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Toxicity in semiarid sediments influenced by tailings of an abandoned gold mine

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Abstract The mining district of El Triunfo (ET-MD) has an estimated 800,000 t of mine wastes scattered in the environment, contaminating the sediment with potentially toxic elements such as As, Cd, Pb, and Zn. In order to estimate the toxicity of the sediment to the adjacent biota, the aims of our study are to calculate the mortality and inhibition through bioassays, using sediment, and test organisms such as *Daphnia magna* and *Selenastrum capricornutum* (*Pseudokirchneriella subcapitata*), respectively. The *D. magna* mortality was 31±12 % and the *S. capricornutum* growth inhibition was 53±24 %. The contamination of the sediment determines the high mortality of *D. magna* and the high inhibition of *S. capricornutum* in the system, indicating risk for the biota in the contaminated system.

Keywords *D. magna* · *S. capricornutum* · Sediment · Arsenic · Heavy metals

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Introduction

Bioassays have been developed to evaluate the presence of toxic substances in water, sediment, soils, and anthropogenic wastes. The toxicity increases as the microbial growth rate decreases (Gabrielson et al. 2002). There are many physiological responses such as growth rate, inhibition, and mortality that have been monitored as toxicity criteria in water, sediment, soil, and tissue of organisms. *Pseudokirchneriella subcapitata*, known also as *Selenastrum capricornutum*, is a unicellular organism belonging to the group of green algae found in eutrophic and oligotrophic aquatic systems (Pica-Granados et al. 2004). These algae are organisms that are sensitive to pollutants which contain metals, and are used to evaluate phytotoxicity (Komjarova and Blust 2008). The algae could also be used to identify the sites impacted by the drainage of the mine effluent, and their incorporation into ecological risk-assessment studies has been recommended (Moreira-Santos et al. 2004; Antunes et al. 2007). *Daphnia magna* is a zooplankton organism with a high sensitivity to environmental changes (Guan and Wang 2004). Several studies have demonstrated the response of *D. magna* to essential and nonessential metals, showing a variable resistance to trace metals (Barata et al. 1998). This organism plays an important role in the food chain because of the link it creates by grazing on primary producers and being food for many fish species (Komjarova and Blust 2008). A combination of element analysis and ecotoxicity tests with *D. magna* was shown to be a potential tool for monitoring and mapping the levels of pollution in aquatic bodies and their sediments (He et al. 1998; Antunes et al. 2007; Lattuada et al. 2009).

Large quantities of metals (Cd, Hg, Pb, Zn) and metalloids (As, Sb), which are potentially toxic elements (PTEs) for adjacent biota, are released to the environment by mining activities. PTEs can be bioaccumulated and biomagnified through the trophic levels, causing irreversible damages (Merian 1991). Those toxic effects are proportional to the exposure period, element concentration, and exposure pathway (Komjarova and Blust 2009).

The El Triunfo mining district (ET-MD) has an area of ~200 km^2 (Romero-Guadarrama 2011) and is located in the mountainous portion (490 m above sea level) of the El Carrizal hydrological basin, which has an area of ~1200 km^2 (Fig. 1). Artisanal gold exploitation started in 1748 and continued intermittently until the end of the last century. Geochemical studies showed high concentrations of As in the tailings (Carrillo 1996; Volke-Sepúlveda et al. 2003) and in the ash and sediments (Marmolejo-Rodríguez et al. 2011). In this desert zone, the arroyo is a dry river which has water for only a few days when a hurricane arrives every 4 or 5 years. The distribution and accumulation of some elements along the arroyo was analyzed before (Romero-Guadarrama et al. 2010; Romero-Guadarrama 2011; Marmolejo-Rodríguez et al. 2011; Sánchez-Martínez et al. 2013). However, the toxicity of this natural sediment was not evaluated.

In addition, the sum of the metals and metalloids in the natural sediment generates different effects from bioassays of an individual element. Therefore, the aims of our study are to evaluate the toxicity of the natural sediment of the Hondo–Las Gallinas–El Carrizal arroyo (H-G-C), which is influenced by the abandoned tailings of the ET-MD enriched with PTEs, using *D. magna* and *S. capricornutum*.

