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BIOLEACHING OF ARSENIC AND ANTIMONY FROM MINING WASTE

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Abstract

This paper is a contribution to quantification of bio-leached arsenic and antimony from mining waste collected from impoundment in Slovinky (Slovakia). Autochtonous fungal strain *Aspergillus niger* was used in all bioleaching experiments. The contents of arsenic and antimony in two different samples from the impoundment were 294.7 and 328.2 $mg.kg^{-1}$ As and 225.3 and 285.7 $mg.kg^{-1}$ Sb, respectively. After 21-day cultivation of *Aspergillus niger* on such contaminated substrates, this strain was capable to bioleach, bioaccumulate and biovolatilize both toxic elements.

Key words: Slovinky, arsenic, antimony, bioleaching, microscopic filamentous fungi, biovolatilization, bioaccumulation

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INTRODUCTION

Microorganisms and their metabolic processes are very important parts of biogeochemical cycles of chemical elements. They significantly affect mobility, toxicity, bioavailability a solubility of potentially toxic metals and metalloids in the environment (FAWCETT et al. 2015). Balance between mobility and immobilization of chemical elements, including arsenic and antimony, is affected mainly by microorganisms, their surrounding environment and physic-chemical conditions of this environment. The mine tailings of abandoned mines contain high levels of various toxic metals and metalloids such as arsenic and antimony, which are accumulated in soils, sediments and vegetation (LEE et al. 2011). The arsenic and antimony contents in mine tailings are known to be higher than their contents in contaminated soils (WANG & MULLIGAN 2009). There are many abandoned mining sites, tailing impoundments and waste piles in Slovakia (JURKOVIČ et al. 2011, 2012; HILLER et al. 2012; TÓTH et al. 2013). One of such highly contaminated sites by arsenic and antimony is the tailing

impoundment Slovinky (Slovakia). To remove various toxic metals from highly metal(loid) contaminated substrates, bioremediation techniques have been introduced and used (NARESHKUMAR & NAGENDRAN 2008). To microbial processes increasing mobility of chemical elements in the environment belongs bioleaching of metals and metalloids by bacteria, yeasts and microscopic filamentous fungi, production of chelates mediated by microbial metabolites and siderophores and alkylation (especially methylation) of metals and metalloids followed by their volatilization into the atmosphere (GADD 2004). Bioleaching of metal(loid)s by microscopic filamentous fungi and yeasts is mainly due to heterotrophic metabolism of microorganisms. Among several mechanisms of bioleaching by heterotrophic microorganisms, production and excretion of organic acids by microorganisms into the surrounding environment is very important. Although, bioleaching increases and accelerate releasing of metals and metalloids from rocks, minerals and other solid substrates, evolved citrate and oxalate anions can produce stabile and hardly soluble complexes with various chemical elements leading to immobilization of such elements (FRANCIS et al. 1992).

The aim of the study was to quantify processes of bioleaching, bioaccumulation and biovolatilization of arsenic and antimony by microscopic fungus *Aspergillus niger*.

MATERIAL AND METHODS

The solid material containing high levels of arsenic and antimony collected from the Slovinky tailing impoundment was used for experiments. All samples were collected using standard methods (SOJÁK et al. 2002). The impoundment material was collected from two different sampling points (S1 and S2) from the depth of 20 cm using hand shovel. Each sample was stored in plastic bags under 4 °C (portable refrigerators were used) in the dark. The samples were air-dried and sieved (0.1 mm) under laboratory conditions. All experiments were carried out in 250 ml conic flasks with addition of 1, 10 and 30 g of the impoundment material, 5 ml suspension of spores of microscopic filamentous fungus *Aspergillus niger* and 45 ml of Sabouraud liquid medium (HiMedia, Mumbai, India). Used *A. niger* strain was isolated from the soil occurred in the farm in Kuwait with highly alkaline pH value reaching 8.49. After 15-day cultivation, fungal biomass was separated from the growth medium, washed by distilled water and dried at 40 °C for 5 days to reach constant weight. Residual solid material after cultivation was filtered using filter paper Whatman No. 1 and dried similarly as fungal biomass. Residual growth medium was transferred to volumetric flasks and treated by adding of 5 ml of concentrated HNO_3 to stabilize arsenic and antimony in the solution and as a prevention of re-growth of *A. niger*. There were three replicated runs for each experiment. Non-inoculated controls containing distilled water and desired amount of impoundment material were prepared to determine background volatilization fluxes of arsenic and antimony. Fungal controls were cultivated in the absence of impoundment material. The biomass, culture medium and residual solid material were analyzed for total arsenic and antimony concentrations by HG AAS (hydride generation atomic absorption spectrometry).

RESULTS AND DISCUSSION

The results of bioleaching of the impoundment solid material are shown in Tabs. 1-3. The original concentration of arsenic and antimony in the samples collected from the impoundment were as follows: S1 and S2 – 294.7 and 328.2 $mg.kg^{-1}$ As and 225.3 and 285.7 $mg.kg^{-1}$ Sb, respectively. Total balance of released arsenic and antimony from these two types of substrates consists of leached amount of arsenic and antimony into the growth medium, accumulated amount of arsenic and antimony in fungal biomass, contents of arsenic and antimony in residual substrates and volatilized amounts of arsenic and antimony. The results of volatilized amounts of arsenic and antimony were

calculated as the difference between the content of total arsenic and antimony before cultivation and the sum of arsenic and antimony in the fungal biomass, growth medium and residual solid substrate. According to obtained results, it can be stated that the *A. niger* strain was capable to mobilize arsenic and antimony from the impoundment material. The released amount of arsenic was significantly higher than the amount of antimony. The highest calculated amounts of arsenic and antimony were released using 1 g of substrate (up to 33% for arsenic). The efficiency of bioleaching, expressed by percentage of total amounts of arsenic and antimony released from original substrates, decreased in case of arsenic with the weight of solid substrate. But in case of antimony, it seems that the weight of substrates is not critical (total amounts of antimony released from substrates at three different weights of substrates were very similar ranging from 10.8 to 13.7%.

Tab. 1: Bioleaching of impoundment material from arsenic- and antimony- contaminated substrates (1 g) (Slovinky, Slovakia) by *Aspergillus niger* (n=3)

Sample	Content of As/Sb in medium after cultivation [μg]	Content of As/Sb in biomass [μg]	Volatilized amount of As/Sb [μg]	Total amount of As/Sb released from substrates [%]
S1 1g/As	20.5	21.1	33.3	25.4
S2 1g/As	33.8	14.4	49.2	33.0
S1 1g/Sb	9.0	15.7	19.7	13.6
S2 1g/Sb	6.4	13.5	11.0	13.7

Comments: S1, S2 – different substrates collected from the tailing impoundment Slovinky, concentrations of As and Sb in S1 and S2 were 294.7 and 328.2 $mg.kg^{-1}$ As and 225.3 and 285.7 $mg.kg^{-1}$ Sb, respectively; 1g – amount of added impoundment substrate

Bioleaching of arsenic and antimony in comparison with ILYAS et al. (2012) is markedly lower. They reached 60% leaching of arsenic from the solid substrates by the *A. niger* KBS4 strain. There is lack of information on fungal bioleaching of arsenic and antimony in the recent literature. Slightly more information can be found on biovolatilization of arsenic and antimony by microscopic filamentous fungi. It seems that microscopic filamentous fungi are capable to grow on even extremely high concentrations of arsenic and antimony (SRIVASTAVA et al. 2011) and subsequently they are able to biovolatilize high amounts of arsenic and antimony. This metabolic process of biomethylation of arsenic and antimony followed by volatilization of these elements into the atmosphere can significantly affect biogeochemical cycles of arsenic and antimony. Moreover, biomethylation and biovolatilization towards with bioleaching of arsenic and antimony could be a promising alternative for bioremediation of arsenic- and antimony- contaminated sites.

Tab. 2: Bioleaching of impoundment material from arsenic- and antimony- contaminated substrates (10 g) (Slovinky, Slovakia) by *Aspergillus niger* (n=3)

Sample	Content of As/Sb in medium after cultivation [μg]	Content of As/Sb in biomass [μg]	Volatilized amount of As/Sb [μg]	Total amount of As/Sb released from substrates [%]
S1 10g/As	54.7	27.7	46.0	4.4
S2 10g/As	55.8	17.5	194.0	9.0
S1 10g/Sb	9.0	15.7	19.7	13.7
S2 10g/Sb	6.4	40.4	11.0	13.7

Comments: S1, S2 – different substrates collected from the tailing impoundment Slovinky, concentrations of As and Sb in S1 and S2 were 294.7 and 328.2 $mg.kg^{-1}$ As and 225.3 and 285.7 $mg.kg^{-1}$ Sb, respectively; 10g – amount of added impoundment substrate

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Tab. 3: Bioleaching of impoundment material from arsenic- and antimony- contaminated substrates (30 g) (Slovinky, Slovakia) by *Aspergillus niger* (n=3)

Sample	Content of As/Sb in medium after cultivation [μg]	Content of As/Sb in biomass [μg]	Volatilized amount of As/Sb [μg]	Total amount of As/Sb released from substrates [μg]
S1 30g/As	10.3	6.8	525.7	6.2
S2 30g/As	3.3	5.3	498.9	5.7
S1 30g/Sb	20.7	19.5	672.5	10.8
S2 30g/Sb	17.2	14.3	792.5	12.2

Comments: S1, S2 – different substrates collected from the tailing impoundment Slovinky, concentrations of As and Sb in S1 and S2 were 294.7 and 328.2 $mg.kg^{-1}$ As and 225.3 and 285.7 $mg.kg^{-1}$ Sb, respectively; 30g – amount of added impoundment substrate

CONCLUSION

Understanding of processes, which affected transformations of potentially toxic elements by microorganisms allows not only their effective application in removal of various potentially toxic elements including arsenic and antimony from highly contaminated substrates but also their influence on the distribution of such elements in the nature and their effects on human health.

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REFERENCES

FAWCETT S. E., JAMIESON H. E., KIRK NORDSTROM D. & BLAINE MCCLESKEY R. 2015. Arsenic and antimony geochemistry of mine wastes, associated waters and sediments at the Giant Mine, Yellowknife, Northwest Territories, Canada. Appl. Geochem., doi: http://dx.doi.org/10.1016/j.apgeochem. 2014.12.012.

FRANCIS A. J., DODGE C. J. & GILLOW J. B. 1992. Biodegradation of metal citrate complexes and implications for toxic metal mobility. Nature 356: 140-142.

GADD G. M. 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma 122: 109-119.

HILLER E., LALINSKÁ-VOLEKOVÁ B., CHOVAN M., JURKOVIČ Ľ., KLIMKO T., JANKULÁR M., HOVORIČ R., ŠOTTNÍK P., FĽAKOVÁ R., ŽENIŠOVÁ Z. & ONDREJKOVÁ I. 2012. Arsenic and antimony contamination of waters, stream sediments and soils in the vicinity of abandoned antimony mines in the Western Carpathians, Slovakia. Appl. Geochem. 27: 598-614.

ILYAS S., RUAN C., LEE J. C. & BHATTI H. N. 2012. One step bioleaching of sulphide ore with low concentration of arsenic by *Aspergillus niger* and Taguchi Orthogonal Array Optimization. Biotechnol. Bioeng. 20: 923-929.

JURKOVIČ, Ľ., HILLER E., VESELSKÁ V. & PEŤKOVÁ K. 2011. Arsenic concentrations in soils impacted by dam failure of coal-ash pond in Zemianske Kostolany, Slovakia. Environ. Contam. Tox. 86: 433-437.

JURKOVIČ, Ľ., ŠOTTNÍK P., LALINSKÁ-VOLEKOVÁ B., VOZÁR J., PETRÁK M. & TÓTH R. 2012. Identifikácia environmentálnych rizík z antropogénnych sedimentov (Odkalisko-Slovinky). In: Sanační technologie 15, Pardubice. Sborník konference, Ekomonitor, 125-127.

LEE K. Y., KIM H. A., LEE B. T., KIM S. O., KWON Y. H. & KIM K. W. 2011. A feasibility study on bioelectrokinetics for the removal of heavy metals from tailing soil. Environ. Geochem. Hlth 33: 3-11.

NARESHKUMAR R. & NAGENDRAN R. 2008. Changes in nutrient profile of soil subjected to bioleaching for removal of heavy metals using *Acidithiobacillus thiooxidans*. J. Hazard. Mater. 156: 102-107.

SOJÁK L., CHMIELEWSKÁ E., KRIŠTÍN J., KUBINEC R. & MÁTEL J. 2002. Monitoring kontaminácie životného prostredia. Bratislava: publ. Comenius University in Bratislava, 143 p.

SRIVASTAVA P. K., VAISH A., DWIVEDI S., CHAKRABARTY D., SINGH N. & TRIPATHI R. D. 2011. Biological removal of arsenic pollution by soil fungi. Sci. Total Environ. 409: 2430-2442.

TÓTH R., PETRÁK M., JURKOVIČ Ľ., ŠOTTNÍK P., VOZÁR J. & PEŤKOVÁ K. 2013. Odkaliská Markušovce a Slovinky – aplikácia metodického postupu pre hodnotenie odkaliskových sedimentov pochádzajúcich z úpravy rúd na modelových odkaliskách. Mineralia Slovaca. 45: 125-130.

WANG S. L. & MULLIGAN C. N. 2009. Enhanced mobilization of arsenic and heavy metals from mine tailings by humic acid. Chemosphere. 74: 274-279.

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ECOLOGICAL RISK ASSESSMENT FRAMEWORK

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Abstract
Purpose of this paper is to draft shot information about framework for ecological risk assessment compile according Guidelines and short description of phases from which this method consists. During description of particular procedures, the meaning of used terms is introduced and explained. The framework for risk assessment is presented as a useful tool for risk management and selection of available cleanup and remedy technologies, and costs of alternative actions.

Key words: risk assessment, ecological effects, risk characterization, framework, outputs

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INTRODUCTION

At present, for environmental quality determination are used such tools as environmental and ecological risk assessment (ERA, EcoRA) which framework involves the examination of risks from natural events (flooding, extreme weather events, etc.), technology, practices, processes, products, agents (chemical, biological, radiological, etc.) and industrial activities that may influence ecosystems, animals and people (ANONYMUS 2010, U.S. EPA 1992, 1996). For correct environmental risk assessment, it is important to understand the concept of hazard and risk. Whereas, hazard is any source (substances, machines, energy forms, or the way work is carried out) which can cause harm or adverse effects, risk is the probability that harm will actually be done during the work or by the way something is used. Risk = hazard x exposure (PAUSTENBACH 2002; SUTER & BARNTHOUSE 2006; U.S. EPA 2012).

METHODS

The methods used for ecological risk assessment come out from U.S. EPA Guidance Document (2008) and U.S. EPA Framework Manual (2012) and their structure and aims are analysed in the main part of the article which describes ideas, framework and outputs of ecological risk assessment.

FRAMEWORK AND OUTPUTS OF RISK ASSESSMENT

Environmental risk assessment

In general, the term *environment* represents physical surroundings common for everything living include air, water, land (ANONYMUS 2004). Environmental risk assessment incorporates the risk to all ecosystems, including man, exposed or impacted through these media. It doesn't involve the risks to individuals or the general public initiated by consumer products or work exposure, where other specific legislation applies (SUTER et al. 2005). There are slightly different steps to risk assessment, depending on whether you are performing a human health risk assessment or an ecological risk assessment.

Human health risk assessment is a process that is used to evaluate the toxic properties of the chemicals and to evaluate the conditions under which people are exposed to that chemical. It is used to determine how a population exposed to a substance could be affected in terms of adverse health effects.

An *ecological risk assessment* (EcoRA) is a scientific process used for evaluation of potential negative effects to plants, animals, and the environment from exposure to toxic contaminants. Ecological risk assessment does not involve the negative effects to man or domestic animals (SUTER et al. 2001). During the risk assessment process, actual and predicted potential influences of contaminants on animal and plant populations or communities that occupy or could occupy affected localities (meadows, forests, lakes, wetlands, streams, estuaries) are evaluated. It is necessary to accept ecological risk assessment as a science for identification of the stressors (physical, chemical, biological) that are able through adverse effects affect and modified ecosystems and their structure and vitality (CORMIER et al. 2000). EcoRA is used as a tool for decision making by regulators. All findings and statements incorporate science into risk-based decision making (U.S. EPA 1998; SETAC 2004).

Ecological risk assessment is relatively new science created in the 1980s as an equivalent to human health risk assessments. Its role is to make environmental decisions. The appropriate theory for ecological risk was developed through the 1980s by the staff of the Environmental Sciences Division of Oak Ridge National Laboratory (ANDERSEN et al. 2005). The system of EcoRA come out from that for human health risk assessment but is more complex on the side of ecological systems because it recognizes and identifies different endpoints. Endpoints selection varied in dependence on populations, communities, and ecosystems. Scale and structure of the original environment are critical to assessment of ecological risks. In the framework of the system of ecological risk assessment are likely involved indirect effects and changes in habitats as well as the direct toxic effects (U.S. EPA 2003).

Environmental risk assessment framework

Ecological risk assessment involves following parts: 1) Problem formulation (hazard identification); 2) Analysis phase and 3) Risk characterization (risk estimation).

Problem formulation (hazard identification) – this part represents systematic planning during which are identified cardinal factors to be evaluated in the risk assessment. In this part are collected all available information about the locality and conceptual model, that identifies the stressor characteristics, ecosystems potentially at risk, and ecological effects to be evaluated, form final product. As a *stressor*, can be consider any physical, chemical, or biological entity that can cause an adverse response. *Assessment endpoints* represent particular components of the ecosystem which reflect presence of the stressor with adverse effect on ecosystem. As endpoints, can be chosen individual organisms, population, ecosystems, or habitants with some common characteristic, such as

ingestion or exposure. *Measurement endpoints* represent the actual measurements by which is ecological risk evaluated. Through these endpoints are described mechanisms of toxicity and exposure pathways and they represent a measurable biological response to a stressor. In some cases they can relate with characteristics chosen as the assessment endpoint. The endpoints are very often determined by using laboratory models which involve the contaminants, soil/sediments, plants, and organisms, however, they can be also measured directly (U.S. EPA 2000, 2003).

