Metals accumulation and As releasing during interaction of clay and iron minerals with heterotrophic bacteria in soil and sediment bioleaching

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***Abstract*— *The soil and sediment samples with different content of metals and clay minerals were investigated during bioleaching. The increasing of clay and metal concentrations with decreasing particle size were found both in contaminated soil and sediment. Heterotrophic bioleaching of the iron rich clay fractions from the soil and the sediment were evaluated for his effectiveness in the cycling of iron bound As by consuming organic nutrients. The treatment involved the use of the indigenous bacteria, whose activity was combined with the chelating strength of EDDS, SDS, Na4P2O7 and fertilizers. Heterotrophic bacteria caused decomposition of iron binding deposition as is adsorption on clay with followed dissolving of Fe mainly by the sediment bioleaching. The concentration of iron decreased by precipitation with As sorption after 19 days of sediment bioleaching. The Cu and Zn extraction was inhibited by bioleaching during the iron and arsenic dissolution and precipitation. By contrast, the additives 3mM Cu and 3mM Zn were applied into medium and thus affected the activity of soil resistant heterotrophic bacteria with followed increasing of the iron and arsenic extraction by the soil clay bioleaching. Therefore, this study confirmed the soil and sediment bioleaching in Fe or As releasing efficiency under different conditions regulated by indigenous bacteria. The bioleaching can be a suitable technology for As removal from the untreated soil and sediment by stimulation of the resistant bacteria activity. The separation of clays from the soil and sediment samples did not decreased of toxic element limits because clay and iron minerals coated on coarse silicate particles and the clay fraction is bearers of metals which contaminate the soil and sediment environment.*

***Keywords*— soil, sediment, clay minerals, heavy metals, bioleaching.**

I. INTRODUCTION

Soil and sediment are major reservoirs for contaminants as they possess an ability to bind various metals. In general many of them contain a wide range of heavy metals with varying concentration ranges depending on the surrounding geological environment and anthropogenic and natural activities occurring or once occurred [1]. Clay minerals are among the major materials that interact with almost all soils and sediments contaminants [2]. Clays often represent a short-term sink of heavy metals in soils and sediments, because of their adsorptive properties [3].

Clay minerals and microbes are ubiquitous in soils, sediments, and sedimentary rocks and they play important roles in environmental processes. Microbes interact with clays via a variety of mechanisms, such as reduction and oxidation of structural iron and mineral dissolution and precipitation through the production of siderophores and organic acids [4]. Along with microorganisms, clays provide some of the most catalytic surfaces in sedimentary environments, which are important to a variety of biogeochemical cycles. Iron (Fe^{+3}) bound in clay minerals should be considered an important electron acceptor supporting the growth of bacteria in soils or sedimentary environments [5]. Clay minerals often account for about half the iron in soils and sediments. Much of the structural iron in clay minerals is ferric iron, which can be reduced either chemically or biologically [4] The reduction of structural Fe(III) to Fe(II) tends to decrease the surface area, interlayer spacing, water swellability, and hydraulic conductivity of clay minerals. In general, reduction increases the negative layer charge and cation exchange capacity [6], [7], while the interlayer cations become less exchangeable. These physical and chemical changes of clay minerals would have profound effects on soil fertility and contaminant mobility. The transport mechanism of heavy metals through soil has long presented great interest to both environmental and soil scientists because of the possibility of groundwater contamination through metal leaching [4]. Many heavy metals are nonessential and potentially toxic to microorganisms. At higher concentrations these heavy metal ions form unspecific complex compounds within the cell, which leads to toxic effects, making them too dangerous for any physiological function. Bioremediation is an option that offers the possibility to destroy contaminants or remove them innocuous using natural biological activity [8]. The presence of bacteria capable of tolerating heavy metals from soil and sea water samples from heavily contaminated sites in Mauritius was investigated by Hookoom and Puchooa [9]. No isolate was able to grow in the presence of copper at concentration higher than 3.0 mM.

The mining waste, mineral processing and industrial treatment processes represent potential source of heavy metals in the soil in close proximity of the industrial area KOVOHUTY Krompachy with production of Cu and Zn. The soil contamination investigated here is located close to the river Hornád which flows into reservoir Ružín and contaminated sediment. Despite the fact mentioned the reservoir is situated in a different aquatic environment and catch contaminants from several sources. In the reservoir, these metals are retained in the sediment layer and during changes of physical-chemical conditions, their immobilization or mobilization can negatively impact aquatic biota through the food chain.

This study was conducted to determine the distribution of heavy metals in two different contaminated samples of soil and sediment in dependence on the particle size distribution of clay and iron minerals. The purpose of the present test was to verify the resistance of bacteria to As and to investigate the effect of indigenous bacteria on mobilization of Fe, Cu, Zn, As by bioleaching.

II. MATERIAL AND METHOD

2.1 Sediment and soil analyses

The soil and sediment used in this study were collected close to the industrial area of Kovohuty Krompachy (source - Z) and at border of the reservoir area of Ružín near town Margecany in Slovakia (outfall - O), where the river Hornád flows into a reservoir. To minimize the possibility of contamination, soil and sediment samples were inserted into UV-sterilized plastic bags immediately after the sampling and all sampling manipulations in laboratory were undertaken using an UV-sterilized laminar flow hood. After the manual homogenization, the soil and sediment samples were sieved through a 2mm sterilized stainless steel sieve (No.1) in order to keep indigenous bacteria. The samples were deep frozen (at – 40 ^{o} C) before the laboratory tests. The separation of the clay fractions (< 2 μm) were performed from the homogenized 4 samples of different depth (soil – ZC, sediment – OC) by sedimentation in the sterilized physiological solution (8.5g NaCl per liter). The clay fractions (< 2 μm) were obtained only from the samples of 30 cm depth from the sediment (OC3), because the soil contained very low amount of clay fraction (< 0.1%) for laboratory tests. The separation of the silt fractions without the clay fraction (< 63 μm) were also performed from samples of 30 cm depth (soil – ZS3, sediment – OS3) and from homogenized 4 samples of different depth (soil – ZS, sediment – OS) by sedimentation in the sterilized physiological solution (8.5g NaCl per liter). The elements of the solid samples were analyzed on the fused beads using an energy dispersive X-ray fluorescence spectrometer (Spectro X-LAB 2000, Germany). The XRD analyses of the clay fraction from sediment and soil were carried out using a Phillips PW 1710 diffractometer (35 kV, 20 mA) with CuKα radiation and a graphite monochromator. All samples were scanned with a 0.02° 2θ step.

2.2 Bacterial identification and resistance

The strains from the natural soil and sediment samples were selected on Tryptone Soya Agar (TSA, Oxoid). The cultivation proceeded under aerobic conditions at 24◦C for 24–72 h. These strains were then isolated in metal-free nutrient agar. The each plate of TSA contained the appropriate metal salt solution 3mM $CuSO_4$ (190 mg/l Cu) and 8mM $CuSO_4$ (508 mg/l Cu), 3mM $Na_2HAsO_4 . 7H_2O$ (225 mg/l As) and 8mM $Na_2HAsO_4. 7H_2O$ (570 mg/l As), 3mM $ZnSO_4 . 7H_2O$ (196 mg/l Zn) and 8mM $ZnSO_4 . 7H_2O$ (523 mg/l Zn). The plates were then incubated at (24°C) for 48 h. The plate method used here gave very consistent results, particularly with respect to the study of the toxicity of metals in solid media, which could be similar to polluted soil and sediment. All isolates were identified based on the morphological features and biochemical properties by using MALDI TOF MS (Microflex LT instrument, Bruker Daltonics GmbH, Leipzig, Germany) with FlexControl software (version 3.0). For transmission electron microscopy of bacterial adherence on clay particles, samples of culture solutions (2 ml) were centrifuged at 5000 x g and the pellet was washed twice with 0.1M ammonium acetate (pH 7.0). The sample were placed on Formvar-coated Cu-grids followed by staining with 1% uranyl acetate (pH 7.2). After washingwith ammonium acetate and distilled water, the grids were examined under a transmission electron microscope JEOL-JEM 1200 (Japan) at an acceleratingvo ltage of 60 kV.

2.3 Bioleaching of sediment and soil

Bioleaching experiments were carried out in 250 ml culture flasks containing 50 g samples and 150 ml medium without metals (denoted as M1, M2, M3) and with 0.2 g/l sodium azid (denoted as A1, A2, A3). Sodium azid was used as biocides in chemical controls. The each media for the next bioleaching contained the metal salt solution 3mM CuSO4 (190 mg/l Cu) and 3mM $ZnSO_4 \cdot 7H_2O$ (196 mg/l Zn). Medium M1 contained (per liter) N and P fertilizers (DAM 390, Pekavit), chelating agent 0.59 g Na_3EDDS, 0.5 g $MgSO_4$, 0.14 g $CaCl_2$, 0.075 g NaCl, 0.35 g KCl, 2 g Na_2SO_4, 0.05 g yeast extract, 20 g glucose and 0.01 g nystatine as a fungal growth inhibitor; pH 6.0 (adjusted with NaOH). Medium M2 and M3 contained besides the listed chemicals 0.28g SDS and 0.53 g $Na_4P_2O_7$, respectively. The experiments with metals additions were performed to assess the potential of indigenous resistant heterotrophic soil and sediment bacteria to metals accumulation. Two replicates were run per experimental onset. The initial pH of all experiments was adjusted to 7.00 ± 0.04 by addition of ultrapure 1M NaOH before the bioleaching. The batch bioleaching of the soil and sediment test was performed under the static conditions at 24°C during 14 - 21 days. The culture solution was separated from the samples after 10 minutes of centrifugation at 10 000 rpm and by means of 0.2 μm pore size membrane filtration. The liquid supernatant was collected for the chemical analyses.

III. RESULTS AND DISCUSSION

3.1 Minerals and heavy metals composition of sediment and soil

The XRD analysis of the fine clay fraction revealed no significant changes in the mineral composition between soil and sediment. It followed from the qualitative determination that the soil (Z) and sediment (O) consist predominantly from quartz, micas and clay minerals (Fig. 1). However, these naturally brown soil samples contain 0.1 % of the iron and clay minerals (< 2 μm) and sediment contain 5 % of the iron and clay minerals (< 2 μm), which either formed the independent fine fraction of samples or are coated or are impregnated on silicate particle surfaces of the sand fraction.

The selected chemical properties of the Z and O samples from the different depth (10, 20, 30 and 40 cm) are shown in Tables 1 and 2. When chemical analyses of the soil from different position are compared with the limit value for loamy - sand soil (Table 3) specified in the Methodical Instructions of the Ministry of Agriculture of Slovakia (MP SR) the very high contamination of the soil samples was found (Table 2). The lower values of metals and higher As contamination were found in the sediment (Table 1) when chemical analyses of samples from different position are compared with the limit value (Table 3) specified in the Methodical Instructions of the Ministry of Environment in Slovakia (MZP SR). The chemical analyses of the fine silt (-S) and clay (-C) fraction (ZS, ZC) separated from the homogenized samples Z and the next samples (OS, OC, OS3, OC3) separated from homogenized samples O and from the sample 30 cm depth shows higher concentration of metals than untreated soil and sediment (Fig. 2A, B; 3 A, B; 4A, B).

The metals in soils are not homogeneously distributed over the various particle size fractions, suggesting that the particle size have a determining influence on the distribution of chemical elements. The distribution characteristics of potentially toxic metals with soil particle size are primarily the function of mineral composition and amount of sorption sites in each size fraction. The increasing metal concentrations with decreasing particle size were found both in contaminated and uncontaminated soils [10], [11] and it indicated that the behavior of metals was governed by the sorption processes in this size range [12]. The accumulation of metals in the clay fraction can be attributed to the enrichment of soil components with high surface area, such as clay minerals, organic matter and Fe-Mn-oxides [13], [14].

According to the comparison of chemical composition of the silt and clay fraction from the Z and O samples, the heavy metals (Cu, Zn, As, Pb) concentrations were increased in the clay fraction (Fig. 2A, 3A). The comparison of chemical analyses of the soil has also shown that the content of metals in the clay fraction of the soil is higher than that in the silt fraction. The content of Fe is about 9 %, Cu is about 6 % , Zn about 40 %, Cd about 41 %, As about 31 %, Hg about 65 %, Pb about 27 %, Ni about 38 % and Al about 33 % higher in the clay fraction, only content of Mn is higher in the silt fraction, about 27 % (Fig. 2A). The content of Si is about 1 % higher in the clay fraction (Fig. 2B). The amount of the clay fraction of soil from 30 cm depth was insufficient for chemical analyses.

In the sediment sample, the content of Fe is about 28 %, Cu about 13 %, Zn about 39 %, Cd about 33 %, As about 25 %, Pb about 32 %, Ni about 26 % and Al about 35 % higher in the clay fraction, only content of Hg is higher in the silt fraction about 27 % and Mn about 27 %. Content of Si is about 19 % higher in the silt fraction (Fig.3).

The comparison of chemical composition of the silt and clay fraction of sediment from 30 cm depth show higher concentration of heavy metals in the clay fraction too (Fig. 4A). The content of Fe is about 36 %, Cu is about 18 %, Zn about 42 %, As about 25 %, Hg about 6 %, Pb about 30 %, Ni about 29 % and Al about 41 % higher in the clay fraction, but content of Cd is much higher in the silt fraction, about 83 % and Mn only about 3 %. Content of Si is about 9 % higher in the silt fraction (Fig. 4B).

The content of heavy metals in the clay fraction is a very good indicator of environmental pollution. In the finest fractions of soil and sediments due to the active surface of their components a continuous enrichment with heavy metals takes place. Unfortunately, it seems to be the most ineffective to apply the separation of clays because the silt fraction after removal the clay fraction are also bearers of metals and metalloids which contaminated the sediment and soil environment. The clay and iron minerals with metals impregnated and coated of coarse silicate particles which formed the sediment and soil contaminated environment.

3.2 Bacterial resistance to heavy metals in clay fraction of sediment and soil

The determination of total soil and sediment metal content alone is not a very useful tool to quantify the contamination and potential environmental and human health risks. Total concentrations of metals in soils are a poor indicator of metal toxicity since metals exist in different solid-phase forms that can vary greatly in terms of their bioavailability [15], [16].

The mobility and bioavailability, and hence potential toxicity of metal in the soil and sediment can depend also on the bacterial concentration in clay fraction. The nature of its association with bacterial resistance on metals, and bacterial ability to release the metal from the solid phase into solution replenish that removed from soil solution by the plants.

The soil is approximately 15 times more contaminated by heavy metals than sediment (Table 1, 2). Although the bacterial amount of isolated heterotrophic bacteria is higher in the clay fraction of sediment than in soil (Table 4). The presence of metals in the growth agar allowed us to maintain the tolerance of bacteria at naturally polluted environments. Cu was the most toxic component for all bacteria in 8mM concentration and only bacteria from the sediment (10^4 CFU/g) tolerated 3mM Cu. The total number of colonies decreased from 10^5 to 10^4 CFU/g with increasing concentration of Zn from 3mM to 8mM [17]. Arsenic was the best tolerated toxic element by the bacteria isolated from the sediment (10^6 CFU/g) and less tolerated by bacteria isolated from the soil (Table 4).

The fresh isolates selected from the sediment and soil, identified as strains tolerant to As, Zn and Cu, were heterotrophic bacteria. The dominant bacterial species tolerant to As, Zn belonged to the genus Bacillus in soil and sediment environment. In the sediment B. mycoides, B. cereus, B. simplex was confirmed. Exiguobacterium was also isolated only from the sediment environment. In the soil B. mycoides, B. cereus, B. idriensis was identified. Therefore, Bacillus spp. isolates appeared as the most tolerant species to As and could serve as bio-indicators of As bioavailability in the polluted environment. The dominant bacterial species tolerant to Cu and Zn belonged to the genus Pseudomonas isolated only from sediment. Bacillus was not tolerant to Cu because these bacteria did not grow on agar plate contaminated with Cu.

The tolerance of soil bacteria to heavy metals has been proposed as an indicator of potential toxicity of metals to other forms of life [18].

3.3 Iron and arsenic releasing by bioleaching of sediment and soil

Iron (Fe^{+3}) bound in clay minerals should be considered an important electron acceptor supporting the growth of bacteria in soils or sedimentary environments. Along with microorganisms, clays provide some of the most catalytic surfaces in sedimentary environments, which are important to a variety of biogeochemical cycles [5].

Ferric minerals also cover clay minerals in sediment and soil, the interaction of ferric minerals and clay minerals is disturbed by bacteria as a result of dissolution of ferric minerals. These processes occurred during the bioleaching and the Fe concentration in the leachate was highest during the bioleaching of sediment OC sample in medium M1 > M3 > M2 than during the leaching of soil sample ZC (Fig. 5). In both cases of bioleaching the difference in the amount of iron in solution between the sediment sample and soil sample is probably caused by bacterial amount of As resistant bacteria because greater amount of bacterial cell was in the clay sediment samples (Table 3), to which bacterial cells are adherent, which give rise to greater Fe extraction. This bacterial adhesion to the surface of clay minerals from sediment was observed by TEM (Fig. 6). The microbial processes and also the kind of Fe-bearing minerals with As bonding may control the dissolution rate of iron minerals. The bacterial activity caused modification of aerobic condition to anaerobic condition and pH change from 7 to 5 during the soil and the sediment bioleaching. The formation of a brown fine-grained fraction of mineral particles precipitated from leachates was observed after 19 days of the sediment bioleaching (Fig. 7). The X-ray analysis of the solid substance confirmed the typical amorphous diffraction pattern in the range of 20 – 40°2θ (data not showed). Scanning electron microscope (15 kV) with attached energy dispersive spectrometer (EDS) revealed that most amorphous particles are composed of Fe and As, Zn coating the particles of clay minerals (Fig. 8). It should be concluded that the precipitated solid substances obtained from the samples after bioleaching in medium M1 and M3 are consisting of ultrafine clay particles covered by ultrafine iron particles with As. In medium M2 the particle contained also Zn after bioleaching (Fig. 8B).

3.4 Metals accumulation by bioleaching of sediment and soil

In previous study [17], the significance of Cu and Zn cations releasing by medium M1, M2 and M3 leaching was the decrease by the presence of heterotrophic resistant bacteria during the soil and sediment bioleaching and not only by the presence of dissolved organic source in abiotic control (A1, A2, A3). These results indicated that live heterotrophic microorganisms can affected the mobilization of Cu and Zn cations from soil and sediment in the presence of organic matter in natural environment as well as in the chemical remediation processes by EDDS and $Na_4P_2O_7$.

The ability of indigenous heterotrophic bacteria to uptake Cu, Zn cations was investigated in the next step of soil (Z) and sediment (O) bioleaching with medium M1, M2, M3 amended with 3mM Cu and 3mM Zn. The soil was more contaminated by heavy metals than sediment (Table 1) and contained less clays than sediment. Thus, the yield of cumulated Cu, Zn was 100% from the amended media M1, M2 and M3 by sediment after 14 days biotic and abiotic leaching. By addition of Cu and Zn cations into medium of sediment bioleaching, Fe dissolution partially decreased and As concentration was below 1 mg/l (data not showed). In the soil leaching by bacteria and by media without bacteria (A1, A2, A3) was observed the difference of qualitative analysis of leachates. This difference in the yield of accumulated Cu, Zn was detected only during soil bioleaching. This immobilization process was negligible in the abiotic samples and the soil bioleaching enabled mainly the Cu cation. It is also observed in Zn accumulation during the soil bioleaching (Fig. 9). The decreasing of Cu and Zn concentration of added cations in medium M3 > M1 > M2 was caused by bacteria because the soil contained the low concentration of clay minerals than the sediment. Cu is immobile in samples with bacteria because of its sequestration with organic matter, precipitates by metabolites or cellular assimilation is taken up for metabolic purposes, or and cell sorption adsorbs onto bacterial surfaces [19]. The bioleaching caused pH change from 5 to 4 during Cu and Zn accumulation and decomposition of iron minerals in the next soil and the sediment bioleaching.

However, the concentrations of Fe and As in the soil leachates were significantly higher in the presence of added cations than in the abiotic controls (Fe and As < 1mg/l) or during the first soil bioleaching without Cu and Zn addition (Fig. 5, Fe < 30 mg/l and As < 1mg/l). The bacterial dissolution of Fe from soil sample was affected by the addition of Cu in medium M3 and by the addition of Zn in medium M1 a M3 (Fig. 10). The gradual bacterial decomposition of iron minerals caused the As extraction from the soil sample. Probably the metabolic activity of soil indigenous bacteria is inhibited by the Cu and Zn additions in medium and only resistant bacteria are responsible for the extraction of As.

In the presence of added chelators, the removal of iron in the bacteria soil test was lower. In the presence of chelators, the bioleaching by indigenous bacteria and strains of Bacillus clearly produce the highest yield of iron dissolution [17].The effectiveness of As mobility in treated soil removal is dependent from addition of chelators, fertilizers and metals to activation of resistant bacteria.

IV. FIGURES AND TABLES

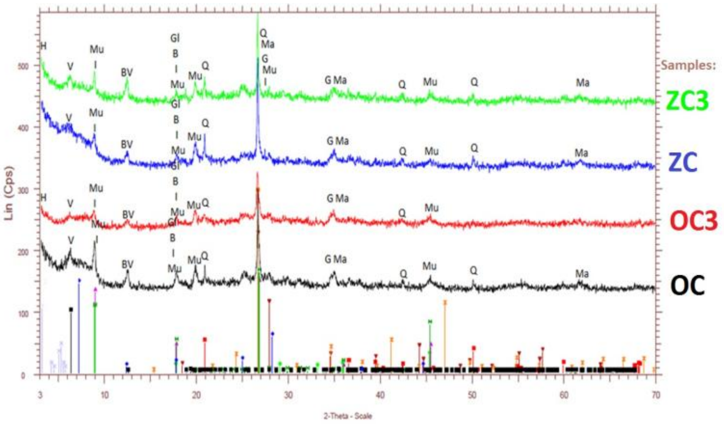

FIG. 1. RÖNTGENDIFFRACTION PATTERNS OF THE CLAY FRACTION OF SOIL (ZC, ZC3) SAMPLES AND SEDIMENT (OC, OC3) (Q-QUARTZ,GL-GLAUKONITE, I-ILLITE, G- GINIITE, MA-MANGANESE BERYLLIUM SILICATE SULFIDE, MU-MUSKOVITE, H-HYDROGEN ALUMINUM SILICATE, V-VERMICULITE, B-BEIDELLITE)

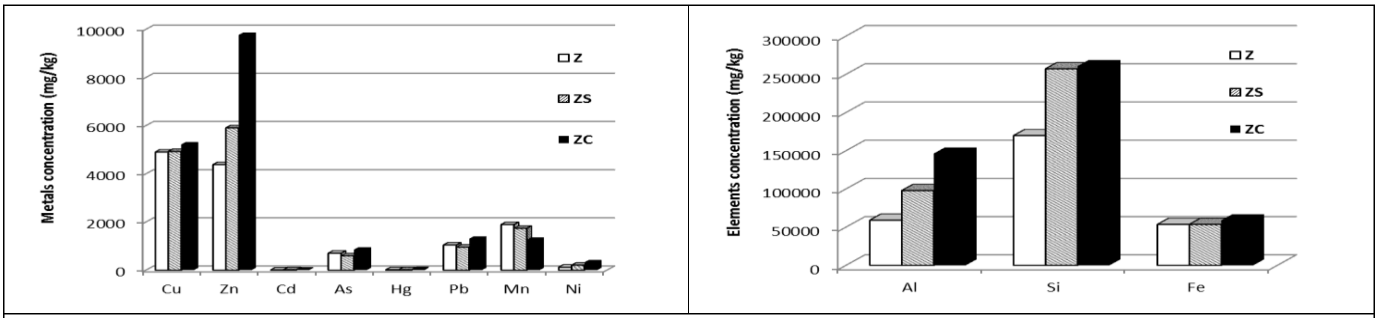

FIG. 2. METALS (A) AND ELEMENTS (B) DISTRIBUTION IN DIFFERENT FRACTIONS OF THE HOMOGENIZED SOIL (Z < 2 MM), THE SILT FRACTION (ZS < 63 MM) AND THE CLAY FRACTION OF SOIL (ZC < 2 MM)

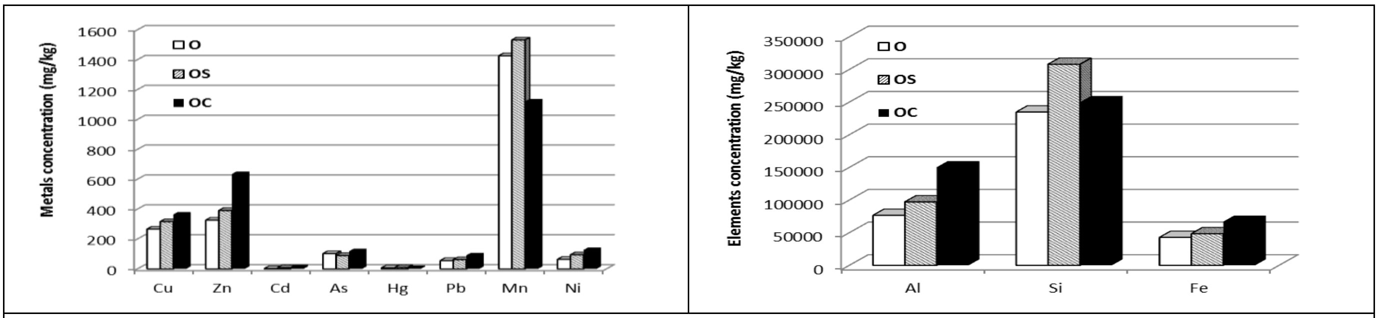

FIG. 3. METALS (A) AND ELEMENTS (B) DISTRIBUTION IN DIFFERENT FRACTIONS OF THE HOMOGENIZED SEDIMENT (O < 2 MM), THE SILT FRACTION (OS < 63 MM) AND THE CLAY FRACTION OF SEDIMENT (OC < 2 MM)

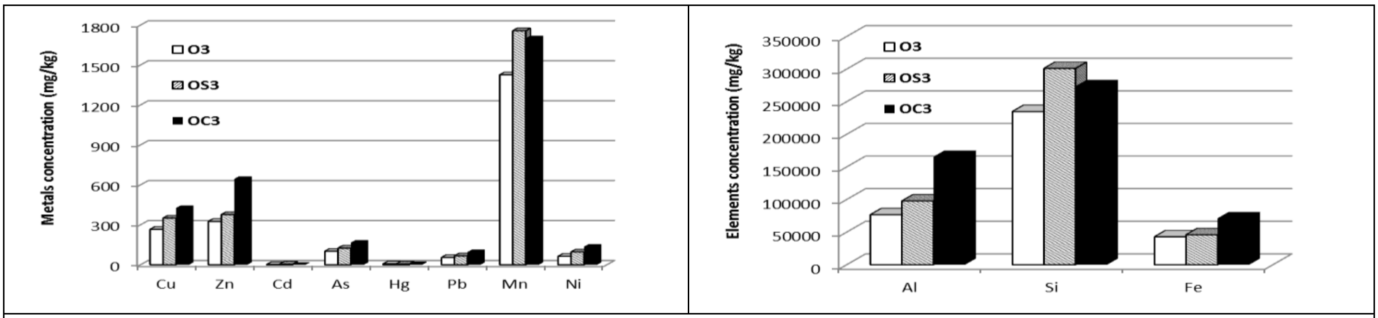

FIG. 4. METALS (A) AND ELEMENTS (B) DISTRIBUTION IN DIFFERENT FRACTIONS OF THE HOMOGENIZED SEDIMENT (O < 2 MM), THE SILT FRACTION FROM 30 CM DEPTH (OS3 < 63 MM) AND THE CLAY FRACTION FROM 30 CM DEPTH OF SEDIMENT (OC3 < 2 MM)

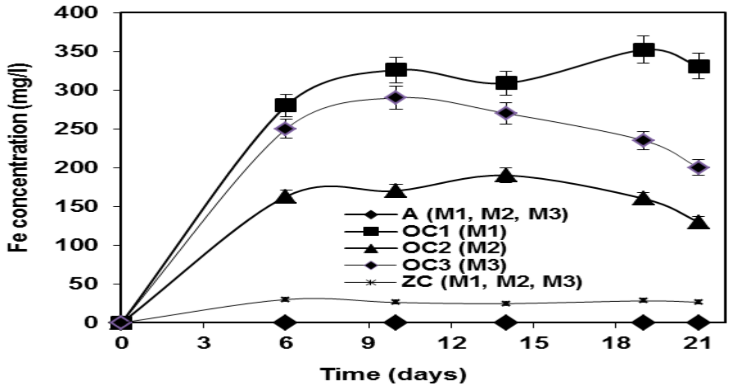

FIG. 5. TOTAL IRON CONCENTRATION IN LEACHATES DURING IRON DISSOLUTION AND BIOLEACHING OF THE CLAY FRACTION FROM SOIL AND SEDIMENT

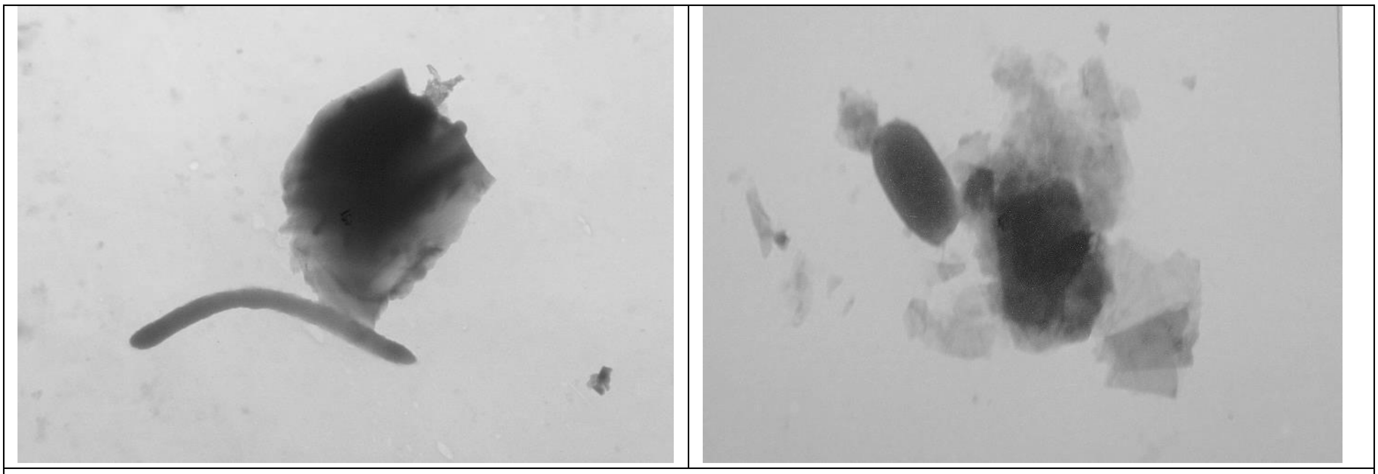

FIG. 6 TEM IMAGES OF BACTERIAL ADHERENCE ON CLAY PARTICLES

FIG. 7. THE FORMATION OF BROWN FINE GRAINED FRACTION OF IRON PRECIPITATE AFTER 19 DAYS OF THE SEDIMENT BIOLEACHING

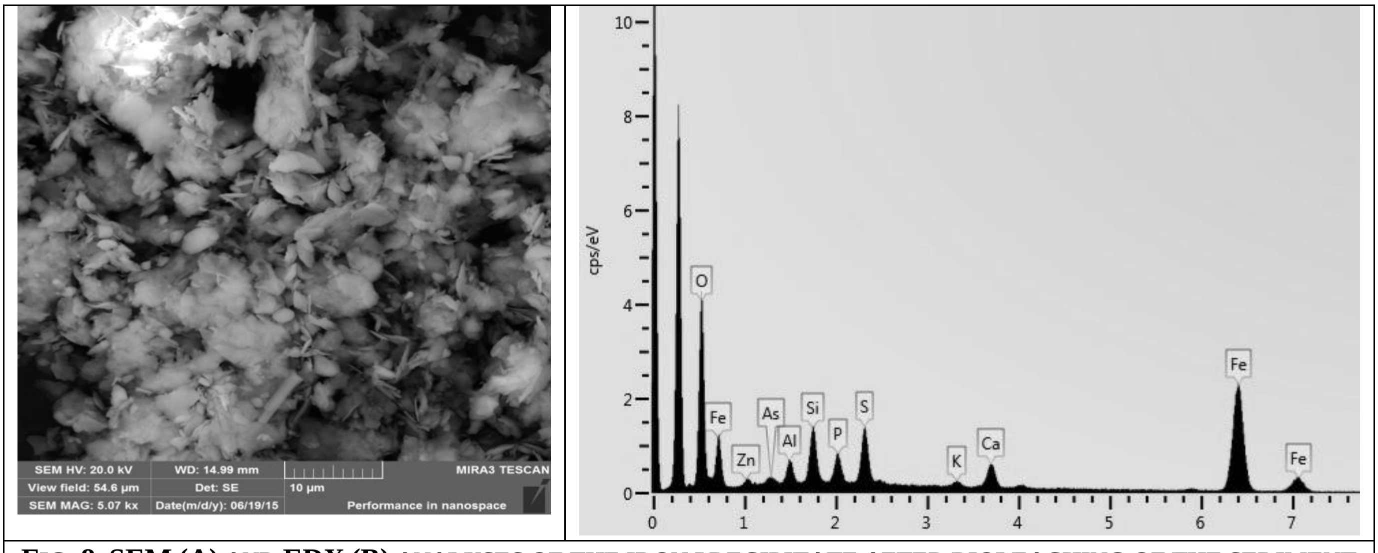

FIG. 8. SEM (A) AND EDX (B) ANALYSES OF THE IRON PRECIPITATE AFTER BIOLEACHING OF THE SEDIMENT SAMPLES

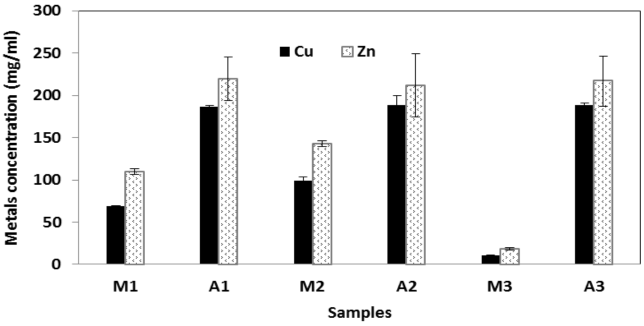

FIG. 9. CONCENTRATION OF CU, ZN IN LEACHATE OF BIOTIC SAMPLE (A) AND ABIOTIC SAMPLES IN MEDIUM M1, M2 AND M3 AFTER 19 DAYS BIOLEACHING OF THE CLAY FRACTION FROM SOIL (ZC)

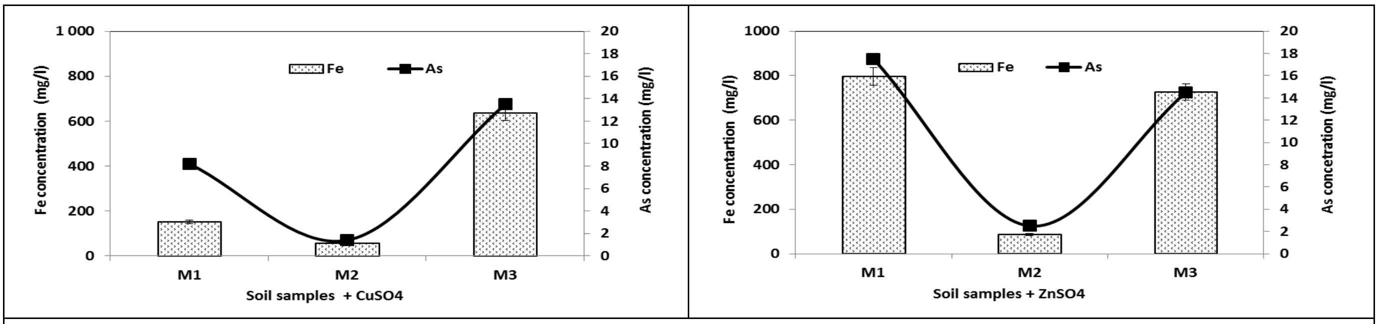

FIG.10. CONCENTRATION OF FE, AS IN LEACHATE OF THE CLAY FRACTION FROM SOIL (ZC) AFFECTED BY THE ADDITION OF CU (A) AND ZN (B) IN MEDIUM M1, M2 AND M3 AFTER 19 DAYS BIOLEACHING

TABLE 1
CHEMICAL COMPOSITION OF SOIL (Z) FROM DIFFERENT DEPTH 10 CM (Z1), 20 CM (Z2), 30 CM (Z3), 40 CM (Z4)

Metals mg/kg	Cu	Zn	Cd	Hg	Pb	Mn	Ni
Z1	9 523	8 681	8.5	12.3	1 605	2 025	80.3
Z2	2 881	3 074	6.5	17	878.5	1 297	65.3
Z3	2 580	1 962	5.8	16.8	820.3	2 457	85.2
Z4	4 628	3 812	5.7	14.3	874.8	1 787	248.3
Elements mg/kg	**As**		**Fe**	**Al**		**Si**	
Z1	742.2		39 460	61 500		200 700	
Z2	712.0		43 470	46 300		122 100	
Z3	625.9		74 540	55 500		175 700	
Z4	732.0		56 870	73 300		180 800	

TABLE 2
CHEMICAL COMPOSITION OF SEDIMENT (O) FROM DIFFERENT DEPTH 10 CM (O1), 20 CM (O2), 30 CM (O3), 40 CM (O4)

Metals mg/kg	Cu	Zn	Cd	Hg	Pb	Mn	Ni
O1	178.5	317.4	2.2	7.7	50.8	1 127	59.6
O2	223.6	364.2	3.7	7.8	55.4	1 290	62.2
O3	314.3	312.2	1.1	7.0	58.1	1 632	65.0
O4	348.8	309.3	0.9	6.5	56.5	1 658	67.8
Elements mg/kg	**As**		**Fe**	**Al**		**Si**	
O1	53.7		41 460	75 900		236 100	
O2	67.2		40 740	75 300		233 900	
O3	140.6		43 400	76 000		232 000	
O4	146.8		45 840	79 500		237 500	

TABLE 3

THE FORMAL RANKING OF METALS BASED ON THE DECISION OF MP SR AND MŽP SR NO. 220/2004 FOR THE LOAM - SAND SOIL AND NO. 188/2003 FOR THE BOTTOM SEDIMENT IS INDICATED.

Metals mg/kg	Cu	Zn	Cd	Hg	Pb	Ni	As
Soil	30	100	0.4	0.15	25	40	10
Sediment	1 000	2 500	10	10	750	300	20

TABLE 4

BACTERIAL COUNTS IN THE CLAY FRACTIONS OF SEDIMENT AND SOIL

Samples	Agar without	3 mM	8 mM
	metalloid	As	As
Soil (ZC)	(8.0±0.3) x 10^5	(1.7±0.4) x 10^3	(1.3±0.3) x 10^3
Sediment (OC)	(4.9±0.1) x 10^6	(3.9±0.3) x 10^6	(1.8±0.2) x 10^6

V. CONCLUSION

Soil is a major source of metals for contamination of sediments. The increasing metal concentrations with decreasing particle size were observed both in contaminated soil and sediment. The enrichment in the clay mineral content of the sediment and soil samples exerts much higher content for Cu, Zn, As, Pb, Ni and Fe except Mn. Consequently, the combined study of mineralogical and bacterial properties of the sediment and soil clay fractions allows to better understand the bioleaching process of potentially toxic metals. The dominant bacterial species belonged to the genus *Bacillus* in the soil and sediment environment. The arsenic tolerance was confirmed in *Bacillus* strains isolated from the clay fraction of soil and sediment. The concentration of dissolved Fe in the soil clay leaching experiment did not exceed 30 mg/l Fe. The iron was solubilized mainly by the sediment clay bioleaching. The bioleaching inhibited Cu and Zn dissolution and the addition of Cu and Zn cations increased Fe dissolution a As extraction from the soil clay sample. The dissolution of Fe by resistant bacteria leads to the extraction of sorbed As from the soil. The presence of heavy metals in the soils represents a significant environmental hazard, and one of the most important problems of contamination to solve. However, it seems to be the most effective to apply the bioleaching in removal of As from the untreated soil and sediment without the separation of clay because clay and iron minerals coated on coarse silicate particles are bearers of metals which contaminate the sediment and soil environment

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