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Bioremediation of a soil contaminated with radioactive elements

S.N. Groudev [a,*], P.S. Georgiev [a], I.I. Spasova [a], K. Komnitsas [b]

[a] *Department of Engineering Geoecology, University of Mining and Geology, Studentski grad-Durvenitza, Sofia 1100, Bulgaria*
[b] *Laboratory of Metallurgy, National Technical University of Athens, Zografos Athens, 15780, Greece*

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Abstract

Some agricultural lands located in the Vromos Bay area, near the Black Sea coast, Southeastern Bulgaria, have been contaminated with radioactive elements (uranium, radium and thorium) and toxic heavy metals (copper, cadmium and lead) as a result of mining and mineral processing of polymetallic ores. Laboratory experiments carried out with soil samples from these lands revealed that an efficient remediation of the soils was achieved by an in situ treatment method based on the activity of the indigenous soil microflora. The treatment was connected with the dissolution of the contaminants in the upper soil horizons and their transfer into the deeply located soil horizons (mainly to the horizon B_2) where they were immobilized as different insoluble compounds. The dissolution of contaminants was connected with the activity of both heterotrophic and chemolithotrophic aerobic microorganisms and the immobilization was due mainly to the anaerobic sulphate-reducing bacteria. The activity of these microorganisms was enhanced by suitable changes in the levels of some essential environmental factors such as water, oxygen and nutrient contents in the soil. On the basis of the above-mentioned laboratory results, the method was then applied under real field conditions in a heavily contaminated experimental plot of land located in the Vromos Bay area. Within 8 months of treatment, the contents of radioactive elements and toxic heavy metals in the soil were decreased below the relevant permissible levels.

Keywords: Bioremediation; Soil; Radioactive elements

1. Introduction

Pollution of soils by radionuclides may be of different kinds. An important type of radionuclides originates from the emission to the atmosphere, e.g. nuclear explosions (^{3}H) or reactor operations (^{85}Kr). Subsequent fallout of radionuclides with precipitation and infiltration causes pollution of different aqueous and terrestrial ecosystems. Such antropogenic pollution is sufficiently well documented worldwide.

Natural radioactivity is due mainly to ^{40}K and the members of the natural ^{238}U decay chain. Natural radionuclides are released to the surface and ground waters from rocks and ores by dissolution and desorption, or by diffusion or atomic recoil, during radioactive decay. Different chemolithotrophic and heterotrophic microorganisms are able to leach ura-

* Corresponding author. Tel.: +359-2-62581; fax: +359-2-962-4940.
E-mail address: groudev@staff.mgu.bg (S.N. Groudev).

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nium and other radioactive elements from different mineral raw materials in both acidic and alkaline water solutions [1–4]. The intensity of these microbial processes in the virgin uranium ore deposits is usually limited by the shortage of oxygen, water and/or dissolved organic compounds. The artificial disturbance of the ore bodies, as a result of human activities, largely enhances the leaching of radioactive elements.

In Bulgaria, for a long period of time uranium was leached commercially in a large number of deposits using different in situ technologies. Most of these commercial-scale operations were connected with the acid leaching of uranium due to the presence of pyrite and the negative net neutralization potentials of the relevant uranium ores. Several years ago, all commercial-scale operations for uranium leaching in the country were stopped due to a complex of different political, economical and environmental reasons. Regardless of some preventive and remedial actions during the uranium recovery, many natural ecosystems were heavily polluted with radioactive elements, mainly through the seepage of acid drainage waters. Such waters are still a persistent environmental problem at many abandoned mine sites. Soils around the water flowpath are polluted with radioactive elements and are unsuitable for agricultural use. In some deposits, alkaline leaching of uranium was applied, using solutions containing carbonate and hydrocarbonate ions. This process also was connected with a heavy pollution of the soils. In some cases, air transportation is also an essential factor for the soil pollution.

