Biotechnology & Metals

Košice 2018

Faculty of Science, Pavol Jozef Šafárik University in Košice
Institute of Geotechnics of Slovak Academy of Sciences
Slovak Mining Society at the Institute of Geotechnics SAS

Book of Abstracts

5th International Scientific Conference on Biotechnology and Metals

Editors: Jana Kisková, Jana Sedláková-Kaduková
Alena Luptáková

ISBN:
October 11 – 12, 2018, Košice, Slovakia

INTERNATIONAL SCIENTIFIC COMMITTEE

Magdaléna Bálintová	Technical University in Košice, Slovakia
Ljudmilla Bokányi	University of Miskolc, Hungary
Vladimír Čáblik	VŠB-TU Ostrava, Czech Republic
Nicolas Kalogerakis	Technical University of Crete, Greece
Daniel Kupka	Slovak Academy of Sciences, Košice, Slovakia
Anna Kinga Nowak	Cracow University of Technology, Poland
Peter Pristaš	Pavol Jozef Šafárik University in Košice, Slovakia
Neil Rowson	University of Birmingham, Great Britain
Mariola Saternus	Silesian University of Technology, Poland
Alexandra Šimonovičová	Comenius University in Bratislava, Slovakia
Stefano Ubaldini	National Research Council, Roma, Italy

ORGANIZING COMMITTEE

Chair

Jana Sedláková-Kaduková Pavol Jozef Šafárik University in Košice, Slovakia

Vice-chair

Alena Luptáková Slovak Academy of Sciences, Košice, Slovakia

Members

P. J. Šafárik University in Košice	**Slovak Academy of Sciences, Košice**
Jana Kisková	Slavomír Hredzák
Mariana Kolesárová	Dominika Behunová
Lenka Maliničová	Jana Jenčárová
Ivana Timková	Eva Mačingová
	Dominika Rudzanová
	Katarína Stuchlá

REVIEWERS

Jana Sedláková-Kaduková
Peter Pristaš
Jana Kisková
Lenka Maliničová
Alena Luptáková

ISBN:

Preface

Dear colleagues and friends,

Biotechnology & Metals

Košice 2018

CONTENT

Biotechnology & Metals Košice 2018

5th International Conference
on Biotechnology and Metals
October 11-12, 2018, Košice
Slovakia

Conference Programme

BIOSORPTION OF Zn AND Cd BY DRIED BIOMASS OF Euglena gracilis FROM AQUEOUS SOLUTIONS

Vanda Adamcová[a], Tomáš Lempochner[a], Martin Valica[a], Dominika Vešelényiová[b], Juraj Krajčovič[b], Miroslav Horník[a]

[a] *Department of Ecochemistry and Radioecology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, Nám. J. Herdu 2, Trnava, SK-917 01, Slovak Republic*

[b] *Department of Biology, Faculty of Natural Sciences, University of Ss. Cyril and Methodius in Trnava, Nám. J. Herdu 2, Trnava, SK-917 01, Slovak Republic*

ABSTRACT

Contamination of the environment with heavy metals, and in particular its removal, has become a subject of concern to many scientific communities around the world in the recent years. A number of remediation methods based on chemical, physical or biological principles have been proposed and tested to removal of the metals to an environmentally acceptable level or at least to decrease their mobility and bioavailability to prevent their entry into living organisms and food chain. In this respect, biosorption represents a promising alternative remediation method that could replace or support the current techniques and processes for removal organic contaminants or metals from contaminated solutions or wastewaters. From the point of view of the selection of heavy metals, the work concentrates on cadmium representing the typical toxic metal, as well as zinc rather belonging to the microelements and the physico-chemical analogue of cadmium.

The aim of the work was to evaluate the possibility to utilize the dried biomass of *Euglena gracilis* var. *bacillaris* as a biosorbent applied for the removal of heavy metals from the wastewaters or contaminated solutions.

For these purposes, analyses characterizing the dried biomass of *E. gracilis* var. *bacillaris* as a potential biosorbent of metals or radionuclides and experiments describing the processes of biosorption of Zn and Cd by dried biomass of euglena from single- and two-component $ZnCl_2$ and/or $CdCl_2$ solutions and under the conditions of batch systems were carried out. As analytical methods for determination of these metals,the atomic absorption spectrometry and scintillation gamma-spectrometry using radioisotopes ^{65}Zn and ^{109}Cd were used.

Prepared dried biomass of *E. gracilis* var. *bacillaris* in the form of particles (< 300 μm) was characterized in terms of the presence of functional groups, concentration of binding sites c_{An} and the value of pH_{zpc} = 6.6 using potentiometric titration and the prediction program ProtoFit. From the kinetics of Zn and Cd binding by dried biomass of euglena from single-

component as well as two-component solutions, it can be assumed that the process of metals binding can be characterized by fast, sorption mechanisms, in which the concentration equilibrium $[Me]_{solution}$:$[Me]_{biomass}$ was stabilized in the first 20 min of interaction. In individual experiments, the influences of the pH value of the solution, the biomass concentration and the sorbate (Zn and/or Cd) concentration on the sorption processes of the both metals binding onto dried biomass of euglena from the single- and two-component solutions were evaluated. Using the Visual MINTEQ speciation modeling program, we found that the Zn and Cd biosorption by dried biomass of euglena decreased practically linearly with the decreasing the proportion of Zn^{2+} or Cd^{2+} in the solution given by the presence and increasing concentration of EDTA-Na_2 as a complexing agent in the solution. According to this finding, it can be suggested that both Zn and Cd will be bound onto biosorbents predominantly in the form of free cations Zn^{2+} and Cd^{2+}. Data on the biosorption of Zn and Cd by dried biomass of *E. gracilis* var. *bacillaris* expressed by the dependence between the equilibrium specific sorption Q_{eq} (in μmol/g; d.w.) and the equilibrium concentration of metal in the solution C_{eq} (in μmol/dm^3) for the both single and binary solutions were better described by adsorption isotherm according to Langmuir than according to Freundlich. From the comparison of maximum sorption capacities Q_{max} values predicted for the single and binary solutions, we found that in the case of single-component solutions ($Q_{max\ Zn}$ = 531±52 μmol/g; $Q_{max\ Cd}$ = 125±8 μmol/g; d.w.) Q_{max} values for dried biomass of *E. gracilis* var. *bacillaris* were significantly higher than in the case of two-component solutions ($Q_{max\ Zn}$ = 13.2±1.1 μmol/g; $Q_{max\ Cd}$ = 18.7±4.8 μmol/g). Also, we found that in terms of the sorption capacity of dried biomass of euglena and its affinity for studied metals, the phase of growth of vital biomass of euglena as a primary material for the preparation of a biosorbent will probably play a significant role.
The obtained results suggest on the possibility to utilize the dried biomass of *E. gracilis* var. *bacillaris* as a biosorbent applied in the removal of heavy metals from wastewaters or contaminated solutions.

Keywords: Zn, Cd, *Euglena gracilis*, biosorbent, biosorption, adsorption isotherms

Acknowledgement
This work was supported by the project of International Visegrad Fund No. 21720055 „*Algal cell biophysical properties as markers for environmental stress in aquatic systems*“.

ENVIRONMENTAL BURDEN BRATISLAVA - VRAKUŇA LANDFILL OF CHEMICAL WASTE

Zuzana Bártová[a], Daniel Kupka[a], Dávid Jáger[a], Róbert Kubinec[b]
[a] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, Košice, Slovakia, bartova@saske.sk*
[b] *Chemical Institute, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, Ilkovičova 6, Bratislava, Slovakia*

ABSTRACT
The toxic waste landfill of former Juraj Dimitrov chemical plant (CHZJD) is located in the districts of Bratislava - Ružinov and Vrakuňa. Approximately 120,000 m^3 of chemical waste is buried under the surface. After the Gabčíkovo waterworks started in 1992, the groundwater level began to rise causing the chemical waste itself to be reached in 1996. At the point of contact there is a dissolving of pollutants which are thus brought into groundwater. Dangerous substances are transported downstream by the groundwater flow at a speed of 300-500 m/year to the densely populated, agricultural and water-borne area of „Žitný ostrov".
Žitný ostrov represents a significant natural accumulation of groundwater, which was declared a protected water management area (CHV) in 1978 - 46/1978 Zb. Without the effective remediation and isolation of the landfill body, leakage of toxic substances can impact this largest reservoir of drinking water in Central Europe.
Pollution of the site dates back to the late 19th century, when Dynamit Nobel, the predecessor of the later CHZJD and today's Istrochem, was established. From 1873, waste water from chemical production of „Dynamitka" was discharged through the open sewage channel to the Small Danube River in Vrakuňa, until 1966.
Probably due to the already existing ecological burden of the site, the decision of the local administrative authorities in Bratislava no. WATER 1059 / 405-66 dated 14 July 1966, established a chemical waste deposition from the CHZJD production in Bratislava in the area of interest. The waste was loaded with tanks and trucks since 1966 with a volume of about 95,000 tonnes per year and deposited in layers into a dry trough of the former lateral arm of the River Danube, named „Mlynské rameno" (Fig. 1)
Analysis of groundwater samples from different monitoring wells in the landfill body showed significant differences of the individual indicators, reflecting the heterogeneous nature of the pollution.
In the surveyed area, anthropogenically unaffected groundwater should be slightly alkaline (pH 7.1-7.5), calcium-magnesium bicarbonate type with

total mineralization values in the range of 400-600 $mg.l^{-1}$. The natural chemical composition of groundwater is influenced by pollution with inorganic and organic substances in the wider area. Higher mineralization and high electrical conductivity values were found in all parts of the landfill. There were also exceeded values for chlorides, sulphate anions and cations of sodium and potassium.

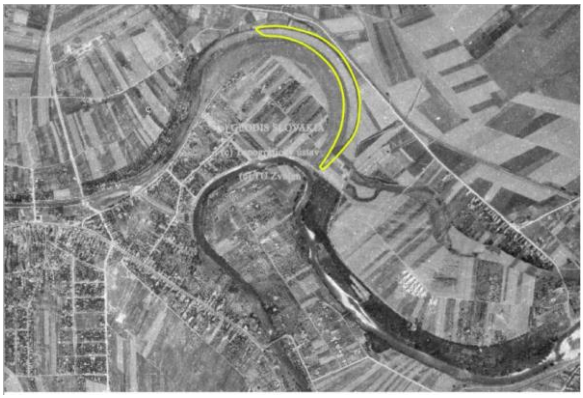


Fig. 1 Historical ortho-photomap from 1950, marking the part of the former lateral arm „Mlynské rameno", in which the chemical waste was deposited in the period 1966-1979. Source: http://mapy.tuzvo.sk/HOFM/

The chemical composition of the contamination is heterogeneous and can be classified into several groups, such as chlorinated hydrocarbons, phenolic compounds, rubber chemicals based mainly on benzothiazole and pesticides. Most of the identified substances are not included in any standards. The content of these substances is approximately 10 times higher than those monitored according to valid standards. The biological effect of the identified chemicals on human health is not yet adequately investigated.
In the groundwater samples from the landfill, the presence of 965 chemicals was found. Of this number, we have identified 282 substances so far.

Keywords: toxic waste landfill, ecological burdens, Bratislava- Vrakuňa

Acknowledgements
The work was supported by the project „Joint project of the Office of Bratislava Self-Governing Region, Slovak Academy of Sciences and Comenius University in Bratislava, concerning environmental burden Bratislava - Vrakuňa landfill of chemical waste of CHZJD and project ITMS: 26220120064 co-financed by EU.

CU(II) AND ZN(II) REMOVAL BY VARIOUS ADSORBENTS BY BATCH AND COLUMN MODE

Alexandra Bekényiová[a], Zuzana Danková[a], Iveta Štyriaková[b]
[a] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovak republic*
[b] *Department of Applied Technology for Raw Materials, State Geological Institute of Dionýz Štúr, Regional center Košice, Jesenského 8, 040 01 Košice, Slovakia*

ABSTRACT
This study presents the possibilities of utilization of natural raw materials siderite (S), kaolin (K), perlite (P), quartz sand (QS), glauconite sand (GS), zeolite (Z) and dolomite (D) and also modified materials as potential adsorbents of Cu(II) and Zn(II) ions. To enhance their adsorption capacity, the surfaces of the most effective materials were coated by MnO_2 particles (samples denoted as SM, KM and GM). The changes of their textural properties after the modification were studied by the low temperature nitrogen adsorption measurement. The most expressive increase of the value of S_{BET} value was observed for the sample SM, from 6 to 36 $m^2 g^{-1}$. The results from the batch adsorption experiments of Cu(II) and Zn(II) ions removal were processed by the Langmuir adsorption isotherm. The calculated value of maximum adsorption capacity increased after the modification from 12.9 to 19.8 mg g^{-1} for siderite and from 10.8 to 39.8 mg g^{-1} for kaolin sample. In the introductory experiments in dynamic regime, the columns filled with mixtures of QS-SM and QS-KM were percolated with binnary Cu(II)/Zn(II) model solution. During the first hour, the effectivity of both mixtures in columns reached between 80- 100 % for both metal ions, except of Cu(II) in case of QS-KM, where removal of 100% was achieved an copper removal remains almost constant up to the end of the experiment (12h) .
With the increasing discharge of industrial wastewater, copper has been listed as one of the most widespread heavy metal contaminants. Zinc is considered as an essential element for life and acts as a micronutrient when present in trace amounts. The WHO recommended the maximum acceptable concentration of zinc and copper in drinking water as 5 mg L^{-1} and 3 mg L^{-1}, respectively.
High surface area of manganese oxides are effective sorbents for the removal of toxic metals from aqueous solutions because of their affinity for several heavy metals. Moreover, nano-MnO_2 has a large number of reactive hydroxyl groups on the surface, which results in large adsorption capacities for heavy metal ions.
The copper and zinc ions have been chose for the adsorption studies with

regard to their wide use in industry and potential pollution impact. Adsorption of Cu(II) and Zn(II) ions from the aqueous solutions on natural materials and also modified materials was investigated and compared in this study. In more detailed were characterized siderite (S) and kaolin (K) samples.
To enhance adsorption capacity of selected natural samples with the highest adsorption capacity, as well as simplify their utilization in the dynamic conditions of the adsorption processes, the coating of surfaces by MnO_2 was used, according to the method of Sivasankar et al. The samples were denoted as siderite modified (SM), kaolin modified (KM) and glauconite modified (GM).
The adsorption of Cu and Zn ions was studied by the batch and column method. The stock solutions were diluted to obtain standard model solutions containing 10 – 300 mg Cu(II)/Zn(II)L^{-1}. The sorption measurements were made by batch technique at the ambient temperature using the rotary shaker (30 rpm). Through the study, the pH was varied from 2 to 9. The adsorbents dose was 1 g L^{-1}. In the preliminary dynamic regime study we used two types of column filled with appropriate amounts of mixture of quartz sand/modified siderite and quartz sand/modified kaolin. Then 50 ml of binnary solution of Cu(II)/Zn(II) ions of concentration 100 mg.g^{-1} for each was passed through the column at a constant flow rate of 6 rpm.
The qualitative phase analyses of natural samples obtained from the XRD measurement confirmed the presence of siderite, quartz, muscovite, chlorite and gyps in S sample and kaolinite, quartz, feldspar, muscovite, rutile, plagioclase and smectite in K sample. In spite of the suppression of synthetic MnO_2 reflections by diffraction lines of mineral phases, its presence in SM and KM was confirmed by the phase analyses. According to that fine MnO_2 particles were assumed what was also proved by SEM.

Table 1 Surface parameters of studied materials

Sample	S_{BET} [m^2 g^{-1}]	V_a [cm^3 g^{-1}]	V_{micro} [cm^3 g^{-1}]	S_t [m^2 g^{-1}]
S	6.89	0.0143	0.001	4.19
SM	36.10	0.0634	0.002	30.95
K	10.76	-	0.00003	10.29
KM	16.09	0.0755	0.00005	15.40

The total pore volume of K, for that case of isotherm type, could not be determined from the value of total volume of adsorbed nitrogen. All surface parameters are summarized in Table 1.
The adsorption of Cu(II) and Zn(II) ions onto various natural and modified samples as a function of pH was studied.
From first series of natural samples (S,P,QS and GS) the most effective

sorbents in the whole pH range were samples S and GS. The S sample achieved the maximum adsorption capacity 9.1 mg.g^{-1} Cu(II) and 11 mg g^{-1} Zn(II) at pH 5.5. For the sample GS 8.2 mg.g^{-1} Cu(II) and 13.1 mg.g^{-1} Zn(II) at pH 5.5 was calculated.
In the second serie of batch adsorption experiments for natural samples (Z,K and D) as a function of pH, the K sample shows the highest affinity to Cu(II) ions at pH 5, for Zn(II) ions at the same pH value the effectivity of sorbents was comparable. The K sample obtained the maximum adsorption capacity 23.2 mg.g^{-1} Cu(II) and 12.1 mg.g^{-1} Zn(II) at pH 5.
The influence of the modification and also the influence of initial ion concentration on sorption properties of the samples show Fig.1 and Fig.2.

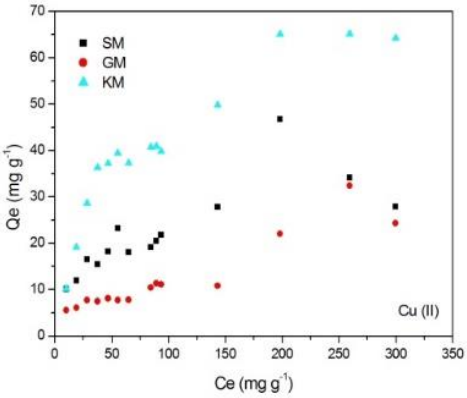


Fig.1 Adsorption isotherms of Cu(II) adsorption onto studied materials

The maximum adsorption of Cu(II) ions appears at pH 5 at the concentration of 200 mg.l^{-1} for all samples. The adsorption of copper ions on SM, GM and KM samples increased with the increasing of initial ion concentration up to 250 mg.l^{-1} , then the equilibrium was established in case of KM sample. In the case of zinc ions with the increasing initial ion concentration increased the adsorption up to 110 mg.g^{-1} of Zn(II).