Material and methods

Twenty-one samples were collected in August 2009 from the ET-MD along the 49 km of the arroyo (H-G-C) to its discharge into the Pacific Ocean (Fig. 1). The sediment was sampled in polyethylene bottles cleaned with HNO_3 (15 %) and HCl (10 %). The samples were dried and pulverized using an agate mortar. For determination of the elements, 0.25 g of the sediment was digested with HF and then with an HNO_3 and $HClO_4$ acid mixture, and then heated in a controlled program with ramping heat cycles to dry the sample. After drying, the samples were reconstituted with HCl and HNO_3. The elements were determined using a PerkinElmer ELAN 9000 inductively coupled plasma mass spectrometer (ICP-MS; Actlabs, Ontario, Canada). Validation of the methods was done using certified reference materials (PACS-2, MESS-3;

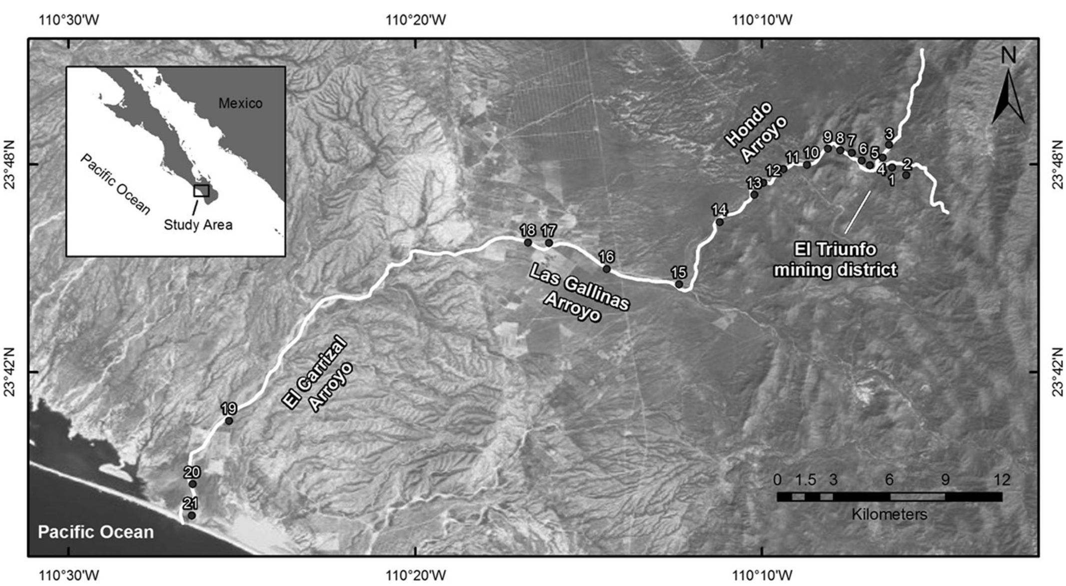

Fig. 1 Map of the study area and the sites sampled

National Research Council Canada Institute for National Measurement Standards). Blanks and duplicates of each ten samples were realized. Results of this validation are presented in Table 1.

The toxicity test was realized with elutriates obtained from the sediment samples according with USEPA (2001). Aliquots of 6–8 g were introduced in beakers of 250 mL, and reconstituted water ($MgSO_4$ $NaHCO_3$; KCl and $CaSO_4$ $2H_2O$, with hardness of 160 mg L^{-1}; NMX-AA-087-1995-SCFI) was added at a 4:1 ratio (water to sediment) stirring the solutions during 1 h; after this process, the solutions were centrifuged obtaining the elutriates.