Different methods for remediation of soils contaminated with radioactive elements are known but only few of them have been applied under large-scale conditions. The excavation and transportation of the heavily polluted soils to specific depositories is still a common practice in most countries.

Recently, a biotechnological method for detoxification in situ of soils contaminated with toxic heavy metals and arsenic was developed and applied in some agricultural lands in Bulgaria [5,6]. This method is connected with the transfer of the soil contaminants into the deeply located soil horizons where the soluble contaminants are turned into the relevant insoluble sulphides. Microorganisms related to different physiological groups, mainly acidophilic iron- and sulphur-oxidising chemolithotrophic bacteria and anaerobic sulphate-reducing bacteria, respectively, carry out both the transfer and precipitation of the contaminants.

The ability of different microorganisms to dissolve uranium in waters with a slightly alkaline pH as well as the ability of the sulphate-reducing bacteria to precipitate the dissolved uranium by reducing it to the tetravalent form are well known [1,7,8]. For that reason, a study was carried out to establish the possibility to apply the above-mentioned remediation method for treatment of soils polluted with uranium and the other radioactive elements from its decay chain. Some agricultural lands located in the Vromos Bay area, near the Black Sea coast, Southeastern Bulgaria, were used in this study. These lands have been contaminated with radioactive elements (uranium, radium and thorium) and toxic heavy metals (copper, cadmium and lead) as a result of mining and mineral processing of polymetallic ores. Data about this study are presented in this paper.

2. Experimental

2.1. Laboratory experiments

A sample of soil heavily polluted with radioactive elements and toxic heavy metals was used in the experimental work. The sample was excavated in a way preserving the natural soil profile. Portions of the sample were used in the bioremediation experiments within 48 h after the excavation to preserve the viable indigenous microflora of the soil.

The freshly excavated soil samples were subjected to bioremediation in plastic rectangular (39×19 cm) lysimeters containing 50 kg (dry weight) of soil each. The soil in the lysimeters was arranged in a way reflecting the natural distribution of the different soil horizons. The soil profile in the lysimeters was 100 cm high (horizon A 25 cm, horizon B 55 cm, and horizon C 20 cm). The soil treatment was connected with the solubilization of the pollutants and their removal from the soil by drainage waters percolating through the soil mass. Leach solutions containing carbonate ions and some dissolved organic compounds (mainly acetate) were used for this purpose. The upper soil layers were ploughed up periodically

to enhance the natural aeration. The pregnant soil effluents were treated by a passive system consisting of an anoxic cell containing a mixture of organic substrates (spent mushroom compost, sawdust, horse and cow manure) and a community of different metabolically interdependent microorganisms. The barren waters from the anoxic cell were recycled to the lysimeters.

In some lysimeters, water solutions of dissolved organic compounds were injected through vertical pipes to the deeply located soil layers (horizon B_2) to enhance the activity of the anaerobic sulphate-reducing bacteria.

2.2. Field experiments

The experimental plot had a rectangular shape and was 400 m^2 in size (20×20 m). The soil profile was 100 cm deep (horizon A 25 cm, horizon B 55 cm and horizon C 20 cm). The soil profile was underlain by intrusive rocks consisting of dense diorite and gabbro. The filtration properties of these intrusives were very low. The filtration coefficient was approximately 6×10^{-8} m/s, and the deeply located rocks were even more impermeable. The ground water level was located about 2–3 m below the surface. The surface and ground waters in the site were separated by the impermeable rocks. It was assumed that both the geologic and hydrogeologic conditions in the site were suitable for the application of in situ methods for soil remediation.

Leach solutions containing carbonate ions and some dissolved organic compounds were used to facilitate the dissolution of the pollutants. The upper soil layers were ploughed up periodically, at least once per 2 months, to enhance the natural aeration. Water solutions of dissolved organic compounds and ammonium phosphate were injected through vertical pipes to the deeply located soil layers (horizon B_2) to enhance the action of the anaerobic sulphate-reducing bacteria.

