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SUSTAINABLE FORESTRY **ODRŽIVO ŠUMARSTVO**
COLLECTION 85-86, 2022 ZBORNIK RADOVA 85-86, 2022

DOI: 10.5937/SustFor2285013M
Original scientific paper

ACTIVITY OF ENZYME CATALASE IN PLANTS FROM METAL TAILINGS OF LEAD-ZINC MINE "TREPČA"

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Abstract *The environment of Northern Kosovo and Metohija has been remarkably influenced for a long time by the huge metallurgical complex "Trepča". The technological process of ore exploitation and processing in the Mining and Metallurgical Chemical Company "Trepča" has resulted in a large amount of discharged material, deposited on metal tailings that are mostly located in inappropriate areas, partially covered with vegetation, mostly consisted of several invasive plants. The activity of the enzyme catalase was investigated in plant species collected from abandoned metal tailings "Žitkovac" and compared with the same plant species from the environment from the vicinity of Niš city. Measurements of enzyme activity were carried out in the underground and above-ground parts of plant species Artemisia vulgaris, Cichorium intybus, Erigeron canadensis, Robinia pseudacacia, Medicago sativa, Teucrium chamaedrys, Plantago lanceolata, Rumex acetosella, Tanacetum vulgare and Euphorbia cyparissias using gasometric method. The results have indicated that an increase of catalase activity in tested plants from the metal tailings is possibly a consequence of stress caused by specific environmental conditions.*

Key words: catalase, metal tailings, plants, mine, Trepča.

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AKTIVNOST ENZIMA KATALAZE U BILJKAMA SA METALNOG JALOVIŠTA RUDNIKA OLOVA I CINKA "TREPČA"

Izvod: *Životna sredina Severnog Kosova i Metohije je dugo bila pod jakim uticajem ogromnog metalurškog kompleksa „Trepča“. Tehnološki proces eksploatacije i prerade rude u Rudarsko-Metalurško Hemijskom Kombinatu „Trepča“, rezultirao je velikim količinama ispuštenog materijala, koji se taložio na metalnim jalovištima, koje se uglavnom nalaze na neprikladnim površinama, delimično su obrasle vegetacijom, i uglavnom su sastavljene od po nekoliko invazivnih biljaka. Ispitivana je aktivnost enzima katalaze kod biljnih vrsta sakupljenih sa napuštenog metalnog jalovišta „Žitkovac” i upoređena sa istim biljnim vrstama iz okoline grada Niša. Merenja enzimske aktivnosti vršena su u podzemnim i nadzemnim delovima biljnih vrsta Artemisia vulgaris, Cichorium intybus, Erigeron canadensis, Robinia pseudacacia, Medicago sativa, Teucrium chamaedrys, Plantago lanceolata, Rumex acetosella, Tanacetum vulgare and Euphorbia cyparissias gasometrijskom metodom. Rezultati su pokazali da je izvesno povećanje aktivnosti katalaze u ispitivanim biljkama sa metalnog jalovišta verovatno posledica stresa izazvanog specifičnim uslovima sredine.*

Ključne reči: katalaza, metalno jalovište, biljke, rudnik, Trepča.

1. INTRODUCTION

The territory of Kosovo and Metohija was an object of numerous studies as a region with extremely disturbed ecological conditions caused mainly by the work of the Mining and Metallurgical Chemical Company (MMCC) “Trepča” (Elezović and Elezović, 2010; Jablanović et al., 1985; Milentijević, 2005; Milentijević et al., 2016; Nedeljković and Milentijević, 2006; UNDP in Kosovo, 2011; Trajković, 1995; Trajković et al., 1998). In the period 1965-1985, the MMCC “Trepča” was one of the biggest producers of lead, zinc, silver and gold in Europe. With the mining capacity of up to 10.000 t of metal ores daily, this giant had produced around 120.000 t of raw lead, 100.000 t refined lead, 100 t of silver, 80.000 t electrolyte zinc, 140.000 t mineral fertilizers, 50.000 t super phosphate.

Althought the Metalurgical complex stoped operating at full capacity in 1999, it has been having strong influence on air pollution and registred strong impact on living world (Jablanović et al.,1985; Trajković, 1995; Trajković et al., 1998). The dire legacy of flotation landfills has remained a serious source of pollutants after cessation of mining activities (Milentijević, 2005; Nedeljković and Milentijević, 2006; Elezović and Elezović, 2010; UNDP in Kosovo, 2011; Milentijević et al., 2016).