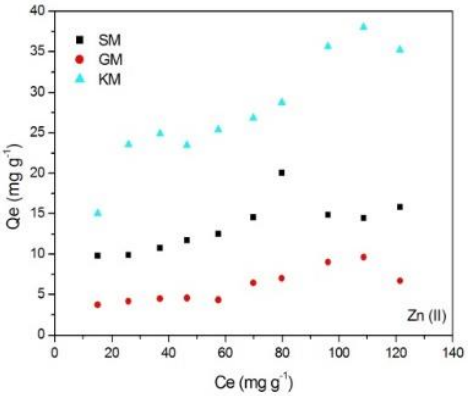


Fig.2 Adsorption isotherms of Zn(II) adsorption onto studied materials

Adsorption effect of modified samples to copper and zinc ions decreased in this order KM > SM > GM. The values of maximum adsorption capacity calculated from the linearized Langmuir model applied to the experimental data increased for selected natural adsorbents after the modification what corresponds with the obtained adsorption isotherms.
The results obtained from this study confirmed that many of non-metallic raw materials located in Slovakia can be utilize as suitable adsorbents of Cu(II) and Zn(II) ions at lower concentration range. From the results of batch adsorption study with natural samples., siderite, kaolin and glaukonite sand with the highest adsorption capacity were modified by manganese oxide.
The highest value of maximum sorption capacity was calculated for KM, which efficiency in the Cu(II) removal from the solutions of lower concentrations reach almost 100 %. In the introductory dynamic conditions (column study) effect of S and K sample modification was compared in a mixture with quartz sand. A maximum removal of 100% was observed for Cu(II) ions in column filled with QS and modified kaolin and with the increase in contact time becomes almost constant up to the end of the experiment (12h). These sorbents can be utilized for the wastewater treatment where the concentration of metal ions are up to 100 mg g^{-1} .

Keywords: adsorption, copper, zinc, siderite, kaolin, quartz sand

Acknowledgement
The authors are thankful for financial support of VEGA grants No. 2/0049/15 and 2/0158/15.

ELECTROCHEMICAL OXIDATION OF ATRZINE

Gergő Bodnár, Daniel Kupka, Dávid Jáger, Miroslava Václavíková, Lucia Ivaničová

Institute of Geotechnics Slovak Academy of Sciences, Watsonova 45, 040 01 Košice, Slovakia. bodnar@saske.sk

ABSTRACT

Atrazine is a commonly used selective herbicide of the triazine class. It has been widely used in the world to control unwanted broadleaf weed. Atrazine was banned in the European Union in 2004, because of its endocrine disruptor effects, possible carcinogenic effect, and negative environmental impact when the contamination in groundwater exceeds the safety levels stated by the EU. Advanced oxidation processes (AOPs) have been widely used to improve biodegradability of many xenobiotic compounds. Electrochemical oxidation (EO) is the most popular procedure among the electrochemical AOPs. The basic principle of electrolytic oxidation of persistent organic compounds is oxidation of the parent molecule to smaller molecules ultimately to carbon dioxide, water and inorganic ions by electrochemically generated hydroxyl radicals (·OH) and other highly reactive species. In this work degradation of atrazine was carried out by electrochemical oxidation using galvanostatic conditions in an undivided cell. Two different types of anodic materials were used for the treatment. Titanium mesh coated by mixed metal oxides RuO_2, IrO_2 and TiO_2 (MMO anode) and niobium mesh coated by boron doped diamond layer (BDD anode). The extent of atrazine mineralization by these two types of anode was evaluated on-line by monitoring the amount of CO_2 liberated in the course of electrolysis. The carbon mass balance was calculated from the initial organic carbon concentration of the atrazine solution and the amount of carbon released as CO_2 in the off gas. The molecular formula of atrazine $C_8H_{14}ClN_5$ comprises eight atoms of carbon which accounts for 44.55% of the molecular mass of atrazine.

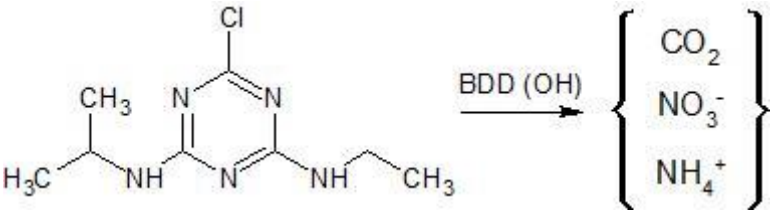


Fig. 1 The structural formula of Atraznie and oxidation products on BDD anode

Accordingly, complete mineralization of 20 mg atrazine yields 8.91 mg of carbon in the form of CO_2. The products of electrochemical degradation of atrazine were analyzed by high performance liquid chromatography (HPLC) using reversed phase column (Acclaim™ 120– C18, 3µm, 120 Å, 2,1x100 mm column (Thermo Scientific)). The separated species were detected by diode array detector (DAD) and electrospray ionization mass spectrometry (ESI-MS).
The results obtained at electrochemical treatment with BDD and DSA anode revealed differences among the electrochemical processes which implies different degradation mechanisms. The oxidation of organic compounds occurs primarily through reactions with hydroxyl radicals. In general, the effectiveness of an electrochemical oxidation is proportional to the ability of anode material to generate hydroxyl radicals.

Keywords: Atrazine, Electrochemical oxidation, Boron doped diamond,

Acknowledgement
This work has been supported by the Marie Curie Programme FP7-People-2013-IAAP-WaSClean project No 612250, the Slovak Grant Agency VEGA, project No 2/0145/15, and the Operational Programme Research and Development through the project: Centre of Excellence for Integrated Research of the Earth's Geosphere (ITMS: 26220120064)

BIOSORPTION OF As(III) BY CHEMICALLY MODIFIED FUNGAL BIOMASS

Slavomír Čerňanský[a], Alexandra Šimonovičová[b]

[a] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Environmental Ecology, Ilkovičova 6, Bratislava 84215, Slovak Republic*

[b] *Comenius University in Bratislava, Faculty of Natural Sciences, Department of Soil Science, Ilkovičova 6, Bratislava 84215, Slovak Republic*

ABSTRACT

Fungal biomass has been identified to possess good biosorption capacity for various chemical elements, especially for metals and radionuclides. Significantly less concern has been focused on biosorption of metalloids such as arsenic, antimony or selenium.

The aim of this paper is to quantify and compare biosorption of trivalent arsenic by non-living, NaOH-treated fungal biomass. The fungal strains of *Aspergillus niger* and *Neosartorya fischeri* used in these experiments, were isolated from arsenic-contaminated mining wastes. All samples were analysed by HG AAS for arsenic contents.

For biosorption experiments, fungi were cultivated onto 50 ml of Sabouraud Broth (HiMedia, Mumbai) on the laboratory shaker at 135 rev.min^{-1} during 72 hours at room temperature. Fungal pellets were killed by boiling them in 0.5 N NaOH solutions for 15 min and then thoroughly washing with huge amount of distilled water till the pH of the eluent was neutral. After washing, the biomass was dehydrated at 5 °C for 24 h and powdered.

Biosorption of trivalent arsenic by NaOH-treated *A. niger* and *N. fischeri* biomass at two different pH (3 and 5) and different contact time (30-240 min) was studied. Initial arsenic content in aqueous solutions were 1 mg which represents 20 mg.l^{-1} of arsenic. The weight of applied fungal dried biomass was 0.5 g. The highest amount of biosorbed trivalent arsenic by the *A. niger* strain was 0.72 and 0.50 at pH 3 and 5, respectively and by the *N. fischeri* strain was 0.43 and 0.47 at pH 3 and 5, respectively. Compared to the results achieved by biosorption of arsenic by unmodified dried fungal biomass (data not shown), biomass treatment with NaOH increased the ability of fungal biomass to bind trivalent arsenic. The highest influence of chemical modification in relation to arsenic biosorption was observed by using of *N. fischeri* (very low arsenic biosorption by untreated biomass).

According to achieved results, it is evident that biosorption of arsenic did not reach comparable results with biosorption of cationic metal ions. Although, biosorption of arsenic in case of chemical modification of fungal biomass was higher compared with unmodified biomass, it is still relatively low. It is

evident that NaOH treatment was not so effective for these types of fungal biomasses, although, some researchers found out it as very effective for arsenic biosorption. It can be caused by different composition of the cell wall of selected fungal strains; it is known that number and quality of functional groups occurring within the cell wall can significantly differ among various fungal strains of the same species.

Keywords: biosorption, arsenic, NaOH treatment

Acknowledgement
The financial support from the Scientific Grant Agency of the Ministry of Education of the Slovak Republic VEGA No. 1/0424/18 is gratefully acknowledged.

THE STUDY OF COPPER REMOVAL FROM WATERS BY WOODEN SAWDUST

Stefan Demcak[a], Magdalena Balintova[a], Maria Demcakova[b], Inga Zinicovscaia[c,d], Nikita Yushin[c], Marina V. Frontasyeva[c]

[a] *Institute of Environmental Engineering, Faculty of Civil Engineering, Technical University of Kosice, Vysokoskolska 4, 04200 Kosice, Slovakia*

[b] *Slovak Academy of Sciences, Institute of Experimental Physics, Watsonova 47, 040 01 Kosice, Slovakia*

[c] *Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Joliot-Curie Str., 6, 1419890 Dubna, Russia*

[d] *Horia Hulubei National Institute for R&D in Physics and Nuclear Engineering, 30 Reactorului Str. MG-6, Bucharest - Magurele, Romania*

ABSTRACT

The heavy metal remediation from wastewater is very important due to their persistency in aquatic environment. Conventional treatment technologies as precipitation for the removal of these toxic heavy metals are not economical and further generate huge quantity of toxic chemical sludge. The using of wooden sawdust is emerging as a potential alternative to the existing conventional technologies for the removal of metal ions from aqueous solutions. Wooden materials or wastes are cheap sorbent materials. The benefits of application of wooden by-products or wastes for wastewater treatment are determined by their high removal selectivity, good adsorption capacity and possibility of regeneration.

The wood sawdust, a low-costs adsorbent, is perspective for removing metal ions, some types of acid and basic dyes as well as other unwanted compounds from wastewaters. The aim of this article is a study of the removal of Cu(II) from water by unconventional waste products including the wooden sawdust of poplar, cherry, spruce and hornbeam.

The efficiency of the adsorption processes strongly depends on the composition of the wastewater showed formation of complex compounds of metal cations with wood sawdust functional groups. The functional groups of poplar, spruce, cherry, and hornbeam were determined using FT-IR spectroscopy (Fig. 1). The metal adsorption capacity of wooden sawdust is influenced with the presence of surface structures of –OH (3,650-3,000 cm^{-1} and 1,700-1,600 cm^{-1}), -COOH (1,750-1350 cm^{-1} and 1,250-1,000 cm^{-1}), and $–NH_x$ (3,337 cm^{-1}) functional groups that are present in organic materials. The structure of wooden sawdust is primarily formed by cellulose, hemicellulose, and lignin.

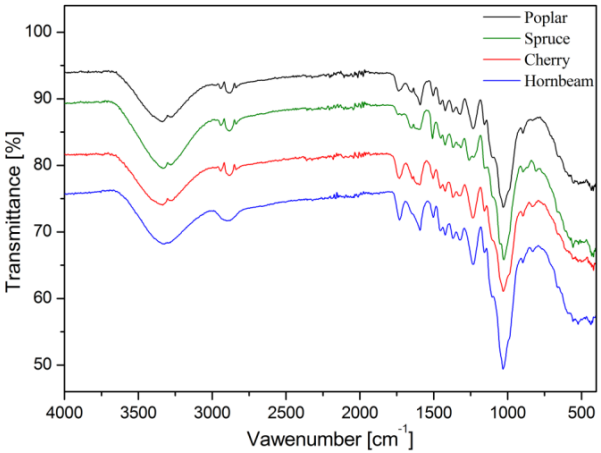


Fig. 1 Infrared spectra of selected wooden sawdust

Data obtained by neutron activation analysis (NAA) revealed that ion exchange is one of the mechanisms underlying metal removal by the selected sawdust from the model solutions (Table 1).

Table 1 Chemical composition of raw and Cu-loaded wooden sawdust

Element	Average content of elements in adsorbents [mg.kg^{-1}]					
	Na	**Mg**	**Cl**	**K**	**Ca**	**Ba**
Poplar	12.5 ± 0.9	264 ± 18.5	12.9 ± 1.42	1790.0 ± 286	1810.0 ± 181	32.1 ± 1.6
Poplar_Cu(II)	12.3 ± 0.9	174 ± 12.2	10.4 ± 1.14	**< 151.0***	**1520.0 ± 152**	24.5 ± 1.23
Spruce	5.8 ± 0.4	497 ± 34.8	51.9 ± 5.71	287.0 ± 45.9	5210.0 ± 521	5.4 ± 0.54
Spruce_Cu(II)	17.9 ± 1.3	37.4 ± 2.62	11.6 ± 1.28	**< 63.0***	**495.0 ± 49.5**	4.0 ± 0.4
Cherry	21.2 ± 1.5	175 ± 12.3	27.8 ± 2.5	606.0 ± 97	1040.0 ± 104	6.5 ± 0.52
Cherry_Cu(II)	15 ± 1.1	8.7 ± 0.61	29.6 ± 2.66	**< 232.0***	**697.0± 69.7**	3.7 ± 0.29
Hornbeam	48.6 ± 3.4	376 ± 26.3	35.0 ±3.85	1570.0 ± 267	4350.0 ± 435	30.2 ± 1.51
Hornbeam_Cu(II)	113 ± 7.9	220 ± 15.4	14.1 ± 1.55	**< 214.0***	**2660.0 ± 266**	26.4 ± 1.32

* Values under detection limits

The NAA obtained a decrease of concentration of the certain elements (K and Ca) after the adsorption, indicating that ion-exchange is one of the mechanisms underlying the interaction of metal with the natural sorbents.

Keywords: heavy metals, water treatment, adsorption, wood sawdust

Acknowledgement
This work has been supported by the Slovak Grant Agency for Science (Grant No. 1/0563/15).

ADSORPTION PROPERTIES OF MODIFIED BENTONITE FROM THE SLOVAK DEPOSIT

Silvia Dolinská, Ingrid Znamenáčková, Zuzana Danková, Slavomír Hredzák

Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 040 01 Košice, Slovak Republic, e-mail: sdolinska@saske.sk

ABSTRACT

This article examined the impact of natrification agents and methods on the adsorption properties of bentonite. The natural bentonite with the most amount of calcium cations in the interlayer space was first modified in the slurry, which contained the activating agent - Na_2CO_3 and distilled water. The second modification of natural bentonite was carried out in suspension through NaOH as activating agent. The shift of peak of the basal plane peak towards the higher values of 2 Theta for modified bentonites was due to the cation exchange of Ca^{2+} with Na^{+} cations with smaller atomic radius. The diffraction pattern of Na_2CO_3/bentonite, especially NaOH/bentonite indicates overlapping peaks, resp. decrease in crystallinity probably due to the unequal natrification of the structure of the montmorillonite.

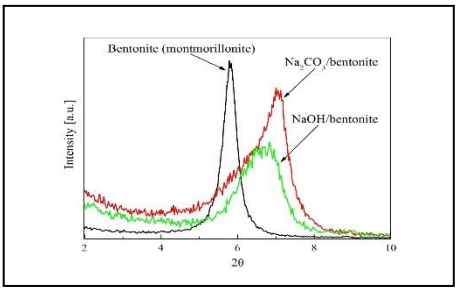


Fig. 1. X-ray diffraction pattern of the natural and modified bentonite

Natrification – decrease of interlayer space: 1.51 nm (bentonite) → 1.24 nm (Na_2CO_3/bentonite) and 1.31 nm (NaOH/bentonite).

The measurements of cation exchange capacity (CEC) by methylene blue method showed that bentonite modified by NaOH achieved higher CEC in meq/100g of clay than bentonite modified by Na_2CO_3 (Table 1). The adsorption properties of activated bentonite samples were compared by the sorption of three different heavy metal cations (cadmium, zinc and lead).

Bentonite modified by NaOH reached the highest maximum adsorption capacities in (mg/g) for each of measured heavy metals (Table 2).

Table 1 Values of CVC for natural and modified bentonites

Sample	**CVC [meq/100g]**
Natural bent.	84.4
Na_2CO_3/bent.	114.1
NaOH/bent.	115.9

Table 2 Maximum adsorption capatities [mg/g]

	Na_2CO_3/bent.	**NaOH/bent.**
Cd^{2+}	72.99	81.97
Pb^{2+}	208.33	249.8
Zn^{2+}	76.34	83.33

Keywords: bentonite, natrification, sorption

Acknowledgements
The work was supported by the Slovak Grant Agency VEGA, Grants No. 2/0055/17 and No. 2/0049/15. This work was supported by the Slovak Research and Development Agency under the contract No. APVV-0252-10.

BACTERIAL LEACHING OF POLLYMETALLIC ORES FROM ZLATÉ HORY LOCALITY

Iva Janáková, Hana Kovaříková, Vladimír Čablík, Sarah Janštová, Dana Rouchalová
VSB-Technical University of Ostrava, Faculty of Mining and Geology, 17. listopadu Str. 15, 708 33 Ostrava-Poruba, Czech Republic

ABSTRACT

This work deals with the use of *Acidithiobacillus ferrooxidans* to bioleach polymetallic ores (Cu, al, Zn, and Fe) from locality Zlaté hory. As for geology, Zlaté Hory is a highly varied locality with frequent, quartz, muscovite, albinite as well as deposits of sulphide ores (pyrite, chalcopyrite). Leaching lasted for 5 weeks in a bioreactor that ensured optimal conditions for bioleaching.