The analysis phase – during this part of ecological risk assessment is evaluated the exposure of ecological receptors to stressors – *exposure assessment* and the relationships between stressors levels and ecological effects – *ecological effects assessment*. *Exposure assessment* quantifies the exposures of ecological receptors (animals, plants and microorganisms) to site contaminants. During this process, quantitative data about contaminant release, its migration and fate are collected; the receptors that may be exposed are determined; and the concentrations to which the receptors are exposed are measured. Contaminant amount entered into the receptor depends on the following factors:

– the contaminant physico-chemical properties,
– the way the contaminant penetrate into the organism (skin absorption, ingestion, inspiration, etc.),
– the nature of the receptors (behavior, life patterns).

Ecological effects assessment determined relations between the contaminant concentration and the intensity of adverse effect on receptors. This is expressed as "dose – response curve" and provide information about *how much* toxicant is associated with *how strong* adverse effect. For this attribute relationship literature reviews, field studies, and toxicity tests are used. Literature reviews provide information about organisms' variability, their ability to tolerate various toxicants in different concentrations and characteristics about species under study. Field studies offer direct evidence of a link between contamination and ecological effects and toxicity tests are used for direct evaluation the contaminated media effects on the survival, growth and reproduction of test organisms (UNEP/IPCS 1999; SUTER et al. 2001; ASKER 2011; BOTSFORD 2002; BREITHOLTZ et al. 2006).

Risk characterization (risk estimation) – is the final part of risk assessment and is based on results comparison from the exposure assessment with those from the ecological effects assessment. Investigators try to answer the following questions:

– Are ecological receptors really at present exposed to site contaminants at levels capable of causing harm, or is future exposure likely?
– If adverse ecological effects are observed or predicted, what are the types and severity of these effects?
– What are the uncertainties associated with the risk assessment?

The risk assessment process finished with control which could seek following measures to reduce or eliminate the risks identified. The risk characterization provides a risk description through presentation of the risk results in both numerical, graphical and descriptive terms. This step provides information on the confidence the risk assessor has in the results, and identifies a threshold for adverse effects. During all ecological risk assessment parts, investigators use new information and insights to refine their hypotheses which used risk managers for management plans preparation. Risk management can advise to reduce or modify the source, by managing or breaking the pathway and/or modifying the receptor (U.S. EPA 1992, 2000; SUTER et al. 2001).

Environmental characterization

For risk assessment quantification, the function expressed the relationship between toxicity and exposure is used and describes basic relationship between concentration and adverse effect intensity, e.g. "dose – response curve". Each risk assessment process consists of multiple steps, begins with

toxicity and exposure estimation, and break up with risk characterization. The most important are data frrom the analysis phase. The science of ecotoxicology wants to catch up the data needs for ERA process that relies on concentration-dependent benchmarks as its primary assessment of risk. Real links between observed effects at a population level and toxicity data (i.e., dose-response, biomarker, bioassay, etc.) are missing for most chemicals and species. As such, most of the current ERAs utilize toxicity data that are questionable in terms of the reality of application to the species or endpoints being assessed. This is a key limitation of the ERA process to contaminated sites (U.S. EPA 2012).

The basis of toxicological and ecotoxicological characterization is in laboratory studies. These studies are internationally administered and reflect adverse effect to animals or plants exposed to a range of concentrations of the stressor being studied. Toxicity can be characterized by mortality or by sublethal effects within the range of doses tested (ASKER 2011).

An important aspect of toxicological evaluation is determination of the relationship between magnitude of exposure and extent and severity of observed effects commonly referred to as dose-response. From this relation, can be obtain not only dose levels at which adverse effects occur, but also no observed effect concentration (NOEC). For risk assessment, the lowest NOEC, LD50, etc. dose is used to estimate risk.

Exposure represents direct contact between receptor and contaminant in the environment, workplace, at home, or in the air, food, water, or soil constitutes. Exposure concentrations may be either estimated or measured, based on the amounts and manner in which the contaminant is used, its physical and chemical properties, and data from laboratory and field experiments. Exposure assessments determine people, wildlife, and plants to stressors present at the environment. The extension of exposure depends on the type of use (crop, lawn, and garden treatment; mosquito control; indoor pest control, etc.), application method, rate and frequency of application or release, along with the contaminant breakdown, partitioning, and movement in the environment (e.g. COVELLO & MERKHOFER 1993; DWYER et al. 1997). Only if exposure approaches or exceeds dose levels are determined as harmful during ecotoxicological studies, an adverse effect is predicted.

Assessment and characterization of the risk to ecological systems, including a large amount of nontarget aquatic and terrestrial organisms as well as surface and ground water, is younger and more complex science than that of human health risk assessment. Ecological risk assessment covers a greater range of complex issues and more species than does human health risk assessment. It follows fish, aquatic invertebrates, aquatic and terrestrial plants, nontarget insects, birds, wild mammals, reptiles, and amphibians.

Each species within an ecosystem fulfils specific ecological role. The primary producers of the energy in any terrestrial or aquatic ecosystem are plants which are able to capture sunlight, convert it to energy used for new plant growth. They are situated at the beginning of the food chain. When organisms consume plant tissues and are, in turn, consumed by another organism, the energy flows through the food chain. For example, one-celled microscopic organisms – green algae are a staple food for invertebrates – water fleas and mysid shrimp, and they become food for young fish and small fish species. The fish are then consumed by predators such as larger fish, amphibians, birds, and aquatic mammals. Because of the dynamics of the flow of energy, perturbations of the most seemingly minor species may lead to observable (measurable) impact on the entire ecosystem. However, because of the ability of organisms and populations to adapt to perturbations, resilient effects on one or more components of an ecosystem may result in minimal ecological change.

Ecological risk assessments outputs

Ecological risk assessment is using to support the regulation of hazardous waste sites, industrial chemicals, and pesticides, and the management of ecosystems affected by chemical, physical, and biological stressors. While chemical stressors involve all chemical substances produced by man, physical stressors include physical factors, such as fires, dust, or barriers, that result from human activity. As physical stressor, might be, for example, introduced sediments released by logging that smother river-bottom invertebrates, or secondarily, warmer water temperatures that kill salmon or trout. As biological stressors introduced in the environment by human activity could be account exotic species or genetically modified organisms, which may either kill, eliminate, or crossbreed with indigenous species, and so change the ecosystem (U.S. EPA 1996; JÄNSCH et al. 2005; LERCHE & GLÄSSER 2006; ASKER 2011).

Evaluation of such versatile spectrum of stressors requires flexibility. Flexibility allows fill in each phase of risk assessment new facts which provide proponents of ecological risk assessment making effective environmental decisions (U.S. EPA 1992). The quality of the whole process is supported by following features:

- The process is iterative, which allows risk assessors to incorporate new information into the risk assessment.
- Like health risk assessment, it expresses changes in ecological effects as a function of changes in exposure to stressors.
- It evaluates uncertainty in the data and models, which allows risk managers to focus research on areas that will reduce uncertainty.
- It provides a basis for comparing and ranking risks, so that risk managers can focus attention on the greatest risks first.
- The analyses consider both scientific issues and management goals.

Ecological risk assessment and its conclusions, recommendations and proposals are very important and useful for risk management and assist in the selection of site remedies. The ecological risk assessment should identify threshold contamination levels for adverse effects (U.S. EPA 2008, 2012). On the basis of threshold values the effectiveness of remedial technics should be suggest and they can be used for cleanup goals arrangement (U.S. EPA 1992; DUPRAS et al. 2012).

The risk assessment affords information whether a risk is present and define a range or magnitude of that risk. With this information, a site risk manager must interconnect the results from risk assessment with other considerations to make and justify risk management decisions. To other considerations are incorporated existing background levels of contamination, available cleanup technologies, and costs of alternative actions and remedy selections.

CONCLUSION

ERA is a scientific process and involves a critical review of available data for identification and quantification of the risks associated with a potential threat.

Human health and ecological risk assessments often produce preliminary proposals and may be based on limited data and/or very conservative assumptions. The precision and comprehension of the risk assessment depends on quantity and accuracy of research data assumptions. The more precise and comprehensive is the risk assessment and the greater confidence in conclusions is drawn, the more specific risk management and regulatory decisions could be done. However, if initial risk assessments indicate no cause for concern, a more refined risk assessment may not be necessary.

During process of ecological risk assessment scientific information are used to identify potential environmental risks associated with some compounds or products released into the environment or

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used by man. The quality of regulatory decisions depends on documented scientific research, an understanding of the strengths and weaknesses of the specific risk assessment, and sound professional judgment in drawing conclusions from compiled data. The role of risk assessments is precise identification of favourable facts and any details required for compound or product critical evaluation. Clear, concise, and thorough ecological risk assessments provide to decision making process more valuable and permit informed debate on compound/product use; ultimately, the registration of a compound must withstand scientific inquiry, public scrutiny, and legal review.

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REFERENCES

ANDERSEN M.C., EWALD M. & NORTHCOTT J. 2005. Risk analysis and management decisions for weed biological control agents: Ecological theory and modeling results. Biol. Control. 35: 330-337.

ANONYMUS 2004. Environmental Protection Act 1990. Published by The Stationery Office Ltd., UK, 245 p.

ANONYMUS 2010. Screening-Level Ecological Risk Assessment (SLEVA). Upper Columbia River., Parametrix, Inc. 421 p.

ASKER S. 2011. Ecotoxicological test methodology for environmental screening of the European Water Framework Directive's priority substances adjusted to Swedish regional. Degree project in biology. Biology Education Centre, Uppsala Universitet, 113 p.

BREITHOLTZ M., RUDÉN Ch., HANSSON S.O. & BENGTSSON B.-E. 2006. Ten challenges for improved ecotoxicological testing in environmental risk assessment. Ecotoxicol. Environ. Saf. 63: 324-335.

BOTSFORD J.L. 2002. A comparison of ecotoxicological tests. Altern. Lab. Anim. 30: 539-550.

CORMIER S., NORTON S., SUTER G. & REED-JUDKINS D. 2000. Stressor Identification Guidance Document. EPA/822/B-00/025. U.S. Environmental Protection Agency, Washington, DC 20460

COVELLO V.T. & MERKHOFER M.W. 1993. Risk Assessment Methods: Approaches for Assessing Health and Environmental Risks. 2nd Edition, Springer, 319 p.

DUPRAS D.M., EDSON R.S., HALVORSEN A.J., HOPKINS R.H. Jr. & MCDONALD F.S. 2012. Problem residents: prevalence, problems and remediation in the era of core competencies. Am. J. Med. 125: 421-425.

DWYER F.J., DWYER J.J., DOANE T.R. & HINMAN M.L. 1997. Environmental Toxicology and Risk Assessment: Modeling and Risk Assessment, Vol. 6. ASTM Committee E-47 on Biological Effects and Environmental Fate. ASTM International, 564 p.

JÄNSCH S., AMORIM M.J. & RÖMBKE J. 2005. Identification of the ecological requirements of important terrestrial ecotoxicological test species. Environ. Rev. 13: 51-83.

LERCHE I. & GLÄSSER W. 2006. Environmental Risk Assessment: Quantitative Measures, Anthropogenic Influences, Human Impact. Springer, Berlin, 343 p.

PAUSTENBACH D.J. (ed.) 2002. Human and Ecological Risk Assessment: Theory and Practice. Hazard identification. Chapter 2. John Wiley & Sons. New York, NY, pp. 85-150.

SETAC 2004. Ecological Risk Assessment. 3rd Edition, Society of Environmental Toxicology and Chemistry, Pensacola, FL, USA, 4 p.

SUTER G.W. & BARNTHOUSE L.W. 2006. Ecological Risk Assessment. 2nd Edition, CRC Press, 643 p.

SUTER G.W., VERMEIRE T., MUNNS W.R. & SEKIZAWA J. 2001. Framework for the integration of health and ecological assessment. In: WHO/PCS/IRA 2001. Integrated Risk Assessment. WHO/UNEP/ILO The International Programme on Chemical Safety, pp. 1-22.

SUTER G.W., VERMEIRE T., MUNNS W.R. & SEKIZAWA J. 2005. An integrated framework for health and ecological risk assessment. Toxicol. Appl. Pharmacol. 207: 611-616.

UNEP/IPCS 1999. Chemical Risk Assessment. Human Risk Assessment and Ecological Risk Assessment. WHO/PCS/99.2 UNEP, Geneva, Switzerland Section A, B, C, 222 p. http://www.chem.unep.ch/irptc/

Publications/riskasse/C2text.pdf; http://www.chem.unep.ch/irptc/Publications/riskasse/B2text.pdf; http://www.chem.unep.ch/irptc/ Publications/riskasse/A2A4Txtab.PDF (Retrieved August 20, 2015)

U.S. EPA 1992. Framework for Ecological Risk Assessment. EPA/630/R-92/001. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington DC 20460, 41 p.

U.S. EPA 1996. Peer Review Workshop Report on Draft Proposed Guidelines for Ecological Risk Assessment. EPA/630/R-96/002. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC 20460

U.S. EPA 1998. Guidelines for Ecological Risk Assessment. EPA/630/R-95/002F. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC 20460

U.S. EPA 2000. Stressor Identification Guidance Document. EPA/822/B-00/025. U.S. Environmental Protection Agency. Office of Water, Washington, DC 20460, 228 p.

U.S. EPA 2003. Generic Ecological Assessment Endpoints (GEAEs) for Ecological Risk Assessment. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC 20460, 59 p.

U.S. EPA 2004. Generic Ecological Assessment Endpoints (GEAEs) EPA/630/P-02/004F. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC 20460

U.S. EPA 2008. Ecological Risk Assessment. Guidance Document DERR-00-RR-031. Environmental Protection Agency, Division of Environmental Response and Revitalization, State of Ohio, 130 p.

U.S. EPA 2012. Sustainable Futures/P2 Framework Manual 2012. EPA-748-B12-001. 13. Quantitative Risk Assessment Calculations. U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC 20460

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STRUCTURE ANALYSIS AND DIVERSITY OF BACTERIAL COMMUNITY AND THEIR RESISTANCE DETERMINANTS IN A NICKEL-CONTAMINATED SOIL IN SOUTHWEST SLOVAKIA

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Abstract

In this study we aimed to analyse the structure and diversity of overall bacterial community and its resistance determinants from nickel-contaminated soil in Slovakia by both, cultivation-dependent and independent approaches. The phylogeny was reconstructed using partial sequences of 16S rRNA (16S rDNA) and heavy-metal resistance genes from separated isolates and bacterial clones. A total of 518 bacterial sequences obtained from both, isolates and clones, represented 266 species belonging to 8 bacterial phyla: Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Gemmatimonadetes, Proteobacteria (α-, β- and γ-classes), Verrucomicrobia, and one yet unclassified group. In addition, among isolates and clones, 49 different *ncc*A-like genes were found in the final output. Majority of them were assigned to a system of transmembrane metal pumps. Our results demonstrate the fact that the nickel-contaminated soil is able to present very specific heavy-metal resistant bacterial community which can be used in different bioremediation processes.

Key words: bacterial community structure, cultivation-dependent and independent approaches, heavy-metal resistance genes, nickel-contaminated soil, phylogenetic analysis

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INTRODUCTION

Bacteria respond to heavy metals in the environment, either from natural sources or due to anthropogenic activities. Bacteria have been interacting with heavy metals since their early evolutionary history. However, industrial and urban wastes, agricultural applications and also mining activities have resulted in an increased concentration of heavy metals in soils (SHERAMETI & VARMA 2010). Copper, chromium, cadmium and nickel are known to be the most common heavy metals used

and widely spread contaminants of the environment (HUSSEIN et al. 2003; VIRENDER et al. 2010). Different environments contain significant concentrations of heavy metals that are not degraded by the conventional processes in nature, so that heavy metals are accumulated and persist for long time in the environment thus affecting its microbial assemblages (ŠMEJKALOVÁ et al. 2003). In high concentrations they react to form toxic compounds in cells (NIES 1999). Presence of high concentration of toxic heavy metals in environment can cause severe problems to human health (KERAMATI et al. 2011). Nickel is one of the most abundant metals in the earth crust (IYAKA 2011) which is necessary in trace amounts for a variety of metabolic processes but in high concentration causing oxidative stress in the cell. However, microorganisms have evolved several mechanisms that regulate metal ion accumulation to avoid heavy metal toxicity in the presence of this metal. The best known mechanisms of nickel resistance are mediated by efflux pumps such as CnrCBA (cobalt-nickel resistance) from *Cupriavidus metallidurans* CH34, NccCBA (nickel-cobalt-cadmium resistance) and NreB (nickel resistance) from *Achromobacter xylosoxidans* 31A, CznABC (cadmium-zinc-nickel resistance) from *Helicobacter pylori* (SALVADOR et al. 2007).

In general, culture-dependent or independent approaches are often used to study the structure and diversity of microbial communities in different environments, including also extreme, e.g. toxic-metal contaminated environments. Although a vast amount of new approaches based on molecular biology comprise more effective tools for the study of bacterial diversity, cultivation is still indispensable for increasing our understanding of specific organisms (PALLERONI 1997). However, sampling of diverse environments shows that only 0.01 – 1% of cells visible under the microscope will form colonies on a Petri dish (CHO et al. 2004; KELLER & ZENGLER 2004). Thus the remaining majority of cells are "uncultivable" (WAGNER & HORN 2006) and thus uncharacterized (MASON et al. 2012). But, when both approaches are combined, this multi-techniques procedure can provide a more complete picture about the bacterial structure in heavy metal contaminated soils. It seems that development of novel technologies based on enormous progress in next-generation-sequencing, such as single-cell sequencing, gives answers to many questions about functions of individual cells in the environment (SHAPIRO et al. 2013).

