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Toxicity assessment of an effluent derived from an inactivated uranium mine: the Poços de Caldas (Brazil) example

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Abstract

Uranium mines can cause environmental impact because of acid mine drainages can reaches the surrounding water bodies. In this work we tested effluent samples from an inactivated uranium mine located in Poços de Caldas city (Minas Gerais, Brazil) for acute and chronic toxicity. Untreated effluents were acutely toxic to *Daphnia similis*, with EC_{50} (48 h) varying from <0.01% to 38%. Both treated and untreated effluent samples presented chronic toxicity to *Ceriodaphnia dubia*. *Vibrio fischeri* acute toxicity tests were negative for all tested samples. Adjustment of pH performed in samples was not sufficient to remove acute toxicity indicating that not only pH was responsible for the observed effect. The physical-chemical treatment applied was not able to remove the chronic toxicity to *C. dubia*. *V. fischeri*, although a rapid test and easy to handle, was not sensitive to the level of toxicants present in the effluent from the mine. To determine the actual impact of the discharge of effluent into receiving waters, both the flow of the effluent and river should be considered. Nevertheless chronic toxicity tests with *C. dubia* could be included in the monitoring program of this facility to provide more information about the impact of this discharge.

Key-words: Acid mine drainage, *Ceriodaphnia dubia*, *Daphnia similis*, ecotoxicity, *Vibrio fischeri*.

Avaliação da toxicidade do efluente proveniente de uma mina de urânio inativada em Poços de Caldas (Brasil)

Resumo

Efluentes provenientes de minas de urânio podem impactar corpos de água adjacentes devido às drenagens ácidas de mina. Neste trabalho, foram testadas amostras de efluentes de uma mina de urânio inativada, localizada na cidade de Poços de Caldas (Minas Gerais, Brasil) para a toxicidade aguda e crônica. Efluentes não tratados foram tóxicos para *Daphnia similis*, com CE_{50} (48 h) variando de <0,01% a 38%. Ambas as amostras de efluentes, tratadas e não tratadas, apresentaram toxicidade crônica para *Ceriodaphnia dubia*. Testes de toxicidade realizados com *Vibrio fischeri* foram negativos para todas as amostras testadas. O ajuste de pH, realizado em algumas amostras, não foi suficiente para remover a toxicidade aguda, indicando que somente o pH não foi responsável pelo efeito observado. O tratamento físico-químico aplicado nesses efluentes dentro da mina, não foi capaz de remover a toxicidade crônica para *C. dubia*. O teste de toxicidade com *V. fischeri*, embora seja um teste rápido e fácil de manusear, não foi sensível para as amostras empregadas. Para determinar o real impacto da descarga desses efluentes, oriundos da drenagem ácida de mina, em águas receptoras, as vazões do efluente e do rio devem ser consideradas. O teste de toxicidade crônica com *C. dubia* poderia ser incluído no programa de monitoramento da mina para fornecer mais informações sobre o impacto dessa descarga.

Palavras-chave: Drenagem ácida de mina, *Ceriodaphnia dubia*, *Daphnia similis*, ecotoxicidade, *Vibrio fischeri*.

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INTRODUCTION

Inactivated and abandoned uranium mines are a significant challenge to manage and a potential risk to environmental and human health. The main impacts of a uranium mine are usually related to its high volume of tailings and acid mine drainages (AMD). AMD are caused by oxidation of natural metallic sulfites in the presence of water and oxygen, resulting in sulfuric acid and several dissociated metals. AMD is a complex mixture of several metals and radioactive species, usually with low pH and significant radioactivity (Fernandes *et al.*, 1998; Wiikman, 1998; Cipriani, 2002). Production of AMD is a high concern, because of the difficulties of its treatment and remediation (Galán *et al.*, 2003; Lin *et al.*, 2007; Antunes *et al.*, 2008). These waters are rich in hazardous chemicals and radioactive elements that can also be transferred to different environmental compartments, such as soil and plants (Neves *et al.*, 2008).

In the literature there are some studies demonstrating the toxicity of uranium mine effluents and their environmental impacts (Antunes *et al.*, 2007a, b). Some authors studied the toxicity of uranium and derived ecotoxicity threshold values to a several terrestrial and aquatic organisms. Uranium is a naturally occurring radioactive element that decays to other elements, such as radon, lead, polonium and bismuth, which are equally toxic to most living organisms (Vera Tome *et al.*, 2002; Sheppard *et al.*, 2005).

