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Preliminary results of ecotoxicological assessment of an Acid Mine Drainage (AMD) Passive Treatment System testing water quality of depurated lixiviates

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Abstract

The current work reports on the preliminary results of a toxicity test using screening experiments to check the efficiency of an innovative passive treatment plant designed for acid mine drainage purification. Bioassays took place with water samples before and after the treatment system and in the river, once treated water is discharged. Due to the high toxicity of the water collected at the mouth of the mine (before the treatment plant), the bioassay was designed and developed with respect to the exposed organism and the characteristics of the water. The results obtained in the screening experiment show the high efficiency of the treatment plant in terms of ecotoxicity, using *Artemia sp.* toxicity test.

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Keywords: Ecotoxicological assessment; Acid Mine Drainage remediation; Odiel watershed

1. Introduction

The Odiel River Basin is generally recognized as a fluvial system with a catastrophic ecological situation due to chronic and severe pollution from AMD, so much so that it affects 37% of the length of the whole drainage network[3]. The Odiel River drains the central part of the Iberian Pyrite Belt (IPB) (SW Spain). This zone contains original massive sulfide reserves of about 1700Mt distributed among more than 50 massive sulfide deposits. Pyrite, sphalerite, galena and chalcopyrite are the main sulfide phases that make up these deposits, which also contain accessory amounts of As, Cd, Co, Ni, Cr, etc. Weathering of these minerals releases to the waters an enormous

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amount of toxic elements which severely affect the Odiel River from its upper section to the Huelva Estuary[4].

There have been numerous studies and pilot projects to neutralize the acidity of the AMD-polluted waters. The objective is to meet the European regulations of water quality (EC Decision 2000/60 / EC) by the horizon of 2027. Nowadays, a Passive Treatment System Plant (PTSP) is currently working and a second plant is being built. The main aim of these plants will be an improvement in the quality of water, which could even be used for irrigation.

The first system of this type was built at the Esperanza Mine (located in the northern part of the IPB, being one of the first mines to pollute the Odiel River). It consists of connected pools of pretreatment, reagent tanks and decanters[2]. The AMD flowing from the abandoned mine gallery has an average pH of 2.64, electrical conductivity between 2.03 - 3.13 mS/cm and about 10% dissolved oxygen. Its net acidity is about 1910 mg/L as $CaCO_3$ equivalent and contains average values of 596 mg/L of Fe, 112 mg/L of Al, 16 mg/L Cu, 12 mg/L Zn and 0.1-3 mg/L of As, Cr, Cd, Co, Ni[2]. The dissolution of reactive material within the PTSP raises the pH of the solution to mean values of 6.46 at the output, with a generation of alkalinity of 320 mg/L in the reagent tanks final disposal.

There is a scientific gap regarding toxicity of these polluted waters and the ecological quality of the Odiel watershed. Several studies have been started, using the bivalve *Corbicula fluminea* as biomonitor of AMD polluted environments[1]. The installation of the PTSP should decrease the acidity and metal load into the watercourses. The water quality is reflected in the aquatic life; hence, the operation of these passive systems should lead to improvements in aquatic ecosystems for the Odiel River basin. This plant will decrease the bioavailable fraction of toxic elements in aquatic systems and, consequently the genotoxic effects at the different levels (biochemical, individual, population and, finally, ecological). Therefore, in addition to the physicochemical characterization in different points of the plant, an ecotoxicological assessment should be helpful.

In this work, preliminary results of a toxicity test are shown for the assessment of the PTSP demonstrating the improved quality of the treated water by the passive treatment system using *Artemia sp.*

Methodology

A PTSP has been built between the Esperanza mine and the Odiel river. Four sampling points were selected (Fig. 1): the mine output (PA sample), the exit of the treatment plant (PB sample) and the Odiel river after the mouth of the PTSP leachate (PC sample). A control sample in the headwaters of the Odiel river was also sampled (CR sample).

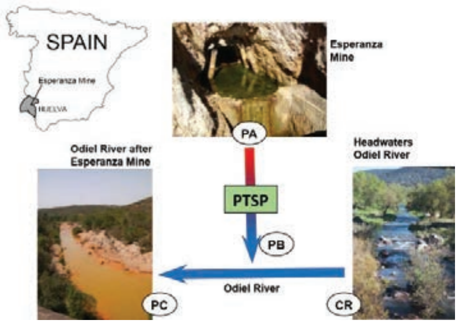

Fig. 1. Diagram of sampling points studied.

