

TOXICITY OF ACID DRAINAGE WATERS TOWARDS *DAPHNIA MAGNA* BEFORE AND AFTER THEIR TREATMENT BY MEANS OF A PILOT-SCALE PASSIVE SYSTEM

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ABSTRACT. The abandoned uranium mine Curilo, situated in Western Bulgaria, about 35 km north from Sofia, is a permanent source of acid drainage waters characterized with acidic pH and concentration of heavy metals (copper, zinc, nickel), toxic elements (cadmium and arsenic), uranium as well as iron and manganese several times higher than the relevant permissible levels. In 2004, a pilot passive system consisting of an alkalizing drain, an anaerobic cell and a surface-flow wetland for chemical neutralization, microbial dissimilatory sulphate reduction and aerobic oxidation of dissolved organic monomers, respectively, was constructed and put in operation. During the next four years the water treatment was studied by means of periodical monitoring on each passive system's section. The efficiency of treatment and resulting changes in water toxicity towards *Daphnia magna* was studied in dependence on the residence time, temperature as well as the time since the start of its operation. The obtained data revealed that by means of pollutants removal the toxicity of drainage waters towards *Daphnia magna* was decreased by more than 99 %. It was determined by the processes of neutralization of acidic pH, oxidation of ferrous iron as well as ferric iron hydrolysis and copper precipitation, mainly. An efficient pollutants removal was achieved even during the winter which revealed the role of (bio)sorption processes to maintain sufficient detoxification of the treated waters towards freshwater organisms.

Keywords: acid mine drainage, multibarrier, toxicity, *Daphnia magna*

ТОКСИЧНОСТ НА КИСЕЛИ ДРЕНАЖНИ ВОДИ КЪМ *DAPHNIA MAGNA* ПРЕДИ И СЛЕД ТЯХНОТО ПРЕЧИСТВАНЕ ПОСРЕДСТВОМ ПИЛОТНА ПАСИВНА СИСТЕМА

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РЕЗЮМЕ. Изоставената уранова мина Курило, Западна България, разположена на около 35 км северно от София е постоянен източник на кисели дренажни води, характеризиращи се с кисело рН и концентрации на тежки метали (мед, цинк, никел), токсични елементи (кадмий и арсен), уран, както и желязо и манган, няколко пъти по-високи от пределно допустимите концентрации. Пилотна пасивна система, състояща се от алкализиращ дренаж, анаеробна камера и мочурище, за пречистване на дренажни води посредством процесите на химична неутрализация, микробна дисимилативна сулфатредукция и аеробно окисление на органични мономери, съответно, беше изградена и пусната в действие през 2004 година. През следващите четири години пречистването на замърсени води беше изследвано посредством прилагане на периодичен мониторинг на всеки елемент от пасивната система. Степента на пречистване и зависещата от това промяна в токсичността на водите спрямо *Daphnia magna* беше изследвана в зависимост от използваното контактно време, температурата на средата, както и продължителността на работа на бариерата. Резултатите показват че посредством задържане на разтворените замърсители, токсичността на дренажните води спрямо *Daphnia magna* беше намалена с повече от 99 %. Това се определяше най-вече от процесите на неутрализация на киселинността, окисление на феро йоните, както и хидролизата на феро йоните и утаяване на медта. Ефикасно задържане на замърсителите беше постигнато дори през зимата, което доказваше ролята на процесите на биосорбция за поддържане на задоволителна детоксикация на пречистваните води спрямо сладководните организми.

Ключови думи: кисели дренажни води, мултибариера, токсичност, *Daphnia magna*

Introduction

Acid mine drainage is one of the most complex local environmental problem arising due to the human activity. The ore body fractures as well as tailing dumps formation strongly enhances water filtration and the oxygen diffusion into depth which stimulate the activity of the indigenous acidophilic chemolithotrophic bacteria. As a result of the bacterial oxidation of sulfide minerals and some secondary chemical/biological process acid mine drainage (AMD) is formed. These waters are characterized with acidic pH (usually in the range 1.9-3.7) and concentration of dissolved heavy metals, toxic and radioactive elements as well as iron, manganese and sulphate ions several times higher than the permissible levels for waters

intended to use in industry of agriculture. When main mining and mineral processing activity still operates, some kind of control on AMD movement and its content is usually applied. The exhaustion of raw materials and mines abandonment arouse devastating effect on the local situated aquatic and terrestrial ecosystems due to the freely mixing of AMD with creek/ river waters or its infiltration through soil profiles. As a result of this, approximately 99 % of the species inhabiting the affected ecosystem are dying and are replaced by microorganisms and few invertebrate species that have adaptation to live in an environment with acidic pH and toxic concentrations of heavy metals and toxic elements (Dsa et al., 2008).