The toxicity test with *D. magna* was done according to Díaz-Báez et al. (2004). Ten neonates of *D. magna* (from the UAM-I stock) were introduced into each beaker (50 mL of capacity with 30 mL of each elutriate) in triplicate tests with one negative control (blank: water reconstituted), and one positive control of a reference (potassium dichromate LC_{50}=0.14 mg L^{-1}). The conditions during the experiment were a temperature of 20±2 °C and a photoperiod of 16 h in the light and 8 h in the dark. Oxygen was measured at >7.8 mg L^{-1}. After 24, and 48 h of exposure to the sediment samples, the surviving organisms of each test were evaluated. With the results obtained, we calculated the percentage mortality at 48 h in each bioassay to determine the toxicity of the sediment.

The bioassays with the microalgae *S. capricornutum* were done according Pica-Granados et al. 2004. The elutriates (2.6 mL) were used in quintuple tests. For each test, we inoculated microalgae (~10,000 cells per milliliter) taken from a stock concentrated of *S. capricornutum*. The solution for the control group was using reconstituted water (for negative control), and $CuSO_4$ (EC_{50}=0.404 mg L^{-1}; positive control). The conditions maintained in the development of the bioassays were continuous light of 1000 lx and a temperature 22±2 °C. At 72 h after the initiation of the experiment, an aliquot of 0.1 mL was taken to count the cell number in the test using a hematocytometer with an optical microscope to calculate the inhibition percentage (%) of each elutriate. This was calculated using the formula:

$$\% \text{ Inhibition} = 100-(\text{average of cells in the test}/\text{average of cells in the control group}) \times 100.$$

Results and discussion

The element contents, the mortality of *D. magna*, and the inhibition of *S. capricornutum* for each sample, are presented in Table 2, including the average of the Upper Continental Crust (UCC, Wedepohl 1995). In the arroyo sediment, the enrichment of elements such as As, Cd, Cu, Pb, and Zn is evident. The enrichment is both natural for the enrichment of elements associated to the gold ore mine, and anthropogenic for the scattered tailings produced with the gold obtaining. The influence of some potentially toxic elements was evaluated before (Marmolejo-Rodríguez et al. 2011; Sánchez-Martínez et al. 2013).

Sediment toxicity and their ratio with the TEC and PEC criteria

The results about the toxicity of PTEs in sediment is evaluated in this study with the reference values for freshwater sediment criteria threshold effect concentration (TEC) and probable effects concentration (PEC)

Table 1 Results of method validations

	As	Cd	Co	Cr	Cu	Pb	Zn
PACS-2 obtained	27.9±3	2.2±0.3	11.6±0.5	100±4.9	290±10	174±9	345±20
Certified	26.2±1.5	2.11±0.15	11.5±0.3	90.7±4.6	310±12	183±8	364±23
%Recovery	106	104	101	110	94	95	94
MESS-3 obtained	22.2±4	0.2±0.1	13.4±3.1	116±9.8	33.6±2.1	20.5±	148±11
Certified	21.2±1.1	0.24±0.11	14.4±+2.0	105±4.0	33.9±1.6	21.1±	159±8
%Recovery	105	83	93	110	99	97	93

It was realized with duplicate of certificate reference materials PACS-2 and MESS-3. Units are in mg kg^{-1}

Table 2 Results of the element contents in surface sediments, bioassays of *D. magna* mortality and *S. capricornutum* inhibition

