

Removal of toxic elements from the minerals particles of contaminated soil by chemical and biological leaching

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Abstract: Four experiments of chemical (CHL) and biological (BL) leaching of toxic elements from the contaminated soil using chelants Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA), Ethylenediamine-*N,N'*-disuccinic acid trisodium salt (Na_3EDDS) and heterotrophic indigenous bacteria was realized with the aim to remove mobile toxic elements from the contaminated soil. The first test of chemical extraction using 10 mM chelants was effective for toxic metal removal in order $\text{Cu(II)} > \text{Zn(II)} > \text{Pb(II)}$, but was very low for the As(V), Sb(III) and Ba(II) extraction. Next the chemically leached sample was treated in the second test combining CHL and BL with 2 mM chelants and nutrients. The second test allowed to enhance the Pb and As extraction to 4 and 6 %, respectively. The extraction of Cu(II) and Ba(II) decreased. Therefore the sample originated from the abiotic control was next treated in the third test only by bioleaching. The effect of leaching was similar as in previous combined test and the As extraction reached 44 %. Based on the obtained result technological method of leaching of contaminants was tested and verified. The effect of Cu(II) and Zn(II) extraction reached 68 and 59 %, respectively. For Zn(II) it was 49 %, for As(V) and Sb(III) 40 and 31 %, respectively and for Ba(II) only 22 %.

Key words: soil, toxic elements, chelants, bacteria, bioremediation

Graphical abstract



Highlights

- The study verified the intensification of toxic elements extraction from the contaminated soils.
- Higher As concentration in soil required longer time of bioleaching and higher nutrients concentration during the leaching cycle.
- The As mobility was influenced by the mineralogical composition of particular soil samples.
- Chemical leaching for metals removal following by bioleaching for maximum As removal seems to be a promising method.

Introduction

Metal pollution of soils is widespread across the globe, as caused by many anthropogenic activities, including mining operations, smelting, waste disposition, coal combustion, application of pesticides, etc. Most hazardous sites are contaminated with mixed heavy metals and metalloids rather than a single heavy metal. One major concern is the treatment of soil contaminated by arsenic. Arsenic as a toxic metalloid occurs in the environment in several oxidation states (-3, 0, +3, +5). Major As species in common environments are predominantly oxyanions, trivalent arsenite and pentavalent arsenate

and their mobility differs from other heavy metals (Chatain et al., 2005; Lee et al., 2013). Many methods have been suggested to eliminate contaminants from soil: stabilization/solidification, vitrification, electrical process, but require long term monitoring and appropriate controls and are limited because of high material costs, regeneration of sludge, high energy requirements, etc. (Yamamura et al., 2005; Yao et al., 2012; Torres et al., 2012; Wang et al., 2009; Bahemmat et al., 2015; Tandy et al., 2004). The detailed overview of different methods for soil remediation and their combination is described by Peng et al. (2018). Soil washing offers a permanent remediation alternative for metal-polluted sites. Washed out metals can

also be recovered from leachate, and then reintroduced into the social material cycle instead of being landfilled (Li et al., 2015). Chemical-enhanced soil washing has shown promising results in removing heavy metals. Soil washing with chelating agents is considered to be an emerging remedial method for removal contaminants from soil rapidly and/or efficiently relative to other methods (Wang et al., 2018). Use of organic metal chelants in the wash formulation offer the advantages of high potential extraction, efficiencies, homogenous treatment of the polluted matrix and specificity for metals. Scientists remain uncertainty as to the optimal choice of chelating agents. Degradability and residues, potential adverse health effects, cost, extraction effect and possibility of chelant recovery need to be considered (Vandevivere et al., 2001). Also, ratio of chelating agent to toxic metals, pH, quantity of major cations extracted and source of contamination should be regarded (Tandy et al., 2004). Ethylenediaminetetraacetic acid (EDTA) is the most popular chelating agent and is used in various industrial fields. EDTA is nominated as a chemical unlikely to be removed during biological sewage treatment even after prolonged exposure. The reason for this might be that it has a slow biodegradation rate under conventional treatment conditions, which usually results in deterioration of soil function (Takahashi et al., 1997; Wang et al., 2018). [S, S]-stereoisomer of ethylenediamine disuccinic acid (EDDS) is a strong transition metal chelant which was originally developed for application in laundry detergents. It was tested as an agent for metal extraction based on its biodegradability, low toxicity, high affinity for most heavy metals ions and low sorption tendency which makes it perspective for environmentally sustainable treatment process (Takahashi et al., 1997; Vandevivere et al., 2001). The extraction of Cu, Zn and Pb using biodegradable chelant EDDS was studied by Hauser et al. (2005). While the Cu extractability was higher in batch experiments, the Zn and Pb extraction was higher in column experiments. Between 18 and 42 % of the applied EDDS was lost through biodegradation after 7 weeks. Kim et al. (2015a, b, 2016) studied the reductive arsenic extraction from soils with various reducing agents in order to remediate arsenic-contaminated soils. Oxalate and ascorbic acid were effective only at extracting arsenic associated with amorphous iron oxide. Extraction by dithionite was not effective because of re-adsorption of arsenic to the newly formed iron oxide phase. But the addition of oxalate enhanced the As extraction. Also, combination of chelating agents (EDTA) with reducing agents greatly improved the As extraction from the soil samples. The increase of the enhancement of EDTA extraction was greatly affected by the reducing conditions and soil-metal binding characteristics. The reductant and chelating agent were applied to remove arsenic and chromium in soil

by electrokinetic technology coupled with permeable reactive barrier (Xu et al., 2019). The pretreatment with reductant (ascorbic acid) enhanced arsenic removal but reduced chromium removal. Moreover, pretreating with chelating agent (EDTA-2Na) slightly enhanced arsenic and chromium removals. For such co-contaminated soils, pretreatment with sodium citrate was a relatively good way, because sodium citrate had both chelation and reducibility. Hashem et al. (2015) studied the effect of phosphate on arsenic mobilization from the contaminated sediment. The leached amount of As(V) was strongly depended on the phosphate concentration. It was expected that during long time leaching under anaerobic, leached As(V) will be reduced to As(III) but after 24 h of the leaching As(III) concentration was decreased. However, EDDS is more expensive than EDTA, therefore the possibility to combine these chelants could be promising for remediation technology.

Bioleaching is second promise technique generally used at industrial scale for low-grade ores. Microbial-based technologies for metal extraction have become attractive because of great potential for future development due to environmental compatibility and possible cost-effectiveness (Chatain et al., 2005; Wang and Zhao, 2009). Bioleaching may be applicable for a large number of inorganic pollutants and can be carried out under either anaerobic or aerobic conditions following specific microbial metabolisms. The stimulation of biochemical processes in decontamination is possible, if resistant bacteria are present in the soil (Štyriaková et al., 2015). Zeng et al. (2015) studied the removal of Cu, Pb and Zn from the contaminated sediment using *Aspergillus niger* strain SY1. The bioleaching efficiencies of heavy metals in the two-step bioleaching were better than that in one-step bioleaching. After the bioleaching, metals remaining in the sediment were mainly found in the stable fractions, and the toxicity of it was reduced to a level for it to be used safely in landfill or in land application. Anaerobic heterotrophic bioleaching batch tests with indigenous bacteria and carbon source addition showed that a fraction of arsenic could be extracted using appropriate biostimulation. When carbon source was added, release of arsenic increased 28-fold (Chatain et al., 2005). Arsenic bioleaching can be a result of direct reduction of arsenite associated with a solid phase to more mobile arsenate, the bio-oxidation of arsenic-containing sulfides or the reductive dissolution of iron hydroxides (Wang et al., 2009). Dissimilatory arsenate-reducing bacterium (DARB), *Bacillus* sp. SF-1 was able to effectively extract arsenic from various arsenic-laden solid via the reduction of solid-phase arsenate to arsenite. Extraction of As from the soil was a little more efficient with rotary shaking than without it. On the other hand, maximum extraction can be achieved even under static conditions by prolonged treatment (Yamamura et al., 2005).

