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BIOLOGICAL PRECIPITATION OF HEAVY METALS IN THE CLAY HORIZON OF A CINNAMON SOIL DURING ITS BIOREMEDIATION

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ABSTRACT. The mulching of an alkaline soil heavily polluted with radionuclides (uranium and radium) and heavy metals (mainly copper, zinc, lead) was effective way to it decontamination. The treatment was connected with solubilization of pollutants located in the horizon A, their transportation through the soil profile as complexes, and precipitation as insoluble compounds in the clay horizon. At this depth the pollutants were precipitated due to the enhanced activity of the indigenous microflora in which the iron and sulphate reducing bacteria were the prevalent groups. Their activity resulted in the creation of layers in the clay with characteristic mineralogical content of iron and sulfur which restrained the leaching out of precipitated heavy metals and uranium.

Keywords: soil, microflora, heavy metals, precipitation

БИОЛОГИЧНО УТАЯВАНЕ НА ТЕЖКИ МЕТАЛИ В ГЛИНЕСТИЯ ХОРИЗОНТ НА КАНЕЛЕНА ПОЧВА ПРИ НЕЙНОТО ТРЕТИРАНЕ

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РЕЗЮМЕ. Мулчирането на алкална почва, тежко замърсена с тежки метали и радиоактивни елементи (уран и торий) беше ефективен начин за нейното пречистване. Процесът беше свързан с разтварянето на замърсителите от хоризонт А, транспортирането им през почвения профил като разтворими комплекси и утаяването им като неразтворими минерали в глинестия хоризонт. На тази дълбочина, замърсителите бяха утаени в резултат повишената активност на местната микрофлора, в която желязо и сулфат редуциращите бактерии бяха основни групи. Тяхната активност водеше до създаване на слоеве в глината с характерен минерален състав на желязото и сярата, които блокираха разтварянето на утаените тежки метали и уран.

Ключови думи: почва, микрофлора, тежки метали, утаяване

Introduction

The mining and mineral processing industries are one of the main sources of soil contamination with heavy metals, toxic elements, and in some cases – radioactive elements (Lottermoser, 2007). Their accumulation in the topsoil has strong negative effect on the soil properties and its productivity. For this reason, the most European countries and the European Community as a whole introduced some permissible levels with the main aim to stop increasing the concentration of these and other pollutants in topsoil and by this way to save its fertility (*Council Directive 86/278/EEC*). At the same time, the needs to apply cheap and effective methods for soil remediation are arising rapidly (*Directive 2004/35/EC*). The main challenge to them is to clean soils polluted with different pollutants presented in different mobility forms. One of the most promising options is the biological methods relying on the activity of the indigenous soil microflora which governs the processes of pollutants dissolution, precipitation or biodegradation (Le Cloirec, Andres, 2005). A preliminary step before their application into practice is a feasibility study with main aim is to determine the most suitable conditions of the relevant soil treatment (Evans, Furloy, 2003). However, this treatment is connected with pollutants migration through the soil profile as a result of the dissolution of inorganic contaminants or the biodegradation of the organic counterparts. For this reason, at this step is obligatory to study the potential negative effect of the applied method towards the whole ecosystem as a part of the risk assessment. The results would allow the including of some additional measures by which the risk to the environment would be decreased (Sistani, Novak, 2006).

This paper presents some results about the precipitation of the leached out from the upper soil horizons non-ferrous metals and uranium in the below lying clay horizon during the treatment of polluted cinnamon forest soil.

Materials and methods

A sample of an alkaline soil was treated in zero suction type lysimeter in which a permanent water layer was maintained on its bottom to prevent the penetration of molecular oxygen from the air. Each lysimeter was charged with 70 kg of soil containing its own living indigenous microflora. The soil profile was underlined by a 5 cm sand layer, which was located on the false bottom of the lysimeter.

Finely cut dried plant biomass (hay) was added to and mixed with the soil horizon 0-30 cm to a final content of 4%. The hay consisted of 36% cellulose, 24% hemicellulose, 18% lignin and 6.1% ash. The feasibility study of the soil treatment was carried out by two different lysimeters. The soil in the first lysimeter was irrigated with solutions containing 0.10 g/l NH_4Cl and 0.02 g/l K_2HPO_4. The soil in the second lysimeter was irrigated with solutions containing the above-mentioned salts plus 0.05 N $NaHCO_3$. The duration of the soil treatment was 18 months.