In our previous works, a phylogenetic analysis was performed either to determine the structure and diversity of cultivable (KARELOVÁ et al. 2010, 2011), hardly cultivable and previously uncultured bacterial isolates by using a diffusion chamber (REMENÁR et al. 2015) or non-cultivable (HARICHOVÁ et al. 2012) fractions of bacterial assemblages in the same heavy-metal contaminated farmland soil in southwest Slovakia using 16S rRNA (16S rDNA) and heavy-metal resistance genes. Thus, the aim of the present study was to characterise overall bacterial assemblage from the same toxic-metal contaminated soil by using a combination of data from both approaches.

MATERIAL AND METHODS

The soil samples, down to 10 cm depth, were collected from farmland situated nearby a dump containing heavy-metal-contaminated waste (48º16'59''N, 17º43'35''E) in southwest Slovakia. They were transported in autoclaved bags, placed in an icebox and stored at 4 ºC in a refrigerator until use. The content of heavy metals in the soil sample was measured using an atomic absorption spectrometer (PerkinElmer model 403, USA) (KARELOVÁ et al. 2011).

A 10 g portion (wet weight) of the soil was mixed in a sterile 250 mL Erlenmeyer flask with 90 mL of a 0.85% (w/v) salt solution and incubated at 30°C on a shaker incubator at 90 rpm for 2 h (KARELOVÁ et al. 2011).

Bacteria were cultivated and isolated by using both, traditional cultivation techniques (KARELOVÁ et al. 2011) and diffusion-chamber-based approach (REMENÁR et al. 2015). As cultivation medium

either soil-extract agar medium (SEA) or Nutrient agar No. 2 (Biomark, India) was used (KARELOVÁ et al. 2011). The diffusion chambers were prepared according to KAEBERLEIN et al. (2002) with some modifications (REMENÁR et al. 2015). The plates (from both, traditional cultivation and diffusion-chamber) were incubated aerobically at 30°C for either 24 – 48 h or 1 – 2 weeks. The numbers of CFUs were repeatedly counted to ensure that, at the time of isolation, the appearance of new colonies had dropped off. Independently growing colonies from both approaches were selected on the basis of their morphology for further analysis.

Bacterial DNA from soil isolates was isolated using the DNeasy purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions as described in KARELOVÁ et al. (2011). The total DNA from soil samples was isolated with PowerSoil DNA Kit (MO BIO Laboratories, Inc., Carlsbad, Canada) according to the manufacturer's instructions as described in HARICHOVÁ et al. (2012). The resulting high-molecular-weight DNA obtained from both, isolates and soil samples, was used as a template in PCR either with universal 16S rRNA gene primers or with non-specific degenerated *ncc*A primer sets (Tab. 1) as described either in KARELOVÁ et al. (2011) or HARICHOVÁ et al. (2012).

In case when the total soil DNA was used in PCR, the relevant 16S rRNA and *ncc*A-like amplicons were separately pooled, after purification ligated into the pDrive Cloning Vector, and transformed into QIAGEN EZ competent cells using the PCR Cloningplus Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Clones containing the potential inserts either of 16S rRNA or *ncc*A-like genes were screened by PCR with primers M13 (Tab. 1) as described in HARICHOVÁ et al. (2012).

Subsamples of either purified 16S rRNA (16S rDNA) correct PCR amplicons of approximately 696 bp from isolates (from diffusion chamber and Petri-dishes) and soil clones or *ncc*A-like correct PCR products of approximately 581 bp, were sequenced by GATC Biotech, Constance, Germany.

The sequences generated or used in this study have been deposited in the GenBank database under accession numbers as follows: a) for bacterial 16S rRNA (16S rDNA) genes from (i) isolates – from GU935266 to GU935334 (KARELOVÁ et al. 2011), from KC809922 to KC809958, from KJ510963 to KJ511005 and from KJ811542 to KJ811562 (REMENÁR et al. 2015), and (ii) clones – from HM038047 to HM038080 (HARICHOVÁ et al. 2012), from JQ756459 to JQ756488 and from JQ772510 to JQ772513 (this study); b) for *ncc*A-like genes from (i) isolates – from GU935257 to GU935265 (KARELOVÁ et al. 2011), from KF218087 to KF218094 and from KF218096 to KF218099 (REMENÁR et al. 2015), and (ii) clones – from HM038081 to HM038096 (HARICHOVÁ et al. 2012) and from JQ916035 to JQ916046 (this study).

Both, bacterial strain and clones identification and identification of *ncc*A-like gene products and phylogenetic analysis were performed as described in KARELOVÁ et al. (2011) with following modifications: multiple sequence alignments and phylogenetic trees were constructed with the MEGA software (version 5.1, TAMURA et al. 2011). Maximum likelihood method with 100 bootstrap replications was chosen with Tamura-Nei model of substitutions and the resulting tree was presented with the Tree Explorer of the MEGA package.

Diversity of bacterial assemblages was estimated on the base of the number and frequency of bacterial taxon occurrences in the nickel-contaminated soil (HARICHOVÁ et al. 2006).

RESULTS AND DISCUSSION

In this study our research is oriented on the structure and diversity determinations of overall bacterial assemblages and their heavy-metal resistance determinants in nickel-contaminated soil sample by using combining of data obtained from both, on cultivation dependent and independent approaches.

Investigated field site is situated nearby a dump containing heavy-metal-contaminated waste in southwest Slovakia. This area is according to environmental monitoring of Slovakia a part of strongly disturbed environment (BOHUŠ & KLINDA 2010) which contained high concentrations of nickel (2109 mg/kg), slightly above the natural occurrence of cobalt (355 mg/kg) and zinc (177 mg/kg), even too low concentration of iron (35.75 mg/kg) for a normal soil and not a toxic amount of copper (32.2 mg/kg) and cadmium (<0.25 mg/kg). In this area black land soil type predominates which emerged on carbonate fluvial sediments. These sediments include a sufficient amount of basic cations in substrate (for Mg in range from 1.52 to 2.51% and for Ca from 3.84 to 7.2%) as well as adequate amount of high-quality organic compounds (NEŠŤÁK et al. 2007). These soil characteristics suggest that such soil system is rich in buffer abilities and it is able to preserve the near-optimal pH of soil environment (pH/KCl = 7.37, NEŠŤÁK et al. 2007). However, in spite of the fact, that these soil characteristics suggested that the heavy metal bio-availabilities of investigated soil sample cannot be so high, the deleterious effect mainly of nickel, (2109 mg/kg), on micro-organisms could be expected.

A total of 518 sequences (isolates and clones) were divided into 266 species belonging to 8 bacterial phyla and one unclassified group: Acidobacteria, Actinobacteria, Bacteroidetes, Cyanobacteria, Firmicutes, Gemmatimonadetes, Proteobacteria (α-, β- and γ-classes) and Verrucomicrobia (Fig. 1a, 2). We demonstrate here that mainly Actinobacteria, Proteobacteria and Firmicutes predominate and the presence of representatives assigned to Bacteroidetes and Acidobacteria, respectively is a common phenomenon in heavy-metal contaminated soils. In addition, the highest abundance level of γ-Proteobacteria inside of the pool of Proteobacteria is not surprising as well. However, higher abundance of β-Proteobacteria in the pool of this phylum in comparison to α-Proteobacteria is evident (Fig. 1a). According to the fact that the phylum Proteobacteria represented mainly isolates (Fig. 1b), distribution of individual classes is more related to use of cultivation media (KARELOVÁ et al. 2011) and/or cultivation conditions (REMENÁR et al. 2015). It is generally known that the representatives of β-Proteobacteria are isolated in larger numbers by the methods mimicking bacterial natural environment (FERRARI et al. 2005; HOHN et al. 2004), because these methods often prefer the cultivation of slowly-growing k-strategists which are able to live in nutrient-poor conditions (FERRARI et al. 2005; WATVE et al. 2000). Similarly to our results, a few of previous studies using both approaches refer to assignment of isolates to same phyla (KOEPKE et al. 2005; ZHANG et al. 2007; WU et al. 2012). In these types of soils also the representatives of Gemmatimonadetes are often included in bacterial structures (ZHANG et al. 2007; WU et al. 2012). The phylum Verrucomicrobia contains only a few described species, e. g. *Verrucomicrobium spinosum*. Evidence suggests that Verrucomicrobia are abundant within the environment and important, especially to soil cultures (CHO et al. 2004). Furthermore, Cyanobacteria are arguably the most successful group of microorganisms on earth. They are the most genetically diverse; they occupy a broad range of habitats across all latitudes, widespread in freshwater, marine, and terrestrial ecosystems, and they are found in the most extreme niches such as hot springs, salt works, and hypersaline bays. Cyanobacteria fulfil vital ecological functions in the world's oceans, being important contributors to global carbon and nitrogen budgets (STEWART & FALCONER 2008). However, the significance of Actinobacteria within the microbial communities of soils contaminated with heavy metals is unresolved (BAMBOROUGH & CUMMINGS 2009). In contrast, other studies on the impact of heavy metal contamination on bacterial community structure have reported a significant decline in the contribution of Actinobacteria to the bacterial community in a forest soil contaminated with cadmium, copper, zinc and lead (FREY et al. 2006). Similarly, VIVAS et al. (2008) found high dominance indices in the other more polluted soils including heavy metals, indicating the supremacy of populations that may be metabolically more active due to

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the presence of pollutants. Thus, this picture about structure of overall bacterial community in nickel-contaminated soil is not surprising.

However, the part of isolates and clones on overall bacterial community is different. Firstly, while cultivable part of the bacterial community was assigned only to four phyla, uncultured fraction was assigned to seven bacterial phyla (Fig. 1b). This result is in accordance with another study using on cultivation dependent approach in which smaller count of the phyla was obtained but with a high level of their abundances (BOLLMANN et al. 2007). Secondly, the majority of the bacteria (74.9%) were obtained exclusively from the Petri dish material in comparison to 25.1% from the uncultured material, and any one of 16S rRNA (16S rDNA) sequences was obtained using both approaches. This result suggests that the bulk of bacterial microflora from both approaches was unique to their used techniques. In addition, most of clones assigned to same phyla as isolates, i. e. Actinobacteria, Bacteroidetes and Proteobacteria respectively, have created an autonomous part of the common phylogenetic tree together with isolates. Only few of them were found inside of common group with isolates although all these clones were situated on the separate branches of the tree (Fig. 2). Moreover, while among isolates Firmicutes predominated, among clones Actinobacteria. The predominance of Actinobacteria in non-cultivable fraction of bacterial community is not surprising, but the absence of Firmicutes and also very low abundance of Proteobacteria in this fraction of bacterial community was not expected with regard to previous data (KARELOVÁ et al. 2011; REMENÁR et al. 2015). On the other hand, the absence of representatives from Acidobacteria, Gemmatimonadetes, Cyanobacteria and Verrucomicrobia, respectively in cultivable fraction of bacterial community is not surprising as well, because only a few individuals from these phyla were isolated up to now (Fig. 1b). These results underlined the fact that only a minor part of bacteria inside of whatever environment is able to form colonies on cultivation media.

Furthermore, although there were only minor discrepancies in diversity level between these two fractions of bacterial community expressed by diversity indices, the diversity of the overall bacterial community exceeded that obtained from individual fractions of bacterial assemblages (Tab. 2).

These differences in the nearest relatives of the 16S rRNA (16S rDNA) genes between isolates and clones even more emphasized the necessity to use multi-technique approach in order to study the bacterial community structures.

All these bacteria which represent the structure of bacterial community in nickel contaminated soil demonstrated metabolic activities mediated via the different heavy-metal resistance genes. Therefore we aimed also on the analysis of heavy-metal resistance genes carried by bacteria occupied this extreme environment. However, we used only degenerative primer set for *ncc*A gene (nickel, cobalt and cadmium resistance) designed only for conserved gene fragments from Gram-negative bacteria (Tab. 1).

49 different *ncc*A-like genes were found in the final output. Corresponding protein sequences were assigned to 15 clusters on phylogenetic tree representing 6 different types with various level of similarity (Fig. 3). Majority of *ncc*A-like genes (36) were after their translation assigned either to cation efflux system protein or heavy metal efflux pump CzcA which pose very similar system of transmembrane metal pumps, one of two basic strategies for a microbe to survive in metal-contaminated environment (NIES 2003). The remaining 13 *ncc*A-like gene products revealed certain homology either to the AcrB/AcrD/AcrF family proteins, or to the two component transcriptional regulator, or to the transcriptional regulator, LysR family, or to the aspartate-semialdehyde dehydrogenase, respectively. These results suggested that such genes could be involved in active protection against heavy metals and also referred to the relatively high degree of variability among resistance determinant products. Similarly, the *czc*+ and/or *ncc*+ strains were detected in a variety of

soil samples highly contaminated by heavy-metals (WUERTZ & MERGEAY 1997; BRIM et al. 1999). However, majority of *ncc*A-like gene products showed only a low level of similarity (40 – 93%) to known proteins encoded by *ncc*A genes (Fig. 3). These sequences may represent new heavy-metal-resistance protein types.

Similarly to bacteria, the products of the resistance determinants from clones and isolates have created autonomous parts of the common phylogenetic tree, expect of three products (EK-I64-hmr, JH-S23-hmr, JH-S44-hmr) of resistance genes either from isolate EK-I64 or clones JH-S23-hmr and JH-S44-hmr, respectively which were found either inside of common group with clone products or inside of common group with isolate products, respectively although all these isolate and clone products were situated on the separate branches of the tree (Fig. 3). It seems that between isolate and clone resistance gene products have not been found any significant sequential similarity, even if (i) all resistance gene products as from isolates as from clones have originated from Gramm-negative bacteria, except of EK-I46-hmr, which was originated from *Arthrobacter chlorophenolicus*, a representant of Gram-positive bacteria (KARELOVÁ et al. 2011) and (ii) the majority of *ncc*A-like gene product from isolates and clones was assigned to very similar system of transmembrane metal pumps, i. e. cation efflux system protein and/or heavy metal efflux pump CzcA, respectively (Fig. 3). In fact, the tested isolates were clustered either to β-Proteobacteria-cluster or γ-Proteobacteria-cluster which were represented either by *Ralstonia* or *Pseudomonads* on phylogenetic tree (KARELOVÁ et al. 2011; REMENÁR et al. 2015). In addition, both, *Ralstonia* and *Pseudomonas* are known to carry heavy-metal-resistance determinants, first of all against nickel, cobalt, zinc and cadmium (MERGEAY et al. 2003; NIES 2003).

Our results exhibit a relatively high degree of bacterial diversity and of variability among resistance determinant products carried by Gram-negative bacteria. A nickel-contaminated soil is able to present very important reservoir for the new and until now partly unknown bacteria, partly heavy-metal-resistance determinants and their products. Microorganisms able to survive in high concentrations of heavy metals are of great interest as bioremediation agents because they can be used in different transformation and immobilization processes.

However, for more realistic study of the bacterial occupation of heavy-metal-contaminated soil and the occurrence of heavy-metal-resistance determinants originated from bacteria in such contaminated environment will be necessary to realize a metagenomic sequencing and/or novel sequencing-based technologies such as single-cell genomics which will uncover cell lineage relationships in more details (SHAPIRO et al. 2013) as well as the function of a single cell inside of indigenous microbial assemblages (MASON et al. 2012).

CONCLUSION

A nickel-contaminated soil presented very specific heavy-metal resistant bacterial community which exhibits important metabolic activities mediated via the different heavy-metal resistance genes carried by bacteria. However, there is a problem how to establish complex bacterial assemblage. Our results suggested that the use of combination on cultivation-dependent and independent approaches could help to resolve this problem. While use of appropriate cultivation techniques enables us to obtain higher numbers not only of previously uncultivable bacteria but also potentially “new” species or genera carrying different heavy-metal resistance genes, the number of bacterial phyla exceeded that obtained from cultured material. However, it is worth mentioning that a metagenomic sequencing and single-cell genomics will be necessary for more realistic study of the bacterial occupation of heavy-metal contaminated soil and the occurrence of heavy-metal resistance determinants originated from bacteria in the environment contaminated as described in the present study. These results also uncover

the advantage to obtain new strains which are specific for particular contaminated sites, are cultivable, and have high pollutant-degradation activity for their possible use in various biotechnologies.

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REFERENCES

BAMBOROUGH L. & CUMMINGS S. P. 2009. The impact of increasing heavy metal stress on the diversity and structure of the bacterial and actinobacterial communities of metallophytic grassland soil. Biol. Fertil. Soils 45: 273-280.

BOHUŠ P. & KLINDA J. 2010. Environmentálna regionalizácia Slovenskej republiky. Bratislava: MŽP SR, Banská Bystrica, SAŽP, pp. 9-21. (in Slovak)

BOLLMANN A., LEWIS K. & EPSTEIN S. S. 2007. Incubation of environmental samples in a diffusion chamber increases the diversity of recovered isolates. Appl. Environ. Microbiol. 73: 6386-6390.

BRIM H., HEYNDRICKX M., DE VOS P., WILMOTTE A., SPRINGAEL D., SCHLEGEL H. G. & MERGEAY M. 1999. Amplified rDNA restriction analysis and further genotypic characterisation of metalresistant soil bacteria and related facultative hydrogenotrophs. Syst. Appl. Microbiol. 22: 258-268.

CHO J., VERGIN K., MORRIS R. & GIOVANNONI S. 2004. *Lentisphaera araneosa* gen. nov., sp. nov, a transparent exopolymer producing marine bacterium, and the description of a novel bacterial phylum, Lentisphaerae. Environ Microbiol 6: 611-621.

FERRARI B. C., BINNERUP S. J. & GILLINGS M. 2005. Microcolony cultivation on a soil substrate membrane system select for previously uncultured soil bacteria. Appl. Environ. Microbiol. 71: 8714-8720.

