ECOTOXICOLOGICAL CHARACTERISTIC OF A SOIL POLLUTED BY HEAVY METALS AND URANIUM BEFORE AND AFTER ITS BIOREMEDIATION

Plamen Georgiev¹, Stoyan Groudev²

¹University of Mining and Geology "St. Ivan Rilski", Sofia 1700; ps_georgiev@mgu.bg ²University of Mining and Geology "St. Ivan Rilski", Sofia 1700; groudev@mgu.bg

ABSTRACT. A sample of an alkaline soil heavily polluted with radionuclides (uranium and radium) and heavy metals (mainly copper, zinc, lead) was subjected to bioremediation under large-scale laboratory conditions. The treatment was connected with creation of conditions enhancing the solubilization of pollutants located in the soil horizon A to the soil solution. The leaching was dependent on the activity of the indigenous microflora and to chemical processes connected with solubilization of pollutants and formation of stable complexes with some organic compounds, chloride and hydrocarbonate ions. These processes were considerably enhanced by adding hay to the horizon A and irrigating the soil with water solutions containing the above-mentioned ions and some nutrients. The dissolved pollutants in the soil solutions were in concentrations highly toxic towards *Daphnia magna*. After 1.6-year treatment period, the total concentration and the bioavailable forms of above-mentioned pollutants in horizon A were considerably decreased which altered their toxicity towards tested species - oats (*Avena sativa*) and earthworms (*Lumbricus terrestris*).

ЕКОТОКСИЧНА ХАРАКТЕРИСТИКА НА ПОЧВА ЗАМЪРСЕНА С ТЕЖКИ МЕТАЛИ И УРАН ПРЕДИ И СЛЕД ТРЕТИРАНЕ Пламен Георгиев¹, Стоян Грудев²

¹ Минно-геоложки университет "Св. Иван Рилски", 1700 София; ps_georgiev@mgu.bg ² Минно-геоложки университет "Св. Иван Рилски", 1700 София; groudev@mgu.bg

РЕЗЮМЕ. Проба от алкална почва, тежко замърсена с радионуклеиди (уран и радий) и тежки метали (главно мед, цинк и олово) беше подложена на третиране в голям мащаб. Третирането беше свързано със създаване на условия, позволяващи разтварянето на замърсителите от почвен хоризонт А в почвения разтвор. Извличането беше зависимо от активността на местната микрофлора и от химични процеси свързани с разтварянето на замърсителите и образуване на стабилни комплекси с някои органични съединения, хлоридни и бикарбонатни йони. Процесите бяха значително ускорени чрез прибавянето на сено към хоризонт А и оросяване на почвата с разтвор, съдържащ гореспоменатите йони и някои макроелементи. Разтворените замърсители в почвения разтвор бяха в концентрации силно токсични към *Daphnia magna*. След 1.6 години период на третиране, общата концентрация и биоусвоимите форми на установените замърсители в хоризонт А беше намалена значително, което промени токсичността на пробата към тестираните видове – овес (*Avena sativa*).

Introduction

Some agricultural lands located in the Vromos Bay area, near the Black Sea coast, Southeastern Bulgaria have been contaminated with radioactive elements (uranium and radium) and heavy metals (copper, zinc, lead) as a result of copper mining and mineral processing applying for decades in the area. The main path for soil contamination was transportation of mineral particles from the drought surface of flotation tailing and their deposition on the surface of the soils situated to the prevalent wind direction. As a result of regular ploughing these fine particles enriched in heavy metals and radionuclides were almost equally distributed in the most fertile soil zone - topsoil. This resulted in increased bioavailability which enhanced accumulation of the pollutants in the plant biomass and the appearance of a toxic effect. The toxicity of each pollutant towards soil organisms depends on the species sensitivity, soil pH, the organic content, soils' exchange capacity, etc. However, the species diversity in contaminated soils is lower than their non-contaminated counterparts. The soil fertility is decreased both as a result of the poorer species variety and/or lower activity. These lead to slower rates of the main processes in the biogeochemical cycles of nitrogen, carbon and phosphorous. As a result, the total content of these elements in the soil is tremendously decreased or they are presented in non-available forms mainly (Alloway, 1995).