The irrigation rate was 50 l/t soil per week. The pregnant effluents were replaced weekly by fresh solutions with the relevant initial composition. The leaching was carried out at the temperatures varying in the range of about 15-23°C.

Nutrient solutions containing equimolar concentration of acetic and lactic acid (total organic carbon 175-200 mg/l), preliminary neutralized to pH 6.1-6.3 and pH 5.1-5.3 were injected in lysimeter 1 and lysimeter 2, respectively. The injection of these solutions was carried out by means of a piezometer at a soil depth of 75 cm.

The migration of the dissolved pollutants from a horizon to a horizon was monitored regularly by means of gravity water solution samplers installed at the relevant soil depths. The collected soil solutions were characterized by pH, Eh, alkalinity, dissolved organic carbon, dissolved hydrogen sulfide. 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 precipitated heavy metals after this treatment were dissolved in HCl and analyzed by ICP spectrophotometry. Uranium concentration was measured photometrically using the Arsenazo III reagent (Savvin, 1961). The specific activity of Ra-226 was measured by means of a gamma-spectrometer (ORTEC-USA). The soil samples below the depth of 50 cm were kept at anaerobic conditions till the moment of the relevant analysis.

Elemental analysis of the soil samples were performed by digestion and measurement of the ion concentration in solution by atomic absorption spectrometry and induced plasma spectrometry. 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). The isolation, identification and enumeration of soil microorganisms were carried out by methods described previously (Groudeva et al., 1993). The basic forms of iron minerals were determined by well-known methods of selective extraction (La Force, Fendorf, 2000).

The mobility fractions of the soil pollutants were determined by means of a sequential extraction procedure (Tessier et al., 1979).The main forms of sulfur in the solid samples were determined by means of sequential chemical extraction method (Tuttle, Goldhaber, 1986). As a part of this analysis the content of the acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) in the clay were determined after its reaction with cold HCl (1N). The produced hydrogen sulfide was entrapped with 0.5 N NaOH and measured spectrophotometrically (Cline, 1969).The pregnant solution from this test was characterized by means of the concentration of dissolved non-ferrous metals, and iron. The sum of dissolved non-ferrous metals (copper, lead, and zinc) was noted as ΣSEM. The ratio ΣSEM/AVS was valued (Van den Hoop et al., 1997).

Results and discussion

The carried out feasibility study showed that effectively removal of the heavy metals and uranium from the soil horizon A were reached when the both processes of dissolution and complexolysis with inorganic and organic ligands have been combined (Georgiev, Groudev, 2009). These processes were greatly depended on the activity of the indigenous soil microflora and for this reason the soils in the tested lysimeters were leached with solutions enriched with assimilable forms of nitrogen and phosphorous (Georgiev, Groudev, 2008). To avoid the sorption of the dissolved cations on the pH-dependent negative charged of the soil sorbent, finely cut hay was added to and mixed with the soil of upper soil horizon in the both lysimeters.

The formation of stable dissolved complexes of heavy metals (copper and lead, mainly) with dissolved organic compounds (monosaccharides and organic acids, as well as humic acids) greatly enhanced their migration as electroneutral or negatively charged complexes through the soil profile (Voegelin et al., 2003). Apart from the non-ferrous metals, the soil was contaminated with radioactive elements (uranium and radium), too. For this reason, the leach solutions for the second soil lysimeter contained $NaHCO_3$ also. It allowed the formation of soluble uranium carbonate complexes and their effectively leaching out of the upper soil horizons.

This article presents some results about the fate of the transported soil pollutants in the clay horizon and how its acid-base properties was changed as a result of the applied one-year-and-half treatment.

As a result of the applied soil treatment the total concentration of the heavy metals and radioactive elements were greatly increased in the clay horizon (Table 1). It was due to interdependent chemical and biological processes that were initiated by the stable redox conditions established there.

Table 1

Data about the content and mobility of pollutants in the soil

Pollutant	Before treatment	After treatment	
		Lysimeter 1	Lysimeter 2
Cu, mg/kg	52.4	177	194
Pb, mg/kg	56.1	365	392
Zn, mg/kg	73.1	550	293
U, mg/kg	2.7	65.4	57.2
Ra, Bq/kg	35 ± 30 %	150 ± 30 %	150 ± 30 %

Solutions enriched with lactic and acetic acids and preliminary neutralized to pH 6.1-6.3 and 5.1-5.3 for lysimeters 1 and 2, respectively, were injected by piezometers at depth of 75 cm in both lysimeters.