One of the main tasks to the environmental biotechnology beside remediation of the affected ecosystems is to elaborate cheap and efficient methods to control formation and/or clean up of already formed AMD (Coldewey and Klinger, 2000). Amongst the latter, the most promising are so called passive systems which are based on natural biological and chemical processes to precipitate dissolved toxic elements and to consume water acidity. Most of the developed methods are tested on bench-scale as well as pilot-scale and received results are promising for technology transfer in industry (Groudev et al., 2008a).

This article presents some results revealing the effect of temperature, residence time of AMD treatment in the passive system, and the time since the start of passive system operation on the pollutants removal as well as the toxicity of effluents to water flea (*Daphnia magna*).

Materials and methods

The passive system was constructed in a ravine collecting a portion of AMD generated in the abandoned uranium mine Curilo situated 35 km north from Sofia. The passive system was consisted of an alkalizing drain, an anoxic section for microbial dissimilatory sulphate reduction (MSDR) and a surface-flow wetland. The first two sections were dug into ravine ground and their bottom and walls was isolated with impermeable plastic material. The alkalizing drain was filled with a mixture of crushed limestone and gravel pieces. Its total volume was 2.5 m³. The section for MSDR had a volume of about 20.4 m³ and was filled with a mixture of biodegradable solid organic substrates (cow manure, plant compost, straw), crushed limestone as well as zeolite saturated with ammonium phosphate. This section of the multibarrier was inhabited by a microbial community consisting mainly of sulphate-reducing bacteria and other metabolically interdependent microorganisms. The surface flow wetland had a total volume of about 5.2 m³ and was filled with grey forest soil, plant compost, plant debris as well as zeolite saturated with ammonium phosphate. The wetland was planted with narrow leaf cattail (*T. angustifolia*) as a dominant species and jointed rush (*Juncus articulatus*), bulrush (*Scirpus spp.*), sedge (*Carex spp.*) and pondweed (*Potamogeton spp.*) as sub-dominant species.

Since the summer of 2004, when the passive system was put in operation, a portion of AMD generated in the ore body is being treated permanently. The quality of the treated waters was monitored regularly in different sampling points located at the inlet and the outlet of each section of the passive system. The parameters measured *in situ* were temperature, pH, Eh, total dissolved solids and dissolved oxygen. *On site* were measured alkalinity, acidity as well as dissolved hydrogen sulfide (Cline, 1969). Water samples for elemental analysis were conserved by means of 2N HCl addition and stored in refrigerator (at 4 °C) till the moment of analysis. It was done by means of atomic adsorption spectrophotometry (AAS) and induced coupled plasma (ICP). The uranium concentrations were determined after the preliminary digestion of dissolved organic compounds by means of 705 UV Digester (Metrohm) followed by photometric analysis with the Arsenazo III as complexing reagent (Savvin, 1961). The concentration of dissolved organic carbon (DOC) was determined after their preliminary chemical oxidation to carbon dioxide at high

temperature and its absorption in NaOH solution, followed by acid base titration with phenolphthalein as indicator (Lurie, 1973). The concentration of sulfate ions was determined by means of BaCl₂ addition followed by the turbidity measurement at $\lambda = 420$ nm.