The technological process of ore exploitation and processing in “Trepča” have resulted in a large amount of discharged material and industrial wastes that remained after the process of extraction of mineral resources. This material was dumped in spoil heaps, mostly located in inappropriate areas (Figure 1). These facilities were without proper construction and monitoring system to keep track on the impacts on the surrounding ecosystems. They have caused an enormous pollution of the local area, including watercourses, rivers, air, and agricultural land

contamination. Flotation tailings “Žitkovac” that was our study area is one of these sites.

The environmental impact of mining waste deposits in the Northern Kosovo and Metohija was studied (Milentijević et al., 2016) and according to these results the tailings “Žitkovac” is the most problematic, covering 26 ha and containing about 3-8.5 million tonnes waste (Elezović and Elezović, 2010). The chemical composition of the tailings “Žitkovac” was investigated showing dominant presence of pyrite (FeS_2, 31.4%), iron (Fe, 22.15%), sulfur (S, 8.2%), arsenic (As, 8.2%), zinc (Zn, 1.62%), manganese (Mn, 7%), lead (Pb, 0.48%) (Milentijević et al., 2010).

Plants that grow at contaminated areas have capacity to adapt to the extreme environmental conditions through changes in morphology and/or physiology. According to Nešić et al. (2005) enzyme catalase takes part in defence mechanism to protect plant from free radicals which are increasingly formed in stressfull conditions, such as exposure to pollution (Radotić and Dugić, 1999). Having in mind harmful effects of contaminated soils on human health, growing interest of scientists but also of the society is development of economically acceptable remediation technologies, including phytoremediation (Stojanović et al., 2010).

Biochemical and physiological changes appear before the morphological and are the first indicators of presence of pollutants in the environment. They are reflected in the higher concentration of certain biochemical parameters, i.e. enzymes, some amino-acids, organic acids, etc. By increasing or decreasing synthesis of certain physiologically active compounds, plans are trying to protect themselves and to survive under adverse conditions.

Biochemical and physiological changes in plants caused by the presence of pollutants are reflected in defense mechanisms that include increased antoxidant activity (Haraguchi et al., 1997) with participaton of enzymes catalase and peroxidase. The substrate of both enzymes is hydrogen-peroxide, produced in different metabolic processes, as reduced form of oxygen, and it can cause several metabolic changes in the plant tissues (Markovic et al., 2015). Because of the high toxicity to living cells, its degradation to nontoxic forms is necessary (Nešić et al., 2005). Catalase breaks down toxic H_2O_2 into H_2O and molecular O_2. This reaction is essentials for plant life. The catalase further oxidizes toxic molecules which include phenol, formic acid, formaldehyde, and alcohol (Markovic et al., 2015). Catalase has an important role in plants defending processes from pollution through elimination of free radicals, which concentration has increased as a response to the presence of heavy metals as pollutants.

Contamination of soil with copper, nickel and other heavy metals had negative influence on the soil enzymes and on the test plants (Wyszkowska et al., 2005a, 2005b, 2008, 2009). The soil enzyme activity use as an indicator of changes in soil properties caused by environmental stress such as heavy metal pollution (Ciarkowska, 2015). Lin et al. (2015) discusse the effect of heavy metal stress on the antioxidant enzymes activities.

While effects of heavy metals on soil enzyme activity in numerous recent studies were observed (Chen et al., 2005; Wyszkowska et al., 2005a, 2008, 2009, 2015; Yang et al., 2006; Khan et al., 2007; Güsler and Erdoğan, 2007; Karaca et

al., 2010; Angelovičovà et al., 2014), lesser investigations were conducted on enzyme activity in plants that grow on heavy metal contaminated soils (Assche and Clijsters, 1990; Clijsters et al., 1999).

The investigations of physiological changes (reflected in the activity of enzyme catalase (CAT; EC 1.11.1.6) in plants that grow in polluted environmental conditions on tailings and comparison to ones from unpolluted area were in the focus of the present study.

2. MATERIAL AND METHODS

2.1. Study area

The study area of the mining waste deposits "Žitkovac" is situated to the north of Kosovo and Metohija on the left bank of river Ibar, in the vicinity of village Žitkovac. Administratively it belongs to the municipality of Zvečan and Kosovska Mitrovica. The landfill was active in period from 1963 to 1974. Flotation tailings are transported from Zvečan, where processed lead and zinc ore from Stari Trg mine (Milentijević et al., 2016).

It has the typical continental climate with long and hot summers and cold winters. According to the meteorological station Kosovska Mitrovica, the average precipitation from 1991-2013 was 637 mm, and average annual temperature 10.3 °C. Mean wind velocity for Kosovska Mitrovica is 1.9 m/s, and the most frequent blowing direction is north with an average velocity of 2.3 m/s and northwesterly wind with an average velocity of 2.1 m/s (Radovanović et al., 2012).