FREY B., STEMMER M., WIDMER F., LUSTER J. C. & SPERISEN C. 2006. Microbial activity and community structure of a soil after heavy metal contamination in a model forest ecosystem. Soil Biol. Biochem. 38: 1745-1756.

HARICHOVÁ J., KARELOVÁ E., CHOVANOVÁ K., STOJNEV T., PROKŠOVÁ M., BRINDZA J., BRINDZA P., TÓTH D., PANGALLO D. & FERIANC P. 2006. Comparison of culturable Gram-negative bacterial community structures in the rhizosphere of three fruit plants. Biologia (Bratislava) 61: 663-670.

HARICHOVÁ J., KARELOVÁ E., PANGALLO D. & FERIANC P. 2012. Structure analysis of bacterial community and their heavy-metal resistance determinants in the heavy-metal-contaminated soil sample. Biologia (Bratislava) 67: 1038-1048.

HOHN M. W., STADLER P., WU Q. L. & POCKI M. 2004. The filtration-acclimatization method for isolation of an important fraction of the not readily cultivable bacteria. J. Microbiol. Methods 57: 379-390.

HUSSEIN H., FARAG S. & MOAWAD H. 2003. Isolation and characterization of *Pseudomonas* resistance to heavy metals contaminants. Arab. J. Biotechnol. 1: 13-22.

IYAKA A. Y. 2011. Nickel in soils: A review of its distribution and impacts. Sci. Res. Essays. 6: 6774-6777.

KAEBERLEIN T., LEWIS K. & EPSTEIN S. S. 2002. Isolating "uncultivable" microorganisms in pure culture in a simulated natural environment. Science 296: 1127-1129.

KARELOVÁ E., HARICHOVÁ J. & FERIANC P. 2010. Štruktúra a diverzita kultivovateľnej zložky bakteriálneho spoločenstva v pôde znečistenej ťažkými kovmi. Acta Environmentalica Universitatis Comenianae (Bratislava) 18: 79-91.

KARELOVÁ E., HARICHOVÁ J., STOJNEV T., PANGALLO D. & FERIANC P. 2011. The isolation of heavy-metal resistant culturable bacteria and resistance determinants from a heavy-metal-contaminated site. Biologia (Bratislava) 66: 18-26.

KELLER M. & ZENGLER K. 2004. Tapping into microbial diversity. Nat. Rev. Microbiol. 2: 141-150.

KERAMATI P., HOODAJI M. & TAHMOURESPOUR A. 2011. Multimetal resistance study of bacteria highly resistant to mercury isolated from dental clinic effluent. Afr. J. Microbiol. Res. 5: 831-837.

KOEPKE B., WILMS R., ENGELEN B., CYPIONKA H. & SASS H. 2005. Microbial diversity in coastal subsurface sediments: a cultivation approach using various electron acceptors and substrate gradients. Appl. Environ. Microbiol. 71: 7819-7830.

LANE D. J. 1991. 16S/23S rRNA sequencing. In: Stackebrandt E., Goodfellow M. (eds), Nucleic acid techniques in bacterial systematics, John Wiley & Sons, New York, pp. 115-148.

MASON O. U., HAZEN T. C., SHARON BORGLIN S., CHAIN P. S. G., DUBINSKY E. A., FORTNEY J. L., HAN J., HOLMAN H.-Y. N., HULTMAN J., LAMENDELLA R., MACKELPRANG R., MALFATTI S., TOM L. M., TRINGE S. G., WOYKE T., ZHOU J., RUBIN E. M. & JANSSON J. K. 2012. Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. ISME J. 6: 1715-1727.

MERGEAY M., MONCHY S., VALLAEYS T., AUQUIER V., BENOTMANE A., BERTIN P., TAGHAVI S., DUNN J., VAN DER LELIE D. & WATTIEZ R. 2003. *Ralstonia metallidurans*, a bacterium specifically adapted to toxic metals: towards a catalogue of metal-responsive genes. FEMS Microbiol. Rev. 27: 385-410.

NEŠŤÁK L., BEJDA M., BEZECNÝ M., HAJDÚ Z. & CHMELO S. 2007. Územný plán mesta Sereď zmeny a doplnky 9c. 02/2007, časť C-Komplexná charakteristika a hodnotenie vplyvov na životné prostredie vrátane zdravia, pp. 27-79. (in Slovak)

NIES D. H. 1999. Microbial heavy metal resistance. Appl. Microbiol. Biotechnol. 51: 730-750.

NIES D. H. 2003. Efflux-mediated heavy metal resistance in prokaryotes. FEMS Microbiol. Rev. 27: 313-339.

PALLERONI N. J. 1997. Prokaryotic diversity and the importance of culturing. Antonie Van Leeuwenhoek Int. J. Gen. Mol. Microbiol. 72: 3-19.

REMENÁR M., KARELOVÁ E., HARICHOVÁ J., ZÁMOCKÝ M., KAMLÁROVÁ A. & FERIANC P. 2015. Isolation of previously uncultivable bacteria from a nickel contaminated soil using a diffusion-chamber-based approach. Appl. Soil Ecol. 95: 115-127.

SALVADOR M., CAROLINA G. & JOSE E. 2007. Novel nickel resistance genes from the rhizosphere metagenome of plants adapted to acid mine drainage. Appl. Environ. Microbiol. 73: 6001-6011.

SHAPIRO E., BIEZUNER T. & LINNARSSON S. 2013. Single-cell sequencing-based technologies will revolutionize whole-organism science. Nat. Rev. Genet. 14: 618-630.

SHERAMETI I. & VARMA A. 2010. Soil heavy metals (Soil Biology). In: SHERAMETI I. & VARMA A. (eds.), Series Soil Biology (Book 19). Springer, 492 pp.

STEWART I. & FALCONER I. R. 2008. Cyanobacteria and cyanobacterial toxins. In: Walsh P. J., Smith S. L., Fleming L. E. (eds), Oceans and human health: risks and remedies from the seas, Academic Press, pp. 271-296.

ŠMEJKALOVÁ M., MIKANOVÁ O. & BORŮVKA L. 2003. Effects of heavy metal concentrations on biological activity of soil micro-organisms. Plant Soil Environ. 7: 321-326.

TAMURA K., PETERSON D., PETERSON N., STECHER G., NIE M. & KUMAR S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Mol. Biol. Evol. 28: 2731-2739.

VIRENDER S., CHAUHAN P. K. & KANTA R. 2010. Isolation and characterization of *Pseudomonas* resistant to heavy metals contaminants. Int. J. Pharm. Sci. Rev. Res. 3: 164-167.

VIVAS A., MORENO B., DEL VAL C., MACCI C., MASCIANDAROC G. & BENITEZ E. 2008. Metabolic and bacterial diversity in soils historically contaminated by heavy metals and hydrocarbons. J. Environ. Monit. 10: 1287-1296.

WAGNER M. & HORN M. 2006. The Planctomycetes, Verrucomicrobia, Chlamydiae and sister phyla comprise a superphylum with biotechnological and medical relevance. Curr. Opin. Biotechnol. 17: 241-249.

WATVE M., SHEJVAL V., SONAWANE C., RAHALKA, M., MATAPURKAR A., SCHOUCHE Y., PATOLE M., PHADNIS N., CHAMPHENKA, A., DAMLE K., KARANDIKAR S., KSCHIRSAGAR V. & JOG M. 2000. The 'K' selected oligotrophic bacteria: a key to uncultured diversity? Curr. Sci. 78: 1535-1542.

WU H., ZHAO H., WEN C., GUO Y., GUO J., XU M. & LI X. 2012. A comparative study of bacterial community structures in the sediments from brominated flame retardants contaminated river and non-contaminated reservoir. Afr. J. Microbiol. Res. 6: 3248-3260.

WUERTZ S. & MERGEAY M. 1997. The impact of heavy metals on soil microbial communities and their activities. In: Van Elsas J. D., Trevors J. T., Wellington E. M. H. (eds), Modern Soil Microbiology, Marcel Dekker, New York, pp. 607-642.

ZHANG H. B., YANG M. X., SHI W., ZHENG Y., TAO T. & ZHAO Z. W. 2007. Bacterial diversity in mine tailings compared by cultivation and cultivation-independent methods and their resistance to lead and cadmium. Microbial. Ecol. 54: 705-712.

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CONTENTS OF POTENTIALLY TOXIC METALS AND MAGNETIC SUSCEPTIBILITY OF SOILS ALONG A RURAL – URBAN – RURAL GRADIENT IN BRATISLAVA CITY (SLOVAKIA)

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Abstract

The concentrations of potentially toxic metals (As, Cd, Cu, Hg, Pb, Zn and Fe) and the values of magnetic susceptibility in surface soils were measured along NW-SE gradient in Bratislava city (rural – urban – rural soils). The results indicate that both the contents of potentially toxic metals (PTMs) and the values of magnetic susceptibility decrease with increasing distance from the city centre in both directions. Urban soils are enriched mainly in Cu, Hg, Pb and Zn. Their elevated concentrations in soils within the city are due to accumulation from anthropogenic activities. There was a statistically significant and positive correlation between the mean values of Tomlinson pollution load index (PLI) and the mean values of magnetic susceptibility in soil samples. This correlation shows that the monitoring of magnetic properties of soils can be used as a rapid and non-destructive tool for the effective determination of environmental pollution in urbanized regions affected by anthropogenic activities.

Key words: potentially toxic metals; magnetic susceptibility; urban and rural soil; pollution

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INTRODUCTION

Potentially toxic metals (PTMs) are occurring commonly in soils but in general, their contents are increased in the urban environment due to anthropogenic activities. Two classes of anthropogenic sources of PTMs can be distinguished: (i) point sources that occur in the vicinity of pollution site, and (ii) diffuse sources, when PTMs are dispersed in the environment farther from pollution sources (ČURLÍK & JURKOVIČ 2012).

ZHU & CARREIRO (2004) documented that nitrogen mineralization and nitrification were significantly higher in urbanized regions than in rural regions, whereas total content of P was lower in urban soils than in rural soils. WAGROWSKI & HITES (1997) found that concentrations of polycyclic

aromatic hydrocarbons (PAHs) in soils exhibited a strong dependence on the urban – rural gradient. Similar trends in PAH concentrations in soils were observed also by HILLER et al. (2015). These changes are attributed to the traffic and industrial activities that are dependent on the urbanization level.

Elevated concentrations of PTMs in urban environments can represent an important input source to the human body through the inhalation of soil dust, and especially for children, through the soil ingestion or dermal contact with soil. Several PTMs have adverse health effects, mostly for children, because of their low body weight and developing nervous system (LJUNG et al. 2006).

Measurements of magnetic susceptibility of surface soil horizons were successfully used for the mapping of anthropogenic pollution level in the vicinity of thermal power plants, cement factories or metallurgical plants (ĎURŽA 1999; PETROVSKÝ et al. 2000; YANG et al. 2012). The mapping of magnetic properties at wide regional scale was performed for different regions in many countries, e.g. United Kingdom (BLUNDELL et al. 2009), Germany (SPITERI et al. 2005) and Argentina (CHAPARRO et al. 2008). Soil magnetic measurements can be also used in studies of urban soils (SHILTON et al. 2005; LU et al. 2011), surface sediments (BRANDAU & URBAT 2000; MILIČKA et al. 2002) or in soil investigations of paleo soils and loesses (ĎURŽA & DLAPA 2009; ORGEIRA et al. 2011).

Previous research has shown that environmental magnetic methods can be used not only for the identification of pollution sources but also for the approximate determination and characterization of the pollution level in urban soils (SCHMIDT et al. 2005; SPITERI et al. 2005; D´EMILIO et al. 2007). Measurements of magnetic parameters were successfully used as a rapid method for the identification of sources of environmental pollution and the mapping of spatial and seasonal distribution of urban areas (PETROVSKÝ et al. 2000; MAHER et al. 2008).

MIKĽAJEV & KUDRJAVCEV (1982) recommended soil magnetic measurements to be used as a proxy method, which allows to identify regions with "elevated geochemical anomalies", to reduce considerably the volume of laboratory works and to change flexibly the mapping methodology. An advantage of the magnetic measurement for the identification of environmental pollution is that it is simple, rapid and non-destructive, allowing it to become controlling, monitoring and cheap tool for precise chemical analyses.

The main aim of this work was: (1) to determine the concentration of selected PTMs (As, Cd, Cu, Hg, Pb and Zn) and magnetic properties of surface soils collected from a depth of 0 – 10 cm along NW-SE gradient in Bratislava city (rural – urban – rural soils) and (2) to provide scientific base for the pollution control and consecutive monitoring of urban soils for PTMs.

Soil magnetic susceptibility

The most magnetic horizon in a soil profile is the humus horizon, in which iron and other transition elements are in ferrimagnetic state (MIKĽAJEV & ŽOGOLEV 1990). Almost all soils contain two strongly magnetic minerals: magnetite (including (titano)magnetites) and maghemite (including (titano)maghemites). Ferrimagnetic particles of iron oxides in ashes, forming during high-temperature combustion of fossil fuels, are the most important source of anthropogenic ferrimagnetic materials found in surface soil horizons (FLANDERS 1994). In addition to magnetite, pyrite is the main source of ferrimagnetic materials in coal, where its content reaches up to 15 wt% of the total inorganic fraction present in bituminous coal (MAGIERA & STRZYSZCZ 2000). Pyrite and other iron sulphides are oxidized during coal combustion. Sulphur is released in a gaseous phase, whereas iron is incorporated into ferrimagnetic minerals that are emitted to the atmosphere with other dust particles. Anthropogenic ferrimagnetic materials are transported as dust and aerosols to different distances, and finally deposited on the soil surface. Anthropogenic ferrimagnetic particles are characterized by specific

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morphology (mostly as spheres with the size ranging from unit μm up to hundred μm). Their content in ashes is relatively high (approximately 10 wt%) and magnetic measurements indicate high values of magnetic susceptibility.

The deposition of atmospheric particulates represents one of the most important contribution to the total environmental load of soils. In addition to gaseous phase, potentially toxic metals are adsorbed on solid particulates that consist mainly of blown soil particles and coal particles. According to PETROVSKÝ et al. (2000), these particulates are generally higher than 5 μm but blown clayey particles have smaller dimensions. The mean size of atmospheric particulates ranges from 0.01 μm to 20 μm.

Urban soils

Urban soils are those that occur in urbanized, industrial, traffic, mining and military regions (SOBOCKÁ et al. 2007). Almost 85% of European population lives in urbanized regions. A typical environmental factor acting in urbanized regions is the higher level of pollution in cities than in ambient rural land. It is due to environmental pollution caused by emissions from traffic and industries, and wastes of industrial, municipal and construction origin.

Urban soils are important indicators of the urban environment and serve as a primary sink for chemical pollutants because anthropogenic PTMs and organic pollutants are normally deposited on the soil surface. The accumulation of pollutants in urban soils leads to degradation of soil quality and poses also a health risk for humans and ecosystems (CHEN 2007; ĎURŽA et al. 2013).

Anthropic soils are predominant soil group (they cover 33.5% of the total area) in the region of Bratislava. Initial soils are the second important soil group (they cover 26%), among which fluvisols are the most abundant. Another soil groups in the region of Bratislava are molic soils (21%), occurring in plain parts of the Danubian Lowland, and brown soils (16%) with the predominant occurrence in the Malé Karpaty Mts. Rendzinas (2%) are found in a wider area of Devínska Kobyla and Záhorská Bystrica. Regarding the soil type, the most abundant are fluvisols (21.8%) and anthrosols (19.1%), followed by cambisols (16.3%), cultizems (14.4%) and chernozems (14.0%) (SOBOCKÁ et al. 2007).

Study area

Bratislava city is situated in SW Slovakia and covers three physico-geographical units (MAZÚR & LUKNIŠ 1980): the Malé Karpaty Mts., the Záhorská and Danubian Lowlands. The climate of the Bratislava region is gently warm with mild winter and warm summer. The mean annual temperature is 10.3 °C. The annual precipitation varies between 500 and 650 mm, and north-western winds are prevailing (SOBOCKÁ et al. 2007). The total area of the city is 367.6 km^2 and almost 500,000 inhabitants live permanently in the city. Important sources of environmental pollution in Bratislava city are considered chemical, energetic, engineering, building and glassware industries, as well as municipal waste incinerator and traffic. Another significant source of atmospheric pollution in the city is secondary dust nuisance, which depends on the meteorological conditions, field and agricultural works and the type of surface (WWW.ENVIROPORTAL.SK).

SOBOCKÁ et al. (2007) divided the soils of Bratislava city into ten regions. In this work, the investigated rural – urban – rural soil gradient along the city included six out of ten regions: the Záhorská Lowland, Devínska Kobyla, Malé Karpaty Mts. and its foothill, Karloveská priekopa, Podunajská rovina and built-up area.

Considerable part of the Záhorská Lowland is formed by Neogene sediments that are covered by quaternary sediments. The most widespread soil types are phaeozems, mainly cultizemic. They have the molic Amč-horizon with oxidation signs (aggregates, Fe and Mn nodules), however, without gleyic reduction horizon.

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Devínska Kobyla is formed by Mesozoic sedimentary complex, which consists of marls, dolomites, limestones and sandstones together with marlaceous limestones and schists. These rocks are parent substrate of rendzinas and pararendzinas that cover almost the whole protected region. Modal luvisols originated from loess occur also in some parts of Devínska Kobyla.

The soils of the Malé Karpaty Mts. come from crystalline rocks, mainly schists, diorites, pegmatites and granitic rocks of Bratislava's type. Modal cambisols are predominant soil types up to 500 metres above sea level. The soils have developed metamorphic cambic Bv-horizon formed by brunification process with the occurrence of metal oxyhydroxides. There are also subtypes of cambisols like stagno-gleyic and luvizemic cambisols in lower parts of the Malé Karpaty Mts.

River terraces of Karloveská priekopa towards the Mlynská dolina Valley are covered by quaternary aeolian sediments (sandy and loamy loesses). The residues of chernozems with the dark mollic A-horizon and carbonate content higher than 0.30 wt% can be found in the region.