The toxicity of AMD and effluents from each section of the passive system was studied to water flea (*Daphnia magna*) in laboratory. The water sample taken from the relevant point was collected in 2.0 l plastic bottles and was stored in dark and at temperature of 4° C. The toxicity testing was started no longer than 24 h towards the sampling time and it was initiated by preparing a set of dilutions between the tested water sample and the basic salt solution used for Daphnids cultivation (EPA, 2002). Ten individuals of water flea no older than 24 h were placed in each dilution as well as in the basic salt solution for Daphnids cultivation that was used as a blank sample. The toxicity of each prepared dilution as well as the organism activity in the blank sample was tested in three repetitions. The toxicity test was carried out at temperatures 18–20° C and 16 h duration of photoperiod. The total duration of the test was 48 hours. The mortality of the Daphnids was accounted at 24 and 48 hour, respectively. The main ecotoxicity parameters of each water sample towards water flea (*Daphnia magna*) — No Observed Effect Concentration (NOEC), the Lowest Observed Effect Concentration (LOEC), LC₅₀ and LC₁₀₀, were determined by the experimental data processing with Shapiro Wilk's test and the Probit method, respectively (EPA 821-B-01-004). The repetitions results for each dilution were used to calculate the average value and the standard deviation to the relevant ecotoxicity parameters. The toxicity results were regarded as acceptable only if the Daphnids mortality in the blank samples were less than 10%.

On the base of LC₅₀ value for the relevant sample, an Acute Toxicity Unit-ATU (1) as well as efficiency of toxicity removal (2) was calculated as follows:

$$ATU = 100/LC_{50} (v/v) \quad (1)$$

$$R = (ATU_i - ATU_e) / ATU_i * 100 \quad (2)$$

where:

ATU_i = toxicity of inlet solutions;

ATU_e = toxicity of outlet solutions;

R = efficiency of toxicity removal for the relevant section of the passive system.

Results and discussion

The toxicity of acid mine drainage (AMD), generated in the abandoned uranium mine Curilo, towards *Daphnia magna* was determined by the concentrations of dissolved copper and iron as the most toxic amongst the presented pollutants as well as the water acidity (De Schampelaere et al., 2002). However, each of these pollutants has a unique mechanism of effect on the test organism. It is well-known, that water acidity as well as the total dissolved solids determined the efficiency of gas exchange in the respiration of aquatic organisms (Alibone and Fair, 1981) which is also strongly dependent on the equilibrium concentrations of dissolved CO₂ and O₂. The respiration of crustacean is strongly impeded in the acidic water due to the very low concentration of dissolved gases which practically

turn the waters as anoxic to that group organism. In addition to this, the higher water acidity greatly enhanced heavy metals and toxic elements solubility which are presented mainly as free cations and/or hydroxy-complexes. Because of their higher concentrations, they passed freely through membranes, replace the essential cations (K, Na, Ca, Mg, etc.) from the organic macromolecules' exchange positions and strong covalent bonds between them are form. As a result of this, the structure and functions of the affected macromolecules are greatly changed which lead to the organism's death. The higher concentration of dissolved iron beside of its negative effect on sub-cell level, it has a negative effect on Daphnids feeding, respiration as well as their movement due to the formation of gelatinous ferric iron precipitates (Randall et al., 1999). The synergetic effect of these three pollutants determined the acute toxicity of the AMD, generated in the abandoned uranium mine Curilo, towards *Daphnia magna* to vary in the range of 2000 – 2500 acute toxicity units (ATU) for the studied period (Table 1, 2).

The efficient removal of dissolved heavy metals and acidity was received due to the applied treatment of a portion of AMD by means of the pilot-scale passive system (Table 1, 2). It was a result of action of different chemical as well as biological processes in different sections which enhanced the selective removal of the relevant class pollutants. For example, the pieces of limestone in the alkalizing drain consumed the water acidity by means of chemical neutralization. During the first year and half of the passive system operation, by this process was consumed 87 – 90 % of the acidity when the drainage waters being treated at 75 h residence time (Table 1). As a result of this, the precipitation of ferric and copper ions was greatly enhanced and their concentrations in the alkalizing drain's effluents were decreased with 58 – 74 and 84 – 87 %, respectively, in dependence of the local temperature. The changes in the content and properties of waters being treated have a strong effect on the water toxicity to *D. magna*. For the studied period (17-22 months) the water toxicity was decreased to 300 – 375 ATU, respectively, which determined 90 – 92 % efficiency of the toxicity removal. The better results were received at 10 – 15°C than 2 – 5°C which was determined mainly by the higher rate of chemical oxidation of ferrous iron by means of the dissolved oxygen. Due to the alkaline drain's operation for longer period of time, the surface of limestone was armored by the ferric iron and aluminum hydroxides as well as some hydroxides of some heavy metals (copper and zinc, mainly). As a result of this, the efficiency of chemical neutralization and pollutants precipitation was lower for the second studied period (26-27 month since the start of the passive system operation). For example, the water acidity was decreased by 74 and 81 % at residence time 50 and 75 h, respectively. In spite of the lower concentration of dissolved iron for that period, the efficiency of its removal was 36 and 52 %, respectively (Table 2). As a result, the toxicity of alkaline drain's effluents towards *D. magna*, in dependence of the applied residence time, was decreased to 550 and 350 ATU, respectively, which determined 71 and 82 % efficiency of the toxicity removal in comparison to the initial AMD toxicity. It mean that after the second year since the start of the passive operation, a longer residence time of the waters being treated was needed to use, which enhanced a higher percent of pollutants as well as toxicity removal in the alkaline drain

