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ISOLATION OF BACTERIA FROM HEAVY METAL CONTAMINATED SOILS AND THEIR APPLICATION IN BIOREMEDIATION

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ABSTRACT:
The presentation deals with the latest results of the experiments focussed on the ability of bacteria to removal heavy metals. Laboratory experiments tested bacteria isolated from soils contaminated by heavy metals, which are presumed to have higher resistance to the metals. The results confirmed the bacterial ability to removal Zn, Pb and Cd and demonstrated that the identified species have potential application in bioremediation of metal pollution. Our experiments identified species with highest removal efficiency: *Bacillus subtilis (*Zn 69%, Pb 89 %, Cd 65 %) *, Bacillus megaterium* (Zn 63%, Pb 84 %, Cd 66 %), *Bacillus licheniformis* (Zn 58%, Pb 81 %, Cd 71 %) and *Corynebacterium jeikeium* (Zn 84 %, Pb 89%, Cd 76 %). Presence of heavy metals on bacterial surfaces was confirmed the results of energy-dispersive X-ray analysis.
KEYWORDS:
heavy metals, zinc, lead, cadmium, metal removal, soil bacteria

1. INTRODUCTION

One of the currently most important environmental problems is the global contamination of the environment by toxic substances. Heavy metals are among the most alien substances found in water and air, representing potential hazard of the contamination of aquifers and food chain, with subsequent impact on human health.

The use of remediation procedures with using of biological systems at redevelopment of soils and waters contaminated with toxic metals is appeared as perspective economic and ecologic alternative of physical-chemic technologies. Advantage of this access is especially low price, minimal amount of secondary wastes and simulateously minimal disturb of environment [5].

Biotechnologies for the environmental decontamination use the ability of microorganisms to immobilisation or transform contaminants. The key microorganisms are bacteria, algae and fungi [8]. Their cells use a broad spectrum of mechanisms for the metals removal, which includes active transport, extracelular production of complexes, precipitation, binding to cell structures, oxidation-reduction reactions and conversion of compounds to volatile or less toxic forms of heavy metals [1,5,8]. As the used are naturally founding microorganisms or genetically modified [5]. Naturaly founding organisms are obtained from longtime contaminated localities, when in these is waiting resistence to heavy metals [6,13]. It was ascertained by studium of the influence of heavy metals on microbial biomass in long time contaminated soils that during long time as well as continually increasing contamination of soils is coming to some adaptation of soil microorganisms to contamination by toxic elements [12,13]. In case of toxic activity of increased concentrations of heavy metals get to decrease of amount, but also diversity of species of soil microorganisms [7,15]. A decreasing diversity is caused by death of species, which have not good resistance to stresses. However, on other site comes to fortification of other species, which at such situation living better and at these comes to change of genetical equipment of microbial cell, what probably leads to obtaining of higher resistance to heavy metals [13,16]. At long time selection pressure of heavy metals is increasing representation of resistant microorganisms in biocenosis [4,12].

Bacterial cells are relatively easily available natural material, which could be interesting from the practical use point of view. Isolation, identification and study of these microorganisms gives a possibility of their use on imobilisation and removal of metals from contaminated environment [12,13].

2. METHODOLOGY

Soil samples

Bacterial species used in the experiments were isolated from the soil samples of Slovakia with an increased content of heavy metals. Heavy metals were evaluated in extract 2M HNO_3 by valid methodise [15]. Contents of heavy metals in soil samples are presented at tables 1.

Table 1. Contents of heavy metals in soil samples [15]

Soil samples	Contents of heavy metals [mg.kg^{-1}]					
	Cr	Cd	Ni	Cu	Zn	Pb
1	7,5	0,6	11,3	65,8	876,5	103,4
2	3,8	0,7	8,7	15,7	93,0	236,8
3	4,4	0,2	15,5	21,3	90,0	185,9

Isolation and identification of bacteria

They were extracted from the soils into a physiological solution and subsequently centrifuged (5,000 rev/min for 5 minutes) and inoculated on nutrient agar medium (Nutrient agar No.2, Imuna, Šarišské Michaľany). Isolation of pure culture was performed on selective agar plates (G^+ Phenylalcohol agar and G^- McConkey agar) under constant conditions at 30 °C. As basic taxonomical criterion for species identification of bacteria was used staining according to Gram. Morphology of isolates was observed by optical microscopy.