	As	Cd	Co	Cr	Cu	Pb	Zn	∑PTEs*	*D.magna*	*S.capricornutum*
1	8890	311	8.0	24.3	1660	92,700	49,600	153,193	66.7	85.5
2	238	6.3	16.8	71.5	28.4	221	350	932	26.7	46.8
3	204	5.0	18.9	68.9	27.7	311	397	1033	40.0	59.3
4	412	7.0	19.2	90.5	44.5	729	882	2184	20.0	97.2
5	251	10.1	7.2	45.6	17.1	301	406	1038	26.7	85.5
6	74	11.7	9.5	37.5	40	1230	1060	2463	26.7	73.3
7	255	9.6	8.2	47.0	43.8	1030	1330	2724	26.7	49.0
8	212	8.1	12.7	70.4	52.6	805	1620	2781	40.0	50.3
9	193	7.5	21.7	107	47.8	523	510	1410	26.7	4.82
10	143	3.9	7.5	50.3	19.7	376	368	968	33.3	35.2
11	27.4	0.1	6.9	35.9	13.5	10.7	35	130	26.7	46.9
12	122	3.8	6.3	42.0	14.2	396	445	1029	13.3	63.4
13	120	4.2	8.3	28.5	23.6	396	475	1056	20.0	37.2
14	250	8.4	10.9	84.4	52.1	869	1950	3225	13.3	51.4
15	33.8	2.0	26.1	134	47.2	131	289	663	40.0	54.7
16	49.9	1.9	11.3	89.1	16.5	161	193	523	26.7	30.3
17	20.8	0.9	9.8	58.9	10.3	100	107	308	46.7	35.8
18	7.0	0.7	17.8	99.6	19.0	70.7	99.2	314	33.3	22.7
19	68.4	2.6	15.1	92.5	29.4	202	244	654	40.0	80.0
20	10.7	0.1	3.2	24.0	4.9	14.8	19.9	77.6	26.7	26.2
21	74.0	0.1	1.4	14.1	4.0	15.4	14.0	123	33.3	78.6
*	2	0.102	12	35	14	17	52	-		

Units for element results are in mg kg^{-1} , and for bioassays the mortality and inhibition are in percentage response

*Upper continental crust, Wedepohl 1995

(MacDonald et al. 2000). The TEC and PEC values have also been used as composite quotients that account for the presence of mixtures of contaminants in different concentrations that may have additive toxicity or other biological effects. The quotients of the elements average/PEC of this system for As, Cd, Pb, and Zn are 17, 3.8, 37, and 6, respectively. According to the reference values, As, Cd, Pb, and Zn are the most enriched in the sediments studied in this system and harmful effects are likely to be observed. Cu is enriched compared with the TEC limits. However, near the abandoned installations, the maximum contents are high for As, Cd, Cu, Pb, and Zn. Here, high toxicity was detected compared with the quality guidelines for metals in freshwater sediment (MacDonald et al. 2000) and are up to 500 times higher compared with the average for the Earth's crust (Wedepohl 1995; Table 2). Chromium is less enriched in the system compared to the TEC and PEC, though if it is compared with the average for the Earth's crust (Wedepohl 1995), it shows enrichment. Cobalt is not enriched in the system. Neither element represents a risk in the system. The contents of the LC_{50} of *D. magna* and EC_{50} of *S. capricornutum* are presented (Table 2; Ecotox database 2004) to be compared with the contents obtained in this study. The contents of As, Cd, Cu, Pb, and Zn of this study are more concentrated than the LC_{50} and EC_{50} compared with them.

PTEs concentration and their ratio with mortality of *D. magna* and inhibition of *S. capricornutum*

The results of the element concentrations in the sediments of each sample and the mortality of *D. magna* and inhibition of *S. capricornutum* are in Table 2. The mortality of *D. Magna* was 10 to 70 % (Table 2; Fig. 2). Despite the great quantities of PTEs, in some cases, they are not toxic for these organisms. This could be due to the chemical species of the PTEs and also

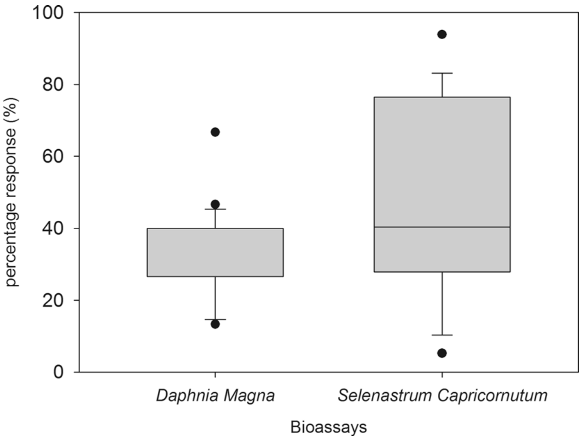

Fig. 2 Percentages of mortality of *D. magna* and inhibition percentages of S. *capricornutum* in the sediments sampled