Zlaté Hory belongs to the geologically very interesting area of the Jeseník District. It is one of the most important mineral deposits in the Czech Republic. The Czech Republic's raw material and mineral resources policy is currently not compatible with proposals for restoring mining activities in this area. Based on statistics compiled by the Ministry of the Environment in 2016, Zlaté Hory is still registered as a source of Au, Ag, Cu, Pb and Zn. Consequently, metal mining is hitting the legislative and economic limits of the present time. From the point of view of the mineral resources of the Czech Republic, there is still the possibility of mining, and the further development of the mining technologies and the development of the world mineral wealth market will be the next activity. The supply of the sediment under investigation is protected by Diamo, a state enterprise. This company manages the treatment of emerging mining waters. The nature of the water is continuously monitored. The area is protected from mineralized water, which poses a potential hazard to groundwater. For this reason, were find here a system of drainage terminals from which water is pumped into a leakage water tank. The second source of water is the water treatment plant for lower galleries with higher acidity.

Waters containing iron, copper, and others are drawn into the channels from which they are pumped. The water is collected in the purification stations, where they are neutralized (neutralizing stations) by slaked lime due to their acidic character. With lime slaked, in the next stage of treatment, settling stations, the components are coagulated to form a dense sludge. Neutralized thickened sludge is pumped into the DORR plant, a settling station that, by its proportions, helps to streamline the process and capture the primary

sludge. The primary sludge is pumped into a sedimentation pond which serves to sediment sludge and once a year this sediment is pumped from the bottom of the pond to the sludge dumps, technologically adapted to the type of material deposited.

The process of bioleaching may be defined as the dissolution of minerals caused by direct or indirect action of diverse microorganisms. In the course of such processes, the natural flora of microorganisms consists of a mixture of acidophilic autotrophic bacteria. Their role is to produce chemical agents vital for leaching. It is a simple and effective method to recover metals from mineral raw materials, where conventional methods cannot be applied. The fundamental mechanism is the direct or indirect oxidation of sulphide ore. Through oxidation, metal releases into solution, from where it can be recovered using conventional chemical-physical methods. In this case, we aim to recover metals from sulphide minerals through the activity of bacteria of *Acidithiobacillus* genus, which convert insoluble metal sulphides into soluble sulphates. With regard to the character of the sample, we selected the most suitable species to leach sulphide ores, i.e. *Acidithiobacillus ferrooxidans*.

The mineralogical composition analysis of the samples was carried out. An X-ray diffraction analysis was done in the laboratories of the Institute of Geological Engineering at VŠB-TU Ostrava. The measurements were carried out on a modernized, fully automated diffractometer URD-6 (Rich. Seifert-FPM, SRN). The following phases were identified on the samples in question: quartz (68.14%), chlorite (5.02%), muscovite (13.96), albite (6.51%), pyrite (5.47%), sphalerite (2.05%). (See Table 1).

The leaching process took place in New Brunswick BIOFLO®&CELLINGEN® 310 (Fig. 1). Having sterilized the bioreactor, 500 g of sample from Zlatý Chlum at 100% grain size under 0.063 mm and 10 litres of 9K medium free of $FeSO_4$ were inserted inside the bioreactor. After one-hour stirring and homogenization of the suspension, a bacterial culture of *Acidithiobacillus ferrooxidans* in the amount of 880 ml was introduced into reactor. A pre-cultivated culture of *Acidithiobacillus ferrooxidans* from the Institute of Microbiology in Brno was applied. The concentration of the introduced bacteria participating in the process of bacterial oxidation was 109 in 1 ml of bacterial solution.

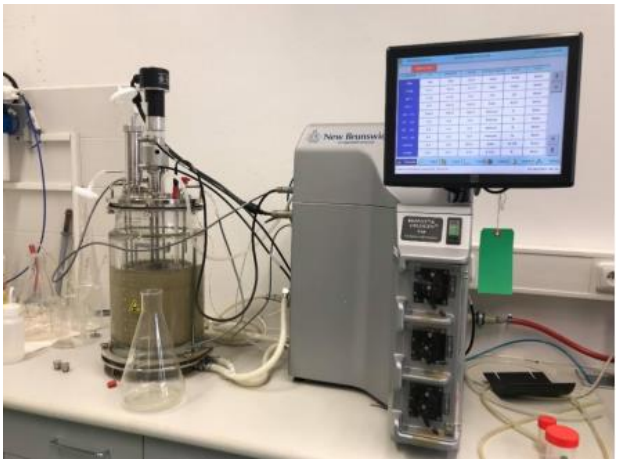


Fig. 1 Bioreactor Bioflo 310

The samples were leached for 5 weeks. In the course of leaching, samples were drawn after the 1st, 2nd, 3rd, 4th and 5th week. All the samples were washed in 0.1.mol HCl and later in distilled water to stabilize pH. The drawn samples were analysed using a portable spectrometer Dynamic XRF DELTA PROFESSIONAL by BAS Rudice s.r.o. In the course of experiment, the colour of the leached sample changed. At the start, the colour was dark brown and it became lighter during the experiment, which was likely caused by the highly acidic environment.
The results imply that the experiments were successful, leaching 54% of Fe, 47% of Zn and 4% of Cu.

Keywords: bacterial leaching, pollymetallic ores, pyrite, chalcopyrite, *Acidithiobacillus ferrooxidans*, bioreactor

Acknowledgement
The authors of the project would like to thank the SGS which financially supports this project, under the SP2018/31

ANALYSIS OF AUTHOTROPHIC SULPHUR OXIDISING BACTERIA IN SLOVAK GOLD MINE HODRUŠA-HÁMRE

Jana Kisková[a], Ivana Timková[a], Lenka Maliničová[a], Jana Sedláková-Kaduková[a], Stanislav Jeleň[b], Peter Pristaš[a]

[a] *Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafarik University in Košice, Šrobárova 2, 04154 Košice, Slovakia*

[b] *Earth Science Institute, Slovak Academy of Sciences, Ďumbierska 1, 974 01, Banská Bystrica, Slovakia*

ABSTRACT

In the past years, our perspective on the Earth's biosphere has expanded from just the terrestrial and oceanic realms to include deep subterranean and subseafloor environments. The deep terrestrial subsurface environments such as those exemplified by deep mines represent an emerging area for exploring microbial populations with bewildering arrays of metabolic capabilities. The subsurface biosphere in gold deposits was very rarely studied, however, according to the newest findings it is obvious that bacteria actively contribute and regulate biogeochemical cycles in this environment. Sulphur-oxidising authotropic bacterial communities in deep biosphere from weathered ore samples collected from active gold mine Hodruša-Hámre, Slovakia using cultivation based analyses followed by DNA extraction, PCR amplification and 16S rRNA gene analyses were studied. The analyses have revealed that the population of sulphur-oxidising bacteria in gold mine is dominated by single species of *Aciditiobacillus*, particularly *A. albertensis* (Fig. 1) suggesting the low level of autotrophic bacterial diversity in deep deposits.

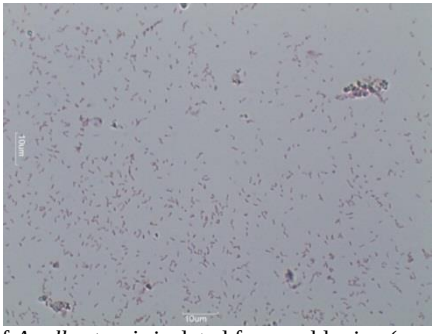


Fig. 2 Photograph of *A. albertensis* isolated from gold mine (magnification 640 x).

This species was for the first time isolated from weathered rocks of subsurface gold mine. Also indirect measurement of pH changes in cultivation media confirmed the presence of acidophilic bacteria with active production of acids, decrease of pH was observed at the beginning of isolation and later pH in range of 2 – 1.5 was maintained in both, sulphuric acid and thiosulphate, media. Presence of gram-negative rods was observed also by optical microscopy. Although *A. albertentsis* is not a new species, still there is very little known about these bacteria and their potential in bioleaching of metals from ores or metal-bearing waste. Further studies of functional bacteria and potential new functional species need to be carried out to explore their specific contributions to gold biogeochemical cycling.

Keywords: autotrophic bacteria, *Acidithiobacillus albertensis*, gold mine

Acknowledgement
The work was financially supported by Research Agency of the Ministry of Education, Science, Research and Sport of Slovak Republic under the VEGA Projects 1/0229/17 and 1/0560/15 and by Slovak Research and Development Agency under the Project APVV-15-0083.

THE FATE OF ANTIBIOTIC RESISTANCE DURING WASTEWATER TREATMENT

Jana Kisková, Adam Juhás, Lenka Maliničová, Jana Sedláková-Kaduková, Ivana Timková, Peter Pristaš

Pavol Jozef Šafárik University in Košice, Faculty of Science, Institute of Biology and Ecology, Department of Microbiology, Šrobárova 2, Košice 04154, Slovak Republic

ABSTRACT

Antibiotics are widely used for the treatment and prevention of bacterial diseases. A serious problem of their intensive use is the development of antibiotic resistant bacteria and resistance genes. Bacterial community including resistant strains can be significantly reduced during wastewater cleaning process but on the other side, many studies documented that wastewater treatment plants can be reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. In our work, we investigated if wastewater treatment plant acts as a barrier for antibiotic resistant bacteria in the wastewater stream. The samples of inlet and outlet water were taken in the wastewater treatment plant for the city Košice in Kokšov-Bakša. After cultivation on R2A medium at 37 °C and laboratory temperature, bacterial isolates were identified by matrix assisted laser desorption/ionization time-of-flight mass spectrometry and their sensibility to ampicillin, kanamycin, tetracycline and chloramphenicol was examined.

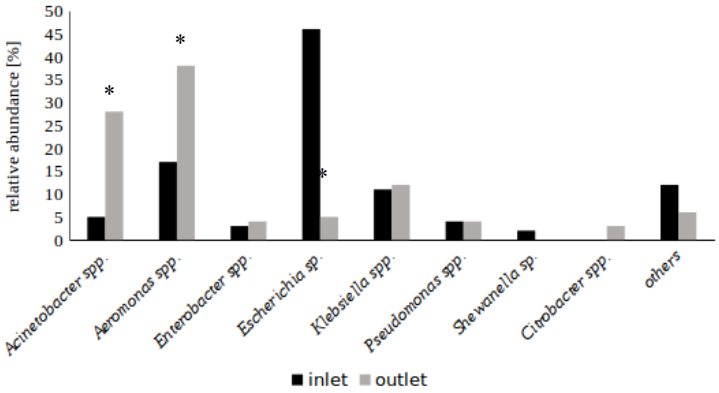


Fig. 1 Relative bacterial abundance at the genus level**.**
* significant changes in the prevalence of bacterial taxa between inflow and outflow water samples (Chi-test, < 0,05)

Escherichia coli was the most common bacterial species in inflow (46%), followed by *Aeromonas* spp. (17%) and *Klebsiella* spp. (11%). In outflow, *Aeromonas* spp. (38%) and *Acinetobacter spp.* (28%) showed the highest abundance (Fig. 1.)

While microbial community was significantly changed during water treatment process, the occurrence of antibiotic resistance was not affected. In inlet water sample, 90% of isolates were resistant to ampicillin, 26% to kanamycin, 31% to tetracycline and 26% to chloramphenicol. In outlet, statistically important reduction was found only within isolates resistant to tetracycline (Fig. 2).

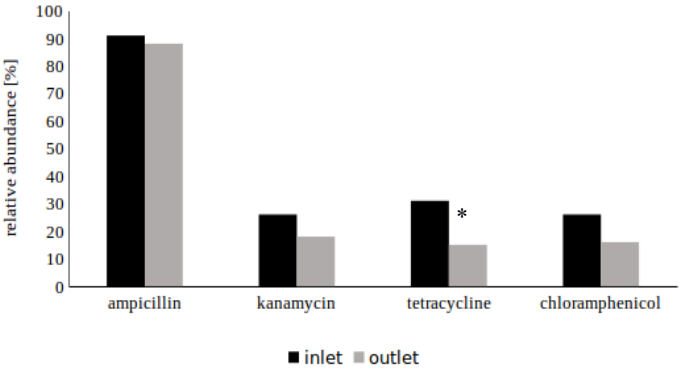


Fig. 2 Relative abundance of antibiotic resistant bacterial isolates**.**
* significant changes in the prevalence of antibiotic-resistant bacterial isolates between inflow and outflow water samples (Chi-test, < 0,05)

Based on these results, we can assume that the bacterial community in the wastewater treatment plant may represent a potential source of antibiotic resistant bacteria and genes, which could be further disseminated in an aquatic environment.

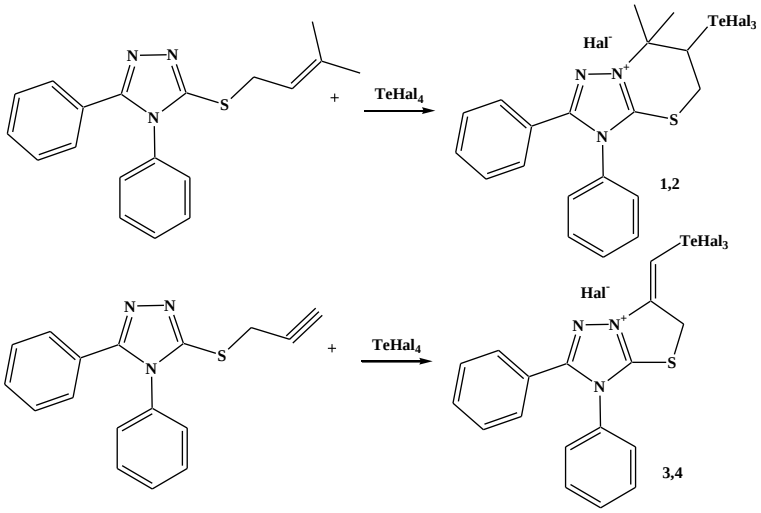
Keywords*:* bacteria, wastewater treatment plant, antibiotics, antibiotic resistance

Acknowledgements
The work was fully supported by an APVV-16-0171 project from the Slovak National Research and Development Agency of Ministry of Education, Science, Research and Sport of the Slovak Republic.

PRODUCTION OF FUNGAL AND ANTIBACTERIAL TELLURIUM-CONTAINING REAGENTS BASED ON 1,2,4-TRIAZOLE MOIETY VIA GREEN CHEMICAL METHODS

Nataliya Korol, Mikhailo Slivka, Valerij Pantyo, Vasil Lendel
Organic Synthesis Laboratory, Uzhhorod National University, Uzhhorod, 88000, Ukraine

ABSTRACT
Modern environmental technologies now are widely used in industry, particularly, in the production of medicines and external disinfecting and antifungal agents. Current trends in organotellurium chemistry are going forward the introduction of these reagents into the field of green chemistry, organocatalysis, as well as into the preparation of biomimetic materials. Development Te-containing bioactive compound is an actual task, because this mineral is essential to the human body with one hand, and on the other hand all know about high toxicity Tellurium compounds.
Tellurium in organic form we have chosen the 1,2,4-triazole system, because this heterocycle has small toxicity; functional and fused derivatives of symmetric triazoles have possess essential bio-activity and they can be synthesized from available reagents and with using of such safe-environmental solvent as water and ethanol.



The results revealed that all Te-containing compounds 1-4 exhibited high activity on fungi and gram-negative bacteria (Table 1). Te-introduction into organic moiety considerably increases the activity of these compounds towards fungi and gram-negative bacteria and decreases their toxicity in comparing with toxicity of known Te-containing remedies.

Table 1 Antimicrobial and fungal activity of compounds **1-4**

Bacteria	Compounds			
	1	**2**	**3**	**4**
Salmonella enteritidis	21,5±3,8	22,2±5,1	21,5±2,7	20,7±3,4
Klebsiella pneumonia	20,6±2,3	25,9±4,6	19,8±3,0	19,9±2,4
Candida albicans	19,8±2,1	17,3±3,4	18,4±2,6	20,3±3,3

For the selected compounds the quantitative data on antimicrobial activity were measured (Table 2).
As a screening test, the Kirby-Bauer method was used in accordance with generally accepted requirements. For substances with pronounced bactericidal activity, the method of serial (2-fold) micro-dilutions in liquid nutrient media was additionally conducted, in which the minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) were determined.

Table 2 Quantitative data on antimicrobial activity of compounds **1,2**

Bacteria	Compound			
	1		**2**	
	MIC (mcg/ml)	MBC (mcg/ml)	MIC (mcg/ml)	MBC (mcg/ml)
K. pneumonia	31,25	62,5	31,25	125
S. enteritidis	62,5	125	-	-

Keywords: 1,24-triazole, Tellurium, antibacterial activity, fungal activity, green chemical methods.

Acknowledgement
Authors express sincere gratitude to Prof., PhD. Jana Sedláková for the opportunity to take part in the conference.