The flowsheet included also a collection system for the soil drainage solutions. These solutions were treated to remove the residual amounts of toxic elements, non-precipitated in the deeply located soil horizons, by means of a passive system of the type of constructed wetlands. The wetland was characterized by an abundant water and emergent vegetation and a varied microflora. *Typha latifolia*, *Typha angustifolia*, *Scirpus* sp. and different algae were the prevalent plant species in the wetland.

Monitoring was done also on a control contaminated plot without the ploughing, irrigation and addition of ammonium phosphate.

2.3. Analytical procedures

A detailed sampling procedure was carried out to characterize the soil and the subsurface geologic and hydrogeologic conditions of the site. Surface and bulk soil samples up to a depth of 2 m were collected by mechanical excavator. Drill hole samples were collected up to a depth of 10 m. Elemental assays in the samples were 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 [9]. True density measurements were carried out in 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 through leaching the samples by DTPA and EDTA [10]. The speciation of the pollutants with respect to their mobility was determined by the sequential extraction procedure [11]. The toxicity of soil samples was determined by the EPA Toxicity Characteristics Leaching Procedure [12].

The dissolved metal concentrations were determined by ICP spectrophotometry. Uranium concentration was measured photometrically using the arsenazo III reagent. Radium was measured radiometrically. Sulphate concentrations were determined photometrically.

The isolation, identification and enumeration of the microorganisms inhabiting the lysimeters and the soil field were carried out by methods described previously [13,14].

3. Results and discussion

3.1. Laboratory experiments

Data about the chemical composition and some essential parameters of the soil are shown in Table 1. The concentrations of contaminants were higher in the upper soil layers (mainly in the horizon A) (Table 2). Considerable portions of the contaminants were present as the relevant inert fractions, which are refractory to solubilization. Regardless of this, the leaching of the contaminants in the upper soil layers was very efficient (Table 2) and was undoubtedly connected with the activity of the indigenous soil microflora. Thus, in a control lysimeter treated by leach solutions containing microbial inhibitor ($HgCl_2$) the leaching of contaminants was negligible.

The analysis of the soil microflora revealed that it included a rich variety of microorganisms (Table 3). In the upper soil layers, the aerobic heterotrophic bacteria were the prevalent microorganisms. Their total number was higher than 10^8 cells/g dry soil. Bacteria related to the genera Pseudomonas and Bacillus were the most numerous in these microbial communities. The fungi were low in number. Some chemolithotrophic bacteria able to oxidise S^0 and soluble inorganic sulphur compounds at neutral and alkaline pH values were also present. *Thiobacillus thioparus* was the main species among these chemolithotrophs but *Thiobacillus neapolitanus*, the anaerobic *Thiobacillus denitrificans* and some mixotrophic bacteria (mainly such related to the species *Thiobacillus novellus*) were also present.

The activity of this microflora was enhanced by suitable changes in the levels of some essential environmental factors such as water, oxygen and nutrient contents in the soil. This was achieved by regular ploughing and irrigation of the soil. The optimum soil humidity was about 45–50% from the moisture capacity of the soil but periodic flushing with leach solutions was needed to remove the dissolved contaminants. Zeolite saturated with ammonium phosphate was added to the soil (in amounts in the range of 2–5 kg/ton dry soil) to provide the microorganisms with ammonium and phosphate ions. The presence of zeolite enhanced to some extent the rate of leaching and decreased considerably the inefficient consumption of the nutrients. The slow-re-

