtures. Compatibility is a concept depending on the scale of a particular experimental method. Thus, a rubber blend can be compatible when tested for mechanical properties or incompatible as revealed by structural assessments (glass transition determination, SEM. For industrial purposes blends as compatible when they show a synergistic behaviour in some valuable properties, incompatible when they show a minimum in some property-composition curves, and semi-compatible when the properties are intermediate for used rubbers [2]. Incompatible blends are heterogeneous, but only the compatible blends on a molecular scale are homogeneous. Different rubbers have specific unsaturation and may require the special crosslinking agents which may have different solubility in continuous or discontinuous domains. The structure of *cis*-1,4-polybutadiene is very similar to that of the natural rubber macromolecule (NR). Both network precursors are non-saturated hydrocarbons but for the natural rubber the double bond is activated by the existence of a methyl group. The polybutadiene macromolecule is without this group, and thus less reactive. The methyl side groups stiff the polymer chains and thus the polybutadiene glass transition temperature (T_g) is consequently smaller than NR glass transition. This lower T_g has a number of limitations to the polybutadiene rubber properties. At room temperature polybutadiene rubber compounds have a higher resilience than natural rubber compounds. On the other hand elastomers based on this network precursor, have poor tensile strength and tear resistance. For example, they are blended with NR in the fabrication of truck tyres and with SBR for passenger car tyres. Rubber compounds based on general purpose network precursors (NR, SBR, BR) or its blends use curing packages based on sulfenamide accelerators. The selection of the crosslinking package is based on processing safety, curing rate index and ultimate modulus buildup per accelerator loading (activity). It is applied to the loss of network structures by thermal nonoxidative ageing. It is associated with isoprene rubber used as network precursor crosslinked by sulfur package. It can be the result of the over-curing or of thick section hot ageing [3]. Sometimes the term reversion is concerned to other types of non-oxidative deterioration (mainly for network precursor not based on isoprene. For example, thermal aging of SBR which can cause increased crosslink point density and hardening is called reversion, since it can be the result of over-curing and can also degrade a elastomeric product. In focus of raising environmental demands manufacturers are now forced to use recycled product. Re-use, recycle, recovery and pyrolysis are the common methods in sustainable management of waste elastomeric products. De-vulcanization is a fabrication process in which crumb rubber is subjected to thermal treatment, pressure or the addition of softening agents to regenerate the rubber compound to its original plastic state. Fabrication of rubber powder from waste tyres is a three-stage procedure primarily shredding, after that granulation and lastly fine grinding. The process of cryogenically freezing scrap rubber and crushing the rubber to the particle size desired. Dry method is procedure that mixes crumb rubber modifier with rubber aggregate before the mixture is combined with asphalt binder. This process applies only to hot-mix asphalt production. Tyre in general consists of pollutants and heavy metals thus there is a potential risk for the introduction of toxins into the soil and ground water. The solubility of these materials varies with the pH of soil and characteristics of local water. Research has estimated that insignificant leaching occurs when shredded tires are used as land fill materials. Sometimes eco-toxicity may be a serious problem. Studies have been assessed that ingredients such as zinc, heavy metals leach into water. Shredded tire particles leach more, forming a bigger concern via the increased surface area on the pieces. Ecology is now the crucial part of the elastomeric materials design. The automobile tire technology is obliged to reduce the fuel consummation via the fabrication of tires with less rolling resistances [4]. The viscoelastic behavior of elastomeric materials results in an energy loss during a cycle of extension-contraction (called hysteretic loss). This property is related to the rolling resistance of tire (the fuel consumption). In the Table 1 are listed the typical rubbers for passenger and truck tire parts manufacturing. The progress in waste rubber recycling is focusing to the incorporation of recycled rubber powder (RRP) or partially de-vulcanized elastomer (PDE) into polymer or rubber matrix and a new sustainable material creation for technological purposes. PDE and RRP are frequently blended with other network precursors such as natural rubber, styrene butadiene rubber, polybutadiene rubber and acrylonitrile butadiene rubber (NBR).

Table1 *Typical rubbers for tire part fabrication*

	Tread	Belt	Carcass	Sidewall
Passenger tire	SBR-BR	NR	NR-SBR-BR	NR-BR or NR-SBR
Truck tires	NR-SBR or BR-BR	NR	NR-BR	NR-BR

The compatibility of these rubbers with these recycled materials should be satisfied as they are among the major rubber components used in tire recipes. The goal of this work was to determine crosslinking behavior of compounds based on recycled rubber powder and triple rubber matrix NR/BR/SBR. It was estimated that the values of optimum vulcanization time (t90) decrease with the increase of recycled rubber powder amount.

EXPERIMENTAL

Network precursors were: (a) Malaysian natural rubber (NR SMR-20) (b) polybutadiene rubber (BR SKD-Nd, producer Nizhnekamsk) (c) Styrene-butadiene rubber (SBR Intol1783). Carbon black was used as active filler. The content of recycled rubber powder was varied. The sulfur vulcanization package was used. Naphthenic oil was used as plastificator (10 phr), The network precursor ratio in triple rubber matrix NR/BR/SBR was 10/10/80 (w/w/w). Rubber compounds were homogenized using laboratory roll mill in the temperature region 60-70 °C. The sheeted rubber compounds were conditioned at 23±2 °C prior to crosslinking behavior assessment at 160 °C using oscillating disk rheometer (Figure 2).

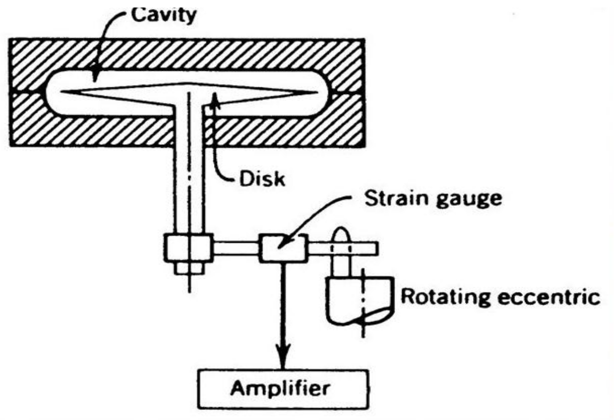

Figure 2 *The scheme of oscillating disk rheometer*

RESULTS AND DISCUSSION

Elastomeric materials based on triple rubber matrix of SBR and highly unsaturated NR and BR are common materials for tire manufacturing (applicative temperature from -40 °C to 70 °C). In the Figure 3 are given the preliminary structures of hybrid materials based either, partially de-vulcanized elastomer (PDE) or on recycled rubber powder (RRP) like in our experimental study.