BACTERIAL OXIDATION OF FERROUS IRON. FROM NATURAL WEATHERING PROCESSES TO INDUSTRIAL BIOMINING

Daniel Kupka
Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001, Košice, Slovakia. dankup@saske.sk

ABSTRACT
The majority of microorganisms that are commonly used for industrial bioleaching of metal sulphides are aerobic, acidophilic Fe(II)–, and/or sulphur oxidizing bacteria or archaea. The ferric ion generated by microbial action is a strong oxidant towards the metal sulphides, liberating the metals into solution. The oxidizing agent iron(III) originates from the microbial iron(II) oxidation. The reduced inorganic sulphur compounds (RISC) that are formed during the reaction of pyrite with ferric iron are further oxidized to sulphuric acid by microorganisms, maintaining low pH, which is essential for acidophiles and ferric iron solubility.
In industrial biomining three technical processes are differentiated: Heap or dump bioleaching, tank bioleaching and in situ bioleaching. Today the recovery of copper from sulphide ores is the most important industrial application, and a significant proportion of the world copper production originates from heap or dump bioleaching. Furthermore, biomining is applied for the recovery of gold, cobalt, nickel, zinc and uranium.
While the oxidation of ferrous to ferric iron is desirable reaction from hydrometallurgical point of view, it is of great importance to pay attention to the biocatalytic action of microorganisms in natural biogeochemical processes, in particular in the production of acid mine drainage waters (AMD) from waste tailings at active as well as derelict sulphidic mines.
Iron is redox active element and readily transforms abiotically or biotically. The E_0 value of the Fe^{3+}/Fe^{2+} redox couple (+771 mV) implies, that this couple can be used by microorganisms both to supply electrons (aerobic respiration) and to accept redox equivalents (e.g., for the oxidation of organic compounds).
The rate of abiotic oxidation of ferrous iron by oxygen at ambient temperatures in acidic solutions (pH < 2) is very slow, is of the first order with respect to the partial pressure of oxygen, and of the second order with respect to Fe^{2+} concentration. As the solution pH is raised above 5 to neutral and basic region, the reaction rate increases and the order changes to second order with respect to hydroxyl.

The rate of bacterial oxidation of ferrous iron released from pyrite surfaces is up to one million times faster than the abiotic oxidation rate at low pH. Because Fe^{3+} is the predominant pyrite oxidant, AMD generation in natural environments are largely determined by microbial activity.
Under aerobic conditions, iron–oxidizing bacteria facilitate ferrous iron and RISC oxidation followed by co-precipitation of iron hydroxysulphate minerals schwertmannite and jarosites (Fig 1 A).
Under anoxic or oxygen limited conditions bacteria that survive in acid mine drainage at the expense of reducing equivalents supplied by organic or inorganic compounds can utilize ferric iron as terminal electron acceptor. Acidophilic iron reducing bacteria cause reductive dissolution of ferric iron minerals in AMD sediments, which promotes mobilization and downstream transportation of iron, sulphate and associated elements.

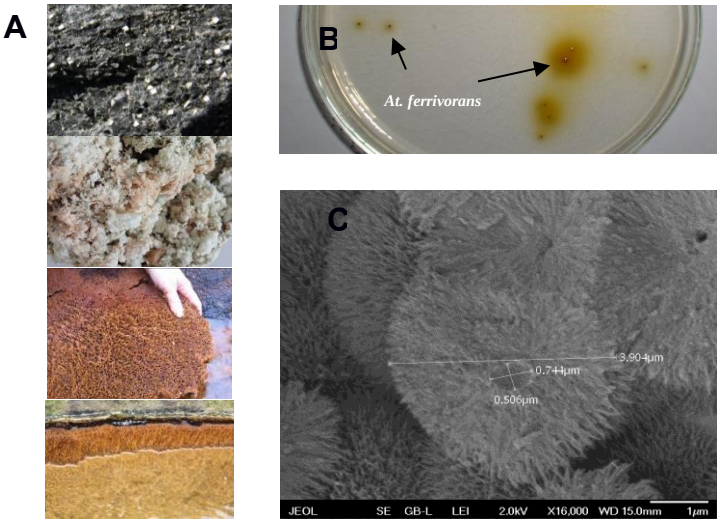


Fig. 1 Paragenetic sequence of iron minerals forming from pyrite oxidation (A). Colonies of Fe-oxidizing bacteria *Acidithiobacillus ferrivorans* on solid medium with ferrous iron (B). SEM microphotography of Schwertmannite mineral (C).

Keywords: acidophilic bacteria, iron, oxidation, reduction, bioleaching

Acknowledgements
This work has been supported by the Marie Curie Programme FP7-People-2013-IAAP-WaSClean project No 612250, the Slovak Grant Agency VEGA, project No 2/0145/15 and project co-financed by EU, ITMS: 26220120064.

THE INFLUENCE OF *A. NIGER* PRE-CULTIVATION ON THE EFFICIENCY OF Li DISSOLUTION FROM Li-ACCUMULATOR

Renáta Marcinčíková[a], Jana Sedláková-Kaduková[b], Alena Lutpáková[c]

[a] *Institute of Materials and Quality Engineering, Faculty of Materials, Metallurgy and Recyclaction, Technical University in Košice, Letná 9, 04200 Košice, Slovakia*

[b] *Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafarik University in Košice, Šrobárova 2, 04154 Košice, Slovakia*

[c] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovakia*

ABSTRACT

Very fast development of mobile electric products leads to an increasing demand for lithium, the main material in rechargeable batteries. The irreplaceable industrial value of lithium increases the global demand for this light metal. Great amounts of lithium ion batteries (LIBs) have been manufactured to meet the global demand for power source resulting in very fast growing amount of spent Li batteries, annually 200 – 500 million tons of Li-containing waste is produced [3]. Li content in such waste exceed the natural occurrence of Li in ores or brines several times what makes a valuable secondary Li resource from spent Li accumulators and batteries, nowadays. Advantage of spent Li batteries processing are not only in Li recovery because they contain several valuable metals such as Co, Li, Mn, Ni and their compounds. There are several technologies suggested for the treatment mostly based on hydrometallurgical and pyrometallurgical processes, however, majority of them is on semi-pilot level. Eco-friendly and cost effective alternative to these processes represent biohydrometallurgical methods based on the ability of microorganisms to transform insoluble solid compounds into soluble ones which can be recovered. The aim of our work was to understand the effect of cultivation on utilisation of *A. niger* on lithium bioleaching from Li-accumulators. The inhibition effect of Co^{2+} ions on fungi spore germination at the beginning of bioleaching period was observed. To avoid the inhibition, *A. niger* biomass was pre-cultivated prior the start of bioleaching experiments. The increase of bioleaching efficiency from 5 to 11% was observed. Bioleaching efficiency using non-cultivated and pre-cultivated biomass of *A. niger* for Li dissolution from Li accumulators is shown in Fig. 1.

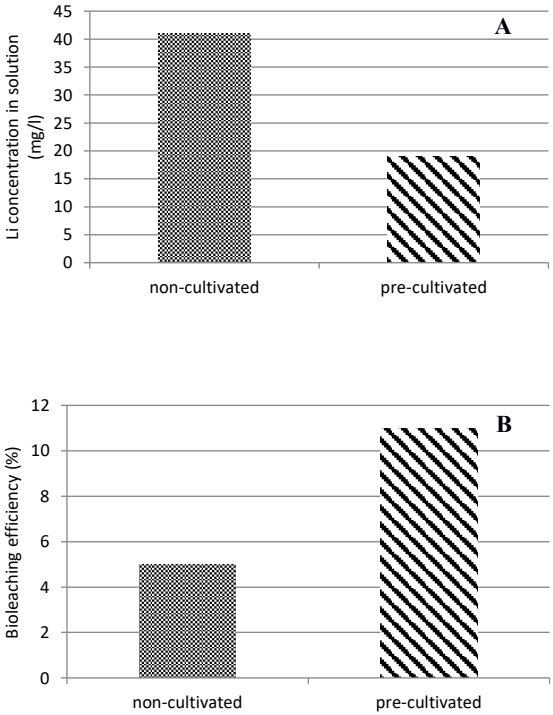


Fig. 1 Differences in Li^+ ions concentration in solution (A) and bioleaching efficiency (B) using non-cultivated and pre-cultivated *A. niger* biomass.

Lower concentration of Li in solution with pre-cultivated biomass was attributed to the fast Li bioaccumulation into the biomass It is visible that pre-cultivation has significant effect on Li bioleaching with *A. niger*, though future study is necessary to optimise bioleaching conditions to reach even higher bioleaching efficiency.

Acknowledgement
The work was financially supported by a grant from the Slovak National Grant Agency under the VEGA Project 1/0229/17.

PLANT BIOTECHNOLOGY TOWARDS METAL TOLERANT PLANTS

Ildikó Matušíková[a], Jana Moravčíková[b], Miroslav Horník[a]

[a] *University of SS. Cyril and Methodius, Department of Ecochemistry and Radioecology, J. Herdu 2, Trnava, SK-917 01, ildiko.matusikova@ucm.sk.*

[b] *University of SS. Cyril and Methodius, Department of Biotechnology, J. Herdu 2, Trnava, SK-917 01*

ABSTRACT

Global mechanization, urbanization and industry release large amounts of toxic compounds into the biosphere. These hazardous pollutants, including heavy metals like cadmium, pose a serious threat to the ecosystem. Developing and optimizing remediation of contaminated soils and water are highly desired, but the conventional remediation approaches are expensive, non-specific and often make the soil unsuitable for agriculture. Therefore, eco-friendly and sustainable approaches such as bioremediation, phytoremediation and rhizoremediation are presently of great interest for the clean-up of contaminated sites. The biotechnological strategies for heavy metal and metalloid containment removal from the environment can use different organisms including microbes and plants. We present our research that reflects to the given problematics by different approaches.

We apply conventional PCR techniques with specific primers to identify metal tolerant varieties of selected crops. Using molecular biology approaches we identify key players of plant metal tolerance, detoxification or sequestration. The genetic transfer of such genes into target species we realize by means of transformation with *Agrobacterium*. The uptake, translocation and accumulation of metals in plants we study by analytical techniques; these include atomic absorption spectrometry, gammaspectrometry, autoradiography and positron emission tomography.

Our screenings for metal tolerant and accumulating varieties characterize the analysed soybean, wheat and flax cultivars that are potentially safe/risky for production of contaminated food (fodder). The results of this screening are provided to the GenBank of Slovakia, and also suggest objects for further studies. More detailed molecular analyses of plants exposed to metal stress revealed several defence components that likely play role in metal tolerance or safe metal accumulation in tissues. For example, three genes for dehydrins, proteins functioning as chaperones mainly under drought stress, were transferred and expressed in tobacco to evaluate their impact on metal uptake and plant tolerance against different metals. The preliminary data revealed

that one of the studied dehydrin genes appears to enhance the transport of cadmium into tobacco shoots without obvious toxicity symptoms, highlighting the advances and implications of obtained transgenic plants in phytoremediation and cleaning-up of contaminated environments.

Keywords: Cadmium, Food Contamination, Genetic Markers, Transgenic Plants

Acknowledgement: The work is financed by the Slovak Research and Development Agency under contract numbers APVV-15-0051, APVV-15-0098 and VEGA 2/0035/17.

MICROBIAL CONSORTIUM IN REMOVAL OF NICKEL FROM ENVIRONMENTAL SAMPLES

Sanja Nosalj[a], Alžbeta Takáčová[b,c], Alexandra Šimonovičová[a], Ivan Šimkovic[a], Milan Semerád[b], Ladislav Dančc, Csilla Tóthová[c], Paula Brandeburová[d]

[a] *Department of Soil Science, Faculty of Natural Sciences Comenius University, Ilkovičova 6, 842 15 Bratislava*

[b] *Department of Environemntal Ecology, Faculty of Natural Sciences Comenius University, Ilkovičova 6, 842 15 Bratislava*

[c] *VÚRUP, a.s., Vlčie hrdlo, P.O.BOX 50, 820 03 Bratislava*

[d] *Department of Environmental Engineering, Institute of Chemical and Environmental Engineering, Faculty of Chemical and Food Technology, Slovak University of Technology, 812 37 Bratislava*

ABSTRACT

Many autothrophic and heterothrophic microorganisms are used to remove or reduce the content of heavy metals and potentionally toxic elements from different environmental samples. In our study we used the consortium of autotrophic microalgae *Chlorella* sp. and heterotrophic microscopic filamentous fungus *Aspergillus niger* in removal of nickel from environmental solid samples. Our experiments allowed to study concurrently biosorption and bioaccumulation of this element.

Algal biomass of C*hlorella* sp. was obtained from Institute of Microbiology of the CAS, v. v. i. (Opatovický mlýn, Třeboň, Czech Republic). Dry biomass of algae was prepared by washing of biomass in deionised water and drying in an oven at 70°C for 6 h. Fungal strain *Aspergillus niger* was isolated from Dystric Cambisol (contaminated and eroded) at the mining locality Šobov. Long-term effect of ultra acidic (pH 3.0) environmental condition combined with presence of metal ions (Fe^{3+} 346 mg/kg) caused mutation of this strain and influenced gene expression, the metabolism and consequently their morphological appearance (Šimonovičová et al. 2013). Fungal pellets were prepared in a 50 ml SDB (Sabouraud Dextrose Broth Liquid Medium, HiMedia, Mumbai, India) enriched with a 5 ml suspension of conidia from a *A. niger* pure culture. Cultivation was carried out using shaker Unimax 2010 (Heidolph, Germany) at 170 rpm. After 3 days the pellets were washed with large amount od distilled water and used in experiment. All experiments were carried out under static conditions and also on the shaker at 170 rpm in combination as show in Table 1.

Table 1 Conditions of all laboratory experiments

sample	solid substrate (g)	*Chlorella* sp.	pellets of *A. niger*	destiled water (ml)	shaker 170 rpm
1a	3	+	-	20	+
1b	3	+	-	-	-
2a	3	-	+	-	+
2b	3	-	+	-	-
3a	3	+	+	-	+
3b	3	+	+	-	-
4a	3	+	+	20	+
4b	3	+	+	20	

Contaminated solid substrate (Ni 317 mg/kg) in amount of 3g were used in all samples as also 1g of dry biomass of *Chlorella* sp. and pellets of *A. niger*. To the samples marked as 1a, 2a and 3a were 20 ml of non-treated destiled water added and experiment was running on shaker Unimax 2010 (Heidolph, Germany) at 170 rpm. Experiment in samples marked as 1b, 2b and 3b was running under static conditions. Temperature conditions during the process were range of 23 ± 2°C. The samples for analysis were taken at defined time interval of 48 hours. Suspension of microbial consortium was separated from the solution (with H_2O) by membrane filtration (Merck Millipore, USA, pore size 0.45 μm). The metal analysis of all samples was carried out on emission spectrometer with inductance coupled plasma (ICP Profile Plus, Teledyne Leeman Labs, USA).

Biosorption of heavy metal ions by microbial biomass is affected by many factors as concentration of metal ions, genus/species of microorganisms used in process, pH, temperature, and the presence of competing ions. The aim of this study is to investigate the variability of the biosorbent and the conditions and their possible effects on metal ions in the solid waste.

Reduction of Ni in % in solid substrate depending on the variability of sorption conditions and biomass showes Fig. 1.

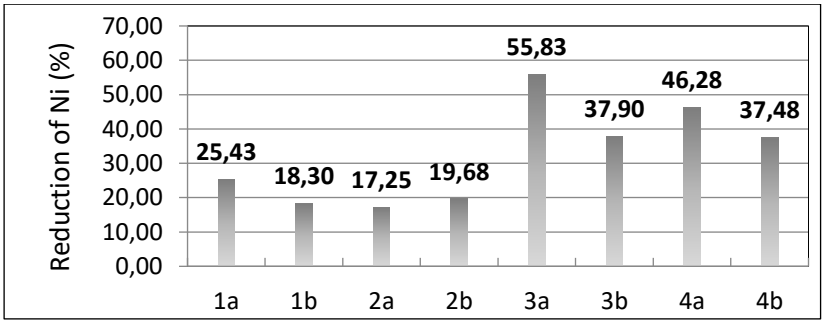


Fig. 1 Reduction of Ni in study samples.

The highest efficiency of 55.83% were achieved using dry biomass of algae with pellets of *A. niger* (3a - without H_2O) and biomass of algae with pellets of *A. niger* (4a - with H_2O) 46.28%. FT-IR analysis can reveal the functional groups on the surface of biosorbent. The FT-IR spectrum of adsorbed strain *Chlorella* sp. and *A. niger* were recorded. After biosorption, the wavelength shifted, showing that functional groups are involved for nickel biosorption. The biosorption mechanism was mainly ascribed to the contributions from functional groups of C-O, C-C, C=O, N-H, C-N.
The present study successfully utilized a new type of non-living biomass *Chlorella* sp. and *Aspergillus nige*r as a highly effective biosorbent to remove nickel from environmental solid samples.

Keywords: Ni, consortium of microorganisms, *Chlorella* sp., *Aspergillus niger*.

Acknowledgements
The work was supported by Slovak Grant Agency, projects VEGA 1/0424/18 and 1/0614/17.

References
Šimonovičová, A., Hlinková, E., Chovanová, K., Pangallo, D. 2013: Influence of the environment on the morphological and biochemical characteristics of different *Aspergillus niger* wild type strains. Indian J Microbiol 53, 2, 187-193.

STUDYING THE UPTAKE OF CADMIUM BY ROOTS OF FLAX (*LINUM USITATISSIMUM* L.)

Mária Pavlovičová[a], Zuzana Gregorová[b], Monika Bardáčová[b], Petra Ranušová[b], Vanda Adamcová[b], Miroslav Horník[b], Ildikó Matušíková[b]

[a] *University of SS. Cyril and Methodius, Department of Biotechnology, J. Herdu 2, Trnava, SK-917 01*

[b] *University of SS. Cyril and Methodius, Department of Ecochemistry and Radioecology, J. Herdu 2, Trnava, SK-917 01, bardacova.monika@gmail.com.*

ABSTRACT

Phytoremediation is an ecologically friendly way for removal of metal pollutants from the polluted sites by using plants. Repeated planting and harvesting of plants grown in contaminated soils can reduce the concentration of toxic metals in soils to an acceptable level. The efficiency of metal uptake and phytoremediation differs among plant species, and depends on their morphological, physiological and anatomical characteristics.

