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PREFACE

Connection of biological sciences with the metal recovery field is maybe not traditional but surely not new. Several centuries before Christ old Chineses already had used bioleaching for metal recovery, Aztecs a bit later used characteristics of plant juices from the family Oxalidaceae in gold processing. Although nowadays these technologies come very slowly to Slovakia they are not new even here. For example Paracelsus in 11th century had written about copper recovery from mine waters originated from the microbial activity in the area of Smolnik, Central Slovakia.

Exhaustion of natural resources of metals, environmental pollution and metal-bearing waste accumulation force us to search for new non-conventional technologies. Knowledge from past together with the newest findings suggest that nature for centuries has unique ways how to cope with negative characteristics of metals. Knowledge of these processes and their application in metal recovery and processing of metal bearing materials is just the point where biotechnology and metals can be successfully connected.

The aim of the conference was to create space for experts from many scientific fields to discuss new possibilities and the newest trends in biotechnology used for metal recovery from primary and secondary sources with the aim to remove them from the environment or to obtain them in the form of the products valuable for practise. The conference concentrated on application of biotechnology to low-grade ores and metal bearing waste processing, cleaning-up biotechnologies for the environment and application of biometallurgical methods in practise.

The conference was organized by two institutions – Faculty of Metallurgy, Technical University of Kosice and Institute of Geotechnics, Slovak Academy of Sciences which cooperate in many different projects several years.

We hope you enjoyed your visit to the conference as well as to Kosice, Slovakia. We believe that we will meet again also at next years at the conference Biotechnology and Metals.

Jana Kadukova
Alena Luptakova

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CONTENTS

THE ROLE OF BACTERIA IN DEGRADATION OF EXPOSED ORE MINERALS AT PEZINOK DEPOSIT

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ABSTRACT
The article reports the chemical characteristic of acid mining drainage waters as well as the results of leaching experiments conducted with and *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* at the same conditions in solution. The experiments were realized using tailing impoundment sediments and ore minerals from the Sb-(Au-) deposit Pezinok (Malé Karpaty Mts., Western Carpathians, Slovakia). The research study shows the oxidation sequence and the leaching progression on surface of the following ore minerals: löllingite, arsenopyrite, stibnite, native Sb, gudmundite, berthierite, pyrite, sphalerite and chalcopyrite. The differences between chemical and biological-chemical leaching activity of various ore minerals on the polished sections surface is discussed. The extent and the kinetics of the biological-chemical leaching of ore minerals is significantly higher than the chemical leaching of ore minerals without bacteria.

KEYWORDS
Acid mine drainage, biological-chemical oxidation, chemical oxidation, etching-patterns, *Thiobacillus ferrooxidans*

1. INTRODUCTION

The supergene oxidation of sulphide minerals is the main source of acidity and increased content of heavy metals and sulphates both in drainage or surface waters as well as in ground waters. Acid mine drainage (AMD) is usually the most important source of contamination in mining regions. The process can by substantially accelerated by influence of the bacterial oxidation, due to the metabolic activity of autochtho-nous, acidophilous and thionic bacteria *Acidithiobacillus ferrooxidans* (*ATF*), *Acidithiobacillus thiooxidans* (*ATT*) and *Leptospirillum ferrooxidans* (*LF*) catalysing the ore minerals oxidation processes.

At the Pezinok deposit two types of ore mineralisation were described: 1 – metamorphosed, exhalation-sedimentary pyrite mineralisation genetically related to Devonian basic volcano-sedimentary cycle which was subsequently metamorphosed and 2 – hydrothermal Sb-Au-As mineralization of epigenetic character which is most frequently localized in beds of ttoecnically deformed black schists [1]. About 20 000 tons of antimony was exploited from this deposit. The reported content of Sb ranges from 1% to 4 %, of As from 0.5 % to 1.5 % and the average content of Au is 3.60 ppm. The mine was closed in 1992. The mining-waste is deposited in several dumps and two sludge lagoons containing 380 000 m^3 of material. As- and Fe- minerals (predominantly arsenopyrite and pyrite) were during the ore dressing process suppressed and moved to the waste. The content of these minerals in the sludge lagoons is considerably higher than that of Sb-minerals. The most frequent sulphide minerals in the sludge lagoons are arsenopyrite (often gold-bearing; the Au content up to 1200 ppm) and pyrite. Gudmundite and stibnite occurs rarely, pyrhotite sporadically. In some samples were determined Sb- and Fe-oxides, tetrahedrite, löllingite and chalcopyrite [1]. The gangue minerals are represented by carbonates and quartz. Schists and chlorite are abundant but caolinite is very rare. The dominant clay mineral is illite. Also Fe-oxyhydroxides and Sb-oxides are formed in the oxidation zone of the sludge lagoons [2].

2. MATERIAL AND METHODS

The AMD waters were analysed by atom absorption analyse for Fe, Mn, As, Cu, Ni, Pb, Sb and Zn. Two types of leaching experiments were realised to study the mobility of previously mentioned metals from the tailing impoundment sediments as well as the oxidation of ore minerals. During the first experiment the tailing impoundment sediment and the polished sections of ore minerals were introduced to solution containing *ATF* bacteria isolated from the mine waters from Pezinok deposit (biological-chemical process) at pH 1.57. Biogenic catalysis of the selected sulphides oxidation was studied using the leaching nutrient medium 9K, part A according to Silverman and Lundgren [3] with the content of nutrients for *ATF* cells growth. Figure 1 describes of the studied area sketch at the Pezinok deposit and the sampling localities.

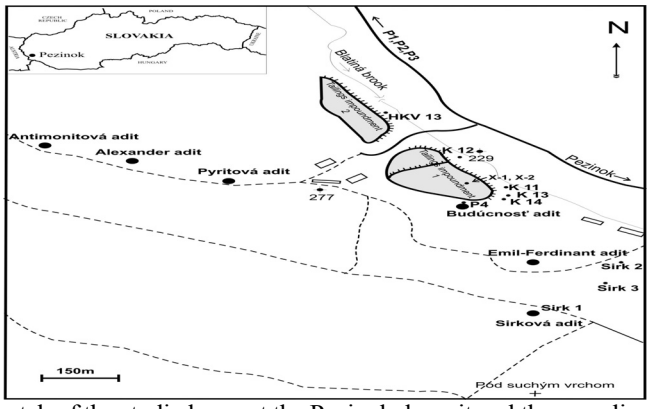

Fig. 1. Sketch of the studied area at the Pezinok deposit and the sampling localities

The second experiment was the abiotic control carried out with the chemically identical leaching agent without bacteria *ATF* (chemical process). The sample of the sediment X-1, the ochre sample X-2 from the tailings impoundments and the AMD of various pH and origin were analyzed by atom absorption analyse (AAS). Polished sections of natural arsenopyrite, löllingite, native Sb, stibnite, gudmundite, berthierite, sphalerite, pyrite and chalcopyrite were studied under conditions of two parallel experiments (biological-chemical and chemical processes). The polished sections of the ore samples were within the both experiments leached in Petri's dishes at the room temperature. The changes on the ore minerals surface were evaluated optically at set time intervals using electron micro-probe JEOL JXA – 840.

3. RESULTS

3. 1. Characterization of AMD water

The investigated sample of sediment from tailing impoundments X-1 used for the next experiments was analyzed by atom absorption analysis (Tab. 1). This sediment sample was employed in experimental works for the investigation of the leaching process by using of both types of drainage waters: acidic and neutral.

The AMD waters contain relatively high contents of Sb, As, Fe, Cu, Cd, Ni, Zn and other metals. The set of the drainage waters from Pezinok was completed with one sample of water from the wider mining ore-field (P 1) (Tab. 2).

The dependence of the leaching activity on leaching medium is presented in Table 3. For this experiment was used drainage water P 2 from Pezinok with autochtonous *ATF* and *ATT* bacteria. The pH of the water was 4.5. The second sample of drainage water used for experiments was drainage water from Pezinok P 4 without bacteria (pH = 6.45). During the study of catalytic influences were realized parallel experiments with rainwater for comparison.

In acidic medium (pH=1.57) *ATF* are active, and vigorously assist the oxidation of ore minerals (Tab. 4). Extraction rate of Fe, As and Sb is highest in the first week of leaching. As it could be expected, concentration of metal cations in the leaching product is highest in the run, where the heavy fraction of the sediment sample was employed.

In the second period of leaching we can observe the gradual decrease of Fe concentration in liquid phase in consequence of precipitation of Fe-oxyhydroxides. After leaching, the solid fraction was examined by XRD. Besides the detritic minerals (quartz, muscovite, phlogopite, chlorite) secondary minerals, such as jarosite, hydrojarosite and gypsum were detected. The activity of *ATF* bacteria considerable accelerated predominantly the extraction process of Fe, As and Sb both from the sample of the sediment (X-1) as well as from the sample of the heavy fraction.

Tab. 1. Atom absorption analysis of the chemical composition of sediment sample from tailing impoundments

Sample	pH	ppm							
		Fe	Mn	As	Cu	Ni	Pb	Sb	Zn
X – 1	1.7	1 071	3.45	0.104	0.69	1.34	0.05	221	0.76
X – 1	1.7	1 014	2.57	0.090	0.48	0.02	0.05	196	0.52

Tab. 2. Characteristic of acid mine waters from Pezinok mining area, including pH, content of investigated elements and presence of acidophilous bacteria.

Sample	pH	g.l^{-1}						mg.l^{-1}	
		As	Cu	Fe	Pb	Sb	Zn	Ag	Au
P 1	5.54	<5	<0.02	24.50	<2	<2	0.16	1.3	<2
P 2	4.50	<5	0.03	0.12	3.1	<2	0.11	0.9	<2
P 3	6.63	<5	<0.02	8.36	<2	3.4	0.12	0.8	<2
P 4	6.64	5.1	<0.02	31.20	<2	6.6	0.12	0.9	<2

Explanatory notes: P 1 – drainage water from the Pernek, P 2 – drainage water from measuring-point 8, P 3 – drainage water from the creek near adit Michal, P 4 – Pezinok, drainage water from adit Budúcnosť. mg/l

Tab. 3. Atom absorption analysis of various liquid media of different pH and rainwater sed for leaching of the sediments from the tailing impoundments

Leaching medium	Time of leaching (weeks)	mg.l^{-1}					
		As	Co	Cu	Fe	Ni	Sb
Drainage water P 2 *ATF+ATT* pH = 4.5	I	17.8	<0.06	0	213.7	2.7	8.0
	II	16.5	<0.06	0	253.4	5.3	8.8
	III	<2.0	0	6.3	195.7	5.2	<0.4
	IV	<2.0	0	5.2	126.5	4.4	<0.4
Drainage water P 4 pH = 6.45 no bacteria	I	16.8	<0.06	0	225.2	3.0	9.4
	II	12.1	<0.06	0	157.0	3.9	7.0
	III	<2.0	0	5.9	156.0	5.4	<0.04
	IV	<2.0	0	5.2	84.2	4.4	<0.04
Rainwater pH = 5.6 no bacteria	I	13.3	<0.06	0	188.9	2.9	8.8
	II	9.6	<0.06	0	142.1	3.5	7.4
	III	<2.0	0	4.1	115.3	3.2	<0.4
	IV	<2.0	0	5.1	95.6	4.1	<0.4

Tab. 4. Leaching of the sediment sample (A) and of its heavy fraction (B) from the tailing impoundments by *Acidithiobacillus ferrooxidans* (*ATF*). a) nutrient medium (pH = 1.57); b) abiotic control without bacteria (pH = 1.57)

Leaching medium	Time of leaching (weeks)	mg.l^{-1}					
		As	Co	Cu	Fe	Ni	Sb
(A) 9K-A *ATF* pH = 1.57	I	23.9	<0.06	0	4259.0	<0.1	9.0
	II	123.2	<0.06	0	2100.0	1.2	12.3
	III	72.0	0	3.3	167.4	1.5	7.5
	IV	58.6	0	3.6	135.6	1.7	9.2
(B) 9K-A *ATF* pH = 1.57	I	72.1	<0.06	0	4576.0	0	12.6
	II	317.3	<0.06	0	3183.0	0	21.6
	III	288.0	0	8.0	280.2	7.2	19.7
	IV	208.1	0	7.5	249.4	6.5	13.0
(A) 9K-A no bacteria pH = 1.57	I	32.6	<0.06	0	167.0	1.2	1.2
	II	36.8	<0.06	0	578.4	4.5	10.1
	III	22.6	0	8.8	378.0	7.1	<0.04
	IV	<2.0	0	7.4	271.1	5.9	<0.04

3. 2. Biological-chemical and chemical oxidation of ore minerals

The experimental study of biogenic catalysis of As-minerals showed that löllingite (mineral with the highest As content) is the first, which is intensively attacked by biological-chemical oxidation. Already after 5 days of leaching was its surface markedly etched.

After 2 days of biological-chemical oxidation of arsenopyrite it was possible to observe the creation of dissolving channels at the cracks and at the contact of individual grains. The first *pearl-string-like-chains* (Fig. 2) began appear

after 10 days. The chains followed along the structural macro-defects of grain are probably formed due to the accumulation of metals at the surface of *ATF* cells. The heterogenous distribution of As within the gold-bearing arsenopyrite grains is a characteristic feature, along with well developed zonal microstructures, which may be interpreted as growth-banding in the oscillatory hydrothermal fluid system. The creation of caves and preferential dissolution of As-rich (and Au-rich) growth zones (Fig. 3) suggest the positive impact of galvanic effect of contact zones with the different As content.

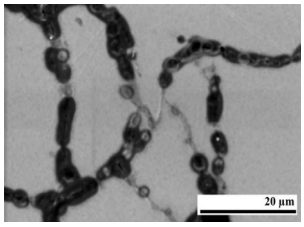

Fig. 2. Pearl-string-like chains at the arsenopyrite surface after 10 days of biological-chemical leaching. SEM image

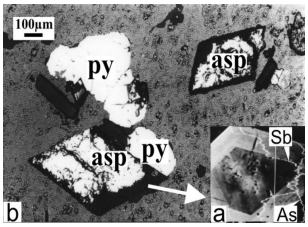

Fig. 3. SEM image of
a) Arsenopyrite crystal with line diagram of As and Sb contents.
b) Preferential dissolution of arsenopyrite (asp) compared with pyrite (py) during biological-chemical oxidation
of sulphide ores (As-abundant arsenopyrite zones show intensive corrosion). Even after 18 days of biological-chemical leaching the surface of pyrite remains intact

This galvanic dissolution allows to explain a complete dissolution of arsenopyrite on the contact with pyrite already after 18 days of leaching. Kinetics of chemical corrosion of pyrite was slower. In comparison with the biological-chemical oxidation the less intensive decomposition of the crystal surface was observed after 18 days of chemical leaching. The first signs of dissolution and subsequent degradation of native antimony surface as a result of *ATF* bacteria activity was possible to observe already after 2 days of oxidation. Etching-patterns reminding the colomorphous structure were formed gradually with the period of leaching, as well as the dissolving cracks at the points of bacteria attachment. The advancing etching gradually denuded the trigonal structure of the mineral.

The interesting concentrically lined spherical shapes were created in consequence of surface dissolution after 10 days of biological-chemical oxidation (Fig. 4). Chemical oxidation of native antimony is not so intensive. After 18 days of oxidation is its surface only planary etched.

The first indications of dissolution by *ATF* bacteria medium were on the stibnite surface observed after 7 days of oxidation. The etching-patterns and triangular-shaped caves appeared after 10 days of leaching arranged along lines parallel with axis c of the stibnite needle. Such progress of leaching reflected the stibnite crystallographic texture. At the contact of stibnite with sphalerite there was observed an intensive dissolution and hollows forming process after 10 days of leaching.

Etching-patterns highlighting the crystal texture were observed after 15 days of chemical leaching of stibnite. The gypsum crystals formation along the carbonate veins and the intensive mineral dissolution was observed after 18 days. The attachment of bacteria on the surface of gudmundite crystal and the creation of dissolving rims along the mineral cracks were possible to observe after 7 days of oxidation. The linear depressions following the gudmundite crystal texture were formed after 18 days of leaching (Fig. 5). The gudmundite surface chemical etching was ascertained only after 15 days. Berthierite is more resistant. The signs of etching appeared at the crystals after 18 days and the intensive biological-chemical degradation started after 21 days. The significant chemical dissolution began markedly after 30 days. The selected sphalerite polished crystal pieces were kept in *ATF* containing solution for a period of 50 days The first signs of dissolution along the individual grains of mineral aggregate were observed after 18 days of leaching but the crystal surface was channelled markedly only after 25 days. However, no significant signs of biodegradation were observed on the euhedral inclusions of pyrite crystals. The final phase of biological-chemical oxidation of sphalerite is the intensive crystal etching after 40 days uncovering the tetrahedral structure of lattice (Fig. 6). The chemical leaching had minor kinetics. Only moderate etching patterns and channels were described after 20 days of leaching. After 10 days of biological-chemical leaching the morphological changes at the pyrite surface were possible to find only along the cracks: cells of bacteria formed chains or aggregates. After 25 days of leaching the surface of pyrite grains was covered with the rough crust of secondary minerals. The microprobe examination of pyrites reveals that the dissolution developed preferentially along preexisting cracks and veinlets, as well as along grain contacts. The point dissolution and the creation of caves in the size of several µm were possible to see on the edges and in the centre of grain. The shape of caves depended on the orientation of individual crystallographic faces. The caves of hexagonal cross-section (Fig. 7) are developed on screw dislocations of the cubic lattice, the triangular caves on (111) faces while square holes

on (001) faces of pyrite cubes. After 30 days of chemical oxidation of pyrite the grains surface seemed to be intact. The linear depressions and oriented tunnels were formed gradually after about 2 months of leaching. They were developed, similarly as in case of arsenopyrite, preferentially in As-rich zones. The pyrite dissolved at first around the inclusions of other sulphide minerals as a result of galvanic effect.

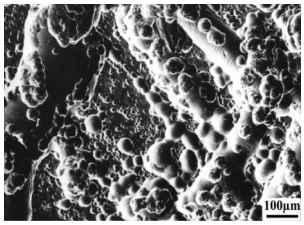

Fig. 4. Concentrically lined globular shapes at the surface of native Sb after 18 days of biological-chemical leaching. SEM secondary electron image.

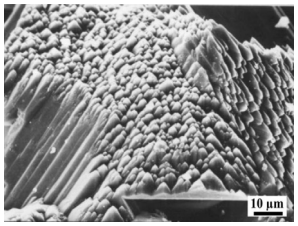

Fig. 5. Etched gudmundite crystal structure after 18 days of biological-chemical leaching.

Fig. 6. Etched texture of sphalerite after 40 days of biological-chemical leaching using *ATF* containing medium.

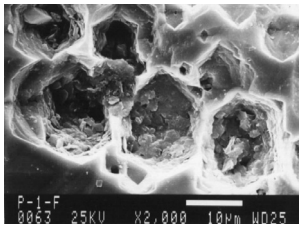

Fig. 7. Hexagonal corrosion pits on pyrite octahedron face after 40 days of biological-chemical leaching, developed on screw dislocations of the cubic lattice.

4. DISCUSSION

There are two types of acid mine drainage in the area of the Pezinok deposit: a) first type - extremely acid (pH < 3) mine waters associated with synsedimentary massive pyrite-pyrhotite ores, b) second type - neutral mine waters (pH 5.5 – 7.0) associated with Sb-carbonate mineralization.

The activity of *ATT* and *ATF* bacteria in the first type of acid mine drainage is much higher. It is the neutral waters that percolate through the sludge lagoons of Sb-ores in the Kolarsky hill area and that is why the leaching intensity is much lower than in the area of pyrite-mineralised parts (Augustin adit and the like). A considerable amount of Fe precipitates in form of ochres during the neutralisation of solutions. Ochres form the geochemical barrier and their surface serve as a sorbent of a considerable amount of metals. It is impossible to exclude that during torrential rains and under other influences the ochres may overcome the barriers of the tailing dams and reach the water flows. According to Luptáková [4] concentration of heavy metals in the water of sludge lagoons may be influenced also by anaerobic sulphate reducing bacteria producing hydrogen sulphide reacting with ions of heavy metals producing the secondary minerals. Luptáková realized the isolation of those bacteria from the solid samples of the reducing zone of the sludge lagoon. The under critical content of metals in waters, if flowing through the country for a long time, intoxicates river-sediments and gradually increases the metal concentrations as well.

Experimental studies of biogenic catalysis from the point of view of changes in the ore mineral surfaces in the sludge lagoons confirmed considerable differences in the kinetics and decomposition of studied minerals. The reaction ability of minerals reflects the distribution of the reactive planes and points at the crystal surfaces and the relation of these points to the energy of mineral crystalline lattice. During the biological-chemical oxidation processes it was possible to observe various signs of dissolution at the mineral surfaces that related to the metabolic processes of bacteria. For instance, the biological-chemical oxidation of pyrite is marked by the formation of characteristic etching-holes described by Morion et al. [5]. Morion described the galvanic interaction between different sulphide minerals during which the electrons are transferred from one sulphide mineral (anode) to less electroactive sulphide mineral. According to Crundwell [6] this effect can be interpreted as the modification of sulphide semiconductive properties accompanied with the tiny changes in the structure and physical properties (reflection, microhardness, conductivity, etc.) of sulphide minerals. The galvanic effect accelerates the dissolution of minerals. Such an effect was also observed during experiments carried out with the selected sulphide minerals from the Pezinok deposit. The preferential dissolution of contact grains was observed at the interface of different sulphide minerals or around the inclusions of sulphide minerals. On the other hand, a considerable deceleration of chemical and biological-chemical oxidation (with

the formation of gypsum as an associate phenomenon) was observed as a result of neutralisation effect of inherent carbonate component for sulphide inclusions in carbonates.

The presented results of native Sb biological – chemical leaching suggest also the possible role of Sb in metabolism of *ATF* bacteria.

5. CONCLUSIONS

The control of acidity is of utmost importance in leaching, because of acidic environment must be maintained in order to keep ferric iron and other metals in solution. Acidity is controlled by the oxidation of iron, sulphur and antimony by the dissolution of carbonate ions and by the decomposition of ferric iron through reaction with water.

The process of studied ore minerals degradation during biological-chemical oxidation in the presence of autochthonous, acidophilous, iron oxidising *ATF* bacteria and during chemical oxidation is principally similar, but the kinetics of both processes is different. Higher kinetics of biological-chemical oxidation processes of studied minerals confirms the bio-catalytic influence of autochthonous bacteria.

The result of experiments confirmed that the biogenic catalysis is the most intensive at löllingite. The other studied minerals are possible to arrange according to the decreasing oxidation kinetics as follows: arsenopyrite, native Sb, stibnite, gudmundite, berthierite, and sphalerite. Pyrite and chalcopyrite seemed to be the most resistant to the biological-chemical as well as chemical oxidation.

The positive correlation between the oxidation rate and As contents was ascertained for löllingite, arsenopyrite and pyrite. At the contact of two different minerals or two mineral growth zones with the different content of isomorphic components the galvanic effect was ascertained. In some minerals (mainly pyrite) the shape of dissolving caves and tunnels depend on the crystallographic orientation of individual crystal faces.

The structures formed during the biological-chemical oxidation in *ATF* containing medium are characterised as follows: the bacterial leaching causes the creation of caves and point etching-patterns formed as a result of direct mechanism of biological-chemical oxidation of the contact dissolution by microorganisms and they relate to the metabolic processes of applied bacteria; the shape of created caves depends on the crystallographic orientation of etched faces; the formation of various oriented tunnels, lines and dissolving planes is the implication of the chemical corrosion.

After a certain time the secondary mineral coatings appear at the mineral surfaces for both types of oxidation mechanisms. The researches show that the influences of the bacterial leaching substantially accelerate the ore minerals degradation. The Au distribution in gold-bearing sulphides is favourable for the exploitation of gold. Probably 90 % of Au is bond in the As-rich rim of the arsenopyrite crystals. The positive correlation between the oxidation rate and As content accelerate dissolution of the gold-bearing crystals. As it is sufficient to dissolve only the rim of the crystals it give a very good starting point for biological-chemical production of gold.

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POSSIBILITY OF USING QUARTZ SANDS IN SORPTION OF HEAVY METALS

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ABSTRACT
This study presents the results of the batch adsorption experiments of copper and zinc using iron oxide coated quartz sands and bacteria. Two samples, natural iron oxide coated sand (ICS-N) and the sand coated with synthetic goethite (ICS-G) were evaluated and compared in adsorption experiments. Metals were added at a concentration of 0.5 mg/l to 2.5 mg/l and adsorbed during the period of 2 hours at room temperature 25°C. Comparing the adsorbability values, there are differences between natural iron oxide coated sand and goethite coated sand as well as with addition of bacteria. Bacteria increased the adsorption efficiency of more than 30%.The result show that more than 27% and 32% of Cu(II) and Zn(II) respectively was removed by ICS-G, but just 16% of Cu(II) and 30% of Zn(II) by ICS-N. In competitive adsorption experiments of Cu(II) and Zn(II), 11.8% and 5.4% of Cu(II) and Zn(II) respectively was removed by ICS-G, and just 7.7% and 3.2% of Cu(II) and Zn(II) respectively by ICS-N. Addition of bacteria increased removal of Cu(II) from 27% to 60% by ICS-G and from 16% to 29% by ICS-N.

KEYWORDS
Iron coated sand, bacteria, heavy metals, adsorption

1. INTRODUCTION

The pollution of an aquatic medium can cause changes in physical, chemical and biological characteristic, compromising the water quality for human consumption [1]. Among the various contaminants, heavy metals have received special attention, since some of them are extremely toxic for a large variety of organism, even at very low concentrations (in the order of milligrams or micrograms per liter) [2,3].

Conventional technology for removing metals from solution involves precipitation of the metals and then separation of the particulate metals by settling, usually aided by a coagulant such as iron hydroxide. It has long been recognized that, if the adsorbent (solid surface) is chosen carefully and the solution chemistry is adjusted appropriately, adsorption- based processes are capable of removing metals over a wider pH-range and to much lower levels than processes based on precipitation [4]. Precipitation is often ineffective if the metals are complexed or if they are present as anions. Sorption to iron-oxide coated sand (ICS) with addition of bacteria should be a promising technology for removal of the heavy metals from wastewater [5].

2. MATERIALS AND METHODS

2.1. Characterization of quartz sand

Quartz sand used in this work was obtained from Šaštín Stráže deposit (Slovakia) and composed of quartz (88-90%), feldspar (8-10%), heavy minerals (1%) and clay minerals of grain size 0-1mm [6]. Quartz sand coated with synthetic goethit was prepared by procedure described below. Natural iron oxide sand was obtained from same deposit but from greater depth of sampling. Both samples were sieved under the grain size of 0.5 mm. The specific surface area of samples was for ICS-G 0.504 m^2/g and for sample ICS-N 0.833 m^2/g.

2.2. Preparation of goethite-coated sand

100 mg of synthetic goethite was mixed with 10 ml $NaNO_3$ solution in a 50 ml polyethylene tube. The pH was fixed at 2.5, adjusted with HNO_3 and the ionic strength at approximately 0.01 M. After shaking the mixture at 25°C for 24 hours in order to obtain a homogenous suspension, 2.5 g of silica sand has been added, and the mixture has been shaken for another 24 h. Finally the coated sand has been washed free from the rest of the goethite traces with the salt solution and then with pure water. The coated sand was oven dried at 60°C for 24 hours [7].

2.3. Bacteria preparation

The bacterial isolate *Bacillus cereus* (obtained from Dr. Iveta Štyriaková) was inoculated into 1 l flask containing Nutrient Broth and anaerobically cultivated at 30°C at static conditions. The cells were harvested from the growth medium after 24 h by centrifugation at 5000 rpm for 10 min. Then the cells were washed five times in 0.1 M $NaNO_3$ (the electrolyte used in experiments). Bacterial suspension was prepared with concentration of bacteria 0.5 g/l.

2.4. Adsorption experiments

Batch equilibrium adsorption experiments were performed in 10 ml test tubes. The model solutions of Cu(II) and Zn(II) were prepared by dissolving of $CuSO_4$.$5H_2O$ and $ZnSO_4$.H_2O in deionized water in various concentrations ranging from 0.5 to 2.5 mg/l. The same concentrations were used in experiments with sorption of Cu(II) and Zn(II) from their mixture. The amount of added adsorbent was 1g. Bacterial suspension added with concentration of bacteria 0.5 g/l. pH value of solution was adjusted before the beginning of experiments. Adsorption suspensions were generated using 0.1 M $NaNO_3$ to maintain a constant pH of about 5 during the course of the experiment. The tubes were capped and shaken for 2 hours at 25°C. Filtered samples were served for analysis. Concentrations of Cu(II) and Zn(II) were measured by atomic absorption spectrofotometer (Varian AA240 Z, AA240 FS, Australia). The percentage adsorption of Cu and Zn was calculated according to:

Percentage adsorption = $\frac{(c - c_0)100}{c_0}$, where c_0 is the initial Cu(II) concentration (mg/l) and c is the final Cu(II) concentration (mg/l).

3. RESULTS AND DISCUSSION

In this study the effect of initial concentration on the removal of copper and zinc on the two different types of iron oxide coated sand and with addition of bacteria was examined and compared. Fig. 1 presents the adsorption of copper on the two samples natural iron oxide coated sand (ICS-N) and goethit coated sand (ICS-G). Figure shows better adsorption for ICS-G. The maximum adsorption efficiency was at concentration of 2 mg Cu/l. Fig. 2 shows the adsorption of zinc on the ICS-N and ICS-G. In this case was the difference between two samples lower but higher adsorption was in case ICS-G. The highest removal of Zn was at concentration of 2.5 mg Zn/l.

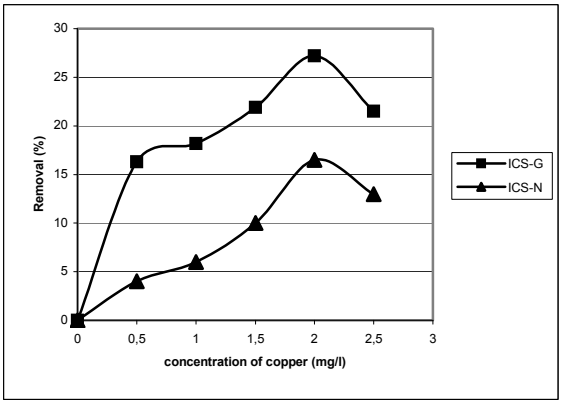

Fig. 1. Effect of influent concentration on the removal efficiency of Cu on ICS-N and ICS-G.

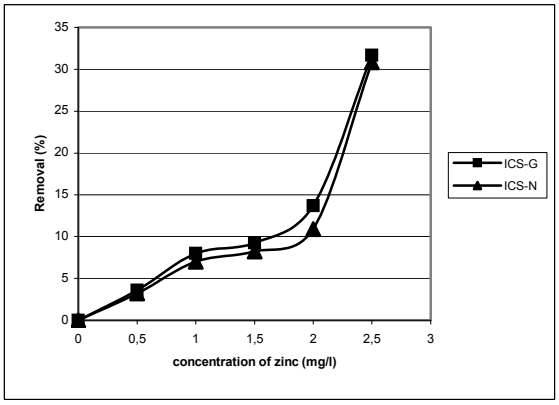

Fig. 2. Effect of influent concentration on the removal efficiency of Zn on ICS-N and ICS-G.

Fig. 3 presents the removal efficiency of Cu(II) and Zn(II) in competitive adsorption experiment onto ICS-G. It can be seen from the figure that percentage removal is twice higher for Cu(II) compare with Zn(II). The maximum removal for Cu(II) was at concentration 1 mg Cu/l ,while in case of Zn(II) it was at 2.5 mg/l which correspond with Fig. 2 for Zn(II). The percentage removal of copper and zinc in mixture system on ICS-N is presented in Fig. 4. Figure shows lower adsorption for both metals. Maximum removal for Cu(II) was at 0.5 mg/l, for Zn(II) at 2 mg/l. The difference in percentage removal of Cu(II) and Zn(II) ions at the same initial metal ions concentration and contact time may be attributed to the difference in their chemical affinity and ion exchange capacity with respect to the chemical functional group on the surface of the adsorbent. It should be done more experiments to study the effect of pH, ionic strength for the adsorption of both metals.

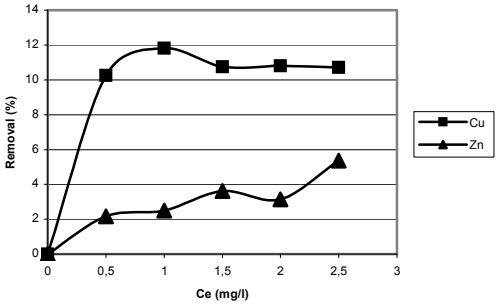

Fig. 3. Effect of influent concentration on the Cu and Zn removal in mixture system onto ICS-G.

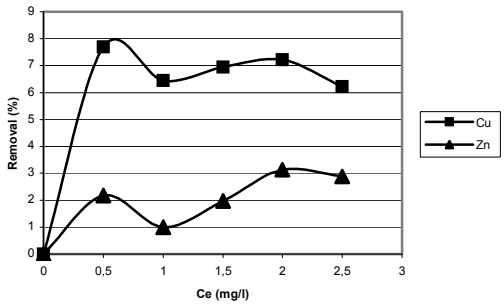

Fig. 4. Effect of influent concentration on the Cu and Zn removal in mixture system onto ICS-N.

For the next experiment with addition of bacteria Cu(II) was chosen due to better adsorption on two different samples (ICS-N, ICS-G). Effect of Cu(II) adsorption on ICS-N and ICS-G in case of addition bacteria is presented in Fig. 5. Figure demonstrate the increase of Cu(II) sorption efficiency due to addition of bacterial suspension (0.5 g/l). In this case adsorption of Cu(II) increased from 28% to 60 % for ICS-G and from 16% to 30% for ICS-N. Attachment of bacteria on mineral surface is strongly influenced by mineralogy, solution pH and ionic strength and similarly, the affinity of metal to adsorb onto bacterial surface is also strongly dependent on surface speciation and solution chemistry.

In this study was found that the removal of Cu(II) and Zn(II) in single and mixture system was greater in case of goethite coated sand compare with natural iron oxide coated sand, as well as with addition of bacteria. We can also demonstrate, that in all experiments was the removal efficiency of Cu(II) higher than Zn(II) removal.

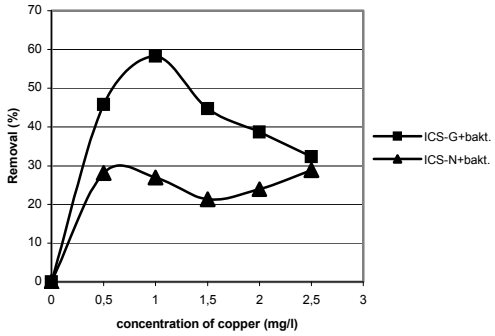

Fig. 5. Effect of influent concentration on the removal Cu onto ICS-N and ICS-G with addition of bacteria.

4. CONCLUSIONS

Results of the adsorption of Cu(II) and Zn(II) by the two different samples of iron oxide coated sand, natural iron oxide coated sand (ICS-N) and goethite coated sand (ICS-G) and with addition of bacteria was examined. The removal efficiency increases with increasing initial concentrations of heavy metal ions in case of Cu(II) and Zn(II) in single adsorption experiments. The maximum removal was 27% for Cu(II) and 32% for Zn(II) on ICS-G at concentration 2 mg/l. The adsorption was one more higher with regard to ICS-N. In competitive adsorption experiments Cu(II) demonstrate higher affinity compare with Zn(II) for both samples. Hence the Cu(II) was used in sorption experiment with addition of bacteria. The positively charged coated sand may change to being negatively charged, causing an electrostatically repulsive interaction between the coated sand and bacteria. The results shown an increase in percentage removal of Cu(II) from 27% without bacteria to 60% with bacteria. There were differences between the iron oxide coatings, the adsorption capacities for copper and zinc were higher for goethit coated sand compared to the natural iron coated sand. We can summarize that goethit coated sand with addition of bacteria demonstrated efficient Cu(II) removal at low concentration as shown our adsorption studies. The results indicated that goethit coated sand with addition of bacteria is an inexpensive, effective and alternative biosorbent for the removal of copper and zinc from aqueous solution. However, the regeneration step needs to be performed to evaluate the economical aspect of sorption process.

ACKNOWLEDGEMENT

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NICKEL, VANADIUM AND MOLYBDENUM EXTRACTION FROM EXHAUST LC-FINER CATALYSTS BY BIOHYDROMETALLURGICAL TECHNOLOGIES

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ABSTRACT
This paper deals with bioleaching of metals from hazardous spent hydro-processing catalyst by means of iron/sulphur oxidizing bacteria. The effects on nickel, vanadium and molybdenum extraction yields of elemental sulphur, ferrous iron and actions contrasting a possible metal toxicity (either the presence of powdered activated charcoal or the simulation of a cross current process by means of filtration stages in series) was investigated. Ferrous iron resulted to be essential for metals extraction and for bacteria adaptation. Nickel and vanadium were successfully bioleached in the presence of iron, reaching extraction yields of 83% and 90%, respectively; on the other hand extractions around 50% for nickel and vanadium were observed both in biological systems in the absence of iron and in the chemical controls with iron. As concerns molybdenum, the highest extraction yields experimentally observed for molybdenum was about 50%, after 26 days bioleaching in the presence of iron, while a maximum extraction of 25 % was observed in the other treatments. In conclusion, a bio-oxidative attack with iron could successfully extract nickel, vanadium and partially molybdenum. Further actions aimed at contrasting a possible metal toxicity resulted not to be effective. The obtained results supported the important role of the indirect mechanism in bioleaching of LC-Finer catalysts and allowed the authors to propose a flowsheet for metal extraction from such waste by means of biohydrometallurgical technologies.

KEYWORDS
LC-finer, industrial waste, bioleaching, nickel, vanadium, molybdenum.

1. INTRODUCTION

Spent hydroprocessing catalysts contain middle-high concentration of base valuable metals, such as nickel (Ni), vanadium (V) and molybdenum (Mo); they represent a large amount of refinery solid waste and have been classified as hazardous waste by the Environmental Protection Agency in the USA. Worldwide, several companies are involved in metal reclamation from spent hydroprocessing catalysts; their technologies are based on two main approaches: either hydrometallurgy or pyrometallurgy. In comparison with this kind of technologies, biotechnological leaching processes offer attractive features: processes are more cost efficient, simpler and more environmentally friendly than their chemical counterparts [1-3].

In this paper, a biotechnological environmentally friendly strategy, involving bioleaching abilities of Fe/S oxidizing bacteria has been applied on Italian refineries spent hydro-processing catalysts. Performed experiments were aimed at finding the best operating conditions for nickel, vanadium and molybdenum extraction from exhaust catalyst by bioleaching.

2. MATERIALS AND METHODS

2.1. The industrial waste

Spent catalysts coming from an Italian refinery LC-Fining units. Ni, V and Mo content was 4.5±0.6, 9.4±0.6 and 4.4±0.2 % (w/w), respectively, as evaluated after acid digestion of the solid samples. X-ray diffraction analysis (Siemens D-500 diffractometer) revealed the presence of the following mineralogical forms, in order of abundance: Al_2O_3 (aluminium oxide), NiV_2S_4 (nickel vanadium sulphide), Mo_4O_{11} (molybdenum oxide), Ni_3S_4 (polydymite). Aliphatic hydrocarbon content in the spent catalyst was about 5 % [4,5].

2.2. Microorganisms

A mixed culture of three strains of Fe/S oxidizing bacteria (*Acidithiobacillus ferrooxidans, A. thiooxidans* and *Leptospirillum ferrooxidans*) isolated from an environmental sample was kindly provided by Prof. Stoyan Groudev (Department of Engineering and Geoecology, University of Mining and Geology "Saint Ivan Rilski",

Sofia, Bulgaria). This culture was cultivated under acidic condition (pH 2), in the ideal liquid growth medium 9K [6].

2.3. Bioleaching experiments

Experiments were carried out in autoclaved 250 mL Pyrex flasks filled to a volume of 100 mL. Incubation was carried out in an orbital thermostated incubator (S150, Stuart), at 30°C and 175 rpm shaking. according to experimental conditions, experimental medium was 9K medium [6], except for experiments performed in the absence of iron source: ferrous sulphate ($FeSO_4$) was not added, while a source of elemental sulphur (5g/L) was provided. Catalyst concentration was 10g/L.

For each bioleaching treatment, a chemical control test was also performed with no bacteria inoculum. Where specified, powdered activated carbon (PAC; Powdered Activated Charcoal Norit, 05100, Fluka) was added in a mass ratio 1:10 carbon:catalyst. Filtration was performed as a possible inhibiting toxicity action by filtering (0,22 μm; Millipore membranes) every 7 days the suspension and re-suspending the cake (bearing both catalysts and bacteria) in fresh medium.

2.4. Analytical determinations

Periodical measurement of pH was carried out by Inolab Multi 720 (WTW). Molybdenum, nickel, vanadium and aluminium were determined by atomic absorption spectrometry (Varian Spectra AA 200)

3. RESULTS AND DISCUSSION

The experimental activity was aimed at finding the best operating conditions for nickel, vanadium and molybdenum extraction from exhaust LC-Finer catalysts by bioleaching. The main factors considered were elemental sulphur (S^0), ferrous iron (Fe^{2+}) and the application of strategies inhibiting an eventual toxic effect of highly concentrated metals. The chosen strategies were two: i) either adding activated carbon as metal adsorbers (like in a carbon-in-leach operation, [7]), ii) or periodically filtering the suspension and resuspending catalysts and bacteria in fresh growth medium (simulating a cross current process, [8]). Table 1 shows in details all experiments performed according to factorial designs.

Tab. 1. Bioleaching treatments and solubilized concentrations (± 5% standard deviation) for nickel, molybdenum and vanadium at the end of the treatments (21 days).