A lot of treatment methods could be used for soil remediation in dependence on the pollutant type, its concentration, structure and properties of the soil horizon, available funds. Among the tested methods the most promising are in situ treatment methods. Their application is not connected with excavation of the soil horizons, their transportation or using of special set-ups in the treatment process. Because of this and relying on the natural biogeochemical processes, their application in real-scale conditions is possible.

Materials and methods

Soil characteristic and treatment

The soil sample used in the experiments belonged to the cinnamon soil type. The soil profile was consisted from the sharply distinguished soil horizons – horizon A (0-30 cm), horizon B (31-70 cm), horizon C (71-90 cm), a clay horizon (91-110 cm). The pH (H₂O) of horizon A was 7.82, the humus content was 3.80 %, and cation exchange capacity (CEC) was 42 meq/ 100 g soil, respectively. The filtration coefficient of the soil profile (0-90 cm) was 5.8 cm/s.

The soil sample was treated in zero suction type lysimeter in which a permanent water layer was maintained on it bottom to prevent the penetration of oxygen from the air through the outflow point for the drainage solutions. The soil treatment was carried out in a lysimeter which was charged with 70 kg of soil keeping the natural soil genesis. A sand layer was located beneath the soil profile. Plant biomass (as a finely cut hay) was added to and mixed with the horizon A to a final content of 4%. The hay consisted of 36% cellulose, 24% hemicellulose, 18% lignin and 6.1% ash. During the treatment the soil was irrigated with solutions containing 0.10 g/l NH₄Cl, 0.02 g/l K₂HPO₄ and 0.05 N NaHCO3 during the treatment. The irrigation rate was 50 l/t soil per week. Each week the pregnant effluents from the lysimeter were replaced by fresh solutions with the relevant initial composition. The leaching was carried out at temperatures varying in the range of about 15-23° C for a period of 18 months.

A nutrient solution containing equimolar concentration of acetic and lactic acid (total organic carbon of 200-220 mg/l), preliminary neutralized to pH 6.1-6.3, was injected weekly at a depth 75 cm during the soil treatment.

Chemical analyses

The transportation of the dissolved pollutants from a horizon to a horizon was monitored regularly by means of drainage solution sampling. The collected solutions were characterized by measurement of pH, Eh, alkalinity, dissolved organic carbon (APHA, 1995). The concentrations of heavy metals and uranium were determined after the preliminary digestion of dissolved organic compounds by means of 705 UV Digester (Metrohm). The heavy metals were analyzed by means of ICP spectrophotometry. Uranium concentration was measured photometrically using the Arsenazo III reagent (Savvin, 1961).

The isolation, identification and enumeration of soil microorganisms were carried out by methods described previously (Groudeva et al., 1993).

Speciation of the dissolved forms of the pollutants during the soil treatment was determined by means of Figura and McDuffie's method (1980).

The distribution of pollutants among the main mobility fractions were determined by means of a sequential extraction procedure (Tessier et al., 1979) and bioavailability test (Lindsay, Norvell, 1978).

The carbonate content was determined by measuring the volume of hydrochloric acid consumed in the neutralization process. The net neutralization potential was determined by a static acid-base accounting test (Sobek et al., 1978). Elemental analysis of the soil sample was performed by digestion and measurement of the ion concentration in solution by atomic absorption spectrometry and induced plasma spectrometry. The specific activity of Ra-226 was measured by means of a gamma-spectrometer (ORTEC-USA).

Ecotoxicity analyses

The toxicity of the soil solutions, which have drained from horizon A during the treatment, was tested towards *Daphnia magna*. This test was performed with fresh soil solution taken not later than 4 hours after the sampling. The toxicity was determined by placing 10 individuals (aged less than 24 hours) in solutions containing different proportions of the tested solution and basic salt solution used for their cultivation (EPA, 2002). In the blank samples individuals were placed in basic salt solution only. Each variant were tested by means of three repetitions. The test was carried out at temperatures 18–20° C and photoperiod of 16 hours. The total duration of the test was 48 hours. The mortality of the tested variants was monitored at 24 and 48 hour.