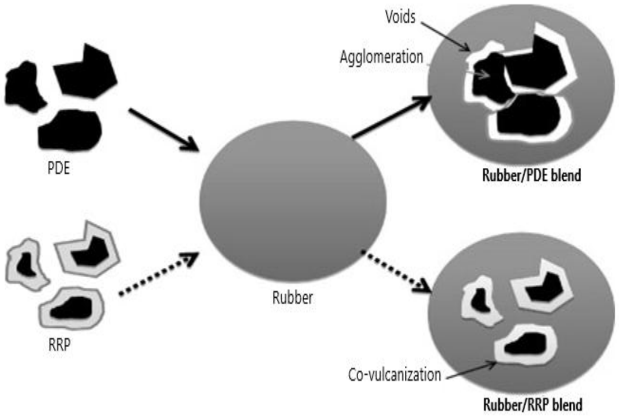

Figure 3 *The typical structure of hybrid elastomeric materials based either on recycled rubber powder (RRP) or partially de-vulcanized elastomer (PDE)*

For saving energy it is important to assess the optimum vulcanization time. With crosslinking time reduction at the same time there is a diminishment of heat losses to the surroundings, which enables the energy consumption to be reduced significantly. In curing behavior assessment the minimum torque is a measure of stock viscosity.

$$M_{t_{90}} = (M_h - M_l) \cdot 0.9 + M_l \qquad (1)$$

where M_h is the maximum torque, and M_{t90} a new torque reading corresponding to 90% cure in the rubber were determined from the cure traces generated at 160. The curing rate index (CRI) is the measure of rate of vulcanization based on the difference between optimum cure time of vulcanization t_{c90} and the scorch time t_{s2}. It can be calculated from the relation:

$$CRI = \frac{1}{t_{c_{90}} - t_{s_2}} \cdot 100 \qquad (2)$$

The data for evaluated optimum crosslinking duration and index CRI are summarized in the Table 2. Scorch safety during vulcanization can be controlled by the selection of the primary accelerator used in the cure system. Sulfenamide accelerators are the most commonly selected primary accelerator for general purpose rubber compounds because, as a class, they give the best processing. It is known that the increase of the minimum torque for RRP/rubber blend compounds is significantly higher compared to PDE/rubber blend compounds. This is related to the substantial structure where RRP is vulcanized material and PDE is partially de-vulcanized network. Due to such structure RRP acts like soft fillers which agglomerates in the rubber matrix and does not flow. The increase in viscosity translates to increase in the minimum torque. The data for evaluated optimum crosslinking duration and index CRI are summarized in the Table 2. Scorch safety during vulcanization can be controlled by the selection of the primary accelerator used in the cure system. Sulfenamide accelerators are the most commonly selected primary accelerator for general purpose rubber compounds because, as a class, they give the best processing. In curing behavior assessment by oscillating disk rheometer the minimum torque is a measure of stock viscosity. The increase of the minimum torque for RRP/rubber blend compounds is significantly higher compared to PDE/rubber blend compounds. This is related to the substantial structure where RRP is vulcanized material and PDE is partially de-vulcanized network. Due to such structure RRP acts like soft fillers which agglomerates in the rubber matrix and does not flow. The increase in viscosity translates to increase in the minimum torque.

Table 2 *Crosslinking characteristics for elastomeric composites based on triple rubber matrix NR/BR/SBR, carbon black particles and recycled rubber powder (RRP)*

Sample	Carbon black (phr)	RRP (phr)	M_L (dNm)	M_H (dNm)	t_{C90} (min)	CRI (min^{-1})
NR/BR/SBR	60	0	1.94	15.83	0.66	303.03
NR/BR/SBR /RRP5	55	5	2.21	15,00	0.70	294.12
NR/BR/SBR/RRP10	50	10	2.05	13.87	0.67	312.50
NR/BR/SBR/RRP20	40	20	1.84	11.29	0.56	434.78

With reduction in the crosslinking time there is a reduction in heat losses to the surroundings. It should be mentioned that used RTR particles contains and some amounts of processing oil which could facilitate the reduction of the minimum torque. Presence of filler such as carbon black in recycled rubber particles also contributes to the increase in the minimum torque. Lower minimum torque of RTR shows, it has better processability compared to GTR. It was assessed that the values of optimum crosslinking time (t90) decrease with the increase of recycled rubber powder content. For saving energy it is obligative to assess the optimum vulcanization time for each rubber compound recipes.

CONCLUSION

The goal of this work was to estimate the influence of recycled rubber powder on the crosslinking characteristics, such as maximum torque (M_h), minimum torque (M_l), torque reading corresponding to 90% cure in the rubber (M_{t90}), curing rate index (CRI) and optimum crosslinking time (t90) on triple rubber matrix NR/BR/SBR to assess the best recipes for rubber compound preparations. Materials based on triple rubber matrix are used for tire tread fabrication. It was estimated that the optimum crosslinking time (t90) decrease with the increase of recycled rubber powder content. For saving energy it is important to assess the optimum vulcanization time. With crosslinking time reduction at the same time there is a diminish of heat losses to the surroundings, which enables the energy consumption to be reduced significantly.

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METHODS FOR REMOVING COPPER FROM WASTEWATER

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Abstract

Copper is one of micro nutrients, essential for life. In high concentrations, it can be harmful to life. In this regard, it is necessary to remove it from wastewater streams. During the development of industry, many methods of removal were developed, and are being developed still. This paper gives a short review of existing methods, and their properties to be considered when choosing them.

Keywords: removal methods, copper, wastewater treatment

INTRODUCTION

Rapid development of industry, which affects the availability and quality of surface and groundwater by pollution, creates a need for wastewater treatment. Heavy metals, due to their different chemical properties, affect living organisms differently and thus the toxicity levels are different for each one. Cadmium and mercury are toxic in extremely low concentrations for humans, while toxic concentrations of silver, chromium, copper, zinc are one order of magnitude higher [1,2].

The permissible limit of copper ions in industrial effluents was reported by the United State Environmental Protection Agency (USEPA) to be 1.3 mg/L while it was stated by World Health Organization that copper ions content in drinking water should not exceed 2 mg/l [3].

This paper provides an overview of methods for removing copper out of wastewaters along with a short list of advantages and disadvantages for their application.

METHODS FOR REMOVING COPPER FROM WASTEWATER

Chemical precipitation

Precipitation is the most effective and most common method used in industry. The process is simple and easy to control. It is comprised of adding a reagent, which in reaction with heavy metal ions, makes an insoluble compound which is removed by sedimentation or filtration in the form of sludge. The most common agents for copper precipitation are hydroxides (NaOH, $Ca(OH)_2$), carbonates (Na_2CO_3), sulphides (FeS), hydrogen peroxide (H_2O_2). The main factor of control is pH, temperature and initial ion concentration [4].

When precipitating with calcium hydroxide, it is necessary to reduce the presence of the cupro-hydroxyl ion, which is under given conditions one of the soluble forms. That is achieved by aeration. It was determined that for both NaOH and $Ca(OH)_2$ the optimal pH is over 9, wherein the reduction of concentration from 48 mg/L to 0.69 mg/L is achieved. It is possible to get a better effect by adding a nucleating agent such as ash. Moreover, better removal effects can be achieved by adding coagulation agents such as alum, iron salts or organic polymers [4].

Despite being used often, hydroxide precipitation has a few flaws. Firstly, during precipitation, the formed precipitate is voluminous, which can be problematic during drying and disposal. The second problem occurs when wastewaters contain other metal ions which are amphoteric and pH value which makes one precipitate causes another to dissolve. Thirdly, when complexing agents are present in water, they may interfere with precipitation [4].