In Brazil, the management of the inactivated uranium mine plant of Poços de Caldas is a new and a challenging reality for both environmental and nuclear agencies. It was one of the first uranium mines explored in Brazilian territory. The management consists of a two- phase physical-chemical treatment: first the precipitation of metals using CaO and $CaCO_3$ and second the use of $BaCl_2$. The final effluents are discharged into receiving water bodies in the surroundings of the mine.

Current Brazilian water legislation does not address specific uranium criteria for the discharge of effluents in water bodies (CONAMA, 2005, 2011). The discharge of uranium mine effluents are mostly assessed by: pH, dissolved metals (Mn, Fe, F, and Al), and both acute and chronic toxicity to aquatic organisms (CONAMA, 2005; Rubio and Silva, 2010). To our knowledge, there is no routine toxicity monitoring of the discharged effluents from this inactivated uranium mine, in order to avoid negative impacts over native organisms, nor any concern about the dilution rates of mine effluent into receiving water bodies.

In the present study, we used three bioassays to assess the toxicity of acid mine drainages samples before and after physical-chemical treatment: acute assays with *Daphnia similis* and *Vibrio fischeri,* and the chronic assay with *Ceriodaphnia dubia*. Both cladocerans are key species in freshwater food chains and standardized test-organisms. The *V. fischeri* assay was also included because of its simplicity and potential on field monitoring programs, which could allow for rapid and easy-to-handle assessments.

MATERIAL AND METHODS

Site characterization and sampling

The studied inactivated uranium mine is located at Poços de Caldas city, Minas Gerais state, Brazil (21º45'S, 46º35'W). Uranium was extracted from this open mine from 1982 to 1995, to supply Angra I reactor power plant and other projects in Brazil. The whole area comprises the mine pit, which has 1.2 km of diameter and 176 m depth. Now it is a pond with the acid mine drainages, besides extensive areas intended for disposing of waste rock piles, several acids mine drainage ponds, settling ponds, and the inactivated uranium production unit. The pond waters comprise a complex mixture of metals with low pH and some radioactivity (Table 1) (Fernandes *et al.*, 1998; Wiikman, 1998; Cipriani, 2002). The overall management system of these effluents is illustrated in Figure 1. Acid drainages are pumped into the mine pit, and then to the chemical plant for the physical-chemical treatment with $CaCO_3$ and CaO. In the past, the slurry from the drainage treatment was deposited in the tailing dam. However, because its full capacity, the precipitate from the chemical treatment is now being deposited in the mine open pit. The effluent from the tailing dam is treated with $BaCl_2$, to remove radium isotopes from the solution, and the precipitated solids settle in two ponds (Fig. 1). The overflow of these two ponds is discharged into the Verde River (Fernandes *et al.*, 2008).

We collected water samples from the five following sites in three sampling events, on September and November 2008 and on March 2009:

P1 – Pond containing untreated acid drainage from waste rock pile 4;

P2 – Mine pit that receives untreated effluent from P1 and slurry from physical-chemical treatment;

P3 – Settling pond that receives treated effluent from P1 and P2, treated with $CaCO_3$ and CaO;

P4 – Settling pond that receives effluents treated with $BaCl_2$, and are then discharged to a local water body (Verde River);

P5 – Reservoir that receives water from a local river and treated effluents from P3, which is then discharged to a local water body (Antas Creek).

Table 1 - Average, minimum and maximum concentrations of pollutants in the P1 site, as determined by Fernandes *et al.* (1998)[a].

Element	Average	Minimum	Maximum
^{226}Ra (Bq L^{-1})	0.29	0.14	0.58
^{238}U (Bq L^{-1})	175	71	315
Al (mg L^{-1})	96	61	161
F (mg L^{-1})	99	5.1	167
Mn (mg L^{-1})	75	6.6	105

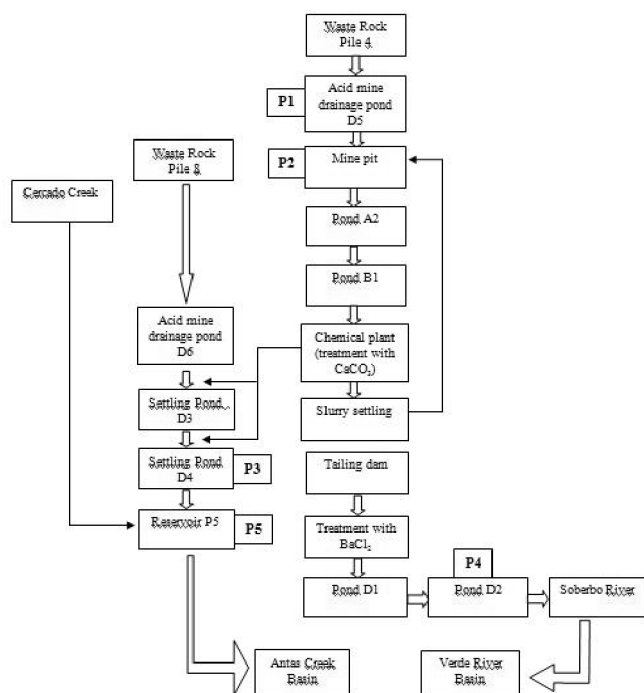[a] Adapted from Fernandes *et al.* (1998).

