BIOREMEDIATION IN SITU OF POLLUTED SOIL IN A URANIUM DEPOSIT

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Abstract. An experimental plot containing acidic soil heavily contaminated with radionuclides (mainly uranium) and heavy metals (mainly copper, zinc and cadmium) was treated by means of an in situ biotechnological method based on the activity of the indigenous microflora, mainly of some acidophilic chemolithotrophic bacteria. The pollutants were located mainly in the upper soil layers (horizon A) and considerable portions of them were present in forms susceptible to bacterial leaching. The treatment was connected with the dissolution of pollutants and their removal from the soil profile by means of water acidified with sulphuric acid to pH of about 3.5. The bacterial activity was enhanced by suitable changes of some essential environmental factors such as pH and water, oxygen and nutrient contents in the soil. The removal of pollutants was very efficient and within 24 months their residual concentrations in the soil were decreased below the relevant permissible levels. The pregnant soil effluents containing the dissolved pollutants were efficiently cleaned up by wetlands located near the experimental plot.

Keywords: soil bioremediation, radionuclides, heavy metals, chemolithotrophic bacteria, wetlands

1. Introduction

The uranium deposit Curilo, located in Western Bulgaria, for a long period of time was a site of intensive mining activities including both the open-pit and underground techniques as well as *in situ* leaching of uranium. The mining operations were ended in 1990 but until now both the surface and ground waters and soils within and near the deposit are heavily polluted with radionuclides (mainly uranium) and heavy metals (mainly copper, zinc and cadmium).

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Laboratory experiments carried out with soil samples from the deposit revealed that an efficient remediation of the soils was achieved by solubilizing the pollutants and washing the soil profile by means of acidified water solutions. The solubilization was connected with the activity of the indigenous soil microflora, mainly with the activity of some acidophilic chemolithotrophic bacteria. It was possible to enhance considerably this activity by suitable changes in the levels of some essential environmental factors such as pH and waters, oxygen and nutrient contents of the soil.

The promising data from the laboratory experiments as well as from the geologic and hydrogeologic investigations of the polluted lands were the main reason for the application of the above-mentioned soil treatment *in situ* under real field conditions in an experimental plot located in the deposit. Some data from these field experiments are shown in this paper.

2. Materials and Methods

A detailed sampling procedure was carried out to characterize the soil and the subsurface geologic and hydrogeologic conditions. Surface and bulk soil samples up to a depth of 2 m were collected by an excavator. Drill hole samples were collected up to a depth of 8 m. Elemental analysis in the samples was performed by digestion and measurement of the ion concentration in solution by atomic absorption spectrophotometry and induced coupled plasma spectrophotometry. Mineralogical analysis was carried out by X-ray diffraction techniques.

The main geotechnical characteristics of the site, such as permeability and wet bulk density, were measured *in situ* using the sand-core method (U.S. Environmental Protection Agency, 1991). True density measurements were carried out in the laboratory using undisturbed core samples. Such samples were also used for determination of their acid generation and net neutralization potentials using static acid-base accounting tests. The bioavailable fractions of the pollutants were determined by leaching the samples with DTPA and EDTA (Sobek et al., 1978). The mobility of the pollutants was determined by the sequential extraction procedure (Tessier et al., 1979). The toxicity of soil samples was determined by the EPA Toxicity Characteristics Leaching Procedure (U.S. Environmental Protection Agency, 1990).

The experimental plot had a rectangular shape and was 80 m^2 in size (10 m × 8 m). Water acidified with sulphuric acid to pH of about 3.5 was used as leach solution. The upper soil layers were ploughed up periodically to enhance the natural aeration.

The flow sheet of the operation included also a system to collect the soil drainage solutions and to avoid their seepage and the distribution of contaminants into the environment. The system consisted of several ditches and wells located in suitable sites in the experimental plot. The soil effluents collected by this system were then treated initially by a natural and since the beginning of the second year by a constructed wetland located near the experimental plot to remove the dissolved contaminants. These wetlands were characterized by an abundant water and emergent vegetation and a diverse microflora. *Typha latifolia, Typha angustifolia, Phragmites australis* and different algae were the prevalent plant species in the wetland.

A plot with the same size and shape was used as a control. This plot also consisted of polluted soil. This soil, however, was not treated during the whole experimental period.