Sulphide precipitation is also a fairly common method. The advantage of this method is that metal sulphides are highly insoluble, far more than hydroxides. Moreover, problems associated with amphoterism do not occur with sulphides. Precipitation with sulphides can be applied over a wide range of pH. Precipitation is good even with low pH values pH 2–3. The attained precipitate is not voluminous and dries more easily than hydroxides do. In case multiple metals are present, selective precipitation is possible. Another advantage, compared to the hydroxide method, is that complexing agents despite interfering with precipitation of sulphides do not do so in a great extent. Sources for sulphide ions can be iron(II) sulphide, sodium sulphide and biological sources, where sulphide ions are formed by the reduction of sulphate. The range of efficient precipitation of copper with sulphide ions is up to pH 6 [5,6].

Studies done on Na_2S precipitation showed that substoichiometric concentration ~ 0.6 of the stoichiometric concentration of sulphide and pH>8 are the most adequate conditions for copper(II). Concentrations after precipitation are 0.05–0.1 mg/L. The speed of precipitation is in the function of pH and sulphide concentrations. The second study showed that calcium sulphide has many advantages as a source of sulphide. By controlling pH, it is possible to control the accessibility of sulphate, calcium sulphide also serves as a nucleating agent, and dissolved calcium serves as a coagulation agent. Calcium sulphide is usually obtained by reacting $Ca(OH)_2$ with H_2S or NaHS [5,6].

Problems that can occur in precipitation of sulphide is, firstly, that there is possibility for evolution of hydrogen sulphide gas which is toxic, and because of that precipitation should be carried out at higher pH values, and secondly, the formed precipitate may be colloidal and difficult to remove. The excess hydrogen sulfide should be removed after completion of the process [5,6].

Carbonate precipitation, compared to sulfide precipitation, does not give such good results. Na_2CO_3 is used as the precipitation reagent. It has been confirmed that as the pH increases, the final concentration decreases until pH 7 and then raises again because of the dissolution of the formed precipitate. Carbonate precipitate is more susceptible to leaching than sulphide precipitation. The good thing about carbonate precipitation is that contact time is much shorter than with hydroxide and the reagent is cheaper [7].

It is also possible to use complexing agents for precipitation. For example, potassium ethyl xanthate is used for copper. It provided satisfying results. Dipropyl dithiophosphate is also used. Its removal efficiency is over 99.9% in the range of pH 3–6. The disadvantage of these methods is the price of the reagent that is used [8].

Coagulation and flocculation methods

Coagulation is a process where, by adding an agent, particle surface charge is neutralizes which leads to their agglomeration. While flocculation represents a physical process of light stirring that leads to the agglomeration of destabilized particles. Various coagulation agents, including alum, iron(II) sulphate, iron(III) chloride, sodium aluminate, poly iron sulfate, polyacrylamide, are used for removing copper as well as other heavy metals. Copper coagulation process is most effective on pH>9. The complexing agents greatly interfere with the process on lower values. This process can reduce the concentration of copper to a satisfactory level. Because of the great amount of chemicals used in the coagulation process and the sludge that is created, this method is not the most favorable for the environment. That is why it requires additional treatment of wastewater and a special way of disposing the resulting toxic sludge [9,10].

Flotation methods

Flotation methods are based on the use of surfactant that makes surface inactive substances, surface active. Such material can then be removed by air bubbling. The method was originally developed for the purposes of concentrating ore, however, it can also be implemented in wastewater treatment. Flotation with dissolved air, ionic flotation and precipitation flotation are the main flotation processes for removing metals from the solution.

In copper flotation experiments as surfactants were used: sodium dodecylsulfate, hexadecyl trimethylammonium bromide, sodium oleate etc. When sodium dodecylsulfate was used, it reached 98% efficiency. Hexadecyl trimethylammonium bromide and other cationic surfactants in same conditions did not give such excellent results. It was concluded that the ideal pH for flotation of copper is around 10, which can be explained by precipitation of $Cu(OH)_2$ on this pH value. Anionic surfactants gave good results up to pH 10, above which their activity weakened. On the other hand, cationic did not produce almost any effect up to pH 10, while they had a satisfactory effect above pH 10. This coincides with the stability of ionic species of copper, which at pH<10 is in the form of Cu^{2+}, $CuOH^{+}$ ions, while at pH>10 it is in the form of $Cu(OH)_4^{2-}$ and $Cu(OH)_3^{-}$ ions. The initial concentration of copper at higher concentrations of 25 mg/L had no significant effect on efficiency, while lower values gave lower efficiency. Adding a small quantity of foaming agents increased the efficiency [11].

In another study, the used agent was LIX65N which gave the best results at pH 5.5 and pH 10. One of the methods was introducing a small amount of sulphide and the flotation of the CuS precipitate. Hijamin 2389 and Amid 23 foam stabilizers were used as collectors. The resulting column efficiency was up to 90% [12].

Ion exchange methods

Ion exchange is a very effective process with a fast kinetics. This process is reversible. Ions from the solution are exchanged with ions from the resin. When the ion exchanger becomes saturated, it needs to be regenerate by solution of base or acid, depending on whether it is cationic or anionic. After regeneration, the salt solution has a high concentration of ions that have been previously exchanged and requires a special treatment.

Synthetic resins are most commonly used because they can be produced to meet certain requirements. Inorganic gels were also studied as ion exchangers due to their ability to withstand drastic physical conditions.

Theoretically, any metal ion can be eliminated in this way, however, with ion exchangers, affordability plays a decisive role in commercial use [13–15].

One of the main setbacks of these systems is the need to regenerate the ion exchanger, which complicates the process. Another factor is the price of chemicals used for regeneration and the need for additional treatment of the solution after regeneration. There are efforts to improve ion exchange by use of recyclable chelatation agents, such as EDTA, NTA, and citric acid. It has been shown that these compounds can be used to regenerate strong acid ion exchangers especially for ions that make stable complexes. Optimal pH for regeneration is between 8 and 9 [13–15].

The most common problems which occur with ion exchangers used for this purpose are: the accumulation of matter on its surface, which leads to loss of capacity, presence of free acids in the treated water and high cost of the process [13–15].

Dowex HCR S/S and Dowex Marathon C were usd in copper removal studies. They are cationic type resins. It was determined that Dowex HCR S/S and Dowex Marathon C have a 26.27 mg/g and 46.55 mg/g capacity. It was also found that the equilibrium constant is 1.81 and 4.71. The separation factor for Dowex HCR S/S is $5x10^4$, and $4.4x10^4$ for Dowex Marathon C. The efficiency for both resins was more than 98%. For both resins, the exchange efficiency is higher at lower pH values. The maximum efficiency for Dowex HCR S/S is at pH 4.5 while for Dowex Marathon C it is at 5.5 [13–15].

The possibility of using ion exchangers made from epoxidized soya oil which was then modified with tetraethyltetramine has been studied. This resin contains a carboxyl group that is active in ion exchange. It has shown effective in removing Cu(II), Ni(II) and Co(II). Resin capacity is 3.5 meq/g. While sorption capacity of copper is 192 mg/g, cobalt 78 mg/g and nickel 96 mg/g. Adsorption is greatly influenced by pH. Bellow pH 3, the resin loses its sorption ability and, therefore, diluted acid solutions may be used for its regeneration. This resin showed a higher affinity for copper sorption, so it can be used to separate it from other methal ions [13–15].