Figure 1. Flowchart of effluent and acid mine drainage treatments inside the inactivated uranium mine in Poços de Caldas, Brazil. Sampling sites are depicted as P1-P5, which are described in detail in the Materials and Methods Section. (Adapted from Cipriani, 2002).

Samples

We collected the water samples in nontoxic plastic containers, and stored them at 4ºC in the dark until the moment of testing. Due to very low pH of samples P1 and P2 observed in the two first sampling events, pH values of samples from the third sampling event were corrected to 7.0 ± 0.5 with addition of NaOH 1N, to reach a biologically acceptable range.

Physical-chemical parameters

We determined physical-chemical parameters at the moment of sampling using a multiprobe equipment (model YSI-556). For each sample, values of pH, conductivity, dissolved oxygen and temperature were registered.

Bioassays

We used bioassays to assess the efficiency of physical-chemical treatments in relation to toxicity, which were used in each sampling event as shown in Table 2.

Vibrio fischeri

We performed *V. fischeri* bioassay with water samples, as detailed in Knie and Lopes (2004), with some modifications. We performed laboratory tests using the field equipment (Biofix® Lumi-10-Macherey-Nagel). For each replicate 300 µL of suspended bacteria was used, in a 1:2 diluting series. Due to dilution caused by the addition of bacteria, the maximum

Table 2 - Bioassays performed in the studied area in three different sampling events.

Sample site		#1	#2	#3
		Sampling events		
Untreated	P1*			Acute tests:
	P2*	Acute test:	Acute test:	*Daphnia similis*
	P3	*Daphnia similis*	*Daphnia similis*	*Vibrio fischeri*
Treated	P4			Chronic test:
	P5			*Ceriodaphnia dubia*

*pH adjusted in the third sampling event.

concentration tested was 63%, followed by 31.5%, 15.75% and 7.88%. Percentages of light inhibition were used to calculate the IC_{50} (15 min) of each sample, by the Trimmed-Spearman Karber method (Hamilton *et al*., 1977). Sensitivity tests were performed with zinc sulfate as the reference toxicant.

Daphnia similis

We kept *D. similis* stock cultures and acute toxicity test according to procedure NBR 12713 (ABNT, 2004). Final concentrations (Table 3) were determined after a preliminary test. After 48 h, the immobilization of test organisms was registered and the EC_{50} (48h) estimated by the Trimmed-Spearman Karber method (Hamilton *et al*., 1977). Test data were considered acceptable if mortality in controls did not exceed 10%.

In the third sampling event, we adjusted pH values of the samples with the lowest pH (P1 and P2) (Table 4), to 7.0 ± 0.5 and tested them before and after adjustment at 0.1, 1, 10 and 100% concentrations.

Ceriodaphnia dubia

We kept the stock cultures and did the chronic toxicity test procedures of *C. dubia* according to NBR 13373 (ABNT, 2005). The endpoint was the total neonate production per female. The final concentrations tested for each sample are detailed in Table 3. These data were used to estimate IC_{25}, using

Table 3 - Concentrations of the tested samples for acute (*D. similis*) and chronic (*C. dubia*) toxicity.

Samples	Tested sample concentrations (%)	
	D. similis	*C. dubia*
P1	0.01; 0.1; 0.5; 1.0; 5.0; 10.0	0.001; 0.01; 0.1; 1.0
P2	0.1; 0.5; 1.0; 5.0; 10.0	0.001; 0.01; 0.1; 1.0
P3	1; 5; 10; 25; 50; 75; 100	0.1; 1; 5; 10;100[a]
P4	10; 25; 50; 100	1; 5; 10; 50;100[a]
P5	10; 25; 50; 100	10; 50; 75; 100

[a] 100% samples were added in the third sampling event.