Vaxevanidou et al. (2008) reported that bacterial activity of *Desulfuromonas palmitatis* can induce the reductive dissolution of Fe(III)-oxides in the contaminated soil and thus enhanced the release the retained arsenic without its reduction. However the pure chemical treatment by EDTA extracted only one third of the arsenic contamination.

The aim of this work is to suggest a method for toxic elements, mainly As(III)/(V), extraction from the contaminated soils, considering the environment and costs requirements. The chemical and biological leaching of soil samples using heterotrophic indigenous bacteria and resistant heterotrophic bacteria from sediments was studied in different leaching tests. Finally, the experiment with higher amounts of contaminated soils were performed to compare and verify the particular results and defined the method of mobile As(III)/(V) extraction.

Materials and Methods

Chemical analyses of soil samples

The soil samples of 20 kg were sampled from three sites of Richnava locality (denoted as R1, R2 and R3). The one of the sampling site was a garden (R1) often flooded by the river Hornád, being through the localities hard loaded by the anthropogenic activities, especially after mining and metallurgical industry. The soils were sieved to grain size < 4 mm. The oversized product consisted of anthropogenic sludge and larger rock grains. The grains below 4 mm were used for the experimental purposes.

X-ray diffraction analysis

The powder X-ray diffraction (XRD) patterns of R1, R2 and R3 soil samples were recorded using a diffractometer D2 Phaser (Bruker, Germany), equipped with a CuK α radiation source (30 kV, 10 mA) and Lynxeye detector. The data were qualitatively and quantitatively analysed using Software DIFFRAC.EVA with PDF-2 Database.

Electron probe micro analyzer

The particular soil grains were observed by electron micro analyzer CAMECA SX-100, allowing to provide the point chemical analyses, line profiles, RTG quantitative and qualitative maps, backscattered electron (BEI) and secondary electron (SEI) images.

Bioleaching experiments

For the bioleaching experiments the heterotrophic bacteria *Bacillus* spp. isolated from the sediment of Ružín water dam were used. The samples were heated at 80 °C for 15 min to kill vegetative cells. The sediment contained the spore-forming bacteria at a concentration of 10⁵ CFU/g active in Fe dissolution (Štyriaková et al., 2016). The isolates were grown in Trypton soya broth at 28 °C for 18 h.

Next the cells were centrifuged at 4000 rpm for 15 min and washed twice with saline solution (0.9 wt % NaCl). These bacteria were inoculated into parallel columns before the medium percolation (to ensure the activity of autochthonous bacteria). The stimulation of indigenous heterotrophic bacteria using nutrients in form of fertilizers verified the mobilization of toxic elements from the soil samples.

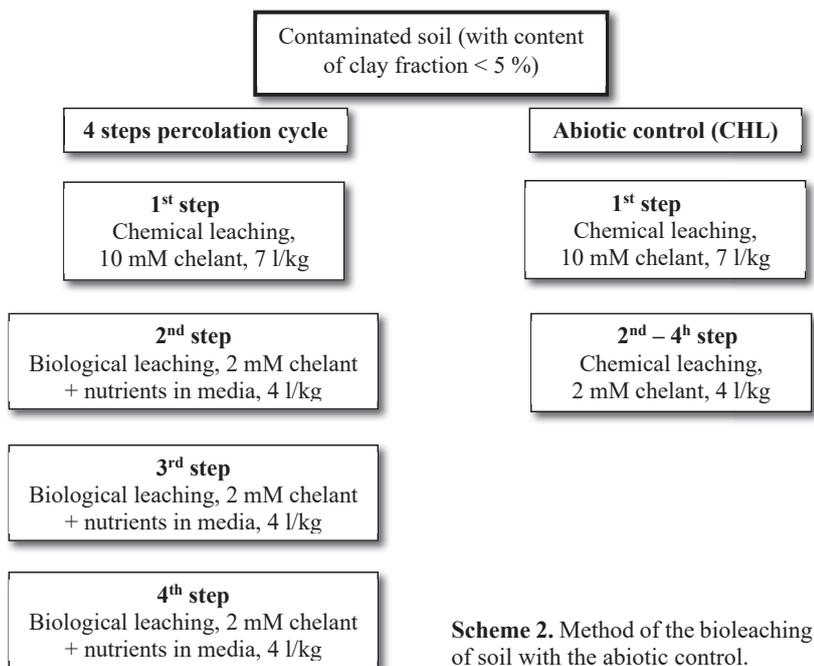
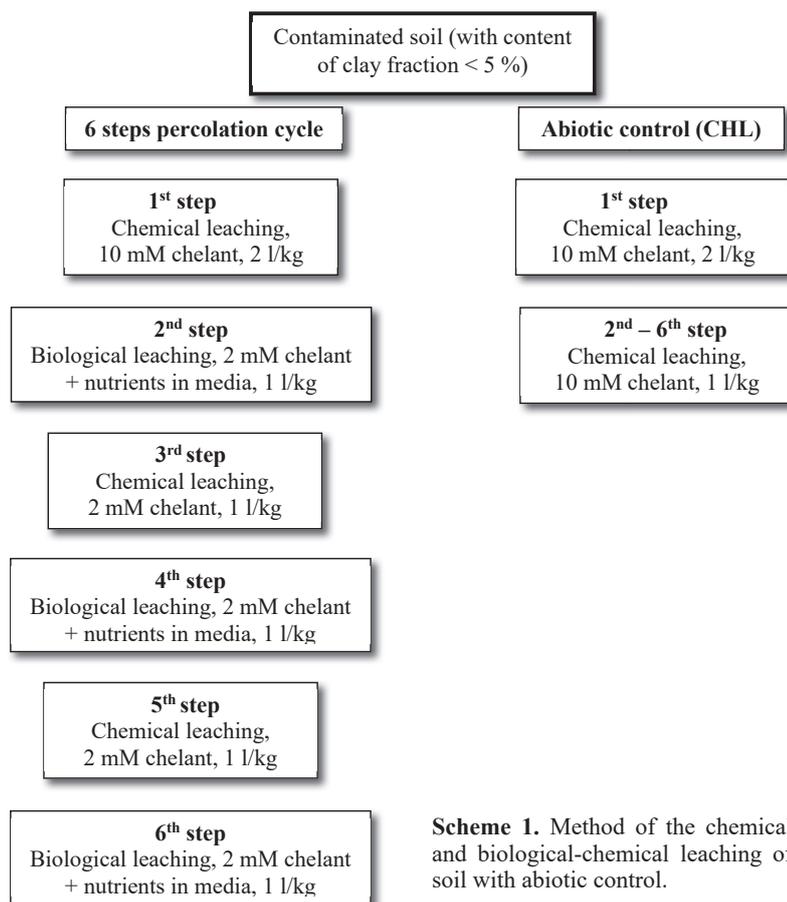
Leaching tests

The first chemical leaching was performed in ex-situ conditions. 600 ml of media percolated through the 300 g of contaminated sample for 5 days. For the experiments three types of media containing different chelants (solution of 10 mM) were used: Na₂EDTA (ch1), Na₃EDDS (ch2) and combination of both chelants in ratio 1 : 1 (ch3). The combination of chelants was used with the aim to decrease EDTA non-degradability according to the Beiyuan et al. (2018). From the chemical analyses of the solutions (leachates) the cumulative concentrations of extracted elements were calculated and compared. The effect of chemical leaching by different chelants was evaluated.