Table 1
Characteristics of the soil used in this study

Parameters	Horizon A (0–25 cm)	Horizon B (26–80 cm)
Chemical composition (%)		
SiO_2	60.6	61.1
Al_2O_3	14.3	14.0
Fe_2O_3	12.5	5.90
CaO	5.72	2.35
MgO	3.21	1.31
K_2O	3.92	3.81
Na_2O	0.88	1.94
S total	0.28	0.21
S sulphidic	0.14	0.10
Humus	3.7	1.4
Bulk density (g/cm^3)	1.51	1.63
Specific density (g/cm^3)	3.14	3.43
Porosity (%)	44	41
Moisture capacity (%)	42	39
Permeability (cm/s)	5×10^{-2}	5×10^{-2}
pH (H_2O)	7.54	7.65
Net neutralization potential (kg $CaCO_3$/ton)	53	+215

Table 2
Toxic elements in the horizon A of the soil before and after the bioremediation under laboratory conditions

Parameters	U	Ra	Cu	Cd	Pb
Content of toxic elements, ppm					
Before treatment	35	540	648	7	275
After treatment	9	90	262	3	114
Bioavailable fraction, ppm					
(a) By DTPA leaching					
Before treatment	2.60	90	268	0.8	0.9
After treatment	0.09	< 30	41	0.1	0.1
(b) By EDTA leaching					
Before treatment	0.10	30	97	< 0.04	1.4
After treatment	< 0.01	< 30	14	< 0.004	0.15
Inert fraction, ppm					
Before treatment	12	210	125	4.1	161
After treatment	8	80	102	2.8	97
Toxic elements solubilized during the toxicity test, ppm					
Before treatment	0.35	< 0.030	1.92	0.01	0.08
After treatment	0.05	< 0.030	0.23	< 0.004	< 0.004

Note: the contents of radium are shown in Bq/kg dry soil and of radium solubilized during the toxicity test—in Bq/l.

Table 3
Concentration of various physiological groups of microorganisms in the soil during the treatment under laboratory conditions

Microorganisms	Horizon A (0–25 cm), cells/g dry soil	Horizon B (26–80 cm), cells/g dry soil
Aerobic heterotrophic bacteria	10^7–10^8	10^5–10^6
Oligocarbophiles	10^4–10^6	10^3–10^4
Cellulose-degrading microorganisms	10^4–10^6	10^2–10^4
Nitrogen-fixing bacteria	10^3–10^5	10^2–10^3
Nitrifying bacteria	10^2–10^5	10^1–10^3
Chemolithotrophic sulphur-oxidising bacteria	10^3–10^6	10^2–10^5
Chemolithotrophic iron-oxidising bacteria	1–10^1	0–10^1
Anaerobic heterotrophic bacteria	10^3–10^5	10^4–10^7
Denitrifying bacteria	10^4–10^5	10^3–10^5
Anaerobic bacteria fermenting carbohydrates with gas production	10^3–10^5	10^3–10^6
Sulphate-reducing bacteria	10^3–10^4	10^4–10^7
Fe^{3+}-reducing bacteria	10^2–10^3	10^2–10^4
Mn^{4+}-reducing bacteria	10^1–10^2	10^2–10^3
Methanogenic bacteria	0–10^1	1–10^2
Streptomycetes	10^3–10^5	10^2–10^3
Fungi	10^3–10^6	10^2–10^4
Total cell numbers	1×10^8–5×10^8	1×10^6–3×10^7

lease solid zeolite made nutrients available to the microorganisms over a period of weeks rather than all being immediately water soluble and subject to rapid washout. This was connected with the maintenance of relatively constant concentrations of ammonium and phosphate ions in the soil solution due to the existence of a stable equilibrium between the dissolved and adsorbed forms of these ions. Furthermore, the zeolite improved the physicomechanical properties of the soil.

The temperature during the treatment was in the range of 16–20°C. However, using control lysimeters it was found that the process was efficient even at temperatures as low as 9–10°C but practically stopped at temperatures lower than 3–4°C.