Table 1.
Effect of temperature on the efficiency of the acid mine drainage treatment by means of a passive system (period-17-19 months since the start of operation and at residence time 75 h) as well as the effluents toxicity towards *Daphnia magna*

Index	AMD ¹	Section of the passive system					
		Alkalizing drain		Section for MDSR		Surface-flow wetland	
		2	3	2	3	2	3
pH	3.22	3.7	4.2	6.6	6.8	6.9	7.3
Acidity, mmol/l	10.4	1.3	1.1	0.4	0.3	0.3	0.2
Alkalinity, mmol/l	-	1.4	1.6	9.5	12.8	10.6	11.3
Cu, mg/l	36.7	5.8	4.6	0.08	0.03	0.02	0.02
Zn, mg/l	41.2	25.7	22.4	0.9	0.5	0.3	0.2
Ni, mg/l	1.4	1.3	1.2	0.03	0.01	< 0.01	< 0.01
Cd, mg/l	0.9	0.8	0.8	0.07	0.05	0.01	0.01
As, mg/l	0.52	0.27	0.21	0.04	0.02	BDL	BDL
U, mg/l	1.23	0.4	0.3	0.06	< 0.06	< 0.06	< 0.06
Fe, mg/l	133	55.3	34.4	1.6	0.9	0.5	0.2
Mn, mg/l	12.5	10.3	9.9	5.5	4.7	0.3	0.2
SO ₄ , g/l	1.6	1.3	1.4	0.9	1.3	1.1	1.1
Al, mg/l	142	95.5	88.3	0.2	0.2	0.1	0.1
DOC, mg/l	-	-	-	135	105	25	17
HS ⁻ , mg/l	-	-	-	0.8	5.5	0.2	-
NH ₄ ⁺ , mg/l	-	-	-	1.7	7.4	0.8	<0.1
Ca, mg/l	108	224	270	233	276	227	270
Mg, mg/l	75	112	125	118	134	112	126
ATU	2500	370	295	5	6	-	-
Toxicity removal efficiency (4), %	-	85	88	99	99	100	100

- (1) – each of the presented values is the average mean of 12 different samples taken in the studied period of the passive system operation;
- (2) – water treatment carried out at temperature 2 – 5° C;
- (3) – water treatment carried out at temperature 10 – 15° C;
- BDL – below detection of the relevant method;
- (4) – the toxicity removal of each section is calculated in comparison to AMD toxicity.

because of armoring of the carbonates' surface with ferric iron precipitates. In spite of the very high percent of the pollutants removal, their residual concentrations in the alkaline drain's effluents were still higher than the relevant permissible levels as well as still very toxic towards *D. magna*. For that reason, the second operating unit for water treatment was the section for microbial dissimilatory sulphate reduction. The main mechanism for pollutants removal was microbial generation of hydrogen sulphide due to the anaerobic oxidation of organic monomers (alcohols and simple organic acids). These organics were used as sources of carbon and energy by sulphate reducing bacteria in the presence of sulphate ions used as their final electron acceptor. The generated hydrogen sulphide was consumed to precipitate dissolved iron as well as