In the second series of experiments the percolation cycle combining CHL and BL in six steps was tested and compared with abiotic control (chemical leaching). The sample from the previous CHL represented the first step of this cycle. Following the medium of 300 mL containing 2 mM chelant and nutrients was percolated through the column (Scheme 1). For the abiotic control, chemical leaching by 10 mM chelant was performed. Also, the chemical leaching using 2 mM chelant as an abiotic control was tested, but the effect of As(III)/(V) extraction was approximately half in comparison with use of 10 mM chelant. The media percolated through the soil sample for 15 and 3 days for BL and CHL steps, respectively. The soil sample contained less than 5 % of clay fraction to ensure sufficient percolation of used media.

In the third series of bioleaching the soil sample after previous leaching in abiotic control (soil leached by 10 mM chelants) was treated in the percolation of 4 steps, Scheme 2. Media of 100 ml with 2 mM chelant with nutrients percolated through the 100 g of soil sample in four cycles during two months. The extraction was controlled after the finish of the experiment. The concentration of toxic elements were compared with abiotic control (CHL) using 2 mM chelants in the same time intervals.

Finally, according to the obtained results from the previous tests, the combination of particular leaching steps was tested and verified. Through the glass column of 80 mm in diameter and 340 mm high containing 1 kg of contaminated soil percolated 2 l of media with 10 mM Na₂EDTA (to eliminate the most content of contaminants such as Cu(II), Zn(II) and Pb(II)). Then,



1.5 l of medium with Na₃EDDS percolated through the glass bottle contained 800 g of chemically leached soil with the aim to remove the non-biodegradable chelant from the soil (and remaining content of inorganic contaminants). After that 700 g of pretreated soil was bioleached using 3 l of media with 2 mM chelant and nutrients.

The percolation rate of the medium was not studied during the experiments, more important for each cycle was the volume of percolated media.

Results

Chemical and mineralogical analyses

Chemical analysis of soil samples showed higher contamination by toxic elements for the sample R1, Table 1, in the order Ba(II) > As(III)/(V) > Sb(III)/(V) > Cu(II). The sites of sampling R2 and R3 soils are far from the flooded locality, where the soil is contaminated by minerals particles of alluvial deposits containing studied heavy metals and metalloids.

The chemical analysis of the plants (onion and potatoes) from the locality R1 showed the presence of over limited concentration of As(III)/(V) (0.7 ppm). Other toxic elements were present in admissible concentrations Cu(II) (0.7–1.5 ppm), Ba(II) (0.5–0.7 ppm), Sb(III) (< 0.1 ppm). From the reason of the highest contamination of R1 sample, the leaching tests were performed with this soil. Because the concentration of Zn(II), Pb(II), Ni(II) and Cd(II) did not exceed the values of permissible limits of their concentration, the leaching of As(III)/(V), Cu(II), Sb(III)/(V) and Ba(II) was studied more detailed and discussed.

Knowledge of the mineralogical composition of soil is important to better explain the mobility of toxic elements in the process of biological treatment. For example, As(III)/(V) is bonded by various bonds in different minerals (arsenopyrite, tetrahedrite, gersdorffite or in the secondary phases of grains coatings), what can influence its liberation into the solution. Therefore, the detailed study of mineralogy of different soil fraction is needed to obtain the information about the As(III)/(V) bonding and mobility in contaminated

Tab. 1

Basic chemical composition of soil sample and concentration of contaminants in studied soil samples.

	As(III)/(V) [mg.kg ⁻¹]	Cu(II) [mg.kg ⁻¹]	Zn(II) [mg.kg ⁻¹]	Pb(II) [mg.kg ⁻¹]	Ni(II) [mg.kg ⁻¹]	Cd(II) [mg.kg ⁻¹]	Sb(III)/(V) [mg.kg ⁻¹]	Ba(II) [mg.kg ⁻¹]
ID	65	500	1500	250	180	10	25	900
IT	70	600	2500	300	250	20	40	1000
R1	364	692	541	143	86	1	61	3303
R2	56	104	329	80	54	< 1	17	465
R3	40	600	187	46	49	< 1	40	478

ID – permissible limit of contaminant concentration in soils

IT – critical limit of contaminant concentration in soils

Tab. 2

Amount of magnetic and non-magnetic fraction in soil sample.

Grain size	Mass yield		Magnetic (MP)	Mass yield of products in relation with grain fraction
	[g]	[%]	Non-magnetic (NP) fraction	[%]
> 0.5	7.62	2.51	MP1	0.96
			NP1	1.55
< 0.5	295.77	97.49	MP2	16.82
			NP2	80.67

soil. For the mineralogical analysis the soil sample was separated to fractions (< 0.5 mm, > 0.5 mm), magnetically and gravitationally separated. The amount of magnetic and non-magnetic fraction is listed in Table 2. The soil fraction of grain size below 0.5 mm was used for the following XRD analysis.

The XRD analysis confirmed the presence of main mineral phase quartz, creating more than 70 % of soil, 12 % of siderite and Mg-siderite (especially siderite grains coated by Fe oxides should be the key factor for As(III)/(V) bioleaching from the soil), next lower content of plagioclase (4.3 %), muscovite (2.4 %), K-feldspar and chlorite (both approximately 1 %), calcite (1.8 %), dolomite (1.4 %) and below 1 % of barite, hematite and illite. The magnetic fraction below 0.5 mm composed of 76 % siderite, 13.5 % quartz and 1.4 % hematite.

In the grains of non-magnetic fraction the point chemical analysis confirmed the content of Fe(III) (almost 50 wt.%), next toxic elements mainly Zn(II) and Co(II), whose content together with other elements (As(III)/(V), Cu(II), Sb(III)/(V)) was more than 0.5 wt.%. The self-standing particles of sulphide minerals (chalcopyrite and pyrite) coated by Fe(II)/(III) layers were observed in the magnetic fraction (Fig. 1). They represented 30–65 % of total content with 1.5 wt.% As(III)/(V). The Ba(II) was bonded in the form of barite, which is very resistant towards chemical and biological leaching (Fig. 2).

On the basis of XRD and electron microanalyses it can be concluded that the mobile form of As(III)/(V) is preferentially bonded on Fe(II)/(III) oxides coating the grains of silicate minerals and carbonates. In the case of Fe(II)/(III) coatings of sulphides more resistant bondings of As(V) towards chemical and biological leaching can be expected (Wolthers et al., 2007).