because other materials in the sediment may modify the toxic effect of the elements, in some cases inhibiting their toxicity. This is documented in the samples evaluated. The sediments affected by the acidic mine drainage (AMD) discharge, which had a high concentration of metals, were shown to be toxic to the *D. magna* (Lattuada et al. 2009). The depuration time evaluated for the PTEs added to *D. magna* in the laboratory shows that Cd is not eliminated by the organism, unlike Zn, which has a significant depuration time of 6 days (Guan and Wang 2004).

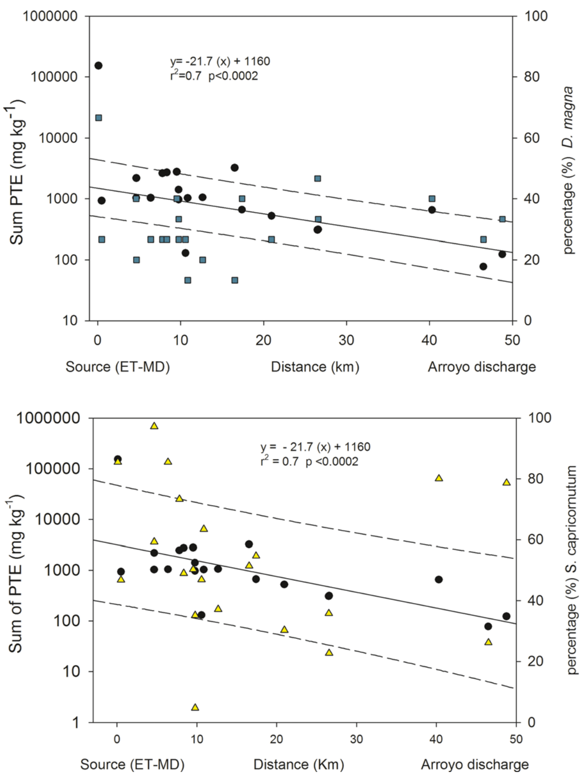

Fig. 3 Sum of the trace elements (PTEs: mg kg^{-1}) in sediments from the source to the destination. *White circles* inside the prediction intervals are the station results that were used to determine the equation and their correlation of potential toxic elements vs. distance. The *squares* are the percentages of mortality of *D. magna*, and the *triangles* are the percentages of inhibition of *S. capricornutum*

Bioassays result vs distance and vs concentration of PTEs

As a general behavior, the sum of the PTEs shows a decrement from the source to destination (Table 2; Fig. 3). The anthropogenic influence and the dilution from the source to the destination are evident.

The results of the mortality and inhibition of *D. magna* and *S. capricornutum* were relatively high in all the samples; their association with the concentration of PTEs (Fig. 4) shows direct ratio (r^2=0.45,