heavy metals as the relevant sulphide minerals. In accordance to uranium, it was reduced from hexa- to tetra-valent state and precipitated as minerals similar to uraninite UO_2 (Senko et al., 2002). These removal processes were very efficient and as a result of this, the residual concentrations of toxic elements and iron in the section's effluents were lower than the relevant permissible levels for waters intended to use in industry/ or agriculture even at temperature lower than 5 °C. Below this temperature threshold, the concentration of dissolved hydrogen sulphide was lower than 1 mg/l (Table 1) due to the strong effect of temperature on the sulfate reduction rate. In that case, the efficient treatment of waters during the winter months was determined by the sorption processes of dissolved metals on the surface of solid biodegradable materials that being used for the section filling up. Due to the hydrolysis of cellulose and hemicellulose by cellulolytic bacteria, a net negative pH-dependable charge was aroused on the solid organics' surface. That charge was compensated with attraction and sorption of cations dissolved in the waters being treated. Because of slightly acidic pH of alkaline drain's effluents, the most of dissolved pollutants (copper, nickel, and iron for example) were presented as hydroxy complexes ($Me(OH)^+$) which were sorbed preferentially on that positions. As a result of this, more than 80 % of pollutants that have been precipitating in the second section were accumulated in its first quarter (Groudev et al., 2008b). With the temperature rising, the rate of microbial sulphate reduction gradually increased which led to higher concentration of dissolved sulphide ion in the section's pore solutions. It allowed the heavy metals and toxic elements that have been sorbed during the winter months to be strongly retained due to their precipitation as the relevant sulphide minerals. From all initially presented pollutants, only the concentration of manganese in the second section's effluents was higher than the relevant permissible levels. When the passive system was operating at residence time in the range of 75 – 100 h, approximately 10-25 % of manganese in waters being treated was retained in the second section. However, it is well-known that bivalent manganese is strongly refractory to any kind of biological or chemical processes at anaerobic conditions and slightly alkaline pH. For that reason, when the passive system turn to operate at shorter residence time (lower than 75 h) higher concentration of dissolved manganese was measured in the section's effluents. That was clear indication that manganese was retained by a reversible sorption process, direction of which is governed by the ratio between sorbed and dissolved cations in the pore solution.

The efficiently removal of heavy metals and toxic elements by means of their precipitation as sulfides was required the higher concentration of dissolved sulphide ion in comparison to the total sum of dissolved pollutants in the treated waters. For that reason, the concentration of sulphide ion in the second section's effluents has being maintained in the range 4-5 mg/l, which was considerably higher than the relevant permissible levels. Beside hydrogen sulfide, the concentrations of ammonia ion, dissolved organic compounds as well as manganese were still higher than the relevant permissible levels. In dependence of the temperature, residence time and continuance of the passive system operation the acute toxicity of the second section's effluents were in the range of 4 – 6 ATU. Those values were mainly determined by the concentrations of dissolved hydrogen sulfide and ammonia ions which are very toxic to aquatic organisms (Hathaway and Stefan, 1995). That mean that the toxicity of

Table 2.

Effect of the applied residence time on the efficiency of the acid mine drainage treatment by means of a passive system (at temperature 10-15° C and period 26 - 27 month since the start of operation) as well as the effluents toxicity towards Daphnia magna

Index	AMD ¹	Section of the passive system					
		Alkalinizing drain		Section for MDSR		Surface-flow wetland	
		2	3	2	3	2	3
pH	3.17	3.8	4.0	6.3	6.6	6.8	7.2
Acidity, mmol/l	10.1	2.6	1.9	0.4	0.3	0.3	0.2
Alkalinity, mmol/l	-	0.2	1.4	9.9	10.2	10.6	11.1
Cu, mg/l	29.4	8.7	5.3	0.06	0.04	0.03	0.03
Zn, mg/l	35.7	22.6	7.2	1.1	0.9	0.4	0.3
Ni, mg/l	1.5	1.1	0.9	0.02	0.02	< 0.01	< 0.01
Cd, mg/l	1.1	0.9	0.8	0.05	0.03	0.01	0.01
As, mg/l	0.46	0.34	0.28	0.06	0.04	BDL	BDL
U, mg/l	1.23	0.3	0.3	0.06	< 0.06	BDL	BDL
Fe, mg/l	112	72	54	1.9	1.4	0.7	0.5
Mn, mg/l	12.1	9.9	9.7	7.1	6.4	0.6	0.4
SO ₄ , g/l	1.5	1.4	1.2	1.3	1.1	1.3	1.2
Al, mg/l	118	87.4	81.5	0.4	0.3	0.2	0.2
DOC, mg/l	-	-	-	100	120	35	20
HS ⁻ , mg/l	-	-	-	2.4	3.3	0.3	-
NH ₄ ⁺ , mg/l	-	-	-	4.4	5.2	<0.1	<0.1
Ca, mg/l	98	148	175	177	202	168	190
Mg, mg/l	65	83	98	96	112	94	110
ATU	2000	500	330	4	5	-	-
Toxicity removal efficiency (4), %	-	75	83	99	99	100	100