Sarmatian-Pannonian Neogene sediments of the Podunajská rovina are covered by a complex of Pleistocene and Holocene fluvial sediments represented by sands, gravels, clays and sandstones with small calcareous and manganese aggregates. A large area of Bratislava city is occupied by elevated plain of the Danubian Lowland with no direct contact with groundwater table. Cultizemic chernozems are predominant soil group of the south-western and south-eastern parts of the region.

Built-up area of Bratislava city is characterized by soils strongly affected by anthropogenic activities. The soils are components of ornamental gardens, street alleys, recreational sites, child and school playgrounds, cemeteries, urban parks, etc. The main soil groups of built-up areas are cultizems and anthrosols. Cultizems are soils with strongly modified surface horizon (more than 30 cm) and the nature of these soils can be identified according to the residual native diagnostic horizon.

Soils of new built-up residential areas, historical centre, commercial or industrial zones are considered as anthrosols, i.e. the soils modified by human interventions with the anthrozemic Ad-horizon, which was formed by transported anthropogenic materials of different origin. All subtypes of anthrosols may be found in Bratislava city but the most abundant is the initial anthrosol with 1 – 10 cm thick Ad-horizon and organic carbon content of 0.30 wt% and more.

In this work, a NW-SE gradient along Bratislava city was investigated, with rural soils in NW part of the gradient, urban soils in central parts, and rural soils in SE part of the gradient. The following nine zones were selected (Fig. 1):

- Zones I and IX (rural area) – zone I is situated on NW margin of Bratislava city, i.e. NW from Devínska Kobyla. It reaches the Záhorská nížina Lowland. Zone IX is on SE margin of the city and reaches the Danubian Lowland. The soils in both zones are agriculturally managed, and therefore less affected by anthropogenic factors.
- Zones III, IV, VII and VIII (built-up area) – zones III and IV are situated NW from the city centre. Zone III is Dúbravka and zone IV is Karlova Ves up to the Mlynská dolina Valley. Zones VII and VIII are located SE from the city centre. They are bordered by the Bajkalská Street and the Little Danube Channel, including Podunajské Biskupice. These zones are used mainly for housing and agricultural soils of the zones serve for gardening. The soils are relatively less impacted by anthropogenic activities.
- Zone V (historical city centre) – this zone is characterized by high population density with more than 2000 years old history of settlement, industrial and commercial utilization, as well as by dense traffic. It is bordered by the Mlynská dolina Valley and the Štefánikova Street.
- Zone VI (wider city centre) – the zone has 50–100 years old history of settlement, industrial and commercial utilization and suffers from dense traffic for the last fifteen years. This zone is bordered by the Štefánikova and Bajkalská Streets.

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– Zone II (vicinity of technical glassworks) – zone II is located close to technical glassworks (SZTS), where leaded glass was produced.

All selected zones are approximately 3 km long, with the exception of zone II, which is shorter – vicinity of SZTS (Fig. 1).

MATERIAL AND METHODS

Measurement of soil magnetic susceptibility

Firstly, plant cover of soil sampling sites (1x1 m) was cleared away, and then the sampling sites, if necessary, were adapted in a manner not to be free air space between the instrument and the soil surface, which could distort the measured values of soil magnetic susceptibility.

Magnetic susceptibility values were determined using a kappameter KT-5. The basic part of an instrument is 10 kHz oscillator inductively coupled with a flat exploring coil in a measuring part of the instrument. The frequency of oscillator is measured by coil placed at the distance higher than 30 cm from the soil surface (free space measuring) and by coil enclosed to the soil surface. The magnetic susceptibility is determined by microprocessor on the basis of difference between frequencies and numerically displayed. The objects at a distance of 30 cm from the instrument may affect the measured values. The measurement uncertainty is ±10%.

Soil sampling and laboratory analysis

Soil samples were collected along NW-SE gradient in Bratislava city from different parts of the city, representing rural soils in NW and SE margins of the gradient and urban soils in central part of the gradient. Totally, 57 soil samples were used in this work and the sampling sites are shown in Fig. 1. After clearing the plant cover and measuring the magnetic susceptibility, approximately the first 10 cm of surface soil was taken. Four soil samples were collected from zone I, 5 soil samples from zone II, 6 soil samples from zone III, 3 soil samples from zone IV, 7 soil samples from zone V, 12 soil samples from zone VI, 9 soil samples from zone VII, 7 soil samples from zone VIII and there were 4 soil samples from zone IX.

Before analyses, the soil samples were dried at room temperature, gently disaggregated and passed through a 2 mm sieve. The soils samples were analysed for the occurrence of As, Cd, Cu, Fe, Pb and Zn after total digestion using an acid mixture of HNO_3, HCl and HF. Only exception was Hg, which was determined directly on the solid samples using a Mercury Analyzer AMA 254. Concentrations of other metals and As in the extracts were measured by inductively coupled plasma-atomic emission spectrometry (ICP-AES; Vista AX, Varian) and atomic absorption spectrometry (AAS; Varian SpectrAA 220) equipped with a hydride generation system (VGA 76), respectively. All chemical analyses were done in accredited testing laboratories EL Ltd. in the city of Spišská Nová Ves.

RESULTS AND DISCUSSION

Fig. 2 shows the changes in concentrations of PTMs (As, Cd, Cu, Hg, Pb, Zn and Fe) in the studied gradient. The concentrations of PTMs indicate a clear trend in the gradient of rural – urban – rural soils. As can be seen from Fig. 2, mainly the contents of typical "urban" metals Cu, Hg, Pb and Zn in soil decrease with increasing distance from the city centre. The soils in the city centre (zones V and VI) exhibit significantly elevated concentrations of PTMs when compared to the soils from rural areas (zones I and IX). The same trend is obtained for soil magnetic susceptibility values (Fig. 2). Urban soils are an important sink for many PTMs, originating from different sources, including industrial wastes, emissions from motor vehicles, wastes from coal combustion and other anthropogenic

activities. Elevated concentrations of PTMs, such as Cd, Cu, Pb and Zn in urban surface soils are an evidence of their accumulation from anthropogenic activities. Similar trends in PTM concentrations in soils across cities were obtained also by other authors (WONG et al. 2004; LU et al. 2009) who documented that concentrations of inorganic and organic contaminants within a given city were lower in soils from rural regions than in soils from highly urbanized regions.

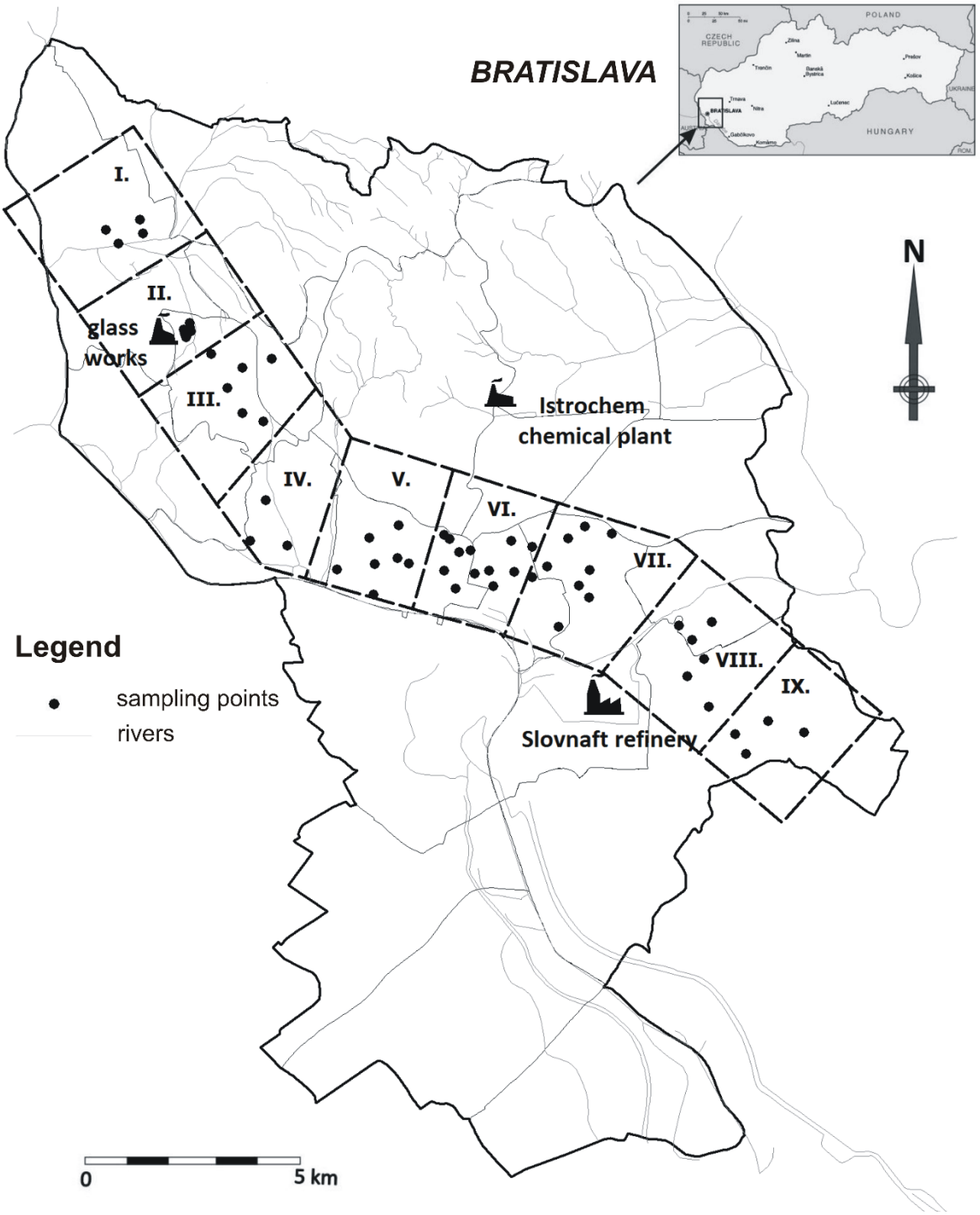

Fig. 1: Location of the soil sampling sites and studied zones along the gradient rural – urban – rural soils in Bratislava city

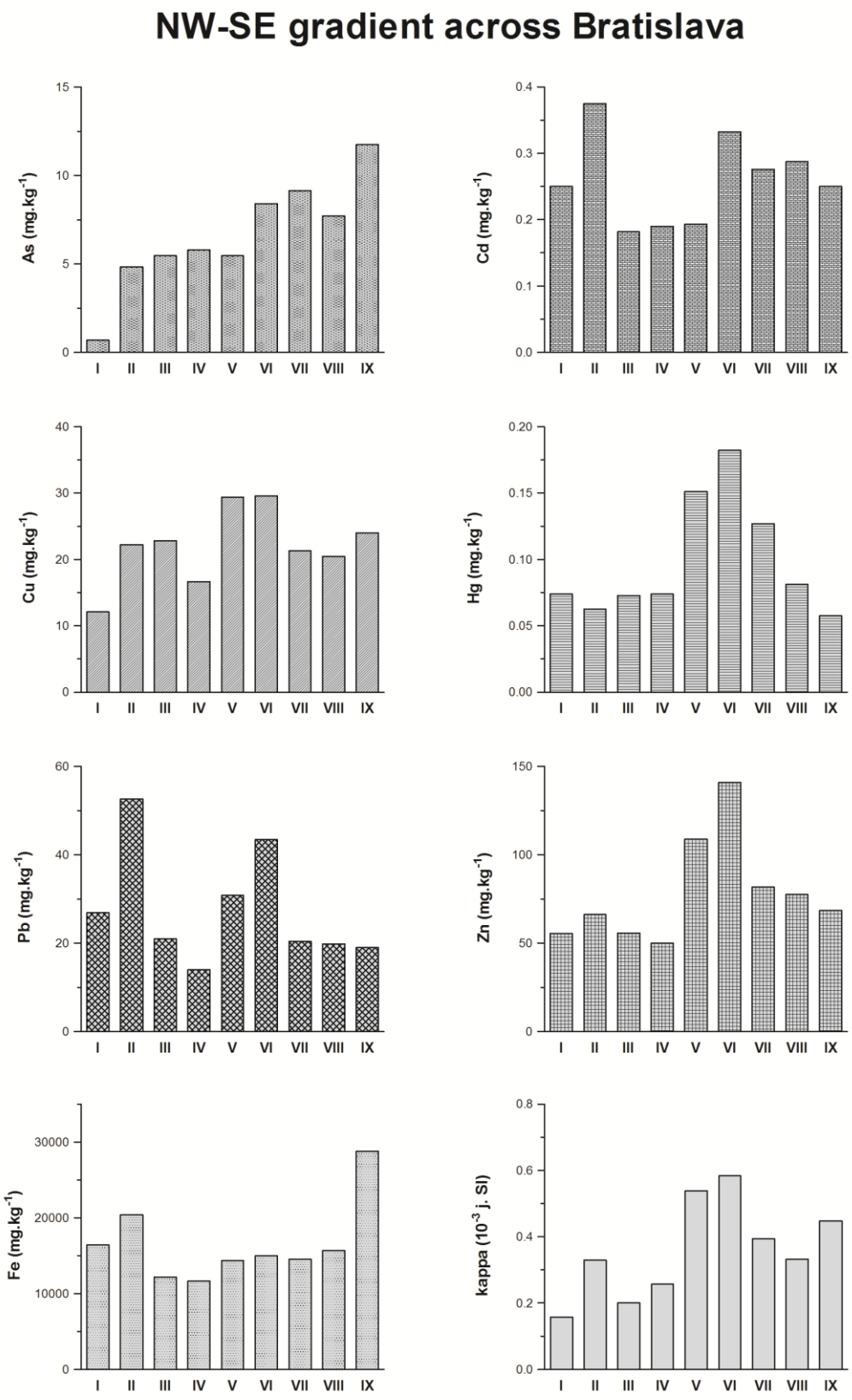

Fig. 2: The effect of distance from the city centre (zones V and VI) on the concentration of potentially toxic metals and the magnetic susceptibility value (kappa) along the gradient rural – urban – rural soils in Bratislava city

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Very interesting are the concentrations of PTMs in soils of zone II, i.e. in the vicinity of technical glassworks, where leaded glass was produced in the past. This is demonstrated by elevated concentrations of Pb in the soils, as well as Cd and Fe. The anomaly in the form of increasing concentrations of Cd, Pb and Fe with increasing distance from the city centre was exhibited also by magnetic susceptibility values (Fig. 2). Another anomaly in general trend of decreasing concentrations of PTMs with increasing distance from the city centre (zones V and VI) was observed for Fe and As in zone IX with the highest mean concentrations of both chemical elements in soils among the selected zones (Fig. 2). This may be explained by long-term effects of petrochemical plant Slovnaft and waste incinerator on the pollution of the surrounding environment.

Notable is also the finding that soil contents of various PTMs (e.g. Cd, Cu, Hg and Zn) and magnetic susceptibility values are higher at SE end of the gradient across the city than at its NW end. This may be due to prevailing NW winds, meaning that the pollution moves from the city centre towards SE, and also due to different soil textures. Sandy soils occur mainly in NW part of Bratislava city, whereas finer, loamy soils are more common in SE part of the city. It is well-known from previous studies (VADJUNINA & BABANIN 1972; HANESCH & SCHOLGER 2005; YANG et al. 2015) that soils with the higher silt content and organic carbon content have usually also higher concentrations of PTMs and values of magnetic susceptibility in comparison to sandy soils.

The change in concentrations of PTMs in soils of different zones across urbanized area can be attributed to the distinct pollution sources and the history of urban land use. Additionally, atmospheric deposition of urban dust, containing elevated concentrations of PTMs, is one of the main sources for the accumulation of toxic metals in urban soils (LI et al. 2001; NG et al. 2003; NORRA & STUBEN 2003). The effect of urban dust on the soil magnetic susceptibility values was studied at sites monitored for the content of solid particulates (PM_{10}) that are freely dispersed in the air (MALOVCOVÁ 2014) (Table 1). Particulate matter PM_{10} is defined as particles, from which 50% has an aerodynamic diameter less than 10 μm. Because of their large surface area, these particles produced by anthropogenic activities are effective adsorbents and accumulators of chemical pollutants like PTMs or organic compounds (e.g. polycyclic aromatic hydrocarbons and polychlorinated biphenyls) (TAN et al. 2006; MENICHINI et al. 2007; MURÁNSZKY et al. 2011).

Tab. 1: Comparison of measured magnetic susceptibility values in the soils at automatic monitoring stations of Slovak Hydrometeorological Institute with the measured air concentrations of PM_{10} particles in 2012

Location	**kappa** (10^{-3} j.SI)	**PM_{10}** ($\mu g.m^{-3}$)
Jeséniova Street	0.58	25.7
Mamateyova Street	0.30	25.1
Trnavské mýto	0.87	35.6
Kamenné námestie	0.39	25.8

With respect to the low number of monitored sites for the content of PM_{10} particles, the data in Table 1 are not sufficiently representative, nevertheless, they provide an approximate pattern about possible relationship between the content of PM_{10} particles and the soil magnetic susceptibility. The relationship between the values of soil magnetic susceptibility and PM_{10} content in air was significant with Pearson correlation coefficient of 0.90. Deposition of particulate matter of anthropogenic origin contains a high proportion (usually 5 – 9 wt%) of strongly magnetic particles (ROBERTSON et al. 2003; KAPIČKA et al. 2004).