Under optimum conditions, portions of the contaminants were removed from the upper soil horizons and their residual concentrations, with the exception of that of the lead, were lowered below the relevant permissible levels within 8 months of treatment. The leaching rate markedly depended on the presence of organic compounds and carbonate ions in the leach solutions. Uranium was leached efficiently in these ecosystems with a slightly alkaline pH probably by two different mechanisms [4]. The first mechanism was connected with the microbial production of peroxide compounds, which turn the tetravalent uranium to the hexavalent state. The U^{6+} is then solubilized as uranyl carbonate or as complexes with some organic compounds. The second mechanisms was connected with the microbial secretion of some organic metabolites, mainly organic acids, which form complexes with this metal.

The toxic heavy metals were solubilized mainly by means of microbially secreted organic acids. Ten different organic acids at least were identified in the soil drainage solutions. It was found that both the species diversity and the concentrations of such acids in the solutions from lysimeters containing soil samples plus bactericide and fungicide agents, i.e. without indigenous soil microflora, were much lower. Experiments carried out by the shake-flask technique using synthetic leach solutions with composition similar to that in the lysimeter tests revealed an efficient solubilization of metals from the soil samples supplied with microbial inhibitors. Iron and manganese were, however, solubilized also as a result of an enzymatic reduction of the Fe^{3+} and Mn^{4+} to the relevant bivalent forms. Control shake-flask experiments with bacteria isolated from the soil and possessing such enzymatic activity revealed an efficient solubilization of these metals from different

mineral specimens. Toxic metals were solubilized, although at low rates, even from the relevant sulphide minerals. This was due to the activity of some chemolithotrophic bacteria, mainly such related to the species *T. thioparus* and *T. neapolitanus*. These bacteria enhance the oxidation of sulphide minerals by removing the passivation films of S^0 deposited on the mineral surface as a result of different chemical, electrochemical and biological processes [1]. Some of the metals, mainly the copper, were solubilized also as complexes with the ammonia produced from the biodegradation of organic matter in these systems.

In some experiments, the dissolved contaminants were removed from the lysimeters by the pregnant effluents. The treatment of these effluents in the anoxic cell resulted in an efficient removal of the contaminants. The toxic heavy metals were precipitated mainly as the relevant insoluble sulphides by the hydrogen sulphide produced by the sulphate-reducing bacteria inhabiting the cell. Uranium was precipitated as uraninite (UO_2) and most of the radium was adsorbed on the solid organic substrates in the cell. The role played by microbial biomass, both viable and dead, in the uptake of these contaminants from aqueous solutions also has been well documented [1].

In other experiments, it was possible to immobilise the contaminants in the deeply located soil layers (mainly in the horizon B_2) as a result of the activity of the indigenous sulphate-reducing bacteria. This activity was enhanced by the water solutions of soluble organic compounds injected to this soil horizon. The precipitated contaminants were further immobilised by their sorption on the clay minerals present in the horizon B_2.

3.2. Field experiments

The permeability of the soil was high and rainwater infiltrated into and created conditions favourable for the dissolution of elements. The pore water quality was poor, with high concentrations of contaminants such as radioactive elements (uranium, radium and thorium) and some toxic heavy metals (copper, cadmium and lead).

The microflora of the soil field was very similar to that of the lysimeters used in the laboratory experiments (Table 4). The activity of this microflora was enhanced by adjusting and maintaining the es-

Table 4
Concentration of various physiological groups of microorganisms in the soil during the treatment under field conditions