Treatment	S^0	Fe^{2+}	Inhibiting toxicity action	Nickel (mg/L)		Vanadium (mg/L)		Molybdenum (mg/L)	
				Bio[a]	Ctrl[b]	Bio[a]	Ctrl[b]	Bio[a]	Ctrl[b]
1	no	no	no	22.8	25.8	44.1	46.5	10.6	11.7
2	yes	no	no	17.9	19.5	31.4	31.3	15.9	8.5
3	no	yes	no	35.5	17.5	84.2	48.8	17.1	0.0
4	yes	yes	no	36.6	18.2	82.6	51.1	13.9	0.0
5	no	no	activated carbon	17.5	22.5	44.0	63.7	2.6	7.0
6	yes	no	activated carbon	14.1	17.4	32.3	41.8	4.0	5.0
7	no	yes	activated carbon	37.3	18.5	86.8	53.2	13.9	0.6
8	yes	yes	activated carbon	33.0	17.9	56.7	49.1	14.4	0.6
9	no	no	filtration	7.6	9.0	20.6	22.5	3.5	3.1
10	yes	no	filtration	8.6	8.0	22.4	19.8	2.7	2.7
11	no	yes	filtration	13.7	4.7	29.4	12.2	2.7	0.2
12	yes	yes	filtration	16.0	3.9	35.6	9.8	4.0	0.2

[a] biological treatments
[b] chemical controls

Figure 1 shows temporal changes of pH during bioleaching in the presence/absence of ferrous ion and elemental sulphur (treatments from 1 to 4 in Table 1). Chemical controls are also reported. Profiles observed with actions for inhibiting metals toxicity (treatments 5 to 12 in Table 1) were similar and they are not shown here. Data in Fig. 1 show trends that are well known for the growth of Fe/S oxidizing strains [9]: after a first

increase, pH decreases during the time as a proof of bacterial activity. This was particularly evident in those biological treatments with ferrous iron (treatments 3, 4, 7, 8, 11, 12 in Tab. 1): after 8 days, pH was lower than 2, as a consequence of bacterial growth and activity. These results were supposed to be associated to the bacterial ability to tolerate high metal concentrations if Fe2+ is present [10]. In the absence of iron (treatments 1, 2, 5, 6, 9, 10 in Tab. 2), pH decrease seemed not to be significant when compared to profiles in ideal conditions (9K medium, no LC-Finer added).

Table 1 also shows the metal extraction yields at the end of each treatment. It was evident that a biological effect on metals solubilization is significant only in the presence of ferrous ion: in all tests with ferrous iron the biological treatments had extraction yields higher than in their controls: Ni and V were successfully bioleached in the presence of iron, reaching extraction yields of 83% and 90%, respectively; on the other hand, extractions around 50% for Ni and V were observed both in biological systems in the absence of iron and in the chemical controls with iron. As concerns Mo, the highest extraction yields experimentally observed about 50%, after 21 days bioleaching with iron, while a maximum extraction of 25 % was observed in the other treatments.

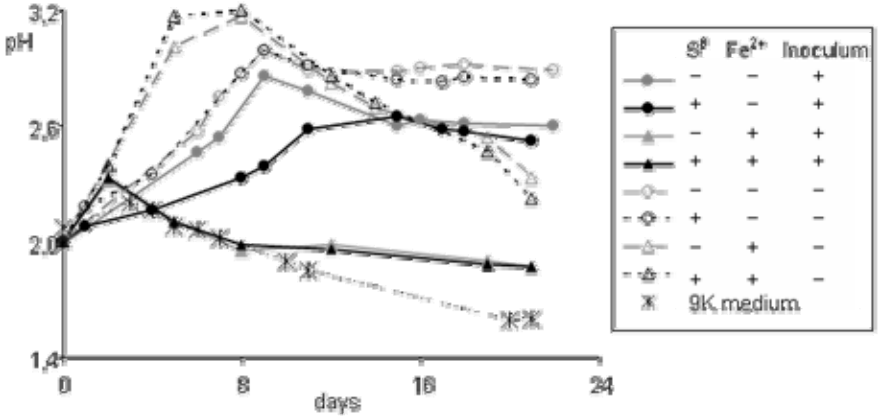

Fig. 1. pH profiles vs. time during bioleaching: effect of Fe^{2+} (160 mM) and S^0 (1g/L). Dotted lines refer to chemical controls (in which no inoculum was added).

The observed results (partially not shown due to space availability) show that Fe/S oxidizing bacteria are very effective in Ni and V extraction from LC-Finer spent refinery catalysts, especially in the presence of ferrous iron. The important role of Fe^{2+} may be associated to its dual biological and chemical function. On the one hand, Fe^{2+} is a key substrate for Fe/S oxidizing bacteria: in fact, several studies have confirmed that Fe^{2+} enhances *A. ferrooxidans* resistance to some metals [11,12]. On the other hand, the ferric iron (Fe^{3+}) produced by bacteria is a strongly oxidizing agent [12], such as the highest extraction yields for Ni and V in biological treatment containing iron (treatment 3, 4, 7, 8, 11, 12 in Tab. 1) may be attributed to a chemical oxidation, mediated by the biologically produced ferric iron. The presence of Fe^{2+} can be responsible of a cycle triggered by Fe/S oxidizing bacteria metabolism: ferrous iron favours bacteria adaptation to the high metal content [12,13], they oxidize Fe^{2+} to Fe^{3+}, which dissolves Ni and V sulphides on the spent catalyst by means of an oxidative attack producing other ferrous iron for the bacterial metabolism. These considerations support the important role of the indirect mechanism in bioleaching of heavy metal contaminated matrices, as already demonstrated by other studies [14].
The negative effect of periodical filtrations on all metal dissolution is a further confirmation of the indirect involvement of microbial consortia on metal leaching, producing either oxidizing Fe3+ for Ni and V extraction or reducing sulphur compounds for Mo extraction. This aspect suggests a possible configuration for metal recovery from LC-Finer exhaust catalysts, which might be more effective with respect to the bioleaching process. A flow diagram of the proposed configuration is shown in Fig. 2. Metal leaching takes place just through the chemical action of ferric iron and intermediate compounds of sulphur bio-oxidation; ferrous iron is subsequently oxidized to ferric iron in a separate biological reactor where Fe/S oxidizing strains have previously been inoculated.

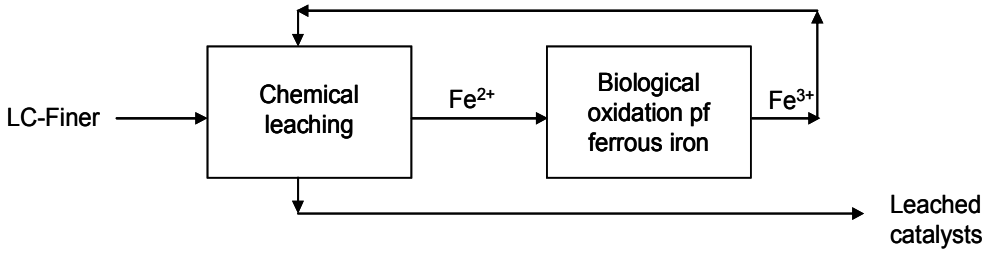

Fig. 2. Flow diagram of a metal recovery process from spent refinery catalyst.

4. CONCLUSIONS

The obtained results supported the important role of the indirect mechanism in bioleaching of LC-Finer catalysts and allowed the authors to propose a flowsheet for effective metal extraction from such waste by means of biohydrometallurgical technologies. Further work will be performed in order to define operating conditions for metal purification and recovery

ACKNOWLEDGEMENT

Authors are grateful to the company ORIM S.p.A. for providing the industrial waste.

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INFLUENCE OF NATURAL ZEOLITE TO THE QUALITY OF CONTAMINATED SOILS AND SEDIMENTS IN INDUSTRIAL LOCALITIES

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ABSTRACT
The results of study of growing certain agricultural plants in contaminated soils with varying dosages of natural zeolite (CT), zeolitic fertilizer and standard NPK fertlizer confirmed the favorable influence of both zeolite and the zeolite based fertilizer. Natural clinoptilolite by ion exchange of heavy metals and sorption of toxic substances into its cavities and channels blocked their reception into the plants.
The article also deals with the possibility of application of natural sorbents, namely zeolite, and with the comparison of sorption capacity and the selection of a suitable sorbent for the sorption of Cu^{+2} ions in contaminated sediments from the locality, the Ruzin No.1 water reservoir. These areas are well-known for its mining and metallurgical activities for several centuries. Heavy metals from the source of pollution is transported by water in river and accumulated in sediments and soils. The metals may come directly from the weathering process of rocks, where the soil-forming process influences their concentration and distribution. Moreover they also come into soil as the result of anthropogenic human activity as well [1-3].

KEYWORDS
Zeolite, fertilizer, heavy metals, copper, sediments, contaminated soils, adsorption

1. INTRODUCTION

Under the present requirements of ecological agriculture there are wide areas of use for a natural, inert and non-toxic material such as the natural zeolite of the clinoptilolite type from the Nizny Hrabovec deposit. Due to its structure and properties this natural, inert and non toxic material can be used as a slowly releasing carrier of fertilizer, as well as other agrochemically, pharmaceutically and biochemically active compounds including disinfectants. Natural zeolite can also be used to improve physical properties of soils and for treatment of contaminated soils. It is also suitable - in very small amounts - as addititive to animal feed [4-7].

Nowadays, adsorption is the most efficient and economical physico-chemical method for the removal of heavy metal from wastewater and contaminated soils. The efficiency of heavy metals removal from wastewaters depends on the quality of used sorbent. The sorption velocity is influenced by pH of environment, the sorption into soil increases at a lower pH value. One of the possibilities of heavy metals immobilisation is the application of sorbents as natural zeolites, bentonites, Slovakit, active coal and the others.

The aim of this work is the research of sorbents influence zeolite from Nizny Hrabovec, zelolite from Majerovce on a decrease of copper content in contaminated sediments from the Ružín No.1 water reservoir, from the River Hnilec branch.

2. MATERIALS AND METHODS

2.1. Samples and methods

The research was carried out with the samples of soils from industrial areas from the East Slovakia. The model plant was spring barley (Hordeum vulgare). The plants were grown on the contaminated soils. The content of heavy metals Zn, Cu, Pb, Co, Cr was defined in the plants and contaminated soils. The heavy metals were carried out by atomic absorption spectroscopy (spectrometer PU 9200X). PCB were carried out by gas chromatography using GC Hewlett-Packard type 428 with column (length 2 m), fill SE30 at the temperature 200°C. The carrier gas was nitrogen (purity more than 99.999%). Delor 103 and Delor 106 were used as standards.

2.2. Sediments

The sorptions of copper ions on 2 types of sorbents were carried out on 2 samples of sediments, which were taken from the Hnilec River branche of the Ruzin No.1 water reservoir in 2007 (Tab. 1). Then the certified

reference material (river sediment LGC 6187 Labe) that contents 83.6 mg/kg of Cu was used. The samples of bottom sediments were sampled into glass bottles by sample device „Multisampler". The samples were dried at room temperature, then quarted and sieved under 1mm. The sorbents zeolite Nizny Hrabovec (fraction 50 μm), zeolite Majerovce (fraction 1 mm), were applied for ion sorption in quantity of 5 wt%, to be precise 1 g of sediment were added to 0.05% of sorbent and 10 ml of destilled water. All components were stirred and time dependence of copper ion sorption in the interval of 21, 90 and 365 days was followed. According to the Supplement no.2 of law no. 220/2004 S.c. [8], the quantity of sorbed Cu^{2+} ions on sorbents were examined in the leachates of 2 M HNO_3 by atomic absorption spectroscopy, flame technique (Varian, Australia).

2.3. The characteristic of the sediment samples

Tab. 1. The values of quality factors of sediments from the chosen localities of the River Hnilec basin sampled in 2007.

Localities	pH	Redox potential (mV)	Organic portion (%)	CEC ($mol.kg^{-1}$)	SEMI analysis (%)
VDR1 estuary Hornád-Hnilec	7.87	262	11.2	0.015	**Al, Si >1** Fe,Ti **1–0.1** Mn, Mg, Ca **0.1–0.01** Cu, Cr, Ni, Zn, Pb **< 0.01**
VDR3 Hnilec branch Vápenka	7.03	256	18.3	0.101	
Zeolite Nizny Hrabovec	-	-	-	0.805	-
Zeolite Majerovce	-	-	-	0.455	-

* (CEC) Cation exchange capacity NH_4^+ according to ČSN 72 1076, (SEMI) The spectral semiquantitative analysis

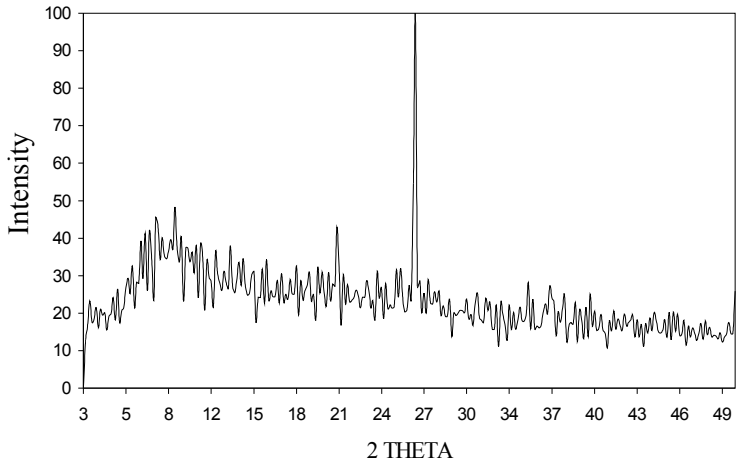

Fig. 1. XRD analysis of sediment VD Ruzin.

Qualitative mineralogical analysis of bottom sediment VD Ruzin was carried out by X-ray diffraction analysis (Fig. 1). **Sediments VDR1-2** contained quartz, sericite, plagioclase as major minerals >15% and chlorite as minor minerals 3-15%. These minerals are the base for the occurrence of heavy metals. Qualitative mineralogical analysis of sorbents confirmed that zeolite contained >15% clinoptilolite and cristobalite as major minerals and 3-15% plagioclase, quartz and dolomite as minor minerals.

3. RESULTS AND DISCUSSION

The use of fertilizers on the basis of natural clinoptilolite as well as other possibilities of application of natural clinoptilolite have been studied in cooperation with practical research workers, mainly with the CHEMKO, a.s. Strazske and ZEOCEM a.s. Bystre industrial enterprises [5,7].

Years of effort and series of vessel and field tests led to the development of two types of zeolite fertilizers with clinoptilolite content up to 40% (ZEOMIX NPK) and 50% (KlinoFert NPK) [5]. These products are multicomponent fertilizers containing the principal nutrients N, P and K together with other biogenic elements, namely sulphur, magnesium, iron, calcium, manganase, boron and molybdenum. ZEOMIX NPK (developed and produced in Chemko a.s., Strazske) contains 8.5% nitrogen, 6.3% phosphorus as P_2O_5, 6.3% potassium as K_2O, 11% sulphur, 0.1% boron, 0.02% molybdenum. KlinoFert NPK (developed and produced in Zeocem a.s. Bystre) contains nitrogen min. 6.0%, phosphorus (P_2O_5) min. 4.5%, potassium (K_2O) min. 7.5% and sulphur (SO_4^{2-}) min.10%.

ZEOMIX NPK and KlinoFert NPK are second generation fertilizers with interesting effects on the quantity and quality of the harvest of crops treated the soil environment and certain aspects of the environment in general. They act as a growth conditioner. Plants in good condition are better prepared to resist fungoid pathogents during the vegetation period.

The decrease of content of heavy metals and other toxic compounds in plants growing on heavily contaminated soils in industrial areas has been studied using natural zeolite as well as zeolitic fertilizers. The results of study of growing certain agricultural plants in contaminated soils with varying dosages of natural zeolite (CT), zeolitic fertilizer and standard NPK fertlizer confirmed the favorable influence of both zeolite and the zeolite based fertilizer. Natural clinoptilolite by ion exchange of heavy metals and sorption of toxic substances into its cavities and channels blocked their reception into the plants.

Table 3 shows the results of vessel experiments in growing the spring barley (Hordeum vulgare) in contaminated soils in different variations with additions of CT, zeolite based fertilizer and standard NPK fertilizer. Analysis of plant material showed that the lowest content of heavy metals (Zn, Cu, Pb, Cd and Cr) as well as of PCB (polychlorinated biphenyls) was found in plants grown in contaminated soils with the application of CT. Plants grown in contaminated soils with the addition of zeolitic fertilizer showed a somewhat higher content. The highest content both of heavy metals and PCB was found in plants grown on untreated contaminated soils.

Analysis of biomass quantity yielded analogous results. The plants that grew best were those growing in contaminated soils with CT added. Good results were obtained also by adding zeolitic fertilizer in low doses (150 kg/ha).

In small plot experiments we investigated the influence of higher doses of CT and zeolitic fertilisers on reduction of intake of heavy metals from contaminated soils. A zeolite of the clinoptilolite type at 600 kg/ha reduced the intake of cadmium from the soil to 0.04 mg/kg in the plants. The effective dose for lead was also 600 kg/ha. Zeolitic fertilizer proved effective in reducing the intake of heavy metals at 700 kg/ha but further icrease of dosage led to a negative influence on the resulting plant biomass.

The results show that adding pure natural zeolite of the clinoptilolite type leads to significant decrease of the content of heavy metals and PCB in plants. Good results may be obtained also using zeolitic fertilizer but in that case it is necessary to find the optimum dosage to balance the blocking influence on heavy metal intake with the growth of the plant biomass.

Tab. 3. Analysis of plant material: spring barley (Hordeum vulgare), grown on: contaminated soil (CS) of an industrial zone with variants of application of natural zeolite (CT), zeolitic fertilizer (ZEOMIX NPK) and standard NPK fertilizer (NPK).

CS with added dose (kg/ha)	Content of the elements (mg/kg)					Content of PCB* (μg/kg)
	Zn	Cu	Pb	Co	Cr	
CS	107	33.1	1.34	0.34	7.5	4765
CS + CT 150	52.4	16.3	1.05	0.24	5.2	2524
CS + NPK 150	65.1	31.0	1.42	0.35	5.4	3477
CS + NPK 250	68.8	30.8	1.84	0.36	6.0	3586
CS + ZEOMIX NPK 150	41.9	24.5	0.95	0.30	4.4	2953

* PCB = expressed as the sum of Delor 103 and Delor 106

As is shown in Tab. 4 the influence of sorbents on the sorption of Cu^{2+} ions in the leachate of sediment from locality VDR1 is obvious the most by the decrease of concentration of Cu after 21-day sorption of zeolite I. (from 136.6 mg/kg to 81.4 mg/kg). In Tab. 1, the efficiency of the sorbents on the elimination of copper ions in the leachates of VDR3 sediments is described. The results confirmed that the decrease of Cu^{2+} ions concentration occurs in zeolite I, II during 365 days, from 88.4 to 109 mg/kg.

Tab. 4. The influence of sorbents on the content of Cu^{2+} ions in the leachates from the sediment VDR1, VDR3 from the confluence of the Hornád - Hnilec River and their decrease after 21, 90 and 365-day sorption.

Time of sorption Cu **VDR1** (days)	I. zeolite ($mg.kg^{-1}$)	II. zeolite ($mg.kg^{-1}$)	Time of sorption Cu **VDR3** (days)	I. zeolite ($mg.kg^{-1}$)	II. zeolite ($mg.kg^{-1}$)
0	136.6	136.6	**0**	128.8	128.8
21	81.4	124.8	**21**	124.4	120.4
90	135.8	98.8	**90**	117.2	126.6
365	114.4	125.4	**365**	109	109.4

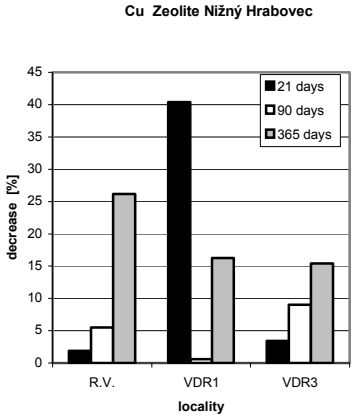

Fig. 2. The kinetic dependence of copper adsorption on zeolite from Nizny Hrabovec locality.

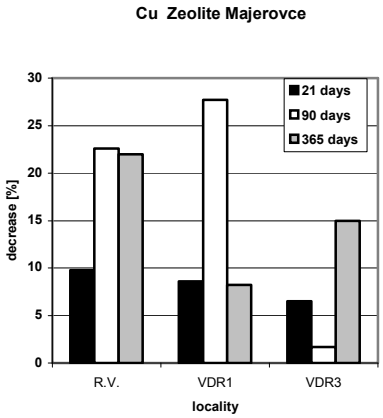

Fig. 3. The kinetic dependence of copper adsorption on zeolite from locality Majerovce.

By the results of the kinetic dependencies (Fig. 2 and 3) of copper adsorption on zeolite I. and II., the highest decrease of Cu^{2+} ions concentration was 40.41% in VDR1 sample sorbed on zeolite I. after 21 days, where the adsorptive capacity of zeolite I. was shown the best. The concentration of 90 and 365-day sorption increased during sorption, thus the decrease was minimal. The highest decrease of Cu^{2+} ions concentration was confirmed in zeolite II. in 90-day interval for sample VDR1 (27.67%). The highest decrease of copper for a reference sample R.V. was after 90-365 -day sorption on zeolite II. (22.56-21.95%).

4. CONCLUSIONS

It was confirmed the application possibility of natural zeolite of the clinoptilolite type as well as zeolitic fertilizer produced on its basis at recultivation of contaminated soils at industrial areas, mainly to decrease the residual content of heavy metals and polychlorinated biphenyls (PCB).

According to obtained results, it can be assumed that bentonite has the most significant sorptive capability from five used sorbents in the order: zeolite I. Nizny Hrabovec > zeolite II. Majerovce. The highest percentual decrease was 40.41% in VDR1 sample sorbed on zeolite I. The kinetic dependence of copper adsorption on each sediments shown that long sorptive experiments have not proved the bond stability of copper ions in sediments. Thus it is necessary to continue in experiments and also test sorbents in shorter time intervals. Until now, it is not possible to recommend some of sorbents for the immobilisation of copper in contaminated bottom sediments VD Ruzin I, the River Hnilec branch.

ACKNOWLEDGEMENT

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BIODEGRADATION OF SOIL FROM LEOŠ JANÁČEK AIRPORT IN MOŠNOV

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ABSTRACT
The paper deals with an examination of possible application of biodegradation in the decontamination of soil samples from Leoš Janáček's Airport in Ostrava. Soils samples were used for laboratory tests of biodegradation taken from oil interceptors. The laboratory biodegradation tests were carried out with a pure bacterial culture of Pseudomonas putida, a pure laboratory culture of Rhodococcus sp, their mixture and a mixture prepared combining their media free of bacteria. The results of the paper imply that for the given purpose, i.e. for biodegradation of airport pollutants, it is most suitable to apply a mixed bacterial culture of Pseudomonas putida and Rhodococcus sp. The results of the paper show that the biodegradation method is applicable for the pollution in question.

KEYWORDS: biodegradation, *Pseudomonas putida, Rhodococcus sp.,* mixed culture

1. INTRODUCTION

Biodegradation (biological decontamination) is grounded in the capacity of certain bacterial strains to make use of hydrocarbons as a source of carbon and energy for their growth and in this way, decomposition of contaminants occur all the way to harmless products - carbon dioxide and water. In short, biodegradation is a special case of degradation during which decomposition of polymers takes place due to the action of biological factors. It makes part of natural processes taking place in water and soil. For example, at contamination of soil by oil substances there is spontaneous degradation of biologically degradable oil substances. However, the process is slow and meanwhile contamination may spread into the surroundings. In the locality some resistant substances remain. In order to speed up the rate of degradation, it was necessary to make the process more intense and to remove resistant substances bacterial mixtures may be utilized.

The ability of microorganisms to degrade hydrocarbons has been known since 1895, when Miyoshi described growth of yeast on paraffin and shortly after the capacity of bacteria to make use of methane as a source of carbon was discovered. Gradually, it was demonstrated that they are able to decompose practically all components of crude oil and many other hydrocarbons. At present, over 200 types of microorganisms have been described that are able to degrade hydrocarbons. Some are able to make use of one hydrocarbon only (e.g. methane), but no microbial strain is known to degrade a whole range of hydrocarbons present in crude oil, for example. Therefore, these are rather microbial associations that participate in degradation.

The objective of the paper was examination of application of bacterial leaching in the decontamination of soil sampled from the Leoš Janáček Airport in Ostrava – these were samples taken from four sampling points.

2. LEOŠ JANÁČEK AIRPORT IN OSTRAVA

The first mention of air traffic at the territory of the present airport is from 1939 when German Luftwaffe built a field aerodrome to attack Poland. After the war the situation calmed down and the area was again used for agricultural purposes as in the past. The modern history began in 1956 when the current airport began to be constructed. The reason for the initiation of construction was the inconvenient state of the airport in Ostrava – Hrabůvka situated in urban development. The construction work finished on 17 October 1959. In that year the first airplane landed on the newly constructed airport and all air traffic from the airport in Ostrava - Hrabůvka was transferred there.

Before 1989, the airport was used mainly for the needs of the air force. Civil aviation was ensured by ČSA, namely for domestic flights, rarely for international ones. A significant turning point was the year of 1993 when the military traffic was terminated at the airport and Česká správa letišť s.p. became the operator.

On 1 July 2004 the Ostrava Airport was transferred from the ownership of Česká správa letišť, s.p. into the ownership of the Moravia-Silesian Region (Krajský úřad, 28. října 117, Ostrava 702 18). The operator is the company Letiště Ostrava, a.s.

On 13 December 2006 the airport was ceremonially christened after the composer of Leoš Janáček and a new departure hall was put into operation. At present, the ever developing airport of Leoš Janáček in Ostrava has been exposed to intense action of anthropogenic impacts, both due to an increasing proportion of traffic as well

as in connection with the own operation of the airport. With regard to its excellent technical parameters, a pronounced development of this traffic junction is expected in the future.

Figure 1 shows the new airport departure hall. Figure 2 shows an aeroplane 737 – 400, which flies from Ostrava to European destinations.

Fig. 1. New departure hall.

Fig . 2. Aeroplane 737 – 400**.**

Fig. 3. Lapol D – leaks of oil products.

It is apparent from the table that accidents with environmental impact prevail, accompanied by leaks of aviation turbine fuel JET A-1.

Tab. 1. Leaks of oil substances at the airport

Date	Leak locality	Substance	Qty of leaked subst. [l]	Material used for disposal	Leak into sewer system
23.6.2005	Central passenger terminal	JET A – 1	200	Vapex, Cansorb	NO
21.6.2006	Central passenger terminal	JET A – 1	Not determ.	Cansorb	NO
28.7.2006	Central passenger terminal	Hydraulic oil	Not determ.	Cansorb	NO
12.9.2006	Central passenger terminal	JET A – 1	Not determ.	Cansorb	NO
18.9.2006	Taxiway	JET A – 1	50	Vapex, Cansorb, water, surface-active agent	NO
27.9.2006	Northern stand	JET A – 1	30	Cansorb	NO
29.3.2007	Central passenger terminal	JET A – 1	30	Cansorb	NO
28.3.2008	Bunkers of airport propellants	Oil products – closely unspecified	Not determ.	Vapex, sorption layer, absorption heaps, sewer seal	YES

3. CHARACTERISTICS OF DRAWN SAMPLES AND THE METHOD OF LABORATORY TESTS

The soil samples from 4 sampling points were taken directly from the airfield of the Leoš Janáček Airport in Ostrava – Mošnov - See Figure 4.

Fig. 4. View of the sampling point

For biodegradation of the samples, pure bacterial cultures of *Pseudomonas putida* and *Rhodococcus sp* were used. The bacterial cultures are shown in Figures 5 and 6.

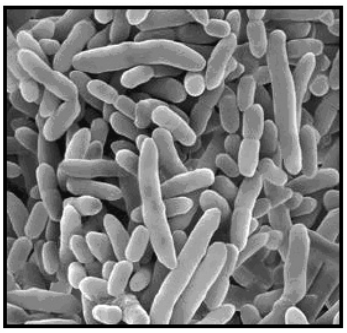

Fig. 5. *Pseudomonas putida.*

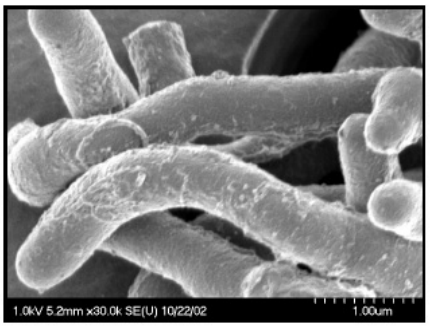

Fig. 6. *Rhodococcus sp.* RHA1.

The culture media were the **liquid medium of M1** for ***Pseudomonas putida*** **and medium of M96** for ***Rhodococcus sp.***

The laboratory experiments were carried out with pure bacterial cultures of Psedomonas putida and Rhodococcus sp., mixed culture and bacterial medium made of 50 % MI medium and 50 % of M96 medium. The experiments were carried out in the laboratories of the Institute of Environmental Engineering at VŠB-TU Ostrava, where 28-day bacterial degradation took place. Each sample was placed into a 2 l glass beaker. Aeration was secured by means of aquarium pumps placed into the beakers. The necks of the beakers were sealed with a foil and then the beakers were moved into the chemical hood. In the course of 4-week degradation the volume in the beakers was regularly filled with distilled water as gradual evaporation occurred. Having finished the experiment, the samples were filtered, dried and sent to further chemical analyses into the Brown Coal Research Institute in Most.

4. SAMPLE CHARACTERISTICS

The mineralogical analyses were implemented in the laboratories of the Institute of Geological Engineering at Mining College - Technical University Ostrava by means of an X-ray diffraction. The results of the mineralogical analyses (Figure 7) imply that the sample contains about 18 % of amorphous phase, majority of quartz - about 57 % and followed by calcite, chlorite, muscovite, orthoclase and albite.

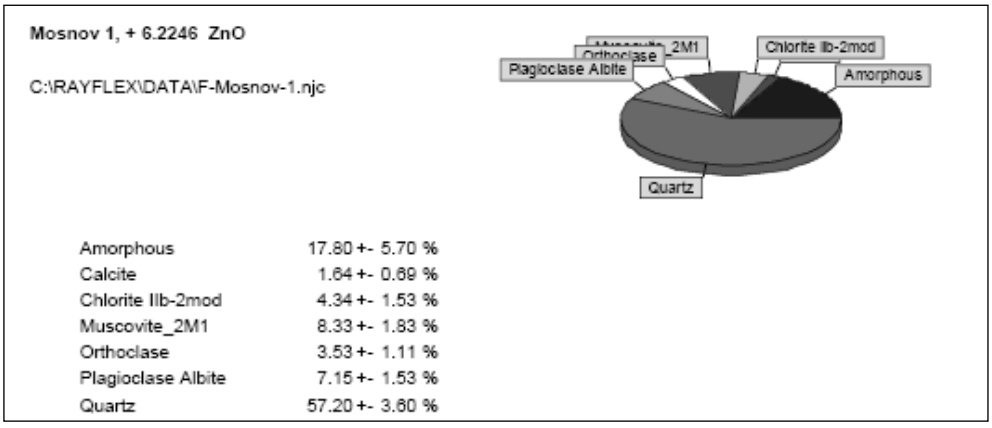

Fig. 7. Mineralogical analysis of the sample

5. BIODEGRADATION TEST RESULTS

The results of laboratory biodegradation tests after one-month biodegradation with applied pure bacterial cultures and mixed culture are stated in **Table 2**. The table implies that in the course of biodegradation tests, gradual degradation of harmful substances content from the sample occurred. For biodegradation the following pure bacterial cultures were used: ***Pseudomonas putida - PP***, ***Rhodococcus sp. - R***, mixed culture ***Rhodococcus sp.*** and ***Pseudomonas putida - R+PP***, **and a *check sample* from media mixtures - *K.***

Tab. 2. Course of degradation the selected contaminants by means of ***Rhodococcus - R***, ***Pseudomonas putida - PP*** and mixed culture ***PP+R***, check test **- K**

Evaluation of the biodegradation test of sample									
Parameter	Input	R	Removal degree	PP	Removal degree	PP+R	Removal degree	Check test - K	Removal degree
-	mg/kg	mg/kg	%	mg/kg	%	mg/kg	%	mg/kg	%
NEL*)	**196**	**127**	**35,2**	**120**	**38,78**	**72**	**63,27**	**89**	**54,59**
anthracene	11,4	1,3	88,6	1,03	90,96	2,22	80,53	4,28	62,46
benzo(a)anthracene	65,8	8,2	87,54	5,83	91,14	12,88	80,43	29,09	55,79
benzo(b)fluoranthene	67,2	11,51	82,87	7,74	88,48	14,61	78,26	40,22	40,15
benzo(k)fluoranthene	61,2	54,16	11,5	5,97	90,25	11,81	80,7	27,36	55,29
benzo(a)pyrene	105	102,29	2,58	3,71	96,47	5,78	94,5	17,66	83,18
benzo(ghi)perylene	56,5	49,76	11,93	3,09	94,53	6,13	89,15	14,31	74,67
Fenantren	208,8	32,03	84,66	22,69	89,13	46,36	77,8	114,38	45,22
fluoranthen	264	24,05	90,89	18,88	92,85	36	86,36	96,96	63,27
chrysene	86,9	0,32	99,63	0,72	99,17	16,41	81,12	42,57	51,01
indeno(1,2,3-cd)pyren	18,7	0,1	99,47	11,98	35,94	4,44	76,26	18,67	0,16
naftalen	12,3	1,35	89,02	0,95	92,28	2,14	82,6	3,71	69,84
pyren	230,9	4,08	98,23	12,45	94,61	25,16	89,1	64,17	72,21
Σ PAH	**1188,7**	**289,15**	**75,68**	**95,04**	**92**	**183,94**	**84,53**	**473,38**	**60,18**
PCB č. 28	0,01	0,01	0	0,01	0	0,01	0	0,01	0
PCB č. 52	0,01	0,01	0	0,01	0	0,01	0	0,01	0
PCB č. 101	0,01	0,01	0	0,01	0	0,01	0	0,01	0
PCB č. 118	0,02	0,01	50	0,01	50	0,01	50	0,02	0
PCB č. 138	0,02	0,02	25	0,02	25	0,01	45	0,02	0
PCB č. 153	0,06	0,02	57,89	0,04	36,84	0,02	59,65	0,04	24,56
PCB č. 180	0,02	0,01	50	0,02	15	0,01	50	0,01	50
Σ PCB	**0,15**	**0,09**	**39,46**	**0,11**	**26,53**	**0,08**	**42,86**	**0,12**	**16,33**

*) NEL – hydrocarbons $C_{10} – C_{40}$

It is apparent from the results of four-week biodegradation test that the most suitable application for the **sample** is that of the pure bacterial cultures of *Pseudomonas putida*, where the degradation of contaminants of **PAH was 92 %.**

In terms of degradation of **PCB** the best was the application of mixed culture, i.e. **42.9 %.** In this case, the efficiency of the mixed bacterial culture was very positive as applying it the following quantities were removed: **63.3 % of NEL**, **84.50 % of PAH** and **42.9 % of PCB.**

CONCLUSION

The objective of the paper was examination of application of biodegradation in the decontamination of soil sample from the Leoš Janáček Airport in Ostrava.

For the laboratory biodegradation tests a soil sample from the airport of the Leoš Janáček in Ostrava – Mošnova was used. The laboratory biodegradation tests were implemented with pure bacterial culture of *Pseudomonas putida*, pure bacterial culture of *Rhodococcus sp*, their mixture and mixture made combining their media free of bacteria.

The efficiency of the biodegradation after one-month leaching with pure bacterial culture of Pseudomonas putida (PP) was 38.8 % for NEL, 92% for PAH, 26.5 % for PCB, by means of pure bacterial culture of Rhodococcus sp.(R) was 35.2 % for NEL, 75.7 % for PAH, 39.5 % for PCB, by means of mixed bacterial culture it was 63.3 % for NEL, 84.5 % for PAH, and 42.9 % for PCB.

The paper results imply that the laboratory sample biodegradation efficiency with the selected contaminants ranged from 35.2 – 63.3 % for NEL, 60.2 - 92 % for PAH and 16.3 – 42.9 % for PCB. The best efficiency was obtained in the laboratory biodegradation of PAH. Intermediate efficiency was reached with biodegradation of NEL and PCB. Best removed NEL and PCB were by means of mixed bacterial culture (PP+R). For soil biodegradation, it is thus the most suitable to apply the mixed bacterial culture of *Pseudomonas putida* and *Rhodococcus sp.*

The results demonstrate that for the given type of contamination the method of biodegradation is suitable.

ACKNOWLEDGEMENTS

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WHEAL JANE TREATMENT TECHNOLOGY: BIOREMEDIATION VERSUS CHEMICAL TREATMENT

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ABSTRACT
This paper reviews the bioremediation and chemical technology considered to treat the acid mine drainage (AMD) produced by the disused Wheal Jane Tin Mine in Cornwall UK. This site is unique in the UK because of the international media attention drawn to the initial outflow of uncontrolled discharge in 1992. Also the information provided by the passive treatment pilot plant and chemical treatment plant built on site have provided a wealth of data on the potential of these techniques for AMD treatment worldwide

KEYWORDS
Acid Mine Drainage, Wheal Jane Incident, Active Treatment, Passive Treatment

1. INTRODUCTION

The immediate consequences of the major discharge in January 1992 are perhaps not wholly unsurprising. Analysis of the drainage identified a low pH and high dissolved metal content. This is clearly illustrated in Table 1 which represents the results taken from site late 1991- early 1992.Comparison of the data highlights the severity of the January 1992 incident. This involved a greater volume of discharge with a flow rate of 50 000 m^3 per day of AMD.

Tab. 1. Chemical Assay of Wheal Jane AMD [1-4].

Minewater Chemical Quality	Jane Adit Drainage (November 1991)	Nanliles Adit Drainage (January 1992)
pH*	2.8	2.6-3.1
Al	-	170-107
As	-	26-29
Cd	0.8-1.6	1.4-1.9
Cu	15-19	14-18
Pb	-	0.2-0.3
Fe	232-975	1720-1900
Mg	-	11-25
Ni	-	4.2-5.1
Zn	346-819	1260-1700

**except for pH, all units expressed as mg/l*

Immediately following the major discharge in January 1992 the receiving waters of the Carnon River experience a noticeable drop in chemical quality. Table 2 shows the pre and post incident water quality in the Carnon River and compares these to the Environmental Quality Standard for the respective metals.

Tab. 2. Carnon River Water Quality and Relevant E.Q.S. [1-4].

	Peak Metal Conc. (January 14th 1992)	Environmental Quality Standards	Pre-incident Quality (January 1990)
pH*	3	6-9	3.5-6.3
As	6 000	50	270
Cd	600	1	30
Cu	7 000	28	900
Fe	600 000	1 000	13 000
Zn	440 000	500	19 500

**except for pH, all units expressed as μg/l*

2. RESULTS AND DISCUSSION

2.1. Passive treatment options

The Wheal Jane Passive Treatment plant (Fig. 1) was the largest controlled and instrumented plant of its type in the world when it was built. It consisted of three treatment schemes, as well as:

1. Five artificial reed beds (aerobic cells) design to cause precipitate of iron as ferric hydroxide and/or oxyhydroxide. With this stage arsenic was also expected to be removed.
2. Single anaerobic cell to promote bacterial sulphate reduction, and increased alkalinity, hence precipitation of copper, cadmium, zinc and iron.
3. Shallow rock filters to promote algal growth and hence high pH which should promote Mn precipitation Some lime dosing of the Wheal Jane discharge of feed streams to the aerobic cells was carried out to monitor the effect of pre treatment on the pH 4.1 raw mine water.

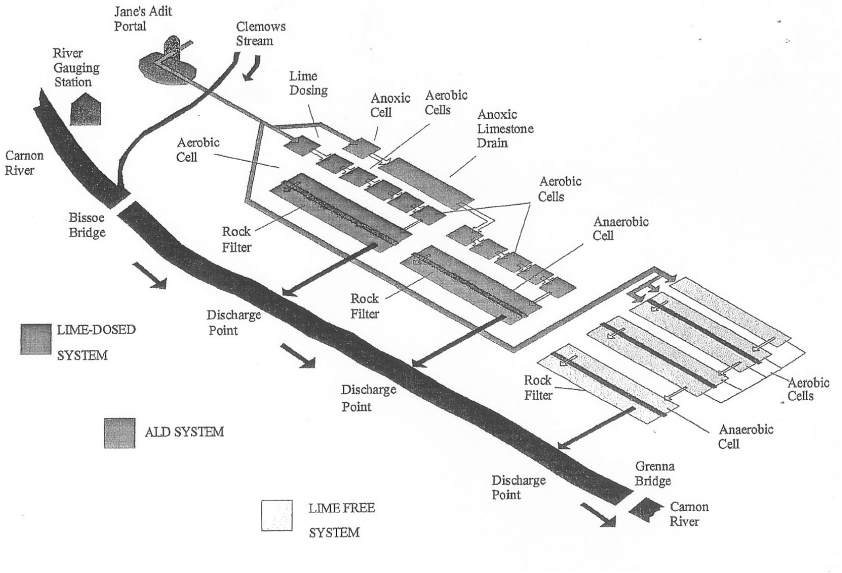

Fig. 1. Schematic Diagram of Wheal Jane Passive Treatment Pilot Plant [4].

Based on an initial 3 year research project carried out between late 1999 to late 2002 the following recommendations were made [1]:

- The process of iron precipitation was demonstrated to have a strong biological component in all 3 systems- this activity was associated with the sediment from the reed beds and was found to be a novel group of moderately acidophilic bacteria whose performance drops off below pH 3.
- Problems occurred with ochre precipitation in the first aerobic cells of all systems- this was associated with the plant root systems and removal would have caused plant disturbance.
- The rock filters (designed to encourage colonisation by algae and hence raise the pH of the water to precipitate Mn) only worked successfully on the non-treated mine water resulting in exit Mn concentrations of below 0.5 mg/l.

2.2. Active Treatment

The Environment Agency in the UK procured a contractor to design and build and operate a treatment plant for Wheal Jane AMD (Fig. 2). Previous research and development had concluded that active treatment using lime dosing and sludge re-circulation was the best option based upon water chemistry and flow rates.

In the first 22 months of operation the plant successfully treated 12 310 000 m^3 of AMD at a rate of 200 l/s, this resulted in the removal of 3 200 tonnes of metal to the tailings dam at a removal efficiency of 99.2% [3].

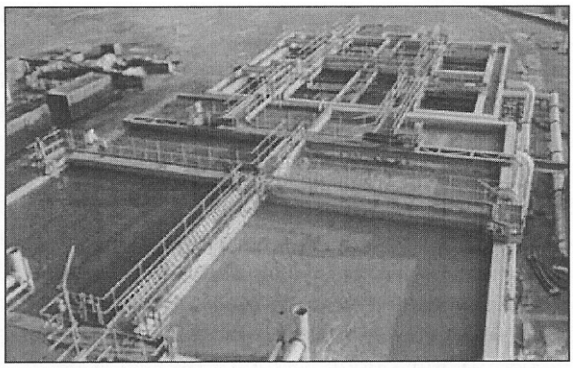

Fig. 2. Photograph of Reaction Chamber and Clarifier/Thickeners [3].