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the ICp software v. 2.0. Values of NOEC (No Observed Effect Concentration), LOEC (Lowest Observed Effect Concentration) and Chronic Value (CV, geometric mean of NOEC and LOEC) were determined for each sample with ANOVA (p<0.05) and Tukey pos test. Test data were considered acceptable if mortality in the controls did not exceed 20% and neonate production per female in the controls was ≥ 15.

RESULTS AND DISCUSSION

Physical-chemical parameters

Values of physical-chemical parameters are depicted in Table 4. Initially, we thought pH values could cause the observed toxicity, but two factors suggested otherwise: high dilution of tested samples naturally elevated pH values to a biologically acceptable range: all tested dilutions had pH values between 6,36 and 7,79; in the third sampling event pH values of P1 and P2 samples were adjusted to 7.0 ± 0.5. In both cases, toxicity was not completely removed from samples (see further sections). As expected, water conductivity was higher in untreated effluents (P1 and P2) than in the treated ones (P3-P5), indicating metal and other ion removal.

Bioassays

Vibrio fischeri

No reduction in light emission was observed for any tested sample. If metals are the cause of the toxicity observed for *D. similis* (see section 3.2.2), it would be expected that *V. fischeri* would provide negative results, because it is known that this bacteria is less sensitive to metals than *Daphnia* (Teodorovic *et al.*, 2009). Other possible explanation for the negative results would be the fact that the highest concentration tested was 63% in the *V. fischeri* assay in contrast to the 100% in *D. similis* test. Initially, we chose *V. fischeri* bioassay because it is easy-to-handle, time- and cost-effective, but it was not useful to us to determine the efficiency of the physical-chemical treatment in relation to toxicity. Antunes *et al.* (2008) used *V. fischeri* bioassay to assess toxicity of soil elutriates from an abandoned uranium mine, and also did not observe toxicity. Lopes *et al.* (1999) also unsuccessfully applied *V. fischeri* assay to test acid mine drainages from an inactivated cupric pyrite mine. They addressed several issues for this unsuccessful use of *V. fischeri* assay: (a) the interaction between metals and the test osmotic regulator might influence bacteria response; and/or (b) the influence of pH on metal speciation. Our results agree with their conclusion that *V. fischeri* bioassay is not adequate to assess the toxicity of acid mine drainages because several interactions can interfere with toxicity results.

Daphnia similis

Results of *D. similis* bioassays showed acute toxicity only in P1 and P2 samples, i.e., to untreated effluent samples (Table 5). Data published elsewhere (Fernandes *et al.*, 2008) showed significant concentrations of metals at P1 sampling site (Table 1), suggesting that these metals can be the cause of toxicity. In the third sampling event, after pH adjustment in two samples, P1 EC_{50} values decreased to up to 17% and P2 EC_{50} up to 38% (Table 5). Even though, these results can also be considered toxic to *D. similis*. Physical-chemical treatment performed in the uranium mine treatment plant comprises of an elevation of pH, with the addition of $CaCO_3$ and CaO. The same procedure was made in the present study. The toxicity of sample P3 also had a significant EC_{50} of 38% in the third sampling event. Samples P4 and P5 did not show acute effect to *D. similis* in any of the sampling events. These results suggest that removal of toxic substances was performed by physical-chemical treatment.

Table 4 - Physical-chemical parameters of tested samples.

Sampling sites	Samplings	Temperature (°C)	Dissolved oxygen (mg L⁻¹)	pH	Conductivity (μS cm⁻¹)
P1	#1	23.3	3.60	3.03	1271
	#2	19.9	4.04	3.51	1631
	#3	22.8	5.28	2.79	1550
P2	#1	23.8	3.42	3.82	1681
	#2	20.8	4.27	4.12	2549
	#3	28.5	5.12	2.38	1864
P3	#1	24.7	4.32	8.44	920
	#2	21.1	4.01	7.13	1100
	#3	25.0	6.30	6.41	700
P4	#1	24.2	4.40	7.35	494
	#2	20.9	4.71	6.58	987
	#3	26.5	5.50	6.79	548
P5	#1	23.3	5.46	7.56	629
	#2	20.9	4.16	6.60	235
	#3	26.1	6.00	5.64	134

Table 5 - EC_{50} (%) values for the acute toxicity test with *D. similis*.

Samples	EC_{50} (48 h) - Sampling events		
	#1	#2	#3
P1	0.4	< 0.01	1.99
P1 - adjusted pH	a	a	17.11
P2	1.05	0.9	2.41
P2 - adjusted pH	a	a	37.75
P3	NT	NT	38.26
P4	NT	NT	NT
P5	NT	NT	NT

a: Not performed. NT: Not toxic.