(1) – each of the presented values is the average mean of 12 different samples taken in the studied period of the passive system operation;

(2) – water treatment at residence time of 50 h;

(3) – water treatment at residence time of 75 h;

BDL – below detection of the relevant method;

(4) – the toxicity removal of each section is calculated in comparison to AMD toxicity.

initially presented pollutants in acid mine drainage being treated was entirely removed due to their retention in the first two sections of the pilot-scale passive system. For that reason, a surface-flow wetland was included as a third element in the constructed passive system. Its main role was to turn these pollutants, which have originated in the second section, to harmless products due to their oxidation at aerobic conditions by means of local microflora. An abundant plant growth, especially after the first year since the start of passive system operation, as well as oxygen diffusion from atmosphere have enabled to maintain permanent aerobic conditions in the upper zone of wetland. It greatly enhanced proliferation of aerobic microorganisms-both chemolithotrophic bacteria and

heterotrophic microorganisms (Groudev et al., 2004). The first oxidized the sulphide and ammonia ions to sulfate and nitrate ions, respectively. The second group turned the dissolved organic compounds and manganese to carbon dioxide and MnOOH/ MnO₂ precipitates mainly (Stultz et al., 1996), respectively, due to organics' oxidation and hydrogen peroxide formation as intermediate metabolite. The chemical or enzymatic degradation of the latter lead to formation of reactive oxygen radicals which oxidized manganese from bivalent to tetravalent state. As a result of this, the surface-flow wetland's effluents were characterized with neutral pH and chemical content in which the alkaline earth metals were dominating and the residual concentrations of heavy metals and toxic elements were below the relevant permissible levels as well as below thresholds which could arise any kind of toxic response in *Daphnia magna*.

Conclusions

1. The acute toxicity of acid mine drainage, generated in the abandoned uranium mine Curilo, towards *Daphnia magna* was determined by the concentration of dissolved copper, mainly, as the most toxic pollutant amongst the dissolved pollutants;
2. The partial consumption of water acidity as well as the partial precipitation of heavy metals, iron, and aluminum in the alkalizing drain decreased the acid mine drainage's acute toxicity towards *D. magna* with more than 70-80 % in dependence of the applied operating conditions;
3. The stable retention of dissolved heavy metals and uranium in the section for microbial dissimilatory sulphate reduction was due to their precipitation as the relevant sulphide minerals and uraninite, respectively. However, during the winter sulphate reduction was strongly limited due to the low local temperature. In that case, very efficient cleaning effect towards water being treated was observed due to the huge working volume of that section as well as the tremendous sorption surface of the used biodegradable substrates. That strongly enhanced participation of biosorption processes as a pollutant removal mechanism. Regardless of the climatic conditions, the acute toxicity of section's effluents towards *D. magna* was decreased by more than 99 %;
4. The residual toxicity of waters being treated in the section for microbial dissimilatory sulphate reduction was determined by the higher concentrations of dissolved hydrogen sulfide and ammonia ion;
5. The sequential removal of the dissolved pollutants in different sections of the constructed passive system have enabled the maintenance of 100 % detoxification of waters being treated even 2.5 years since the start of passive system operation and regardless of the local climatic conditions;
6. The acid mine drainage cleanup by means of passive system will cease the migration of dissolved pollutants in the environment which would make possible the gradual recovery of biodiversity in the affected aquatic as well as terrestrial ecosystems.

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