The Ibar River's alluvial plain is the landscape where the metal tailings is situated (Nikić, 2003). Tailing material of the study area shows permanent toxic pollution of water and agricultural land (Nedeljković and Milentijević, 2006).

2.2. Plant material

Enzyme activity assessment was carried out on the woody branches and leaves of invasive plant species *Robinia pseudoacacia* and on the underground and above-ground parts of other plants (*Artemisia vulgaris, Cichorium intybus, Erigeron canadensis, Medicago sativa, Teucrium chamaedrys, Plantago lanceolata, Rumex acetosella, Tanacetum vulgare* and *Euphorbia cyparissias*). The plant material was identified using the key for the regional flora (Josifović, 1970-1986; Velchev, 1982-1989) and the voucher specimens was deposited in the Herbarium Moesiacum Niš (HMN), Department of Biology and Ecology, Faculty of Science and Mathematics, University of Niš (*Table 1*).

Table 1. *Repository data of herbarium specimens of plants used in this study*

Inventory number	Plant species	Location	Habitat	Date
12495	*Artemisia vulgaris* L.	Žitkovac	metal tailings	27.9.2016.
12496	*Cichorium intybus* L.	Žitkovac	metal tailings	27.9.2016.
12500	*Erigeron canadensis* L.	Žitkovac	metal tailings	27.9.2016.
12509	*Robinia pseudacacia* L.	Žitkovac	metal tailings	27.9.2016.
12510	*Medicago sativa* L.	Žitkovac	metal tailings	27.9.2016.
12514	*Teucrium chamaedrys* L.	Žitkovac	metal tailings	27.9.2016.
12516	*Plantago lanceolata* L.	Žitkovac	metal tailings	27.9.2016.
12517	*Rumex acetosella* L.	Žitkovac	metal tailings	27.9.2016.
13114	*Tanacetum vulgare* L.	Niš	ruderal places	29.9.2016.
13115	*Euphorbia cyparissias* L.	Niš	ruderal places	29.9.2016.
16428	*Artemisia vulgaris* L.	Niš	ruderal places	29.9.2016.
16429	*Cichorium intybus* L.	Niš	ruderal places	29.9.2016.
16430	*Erigeron canadensis* L.	Niš	ruderal places	29.9.2016.
16431	*Robinia pseudacacia* L.	Niš	ruderal places	29.9.2016.
16432	*Medicago sativa* L.	Niš	ruderal places	29.9.2016.
16433	*Teucrium chamaedrys* L.	Niš	ruderal places	29.9.2016.
16434	*Plantago lanceolata* L.	Niš	ruderal places	29.9.2016.
16435	*Rumex acetosella* L.	Niš	ruderal places	29.9.2016.
16436	*Tanacetum vulgare* L.	Žitkovac	metal tailings	27.9.2016.
16437	*Euphorbia cyparissias* L.	Žitkovac	metal tailings	27.9.2016.

2.3. Methodology

The plant material was collected in the autumn from the metal tailings "Žitkovac". The same plant species were collected from the uncontaminated area near city of Niš (eastern Serbia). They have served as the control plant group. Plant samples were put into liquid nitrogen in which they transported and then put into a freezer kept at -20ºC where they were stored until the analysis. The underground and above-ground plant parts were separated before the analysis and cut into little pieces.

Catalase activity was measured using the gasometric method (Mosheva, 1982) and expressed as ml of O_2. Detailed description of the method is given in Markovic et al. (2015).

3. RESULTS AND DISCUSSION

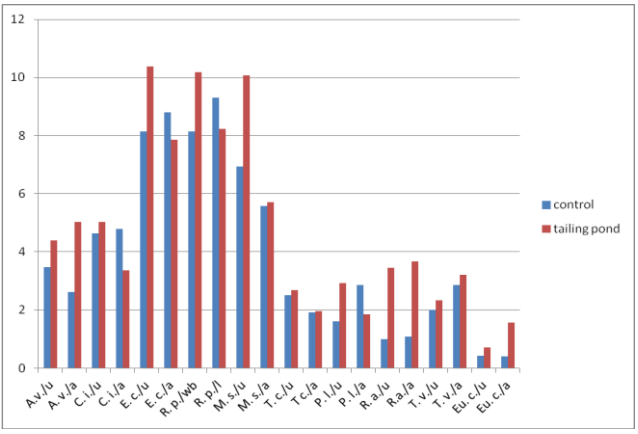
Activity of enzyme catalase was measured in underground and above-ground plant parts from the spoil heaps and from the uncontaminated area which represent a control group. The results, given in *Figure 1*, show that catalase activity is mostly increased in the underground parts of the experimental samples from the tailings, compared to the control samples from the uncontaminated habitats. In

above-ground plant parts the activity of catalase is different in the experimental samples in comparison the control samples. These differences may be related to morphological, anatomical, and chemical structure of investigated plants which are probably genetically conditioned which had been confirmed in previous investigations (Nešić et al., 2005).