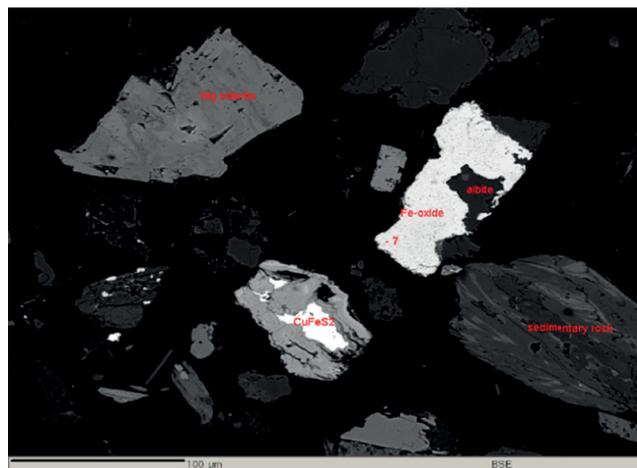


Fig. 1. Electron probe BSI image of selected chalcopyrite grain in magnetic fraction of soil sample.

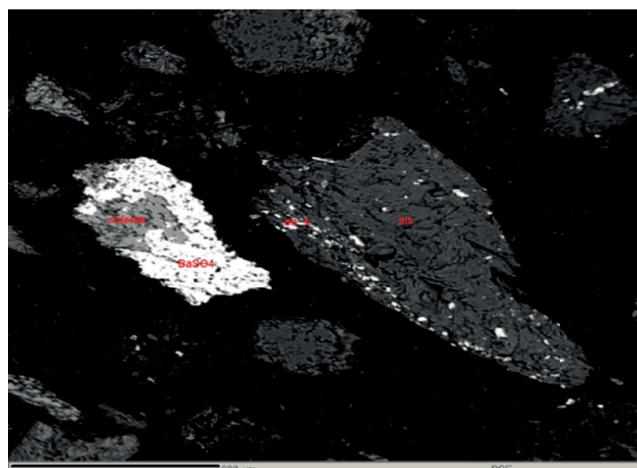


Fig. 2. Electron probe BSI image of selected barite grain in magnetic fraction of soil sample.

Chemical leaching of soil

Using the chemical leaching the extraction of studied elements decreased in the order Cu(II) > Zn(II) > Pb(II). However, the concentration of extracted As(III)/(V), Ba(II) and Sb(III)/(V) was very low. Higher effect of extraction of Pb(II) was observed for chelant ch1, while for the Zn(II) and Cu(II) leaching the chelant ch2 was more effective. Effect of extraction of other elements decreased in order Mn(II) > Fe(III) > Ni(II) > As(III)/(V) > Cd(II) > Ba(II) > Sb(III)/(V). The chelant ch2 was more effective for Mn(II), Fe(III), Ni(II), As(III)/(V) and Sb(III)/(V) extraction (Fig. 3). Ba(II) and As(III)/(V) are strongly bonded in minerals, their cumulative amount was below 1 mg.kg⁻¹ after the first cycle of media percolation. The highest effect of extraction was obtained for Cu(II) removal. For Sb(III)/(V) and As(III)/(V) leaching more washing cycles are needed to decrease their over-limited concentrations in soil.

Chemical and biological-chemical leaching of soil

The 3 days chemical leaching with medium of 2 mM chelant was combined with 15 days bioleaching (Scheme 1). The toxic elements extraction was studied after each step and compared with the abiotic control (chemical leaching of soil sample using 10 mM chelant in the same time intervals). In the abiotic control the extraction of Cu(II) decreased after the second percolation cycle for all chelants. The effect of bioleaching was very low, the extraction decreased after the first step of chemical leaching and leaching by bacteria was not effective (Fig.

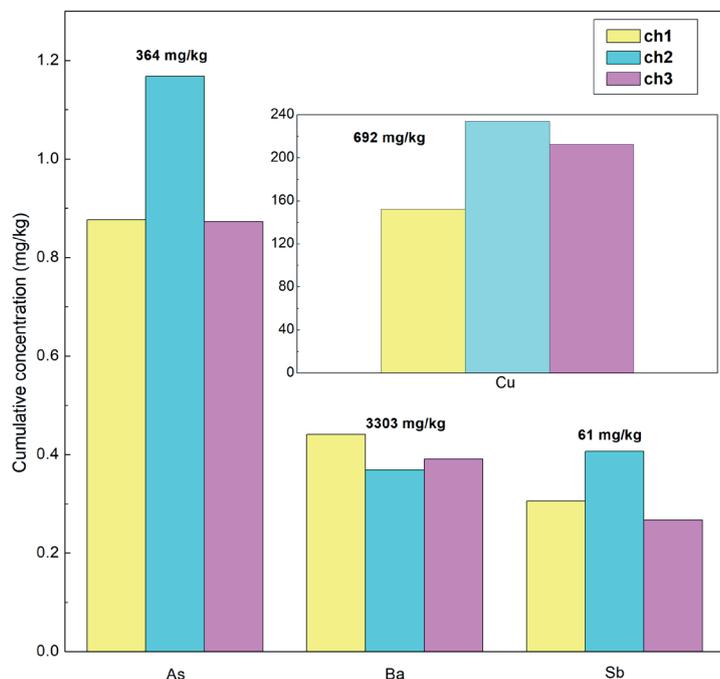


Fig. 3. Cumulative amounts of extracted toxic elements Cu, Sb, As and Ba.

4). After the last step, the Cu(II) concentration in the soil sample was decreased up to 36 %, what was at 4 % less than in case of abiotic control. Similarly, the effect of Zn(II) removal was lower in comparison with CHL (Tab. 3). Also, biological extraction of Sb(III)/(V) and Ba(II) was very low. The effect of extraction slightly increase in the 3rd and in the 5th step (after the nutrients addition in the previous BL step) for chelants ch1 and ch3, but it was comparable with CHL (Figs. 5 and 6). The combination of CHL and BL resulted in 43 % effect of As(III)/(V) extraction, what was at 6 % higher in comparison with CHL using 10 mM chelant. Positive running of the extraction curve can be observed especially in the 3rd and 5th step (Fig. 7). Addition of nutrients in BL step enhanced the mobilization of toxic elements by bacteria and they were following removed in CHL step in higher amounts. The process of bioleaching seems to be effective only for As(III)/(V) and Pb(II) (Tab. 3). But, comparing the results from CHL and BL, the concentrations of extracted toxic elements and heavy metals are similar, what pointed at the propriety of combined method of soil leaching, mainly from the reason of using of lower amounts of chelants (especially non-biodegradable EDTA).