Trnavské mýto belongs to crossroads with the highest traffic volume, corresponding also to the content of PM_{10} particles (35.6 $\mu g.m^{-3}$), which was the highest among the above mentioned monitored

sites (Table 1). The highest value of soil magnetic susceptibility was determined at the site ($0.87x10^{-3}$ j.SI). On the other hand, the lowest content of PM_{10} particles (25.1 $\mu g.m^{-3}$) among all stations and also the lowest values of soil magnetic susceptibility ($0.30x10^{-3}$ j.SI) were found in Mamateyova Street. A transported sandy soil occurs at the site, which contains only low amounts of PTMs likely due to low adsorption capacity of quartz grains (ALLOWAY 1990). However, at site Jeséniova, clayey soils occur that might have an effect on the higher values of soil magnetic susceptibility ($0.58x10^{-3}$ j.SI) in comparison with PM_{10} content (25.7 $\mu g.m^{-3}$). Similar values of PM_{10} content (25.8 $\mu g.m^{-3}$) were on Kamenné námestie, where lower values of magnetic susceptibility in soils were recorded ($0.39x10^{-3}$ j.SI) than in soils at site Jeséniova.

A complex pollution index, i.e. Tomlinson pollution load index (PLI) was calculated for all seven PTMs investigated in this work to assess the environmental quality of the soils (TOMLINSON et al. 1980). Tomlinson pollution load index is defined as the n-th root of ratios between the actual concentration of a metal in soil (C_n) and the background concentration of the same metal in soil (B_n) ($CF_n = C_n/B_n$):

$$PLI = \sqrt[n]{CF_1 \times CF_2 \times ...CF_n}$$

As the geochemical background of PTMs, their mean concentrations in C horizons of soils from Bratislava city were used and they were obtained from the database of Soil Geochemical Atlas of Slovakia (ČURLÍK & ŠEFČÍK 1999). This pollution index is a measure of the overall toxicity of soil samples and results from the contribution of all seven PTMs. The values of PLI higher than 1.0 indicate deterioration in soil quality. As can be seen from Fig. 3a, there was a significant and positive relationship between the mean values of soil magnetic susceptibility and mean PLI values. The significant correlation (Fig. 3a) with the data shown in Fig. 2 suggest that measurements of magnetic properties in soils can be used as a rapid and cheap tool for the monitoring of soil pollution with potentially toxic elements caused by anthropogenic and industrial activities. The utilization of magnetic susceptibility measurements for tracing of pollution in urban soils with PTMs was also supported by strong correlations of magnetic susceptibility values to the contents of individual PTMs in soils (Table 2). These findings are explained by the fact that PTMs adsorb preferentially on the external surface of ferrimagnetic grains, containing frequently a considerable amount of ferric oxyhydroxides (LU et al. 2008). Magnetic components are admixed with other PTMs. Coexistence of PTMs with magnetic components present in soils can explain elevated concentrations of PTMs in surface soils of urbanized regions along the urban – rural gradient. Observed correlations of PLI values and PTM concentrations with magnetic susceptibility of the soils along NW-SE gradient in Bratislava city are consistent with the results from other cities of different countries in the world, e.g. Hangzhou, Shanghai and Kaifeng cities in China (LU & BAI 2006; HU et al. 2007; LIU et al. 2016), Beni Mellal city in Morocco (EL BAGHDADI et al. 2012), Mexico city (MORTON-BERMEA et al. 2009) or Wien in Austria (SIMON et al. 2013). Additionally, according to the mean values of PLI (Fig. 3b), the environmental quality of the soils is the worst in the city centre (zone V) and wider surrounding of the central zone (zone VI), whereas the soils of NW rural area (zone I) and residential areas (zone III and IV) have much better quality.

Tab. 2: Pearson correlation coefficients for relationships between soil magnetic susceptibility and soil contents of PTMs

	As	Cd	Cu	Hg	Pb	Zn	Fe
kappa	0.41**	0.52***	0.50***	0.45***	0.64***	0.70***	0.22ns

Symbols ** indicate correlation significant at P <0.01, *** significant at P <0.001, ns not significant

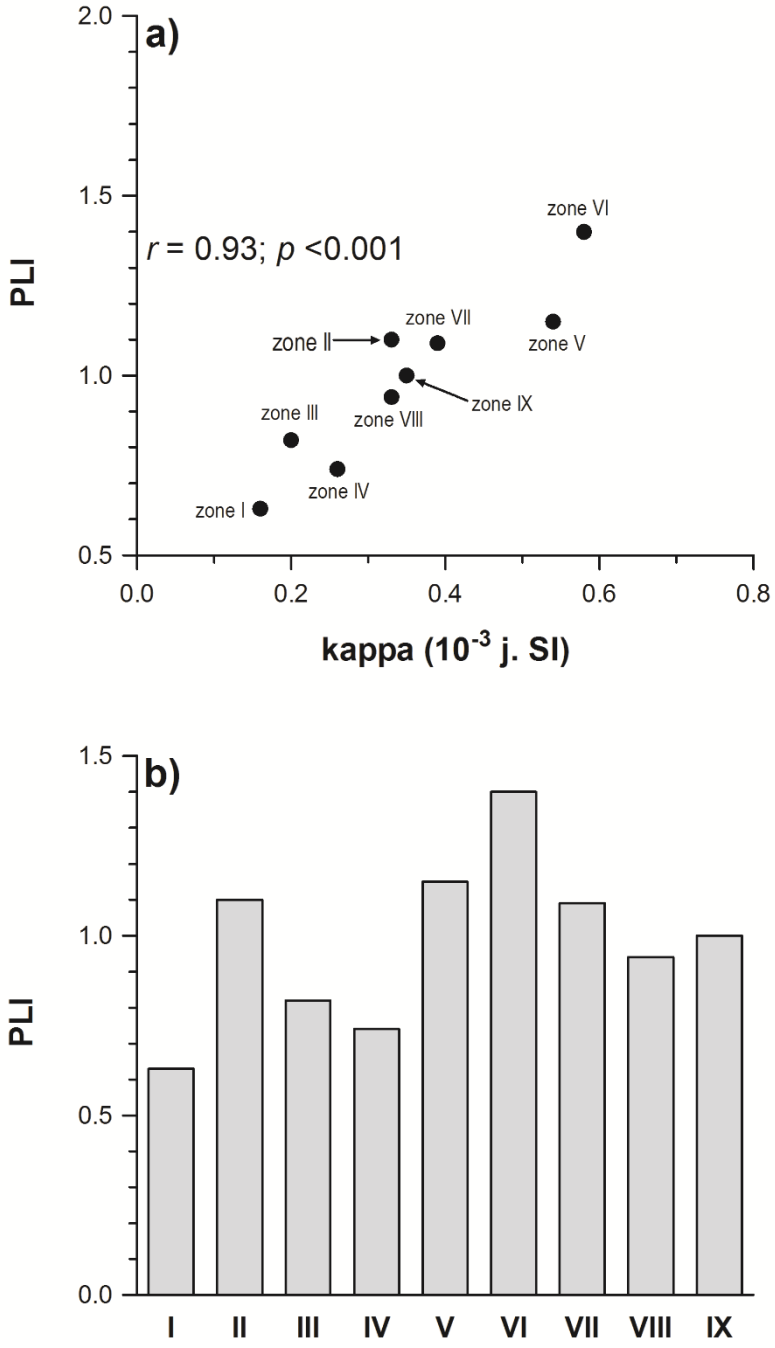

Fig. 3: **a)** Relationship between the mean values of magnetic susceptibility (kappa) and the values of Tomlinson pollution load index (PLI) for soils of the selected zones and **b)** mean values of PLI for soil samples in individual zones

CONCLUSION

This work showed that concentrations of PTMs (As, Cd, Cu, Hg, Pb, Zn and Fe) and magnetic susceptibilities in surface soils can change significantly along the urban – rural gradient in Bratislava city. Elevated concentrations of PTMs, mainly Hg, Pb and Zn, and higher values of magnetic susceptibility were found in the soils of highly urbanized areas of Bratislava city and they decrease with increasing distance from the city centre and adjacent sites. The enrichment of surface soils in PTMs in wider surrounding of the city centre is due to long-term urbanization and industrialization of these areas. Strongly significant correlation between Tomlinson pollution load index (PLI) and magnetic susceptibility values was found for soils polluted by PTMs. This type of correlation indicated the possibility of using magnetic measurements for simple and rapid indication of surface soil enrichment with potentially toxic metals. Therefore, the determination of magnetic susceptibility in soils can serve as an efficient "preliminary screening" method for the optimal selection of soil sampling sites and replace frequently used expensive and labour chemical analyses. From this reason, mapping of magnetic susceptibility of surface soils may also be used as a rapid method for the determination of the effect of urbanization on the pollution status of soils in relatively large areas.

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REFERENCES

ALLOWAY B. J. 1990. Heavy metal in soils. Blackie and Son Ltd., Glasgow and London, 339 p.

BLUNDELL A., HANNAM J. A., DEARING J. A. & BOYLE J. F. 2009. Detecting atmospheric pollution in surface soils using magnetic measurements: A reappraisal using an England and Wales database. Environ. Pollut. 157: 2878-2890.

BRANDAU A. & URBAT M. 2000. Rock magnetic signature of sediment near the active Dead Dog Mound, Juan De Fuca Ridge. XXV EGS General Assembly, Geophysical Research Abstract, vol. 2, CD Rom.

ČURLÍK J. & ŠEFČÍK P. 1999. Geochemical atlas of Slovak Republic. Part V – Soils. Soil Science and Conservation Research Institute, Bratislava.

ČURLÍK J. & JURKOVIČ Ľ. 2012. Pedogeochémia. Univerzita Komenského v Bratislave, Bratislava, 228 p.

D'EMILIO M., CHIANESE D., COPPOLA R., MACCHIATO M. & RAGOSTA M. 2007. Magnetic susceptibility measurements as proxy method to monitor soil pollution: Development of experimental protocols for field survey. Environ. Monitor. Assess. 125: 137-146.

ĎURŽA O. 1999. Heavy metals contamination and magnetic susceptibility in soils around metallurgical plant. Phys. Chem. Earth (A) 24: 541-543.

ĎURŽA O. & DLAPA P. 2009. Magnetic susceptibility record of loess/paleosol sequence: Case study from south-west Slovakia. Contrib. Geophys. Geodesy 39: 83-94.

ĎURŽA O., HILLER E., LACHKÁ L. & TÓTH R. 2013. Magnetická susceptibilita a znečistenie pôd v areáloch materských škôlok Bratislavy potenciálne toxickými prvkami (Slovensko). Acta Geol. Slov. 5: 155-162.

EL BAGHDADI M., BARAKAT A., SAJIEDDINE M. & NADEM S. 2012. Heavy metal pollution and soil magnetic susceptibility in urban soil of Beni Mellal City (Morocco). Environ. Earth Sci. 66: 141-155.

FLANDERS P. J. 1994. Collection, measurements, and analysis of airborne magnetic particulates from pollution in the environment. J. Appl. Phys. 75: 5931-5936.

HANESCH M. & SCHOLGER R. 2005. The influence of soil type on the magnetic susceptibility measured throughout soil profiles. Geophys. J. Int. 161: 50-56.

HILLER E., LACHKÁ L., JURKOVIČ Ľ. & VOZÁR J. 2015. Polycyclic aromatic hydrocarbons in urban soils from kindergartens and playgrounds in Bratislava, the capital city of Slovakia. Environ. Earth Sci. 73: 7147-7156.

HU X. F., SU Y., YE R., LI X. Q. & ZHANG G. L. 2007. Magnetic properties of the urban soils in Shanghai and their environmental implications. Catena 70: 428-436.

CHAPARRO M. A. E., CHAPARRO M. A. E., MARINELLI C. & SINITO A. M. 2008. Multivariate techniques as alternative statistical tools applied to magnetic proxies for pollution: a case study from Argentina and Antarctica. Environ. Geol. 54: 365-371.

CHEN J. 2007. Rapid urbanization in China: A real challenge to soil protection and food security. Catena 69: 1-15.

KAPIČKA A., PETROVSKÝ E., FIALOVÁ H. & PODRÁZSKÝ V. 2004. Využití magnetické susceptibility lesních půd pro mapování imisní zátěže v regionu KRNAP. In: Štursa J., Mazurski K.R., Palucki A. & Potocka J. (Eds.): Geoekologické problémy Krkonoš. Szklarska Poreba. Opera Corcontica, 41, pp. 55-59.

LI X., POOM C. & LIU P. S. 2001. Heavy metal contamination of urban soils and street dusts in Hong Kong. Appl. Geochem. 16: 1361-1368.

LIU D., MA J., SUN Y. & LI Y. 2016. Spatial distribution of soil magnetic susceptibility and correlation with heavy metal pollution in Kaifeng City, China. Catena 139: 53-60.

LJUNG K., SELINUS O. & OTABBONG E. 2006. Metals in soils of children's urban environments in the small northern European city of Uppsala. Sci. Total Environ. 366: 749-759.

LU S. G. & BAI S. Q. 2006. Study on the correlation of magnetic properties and heavy metals content in urban soils of Hangzhou City, China. J. Appl. Geophys. 60: 1-12.

LU S. G., BAI S. Q. & FU L. X. 2008. Magnetic properties as indicator of Cu and Zn contamination in soils. Pedosphere 18: 479-485.

LU S. G., WANG H. Y. & BAI S. Q. 2009. Heavy metal content and magnetic susceptibility of soils along an urban-rural gradient in rapidly growing city of eastern China. Environ. Monitor. Assess. 155: 91-101.

LU S. G., WANG H. Y. & GUO J. L. 2011. Magnetic enhancement of urban roadside soils as a proxy of degree of pollution by traffic-related activities. Environ. Earth Sci. 64: 359-371.

MAGIERA T. & STRZYSZCZ Z. 2000. Ferrimagnetic minerals of anthropogenic origin in soils of polish national parks. Water Air Soil Pollut. 124: 37-48.

MAHER B. A., MOORE C. & MATZKA J. 2008. Spatial variation in vehicle-derived metal pollution identified by magnetic and elemental analysis of roadside tree leaves. Atmos. Environ. 42: 364-373.

MALOVCOVÁ E. 2014. Využitie pôdnej magnetometrie pri štúdiu kontaminácie urbánnych pôd potenciálne toxickými stopovými prvkami (príklad Bratislavy). Manuskript, PriF UK Bratislava, 82 s.

MAZÚR E. & LUKNIŠ M. 1980. Regionálne geomorfologické členenie SSR 1:500 000. Atlas SSR. Geografický ústav SAV, Bratislava.

MENICHINI E., IACOVELLA N., MONFREDINI F. & TURRIO-BALDASSARRI L. 2007. Atmospheric pollution by PAHs, PCDD/Fs and PCBs simultaneously collected at a regional background site in central Italy and at an urban site in Rome. Chemosphere 69: 422-434.

MIKĽAJEV J. V. & KUDRJAVCEV JU. I. 1982. Izučenije magnitnoj vosprijimčivosti počv primeniteľno k zadačam geologičeskogo kartirovanija. Vest. Leningr. Universit. 18: 79-84.

MIKĽAJEV J. V. & ŽOGOLEV S. L. 1990. O prostranstvennoj svjazi anomalij magnitnoj vosprijimčivosti počv s litogeochemičeskimi oreolami rudnych elementov. Vest. Leningr. Universit. 7: 26-33.

MILIČKA J., VASS D. & PERESZLÉNYI M. 2002. Organická hmota v neskoromiocénnych sedimentoch Lučeneckej kotliny, južné Slovensko. Miner. Slov. 34: 93-98.

MORTON-BERMEA O., HERNANDEZ E., MARTINEZ-PICHARDO E., SOLER-ARECHALDE A. M., LOZANO SANTA-CRUZ R., GONZALEZ-HERNANDEZ G., BERAMENDI-OROSCO L. & URRUTIA-FUCUGAUCHI J. 2009. Mexico City topsoils: Heavy metals vs. magnetic susceptibility. Geoderma 151: 121-125.

MURÁNSZKY G., ÓVÁRI M., VIRÁG I., CSIBA P., DOBAI R. & ZÁRAY G. 2011. Chemical characterization of PM_{10} fractions of urban aerosol. Microchem. J. 98: 1-10.

NG S. L., CHAN L. S., LAM K. C. & CHAN W. K. 2003. Heavy metal contents and magnetic properties of playground dust in Hong Kong. Environ. Monitor. Assess. 89: 221-232.

NORRA S. & STUBEN D. 2003. Urban soils. J. Soil. Sediment. 3: 230-233.

ORGEIRA J. M., EGLI R. & COMPAGNUCCI H. R. 2011. A quantitative model of magnetic enhancement in loessic soils. In: PETROVSKÝ E., HERRERO-BERVERA E., HARINARAYANA T. et al. (eds.): Earth's Magnetic Interior. Book Series: IAGA Special Sopron Book Series, 1, pp. 361-397.

PETROVSKÝ E., KAPIČKA A., JORDANOVA N., KNAB M. & HOFFMANN V. 2000. Lowfield magnetic susceptibility: a proxy method of estimating increased pollution of different environmental systems. Environ. Geol. 39: 312-318.

Available online at: www.degruyter.com/view/j/aeuc
doi: 10.1515/aeuc-2016-0004

ROBERTSON D. J., TAYLOR K. G. & HOON S. R. 2003. Geochemical and mineral magnetic characterisation of urban sediment particulates. Manchester, UK. Appl. Geochem. 18: 269-282.

SHILTON V. F., BOOTH C. A., SMITH J. P., GIESS P., MITCHELL D. J. & WILLIAMS C. D. 2005. Magnetic properties of urban street dust and their relationship with organic matter content in the West Midlands, UK. Atmos. Environ. 39: 3651-3659.

SCHMIDT A., YARNOLD R., HILL M. & ASHMORE M. 2005. Magnetic susceptibility as proxy for heavy metal pollution: A site study. J. Geochem. Explor. 85: 109-117.

SIMON E., VIDIC A., BRAUN M., FÁBIÁN I. & TÓTHMÉRÉSZ B. 2013. Trace element concentrations in soils along urbanization gradients in the city of Wien, Austria. Environ. Sci. Pollut. Res. 20: 917-924.