Electrochemical methods

The electrolytic extraction of metal from wastewater is based on an electrical current passing through water and depositing metals in elemental form. In addition, there is an electrocoagulation method based on the use of electrodes from which a coagulation agent is formed by passing of the current. Electrochemical wastewater treatment plants require high initial investment and consume a high amount of electricity. However, in recent times they are used more and more because of their efficiency. Many parameters of solution effect the process. For example, pH, temperature, current density, electrode surface area, initial concentration of metal ion etc. A common problem is cathodic polarization due to depletion of the cathode solution. This results in a slower deposition on the cathode, which is eliminated by stirring, heating or increasing the surface of the cathode, or reducing the density of the current.

Cathodic and anodic processes are oxido-reduction processes. When depositing copper, the cathodic and anodic reactions are:

$$Cu^{2+} + 2e^- \rightarrow Cu \quad (1)$$

$$H_2O \rightarrow 2H^+ + 1/2O_2 + 2e^- \quad (2)$$

During Bennion and Newman experiments, they developed a cell with fixed, flowing, porous electrodes, which reduced the concentration of copper in solution from 670 mg/L to a concentration of 1 mg/L. The main limitation was the price of the cell. Bishop and Breton studied the affect of the electrode surface on the removal of copper from diluted solutions with chelated copper. Efficiency was 80–85%. The pH had little influence in the range of 3–11. The initial concentration and solution‘s flow rate had the greatest influence. With the electrochemical process it is possible to reduce the concentration of copper to 0.05 mg/L. Akbal and Camc performed electrocoagulation of copper, where they used Fe–Al electrodes. They achieved a 100% efficiency of copper removal with contacting time of 20 min, current density of 10 mA/cm^2 and pH 3. Escobar et al. used electrodes made of laminated steel to extract copper from natural ground water and wastewaters. Optimal conditions they got were pH 7, flow rate 6.3 cm^3/min and density of current 31–54 A/m^2 [16,17].

One of the electrochemical processes for copper removal is cementation. Through cementation, copper is obtained from the solution in elemental state, by sacrificing a more reactive metal, most often iron. The reaction in this process is:

$$Cu^{2+} + Fe \rightarrow Fe^{2+} + Cu \quad (3)$$

Which results in coating of the iron surface with copper that can easily be removed. Electrode potentials of half–reaction can be used to predict the cementation process. The advantages of the process are: easily controllable, low energy consumption and high purity of the obtained metal.

Patterson and Jancuk have determined that copper cementation is a first order reaction, dissolved oxygen has no effect on the reaction and pH has no effect on the reaction. However, pH over 3 leads to the deposition of iron hydroxide that interferes with copper extraction. In recent times, cementation is done by suspending waste iron in drums through which water that is treated flows. Copper which is formed on the surface is scraped in the form of fine particles while the pieces of iron tumble in side drum [18].

Biological methods

A great amount of studies were done on the topic of biological adsorption of copper. Mostly, they were studies on activated sludge, which gave very good results. They have shown that microorganisms‘ ability to absorb copper increases with pH; however, it is believed that the efficiency of pH higher than 5 is better due to precipitation, not absorption. Copper intake is relatively fast. It has been discovered that biomass has the ability to absorb 0.17 g Cu/g in 10 min.

Shumat *et al*. [19] have found that if the initial concentration of copper is up to 50 mg/L it is possible to remove 80–85% of copper with this procedure. The greatest level of copper absorption is in the pH 7–8 range.

Recently, the influence of higher plants on bio–film and copper absorption in artificially created wetlands has also been studied. They have shown that higher plants do not gave a great influence, some even negatively influence copper absorption, like *J. Articulatus.* Seasons had a greater effect on adsorption due to the influence of temperature on microorganisms activity. There was a decrease in the concentration of copper from 158.5 μg/l to 48 μg/l in the early spring and 13 μg/l was achieved during growing season. Nonetheless, this area requires more research [20].

Adsorption methods

Adsorption of heavy metals has recently gained importance since the available adsorbents and the quality of the resulting water is satisfactory. Sometimes, it is possible to perform desorption and thus regenerate the adsorbents.

Activated carbon is one of the most commonly used adsorbents. Because it has a big amount of micro and meso–pores, it has a large surface area, which is the main factor in adsorption. Since the price of activated carbon has slightly increased due to reduced production, other possibilities are also explored, including activated carbon blends with surfactants, tannins, etc. It is also possible to obtain activated carbon from alternative sources. Kongsuwan *et al*. have researched copper adsorption on activated carbon obtained from the bark of eucalyptus and achieved a maximum copper adsorption of 0.45 mmol/g. Saito *et al*. used activated carbon from sulphonated coal. The adsorption efficiency was over 98%, at a concentration of 312 mg/l, the concentration was decreased to 0.08 mg/l after a two–step extraction [20,21].

CONCLUSION

Depending on the type of industry, economics and level of purification needed, different methods can be applied. One of the fastest and cheapest methods is sulfide precipitation. This method has problems with disposal of precipitated material. Ion exchange and adsorption methods are much cleaner and create a possibility of recycling copper. They need frequent regeneration of the sorbent, sorbent also is not cheap and these methods create high concentration solutions that need treatment, also disposal of the sorbent can be an issue. Electrochemical methods are also clean methods, they have a possibility to recycle copper and do not produce secondary contaminants. Only downside is huge electric energy consumption. Flotation and flocculation methods are very efficient but produce a lot of material that needs adequate treatment. Biological methods are cheap and easy for implementation, however they have a need for a lot of space and they are sensitive to spikes of copper concentration in inflow. When creating heavy metal removal facilities there are many criteria to consider. Also the feasibility of chosen methods depends from case to case.

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REMOVAL OF Cr^{3+} FROM ELECTROPLATING WASTEWATER USING DIFFERENT ADSORBENTS

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Abstract

Many anthropogenic activities produce a large quantity of wastewater with high concentration of harmful substances. In order to avoid harmful consequences on the environment, it is necessary to purify wastewater coming from industrial processes to the required level necessary for its safe discharge into water streams. When choosing a wastewater treatment method, the following should be considered: the method should be eco–friendly, cost effective, easy to use and be highly efficient in removing unwanted substances. In this study, Cr^{3+} *removal from synthetic aqueous solutions was investigated by using the batch adsorption method. The efficiency levels of* Cr^{3+} *adsorption from solutions, which were in chemical composition similar to the chromium bath based on trivalent chromium, were compared using three different adsorbents. The results indicate that the highest efficiency of the process is achieved using synthetic adsorbent (sulfonated polystyrene).*

Keywords: adsorption, Cr^{3+}, phenol-formaldehyde resin, sulfonated polystyrene, bentonite

INTRODUCTION

In developing countries, the industry is concentrated along water streams, meaning that harmful substances are discharged into surface waters without prior treatment [1]. Harmful substances include organic compounds such as textile dyes and pesticides, as well as inorganic chemicals species such as metal ions [2]. The increased quantities of such metals can seriously impair the quality of the environment because they are toxic, persistent and non-degradable [3].

Wastewaters with increased concentrations of chromium come from various industrial processes such as: electroplating, wood protection, leather and textile production, stainless steel production, anodizing and chrome mining [4–6]. A large number of these industries emit large amounts of wastewater in which chromium concentrations vary from 1-10 ppm [5].

The toxic effect of chromium ions on aquatic organisms and mammals has been confirmed. In mammals, the toxic effect of Cr^{3+} is a consequence of its low solubility and mobility in the ecosystem. The International Agency for Research on Cancer (IARC) has identified chromium ions as strong carcinogenic agents that can lead to changes in the DNA transcription process [7]. In humans, chromium ions reach the body through the food chain and can lead to brain damage, edema of the lungs and lung damage [8].