The flow-sheet for the plant can be seen in Fig 3. The operation consists of number 1 reaction chamber which is used to raise the influent mine water pH from 3.5 to between 6-8. These stage will precipitate most of the dissolved metals from the AMD. The water then passed to number 2 reaction chamber where the slurry pH is raised to 9.2 by the addition of lime slurry. This is to facilitate near total removal of Mn. Air blowers are used to ensure complete oxidation of ferrous to ferric iron [3].

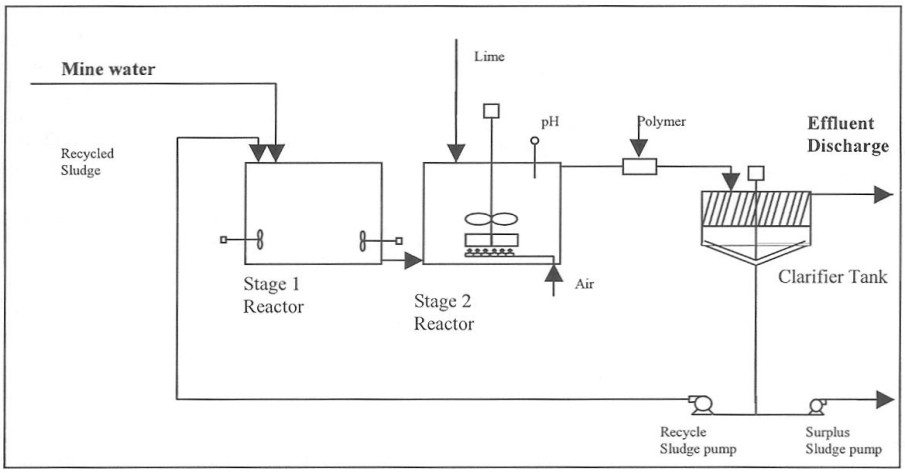

Fig. 3. Flow-sheet of Wheal Jane active treatment plant [3].

Solid/liquid separation is achieved in a lamellar clarifier/thickener operated in parallel. Some thickened solids are recycled to reaction chamber1 to enhance subsequent thickening. Excess sludge from the process is pumped to a holding tank prior to disposal to the tailings dam where the solids settle to about 50% w/w.

3. DISCUSSION

The choice of which option to select when considering AMD abatement will be influenced by a number of factors both technical (discharge quantity and chemistry) and economic/environmental. However, when considering the latter it is important to consider the true environmental impact of the remediation technology selected.

For example, whilst chemical treatment technology was eventually chosen for the Wheal Jane application there is no local supply of lime near the site so the environmental impact of transport costs must be considered [5].

History indicates that large AMD discharge quantities of an acidic nature are treated by chemical neutralisation techniques. It is the traditional view that passive treatment techniques such as those used in the Wheal Jane Pilot Plant would take up too much land area to treat the high quantity of AMD discharge on a daily basis. However, the contact area required for passive reed bed treatment systems can be dramatically reduced by optimisation and a better understanding of the chemical and biochemical reactions taking place.

It is worth noting that passive systems are not totally maintenance free, considerable problems with iron hydroxide coating and sediment build up were reportrd by researchers at Wheal Jane [5].

Recycling and Reuse of any precipitated metals would greatly benefit the environmental model for this type of process. Unfortunately despite the recent high metal prices on the worlds market it has not proved viable to extract and sell the iron, zinc, copper and manganese from the Wheal Jane operation. Economic viability would be greatly enhanced if their were significant quantities of precious metals present in the precipitated metal product as the market price of gold and platinum have been at record levels in the past few years.

4. CONCLUSIONS

The eventual choice of the chemical treatment system for the high quantity acidic discharge from Wheal Jane has proved to be good one. The total cost of the neutralisation and clarifying plant was in the region of £17 million with some saving being made because the proposed second phase was not required due to excellent plant performance. The annual operating cost for the plant is £1 million and the typical operating cost is 18 pence per m^3 of water treated [3].

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UPTAKE AND TRANSLOCATION OF METAL COMPLEXES IN VASCULAR PLANTS

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ABSTRACT
In this work the effect of chelating agents ethylenediaminetetraacetate (EDTA) and nitrilotriacetate (NTA) on uptake and translocation of Cd, Zn and Co in tobacco (*Nicotiana tabacum* L.) hydroponically grown in diluted Hoagland media (HM) spiked with ^{109}Cd, ^{65}Zn and ^{60}Co was analysed. Speciation analysis using a program Visual MINTEQ showed, that the portion of bioavailable ionic Me^{2+} forms for all studied metals significantly decreased with increasing concentration of EDTA or NTA in 25% HM for account of $[Me\text{-}EDTA]^{2-}$ or $[Me\text{-}NTA]^{-}$ complexes. We found that Zn and Co uptake by tobacco roots decreased by increasing concentration EDTA or NTA in cultivation media as a consequence of metal-ligand complexation. However Zn uptake but not Co uptake in the presence of EDTA was diminished. Cd uptake by roots in the presence of EDTA or NTA was not changed. Metal mobility in plant tissues, i.e. intensity of root-to-shoot transport increased in the order: Co < Zn < Cd and increased with increasing concentration of chelates in media, except for Zn in the presence of NTA and Co in the presence of EDTA. Obtained data suggest the possibilities and constraints in the use of chelating agents in phytoextraction technologies in term of affecting metals bioavailability as well as in the term of affecting metals uptake and translocation in plant tissues.

KEYWORDS
Cadmium, zinc, cobalt, *Nicotiana tabacum*, uptake, chelating agents, speciation

1. INTRODUCTION

Global industrialization has resulted in the release of large amounts of potentially toxic compounds into the biosphere, among which are trace elements, like cadmium, mercury, lead, arsenic, zinc, nickel and cobalt, which are commonly addressed as heavy metals. Traditional techniques of contaminated environment remediation are expensive and may cause secondary pollution. There is a growing body of evidence that cost-effective and environmentally-friendly phytoremediation methods (such as phytoextraction, phytostabilization, phytovolatilization and rhizofiltration), using green plants to remove toxic metals or radionuclides from contaminated environment, are a promising approach applicable to metal-contaminated soils. These methods are described in numbers of review papers [1,2] and monographs [3,4].

However, efficiency of phytoextraction method depends mainly on the bioavailability of toxic metals or radionuclides in the soil and the plant capacity to accumulate these contaminants. Considering that metal uptake is related to the availability of metals in soils, some researchers have found that chelating agents (e.g. EDTA, NTA, EDDS and others) can form complexes with toxic metals or radionuclides and improve their solubility or ionic charge, thereby making them more readily available for plant uptake and translocation [5].

In our previous papers we studied the uptake of Zn, Co, Cd or Cs from nutrient solutions by roots of hydroponically cultivated sunflower (*Helianthus annuus* L.), tobacco (*Nicotiana tabacum* L.) and celery (*Apium graveolens* L.) plants [6,7] or *via* leaf surface of ivy (*Hedera helix* L.) and tobacco (*N. tabacum* L.) [8,9]. Also we studied the ability of autochthonous vegetation of plants to accumulate radiocesium ^{137}Cs from contaminated soil [10]. The aim of this work was to characterize the effect of chelating agents ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA) on Cd, Zn and Co uptake and translocation in tissues of hydroponically cultivated vascular plants of tobacco (*N. tabacum* L.). For this purpose radiometric analysis using ^{109}Cd, ^{65}Zn and ^{60}Co was used in experiments.

2. MATERIALS AND METHODS

2.1. Plant material and growth conditions

Seeds of tobacco (*N. tabacum* L.) were germinated and grown in pots filled with granulated perlite as an inert carrier and watered with 25% strength Hoagland nutrient solution [11] at light/dark photoperiod 12/12 h (2 000 lx) and 22°C. The composition of the full strength (100%) nutrient solution was (in mg/dm^3) $MgSO_4.7H_2O$ –

370; KNO_3 – 404; $CaCl_2$ – 444; $NaH_2PO_4.2H_2O$ – 292; $Na_2HPO_4.12H_2O$ – 46.4; $FeSO_4.7H_2O$ – 18; $NaNO_3$ – 340; NH_4Cl – 214; NH_4NO_3 – 160; H_3BO_3 – 8.4; $Na_2MoO_4.2H_2O$ – 0.06; $MnSO_4.5H_2O$ – 5.0; $ZnSO_4.7H_2O$ – 0.64; $CuSO_4.5H_2O$ – 0.8 (pH 6). After 8 weeks of pre-cultivation seedlings of tobacco were gently removed from perlite and roots were washed free of any adhering perlite granules by deionized water and used in bioaccumulation experiments.

2.2. Establishment of the bioaccumulation experiments and cultivation conditions

Plants from pre-cultivation phase were transferred into vessels with a cover to protect plant roots against lights and cultivated for 8 days in 25% strength Hoagland nutrient solution containing Cd, Zn or Co (as chloride salts) spiked with ^{109}Cd, ^{65}Zn or ^{60}Co. There were two treatments without or with chelating agents EDTA or NTA. The pH of nutrient solutions was adjusted to 6.0 using 1 M NaOH. Bioaccumulation experiments were carried out in triplicate series at photoperiod 12/12 h (2000 lx) and 22°C. In time intervals samples of nutrient solution were taken and ^{109}Cd, ^{65}Zn or ^{60}Co radioactivity was measured by gamma-spectrometry. At the end of the experiments plants were harvested, roots were carefully rinsed in deionized water and incorporated radioactivity in roots, stems and leaves was measured. Plant parts were then oven dried (at 60°C for 24 hours) and dry weights were determined.

Growth value (GV) was calculated during the experiments as a ratio between m(i) – m(t) and m(i), where m(i) or m(t) are fresh weight of plants at the start or end of experiments, respectively.

2.3. Speciation modelling

For the prediction of Cd, Zn or Co speciation in nutrient solutions as a function of total salt concentration, the presence or absence of chelating agents (EDTA, NTA or citric acid), solution pH and temperature Visual MINTEQ (version 2.53) program was used. This speciation modelling program allows the calculation of complexes [Me-EDTA] or [Me-NTA] portion in cultivation media for specified conditions.

2.4. Radiometric analysis

Gamma spectrometric scintillation detectors 54BP54/2-X and 76BP76/3 with well type crystal NaI(Tl) (Scionix, Netherlands) and data processing software Scintivision32 (Ortec, USA) were used for ^{109}Cd, ^{65}Zn and ^{60}Co determination in plant parts and cultivation media. A library of radionuclides was built by selecting characteristic γ-ray peaks (88.04 keV for ^{109}Cd, 1115.52 keV for ^{65}Zn, 1173.24 keV and 1332.50 keV for ^{60}Co) for energy and efficiency calibration. Standardized $^{109}CdCl_2$, $^{65}ZnCl_2$ and $^{60}CoCl_2$ solutions were obtained from Czech Metrological Institute (Czech Republic).

3. RESULTS AND DISCUSSION

It is generally known that metals uptake by root system and their subsequent translocation to aboveground parts of plants depend mainly on metal concentrations in bioavailable Me^+ or Me^{2+} forms in soil and soil solution. In this context, it is very important to know all metal speciation forms in the vicinity of plant roots.

For qualitatively and quantitatively prediction of Cd, Zn or Co speciation forms in nutrient media the program Visual MINTEQ version 2.53 was used in our work. For the purpose of this work, the addition of EDTA or NTA was optimized so that the complex of $[Me\text{-}EDTA]^{2-}$ or $[Me\text{-}NTA]^-$ represented about 0%, 25%, 50%, 75% and close to 100% of the total amount of Me (Cd, Zn or Co) in cultivation media. The addition of 20 μmol/dm^3 EDTA or 50 μmol/dm^3 NTA in 25% Hoagland nutrient solution (pH 6.0 and 22°C) containing 10 μmol/dm^3 of $MeCl_2$ caused, that more than 95% of the total amount of Cd, Zn or Co in solution were in the form of complexes $[Me\text{-}EDTA]^{2-}$ or $[Me\text{-}NTA]^-$. Also we found, that the portion of bioavailable ionic forms of bivalent cations significantly decreased with increasing concentration of EDTA or NTA in cultivation medium for account of $[Me\text{-}EDTA]^{2-}$ or $[Me\text{-}NTA]^-$ complexes (data not shown).

Figure 1 shows, that the addition of EDTA or NTA in cultivation media positively diminished phytotoxicity effect of Cd and Co on growth of tobacco plants evaluated on the basis of visually observed chlorosis or die-back of leaves (data not shown) and growth values (GV). Similar effect was also observed in the case of Zn. We suggest that the decrease of metal concentration in Me^{2+} form caused by chelating agents in nutrient media and the increase of [Me-ligand] form could diminish phytotoxic effect. Also others authors found, that free ionic forms of toxic metals Me^{2+} are more phytotoxic than form of complexes [Me-ligand] [12]. This fact could be helpful in phytoremediation of contaminated soils with high concentration of toxic metals, where most plants can not grow under normal conditions.

Bioaccumulation of Cd by tobacco roots during 8 days cultivation was minimally affected in the presence of EDTA or NTA (Fig. 2). On the contrary, EDTA or NTA added into Hoagland media caused considerable

decrease of Zn and Co uptake by roots of tobacco and this effect increased with increasing concentration of chelating agents in media. This fact can represent considerable disadvantage at phytoremediation of soils contaminated both by toxic metals and chelating agents. However, several papers showed that chelating agents solubilized metals from soil matrix and therefore can contribute to the increase of phytoremediation efficiency [5,13].

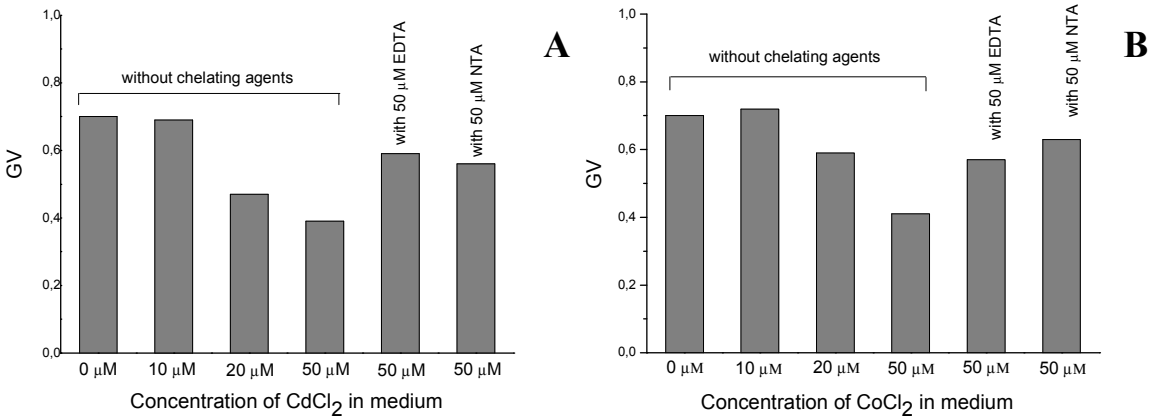

Fig. 1. The evaluation of phytotoxicity effect of Cd (**A**) and Co (**B**) on the basis of growth value (GV) of tobacco plants (*N. tabacum* L.) cultivated during 8 days in 25% Hoagland medium without or with addition of EDTA or NTA at illumination 12/12 day/night period (2 000 lx), pH 6.0 and 22°C.

For evaluation of metals mobility in conductive tissues of plants we established non-dimensional transport ratio (*TR*), which represent the ratio of metal concentration in aboveground part of plants $[Me]_{shoot}$ to metal concentration in root system of plants $[Me]_{root}$. The highest mobility in conductive tissues of tobacco plants was observed in the case of Cd and this mobility decreased in the order: Cd > Zn > Co. Transport ratios *TR* showed, that Zn mobility increased with increasing concentration of EDTA in cultivation media. At concentration ratio [EDTA]:[$ZnCl_2$] = 0.9:1, when in cultivation media about 76% of total Zn was occurred in $[Zn\text{-}EDTA]^{2-}$ form, Zn mobility was 10-times higher in comparison with control experiment (without addition of chelating agents), In the case of Cd, this effect was observed in less extend and in the case of Co this effect was not observed (Fig. 3A). Figure 3B shows, that 3-times increase of Cd mobility was observed at ratio [NTA]:[$CdCl_2$] = 1:1 in media, when about 50% of the total Cd amount was occurred in $[Cd\text{-}NTA]^-$ form in cultivation media. Mobility of Co in tobacco tissues in the presence of NTA increased 2-times in comparison with control experiment. In the case of Zn, dependence of transport ratio (*TR*) on portion of $[Zn\text{-}NTA]^-$ in cultivation medium was fluctuated and not significant. Sun *et al.* [14] observed that addition of EDTA into contaminated soil caused increase of Cd and Zn accumulation in aboveground parts of *Sedum alfredii* plants.

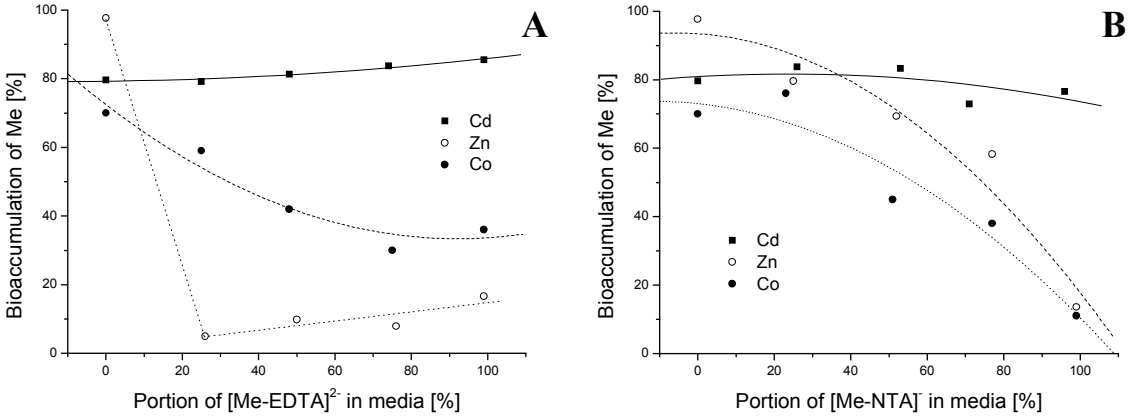

Fig. 2. Bioaccumulation of Cd, Zn and Co by roots of tobacco (*N. tabacum* L.) in dependence on portion of complexes $[Me\text{-}EDTA]^{2-}$ (**A**) or $[Me\text{-}NTA]^-$ (**B**) in 25% Hoagland medium containing 10 μmol/dm^3 $MeCl_2$ (Me = Cd, Zn or Co) without or with addition of EDTA or NTA at pH 6.0 and 22°C. Plants were cultivated during 8 days at illumination 12/12 day/night period (2 000 lx). Speciation of metals in cultivation media was analyzed by program Visual MINTEQ ver. 2.53.

Bell *et al.* [15] explained the influence of chelating agents on metal uptake using of ^{14}C-labeled ligands and translocation in plant tissues by two scenarios. Inhibition could result from the repulsion between chelate

and the negatively charged cell wall preventing movement of the chelate toward the plasmamembrane. Conversely, increased uptake could occur if a phenomenon similar to that used in HPLC (high pressure liquid chromatography) occurs where ions with the greatest and similar charge to that of the adsorbing surface passes through the columns fastest a similar phenomenon occurs in some soils with faster movement of anions than cations through the soil profile. However more studies will be necessary for complete understanding of the role of the individual transport path in upstream and downstream transport of metal species in vascular plants.

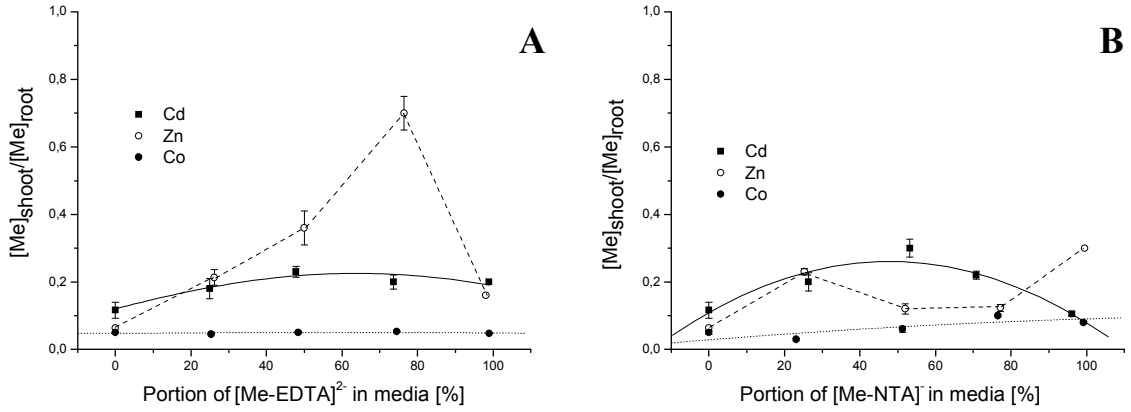

Fig. 3. Translocation of Cd, Zn and Co from roots to shoots (*TR*) of tobacco (*N. tabacum* L.) in dependence on portion of complexes $[Me\text{-}EDTA]^{2-}$ (**A**) or $[Me\text{-}NTA]^{-}$ (**B**) in 25% Hoagland medium containing 10 μmol/dm^3 $MeCl_2$ (Me = Cd, Zn or Co) without or with addition of EDTA or NTA at pH 6.0 and 22°C. Plants were cultivated during 8 days at illumination 12/12 day/night period (2 000 lx). Speciation of Co in cultivation media was analyzed by program Visual MINTEQ ver. 2.53.

4. CONCLUSIONS

Chelating agents are able to decrease of Cd, Zn and Co phytotoxicity, and on the other side these agents can increase of metal translocation from roots to shoots of tobacco plants. This fact can positively affect all processes involved in phytoremediation of contaminated environment with toxic metals or radionuclides.

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EFFECT OF COPPER ON ZINC AND NICKEL BIOSORPTION

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ABSTRACT
Heavy metals are major pollutants in marine, lake and groundwaters as well as in industrial. Traditional metal removal methods like chemical precipitation, electrochemical treatment, membrane processes and ion exchange can be extremely expensive or inefficient, especially for large solution volumes at relatively very low concentrations. In this work biosorption, an inexpensive and reliable method to remove zinc and cooper ions from solution using dry alga biomass as biosorbents, was investigated. Alga *Chlorella kessleri* as biosorbent in the powder form was used. The effect of Cu ions on biosorption of Zn and Ni, was investigated. Results has showed that presence of other metal in solution affects process of biosorption. The isotherms studies revealed that the maximum zinc ions adsorption 46.6 mg/g *Chlorella kessleri* was obtained from 1-ion solution and 15.6 mg/g from 2-ions solution.

KEYWORDS
Biosorption, *Chlorella kessleri*, heavy metals, wastewater

1. INTRODUCTION

The intensification of industrial activities during the last decades contributed greatly to the level of current environmental pollution [1]. The presence of heavy metals in the aquatic ecosystem poses human health risks and causes harmful effects to living organisms in water and also to the consumers of them [2]. The contamination of wastewater by toxic metal ions is a worldwide environmental problem. The source of these environmental pollution with heavy metals is mainly industry, i.e. metallurgical, electroplating, metal finishing industries, tanneries, chemical manufacturing, mine drainage and battery manufacturing [3]. Traditional technologies for the removal of heavy metals, such as precipitation, adsorption, ion exchange, filtration, solvent extraction and separation by membranes, are often ineffective or very expensive in the reduction of heavy metal ions at very low concentrations [4,5]. Therefore, there is the need to search for other methods that would be efficient at low concentration of pollutants. Such a possibility offers a biosorption [3].

Biosorption is an innovative technology using inactive and dead biomass to remove heavy metals from aqueous solutions. This biological phenomenon can be explained by considering different kinds of chemical and physical interactions among the functional groups present on the cell wall and the heavy metals in solution. The active sites present on cell wall can be very different according to the nature of the biosorbent: carboxylic, phosphate, sulfate, amino, amide and hydroxyl groups are the most commonly found [6]. The mechanism of binding metal ions by inactivated algal biomass may depend on the species and ionic charges of metal ion, the algal organism and the chemical composition of the metal ion solution [7-9]. The most frequently studied biosorbents are bacteria, fungi and algae.

Wastewaters usually contain more than single metal. The presence of more than one metal in wastewater is expected to cause interactive effects depending on many reasons, such as the number of metals competing for binding sites; the metal concentrations; and the nature and dose of the biosorbent [10]. Thus is important study the effect other ions on biosorption.

In the present paper it is proposed to apply algae *Chlorella kessleri* as powerful biosorbent of metal ions. The aim of this work was to study effect of Cu ion on biosorption Zn and Ni ions. The effect of Cu was evaluated by comparison of biosorption capacities of alga for Zn and Ni in single-metal solution and in the presence of Cu.

2. MATERIALS AND METHODS

2.1. Biosorbent and solution preparation

Chlorella kessleri, an unicellular green alga, obtained from Institute of Botany of Slovak Academy of Science was used in this study. It was grown at 25°C in aerated and lighted by 4x40 W fluorescent tubes liquid medium Milieu Bristol [10]. The pH of the media of *C. kessleri* was adjusted to 7.0 with 10% NaOH. In the Log

phase of growth, *C. kessleri* cells were cooled, centrifuged at 3000 rpm for 5 min, washed twice with distilled water and then dried at 100°C. Powder cell were used as biosorbent.

2.2. Equilibrium sorption experiments

Exactly 0,1 g of dry and powdered algae was added into 50 ml of metal solution in 100 ml flasks. The solution was gently mixed for 24 hrs. Uptakes of metals were determined from the difference of metal concentrations in the initial and final solutions. The kinetic experiments were carried out at initial pH of 5.0. The pH of the solutions before the sorption experiments was adjusted with 10% NaOH or 10% H_2SO_4. The samples were withdrawn in the following intervals 10, 30, 60, 90, 120 minutes, 24 hours. All experiments were performed in triplicate and the average value was taken for calculations. The metal concentration of liquid samples was determined by the AAS (Varian AA20+).

2.3. Adsorption isotherms

The effect of the initial Zn^{2+} ions concentration on the biosorption was studied at pH 5.0. The initial concentration of metal ions species in the biosorption medium has varied between 5 and 500 mg/l. The pH of the solutions before the sorption experiments was adjusted with 10% NaOH or 10% H_2SO_4. Exactly 0.04 g of dry, powdered algae was added into 20 ml of metal solution in flasks. The samples were withdrawn after 24 hours. The metal concentration of liquid samples was determined by the AAS (Varian AA20+). Each experiment was repeated twice and the results given are the average values.

The metal uptake q was calculated from the mass balance equation as follows:

$$q = \frac{V(Co - Ce)}{m} \tag{1}$$

where q is the quantity of metal uptake by biomass [mg/g]; C_0 [mg/l] and Ce [mg/l] are the initial and final (after sorption at equilibrium) metal concentration, respectively; V is the volume of solution in l and m [g] is the dry weight of the biomass added.

3. RESULTS AND DISCUSSION

The Fig. 1 represents biosorption of Zn^{2+} from an aqueous solution containing a single ion solution and from Zn-Cu solution. Effect of copper on zinc biosorption was negative. Values of specific adsorption was 20.7 and 15.5 mg/g Zn from 1-ion solution and 2-ions solution, respectively.

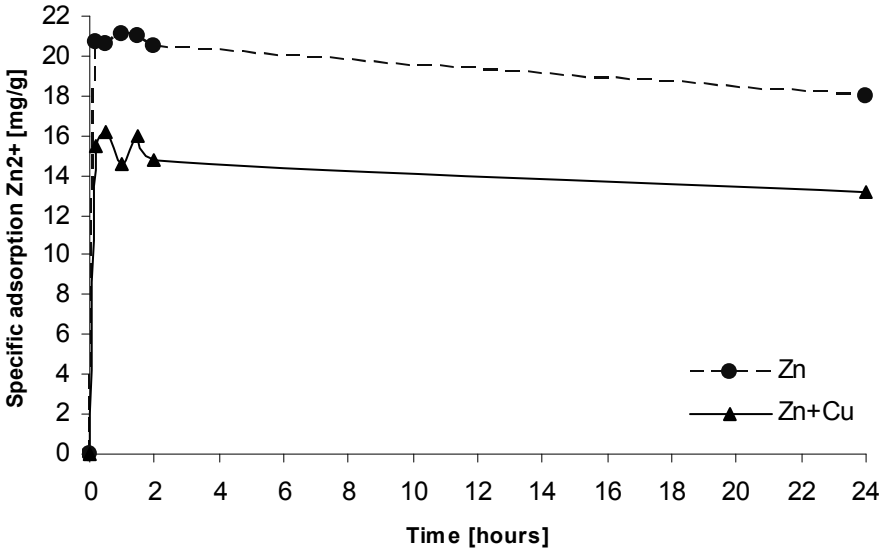

Fig. 1. Effect of Cu^{2+} on biosorption Zn^{2+} by *Ch. kessleri.*

In the Fig. 2 is show biosorption Ni^{2+} from 1-ion and 2 ions solutions nickel and copper. During the Ni biosorption from 2-ions solution, it was found that Cu had significant negative effect on Ni biosorption capacity. In the presence of Cu it was removed only 15% of Ni from solution but from 1-ion solution Ni was removed 35% after 10 minutes. Values of specific adsorption of nickel were 9.8 and 5.7 mg/g Ni from 1-ion and binary metal solutions, respectively.

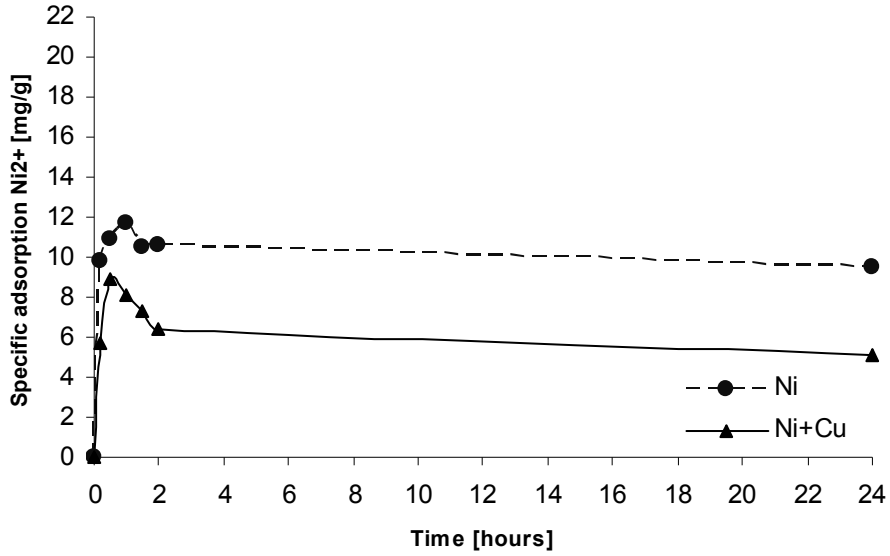

Fig. 2. Effect of Cu^{2+} on biosorption Ni^{2+} by *Ch. kessleri.*

Effect of the presence of Cu^{2+} ions on the biosorption Zn^{2+} ions was investigated in terms of equilibrium isotherm. As shown in Fig. 3, the results indicated that the equilibrium uptake of Zn^{2+} ions decreased with presence Cu^{2+} ions. In the single-ion solution, the maximum uptake obtained at initial concentration of Zn^{2+} ions 115 mg/l was found to be 39.6 mg/g, while the uptake obtained in the binary metal solutions at the same initial concentration of Zn^{2+} ions and biosorption conditions, was found to be 16.9 mg/g.

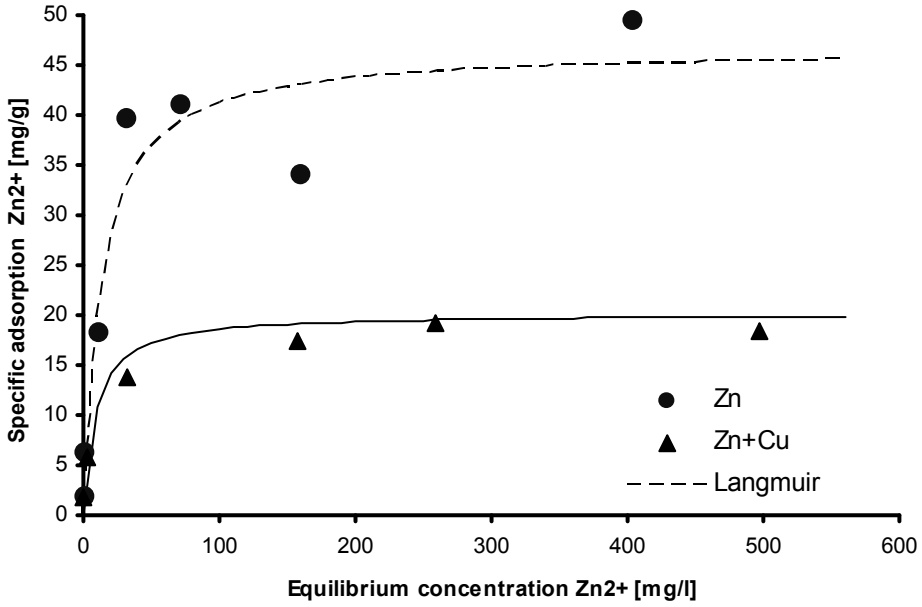

Fig. 3. Comparison of adsorption isotherms of Zn^{2+} ions on *Ch.k.*, between the solutions with Zn^{2+} present as the single metal and with the presence of Cu^{2+} ions.

Langmuir model was used to describe the experimental data of adsorption isotherms in this study. The obtained isotherm models constants and r^2 values are listed in Table 1. Langmuir model fit the data very well. The fact that the fit obtained with Langmuir model showed the well results suggests that the binding of zinc does occur as a monolayer on the surface of the biomass [12]. The maximum biosorption capacity were calculated as 15.6 mg/g for Zn^{2+} (single metals) and 0.15 mg/g for Zn^{2+} and were consistent with the experimental data.

Tab. 1. Isotherm model parameters for the biosorption of Zn^{2+} ions from 1-ion and binary metals solution onto *Ch.k.*

Metal	Langmuir isotherm		
	q_{max}	b	R^2
Zn	46.6	13.3	0.95
Zn-Cu	15.62	0.15	0.99

4. CONCLUSION

As a potentially attractive technology for removal of toxic heavy metals or recovery of precious heavy metals from industrial wastewater, biosorption is a process that utilizes low-cost biosorbents to sequester toxic heavy metals. Abundant natural biomass or agriculture waste products can be economically used as potential biosorbents for heavy metals.

This work indicated that the *Ch. kessleri* could be used as an effective biosorbent material for the treatment of copper and zinc bearing wastewater streams. Copper has negative effect on Zn and Ni biosorption.

ACKNOWLEDGEMENT

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THE INFLUENCE OF ACID-BASE CHARACTER OF SOLUTIONS ON THE STABILITY OF CALCIUM ALGINATE

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ABSTRACT
Immobilizing biomass in a polymeric matrix may improve biosorption capacity and facilitate the separation of biomass from metal-bearing solutions. Many polymers are studied as immobilizating agents for biosorption including biopolymers such as sodium alginate.
Swelling behaviour of gel and dry beads has been studied in aqueous solutions with different acid-base character. Swelling of gel or dry calcium alginate beads was found in all acidic and basic solutions except for the gel beads, which exhibited the tendency to shrink. Dry beads in diluted acidic solutions possessed the greatest stability because they exhibited minimum swelling. Dry and gel beads were completely dissolved in concentrated solutions of sodium and potassium hydroxides.

KEYWORDS
Calcium alginate beads, pH, stability, dissolution, swelling, biosorption

1. INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today. One of the processes used for heavy metal removal and even recovery can be biosorption that utilizes various certain natural materials of biologic origin [1,2].

Biosorption can be defined as the passive sequestering of metal ions by metabolically inactive biomass via various physicochemical mechanisms. Metal uptake may take place by different metal-binding mechanisms such as physical adsorption, chemisorption (complexation, coordination, chelation), ion exchange, microprecipitation and entrapment or their combination [1,2].

Immobilization of biosorbents is a key aspect for the purpose of biosorption application. For industrial application of biosorption, it is important to utilize an appropriate immobilization technique to prepare commercial biosorbents. Disadvantage of the free microbial cells used in laboratory conditions is that they are basically small particles, with low density, poor mechanical strength and little rigidity. In real application they may come up with the solid-liquid separation problems, possible biomass swelling, inability to regenerate/reuse and development of high pressure drop in the column mode. High pressures can cause disintegration of free biomass. The immobilization of the biomass in solid structures would create a biosorbent material with the right size, mechanical strength, rigidity and porosity necessary for use in practical processes. Various methods are available for biomass immobilization. These include entrapment in polymers, adsorption, cross-linking and covalent linkage [3]. The polymeric matrix determines the mechanical strength and chemical resistance of the final biosorbent particle to be utilized for successive sorption-desorption cycles [2].

Important immobilization matrices used in biosorbent immobilization include sodium or calcium alginate. Alginate is a linear polysaccharide isolated from many strains of marine brown algae. Alginates constitute a family of unbranched binary copolymers of 1-4 linked □-D-mannuronic acid (M) and □-L-guluronic acid (G) residues [4-8].

The aim of this work was to study the stability of calcium alginate beads in different acidobasic conditions with respect to application in biosorption and desorption experiments.

2. Materials and methods

2.1. Preparation of calcium alginate beads

Sodium alginate was dissolved in distilled water at a concentration of 3wt.%. After sodium alginate was completely dissolved, the solution was left undisturbly for 30 minutes to eliminate the air bubbles. The solution was then dropped from a height of 20 cm into gelling medium of 0.2 M calcium chloride solution using a syringe with a needle. On the principle of simple ion exchange water soluble sodium alginate was converted to water insoluble and stable calcium alginate salt. The formed beads were cured in the gelling medium for 30 min.

Calcium alginate beads were divided to two fractions:

I gel beads: fresh prepared calcium alginate beads were washed with distilled water and used for experiments. The average size of the bead was found to be 3.03 mm.

II dry beads: prepared calcium alginate beads were rinsed with distilled water, dried and dipped in distilled water for 5 days. The average size of the bead was found to be 1.02 mm.

2.2. Experimental set-up

The concentrated and diluted solutions of hydrochloric, nitric and sulphuric acids and sodium and potassium hydroxides were prepared. pH values were measured for each solution.

Approximately 8-16 beads from each fraction were immersed in the tubs with 10 ml of acidic and basic solutions and stored at room temperature and changes in the form of beads were observed.

During the whole experiment average diameter of beads were measured by using the micrometer screw gauge and these data were used for % volume change calculation according to the formula:

$$V_c = \frac{V_f - V_i}{V_i}.100$$

where V_c - volume change (%), V_f - final volume (mm^3), V_i - initial volume (mm^3).

3. RESULTS AND DISCUSSION

Obtained characteristics of the behaviour in acidic conditions are shown for gel beads (Tab. 1) and dry beads (Tab. 2). The changes of the gel beads and dry beads in the basic solutions are listed in (Tab. 3) and (Tab. 4).

Tab. 1. pH values of the acidic solutions, average diameter of the beads, their volume with % volume change before and at the end of experiment with gel beads for fraction I.

pH of the solution		HCl	HNO_3	H_2SO_4	HCl	HNO_3	H_2SO_4
initial		1.13	1.14	1.29	2.64	2.78	2.79
with fraction I		1.13	1.14	1.3	3.23	3.34	3.32
diameter of beads (mm)	3.03	2.82	2.38	2.25	3.6	3.5	3.9
volume (mm^3)	14.57	11.74	7.06	5.96	24.43	22.45	31.06
volume change (%)		**-19.4**	**-51.5**	**-59.1**	**67.7**	**54.1**	**113.2**

Tab. 2. pH values of the acidic solutions, average diameter of the beads, their volume with % volume change before and at the end of experiment with dry beads for fraction II.

pH of the solution		HCl	HNO_3	H_2SO_4	HCl	HNO_3	H_2SO_4
initial		1.13	1.14	1.29	2.64	2.78	2.79
with fraction II		1.15	1.15	1.31	3.77	3.68	3.99
diameter of beads (mm)	1.02	1.3	1.23	1.3	1.07	1.07	1.03
volume (mm^3)	0.56	1.15	0.97	1.15	0.64	0.64	0.57
volume change (%)		**107**	**75.4**	**107**	**15.4**	**15.4**	**3**

Tab. 3. pH values of the basic solutions, average diameter of the beads, their volume with % volume change before and at the end of experiment with gel beads for fraction I.

pH of the solution		KOH	NaOH	NaOH	KOH
initial		10.73	11.12	12.73	12.98
with fraction I		8.07	8.33	12.04	12.36
diameter of beads (mm)	3.03	3.6	4.1	dissolved by 3 days	
volume (mm^3)	14.57	24.43	36.09		
volume change (%)		**67.7**	**147.8**		

Tab. 4. pH values of the basic solutions, average diameter of the beads, their volume with % volume change before and at the end of experiment with dry beads for fraction II.

pH of the solution		KOH	NaOH	NaOH	KOH
initial		10.73	11.12	12.73	12.98
with fraction II		8.02	8.43	12.24	11.5
diameter of beads (mm)	1.02	1.13	1.25	dissolved by 3 days	
volume (mm^3)	0.56	0.76	1.02		
volume change (%)		**36**	**84.1**		

Schematic comparison of initial and final diameters of the gel beads (Fig. 1) and the dry beads (Fig. 2) is illustrated.