Microorganisms	Horizon A (0–25 cm), cells/g dry soil	Horizon B (26–80 cm), cells/g dry soil
Aerobic heterotrophic bacteria	10^6–10^8	10^4–10^6
Oligocarbophiles	10^3–10^7	10^2–10^5
Cellulose-degrading microorganisms	10^3–10^6	10^2–10^4
Nitrogen-fixing bacteria	10^3–10^5	10^2–10^4
Nitrifying bacteria	10^2–10^5	10^1–10^3
Chemolithotrophic sulphur-oxidising bacteria	10^3–10^7	10^2–10^5
Chemolithotrophic iron-oxidising bacteria	1–10^1	0–10^1
Anaerobic heterotrophic bacteria	10^3–10^6	10^3–10^7
Denitrifying bacteria	10^3–10^5	10^3–10^5
Anaerobic bacteria fermenting carbohydrates with gas production	10^3–10^5	10^3–10^6
Sulphate-reducing bacteria	10^3–10^5	10^4–10^7
Fe^{3+}-reducing bacteria	10^2–10^4	10^2–10^5
Mn^{4+}-reducing bacteria	10^1–10^3	10^2–10^4
Methanogenic bacteria	0–10^2	1–10^5
Streptomycetes	10^3–10^6	10^2–10^4
Fungi	10^3–10^6	10^2–10^4
Total cell numbers	1×10^8–6×10^8	1×10^6–5×10^7

sential environmental factors such as water, oxygen and nutrient contents of the soil at the optimum levels established during the laboratory experiments. This was achieved by the regular ploughing up and irrigation of the soil and by the addition of zeolite saturated with ammonium phosphate.

The treatment of the soil was started in March 1998 and at the end of October 1998 it was found that considerable portions of the contaminants were removed from the upper soil horizons and their residual concentrations, with the exception of those of the radium and lead, were lowered below the relevant permissible levels (Table 5).

The temperature of the soil also was an essential factor affecting the microbial growth and activity. The highest rates of contaminant solubilization were achieved during the summer months (June–August 1998) when the temperature inside the soil profiles was in the range of 19–35°C. However, the process was efficient even at temperatures as low as 10°C (in March 1998) when the well exposed and the most easily soluble forms of the above-mentioned toxic elements were solubilized.

Considerable portions of the contaminants removed from the upper soil horizons were transferred into the deeply located soil horizons (mainly to the horizon B_2) where they were immobilized as a result of the activity of the anaerobic sulphate-reducing bacteria inhabiting this soil horizon (Table 5). This activity was enhanced by the water solutions of soluble organic compounds and ammonium phosphate injected to the horizon B_2. Uranium was precipitated as uraninite and most of the radium was adsorbed on the clay minerals present in the horizon B_2. The toxic heavy metals were precipitated mainly as the relevant insoluble sulphides.

Table 5
Content of pollutants in the soil before and after the treatment under field conditions

Parameters	U	Ra	Cu	Cd	Pb
Content of pollutants in the soil before treatment					
Horizon A	35	540	648	7.0	275
Horizon B	17	280	230	3.0	190
Content of pollutants in the soil after treatment					
Horizon A	9	90	240	3.0	95
Horizon B	27	460	378	4.6	247
Removal of pollutants from the whole soil profile (horizon A + horizon B), %	6.2	4.6	7.1	4.1	8.0
Permissible levels for soils with pH > 7.0	10	65	280	3.0	80

Note: the content of Ra is shown in Bq/kg dry soil; the content of all other pollutants is shown in mg/kg dry soil.

The concentrations of pollutants in the soil effluents were low, and in most cases, below the relevant permissible concentrations for waters used in agriculture and/or industry. These effluents were efficiently treated by the passive system located near the experimental plot.

The monitoring of the control plot without ploughing, irrigation and addition of ammonium phosphate revealed that the content of contaminants in the soil was only slightly decreased after the end of the 8-month period.

4. Conclusions

The results obtained during this study revealed that the above-mentioned bioremediation in situ method can be very efficient under real field conditions for treatment of soils contaminated with radioactive elements and toxic heavy metals. It must be noted that the field application of this method is connected with a detailed characterization of the subsurface geologic and hydrogeologic conditions of the relevant site and with the construction of an effective collection system to prevent soil effluent migration and pollution of surface and ground waters. Furthermore, some conventional remediation procedures such as grassing of the treated soil, addition of certain fertilisers and animal manure as well as with periodical ploughing up, liming (in the case of acidic soils) and irrigation are needed to restore completely the physical, water and biological properties of the soil.

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