Wastewater remediation costs are high and do not enable the recycling of harmful substances. By using techniques for removing hazardous substances from water streams with the likelihood of them being recycled, the environment is protected and economic profit is achieved [9].

Among the various methods of wastewater treatment, adsorption is mostly applied due to its simple application and high level of efficiency. Natural minerals, agricultural and industrial biosorbents and waste materials as well as synthetic materials are used as adsorbents [10–12].

In this paper, a comparison of efficiency levels in removing chromium ions from spent chromium baths was made using one natural and two synthetic adsorbents. Kaolinite mineral was used as the natural adsorbent and sulfonated polystyrene and phenol-formaldehyde resin as the synthetic adsorbents.

MATERIALS AND METHODS

Preparation of adsorbents

Place 20 mL of concentrated sulfuric acid in a 100 mL round bottom flask. Add 0.03 g of silver sulfate and carefully stir. Warm the mixture to 90ºC on a steam bath, and add 2.0 g of polystyrene beads. Stopper the flask with a cork. Heat the mixture for two hours with occasional stirring. When the reaction period is complete, carefully add the mixture, with stirring, to 100 mL of cold 6 M sulfuric acid. Filter the slurry in a Buchner funnel, and wash with five 10 mL portions of distilled water; after the fifth washing, a portion of the filtrate should be checked with pH paper to ensure the residual sulfuric acid has been removed from the resin. The polymer is then rinsed with two 10 mL portions of anhydrous methanol. Spread the sulfonated resin on a watch glass, and dry to a constant weight by placing in a drying oven held at 105°C for 10-15 min. The solid should be stirred occasionally to prevent charring and facilitate drying. Figure 1 shows the synthesis chemical process of the sulfonated polystyrene.

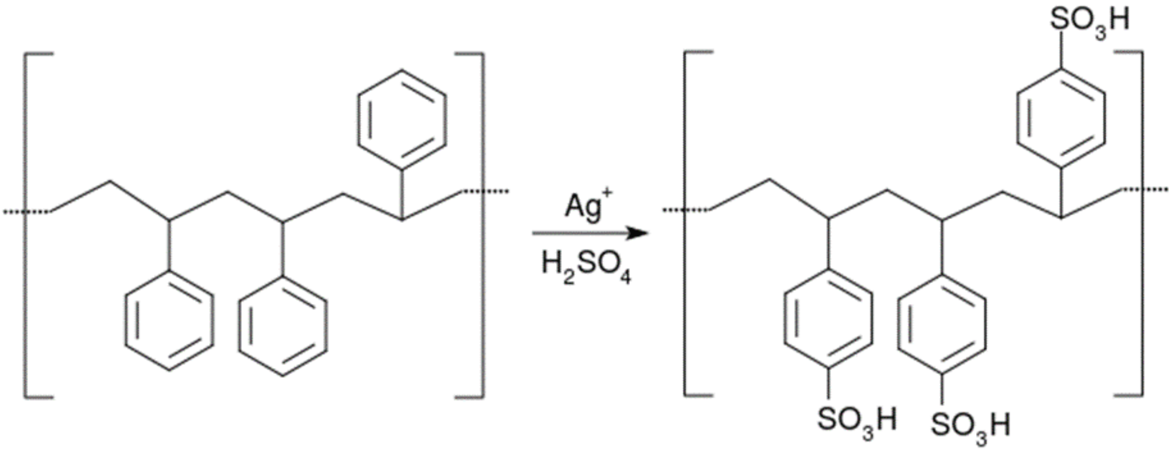

Figure 1 *Sulfonation reaction*

The phenol-formaldehyde resin was synthesized using initial formaldehyde/phenol ratio of 2:1. Sodium-hydroxide 10% solution was used as the catalyst in the synthesis. The synthesis was conducted at 90°C for 2 h in a refluxing flask. After synthesis, the resinous prepolymer products were further cured with heat treatment for 2 h at 100°C. Figure 2 shows the synthesis chemical process of phenol-formaldehyde resin.

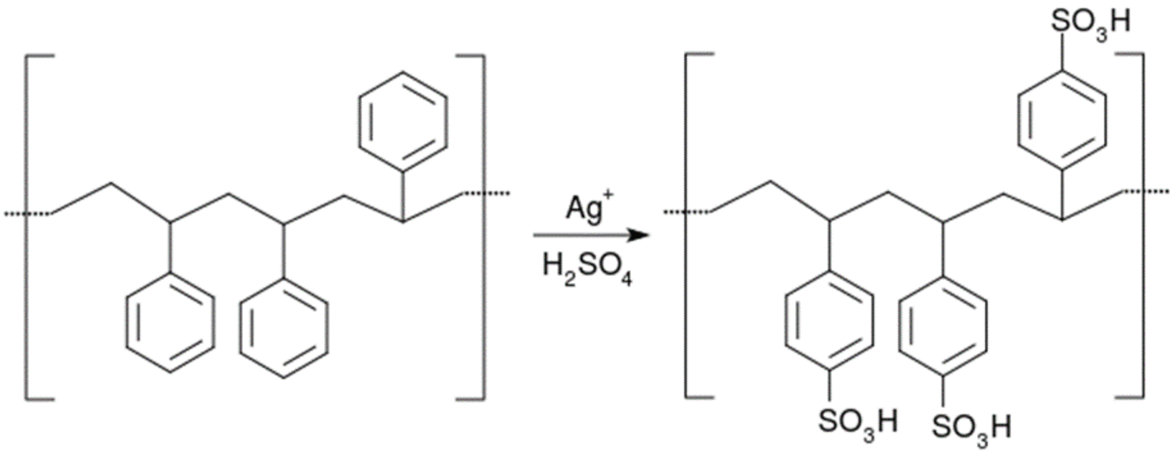

Figure 2 *Preparation of phenol-formaldehyde resin*

The clay used was bentonite produced by Riznica Prirode from Serbia. Clay contains: montmorillonite, kaolinite, illite and muscovite.

Adsorption studies

Stock solutions of 157 mg/L of Cr^{3+} were prepared by dissolving required amount of chromium(III) sulfate ($Cr_2(SO_4)_3$) in double distilled water in a 1L standard flask. To simulate the chrome bath, K_2SO_4, Na_2SO_4, H_3BO_3, maleic acid and thiocarbamide were added to obtain respective concentrations of 150, 100, 60, 10, 0.1 g/L. The pH values of the solutions were adjusted to 3.5 by adding 0.5 M H_2SO_4 or 1 M NaOH. Solution's pH was measured using pH-EC-TDS meter, HI 9812, Hanna instruments. All reagents used in this study were of analytical grade. The stirring speed was constant and fixed at 400 rpm during the test at room temperature (20°C). The experiments were terminated at a contact time of 25 min. At the end of each adsorption period, the adsorbents were filtered through filter paper (Blue ribbon, Chmlab) and the residual Cr^{3+} concentration was analyzed by an Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES) (Optima 8300, Perkin Elmer, USA).