The experimentally data from all repetitions of each toxicant concentration were statistically assessed by means of determination of average values and standard deviation. The main ecotoxicity parameters-No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), LC₅₀ and LC₁₀₀ were determined by processing of experimental data by means of Shapiro Wilk's test and the Probit method, respectively (*EPA*, 2002).

The toxicity of the upper soil layer to oats (Avena sativa) was in accordance to well-known methods (OECD, 1984). The test was carried in pots which were filled with mixtures produced by the mixing of the sample and composted biomass in different proportions. Each pot was planted with 10 seeds. The blank samples consisted of composted biomass. Each variant was tested by means of three repetitions. The test was carried out in a greenhouse at temperatures 16–22° C, photoperiod of 16 hours and the humidity was maintained by means of distilled water. The duration of the test was 30 days and during this period the rate of growth, signs of chlorosis,and mortality were measured. At the end of test, the lengths of root and stem, as well as mortality were determined.

The toxicity of upper soil layer to earthworm (*Lumbricus terrestris*) was carried out with worms produced from synchronized population preliminary cultivated for 1 year at temperature 18-20 °C, at relative humidity of 65-70 % and darkness. They were fed by means of dried maple leaves added to and mixed with brown forest soil. The toxicity of the soil sample was determined by range-finding and definitive tests (*EPA*, 1996).

Table 1

Data about the total content of heavy metals and radionuclides in soil horizon A before and after the treatment

Pollutant	Before After		Permissible	
	treatment	treatment	levels for soil	
			with pH>7.0	
Pb, mg/kg	272	149.7	80	
Zn, mg/kg	241	166.2	370	
Cu, mg/kg	649	551	280	
Ni, mg/kg	71	65.7	-	
Co, mg/kg	98	88	-	
U, mg/kg	34.5	11.6	10	
Ra-226, Bq/kg	400	250	65	

Table 2

Data about the main soil properties of the soil horizon A before and after the treatment

Index	Before	After	
	treatment	treatment	
pH (in H ₂ O)	7.82	10.47	
Carbonate content, %	8.42	4.85	
Content of sulphidic	3.36	2.63	
sulphur, g/kg			
Net neutralization	+ 136.8	+ 66.6	
potential, kg CaCO ₃ /t			
Humus content, %	3.80	3.0	

Table 3

Data about the microorganisms in the drainage solutions produced from soil horizon A (0-30 cm) during soil treatment

Microorganisms	Before	During
	treatment	treatment
Aerobic heterotrophic	10 ³ - 10 ⁴	10 ⁴ - 10 ⁵
bacteria		
Fungi	10 ³ - 10 ⁴	10 ³ - 10 ⁴
S ₂ O ₃ ² -oxidizing	10³ - 10⁵	10 ⁵ - 10 ⁶
chemolithotrophs (at pH 7)		
Nitrifying bacteria	10 ¹ - 10 ²	10 ² - 10 ⁴
Fe2+- oxidizing bacteria (at	10 ⁴ - 10 ⁵	10 ¹ - 10 ²
pH 7)		
Cellulose-degrading	10 ¹ - 10 ²	10 ² - 10 ³
bacteria		
Anaerobic heterotrophic	10 ³ - 10 ⁵	10 ⁴ - 10 ⁵
bacteria		
Denitrifying bacteria	10 ³ - 10 ⁴	10 ³ - 10 ⁴
Fe ³⁺ -reducing bacteria	10 ³ -10 ⁴	10 ⁴ - 10 ⁵
Sulfate-reducing bacteria	10 ² - 10 ⁴	10 ⁴ - 10 ⁵

The ecotest was carried out in 1.0 I plastic boxes which were filled with the tested sample and brown forest soil in different proportions. The blank sample consisted of brown forest soil only. Ten worms with similar length (5.5-6 cm) were added to each variant. Each variant were tested by means of three repetitions. The relative humidity during the test was maintained by means of distilled water. The total duration of the test was 30 days. At the end worm survival and marks of their activity was determined.

The experimentally data from all repetitions of each toxicant concentration to the relevant species were statistically assessed by means of determination of average values and standard deviation. The main ecotoxicity parameters-No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), LC₅₀ and LC₁₀₀ were determined by processing of experimental data by means of Shapiro Wilk's test and the Probit method, respectively (*EPA*, 1994).