Bioleaching of soil

The sample after chemical leaching using 10 mM chelant (from the previous abiotic control) was bioleached in 4 steps by percolation of medium containing 2 mM chelant and nutrients during 2 months and compared with the abiotic control (according to the Scheme 2). The partial Cu(II) extraction was observable only in the first step of bioleaching. The concentration of extracted Cu(II) was minimal (Fig. 8). In spite of relatively high concentration of Ba(II) in the as-obtained sample, the concentration of extracted Ba(II) was only 2 mg/L (Fig. 9). The highest extraction was observed for chelant ch1 after the first step of bioleaching. Then the extraction decreased for ch1 and ch2. In the next steps it has slightly increased. For chelant ch3, the Ba(II) extraction increased for the 2nd and the 3rd step of BL. As was mentioned above, Ba(II) is strongly bonded in the mineral phase of barite, that is resistant towards chemical and biological leaching. The mobile phase of Ba(II) in the tested sample represents only 20 %. The extraction of As(III)/(V) increased in the 2nd step for chelant ch1 and ch2. The most expressive increase of extraction can be observed for the 3rd step of BL for chelant ch3. Comparing the extraction curves with the abiotic control, BL of As(III)/(V) is more effective in each BL step for each used chelant (Fig. 10). The concentration of As(III)/(V) 24 mg.l⁻¹ was obtained after the

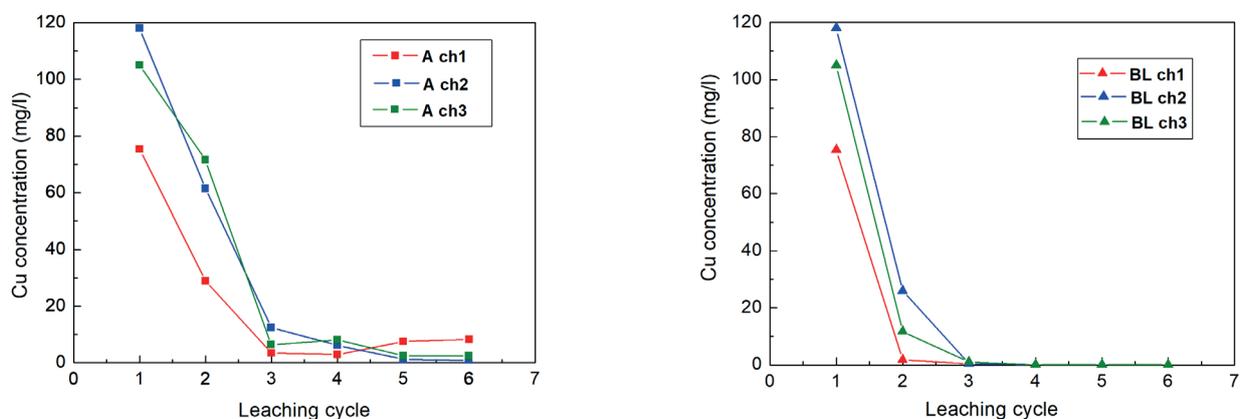


Fig. 4. Chemical (A) and biological-chemical (BL) extraction of Cu from the soil sample.

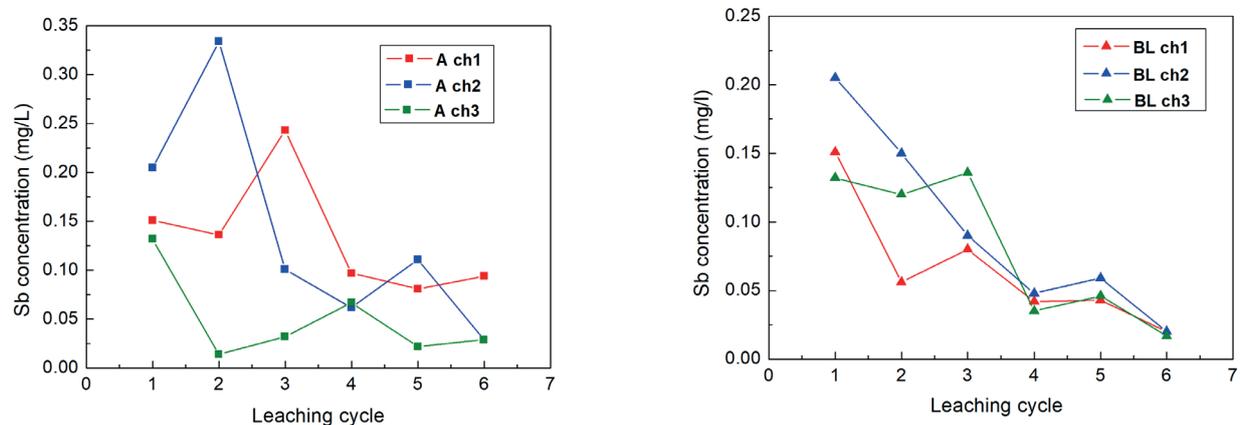


Fig. 5. Chemical (A) and biological-chemical (BL) extraction of Sb from the soil sample.

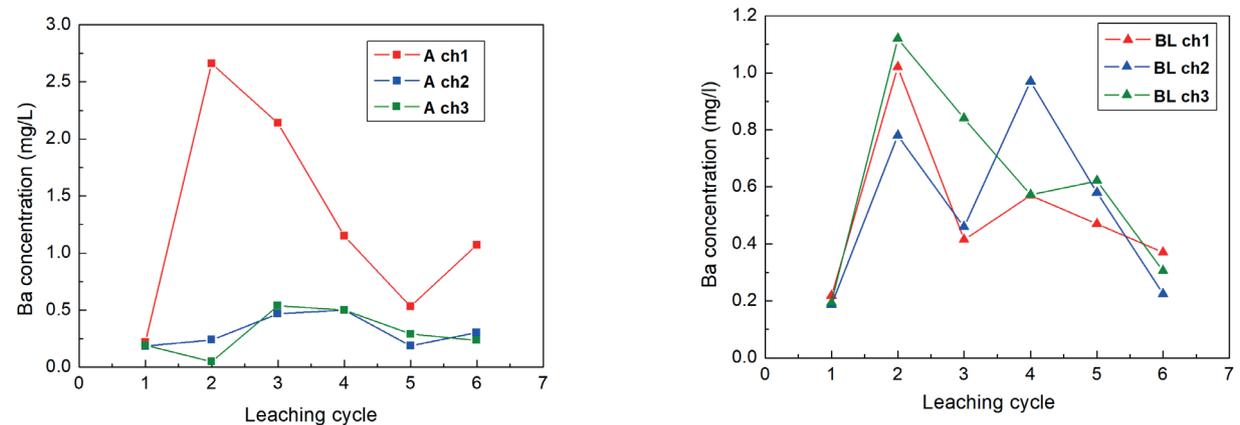


Fig. 6. Chemical (A) and biological-chemical (BL) extraction of Ba from the soil sample.

leaching of soil using chelant ch3. The maximal effect of As(III)/(V) removal was 44 %, what confirmed that the BL enhanced the effect of As(III)/(V) extraction from the contaminated soil.

The over-limited concentration of Cu(II) and partially Sb(III)/(V) in soil can be decreased by chemical and biological leaching. Also, the decrease of other toxic elements and heavy metals was observed, such as Pb(II), Zn(II), Ni(II), Co(II). The concentration of these elements

did not exceed the permissible values. After the CHL and BL the toxic elements As(III)/(V) and Ba(II) are still present in the over-limited concentrations in the soil. Effect of their extraction was 44 and 21 %, respectively (Tab. 4). By the combination of biological and chemical leaching as an activity of heterotrophic bacteria and chelants it is possible to remove only mobile amounts of elements, whose can come into food chain through the plant growing in the contaminated soil.