The removal percentage of (R%) Cr^{3+}ions by clay mineral, sulfonated polystyrene, phenol-formaldehyde resin and the adsorption capacity q_e(mg/g) after equilibrium was calculated by equations as follows:

$$R(\%) = \frac{Ci - Ce}{Ci} \cdot 100 \tag{1}$$

$$q_e = \frac{Ci - Ce}{m} \tag{2}$$

where:

C_i is the initial concentration of metal ions in the solution (mg/dm^3),

C_e is the final concentration of metal ions in the solution (mg/dm^3),

V is the volume of the solution (dm^3),

m is the mass of the adsorbent (g).

RESULTS AND DISCUSSION

In Table 1 and Figure 3 a comparison of the levels of Cr^{3+} ion removal was performed using three different adsorbents. The obtained results indicate that the highest removal efficiency percentage of Cr^{3+} was achieved using sulfonated polystyrene at 99%, followed by bentonite with a removal rate at 59.6%, while the lowest level of adsorption was achieved using phenol-formaldehyde resin at 57.67%. Tabekh at al. [13] had achieved a high level of Cr^{3+}removal from contaminated wastewaters, using sulfonated polystyrene as the adsorbent. They have shown that sulfonated polystyrene can also be used for adsorption of other ions from wastewater such as: Cu^{2+}, Co^{2+} i Ni^{2+}.

Table 1 *Removal percentage (R%) Cr^{3+} ions and adsorption capacity (q_e) using different adsorbents*

Type of adsorbent	R (%)	q_e (mg/g)
Sulfonated polystyrene	95	138.6
Bentonite	59.6	3.4
Phenol-formaldehyde resin	57.67	66.45

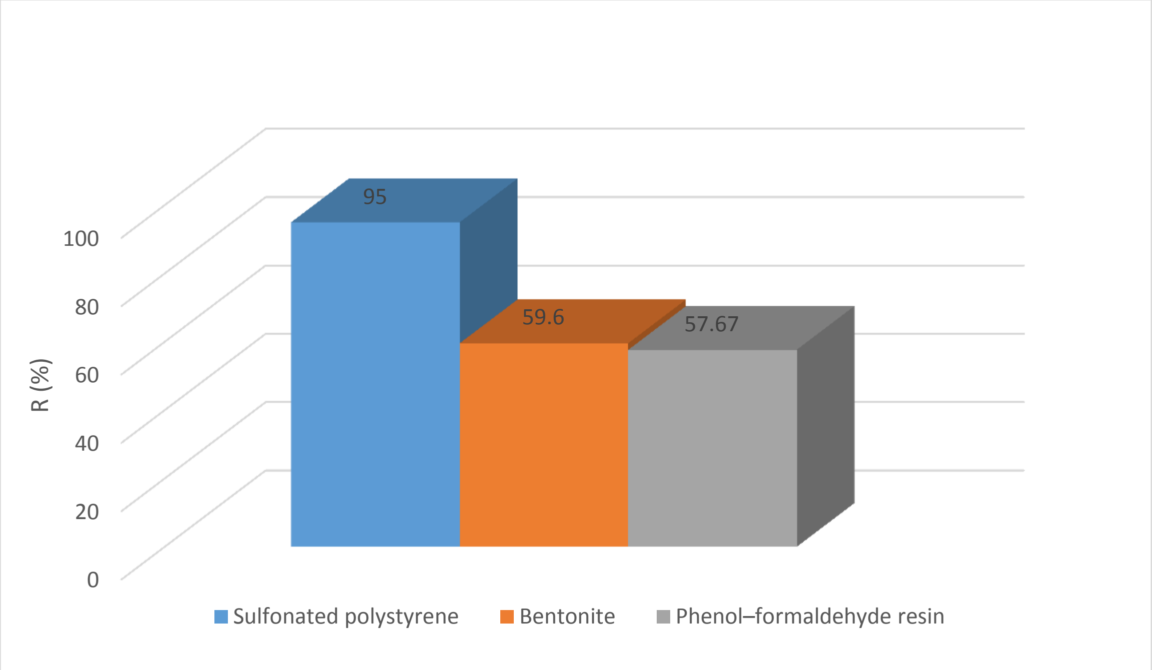

Figure 3 *Removal percentage (R%) Cr^{3+} ions using different adsorbents*

A high level of efficiency in sulfonated polystyrene can be explainedby ion exchange on strongly acid sulfonic groups. Ion exchange also occurs with bentonite, but at low pH values the exchange places on bentonite are less available.Low level of efficiency with phenol-formaldehyderesin can be attributed to the fact that only surface adsorption occurs.

CONCLUSION

The negative characteristics of the chromium bath based on Cr^{6+}, such as high current density and small coverage of the resulting coatings, led to the development of modern baths with trivalent chromium ions. However, the high toxicity of chromic acid used in these baths requires the complete removal of chromium ions from wastewater. This paper examines the possibility of using various adsorbents in order to purify spent water baths. Sulfonated polystyrene showed a high level of Cr^{3+}adsorption efficiency at 95%, but further research is needed in order for the adsorbent to be used in real systems.

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TOXIC FUMES FROM BLASTING

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Abstract

The paper deals with the toxic fumes that may be present in the gaseous products of the detonation process of explosives and blasting agents that pose a health or environmental hazard. The gases that are produced, in theory, during the detonation of commercial explosives are: carbon dioxide, nitrogen and water vapour. However, in practice, there can be deviations from the ideal conditions so detonation may also produce toxic fumes. The issue of toxic fumes from blasting is not only present in underground operations, where the greatest care must be taken when designing and using explosives, but also in surface mine operations.

Keywords: toxic fumes, rock blasting, surface mine, detonation, gaseous product

INTRODUCTION

Explosives are materials that are intended to produce an explosion, i.e., to have the ability to rapidly decompose chemically, thereby producing hot gas which can do mechanical work on the surrounding material [1]. In an ideal detonation, an oxygen-balanced explosive with the atomic constituents carbon, hydrogen, nitrogen and oxygen (CHNO explosives) would form only the gaseous reaction products carbon dioxide (CO_2), water vapor (H_2O) and nitrogen (N_2) [1]. Gaseous products are formed as a result of the very rapid decomposition of explosives in which thermochemical unstable substances are transformed into stable products. Oxidation processes used to burn combustible components of explosive result in large-scale heating of gas explosive products [2].

During the application of commercial explosives in mining operations, there is a greater or lesser deviation from an ideal chemical decomposition of explosives as well as the expected gaseous products of detonation process. The most significant consequences of these deviations are the occurrence of toxic fumes such as carbon monoxide and nitrogen oxides. Sulphur gases, mercury and lead vapors may also occur in relatively small amounts in gaseous products of blasting process when blasting the rocks containing sulphur minerals or ores of mercury, arsenic or lead.

FACTORS AFFECTING THE FORMATION OF TOXIC FUMES BY ROCK BLASTING

B.D. Rossi [2], systematized the causes of formation of toxic fumes by the magnitude of impact, on the basis of laboratory tests carried out in 1966, as follows:

- the properties of rock mass surrounding explosive charge,
- chemical composition of explosives,
- the coating of the explosive cartridge,
- blasting conditions.

Factors affecting the formation of toxic fumes by rock blasting can be generally classified into three basic groups: rock properties, the properties of explosives and blasting conditions.