Flax (*Linum usitatissimum* L.) is a plant used for industrial purposes as fiber crop, while oil from seeds is also rich in healthy polyunsaturated fatty acids. Flax plants have been shown to tolerate heavy metals and accumulate them at relatively high rates. The current study compares six different flax varieties for their ability to take up and accumulate cadmium. We aim to identify the mechanisms that might be responsible for the observed differences, therefore we investigate the impacts of Cd presence on physiology, biochemistry and enzyme activities of flax plants exposed to cadmium stress.

Sterilized seeds were germinated, and after 5 days the seedlings were transformed into standard Hoagland media with cadmium (50 $mg.l^{-1}$ Cd^{2+}), spiked with $^{109}Cd^{2+}$ (Fig. 1A). Isotope depletion from solution by roots was measured at 24 h intervals for 10 days by scintillation gammaspectrometry (76BP76/3, Scionix, The Netherland). At the end of the experiment, cadmium content in the tissues was determined.

Our results showed that the flax plants were negatively impacted by presence of cadmium ions by means of fotosynthetic pigment contents, and oxidative stress occured as well (data not shown). The kinetics of metal depletion was similar in varieties Escalina, Flanders, Marina and Krasnoder but differed in varieties Jitka and Belinka. This was reflected also in final metal cadmium content in roots (Fig. 1B). This experiment showed that flax viarieties greatly differ in tolerance to cadmium, and also in capability to accumulate it in their tissues. We identified the variety Jitka as accumulating the most-, and the variety Belinka as accumulating the least cadmium from the media. Our

results are relevant not only in context of soil remediation but also in regard of food safety.

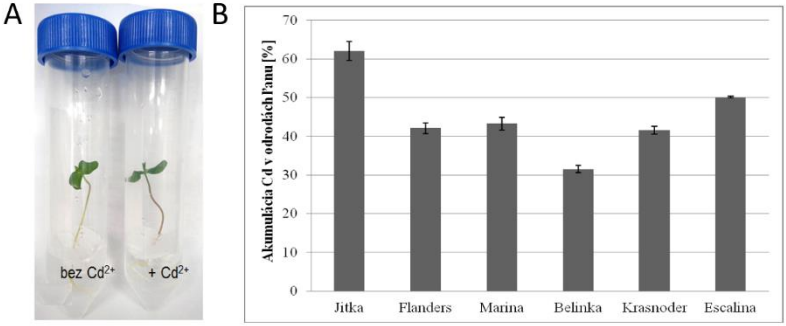


Fig. 1 Accumulation of cadmium by roots of different flax varieties. Plants were individually cultured hydroponically in presence or absence of Cd^{2+}, spiked with isotope ^{109}Cd (A). After 10 days the total cadmium content was determined (B). The values correspond to the arithmetic average ± SD (n = 4).

Keywords: Cadmium, Flax, Hyperaccumulators, Metal Tolerance, Phytoremediation

Acknowledgement: The work is financed by the Slovak Research and Development Agency under contract number APVV-15-0051.

REMOVAL OF Cs AND Co BY BACTERIAL BIOFILM FORMED ON STAINLESS STEEL

Martin Pipíška[a,b], Alexandra Vanková[b], Miroslav Horník[b]

[a] *Department of Chemistry, Trnava University in Trnava, Priemyselná 4, 918 43 Trnava, Slovakia (martin.pipiska@truni.sk)*

[b] *Department of Ecochemistry and Radioecology, University of SS. Cyril and Methodius, Nám. J. Herdu 2, 917 01 Trnava, Slovakia*

ABSTRACT

The formation of microbial biofilms in spent nuclear fuel pools is one of the major concerns in safety assessment of temporary spent nuclear fuel disposal. It is well known that chemical processes mediated by microorganisms can lead to (i) deterioration of metal and non-metal surfaces; (ii) accumulation of radionuclides by biofilms and (iii) biofouling and plugging of armatures. In present study, the ability of bacteria *Kocuria palustris* and *Micrococcus luteus* isolated from spent nuclear fuel pools in JAVYS Inc. (Slovakia) to form biofilms on stainless steel under laboratory conditions were investigated. Bacterial biofilm was produced aerobically in stirred flasks containing submerged stainless steel (1.4301/AISI 304) coupons (11×22×0.5 mm) in nutrient broth (No.2) during batch culture for 72 h at 30°C. Accumulation of radionuclides ^{137}Cs and ^{60}Co by developed bacterial biofilms was studied.

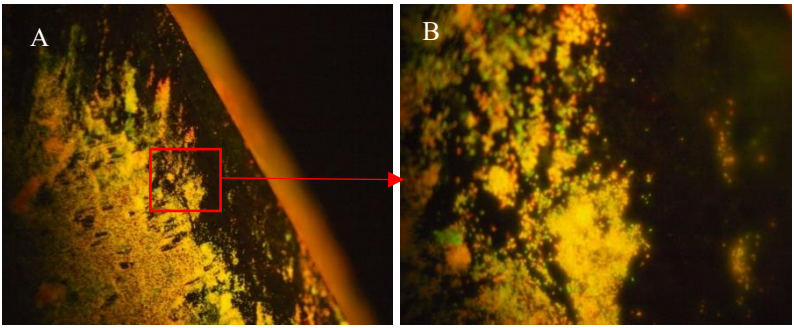


Fig. 1 Epifluorescence micrographs of *M. luteus* biofilm on stainless steel surface. Magnification 40 × (A), 100 × (B). Visualized using LIVE/DEAD BacLight Viability Kit.

Results showed that both bacterial isolates were able to adhere on stainless steel surfaces. *M. luteus* formed a multilayer biofilm continuously covering both polish and rough surfaces of stainless steel coupons under aerobic

conditions (Fig. 1). In contrast, *K. palustris* did not form a typical biofilm and only individual cells were adhered on irregularities and fissures of stainless steel surface (not shown). Cell attachment is due to the fact that both bacteria have a net negative surface charge and therefore interact with positively charged stainless steel coupon surfaces (electrostatic attraction) followed by van der Waals interactions between the hydrophobic region of the outer cell wall and the surface.

Table 1. Uptake of Cs and Co ions by bacterial adhered cells and biofilms formed on stainless steel coupons after 24h incubation in solution (c_0 = 50µmol/L CsCl or $CoCl_2$, 108 Bq/mL $^{137}CsCl$ or 100 Bq/ml $^{60}CoCl_2$) at pH 4.0.

Coupons	Me	c_{24} (µmol/L)	Uptake (%)	Uptake (Bq/cm^2)	Uptake (µmol/mg)*
M. luteus biofilm		46.5 ± 0.2	7.1 ± 1.6	4.9 ± 0.4	0.039 ± 0.009
K. palustris cells	Cs	49.4 ± 0.7	1.25 ± 0.7	0.87 ± 0.53	0.011 ± 0.004
without biofilm		49.7 ± 0.1	0.5 ± 0.1	0.34 ± 0.05	-
M. luteus biofilm		33.9 ± 2.8	32.1 ± 5.4	20.0 ± 3.1	0.243 ± 0.013
K. palustris cells	Co	49.7 ± 0.1	0.60± 0.26	0.37 ± 0.11	0.099 ± 0.049
without biofilm		50	0	0	-

* accumulation of metal/bacterial protein

Stainless steel coupons with developed biofilm (*M. luteus*) and adhered cells (*K. palustris*) were submerged into unbuffered Cs or Co solutions (50 µmol/L) spiked with ^{137}Cs or ^{60}Co, respectively. Table 1 shows that both bacteria have the ability to remove metal ions from solution. After 24h incubation Cs uptake reached 0.039 ± 0.009 and Co uptake 0.243 ± 0.013 µmol/mg (*M. luteus* biofilm). Lower uptake was observed by adhered cells of *K. palustris*. Significantly higher uptake of Co^{2+} ions compared to Cs^+ ions indicates that the uptake process consists of both metabolism independent extracellular binding (biosorption) and passive or metabolism dependent intracellular uptake (bioaccumulation). Cs^+ showed only weak affinity to negatively charged surface functional groups (e.g. –COOH, PO_3H_2) and therefore transport to cytoplasm dominates in both *M. luteus* biofilm and *K. palustris* adhered cells. Co^{2+} ions were predominantly associated with cell surface, suggesting crucial role of surface complexation and electrostatic attractions in Co removal.

Keywords: biofilm, bacteria, stainless steel, cesium, cobalt, accumulation

ACIDIC BIOBENEFICATION OF KAOLINE BY MICROSCIPIC FUNGUS *ASPERGULLUS NIGER*

Filip Polák[a], Martin Urík[a], Marek Bujdoš[a], Kim Hyunjung[b]

[a] *Institute of Laboratory Research on Geomaterials, Faculty of Natural Sciences, Comenius University in Bratislava, Mlynská dolina, 84215 Bratislava, Slovakia*

[b] *Department of Mineral Resources and Energy Engineering, Chonbuk National University, 567, Baekje-daero, Deokjin-gu, Jeonju, Jeonbuk 54896, Republic of Korea*

ABSTRACT

In recent years, biobenefication showed promising results as a method increasing the value of raw material through use of microorganisms. Therefore, in this study, we are considering the use of microscopic filamentous fungus *Aspergillus niger* for biobenefication of kaoline to decrease unwanted iron colouring.

To determine the influence of *A. niger* induced iron bioextraction from kaoline, we supplemented polypropylene Erlenmeyer flasks containing 1 g of sterile kaoline with 50 ml of growth medium that was adjusted to maximise the capability of fungus to maintain acidic pH. Subsequently, it was inoculated with fungal spores and cultivated in static conditions at 25°C for 19 days. To determine the amount of iron bioextracted by *A. niger*, we used atomic absorption spectrometry (Perkin Elmer 1100, USA) for filtered culture media (0.45 µm MCE membrane filter) and acid digested biomasses (**Fig. 1**) that were collected 2 or 3 days apart during the cultivation. Production of organic acids by *A. niger* represents influential factor in bioextraction, therefore, we used capillary isotachophoresis (Villa Labeco, Slovakia) to determine the presence of organic acids in media as well.

Surprisingly the concentration of organic acids produced by *A. niger* was lower than 0.1 mmol/l during experiment. This effect could occurred as a result of macro- and micronutrients being in excess. Bioextraction of iron in this study is therefore most probably pH induced. Production of H^+ during the cultivation of *A. niger* was enough to keep the pH of growth medium at average level of 1.4. This led to 38% of iron being bioextracted.

These findings conclude that activity of H^+ cations produced by *Aspergillus niger* during cultivation significantly influences the mobility of iron and helps to remove it from kaoline during biobenefication process.

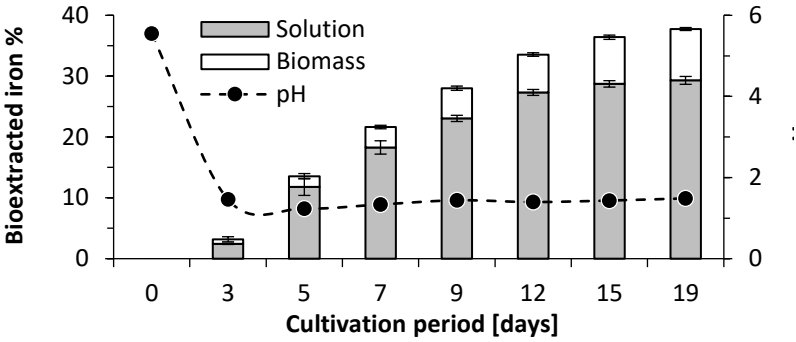


Fig. 1 Bioextraction efficiency of iron from kaoline by *A. niger*'s acidic activity and consecutive accumulation in biomass during cultivation

Keywords: *Aspergillus niger*, pH, biobenefication, bioextraction, iron

Acknowledgement
The work was supported by Scientific Grant Agency of the Ministry of Education, Science, Research and Sport of the Slovak Republic and the Slovak Academy of Sciences under the contracts VEGA 1/0146/18; by Comenius University Grant No. UK/120/2018 and the bilateral project between Slovakia and South Korea No. SK-KR-18-0003.

ACIDITHIOBACILLUS SPP. - A WORKING HORSE OF MINERAL BIOTECHNOLOGIES

Peter Pristaš, Jana Kisková, Ivana Timková, Lenka Maliničová, Jana Sedláková-Kaduková

Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafarik University in Košice, Šrobárova 2, 04154 Košice, Slovakia

ABSTRACT

The genus *Acidithiobacillus* comprises a group of Gram-negative obligatory acidophilic chemolithotrophic bacteria that derive energy mainly from the oxidation of reduced sulphur compounds. Bacteria from the genus *Acidithiobacillus* are often associated with mineral biotechnologies (biomining) and acid mine drainage. Biomining utilises these bacteria for recovery of metals from sulphidic low grade ores and concentrates. Acid mine drainage results in acidification and metal contamination of soil and water emanating from the dissolution of metal sulphides from deposits and mine waste storage. Acidophilic microorganisms play a central role in these processes by catalysing aerobic oxidation of sulphides. While acceleration of mineral solubilisation is a positive aspect in mineral biotechnologies, it is undesirable in acid mine drainage with strong negative ecological impact and there is profound interest in genetics and genomics of these bacteria.

The *Acidithiobacillus* genus was formed when the former *Thiobacillus* genus was divided into the genera *Acidithiobacillus*, *Halothiobacillus* and *Thermithiobacillus*. The type species of the genus *A. thiooxidans* was described nearly 100 years ago and up to now 7 species within the genus were validly described. All species are autotrophs capable of growth utilising inorganic sulphur compounds as sole energy substrate. Four of the species also catalyse the dissimilatory oxidation of ferrous iron while three (*A. thiooxidans*, *A. albertensis*, and *A. caldus*) do not. Representatives of *Acidithiobacillus* genus occur world-wide in a diverse range of natural (acid rock drainage, sulfur springs, etc.) and industrial settings (ore concentrates, leaching solutions of the mining industry, etc.), with varying physicochemical characteristics. Phylogenetic analyses confirmed pronounced genetic diversity within *Acidithiobacillus* genus, especially within *A. ferrooxidans* complex, and description of multiple new species is very probable.

Recent advances in genomics revealed the common and distinctive features some of *Acidithiobacillus* species isolated from similar habitats and provided data to understand potential mechanisms that enable acidophiles to adapt to extremely acidic environments. Based on the genomic contents of

Acidithiobacillus species, it was shown that different metabolic pathways might allow these microorganisms to acquire energy, carbon sources, and nitrogen sources from acidic environments, ensuring their survival and proliferation in these harsh environments.

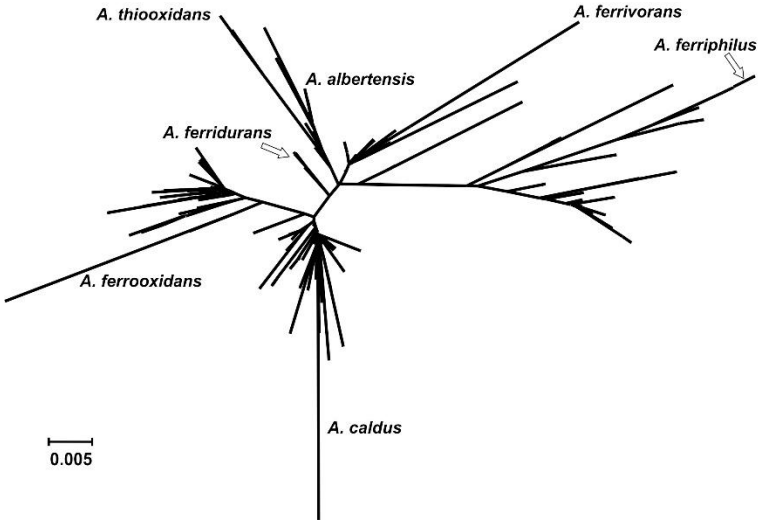


Fig. 1 Phylogenetic tree documenting phylogenetic diversity of *Acidithiobacillus* spp inferred using the Neighbor-Joining method.
The placement of validly described species is shown. The bar represents genetic distance shown in number of base substitutions per site.

Keywords: *Acidithiobacillus*, genetic variability, genomics

Acknowledgement
The work was financially supported by Research Agency of the Ministry of Education, Science, Research and Sport of Slovak Republic under the VEGA Project 1/0229/17.

BIODEGRADATION OF PHENOL UNDER LOW CONCENTRATION FROM MODEL SOLUTIONS BY SULPHATE-REDUCING BACTERIA

Dominika Rudzanová, Alena Luptáková, Eva Mačingová
Department of Mineral biotechnology, Institute of Geotechnics of Slovak Academy of Sciences, Watsonova 45, 040 01 Kosice, Slovak Republic, e-mail:rudzanova@saske.sk, luptakal@saske.sk, macingova@saske.sk

ABSTRACT
The aim of the present study is to investigate the possibility of adaptation and biodegradation of phenol at low concentration under sulphate-reducing conditions. Phenol as toxic compound is present in the liquid effluents from coal gasification and coking plants, petroleum refineries and many other industries. Biological treatment of phenolic effluents is limited of phenol toxicity to microorganisms, but is eco-friendly and cost-effective. Sulphate-reducing bacteria (SRB) are facultative anaerobes that utilize organic substrates as a carbon source and sulphates as a terminal electron acceptor to produce hydrogen sulphide. SRB utilized in experiments were obtained from potable mineral water (Gajdovka spring, located in Kosice, Slovakia). For biodegradation study, the Postgate's medium C was modified. Instead of lactate, phenol with concentration 10 mg/l, was used as sole carbon source. 2 biotic sets (1 biotic set with previously cultivated SRB and 1 set of mix bacterial culture without prior cultivation), and 1 abiotic control set were carried out, simultaneously. All experiments were performed under strictly anaerobic conditions, at 30°C, with pH 7.5, in dark, without shaking, for 21 days. In all, 3 transfers were carried out into fresh modified medium. Results from Cycle 1 are shown at Fig. 1. The best results of phenol degradation were obtained after 35 days. Phenol concentration declined from initial concentration 10 mg/l to 0.6 mg/l and concentration of sulphates decreased from 1650 mg/l to 1547 mg/l, for mix culture (F1-G). This fast decrease can be caused by other microorganisms present in mineral water Gajdovka. Results from Cycle 2 are shown at Fig. 2. After 139 days, phenol concentration declined from initial concentration 10 mg/l to 0 mg/l, and concentration of sulphates decrease from initial 1652 mg/l to 1589 mg/l, for sample with cultivated bacterial culture (F2-1).
Adaptation is challenging and long process. Because of, SRB were isolated from natural source without prior organic contamination.Iit was necessary to adapt SRB to organic substrate and adaptation mechanisms responsible for degradation of phenol, have to be created. For this reason, initial concentration of phenol was very low, only 10 mg/l. Results showed that it is possible to adapt SRB from

natural sources at low phenol concentration. Results obtained provide bases for further biodegradation study with higher phenol concentration.