In the roots of *Erigeron canadensis* and *Medicago sativa*, and in woody branches of *Robinia pseudoacacia* from the metal tailings the activity of catalase is highest (> 10 O_2/g of fresh matter). In all other samples the catalase activity is lower (< 10 O_2/g of fresh matter).

It was noticed that the activity of catalase in the invasive species *Erigeron canadensis* is increased in the root of the experimental samples, compared to the control samples from the uncontaminated habitat, while the catalase activity in the above ground parts is decreased in experimental samples. The activity of catalase in the woody branches of the invasive species *Robinia pseudoacacia* is increased in the experimental samples in comparison the control samples, while the catalase activity in the leaves is decreased of the experimental samples. The results show a significant increase in catalase activity in the roots of invasive species *Erigeron canadensis* and woody branches of invasive species *Robinia pseudoacacia*. Increased catalase activity in underground parts of invasive plants is probably a consequence of stress caused by chemical changes in soil on the tailings (Jakšić et al., 2017).

Negruckaja and Ermukova (1990) found that the catalase activity in experimantal intoxication increases only in young plants and does not change in plants which are premanently exposed to pollution. In contrast, studies of Trajković (1995) and Trajković et al. (1998) suggest an increase in catalase activity in some plants that are permanently exposed to pollution.



Graph 1. *Activity of enzyme catalase [ml O_2/g of fresh matter] in plants on metal tailings and the uncontaminated area (control).*

Legend: Tested plants: Artemisia vulgaris – A.v., Cichorium intybus – C.i., Erigeron canadensis – E.c., Robinia pseudoacacia – R.p., Medicago sativa – M.c., Teucrium chamaedrys – T.c., Plantago lanceolata – P.l., Rumex acetosella – R.a., Tanacetum vulgare – T.v., Euphorbia cyparissias – Eu.c. Plant parts: u – underground plant parts; a – above-ground plant parts; wb – woody branches; l – leaves

4. CONCLUSIONS

Plants growing at contaminated areas have capacity for adaptation to the environmental conditions through changes in physiology. In present study was noticed that the activity of catalase in underground parts is higher in all samples from the metal tailings, while in above-ground parts is different in comparison to the control samples. An increased catalase activity is a result of stress that is caused by chemical changes in the soil on tailings representing good metabolic way of detoxification, which belongs to the mechanisms of defence and acquiring resistance. The future investigations of biochemical and physiological changes in plants on metal tailings are necessary to better understand impact of soil contamination on machanisms of acquiring resistance on polluted areas. Additional studies are also needed to determine the fate of various compounds in the plant metabolic cycle to ensure that plant droppings and products do not contribute toxic or harmful chemicals into the food chain.

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ACTIVITY OF ENZYME CATALASE IN PLANTS FROM METAL TAILINGS OF LEAD-ZINC MINE "TREPČA"

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Summary

The territory of Kosovo and Metohija has been the subject of numerous studies as a region of extremely disturbed ecological conditions, mainly caused by the work of the Mining and Metallurgical Chemical Combine (RMHK) “Trepča”. The technological process of ore exploitation and processing at RMHK "Trepča" has resulted in a great deal of industrial waste that remains after the extraction of mineral raw materials. This material is disposed of in waste dumps, mostly located in unsuitable areas. The facilities are without appropriate systems for monitoring the surrounding ecosystems. They have caused massive pollution of local areas, including waterways, rivers, air and agricultural land. One of these sites is the flotation tailings pond “Žitkovac”, which is the area of our research.

Catalase activity was determined in the woody branches and leaves of the invasive plant species *Robinia pseudoacacia* and in the underground and aerial parts of other plants (*Artemisia vulgaris, Cichorium intybus, Erigeron canadensis, Medicago sativa, Teucrium chamaedrys, Plantago lanceolata, Rumex acetosella, Tanacetum vulgare* and *Euphorbia cyparissias*). The plant material was identified using keys in the Flora of Serbia, and specimens were deposited in the herbarium of the Department of Biology and Ecology of the Faculty of Science and Mathematics in Niš – Herbarium Moesiacum Niš (HMN).