Bacterial isolates were identified by BBL CRYSTAL Identification System (Becton-Dickinson, USA). Test is based on biochemical and enzymatic reactions of specific substrates by tested bacteria, cell morphology and Gram reaction [3].

Metal removal studies

For the assessment of the ability to remove heavy metals from the solution, the isolated bacterial strains were cultivated in the heavy metals solution:1 mmol of the $Zn(NO_3)_2 \cdot 6H_2O$, $Pb(NO_3)_2$ and $Cd(NO_3)_2$ solution during 48 hours by the temperature of 30 °C. The sorption occurred in 50 ml of the metal solution mixed with additional 0.3 g (wet weight) of living bacteria. After the cultivation, the samples were centrifuged (12,000 rev/min for 10 minutes) and the changes in the concentration of the studied metals were measured with the help of atomic absorption spectrophotometer (Perkin-Elmer 3100).

Data evaluation

For the purpose of the ability the bacteria to removal metals, the experimental results were mathematically analysed to arrive at the removal capacity of the bacterial cells and the removal efficiency.

Removal (binding) capacity q [mg.g^{-1}] expresses the amount of pollutant, which is bonded, on one gram of bacteria during the experiment. It is calculated according to the following equation [2]:

$$q = V\,(c_i - c_f)\,/\,S, \qquad (1)$$

where V [l] is the volume of medium, c_i [mg.l^{-1}] input and c_f [mg.l^{-1}] equilibrium metal concentration, S [g] is the weight of added bacteria.

Removal efficiency E [%] represents perceptual value of the metal removed from the solution. It is calculated according to the following equation [2]:

$$E = ((c_i - c_f) / c_i)*100 \tag{2}$$

Energy-dispersive X-ray analysis

For evaluation of ability to bind heavy metals by bacterial cells was used energy-dispersive X-ray analysis. There were compared species cultivated in solutions of heavy metals with species cultivated in nutrient broth. Bacterial suspension was collected on Formvar carbon-coated copper grids. The cells were lightly stained with 2 % uranyl acetate for 2 minute to provide better visualization of these cells [11]. Samples were examined by electron microscopy (TESLA 300 MESH) with an EDAX energy-dispersive X-ray analyser.

3. RESULTS

Experiments were directed on isolation and identification of bacteria from soils contaminated by heavy metals as well as their ability to cumulate and remove heavy metals – zinc, lead and cadmium.

Individual bacterial species were isolated from the soils which were long-term contaminated by heavy metals, and which therefore can be expected to have resistance to higher concentrations of these elements [14]. Increased heavy metals content in the soils causes genetically changes of the microbial cells, which in turn most likely causes increased resistance [16]. Such resistant bacteria are usable for the heavy metal decontamination purposes [4,10,13].

Extracted bacterial strains were tested in the model solutions of zinc, lead and cadmium. Obtained results, which are presented graphically on Figure 1 confirmed ability of isolated bacterial strains to remove heavy metals, this fact was confirmed by obtained effectivity of removal of Zn (16 – 84 %), Pb (19 – 89%) and Cd (7 – 75 %).