The isolation, identification and enumeration of microorganisms were carried out by methods described elsewhere (Karavaiko et al., 1988; Groudeva et al., 1993).

The bacterial activity *in situ* in the soil was determined by following the rates of ferrous iron oxidation in samples of drainage waters collected from different sections of the experimental and control plots as well as in 9K nutrient medium (Silverman and Lundgren, 1999) inoculated with freshly collected soil samples. These experiments were carried out in 300 ml Erlenmeyer flasks containing 100 ml liquid phase. The flasks were incubated *in situ*, at different depths in the plots, at the relevant natural temperatures, for 5 days. The technique described by Karavaiko and Moshniakova (1971) was used with some modifications (Groudev and Groudeva, 1993) to determine the $^{14}CO_2$ fixation *in situ*.

3. Results and Discussion

The soil profile was approximately 90 cm deep (horizon A, 30 cm; horizon B, 40 cm; horizon C, 20 cm). The soil profile was underlined by intrusive rocks with a very low filtration coefficient (approximately 7×10^{-8} m/s).

The groundwater level was located at about 10 m below the surface. The surface and groundwaters in the site were separated by the impermeable rocks.

Data about the chemical composition and some essential geotechnical parameters of the soil are shown in Tables 1 and 2. The concentrations of contaminants were higher in the upper soil layers (in the horizon A). Considerable portions of the contaminants were present in fractions susceptible to biological leaching and within the experimental period of 24 months their residual concentrations in the horizon A were decreased below the relevant permissible levels.

At the same time, the decrease in the concentrations of contaminants in the control plot as a result of the processes of natural attenuation was negligible.

The analysis of the microflora in the experimental plot showed that it contained a rich variety of microorganisms (Table 3). The mesophilic acidophilic chemolithotrophic bacteria related to the species *Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* were the prevalent microorganisms in the top soil layers. These bacteria were able to oxidize the sulphide minerals present in the soil and to solubilize their metal components. The non-ferrous metals, i.e. copper, zinc, cadmium, were removed mainly in this way. Uranium was also solubilized as a result of its prior bacterial oxidation from the tetravalent to the hexavalent state. The activity of these acidophilic chemolithotrophic bacteria *in situ* was quite high (Table 4). Thermophilic chemolithotrophic bacteria able to oxidize sulphide minerals and uranium were not found in the soil. However, some basophilic chemolithotrophic species (mainly *Thiobacillus thioparus, T. denitrificans* and *Halothiobacillus neapolitanus*) as well as some acidophilic heterotrophs (mainly such related to the genus *Acidiphilium*) were also present but in lower numbers. In the deeply located soil layers (in the subhorizon B_2) both the total number and diversity of the microorganisms were much lower.

TABLE 1. Characteristics of the soil treated in this study

Parameters	Horizon A (0 – 30 cm)	Horizon B (31 – 70 cm)
Chemical composition (in %):		
- SiO_2	64.4	67.5
- Al_2O_3	12.2	14.1
- Fe_2O_3	10.4	9.1
- CaO	0.41	0.37
- MgO	0.44	0.35
- K_2O	1.45	1.27
- S total	1.04	0.80
- S sulphidic	0.86	0.75
- Humus	2.1	0.8
Bulk density, g/cm^3	1.22	1.40
Specific density, g/cm^3	2.80	3.02
Porosity, %	51	46
Moisture capacity, %	48	44
Permeability, cm/s	8×10^{-2}	6×10^{-2}
pH (H_2O)	4.6	4.8
Net neutralization potential, kg $CaCO_3$/t	- 170	- 134

TABLE 2. Content of contaminants in the horizon A of the soil before and after the treatment

Parameters	Cu	Zn	Cd	U
Content of contaminants, ppm				
- before treatment	170	194	4.1	59
- after treatment	32	44	0.9	8.2
Permissible levels for soils with pH 4.1 – 5.0	40	60	1.5	10
Permissible levels for soils with pH <4.0	20	30	0.5	10
Bioavailable fraction, ppm:				
a. by DTPA leaching				
- before treatment	28	25	0.7	9.1
- after treatment	3.7	3.2	0.05	2.8
b. by EDTA leaching				
- before treatment	23	14	0.4	4.6
- after treatment	1.5	1.4	0.01	0.8
Easily leachable fractions – exchangeable + carbonate, ppm				
- before treatment	64	71	1.0	17
- after treatment	3.5	4.4	0.03	2.1
Inert fraction, ppm				
- before treatment	71	82	2.1	14
- after treatment	27	37	0.8	6.4
Pollutants solubilized during the toxicity test, ppm				
- before treatment	5.94	7.52	0.14	1.35
- after treatment	0.35	0.41	0.01	0.17