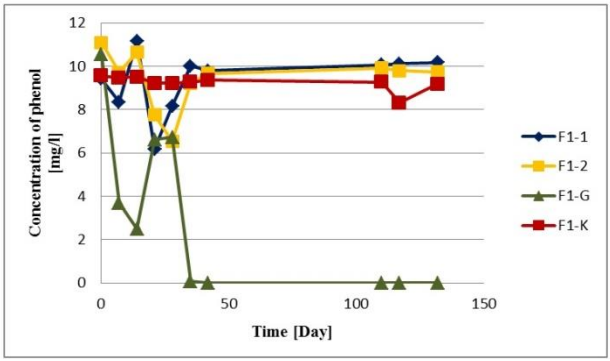


Fig. 1 Biodegradation of phenol-Cycle 1. F1-1 and F1-2: biotic samples-cultivated SRB, F1-G: biotic sample-no cultivated SRB, F1-K: abiotic control.

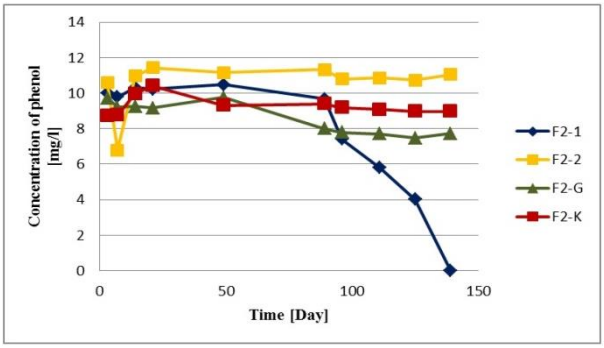


Fig. 2 Biodegradation of phenol-Cycle 2. F2-1 and F2-2: biotic sample-cultivated SRB, F2-G: biotic sample-no cultivated SRB, F2-K: abiotic control.

Keywords: Adaptation, biodegradation, phenol, sulphate-reducing bacteria

Acknowledgements

This work has been supported by the project VEGA 2/0145/15 and Slovak R&D Agency project No APVV-10-0252-WATRIP.

POSSIBILITIES OF CRITICAL METALS RECOVERY FROM WASTE MATERIALS USING BIOHYDROMETALLURGICAL METHODS

Mariola Saternus, Joanna Willner, Agnieszka Fornalczyk
Silesian University of Technology, Faculty of Materials Engineering and Metallurgy, Department of Extractive Metallurgy and Environmental Protection,, Krasińskiego 8, 40-019 Katowice, Poland, Mariola.Saternus@polsl.pl

ABSTRACT

Critical raw materials are defined as materials of which the risk of supply shortage and their impacts on the economy are higher compared to most of other raw materials. The outdated technologies and end-of-life products causing creation of constantly growing stream of waste, which are treated as a potential source of metals. These metals can be recovered by hydro- or pyrometallurgical methods. However, currently biohydrometallurgy becomes more and more attractive due to economic factors. Bioleaching is not so expensive method and requires considerably lower investment inputs taking into account the traditional refining and melting methods. If bioleaching method is used the following advantages are observed: no emission of sulfur dioxide, low energy consumption or possibilities of applying for not easily accessible sources containing metals. Figure 1 shows the schematic diagram presenting the sources of critical metals, which can be recovered by use of microorganisms.

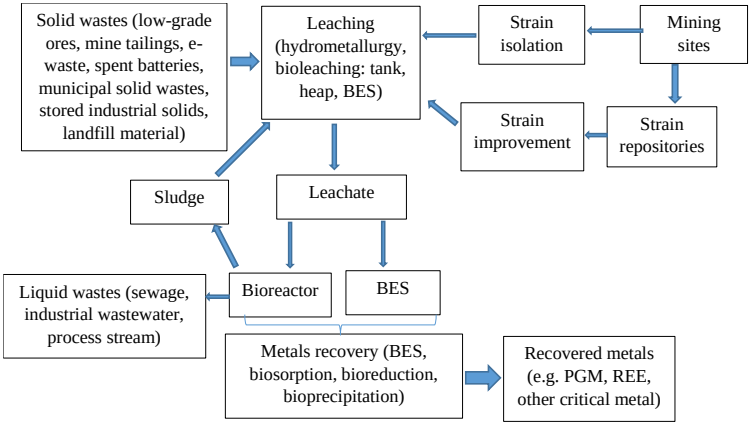


Fig. 1. Schematic diagram presenting the sources of critical metals, which can be recovered applying microorganisms in biomining, bioleaching and BES**.**

Microbial technologies become more and more promising for the selective recovery of metal ions due to low cost, technical feasibility for large scale applications and no generation of hazardous wastes. Such methods as bioprecipitation, biosorption, bioreduction and bioaccumulation have been examined for the removal of metal ions from the leachates and aqueous wastewater. Table 1 shows the exemplary of microbe-metal interactions for immobilization of some of critical metals. The focus concentrated mainly on PGM and REE metals; however in the last years also other critical metals like gallium or indium start to attract researcher.

Table 1. Microbe–metal interactions for immobilization of critical metals

Metal/source	Organism/Mixed Culture/ Composites	Biochemical Process
$Ag/AgNO_3$	*Aeromonas sp. SH10, Bacillus cereus, B. subtilis, B. megaterium, B. licheniformis, Brevibacterium casei, Corynebacterium sp. SH09, Enterobacter cloacae, Escherichia coli, Klebsiella pneumonia, Lactobacillus fermentum, Proteus mirabilis, Pseudomonas putida, P. stutzeri, Serratia nematodiphila*	Bioreduction
$Au/HAuCl_4$	*B. megatherium, B. subtilis, D. desulfuricans, D. vulgaris, Escherichia coli, P. aeruginosa, P. fluorescens, Shewanella algae*	Bioreduction
Pd/Na_2PdCl_4	*Anabaena, Bacteroides vulgatus, Bacillus sphaericus, Calothrix, Clostridium pasterianum, Cupriavidus metallidurans,C. nector, Citrobacter braakii, D. desulfuricans, D. fructosivorans, D. vulgaris, Paracoccus denitrificans, Pseudmonas putida, Plectonema boryanum, Geobacter sulfurreducens*	Bioreduction
$Pt/PtCl_6^{2-}$	*D. sulfuricans, Shewanella algae, Pseudomonas sp.*	Bioreduction
Ru/Ru-acetate	Bacterial biomass/chitosan	Biosorption
$Dy/DyCl_3$	*Penidiella* sp. T9	Bioaccumulation
	P. aeruginosa	Biosorption
$La/La(NO_3)_3$	*P. aeruginosa, B. subtilis, M. xanthus, M. smegmatis, E. coli, Pseudomonas sp.*	Biosorption
$Nd/NdCl_3$	*P. aeruginosa*	Biosorption
$Sc/ScCl_3$	*Saccharomyces cerevisiae, Rhizopus arrhizus, Aspergillus terreus*	Biosorption
$Eu/EuCl_3$	*P. aeruginosa, Myxococcus xanthus*	Biosorption
$Yb/YbCl_3$	*M. smegmatis*	Biosorption

Keywords: biohydrometallurgy, critical metals, bioleaching, biosorption, waste treatment.

PRACTICAL APPLICATIONS OF BACTERIA ISOLATED FROM EXTREME ENVIRONMENTS: CASE STUDY – SLOVAKIA

Jana Sedláková-Kaduková[a], Jana Kisková J[a], Lenka Maliničová[a], Ivana Timková[a], Simona Kvasnová[b], Peter Pristaš[a]

[a] *Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafarik University in Košice, Šrobárova 2, 04154 Košice, Slovakia*

[b] *Department of Biology and Ecology, Faculty of Natural Sciences, Matej Bel University, Tajovského 40, 97401 Banská Bystrica*

ABSTRACT

Microorganisms are ubiquitous in all known ecosystems on the Earth forming a vital part of nature. Microorganisms that inhabit extreme environments are classified as extremophiles. The fascinating abilities of these organisms to adapt and survive in environments where other living forms are not able to live for longer time make them special candidates not just for research but also for technological applications. For research they represent an interesting reservoir of the hidden genetic variations because they have evolved a number of strategies through various mechanisms to cope up with the effect of the prevailing adverse factors including modification in their cell structure and biochemical activities. In technology they can have broad applications in both environmental (such as bioremediation) and industrial biotechnology (as a source of new enzymes or various bioactive compounds), their potential applications in biotechnology are almost unlimited. The aim of our work was to study microbial populations living under Slovak "extreme" conditions including high metallic pollution, high and low pH, elevated salinity, high radiation etc. Bacterial diversity in these places was according to intensity of extreme parameters of physico-chemical conditions significantly reduced in comparison with normal environmental conditions. We found several microorganisms that are probably belonging to novel species of the genera *Streptomyces*, *Methylobacterium* or *Oceanobacillus*. Several new bacterial species were isolated which can be potentially exploited in bioremediation studies because of their specific abilities to e.g. dissolve metals (*Acidithiobacillus albertensis*), bioaccumulate Zn (*Arthrobacter sulfureus, Streptomyces* K11), produce bioactive compounds or antibiotics (streptomycetes from High Tatras), utilise methanol and C_2 to C_4 compounds (*Methylobacterium* sp.) or act in travertine formation (*Oceanobacillus*). However, majority of these bacteria needs further study to understand their potential in biotechnological applications.

Keywords: extreme environments, extremophiles, applied microbiology, biotechnology

Acknowledgement
The work was financially supported by a grant from the Slovak National Grant Agency under the VEGA Project 1/0229/17.

SELECTIVE PRECIPITATION OF METALS AFTER BIOLEACHING

Jana Sedláková-Kaduková[a], Anna Mražíková[b], Alena Lutpáková[c]

[a] *Department of Microbiology, Institute of Biology and Ecology, Faculty of Science, Pavol Jozef Šafarik University in Košice, Šrobárova 2, 04154 Košice, Slovakia*

[b] *Institute of Materials and Quality Engineering, Faculty of Materials, Metallurgy and Recyclaction, Technical University in Košice, Letná 9, 04200 Košice, Slovakia*

[c] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovakia*

ABSTRACT

In the present time world faces the increasing amount of waste, only in 2010 the total waste production in Europe was 2.5 billion tons, on the other hand there is just limited amount of primary sources suitable for metal recovery to fulfil growing needs of human population. For these reasons there is increasing pressure to develop new technologies which would be cost effective, energy and environmentally friendly but also able to adapt to changing quality of initial materials. Among various possible technologies there is a growing interest on biotechnological application to recover metals not only from low grade ores but also from waste materials. Biohydrometallurgy is generally regarded as a green technology for the recycling of metal-bearing waste, including various types of electronic waste. It offers several possibilities not only to dissolve metals but also to recover them from leachates and form fully biological processes producing just limited amount of waste. In the present work we dealt with a study of metal recovery from leachates obtained after bioleaching of printed circuit boards. We developed biological-chemical method based on selective precipitation process (Fig. 1) to recover Fe, Cu, Al, Zn and Ni. Iron in the form of $Fe(OH)_3$ and copper in the form of CuS were precipitated from leachate with very high purity.

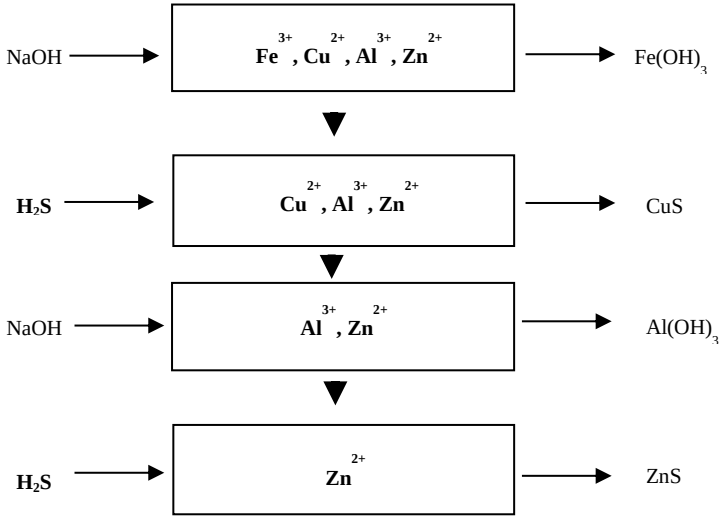


Fig. 1 Scheme of the selective biological-chemical process**.**

No impurities were detected in precipitates. Al and Zn were also successfully removed from leachate; however, they contained several metallic impurities from original solution. Purity of all precipitates was studied using EDX analysis. During the process, all metals were removed from solutions with very high efficiency exceeding 98%. To recover Ni further research is necessary.

According to obtained results selective precipitation may represent a powerful tool for metal recovery from solutions after bioleaching using environmentally friendly combination of biological-chemical precipitation, however future study is necessary to optimise the final part of the precipitation so all of the formed precipitates would have high purity and would be suitable for consequent utilisation in the commercial processes.

Keywords: bioleaching, selective bioprecipitation, printed circuit boards, sulphate-reducing bacteria

Acknowledgement

The work was financially supported by a grant from the Slovak National Grant Agency under the VEGA Project 1/0229/17.

TOXICITY OF RARE EARTH ELEMENTS

Petr Soudek[a], Šárka Petrová[a], Joanes Landa Lekuberria[a], Ivana Tomášková[b], Tomáš Vaněk[a]

[a] *Laboratory of Plant Biotechnologies, Institute of Experimental Botany AS CR, v.v.i., Rozvojová 263, 165 02 Prague 6 – Lysolaje, Czech Republic*

[b] *Czech University of Life Sciences Prague, Department of Genetics and Physiology of Forest Trees, Faculty of Forestry and Wood Sciences, Kamýcká 1176, CZ-165 21 Praha 6 – Suchdol, Czech Republic*

ABSTRACT

Rare earth elements (REEs) are a group of 15 chemical elements in the periodic table, specifically the lanthanides. Two other elements, scandium and yttrium, have a similar chemistry and toxicology to the lanthanides, are commonly found in the same mineral assemblages, and are often referred to as REEs (EPA, 2012).

Despite the fact that their distribution in the Earth's crust is poor and their solubility and bioavailability are low, these metals are a potential environmental problem. They are widely used in electrical and electronic devices used for green and communication technologies. The devastation of the environment has already occurred greatly during their extraction. It can also be assumed that they will get back to environment after the end of the life cycle of the products they contain.

REE toxicity for human is relatively well documented (EPA, 2012; Hirano and Suyuki, 1996). Less information is about plant toxicity and the plant's ability to metals bioconcentration and thus bring them into the food chain. Li et al. (2013) to assess the risks arising from the consumption of vegetables containing REE should not lead to an exceedance of the estimated daily intake that is already harmful to human health for adults and children. It seems that REE transfer to plants is not very significant. Liang et al. (2005) reports that the REE content in wheat seeds was 3-4 orders lower than in soil. Availability depends on the physico-chemical properties of the soil. It can be increased, for example, by the addition of EDTA (Lihong et al., 1999). Root uptake is not the only way how REEs can get into the plant. Chua et al. (1998) has shown that the application of cerium onto the surface of the leaves of sugarcane results in its rapid distribution to different parts of the plant.

One of the other ways REEs can get into the soil is the intensive use of fertilizers enriched with REE in China (Zhang and Shan, 2001). At low concentrations REEs have a promoting effects on seed germination, growth of roots, total biomass accumulation, production of secondary metabolite and

absorption of minerals and metals for medicinal plants (Chunhong et al., 2013).
Some REE toxicity studies on living organisms have been performed. Most of these works either test only one of the elements (Oral et al., 2010; Saitoh et al., 2010; Qu et al., 2004; Barry and Meehan, 2000) or used to test a variety of animal species, such as e.g. *Caenorhabditis elegans* (Zang et al., 2006), *Tenebrio molitor* (Zhao et al., 2005), *Holotrichia parallela* (Li et al., 2006) or *Daphnia carinata* (Barry and Meehan, 2000).
The aim of the present work was to evaluate the effect of REE on the germination of white mustard (*Sinapis alba* L.) seeds, on grow of duckweed (*Lemna minor* L.) and on viability of suspension culture of *Arabidopsis thaliana* L. Toxicity of Sc, Y, La, Ce, Pr, Nd, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb and Lu, at five different concentrations (in the range 0.05 - 5 mM) was tested by standard ecotoxicity tests. The root length of *S. alba* was measured after 72-h incubation. The elongation inhibition, EC_{50} value and slope values were calculated. The exposure of suspension culture of *A. thaliana* took 96 h under dark condition. The tolerance of suspension cultures to REE was assessed using the reduction of 2,3,5-triphenyltetrazo-lium chloride (TTC). Grow of *L. minor* plant was test by 7 days exposition and increasing the thallus area was measured. The results showed that REE toxicity decreased in the order: Lu > Er > Yb > Sc > Tm > Y > Ce > Ho > La > Nd > Pr > Dy > Gd > Tb > Eu > Sm.