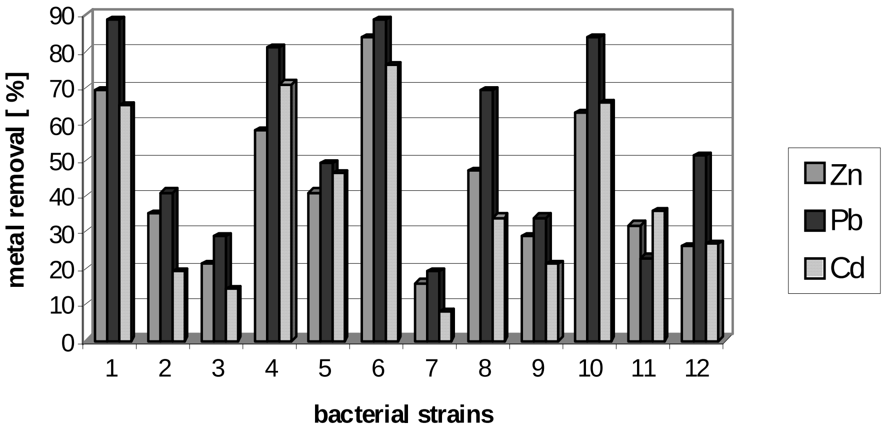

Fig.1. Zinc, lead and cadmium removal percentage by bacterial strains

Identificated were bacterial strains which showed the most high ability to remove metals from model solutions and can be practically used at removal of contaminants from polluted waters and soils. The bacterial isolates were classified as the strains of the genus *Bacillus* and *Corynebacterium*. The *Bacillus* strains were identified as *Bacillus subtilis* (bacterial strain No.1), *Bacillus licheniformis* (bacterial strain No.4), *Bacillus megaterium* (bacterial strain No.10). Of the genus *Corynebacterium* was identified the species *Corynebacterium jeikeium* (bacterial strain No.6).

Identified bacteria are gram-positive species, which are more resistant to higher concentrations of heavy metals in comparison with gram-negative bacteria [14]. Studies of many authors confirmed that gram-positive bacteria have higher ability to bind heavy metals as gram-negative species. This ability is caused by different composition of cell surfaces G^+ and G^- species [9,11].

The tested metals were most efficiently removed by the *Corynebacterium jeikeium* species (Tab.1). The efficiency of zinc and lead removal progressively decreased in the following series: *Corynebacterium jeikeium* (Zn 84 %, Pb 89%) > *Bacillus subtilis* (Zn 69%, Pb 89 %) > *Bacillus megaterium* (Zn 63%, Pb 84 %) > *Bacillus licheniformis* (Zn 58 %, Pb 81 %). The efficiency of cadmium removal progressively decreased in the following series: *Corynebacterium* jeikeium (76 %) > *Bacillus licheniformis* (71 %) > *Bacillus megaterium* (66 %) > *Bacillus subtilis* (65 %). The highest removal capacity of cells was demonstrated for lead, and reached values from 25,61 to 28,23 $mg.g^{-1}$ for gram of bacterial biomass. The removal capacity of cells for tested metals decreased in order: lead (25,61-28,23 $mg.g^{-1}$) > cadmium (9,81-11,38 $mg.g^{-1}$) > zinc (5,82-8,37 $mg.g^{-1}$). By comparison of the experimental results we have observed that bacterial species have higher removal ability for lead than cadmium and zinc, and that they removed lead from the model solutions with high efficiency.

Tab.1 Comparison of the efficiency a removal capacity of the bacterial species in Zn, Pb and Cd removal from model solutions

Bacterial species	Removal efficiency, [%]			Metal removal capacity, [$mg.g^{-1}$]		
	Zn	Pb	Cd	Zn	Pb	Cd
Bacillus subtilis	69,13	88,68	65,38	6,91	28,08	9,81
Bacillus licheniformis	58,20	80,86	70,80	5,82	25,61	10,62
Bacillus megaterium	62,83	83,55	66,46	6,28	26,46	9,97
Corynebacterium jeikeium	83,65	89,16	75,84	8,37	28,23	11,38

Presence of heavy metals on bacterial surfaces was investigated by energy-dispersive X-ray analysis. Species, which were cultivated in solutions of heavy metals, were investigated and compared with species that were not liable to effect of metals (Fig. 2-5). Results confirmed presence of metals on surfaces of bacteria cultivated in solutions of metals and by this way also ability of binding of heavy metals by bacterial cells.