Catalase activity was measured in the underground and aboveground parts of plants from the “Žitkovac” metal tailings pond and from an uncontaminated area in the vicinity of Niš, representing the control group of samples. The results showed that the activity of catalase was slightly increased in the underground parts of samples from the tailings pond compared to the control samples from unpolluted habitats. In the aerial parts of plants, catalase activity in the experimental samples differed from that in the control

samples. These differences may be related to the morphological, anatomical and chemical structure of the examined plants, which are probably genetically determined.

Plants growing in contaminated areas have the ability to adapt to environmental conditions through changes in their physiology. In this research, it was observed that catalase activity in the underground parts was higher in all samples from the metal tailings pond, while in the aboveground parts it was different compared to the control samples. The increased catalase activity is the result of stress caused by chemical changes in the soil at the tailings dump and is a metabolic pathway of detoxification, which is part of the defense mechanisms and acquisition of resistance. Future studies of biochemical and physiological changes in plants at metal tailings dumps are necessary to better understand the impact of soil contamination on resistance acquisition mechanisms in polluted areas.

AKTIVNOST ENZIMA KATALAZE U BILJKAMA SA METALNOG JALOVIŠTA RUDNIKA OLOVA I CINKA "TREPČA"

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Rezime

Teritorija Kosova i Metohije bila je predmet brojnih proučavanja kao region sa izuzetno poremećenim ekološkim uslovima, uzrokovanim uglavnom radom Rudarsko-Metalurško Hemijskog Kombinata (RMHK) „Trepča". Tehnološki proces eksploatacije i prerade rude u RMHK „Trepča" rezultirao je velikom količinom industrijskog otpada, koji je zaostao nakon procesa vađenja mineralnih sirovina. Ovaj materijal je odložen na gomile otpada, uglavnom locirane na neprikladnim područjima. Ovi objekti su bili bez odgovarajućih sistema za praćenje na okolne ekosisteme. Oni su izazvali ogromno zagađenje lokalnoih područja, uključujući vodotoke, reke, vazduh i zagađenje poljoprivrednog zemljišta. Jedno od ovih nalazišta je flotaciono jalovište „Žitkovac", koja predstavlja naše područje istraživanja.

Aktivnost enzima katalaze određena je u drvenastim granama i listovima invazivne biljne vrste *Robinia pseudoacacia* i u podzemnim i nadzemnim delovima drugih biljaka (*Artemisia vulgaris, Cichorium intybus, Erigeron canadensis, Medicago sativa, Teucrium chamaedrys, Plantago lanceolata, Rumex acetosella, Tanacetum vulgare* и *Euphorbia cyparissias*). Biljni materijal je identifikovan pomoću ključeva u Flori Srbije, a herbarijumkski primerci deponovani su u Herbariumu Departmana za biologiju i ekologiju Prirodno-matematičkog fakulteta u Nišu- *Herbarium Moesiacum Niš* (HMN).

Merena je aktivnost enzima katalaze u podzemnim i nadzemnim delovima biljaka sa metalnog jalovišta „Žitkovac" i sa nekontaminiranog područja u okolini Niša, koji predstavlja kontrolnu grupu uzoraka. Rezultati su pokazali da je aktivnost katalaze neznatno povećana u podzemnim delovima oglednih uzoraka iz jalovišta, u poređenju sa kontrolnim uzorcima sa nezagađenih staništa. U nadzemnim delovima biljaka aktivnost katalaze je drugačija u oglednim uzorcima u poređenju sa kontrolnim uzorcima. Ove razlike mogu biti povezane sa morfološkom, anatomskom i hemijskom strukturom ispitivanih biljaka, koje su verovatno genetski uslovljene.

Biljke koje rastu na kontaminiranim područjima imaju sposobnost prilagođavanja na uslove sredine kroz promene u fiziologiji. U ovom istraživanju uočeno je da je aktivnost katalaze u podzemnim delovima veća u svim uzorcima sa metalnog jalovišta, dok je u nadzemnim delovima različita u odnosu na kontrolne uzorke. Povećana aktivnost katalaze je rezultat stresa koji je uzrokovan hemijskim promenama u zemljištu na jalovištu, što

predstavlja metabolički način detoksikacije, koji spada u mehanizme odbrane i sticanja otpornosti. Buduća istraživanja biohemijskih i fizioloških promena u biljkama na metalnom jalovištu su neophodna da bi se bolje razumeo uticaj kontaminacije zemljišta na mehanizme sticanja otpornosti u zagađenim područjima.