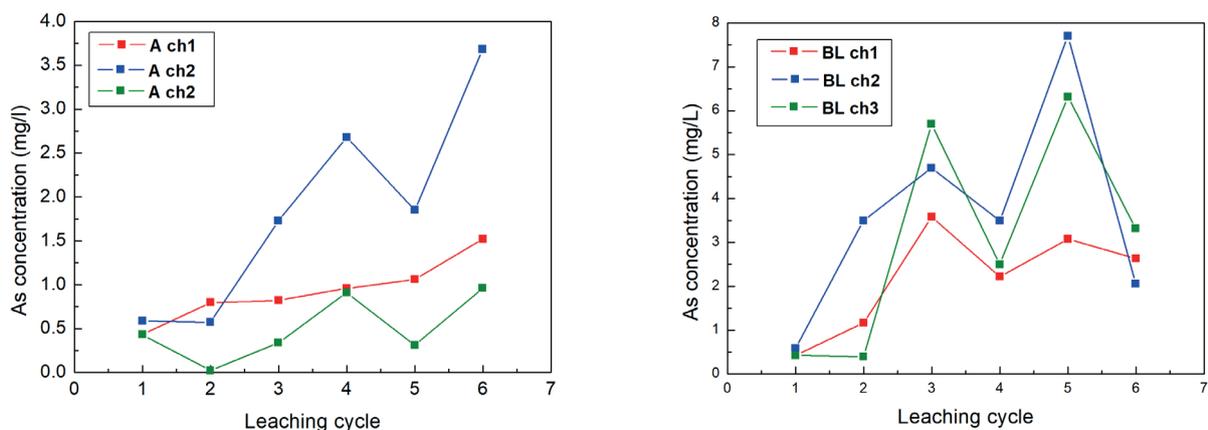


Fig. 7. Chemical (A) and biological-chemical (BL) extraction of As from the soil sample.

Tab. 3

Effect of toxic elements extraction by chemical leaching using ch2 chelant in comparison with bioleaching.

	Initial concentration [mg.kg ⁻¹]	Concentration after CHL [mg.kg ⁻¹]	Effect of CHL [%]	Concentration after BL [mg.kg ⁻¹]	Effect of BL [%]	Difference in CHL and BL [%]
Cu(II)	692	219	68	267	64	- 4
Zn(II)	541	261	52	287	47	- 5
Sb(III)/(V)	61	43	30	43	30	0
Ba(II)	3303	2592	22	2632	20	- 3
As(III)/(V)	364	229	37	208	43	+ 6
Pb(II)	143	88	39	82	43	+ 4

Verification of the process of chemical and biological leaching of soil

On the basis of the results obtained from the previous test the verification of the CHL and BL of soil sample of weight 1 kg was performed. The applied volume of solution was equal with previous tests, but during the CHL leaching it was decreased up to half (with the aim to decrease amounts of used chelants). It was shown above that the extraction of Cu(II) (partially also Pb(II)) is inhibited by heterotrophic bacteria. On the other hand, higher As(III)/(V) extraction was achieved by BL. For that reason the soil was at first leached by chelant ch1 with the aim to extract heavy metals. Subsequently the chelant ch2 was used to remove the non-biodegradable chelant ch1 as well as the rest of toxic elements from the soil. Finally, the BL was performed to ensure the maximal possible extraction of mobile As(III)/(V).

The chemical analyses of leachates after each cycle pointed at the chemical extraction of Cu(II), Zn(II) and Pb(II). Higher extraction of As(III)/(V), Sb(III)/(V), Ba(II) and Ni(II) was obtained by BL (Fig. 11). The effect of Ba(II) extraction was minimal in comparison with its concentration in the soil. Because the barite is very stable form towards the CHL and BL, and as such is not accessible for plants, it should not be dangerous for underground waters.

This test of chemical and biological leaching in three steps showed the enhance of Cu(II) extraction at 5 %, but a small decrease in As(III)/(V), Ni(II) and Co(II) extraction in comparison with the biological leaching (previous 4 steps test; Tab. 5). The chemical leaching, using chelant ch2, did not influence the As(III)/(V) and Sb(III)/(V) extraction. On the other hand, the bioleaching enhanced the As(III)/(V) removal at 7 %. The effect of leaching obtained from this test is a little bit lower in comparison with 4 steps bioleaching. Considering the decrease of chelants consumption, the difference is not so significant. The concentration of toxic elements As(III)/(V), Ba(II) and Sb(III)/(V) remained still over limited in the soil. But, their mobile phases, whose are the potential source of the contamination of groundwater and agricultural plants were removed successfully.

Conclusion

The study was focused on testing the chemical and chemical-biological methods of toxic elements extraction from the soil. The aim of the study was to outline environmentally friendly as well as cost effective method of the soil decontamination. Four different leaching tests showed that Cu(II), Zn(II) and Pb(II) can be removed by chemical leaching. The biological leaching combined

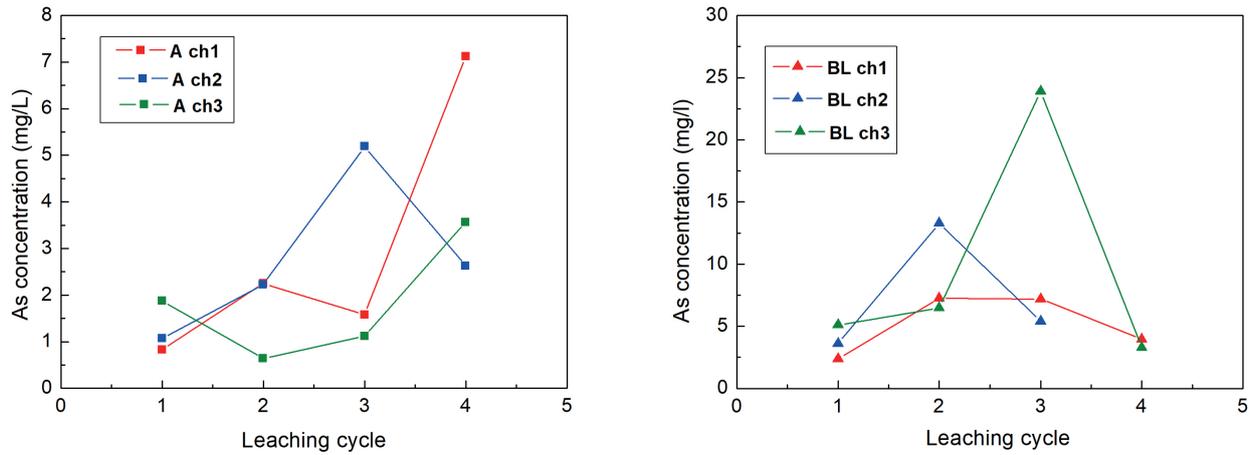


Fig. 8. Extraction of Cu from the soil sample during the bioleaching (BL) in comparison with abiotic control (A).