TABLE 3. Microorganisms in the horizon A of the experimental plot before and after the treatment

Microorganisms	Before treatment	During treatment
	Cells/g dry soil	
Aerobic heterotrophic bacteria	$10^3 – 10^7$	$10^3 – 10^6$
Fe^{2+} - oxidizing chemolithotrophs (at pH 2.5)	$10^1 – 10^3$	$10^4 – 10^7$
S^0 - oxidizing chemolithotrophs (at pH 2.5)	$10^2 – 10^4$	$10^4 – 10^7$
$S_2O_3^{2-}$ - oxidizing chemolithotrophs (at pH 7)	$10^2 – 10^5$	$10^1 – 10^4$
Nitrifying bacteria	$10^1 – 10^4$	$< 10^2$
Nitrogen – fixing bacteria	$10^2 – 10^4$	$< 10^2$
Streptomycetes	$10^2 – 10^5$	$10^1 – 10^4$
Fungi	$10^2 – 10^6$	$10^1 – 10^4$
Anaerobic heterotrophic bacteria	$10^3 – 10^5$	$10^2 – 10^4$

TABLE 4. Microbial activity in situ at different environmental conditions

Sample tested	Fe^{2+} oxidized for 5 days, g/l	$^{14}CO_2$ fixed for 5 days, counts/min.ml (g)
Soil effluents with a pH of 4.6 + Fe^{2+} (9 g/l) at 8 – 10°C	0.46 – 1.32	1400 – 3500
Soil effluents with a pH of 3.7 + Fe^{2+} (9 g/l) at 8 – 10°C	0.86 – 2.75	2100 – 6400
Soil effluents with a pH of 3.7 + Fe^{2+} (9 g/l) at 17 – 20°C	1.45 – 4.10	3700 – 10400
Ore suspensions in 9K nutrient medium (with 9 g/l Fe^{2+} and pH 3.7) at 8 – 10°C	0.55 – 1.59	1500 – 4100
Ore suspensions in 9K nutrient medium (with 9 g/l Fe^{2+} and pH 3.7) at 17 – 20°C	0.95 – 2.99	2300 – 7100
Ore suspensions in 9K nutrient medium (with 9 g/l Fe^{2+} and pH 4.6) at 17 – 20°C	0.68 – 2.08	1700 – 5100

The number and activity of the acidophilic chemolithotrophs in the soil were limited by some essential environmental factors such as the relatively high soil pH, shortage of oxygen and of some important nutrients such as nitrogen and phosphorus sources in the soil, insufficient soil moisture during relatively long periods of time. The treatment of the contaminated soil was connected with the increasing number and activity of the indigenous acidophilic chemolithotrophic microorganisms by suitable changes in the levels of the above-mentioned environmental factors. This was achieved by regular ploughing up and irrigation of the soil and by addition of some essential nutrients. The optimum soil humidity was about 50% of the moisture capacity of the soil, but periodic flushing with slightly acidified water (with pH of about 3.5) was needed to remove the soil contaminants. Zeolite saturated with ammonium phosphate was added to the soil (in amounts of 5 kg/t dry soil) to provide the microorganisms with ammonium and phosphate ions to improve the physico-mechanical properties of the soil.

The rate of soil clean-up markedly depended on the temperature. The highest rates were achieved during the warm summer months at soil temperatures, which exceeded 25°C. It must be noted, however, that the clean-up was efficient even at temperatures as low as 5 – 10°C, and practically stopped only during the cold winter months (December – February) when the temperatures were often close or even below 0°C.

The soil in the control plot was characterized by much lower content and activity of microorganisms.

Some amounts of contaminants solubilized in the upper soil horizon A were retained in the deeper horizons, mainly due to processes such as the microbial dissimilatory sulphate reduction and sorption on the clay minerals.