Water for bioassays were collected and carried to the laboratory in 100 mL plastic bottles (pre-washed with 10 percent HNO_3). Aliquots of water samples were filtered immediately in the field through 0.45 µm Millipore filters fitted on Sartorius polycarbonate filter holders. Samples for cations and metal analysis were acidified in the field to pH<2 with superpure? HNO_3 (2%) and then stored in the dark at 4 ºC in polyethylene bottles until analysis. Samples collected for alkalinity and anion determinations were filtered but not acidified.

Due to the extreme acidity of the AMD (point PA), several dilutions were performed before to bioassay attending ranges of pH: PA_{100} (100% AMD, no dilution; pH= 2.72), $PA_{12.5}$ (12.5% AMD, pH 4-5) and $PA_{6.25}$ (6.25% AMD, pH > 6). Several physicochemical parameters were measured in the field and in the laboratory along with the bioassays. Temperature, pH and redox potential were measured using a portable MM40+ meter (Crison). Electrical conductivity was measured by a Conductivity 3000 H meter. Dissolved oxygen was measured with an auto-calibrating Hanna® portable meter and gross alkalinity was determined using CHEMetrics® Total Titrets®. Concentration of As, Cd, Co, Cr, Cu, Mn, Pb, Se and Zn were determined by inductively coupled plasma-mass spectrometry (ICP-MS) (Thermo Elemental Series-X) performed at the Spectroscopy Division (ICP/AAS), and Al and Fe concentration were measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) of the "Servicios Centrales de Ciencia y Tecnología" of the University of Cadiz.

A standardized toxicity test was performed using *Artemia sp.* (<24 hours). It is a simple and cost-effective bioassay based on the facilities of the University of Cádiz to obtain screening results. Survival tests with Artemia (N=10 by triplicate) were monitored with 17 bioassays and two controls conducted in a disposable multiwell test plate with their correspondence serial dilution. Salinity was adjusted from Microtox® osmotic diluent until 10 ppt was reached.

Recount of individual survival was recorded exponentially over 24 hours by using magnifier equipment. Mortality in the negative control (NC, commercial water with osmotic diluent) and the positive control (PC, water from non polluted river) samples was recorded as 2% and 6% respectively.

Results from survival tests were statistically analyzed using the program GraphPad Prism version 5.00 software (San Diego California, USA) for Windows: median survival (LT_{50}) was obtained from Kaplan-Meier graphs and compared survival curves using both the logrank (Mantel-Cox) test and the Gehan-Wilcoxon test; percentage of survival at the end of the bioassays were analyzed by one-way ANOVA with Dunnett to find significant differences among the controls and the treatments. Cluster analysis was based on the correlation matrix created by computing the Pearson‘s r statistic, using Bray-Curtis as the similarity measure.

3. Results

Physicochemical parameters and concentrations in water samples and the different dilutions are summarized in Table 1. Comparing metal concentrations with values established by the Water Framework Directive (WFD) and EPA 2002 for freshwater and drinking water, water quality can be assessed (Table 1). Metal concentrations of the AMD lixiviates (PA) are exceeded for all the metals except Pb. However, concentrations are less than the established values once the water is treated (PB), only exceeded by the Mn concentration (3.8 mg/L).

Data derived from Kaplan-Meier graphs provides the LT_{50} for samples PA, PB, PC and their dilutions (Fig. 2). No significant differences were obtained between the original AMD sample (PA_{100}) and its first dilution (PA_{50}), due to null pH differences (pH PA_{100}= 2.72 and pH PA_{50}=2.87) and mortality results (LT_{50} 267 min, Fig.2). After 48 hours, the percentage of survival for sample PA and its dilutions was zero, except the highest dilution ($PA_{6.25}$), which registered a survival of 83% (Table 1). The survival of the treated sample (PB) was 40%; and the mixture of this with the Odiel River (PC) showed an average survival of 69%. Lethal time for the control was superior to 48 hours. The ANOVA test found significant differences in the samples and their dilutions compared to the control samples for PC_{100}, PC_{25} ($p<0.05$) and all the dilutions of the samples PA (except 6.25%) and PB (except 12.5 and 6.25%) ($p<0.001$).