The lower pH of the solutions in the second lysimeter was needed to neutralize highly alkaline pH of the solutions drained from the upper soil horizons. By this way, pH was optimal (slightly acidic to neutral) for most groups microorganisms, as well as the stability of some dissolved organo-metallic complexes had drained to the clay horizon were lowered. These factors enhanced the biodegradation of their organic ligands, and respectively, the sorption and precipitation of the released heavy metals cations. However, the bidentate chelate complexes of humic compounds with copper and lead were quite stable which allowed them to leach out of the clay horizon. It is well known that this is one of the main disadvantages of bicarbonate leaching of alkaline soils polluted with heavy metals which limit the widely application of method into the practice (Xu et al., 2005).

The permanent supply of easily assimilable organic compounds in the clay horizon by means of the injection and drained solutions from the upper soil horizons greatly increased the number and activity of the soil microflora (Table 2, 3). Due to the existed conditions at this depth, the available oxygen was consumed rapidly and the anaerobic heterotrophic bacteria were prevalent groups during the soil treatment. Amongst them, the iron and sulphate-reducing bacteria played the main role in the degradation of dissolved organic compounds and the followed pollutants precipitation. Because of the higher content of the ferric iron minerals in the clay, initially the iron-reducing bacteria were the prevalent microbial group. As a result of their activity, the ferric iron was reduced to ferrous state, which was resulted in the iron dissolution, changes in the iron mineralogy and turn from yellow-brown to grey color of some zones in the clay horizon. It is well known, that the process of bacterial iron reduction is carried out by means of direct and indirect contact between the microbial cell and the ferric iron minerals (Hansel et al., 2003). In lysimeter 2, the indirect mechanism was more important process because of the higher concentrations of dissolved humic acids in the soil solutions, which were acting as an electron shuttle between bacterial cells and ferric iron minerals (Kostka et al., 2002). Amongst them, ferric hydroxide $Fe(OH)_3$, ackagenite β-FeOOH, and lepidocrocite γ-FeOOH were the prevalent electrons` acceptor because of their higher specific surface. As a result of this, the content of iron presented in easily reducible iron oxides was decreased with 36.2% and 25.5% for lysimeters 1 and 2, respectively (Table 4). The content of iron in the secondary iron minerals (presented mainly as siderite $FeCO_3$ and vivianite $Fe_3(PO_4)_2.8H_2O$ was increased 3.3 and 2.2 times for lysimeters 1 and 2. The content of iron in the crystalline iron oxides (presented mainly as magnetite Fe_3O_4) was increased 1.2 and 1.1 times respectively for lysimeters 1 and 2, and in both lysimeters the total content of iron in the clay was decreased by 1.5%. However, the main part of the iron was capsulated in the crystal lattice of the silicates, which were microbial inaccessible (Table 4), and for that reason the bacterial iron reduction was steadily ceased after the seven month of the soil treatment. The higher concentration of sulphate ions in the pore solutions, formed as a result of the sulphides` oxidation in the horizon A, determined the microbial sulfate reduction as the key process in the anaerobic degradation of dissolved organic compounds in the clay horizon when ferric reduction waned.

Table 2

Data about the microorganisms in the lysimeter effluents during the soil treatment (3-8 months)

Microorganisms	After treatment	
	Lysimeter 1	Lysimeter 2
Aerobic heterotrophic bacteria	10^2 - 10^3	10^2 - 10^3
$S_2O_3^{2-}$-oxidizing chemolithotrophs (at pH 7)	10^6 - 10^8	10^4 - 10^5
Fe^{2+}- oxidizing bacteria (at pH 7)	10^5 - 10^6	10^5 - 10^6
Anaerobic heterotrophic bacteria	10^5 - 10^7	10^5 - 10^6
Microorganism fermenting sugars with gas production	10^4 - 10^5	10^4 - 10^5
Denitrifying bacteria	10^5 - 10^7	10^5 - 10^6
Fe^{3+}-reducing bacteria	10^5 - 10^6	10^4 - 10^5
Sulfate-reducing bacteria	10^4 - 10^5	10^3 - 10^4