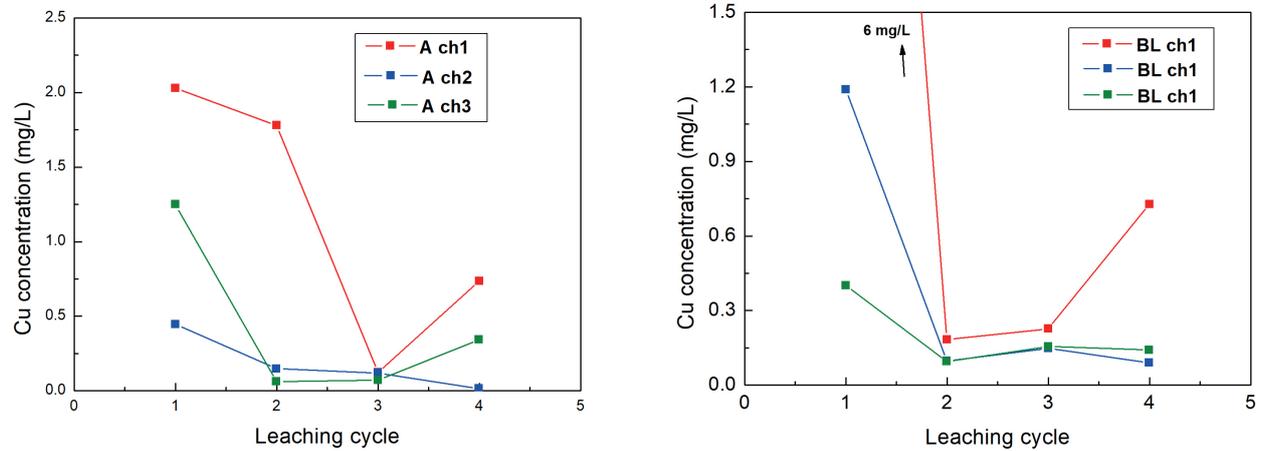


Fig. 9. Extraction of Ba from the soil sample during the bioleaching (BL) in comparison with abiotic control (A).

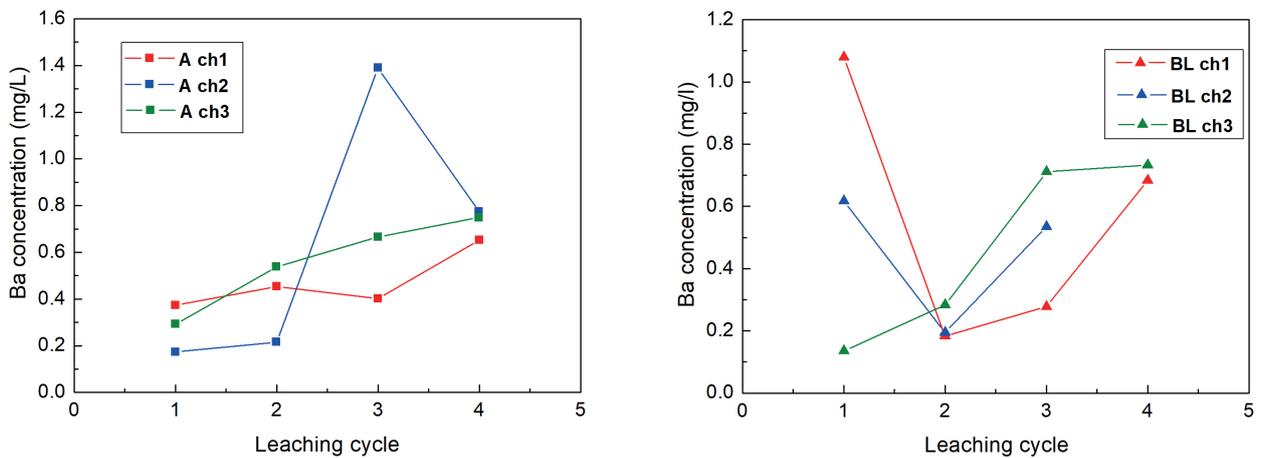


Fig. 10. Extraction of As from the soil sample during the bioleaching (BL) in comparison with abiotic control (A).

Tab. 4

Effect of chemical and biological leaching in extraction of different toxic elements concentrations.

	As(III)/(V) [mg.kg ⁻¹]	Cu(II) [mg.kg ⁻¹]	Zn(II) [mg.kg ⁻¹]	Pb(II) [mg.kg ⁻¹]	Ni(II) [mg.kg ⁻¹]	Co(II) [mg.kg ⁻¹]	Sb(III)/(V) [mg.kg ⁻¹]	Ba(II) [mg.kg ⁻¹]
Soil	364	692	541	143	86	25	61	3303
CHL + BL	204	258	276	57	50	7	42	2598
Effect [%]	44	63	49	60	42	72	31	21

with the chemical leaching resulted in increase of As(III)/(V) extraction, its mobile forms were leached from the soil. Moreover, for this test, lower amounts of chelants were needed, what decreases the costs on treatment of the contaminated soil. On the basis of obtained results, simply method of bioremediation was draft and tested with positive results:

- chemical leaching with Na₂EDTA [with the aim to remove Cu(II), Zn(II) and Pb(II)],
- chemical leaching with Na₃EDDS (with the aim to remove non-biodegradable chelant and rest of the toxic elements and heavy metals),
- bioleaching of the contaminated soil using less concentrated chelant in the leaching medium with nutrients for the maximal removal of mobile As(III)/(V).

These three steps of soil decontamination should be realized on heaps, where the contaminated soil would be sprinkled by media. The leachates would be collected in basins for further leaching media recycling and heavy metals concentration.

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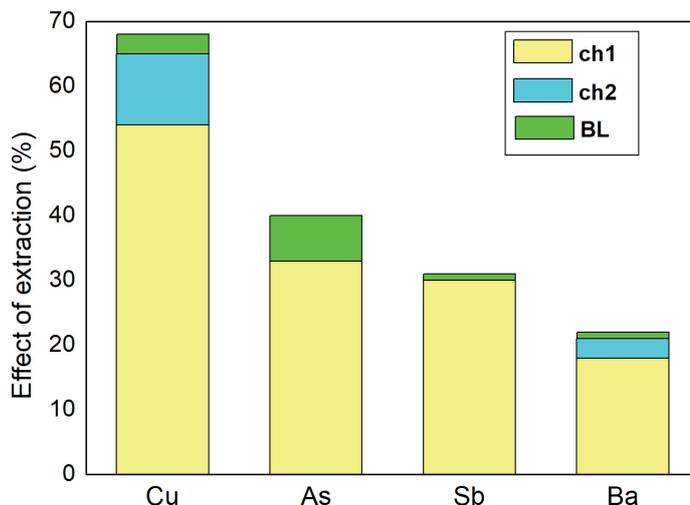


Fig. 11. Effect of chemical and biological leaching on extraction of studied toxic elements.

Tab. 5

Chemical analysis of soil sample after the chemical leaching with chelants ch1 and ch2 and after the biological leaching.