Influence of rock properties on the quantity of toxic fumes produced by blasting

Z.G. Pozdnjakov and B.D. Rossi [2] were examining the influence of rock properties on the quantity of toxic fumes produced by blasting in 1971. They came to the conclusion that there was no single standard on this basis for different types of explosives. For the Ammonite 6ŽV explosive, taken as an etalon, three classes of rocks have been formed:

a) the first class of rocks (apatite-nepheline ore, potassium salts, copper and molybdenum ores, polymetallic ore) that produce up to 40 l CO /kg of explosives,

b) the second class of rocks (coal, shale, Pb-Zn ore, andesite, Fe ore) that produce 40-100 l CO/kg of explosives,

c) the third class of rocks (the hardest Fe ore) more than 100 l CO/kg of explosives.

Influence of properties of explosives on the quantity of toxic fumes produced by blasting

If a single molecular explosive or a composite explosive composition contains just enough oxygen to oxidize all the hydrogen atoms in the composition to water, all the carbon to carbon dioxide, and all the other fuel components to their highest degree of oxidation, while all nitrogen becomes dinitrogen N_2, then that composition is said to be oxygen balanced, and its oxygen balance is said to be zero [1]. Explosives have a positive oxygen balance if there are free oxygen molecules after complete oxidation of carbon to carbon dioxide and hydrogen to water. Explosives have a negative oxygen balance if there is not enough oxygen in the combustion products to fully oxidize carbon into carbon dioxide, so there is a higher concentration of carbon monoxide. An explosive with excess oxygen produces toxic NO and NO_2, an explosive with an oxygen deficiency produces toxic CO [1].

Table 1 *Oxygen balance of explosive and non explosive materials [2]*

Oxygen balance, (%)			
Ammonium nitrate	+20	Nitroglycol	0
Hexogen	- 21.6	Aluminium powder	- 89
Trinitrotoluene	- 74.0	Tetryl	- 47.4
Nitroglycerin	+ 3.52	Potassium nitrate	+ 39.6

Table 2 shows some calculated results for ANFO when the oxygen balance is shifted from a deficit to an excess of oxygen. The program used was NITRODYNE which does not take into account the formation of NO [1].

Table 2 *Fumes from ANFO with various oxygen balance calculated by thermodynamic computer codes [1]*

Explosive mixture ANFO	Oxygen balance (%)	Calculated gaseous detonation products, (mol/kg)							Heat of reaction at const. pressure (MJ/kg)
		H_2O	CO_2	N_2	CO	H_2	O_2	CH_4	
92/8	- 9.41	26.46	2.42	11.49	3.19	2.58	-	0.03	3.34
94/6	- 2.06	27.59	3.42	11.74	0.81	0.48	-	-	3.69
94.56/5.44	0	27.78	3.83	11.81	-	-	-	-	3.79
95/5	1.61	27.55	3.52	11.87	-	-	0.50	-	3.72
96/4	5.29	27.04	2.82	11.99	-	-	1.65	-	3.18

Explosive containing a stoichiometric mix of fuel and oxidizer minimizes the production of CO and NO_x. If there is an excess of fuel, detonation of the explosive or blasting agent will generate increased quantities of CO. If there is not enough fuel, detonation of the explosive or blasting agent will generate increased quantities of NO_x. Figure 1 illustrates the effect of ANFO fuel oil content on CO and NO_x production, [3].

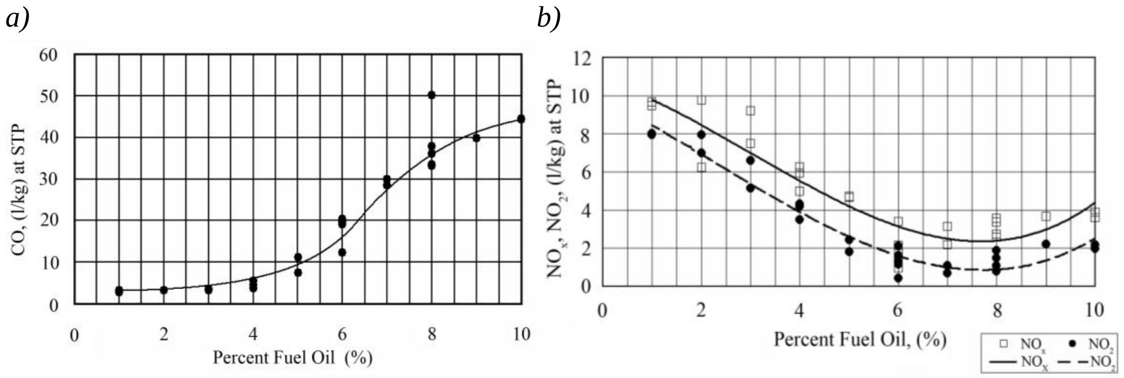

Figure 1 *The effect of fuel oil content on the quantity of carbon monoxide (a) and nitrogen oxides (b) produced by detonating ANFO, (STP- standard temperature and pressure), [3]*

Figure 2 illustrates the cloud of gas and dust from a surface blast in a case when there is no NO_x and when the greatest concentration of NO_x is produced from a blast.

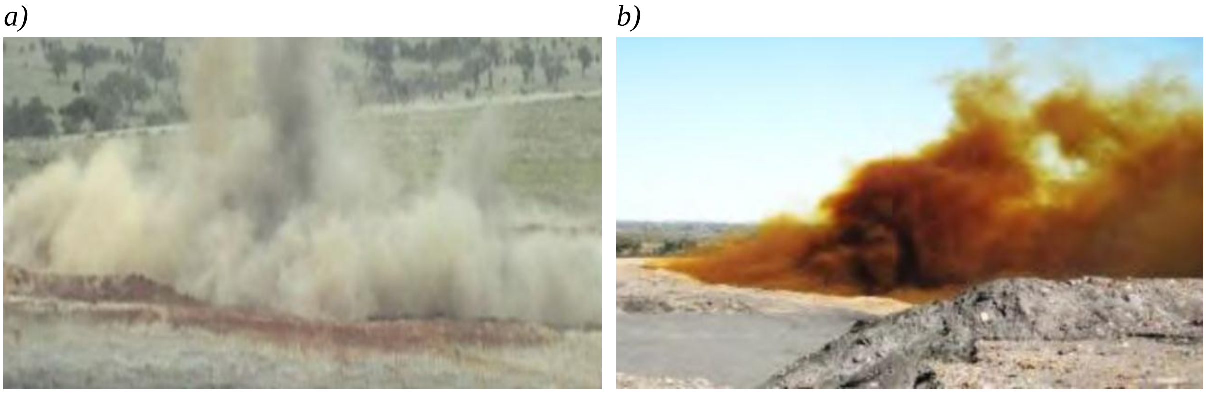

Figure 2 *The cloud of gas and dust from the surface blast; a) no NOx, b) the greatest concentration of NO_x produced from the blast, [4]*

Influence of blasting conditions on the quantity of toxic fumes produced by blasting

Influence of confinement

The creation of the toxic fumes NO_x is heavily influenced by the confinement of the charge. Figure 3 shows the influence of confinement upon the formation of NO_x from the detonation of dynamite.

