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Influence of bacteria on toxic elements leaching from the contaminated soil

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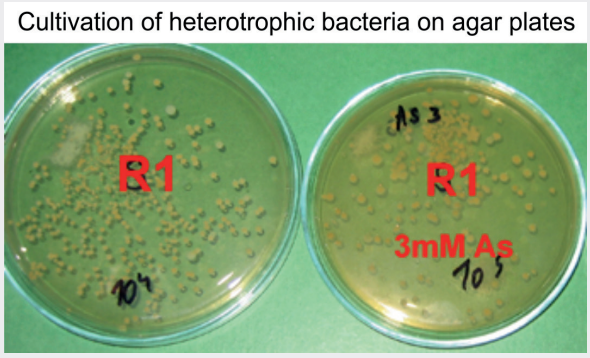
Abstract: The study is focused on better understanding of bacterial activity in biological-chemical leaching in soil remediation process. The heterotrophic bacteria isolated from the contaminated soil from the locality Richnava (Eastern Slovakia) showed good resistance towards As, in both experiments with bacterial growth on solid nutrient media (agar plates), as well as in liquid nutrient media (glass tubes), especially to molarity of 0.3 mM As. Even though the toxic character of elements presented in studied soil was not proven by the method of Toxic Characteristic of Leaching Process (TCLP), the microbiological study pointed at the bacterial activity in disruption of bounds between the mineral grains and toxic elements, especially of As bounded on Fe coatings of mineral grains. That was confirmed by changes of mineral grains surface observed by optical and scanning electron microscopy. The Simple Bioavailability Extraction Test (SBET) pointed at the bacteria influence in biological-chemical leaching of the soil, where bioassessment of studied toxic elements decreased after the soil treatment, except of Fe and As. The results from the sequential extraction analyses showed the decrease of As content in the residual fraction as well, contrary to the other toxic elements, after the biological-chemical leaching and its increase in the bioavailable fractions.

Key words: contaminated soil, sequence extraction analysis, bacteria resistance

Graphical abstract



Highlights

- Heterotrophic indigenous bacteria showed good resistance towards As.
- Biological-chemical leaching decreased the content of As in the residual, non-biodegradable, fraction of the contaminated soil.
- Repeating of cycles of chemical and biological-chemical leaching is promising way for the soil remediation.

1. Introduction

Soil contamination by toxic elements is one of the main environmental problem in the world. An international trend of enforcing more stringent legislation on landfill disposal, e.g. European Union Landfill Directive (Council of the European Union 1999), has prompted a strong drive on the land remediation industries to develop remedial technologies for sustainable resource recycling/conservation (Tsang & Yip, 2014).

Heavy metals are natural elements, in their basic level being just atoms. That is why their degradation and metabolism is not possible. Instead, microorganisms have evolved coping strategies to either transform the element to a less-harmful form or bind the metal intra- or extracellularly, thereby preventing any harmful interactions in the bacterial cell. Plus, they are able to actively transport the metal out of the cell cytosol (Nwagwu et al., 2017).

The bioleaching process can be a promising alternative technology for heavy metal polluted soils remediation due to the simplicity of the operation, low costs and eco-friendliness. Certain types of bacteria have been used in remediation processes of contaminated soil for decades due to their capacity to detoxify certain heavy metals,

their high surface area to volume ratio, and their capacity to promote plant growth and metal accumulation on plant tissues (Pires et al., 2017). Soil bacteria communities play an important role in nutrient cycling, plant symbioses, decomposition, and other ecosystem processes. Selection of the proper microbial agent is one of the most critical steps in order to remove heavy metal from the soil (Xu et al., 2020).

Microbes deal with poisonous chemicals by applying enzymes to convert one chemical into another form and taking energy or utilizable matter from this process. Despite its toxicity, the ancient and constant exposure of bacteria to arsenic has led to the microbe colonization of arsenic-rich environments throughout the development of metabolism coupled biotransformation processes, i.e. reduction, oxidation, that affects geochemistry, speciation and toxicity of this element. Due to the ability of bacteria to metabolize highly toxic arsenic compounds into a less toxic form, the isolation and study of arsenic resistant bacteria is attractive for the establishment of processes to ameliorate the bioavailability of arsenic in contaminated soil and water (Alaniz-Andrade et al., 2017).

Bioaccumulation mainly involves the biosorption or physiological uptake of arsenic by microbial metabolically active and passive processes. Microbial-mediated arsenic reactions may occur thereafter, which is part of the most important phenomena involved in arsenic metabolism. Because of its high efficiency, low cost, and most importantly its eco-friendly nature, bioaccumulation presents an interesting option for the removal and recovery of arsenic from the contaminated environments (Pandey & Bhatt, 2015).

This research study deals with the explaining of the bacterial contribution in the soil leaching and continues on the results from the research of contaminated soil bioleaching published by Štyriaková et al. (2019). The resistance of heterotrophic autochthonous bacteria isolated from the contaminated soil towards the As was studied. The toxic characteristic of leaching process, bioassessment of studied toxic elements as well as sequential extraction analyses were provided to evaluate the influence of bacteria onto toxic elements leaching. The experimental results were completed by the electron microanalyses.

2. Materials and Methods

2.1. Soil sample

The soil sample was taken from the Richnava locality (denoted as R1). The sampling site was a garden often flooded by the Hornád river. The river was polluted because flowing through the localities, heavy loaded with products of anthropogenic activities – especially mining and metallurgical industry. The soil was sieved to grain size below 4 mm. The oversized product consisted of anthropogenic sludge and larger rock grains. The grains below 4 mm were used for experimental purposes. According to the XRD analysis the main mineral phase of studied soil was quartz, creating more than 70 %, than siderite and Mg-siderite (12 %). Other minor phases were plagioclase (4.3 %), muscovite (2.4 %), K-feldspar and chlorite (both approximately 1 %), calcite (1.8 %), dolomite (1.4 %) and barite, hematite and illite (below 1.0 %) (Štyriaková et al., 2019).

2.3. Microbiological analysis of soil

Microbiological analyses were performed with the aim to determine the count of heterotrophic bacteria in contaminated soil that are tolerant towards high concentration of toxic As. The resistance of heterotrophic bacteria isolated from the contaminated soil towards As was tested by bacterial cultivation on the solid nutrient media – agar plates (Trypton soya agar – TSA) and in the liquid nutrient media TSB (Trypton-soya broth) with the addition of 0.3 mM and 3 mM As respectively. The bacterial turbidity was determined using the McFarland standard (2002).

McFarland standard is a chemical solution of barium chloride and sulphuric acid. The result of the chemical reaction is a fine precipitate of barium sulphide. After the suspension shaking its turbidity is visually comparable with the bacterial suspension of known concentration. The degree of turbidity is in the range of 0.5–10 and represents the different bacterial density, count of bacterial cells (Tab. 1). The measured value of absorbance corresponds with the particular value of McFarland standard. On this basis it is possible to determine the approximated count of bacterial cells for each sample.