The population of sulphate-reducing bacteria in these deeply located soil layers was quite diverse but not numerous during the treatment (Table 5). This was due to the acidic pH and relatively low concentration of dissolved organic compounds, which were used as sources of energy and carbon by these bacteria. At the same time, the contents of pollutants in the deeply located soil layers were also decreased as a result of bioleaching processes carried out by different microorganisms, mainly by the chemolithotrophic sulphide-oxidizing acidophilic bacteria and the products from their oxidative activity (ferric ions and sulphuric acid). Some role was played also by the basophilic chemolithotrophs *H. neapolitanus* and *T. thioparus*, which were found in some microzrones with pH level of about 3 – 3.5, i.e. at the limit allowing the growth and activity of these bacteria. They oxidized the elemental sulphur formed as a result of different chemical, electrochemical and biological processes and deposited on the surface of the sulphides. It must be noted that both the acidophilic and basophilic chemolithotrophs in deeply located soil layers were active at microaerophilic conditions created by the oxygen transported to these layers by means of the drainage waters.

Some anaerobic heterotrophic bacteria, mainly such possessing iron and manganese respiration, were able to solubilize iron and manganese hydroxide and oxide minerals and in this way to liberate the non-ferrous metals and uranium encapsulated in these minerals. Portions of the non-ferrous metals were solubilized as complexes with some organic compounds. Most of the lead was solubilized in this way.

TABLE 5. Sulphate-reducing bacteria in the soil subhorizon B_2 during the treatment

Sulphate-reducing bacteria	Cells/ml pore solution
Desulfovibrio (mainly *D. desulfuricans*)	$10^1 – 10^3$
Desulfobulbus (mainly *D. elongatus*)	$10^1 – 10^3$
Desulfococcus (mainly *D. postgatei*)	$0 – 10^2$
Desulfobacter (D. multivorans)	$0 – 10^2$
Desulfotomaculum (mainly *D. nigrificans*)	$0 – 10^1$
Desulfosarcina (D. variabilis)	$10^1 – 10^2$
Desulfomonas (non-identified species)	$0 – 10^1$

As a result of the microbial activity not only the horizon A but, to some extent, the whole soil profile was efficiently cleaned at the end of the experimental period from the easily leachable forms of the pollutants. Regardless of

the precipitation of portions of the pollutants in the subhorizon B_2, the soil effluents usually still contained most pollutants in concentrations higher than the relevant permissible levels for water intended for use in the agriculture and/or industry. These effluents were efficiently treated by means of the wetlands located near the experimental plot (Table 6). The removal of contaminants in these wetlands was connected with different processes but the microbial sulphate reduction and the sorption of contaminants on the organic matter (mainly living and dead plant biomass) and clay minerals present in the wetlands played the main role. Portions of iron and manganese were removed as a result of the prior oxidation of Fe^{2+} and Mn^{2+} to Fe^{3+} and Mn^{4+}, respectively, followed by precipitation of these higher valency forms as $Fe(OH)_3$ and MnO_2. Data about the microflora of the soil effluents and wetlands are shown in Table 7.

TABLE 6. Composition of the soil effluents before and after treatment in the wetlands

Parameters	Before treatment	After treatment	Permissible levels for waters used in agriculture or industry
pH	3.0 – 3.7	7.1 – 7.7	6 – 9
Eh, mV	(-95) – (-203)	(+235) – (+307)	–
Dissolved oxygen, mg/l	0.3 – 0.9	2.1 – 5.0	2
Total dissolved solids, mg/l	482 – 1294	275 – 655	1500
Solids, mg/l	38 – 82	25 – 73	100
Dissolved organic carbon, mg/l	14 – 41	17 – 46	20
Sulphates, mg/l	230 – 648	140 – 320	400
Uranium, mg/l	0.05 – 0.88	< 0.05	0.6
Copper, mg/l	0.35 – 2.42	< 0.2	0.5
Zinc, mg/l	0.41 – 9.50	0.05 – 0.91	10
Cadmium, mg/l	< 0.01 – 0.07	< 0.01	0.02
Lead, mg/l	0.15 – 1.22	< 0.1	0.2
Iron, mg/l	15 – 38	0.7 – 2.3	5
Manganese, mg/l	4.1 – 18.1	< 0.5	0.8

The chemical composition, structure and main physical and water properties of the soil after the treatment were altered to a small extent. However, the pH of the soil was decreased from the initial 4.6 to 3.5. After the two-year treatment period the experimental plot was subjected to some conventional remediation procedures such as grassing of the treated soil, addition of suitable fertilizers and animal manure as well as with periodical ploughing up, liming (to increase and maintain the pH to about 5) and irrigation. As a result of this, the quality of the soil was completely restored. No soluble forms of the above-mentioned

contaminants in concentrations higher than the relevant permissible levels were detected so far (approximately five years after the end of the treatment) in the soil pore and drainage waters after rainfall.