SOBOCKÁ J., JAĎUĎA M., RUŽEKOVÁ-POLTÁRSKA K. & ŠURINA B. 2007. Urbánne pôdy (Príklad Bratislavy). Výskumný ústav pôdoznalectva a ochrany pôdy, Bratislava, 174 s.

SPITERI C., KALINSKI V., ROSLER W., HOFFMANN V. & APPEL E. 2005. Magnetic screening of a pollution hotspot in the Lausitz area, Eastern Germany: correlation analysis between magnetic proxies and heavy metal contamination in soils. Environ. Geol. 49: 1-9.

TAN J.-H., BI X.-H., DUAN J.-C., RAHN K.A., SHENG G.-Y. & FU J.-M. 2006. Seasonal variation of particulate polycyclic aromatic hydrocarbons associated with PM_{10} in Guangzhou, China. Atmos. Res. 80: 250-262.

TOMLINSON D. L., WILSON J. G., HARRIS C. R. & JEFFNEY D. W. 1980. Problems in the assessment of heavy metal levels in estuaries and the formation of a pollution index. Helgoländer Wissenschaftliche Meeresuntersuchungen 33: 566-572.

VADJUNINA A. F. & BABANIN V. F. 1972. Magnitnaja vosprijimčivosť nekotorych počv SSSR. Počvovedenije 10: 55-66.

WAGROWSKI D. M. & HITES R. 1997. PAH accumulation in urban, suburban and rural vegetation. Environ. Sci. Technol. 31: 279-282.

WONG F., HARNER T., LIU Q. T. & DIAMOND M. L. 2004. Using experimental and forest soils to investigate the uptake of polycyclic aromatic hydrocarbons (PAHs) along an urban-rural gradient. Environ. Pollut. 129: 387-398.

YANG P., YANG M. & SHAO H. 2012. Magnetic susceptibility and heavy metals distribution from risk-cultivated soil around the iron-steel plant, China. Clean - Soil Air Water 40: 615-618.

YANG P., YANG M., MAO R. Z., YANG P., YANG M. & BYRUE J. M. 2015. Impact of long-term irrigation with treated sewage on soil magnetic susceptibility and organic matter content in North China. Bull. Environ. Contam. Toxicol. 95: 102-107.

ZHU W. X. & CARREIRO M. M. 2004. Variations of soluble organic nitrogen and microbial nitrogen in deciduous forest soils along an urban-rural gradient. Soil Biol. Biochem. 36: 279-288.

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ON THE OCCURRENCE OF THE INTRODUCED PEST *THRIPS SIMPLEX* (MORISON 1930) (THYSANOPTERA: THRIPIDAE) IN SLOVAKIA

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Abstract

Exotic species introduction has recently increased European insect diversity in accordance with global climate change and international biological commodity trade, often with serious environmental and economic consequences for natural ecosystems as well as urban and farmland area. This short communication deals with the first official faunistic record of the gladiolus thrips *Thrips simplex* (Morison, 1930) (Thysanoptera, Thripidae) in Slovakia.

Key words: Gladiolus thrips, Slovakia, pest, *Thrips simplex*, Thysanoptera

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INTRODUCTION

Introduced exotic species have recently increased European insect diversity, often with serious environmental and economic consequences for natural ecosystems as well as urban and farmland area. Approximately 580 thrips (Thysanoptera) species, including the pests with invasive and economic potential, are known from Europe (ZUR STRASSEN 2003), however, the species richness has grown up due to the synergic complex of many natural and human-induced phenomena (GOLDARAZENA 2011; KARADJOVA & KRUMOV 2003; RODIKATIS et al. 2006; TRDAN et al. 2003, 2005; VIERBERGEN et al. 2006; FEDOR & VARGA 2007; VARGA 2008; VARGA & FEDOR 2008, etc.). Exotic species, originally from tropical and subtropical regions, can more easily spread into temperate countries, especially due to the globalized trade with biological commodities and global climate change (COLLINS 1998; FEDOR & VARGA 2007; JENSER & CZENZ 1988; LEWIS 1997; PELIKÁN 1989, 1991; VARGA & FEDOR 2008; VIERBERGEN et al. 2006; VIERBERGEN & DE JONG 2013), continuously adapt to local climatic and

ecological conditions and even expand to other regions (MASAROVIČ et al. 2014). Research on their ecology, distribution and invasion potential is often a key to reduce consequential economic damage.

The gladiolus thrips was firstly described by Morison (1930) as *Physothrips simplex* from flowers of *Dianthus carryophylus* (Urrbrae, South Australia). Its origin still remains discussed (MORISON 1957; LEWIS 1973; MOUND 1997). The species has now rather cosmopolitan distribution and is found wherever *Gladiolus* plants are grown or cultivated: in Africa, southern Asia and Japan, Australia, several Pacific Islands, Europe and North and South America (DENMARK & PRICE 1998). It occurs mostly on *Gladiolus* cultivars, but also on *Calla* sp., *Pancratium* sp., *Protea* sp. and *Vitis* sp. (ZUR STRASSEN 2003). Due to its widespread distribution this is the first official faunistic record from Slovakia.

Thrips simplex (Morison 1930) is characterized by the following description: antennae composed of 8 segments, interocellar setae placed inside the ocellar triangle, a netlike structure between S1 setae of metanotum and reticles characterised by typical internal microscopic markings, body and legs dark brown, antennal segment 3 and tarsi paler, 3 (sometimes 4) pairs of posteromarginal setae on pronotum, wings paler basally.

Adults emerge milky white, but soon turn brown and being feeding. Eggs are deposited in the leaf tissue and corms. The two larval stages are light yellow and are usually found beneath the leaves or bracts. The fully developed second instar larva is about the size of the adult (DENMARK & PRICE 1998).

METHODS

The thrips were sampled within our complex research on Thysanoptera diversity in Slovakia, individually, using the pincers and brush as well as shaking the flowers. AGA solution (alcohol, glycerol, aqua) and ethanol were used as a conservation liquid. Thrips were mounted according to standard preparatory techniques using for thrips (FEDOR et al. 2012; SIERKA & FEDOR 2004). *Thrips simplex* specimens were determinated according to ZUR STRASSEN (2003). The material has been deposited in the collections of the authors.

RESULTS AND DISCUSSION

The first official faunistic record of the introduced gladiolus thrips Thrips simplex (Morison 1930) in Slovakia:

Material examined: 10 specimens were found in SW Slovakia, in the city of Trnava (Fig 1, GPS: 48°21′47,19′′ N, 17°34′59,52′′ E; DFS grid square 7671). August 4, 2015, 2 ♂ 8 ♀, collected with the brush and pincers from pink-violet gladioli flowers. 28 specimens of the gladiolus thrips were also discovered in the village of Miloslavov (Fig. 1, GPS: 48°06′16,95′′ N, 17°17′56,57′′ E, DFS grid square 7969). August 5, 2015, 14 ♀, sampled by shaking the violet gladioli flowers and using the brush. September 4, 2015, 14 ♀, obtained by shaking the yellow gladioli flowers and applying the brush. Leg.: R. Masarovič, J. Sigmund, det.: R. Masarovič, coll. R. Masarovič.

The species breeds on gladiolus corms, leaves and flowers and appears to be more numerous on dark flowered cultivars (HERR 1934; HAGREAVES & COOPER 1980; MILEVOJ et al. 2008). Adults and larvae feed on gladiolus foliage and stalks causing blasting and silvering of leaves. Larvae are full of green chlorophyll, which is clearly visible through the body wall (HERR 1934). The flowers are also deformited and discolorated (HERR 1934; DENMARK & PRICE 1998). Thrips create silvery white spots on gladiolus flowers which later turn brown (DENMARK & POE 1972). The gladiolus thrips is probably the most dangerous pest that infests gladioli as it causes damage by sucking and can completely destroy flowers (ZGONEC 1990).

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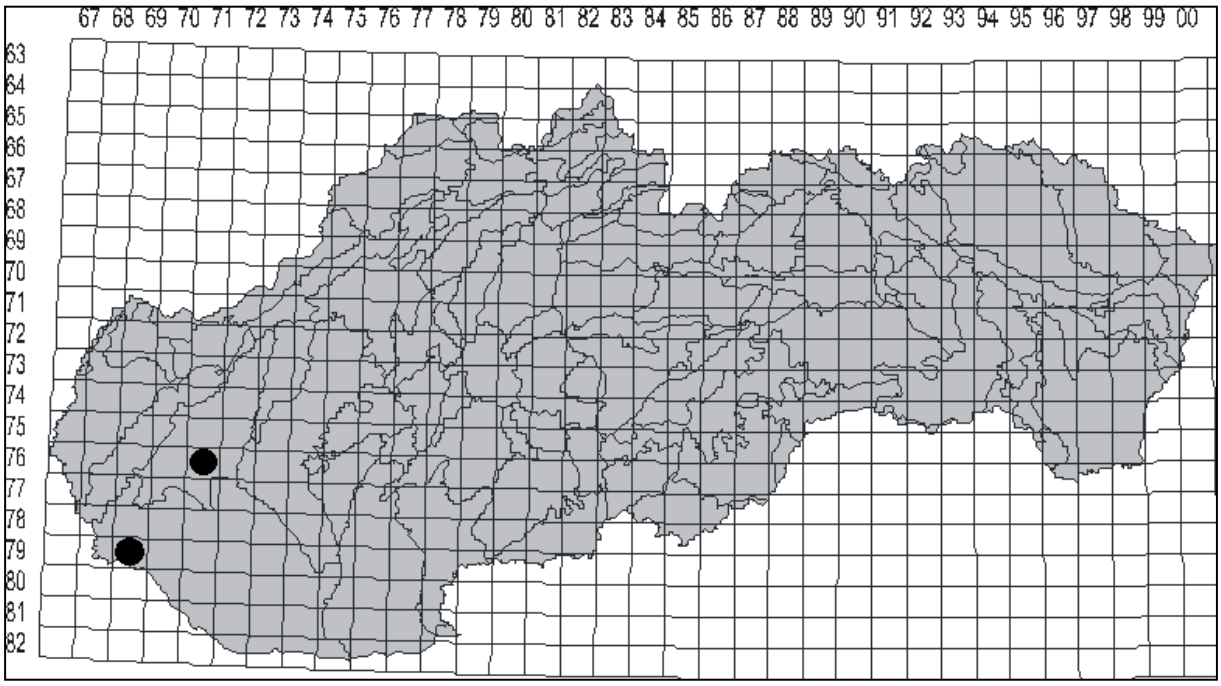

Fig. 1: Study sites with first records of *T. simplex* in the map of Slovakia

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REFERENCES

COLLINS D. W. 1998. Recent interceptions of *Echinothrips americanus* Morgan (Thysanoptera, Thripidae) imported into England. Entomol. Month. Magaz. 134: 1-3.

DENMARK H. A. & POE S. L. 1972. The gladiolus thrips, *Taeniothrips simplex* (Morison) in Florida (Thysanoptera: Thripidae). Florida department of agriculture and consumer services, Division of plant industry. Entomol. Circ. 119: 1-2.

DENMARK H. A. & PRICE J. F. 1998. Gladiolus thrips, *Thrips simplex* (Morison) (Insecta: Thysanoptera: Thripidae). University of Florida, DPI Entomol. Circ. 322: 1-3.

FEDOR P. J. & VARGA L. 2007. The first record of *Gynaikothrips ficorum* Marchal, 1908 (Thysanoptera) in Slovakia. Thysanopteron. Pismo Entomol. 3(1): 1-2.

FEDOR P., DORIČOVÁ M., MASAROVIČ R. & SIERKA W. 2012. Strapky (Thysanoptera) Slovenska. Comenius University, Bratislava. 184 p.

GOLDARAZENA A. 2011. First record of *Thrips hawaiiensis* (Morgan, 1913) (Thysanoptera: Thripidae), an Asian pest thrips in Spain. Bulletin OEPP/EPPO (Organisation Europeene et Mediterraneene pour la Protection des Plantes/European and Mediterranean Plant Protection Organization) 41(2): 170-173.

HAGREAVES, J. R. & COOPER L. P. 1980. Differential susceptibility of some gladiolus cultivars to gladiolus thrips, *Taeniothrips simplex* (Morison). Queensl. J. Agric. Anim. Sci. 37: 161-163.

HERR E. A. 1934. The gladiolus thrips, *Taeniothrips gladioli* M. and S. Bulletin, Ohio Agricultural Experiment Station 537: 64.

JENSER G. & CZENZ K. 1988. Thysanoptera species occurring frequently on cultivated plants in Hungary. Acta Phytopathol. Hun. 23(3-4): 285-289.

KARADJOVA O. & KRUMOV Y. 2003. *Echinothrips americanus* Morgan (Thysanoptera: Thripidae), a new pest of the Bulgarian Greenhouses. In: Proceedings of the International Scientific Conference at the 50th Anniversary of the University of Forestry, Plant Protection Section, 1-2 April 2003, Sofia., pp. 122-125.

LEWIS T. 1997. Thrips as Crop Pests. CAB International, Wallingford, 740 p.

LEWIS T. 1973. Thrips, their biology, ecology, and economic importance. Academic Press London and New York, 349 p.

MASAROVIČ R., DORIČOVÁ M., PROKOP P. & FEDOR J. 2014. „Testing the limits" – an interesting record of the exotic banded greenhouse thrips *Hercinothrips femoralis* (Thysanoptera: Thripidae: Panchaetothripinae) at the high Carpathian mountain altitudes. Biologia 69(11): 1631-1634.

MILEVOJ L., ZDEŠAR M. & TRDAN S. 2008. Susceptibility to gladiolus thrips (*Thrips simplex* [Morison]) in four different coloured gladiolus cultivars. Acta Phytopathol. Hun. 43(2): 323-327.

MORISON G. D. 1930. On a collection of Thysanoptera from South Australia. Bull. Entomol. Res. 21: 9-14.

MORISON G. D. 1957. A review of British glasshouse Thysanoptera. Transactions of the Royal Entomological Society of London 109: 467-534.

MOUND L. A. 1997. Biological diversity. In: LEWIS T. (ed.), Thrips as crop pests. CAB International, Oxford and New York, pp. 197-215.

PELIKÁN J. 1989. Nově importovaný škůdce skleníkových rostlin, třasněnka západní, *Frankliniella occidentalis* (Pergande, 1895). Ochrana Rostlin 25: 271-278.

PELIKÁN J. 1991. Truběnka fikusova (*Gynaikothrips ficorum* Marchal, 1908) ve sklenících v Československu. Ochrana Rostlin 27: 287-291.

RODITAKIS E., MOUND L. A. & RODITAKIS N. E. 2006. First record in Crete of *Hercinothrips femoralis* in greenhouse banana plantations. Phytoparasitica 34(5): 488-490.

SIERKA W. & FEDOR P. 2004. Wciornastki (Insecta, Thysanoptera). Universytet Slaski, Katowice.

TRDAN S., JOVIĆ M. & ANDJUS L. 2005. Palm thrips, *Parthenothrips dracaenae* (Heeger) (Thyanoptera: Thripidae), in Slovenia: still a pest of minor importance? Acta Agric. Slov. 85(2): 211-217.

TRDAN S., MILEVOJ L., RASPUDIĆ E. & ŽEŽLINA I. 2003. The first record of *Echinothrips americanus* Morgan in Slovenia. Acta Phytopathol. Hun. 38(1): 157-166.

VARGA L. & FEDOR P. J. 2008. First interception of the greenhouse pest *Echinothrips americanus* Morgan, 1913 (Thysanoptera: Thripidae) in Slovakia. Plant Protect. Sci. 44(4): 155-158.

VARGA L. 2008. *Hercinothrips femoralis* (Reuter, 1891) – a New Pest Thrips (Thysanoptera: Panchaetothripinae) in Slovakia. Plant Protect. Sci. 44(3): 114-118.

VIERBERGEN G. & DE JONG Y. 2013. Fauna Europaea: Thysanoptera, Thripidae. Fauna Europaea (version 2.6.2). http:// www.faunaeur.org. (accessed 10.05.2014).

VIERBERGEN G., CEAN M., SZELLER I. H., JENSER G., MASTEN T. & ŠIMALA M. 2006. Spread of Two Thrips Pests in Europe: *Echinothrips americanus* and *Microcephalothrips abdominalis* (Thysanoptera: Thripidae). Acta Phytopathol. Hun. 41(3-4): 287-296.

ZGONEC S. 1990. Bolezni in škodljivci v okrasnem vrtu. Ljubljana, ČZP Kmečki glas, 112 p.

ZUR STRASSEN R. 2003. Die terebranten Thysanopteren Europas und des Mittelmeer-Gebietes. Goecke & Evers, Keltern.

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RESPONSE OF TOMATO PLANTS (*SOLANUM LYCOPERSICUM*) TO STRESS INDUCED BY SB(III)

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Abstract

Presented study evaluates effects of various Sb(III) concentrations on tomato plants (*Solanum lycopersicum*) cultivated hydroponically. Visual symptoms of antimony toxicity were observed only at two highest applied concentrations (50 and 100 mg/L). Dry weight of aboveground parts decreased significantly in variants treated with 25, 50 and 100 mg/L Sb(III), by ~12, 35 and 65 %, respectively, in comparison to the control. Statistically significant decrease of chlorophyll a and b was observed only after application of two highest studied concentrations 50 and 100 mg/L Sb(III). On the other hand concentration of total carotenoids in leaves rose with increasing external Sb(III) concentration. High concentrations (50 and 100 mg/L) of Sb(III) in nutrient solution caused that protein content in leaves dropped by ~20 and 39% relative to control. Accumulation of antimony in roots was about 5- (10 mg/L) to 27-times (25 mg/L) greater than that in shoots. The highest BAF factor value determined for shoots was ~55 at 10 mg/L Sb(III) and for roots it was ~821 at 50 mg/L Sb(III). Translocation factor values were in whole studied concentration range 5 – 100 mg/L Sb(III) < 1.The most effective translocation of antimony from roots to shoots was observes for variants treated with 10 mg/L of Sb(III).