Table 3

Data about the microorganisms in the lysimeter effluents during the soil treatment (9-16 months)

Microorganisms	After treatment	
	Lysimeter 1	Lysimeter 2
Aerobic heterotrophic bacteria	10^3 - 10^4	10^2 - 10^3
$S_2O_3^{2-}$-oxidizing chemolithotrophs (at pH 7)	10^7 - 10^8	10^5 - 10^6
Fe^{2+}- oxidizing bacteria (at pH 7)	10^5 - 10^6	10^4 - 10^5
Anaerobic heterotrophic bacteria	10^6 - 10^7	10^5 - 10^6
Microorganism fermenting sugars with gas production	10^4 - 10^5	10^4 - 10^5
Denitrifying bacteria	10^6 - 10^7	10^4 - 10^6
Fe^{3+}-reducing bacteria	10^3 - 10^5	10^3 - 10^4
Sulfate-reducing bacteria	10^5 - 10^6	10^4 - 10^5

Table 4

Data about the content of the main types iron minerals in the clay samples before and after treatment determined by chemical tests

Fraction	Before treatment	After treatment	
		Lysimeter 1	Lysimeter 2
Iron in secondary iron minerals, g/kg	0.4	1.3	0.9
Iron in easily reducible iron oxides, g/kg	4.7	3.0	3.5
Iron in crystalline iron oxides, g/kg	2.6	3.2	3.0
Iron capsulated in the silicate minerals, g/kg	18.7	18.6	18.6
Total iron, g/kg	26.4	26.1	26.0

Table 5
Data about the content of the main forms of the sulfur in the clay samples before and after treatment

Fraction	Before treatment	After treatment	
		Lysimeter 1	Lysimeter 2
Sulfate sulfur, g/kg	0.5	0.8	0.8
Acid volatile sulfur, g/kg	0.3	1.9	0.9
Disulfide sulfur, g/kg	1.2	10.0	6.5
Total sulfur, g/kg	2.2	13.0	9.3

Table 6
Data about the values of SEM, ∑ (SEM)/AVS and the content of the non-ferrous metals and iron in oxidisable fraction

Index	Lysimeter 1		Lysimeter 2	
	SEM, mg/kg	Oxidisable fraction, mg/kg	SEM, mg/kg	Oxidisable fraction, mg/kg
Cu	14.3	79.7	28.5	73.5
Pb	22.6	103.4	72.4	108.3
Zn	36.2	138.2	66.7	110.4
∑ (SEM)	73.1	-	182.1	-
∑ (SEM)/AVS	0.04	-	0.19	-
Fe	1550	6800	1200	5100
U	5.9	18.4	23.6	12.2

Table 7
Data about the content of pollutants in the horizon A before and after treatment

Index	Before treatment	After treatment	
		Lysimeter 1	Lysimeter 2
pH (in H_2O)	7.90	9.34	8.53
pH (in KCl)	7.20	8.48	8.15
Carbonate content, %	3.73	5.56	4.64
Content of sulphidic sulphur, g/kg	1.3	11.4	9.6
Net neutralization potential, kg $CaCO_3$/t	+ 41.1	+ 22.5	+ 18.3

The sulphate-reducing bacteria are metabolically versatile microorganisms using a wide range of organic compounds as a source of carbon and energy as well as different electron acceptors. It allowed thriving in the aquatic and terrestrial environments worldwide (Faugue, 1995). During the soil treatment the produced hydrogen sulfide was immediately consumed for the precipitation of ferrous iron and non-ferrous metals presented as cations in the pore solution. As a result of this, the concentration of the heavy metals and uranium in the solutions draining out of the clay horizon were below the relevant permissible levels for waters intended for use in industry or agriculture. Although the higher leaching of the pollutants from horizon A in lysimeter 2, the better sorption and precipitation of the non-ferrous metals and uranium in the clay horizon were measured in lysimeter 1. The formation of bidentate complexes of the dissolved heavy metals as well as the bubbles` accumulation of carbon dioxide in the soil pores, formed as a result of the chemical neutralization, were the main factors that have influenced on the pollutants behavior in lysimeter 2. It diminished their diffusion by means of the soil solution and the sorption of the dissolved pollutants on surface of the clay particles later.