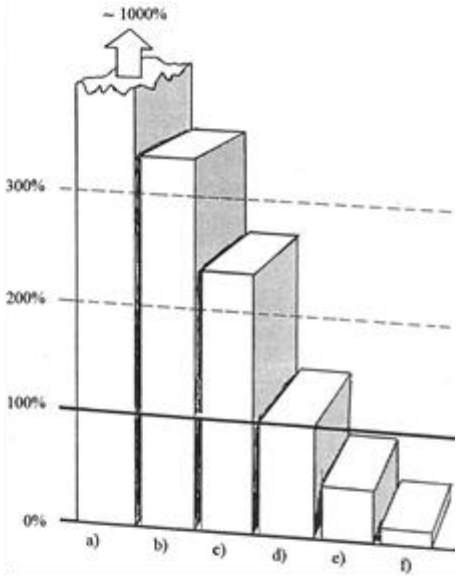

Figure 3 *Influence of confinement upon the formation of NO_x from the detonation of dynamite. 100% is equal to the NO_x formed at blasting a 10 m^2 tunnel round with a specific charge of 3 kg/m^3; a) unconfined charge b) steel tube 11 mm wall thickness, c) steel tube 20 mm wall thickness, d) tunnel round, e)single shot with burden breakage, f)single shot without burden breakage, [5]*

The highest NO_x concentrations are found when firing an unconfined charge in air. The tendency to form CO is also highest for the unconfined charge, but CO formation is less affected than the formation of NO by the degree of confinement. It is obvious that confinement must be considered for any type of fume characterization of explosives.

FUMES CLASSIFICATION OF EXPLOSIVES

In the USA, there are two different fume classifications for explosives. One concerns permissible explosives for use in underground coal mines and one concerns non-permissible. Table 3 shows the US allowable limit values. The US Bureau of Mines measures CO, NO_2, NO, and H_2S. The Institute of Makers of Explosives (IME) values are only for CO and H_2S, [1]. Figure 4 shows two methods proposed by G. Persson for fume classification of explosives.

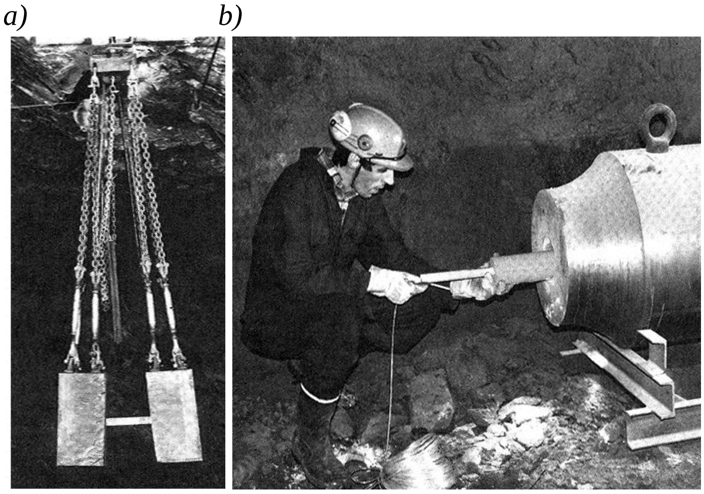

Figure 4 *Methods suggested for fume classification of explosives: a) gas pendulum, b) gas mortar, [5]*

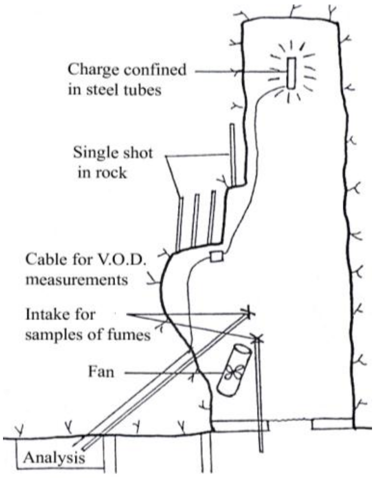

Figure 5 *Test chamber in Stripa Mines [5]*

Table 4 shows the maximum allowable limit values of toxic fumes in air according to JUS Z.B0.001–1971 [6].

Table 3 *Fumes classification of explosives in the USA, [1]*

A. Permissible explosives rated according to the US Bureau of Mines classification (Noxious gases measured: CO, NO_2, NO and H_2S)		B. Non - permissible explosives rated according to the Institute of Makers of Explosives classification (Noxious gases measured: CO and H_2S)	
Fume Class	Noxious Gases (l/kg)	Fume Class	Noxious Gases (l/kg)
A	< 78	1	< 22.5
B	78 - 156	2	22.5 - 46.8
		3	46.8 - 94.9

Table 4 *Maximum allowable limit values of toxic fumes in air according to JUS Z.B0.001–1971, [6]*

Toxic fume	The value of the maximum permitted concentration of toxic fumes in the air	
	mg/m^3	**ppm**
Carbon dioxide	9000	5000
Carbon monoxide	58	50
Nitric oxide	30	25
Nitrogen dioxide	9	5
Hydrogen sulfide	10	7
Sulfur dioxide	10	4
Lead vapor	0.15	-
Mercury vapor	0.10	-
Ammonia	35	50

Gunnar Persson [5] at the Swedish Detonic Research Foundation carried out extensive measurements of toxic fumes and developed a test method which has found extensive use in Sweden. A test chamber in the Stripa Mine in Sweden (Figure 5) was established in 1976. The rock chamber had a volume of 240 m^3. Blasting experiments were carried out at these sites using unconfined charges, charges confined in steel tube, single charges in drill holes in rock with and without breakage, and in full-scale tunnel rounds. Immediately after a blast, the chamber was sealed off and a fan was started to thoroughly mix the fumes with the air inside the chamber. Average emission of fumes from tested commercial explosives is presented in Table 5 [5].

***Table 5** Average emission of fumes from tested commercial explosive, [5]*

Type of gas	% of total volume emitted gas	Comment
H_2O, steam	35 - 65	
N_2, nitrogen	20 - 30	
CO_2, carbon dioxide	10 - 40	Stable gas
H_2, hydrogen	0.5 - 10	Explosive with an oxygen excess
O_2, oxygen	0.1 – 3.0	
CH_4, methane	< 0.5	
CO, carbon monoxide	0.5 - 10	Stable gas
NO_X, nitric oxide	0.1 - 2.0	NO is oxidized to NO_2 which reacts with H_2O and forms HNO_3 and HNO_2
NH_3, ammonia	0.0 - 0.1	
Other gaseous organic species	<0.1	
Lead dust	40 mg/cap	From the caps

MEASURES TO REDUCE THE AMOUNT OF TOXIC FUMES

The prevention of the creation of toxic fumes during blasting can be achieved, as follows:

- using the oxygen balanced explosives with good fumes characteristics,
- making an explosive of such recipes and properties that will correspond to a particular working environment,
- using special neutralizers in blast hole stemming or cartridge coating which are spraying during the detonation and disabling the creation of toxic gases,
- using special agents, for example water curtains in underground blasting operations in order to absorb gaseous products, especially nitrous gases,
- improvement of the methodology of testing and the application of certain types of explosives in special working environments.

CONCLUSION

Toxic fumes which may be contained in reaction products of explosive detonation pose environmental and health hazard not only in underground mine but also in surface mining operations. The increased production in surface mines worldwide led to large-scale blasts where a large quantity of explosives and explosive agents are initiated forming the great clouds of gas and dust.

Toxic fumes are not always present in these clouds and their formation depends on many factors. However, there must be awareness that certain conditions may result in the products of detonation process that may be harmful to humans and the environment. Certain measures must be carried out to reduce the amount of toxic fumes from blasting in underground and surface mining.

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