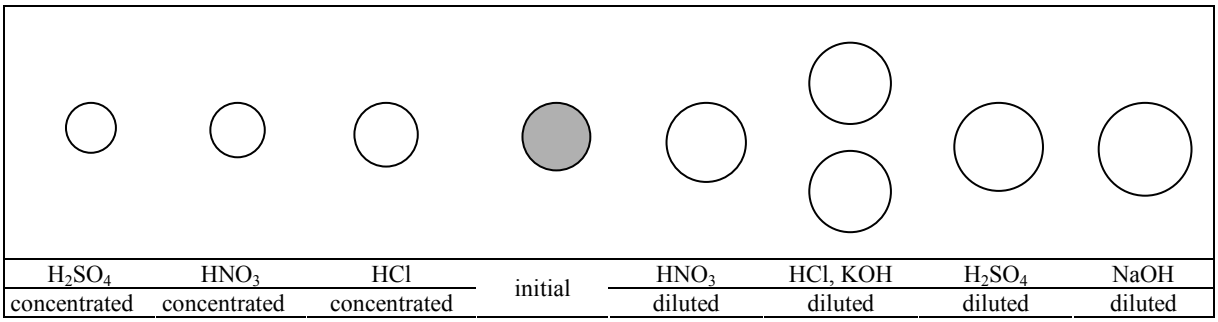

Fig. 1. The gel beads according to increasing diameter of the beads (double extension)

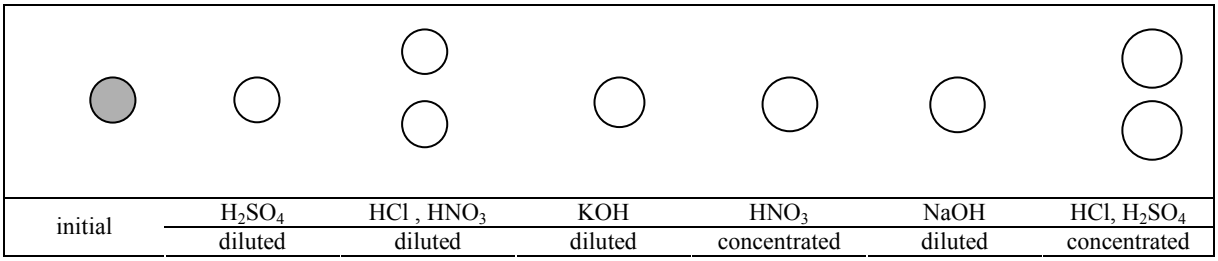

Fig. 2. The dry beads according to increasing diameter of the beads (six times extension).

From the presented results it is obvious that diameter and volume of the gel and dry beads of both fractions in diluted acidic solutions of HCl, HNO_3, H_2SO_4 and in concentrated and diluted basic solutions of NaOH, KOH have increased in comparison with initial state and exhibited the tendency to swell. The gel beads in diluted solutions of NaOH and H_2SO_4 reached the highest swelling. From figures it is visible that the dry beads in the concentrated solutions of HCl, H_2SO_4 and in the diluted solution of NaOH were the most swollen. The dry beads in diluted acidic solution with pH values 2.64-2.79 have showed out minimal volume changes - from 3-15.4% (Tab. 2).

The changes of pH values were observed in the all solutions, excepting the concentrated acidic solutions. pH values in acidic solutions were higher and in alkaline solutions were lower after the swelling of calcium alginate beads. Whether with gel or with dry beads, pH values in concentrated acidic solutions remained without changes.

The gel and dry beads were dissolved by 2 days in concentrated solutions of sodium and potassium hydroxides. Observed degradability of alginate beads is caused by the presence of high concentrations of non-gelling ions, such as Na^+ and K^+ [9]. The swelling mechanism and subsequent degradation of the beads in presence of the solution of NaOH/KOH is related with the Ca^{2+} and Na^+/ K^+ exchange.

Swelling of the dry beads is mainly attributed to the hydration of the hydrophilic groups of alginate. Free water penetrates inside the beads in order to fill the inert pores among the polymer chains, contributing to a greater swelling [10].

The same gel beads tended to shrink when exposed to the concentrated acidic solutions of HCl, HNO_3 and H_2SO_4 (Tab. 1). At low pH values the carboxylic groups of alginate are protonized and hence the electrostatic repulsion among these groups lessens and shrinkage is favoured [10].

4. CONCLUSIONS

This study shows that the stability of calcium alginate beads depends on pH values of the aqueous solutions and the initial physical state of the beads.

Behaviour of gel or dry beads is interesting because of the influence of individual metal ions in solutions. Calcium alginate beads are sensitive to the solutions containing sodium and/or potassium ions. In these solutions they are swelled and subsequently dissolved by ion-exchange process taking place between Na^+/K^+ ions in solution and Ca^{2+} ions in the beads. Their presence is the limiting factor for utilization of calcium alginate in metal biosorption.

Dry calcium alginate beads treated with diluted acidic solutions exhibited minimum swelling and is preferable in bioreactors because of their high mechanical stability.

In contrast to the dry beads with the minimal swelling, the beads with tendency to increase are suitable when swelling and dissolution of the gels is required – an important factor in all applications where recovery of the cells is essential.

ACKNOWLEDGEMENT

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SORPTION OF CADMIUM AND ZINC BY BIOGENIC SULPHIDES

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ABSTRACT
Biogenic iron sulphides are good sorbents of metal ions and they can be used for the elimination of metals from polluted waters. One of the principles of the iron sulphides preparation is the sulphate-reducing bacteria (SRB) utilization.
This study is oriented on biogenic sorbent preparation by SRB under different modes of bacteria cultivation and realization some sorption experiments.

KEYWORDS
sulphate-reducing bacteria, biosorption, iron sulphides

1. INTRODUCTION

One of techniques that can be used for the removal of pollutants from waters, especially those that are not easily biodegradable such as metals is biosorption [1]. Biosorption is the term given to the passive sorption and/or complexation of metal ions by biomass. The mechanisms of biosorption are generally based on physicochemical interactions between metal ions and the functional groups present on the cell surface, such as electrostatic interactions, ion exchange and metal ion chelation or complexation [2]. The metal sequestering capability is caused by number different mechanisms, depending on a number of external environmental factors as well as on the type of a metal, its ionic form in the solution, and on the type of a particular active binding site responsible for sequestering the metal [3].

Some microorganisms are during shorter or longer period able to synthesize sorbents. One of possibilities sorbents synthesis is bioprecipitation. It is biological-chemical method, which exploits metabolic processes of organisms to produce biosorbents [4].

Sulphate-reducing bacteria are a unique group of prokaryotes with the common ability to conserve energy by the dissimilatory reduction of SO_4^{2-} to H_2S in anoxic environments [5]. The hydrogen sulphide produced by the SRB, as part of their metabolic processes is capable to react with metal cations to form stable sulphide precipitates. Microbial iron sulphide is well known as a sorbent for the treatment of metallic ion polluted waters [6].

2. MATERIALS AND METHODS

2.1. Biogenic sorbent preparation

Sulphate-reducing bacteria were obtained and isolated from a mixed culture from mineral water collected at Gajdovka spring (Kosice). For sulphate-reducing bacteria cultivation was modified nutrient medium DSM-63 [7] with the addition of $Fe_2(SO_4)_3.9H_2O$ used. This medium creates optimal conditions for growth of SRB, mainly genera *Desulfovibrio* and *Desulfotomaculum.*

The preparation was realized in the bioreactor filled with 400ml of modified nutrient medium DSM-63 and inoculated with 100ml of a culture of SRB during 21 days at 30° C under anaerobic conditions. These conditions were generated by introducing an inert gas (N_2) and chemically with sodium thioglycollate. The pH of the medium was adjusted to the value 6.8 with sodium hydroxide.

Preparation was realized under 2 different modes. During discontinuous mode the bioreactor worked without addition of fresh nutrient medium. During semicontinuous process of preparation the bioreactor worked 4 days in batch mode and then 3 days in continuous mode (i.e. fresh medium was supplied into reactor for more intensive SRB growth and iron sulphides production). 3 periods were realized.

2.2. Sorption experiments

For sorption experiments were analytical grade $3CdSO_4.8H_2O$ and $ZnSO_4.7H_2O$ used. Solutions were prepared by dissolving the salt in distilled water. Model solutions contain 50 and 100mg/l of Zn^{2+} and Cd^{2+}.

Batch sorption experiments were performed in 100 ml Erlenmeyer flasks. The sorbent dose was 1g/l. For the best contact between sorbent and model solution were samples agitated by mechanical laboratory shaker at 250

oscillations per minute. Sampling was conducted during 90 minutes. The concentration of zinc and cadmium was determined by atomic absorption spectrometry.

3. RESULTS AND DISCUSSION

The process of the iron sulphides preparation by SRB was in both modes successful, demonstrated by black precipitates and the sensorial detection of H_2S odour. They were removed from liquid phase by filtration, next dried and used for sorption experiments.

Sorption of cadmium and zinc ions from model solutions by biogenic sorbents was studied. Figure 1 shows sorption of Zn^{2+} and Cd^{2+} during 90 minutes by semicontinuous and discontinuous sorbents, when initial concentration of metal ions in model solutions was 50mg/l.

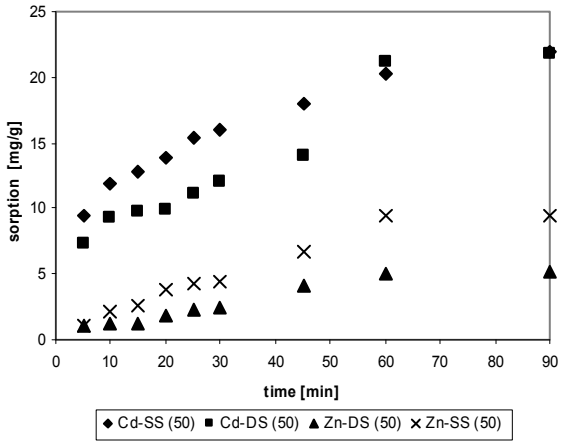

Fig. 1. Sorption of cadmium and zinc ions from model solutions

The quantities of metal ions that iron sulphides captured from 100 ml of solution are in calculation on 1g weights of dry the sorbent. We can see that values for Zn^{2+} are less than for Cd^{2+}. In this case is our sorbent more selective for cadmium. The sorption value for cadmium after 90 minutes for semicontinuous sorbent (Cd-SS) is 21,96mg/g and 21,83mg/g for discontinuous sorbent (Cd-DS). Values of zinc sorption are 9,49mg/g for semicontinuous sorbent (Zn-SS) and 5,22mg/g for discontinuous sorbent (Zn-DS).

Figure 2 compares sorption of Zn^{2+} and Cd^{2+} during 90 minutes by semicontinuous and discontinuous sorbents, when initial concentration of metal ions in model solutions was 100mg/l.

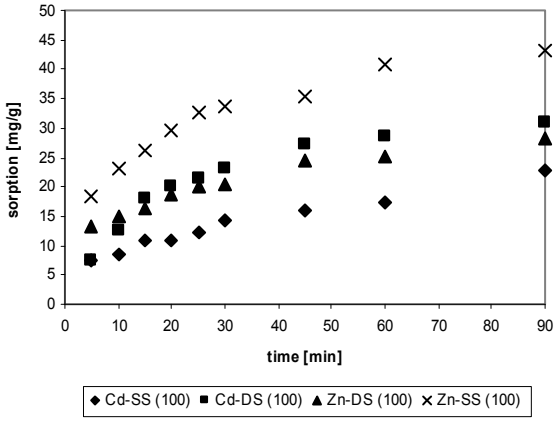

Fig. 2. Sorption of cadmium and zinc ions from model solutions

In this case the highest value after 90 minutes was obtained for zinc sorption by semicontinuous sorbent (Zn-SS) - 43,13mg/g, then for cadmium sorbed by discontinuous sorbent (Cd-DS) - 31,00mg/g, zinc sorbed by discontinuous sorbent (Zn-DS) - 28,09mg/g and the lower value belong to cadmium sorption by semicontinuous sorbent (Cd-SS) - 22,75mg/g.

Figure 3 illustrates removal of metal ions from model solutions for individual sorbents after 90 minutes of sorption experiments. These values are calculated in percentage.

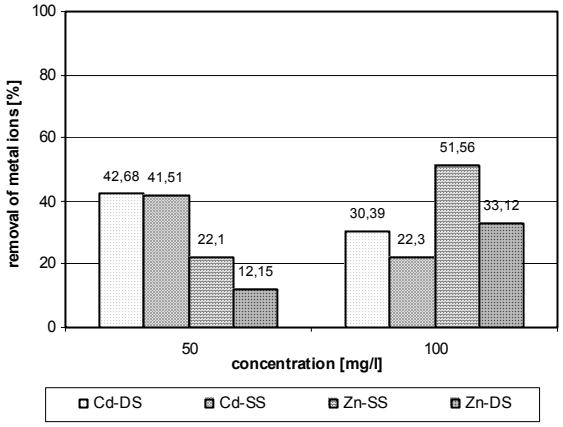

Fig. 3. Removal of cadmium and zinc ions from model solutions

The highest removal - 54,56% was achieved for zinc sorption by semicontinuous sorbent (Zn-SS), with initial concentration of Zn^{2+} 100mg/l, the lowest – 12,15% for zinc sorption by discontinuous sorbent (Zn-DS), with initial concentration of Zn^{2+} 50mg/l.

4. CONCLUSIONS

This work oriented on iron sulphides sorbents production by sulphate-reducing bacteria from Gajdovka spring under 2 modes of cultivation using suitable nutrient medium confirm that these bacteria can be used for their preparation.

Adsorption experiments showed that the iron sulphides remove cadmium and zinc ions from aqueous solutions and we expect that with optimization of sorption conditions (sorption time, pH value, temperature, sorbent dose) they will achieve better results.

We can also conclude that different mode of iron sulphides preparation reflects in sortpive capability of sorbents. In some cases was semicontinuous sorbent more effective than discontinous sorbent and in other cases opposite phenomenon was seen. It depends probably also on experiment conditions (concentration of solution, metal, sorbent dose, time, pH, temperature, etc.).

ACKNOWLEDGEMENT

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REMOVAL OF PRECIOUS METALS FROM SOLUTIONS BY BIOLOGICAL METHODS

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ABSTRACT
Precious metals due to their exceptional and specific characteristics are very often used in industry. Waste produced during their processing or use often contain interesting amount of these metals. One of the possibilities how to recover precious metals is to use biological methods. In the article results of living (bioaccumulation) and non-living (biosorption) biomass for precious metals recovery are presented. Very high capability to sequester precious metals from aqueous solutions was observed in non-living biomass.

KEYWORDS
Precious metals, silver, gold, platinum, *Chlorella kessleri*, biosorption, bioaccumulation

1. INTRODUCTION

Recovery of precious metals from aqueous solutions is interesting due to their high market prices and steadily increasing demand for them. Conventional physical chemical methods applied for their recovery from mine and industrial effluents can be inefficient or expensive especially when these metals are present in very low concentration [1,2]. Biological methods can be used for precious metals recovery as an alternative with several advantages. There are usually cheap, efficient and environmentally friendly. Biomass is generally known to have high binding capacity for precious metals - for gold it was reported from 33 mg/l - 8 g/l, for platinum from 44-346 mg/l [1], for silver 199-300 mg/g [3-5]. Biological methods suitable for precious metals recovery can be divided into three groups:

Bioaccumulation (intracellular accumulation) - is metal sequestration inside the cell which may be accomplished by several ways, e.g. metal binding onto intracellular compounds, metal precipitation in the cell, methylation of the metals, intracellular accumulation of metal in higher organisms etc.. This process depends on metabolism and usually is slow with low capacity [6].

Biosorption is the process of passive uptake of metal ions onto the surface of biological materials - cells, plant or animal debris etc. It is based on physical- chemical interactions of cell wall with metal ions, thus, metal does not enter inside the cell but it is sequestered on the cell surface [7, 8]. Several factors quantitatively and qualitatively influence the biosorption system: these include the biomass used, the targeted metal species, and the solution chemistry as well as the operating conditions [9].

Bioprecipitation - is based on metal precipitation in the form of poorly soluble compounds (e.g. organometallic complexes, sulphides or oxides of metals) [10]. Extracellular precipitation may, but needs not to be metabolism dependent [11], in most cases it is slow, irreversible and temperature dependent [12]. Organisms frequently protect themselves from toxic metals by producing various compounds facilitating the precipitation of metals on the cell surface or in the solution (metabolism dependent mechanism).

In fact it is very difficult to distinguish biological mechanisms responsible for metal removal because there is very little knowledge about them. Probably combination of several mechanisms takes place in the process. For this article the easiest dividing into two groups - biosorption (using non-living cells) and bioaccumulation (using living cells) was used although especially when living cells are used several different mechanisms can take place at the same time.

The aim of the article was to study possibilities of gold, silver and platinum recovery from aqueous solutions by living and non-living cells of green microscopic unicellular alga *Chlorella kessleri* and compare the efficiency of using living and non-living biomass.

2. MATERIALS AND METHODS

2.1. Biomass preparation and chemicals

Platinum and gold were dissolved in aqua regia. Standard solutions with concentration 1 g/l were prepared and diluted into 100 mg/l for experiments. Analytical grade $AgNO_3$ was used for experiments. Metal solution of Ag^+ ions was prepared by dissolving the salt in distilled water.

The biomass of *Chlorella kessleri* was obtained from Institute of Botany of the Slovak Academy of Science. The biomass was cultivated in Milieu Bristol medium (composition of medium is in [7]). Biomass was aerated and lighted by 4x40 W fluorescent tubes. Cultivation took 13 days. If it was use in living form it was after cultivation filtered through membrane filters, gently washed and added into metal solution. If it was used in non-living form it was cooled, washed, separated from solution by centrifugation and dried at 80°C. Dry biomass was powdered and used in batch equilibrium biosorption experiments.

2.2. Metal binding experiments

Experiments were performed in Erlenmeyer flasks. Initial metal concentrations were 10, 35 and 100 mg/l for Au, Ag and Pt, respectively. (For biosorption experiments 50 mg/l of silver ions was used.) pH values of solutions were 2.5, 5 and 1.4 for Au, Ag and Pt, respectively. Non-living biomass was added in concentration 2 g/l. Living biomass was added in the amount equivalent to 2 g/l. In chosen time intervals samples of supernatant liquid were withdrawn. The concentration of unadsorbed Au, Ag and Pt ions in filtrate was determined using an atomic absorption spectrophotometer (Varian AA20+) with an air-acetylene flame. Biosorbent-free blanks were used as controls.

3. RESULTS AND DISCUSSION

After addition of algal biomass fast decrease of silver concentration was reported in both cases - when living and non-living biomass was used (Fig. 1). Although generally it was observed that 10-15 minutes is enough time for biosorption equilibrium in the case of silver more than 2 hours were necessary and even after that slight increase of silver concentration was reported. Increase of the silver concentration was observed also when living biomass was used but in this case very little amount of silver was taken up by biomass (Tab. 1).

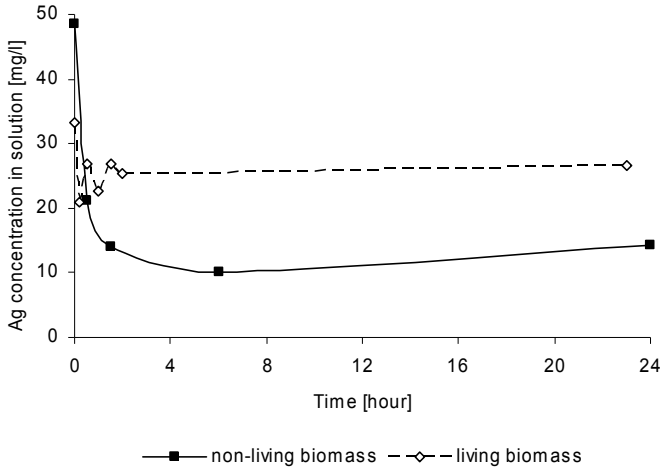

Fig. 1. Decrease of silver concentration in solution after addition of living algal cells.

Gold bioaccumulation and biosorption (Fig. 2) was again very fast but living cells of *Chlorella kessleri* released big part of gold back into solution whereas dead cells kept it without significant changes. Very high efficiency of gold recovery was reached by dead cells because after 10 minutes only traces of gold were measured in solution. High efficiency (almost 100%) using living biomass of seawater algae for gold bioaccumulation reported also Yazawa and Kuwabara [13]. Biomass was successfully used also for recovery of gold when gold was in the form of $[Au(CN)_2]^-$ [14].

Pt was taken up very fast and with very high capacity with dry cells of alga but when living cells were used it needed longer time but after 24 hours Pt concentration in solution did not change (Fig. 3). Probably when living cells were added Pt was accumulated in inner parts of organisms. Fast biosorption of platinum was reported by Mack [15]. They needed 20 minutes to remove 82% of Pt by biomass of *Saccharomyces cerevisae*.

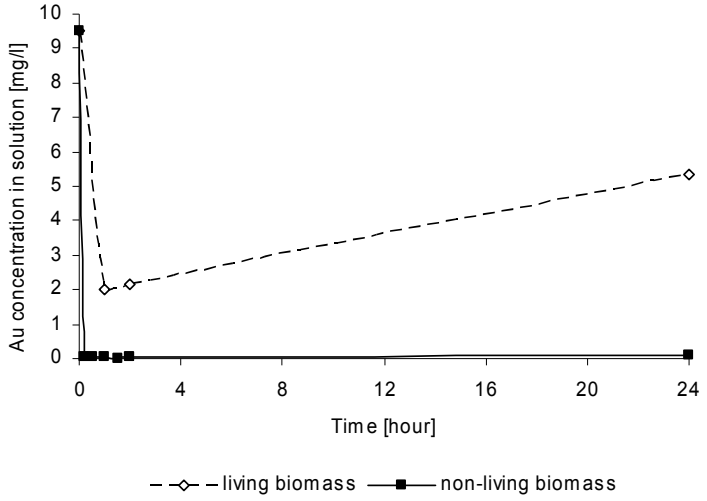

Fig. 2. Decrease of gold concentration in solution after addition of living algal cells.

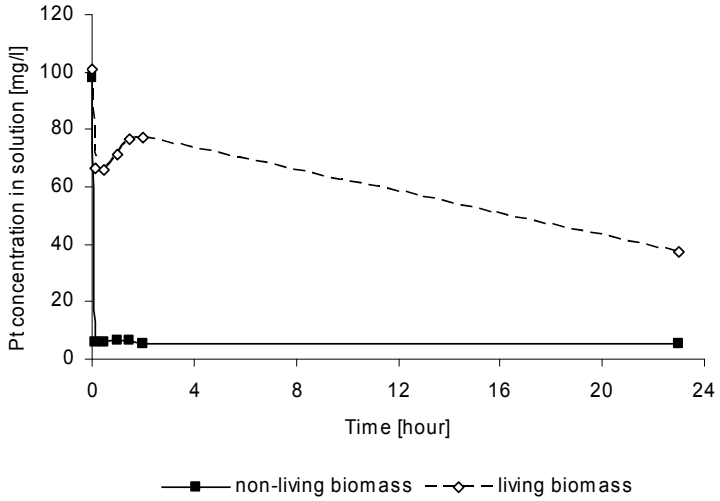

Fig. 3. Decrease of platinum concentration in solution after addition of living algal cells.

From the comparison in Table 1 is visible that living cells have lower capacity to bind precious metals than non-living cells. Also they are more efficient during first hour (in the case of silver and gold). Probably part of the metals is released back into solution till equilibrium is reached. Only in the case of platinum during first hour significantly less Pt was accumulated in comparison with equilibrium. Differences in biosorption (using non-living cells) efficiency between first hour and in equilibrium were not very big.

Tab. 1 Efficiency of precious metals bioaccumulation and biosorption

		Ag	**Au**	**Pt**
Bioaccumulation efficiency (%)	After 1 hour	31.9	78.8	29.5
	Equilibrium	**20**	**44.1**	**62.8**
Biosorption efficiency (%)	After 1 hour	63.8	99.5	93.5
	Equilibrium	**70.5**	**98.8**	**94.3**

4. CONCLUSIONS

Biological methods represent good alternative to traditional physico-chemical methods for metal recovery and wastewater treatment. Nowadays using of biological methods for recovery of several metal ions such as Cu, Zn, Cd, Ni is more studied and applied but the recovery of precious metals via this processes is still in the beginning. From the presented results it is obvious that precious metals can be sequestered from aqueous solutions with very high efficiency when dead biomass is used. Thus, biosorption offers good way for studying precious metals recovery.

ACKNOWLEDGEMENT

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PHYTOREMEDIATION RESEARCH AT THE TU-CRETE USING HALOPHYTES

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ABSTRACT

Phytoremediation research at TU-Crete is focusing on the remediation of soils decontaminated with heavy metals and in particular on halophyte species which are of special interest since these plants are naturally present in environments characterized by an excess of toxic ions, mainly sodium and chloride, and can tolerate additional stresses such as chilling, freezing, heat and drought. It has been speculated that salt-tolerant plants may also be heavy metal tolerant and further, may be able to accumulate metals. Therefore, halophytes are suggested to be better adapted to cope with environmental stresses, including heavy metals and hence, halophytes are expected to receive more attention from phytoremediation researchers in the future. The use of such plants can be also viewed as an alternative method for soil desalination since soil deterioration resulting from salinity is a major impediment to optimal utilization of land resources and salt-affected soils exist in more than 100 countries and on all continents. Examples of halophytic species under investigation are *Atriplex halimus* L. (Mediterranean saltbush), *Nerium oleander* L. (oleander) and *Tamarix smyrnensis* Bunge (salt cedar). Data are presented here for the later. We have recently shown that same holophytes have the ability to translocate heavy metals from the soil and excrete them on the surface of their leaves. We have called this "new" phytoremediation process "Phytoexcretion" to emphasize the fact that it is an alternative phytoremediation process that should be further explored.

KEYWORDS

Tamarix smyrnensis, lead, cadmium, phytoremediation, salt glands, metal excretion

1. INTRODUCTION

Heavy metal contamination is one the most serious environmental problems limiting plant productivity and threatening human health. Amongst the substances that contribute to anthropogenic pollution of the biosphere, trace elements are one of the most toxic. Lead (Pb) and cadmium (Cd) are toxic metals of increasing environmental concern as they enter the food chain in increasingly significant amounts. Among heavy metals, lead is the most abundant, globally distributed, best-recognized hazardous element that has been used since ancient times. Lead is a major contaminant in soil and waste deposits; however, other metals including cadmium are typically found in lower concentrations. Cadmium is one of the most toxic metals in the environment and it is more toxic to humans than lead.

Phytoextraction offers great opportunities to clean-up polluted environment in an economical and environmentally friendly fashion. Studies of phytoextraction have mainly focused on metal hyperaccumulating plants, as they accumulate 100 to 1000–fold the levels normally accumulated in plants, with no adverse effects on their growth. However, hyperaccumulators are often small plants with slow growth, and they have no apparent economic value. In comparison to herbaceous hyperaccumulator species, trees tend to take up relatively small amounts of heavy metals; however, they provide an economic return from the contaminated land through the production of biomass [1]. In addition, a number of tree species can grow on land of marginal quality. This allows the establishment of trees on sites with low fertility and poor soil structure, keeping the costs of soil reclamation low.

2. MATERIALS AND METHODS

2.1. Plant collection and propagation

Cuttings of *Tamarix smyrnensis* were collected from the coastal cliffs of Ag. Apostoloi located 4 km west of the city of Chania (Crete, Greece). Cuttings 10 – 15 cm long were placed into sand in a mist propagator for 21 days. To enhance rooting, auxins were used. Rooted cuttings of *T. smyrnensis* were transferred into plastic pots (one cutting per pot) and were filled with pre-weighted amount of organic substrate mixture (commercially available with the trade name Blumenerde, Capriflor, Germany).

For the hydroponic experiments, the rooted plants were removed from soil, washed carefully to remove the soil from their roots, taking care to prevent damage of the root system. Every plant was transferred into a 3 litre plastic tank filled with the nutrient solutions for hydroponic growth. The plants were divided into nine experimental groups with 3 plants per group-treatment. The nutrient solution was adapted from modified Arnon and Knop culture solution with the following composition in mg/l: 143.0 $Ca(NO_3)_2$, 35.75 KNO_3, 17.75 KCl, 35.75 KH_2PO_4, 35.75 $MgSO_4$, 2.86 H_3BO_3, 1.86 $MnCl_2.4H_2O$, 0.22 $ZnSO_4.7H_2O$, 0.079 $CuSO_4.5H_2O$ and 0.6 $FeSO_4.7H_2O$. Lead and cadmium were added to the medium at concentrations of 100 ppm and 5 ppm as $Pb(NO_3)_2$ or $Cd(NO_3)_2.4H_2O$, respectively, and NaCl at concentrations of 100 and 200 mM according to the experimental design. The nutrient solution was continuously bubbled with air and its amount in each tank was readjusted daily with deionised water.

2.2. Metal excretion determination on the leaf surface

The excreted metals were determined according to the method of Hagemeyer and Waisel [2]. Above ground parts of plants were washed with 100 ml of 0.1% HNO_3 for 2 minutes. Such a thorough rinsing procedure was applied in order to dissolve all compounds on the leaf surface and inside the crypts. Rinsing the branches of tamarix in such a weak acid for 2 min did not cause any apparent damage. The rinse water was collected, filtered and stored until ICP analysis was carried out.

2.3. Chlorophyll content measurement

At the last day of the experiment, 0.2 g fresh leaves were randomly selected from each plant, washed with deionised water and stored at -22 °C until the measurement of chlorophyll content (not longer than a day) which was performed according to the method of Harborne[33].

2.4. Water content and biomass measurement

At the end of the experiment, shoots and roots were separated, washed with tap water and deionised water in order to remove any dust deposits and nutrient solutions, respectively, and their fresh weights (FW) were determined. Dry weights (DW) were determined after oven-drying for 48 hours at 70°C and cooled down to room temperature. Water content (WC, %) was calculated by the formula: $WC = (FW - DW)/FW \times 100$

2.5 Determination of lead and cadmium in the plant tissue and nutrient solution

All dry plant tissue samples were milled, air dried and metal content analysis in the plant tissue was performed by a Leeman Labs PS1000AT Inductively Coupled Plasma Atomic Emission Spectroscope according to the method of Soon[34]. Additionally, 10 ml sample of the nutrient solution from each plant was taken at the beginning of the experimental phase and after 5 and 10 days of the experiment for the determination of Pb and Cd content in the nutrient medium by ICP-AES spectroscopy.

3. RESULTS AND DISCUSSION

3.1. Cadmium accumulation by *Tamarix Smyrnensis* at different salinities

The data from these experiments suggest that increasing salinity increases the cadmium uptake by *T. smyrnensis* shown in Figure 1. Cd accumulation in the roots increases with increasing salinity reaching the amount of 2.45 ppm from the treatment with 3% salinity. Correspondingly, the accumulation in the shoots increases with increasing salinity reaching the amount of 3.3 ppm from the treatment with 3% salinity but the toxic level of Cd in leaves of plants was not exceeded which is 5-30 ppm dry weight [3]. The same conclusion is reached by examining the total removal of cadmium (phytoextraction) by the plant. From the treatment without salt addition, Cd total removal by the whole plant was 9.4 μg; for the treatment with 0.5% salinity the removal was 19.7 μg and for plants treated with 3% salinity the removal was 38.3 μg. Hence, it is obvious that the total Cd accumulated into the whole plant increases by a factor of 4 when salinity increases from 0 to 3%. These results are in general agreement with other researchers which found increased concentrations of metals in most plant parts under saline conditions and it was speculated that this may be related to higher mobility of metals in the sediment and/or higher water uptake (due to increased transpiration), leading to a higher flux of metals into the plant [4,5].

3.2. Release of Cd by the leaves of *Tamarix Smyrnensis*

Salt excretion through salt glands is considered to be an important mechanism which contributes to the increased resistance of halophytes to salinity. Salt-secreting halophytes are adapted to saline environments by three different mechanisms; salt avoidance, where the roots have low permeability to salts; salt tolerance, which is the capability to cope with the presence of high intracellular salt levels; and salt evasion, i.e. excretion of some of the penetrating ions and retention of others. The main function of salt glands is the secretion of excess stress-inducing ions that invade the plant. Our work [6,7] supports that *T. smyrnensis* excretes cadmium which is associated with salt crystals exuded from glandular tissue confirming the fact that the roots of this plant have a

low selectivity to the uptake of ions from the soil. Additionally it could be proposed that the low concentrations of cadmium in the shoots of the plant could be connected with the salt excretion mechanism which allows the plant to resist metal toxicity. Moreover cadmium release rises with increasing salinity and there is higher salt excretion as shown in Figure 2. The concentration of Cd excreted by plants grown in saline environment with 0.5% salinity is 3.4 times higher than plants grown in non saline environment.

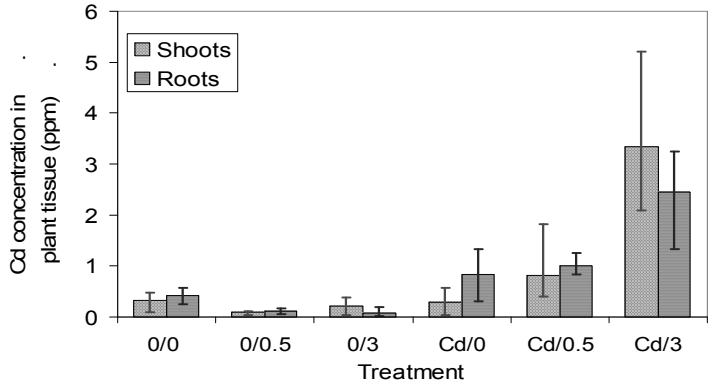

Fig. 1. Cadmium concentration (mg kg^{-1} dry weight) in individual parts of *tamarix smyrnensis* treated with 16 ppm Cd of dry weight of soil at different soil salinities. Values shown are means (n = 4) with min and max values.

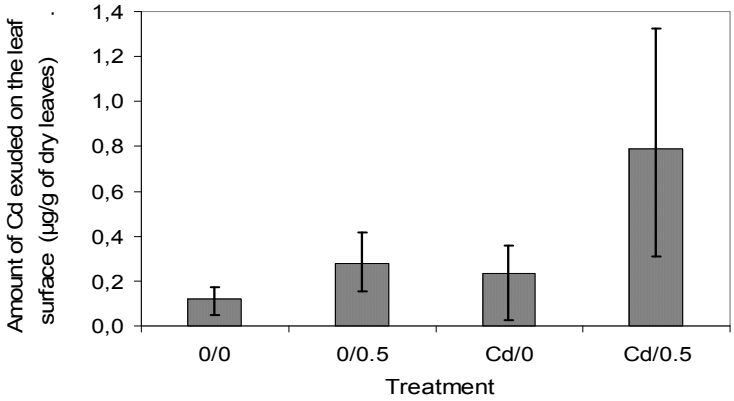

Fig. 2. Cadmium excretion from leaf tissue of *tamarix smyrnensis* treated with 16 ppm Cd of dry weight of soil at different soil salinities. Values shown are means (n = 6) with minimum and maximum values.

3.3. Correlation between Cd accumulation and physiological response

In our study we found that *T. smyrnensis* developed no visible signs of metal toxicity, only salt toxicity symptoms were observed. Plants grown in saline environment have dry bottom branches which is one of the symptoms of salt toxicity. Furthermore, all plants treated with the 3% salinity dried before the end of the experiment period. In addition, in this work the amount of photosynthetic pigments of *T. smyrnensis* was not found to change with cadmium or salinity. Total chlorophyll, chlorophyll *a* and chlorophyll *b* concentrations were not significantly affected by either the presence of cadmium or salty conditions. Moreover, the growth of plants expressed as shoot length and biomass (dry weight) shown in Figure 3, were not significantly affected by the cadmium addition into the soil. Instead, there is an apparent correlation between the increase of salinity and degrease of Tamarix biomass and growth except in the case where no salt was added. The fact that increased salinity caused a reduction in plant growth but not a reduction of the chlorophyll content has also been observed in previous studies in which *Tamarix ramosissima* showed a marked diminution in growth in response to salinity but no diminution in photosynthesis over a salinity gradient from 0 to 200 mM NaCl. It was concluded that growth was negatively affected by salinity due to diversion of energy for increased respiration and salt pumping [8]. All the above observations considered together indicate that *Tamarix smyrnensis* is a cadmium tolerant plant.

3.4. Hydroponic experiment

The data from the performed hydroponic experiments [9] suggest that *T. smyrnensis* is not a Pb and Cd hyperaccumulator considering not only the metal concentrations in plant shoots but also the growth and survival of the plants. However, the accumulation of lead and cadmium in shoots ranged from 150 – 270 ppm and 7.5 - 42 ppm, respectively, namely in concentrations high enough and considered together with its high biomass production it can be inferred that *T. smyrnensis* can be used for phytoextraction applications. Moreover, salinity was found to increase Pb and Cd accumulation in the shoots of the plants. However, the presence of high concentrations of the metals in the plant tissues and high salt concentrations affected negatively the plant health

and *Tamarix* developed visible signs of metal and salt toxicity. Additionally, metals and salt as stressors were found to decrease the chlorophyll content, the biomass and finally the survival of the plants which particularly in the case of Cd was found to be zero after a 10 days exposure of plants to the metal. In conclusion, it is suggested that the roots of *T. smyrnensis* are not selective in the uptake of ions from the root environment and furthermore it seems that the plant does not use a restriction of the internal transport of the metals from the roots to the shoots.

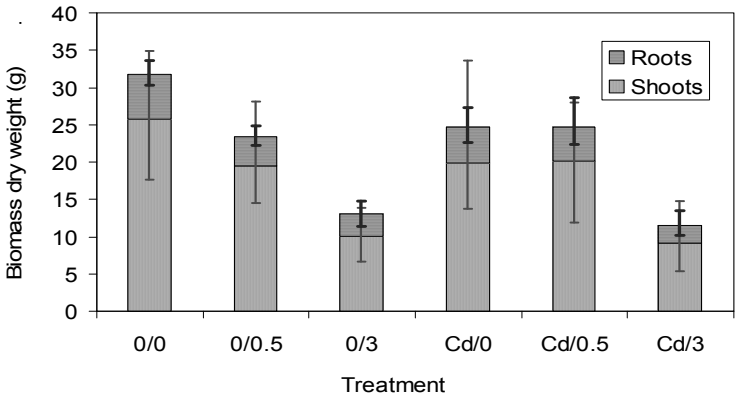

Fig. 3. Biomass of *tamarix smyrnensis* exposed to 16 ppm Cd of dry weight of soil at different soil salinities. Values shown are means (n = 6) with minimum and maximum biomass values.

4. CONCLUSIONS

Tamarix smyrnensis was not found to be a cadmium hyperaccumulator; however, it was found that cadmium uptake by *Tamarix* increases with increasing salinity. Moreover, it was found that salinity affects significantly the translocation of Cd from the roots to the aerial parts of the plant. The toxic level of Cd in the leaves of plants was not exceeded suggesting that *T. smyrnensis* uses its salt excretion mechanism to excrete excess metals on its leaf surface as a possible detoxification mechanism.

ACKNOWLEDGEMENT

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THE EFFECT OF ADAPTION AND TEMPERATURE ON THE BIOLEACHING OF A CHALCOPYRITE CONCENTRATE

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ABSTRACT
This study investigates the role of key parameters such as temperature and duration of bacteria adaption on the bioleaching rate of a chalcopyrite concentrate from Palabora in South Africa. The bacteria *Acidithiobacillus ferroxidans* was contacted with the chalcopyrite in a temperature range from 30°C to 40°C. Copper leaching efficiency was found to be higher at 30°C than at 40°C, confirming literature evidence indicating 32°C to be the optimum temperature for this bacteria.
The controlled adaption of bacteria to grow on the chalcopyrite was found to significantly improve the copper leaching rate and efficiency with copper extraction being reported at 19.5% after 600 hours exposure for un-adapted bacteria and 30% for adapted bacteria

KEYWORDS
Bioleaching, chalcopyrite, copper, iron, role of temperature

1. INTRODUCTION

A. ferrooxidans are generally characterised by five main properties:
- Chemolithotrophic,
- Autotrophic,
- Aerobic,
- Mesophilic,
- Acidophilic.

It is reported in literature that they have an optimum pH range of 1.5-4 and an optimum operating temperature of 20-35°C [1,2].
This study looked at the role of operating temperature and bacteria adaption on the bioleaching rate and efficiency when the bacteria were contacted with a chalcopyrite concentrate.

2. MATERIALS AND METHODS

The bacteria were obtained from the Centre for Bioprocess Enzymes (Strain ATCC 19859). This strain was grown on ATCC64 medium and then used for bacterial growth studies. The optimum pH for the medium was initially studied in this work to provide maximum efficiency in cell growth, iron oxidation and the bioleaching process itself. From these studies the pH was optimised at 2.2 which is lower than the 2.8 recommended by ATCC [3].

The chalcopyrite concentrate used for this study was obtained from Rio Tinto Palabora Mine in South Africa. It was ground in a ball mill and then screened to give a close particle size distribution for all tests. The size fraction used in these tests was +106 micron,-150 micron. The concentrate contained 28.2% copper and 26.3% iron measured by Atomic Adsorption Spectrometry with low reported levels of galena, arsenopyrite and chromite.

3. RESULTS AND DISCUSSION

Shake flask experiments were carried out at 30°C and 40°C using an un-adapted strain of bacteria (Fig. 1). The data shows that copper and iron dissolution over the leaching period of 600 hours decreased as the operating temperature increased. It can be seen that this decrease in leaching is more pronounced for copper than it is for iron. The copper extraction dropped from 21.5% to 5.9% over 26 days indicating very poor bacteria performance at 40°C. The iron extraction was significantly lower than copper in all operating conditions.

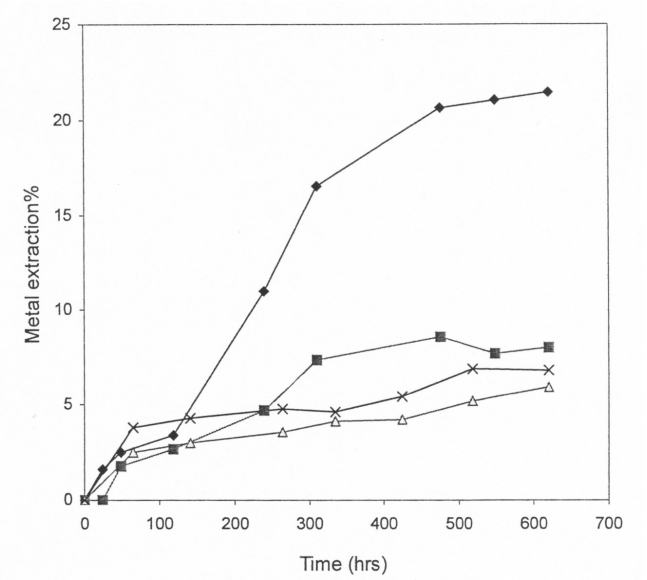

Fig. 1. Bio-Leaching of Chalcopyrite with un-adapted *A. ferrooxidans* Cu extraction at 30°C(♦) and 40°C (Δ), Fe dissolution at 30°C (■) and 40°C (x).

The effect of bacteria adaption is presented in Fig. 2. As would be expected both copper and iron extraction levels increased when adapted bacteria were used at an operating temperature of 40°C.For copper less than 5% extraction was obtained using un-adapted bacteria after 700 hours bioleaching time. Adapted bacteria gave a significant increase in copper extraction up to 23% in a similar time. Iron extraction showed a similar trend re-enforcing the importance of suitable bacterial adaption for an industrial leaching operation.

