TREATMENT OF ACID MINE DRAINAGE BY MEANS OF A NATURAL WETLAND

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ABSTRACT. Acid mine drainage waters generated in the uranium deposit Curilo were treated by means of a natural wetland located in the deposit. The waters had a pH in the range of about 2.5–4.5 and contained radionuclides (mainly uranium and radium), heavy metals (copper, zinc, cadmium, lead, nickel, cobalt, iron, manganese), arsenic and sulphates in concentrations usually much higher than the relevant permissible levels for waters intended for use in agriculture and/or industry. The wetland was characterized by an abundant water and emergent vegetation and a diverse microflora. *Typha latifolia* and *Typha angustifolia* were the main plant species in the wetland but representatives of the genera *Juncus, Eleocharis, Potamogeton, Carex* and *Poa* as well as different algae were also present. An efficient cleanup of the polluted waters was carried out in the wetland during the different climatic seasons, even during the cold winter months at temperatures close to the freezing point. The water cleanup was due to different mechanisms but the microbial dissimilatory sulphate reduction and biosorption played the main role.

ТРЕТИРАНЕ НА КИСЕЛИ ДРЕНАЖНИ ВОДИ ПОСРЕДСТВОМ ЕСТЕСТВЕНО МОЧУРИЩЕ

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РЕЗЮМЕ. Ккисели дренажни води, генерирани в урановото находище Курило, бяха третирани посредством естествено мочурище, разположено в находището. Водите бяха с pH в границите от около 2.5-4.5 и съдържаха радионуклиди (главно уран и радий), тежки метали (мед, цинк, кадмий, олово, никел, кобалт, желязо, манган) арсен и сулфати в концентрации обикновено много по-високи от съответните допустими нива за води, предназначени за използване в селското стопанство и промишлеността. Мочурището се характеризираше с богата водна и блатна растителност и разнообразна микрофлора. *Typha latifolia* и *Typha angustifolia* бяха главните растителни видове в мочурището, но представители на родовете *Juncus, Eleocharis, Potamogeton, Carex* и *Poa,* както и различни водорасли, бяха също представени. Ефикасно пречистване на замърсените води бе осъществено в мочурището през различните климатични сезони, дори през студените зимни месеци при температури близки до точката на замръзване. Пречистването на водите се дължеше на различни механизми, но микробната дисимилативна сулфатредукция и биосорбцията играеха главната роля.

Introduction

The uranium deposit Curilo, Western Bulgaria, for a long period of time was a site of intensive mining activities including both the open-pit and underground mining techniques as well as in situ bioleaching of uranium. The ore was rich in pyrite and, apart from uranium, contained some non-ferrous metals and arsenic. The mining operations in the deposit were ended in 1990 but since that time the deposit is a permanent source of acid mine drainage waters. These waters have low pH (usually in the range of 2.5–4.5) and contain uranium, radium, several heavy metals (copper, zinc, cadmium, lead, nickel, cobalt, iron, manganese), arsenic and sulphates in concentrations usually much higher than the relevant permissible levels for water intended for use in agriculture and/ or industry.

The generation of acid mine drainage waters is connected mainly with the oxidation of pyrite, other sulphides and uranium-bearing minerals by the indigenous acidophilic chemolithotrophic bacteria (Groudev et al., 2001).

Different methods to cleanup the above-mentioned polluted waters were tested under laboratory and pilot-scale conditions. Most of these methods were connected with the application of various passive systems such as alkalizing drains, natural and constructed wetlands, permeable reactive barriers and rock filters, used separately or in different combinations (Groudev et al., 2002; 2004; 2005). Some data about treatment of such waters by means of a natural wetland are present in this paper.

Materials and Methods

The wetland was located in a ravine, which collected a portion of the acid drainage generated in the deposit. The wetland covered an area of about 500 m^2 (approximately 75 m long and 6–7 m wide). Intrusive rocks with a very low hydraulic conductivity served as a bottom of the wetland. The bottom was cover by a 0.3–0.5 m layer consisting of a mixture of soil, silt, sand and different sediments. The wetland was characterized by an abundant water and emergent vegetation and a diverse microflora. *Typha latifolia* and *Typha angustifolia* were the prevalent plant species in the wetland but species of the genera *Juncus, Eleocharis, Potamogeton, Carex* and *Poa* as well as different algae were also well present. Data about the microflora of the wetland are shown in Table 1.