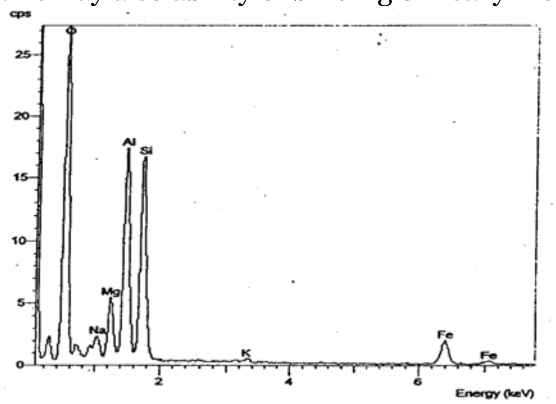

Fig.2 EDAX analyse of surface of bacterial cells cultivated in nutrient medium (without metals)

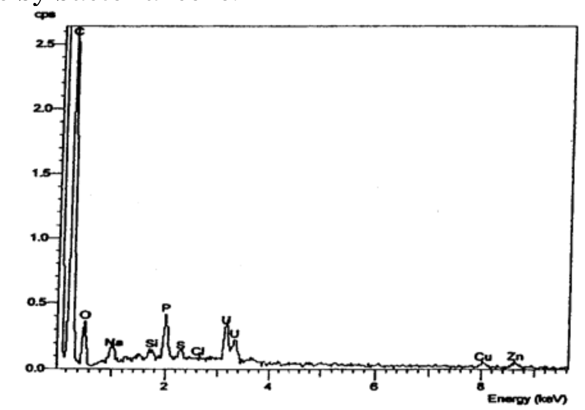

Fig.3 Detection of Zn on surfaces of bacterial cells cultivated in Zn model solution (EDAX analyse)

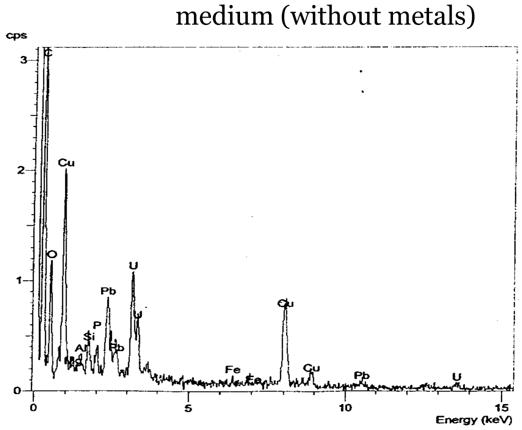

Fig.4 Detection of Pb on surfaces of bacterial cells cultivated in Pb model solution (EDAX analyse)

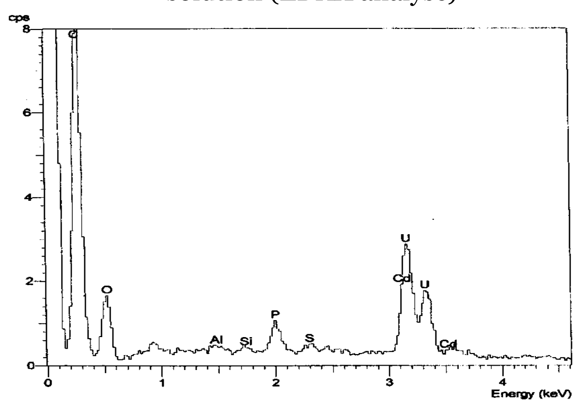

Fig.5 Detection of Cd on surfaces of bacterial cells cultivated in Cd model solution (EDAX analyse)

4. CONCLUSIONS

The bacteria were isolated from heavy metal contaminated environments, and the applicability of their heavy metal removal from polluted environment was evaluated at a laboratory scale. The experimental results can be summarised in the following way:

- We have confirmed the bacterial ability to removal zinc, lead and cadmium. At the same time, our experiments have shown that the heavy metal removal by the bacterial cell is species dependent.
- We have identified species with the highest removal efficiency and capacity: *Bacillus subtilis, Bacillus megaterium*, *Bacillus licheniformis* and *Corynebacterium jeikeium*. The best results for both metal removals were achieved by the *Corynebacterium jeikeium* species.
- The zinc removal by identified bacterial species is demonstrated by 58 – 84 % decrease in the concentration of zinc in the model solutions. The removal capacity of cells of the tested bacterial species ranged from 5,82 to 8.37 mg for each gram of bacterial cells.
- The high ability of metal removal is evident in the case of lead removal by bacterial species. Lead was removed from solutions from 81 to 89 %. The sorption capacity of cells for lead reached values from 25,61 to 28,23 $mg.g^{-1}$ of bacterial biomass.
- The cadmium removal by bacteria ranged from 65 to 76 %, the capacity of bacterial cells ranged from 9,81 to 11,38 $mg.g^{-1}$.
- Presence of heavy metals on bacterial surfaces was confirmed the results of energy-dispersive X-ray analysis.