Integration of biological responses and chemical characterization of water samples showed that the treated sample (PB) has a toxic effect (LT_{50} 265 min for undiluted sample). Considering the low content of other metals, the toxic effect is possibly due to elevated concentration of Mn (3.8 mg/L) and/or to low dissolved oxygen concentration (2 mg/L) flowing from the PTSP. This assumption is supported by the fact that after the first dilution the LT_{50} increases more than 77% (Fig. 2).

4. Conclusions

The screening results for chemical and biological characterization of the AMD and their effluents after improvement from a Passive Treatment System Plant were found once discharges are discharged into the Odiel River. The preliminary conclusions based on the results obtained from the sample dilutions show the extreme sensitivity of species under these polluted waters. The results of toxicity bioassays using *Artemia sp.* reveal improvement of water quality close to that of unpolluted water. The installed system clearly indicates an improvement in water properties; however, water reached better quality in the confluence with the Odiel River, probably due to aeration processes, adding dissolved oxygen favorable for life.

Table 1. Measured parameters in the water samples, the dilutions and the control samples (CN: negative control; CR: positive control; EC: electrical conductivity; DO: dissolved oxygen; <dl: below detection limit). Water quality values for drinking water under the Water Framework Directive (WFD) and EPA2002.

	CN	CR	PA$_{100}$	PA$_{12.5}$	PA$_{6.25}$	PB	PC	EPA2002	WFD
pH	8.06	7.42	2.72	4.65	7.64	7.11	7.25		
Eh (mV)	344	395	638	360	365	196	353		
EC(mS/cm)	0.47	0.36	2.77	0.65	0.54	1.45	0.27		
T (ºC)	20.3	15.0	14.8	20.3	20.3	17.3	17.9		
DO (mg/L)	7.24	7.5	2.90	7.86	8.33	2.08	7.70		
Alk (mg/L)	197	200	<dl	<dl	160	225	100		
Al (µg/L)	<dl	<dl	111790	17007	31.0	106	<dl	5000	
As (µg/L)	1.74	5.90	306	13.0	2.15	78.9	257	100	10
Cd (µg/L)	0.50	<dl	63.0	11.4	1.38	0.24	0.61	10.0	3
Co (µg/L)	<dl	5.20	417	82.5	8.68	5.94	9.34	50	
Cr (µg/L)	0.40	<dl	24.7	4.28	0.84	<dl	<dl	50	
Cu (µg/L)	<dl	1.56	13509	2252	237	17.2	14.3	500	2000
Fe (µg/L)	5.28	13.0	348663	61235	6247	220	32.3	2000	
Mn (µg/L)	<dl	93.0	3171	713	75.0	3769	140		50
Pb (µg/L)	<dl	<dl	19.1	2.96	0.31	<dl	<dl	50	40
Se (µg/L)	<dl	<dl	15.8	2.35	0.25	<dl	<dl	20	20
Zn (µg/L)	1.29	7.00	15060	3003	317	349	176	2000	
%Survival	100	100	0.00	0.00	82.7	39.9	69.2		

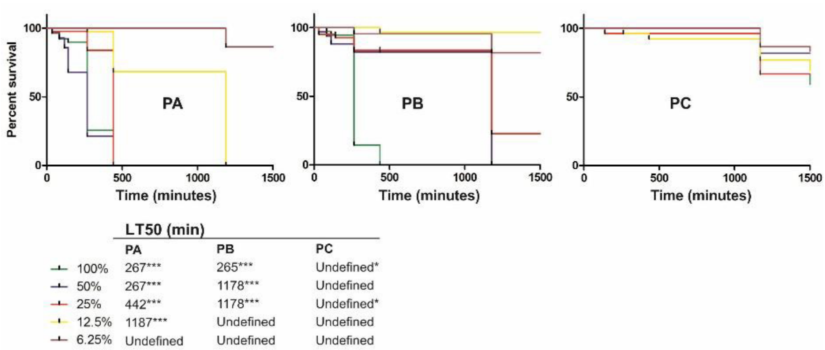

Fig. 2. Kaplan-Meier survival curves obtained for the different treatments in the first 24 hours for the different samples (PA, PB, PC and the serial dilutions). The median survival (LT$_{50}$ in minutes) calculated by the GraphPad Prim program are written next to each leachate in the first 24 hours exposure time. Asterisks (*) point the significance level reached compared through the Dunnett test.

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