Results and Discussion

The content of copper, zinc and lead in the horizon A of the treated soil was 3.4, 2.3 and 3.4 times above the relevant permissible levels, respectively. In accordance to uranium and radium-226 their concentrations were 3.4 and 6.2 times higher than the accepted standards (Table 1). The initial research revealed that these pollutants were presented mainly as carbonate, reducible and oxidisable fractions in the contaminated soil. The carbonate and reducible fractions presented ions sorbed on or entrapped in the surface of the carbonate and ferric and manganese hydroxide minerals, respectively. The pollutant's availability from the both fractions to plants in a short term period is determined by the process of complexolysis by means of secretion of organic compounds (mainly organic acids) and forming of stable organo-metallic complexes. The oxidisable fraction presented sulfide minerals of the heavy metals and tetravalent minerals of uranium. A common feature of these minerals is needed of molecular oxygen for their oxidation. The rates of these processes limited their availability and toxicity to organisms in a long term period. The quite low content of exchangeable fraction of the pollutants was due to alkaline pH which determined the negative surface charge of the soil sorbent. This resulted in active sorption of all cations presented in soil solutions. As a result of aging process, the sorbed cations were capsulated in the crystal lattice of the secondary minerals presented in the soils. The content of the inert fraction of the heavy metals was in the range 31-39%. It presented finely disseminated sulfide minerals among the minerals of aluminum and silicon.

The main approach applied during the soil treatment was enhanced solubilization of soil pollutants from upper horizons and their transportation into soil depth by means of the drainage soil solutions. A classical method applied in hydrometallurgy, for recovery of uranium from raw materials as well as in remediation of uranium contaminated soils and sediments with positive neutralization potential and high content of carbonate minerals, is alkaline leaching (Suzuku, Suko, 2006). For this reason, the concentration of hydrocarbonate ions was maintained constant during the soil treatment. However, at alkaline pH the surface charge of the soil sorbent was increased because of almost completely deprotonation of its reactive groups. As a result of this, the mobility of all pollutants (including heavy metals) presented as cations in soil solutions will be steadily decreased because of their attraction and sorption on the sorbent surface. For this reason, cut hay enriched in easily degradable biopolymers (cellulose and hemicellulose mainly) was added to and mixed with soil from upper soil horizon. These polymers were degraded by means of chemical and biological mechanisms and the soil solution was enriched with sugar acids which formed stable organo-metallic complexes with the heavy metals. For example, It is well known that at alkaline pH cellulose and hemicellulose produced sugar acids as a result of chemical hydrolysis and peeling off reactions (Fisher, Bipp, 2002). This process had to some extent a protective role towards soil humus compounds (Table 4) because of it easily dissolution at alkaline pH. The sugar acids and dissolved humic acids formed stable complexes with heavy metals as a result of the preliminary process of deprotonation of their hydroxilic or phenolic groups. These processes enhanced the formation of bidentate chelate complexes which were more stable and mobile through the soil profile (Sauve et al., 2000). By this way, it was possible to decrease the sorption of heavy metals and to improve their leachability considerably (Table 4).

Table 4

Data about the properties and content of pollutants in the drainage solutions produced from soil horizon A during the soil treatment

Index	Before During	
	treatment	treatment
pН	7.51-7.73	8.82 – 9.66
Eh, mV	(+85)-(+110)	(+41)– (+72)
Alkalinity, mmol/l	9.5-14.0	24.0 - 30.5
Dissolved organic carbon, mg/l	18.6-25.4	92.7 – 148.4
Pb, mg/l	< 0.01	0.10 – 3.66
Zn, mg/l	0.07-0.12	0.67 – 3.5
Cu, mg/l	< 0.01	0.29 – 3.17
Ni, mg/l	< 0.01	0.04 – 0.37
Fe, mg/l	0.1-0.22	0.52 – 7.81
Mn, mg/l	0.3-0.5	0.08 – 5.2
SO4 ²⁻ , mg/l	145-210	2140 – 2420
Co, mg/l	0.03-0.05	0.11 – 0.61
U, mg/l	0.08-0.22	0.24 – 1.18
Ca, mg/l	42-56	360 - 650