Tab. 1
McFarland standard for determination of count of bacterial cell in media in dependence on measured value of absorbance.

McFarland standard	Absorbance	Grown bacterial cells [ml]
0.5	0.125	1.5 x 108
1	0.25	3.0 x 108
2	0.5	6.0 x 108
3	0.75	9.0 x 108
4	1	1.2 x 109
5	1.25	1.5 x 109
6	1.5	1.8 x 109
7	1.75	2.1 x 109
8	2	2.4 x 109
9	2.25	2.7 x 109
10	2.5	3 x 109

The value of absorbance of media was measured in selected time intervals by UV VIS spectrometer Spectroquant Pharo 300 (Merck, Germany) at wavelength of 540 nm. As the blank control the uninoculated TSB medium was used.

2.3. Chemical and biological chemical leaching of soil

According to the method of soil remediation published by Štyriaková et al. (2019), the studied sample was leached in three steps. Through the glass column of 80 mm in diameter and 340 mm high containing 1 kg of the contaminated soil percolated with 2 l of media containing 10 mM Ethylenediaminetetraacetic acid disodium salt dihydrate (Na_2EDTA, denotes as chelant ch1). Subsequently, 1.5 l of medium with Ethylenediamine-*N*,*N*′-disuccinic acid trisodium salt (Na_3EDDS, denoted as chelant ch2) percolated through the glass bottle containing 800 g of chemically leached soil. After that 700 g of as-treated soil was bioleached using 3 l of media with 2 mM chelant ch1 and nutrients.

For the bioleaching experiments the heterotrophic bacteria *Bacillus* spp. isolated from the sediment of water dam Ružín 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 10^5 CFU/g active in Fe dissolution (Štyriaková et al., 2016). The isolates were grown in Trypton soya broth at 28 °C for 18 h. Following the cells were centrifuged at 4 000 rpm for 15 min and washed twice with the saline solution (0.9 wt % NaCl). These bacteria were inoculated into the parallel columns before the medium percolation (to ensure the activity of autochthonous bacteria). The stimulation of indigenous heterotrophic bacteria using nutrients in the form of fertilizers verified the mobilization of toxic elements from the soil samples.

2.4. Toxicity and bioassessment of soil

Soil toxicity was determined using Toxic Characteristic of Leaching Process (TCLP) according to the US EPA 1311 method (Khorasanipour & Eslami, 2014). The soil sample of 50 grams was treated by solution of 1 N NaOH and vinegar acid of pH 4.2 for 18 hours under the vigorous stirring. The permissible concentration of elements extracted from the soil or waste are listed in Tab. 2. The limits are supplemented by values according to the solid-waste extraction procedure for leaching toxicity HJ/T 300-2007 (IEPT) (Xu et al., 2019).

Tab. 2

TCLP and IEPT limits.

	Cu	Pb	Zn	As	Ba	Cd	Cr
TCLP limit [mg/l]	–	5	–	5	100	1	5
IEPT [mg/l]	50	3	50	1.5	–	0.3	10

The bioassessment of the toxic elements present in the contaminated soil was tested by Simple Bioavailability Extraction Test (SBET). The soil sample of 5 grams was treated by solution of 0.4 M glycine of pH 1.5 (adjusted by HCl) under the vigorous stirring for 1 hour at 37 °C (Report No.: 1542820-003-R-Rev0, 2016; Kim et al., 2009).

2.5. Sequential extraction analysis of soil

The as-received soil sample, chemically leached samples by chelants ch1, ch2 and biological-chemical leached sample were subjected to sequential extraction analysis with the aim to determine the content of toxic elements in biologically available and unavailable fractions. The sequential extraction analysis was provided according to the method described by Mackových et al. (2000).

2.6. Optical and electron microscopy, electron microanalysis

The changes of separated grains after the biological-chemical leaching were observed by binocular optic microscope with camera Nicon P-FMD (Japan).

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

More detailed study of morphology and grain surface were studied by scanning electron microscopy FE MIRA 3 (Tescan, Czech Republic) equipped by XRD energy-dispersive (EDX) analyser of chemical composition (Oxford Instruments).

3. Results

3.1. Microbiological analysis of soil

The detailed chemical and mineralogical analysis of the soil sample R1 was described by Štyriaková et al. (2019). The contamination by studied toxic elements decreased, according to the contamination criteria, in order Ba > As > Sb > Cu (Tab. 3). Also, the plants growing in this locality showed the presence of higher As concentrations (Štyriaková et al., 2019).

Tab. 3

Concentration of studied elements present in the contaminated soil.

R1 [mg/g]	As	Sb	Ba	Cu	Zn	Pb	Ni	Cd	Hg
	364	61	3 303	692	541	143	86	1	31
IT [mg/g]	65	25	900	500	1 500	250	180	10	2.5
ID [mg/g]	70	40	1 000	600	2 500	300	250	20	10

ID – Permissible limit of contaminant concentration in soils
IT – Critical limit of contaminant concentration in soils

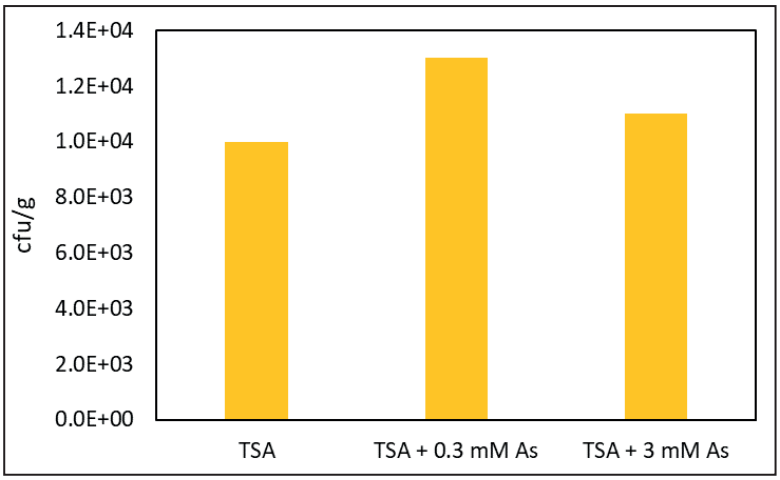


Fig. 1. Comparison of heterotrophic bacteria cultivation on agar plates (TSA), and on plates with addition of As.

High concentration of As should be released from the soil matrix by the biological-chemical processes and lead to gradual contamination of plants and. From this reason the microbiological analyses were performed with the aim to determine the count of the heterotrophic bacteria in contaminated soil that are tolerant towards high concentration of toxic As.

The highest resistance towards As was observed for the TSA medium with 0.3 mM As, 1.3 x 10^4 colony forming unit (cfu/g). For the TSA with 3mM As the bacteria growth reached 1.1 x 10^4 cfu/g (Fig. 1). The resistance of bacteria does not correspond with the As concentration in the soil sample. It is influenced by the soil utilization for agriculture, behind with the continuous supply of organic matter and biogenic elements in the form of fertilizer relates.