The water flow rate through the wetland varied in the range of about 0.2–1.2 l/s reflecting water residence times from about 70 to 12 hours.

Table 1

Microflora of the drainage waters and the natural wetland during the warmer months

Microorganisms	Samples		
	Drainage waters before treatment	Waters from the wetland	Sediments from the wetland
	Cells/ml (g)		
Aerobic heterotrophic bacteria	10^1-10^4	10^4-10^7	10^2-10^5
Cellulose – degrading microorganisms	0-10^2	10^2-10^6	10^1-10^4
Fungi	0-10^2	10^1-10^3	0-10^2
Fe^{2+} – oxidizing chemo-lithotrophs (at pH 2)	10^4-10^7	10^1-10^3	0-10^2
$S_2O_3^{2-}$ – oxidizing chemo-lithotrophs (at pH 7)	0-10^2	10^2-0^4	0-10^2
Fe^{2+} – oxidizing heterotrophs (at pH 7)	0-10^1	10^1-10^4	0-10^2
Anaerobic heterotrophic bacteria	0-10^2	10^2-10^5	10^3-10^7
Bacteria fermenting sugars with gas production	ND	10^1-10^4	10^3-10^6
Sulphate-reducing bacteria	0-10^1	10^2-10^6	10^3-10^7
Denitrifying bacteria	0-10^1	10^2-10^4	10^2-10^5
Fe^{3+} - reducing bacteria	0-10^1	10^1-10^3	10^3-10^6
Methanogenic bacteria	ND	0-10^1	10^1-10^4

The quality of the waters was monitored at least twice per month in a period of about six years at different sampling points located in the wetland. The parameters measured in situ included: pH, Eh, dissolved oxygen, total dissolved solids and temperature. Elemental analysis was done by atomic absorption spectrometry and induced coupled plasma spectrometry in the laboratory. The radioactivity of the samples was measured, using the solid residues remaining after their evaporation, by means of a low background gamma-spectrophotometer ORTEC (HpGe-detector with a high distinguishing ability). The specific activity of Ra-226 was measured using a 10 l ionization chamber. The total β-activity was measured by a low background instrument UMF-1500 M. Mineralogical analysis was carried out by X-ray diffraction techniques. The mobility of the pollutants was determined by the sequential extraction procedure (Tessier et al., 1979).

Sediment samples from the wetland were subjected to microbial leaching using different mixed enrichment cultures of indigenous microorganisms: No 1 – mixed culture of aerobic heterotrophic microorganisms enriched in nutrient medium containing 0.2 % glucose and 0.2 % peptone as sources of carbon and energy, at pH 7.0; No 2 – mixed culture of anaerobic heterotrophic microorganisms enriched in nutrient medium containing 0.2 % lactate and 0.2 % acetate as sources of carbon and energy, at pH 7.0; No 3 – mixed culture of basophilic chemolithotrophic bacteria enriched in nutrient medium containing 1.0 % $S_2O_3^{2-}$ as energy source, at pH 7.0; No 4 – mixed culture of acidophilic chemolithotrophic bacteria enriched in nutrient medium containing 1.0 % Fe^{2+} as energy source, at pH 2.5; No 5 – natural water from the wetland not supplemented with any additives and containing only natural community of indigenous microorganisms, at pH 7.0, anaerobically. The leaching of sediments by these cultures was carried out in mechanically stirred reactors (300 rpm) containing 500 g sediment (dry weight) and 2 l leach solution each, at 20 °C for 14 days. The anaerobic leaching was carried out in nitrogen atmosphere.

The isolation, identification and enumeration of microorganisms were carried out by methods described elsewhere (Karavaiko et al., 1988; Widdel and Hansen, 1991; Widdel and Bak, 1991; Groudeva and Tzeneva, 2001).