Table 5

Data about the toxicity¹ of the drainage soil solutions towards Daphnia magna

Index	Before	During the	
	treatment	treatment	
NOEC	4.0	0.25	
LOEC	5.0	0.5	
LC ₅₀	20.0	3.0	
LC ₁₀₀	27.5	4.5	
Toxicity units	5.0	33.3	

¹ Expressed as volume of solution during the test at which the relevant characteristic was determined

The soil irrigation with solution enriched in ammonia and phosphate ions allowed the maintenance of higher microbial activity. The microbial population of some groups' microorganisms even increased in comparison with the nontreated soil. For example, microbial cells of nitrifying and S₂O₃²-oxidizing chemolithotrophs (at pH 7) was increased because of the presence of suitable electron donors assimilated by them (Table 1). The S2O32-oxidizing chemolithotrophs related to the species Thiobacillus thioparus, T. denitrificans oxidized the polysulfide forms of sulfur produced as a result of chemical oxidation of sulphide sulfur and by this way the passivation layer on the mineral surface was removed. Higher concentration of hay maintained during the treatment allowed to proliferate all group interdependent aerobic and anaerobic heterotrophic microorganisms (Table 3). The intensity of these processes was strongly dependent on

the permanent supply of organic acids from the added biomass. For this reason, finely cut hay was added to and mixed within the soil horizon A monthly to maintain it concentration at about 3-4% during the treatment.

Because of the same charge, the formed organo-metallic complexes were repulsed from the negative soil surface to the soil solution (Voegelin et al., 2003).

As a result, a relatively constant concentration of dissolved heavy metals (3.4-6.8 mg/l) in the drainage solutions of horizon A was measured and the migration of these pollutants through the soil profile was considerably enhanced. The permanent supply of bicarbonate ions enhanced the chemical oxidation of tetravalent uranium to the hexavalent state by the molecular oxygen. The uranyl ion formed stable and easily dissolved uranyl-carbonate complexes as well as uranyl-humate complexes. Their sorption to the soil surface was also negligible because of their negative charge which determined the higher concentration of uranium in the drainage solutions.

The concentrations of the dissolved pollutants, the alkaline pH and some higher molecular organic acids produced from the lignin leaching determined the determined value of LC_{50} and LC_{100} . In comparison to the value of non-treated soil the toxicity was of the drainage solutions was increased (Table 5). However, the higher alkalinity, hardness and presence of dissolved organic carbon to some extent detoxify the dissolved pollutants towards *Dapnhia magna* by means of decreasing of their biosorption (De Schamphelaere, Janssen, 2004). For example, all ecotoxicological parameters were determined at higher concentrations of copper and lead than the values determined when the same pollutants were presented as free ions.

At a depth of horizon C (~75 cm) were injected solutions enriched in lactic and acetic acids (preliminary neutralized to pH 6.4-6.7) by piezometer. These organic compounds were used as electron donors by sulfate-reducing bacteria and iron reducing bacteria for their growth. By this way, the non-ferrous metals and uranium which have been dissolved from above lying soil horizons were precipitated as relevant sulphide minerals and uraninite or entrapped in the crystal lattice of magnetite.

As a result of the soil treatment for a period of 18 months the total content of lead, zinc, copper and uranium were decreased by 55.3, 31.0, 15.1 and 66.3%, respectively (Table 3). The concentration of Ra-226 was decreased by 37.5% to the range of 250 Bg/kg soil. In comparison to the relevant permissible levels, the residual concentrations of lead and copper were 1.9 and 2.0 times higher. The residual concentration of uranium was on the brink of the relevant permissible level of 10 mg/kg. The residual activity of radium-226 was still 3.8 times higher than the accepted norm. The content of the exchangeable fraction of lead and copper were increased in comparison with the soil sample before treatment due to the additional sorbent surface that were formed as result of chemical hydrolyses of the added hay. As a result of this, the exchange capacity of the soil sample was increased as a whole (Tiemann et al., 1999). On the other hand, the content of carbonate fraction of the above-mentioned elements were considerably decreased which determined their lower bioavailability to the soil inhabitants. However, the toxicity of the treated soil towards the tested plant (mainly oats) was even increased (Table 7). This response was a result of a high alkaline pH of the soil at the end of treatment which arouse some difficulties with the mineral uptake and transport of some nutrients from soil solution into plant tissues. For example, it is well known the strong negative effect of the alkaline pH on the availability of iron into soil. This lead to extra accumulation of organic acids into the plant biomass, insufficient production of