Approximately nine months after the beginning of the soil treatment three distinct layers were formed in the clay horizon of the first lysimeter. The first layer was product of the ferric iron reduction and it was characterized with a pale grey color. When sulfate reduction was the prevalent microbial process of anaerobic degradation of the dissolved organic compounds (8-16 months, Table 3), the second layer (with a distinct bright black color) was formed in the middle of the first one. It was a result of the accumulation of amorphous iron sulfide minerals (such as pyrrothine FeS, greygite Fe_3S_4), and magnetite Fe_3O_4 formed there. After it, the third (post-reduction) layer was formed in which bands with grey color were distributed scatterly in the clay horizon. In the second lysimeter the first two layers were distinguishable, however their distribution were quite narrow in comparison to their counterparts in lysimeter 1. The results presented in the article about the total content of the pollutants, the mobility fractions of iron and sulfur, and some parameters of the clay horizon (Tables 1, 4, 5, 6, and 7) are referred to the second layer in the clay horizon of each lysimeter. Apart from the transformations of the iron mineralogy, the fate of the non-ferrous metals in the clay horizon was assessed by means of the ratio SEM/AVS. It characterized the extent of their precipitation, the redistribution and stability of presented mobility fractions when microbial sulfate reduction was dominant process in the clay horizon. For example, copper as a typical chalcophilic element was mainly accumulated as low leachable sulphides in both lysimeters independently of the soil pH. However, the bioavailability of this metal in lysimeter 1 was two times lower than lysimeter 2 (Table 6). On other hand, the total content of zinc and lead in the solid samples were similar but leachability of both elements from the clay sample of lysimeter 2 was considerably higher than that of sample of lysimeter 1. However, the higher content of the dissolved iron in the pregnant solution of this analysis showed that in the both lysimeter the main part of the hydrogen sulphide was consumed in reactions with the iron.

The microbial iron and sulfate reduction are both alkaline generating processes and for this reason the soil pH and carbonate content in both lysimeter was increased (Table 6). The accumulation of sulfide sulfur (presented mainly as iron sulphides) determined the lower value of the net neutralization potential of the clay horizon if the oxidation process would be initiated. Because of the soil depth and mineralogical content, the penetration of strong acids or oxidants (such as molecular oxygen) into the clay horizon is minimal which determined the possibility of leaching of the precipitated non-ferrous metals as insignificant.

Conclusion

The permanent injection of solutions enriched with acetate and lactate in the clay horizon of the treated soil enlarged the number and increased the activity of anaerobic bacteria. Amongst them, the prevalence of iron reducing bacteria was changed with the domination of sulfate reducing during the treatment. It allowed the non-ferrous metal, leached out from the upper horizons and drained to the clay horizon, to be precipitated as the relevant sulfides. However, a large amount of produced hydrogen sulfide was consumed by ferrous iron which allowed accumulation of the relevant acid volatile sulfides (AVS). Their formation determined the lower leachability of the precipitated non-ferrous metals even in the presence of strong acid or oxidant.

References

Cline, J. D. 1969. Spectrofotometric determination of hydrogen sulfide in natural waters. – *Limnology Oceanography, 14*, 454-458.

Crowe, S., J. Roberts, C. Weisner, D. Fowle. 2006. Alteration of iron rich lacustrine sediments by dissimilatory iron-reducing bacteria. – *Geobilogy*, 1-11.

Council Directive 86/278/EEC on the Protection of the Environment and in Particular of the Soil, when Sewage Sludge is Used in Agriculture, available at www: http://eur-lex.europa.eu/.

Directive 2004/ 35/ EU. European Parliament and of the Council of 21 April 2004 on Environmental Liability with regard to the Prevention and remedying of Environmental Damage. *Official Journal L143*, 30, 0056-0075.

Evans, G. M., J. C. Furloy. 2003. Contaminated land and bioremediation. – In: *Environmental Biotechnology – Theory and Application.* John Wiley & Sons, West Sussex, England, 196-223.

Faugue, G. 1995. Ecology of sulfate-reducing bacteria. – In: *Sulfate-reducing bacteria (Ed. L. Barton), series: Biotechnology Handbook, vol. 8*, Springer.

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. – *Analytical Chemistry, 52*, 1433-1439.

Francis, A. J., C. J. Dodge, J. B. Gillow. 1992. Biodegradation of metal citrate complexes and implications for toxic metal mobility. – *Nature, 356*, 140-142.