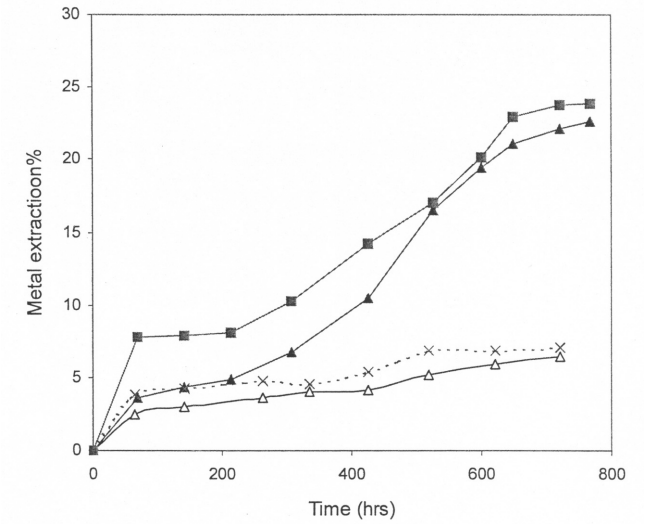

Fig. 2. The effect of adaption time on chalcopyrite bio-leaching with *A. ferrooxidans* at 40°C, Cu extraction with adapted bacteria (▲) and un-adapted bacteria (Δ). Fe extraction with adapted (■) and un-adapted culture (x)

As part of this study a number of copper tolerant bacteria strains were developed. Bacteria were exposed to solutions of 2 g/l and 5 g/l copper and subsequently used for bioleaching experimentation.
Fig 3 shows the improvement in copper extraction using these copper tolerant bacteria over the un-adapted bacteria previously used. Interestingly there is no significant difference in the extraction efficiency between the 2 g/l and 5 g/l adapted bacteria, but the adapted bacteria were 50% more effective than the un-adapted (30% extraction compared to 20% after 600 hours).

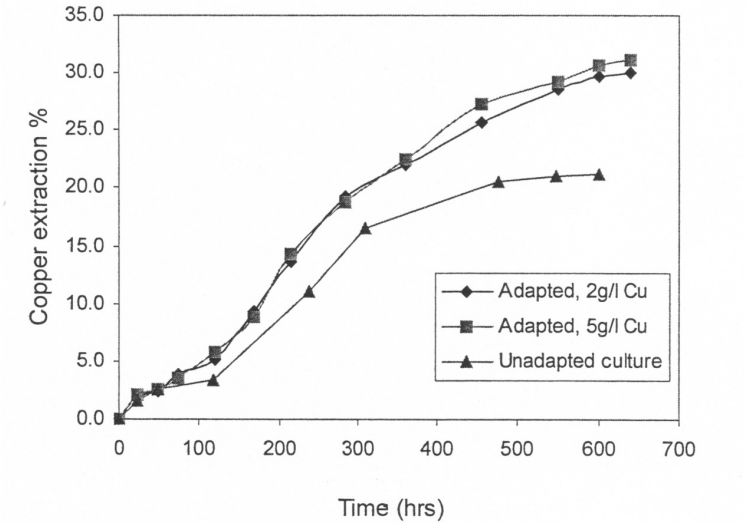

Fig. 3. Effect of adaption on copper extraction during bioleaching at 40°C.

The adaption of bacteria to chalcopyrite mineral surfaces was also carried out. This was shown (Fig 4) to improve the culture capability even further with enhanced copper extraction under similar operating conditions (temperature, cell concentration, etc) when compared to bacteria adapted to copper solutions of 2 g/l and 5 g/l.

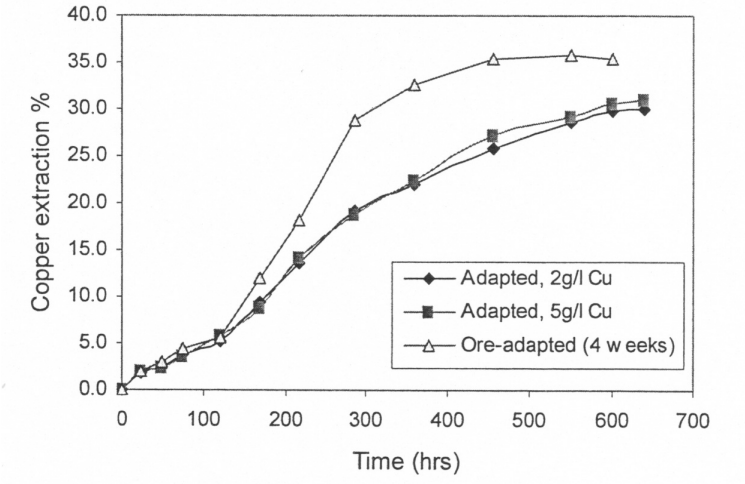

Fig. 4. The effect on adaption protocol on bio leaching at 40°C.

The total copper extraction using chalcopyrite adapted bacteria was 35% after 600 hours leaching time this compared to 30% for the copper solution adapted bacteria in a similar time period. Thus indicating that adaption of bacteria on chalcopyrite concentrate surface gives enhanced leaching kinetics for the bacteria studied in this work.

4. CONCLUSIONS

The bioleaching of chalcopyrite is a problematic process with slow leaching kinetics that can inhibit the application of this technology in an industrial context. This study has investigated the bioleaching of a chalcopyrite concentrate and the effect of temperature and adaption protocol on the metal extraction efficiency. The mesophillic bacteria used (*Acidithiobacillus ferrooxidans* ATCC19859) was found to be give optimum leaching performance at an operating temperature of 30°C and when the adaption protocol involved contact with chalcopyrite mineral surfaces.

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EFFECT OF HEAVY METALS ON OXIDATIVE STRESS IN *LINUM USITATISSIMUM*

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ABSTRACT
Pollution of the environment by heavy metals, which is one of the serious problems today, has negative influence on all ecosystem, including plants. Stress evaluation caused by heavy metals is very complex issue. In presented article the effect of mixture of cadmium, copper and zinc on *Linum usitatissimum* physiology was evaluated. Antioxidant activity of guaiacol peroxidase and chlorophyll content were measured. Studied metals did not have any effect on chlorophyll content but the guiacol peroxidase activity was several times higher.

KEYWORDS
Biochemical analyses, heavy metals, oxidative stress, *Linum usitatissimum*

1. INTRODUCTION

Heavy metals are the most abundant and persistent environmental inorganic pollutants. Unlike other pollutants, metals cannot be degraded but the cleanup usually requires their removal. Moreover, most of them have a very long half-life and they can be bioaccumulated through multiple trophic levels in food chains [1].

High heavy metal concentrations in soils could be very toxic for plants and affect the plant metabolism. Three molecular mechanisms of heavy metal toxicity can be outlined according to their distinct chemical and physical properties: (a) production of reactive oxygen species (ROS) by autoxidation and Fenton-reaction (b) blocking of essential functional groups in biomolecules and (c) displacement of essential metal ions from biomolecules [2,3].

The most often studied mechanisms is production of ROS, when metal presence causes uncontrolled redox reaction in cells, resulting in oxidative stress. ROS can react with lipids, proteins, pigments and nucleic acid and cause lipid peroxidation, membrane damage and inactivation of enzymes, thus affecting cell viability [4].

Plant cells contain many protective and repair systems that minimize the occurrence of oxidative damage. These systems can be divided into two categories: those that interact with active forms of oxygen and keep them at low levels [superoxide dismutases (SODs), catalases (CATs), and ascorbate peroxidases (APXs), guaiacol peroxidases (GPXs)], and those that regenerate oxidized antioxidants [glutathiones (GSHs), glutathione reductases (GRs), ascorbates and mono- and dehydroascorbate reductases]. The first group of enzymes are involved in the detoxification of $O_2^{\bullet-}$ radicals and H_2O_2, thereby preventing the formation of $OH^{\bullet}$ radicals. GR and GSH are important components of the ascorbate-glutathione pathway responsible for the removal of H_2O_2 in different cellular compartments [5]. Various antioxidative responses and degrees of tolerance to metal-induced oxidative stress are exhibited by different metal accumulating species [4].

For experimental work was chosen flax (*Linum usitatissimum* L.), which is an annual plant species widely cultivated in temperate climates and grown in industrially polluted regions. Flax (*Linum usitatissimum* L.) is primarily utilised to produce industrial oil, with the meal being used in animal feed and for human consumption [6]. Flax is considered to be metal tolerant plant without evident yield depression or decrease of quality of harvested products. Among the crops, flax is known accumulator of heavy metals, i.e., *Linum usitatissimum* accumulates Cd, frequently containing more than 0,10 mg Cd. kg^{-1} dry matter [7-10] and Pb, Cu and Zn [7].

The aim of our study was to evaluate the toxic effects induced by the mixture of three heavy metals-copper, cadmium and zinc on *Linum usitatissimum* by observing changes in physiological parameters such as chlorophyll content and guaiacol peroxidase (GPX) activity in order to determine the occurrence of oxidative damage and reducing photosynthesis (chlorofyll degradation). These results were used to evaluate the suitability of the selected plant for the removal of heavy metals from soils by means of the phytoremediation.

2. MATERIALS AND METHODS

2.1. Experimental set-up

Linum usitatissimum (variety called Merkur*)* seeds were germinated and grown in laboratory conditions in plastic pots (four per each pot) filled with organic substrate. Organic substrate used for plants was produce in Slovakia with the trade name Florcom, garden substrate. The weight of the organic substrate was measured after drying at 80°C for 24 hours and each pot was filled with the same amount of soil (1130 g). Plants were watered two times per week with tap water (or more if it was necessary) not to overflow from the pots.

The experiment was carried out from half of May until the beginning of September 2008 and lasted for 120 days. At the beginning of the experimental phase plants were divided into three groups 5 plants per each group. Five plants were used as controls in the uncontaminated soil and 10 plants were used for the treatment with Cu, Cd and Zn in contaminated soils. Copper, cadmium and zinc were separately added into each pot in concentrations listed in Table 1 per dry weight of soil as an aqueous solutions of $CuSO_4$, $Cd(NO_3)_2$ and $Zn(NO_3)_2$ in one dose on the first day of the experiment. The volume of water added to the pots during the watering was approximately 200 ml not to overflow from the pots.

Tab. 5. Experimental design.

Soil		Control	Contaminated soil	
Group		**A**	**B**	**C**
metal	Cu	–	144.3	721.5
concentration	Cd	–	0.9	4.3
($mg.kg^{-1}$)	Zn	–	220	1100

2.2. Measurements of biochemical parameters

The chlorophyll content was measured at the tenth week of experiment by the method of Harborne [11]. 0.05 g of fresh leaves were taken from each plant randomly, washed with distilled water and small cuttings homogenized in 80% acetone. After double centrifugation (14 000 g, 1 min), direct determination of the absorbance of the supernatant was measured at wavelengths 663 and 646 nm.

For measuring the GPX activity and the soluble protein content according to Erdelský, Frič [12], 0.5 g fresh of plant material was homogenised in 4 ml of cold 0.05M phosphate buffer (pH 5,8). The homogenate was centrifuged at 14 000 g for 20 min. Supernatant was used for determination of soluble protein in leaf extracts and guaiacol peroxidase assay to determine enzyme activity.

The soluble protein content was measured using Lowry [13] method by Modified Lowry Protein Assay Kit from Pierce, Rockford IL (product No. 23240) using bovine albumin serum as a standard.

The assay of guaiacol peroxidase (GPX) activity is based upon a modification of the assay developed by Erdelský and Frič [12].

3. RESULTS AND DISCUSSION

3.1. Chlorophyll content measurement

Usually a common response of plants to metal stress is a decrease of the chlorophyll content in leaves of plants and subsequently the reduction photosynthesis and finally leads to a lower biomass production [14].

Ghnaya at al. [15] found a decreased chlorophyll *a*, chlorophyll *b* content and the total amount of chlorophyll in *Brassica napus* L. treated with different Cd and Zn concentrations. Likewise Monferrán et al. [16] observed symptoms of changes in the photosynthetic apparatus in *Potamogeton pusillus* after exposure to copper, these include a decrease in chlorophyll *a* and chlorophyll *b*.

But during our experiment in the presence of metal mixture (Cd, Zn, Cu) total chlorophyll, chlorophyll *a* and chlorophyll *b* contents were not different from the controls for *Linum usitatissimum* under investigation (Fig.1). The results presented in Figure 1 indicate that at low metal content in soil non-significant chlorophyll increase was determined. Similarly Gupta et al. [17] observed only very slight increase of chlorophyll content in the leaves of *Sesamus indicum* L. treated with the mixture of cadmium, copper and zinc. In our study only at high metal content in soil 8.3% decrease of chlorophyll content was observed. There is possible explanation of this occurrence that combination of a essentials nutrients (Zn, Cu) and a non-essential metal (Cd) could not cause decrease of chlorophyll due to the fact that Zn is known by its protective function in organism against impact of the other metals. Zn is also known as the antagonist of Cd and Cu toxicity [18]. Probably this was the reason that chlorophyll content did not change at low metal concentration in soil. But probably the protective effect of Zn is

limited because in soil with high metal content toxicity symptoms such as slight decrease in chlorophyll content were found.

But in according to findings of Angelova et al. [7] and Gangrong [9] very little presence of toxicity symptoms may be caused by the higher metal tolerance of flax plants.

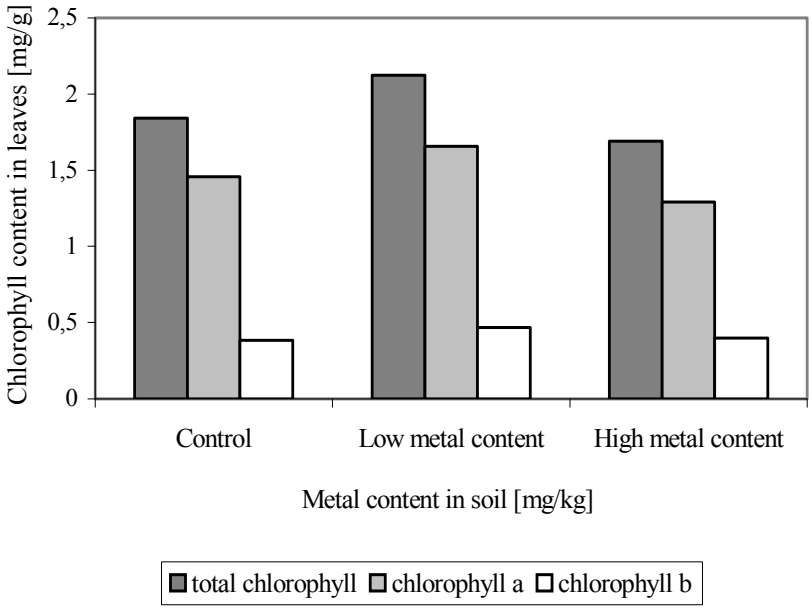

Fig.1. Chlorophyll contents in fresh leaves of *Linum usitatissimum* (per g of fresh weight of leaves).

3.2. Guaiacol peroxidase (GPX) activity

Amongst various enzymes involved in scavenging of the ROS activity, guaiacol peroxidase (GPX) plays a vital role in scavenging destructive oxidant species. Measuring of the peroxidase activity can be used as a biomarker to evaluate the intensity of stress [**Chyba! Nenašiel sa žiaden zdroj odkazov.**]. The results of measurements of the activity of guaiacol peroxidase in plants are given in Figure 2.

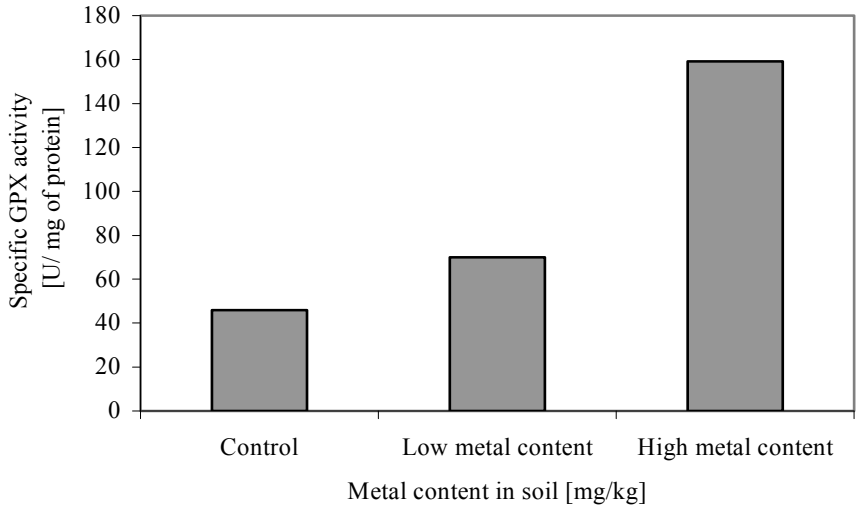

Fig. 2. Specific GPX activity in *Linum usitatissimum* treated with metal concentration.

In our study, guaiacol peroxidase activity strongly response to metal exposure. Specific activity of peroxidase (GPX) was found to be significantly higher (1.5 times) at plants treated with low metal content in soil in comparison with control plants (Fig. 2). Thus already lower metal content above standards in soil caused such a strong oxidative stress. At high metal content (5 times higher concentration above standard in soil) was GPX

activity 3.5 times higher than in control. Our results indicate a considerable enhancement in the activity of guaiacol peroxidase, suggesting that this antioxidative enzyme acts to reduce the impact of metal toxicity.

4. CONCLUSIONS

At the present, due to the development of phytoremediation methods, plants are even more used in the environment with high concentrations of metals. Therefore it is necessary to study effect of these metals on health of the plants. Stress of the plants is evaluated by several factors. In our study we have observed, that in *Linum usitatissimum*, more sensitive to stress is guaiacol peroxidase. The 3.5 times higher value of GPX activity was recorded in plants with high metal content in soil, while the chlorophyll content was decreased only very little.

ACKNOWLEDGEMENT

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RETENTION OF METAL IONS FROM AMD IN THE FORM OF IRON-HYROXYSULFATE PRECIPITATES

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ABSTRACT
The weathering of metal sulfides results in the dissolution of metal cations, sulfates and other oxyanions from the minerals to drainage waters, which contaminate the surrounding environment. The fate of iron, sulfate and other dissolved products of sulfide weathering depends on particular environmental conditions. As ferric iron in acid sulfate solutions becomes fully oxidized, it eventually reaches saturation with respect to a variety of less soluble iron minerals. Fe^{II} oxidation and Fe^{III} and sulfate co-precipitation was monitored from $FeSO_4$ solutions containing iron-oxidizing bacteria at 4 and 25ºC in the pH range from 2 to 3. The chemical composition of the solutions directed the precipitation of bacterially produced ferric iron towards minerals schwertmannite, NH_4–jarosite and K–jarosite respectively. These minerals are commonly found in sediments of many streams impacted by mine drainage. Analysis of the "ochreous precipitates" taken from AMD in Smolnik abandoned mine, revealed scavenging potential of the minerals for some metal species. Formation of these iron precipitates may play an important role in the transient storage of metals.

KEYWORDS
Acid mine drainage AMD, iron, oxidation, metal precipitation, schwertmannite, jarosite.

1. INTRODUCTION

The weathering of sulfide minerals produces extremely acidic and metal-rich effluents that contaminate waters draining mine bodies and waste dumps. Acid mine drainages (AMDs) cause serious environmental problems in many countries. Uncontrolled discharges of AMDs from abandoned (flooded) mines contaminate surface waters by heavy metals and devastate adjacent environment. Recovery of mine impacted environments requires the retention of the contaminants from AMD effluents. Formation of iron precipitates may play an important role in the transient storage of metals and could be included in long-term bioremediation technologies

The oxidation of pyrite (FeS_2) is followed by the dissolution of iron and sulfur entities to drainage waters (eqn. 1) [1]. To keep the continuity of pyrite leaching, the oxidant, Fe^{3+}, which is reduced in reaction with sulfide, needs to be regenerated.

$$FeS_2 + 14Fe^{3+} + 8H_2O \rightarrow 15Fe^{2+} + 2SO_4^{2-} + 16H^+ \quad (1)$$

The oxidation of ferrous iron by molecular oxygen (eqn. 2) is the rate determining step [2] which returns the oxidizing agent consumed in reaction 1. This allows keeping the oxidation of sulfide mineral and the production of AMD on.

$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (2)$$

The fate of iron, sulfate and other dissolved products of sulfide weathering depends on particular environmental conditions. In acid mine drainages ferric ion undergoes hydrolysis and precipitates in the form of FeIII-hydroxysulfates and oxihydroxides [3]. The following reactions describe the formation of secondary minerals Schwertmannite (eqn. 3) and jarosites (eqn. 4) respectively [4-6].

$$8Fe^{3+} + SO_4^{2-} + 14H_2O \rightarrow Fe_8O_8(OH)_6SO_4 + 22H^+ \quad (3)$$

$$3Fe^{3+} + K^+ + 2SO_4^{2-} + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (4)$$

Schwertmannite, $Fe_8O_8(OH)_6SO_4$; goethite, α-FeOOH; and jarosite, $MFe_3(OH)_6(SO_4)_2$ are commonly found as a secondary precipitates in sediments of AMD recipients. Normally, formation of these minerals takes place at very low pH values. In the environment, there is an evidence that aged schwertmannite converts to goethite over the time (eqn. 5). During this process the pH and Fe concentration decreased, whereas the sulfate concentration increases [7].

$$Fe_8O_8(OH)_6SO_4 + H_2O \rightarrow 8FeOOH + H_2SO_4 \quad (5)$$

Goethite increased in abundance relative to schwertmannite in precipitates formed at higher pH. On the other hand, lower pH promotes jarosite precipitation (eqn 4) and the transformation of schwertmannite to jarosite phase (eqn. 6) [8].

$$Fe_8O_8(OH)_6SO_4 + SO_4^{2-} + K^+ + 16H^+ \rightarrow KFe_3(SO_4)_2(OH)_6 + 5Fe^{3+} + 8H_2O \quad (6)$$

Bigham et al. [8] demonstrated that relatively minor changes in pH and solution composition can have important impacts on mineral speciation within acid sulfate systems. Paragenetic sequence of minerals forming as products of pyrite oxidation, which is governed mainly by the pH and the sulfate and iron concentrations of the stream, relationship between minerals schwertmannite, jarosite and goethite/ferrihydrite and their scavenging potential for some metal species, have recently been documented [3,6,9,10].

The objective of this study is to demonstrate the iron speciation and Fe^{3+}, K^+ and SO_4^{2-} precipitation in the course of bacterial Fe^{2+} oxidation in laboratory conditions. Changes in pH and redox potential of the solutions in the course of iron oxidation/precipitation were also measured. A natural sample of Fe-precipitates formed from AMD in Smolnik and physico-chemical parameters of related AMD water are also presented. The elemental analysis of naturally occurring precipitates indicates that formation of these minerals may play an important role in the transient storage of metals.

2. MATERIALS AND METHODS

2.1. Bacterial iron oxidation and Fe^{III} precipitation

The ferric-iron-hydroxysulfates were prepared from ferrous sulfate ($FeSO_4.7H_2O$) by its biological oxidation to ferric sulfate. Oxidation was carried out at 4ºC and 25ºC respectively. Psychrotolerant iron-oxidizing bacterium *Acidithiobacillus ferrooxidans* SS3 [11] was inoculated to all flasks. The incubation was carried out in 500 ml flasks stored in cooling thermo cabinets and magnetically stirred at 180 rpm.

The formation of schwertmannite was achieved in the absence of monovalent cations, which would otherwise direct the precipitation toward jarosite [12,13]. The modified mineral salts medium for schwertmannite synthesis contained 1.6 mM $MgSO_4$ $7H_2O$, 6.1 mM $NH_4H_2PO_4$ and 160 mM $FeSO_4$ 7 H_2O in 4.05 mM H_2SO_4. In contrast, K–jarosite was precipitated in media supplemented with K^+ salts. For ammonio jarosite experiments medium was reformulated to avoid the precipitation of K–jarosite [13]. The initial pH of all solutions was 2.6. Samples of the solutions were taken in intervals for iron speciation, sulfate, potassium, pH and redox analysis.

2.2. Chemical analysis

Ferrous iron was determined by titration with potassium dichromate, ferric iron by UV spectrophotometric method. Sulfate concentration was determined by $BaCl_2$ nephelometric method. Concentrations of total iron and potassium were determined by AAS and pH and redox potential of the solutions by potentiometric method.

3. RESULTS AND DISCUSSION

Bacterial Fe^{2+} oxidation (Fig. 1) showed linear pattern due to intentional incubation with oxygen mass transfer limitation. Thus the iron oxidation rate was similar in both 4ºC and 25ºC incubation modes and the ferrous iron was completely oxidized within 15 (flasks with schwertmannite) to 35 days (NH_4-jarosite flasks) respectively. Over the same time intervals, Eh increased from 530 mV to values of 870 mV. The solution pH initially increased due to the acid-consuming iron oxidation (reaction 2). As ferric iron in the solutions becomes fully oxidized, it eventually reached saturation with respect to insoluble hydroxysulfates. Subsequent reactions of iron hydrolysis, leading to schwertmannite (eqn. 2) and jarosite (eqns. 3 and 4) formation are acid producing and the final pH value depends on the extent of equilibration and type of ferric iron precipitation. All synthesis conditions involved 10 days of equilibration before the samples of precipitates were taken washed and air dried.

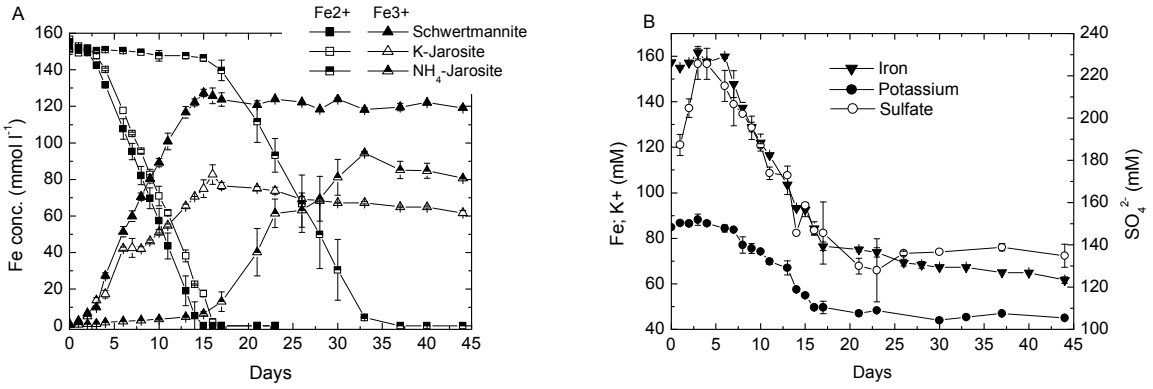

Fig. 1. Time course of bacterial Fe^{2+} oxidation during schwertmannite, K-jarosite and NH_4 jarosite synthesis (A), and Fe^{3+}, SO_4^{2-} and K^+ precipitation in medium with K-jarosite synthesis at 4ºC. Symbols indicate averages ± standard deviations from triplicate flasks.

Schwertmannite formation, (Fig. 1A closed symbols), does not require the presence of monovalent or divalent cations and removes less sulfate from the solution phase as compared to jarosite precipitation. In flasks with K–jarosites formation (Fig. 1A, B), the concentrations of ferric iron, sulfate and potassium ions gradually decreased in almost congruent ratio K:Fe:S = 1:3:2, with respect to ideal formula $KFe_3(SO_4)_2(OH)_6$. In media with NH_4–jarosite formation (Fig. 1A half-open symbols), the bacterial oxidation was delayed by a distinct lag period, because of higher ammonium sulfate concentration. The net Fe^{III} and sulfate yield in precipitates from solutions, in that the synthesis was directed towards schwertmannite was almost similar in both 4 ºC and 25 ºC incubation modes. On the other hand, higher incubation temperature brought about higher yield of ferric iron and sulfate ions in precipitates from flasks with K–type and NH_4–type jarosite synthesis.

In the environment, there is evidence that aged schwertmannite converts to goethite over the time Schwertmannites are yellowish brown. (yellow-orange) and tend to be intermediate in color to goethite and jarosite [14]. Paragenetic relationship between schwertmannite, jarosite and goethite/ferrihydrite as products of pyrite oxidation is governed mainly by the pH and the sulfate and iron concentrations of the stream.

Table 1 indicates the content of major elements in natural sample of precipitates taken from AMD in Smolnik mine site. The concentration of iron and sulfate in the AMD was 380 mg l^{-1} and 2 320 mg l^{-1} respectively. The pH of the water was 3.9 and temperature 14 ºC. Natural precipitates in sediments of streams, where the water has attained a pH of 3 to 4, are dominated by schwertmannite. Schwertmannite was the major component of all collected and studied AMD precipitates formed between pH 2.5 and 4 by Murad et al. [7].

Tab. 1. Chemical analysis of precipitates taken from AMD (shaft PECH) in Smolnik abandoned mine (April, 2009).

Fe	S	Al	Ca	Mg	Na	K	As	Cu	Zn	Mn	Pb
%	%	%	%	%	%	ppm	%	ppm	ppm	ppm	ppm
44.89	5.53	0.38	0.1	0.07	0.02	47.5	0.28	112.5	72.5	35	16.8

Schwertmannite, with ideal formula $Fe_8O_8(OH)_6(SO_4)$, implies an Fe/S molar ratio of 8. However, the sulfate content is variable in natural samples of schwertmannite and varies from 10 to 15 wt %, yielding Fe/S mole ratios ranging from 8 to 4.6 [4,7,8]. Stoichiometric jarosite, by comparison, contains 38.4% SO_4 and has an Fe/S mole ratio of 1.5. The weight % of iron and sulfur in the "Smolnik" precipitate (Table 1) are 44.89 and 5.53 respectively, yielding Fe/S molar ratio of 4.7. This value belongs to the range of naturally occurred schwertmannites published by Murrad et al [4].

Fe(III)-hydroxysulfates from acid mine drainages have been shown to contain As, Cr, and a variety of rare earth elements [9, 10, 18]. Relatively high content of arsenic (0.28 wt %) in the "Smolnik" precipitates indicates their ability to accumulate oxyanions from surrounding water. The concentration of arsenic in the AMD stream in which the precipitates were formed was 30 μg l^{-1}.

4. CONCLUSIONS

Solution parameters such as pH and SO_4^{2-} play an important role in determining the mineralogical fate of iron, and the paragenetic sequence of minerals forming from acid mine drainage from pyrite oxidation products. Ferric iron in many natural acid sulfate systems reaches saturation with respect to a variety of less soluble iron minerals – oxides, oxihydroxides and hydroxysulfates. Such situation is likely to occur in oxic conditions in the presence of acidophilic iron-oxidizing bacteria that thrive in those environments.

Iron-reducing bacteria and sulfate-reducing bacteria have been shown to reduce the Fe and S entities in schwertmannite and jarosite, coupling the process to anaerobic oxidation of organic compounds [15-17]. The cycling of Fe between oxidized and reduced solid phases may also involve solubilization or immobilization of potentially toxic metals or other ions if they are associated with precipitates through sorption or solid solution. Fe(III)-hydroxysulfates from acid mine drainage and acid sulfate soils have been shown to contain As, Cr, and a variety of rare earth elements [9,10,18].

This work demonstrates that relatively minor changes in pH can have important impacts on mineral speciation within acid sulfate systems. Consequently, the individual minerals formed, differ in their ability to scavenge iron and sulfate from the solution. Our results display different yield of iron and sulfate in the solid phase according to minerals being formed. The type of precipitate may have a substantial impact on the extent and quality of co-precipitated species, which affect the persistence and mobility of metals, oxyanions, radionuclides, and, even organic pollutants in the environment.

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RECOVERY OF METALS FROM ACID MINE DRAINAGE BY COMBINATION OF CHEMICAL AND BIOLOGICALLY PRODUCED AGENTS APPLICATION

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ABSTRACT
AMD generation is the most serious environmental problem created mainly by the mining industry of the sulphide minerals. These waters has to be contained and treated before it can be discharged. Nowadays is pay attention to treatment of AMD by selective sequential precipitation to recover metals as hydroxides and sulphides. Involved methods constitute the possibility of the metals recovery in the suitable forms for commercial value.
The objectives of the present work were to relate chemical and biological-chemical methods to separate metals from AMD out-flowing from the shaft Pech of the deposit Smolník (Slovak republic). Experiments were oriented on to study of the chemical selective precipitation metals by the neutralization with NaOH; to develop the system for the heavy metals precipitation by bacterially produced hydrogen sulphide; and the combination of the aforementioned methods.

KEYWORDS
Acid Mine Drainage, heavy metals, neutralization, bacterially produced hydrogen sulphide

1. INTRODUCTION

Acid Mine Drainage (AMD) is unique industrial contaminants occuring mainly in the cours of sulphide minerals mining. These waters can occur in underground mine workings, waste rock dumps, mill tailings piles, ore stockpiles, spent ore piles from heap leach operations and in other residue deposits which present a high surface area for oxidation. The AMD generation proceeds during the active mining operations in the sulphide ore deposit but usually is increased considerably after the closure of mines. Acid mine waters have low pH-values (pH 2 - pH 4), and typically high concentrations of sulphates, iron and non-ferrous metals [1], which serve as buffering systems. The impacts of mine water pollution on biological systems are mostly severe.

In Slovak republic there are some localities with existing AMD generation conditions. Our previous research demonstrates the biological catalysis of oxidation „in situ“ processes in six deposits: Smolnik, Pezinok, Sobov, Slovinky, Roznava and Rudnany [2]. The critical values were observed in the abandoned Cu-Fe ore deposit Smolnik. The continuation of AMD generation at the locality of Smolnik is not possible to stop and there is no chance for situation self-improvement. It is necessary to develop methods for their treatment. That was the reason for starting a systematic monitoring of geochemical development in acid mine drainage in 2004 in order to prepare a prognosis in terms of environmental risk and use of these waters as an atypical source of a wide range of elements.

Development of cost-effective and sustainable remediation solutions for the mine water problem has been the subject of extensive review [3-5]. In addition to monitored natural attenuation, the two broad philosophies which have been pursued in the treatment and abatement of mine water pollution include measures directed towards prevention at source, usually involving physical intervention of one form or another, and measures directed at the resulting effluent, including active or passive remedial systems [6]. Both active and passive systems may be implemented using physicochemical or biological treatment technologies.

Nowadays is pay attention to bio treatment of AMD by selective sequential precipitation to recover metals as hydroxides and sulphides. It is the process of the heavy metals precipitation by bacterially produced hydrogen sulphide with the combination of the metals precipitation by sodium hydroxide at the various values of pH AMD [7,8]. The base of this method was the application of sulphate-reducing bacteria (SRB). The metabolic process of SRB is the anaerobic reduction of sulphates by the formation of hydrogen sulphide reacting in the water with cations of metals forming sparingly soluble sulphides. Investigated was the process of the heavy metals precipitation by bacterially produced hydrogen sulphide with the combination of the metals precipitation by sodium hydroxide at the various AMD pH values. In the literature this methods is so called selective sequential precipitation (SSP) [7].

AMD are characterized by low pH and high acidity due to strong buffering systems, which are of importance for environmental studies and treatment of acidic mining waters. Information about the strength of the buffering systems provides the titration curves that are determined by endpoint titration.

Experiments were oriented on to study of titration curves for acidity determination and characterization of chosen AMD; to study of the chemical selective precipitation metals by the neutralization with NaOH; to develop the system for the heavy metals precipitation by the bacterially produced hydrogen sulphide; and the combination of the aforementioned methods.

2. MATERIALS AND METHODS

2. 1. Acid mine drainage

The experiments were conducted with AMD coming from the abandoned and flooded deposit of Smolnik (Slovak Republic). The average values of pH and the major metals composition of acid mine drainage was following: pH 3.9; SO_4^{2-} - 2 938 mg l^{-1}; Fe - 307 mg l^{-1}; Mn - 26 mg l^{-1}; Cu - 5 mg l^{-1}; Zn - 11 mg l^{-1}; Al - 77 mg l^{-1}.

2.2. Titration of Acid mine drainage

Titration curves were collected using a TitraLab 854/10 autotitrator (Radiometer Analytical SAS) through addition of 0.2 M NaOH. The same titrator carried out endpoint titrations. The parameters for the endpoint method were adjusted for high precision and a waiting time of 5 min at the end of the measurement. The titration tests were carried out by raw AMD samples of 100 ml.

2.3. Metals precipitation by NaOH

The precipitation by 1M NaOH solutions was used for the removal of metals as hydroxides from AMD. Experiments were carried out by raw AMD samples of 100 ml and each were titrated to pH end points ranging from 5 to 9 using 1M NaOH. When the preset pH end point was reached, the titrated solution was filtered to precipitated metals removing. During titration the AMD solution was continuously stirred, the pH was monitored and the concentration of metals was determined too.

2.4. Metals precipitation by bacterially produced H_2S and NaOH

For the production of the bacterially H_2S the cultures of SRB (genera *Desulfovibrio*) were used. These bacteria were isolated from a mixed culture obtained from the potable mineral water (Gajdovka spring, the locality Kosice-north, Slovakia).

Used biological-chemical method contains several process steps and can be divided in to these main steps, as well as: the bacterially H_2S production by SRB; the heavy metals precipitation by the bacterially produced H_2S; the heavy metal sulphides separation by filtration; the setting pH of filtrate by 1M NaOH with simultaneously precipitation of metals as hydroxides; the heavy metal hydroxides separation by the filtration; the subsequent precipitation of the heavy metals by bacterially produced H_2S. Values of pH for the heavy metals precipitation were assigned on the ground of the study of the orientation conditions for the selected metal removal from AMD by precipitation using NaOH and our previous works and calculations [8]. The concentration of metals was determined by atomic absorption spectrometry (AAS) using Spectrometer AA - 30 Varian (Australia). A glass pH electrode combined with the reference Ag/AgCl electrode was used to measure pH by digital pH- meter GPRT 144 AGL (Germany).

3. RESULTS AND DISCUSSION

Results of the titration test AMD by 0,2M NaOH are shown on the Figure 1. It is documents the titration curve and elemental AMD analysis. The titration curve of AMD can be divided into number of by its different slopes. Its show that the optimum pH for metals precipitation is different for each metal: for Al - pH 5; for Cu - pH 6; for Zn - pH 7; for Fe - pH 8; for Mn - pH 9.

Table 1 demonstrates results of metals precipitation by NaOH that is titration of AMD to pH end points for the individual metals ranging from 5 to 9 using 1M NaOH. As it is seen from Table 2 was removed 97% of Al at pH 5; 99.9% of Cu at pH 6; 99.9% of Zn at pH 7; 99.9% of Fe at pH 8 and 99.9% of Mn at pH 9. Initial assumption about precipitation of followed metals up to pH 9 has been confirmed.

The selective sequential precipitation of heavy metals form AMD sample was performed in two interconnected bioreactors with a capacity 1000 ml (the first bioreactor) and 250 ml (the second bioreactor), which operated at the semi-continual conditions. Using the operating conditions and obtained results of the selective sequential precipitation of heavy metals form AMD sample illustrated in Table 2. The bacterially produced hydrogen sulphide by SRB at pH 3.9 (initial pH of AMD), 4.5 and 6.0 realized the selective sequential

precipitation of Cu, Zn and Fe respectively (i.e. steps 1, 3 and 5). Al and Mn were precipitated as aluminium and manganese hydroxide at pH 6.0 and 9.0 respectively (i.e. steps 4 and 6). Fe was precipitated predominantly as hydroxide (steps 2, 4 and 6).

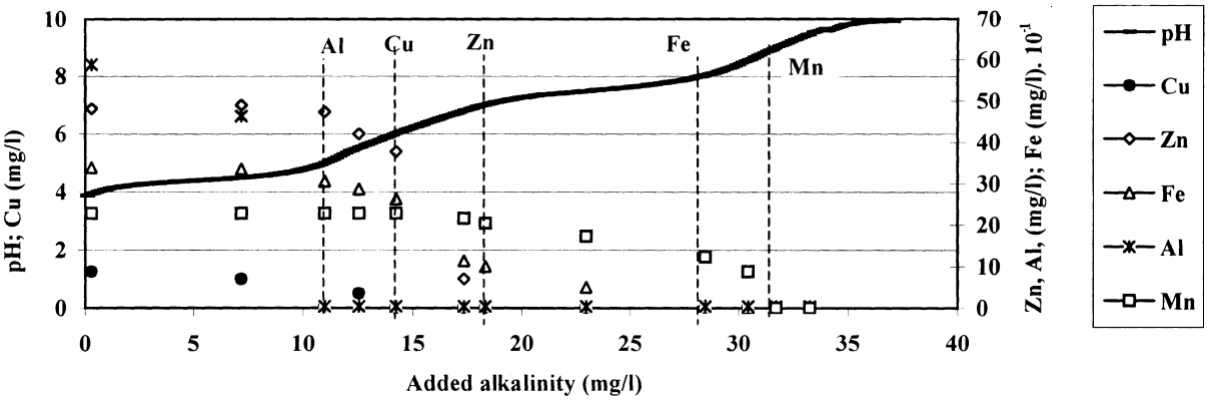

Fig. 1. Titration curve and metal AMD analysis of AMD from the deposit of Smolnik.

Tab. 1. Precipitation of Al, Cu, Zn, Fe and Mn in dependence on pH (initial pH of AMD was 3.9).

pH	Al (mg l^{-1})	Cu (mg l^{-1})	Zn (mg l^{-1})	Fe (mg l^{-1})	Mn (mg l^{-1})
3.9	58.8	1.38	6.88	338.6	22.88
5	**0.4**	0.65	6.75	268.8	22.88
6	0.4	**<0.02**	5.38	9.8	22.88
7	0.4	<0.02	**<0.03**	2.5	20.38
8	0.4	<0.02	<0.03	**<0.03**	12.25
9	0.4	<0.02	<0.03	<0.03	**0.03**

Tab. 2. Metals precipitation by bacterially produced H_2S and NaOH (initial pH of AMD was 3.9).

	Step 1	**Step 2**	**Step 3**	**Step 4**	**Step 5**	**Step 6**
pH	3.9	4.5	4.5	6.0	6.0	9.0
Precipitating agent	H_2S	NaOH	H_2S	NaOH	H_2S	NaOH
Removed metals	Cu	Fe, Al	Zn	Al, Fe	Fe	Mn, Fe
Form of removed metals (solid phase)	CuS	$Fe(OH)_3$ $Al(OH)_3$	ZnS	$Al(OH)_3$ $Fe(OH)_3$	FeS	$Mn(OH)_2$ $Fe(OH)_2$
Filtrate composition concerning of metals presence (liquid phase)	Fe, Al, Zn, Mn	Fe, Al, Zn, Mn	Fe, Al, Mn	Fe, Mn	Fe, Mn	-

4. CONCLUSIONS

The major metal ions in the AMD from the abandoned and flooded deposit of Smolník (Slovak Republic) were Fe, Al, Ca and Mg, among which Fe and Al were potentially valuable, while others such as Cu, Zn and Mn were present as minor metals at significantly low concentrations. The value of pH, contend of Fe, Al, Zn, Cu, Mn and Mg of AMD did not meet the effluent limitation of NV SR è. 296/2005 Z.z. Up to now AMD was not treated at the site.

Results of the AMD titration test document that the titration curve can be divided into four ranges (I to IV). Its show the different optimum pH for metals precipitation of each metal: for Al - pH 5; for Cu - pH 6; for Zn - pH 7; for Fe - pH 8; for Mn - pH 9. The metals precipitation using 1M NaOH confirms that was removed 97% of Al at pH 5; 99.9% of Cu at pH 6; 99.9% of Zn at pH 7; 99.9% of Fe at pH 8 and 99.9% of Mn at pH 9. Metals removal by precipitation is possible up to pH 9.

The study of the selective sequential precipitation and bio recovery of metals from aforementioned AMD was realized by the combination of the metals precipitation by the bacterially produced hydrogen sulphide and the precipitation of metals by sodium hydroxide at the various AMD pH values. For the removal of Cu and Zn in the form of sulphides were received excellent results. Was not come to good results point of view of the Fe, Al and Mn selective precipitation, because was determined the co-precipitation of Fe and Al or Fe and Mn.

Obtained results can be used for suggestion of technology for selective metal recovery from acid mine drainage from Smolník. The base of this technology will be the combination of chemical and biologically produced agents aplication.

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PARTICIPATION OF BIOLOGICAL AND ABIOLOGICAL PROCESSES IN Co^{2+}, Zn^{2+}, Cd^{2+} AND Cs^{+} SORPTION BY ACTIVATED SLUDGE OF SEWAGE TREATMENT PLANT

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ABSTRACT
Sewage sludge is a byproduct of sewage treatment process. Land application of sewage sludge is one of the final step of waste water treatment, but solubilization of toxic metals restrict this method of sewage disposal. In our paper cobalt, zinc, cadmium and cesium sorption by suspension of non-stabilized activated sewage sludge (14 g/L, dry wt.) from waste water (0.5-50 mmol/L) spiked with ^{60}Co, ^{65}Zn, ^{109}Cd and ^{137}Cs were determined in laboratory experiment at 20°C. Activated sludge supplied by the municipal sewage treatment plant in Zeleneč (Trnava region, Slovakia) showed high efficiency to sorb Co^{2+}, Zn^{2+}, Cd^{2+} and Cs^{+} ions added to waste water pH 6-7. The process can be characterized by the concentration equilibrium (C_{solid}/C_{liquid}) typical for sorption processes. Efficiency of the sorption increased in the order Cs < Co < Zn < Cd. Metal sorption was not inhibited by pretreatment with 0.2% formaldehyde or thermal inactivation at 60°C, what confirms that the process was not dependent on metabolic activity of activated sludge. The metal ions are easily removable from the sludge by washing with diluted HCl, EDTA or water solutions of corresponding metal ions, forming new concentration equilibrium. This study underline the risk of utilization of non-treated sewage sludge contaminated with cobalt, zinc and cadmium as well as with low level radioactive ^{137}Cs wastes.

KEYWORDS
Activated sludge, sorption, desorption, Co, Zn, Cd, Cs, radiotracer analysis

1. INTRODUCTION

Sewage sludge is by-products of urban and industrial wastewater treatment plant activities. Land application of sewage sludge is one of the important disposal activities. Being rich in organic and inorganic plant nutrients, sewage sludge may substitute for fertilizers, but availability of potential toxic metals often restricts its uses [1]. In activated wastewater sludge treatment, sludge tends to accumulate heavy metals existing in the wastewater [2]. Heavy metals such as zinc, copper, nickel, cadmium, lead, mercury and chromium are principal elements restricting the use of sludge for agricultural purposes [2,3]. Sludge also retains substantial amounts of radionuclides from contaminated waste waters. The most hazardous units of sewage works are sludge areas, filtration fields and biological ponds, where more than 90% of the radioactivity is accumulated. In the technological units of sewage works, the level of radioactive contamination of dewatered sewage-water sediments can correspond in the activity of ^{137}Cs to the level of radioactive contamination of conditionally radioactive wastes and radioactive wastes [4]. Much experimental work is performed worldwide to determine the extractable trace metals in sludge to assess the bio-available metal fraction and the potential mobility of trace metals from polluted sludge [5-8]. The objective of this study is to obtain quantitative data of cobalt, zinc, cadmium and cesium sorption by non-treated activated sludge of the municipal wastewater treatment plant.

2. MATERIALS AND METHODS

2.1. Biomass and chemicals

An activated sludge supplied by a municipal sewage treatment plant in Zeleneč (Trnava region, Slovakia) was kept in refrigerator at 4°C and used for experiments. Suspension of activated sludge pH 6.9 preconcentrated by centrifugation to 250 g /L (wet wt.) contained 13.95 g/L (dry wt.) or 55.8 g (dry wt.) per one kg of sediment was used for sorption and desorption study. Batch equilibrium sorption experiments were performed in shaken test tubes at 20°C.

2.2. Radiometric analysis

A gamma spectrometric assembly using the well type scintillation detector 54BP54/2-X, NaI(Tl) (Scionix, the Netherlands) and data processing software Scintivision 32 (ORTEC, USA) were used for ^{60}Co, ^{65}Zn, ^{109}Cd and ^{137}Cs determination in the sediment of sludge and supernatant fluids (14 500 RPM for 20 min). Counting

time 600 s allowed obtaining data with measurement error < 2%, which do not reflect other source of errors. Standardized solution of $^{60}CoCl_2$ (5.181 MBq/cm^3, 20 mg/dm^3 $CoCl_2$ in 3 g/dm^3 HCl), $^{65}ZnCl_2$ (4,901 MBq/cm^3, 50 mg/dm^3 $ZnCl_2$ in 3 g/dm^3 HCl), $^{109}CdCl_2$ (3.937 MBq/cm^3, 50 mg/dm^3 $CdCl_2$ in 3 g/dm^3 HCl), $^{137}CsCl$ (5.723 MBq/cm^3, 20 mg/dm^3 CsCl in 3 g/dm^3 HCl) were obtained from The Czech Institute of Metrology (Prague, Czech Republic). The energy of γ- photons [keV]: ^{60}Co - 1173.24, ^{65}Zn – 1115.52, ^{109}Cd – 88.04, ^{137}Cs – 661.62.

3. RESULTS AND DISCUSSION

3.1. Sorption

The mobility of trace metals, their bioavailability and related eco-toxicity to plants, depend strongly on their specific chemical forms or ways of binding. Sorption experiments showed that activated sludge is able to bind remarkable amounts of cobalt, zinc, cadmium and cesium (Fig. 1). At C_0 = 50 mmol/L sorption Q (μmol/g, dry wt.) 611, 995, 1076 and 374 for cobalt, zinc, cadmium and cesium respectively, of non-treated sludge was obtained. However at the initial concentration C_0 = 50 mmol/L, the system was not saturated and higher Q_{max} values can be expected.

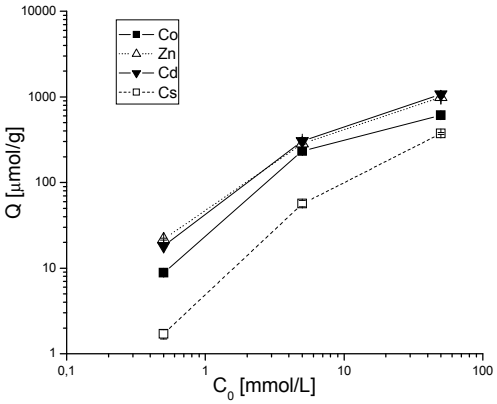

Fig. 1. Cobalt, zinc, cadmium and cesium uptake Q (μmol/g; dry wt.) by non-treated activated sludge from waste water pH 6.98, spiked with $^{60}CoCl_2$, $^{65}ZnCl_2$, $^{109}CdCl_2$ or $^{137}CsCl$ in dependence on the initial concentration of $CoCl_2$, $ZnCl_2$, $CdCl_2$ and CsCl. Data after 1 h reaction at 20°C. Calculated from the decrease of volume radioactivity of supernatant after 1 h reaction at 20°C under agitation. Initial volume radioactivity: ^{60}Co – 195; ^{65}Zn – 156; ^{109}Cd – 192; or ^{137}Cs – 172 Bq/ml.

3.2. Role of metabolism in the metal uptake

Broad consortium of vital aerobic microbial population in activated sludge can participate in metal uptake driven by metabolic processes. On the other hand dead microbial biomass and polymers such as proteins and polysaccharides can bind metals on the basis of sorption processes or complexing. Short term experiments showed that treatment of activated sludge by formaldehyde or thermal inactivation at 60°C had minimal effect on cobalt, zinc, cadmium and cesium uptake (Fig. 2).

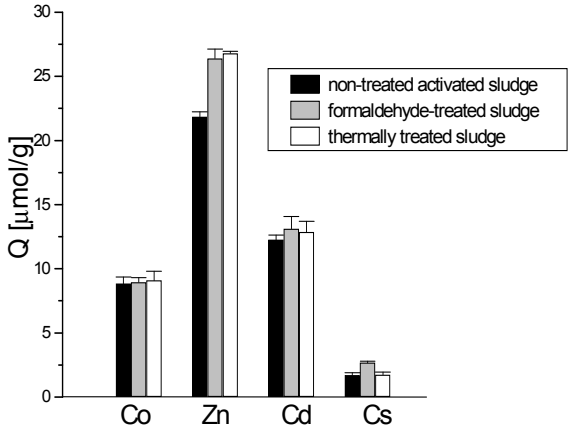

Fig. 2. Uptake of cobalt, zinc, cadmium and cesium Q (μmol/g) by non-treated activated sludge ■, formaldehyde-treated sludge (0.5%, 30 min at 20°C, under wortexing) ■, and thermally treated sludge (30min at 60°C) □.
Sorption 1 h under agitation at 20°C. Reaction conditions see Fig. 1.

3.3. Desorption

High percentage of bound metals can be solubilized by washing of the sludge with salt, EDTA and HCl solutions (Tab. 1., Fig. 3.). Cadmium showed the highest affinity to sorbent and the lowest extractability with mineral acids, salts and salt solutions.

The explanation of this phenomenon will required a more detailed study oriented toward speciation of cadmium in individual components of the sludge flocks. In the sorption processes can participate mainly cell walls fraction of microbial population [9], and both intracellular and extracellular polymer compounds containing negatively charged functions such as carboxylic, phosphate and sulfate groups [10]. Metal that is not extracted by acid might be trapped intracellularly [11].

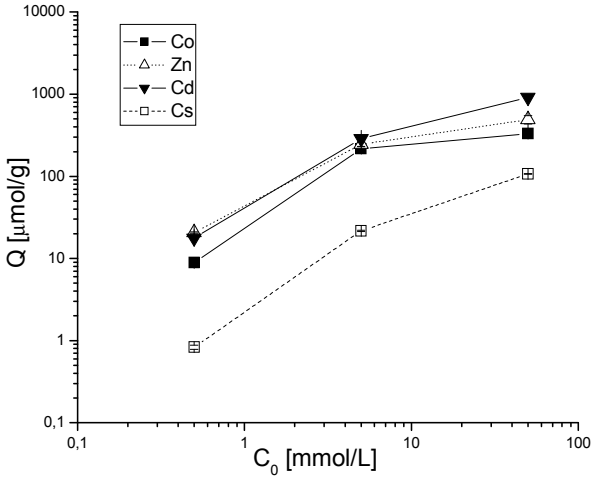

Fig. 3. Concentration of cobalt, zinc, cadmium and cesium (µmol/g, dry wt.) in 14 500 CPM sediments of sludge washed for 30 min with 0.5; 5.0 and 50 mmol/L $CoCl_2$, $ZnCl_2$, $CdCl_2$ or CsCl. Uptake conditions and Q values of non-washed activated sludge are shown in Fig. 1.

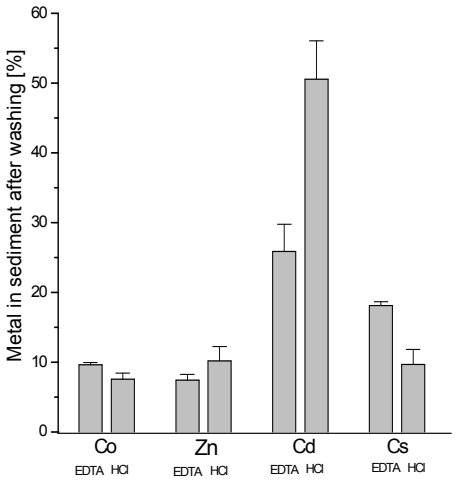

Fig. 4. Percentage of cobalt, zinc, cadmium and cesium remaining in activated sludge after washing with 0.1 mol/L EDTA or HCl. Sorption data see in Fig. 1.

Tab. 1. Recoverable portion of metals by single step washing of sludge with 0.1M HCl, 0.1 M EDTA or 0.05 M $CoCl_2$, $ZnCl_2$, $CdCl_2$ and CsCl. Calculation based on the volume radioactivity of 14 500 RPM supernatant.

	Co	Zn	Cd	Cs
Metal sorbed [%]*	100	100	100	100
Removed with 0.5 mM salt [%]	46	50	15	72
Removed with 0.1 M HCl [%]	81	91	30	83
Removed with 0.1 M EDTA [%]	90	92	73	81

* Biosorption Q (μmol/g, dry wt.): Co – 611; Zn – 995; Cd – 1076; Cs – 374; C_0 = 5 mmol/L.

It should be noted that EDTA and salt extraction provides a chemical evaluation of the amount of metals that are available for plant uptake. According to Hsiau and Lo [2], sequential extraction revealed that the percentages of the heavy metals of organically bound form and exchangeable form in chemically fixed sludge samples were in the order of Cu > Pb > Cr > Zn.

Cs^+ ions show the highest mobility in biological systems resembling behaviour of K^+ ions. In the presence of clay materials in waste water, cesium will be bound irreversibly on clay mineral fractions and will not be bio-available or leachable in natural environment.

4. CONCLUSIONS

Capacity of non-treated activated sludge for biosorption of Co^{2+}, Zn^{2+}, Cd^{2+} and Cs^+ metal ions from waste water spiked with $CoCl_2$, $ZnCl_2$, $CdCl_2$ and CsCl is based mainly on physical processes independent on the metabolic activity of microflora of the sludge. Recovery of the metals by washing with diluted HCl, EDTA and salt solutions decreases in the order: Cs > Co = Zn > Cd. Obtained data can serve as a criterion for quantification of possible negative effect of heavy metal contaminated sludge used as soil fertilizers.

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BIOSORPTION OF HEAVY METALS BY PLANT BIOMASS *(Reynoutria japonica)*

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ABSTRACT
In the present study, the sorption capacity of plant biomass has been studied; particularly the ability of biomass of roots, stems and leaves of an invasive plant *Reynoutria japonica* to bind up Cu^{2+} and Zn^{2+}.
The results of this biosorption study revealed that the rate and extent of uptake were affected by pH level, contact time and initial metal concentration. The maximum uptake of metal ions was obtained at pH 6.0. The equilibrium sorption data for metal system at pH 6 were described by the Langmuir isotherms model. For Zn^{2+}, sorption capacity q_{max} of 17 mg/g was achieved using biomass from leaves. Removal of Zn^{2+} with 1g biosorbent from leaves was almost 77% when present in low concentrations, whereas it is lower at higher concentrations.

KEYWORDS
Reynoutria japonica, biosorption, copper, zinc, isotherm

1. INTRODUCTION

Heavy metal pollution has today become one of the most serious problems. Pollution of the environment by toxic metals arises as a result of many human activities like mining, metallurgy, electroplating, leather tanning, metal finishing, textile industry, and paper industry. Heavy metals are discharged or transported into the atmosphere and aquatic and terrestrial environments mainly as solutes or particulates and may reach high concentrations, especially near the site of entry [1]. Heavy metals have several characteristics that increase their harmful nature: high toxicity, ability to accumulate in living organisms and long time of staying in ecosystems [2]. Effects of these metals on ecosystems are of large economic and public-health significance [3].

Biosorption, as it has been perceived thus far, could be considered for its economic edge as a possible alternative technique for metal recovery. By far the greatest demand for metal sequestration comes from the need for immobilizing the metals "mobilized" by and partially lost as a result of human technological activities [3].

Previous studies have shown that a variety of biosorbents can be used for this purpose [4]. In this study we have chosen roots, stems and leaves of a plant species *Reynoutria japonica*. This plant species is an invasive ubiquitous plant investigated currently as a possible energetic plant. It is also known that the species of this genus are able to accumulate heavy metals from soil [5,6].

2. MATERIAL AND METHODS

2.1. Preparation of biosorbents

All the samples of *Reynoutria japonica* used were collected from the same non-urban area in foothills of Lysá hora mountain, in the area of the Moravskoslezské Beskydy. This sampling area does not have any prior history of contamination by heavy metals. Roots, stems and leaves of this plant were air-dried at room temperature. Dried samples were ground and screened using a sieve shaker; uniform particle size fraction < 0.5 mm was obtained. Particles of roots, stems and leaves were twice washed with 0.01 M HCl (10 g / 1 litre), then with extensive volume of de-ionized water in order to remove soil or debris, and finally washed with distilled water. The biomass samples were then oven-dried at 90°C for one day.

2.2. Chemicals

The heavy metals used in this study were copper ions Cu^{2+} ($CuSO_4.5H_2O$) and zinc ions Zn^{2+} ($ZnSO_4.7H_2O$). The test solutions containing single ions were prepared by diluting proper amount of 1g/l stock solutions of above mentioned metal ions to obtain desired concentrations.

2.3. Procedures of experiments

2.3.1 Time course

The time course of Cu^{2+} and Zn^{2+} uptake by *Reynoutria japonica* was investigated. Two samples of 0.5 g and 5 g of sorbent biomass were each suspended in 500 ml of heavy metal solution. The flasks were placed (24 hours for Cu^{2+} and 6 hours for Zn^{2+}) on a shaker at 120 rpm, in room temperature. pH of solutions tended to drop during the equilibration and therefore it was, during the sorption experiments, adjusted with 0.1 M solution of NaOH. Temperature and pH were measured by a microcomputer meter *pH VISION 6071*. Samples were taken from the solution at intended intervals and were filtered through filter paper. Heavy metal concentrations in the resulting supernatant were measured by the Atomic Absorption Spectrometry (AAS). All samples were duplicated.

2.3.2 Determination of adsorption isotherms

Amounts of 0.1 g of dry acid-pre-treated plant biomass were suspended in 100 ml samples of various concentrations of Zn^{2+} solutions. pH of solutions before and during equilibration was adjusted with 0.1 M solution of NaOH. After 60 minutes of incubation, zinc samples were filtered in order to remove the biomass and metal concentration in supernatant was measured with AAS. Uptake of heavy metal ions was determined from the difference of metal concentrations in initial and final solutions.

3. RESULTS AND DISCUSSION

3.1. Time-course of sorption and effect of pH

Time-course profiles of sorption of Cu^{2+} and Zn^{2+} (at concentrations 100 mg/l for Cu^{2+} and 10 mg/l for Zn^{2+}) by *Reynoutria japonica* are illustrated in Fig. 1 and 2. The sorption rate for both zinc and copper was distinctly divided into two stages: fast initial sorption stage was followed by a much slower sorption stage. At pH 6 metal concentration dropped rapidly during the first 10 minutes. Biosorption equilibrium could be achieved in 60 minutes; after that further biosorption was negligible (Fig. 2). These results are in accordance with biosorption studies using various groups of microorganisms and plant where fast initial rates of metal binding, followed by slower onset of equilibria have been reported [7].

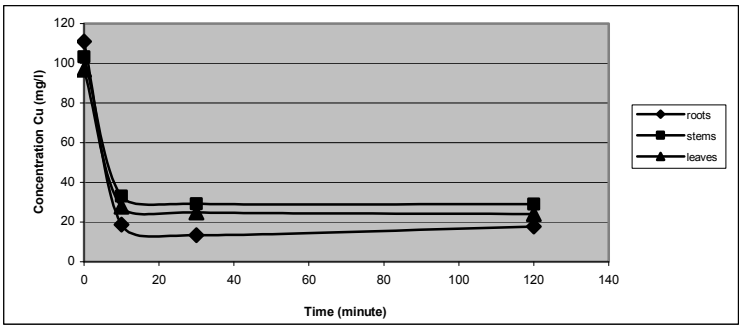

Fig.1. Time course of copper biosorption by biomass of roots, stems and leaves of *Reynoutria japonica*, (biomass dosage 10g/l, initial metal concentration 100 mg/l, room temperature, pH 6).

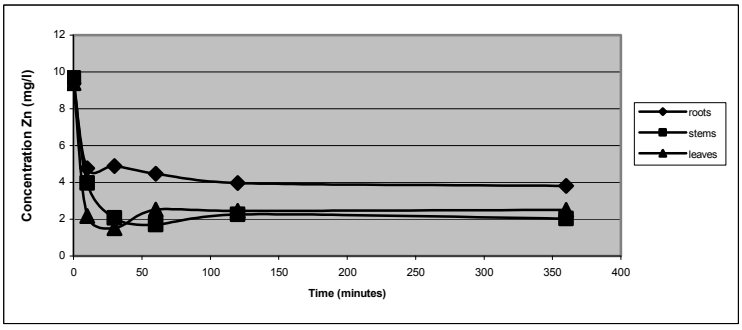

Fig.2. Time course of metal sorption of divalent zinc by roots, stems and leaves of *Reynoutria japonica* (biomass dosage 1g/l, initial metal concentration 10 mg/l, room temperature, pH 6).

For Cu^{2+}, the experiment showed increase of sorption with pH values growing from 2 to 6 (Fig. 3). For Zn^{2+}, the same increase of sorption occurred at pH values growing from 3 to 6; at pH 7 steep decrease of sorption occurred for all three types of biomass - roots, stems and leaves (Fig. 4). Similar results were obtained with crab shell particles biosorbent where metal uptake grew with increasing pH values, and optimal pH for copper was found to be 6 [7].

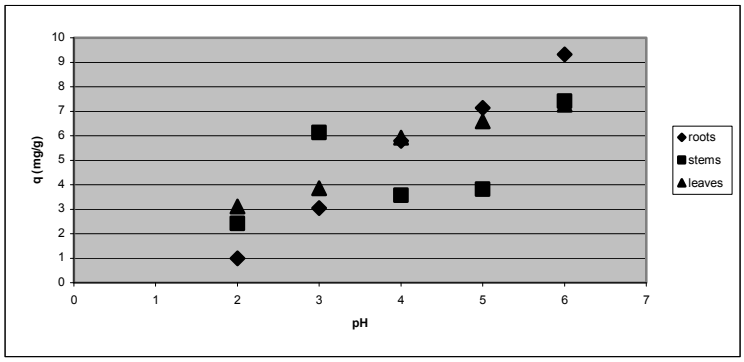

Fig. 3. Effect of pH on the sorption of Cu^{2+} by *Reynoutria japonica* (biomass dosage 10g/l, pH 2-6, initial Cu^{2+} concentration 100 mg/l, room temperature).

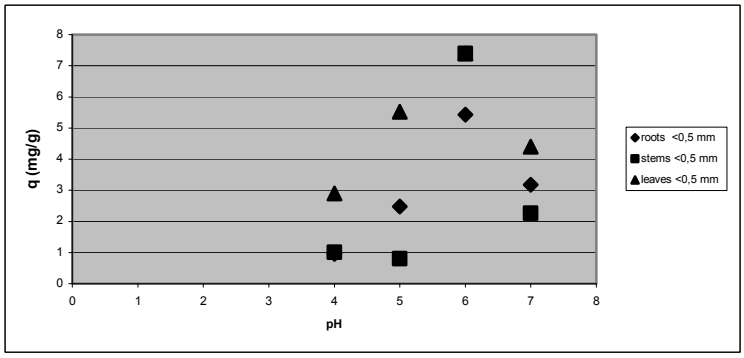

Fig. 4. Effect of pH on the sorption of Zn^{2+} by *Reynoutria japonica* (biomass dosage 1g/l, pH 2-6, initial Zn^{2+} concentration 10 mg/l, room temperature).

3.2. Biosorption equilibrium of zinc uptake at pH 6

In order to describe the sorption of Zn^{2+} by *Reynoutria japonica* at pH 6, experiments were carried out with concentrations ranging from 10 to 100 mg/l. In the present study the experimentally observed uptake capacity was 19.479 mg/g of leaves of *Reynoutria japonica* (1g of leaves biomass per 1 l of sample, initial zinc concentration 50 mg/l). However, in both low and high concentrations, leaves showed the best results (Fig. 5).

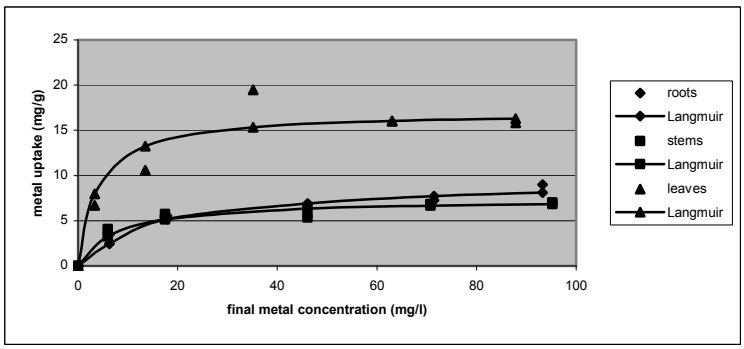

Fig. 5. Sorption isotherms for the sorption of Zn^{2+} onto roots, stems and leaves *Reynoutria japonica* (biomass dosage 1g/l, concact time 60 minute, pH 6, room temperature).

The proces of zinc sorption on the biosorbent was described by the Langmuir model. The best results were obtained with biosorbent from leaves of *Reynoutria japonica*. Maximal metal uptake sorption capacity of leaves was 17 mg/g at pH 6. For comparison, absorption capacity for waste tea leaves were found to be 11.77 mg/g

[9]. Use of biomass of submersed aquatic plant *Ceratophyllum demersum* resulted in capacity of 13.98 mg/g. [10]. The Langmuir constants (q_{max}, b, R^2) for the zinc biosorption onto biomass of *Reynoutria japonica* are presented in Table 1.

Tab. 1. Langmuir constants and correlation coefficient for biosorption zinc on biomass

Metal	Biosorbent	Langmuir adsorption isotherms		
		q_{max}	b	R^2
Zn	Roots	7.36	0.05	0.9128
	Stems	9.73	0.13	0.9779
	Leaves	17	0.26	0.9807

4. CONCLUSIONS

This work has demonstrated the possibility of utilization of biomass of *Reynoutria japonica* for biosorption of heavy metals. Capacity experiments have demonstrated:

Kinetics of copper and zinc biosorption by inactive biomass was fast. Biosorption equilibrium could be achieved in 60 minutes. The maximum decrease of the copper concentrations (removal efficiency) was detected in sample of root biomass (87.9%) and the maximum decrease in the zinc concentrations was detected in sample of leaves biomass (77%).

pH had a strong effect on copper and zinc biosorption capacity.

The capacity of copper and zinc biosorption by biomass increased with the increasing of pH values in the range from 2 to 6.

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EQUILIBRIUM MODELLING OF METAL BIOSORPTION FROM MULTICOMPONENT SYSTEMS

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ABSTRACT
A process of cadmium, cobalt and zinc removal from single, binary and ternary solutions by biosorbent prepared from moss *Rhytidiadelphus* sp. was described in this work. Experimental equilibrium biosorption data for cadmium, cobalt and zinc ions were analysed by the Langmuir and Freundlich isotherm models. According to the Akaike's information criterion (*AIC*), residual sum of squares (*RSS*) and coefficient of determination (R^2), the Langmuir isotherm was found to well represent the measured sorption data in single systems and the maximum metal sorption capacities Q_{max} onto moss biosorbent were 298 μmol/g for Zn^{2+}, 308 μmol/g for Cd^{2+} and 208 μmol/g for Co^{2+}. Competitive Langmuir equations were used to fit the experimental data from the Zn^{2+}-Co^{2+} binary and Cd^{2+}-Co^{2+}-Zn^{2+} ternary system and simple isotherm curves were replaced by three-dimensional sorption isotherm surfaces and triangular equilibrium plot. Results revealed that the presence of Zn^{2+} more significantly decreased the sorption of Co^{2+} in binary Zn^{2+}-Co^{2+} mixtures than vice versa. In ternary system Cd^{2+}-Co^{2+}-Zn^{2+} the affinity to biosorbent decreased in the order Cd^{2+}>Zn^{2+}>>Co^{2+}.

KEYWORDS
Rhytidiadelphus sp., toxic metals, multi-component biosorption, isotherms, modelling

1. INTRODUCTION

Biosorption represents one of the possible interactions of toxic metals with biological systems in contaminated environment. Sorption with biomaterials has became an alternative to traditional methods of industrial wastewater treatment and it is relatively inexpensive and nonhazardous [1]. The process of metal ions removal by various kinds of biomass e.g. agricultural and fermentation wastes, microorganisms is a well documented phenomenon [2,3].

While much research has been carried out on the uptake of single metal species, little attention seems to have been given to the study of multi-metal systems. A practical consideration to the problem reveals that most of the effluents represent a case of multi-metal situation rather than mono-metal situation [4]. In this regard it is necessary to investigate the simultaneous biosorption of two or more metal ions because these studies may provide additional information on the nature of biosorption process, such as the fraction of adsorption sites being shared with each species, their relative affinities toward these sites, and the mutual interaction between the adsorbed species [5]. That objective can be achieved by investigating the equilibrium using multi-component isotherm models [6] or by using the artificial neural networks [7] and experimental design methodologies [8].

The aim of our work was to study the sorption of cadmium, cobalt and zinc ions by biosorbent prepared from moss *Rhytidiadelphus* sp. from single, binary and ternary systems using radiometric analysis. Competitive Langmuir equations were chosen for analyzing equilibrium data and describing mutual competitive effect of Cd^{2+}, Co^{2+} and Zn^{2+} ions in binary and ternary systems. The choice of metals was made with regard to their industrial use and potential pollution impact.

2. MATERIALS AND METHODS

2.1 Biosorbent preparation

Biosorbent was prepared from the moss *Rhytidiadelphus* sp. collected from the forests of High Tatras Mountains, Slovak Republic. The biomass was washed twice in deionised water, oven-dried for 72 h at a maximal temperature 45°C to avoid the degradation of binding sites. After drying the biomass was milled and sieved. Particle size 300 - 600 μm was used in biosorption experiments.

2.2 Batch biosorption experiments in single and multi-metal systems

Batch biosorption experiments in single-metal systems were carried out in aqueous solutions containing $CdCl_2$, $CoCl_2$ or $ZnCl_2$ in concentration range 100 to 4000 μM and spiked with $^{109}CdCl_2$, $^{65}ZnCl_2$ or $^{60}CoCl_2$.

Biosorption experiments in binary and ternary metal systems were carried out in duplicate series of solutions containing each metal in concentrations varying from 100 to 4000 μM and in various molar ratios 2:1, 1:1, 1:2 in case of Co^{2+}-Zn^{2+} binary system and 1:1:1, 1:1:2, 1:2:1 or 2:1:1 in case of Cd^{2+}-Co^{2+}-Zn^{2+} ternary system. The pH was adjusted to 6.0 with 0.1 M NaOH. Biosorbent (2.5 g/L, d.w.) was added, and the content in Erlenmeyer flasks was agitated on a reciprocal shaker (120 rpm) for 4 h at 20°C. At the end of each experiment biosorbent was filtered out, washed twice with deionised water and radioactivity of both moss biomass and liquid phase was measured. The metal uptake was calculated as $Q = V (C_0 – C_{eq}) / m$ where Q is the uptake (μmol/g, dw), C_0 and C_{eq} is the initial and the final metal concentrations in solution (μmol/L) and m is the amount of dried biosorbent (given in grams).

2.3 Radiometric analysis

For radiometric determination of ^{109}Cd, ^{60}Co and ^{65}Zn in liquid samples and biomass, gamma spectrometric scintillation detectors 54BP54/2-X and 76BP76/3 with well type crystal NaI(Tl) (Scionix, Netherlands) and data processing software Scintivision32 (Ortec, USA) were used.

2.4 Data analysis

To calculate the maximum sorption capacities Q_{max} values and the corresponding parameters of adsorption isotherms non-linear regression analysis was performed by the software ORIGIN 8.0 Professional (OriginLab Corporation, Northampton, USA). The 3-D sorption surfaces for binary system were obtained by plotting the experimental metal equilibrium concentrations C_{eq} on the X and Y axes, against the metal uptake Q_{eq} on the Z axis. To graphically depict the equilibrium in ternary system Cd^{2+}-Co^{2+}-Zn^{2+} triangular diagram was used. The TableCurve 3D 4.0 and SigmaPlot 9.0 (Systat Software, Inc., Chicago, USA) were used for this purpose.

3. RESULTS AND DISCUSSION

3.1 Biosorption from single systems

Biosorption of Cd^{2+}, Co^{2+} and Zn^{2+} ions by biosorbent prepared from moss *Rhytidiadelphus* sp. from single systems occurs mainly within the first 20 min. Removal of metal ions is faster at the initial stage and gradually decreases with the time until saturation. The equilibrium was reached within one hour (data not shown). Equilibrium biosorption data are generally represented in the form of adsorption isotherms. Langmuir and Freundlich isotherms were fitted to the equilibrium data for Cd^{2+}, Co^{2+} and Zn^{2+} ions biosorption on moss biosorbent. Model parameters (Q_{max}, b, K, $1/n$) obtained by non-linear regression analysis as well as statistical parameters (R^2, RSS, AIC_c) are reported in Table 1 and Table 2.

Tab. 1. Langmuir and Freundlich equilibrium parameters for Cd^{2+}, Co^{2+} and Zn^{2+} biosorption by moss *Rhytidiadelphus sp.* from single systems at pH 6.0 obtained by non-linear regression analysis.

Metal	Langmuir		R^2	Freundlich		R^2
	Q_{max} (μmol/g)	b (L/μmol)		K (L/g)	$1/n$	
Zn^{2+}	298 ±7	0.004 ±0.001	0.992	27.7 ±9.2	0.29 ±0.05	0.906
Co^{2+}	208 ±2	0.008 ±0.001	0.996	32.2 ±10.1	0.24 ±0.04	0.855
Cd^{2+}	308 ±14	0.005 ±0.001	0.982	32.1 ±8.0	0.29 ±0.03	0.956

The adequacy of the two models was compared by using the corrected Akaike`s information criterion (AIC_c) and residual sum of squares (RSS). AIC_c is able to answer the question which model is better for mathematical description of Co^{2+}, Cd^{2+} and Zn^{2+} biosorption by *Rhytidiadelphus* sp. The isotherm model with the lower AIC_c value is considered most likely to be correct.

Tab. 2. Comparison of corrected Akaike`s information criterion (AIC_c) and residual sum of squares (RSS) values of Langmuir and Freundlich isotherms for Co^{2+}, Cd^{2+} and Zn^{2+} biosorption by *Rhytidiadelphus* sp. from single systems.

Metal	Langmuir			Freundlich		
	RSS	AIC_c	Akaike´s weight	RSS	AIC_c	Akaike´s weight
Zn^{2+}	**617**	**48.85**	**0.9999**	7045	70.77	$1{,}7\times10^{-5}$
Co^{2+}	**138**	**36.28**	**1**	5508	73.11	1×10^{-8}
Cd^{2+}	**1152**	**49.72**	**0.9564**	2784	55.90	0.0436

As can be seen from Table 2 the Langmuir isotherm fits the data better than the Freundlich isotherm, as is demonstrated by the more homogeneous standard deviation of each observed parameter (Table 1) and by the lower the AIC_c values obtained as well as sum of squares. The maximum sorption capacity Q_{max} at 6.0 obtained from Langmuir isotherm in single systems followed the order $Cd^{2+}>Zn^{2+}>>Co^{2+}$ (Table 1). This indicates higher affinity of *Rhytidiadelphus* sp. for Cd^{2+} and Zn^{2+} than Co^{2+} sorption from single metal solutions. Moreover, this is consistent with the idea that difference in sorption capacity under similar environmental conditions could be attributed to different ionic characteristics of metal ions [9]. Zamil *et al*. [10] demonstrated that metal uptake capacities Q_{eq} of divalent cations by *Staphylococcus saprophyticus* were significantly influenced by covalent index and ionic radius and increased in the order $Ni^{2+} < < \mathbf{Co^{2+}} < Cu^{2+} < Hg^{2+} < \mathbf{Zn^{2+}} < Cr^{3+} < \mathbf{Cd^{2+}} < Pb^{2+}$.

3.2 Biosorption from multi-component systems

The equilibrium data were analyzed using the Langmuir-type equations for multi-component systems [11]. Parameters obtained are presented in Table 3. Biosorption of Co^{2+} and Zn^{2+} ion from binary systems is depicted on Fig. 1 A-C in the form of sorption isotherm surfaces. When Co^{2+} and Zn^{2+} were presented in the solution together, significant reduction of Co^{2+} uptake (Q_{eq}) was observed with increasing concentration of Zn^{2+} ions in solution (Fig. 1A). On the contrary, the presence of Co^{2+} caused only moderate decrease of Zn^{2+} sorption capacity (Fig. 1B).

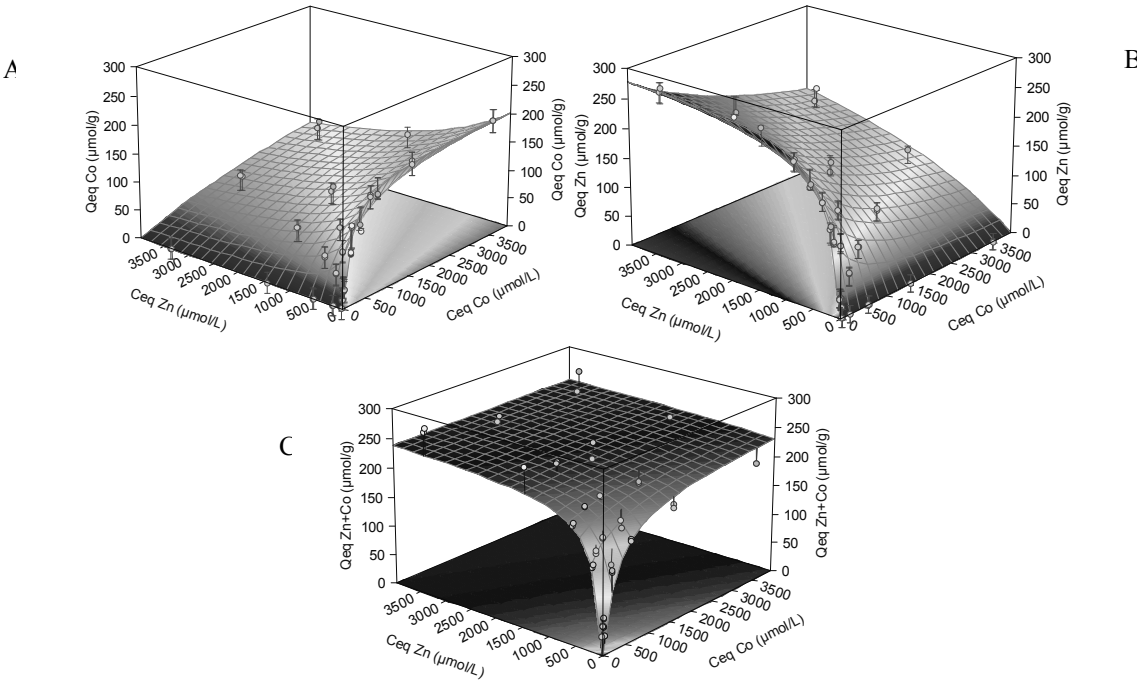

Fig. 1. 3-D sorption isotherm surfaces of Co-Zn binary system: (A) Co sorption (µmol/g) (B) Zn sorption (µmol/g); (C) total metal (Co+Zn) sorption (µmol/g). The surfaces are predicted by the competitive Langmuir model and the symbols are experimental data obtained at pH 6.0 and 20°C. The vertical bars represent 95% confidence interval.

Tab. 3. Equilibrium parameters for Co^{2+} and Zn^{2+} sorption by *Rhytidiadelphus* sp. at pH 6.0 from the system Co-Zn calculated from binary Langmuir type equations.

Model	Q_{max} (µmol/g)	b_{Co} (L/µmol)	b_{Zn} (L/µmol)	R^2	*RMSE*
$Q(Co+Zn)=\frac{Q_{\max}(b_{Co}C_{eqCo}+b_{Zn}C_{eqZn})}{1+b_{Co}C_{eqCo}+b_{Zn}C_{eqZn}}$	248 ± 5	0.003 ± 0.001	0.006 ± 0.001	0.941	18.74
$Q(Co)=\frac{b_{Co}Q_{\max Co}C_{eqCo}}{1+b_{Co}C_{eqCo}+b_{Zn}C_{eqZn}}$	211 ± 5	0.006 ± 0.001	0.009 ± 0.001	0.978	8.91
$Q(Zn)=\frac{b_{Zn}Q_{\max Zn}C_{eqZn}}{1+b_{Zn}C_{eqZn}+b_{Co}C_{eqCo}}$	294 ± 5	0.004 ± 0.001	0.004 ± 0.001	0.992	7.37

The b values reflect the affinity between moss biosorbent and the Co^{2+} and Zn^{2+} ions in binary system. The higher b represents the higher affinity. Almost in all cases b_{Zn} was higher than b_{Co}, what indicates higher affinity of metal binding sites of moss biosorbent for Zn^{2+} ions comparing with the affinity to Co^{2+} ions (Tab. 3). The total metal ions uptake by biosorbent calculated from binary Langmuir model was 248 ± 5 µmol/g.

Sorption equilibrium in ternary system Cd^{2+}-Co^{2+}-Zn^{2+} was well described by the multi-component Langmuir equation [12] (Fig. 2). Total sorption capacity Q_{eq} (Cd+Co+Zn) calculated from the model was 271 ± 4 μmol/g. Similarly as in the binary system, *b* values represent affinity of sorbent to sorbate and the affinity of biosorbent to metal ions decreased in the order Cd^{2+} (b_{Cd} = 0.0038) >Zn^{2+} (b_{Zn} = 0.0035)>>Co^{2+} (b_{Co} = 0.0021). These results confirm that biosorption of Cd^{2+} was most favored by the biosorbent which correlate with single component data.

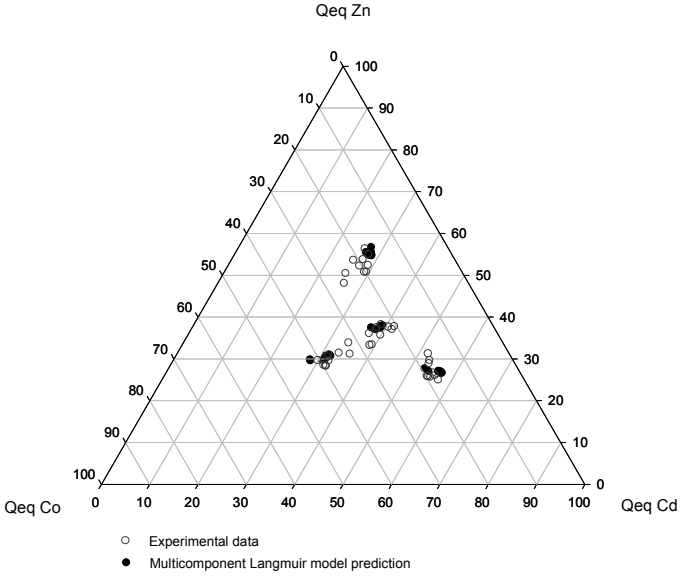

Fig. 2. Graphical representation of Cd^{2+}, Co^{2+} and Zn^{2+} biosorption (μmol/g) from ternary system by *Rhytidiadelphus* sp. (•) experimental data – normalized values, (○) data calculated from multi-component Langmuir model – normalized values.

4. CONCLUSIONS

Investigation of the metal ions removal from aqueous solution by moss biosorbent showed that Cd^{2+}, Co^{2+} and Zn^{2+} biosorption is a rapid process. The experimental equilibrium data of single systems have been well described by the Langmuir isotherm and the maximum sorption capacity Q_{max} decreased followed the order Cd^{2+}>Zn^{2+}>>Co^{2+}. The competitive Langmuir model fits adequately the experimental biosorption data for Co^{2+}-Zn^{2+} binary system. The presence of Zn^{2+} more significantly decreased the sorption of Co^{2+} in binary Co^{2+}-Zn^{2+} mixtures than vice versa. In a ternary system, *Rhytidiadelphus* sp. displayed the sorption of metal ions in the preferential order Cd^{2+}>Zn^{2+}>>Co^{2+}.

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REMOVAL OF COPPER AND ZINC CATIONS BY BIO-MODIFIED BROWN COAL

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ABSTRACT
Knowledge on the mechanism of microorganisms participation in the elements cycle and their role in the environmental equilibrium establishment that leads to new generation of environmental biotechnologies evolution is significantly growing. Biotechnological methods have wide application area in the remediation of heavy metals from wastewaters. We studied bio-modification of brown coal sorption materials with the aim to anhance its sorption properties. The micromycetes (*Aspergillus niger*, *Aspergillus clavatus*, *Penicillium glabrum* and *Trichoderma viride*) have been selected for biological activation of coal samples. The quantitative maceral composition and specific surface of prepared sorbents has been investigated. The sorption of copper(II) and zinc(II) ions from aqueous solutions by this unconventional sorbents was studied. It was found that, for each sorbent the equilibrium establishing time was about 1 hour. The Langmuir adsorption model was applied to describe isotherms and isotherm constants. The isotherms studies revealed that prepared sorbents exhibited relatively high capacity for the metals removal.

KEYWORDS
Brown coal, micromycetes, heavy metals, sorption

1. INTRODUCTION

Numbers of studies of metal accumulation from the metal recovery and/or removal of metal from aqueous solutions have been launched due to a great awareness of the ecological effects of toxic metals released into the environment.

Conventional methods of heavy metals removal from aqueous solutions include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, membrane technologies, and evaporation technology [1,2]. These processes may be ineffective or extremely expensive, especially when the metals occur in low concentrations (1 to 100 mg/L) [1].

In recent years, numerous low-cost natural materials have been proposed as potential source of commercial synthetic sorbents [3]. Natural low-cost materials that have been studied include leaves, mould, peat moss, green algae, fungi, activated carbon fibers, coconut waste, etc. [4-10].

However, new economical, easily available and highly effective sorbents are still needed. Although the adsorption capacities of low-rank coal are lower than of synthetic ion-exchange materials, the substantially lower costs shows great potential for the use of low-rank coal for removing heavy metal ions from wastewater [4].

Knowledge of the porous structure of different coal types is of relevance if the organisms involved are sufficiently small to penetrate part of the structure. This might mean that the surface area available for reaction is considerably increased and could indicate, which coal type would be more susceptible to bio-attack. Considering the possibility of in situ coal conversion in deep streams by either conventional gasification or microbial action, knowledge of the physical and chemical properties of the coal under the prevailing conditions is essential. The fact that microorganisms can be developed in fine pores was reported within the research on the aerobic microbial conversion of phenols adsorbed on active carbon [11].

Micromycetes belong to soil microorganisms, which take part in decomposition processes in the soil. Micromycetes are saprophytes, sponges and live in commensalisms. They attack plastics, glass, the walls of buildings, etc. Micromycetes can decompose the organic matter of vegetable and animal origin in the nature [12-15]. In accordance with theoretical knowledge there was an effort to choose microorganisms for the biological activation of coal samples, of which bearing on the coal matrix was not studied so far. Finally, 4 types of microorganisms were chosen for bio-activation of brown coal samples, such as *Aspergillus niger*, *Aspergillus clavatus*, *Penicillium glabrum* and *Trichoderma viride*. Studied brown coal was after its bio-activation applied as sorbent in removal of Cu(II) and Zn(II) from water solutions [16].

2. MATERIALS AND METHODS

2.1. Brown coal

A sample of Slovak brown coal from Novaky deposit (Hornonitrianske mines, Prievidza), with diameter under 1 mm was selected for an activation by microorganisms. The chemical analysis of the sample showed Ash^d 20.88%, W^a 24.56%, Fe^d_{dat} 1.44%, S^d 3.00%, As^d 380 ppm, HA^{dat}_{total} 12.94%.

The quantitative maceral analysis was performed using the NU 2 microscope by Carl Zeiss-Jena in oil immersion with the refraction index 1.515, at 20°C and wave length 564 nm. Samples of the investigated coal were embedded in to the epoxy resin and were prepared as polished specimen in accordance with ISO 7404-2.

2.2. Sorbent preparation and adsorption experiments

The cultures of selected micromycetes (cultures obtained from Department of Soil Science, Faculty of Natural Sciences of Comenius University in Bratislava) were grown in Sabouraud agar medium (SAB - mycological peptone, maltose, agar, distilled water). Suspension was injected (5 ml) of every 14-day-old culture spores in SAB to the 10 g of brown coal mixed with 10 ml of SAB medium. The cultivation was executed in the dark at ambient temperature.

The leaching process took 7 weeks. After leaching, the suspensions were filtered, washed with distilled water, dried and prepared for adsorption experiments.

Stock solutions of each metal: Cu(II) containing 30-300 mg/L and Zn(II) containing 30-300 mg/L were prepared by dissolving $CuSO_4.5H_2O$ and $ZnSO_4.H_2O$ of analytical grade in distilled water.

All the adsorption experiments were conducted at ambient temperature in a laboratory shaker. Metal solutions of known concentrations were introduced into the glass Erlenmeyer bottles (25 ml) containing defined amounts (10 g/L) of the adsorbent. The bottles were shaken horizontally and the adsorbent was removed by filtration after 1 hour adsorption.

The equilibrium concentrations of heavy metals were determined by atomic adsorption spectroscopy (Varian Spectr AA-30) and the metal uptake was calculated from the difference.

The Langmuir adsorption isotherms have been constructed and the maximum adsorption capacity of the adsorbents has been determined. The pH measurements were carried out with a laboratory pH meter GPRT 1400 A-GL. For Cu(II) sorption the pH reached 5, as Cu(II) ions undergo hydrolysis reactions in water and form insoluble aqueous complexes with increasing pH. Zn(II) sorption experiments were performed at pH 7 for the same reason as in case of Cu(II) adsorption. The surface characteristics of prepared adsorbents were measured by the BET method using the Micrometrics Gemini 2360 apparatus and the surface morphology was studied using the Scanning Microscope TESLA BS 300.

3. RESULTS AND DISCUSSION

Specific surface area of brown coal was determined by the BET method before and after the activation by microorganisms and the results are shown in the Table 1.

Tab. 1. Specific surface area of brown coal sorbents activated by different species of microorganisms

Sample	S1	S2	S3	S4	S6
Microorganism	*Aspergillus niger*	*Aspergillus clavatus*	*Penicillium glabrum*	*Trichoderma viride*	-
Specific surface (m^2/g)	4.94	3.60	3.93	4.34	3.23

The Figures 1 and 2 present the scanning electron micrographs of the samples S1 and S3. SEM micrographs were taken using the Tesla BS 340 electron microscope. Scanning electron micrograph of modified brown coal shown in Figure 1 presents sample S1, which was treated by *Aspergillus niger*, while Figure 2 presents the S3 modified by *Penicilium glabrum*. From both figures is obvious that the aggregates were developed.

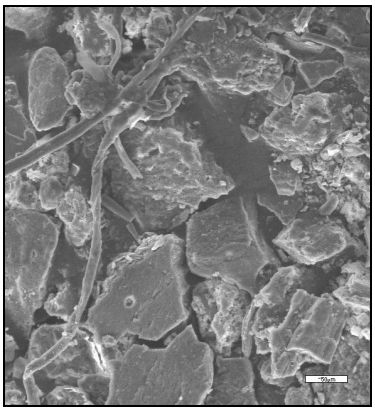

Fig. 1. SEM micrograph of brown coal treated by *Aspergillus niger* (S1)

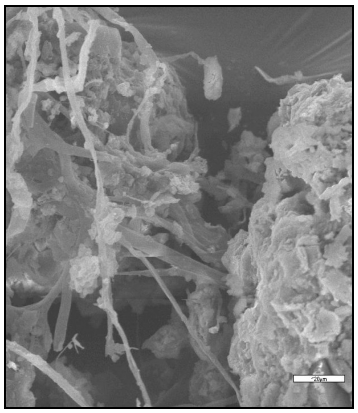

Fig. 2. SEM micrograph of brown coal treated by *Penicillium glabrum* (S3)

The microscopical evaluation confirmed that the investigated sample is brown coal. Maceral groups of huminite were hardly gelified. According to the intensive colouring of liptinite maceral group (dark brown) we can state that this represents a coal rank with low coalification. The content of inorganic materials as well as of clay minerals, sulphides, carbonate minerals etc., was very high, because the polished specimens were prepared from the original non-washed samples.

Table 2 shows the results of maceral analysis of coal affected by different micromycetes species. The results are corrected to the clear coal mass. From the results of maceral analysis we can say that the most notable changes in maceral composition were in the sample affected by microorganisms from the species *Penicillium glabrum*.

Tab. 2. Maceral analysis of coal samples affected by different micromycetes species

Sample	HT (%)	HK (%)	HD (%)	L (%)	I (%)
Coal	28.0	57.6	1.0	11.3	3.1
Coal + *Aspergillus niger*	17.7	57.8	10.2	8.6	5.7
Coal + *Aspergillus clavatus*	19.2	58.4	8.4	11.3	2.7
Coal + *Penicillium glabrum*	1.9	63.2	10.9	19.8	4.2
Coal + *Trichoderma viride*	4.2	62.8	24.0	2.6	6.4

* HT – humotelinite, HK – humocollinite, HD – humodetrinite, L – iptinite, I – inertinite

Figures 3 and 4 present adsorption isotherms of copper and zinc adsorption by bio-modified adsorbents. Experimental data were fitted by Langmuir equation. The correlation coefficient ranged from 0.96 to 0.99.

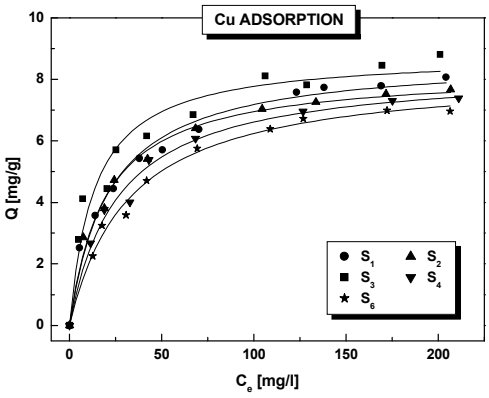

Fig. 3. Langmuir adsorption isotherms of Cu adsorption on brown coal treated by different microorganisms (samples S1 – S6)

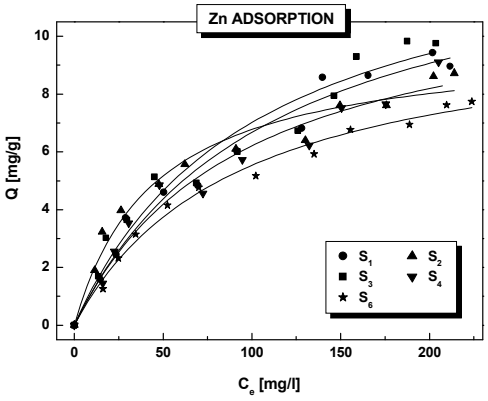

Fig. 4. Langmuir adsorption isotherms of Zn adsorption on brown coal treated by different microorganisms (samples S1 – S6)

Experimental results showed that maximum increase of metals uptake for biologically activated brown coal was achieved by the activation by *Penicillium glabrum* (sample S3), i.e. 8.8 mg Cu(II)/g of sorbent and 14.3 mg Zn(II)/g of sorbent.

4. CONCLUSION

The micromycetes evoked certain changes in maceral composition of coal. The most notable changes were influenced by *Penicillium glabrum*. The results of maceral analysis can be used only as orienting as they are strongly influenced by inorganic material in the original non-washed sample of coal from Novaky deposit, hence all results can be referred only to used coal sample.

The results of adsorption experiments showed that selected type of sorbent preparation had positive influence on sorption properties of activated material and prepared sorbents had good affinity to selected metals.

The results confirmed that there is a possibility to prepare the sorbents by new non-conventional methods and the obtained results appear as promising for the research and development in utilisation of non-energetic coal.

ACKNOWLEDGEMENTS

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TALVIVAARA SOTKAMO MINE – BIOLEACHING OF A POLYMETALLIC NICKEL ORE IN SUBARCTIC CLIMATE

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ABSTRACT
The main activity of the Talvivaara Mining Company Plc. (the Group) is the development and exploitation of the Talvivaara deposits in Sotkamo, Finland using bioheapleaching. The Talvivaara deposits comprise one of the largest known sulfide nickel resources in Europe with 642 million tonnes of ore in measured and indicated categories, sufficient to support anticipated production for a minimum of 60 years. The mine started in late 2008 and will have an annual nickel output of approximately 33,000 tons when it reaches full production. In addition, the mine will also produce zinc (approximately 60,000 tpa), copper (approximately 10,000 tpa) and cobalt (approximately 1,200 tpa) as by-products of the process. The Group has demonstrated the viability of bioheapleaching technology for the extraction of nickel in a large on-site pilot trial using the Talvivaara ore. The three year pilot has shown that the leaching process also works well in the subarctic climatic conditions of Eastern Finland.

KEYWORDS
Bioheapleaching, black schist, nickel, zinc, copper, cobalt, Talvivaara

1. INTRODUCTION

1.1. General

The Talvivaara deposits are located in the southern part of the Kainuu belt, also known as the Kainuu schist zone in Eastern Finland approximately 35 kilometres south-east of the town of Kajaani and approximately 30 kilometres south-west of the town of Sotkamo. The belt is approximately 200 kilometres long with a maximum width of approximately 40 kilometres.

The Talvivaara deposits comprise two different polymetallic orebodies, Kuusilampi and Kolmisoppi. The deposits are located approximately three kilometres apart. The deposits are outcropping and relatively easy to mine. The mineral resources have been classified by Australian JORC code with 0.07% Ni cut-off at 1004 million tons, containing 0.23% of nickel, 0.51% of zinc, 0.13% of copper and 0.02% of cobalt. 80% of the mineral resource is in measured and indicated categories.

1.2. History of geological work and exploration

Geological work in the Talvivaara area commenced in the early 1900's when geological mapping of bedrock was conducted by the Geological Survey of Finland ("GSF") and continued through 1951. Between 1951 and 1962, various Finnish companies carried out exploration activities in the area. The result of this phase led GSF to commence detailed exploration in the area in 1977. As a result of the work done by GSF, two polymetallic deposits, Kuusilampi and Kolmisoppi, were established. Outokumpu acquired the deposits from the Finnish State in 1985 and the mining license covering the Talvivaara deposits was granted to Outokumpu in 1986. Between 1989 and 1992, Outokumpu focused on further geological and metallurgical work on the Talvivaara deposits.

1.3. Focus on the environment

The Environmental Impact Assessment relating to the Talvivaara Project was carried out in 2003-2005. The Environmental and Water Management permit was granted in 29 March, 2007 by the Northern Finland Environmental Agency.

An extensive environmental monitoring plan includes ore excavation, leaching, traffic, emergency situations etc. Close look is in water and air discharges in addition to other environmental impact monitoring. Special monitoring is in place for some rare species including the Russian flying squirrels, bats, rufous milk-caps and ants.

2. PRODUCTION PROCESS

2.1. Overview

The mining method selected at Talvivaara is large scale open pit mining. Materials handling covers all physical ore processing steps from the primary crusher to the heaps. After one and one-half years of bioleaching on the primary pad, the leached ore will be reclaimed, conveyed and restacked onto the secondary heap pad. After secondary leaching, the barren ore will remain permanently on the secondary heaps. In the metals recovery process, the metals will be precipitated from the pregnant leaching solution ("PLS") using hydrogen sulfide. The resulting products will be intermediates to be transported for further processing in refineries operated by the Group's customers.

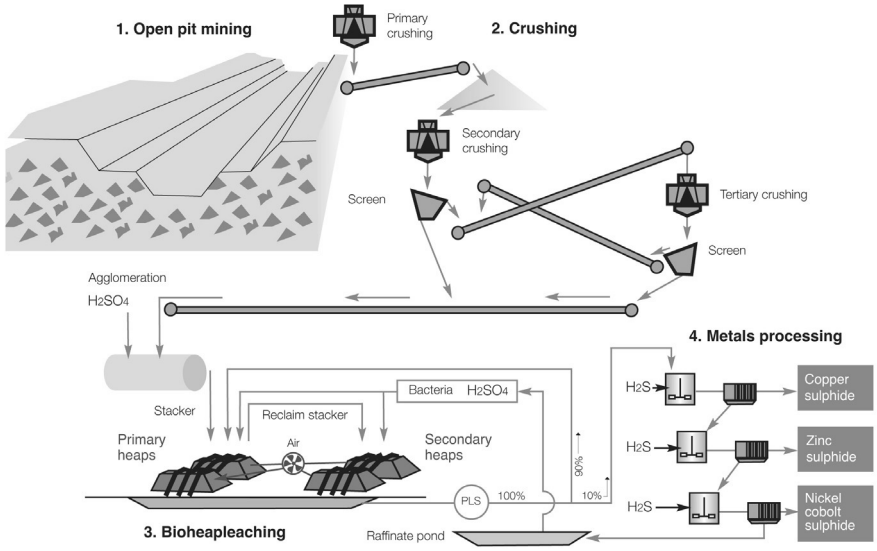

Fig. 1. Talvivaara process chart.

2.2. Open pit mining

Annual ore production is approximately 15 million tons. Sufficient areas to extend the pit, will be prepared in subsequent years, normally a year prior to when mining is scheduled to commence. Any overburden or moraine not required for road, perimeter wall or other construction, will be stockpiled for later use in rehabilitation. Ore and waste are extracted using conventional large scale open pit drill and blast methods. A fleet of self-propelled diesel hydraulic track drill rigs are used. Fragmentation by blasting is preferred to crushing because of lower costs. It is also beneficial to create more fracturing at blasting stage, and it increases the surface area and therefore improves leaching solution entry and consequently leaching efficiency. As a result, the drilling pattern and hole sizes are on average smaller than in mines with similar production rates.

2.3. Materials handling and bioheapleaching

Materials handling in Talvivaara cover all the physical ore processing steps from the primary crusher to the final locations on the secondary heaps. During the process, the ore is crushed and screened in three stages. After primary crushing, the ore is conveyed to primary and secondary crushing, screening and is agglomerated for bioheapleaching. Agglomeration takes place in a rotating drum, where diluted sulfuric acid is added to the ore in order to consolidate the fine ore particles with coarser ore particles. This preconditioning step makes the ore permeable to air and water for bioheapleaching. After agglomeration, the ore is conveyed and stacked ten meters high on the primary heap pad for one and one-half years of bioheapleaching. The heap pad is equipped with piping, laid on the bottom of the pad, through which low-pressure fans supply air to the stacked ore. From the top, the heap is irrigated with leaching solution, which is collected from the bottom of the heap. A ten percent side flow is taken for metals recovery and the rest of the solution is diluted with pure water in order to keep the amount of solution constant.

After one and one-half years of leaching on the primary pad, the leached ore is expected to be reclaimed, conveyed and restacked onto the secondary heap pad, where it will be leached further in order to recover metals from those parts of the primary heaps, where leaching solution has had poor contact. Such areas include, for

example, the slopes of the heaps and areas between channels formed by the circulating leaching solution. After secondary leaching, the barren ore is expected to remain permanently on the secondary heaps.

2.4. Metals recovery

In the metals recovery process, the metals are precipitated from the PLS using hydrogen sulfide. The resulting products are intermediates, such as copper and zinc sulfides and a mixed nickel cobalt sulfide. These intermediates are transported for further processing in refineries operated by Talvivaara's customers. The recovery process also produces gypsum as a secondary product which will be collected and remain in a separate pond on the mining site.

2.5. Water management

The water management plan for the Talvivaara Project area includes all relevant pipelines, ponds and pump stations related to the processes outside the metals recovery plant area. It also includes surface water management, effluent treatment and other surface water construction projects. Water management plays an important role at the operation. The most important component is the recycling of the leaching solution from the irrigation pond to the heap and thereafter to the PLS pond. From the PLS pond, approximately 90 per cent of the solution is recycled back to irrigation to increase the metal grade, while about 10 per cent is lead to metals recovery. After metals precipitation, the remaining solution goes into the raffinate pond to pH adjustment and is reused to irrigate the heaps.

2.6. Land and Infrastructure

Prior to construction of the pilot operation in 2005, the Talvivaara deposits had no existing mining facilities. The development of the infrastructure and services included:

- Access roads, internal roads and railway;
- Power supply;
- Fuel services;
- Drinking water supply/water management;
- Metal recovery facility;
- Sewage and waste management.

The first construction phase commenced in February 2007 with construction of roads to allow good access to the site and between the construction areas. The majority of all earthworks were completed by the end of 2008. The Talvivaara Project operational area totals 61 km^2 and requires construction of buildings with total space of approximately 63,000 square meters.

3. BIOHEAPLEACHING AT TALVIVAARA

Bioheapleaching has been chosen for the Talvivaara Project based on its favourable capital and operational cost profile and the good performance data obtained with the technology in earlier trials with the Talvivaara ore. The Group's application of the bioheapleaching technology has its origins at Outokumpu Research, where it has been developed in conjunction with the Talvivaara ore since 1987. All the rights relating to the accumulated research and development data on bioheapleaching were transferred to the Group in connection with the acquisition of the mining licenses in February 2004.

The Group has developed bioheapleaching and metals recovery from leaching solutions in collaboration with Tampere, Helsinki and Lappeenranta Universities of Technology and OMG Kokkola Chemicals Oy. The research and development work has been partly funded by Tekes (Finnish Funding Agency for Technology and Innovation), through capital loans and grants since early 2004, and the Group has benefited from cooperation with the international, European Union funded Bioshale Project. For example, GSF and the Group set up, as part of the Bioshale project, a 110 tonne pilot trial in March 2005 to test bioheapleaching of Talvivaara ore. The trial was successfully started in -20ºC conditions, thus serving as an important indicator of the feasibility of the process in subarctic conditions prior to moving to larger scale on-site trials [1]. Smaller scale laboratory trials have provided the Group with an understanding of the key parameters of bioheapleaching e.g. particle size, pH value, temperature, oxidation and aeration rate.

In order to validate the bioheapleaching process on a larger scale, a demonstration scale on-site pilot study was commenced at Talvivaara in May 2005. Heap irrigation was started in August 2005 and metals recovery in November 2005. The demonstration heap was constructed of 17,000 tons of Talvivaara ore. Similar to the laboratory scale trials, the Group varied the key parameters to confirm the optimum conditions. The results from the demonstration plant were substantially better than anticipated based on laboratory and other pilot results and

confirmed the viability of the Talvivaara Project. It is assumed that most of nickel and zinc will be leached in the primary heap within one and one-half years. Metal recovery started in November 2005. The leaching rates were calculated from the chemical analyses of the solution taken away for metal recovery. Every tenth sample was sent to an accredited laboratory in order to be sure that the results are correct.

The recovery rates were higher than anticipated. Nickel recovery reached 80% within 400 days. The corresponding zinc recovery was 80% in 480 days. The demonstration plant has shown that the assumed recoveries for nickel and zinc can be reached [2,3].

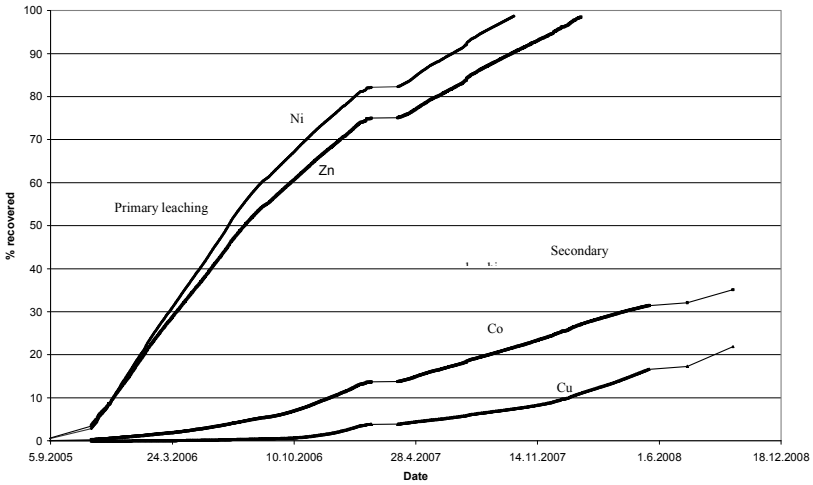

Fig. 2. Leaching recoveries in Talvivaara pilot.

The recovery of cobalt remained low and the leaching rate was progressing slowly over the first 500 days. Only 2.5% of copper were recovered. Copper is in chalcopyrite. The main part of cobalt is in pyrite. As sulphide minerals have semiconductor properties, galvanic interactions appear when there is an electrical contact among mineralogical phases. During dissolution of a mineral assembly of different sulphides, those minerals that have the highest rest potentials behave as cathodes which mean that they are galvanically protected and their leaching is hindered until the minerals with lower rest potentials have been leached. The electrochemical potentials of chalcopyrite and pyrite are higher than the ones of pyrrhotite, pentlandite and sphalerite. The leaching of copper and cobalt proceeded well in the secondary leaching phase of the demonstration plant.

The on-site pilot trial also demonstrated that the leach solution temperature and temperatures inside the heaps are practically independent of the surrounding environmental conditions. The outside temperature in the area varied between approximately -30ºC and +30ºC during the trial, but the leach solution flowing out of the heaps remained steady at +40-50ºC, and the temperatures measured inside the heap at approximately +50ºC, with temperatures as high as +90ºC measured in some parts of the heaps.

4. CONCLUSIONS

Extensive research and pilot trials have proven bioleaching a feasible and environmentally sound technology in treating Talvivaara low grade sulfide nickel deposits in subarctic conditions. Talvivaara Project has progressed in the set timetable and budget, starting in late 2008. In July 2009 Talvivaara Project Ltd. changed its name to Talvivaara Sotkamo Mine Ltd. The mine and the plant are in industrial use since the beginning of year 2009.

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BEHAVIOUR OF Fe, Mg AND Ca IN ACID MINE DRAINAGE AND VARIOUS EXPERIMENTAL SOLUTIONS BY DIFFERENT STRAINS OF *ASPERGILLUS NIGER* SPECIES

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ABSTRACT
This article analyzes the ability to accumulate Fe, Mg and Ca from acid mine drainage (AMD) at the locality Smolník by micromycetes. Four strains of the *Aspergillus niger* (*An*) species originated from different types of environment in the experiments were used: the *An*-G strain (the locality of Gabčíkovo, Eutric Fluvisol), the *An*-P strain (the locality of Pezinok-Kolársky vrch, mining region with elevated amounts of As and Sb), the *An*-N strain (the locality of Nováky, mining region with elevated amounts of As and S), the *An*-Š strain (Banská Štiavnica-Šobov, the locality impacted by an acid sulphate weathering and extremely low pH).
In the most cases the accumulation of Mg was the highest in comparison to accumulation of Fe. Accumulation of Ca was very low. Among the tested microfungi, the highest accumulation was noted by the strain *An*-N 55 % of Mg and by the strain *An*-Š 54 % of Fe from the solution 3.

KEYWORDS
Acid mine drainage, experimental solutions, micromycetes, *Aspergillus niger* strains, Fe, Ca, Mg accumulation

1. INTRODUCTION

Mining activity is one of those antropogenic activities that cause the heavy metals and chemical elements to penetrate in the environment. The by-products of mining, as the disposal sites and mullock tips with high amount of minerals as well as flooded mines are the source of an acid mine drainage (AMD). AMD poses a serious environmental problem with a negative impact on the surrounding aqueous environment and the stream sediments in terms of the increased acidity, decreased oxygen and the heavy metal release. The region of Smolník is a typical example of the production and occurrence of the AMD. Their composition is strongly affected especially by weathering of sulphides. The evolution of sulphuric acid leads to the leaching of copper and other elements from ores. At the Pech mine locality, very high concentrations of the Fe, Mg and Ca elements had been found, often exceeding the limits set by the government ruling (NV SR No. 296/2005 Z.z.) [1]. The utilization of the removal or accumulation ability of micromycetes in the process of the heavy metal ions elimination, respectively reduction, from waste water, should be one option of the soil and water decontamination.

2. MATERIAL AND METHODS

2.1. Micromycetes

The *Aspergillus niger* TIEGH. species is one of the filamentous fungi that are commonly found in different components of the environment. In our experiments we used four different strains of this species, isolated from various types of the environment.

The 1st *Aspergillus niger* strain (*An*-G) was isolated from Eutric Fluvisol, pH H_2O/KCl = 7.7/7.4), in the Gabčíkovo region. The 2nd *Aspergillus niger* strain (*An*-P) was isolated from the stream sediment of the Blatina river with elevated natural amounts of As (363 mg/kg) and Sb (93 mg/kg), pH H_2O/KCl = 5.3/4.8, in the Pezinok mining region. The 3rd *Aspergillus niger* strain (*An*-N) was isolated from the coal dust influenced by As (400 mg/kg), pH H_2O/KCl = 3.3/2.9, in the Novaky mining region. The 4th *Aspergillus niger* strain (*An*-Š) was isolated from the probe No. 15 in the 24 m long sampling line at the Šobov locality. This locality is impacted by an acid sulphate weathering, extremely low pH (H_2O/KCl = 3.0/2.7) and high exchangeable contents of Al and Fe [2,3].

2.2. Biomass preparation

The strains were cultivated in pure culture on Sabouraud agar (SAB, Himedia, Mumbai, India). For the preparation of mycelia biomass 5 ml of conidia suspension in distilled water added to 45 ml of liquid SAB medium were used. The cultivation of mycelia took place at 25°C for 10 days. After then, mycelia were filtered, flushed in de-ionized water (Water Pro LS, Labconco, USA) and brought to 50 ml of the AMD or experimental solutions. The stationary accumulation went on for 24 hours at the 25°C temperature. All experiments were performed in three repetitions, and the results reflect the average values.

2.3. Removal of elements

In the experiments we used natural AMD from the Pech mine at the Smolník locality (year 2008) and three experimental solutions. Experimental solution 1 present a mixture of Fe [176 mg/l], Mg [234 mg/l] and Ca [98 mg/l] also with concentration of elements similar to AMD, experimental solution 2 present 10x diluted original solution 1, Fe [17.4 mg/l], Mg [22.6 mg/l] and Ca [9.2 mg/l], experimental solution 3 present 100x diluted original solution 1, Fe [1.67 mg/l], Mg [2.35 mg/l] and Ca [1.14 mg/l]. Concentrations of the individual elements Fe [201 mg/l], Mg [229 mg/l] and Ca [88 mg/l] prepared separately were also used for accumulation of the mentioned elements.

As a control sample de-ionized water (Water Pro LS, Labconco, USA) were used. The amounts of the Fe, Ca and Mg elements accumulated by microscopic fungi from the solutions was determined by using the method of the atomic absorption spectrometry (AAS). We evaluated the amounts of the elements in the samples according to the calibration curves of the selected elements.

3. RESULTS AND DISCUSSION

The *Aspergillus niger* species belong to the filamentous microfungi which can accumulate various heavy metals from waste waters [4,5], from mine waters [6] and from different experimental solutions [7,8].

The valley of the Smolnik creek is the historical mine region where Fe, Cu, Ag and Au were mined since the 14th century till the 1990 year. The surface waters penetrating through the mine region are enriched by metals leading to decrease of their pH values. The acidity of the mine waters is caused by sulphure mineral oxidation as well as by presence of bacteria *Acidothiobacillus* and *Leptospirillum* genera, which accelerate the bio-chemical oxidation of pyrite and sulphure [1].

The aim of our experiments was to study the accumulation of the Fe, Mg and Ca, secondary biogenic elements, the quantities of which in the AMD exceed the limits (Tab. 1).

Tab. 1. The values of the AMD indicators from the Pech mine.

Indicator	2006	2008	2009*	Limits
pH	3.88	3.95	2.75	6 - 8.5
Fe (mg/l)	463	365.6	214	2
Mg (mg/l)	344	333.4	265	100
Ca (mg/l)	176	144.2	133	200

*values introduced in the text

Accumulation of studied elements from AMD is relatively low (Tab. 2) with the greatest ability by *Aspergillus niger* strains to accumulate of Mg (25 mg/l on average), less of Fe (22 mg/l on average). All strains under the study showed very limited ability for accumulation of Ca (2.7 mg/l on average).

From experimental solution 1 we have noted accumulation of the studied elements on the same level like from natural AMD, Mg (21 mg/l on average), Fe (14.25 mg/l on average) and the lowest value Ca (5.25 mg/l on average). The statement of accumulation in percentage is relatively higher from the solution 2 (10x diluted original solution 1) with the maximum amount of Fe (5.1 mg/l = 29%) and from solution 3 (100x diluted original solution 1) with amount of Fe (0.9 mg/l = 54%) and Mg (1.28 mg/l = 55%), see Tab. 3.

Tab. 2. The accumulation of Fe, Mg, Ca from the natural AMD by *A. niger* strains in (mg/l) and in %.

Aspergillus niger strain	Fe (mg/l)	Mg (mg/l)	Ca (mg/l)
An - G	33	30	2
An - P	35	25	3
An - N	6	23	3
An - Š	14	22	3
average	22	25	2.7
%	3 - 16	8-11	2

On the other hand, from experimental solution 2 and 3 it was not possible to notice the accumulation of Ca by *Aspergillus niger* strains (Tab. 3). This element is a component of cell walls and mitochondrial membranes. On Ca surplus microorganisms react by excreting of Ca from cells in to the environment. Therefore the amount of Ca after accumulation was in the solutions 2 and 3 higher than in the original solution. This fact we suppose is caused by the release of Ca from the cell walls and mitochondrial membranes into the measured solutions.

Tab. 3. The accumulation of Fe, Mg, Ca from the solution 1 by *A. niger* strains in (mg/l) and in %.

Aspergillus niger strain	Solution 1			Solution 2		Solution 3	
	Fe (mg/l)	Mg (mg/l)	Ca (mg/l)	Fe (mg/l)	Mg (mg/l)	Fe (mg/l)	Mg (mg/l)
An - G	17	23	6	5.1	2.8	0.66	0.85
An - P	12	22	6	3.5	3.4	0.68	0.93
An - N	14	21	2	3.2	2.8	0.71	1.28
An - Š	14	18	7	3.1	3.3	0.9	1.07
average	14.25	21	5.25	3.7	3.1	0.74	0.78
%	7-10	7-10	2-7	18-29	12-15	41-54	36-55

In the accumuluation of studied elements from the individual solutions prepared separately (Tab. 4) we have noticed the same situation like in natural AMD (Tab. 2) or in solution 1 (Tab 3). It means, the highest was the accumulation of Fe (23.5 mg/l on average) in the range from 7 to 15%, less of Mg (12.5 mg/l on average) in the range from 4 to 7% and the lowest was accumulation of Ca (5.25 mg/l on average) in the range from 5 to 7% (Tab. 4).

Tab. 4. The accumulation of Fe, Mg, Ca from individual solutions by *A. niger* strains in (mg/l) and in %.

Aspergillus niger strain	Fe (mg/l)	Mg (mg/l)	Ca (mg/l)
An - G	30	16	6
An - P	23	11	6
An - N	15	9	5
An - Š	26	14	4
average	23.5	12.5	5.25
%	7-15	4-7	5-7

The primary interaction between micromycetes and the chemical elements occur at the cell wall level. The main components of the cell wall are polysaccharides, primarily chitin and chitosane as well as proteins, lipids and other substances. The cell wall contains phosphate, carboxyl and hydroxyl biosorption sites which offer extensive possibilities for binding metals through different mechanisms, predominantly ion exchange and coordination. A lot of metals such as Zn, Pb, Cu, Cd, Hg, Fe have been reported as being readily taken up by chitin what is also dependent on pH of the solution with the 3 - 4 optimum. When two or more transition metal ions are present in the solution together, the cation that forms the most stable complex with the cell wall polymer will be preferentially collected leaving most of the other cations in solution. Alkali metals, ammonium, Mg and Ca are not sequestered by chitin [9,10]. Primarily amine and carboxyl groups of *Aspergillus niger* species are active by the heavy metal bindings to the cell wall, unlike phosphate groups and lipid fractions [11].

The aim of our next study will be the relationship between pH of solutions before and after accumulation by the *Aspergillus niger* strains and the amount of accumulation of study elements.

4. CONCLUSIONS

We observed the accumulation of the Fe, Mg and Ca elements by four strains of *Aspergillus niger* species, which were extracted from various types of the environment. In average the values for the accumulation of the studied elements from AMD were in the order: Mg (25 mg/l) > Fe (22 mg/l) > Ca (2 mg/l); from the solution 1: Mg (21 mg/l) > Fe (14.25 mg/l) > Ca (5.25 mg/l); from the solution 2: Fe (3.7 mg(/l) > Mg (3.1 mg/l); from the solution 3: Mg (0.78 mg/l) > Fe (0.74 mg/l). The accumulation from the individual solutions prepared for each element separately was in the order: Fe (23.5 mg/l) > Mg (12.5 mg/l) > Ca (5.25 mg/l). In most cases the accumulation of Mg was the highest in comparison to accumulation of Fe and Ca.

With regard to the studied strains of microfungi the accumulation of the studied elements in percentage was in the order: *An*-P 16% of Fe and *An*-G 10% of Mg from AMD; *An*-G 10% of Mg and Fe (from solution 1); *An*-P 20% of Fe and 15% of Mg from solution 2; *An*-N 55% of Mg and *An*-Š 54% of Fe from solution 3. The strain *An*-G 15% of Fe and 7% of Mg from the individual solutions prepared for each element separately.

ACKNOWLEDGEMENT

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BIOGEOTECHOLOGY OF ANTIMONY ORES AND CONCENTRATES

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ABSTRACT
Thiobacillus ferrooxidans was used for selection of Hg minerals from antimonite and for transformation of antimonite into commercial product of antimony trioxide. Bacterial accumulated culture *Desulfovibrio desulfuricans* have been employed as chemical agents of the flotation and leaching. Encouraging results have been obtained in isolation antimony into the solution (recovery 96-98%).

KEYWORDS
Ore, antimony, flotation, leaching, biooxidation.

1. INRODUCTION

One of acute problems is rational use gold of antimony ores. The given problem is characterized by two basic facts distinguishing her from problems of extraction of gold from persistent pyrite - arsenopyrite and coal-bearing of ores. Antimony in gold ores is not only chemical depressor gold in cyanic process (one of attributes of technological persistence of ores), but also and accompanying valuable component. Forms of presence of antimony in gold raw material are extremely various: they are submitted more than 18 minerals, each of which differently behaves at enrichment and at cyanidation concentrates.

The abundance of variants of processing used in the industry antimony gold ores is observed. These variants reflect 2 basic directions of technology.

1. Preliminary removing antimony from ores methods of flotation or chemical enrichment (sulfide -alkaline, ferrochlorine leaching) with the subsequent extraction of gold from antimonyless products cyanidation.
2. Direct cyanidation a concentrate in a special mode with translation in a solution of gold or hydrochlorination of slags with translation in solutions of both metals (Au, Sb) with their subsequent division into stages of processing of solutions up to the appropriate commodity products.

The major direction is creation of highly effective, ecologically safe processes of complex processing antimony, gold - antimony ores and concentrates, including new technologies of processing, Hg-Sb, Sb, Au-Sb the ores, allowing to take Sb, precious metals with high technical -economic parameters on a basis achievement of fundamental physical and chemical sciences, with the purpose of improvement of ecological conditions at the metallurgical enterprises.

To actual problems can also are referred:
Hydrometallurgy processing flotation concentrates after preliminary biochemical oxidation antimonite;
For increase of contrast physical and chemical, sorption and flotation properties sulfide, volume number of antimony minerals, applied processing by their microorganisms and carried out oxidizing and regenerative bacterial reactions.

A widely available cyanidation process is not applicable for gold extraction from Sb_2S_3-Au ores and concentrates, because of large cyanide consumption due to Sb reaction with NaCN and the locking of Au in the sulfide matrix. The problems of antimony leaching can be solved by applying and oxidizing acid leach. Other investigators have used ferric chloride (to leach antimonite (stibnite) obtaining antimony trioxide [1,2].

A number of enzymes participate in the above reactions. Many studies have been conducted using sulfate - reducing bacteria (SRB) in flotation of oxidized antimony and lead ores, as a desorbent and depressor in flotation of concentrates, for selection of lead from zinc minerals, and for the separation of molybdenite from chalcopyrite [3].

2. Materials and Methods

An apparatus, specially designed for bacterial leaching, was also employed. Ores and concentrates for study were sterilized by boiling. In some experiments the solutions were filtered from insoluble residue. pH and the number of bacterial cells and antimony content were measured.

For research purposes a SRB strain was isolated from the Tyrny-Auz molybdenum-wolfram deposit. Postgate medium was used to cultivate the SRB under laboratory conditions. Using a direct observation method a maximum of $220x10^6$ cells$\cdot$ ml^{-1} of SRB were found out to grow on the fourth day of culturing. During this

period of time 400 mg· 1^{-1} of soluble H_2S was obtained. SRB a ready-to-employ reagent solution of H_2S for the leaching process after four days of growth. *Thiobacillus ferrooxidans* were cultured from the Bakarchinsk and Olimpiadinsk deposits. The experimental testwork was performed in 250-ml Erlenmeyer flasks using 9K medium.

3. RESULTS AND DISCUSSION

3.1. The selective flotation and separation of cinnabar from antimonite

The selective flotation and separation of cinnabar from antimonite minerals using *T. ferrooxidans* is studied. Bacterial conditioning of 5 h did not affect cinnabar flotation (recovery 89.6%), while the antimonite recovery by flotation decreased from 89 to 6.2%; this led to almost complete selection of the minerals. As a result of bacterial oxidation, antimonite is converted to antimony trioxide, the mineral senarmontite. *T. ferrooxidans* oxidizes the surface of antimonite crystals while cinnabar remains intact. As a result, HgS is floated and extracted into the concentrate, while Sb_2S_3 is coated by a fine film of oxides (Sb_2O_3) and removed in the tailings.

Kenzhalov et al. [4] from the Institute of Metallurgy and Mineral Processing, Republics Kazakhstan, have selected for a heterothroph, *Pseudomonos aureofaciens*, from a Kazakhstan mineral deposit. They have studied in detail the influence of this heterothroph on antimonite, defining of the kinetics of the reaction, the order of reaction, the velocity constant, and the maximum velocity of product formation – (M_{max} and the Michael's constant, K_m). Rapid dissolution of antimony from antimonite was obtained in the presence of the bacteria ($0.11X10^{-4}$); without the bacteria the rate was $0.29X10^{-5}$. Bacterial affinity to antimony was quite high ($К_м$ = 0.07-0.37). Dissolution of sulfur surpassed that of antimony.

At bacterial processing during 30 mines the mercury -antimony concentrate with the contents of 4.0% Hg, extraction of mercury of 85.2% is allocated. The content of mercury is almost higher 1.15%, than at use of a mix of peroxide of hydrogen and bichromate sodium (contents) Hg of 2.85% and extraction of mercury of 84.5%).

Restoration of the oxidized surface of a mineral up to sulfide of antimony carried out SRB bacteria *Desulfovibrio desulfuricans*. Probably, in addition to take 7-11% due to the oxidized forms of antimony. Processing by these bacteria of the oxidized minerals of antimony allows taking them on 85-92%.

3.2. Mechanism of transformation of antimonite to antimony trioxide by Th. ferrooxidans

Information on antimonite biooxidation is of certain interest. According to the results of investigations carried out by Irgiredmet, Sb_2S_3 oxidation with Th. ferrooxidans was described:

$$2Sb_2S_3 + 12O_2 + 6H_2O = Sb_4O_6 + 6H_2SO_4 \quad (1)$$

Antimonite biooxidation realized in relatively pliant regime is to improve the technological characteristics of cyanided material due to the release of gold associated with Sb_2S_3 and transformation of antimony to the less active chemical form. It was established that upon bacterial oxidation of gold-arsenic concentrates by *Th. ferrooxidans* occurred within 100-120 h, the high degree of sulfide oxidation is achieved (%) 96-98 of arsenopyrite, 97-98 of pyrrhotite, 92-95 of antimonite, and 65-84 of pyrite .

Under biochemical leaching was observed greater amount of the oxidized forms of stibium and also its oxides of high valences regardless of initial contents of stibium minerals. After bacterial influence an intensity of lines D 5.05 D 5.66 A, belonging to Sb_2S_3.

Thionic bacteria oxidized animonite sulfur and formed antimony trioxide of cubic syngony of senarmontite type according to the following reactions:

$$3\ Sb_2S_3 + 3Fe_2(S0_4)_3 + 6H_2O = Sb_4O_6 + 12FeS0_4 + 3S \quad (2)$$

$$S + H_20 + 3/20_2 = H_2SO_4 \quad (3)$$

$$2FeS0_4 + l/20_2 + H_2SO_4 = Fe_2(S0_4)_3 + H_20 \quad (4)$$

$$Sb_4O_6 + H_2SO_4 = 2Sb_2(S0_4)_2 + 6H_2O \quad (5)$$

The rhombic form of Sb_2O_3 is obtained in hydrolysis of antimony-chloride solutions. The action of diluted H_2SO_4 on antimony sulfate results in hydrolysis with formation of antimonyl sulfate:

$$Sb_2(S0_4)_3 + 2H_20 = (SbO)_2S0_4 + 2H_2SO_4 \quad (6)$$

3.3. Sulfate - reducing bacteria at flotation and irishmen ores

Three ways of application sulfate - reducing bacteria (SRB) are considered as modifying as flotation regulators agent in flotation of minerals as sulfider, depressioner, and desorptioner the collector from a surface of sulfide minerals. It is in details investigated flotation minerals with SRB.

The flotation minerals and ores depends on concentration SRB, efficiency desorption of xanthogenate from the mineral surfaces by SRB. Results of selective division $CuFeS_2$ from MoS_2 are submitted. As desorption of xanthogenate from a surface of sulfide minerals by sulfate - reducing bacteria more effectively, than sulphurous sodium.

Sulfate - reducing bacteria were checked up at division of a mix of minerals from galena (3гр) and sphalerite (3гр), and also mixes molybdenite and chalcopyrite after their preliminary flotation with the maximal extraction of minerals. After processing a concentrate by microorganisms about the contents in 1 ml of a bacterial liquid ($23.7 \cdot 10^6$) cells, extraction galena in a concentrate has made 83.63% at the contents of him -94.65. Extraction sphalerite in these conditions was -4.48%. Redox of the potential of a pulp changed from -20 up to 90 мВ.

In control experiences of selective division lead - the zinc concentrate did not occur. Bacteria do not render depressoring influence at selective separation molybdenite from chalcopyrite. At long bacterial processing (1.5 hour) flotation of the molybdenite practically is not reduced. In table 1 results of selective division of mix $CuFeS_2$ and MoS_2, PbS and ZnS are submitted.

Successful division Pb-Zn and Cu-Mo concentrates is possible is directed to adjust in the way creation of the certain conditions for duplication of a bacterium and quantity shtamma, brought in process desorption.

Tab. 1. Selection of the molybdenite and chalcopyrite, galena and sphalerite mixture (1:1) depending on SRB treatment.

Time of bacterial treatment (min)	Yeald of minerals (%)			
	$CuFeS_2$	MoS_2	PbS	ZnS
5	59.2	88.8	98	37
10	38.8	86.9	98	18.5
15	27.7	85.1	96.2	11.1
20	20.3	83.2	92.5	7.4
30	14.8	79.5	85	1.8

3.4. Sulfate-reducing bacteria in hydrometallurgy

3.4.1. Sulfate-reducing bacteria as antimonite and tin-bearing materials solvents

However researchers pay little attention to utilizing SRB to produce H_2S reagents for ore leaching and especially for leaching antimony-bearing materials. Only alkaline leaching of antimony sulfides has achieved industrial application. This hydrometallurgical process results in high selectivity for the noble-metal groups, which remains in a leaching cake. Dissolving antimony sulfide and antimony oxide in sodium sulfide and caustic soda results in sodium thioantimonite and thioantimonate formation according to the following reactions:

$$H_2S + 2NaOH = Na_2S + 2H_2O \quad (7)$$

$$Sb_2Sb_3 + 3H_2S + 6NaOH = 2Na_3SbS_3 \quad (8)$$

$$Sb_2S_3 + 4NaOH = Na_3SbS_3 + NaSbO_2 + 2H_20 \quad (9)$$

$$Sb_20_3 + 5Na_2S + 3H_2O = NaSb_2S_3 + 6NaOH \quad (10)$$

At bacterial leaching antimony-bearing a material as solvents used solution caustic sodium (120 g/L) with various concentration of memory culture sulfate - reducing bacteria (SRB). A pulp warmed up up to 90°C at S: L=1:16. The leaching antimonies carried out in two stages with fractional submission SRB. Extraction of antimony in a solution has made about 96.0 - 98.04% [5].

The offered technology, in comparison with use Na_2S, has a number of advantages. Presence in electrolyte of free alkali and SRB results in substantial increase electro current a solution and to decrease of ions of polysulfides that promotes improvement of parameters of the subsequent electrolyze a solution. Use SRB is shown at leaching tin-containing concentrates.

3.4.2. Sulfate - reducing bacteria and bacteria of type Lehtospirillum, Sulfobacillus, archie type Ferroplasma in hydrometallurgy gold - antimony concentrates

Recovery of gold from refractory ores requires a pretreatment to liberate the gold particles from the host mineral. The antimony forms stable compound with NaCN during the cyanidation process. Pretreatment is usually an oxidation step. As an alternative, chemical or bioleaching can be applied to liberate the gold particles from the sulfur matrix.

Emphases deserve an operation of sulfide-alkaline leaching as a way of selective separation of stibium from gold-bearing concentrates. Known developed by Irgiredmet (Irkutsk) technology of metallurgical conversion of rich gravity concentrates of Sarylachsk dressing plant. The scheme includes sulfide-alkaline leaching with the following electrolytic extraction of stibium from solutions. Three-phase recleaning of stibium leaching tailings from concentration tables, melting of secondary gravity concentrates (contents Au - 48 kgt^{-1}) on the Dor metal and cyanidation of tailig from final gravity concentration, received total extraction in corresponding commodity products Au - 98%, Sb - 96.5%.

The authors demonstrated the technical feasibility recovery of Sb by Na_2S and NaOH leaching, the successive gold solubilisation by conventional cyanidation process and the recovery of Sb and Au from the respective leach solutions by electrowinnig. It was reasonable to check a possibility of leaching stibium by bacteria.

Antimony leaching was done by somewhat different technique. Antimony-containing portion was mixed up with SRB of different hydrogen sulfide concentration and with caustic soda solution. The pulp was heated up to 90°C and it was stirred with S : L = 1 : 16 ratio. Antimony solubility is most effective at 120 g l^{-1} caustic soda concentration and maximum hydrogen sulphide concentration in SRB. Special experiments established that the time necessary for leaching of antimony is 1 or 1.5 h. To increase antimony transition into the solution, it is necessary to increase contact time with SRB during leaching. However, when the time of contact was increased, it was necessary to control antimony ions in the solution whose optimal concentration slowed down the leaching process. Therefore, antimony leaching was done in two stages with gradual addition of reagents. The first stage lasted 1 h., after that the solution was decanted, then once again necessary reagents were added and contacted for 0.5 h. Effective antimony leaching was observed under maintained optimal conditions. Isolation of antimony into the solution under those conditions was about 96.5-98.0%.

Traditional technology of antimony leaching requires higher concentrations of sodium sulfide, than in the case of SRB leaching, the latter reducing yield on the electric current. Given cake is processed by usual methods:

- Cyanidation, since contents of stibium does not render influences upon the leaching of gold;
- Presence in cake sulfur (more than 14%) must be sodium neutralized;

Chemical basic leaching of pure stibnite by Na_2S and NaOH under different experimental conditions at 40°C has been studied in order to optimize the reagents concentrations for the antimony dissolution process. Response surface methodology has been used to find the best experimental concentrations to maximize the Sb extraction yield. 98-100% of antimony recovery was obtained by using 1g Na_2S and 1g NaOH per gram of pure stibnite. At laboratory scale was investigated the best conditions for alkaline leaching of a refractory gold-bearing Sb_2S_3 (13.25 Sb_2S_3; 30g t^{-1}Au) coming from South America [6-8]. The solution was constituted by sodium sulfide and sodium hydroxide. Main parameters studied were: Na_2S concentration, NaOH concentration, pulp density and temperature.

Also, conventional bacterial leaching is promising. Treatment of a gold-bearing stibnite ore with cyanide yielded only 4% Au extraction; however, after seven days of bioleaching 85.5% Au recovery was attained with 24 hours of cyanidation.

Biotechnological processing gold-arsenic-antimony concentrates of ores Olympiada of a deposit is mastered and develops in industrial scale. Capacity of manufacture at the present moment 8 million tons of initial ore per one year. One third of the made quantity of gold of -26 tons falls to biotechnology. Oxidation the steady association moderately termofil microorganisms with the specific structurally functional organization, actively oxidizing conducts sulphidic minerals of a concentrate. The structure of community includes bacteria of type *Lehtospirillum*, *Sulfobacillus*, archei type *Ferroplasma*.

Process of biooxidation of a concentrate occurs in a continuous mode. Average productivity of bioplant 300 t/days. Time of process 100-120 hours. Density of a pulp 120-150 g/l. Working temperature 38-39°C. Concentration of cells in a pulp 3-5 g/l [9].

3.5. Clearing of industrial streams of ions of heavy metals at use of hydrogen sulfide of a biogenic origin

Numerous results on application of culture SRB for extraction of metals and clearing of industrial streams from cations heavy nonferrous metals are analyzed.

The description of application of the technology developed by company Paques which realized about 500 projects of described biological processing is given. The technology is sold under trade mark THIOPAQ ®.

Diluted gas H_2S made on a place a natural biomass which restores concentrated sulfur-bearing compounds turns out. The equipment allows receiving from 25 kg/day H_2S up to plenty - in limits t/days. The side benefit of process is that thus, probably, simultaneously to remove sulfates up to a level of 100-200 ppm/m^3. In this case superfluous sulfates are restored up to elementary sulfur.

In Kovohute Pribram (the Czech Republic) in exchange neutralization by alkaline carbonates of drains of sulfate of the sodium, containing lead, zinc, tin and high concentration of arsenic and antimony was applied technology THIOPAQ ® with use biogenic H_2S. Biogenic hydrogen sulfide can be received or from elementary sulfur, or from a sulfuric acid of the used accumulators.

3.5.1. Removal of cadmium

In institute of geotechnics of Slovak Academy of sciences significant works are carried out by A. Luptakova and M. Kusnierova on application SRB in hydrometallurgy [10]. Authors have shown that in case of sedimentation Cu at pH 2.8 and Cd at pH 3.5 it is possible to receive pure sulfides cupper and cadmium.

4. CONCLUSIONS

Application *Th. ferrooxidans* for oxidation of minerals of antimony and selective separation of cinnabar from antimonite is shown. The mechanism of oxidation *Th. ferrooxidans*, by bacteria of type *Lehtospirillum*, *Sulfobacillus*, archei type *Ferroplasma* of various minerals of antimony and their dissolution in the sour environment is submitted.

Application sulfate - reducing bacteria is shown- bacteria *Desulfovibrio* as regulators of flotation of minerals of nonferrous metals. At processing by solutions SRB during 30 mines probably selective flotation and branch of molybdenum from chalcopyrite, galena from sphalerite.

Alkaline solutions SRB are used for in two stages leaching antimonies from antimony-bearing materials. The biotechnology of processing gold of antimony concentrates about reception of cathodic antimony and translation of gold in a solution cyanidation is offered.

Industrial experience of application SRB in process cations metal and preservation of the environment is shown.

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BIOHYDROMETALLURGICAL PROCESSES FOR HEAVY METALS REMOVAL FROM ACID MINE DRAINAGE

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ABSTRACT
The present experimental work has been carried out in the framework of the agreement of scientific cooperation between the Institute of Environmental Geology and Geoengineering of the CNR, Italy and the Institute of Geotechnics of Slovak Academy of Sciences, Slovakia (years 2007-2009). The main scope was to remediate Acid Mine Drainage (AMD) by application of biohydrometallurgical processes, environmentally friendly, to remove heavy metals such as Zn, Cu, Mn, Cd, Al and Fe. The processes studied have been electrowinning and bioprecipitation. The samples utilised were collected from the zinc mine located in Italy and from a cooper – iron ore deposit in Slovakia. By electrochemical experiments, high metals removal, with a low energetic consumption, has been achieved: in particular, by Zn electrodeposition, it was possible to achieve 95-99% Zn removal. Culture of sulphate-reducing bacteria (SRB) of genera *Desulfovibrio sp.* was used for the bioprecipitation tests. The kinetic of the selective precipitation of Cu and Zn of AMD by SRB has been investigated. This method has been performed in two interconnected reactors. Achieved results demonstrate the about 98-99% elimination of Cu and Zn by bacterially produced H_2S. Both the electrowinning and bioprecipitation processes have been demonstrated the technical feasibility to decrease the heavy metals concentration.

KEYWORDS
Biohydrometallurgical processes, Acid Mine Drainage, heavy metals, microorganisms.

1. INTRODUCTION

In contrast to most organic pollutants, heavy metals are never degraded. The only ways to remedy heavy metals-polluted lands are stabilization or extraction using the suitable methods [1,2].

Various methods are used for redevelopment of soils and waters in the world, but any of them are universal [3,4]. Classical treatments for the removal of heavy metals from contaminated waters are precipitation with lime or more expensive chemicals. However, these methods present negative drawbacks - the production of secondary wastes (e.g. lime precipitation generates high volumes of solid wastes) [3]. There is a need for new and low-cost technologies in the field of elimination metals from environment. With respect to involved proposition various authors have studied, at laboratory scale, the application of physicochemical and biological chemical processes. Between the innovative and unconventional technologies belong for example the electrowinning and the bioprecipitation. Electrowinning process is currently used at large scale to purify process solutions and to recover precious metals. Microorganisms play important roles in the environment fate of heavy metals with a multiplicity of physical, chemical and biological mechanisms effecting transformations between soluble and insoluble phases [5].

The biggest environmental problems relating to mining and processing activities in the worldwide is the formation and treatment of acid mine drainage. The source of acid mine drainage (AMD) is the residues of mining activity mainly after the mining of deposits containing of sulphide minerals. AMD contains sulphuric acid, metals in the soluble form and its pH can be very low [6]. In Italy and Slovakia there are some localities with existing AMD generation conditions [7,8].

2. MATERIALS AND METHODS

2.1. Samples

The investigation has been carried out at laboratory scale by synthetic solutions, starting from AMD from the zinc mine located in Montevecchio Mine (Italy) and Slovak Mine located in Smolník. The AMD characterisation is reported in Tables 1 and 2.

Tab. 1. Composition of the AMD Italian sample.

Components	Zn	Cd	Cu	Ni	As	Sb	Pb	Mn	Fe	SO_4^{2-}	pH
Concentration (mg/l)	1600	3.50	0.50	4.00	0.006	0.005	0.076	86	190	1800	4.6

Tab. 2. Composition of AMD Slovak sample.

Components	Zn	Cd	Cu	Ni	As	Al	Pb	Mn	Fe	SO^{4-}	pH
Concentration (mg/l)	10.13	0.1	4.31	0.32	0.042	79.50	0.019	20	270	2938	3.5

2.2. Physical-chemical process: electrowinning

Nitric acid (HNO_3) has been added to the synthetic solution, with the aim to oxidise Fe^{2+} to Fe^{3+}. In a subsequent step, sodium hydroxide (NaOH) was added to reach pH 4.0. Successively, the deposit has been separated by filtration.

Electrowinning tests have been performed in a cylindrical glass laboratory cell of 200 cm^3 volume according to Ubaldini et al., 2008 [9]. The cell was connected to a potentiostat-galvanostat. With the scope to study the electrodeposition kinetic, liquid samples of 3 cm^3 have been whit drawn and submitted to chemical analysis by ICP-MS, while the purity of the solid deposit was determined by X-Ray Diffraction technique (XRD). Metallic content of the deposit was analysed by ICP-MS.

2.3. Biological-chemical processes: bioprecipitation

The cultures of SRB (genera *Desulfovibrio sp.*) were used which were isolated from a mixed culture obtained from the potable mineral water [10,11]. Scanning electron micrographs of sulphate-reducing bacteria is reported in Figure 1.

The precipitation of heavy metals form AMD sample was performed in two interconnected bioreactors with a capacity 1000 ml (the first bioreactor) and 250 ml (the second bioreactor) [6,10].

The heavy metals concentration in the liquid samples taken from the bioreactor was determined by atomic absorption spectrophotometry. The qualitative analysis of precipitates obtained by bacterial produced hydrogen sulphide was realized by energy dispersive spectrometry (EDS) analysis. Samples of precipitates were dried and coated with gold prior to the EDS analysis.

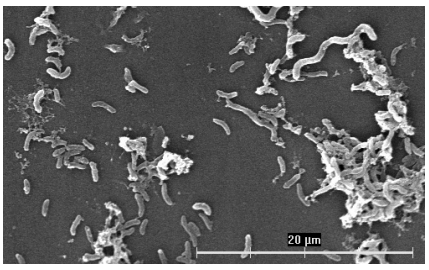

Fig. 1. Scanning electron micrographs of sulphate-reducing bacteria (*Desulfovibrio sp.).*

3. RESULTS AND DISCUSSION

3.1. Physical-chemical process: electrowinning

Preliminary precipitation step has been carried out before electrowinning. During this phase, also Al deposition has been achieved. The liquor prepared was treated by using an electrowinning lab-scale operation, to verify the technical feasibility of the metals deposition.

The average results of the electrowinning tests on Montevecchio (initial pH 4.6) and Smolnik samples (initial pH 3.5), show that, after 30', Zn deposit was of low quality. After 1 h, Zn deposit was uniform, while on counter electrode surface MnO_2 deposited. Manganese deposited to the anode as MnO_2 and to the cathode as Mn^+. After 2 hours, 90-95% of the metals have been removed by a quantitative cathodic deposition. The high grade purity of the metallic deposit has been achieved, such as it was demonstrated from the results of analysis conducted by XRD.

At the end of the processes, all the metals concentrations decrease under the recommended limit suggested from EC (data not shown here) [6, 12]. Table 3 and Table 4 show the main results of Zn electrowinning from

synthetic solution (AMD Italian and Slovak samples, respectively), while Table 5 shows the results attained after chemical precipitation by NaOH (AMD from Slovak sample).

Tab. 3. Main results of Zn electrowinning of synthetic solution (AMD Italian sample).

Time (min)	R (%)	η* (%)	E* (kWh/kg)
90	97.98	21.65	16.23
120	99.85	13.05	30.21

** η- Faradic current efficiency, E - energetic consumption*

Tab. 4. Main results of Zn electrowinning of synthetic solution without chemical precipitation (AMD Slovak sample).

Time (min)	R (%)	η* (%)	E* (kWh/kg)
90	92.18	20.10	17.13
120	96.89	11.92	31.21

** η- Faradic current efficiency, E - energetic consumption*

Tab. 5. Main results of Zn electrowinning of synthetic solution after chemical precipitation (AMD Slovak sample).

Time (min)	R (%)	η* (%)	E* (kWh/kg)
90	97.07	25.94	12.00
120	99.71	14.99	24.91

** η- Faradic current efficiency, E - energetic consumption*

3.2. Biological-chemical processes: bioprecipitation

During the metals bioprecipitation at the original pH of aforementioned AMD, only the precipitation of Cd (in the case of the AMD sample from Montevecchio Mine) and Cu (in the case of AMD sample from Smolnik - adit Pech) were observed. Figure 2 presents that at pH 4.6 Cd was effectively recovered from AMD of Montevecchio Mine using biologically produced H_2S. After 30 minutes the concentration of Cd was 0.03 mg/L. On the basis of the results of EDS qualitative analysis, Cd was precipitated in the form cadmium sulphides.

In the event of the AMD Smolnik at pH 3.5, the initial copper concentration 4.31 mg/l was decreased to less than 0.05 mg/l after 4 hours (Fig. 3). EDS qualitative analysis of precipitates demonstrates that Cu was precipitated in the form sulphides CuS.

Concentration changes of other metals (Zn, Fe, Ni, Mn), were not significant or remained without changes in case of both aforementioned AMD.

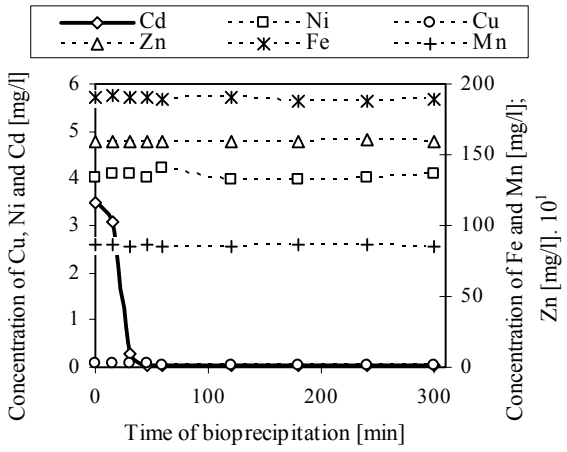

Fig. 2. Precipitation of heavy metals by biologically produced H_2S by SRB from AMD - Italian mine.

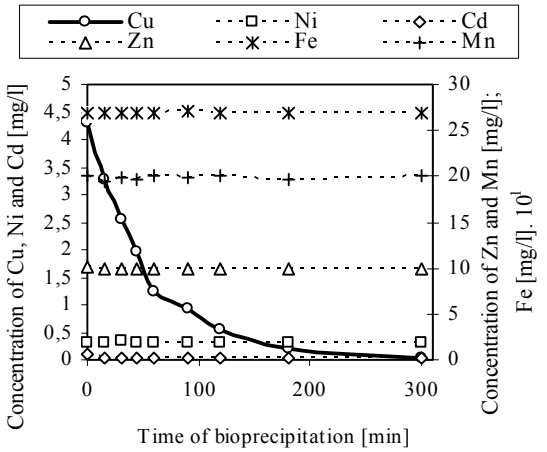

Fig. 3. Precipitation of heavy metals by biologically produced H_2S sulphide by SRB from AMD – Slovak mine.

4. CONCLUSIONS

Biohydrometallurgical processes have demonstrated the technical feasibility to decrease the heavy metals concentration on the AMD studied to remove toxic metals from AMD samples.As far as Zn electrowinning, it was possible to achieve high Zn removal with a low energetic consumption. Bioprecipitation process demonstrates the selective precipitation of heavy metals by SRB from the AMD samples. From AMD of Italian Mine was achieved only the precipitation of Cd. From AMD of Slovak mine was achieved only the precipitation of Cu. Obtained results indicate the 98-99% elimination of Cd or Cu by bacterially produced H_2S.

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APPLICATION OF SULPHATE REDUCING BACTERIA IN REMEDIATION OF ENVIRONMENT CONTAMINATED BY METALS

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ABSTRACT
Migration of heavy metals as well as their uncontrolled appearance in the environment is greatly influenced by anthropogenic processes. Industrial activity, e.g. exploitation of metal ores, transport of ore to processing plant, transformation, final management and utilization of used products are the main anthropogenic sources of heavy metals. The resulting solid and gaseous wastes as well as sewage are accumulated in water, soil and atmosphere.
Nowadays it looks for ecological methods that can use in bioremediation environment contaminated by metals but in natural environment seldom we can observe pollution by only one organic or inorganic compound. Very often together with metal pollution occurs contamination by petroleum products.
Sulphate reducing bacteria (SRB) can be thus be utilized in treatment of sewage and mine water from toxic metals, and the microorganisms applied in this process should effectively reduce sulphates, tolerate changes in pH and be resistant against toxic metals.

KEYWORDS
Sulphate reducing bacteria, heavy metals, environments contaminated by heavy metals

1. INTRODUCTION

Human activity is strictly linked with the production of waste i.e. materials and substances that are undesired and cannot be used further. On the one hand these substances are natural to the environment, eliminated from further technological process by their uselessness e.g. mining waste, or represent new products i.e. anthropogenic waste, being the by-product of industrial and agricultural activities. A separate group comprises municipal waste that is not linked with production but results from human dwelling. Very often it can be observe pollution of soil and water by petroleum products and metals together. Migration of heavy metals as well as their uncontrolled appearance in the environment is greatly influenced by anthropogenic processes.

High content of heavy metals can be found in industrial waste containing heavy metals derive mainly from: smelters, electroplating plants, tanning, fertilizer, pesticide and electrochemical industries, from plants producing batteries, accumulators, catalysts, etc. However high concentration of heavy metals it can be observe in soil contaminated by petroleum products. Heavy metal ions, such as lead, tin, arsenic, mercury, germanium, antimony, thallium, vanadium, iron, aluminium, nickel, calcium, and magnesium included in the crude oil.

Pollution of environment by petroleum products and metals is a very complicate global problem and it hasn't solved yet. This in turn points to a multidisciplinary approach to the issue and the need to involve not only scientists working on bioremediation such as e.g. microbiologists, but also specialists in geology, soil science or hydrogeology.

Thus it seems crucial to search for pro-ecological methods focused on the utilization of different pollution that are hazardous to the natural environment. To such methods belong biological methods that use microorganisms to remove organic and some inorganic compounds e.g. metals from environment.

With the use of microbiological methods are used to characterize strains of microorganisms capable of good growth even in adverse environmental conditions. Very often use the mixed microbial populations, earlier adapted to extreme condition of contaminated of environment.

2. SULPHATE REDUCING BACTERIA

Sulphate reducing bacteria (SRB) are heterotrophs and absolute anaerobes that can utilize sulphates, as well as other partly oxidized sulphur compounds and elemental sulphur as the final electron acceptor in the respiration processes [1]. Electron donors for this microorganisms group are organic compounds such as e.g. alcohols, phenols, aliphatic and aromatic hydrocarbons [2]. Diverse SRB physiology influences their distribution in the natural environment as well as in anthropogenic environments e.g. polluted by crude oil and oil products [3,4]. SBR occur in soils, deposits of fresh water and marine reservoirs, in silts at the moutha of river deltas etc., thermal springs and in geothermal regions, in crude oil, refining and petrochemical waste, natural gas intakes and on corroding steel. They may be found in all types of bioreactors purifying sewage in anaerobic conditions, from which they can be isolated [5]. The most characteristic environments of SRB occurrence are marine deposits, as

well as oil fields and crude oil reservoirs [6,7]. Their presence has also been noted in environments polluted by crude oil and oil products, dairy work sewage, whey, refining-petrochemical waste, acid marine drainage [8-10]. Sulphate reducing bacteria occur in extreme environments like acid like or acid mine drainage [11].

A large number of SRB species are known to be able to biodegrade crude oil and oil-derived products. The activity of this group of microorganisms is not without effect on the natural environment, moreover, it plays a crucial role particularly due to their ability to biodegrade aromatic compounds and the ability to form in anoxic conditions different mineral phases often largely influencing the entire process of remediation of soils polluted by oil-derived products [12].

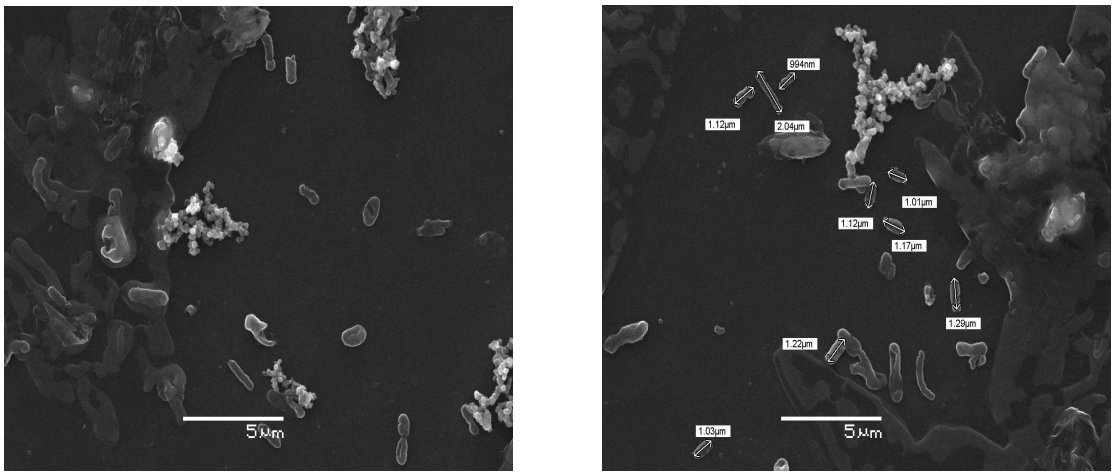

Fig. 1. Sulphidogenic anaerobic bacterial communities isolated from soil contaminated by petroleum products and metals (Wolicka).

2.1. Application of Biological methods in removal of heavy metals

Biological methods are more frequently applied these methods allow recovery of heavy metals and cause the transformation of toxic cations of heavy metals into sparingly soluble sulphides, a desired effect particularly in solid waste management [13,14].

This process comprises: linking metal ions by reactive groups of biopolymers occurring in cell membranes of microorganisms, their preservation on surfaces of non-soluble hydroxides, salts or metal complexes, chemical reactions with released metabolites, formation of non-soluble metal compounds, followed by their accumulation and crystallization within cell membranes. Key role in this process is played by extracellular polymer substances (EPS) abundantly released by some microorganisms.

Biosorption has several limitations linked with high costs caused by the need to separate biomass from the post-culture fluid after sorption and low stability of biomass, precluding multiple applications of microorganisms in subsequent cycles of sorption and desorption. Immobilization of organisms hampers their passage to the mobile phase transporting the substrate and product, allows the increase of microorganism population density in the reactor and effective separation of biomass from the solution. It also has positive influence on the stability and resistance of biomass, as well as the increase of its application in continuous processes.

Nowadays, sulphate reducing bacteria are becoming more frequently applied in the biodegradation of anthropogenic waste. These bacteria in course of anaerobic respiration produce hydrogen sulphide, which can bind heavy metals in poorly soluble and non-toxic sulphides of metals. This is one of the many advantages of sulphidogenesis application in environmental biotechnology [15].

Additionally, due to the toxic activity of hydrogen sulphide, SRB may eliminate various microorganisms from the environment, including pathogenic forms, what causes their domination in a sulphate rich environment. The bacteria may be utilized during the biodegradation of two industrial wastes, of which one may be solid waste as the sulphate source, and the second – liquid waste as the carbon source. Application of such process allows simultaneous biodegradation of two arduous industrial wastes, thus shortening the biodegradation time. Sulphate reducing bacteria among the microorganisms that can be used in bioremediation of contaminated soil and water by crude oil and heavy metals at the same time. As a result of their metabolic activity of the oxidation of organic compounds is CO_2 and H_2O and reduction sulphates are insoluble sulphides of metals.

Metals are precipitated from solution as the insoluble metal sulphide. The basin reactions mediated by SRB as are follow (M^{2+} - metal ion such as Zn [16]):

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^-$$

$$M^{+2} + S^{-2} \rightarrow MS\downarrow$$

Microorganisms conducting dissimilative sulphate reduction contribute to the treatment of mine and metallurgy sewage, as well as effluents from municipal and industrial waste dumps. Application of SRB in the demobilization of various heavy metals has been described in a number of papers, e.g. The influence of chromium, nickel, manganese, copper, and zinc on *Desulfovibrio vulgaris* and *Desulfovibrio* sp. has been described by Cabrera et al. [17]. Removal of heavy metals in short-term bench scale upflow anaerobic packed bed reactor was described by Jong & Parry [18], and the influence of copper and zinc on the mixed SRB population was studied by Utgikar et al. [19]. Rafida [20] notes the significant role of biofilm formed during SRB activity.

Selected anaerobic sulphidogenic bacterial communities can be use to bioremediation process. Bioremediation is a process in which autochtonous microorganisms of contaminated soli or water are used to reduce the concentration of heavy metals in the ground to a safe level. As soon as circumstances are favorable, the method is applicable, since it does not require additional interventions, in addition to monitoring the natural process of biodegradation of contamination.

4. CONCLUSIONS

Biological methods and their application must be preceded by extensive microbiological research, with particular attention to all elements of the environment, which may have an impact on the course of remediation. Application selected sulphate reducing bacteria in bioremediation environments contaminated by petroleum and metals seems reasonable.

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Institute of Geotechnics

Slovak Academy of Sciences

The Institute of Geotechnics SAS has a dominant position in Slovak Republic within the basic and applied research in the area of rock disintegration, mineral processing, mechanochemistry, mineral biotechnologies and environmental protection.

Activities of the Institute of Geotechnics SAS

* basic research of processes in the field of continuous disintegration of the rock mass and underground constructions stability, the transport of energy and mass in the rock disintegration processes;

* basic research of the patterns of solid dispersions origin, and modification of their properties by physical, mechanical, chemical and biotechnological processes;

* qualitative and quantitative evaluation of phase interactions at the disperse systems origin and at their spreading in working and living environment;

* focus on application of theoretical knowledge from presented areas for improvement of the top technologies concepts in the following fields: rock disintegration, mineral processes, monitoring of selected components of working and living environment, monitoring of environmental, chemical and geological changes in waste repositories aimed on ecological revitalizations;

* advisory and expertise services related to main activities;

* scientific education in terms of generally valid legislation;

* publishing of scientific-research activities using the periodic and non-periodic press. The publishing of periodic and non-periodic press follows the regulations of the SAS Presidium.

Scientific departments of the Institute of Geotechnics SAS

Department of destructional and constructional geotechnics
Department of mineral biotechnologies
Department of physical and physico-chemical mineral processing methods
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Department of mineral biotechnologies

studies all the technological, mining, treatment processes, in which the microorganisms or the products of their metabolic activities are used in order to achieve qualitative changes of inorganic as well as of organic mineral raw materials and their wastes. Fundamental bases of the mineral biotechnologies are environmentally correlative, cyclic, biological-chemical processes at the contact of abiotic and biotic systems in biosphere, i.e. minerals - raw materials - rocks and autochthonous microorganisms.

Research course was founded by
Prof. Dr.h.c. Ing. Frantisek Spaldon, DrSc. in 1985.
In 1988, an autochthonous microorganisms internal bank was founded,
with 60 various tribes, species, and phylums stored at the present time.

In 1992, a detached department was established, headed by assoc. prof. Ing. Maria Kusnierova, PhD., with specialists working in various research areas of mineral biotechnologies with specific orientation on the problems of Slovak raw materials basis and environment. The department has been lead by MVDr. Daniel Kupka, PhD. since 2006.

Study of fundamental knowledge and possibilities of its application is focused on following research areas:

Explanation of biological-chemical oxidation processes and sulphide transformation for the development of environmental technologies, their processing with utilization of autochthonous and physiologically adapted bacteria *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. Technology of biological - chemical treatment and Au obtaining from refracted Au-As-Sb-Fe ores at Pezinok, and methods of non-extraction application of microorganisms in the processes of selective bioflotation were developed.

- Determination of factors affecting the metabolic functions and the interactions of bacteria with mineral surface. These factors enable to rectify the bioleaching processes rate and efficiency in Fe extraction, which is main ineligible component of non-metallic raw materials (kaolines, quartz sands, feldspars, zeolites, and fly-ashes).
- Research of sulfate reducing-bacteria metabolism and their application in the processes of heavy metals and sulfates elimination from acid mine drainage; study of mineral raw materials and their wastes treatment and processing; study of nanosorbents biological-chemical preparation on sulphide base.
- Research of biodiversity of autochtonous acidophilus bacteria *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* in the areas of abandoned and flooded mines as well as of old mine loadings after mining activities of sulphide ores in Slovakia.
- Study of organic pollutant biodegradation, mainly oil hydrocarbons in soils and waters. Monitoring of kinetics of biodegradation processes in laboratory and in-situ conditions in surroundings of ecological disaster or decontamination station was developed within the cooperation with Evironcentrum Ltd.
- Study of autochthonous microflora influence on the exogenous process behaviour in energetic wastes, fly-ashes and at the coal-mass transformation related to its sorption characteristics.
- Investigation of biological-chemical oxidation of metallurgical wastes with Fe content with a view to prepare Fe nanodispersions usable industrially as sorbents and pigments

Technical University of Košice **Faculty of Metallurgy**

The Faculty of Metallurgy is an integral part of Technical University of Kosice. The main aim of the Faculty is to prepare new specialists in metallurgy and related fields. Scientific research is oriented on metallurgy and material technologies.

Scientific and applied research is oriented on the following fields:

- pig iron and steel production
- ladle metallurgy of steel and continuous casting
- metallurgy of non-ferrous metals
- modern foundry technologies
- metals forming
- metals finish and protection
- refractory materials production and application
- energy balances of the thermal processes and aggregates
- energy resources efficient utilization
- environmental aspects of the metallurgical production
- wastes recycling and utilization
- research and development of new materials and technologies
- development of new methods for the material properties evaluation
- physical and mathematical process modeling
- quality control and environment protection in the industrial enterprises

The Faculty publishes a specialized journal Acta Metallurgica Slovaca, which is distributed to 23 countries.

Departments of the Faculty of Metallurgy

Department of Chemistry
Department of Non-Ferrous Metals and Waste Treatment
Department of Furnaces and Thermal Technology
Department of Materials Science
Department of Ferrous and Foundry Metallurgy
Department of Integrated Management
Department of Metal Forming
Department of Ceramics

Department of Non-Ferrous Metals and Waste Treatment

Department of Non-Ferrous Metals and Waste Treatment has been established in 1954. Its original name was Department of Non-Ferrous Metals and Foundry and its main specialization was concerned with wide field of metallurgy of non-ferrous metals. Nowadays, two accredited fields of study can be studied at the Department, namely Metallurgy of Non-ferrous Metals and Waste Processing and Recycling. The basic idea of the existence of study program Waste Processing and Recycling is to connect ecological and environmental activities of legislation and separation of waste with a metallurgical processing.
Department of Non-Ferrous Metals and Waste Treatment provides the lectures on the theory of non-ferrous metals production from primary and secondary raw materials as well as lectures on the consequential disciplines like powder metallurgy, production of high purity metals, biometallurgy, separation of phases and facilities in hydrometallurgy, production of heavy, light and rare metals, etc., in the educational program Metallurgy.
In the research field, department is concerned mostly with the study of thermodynamic and kinetic parameters of pyrometallurgical and hydrometallurgical systems, the study of complete utilization of primary and secondary raw material sources of non-ferrous metals, the study of relationship between technological processes and crystal structure of input materials and byproducts, etc.
At the present time, the biometallurgy is included in the research at the Department. The main idea is to connect biological and metallurgical fields. The aim is to use living or non-living biomass to recover metals, clean the environment and use microorganisms to produce valuable products. Since September 2009, the new Laboratory of Biometallurgy and Environmental Biotechnology was established at the Department.

Laboratory of Biometallurgy and Environmental biotechnology

The main research areas are:

- Study of phytoremediation and its application in soil clean up
- Study of bioleaching in waste processing, particularly spent batteries and printed circuit boards processing
- Study of metal ions biosorption from water
- Study of noble metals recovery from solutions by living organisms

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