Table 6

~	Mobility	Before		After	
qe	fraction	treatment		treatment	
Ч		mg/kg	%	mg/kg	%
Pb	Exchangeable	0.3	0.1	2.5	1.7
	Carbonate	1.7	0.7	14.3	9.6
	Reducible	27.6	10.1	59.5	39.7
	Oxidisable	156.3	57.5	47.7	31.9
	Inert	86.1	31.6	25.7	17.1
Total	content	272	100	149.7	100
Bioav	ailable fraction	28.7	10.5	22.5	15.0
deter	mined by DTPA				
test					
Cu	Exchangeable	1.0	0.2	6.4	1.1
	Carbonate	65.6	10.1	34.0	6.1
	Reducible	16.0	2.5	13.6	2.5
	Oxidisable	312.0	48.0	259	46.8
	Inert	254.4	39.2	240	43.5
Total	content	649.0	100	551	100
Bioav	ailable fraction	22.7	3.5	14.5	2.6
determined by DTPA					
test					
U	Exchangeable	1.7	4.9	0.2	1.7
	Carbonate	13.8	40.0	0.6	5.2
	Reducible	7.9	22.9	4.0	34.5
	Oxidisable	4.7	13.6	1.9	16.4
	Inert	6.4	18.6	4.9	42.2
Total content		34.5	100	100	11.6
Bioavailable fraction		17.8	51.6	1.3	11.2
deter	mined by DTPA				
test					

Data about the pollutants` fractions and mobility in soil horizon A before and after treatment

Table 7

Data about the toxicity¹ of soil horizon A to different test organisms before and after treatment

Test organism	Before	After	
_	treatment	treatment	
Oats (Avena sativa)			
NOEC	35	30	
LOEC	40	40	
LC ₂₅	55	50	
Earthworms (Lumbricus terrestris)			
NOEC	25	50	
LOEC	30	60	
LC ₅₀	55	70	
Toxicity unit	1.8	1.4	
Efficiency of treatment, %	-	22.2	

 $^{\rm 1}$ Expressed as the weight sample at which the relevant characteristic was determined

chlorophyll, and disturbed pH balance of the plant tissue (Yang et al., 1994; McBride, 2001). The visible sign of these processes is chlorosis which was observed during the test with oats. The earthworms valued the toxicity of the treated soil at 1.4 units which meant 22 % reduction in comparison with the toxicity of the non-treated soil. However, the chronic toxicity of the treated soil was still higher because of negative effect of alkaline pH on the earthworms' activity.

These results showed the need to include the additional stage in the soil treatment. At this stage, the soil pH must decrease to about neutral point by means of an introduction of sources of hydrogen ions or by stimulation of suitable proton-generating process.

Conclusions

The results obtained during this study, revealed that by means of moulching of horizon A with hay, an efficient bioremediation of soil contaminated with heavy metals and uranium could be achieved. This was a result of interdependent chemical and biological processes leading to dissolution of pollutants and formation of stable complexes with organic and inorganic compounds with net negative charge. However, the higher concentration of dissolved pollutants they were presented as highly mobile complexes which were biosorbed at a lower extent. For this reason, during the soil treatment in a real scale an efficient control on the drainage solutions have to be carried out. Despite of the residual content of copper and lead in the treated soil, in comparison to the relevant permissible levels, these non-ferrous metals were presented mainly as non-available forms to the soil biota at the end of soil treatment. As a result of this, the toxicity of the treated soil sample was a considerably lesser towards tested typical soil inhabitants.