Lopes *et al*. (1999) successfully used an acute toxicity test with *C. dubia* in samples of acid mine drainages from an inactivated cupric pyrite mine. The authors also corrected pH values prior to testing, intending to separate toxicity due to low pH from that due to metals. Lin *et al*. (2007) reported acute toxicity to *Daphnia carinata* in river water up to 25 km downstream of an iron and cupric ore mines, after a major flood event. They addressed both the impact of an acid mine drainage discharging into the environment, and possible flood impacts on surrounding environments of an inactivated mine.

Antunes *et al*. (2007a and 2007b) reported acute toxicity (EC_{50} (48 h)) for D*aphnia longispina* from 20.5 to 28.4% and for *Daphnia magna* from 35.8 to 50.4% in ponds of an abandoned uranium mine in Portugal. They also found no acute toxicity in a pond that had its effluent treated with $BaCl_2$, similarly to results found at P4 site, which also has treated effluent with the same reagent.

Ceriodaphnia dubia

Chronic toxicity results of *C. dubia* for the third sampling event are detailed in Table 6. All tested samples caused chronic toxicity to *C. dubia*, with NOEC ranging from 0.01 to 5%, and LOEC from 0.1 to 10%. This chronic bioassay also showed higher toxicity in untreated effluents, as expected, but also showed that physical-chemical treatment was not able to completely remove chronic toxicity to this organism. Even the lowest tested concentration of sample P5 (10%) caused chronic toxicity which did not allowed NOEC estimation. IC_{25} (7 d) values varied from 0.04% to 7.05%. Chronic Values varied from 0.03 to 7.07%. Values of NOEC and CV from Sample P5 could not be estimated, because even the lowest tested concentration was toxic to *C. dubia*. IC_{50} were also estimated, but only IC_{25} values were reported, for we considered them more protective of aquatic life.

Antunes *et al*. (2007b) reported chronic toxicity to *D. magna* to different endpoints (total number of offspring, number of broods, age at first reproduction, somatic growth rate, and rate of population increase) when they assessed an untreated effluent pond of a Portuguese uranium mine. However, they found both deleterious and stimulatory effects with *D. longispina* and *D. magna*. The same authors also reported that receiving water bodies with less water flow might not be able to sufficiently dilute effluents to avoid chronic toxicity to indigenous aquatic organisms. In our study we

observed chronic toxicity to *C. dubia* even after the treatment but unfortunately we did not have access to water flow rates of the receiving water bodies influenced by the discharge of the effluents from the inactivated uranium mine plant. Thus, we could not estimate the impact that these effluents might have on aquatic life.

Based on metal determinations from the untreated acid drainage basins published by Fernandes *et al*. (2008), it is possible that uranium concentrations had a significant contribution to the toxicity of P1 and P2 samples, although the metal determinations were not performed in the samples that toxicity was measured. The ^{238}U concentrations of these sites were 16.6 and 24.8 mg L^{-1}, respectively. However, more studies would be required to better determine which compounds are causing the toxicity in the effluents from the studied mine. Antunes *et al*. (2007b) reported that mine effluents are complex mixtures, and toxicity cannot be entirely attributed to a single toxicant, because even non toxic substances might have a synergistic effect and cause toxicity. Toxicity Identification and Evaluation (TIE) could be a proper tool to assess the studied effluent.

CONCLUSIONS

Adjustment of pH was not enough to completely remove acute toxicity to *D. simils,* demonstrating that other substances are present at toxic levels in the uranium mine effluent. *V. fischeri*, although an easy-to-handle, time- and cost-effective bioassay, was not sensitive to detect toxicity of the mine effluent samples. The physical-chemical treatment applied by the facility was not efficient to remove the toxicants at non-toxic levels to *C. dubia*, suggesting that treatment improvement would still be necessary. Surface water Brazilian regulations CONAMA 357/2005 and CONAMA 430/2011 requires that effluents must not have the potential to impact the aquatic life of the water bodies classified as 1, 2 or 3. To determine the possible impact caused by the discharge of these effluents both effluent and river flow need be considered.

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Table 6 - Chronic toxicity values (%) for *C. dubia*.

Samples	IC_{25} (7 days)	NOEC	LOEC	CV
P1	0.04	0.01	0.1	0.03
P2	0.05	0.01	0.1	0.03
P3	1.04	1.0	5.0	2.23
P4	7.05	5.0	10.0	7.07
P5	4.11	< 10.0	10.0	< 10.0

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