The experiments in TSB were performed in the glass tubes containing nutrient medium and in the glass reagent flasks containing nutrient medium with 5 g of contaminated soil.

During the whole experiment more prominent bacterial growth was observed in the flasks. The values of absorbance reached higher values (higher medium turbidity). The available nutrients for bacteria growth in flasks were provided not only from the TSB media but also from the present soil. The process was finished after 90 hours with higher bacteria growth. In the glass tubes, the experiment was finished earlier, after 70 hours.

During the first 40 hours the more expressive bacterial growth was observed in the glass tubes with the As addition. After 43 hours the stagnation of growth can be observed for all tubes and after 48 hours the slight decrease was detected. While the measured values of absorbance were similar for TSB and TSB with 0.3 mM As, the highest values were obtained for TSB with 3 mM As (Fig. 2).

The bacterial growth in flasks was of jump character. After 27 hours the slight increase was observed for all samples. More expressive increase was detected after 50 hours and after 69 hours the slowing down of the growth occurred (Fig. 3). In the first

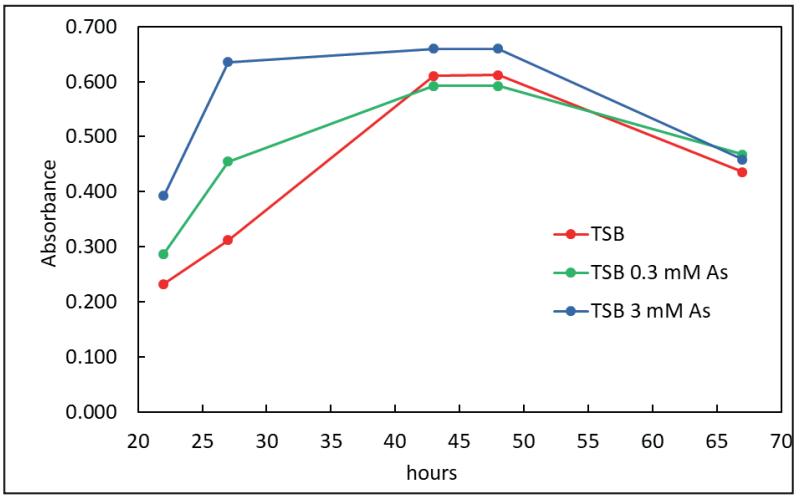


Fig. 2. Comparison of growth of bacteria isolated from the contaminated soil in glass tubes containing TSB and TSB with addition of 0.3 and 3 mM As by absorbance measuring using UV VIS spectrometer.

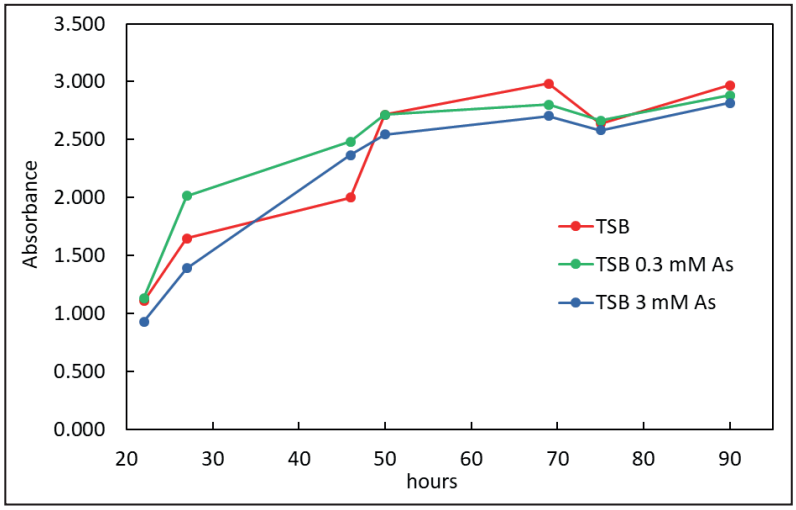


Fig. 3. Comparison of growth of bacteria isolated from the contaminated soil in glass flasks containing soil and TSB and TSB with addition of 0.3 and 3 mM As by absorbance measuring using UV VIS spectrometer.

hours of experiment the more expressive increase was observed for flask contained TSB with 0.3 mM As what also corresponds with the results obtained from the experiments of isolated bacteria growth provided on agar plates. For all studied media the maximum bacteria growth was reached after 43 hours and was in the range 6 x 10^8 – 9 x 10^8 cfu/ml.

In flasks the highest value of absorbance was measured after 69 hours and it was higher than 2.7, what represents more than 3 x 10^9 cfu/ml. The mineral particles present in the soil are a source of biogenic elements that stimulate bacterial metabolism, reproduction and growth. Thus the growth of the resistant bacterial cells was higher in the flask than in the tubes containing only the TSB medium. In this case it is not possible to determine the count of bacterial cell by the McFarland standard. More appropriate method is direct counting under the microscope in Burker chamber. From the reason of unavailable device equipment this method was not applied to evaluate the measurements of absorbance.

3.2. *Toxic characteristics and bioassessment of soil*

The TCLP test was applied on leaching of the as-received soil (R1) and soil after the biological-chemical leaching (R1 L). Except the Ba, higher concentrations of studied elements were released from the R1 L sample, but they were under the limited values according to the TCLP and IEPT limits (Fig. 4).

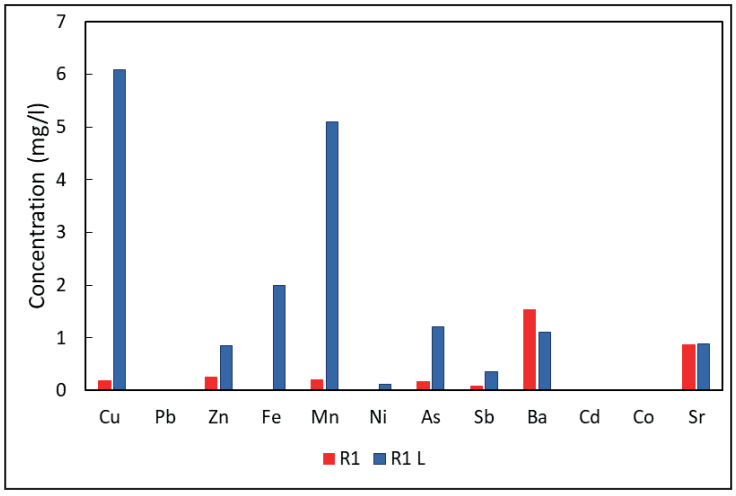


Fig. 4. Concentrations of studied toxic elements in leachates after the TCLP test applied on as-received and biological-chemical leached soil sample.