	As(III)/(V) [mg.kg ⁻¹]	Cu(II) [mg.kg ⁻¹]	Zn(II) [mg.kg ⁻¹]	Pb(II) [mg.kg ⁻¹]	Ni(II) [mg.kg ⁻¹]	Co(II) [mg.kg ⁻¹]	Sb(III)/(V) [mg.kg ⁻¹]	B(II) [mg.kg ⁻¹]
Soil	364	692	541	143	86	25	61	3303
CHL, ch1	244	317	296	70	65	14	43	2721
Effect [%]	33	54	45	51	24	44	30	18
CHL, ch2	245	242	281	63	68	11	42	2618
Effect [%]	33	65	48	56	21	56	31	21
BL	218	224	275	59	53	8	42	2562
Effect [%]	40	68	49	59	38	68	31	22

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Odstraňovanie toxických prvkov z minerálnych častíc kontaminovanej pôdy chemickým a biologickým lúhovaním

S cieľom odstrániť toxické prvky z kontaminovanej pôdy z lokality Richnava sa realizovali testy chemického a biologického lúhovania pôdy. Vzorky pôdy boli odobrané z lokality Richnava (R1, R2 a R3). Vzorka pôdy R1 pochádzala z oblasti kontaminovanej nánosmi rieky Hornád, ktorá preteká lokalitami zaťaženými

metalurgickým priemyslom, ako aj niekdajšou banskou činnosťou. Chemická analýza poukázala na jej znečistenie toxickými prvkami v poradí Ba > As > Hg > Sb > Cu. Koncentrácia Zn, Pb, Cd a Ni neprekračovala hodnotu stanovených koncentračných limitov. V ďalších dvoch vzorkách sledované toxické prvky neprekračovali

povolené limitné hodnoty ich koncentrácie v pôde (tab. 1). Preto na ďalšie experimenty bola zvolená vzorka pôdy R1. Röntgenovodifrakčná (rtg.) analýza a elektrónová mikroanalýza ukázali, že As sa prioritne viaže s oxidmi železa (hydroxidmi) pokrývajúcimi zrná silikátových a karbonátových minerálov (obr. 1 a 2). Na základe uvedených analýz sa predpokladala aj vyššia odolnosť Fe povlakov sulfidov proti chemickému a biologickému lúhovaniu. Chemické lúhovanie sa realizovalo použitím troch rôznych chelátov: Na₂EDTA (médiu ch1), Na₃EDDS (ch2) a ich zmes v pomere 1 : 1 (ch3). Počas chemického lúhovania klesala koncentrácia extrahovaných sledovaných prvkov v poradí Cu > Zn > Pb, pričom v prípade As a Ba sa získala vo výluhoch len veľmi nízka koncentrácia. Pri lúhovaní chelátom ch1 sme pozorovali viac extrahovaného Pb, zatiaľ čo pri lúhovaní Cu a Zn bol účinnejší chelát ch2. Výsledky extrakcie pri ostatných prvkoch klesali v poradí Mn > Fe > Ni > As > Cd > Ba > Sb, pričom vyšší účinok odstránenia Mn, Fe, Ni, As a Sb bol dosiahnutý pri použití chelátu ch2 (obr. 3). Väzby Ba a As v mineráloch boli silné a ich kumulatívna koncentrácia v roztoku po prvej perkolácii média predstavovala hodnotu nižšiu ako 1 mg · kg⁻¹. Najvyššia účinnosť lúhovania sa dosiahla pri Cu. Na zníženie nadlimitnej koncentrácie Sb a As v pôde by bolo potrebné opakovanie niekoľkých cyklov chemického lúhovania.

Druhý lúhovací test sa realizoval použitím autochtých baktérií a heterotrofných baktérií izolovaných zo sedimentov (Štyriaková et al., 2016). Extrakcia ťažkých kovov a toxických prvkov sa sledovala kombinovaným testom 15-dňového biolúhovania a 3-dňového chemického lúhovania s 2 mM chelátom. Výsledky sa porovnávali s abiotickou kontrolou (chemické lúhovanie pôdy 10 mM chelátom) v rovnakých časových intervaloch (schéma 1). V prvých dvoch perkolačných cykloch sa prejavil výrazný vplyv chemického lúhovania na odstránenie Cu. Počas biolúhovania koncentrácia Cu poklesla už v druhom perkolačnom cykle. Kombinácia chemického a následného biologického lúhovania umožnila pokles obsahu Cu vo vzorke pôdy o 64 %. Účinnosť odstránenia Sb a Ba bola rovnaká ako pri použití 10 mM chelátu v chemickom lúhovaní. Kombinované lúhovanie však umožnilo odstránenie 43 % As, čo predstavovalo o 6 % viac ako pri úprave pôdy chemickým lúhovaním 10 mM chelátom (tab. 3). Aj keď účinnosť odstránenia sledovaných prvkov touto kombinovanou metódou bola porovnateľná s účinnosťou chemického lúhovania pôdy, kombinovaná metóda je oveľa vhodnejšia. Dôvodom je použitie menšieho množstva chelátov, čo je dôležité z environmentálneho hľadiska, najmä pri použití biologicky nedegradovateľného Na₂EDTA (Takahashi et al., 1997; Wang et al., 2018).

Vzorka po chemickom lúhovaní 10 mM chelátom z predchádzajúceho testu (abiotickej kontroly) sa počas dvoch mesiacov biologicky lúhovala v štvorstupňovom perkolačnom cykle s použitím média obsahujúceho 2 mM chelát a živiny a porovnávala sa s abiotickou kontrolou (chemické lúhovanie 2 mM chelátom; schéma 2). Iba v prvom lúhovacom cykle sme pozorovali čiastočnú extrakciu Cu (obr. 8). Koncentrácia Ba aj napriek jeho vysokému obsahu v pôde dosiahla iba 2 mg · l⁻¹ (obr. 9). Bárium je pevne viazané v minerálnej fáze baritu, ktorý je veľmi odolný proti biologickému lúhovaniu. Mobilná fáza v testovanej vzorke predstavovala iba 20 % Ba. Najvyššia koncentrácia As bola extrahovaná pri použití média ch3 (obr. 10). Maximálna účinnosť odstránenia As dosiahla 44 %. Chemické a biologické lúhovanie viedlo k poklesu koncentrácie Cu v pôde a k čiastočnému poklesu koncentrácie Sb, Pb, Zn, Ni a Co. As a Ba naďalej ostávali v koncentrácii prekračujúcej ich dovolené limitné hodnoty v pôde (tab. 4). Biologicko-chemické lúhovanie ukázalo, že týmto spôsobom je možné odstrániť iba kontaminanty v mobilnej fáze, ktoré môžu vstupovať do potravinového reťazca prostredníctvom plodín pestovaných v kontaminovanej pôde.

Posledný test sa uskutočnil na overenie výsledkov získaných z predchádzajúcich lúhovacích experimentov. Realizoval sa na vzorke pôdy s hmotnosťou 1 kg, pričom objem jednotlivých médií korešpondoval s objemom v predchádzajúcich testoch. Pri chemickom lúhovaní sa však použil polovičný objem roztokov s cieľom znížiť spotrebu chelátov. Vzorka sa najprv lúhovala chemicky chelátom ch1 s cieľom odstrániť ťažké kovy ako Cu, Zn a Pb. Následne sa použilo médium s chelátom ch2, aby sa odstránili zvyšné mobilné fázy kontaminantov, ale najmä, aby sa z pôdy odstránil biologicky nedegradovateľný chelát. Posledným krokom bolo biologické lúhovanie zamerané na maximálne odstránenie As z pôdy. Opakovaný trojstupňový test na jednej strane zvýšil extrahované množstvo Cu o 5 % v porovnaní so štvorstupňovým perkolačným testom, na druhej strane sme pozorovali čiastočný pokles v extrakcii As, Ni a Co po chemickom lúhovaní. Biologické lúhovanie zvýšilo odstránenie As ešte o 7 % (tab. 5). Táto kombinovaná metóda je veľmi perspektívna, pretože na extrakciu toxických prvkov využíva menšie množstvo chelátov. Navyše, je aj finančne nenáročná a umožňuje odstránenie mobilných fáz kontaminantov z pôdy, ktoré sú potenciálnym zdrojom znečistenia vody a kontaminácie rastlín.

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