Georgiev, P., S. Groudev. 2009. Effect of the treatment method on pollutants leaching and drainage's soil toxicity during bioremediation of an alkaline soil polluted with radionuclides and heavy metals. – *Paper presented at the 13th Conference on Environment and Mineral Processing held in Ostrava, Czech Republic, June, 4-6*.

Georgiev, P., S. Groudev. 2008. Bioremediation of an alkaline soil polluted with radionuclides and heavy metals by enhancing the activity of its indigenous microflora. – *Paper presented at the Twelfth Conference on Environment and Mineral Processing, Ostrava, the Czech Republic June, 5-6*.

Groudeva, V. I., I. A. Ivanova, S. N. Groudev, G. C. Uzunov. 1993. In: A. E. Torma, H. L. Apel, C.L. Brierley (Eds.), *Biohydrometallurgical Technologies. Vol. II. The Minerals*. Metals and Materials Society, Warrandale, PA, 349-356.

Hansel, C., S. Benner, J. Neiss, A. Dohnalkova, R. Kukkadapu, S. Fendorf. 2003. Secondary mineralization pathways induced by dissimilatory iron reduction of ferrihydrite under advective flow. – *Geochemica et Cosmochimica Acta, 67*, 2977-2992.

Jang, A. J., S. W. Lee, Y. Seo, K. W. Kim, I. S. Kim, P. L. Bishop. 2007. Application of mulch for treating metals in urban runoff: batch and column test. – *Water Science and Technology, 55*, 95-103.

Kostka, J. O. Dalton, H. Skelton, S. Dollhopf, J. Stucki. Growth of iron (III)-reducing bacteria on clay minerals as the sole electron acceptor and comparison of growth yields on a variety of oxidized iron forms. – *Applied and Environmental Microbiology, 68*, 6256-6262.

La Force, M., S. Fendorf. 2000. Solid-phase characterization during common selective sequential extractions. – *Soil Science Society of American Journal, 64*, 1608-1615.

Le Cloirec, P., Y. Andres. 2005. Bioremediation of heavy metals in soils using microorganisms. – In: *Bioremediation of Aquatic and Terrestrial Ecosystem (Eds. Fingerman, M. and R. Nagabhushanam),* Science Publishers, Plymouth, UK, 97-141.

Lottermoser, B. G. 2007. *Mine Wastes. Characterization, Treatment, Environmental Impacts*. Springer–Verlag, Berlin Heidelberg, 4-11.

Savvin, S. 1961. Analytical use of Arsenazo III; determination of thorium, zirconium, uranium and rare earth elements. *Talanta, 6*, 673-685.

Sistani, K. R., J. M. Novak. 2006. Trace metals accumulation, movement, and remediation in soils receiving animal manure. – In: *Trace Elements in the Environment. Biogeochemistry, Biotechnology and Bioremediation. (Eds. M. N. V. Prasad, K. S. Saiwan, R. Naidu),* Taylor& Francis Group, Boca Raton, 689-703.

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.

Tessier, A., P. G. G. Campbell., M. Bison. 1979. Sequential extraction procedure for speciation of particulate trace metals. – *Analytical Chemistry, 51*, 844-851.

Tuttle, M. L., M. B. Goldhaber. 1986. An analytical scheme for determining forms of sulfur in oil shales and associated rocks. – *Talanta, 33*, 953-961.

Wasay, S. A., Barrington, S. F., Tokunaga, S. 1998. Remediation of soils polluted by heavy metals using salts of organic acids and chelating agents. – *Environmental Technology, 19*, 369-380.

Van den Hoop, M. A. G. T., H. A. den Hollander, H. N. Kerdijk. 1997. Spatial and seasonal variations of acid volatile sulfide (AVS) and simultaneously extracted metals (SEM) in Dutch marine and freshwater sediments. – *Chemosphere, 35*, 2307-2316.

Voegelin, A., K. Barmettler, R. Kretzschmar. 2003. Heavy metal release from contaminated soils: comparison of column leaching and batch extraction results. – *Environmental Science & Technology, 32*, 865-875.

Xu, J., J. Han, S. Sun, F. Meng, S. Dai. 2005. Leaching behavior of copper (II) in a soil column experiment. – *Bull. Environmental Contamination Toxicology, 75*, 1028-1033.

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