According to the results of the SBET test, the biological-chemical leaching of the sample led to the decrease of bioassessment of Cu, Pb, Zn, Mn, Ba and Sr (Fig. 5). On the other hand, the bioassessment of Fe and As increased, probably due to the disruption of their bounds in the structure of leached mineral grains. This result pointed at the further possibility of As extraction by the process of acid leaching (pH lower than 2), that should be effective in As concentration, lowering in the soil up to limited values.

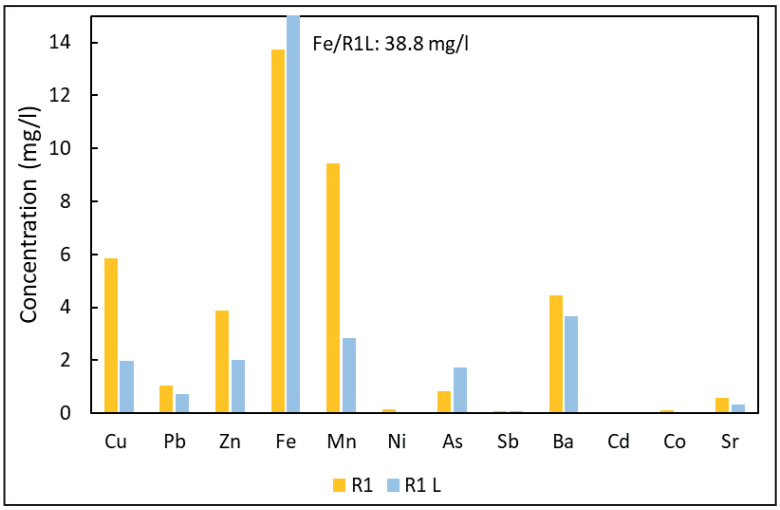


Fig. 5. Concentrations of studied toxic elements in leachates after the SBET test applied on as-received and biological-chemical leached soil sample.

3.3. *Sequential extraction analysis*

To explain the effect of combined three step chemical and biological-chemical leaching on soil decontamination, the sequential extraction analysis was provided.

The fractions (1) to (4) represent the forms of risk chemical elements that are available for organisms in their living environment and they are the most hazardous for their contamination. The used reagents imitate the nature processes of liberation of elements from the primary bounds into the solutions and their recombination into secondary minerals. The fraction (1) is soluble in water, (2) exchangeable and/or carbonate, (3) reducible, and (4) oxidizable/organo-sulfide. The fraction (5) representing residual, encompasses the elements in form of no real risk for organisms present in given environment.

In the R1 sample the content of Cu (49.2 %), Pb (85.1 %), Zn (40.3 %) and Co (33.0 %) was the highest in the reducible fraction, content of Ni (51.1 %), As (52.0 %), Sb (92.0 %), Ba (65.5 %) and Cr (77.6 %) in the residual fraction and Hg (93.8 %) in the organo-sulfide fraction (Fig. 6). After the chemical leaching by chelant ch1, the highest ratio of Pb (78.6 %), Zn (40.2 %) and Co (26.1 %) was still in the reducible fraction (the same ratio of Co

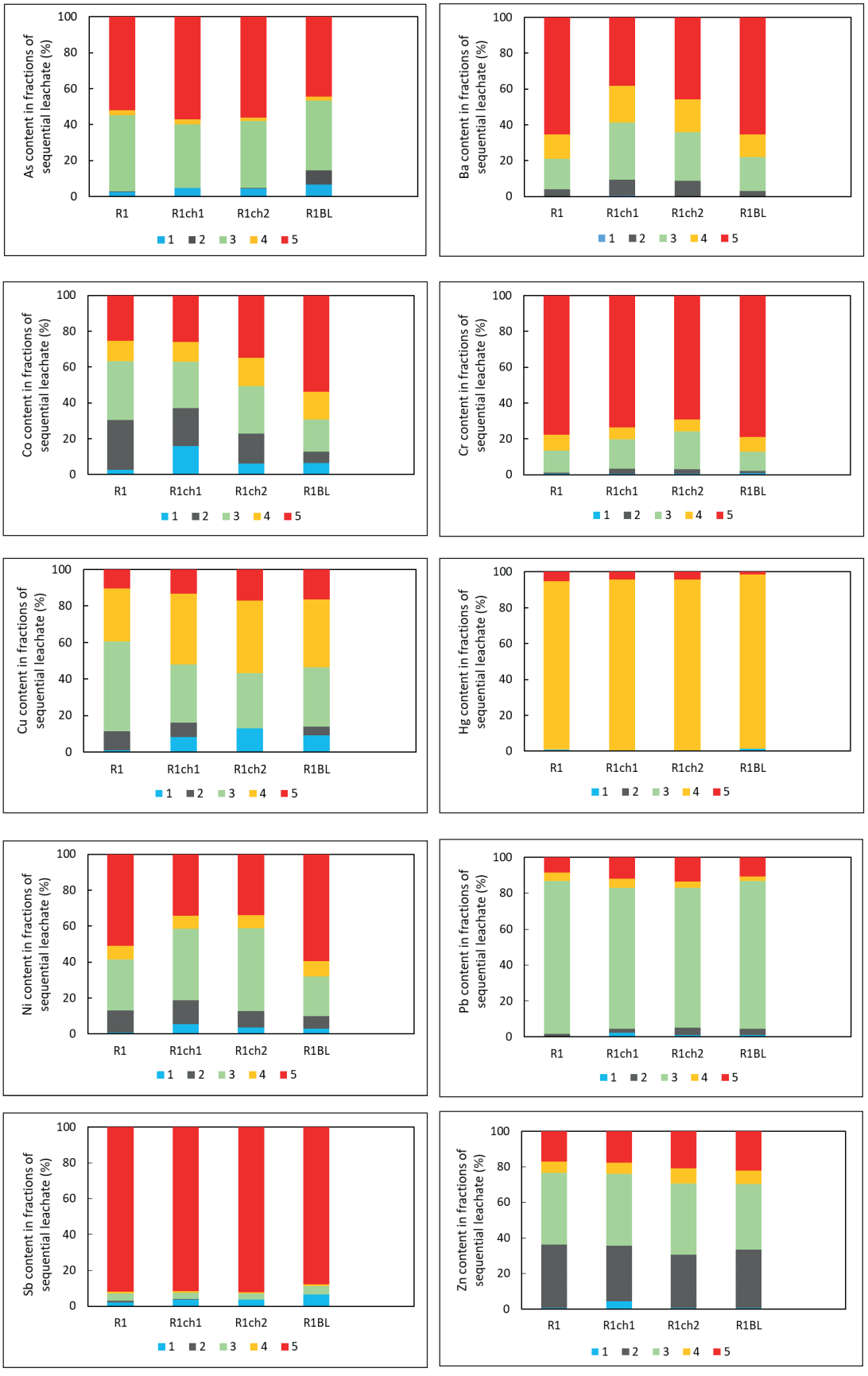


Fig. 6. Content of studied toxic elements in the fractions of sequential leachate as received in soil, soil after chemical (R1ch1, R1ch2) and and biological-chemical leaching (RIBL).

also in residual), but their content decreased after chemical leaching in behalf of water soluble fraction (Pb, Zn, Co), ion exchangeable and carbonate fraction (Pb) and organo-sulfide (Pb, Zn) fraction. For Cu the increase of its content in the fractions (1), (4) and (5) was observed. The highest ratio of Cu was in the organo-sulfide fraction. The chemical leaching by chelant ch1 also led to increase of content of Ni, Ba and Cr in the fractions (1), (2) and (3), of As in (1) and (2), of Sb, Co in the fraction (1). Also, the ratio of As and Co was increased in the reducible fraction, As for more than 5 % and Co 0.5 % (Fig. 6).

After the chemical leaching by chelant ch2 the highest content of Cu (39.5 %) and Hg (95.3 %) stayed in the organo-sulfide fraction, Pb (78.6 %), Zn (40.1 %) and Ni (45.9 %) in the reducible fraction and As, Sb, Ba, Co and Cr in the residual fraction. For all studied toxic elements, except of Ni and As, the increase of their concentrations in the residual fraction was observed (for Ba of 7.39 %). The content of Cu increased in the fractions (1) and (4), Pb in (2), Zn in (4), Ni, Co in the fractions (3) and (4), As and Cr in the fraction (3) (Fig. 6).

After the biological-chemical leaching the highest ratio of Cu (37.3 %) and Hg (97.0 %) was again in the organo-sulfide fraction, content of Pb (82.4 %) and Zn (36.7 %) in the reducible fraction and other studied elements in the residual fraction. In comparing with R1ch2, the concentration of Cu, Pb, As, Sb and Hg decreased in the biologically unavailable fraction. The most significant decrease was observed for As (of 11.5 %) in behalf of fractions (1), (2) and (3) (Fig. 6).

From these results it can be concluded that the repeated chemical and biological-chemical leaching of contaminated soil should lead to more expressive decrease of As, but also of Cu, Pb and Sb concentrations in the studied soil.

3.4. Optical and electron microscopy, electron microanalysis

Grain body was composed mostly of aluminosilicates with different content of Fe with admixture of quartz, feldspars, chlorite, siderite, and also with minor ratio of apatite and rutile (Štyriaková et al., 2019).

Disrupted structure of Fe coatings was observed on the marginal sites of grains after the biological-chemical leaching. The heterotrophic bacteria used in the leaching process are able to disrupt a less resistant structure of Fe oxides and hydroxides. The point analysis in the sites with higher content of Fe did not show and presence of significant content of As and other studied toxic elements (Fig. 7, Tab. 4). This pointed at their effective removal from the grain surface by biological-chemical leaching.

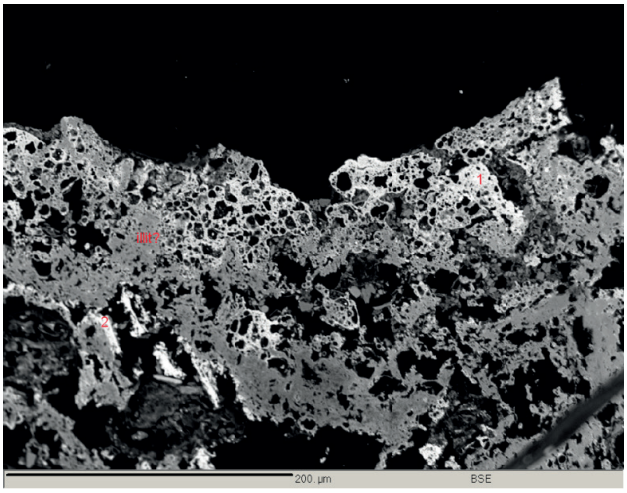


Fig. 7. BSE image of separated grain (11R) with the marked place of point EDX analysis.

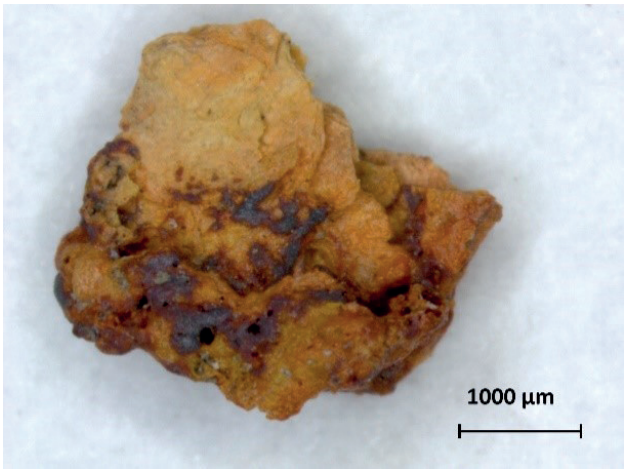


Fig. 8. Separated grain 7R (left) and grain after the biological-chemical leaching (right) observed by optical microscope.

The disruption of less resistant forms of Fe oxides and hydroxides were also observed by optic microscope. The surface of separated grain 7R after the biological-chemical

leaching changed visibly (Fig. 8). The application of heterotrophic bacteria caused the Fe dissolution from the structure, with which the As removal (and other toxic elements bounded in Fe coatings) is expected as well.

Tab. 4

Point analysis of separated grain from the Figure 7.

11R wt. %	Fe	Mn	Si	Al	Mg	Na, Ca, K	Cu, Ni, Zn, Pb, Co, Cr, Sb, As	O, ost.
an1	25.18	0.01	10.75	5.39	0.97	1.14	0.08	56.48
an2	27.95	0.08	2.94	9.42	5.03	0.40	0.38	53.80

Detailed morphology of surface of separated grain 7R after the leaching was observed by scanning electron microscope. The mentioned surface structure disruption was observed as a presence of expressive pores on the grain surface (Fig. 9). More resistant forms of Fe oxides were observed in the form of spherical particles created agglomerates on the grain surface (Fig. 10).

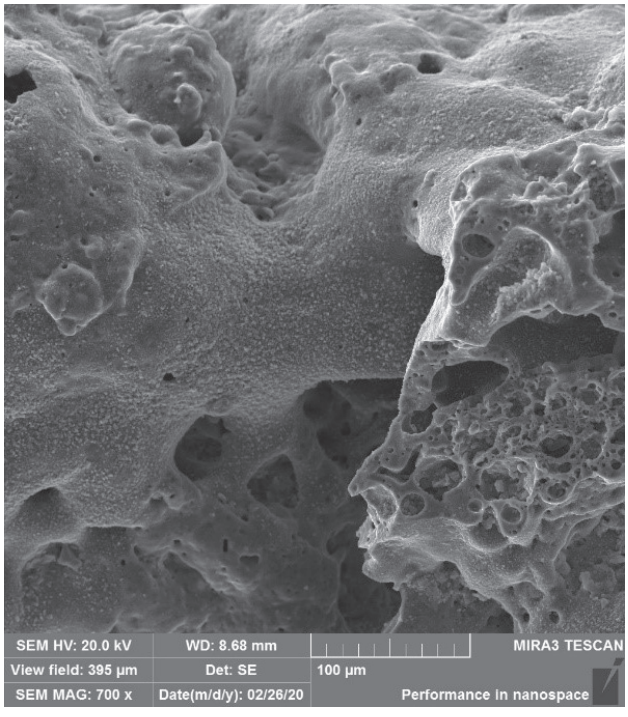


Fig. 9. Morphology of the surface of separated grain 7R after the biological-chemical leaching at magnification 700x.

Chemical analysis of separated grain 7R by EDX confirmed the presence of basic structural elements of matrix, as well as Fe particles on its surface (Fig. 11). The presence of As or other toxic elements was not detected, what corresponds with the results from the electron microanalysis (Fig. 12).

The using of three different microscopic methods to analyse the separated grains allows to confirm the effect of biological-chemical leaching on toxic elements removal from the contaminated soil, especially As.

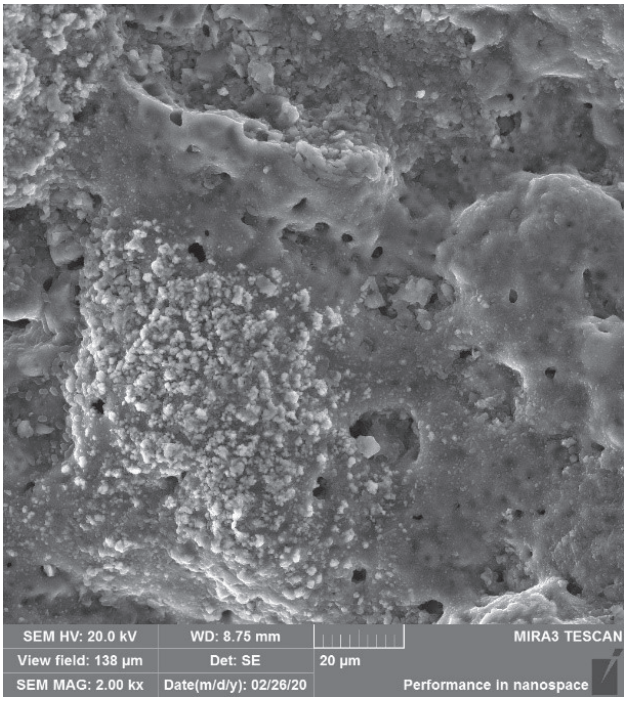


Fig. 10. Morphology of the surface of separated grain 7R after the biological-chemical leaching at magnification 2 000x.

4. Conclusion

The study was focused on more detailed characterization of the bacterial influence on the toxic elements leaching from the contaminated soil. The analysed toxic elements did not show expressive toxic character. On the other hand, the studied soil was often fertilized, what represents good conditions for bacteria activities in disruption of bonds between the minerals and toxic elements in the soil after the nutrient supply. Also, they showed good resistance onto As in their environment. The series of experiments were carried out in order to study the bioavailability of toxic elements in contaminated and treated soil. The bioavailability of Cu, Pb, Zn, Mn, Ba, Co and Sr decreased after the soil treatment, but the bioavailability of Fe and As increased. Also, the sequential extraction analyses pointed at the decrease of As content in the residual fraction after the biological-chemical leaching. The results presented in the study confirmed the possibility of soil decontamination by repeated cycles of chemical and biological-chemical leaching, what is promising way for the soil remediation with regard to the natural environment.

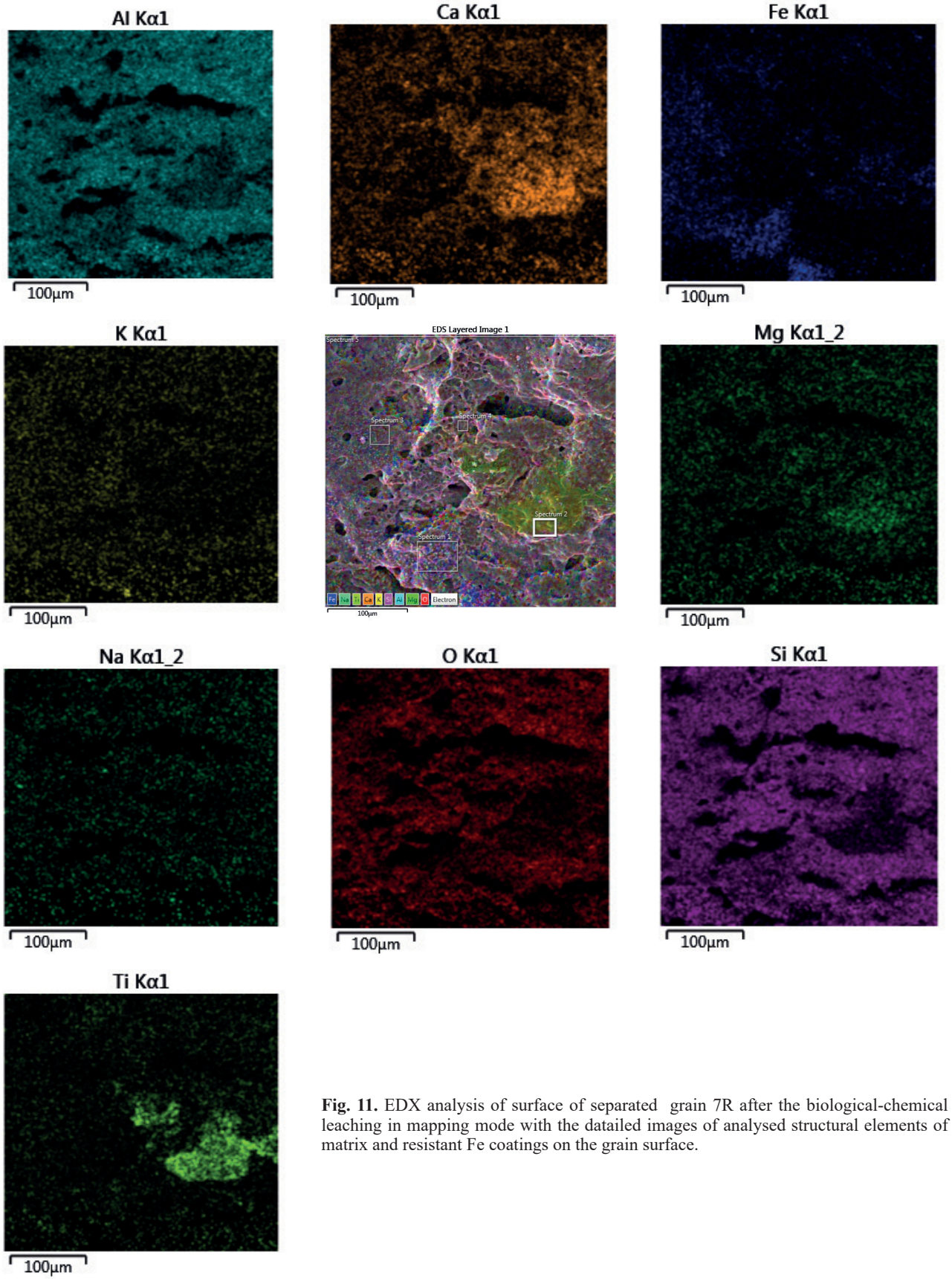


Fig. 11. EDX analysis of surface of separated grain 7R after the biological-chemical leaching in mapping mode with the datailed images of analysed structural elements of matrix and resistant Fe coatings on the grain surface.

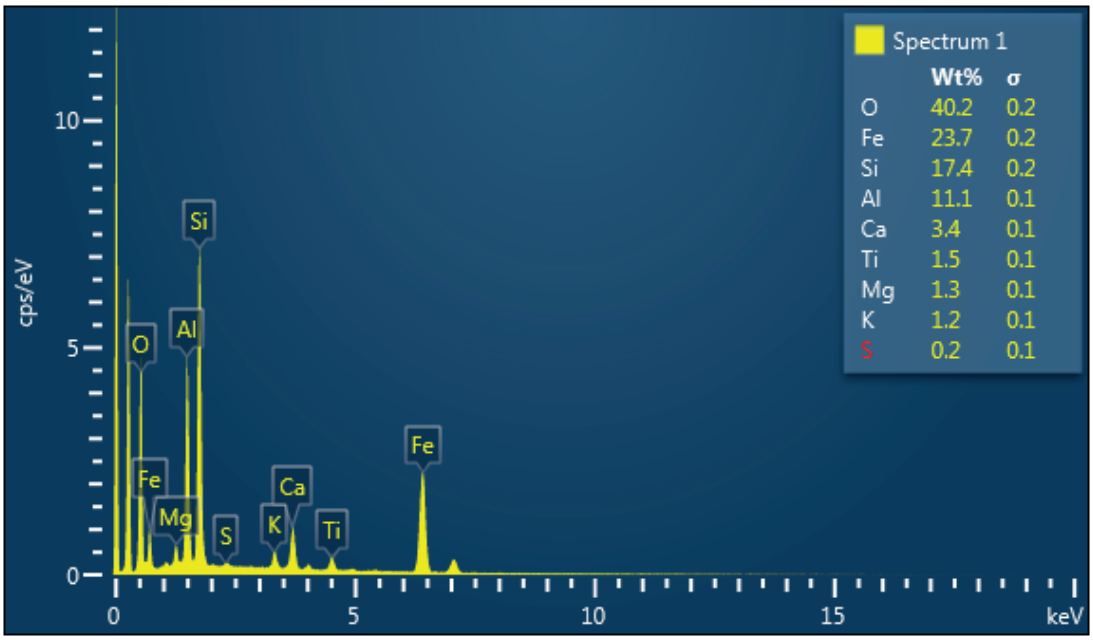


Fig. 12. EDX spectrum of separated grain 7R after the biological-chemical leaching – analysis in the point 1 on Fig. 11.

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Vplyv baktérií na lúhovanie toxických prvkov z kontaminovanej pôdy

Cieľom štúdie bolo bližšie charakterizovať vplyv baktérií na uvoľňovanie toxických prvkov z kontaminovanej pôdy v procese biologicko-chemického lúhovania. Experimenty sa realizovali na vzorke pôdy z lokality Richnava. 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. Na experimentálne účely sa použila kontaminovaná pôda a pôda po procese trojstupňového biologicko-chemického lúhovania, opísaného bližšie v príspevku Štyriakovej et al. (2019).

Vzorka kontaminovanej pôdy s hmotnosťou 1 kg bola umiestnená v sklenenom valci s priemerom 80 mm a výškou 340 mm, ktorým perkoloval roztok 10 mM etyléndiamíntetraoctanu sodného (Na_2EDTA, ďalej označený ako ch1) s objemom 2 l. Potom chemicky lúhovanou vzorkou s hmotnosťou 800 g perkolovalo 1,5 l roztoku 10 mM etyléndiamínsukcínu sodného (Na_3EDDS, ďalej označený ako ch2) a následne sa vzorka (700 g) biologicko-chemicky lúhovala 3 l média s obsahom 2 mM chelátu ch1 a živín. Pôda po jednotlivých stupňoch lúhovania bola neskôr analyzovaná pomocou sekvenčnej extrakčnej metódy.

Mikrobiologickými analýzami sa zisťovali počty heterotrofných baktérií nachádzajúcich sa v kontaminovanej pôde, ktoré tolerujú prítomnosť vysoko toxického prvku arzénu. Arzén sa môže uvoľňovať z pôdneho matrixu biochemickými procesmi a spôsobovať postupnú kontamináciu rastlín alebo podzemnej vody, a tak spolu s inými prvkami ohroziť životné prostredie.

Rezistencia heterotrofných baktérií izolovaných z pôdy proti As sa overovala kultiváciou baktérií na tuhom živnom médiu – agarových platniach (tryptónovo-sójový agar – TSA) s prídavkom 0,3 mM a 3 mM As a v tekutom živnom médiu TSB (tryptónovo-sójový bujón) s prídavkom 0,3 mM a 3 mM As. Experimenty sa realizovali v skúmavkách obsahujúcich živné médium a vo fľašiach obsahujúcich živné médium a 5 g skúmanej pôdy. Absorbancia médií sa merala vo vybraných časových intervaloch na UV VIS spektrometri Spectroquant Pharo 300 pri vlnovej dĺžke 540 nm.

Heterotrofné baktérie izolované z kontaminovanej pôdy preukázali dobrú odolnosť [počet baktérií vyše $1,2 \cdot 10^5$ kolóniu tvoriacich jednotiek (KTJ)/g) proti koncentrácii As (0,3 mM)] v tuhom médiu – na agarových platniach (obr. 1). V tekutom živnom médiu sa získali len veľmi malé rozdiely v raste počtu baktérií v závislosti od koncentrácie As v médiu, pričom v prvých hodinách priebehu experimentu bol najvýraznejší rast baktérií v médiu s 0,3 mM As. Zodpovedá to aj výsledkom získaným na agarových platniach (obr. 2). Rast baktérií bol výraznejší vo fľašiach, ktoré okrem živného média obsahovali aj prídavok kontaminovanej pôdy. To umožnilo zvýšenie prísunu živín na rast počtu heterotrofných baktérií (obr. 3). Výsledky poukázali na fakt, že rezistencia baktérií proti As v pôde nesúvisí s jeho koncentráciou, ale je ovplyvnená využívaním pôdy na pestovanie plodín, ktorým je zabezpečený pravidelný prísun organických látok a biogénnych prvkov pre prítomné mikroorganizmy vo forme hnojív.

Test toxicity metódou US EPA (1311) aplikovaného procesu lúhovania pri vzorkách vstupnej pôdy a pôdy po biologicko-chemickom lúhovaní neviedol k prekročeniu limitných hodnôt koncentrácie vybraných toxických prvkov v zmysle kritérií TCLP a IEPT (obr. 4, tab. 2).

Test bioprístupnosti s využitím kyseliny chlorovodíkovej preukázal zníženie bioprístupnosti sledovaných toxických prvkov vo vzorke pôdy po biologicko-chemickom lúhovaní, s výnimkou Fe a As (obr. 5). Ich bioprístupnosť v upravenej vzorke pôdy sa zvýšila. Naznačuje to ďalšiu možnosť odstránenia As a Fe procesom kyslého chemického lúhovania, ak by sa zmenili podmienky pH na menej ako 2, pričom by bolo možné znížiť hlavne nadlimitnú koncentráciu As.

Vstupná vzorka a vzorky z jednotlivých stupňov chemického a biologicko-chemického lúhovania (podľa postupu uvádzaného Štyriakovou et al.) bola následne podrobená päťstupňovej sekvenčnej extrakčnej analýze na zistenie mobility sledovaných toxických prvkov a ich bioprístupnosti (Mackových et al., 2000).

Vo vstupnej vzorke pôdy sa nachádzali Cu (49,2 %), Pb (85,1 %), Zn (40,3 %) a Co (33,0 %) v najväčších podieloch v redukovateľnej frakcii, Ni (51,1 %), As (52,0 %), Sb (92,0 %), Ba (65,5 %) a Cr (77,6 %) v reziduálnej frakcii a Hg (93,8 %) v organicko-sulfidickej frakcii (obr. 6). Po chemickom lúhovaní pôdy chelátom ch1 ostáva najväčší podiel Pb (78,6 %), Zn (40,2 %) a Co (26,1 %) v redukovateľnej frakcii (Co rovnako 26,1 % aj v reziduálnej frakcii), no pri všetkých troch prvkoch sa ich podiel oproti vstupnej vzorke pôdy vo frakcii (3) znížil v prospech frakcie rozpustnej vo vode (Pb, Zn, Co), ionovymeniteľnej a karbonátovej (Pb) a organicko-sulfidickej (Pb a Zn) frakcie. Pri Cu je možné pozorovať nárast koncentrácie vo frakciách (1), (4) a (5), pričom najväčší podiel Cu sa nachádzal v organicko-sulfidickej frakcii. Chemické lúhovanie chelátom ch1 viedlo ďalej k zvýšeniu podielu Ni, Ba a Cr vo frakciách (1), (2) a (3), As

v (1) a (2), Sb a Co vo frakcii (1). V redukovateľnej frakcii sa okrem spomínaného obsahu Cu, Pb a Zn zvýšil aj obsah As (o vyše 5 %) a Co (0,5 %) (obr. 6). Po chemickom lúhovaní pôdy chelátom ch2 ostávajú v najväčšom podiele Cu (39,5 %) a Hg (95,3 %) v organicko-sulfidickej frakcii, Pb (78,6 %), Zn (40,1 %) a Ni (45,9 %) v redukovateľnej frakcii a As, Sb, Ba, Co a Cr v reziduálnej frakcii. Pri všetkých sledovaných prvkoch okrem Ni a As pozorujeme nárast ich koncentrácie v reziduálnej frakcii (Ba o 7,39 %), v prípade Cu nárast koncentrácie vo frakciách (1) a (4), Pb vo frakcii (2), Zn v (4), Ni a Co v (3) a (4) a pri As a Cr vo frakcii (3) (obr. 6). Po biologicko-chemickom lúhovaní pôdy (BL) ostáva najvyšší podiel Cu (37,3 %) a Hg (97,0 %) v organicko-sulfidickej frakcii, Pb (82,4 %) a Zn (36,7 %) v redukovateľnej frakcii a ostatných sledovaných prvkov v reziduálnej frakcii (obr. 6). V prípade Cu, Pb, As, Sb a Hg však v porovnaní s pôdou po lúhovaní s ch2 sa znížila ich koncentrácia v biologicky neprístupnej frakcii, pričom významným znížením je koncentrácia As (o 11,5 %) v prospech frakcií (1), (2) a (3) (obr. 6).

Po chemickom lúhovaní vstupnej pôdy chelátmi ch1 a ch2 ostali vo vzorke pôdy vo zvýšenej koncentrácii As, Sb, Ba a Hg. Sú to prvky, ktorých najvyšší podiel podľa výsledkov sekvenčnej extrakčnej analýzy je viazaný v reziduálnej frakcii, teda biologicky neprístupnej. Vo vzorkách po trojstupňovom lúhovacom cykle však táto analýza preukázala preskupenie ich obsahu, najmä As, do biologicky prístupných frakcií. Zvýšila sa ich mobilita, a teda aj možnosť ďalšej extrakcie použitím chemických a biologicko-chemických postupov. Opakovanou aplikáciou navrhnutého procesu by teda bolo možné znížiť najmä koncentráciu As a Sb na požadované hodnoty v zmysle indikačných kritérií.

Pozorovaním jednotlivých separovaných zŕn z pôdy pred biologicko-chemickým lúhovaním a po ňom sa binokulárnym optickým mikroskopom potvrdilo odstránenie menej odolných Fe povlakov na povrchu zŕn po biologicko-chemickom lúhovaní, v ktorých je prioritne viazaný arzén (obr. 8). Elektrónovým mikroanalyzátorom bolo možné po okrajoch biologicko-chemicky lúhovaných zŕn pozorovať narušené štruktúry Fe povlakov, keďže heterotrofné baktérie využité v procese biochemického lúhovania sú schopné rozrušiť menej odolné štruktúry oxidov a hydroxidov železa (obr. 7). Bodová analýza v miestach s vyšším podielom Fe nepreukázala prítomnosť vysokého obsahu As ani ostatných sledovaných toxických prvkov. Poukazuje to na fakt, že tieto prvky boli procesom biologicko-chemického lúhovania odstránené z povrchu separovaného zrna (tab. 4). Detailnejšia morfológia povrchu vybraného separovaného zrna po biologicko-chemickom lúhovaní bola pozorovaná skenovacím elektrónovým mikroskopom. Už spomínané narušenie štruktúry menej odolných Fe povlakov na povrchu zrna vplyvom heterotrofných baktérií sa prejavilo prítomnosťou výrazných pórov (obr. 9, 10).

Štúdia bola zameraná na detailnejšiu charakterizáciu vplyvu baktérií na lúhovanie toxických prvkov z kontaminovanej pôdy, pričom sa preukázala relatívne vysoká rezistencia heterotrofných autochtónnych baktérií proti As v ich prirodzenom prostredí. Výsledky prezentované v tejto štúdii zároveň poukazujú na možnosť efektívnej dekontaminácie pôdy prostredníctvom opakovaných cyklov chemického a biologicko-chemického lúhovania s využitím heterotrofných baktérií. Predstavuje to perspektívnu metódu remediácie pôdy znečistenej anorganickými kontaminantmi s ohľadom na životné prostredie.

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