Results and Discussion

An efficient clean up of the polluted waters was achieved by the wetland and the residual concentrations of the pollutants were decreased below the relevant permissible levels for waters intended for use in the agriculture and/or industry (Table 2). The removal of the pollutants markedly depended on the water temperature (Table 3). However, good results were achieved even during the cold winter months (December-February) at temperatures close to 0°C, although at higher residence times.

Table 2

Data about the drainage waters before and after their treatment by the natural wetland

Parameters	Before treatment	After treatment
Temperature, °C	(+0.1)-(+25.1)	(+0.1)-(+27.7)
pH	2.4- 4.15	6.8-7.5
Eh, mV	(+350)-(+572)	(+235)-(+385)
Dissolved O_2, mg/l	1.5-4.4	2.3-5.1
Total dissolved solids, mg/l	614-2822	370-1392
Solids, mg/l	28-114	23-77
Dissolved organic carbon, mg/l	0.6-4.4	17-68
Sulphates, mg/l	352-1630	181-590
Uranium, mg/l	0.32-3.41	<0.1
Radium, Bq/l	0.08-0.45	<0.05
Total β-activity, Bq/l	0.45-2.30	<0.5
Copper, mg/l	0.77-9.54	<0.5
Zinc, mg/l	1.25-23.5	0.05-1.9
Cadmium, mg/l	0.02-0.10	<0.01
Lead, mg/l	0.17-0.95	<0.1
Nickel, mg/l	0.41-2.08	<0.2
Cobalt, mg/l	0.32-1.70	<0.2
Arsenic, mg/l	0.01-0.37	<0.01
Iron, mg/l	80-914	0.2-1.9
Manganese, mg/l	1.0-35	<0.5

The removal of sulphates and of the largest portions of the heavy metals and arsenic present in the waters was due to the process of microbial dissimilatory sulphate reduction. The anaerobic sulphate-reducing bacteria were a quite numerous and diverse population, particularly in the bottom zone of the wetland, mainly in the sediments (Table 4). These bacteria were well adapted to the environmental conditions existing in the wetland and were not inhibited by the concentrations of pollutants present in the waters. The sulphate-reducing bacteria reduced the sulphate ions to hydrogen sulphide, using different organic monomers as donors of electrons for this reduction. The hydrogen sulphide precipitated the dissolved heavy metals and arsenic as the relevant insoluble sulphides. The dissolved hexavalent uranium was reduced to the tetravalent state and was precipitated mainly as the mineral uraninite (UO_2). The hydrocarbonate ions produced during the sulphate reduction, together with the chemical alkalizing agents (mainly carbonates) present in the wetland, increased the pH and stabilized it around the neutral point.

Table 3
Removal of pollutants from the drainage waters during the different climatic seasons

Pollutant	Pollutant removal, g/24h	
	During the warmer months	During the cold winter months (at 0 - 5 °C)
Uranium	62 - 242	23 - 82
Copper	91 - 451	32 - 154
Zinc	190 - 974	41 - 387
Cadmium	1.4 - 5.9	0.5 - 1.7
Lead	21 - 77	8.2 - 24
Nickel	48 - 145	21 - 53
Cobalt	41 - 122	15 - 44
Manganese	109 - 1090	51 - 334
Arsenic	14 - 41	0.5 - 18

Table 4
Sulphate-reducing bacteria in the sediments in the natural wetland

Sulphate-reducing bacteria	Cells/g dry sediments
Desulfovibrio (D. vulgaris, D. desulfuricans, D. saprovorans)	10^3 - 10^7
Desulfobulbus (D. elongatus, D. propionicus)	10^2 – 10^6
Desulfococcus (D. postgatei)	10^1 – 10^4
Desulfobacter (D. multivorans)	10^2 - 10^6
Desulfobacterium (D. autotrophicum, D. vacuolatum)	10^1 - 10^3
Desulfotomaculum (D. nigrificans, D. orientis)	0 – 10^2
Desulfosarcina (D. variabilis)	10^1 – 10^3
Desulfomonas (Non-identified species)	10^1 – 10^3