Key words: accumulation, antimony, chlorophyll, proteins, *Solanum lycopersicum*, TBARS

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INTRODUCTION

Antimony, a metalloid, is widely spread in environment and considered to be highly toxic for various organisms including humans. Even low environmental concentrations can be threatening. The main sources of Sb are mining sites where the metalloid is released to the environment mainly via oxidative weathering of sulfide minerals (predominantly stibinite (Sb_2S_3)) included in waste products. In Slovakia, such sites are located in vicinity of villages Dúbrava, Medzibrod, Poproč and Čučma (district of Rožňava town). All mines had been closed in 90´s but the remained waste could be marked as potentially health risky (HILLER et al. 2012).

The mechanisms of uptake, assimilation, toxicity and detoxification of Sb in plants are not quite clear as those in animals and humans. The ability of plants to take up Sb depends on these following

factors: (1) phytoavailability of antimony in soils; (2) speciation of Sb; (3) differences in the concentration of other ions in soils, such as P and Ca. Uptake of Sb also varies with plant species (GEBEL 1997; SHTANGEEVA et al. 2011; WAN et al. 2013).

Large amounts of Sb accumulated in plants can be responsible for various negative effects such as excessive production of reactive oxygen species (ROS), micronutrient uptake retardation, decrease in photosynthesis and synthesis of soluble proteins (DING et al. 2015).

Presented study evaluates effects of Sb(III) on tomato plants (*Solanum lycopersicum*). Effect on growth (dry mass and length of plants organs) and biochemical (content of photosynthetic pigments, soluble proteins, thiobarbituric acid reactive substances in leaves) parameters of plants were studied. Concentration of Sb in individual plant parts and corresponding bioaccumulation and translocation factor values were estimated as well.

MATERIAL AND METHODS

Seeds of *Solanum lycopersicum* were germinated at 25 °C for three days in dark on filter paper in Petri dishes filled with 15 mL of demineralized water. After 72 h seedlings were transferred to 1 L beakers filled with Hoagland nutrient solution of pH = 5.5 and cultivated in growing chamber at constant conditions for 17 days. After that plants were transferred to nutrient solution with addition of antimony potassium tartrate ($K_2Sb_2(C_4H_2O_6)$; 5, 10, 25, 50, 100 mg/L) were they last for another 10 days. All beakers were well aerated by air pump. Conditions in growing chamber were as follows: relative humidity 80 ± 5%, mean temperature 25 ± 1 °C, photoperiod: 16h light / 8h dark, light energy 80 μmol.m^{-2}.s^{-1} PAR. After 10 days roots and shoots of control plants as well as Sb(III) treated variants were well dried at 50 °C in order to determine the dry weight and the content of antimony.

Concentrations of chlorophyll *a*, *b* and total carotenoids (chl *a*, chl *b*, carot) in plant leaves were determined spectrophotometrically (chl *a* at 663.2 nm, chl *b* at 646.8 nm, and carot at 470.0 nm) after extraction into 80% (v/v) acetone (Genesys 6, Thermo Scientific, U.S.A) according to LICHTENTHALER (1987). Concentration of thiobarbituric acid reactive substances (TBARS) in leaves of tomato plants was determined according to the method described in detail in PEŠKO et al. (2012). Briefly: 2 mL of supernatant was incubated at ~95 °C for 30 min with 1 mL of mixture containing 0.5% (w/v) thiobarbituric acid, 20% (v/v) trichloroacetic acid, and 100 μL of 4% butylated hydroxytoluene, followed by cooling in an ice bath for 10 min and centrifuged 2 min at 2900g. The absorbance of the solution was determined at λ = 532 nm spectro-photometrically (Genesis 6, Thermo Scientific) and concentration of TBARS was calculated using extinction coefficient ε = 155 L/mmol.cm.

Soluble protein concentration in leaves was determined spectrophotometrically (Genesys 6, Thermo Scientific, USA) according to BRADFORD (1976) using Bradford reagent prepared by dissolving of 20 mg Coomassie Brilliant Blue G-250 in 10 mL of 95% ethanol, thereafter 20 mL H_3PO_4 and 70 mL deionized water was added, respectively. The reaction mixture (200 L of supernatant and 2 mL of Bradford reagent) was incubated at room temperature for 5 min. Absorbance of incubated mixture was measured at λ = 412 nm and protein concentration evaluated by using calibration curve. Serum bovine albumin was used as a standard.

Dried plant samples from control and Sb(III) treatments were digested in solution containing HNO_3 and H_2O_2 (4:1), than heated in the oven at 160 °C for 1 h and diluted with deionized water. Antimony concentration in shoots and roots was determined by means of galvanostatic dissolving chronopotentiometry on EcaFlow 150 GLP (Istran, Slovakia).

The results were evaluated by the multifactorial ANOVA algorithm ($p \leq 0.05$) after verification of normality and homogeneity of the variance.

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RESULTS AND DISCUSSION

Thirty days old tomato plants exposed to different Sb(III) concentrations for 10 days exhibited visual symptoms of antimony toxicity only at two highest studied concentrations (50 and 100 mg/L). Leaves of these plants were chlorotic, wilted and some of them even desiccated. Roots were slightly brownish. Growth of plants treated with 100 mg/L Sb(III) was stunt. Slight stimulation of biomass growth (Fig. 1) of plants was observed for variants were Sb(III) was applied in low concentrations (5 and 10 mg/L).

Application of concentrations 25, 50 and 100 mg/L (Sb(III)) caused that the dry weight of aboveground parts (leaves + stems) decreased by ~12, 35 and 65%, respectively, in comparison to the control. On the other hand, adverse effect of Sb(III) on dry weight of roots was observed only in variants treated with 100 mg/L (Fig. 1). Decrease in root weight of these plants was about ~30%, comparing to the untreated variants. Inhibition of plant growth as a result of application of Sb(III) was observed also in experiments with other species, e.g. paddy rice (DING et al. 2015), fern plants (FENG et al. 2009), maize (PAN et al. 2011), and lichen (PAOLI et al. 2013).

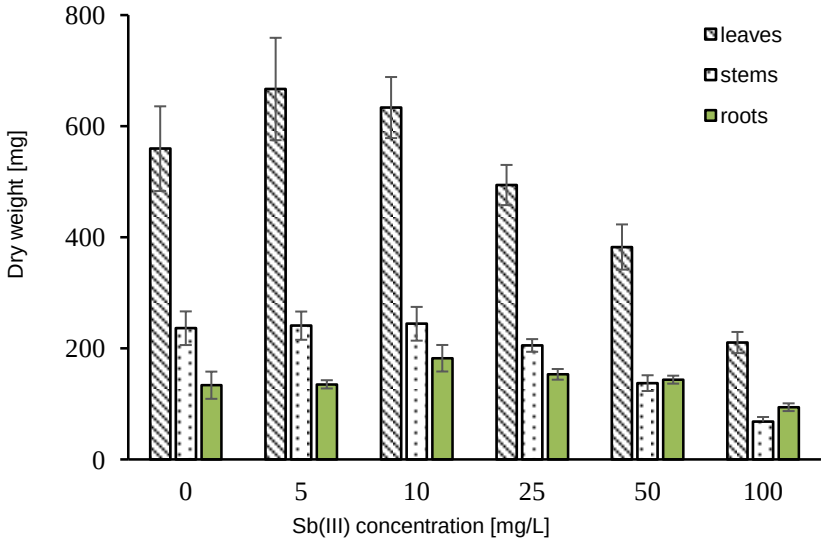

Fig. 1: Dependence of dry weight of leaves, stems and roots of tomato plants on different Sb(III) concentrations. Mean ± S.E.; n = 5; S.E. – standard error

Relationship between external concentration of antimony potassium tartrate and concentration of photosynthetic pigments in leaves of tomato plants is shown in Fig. 2. Statistically significant decrease of chlorophyll *a* and *b* was observed only after application of two highest studied concentrations 50 and 100 mg/L Sb(III). Comparing to the control plants, chlorophyll *a* dropped by ~31 and 55 %, and chlorophyll *b* by ~39 and ~59%, respectively. Similar results were observed also by PAN et al. (2011) in their experiments with *Zea mays* plants. This suggest that Sb is interfering with biomolecules involved in synthesis of chlorophylls. Antimony could also interfere with or damage important molecules and enzymes within chloroplasts, which is supported by results from experiments of DING et al. (2015). They found that more than 10% of Sb accumulated in shoots of paddy rice plants was located in chloroplasts. Concentration of total carotenoids in leaves rose with increasing concentration of Sb(III) in nutrient solution. Application of 100 mg/L Sb(III) caused that concentration of total carotenoids was almost twice the control. Excessive production of carotenoids is probably a part of protection mechanism against oxidative stress.

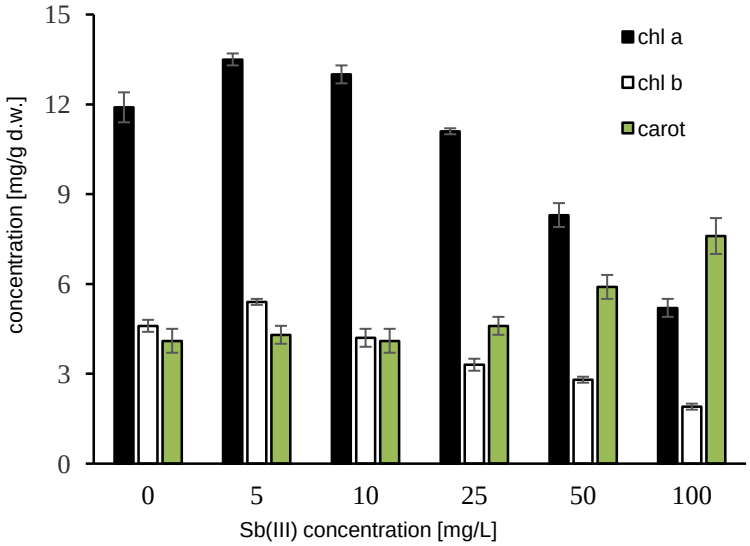

Fig. 2: Dependence of chlorophyll *a* (chl a) and *b* (chl b) as well as carotenoids (carot) concentration in leaves of tomato plants on different Sb(III) concentrations. Mean ± S.E.; n = 5; S.E. – standard error; d.w. – dry weight

Concentration of TBARS and soluble proteins in leaves of plants treated with different concentrations of Sb(III) are summarized in Tab. 1. In our study concentration of these products (Tab. 1) in leaves of tomato plants rose as the external concentration of Sb(III) increased. Massive accumulation of TBARS in leaves was observed for variants treated with two highest studied concentrations (50 and 100 mg/L Sb(III)). Accumulation of these products reached ~129 and 240% of control. Thiobarbituric acid reactive substances (TBARS), products of peroxidation of membrane lipids, are very good marker of oxidative stress caused by the presence of metals. Our results are in good conformity with experiments of many other authors. For example accumulation of TBARS in shoots of *Hedysarum pallidum* plants grown on highly contaminated Sb-mining sites was 5-times greater than in those grown on uncontaminated control sites (BENHAMDI et al. 2014).

High concentrations (50 and 100 mg/L) of Sb(III) in nutrient solution caused that protein concentration in leaves dropped by ~20 and 39% relative to the control. Reactive oxygen species (ROS) that are produced as a result of oxidative stress, are responsible not only for membrane lipid peroxidation but cause protein inactivation and degradation as well. The bulk of the oxidized proteins is then degraded by proteolysis (SHRINGARPURE et al. 2003).

Tab. 1: Concentration of TBARS and soluble proteins in leaves of tomato plants treated with different concentrations of Sb(III)

c Sb(III) [mg/L]	TBARS [µmol/g d.w.]	Soluble proteins [mg/g d.w.]
0	1.7 ± 0.10	41.4 ± 2.1
5	2.3 ± 0.10	43.3 ± 2.0
10	2.2 ± 0.05	42.2 ± 1.6
25	2.6 ± 0.11	40.4 ± 3.1
50	3.9 ± 0.12	33.3 ± 2.9
100	5.8 ± 0.15	26.1 ± 2.3

Concentration of Sb in roots and shoots increased with increasing external Sb(III) concentration. Accumulation of antimony in roots was about 5- (10 mg/L) to 27-times (25 mg/L) greater than that in shoots. The fact that tomato plants were able to accumulate in their shoots more than 10 μg Sb/g d.w. already at the lowest applied external concentration, 5 mg/L Sb(III), suggests that *Solanum lycopersicum* is Sb-tolerant. But it can't be marked as an accumulator of this metalloid because most of Sb remained accumulated in roots. Higher amount of Sb accumulated in shoots was observed by HAMMEL et al. (2000). Spinach plants grown on contaminated soil (100 μg Sb(III)/g) were able to accumulate 399 μg Sb /g d.w. in shoots.

Tab. 2: Concentration of antimony in shoots and roots as well as corresponding bioaccumulation factor (BAF) and translocation factor (TF) values, and percentage share of Sb accumulated in shoots of tomato plants treated with different Sb(III) concentrations

c Sb(III) mg/L	c Sb [μg/g d.w.]		BAF		TF	% of Sb in shoots
	Shoots	Roots	Shoots	Roots		
5	43.9	688.6	46.2	577.7	0.064	19.0
10	105.1	548.8	55.3	362.4	0.192	26.8
25	132.3	3 612.5	27.8	760.5	0.037	6.8
50	308.1	7 798.6	32.4	820.9	0.040	7.4
100	998.5	13 162.9	54.8	692.8	0.090	9.2

The highest BAF factor value determined for shoots was ~55 at 10 mg/L Sb(III) and for roots it was ~821 at 50 mg/L Sb(III). This suggests that bioaccumulation of antimony within aboveground parts is most effective at lower external concentrations.

Translocation factor values were in whole studied concentration range 5 – 100 mg/L Sb(III) < 1, suggesting restricted translocation of Sb into the aboveground parts. The most effective translocation of antimony from roots to shoots was observed for variants treated with 10 mg/L of Sb(III). TF value was in this case 0.192.

The amount of antimony accumulated in shoots depends not only on applied external concentration but also on dry weight of plant organs. The percentage share of Sb accumulated in shoots from total amount accumulated by tomato plants was for low external concentrations relatively high, 19% (5 mg/L) and 26.8% (10 mg/L). In case of variants treated with higher concentrations, the percentage share was below 10%.

CONCLUSION

According to the presented results it can be said that toxic effects of antimony on tomato plants (*Solanum lycopersicum*) were exhibited only in variants treated with higher concentrations (50 and 100 mg/L) of studied metalloid. These variants exhibited moderate to severe signs of Sb toxicity, including chlorosis, stunt plant growth, decreased concentration of chlorophylls, soluble proteins, and increased accumulation of products of lipid peroxidation in leaves, nevertheless *Solanum lycopersicum* could be marked as Sb-tolerant because in presence of lower Sb(III) concentrations (5, 10 and 25 mg/L) was the accumulation of antimony in aboveground parts relatively high, which in turn could be utilized in phytoremediation of lightly to moderate polluted sites, but further field experiments should be done to support our results.

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REFERENCES

BENHAMDI A., BENTELLIS A., RACHED O., DU LAING G. & MECHAKRA A. 2014. Effects of antimony and arsenic on antioxidant enzyme activities of two steppic plant species in an old antimony mining area. Biol. Trace. Elem. Res. 158: 96-104.

BRADFORD M.M. 1976. Rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72: 248-254.

DING Y., WANG R., GUO J., WU F., XU Y. & FENG R. 2015. The effect of selenium on the subcellular distribution of antimony to regulate the toxicity of antimony in paddy rice. Environ. Sci. Pollut. Res. Int. 22(7): 5111-5123.

FENG R. W., WEI C. Y., TU S. X., WU F. C. & YANG L. S. 2009. Antimony accumulation and antioxidative responses in four fern plants. Plant Soil 317: 93-101.

GEBEL T. 1997. Arsenic and antimony: comparative approach on mechanistic toxi-cology. Chem. Biol. Interact. 107: 131-144.

HAMMEL W., DEBUS R. & STEUBING L. 2000. Mobility of antimony in soil and its avail-ability to plants. Chemosphere 41: 1791-1798.

HILLER E., LALINSKÁ B., CHOVAN M., JURKOVIČ L., KLIMKO T., JANKULAR M., HOVORIČ R., ŠOTTNIK P., FĽAKOVÁ R., ŽENIŠOVÁ Z. & ONDREJKOVÁ I. 2012. Arsenic and antimony contamination of waters, stream sediments and soils in the vicinity of abandoned antimony mines in the Western Carpathians, Slovakia. Appl. Geochem. 27: 598-614.

LICHTENTHALER H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. Method. Enzymol. 148: 350-382.

PAN X., ZHANG D., CHEN X., BAO A. & LI L. 2011. Antimony accumulation, growth performance, antioxidant defense system and photosynthesis of *Zea mays* in response to antimony pollution in soil. Water Air Soil Pollut. 215: 517-523.

PAOLI L., FIORINI E., MUNZI S., SORBO S., BASILE A. & LOPPI S. 2013. Antimony toxicity in the lichen *Xanthoria parietina* (L.) Th. Fr. Chemosphere 93: 2269-2275.

PEŠKO M., KRÁĽOVÁ K. & BLAŠKO J. 2012. Phytotoxic effects of trivalent chromium on rapeseed plants. Fresen. Environ. Bull. 21(3A): 761-768.

SHRINGARPURE R., GRUNE T., MEHLHASE J. & DAVIES K. J. A. 2003. Ubiquitin conjugation is not required for the degradation of oxidized proteins by proteasome. J. Biol. Chem. 278: 311-318.

SHTANGEEVA I., BALI R. & HARRIS A., 2011. Bioavailability and toxicity of antimony. J. Geochem. Explor. 110: 40-45.

WAN X. M., TANDY S., HOCKMANN K. & SCHULIN R. 2013. Changes in Sb speciation with waterlogging of shooting range soils and impacts on plant uptake. Environ. Pollut. 172: 53-60.