References

- Alloway, B. J. 1995. *Heavy Metals in Soils*. Chapman & Hall, London.
- APHA. 1995. Standard methods for the Examination of Water and Wastewater. American Public Health Association, Washington, D.C.
- De Schamphelaere, K. A. C, Janssen, C. R. 2004. Effects of dissolved organic carbon concentration and source, pH, and water hardness on chronic toxicity of copper to Daphnia magna. – Environ. Toxicol. Chem. 23, 1115– 1122.
- Figura, P., B. McDuffie. 1980. Determination of labilities of soluble trace metal species in aqueous environmental samples by anodic stripping voltammetry and Chelex column and batch methods. – Anal. Chem., 52, 1433-1439.
- Fisher, K., H. P. Bipp. 2002. Removal of heavy metals from soil components and soils by natural chelating agents. Part II: Soil extraction by sugar acids. - *Water, Air, and Soil Pollution.* 137, 267-286.
- Groudeva, V. I., I. A. Ivanova, S. N. Groudev, G. C. Uzunov. 1993. In: *Biohydrometallurgical Technologies, vol. II, The*

Minerals, Metals and Materials Society (Eds A. E. Torma, H. L. Apel, C. L. Brierley). Warrandale, PA, 349-356.

- Yang, X., Römheld, V., Marschner, H. 1994. Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (Oryza sativa L.). – *Plant Soil*, 164, 1–7.
- Lindsay, W. L., W. A. Norvell. 1978. Development of DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci. Soc. Am. J.*, 42, 421-428.
- McBride, M. C. 2001. Cupric ion activity in peat soil as a toxicity indicator for maize. *J. Environ. Qual.* 30, 78-84.
- OECD. 1984. Guideline for testing of chemicals. 208. Terrestrial plant, growth test. 1984.
- Phillips, E. J. P., E. R. Landa, D. R. Lovley. 2005. Remediation of uranium contaminated soils with bicarbonate extraction and microbial U(VI) reduction. – *Journal of Industrial Microbiology*, 14, 203-207.
- Sauve, S., W. Hendershot, H. E. Allen. 2000. Solid-solution partitioning of metals in contaminated soils: dependence on pH, total metal burden, and organic matter. – *Environ. Sci. Technol.*, *34*, 1125-1131.
- Savvin, S. B., 1961. Analytical use of arsenazo III: determination of thorium, zirconium, uranium and rare earth elements. *Talanta*, 6, 673-685.
- Sheppard, S. C., W. C. Evenden. 1992. Bioavailability indices for uranium: effect of concentration in eleven soils. – Arch. Environ. Contam. Toxicol., 23, 117-124.
- Sobek, A. A., Schuller, W. A., Freeman, J. R., Smith, R. M. 1978. Field and laboratory methods applicable to overburden and mine soils. US EPA Report 600/ 2–78– 054.

- Suzuku, Y., T. Suko. 2006. Geomicrobiological factors that control uranium mobility in the environment: update on recent advances in the bioremediation of uraniumcontaminated sites. – *Journal of Mineralogical and Petrological Sciences*, 101, 299-307.
- Tessier, A., Campbell P. G. C., Bisson, M. 1979. Sequential extraction procedure for speciation of particulate trace metals. – Anal. Chem., 51 (7), 844 - 851.
- Tiemann, K. J., G. Gamez, K. Dokken, J. G. Parson, M. W. Renner, L. R. Furenlid, J. L. Gardea-Torresday. 1999. Copper (II) and lead (II) binding by alfalfa biomass: use of chemical modification and X-Ray absorption spectroscopy to determine the metal binding mechansism. Proceedings of the 1999 Conference on Hazardous Waste Research.
- U.S.Environmental Protection Agency. 1994. Methods for assessing the toxicity of sediment-associated contaminants with etuarine and marine amphipods. EPA 600/R-94/025.
- U.S.Environmental Protection Agency. 1996. Ecological effects test guidelines. OPPTS 850.6200. Earthworm subchronic toxicity test. EPA 712-C-96-167.
- U.S. Environmental Protection Agency. 2002. Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. EPA-821-R-02-012.
- Voegelin, A., Barmettler K., Kretzschmar, R. 2003. Heavy metal release from contaminated soils: comparison of column leaching and batch extraction results. – *Environmental Science & Technology*, 32, 865-875, 2003.

Recommended for publication by Department of "Engineering Geoecology", Faculty of Geology and Prospecting