Portions of the uranium, heavy metals and arsenic as well as most of the radium were removed by their sorption by the plant biomass (Table 5) and clay minerals present in the wetland. The dead plant biomass was more efficient sorbent than the living plants. Larger contents of pollutants were also found inside the dead plant cells. Relatively small portions of the pollutants were accumulated inside the living plant, mainly in their root systems. Some algae (mainly related to the genera *Pediastrum, Eudorina, Volvox, Melosira* and *Scenedesmus)* were efficient sorbents and accumulators of pollutants. The total content of heavy metals in some specimens of these algae exceeded 10 g/kg biomass, and that of radium exceeded 500 Bq/kg. Some microorganisms (mainly such related to the genera *Aspergillus, Penicillium, Pseudomonas* and *Bacillus*) also adsorbed pollutants. Negative effects of the pollutants on the growth and activity of the indigenous plant and microbial communities were not observed.

Table 5
Content of radionuclides, heavy metals and arsenic in living and dead biomass from the natural wetland

Pollutant	Typha latifolia		Typha angustifolia	
	I	II	I	II
	mg/kg dry biomass			
Uranium	64-262	64-325	41-222	60-321
Copper	109-352	125-545	80-284	99-398
Zinc	71-370	77-710	64-302	95-515
Cadmium	12-60	21-82	10-51	15-71
Lead	23-140	28-212	24-125	35-190
Nickel	48-203	59-321	35-170	32-280
Cobalt	44-208	60-284	35-145	62-244
Manganese	109-410	99-820	91-321	107-684
Arsenic	28-82	37-125	23-77	41-109

Notes: I – Living biomass;
II – Dead biomass during the winter months.

The total content of non-ferrous metals in some clay specimens exceeded 15 g per kg dry clay, and the contents of uranium and radium exceeded 1 g and 1000 Bq per kg, respectively.

The role of the sorption mechanisms was particularly essential during the cold winter months when the growth and activity of the plant and microbial communities in the wetland were negligible or completely inhibited. In this period the pollutants were present mainly in the sediments precipitated in easily soluble (exchangeable and carbonate) mobility fractions whereas the concentration of sulphides and uraninite was much lower compared to that measured during the warmer periods.

The removal of portions of iron and manganese was connected with the prior oxidation, mainly bacterial, of the Fe^{2+} and Mn^{2+} ions to Fe^{3+} and Mn^{4+} ions. The Fe^{3+} and Mn^{4+} ions precipitated as $Fe(OH)_3$ and MnO_2, respectively.

The leaching tests revealed that the sediments from the wetland were relatively refractory to microbial leaching, especially under conditions similar to the real conditions in the wetland, i.e. prevalent anaerobic conditions in the bottom zone, with pH around the neutral point, and microbial community consisting mainly of sulphate-reducing bacteria and other metabolically interdependent microorganisms (Table 6). Only small portions of manganese and iron were solubilized as a result of enzymatic reduction of Mn^{4+} and Fe^{3+} to the relevant bivalent forms. However, the dissolved Mn^{2+} and Fe^{2+} were again turned into insoluble compounds such as MnS, $MnCO_3$ and FeS. The results from these leaching experiments confirmed the data from the chemical analyses of the wetland effluents and revealed that the solubilization of the precipitated pollutants in the natural wetland was negligible.

The data from this study revealed that acid drainage waters heavily polluted with radionuclides, heavy metals and arsenic can be efficiently treated by means of natural wetlands with a proper size and suitable plant and microbial communities and located in areas with suitable geological, hydrogeological and climatic conditions. Such water clean up can be regarded as one of the most typical examples of sustainable development of natural ecosystems.

Table 6
Microbial leaching of pollutants from sediments from the wetland

Pollutants	Microbial cultures				
	No 1	No 2	No 3	No 4	No 5
	Pollutant solubilized within 14 days, %				
U	22.4	2.5	4.6	70.7	2.3
Cu	2.3	0.7	1.9	55.8	0.9
Zn	5.0	0.9	4.1	57.2	1.2
Cd	8.6	1.9	4.8	88.2	2.8
Pb	14.0	1.0	2.3	1.9	2.5
Mn	3.2	8.2	2.1	15.4	9.5

Acknowledgements: A part of this work was financially supported by the National Fund "Scientific Research" under the project CENBIOHEALTH.

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

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