Keywords: heavy metals, phytoremediation, accumulation, stress, rare earth elements (REE).

Acknowledgement
This work was supported by project COST ES1407 (project No. LTC17046).

INFLUENCE OF ZnO NANOPARTICLES ON FILAMENTOUS FUNGUS ASPERGILLUS NIGER AND CREATION OF ZINC BIOMINERALS

Martin Šebesta[a], Martin Uríka, Filip Poláka, Marek Kolenčíkb, Marek Bujdoša, Kim Hyunjungc, Ivo Vávrad, Edmund Dobročkad, Peter Matúša

[a] *Comenius University in Bratislava, Faculty of Natural Sciences, Institute of laboratory research on geomaterials, Mlynská dolina, Ilkovičova 6, Bratislava 84215, Slovak republic*

[b] *Slovak University of Agriculture in Nitra, Faculty of Agrobiology and Food Resources, Department of soil science and geology, Trieda A. Hlinku 2, Nitra 94976, Slovak republic*

[c] *Department of Mineral Resources and Energy Engineering, Chonbuk National University, 567, Baekje-daero, Deokjin-gu, Jeonju, Jeonbuk 54896, Republic of Korea*

[d] *Institute of Electrical Engineering, Slovak Academy of Sciences, Dúbravská cesta 9, Bratislava 841 04, Slovak republic*

ABSTRACT

There is an increase in usage of nanoparticles in commercial products, including pharmaceutical and personal care products. ZnO nanoparticles are among the most widely used nanoparticles in both commercial products and industrial applications; and they are subsequently released to the environment. Through sewers, they are transported to the wastewater treatment plants. There, they bind with activated sludge, which, under right conditions, is used as a fertilizer in agriculture. ZnO nanoparticles show a great potential to be used as an effective source of zinc for micronutrient fertilizers. Their application could help to alleviate the problem of zinc deficiency in agricultural soils, especially in subtropic and tropic regions. The bioavailability of zinc to plants is affected significantly by activity of filamentous fungi which have an ability to mobilize micronutrients in soils. However, presence of ZnO may also affect fungal growth.

To see how the ZnO nanoparticles influence the growth of a common soil fungus *Aspergillus niger*, 7-day laboratory experiments were conducted in their presence. The effect of the ZnO nanoparticles was compared with bulk ZnO and aqueous solution of $ZnSO_4$. Weight of the dry fungal biomass, concentration of Zn in the biomass, and pH of the growth media were measured at the end of the experiment. Another set of experiments was conducted to find the effect of fungal exometabolites on the transformation of ZnO and potential formation of new biogenic mineral. These were studied by X-ray diffraction (XRD) and transmission electron microscopy (TEM).

A. niger had different responses to the three forms of Zn. ZnO nanoparticles had greater effect on dry weight of fungus compared to the bulk ZnO and comparable to the aqueous solution of $ZnSO_4$ (Fig. 1). However, there is visible positive and statistically significant influence of zinc on the growth of filamentous fungus. The bioaccumulation of Zn from the three forms of Zn was very similar and showed no statistical significance.Using the TEM and XRD, a newly formed biogenic mineral phase of zinc oxalate was identified after the experiment.

These findings increase our understanding of ZnO nanoparticles' behavior and may help us to evaluate the potential risk and benefits of their intentional use, unintentional release, and ecotoxicity in soil environments.

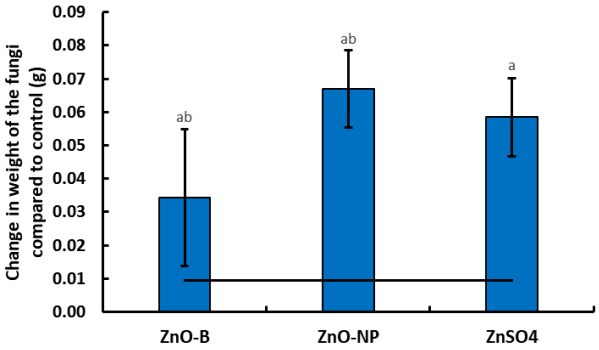


Fig. 1 Change in the dry weight of fungus with different treatments compared to the control after seven days of growth. The line below 0.01 represents standard deviation of the control. a represents statistically significant ($\alpha < 0.05$) difference compared to control. b represents statistically significant ($\alpha < 0.05$) difference compared to ZnO-B.

Keywords: Zinc oxide nanoparticles, micronutrients, heavy metals, fungal leaching, biomineralizations

Acknowledgement

This work was supported by Comenius University in Bratislava under grant UK/112/2018; the Scientific Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences under VEGA contracts No. 1/0146/18, No 1/0164/17 and No. 1/0836/15; and by the bilateral project between Slovakia and South Korea funded by Slovak Research and Development Agency under contract No. SK-KR-18-0003.

INDUSTRIALIZED SOILS TOXICITY ASSESSMENT USING EARTHWORMS

Oľga Šestinová, Jozef Hančuľák, Tomislav Špaldon
Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 040 01 Košice, Slovak Republic

ABSTRACT
In present study, we used ecotoxicity tests with *Dendrobaena veneta* to infer about potential toxic risks to the agricultural and permanent grass vegetation of the urban soils around the Košice, city in eastern Slovakia were performed. The total Fe, Cu, Zn, Pb, Cd, Cr and As concentrations and eco-toxicological tests of industrialized soils from the around U. S. Steel Košice were determined of 12 sampling sites in 2016-2017 years. The combination of chemical measurements with the calculation of BAFs and *r*-Person matrix correlations can be a useful tool in risk assessment. Bioaccumulation factor for earthworms (BAF) were calculated according to (OECD, 2010): BAF= (metal)earthworm /(metal)soil.

An influence of the sampling sites distance from the largest steel producer U. S. Steel Košice on the total concentrations of heavy metals was recorded for iron, cadmium, chromium and arsenic. The highest concentrations of studied metals were measured at sites located south of the ironworks in their vicinity (4 U.S.Steel-plant main gate for **PGV**: Fe 10.5mg/kg, Zn 1084mg/kg, Pb 379mg/kg, Cd 21,4mg/kg, Cr 278mg/kg and As 94mg/kg. For the **A**-soils were determined the highest values of metals in the areas: 4 U.S.Steel-plant west mainly for As 34.8mg/kg and 3 Gomboš for Cr 160mg/kg. The mainly sources of metals contamination in soils are represented by neighbouring the iron and steel works.

It was found that earthworms (*D. veneta*) in some cases caused decrease of metals concentration in contaminated soils. The largest metal concentration differences were recorded in the samples **PGV**: (4) U.S.Steel-plant-main gate, (As=67.3mg/kg, Cr=258.5mg/kg, Cd=19.5mg/kg, Pb=368,4mg/kg, Zn=1022mg/kg and Fe=9.1mg/kg); and in the samples **A**: (4) U.S.Steel-plant west As=30.3mg/kg**,** (3) Gomboš Cr=152.1mg/kg, after 7 days earthworms exposure.

The results of the BAFs were lower than 1 for more studied metals: Fe, Cu, Pb, Cr and As and the higher values BAFs for Zn and Cd, that are presented in Fig.1. The correlation between mortality of earthworms (%) and heavy metal concentrations were calculated using the Pearson matrix correlation shown in Fig. 2. The results obtained show the relatively highest positive correlation between mortalities of earthworms and concentrations

with Fe (r= 0.25 to 0.85) and the highest negative correlation between mortalities of earthworms and concentrations with Cu (r= -0.17 to -0.82).

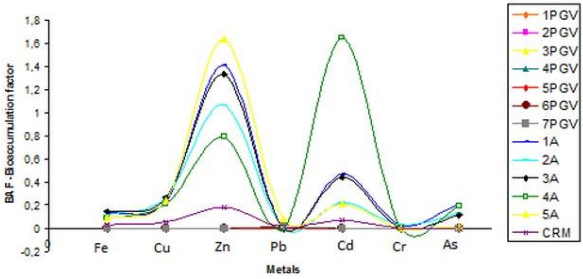


Fig. 1 Bioaccumulation factor for studied metals in earthworm tissues and soils

The relatively lower values of the correlation coefficient were calculated for these metals with Zn (r= -0.37 to 0.48), As (r= 0.09 to 0.54); Pb (r= -0.22 to -0.78) and the lowest values were detected in case of Cd (r= -0.13 to 0.32) and Cr (r= -0.21 to 0.28). It is evident that samples with the high concentrations of metals don't influence significantly the mortality of earthworms.

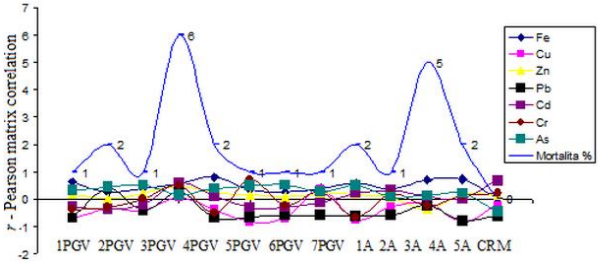


Fig. 2. Effect of metals on mortality *D. veneta* after 7 days of exposure of tests and correlation between mortality of earthworms and concentration of metals for all soils

Keywords: industrialized soils, *Dendrobaena veneta*, heavy metals, potential toxic risk, bioaccumulation factor.

Acknowledgements
The work was supported by the Slovak Grant Agency VEGA, Grants No. 2/0194/15

HEAVY METALS FROM THE ENVIRONMENT OF MINE AND DUMP IN THE LIGHT OF BACTERIAL ANTIBIOTIC TOLERANCE

Ivana Timková, Miroslava Lachká, Peter Pristaš, Jana Sedláková-Kaduková

[a] *Pavol Jozef Šafárik University in Košice, Faculty of Science, Department of Microbiology, Šrobárova 2, Košice, 04154, Slovak Republic*

ABSTRACT

It was proved that environment could act as a reservoir of antibiotic resistance genes allowing them to spread among bacterial species via horizontal gene transfer resulting in antibiotic resistance also in pathogen microorganisms. Heavy metals could play an important role in this process because of their potential to create selective pressure with subsequent selection of antibiotic resistant bacteria through linkage between antibiotic resistant genes and heavy metal resistance genes. This study was conducted to investigate the antibiotic tolerance profile of bacteria isolated from contaminated environment of mine and dump; the environment where, we did not expect the presence of antibiotics.

Two soil samples from gold mine (labelled RB1 and RB2) and one sample from the dump located near the mine (labelled H1) were collected from active gold mine Hodruša – Hámre, Slovakia (GPS N 48°45.658´ E 18°85.166´). 1 g of thoroughly homogenized sample was suspended in 10 ml PBS-T and intensively shaken for 30 minutes. Then, 50µl of appropriate serial 10-fold dilutions of soil suspensions were plated onto four different agar media, which were cultivated at 25°C for 48 hours to determine CFU (colony forming units) per gram of the sample. Based on the variable phenotype of bacterial colonies, 40 isolates from each type of cultivation media from one sampling site were selected for MALDI – TOF MS (Matrix – Assisted Laser Desorption / Ionisation Time of Flight Mass Spectrometry) identification. After the MALDI – TOF MS identification, representative isolates from each clade of phylogenetic tree were tested for their antibiotic tolerance. Antibiotic tolerance testing of bacterial isolates was performed on MH (Mueller-Hinton) agar using dilution method with the addition of antibiotics- ampicillin (AMP), chloramphenicol (CHLOR), tetracycline (TET) and kanamycine (KAN). Concentrations of antibiotics were as follows: AMP - 10, 20, 50, 100 µg/ml; CHLOR – 1, 2, 5, 10, 20 µg/ml; TET – 2, 5, 10, 20, 50 µg/ml and KAN – 5, 10, 20, 50, 100, 200 µg/ml. The plates were incubated in the dark at 25°C for 48 hours and then the growth of bacterial isolates was evaluated.

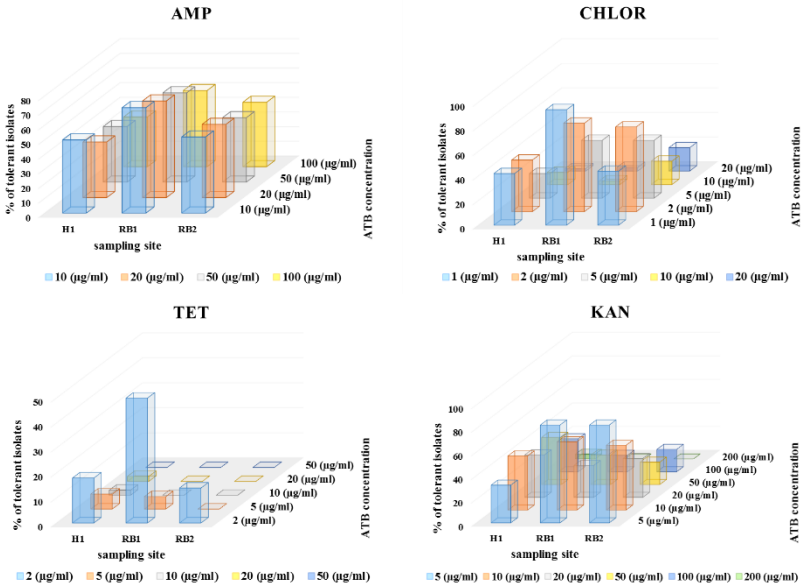


Fig. 1 Minimal inhibitory concentrations of ampicillin (AMP), chloramphenicol (CHLOR), tetracycline (TET) and kanamycine (KAN) found for selected isolates.

In our experiment we showed the presence of antibiotic tolerance of bacteria which could arise from selective pressure created just by heavy metals. There are concerns about the probability of metal contaminated soils acting as favourable sites for antibiotic resistant bacteria and thereby pool of antibiotic resistant genes in both natural and clinical settings.

Keywords: antibiotic resistance, heavy metals, bacteria, mine, dump

Acknowledgements
The work was fully supported by a grant from the Slovak National Grant Agency under the VEGA Project no. 1/0229/17.

.

DETERMINATION OF POLYCYCLIC AROMATIC HYDROCARBONS AFTER BIOLOGICAL DEGRADATION OF CONTAMINATED SOIL

Jana Tomčová, Daniel Kupka, Eva Mačingová, Miroslava Václavíková
Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovakia, vaclavik@saske.sk

ABSTRACT
Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants. The increased scientific interest in PAHs is related to the fact that many of these compounds have carcinogenic and mutagenic effects and pose a hazard for human health. 16 PAHs have been listed as the priority pollutants by US Environmental Protection Agency (EPA). PAHs are highly recalcitrant molecules that can persist in the environment due to their hydrophobicity and low water solubility.
Many microorganisms have been isolated and characterized for the ability to degrade different PAHs aerobically and anaerobically. The determination of polyaromatic hydrocarbons in soils generally includes extraction, fractionation and final identification and quantification.
The aim of this study was to determine degradation products (PAHs) in the coal tar contaminated soil sample after 40 days of biodegradation. The soil degradation was studied under aerobic conditions in laboratory glass columns using the indigenous microorganisms. Soil was air-dried, homogenized, sieved through a 2-mm sieve and placed in glass columns which were aerated with atmospheric air flow. The oxidation kinetic of organic carbon was monitored using a respirometer by measuring the rate of O_2 consumption and the rate of CO_2 production. 12 500 mg kg^{-1} of total organic carbon was completely mineralized to CO_2 within 40 days (Fig. 1). Identification and quantification of PAHs was performed by HPLC chromatography with a diode array detector. Soil sample was extracted in Soxhlet extractor with petroleum ether for 20 hours. The SPE extraction was performed according Macherey-Nagel Appl. No. Separation of the 16 PAHs was performed with a column 250 x 3 mm Nucleosil 100-5 C18 PAH. The total concentration of these pollutants was 34 353.66 mg per kg of soil. The amount of PAHs was decreased by 4909 mg kg^{-1} during biodegradation, which was approximately 14% of the initial value. The individual PAH content before and after the biodegradation test is shown in Fig. 2.

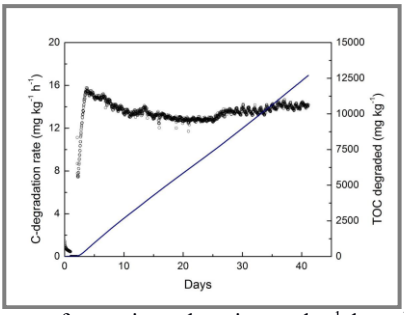


Fig. 1. Degradation rate of organic carbon in mg kg^{-1} $hour^{-1}$ and the cumulative amount of degraded organic carbon in mg kg^{-1}.

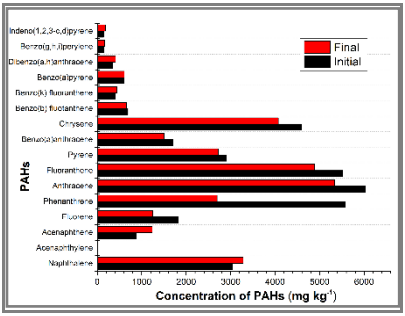


Fig. 2. Individual PAH content (mg kg^{-1}) in soil sample before and after the biodegradation experiment.

During the biological degradation, the total organic carbon content calculated from the CO_2 production decreased by 12.500 mg kg^{-1} and the amount of 16 selected PAHs converted to total carbon was reduced by 4629 mg kg^{-1}. This can be explained by the fact that other organic substances, polyaromatic compounds not monitored within the 16 US EPA PAHs and their derivatives are present in the soil. These compounds are likely to be transformed into other PAHs (including the monitored 16 PEA PAHs) of lower molecular weight by cleaving a portion of the molecule by bacteria.

Keywords: PAHs, biodegradation, HPLC analysis.