TABLE 7. Microflora of the fresh soil effluents and the constructed wetland

Microorganisms	Samples		
	Soil effluents before treatment	Waters from the wetland	Sediments from the wetland
	Cells/ml (g)		
Aerobic heterotrophic bacteria	$10^1 – 10^3$	$10^2 – 10^6$	$10^1 – 10^4$
Fe^{2+} - oxidizing chemolithotrophs (at pH 2.5)	$10^3 – 10^5$	$0 – 10^1$	0
$S_2O_3^{2-}$ - oxidizing chemolithotrophs (at pH 7)	$10^1 – 10^3$	$0 – 10^2$	$0 – 10^1$
Fe^{2+} - oxidizing heterotrophs (at pH 7)	$0 – 10^2$	$10^2 – 10^4$	$0 – 10^2$
Cellulose-degrading aerobes	$0 – 10^1$	$0 – 10^4$	$0 – 10^2$
Anaerobic heterotrophic bacteria	$10^1 – 10^4$	$10^2 – 10^4$	$10^2 – 10^5$
Fe^{3+} - reducing bacteria	$10^1 – 10^3$	$0 – 10^3$	$10^2 – 10^5$
Sulphate-reducing bacteria	$10^1 – 10^3$	$0 – 10^2$	$10^2 – 10^4$
Cellulose-degrading anaerobes	$0 – 10^1$	$0 – 10^2$	$10^1 – 10^4$

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References

Groudev, S.N. and V.I. Groudeva, 1993. “Microbial Communities in Four Industrial Copper Dump Leaching Operations in Bulgaria”, FEMS Microbiological Reviews, 11:261–268.

Groudeva, V.I., I.A. Ivanova, S.N. Groudev and G.C. Uzunov. 1993. “Enhanced Oil Recovery by Stimulating the Activity of the Indigenous Microflora of Soil Reservoirs”. In A.E. Torma, M.L. Appel and C.L. Brierley (Eds.), Biohydrometallurgical Technologies, vol. II, pp. 349-356. TMS Minerals, Metals & Materials Society, Warrendale, PA.

Karavaiko, G.I. and S.A. Moshniakova, 1971. “A Study on Chemosynthesis and Rate of Bacterial and Chemical Oxidative Processes Under Conditions of Copper-nickel Ore Deposits of Kolsky Peninsula”, Mikrobiologiya, 40:551–557 (in Russian).

Karavaiko, G.I., G. Rossi, A.D. Agate, S.N. Groudev and Z.A. Avakyan, (Eds.) 1988. Biotechnology of Metals. Manual, Center for International Projects GKNT, Moscow.

Silverman, M.P. and D.G. Lundgren, 1959. “Studies on the Chemoauthotrophic Iron Bacterium Ferrobacillus ferrooxidans. I. An improved medium and a harvesting procedure for securing high cell yields”, Journal of Bacteriology, 77:642–647.

Sobek, A.A., W.A. Schuller, J.R. and R.M. Smith, 1978. Field and Laboratory Methods Applicable to Overburden and Mine Soils, US Environmental Protection Agency, Report 600/2-78-054.

Tessier, A., P.G.C. Campbell and M. Bisson, 1979. “Sequential Extraction Procedure for Speciation of Particulate Trace Metals”, Analytical Chemistry, 51(7):844–851.

U.S. Environmental Agency, 1990, Characteristics of EP Toxicity, Paragraph 261.24, Federal Register 45 (98).

U.S. Environmental Protection Agency, 1991. Description and Sampling of Contaminated Soils – A Field Pocket Guide. EPA/625/12-91/002 Technology Transfer, Centre for Environmental Research Information, U.S. Environmental Protection Agency, Cincinnati, OH.