The study demonstrated that the identified *Bacillus* and *Corynebacterium* species have potential application for the immobilization and removal of zinc, lead and cadmium from soil and water.

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REFERENCES

[1] AHALYA N., RAMACHANDRA T.V., KANAMADI R.D.: *Biosorption of heavy metals*. Res.J.Chem.Environ., 7 (4), 2003, 71-79

[2] BARROS L. M., MACEDO G. R., DUARTE M. M. L., SILVA E. P., LOBATO A.K.C.L.: *Biosorption of cadmium using the fungus Aspergillus niger*. Braz.J.Chem.Eng., 20 (3), 2003, 229-239

[3] *BD BBL Crystal™ Identification Systems*. Becton, Dickinson and Company, Maryland, USA, 2004

[4] CONGEEVARAM S., DHANARANI S., PARK J., DEXILIN M., THAMARAISELVI K.: *Biosorption of chromium and nickel by heavy metal resistant fungal and bacterial isolates*. J.of Hazardous Materials, 146, 2007, 270-277

[5] DERCOVÁ K., MAKOVNÍKOVÁ J., BARANČÍKOVÁ G., ŽUFFA J.: *Bioremediácia toxických kovov kontaminujúcich vody a pôdy*. Chem. Listy 99, 2005, 682-693

[6] DUXBURY T., BICKNELL B.: *Metal – tolerant bacterial populations from natural and metal – polluted soil*. Soil Biol.Biochem., 15 (3), 1983, 243–250

[7] FROSTEGARD A., TUNLID A., BAATH E.: *Changes in microbial community structure during long – term incubation in two soils experimentaly contaminated with metals soil*. Soil Biol.Biochem., 28 (1), 1996, 55–63

[8] GADD G.M.: *Microbial influence on metal mobility and application for bioremediation*. Geoderma, 122, 2004, 109–119

[9] GOURDON R., BHENDE S., RUS E., SOFER S.S.: *Comparison of cadmium biosorption by Gram-positive and gram-negative bacteria from activated sludge*. Biotechnology Letters, 12 (11), 1990, 839-842

[10] KIM S.U., CHEONG Y.H., SEO D.C., HUR J.S., HEO J.S., CHO J.S.: *Charakterisation of heavy metal tolerance and biosorption capacity of bacterium strain CPB4 (Bacillus spp.)*. Water science&Technology, 55 (1), 2007, 105-111

[11] MULLEN M.D., WOLF D.C., FERRIS F.G., BEVERIDGE T.J., FLEMING C.A., BAILEY G.W.: *Bacterial sorption of heavy metals*. App.Env.Microbiology, 55 (12), 1989, 3143–3149

[12] NIES D.H.: *Microbial heavy-metal rezistence*. Appl.Microbiol.Biotechnol., 51 (6), 1999, 730-750

[13] SHAKORI A.R., MUNEER B.: *Copper-resistant bacteria from industrial effluents and their role in remediation of heavy metals in wastewater*. Folia Microbiol., 47 (1), 2002, 43-50

[14] ŠTYRIAK I., ALÁČOVÁ A., DUTKOVÁ M., ŠTYRIAKOVÁ I., KOŠČOVÁ M.: *Možnosti využitia pôdnych mikroorganizmov na adsorpciu olova*. Mineralurgy and environmental technology, II. International Conference, Herľany, Slovakia, 2001, 127-132

[15] ŠTYRIAK I., SZABOVÁ T., ALAČOVÁ A., KOŠČOVÁ M., ŠTYRIAKOVÁ I.: *Vplyv ťažkých kovov na pôdnu mikroflóru*. Acta Montanistica Slovaca, 7 (4), 2002, 271-273

[16] VITI C., PACE A., GIOVANETTI L.: *Charakterization of Cr(VI)-resistant bacteria isolated from chromium-contaminated soil by Tannery activity*. J.Current Microbiology, 46 (1), 2003, 1-5