Acknowledgement: This work has been supported by the MarieCurieProgramme FP7-People-2013-IAAP-WaSClean project No 612250, VEGA-2/0158/15 and 2/0055/17 and ERDF GeoCex project No ITMS 26220120064 – Centre of Excellence for Integrated Research of the Earth's Geosphere.

FUNGAL EXTRACELLULAR METABOLITES AND THEIR EFFECTS ON MOBILITY OF ELEMENTS

Martin Uríka[a], Marcel B. Miglierini[b], Marek Bujdoš[a], Hyunjung Kim[c]

[a] *Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Laboratory Research on Geomaterials, Ilkovičova 6, Mlynska dolina, 842 15 Bratislava 4, Slovakia*

[b] *Slovak University of Technology in Bratislava, Faculty of Electrical Engineering and Information Technology, Institute of Nuclear and Physical Engineering, Ilkovičova 3, 812 19 Bratislava, Slovakia*

[c] *Chonbuk National University, Department of Mineral Resources and Energy Engineering, 567, Baekje-daero, Deokjin-gu, Jeonju, Jeonbuk 54896, Republic of Korea*

ABSTRACT

Our extensive research on fungal transformation of selenium, mercury, aluminium, arsenic, antimony and other hazardous substances highlighted that metabolic activity of filamentous fungi do affect the environmental behavior of these metals and metalloids. To quantify the fungal impact on mobile metals and metalloids' fraction, we have applied various experimental methods, including studies on biovolatilization, biosorption and bioextraction. Our research confirmed that filamentous fungi naturally produce various acidifying, redox active and chelating metabolites into their extracellular space. It has also highlighted the idea that the chemical properties of naturally occurring fungal extracellular metabolites undeniably affect translocation of environmental contaminants.

Keywords: filamentous fungi, bioaccumulation, bioextraction

Acknowledgement

This work was supported by the Scientific Grant Agency of the Slovak Republic Ministry of Education and the Slovak Academy of Sciences under VEGA contract Nos. 1/0164/17, 1/0836/15, and 1/0146/18, and SRDA bilateral Slovak-Korean project No. SK-KR-18-0003.

INFLUENCE OF TEMPERATURE ON INDIUM AND TIN EXTRACTION FROM LCD GLASS DURING CHEMICAL AND BIOLOGICAL LEACHING

Joanna Willner[a], Kinga Wyspiańska, Agnieszka Fornalczyk[a], Bernadeta Gajda[b], Mariola Saternus[a]

[a] *Silesian University of Technology, Faculty of Materials Engineering and Metallurgy,, Department of Extractive Metallurgy and Environmental Protection, ul. Krasińskiego 8, Katowice, Poland;*

[b] *Czestochowa University of Technology, Department of Extraction and Recirculation of Metals, ul. Armii Krajowej 21, Częstochowa, Poland*

ABSTRACT

Indium is considered as one of the critical raw materials for the EU. It is expected that the demand for special metals as well as other rare raw materials will increase by three times up to 2030. This situation is conditioned by the growing demand for critical metals, such as lithium, indium, tantalum or germanium, needed for the production of photovoltaic modules. In this situation, the importance of recycling increases more and more. Indium as metal is used in electronics in the form of alloys and low-melting solder. However the main indium raw materials are indium oxide and In-Sn oxide (ITO: 90% In_2O_3, 10% SnO_2, the contents of other elements are less than 20 ppm), which ultrathin, transparent coatings are used in the production of liquid crystal displays (LCD). The amount of indium in LCD screens varies in different models of devices and is approximately 102 mg/kg. This value can be even higher (1400 mg In/kg) when the polymer film attached to the LCD screen is previously removed.

Article presents results of the indium and tin extraction from the LCD glass. Biological and chemical leaching were used for the ground LCD material. Experiments were carried out in temperature 20°C and 30°C in two separated leaching tests: i) bioleaching during 32 days in 9K medium and mixed bacteria *Acidothiobacillus ferrooxidans* and *Acidothiobacillus thiooxidans* ii) chemical leaching during 24 hours with $Fe_2(SO_4)3{\cdot}5H_2O$ solution and Fe(III) concentration 9 g/dm^3. Results of indium and tin concentration in leaching solution after biological and chemical tests are presented in Fig. 1 and Fig. 2.

The temperature enhanced dissolution of indium and tin both in the chemical and biological leaching. However higher concentrations of these metals were achieved under biological leaching conditions at the temperature of 30°C: respectively 11,1 mg/dm^3 for In and 6,1 mg/dm^3 for Sn

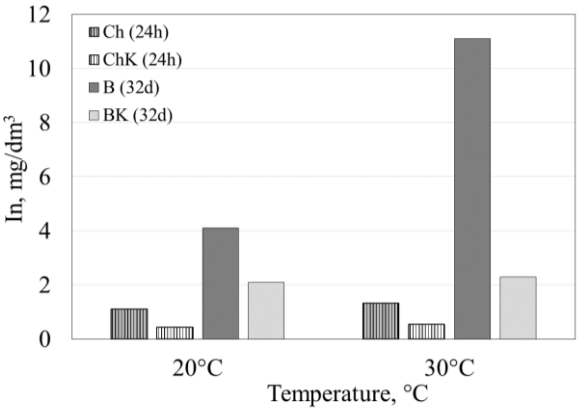


Fig. 1 Indium concentrations in the chemical leaching with $Fe_2(SO_4)_3 \cdot 5H_2O$ solution (Ch) and in control system (ChK) after 24 h and in bioleaching (B) and control system (BK) after 32 days,, depending on the temperature

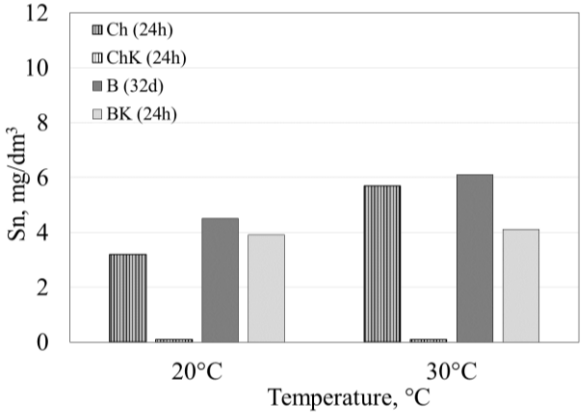


Fig. 2 Tin concentrations in the chemical leaching with $Fe_2(SO_4)_3 \cdot 5H_2O$ solution (Ch) and in control system (ChK) after 24 h and in bioleaching (B) and control system (BK) after 32 days, depending on the temperature

Keywords: indium recovery, LCD panels, bioleaching, leaching

SORPTION PROPERTIES OF MAGNETICALLY SEPARATED CARBON-RICH COAL FLY-ASH

Anton Zubrik, Michal Lovás, Zuzana Danková, Marek Matik, Slavomír Hredzák
Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovakia

ABSTRACT
Utilization of fly-ashes is given by physical, chemical and mineral properties. Fly-ash contains silicates, alumosilicates, unburned carbon and iron. Fe-bearing minerals can be separated by applying of external magnetic field. On the other hand, unburned carbon (concentrated to "non-magnetic" product) with a porous structure can be used as a cheap adsorbent of toxic substances from aqueous solution. The most popular studies are devoted on sorption experiments towards heavy metals, nevertheless many studies showed that fly-ashes are effective adsorbents of organic pollutants.
The paper deals with the study on the sorption properties of physically treated coal fly-ash towards cationic dye (methylene blue). Firstly, carbon rich coal fly-ash (C^d = 21.54 %) from the Heating plant was magnetically separated at two inductions of magnetic field, i.e. 0.1 T and 0.4 T, respectively. Thus, three products, two magnetic and one "non-magnetic" different in carbon content and volume magnetic susceptibility were obtained. Increasing of carbon content to 23.2 % was achieved in the "non-magnetic" product.
Secondly, the basic sample and selected products of separation was tested as a sorbent of methylene blue. Initial pH adsorption tests showed that adsorption capacity is similar for all studied samples and the adsorption capacity increased with pH (to alkaline region). The best sorption process was observed at pH 10.7. Since the composition of physical separation products has no influence to sorption of methylene blue, basic sample of fly-ash was chosen for determination of maximum sorption capacity using Langmuir model (Q_m = 83.33 mg/g).

Keywords: coal fly-ash, magnetic separation, adsorption, methylene blue

Acknowledgements
This work has been supported by the Slovak Grant Agency for Science VEGA (project No. 2/0158/15).

UTILISATION OF FERROFLUD FOR SYNTHESIS OF EFFECTIVE MAGNETIC ADSORBENTS

Anton Zubrik[a], Marek Matik[a], Michal Lovás[a], Katarína Štefušová[a], Zuzana Danková[a], Slavomír Hredzák[a], Libor Machala[b], Jiři Pechoušek[b]

[a] *Institute of Geotechnics, Slovak Academy of Sciences, Watsonova 45, 04001 Košice, Slovakia*

[b] *Department of Experimental Physics, Faculty of Science, Palacký University, 17. listopadu 1192/12, 771 46 Olomouc, Czech Republic*

ABSTRACT

Permanent decreasing of water quality requires the development of novel, effective and cost available adsorbents. In recent years, low cost materials such as waste biomass are often used as a feedstock for synthesis of effective adsorbents. The most suitable biomass based materials used as a sorbents are raw biomass, bio-char and activated carbon. However, the carbon based materials are less effective for removal of metal species in oxoanionic form (i.e. As, Cr). Iron-based materials such as magnetic carbon are considered to be good candidates for oxyanion removal from water. This is due to electrostatic forces between the negatively charged arsenic and the positively charged iron surface.

Herein, a simple procedure for preparation of magnetic adsorbent from agricultural waste biomass and ferrofluid has been introduced. Specifically, ferrofluid mixed with wheat straw was directly pyrolysed by microwave irradiation (900 Watt, 30 min). Microwave conversion allowed obtaining material with different chemical composition (various Fe-bearing phases identified by Mössbauer spectroscopy), developed porosity and texture (S_{BET} = 119.3 m^2/g). Results showed that microwave pyrolysis produces magnetic char with high adsorption capacity towards the As(V) (Q_m = 25.6 mg/g at pH 4). Moreover, solid/liquid magnetic filtration as a rapid and effective technique can be applied in removal of used magnetic biochar from aqueous solution after sorption process.

Keywords: magnetic bio-char, microwave pyrolysis, wheat straw, ferrofluid, sorption

Acknowledgements

This work has been supported by and Scientific Grant Agency VEGA (projects No. 2/0158/15).

CHARACTERIZING CADMIUM UPTAKE IN WHEAT CULTIVARS FOR FOOD SAFETY

Laura Žideková[a], Zuzana Gregorová[b], Monika Bardáčová[b], Petra Ranušová[b], Vanda Adamcová[b], Miroslav Horník[b], Ildikó Matušíková[b]

[a] *University of SS. Cyril and Methodius, Department of Biotechnology, J. Herdu 2, Trnava, SK-917 01*

[b] *University of SS. Cyril and Methodius, Department of Ecochemistry and Radioecology, J. Herdu 2, Trnava, SK-917 01, pranusova@gmail.com.*

ABSTRACT

Plants face adverse environmental conditions that include presence of toxic heavy metal ions such as cadmium. Intake and accumulation of cadmium by wheat plants (grains) bears risk for production of contaminated food. Some durum wheat cultivars accumulate high amounts of cadmium in their grains, while common wheat accumulates cadmium at much lower rates. Soil properties, including presence of different concentration and form of fertilizers, can affect the accumulation. Mechanisms for metal translocation and accumulation in grains are not fully described. Molecular, biochemical and physiological analyses enable to identify varieties accumulating low amounts of cadmium.

The SCAR marker *ScOPC20* localized on 5B chromosome for cadmium accumulation capacity is being used to determine the genetic potential of different tetraploid and hexaploid wheat cultivars. More than 60 wheat (*Triticum*) cultivars of different origin are being analyzed by detecting the PCR fragment of expected size 394 bp when "high" cadmium accumulating allele is present in genome. For selected cultivars, the genetic analyses are complemented with analyses of physiological parameters (e.g. content of photosynthetic pigments), content of proline or activity of defense proteins (data not shown). More importantly, the kinetics of cadmium uptake from solution of 50 $mg.l^{-1}$ Cd^{2+}, spiked with $^{109}Cd^{2+}$, and also the amount of accumulated cadmium in tissues are determined by scintillation gammaspectrometry (76BP76/3, Scionix, The Netherland). The experimental wheat plants are cultivated in hydrophonics in standard Hoagland media, supplemented with various amounts of ammonium nitrate as fertilizer, under standard growth conditions (Fig. 1A).

Our analyses identified so far the genetic potential for accumulation of low amounts of cadmium for 5 accessions of *T. durum*, 6 accessions of *T. dicoccum*, 1 accession of *T. turgidum*, 0 accessions of *T. compactum*, 2 accessions of *T. aestivum* and 11 accessions of *T. spelta*. Selected cultivars

were further analysed for cadmium uptake. These results point on variability in intake of metal. Moreover, the nutrition conditions affected the kinetics of metal depletion from medium by roots. For example, the plants of the cultivar Slommer grown at surplus of nutrition exerted limited cadmium uptake comparing to optimal fertilizer dose, but more than starving plants (Fig. 1B). This effect was not observed for cultivar Katya.

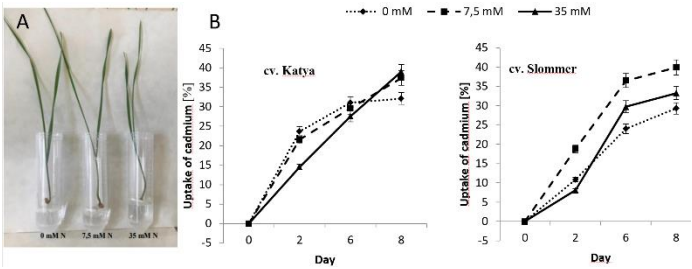


Fig. 1 Wheat plants cultured hydroponically in presence or absence of Cd^{2+} spiked with isotope ^{109}Cd, under different fertilizer regimes (A). Kinetics of cadmium depletion from medium demonstrated for two wheat cultivars (B). The values correspond to the arithmetic average ± SD (n = 4).

Our results show that genetic markers can identify wheat cultivars that bear risk for production of contaminated food. The fertilizer dose applied should carefully be considered since can influence the metal uptake rate.

Keywords: Cadmium, Food Contamination, Genetic Markers, *Triticum aestivum*

Acknowledgement: The work is financed by the Slovak Research and Development Agency under contract numbers APVV-15-0051, APVV-15-0098 and supported by the Operational Program Research and Development of the project "Implementation of the Research on Plant Genetic Resources and its Support in the Sustainable Development of the Slovak Economy" (ITMS: 26220220192) co-financed by the European Regional Development Fund.

Faculty of Science
Pavol Jozef Šafárik University
in Košice

The Pavol Jozef Šafárik University in Košice belongs to the significant and recognised teaching and educational facilities not only in the Slovak Republic but in the developed Europe as well. The University belongs to the institutions, which present a long tradition of scientific research, which follows the mission of the university and which belongs to its priorities.
The Faculty of Science of the Pavol Jozef Šafárik University in Košice is a significant scientific institution, which offers courses in undergraduate, graduate and post-graduate level of studies in the field of natural sciences, mathematics, computer science and teacher studies of select academic courses. The Faculty, amid natural science oriented faculties, is long considered as one of the top scientific, research and teaching institutions in the Slovak Republic (it has been accredited in 8 out of the 16 scientific fields of accreditation at the Pavol Jozef Šafárik University). The achieved results in all of the fields of natural science: biology, physics, chemistry, computer science and mathematics, which are taught either as one major or two major courses in teaching, a wealth of publications, international connections and cooperation with people in the field elevate its position among scientific institutions not just at home but in Europe and in the world as well. The faculty's main role in scientific research activities is the support of successful grant schemes, strengthening of the interdisciplinary and multidisciplinary character of science and research and the expansion of international cooperation, mainly by taking part in European scientific and scientific and technological projects.

Institute of Biology and Ecology

Institute of Biology and Ecology is an institution with a complex approach to the solution of broad range of biological and ecological problems of the current science using the newest molecular methods. Highly educated researchers and up-to-date equipment provide education in main study branches – Biology and Ecology. Institute is part of the Faculty of Science and consist of 8 Departments.

Department of Botany
Department of Cell Biology

Department of Animal Physiology
Department of Genetics
Department of Microbiology
Department of Zoology
Laboratory of Molecular–biological Diagnostics
Division of Didactics of Biology

Department of Microbiology

Department of microbiology covers several scientific topics from the field of clinical, environmental and applied microbiology. The main emphasis in the field of environmental and applied microbiology is on the metal-microbe interactions study on population as well as individual levels. It deals with a characterisation of microorganisms from extreme conditions (including high metal contamination), with a study of metal resistance and cross metal and antibiotic resistance with the aim to shed light on mechanisms which could lead to the development of bioremediation technologies.

Knowledge obtained from the research is spread in educational process within several study courses such as Microbiology and Virology, Environmental Microbiology, Applied Microbiology, Bioinformatics, Genetic Engineering, Laboratory Diagnostics in Microbiology and Environmental Biotechnology

SPONSORS

SPONSORS

SPONSORS

4th International Scientific Conference on Biotechnology and Metals


Biotechnology
Metals
Kosice 2016

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Conference Notes

Proceedings: Book of Abstracts of the 5th International Scientific Conference on Biotechnology and Metals

Editors: Jana Kisková, Jana Sedláková-Kaduková, Alena Luptáková

Publishers: Faculty of Science, Pavol Jozef Šafárik University &
Institute of Geotechnics, Slovak Academy of Sciences
Slovak Mining Society at the Institute of Geotechnics SAS

Printed: EQUILIBRIA, Letná 42, 040 01 Košice

Year: 2018

Edition: First edition

Print run;

Pages:

ISBN: