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IN SITU BIOREMEDIATION OF A SOIL HEAVILY CONTAMINATED WITH NON-FERROUS METALS AND ARSENIC

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ABSTRACT. Two experimental plots of an acidic soil heavily contaminated with non-ferrous metals (mainly copper, zinc and cadmium) were treated in situ under real field conditions using the activity of the indigenous soil microflora. This activity was enhanced by suitable changes of some essential environmental factors such as pH and water, oxygen ad nutrient contents of the soil. The treatment was connected with solubilization and removal of contaminants from the top soil layer (horizon A) due to the joint action of the soil microorganisms (mainly of the acidophilic chemolithotrophic bacteria) and the leach solutions used to irrigate the soil (diluted sulphuric acid). The dissolved contaminants were removed from the soil profile of one of the plots (№ 1) through the drainage effluents. The dissolved contaminants in the plot № 2 were transferred to the deeply located soil subhorizon B_2 where they were precipitated as the relevant insoluble sulphides as a result of the activity of the sulphate-reducing bacteria inhabiting this soil subhorizon. This activity was enhanced by injecting water solutions of dissolved organic compounds (lactate and acetate) and ammonium and phosphate ions through vertical boreholes to this soil subhorizon).

Keywords: soil cleaning, heavy metals, arsenic, soil micloflora

БИОРЕМЕДИАЦИЯ IN SITU НА ПОЧВА ТЕЖКО ЗАМЪРСЕНА С ЦВЕТНИ МЕТАЛИ И АРСЕН

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РЕЗЮМЕ. Два експериментални участъка на кисела почва, тежко замърсена с цветни метали (главно мед, цинк и кадмий) и арсен бяха третирани in situ при реални полеви условия, използвайки активността на естествената почвена микрофлора. Тази активност беше повишена посредством подходящи промени на някои основни екологични фактори като pH и съдържания на вода, кислород и хранителни вещества в почвата. Третирането на почвата беше свързано с разтваряне и отстраняване на замърсители от горните почвени пластове (хоризонт А) поради съвместтното действие на почвените микроорганизми (главно на ацидофилните хемолитотрофни бактерии) и на излугващите разтвори, използвани за оросяване на почвата (разредена сярна киселина). Разтворените замърсители бяха отстранени от почвения профил на единия от участъците (№ 1) чрез дренажните води. Разтворените замърсители в участък № 2 бяха оставени да се придвижат до дълбоко разположения почвен подхоризионт B_2, където бяха утаени като съответните неразтворими сулфиди в резултат на активността на сулфатредуциращите бактерии, обитаващи този почвен подхоризонт. Тази активност беше повишена чрез инжектиране на водни разтвори на разтворени органични съединения (лактат и ацетат) и амониеви и фосфатни йони през отвесни сондажи, достигащи до този почвен подхоризонт.

Ключови думи: пречистване на почви, тежки метали, арсен, почвена микрофлора

Introduction

The large commercial-scale operations for dump and even for heap bioleaching of ores of non-ferrous metals and uranium are often connected with contamination of environment with several toxic elements. The remediation of waters and especially of soils contaminated with such pollutants is a difficult problem (U.S. Environmental Protection Agency, 1991). Traditional efforts to manage such soils until recently focused on their removal by extraction followed by off-site treatment/disposal as well as on the in situ monitored natural attenuation or passive capping using the installation of clean, inert material over the contaminated soil. However, the application of some in situ methods based on the chemical and/or biological removal of such toxic contaminants is steadily increasing due to their effectiveness and less expensive and less risky management (Knox et al., 2008; Groudev et al., 2010). Some data about the in situ bioremediation of a soil heavily contaminated with non-ferrous metals and arsenic are shown in this paper.

Materials and Methods

The soil used in this study was a typical leached cinnamonic soil heavily polluted with non-ferrous metals (mainly Cu, Zn and Cd) and arsenic. The soil profile was 125 cm deep (horizon A, 25 cm; horizon B, 75 cm; horizon C, 20 cm) and was underlined by intrusive rocks with a very low permeability. Data about the chemical composition and some essential geotechnical parameters of the soil are shown in Table 1. The contaminants were located mainly in the horizon A (Table 2).

Two experimental plots of the soil with a size of 120 m^3 each were treated under real field conditions. The treatment was connected with solubilization and removal of contaminants from the horizon A due to the joint action of the soil microorganisms (mainly of the acidophilic chemolithotrophic bacteria) and the leach solutions used to irrigate the soil. These solutions consisted of water acidified to pH 2.5 - 3.5 and containing ammonium and phosphate ions in amounts sufficient to maintain their concentrations in the soil pore solutions at about 30 and 15 mg/l, respectively.

Table 1.
Characteristics of the soil in the plot before and after the treatment

Parameters	Before treatment	After treatment	Parameters	Before treatment	After treatment
Chemical composition, %			pH (H_2O)	4.11	2.85
SiO_2	76.5	77.2	Net neutralization potential (kg $CaCO_3$/t)	-35.0	-18.7
Al_2O_3	14.6	14.2	Bulk density (g/cm^3)	1.54	1.50
Fe_2O_3	3.20	2.62	Specific density (g/cm^3)	2.64	2.58
P_2O_5	0.05	0.07	Porosity (%)	54	51
K_2O	2.26	1.94	Permeability (cm/h)	15.4	12.5
N total	0.10	0.11	Particle size, mm (%)		
S total	1.50	0.80	>1.00	3.2	2.1
S sulphidic	1.20	0.62	1.00 - 0.25	28	19.8
Carbonates	0.17	0.03	0.25 - 0.01	50.9	53.2
Humus	1.69	1.43	<0.01	25.1	24.9

Table 2.
Content of contaminants in the horizon A of the soil treated in the experimental plots

Parameters	Cu	Zn	Cd	As
	Contents of contaminants, ppm			
Before treatment	484	520	6.4	82
After treatment	64	82	0.8	15
Permissible levels for soils with pH<6.0	80	200	1.5	25
Permissible levels for soils with pH 6.0 - 7.4	150	320	2.0	25
Bioavailable fractions, ppm				
a) By DTPA leaching				
Before treatment	195	137	0.8	32
After treatment	19	11	0.07	3.5
b) By EDTA leaching				
Before treatment	156	80	0.41	23
After treatment	7.7	6.2	0.07	2.1
Easily leachable fractions, ppm				
Before treatment	305	245	1.7	42
After treatment	15	19	0.12	2.8
Inert fractions, ppm				
Before treatment	82	73	0.65	12
After treatment	77	70	0.59	11

The composition and irrigation rate of the leaching solutions were adjusted in connection with the levels of the local natural rain fall and temperature to maintain the water-filled porosity in the horizon A at about 60 - 70% and the pH of the soil pore solutions within the range of about 2.8 - 3.2. The upper layers (horizon A) were ploughed periodically to enhance the natural aeration.

The contaminants dissolved in the first plot (№1) were removed from the soil profile through the drainage effluents. The pregnant drainage effluents were collected in a collector pond and then were treated by a constructed wetland located near the soil plots. The contaminants dissolved in the plot №2 were transferred to the deeply located soil horizon B where they were precipitated as the relevant insoluble sulfides as a result of the activity of the indigenous sulfate-reducing bacteria. This activity was enhanced by injecting water solutions of dissolved organic compounds (lactate and acetate) and ammonium and phosphate ions through vertical boreholes to this soil horizon. Regardless of the removal of contaminants, the relatively barren drainage solutions from the plot №2 were also treated by the constructed wetland.

The isolation, identification and enumeration of microorganisms, as well as the determination of the bacterial activity in situ, were carried out by methods described elsewhere (Groudev et al., 2010; Karavaiko et al., 1988; Escobar et al., 2008; Sanz and Köchling, 2007).

Results and Discussion

The leaching of contaminants in the horizon A was very efficient and within a period about 9 months (from March to November to avoid the cold winter months in Bulgaria) their concentrations were decreased below the relevant permissible levels (Table 2). The treatment was connected with considerable changes in the composition of the soil microflora (Table 3). The mesophilic acidophilic chemolithotrophic bacteria related to the species *Acidithiobacillus ferrooxidans*, *At. thiooxidans* and *Leptospirillum ferrooxidans* became the prevalent microorganisms in the horizon A (Table 3). These bacteria were able to oxidize the sulphide minerals present in the soil and to solubilize their metal components. The non-ferrous metals (Cu, Zn, Cd) and arsenic were solubilized mainly in this way and as the relevant cations were removed from this soil horizon.

Table 3.
Microorganisms in the soil horizon A before and during the leaching of contaminants

Microorganisms	Before treatment	During treatment
	Cells/g dry soil	
Aerobic hetertrophic bacteria	10^4 - 10^7	10^3 - 10^5
Acidophilic chemolithtrophs oxidizing Fe^{2+} and/or S^0	10^2 - 10^4	10^5 - 10^7
Chemolithotrophs able to oxidize S^o at pH 4 - 9	10^3 – 10^5	10^1 - 10^3
Nitrifying bacteria	10^2 – 10^3	10^1 - 10^2
Fungi	10^1 - 10^3	10^1 - 10^2

The activity of the chemolithotrophic bacteria depended on some essential environmental factors such as pH and temperature (Table 4), presence of essential nutrients (mainly sources of N and P), as well as on the levels of soil humidity and aeration.

Table 4.
Microbial activity in situ at different environmental conditions in the experimental plots

Sample and conditions of testing	Fe^{2+}oxidized for 5 days, g/l	$^{14}CO_2$ fixed for 5 days, counts/min.g
Soil suspensions in 10K nutrient medium with pH 3.7 +Fe^{2+} (10g/l) at 9 – 12 °C	0.71 – 1.59	1700 - 3200
Soil suspensions in 10K nutrient medium with pH 3.7 +Fe^{2+} (10g/l) at 15 – 18 °C	1.98 - 4.86	3600 - 8100
Soil suspensions in 10K nutrient medium with pH 3.7 +Fe^{2+} (10g/l) at 20 – 23 °C	2.55 - 6.82	4100 - 12200
Soil suspensions in 10K nutrient medium with pH 3.0 +Fe^{2+} (10g/l) at 9 – 12°C	1.55 - 3.32	3700 - 7200
Soil suspensions in 10K nutrient medium with pH 3.0 +Fe^{2+} (10g/l) at 15 – 18 °C	2.84 - 6.44	4300 - 12000
Soil suspensions in 10K nutrient medium with pH 3.0 +Fe^{2+} (10g/l) at 20 – 23°C	3.07 - 8.15	4600 - 15500

The removal of contaminants from the pregnant drainage solutions generated in the horizon A of the experimental soil plots by treatment outside the soil plot № 1 and by treatment inside the soil plot № 2 was very efficient and the exact choice of the more attractive variant depends on several factors, some of which are not directly connected with the type of the soil and the kind and the concentration of the contaminants. In any case, after the end of the operation for removal or detoxification of the contaminants, the treated soils are subjected to some conventional melioration procedures such as liming (if necessary), grassing, moulching, addition of fertilizers and animal manure as well as periodic ploughing and irrigation.

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The article has been recommended for publication by department “Engineering geoecology”