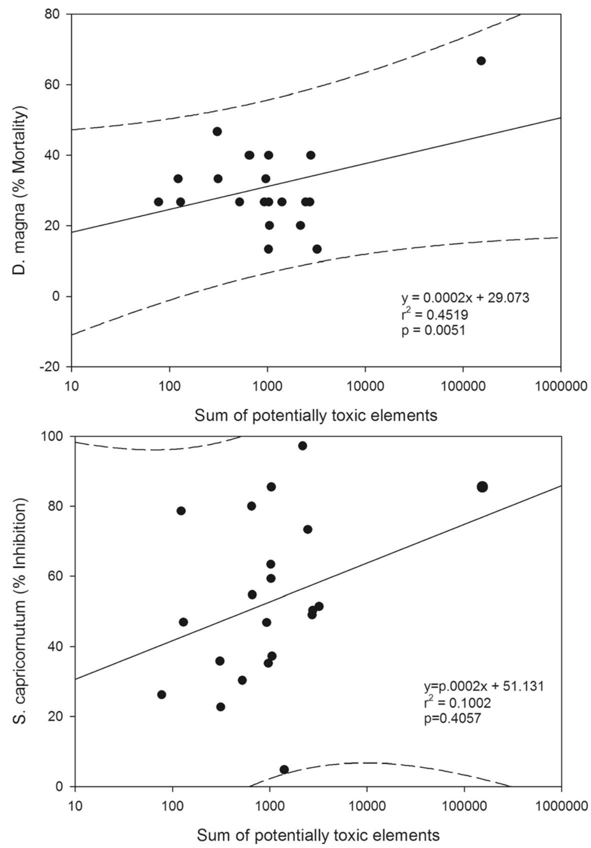

Fig. 4 Sum of the trace elements (PTEs: mg kg^{-1}) in sediment of the trace elements, and their ratio with the *D. magna* mortality and *S. capricornutum* inhibition

$p<0.005$; Fig. 4) *D. magna* mortality and the PTEs. However, *S. capricornutum* did not show the same behavior ($r^2=0.10$, $p=0.405$; Fig. 4). This could be caused because the oxidation states of the PTEs have different effects with the organisms studied here. In PTEs such as As, its natural form is mineralized into arsenopyrite, which includes high concentrations of S and Fe and is less toxic than the PTEs in tailings. In the tailings, it forms oxides, and in the ash, arsenolite, which is the most toxic form because of its high solubility in water. Thus, the toxicity depends on the process. In this study area, high concentration of As, Cd, Sb, Hg, Pb, Zn, and other potentially toxic elements to the gold mining are associated. The total concentration of PTEs in sediment is not an indicator of toxicity (MacDonald et al. 2000); they can be associated with stable mineral phases, or by the contrary, bioavailable in the environment (López-González et al. 2006). Although the bioavailability of PTEs in those sediments is different than in controlled conditions, the bioassays with *S. capricornutum* provide information about the bioavailability of PTEs. Thus, the results of the mortality and inhibition of the organism in ratio with the PTEs show synergy (sample 21; Table 2), and antagonism processes (sample 9; Table 2) probably are occurring. However, the low inhibition (sample 9) also could be due the elements are in stable mineral phases.

Studies of *S. capricornutum* to evaluate anthropogenic effects show that the growth inhibition test with *S. capricornutum* occurs on dilution of the sample; therefore, it is suitable for determination of heavy metal toxicity of a polluted matrix (Ivanova and Groudeva 2006). More studies on the natural sediments impacted by mining activities are necessary to evaluate the bioaccumulation of the PTEs in organisms and to determine which of them are concentrated in the food chain.

Conclusions

The variability of the concentration and the complex association of the potentially toxic elements are different in each environment, which is why the bioassays are necessary applied directly to evaluate the sediment toxicity in a determined system. In this study area, As, Cd, Cu, Pb, and Zn are more enriched than PEC in the sediments along the arroyo (Table 3). Those elements are more concentrated in sediments near the abandoned installations of the mining district, representing more risk in the system. Copper is more enriched than the TEC in the whole of the system, and also represents a risk. *D. magna* had percentage mortality in all the samples, with the maximum percentage mortality occurring in the sediments of the mining district. The

Table 3 Mean, standard deviation, maximum, and minimum concentration (mg kg^{-1}) of the elements determined

	As	Cd	Co	Cr	Cu	Pb	Zn
Av±*s*	555±1913	19±67	12±6	63±32	106±357	4790±20,146	2876±10,719
Min–Max[a]	7–8890	0.1–311	1–26	14–134	4–1660	11–92,700	14–49,600
Av/TEC[a]	57	19	nr	1.45	3.35	133	23.7
Av/PEC[a]	17	3.8	nr	0.56	0.71	37	6.3
TEC[b]	9.79	0.99	nr	43.4	31.6	35.8	121
PEC[b]	33.0	4.98	nr	111	149	128	459
Earth's crust[c]	2	0.102	12	35	14	17	52
D. magna LC_{50}48h μg L^{-1}	3300–4300	27–40	4400	22	33	3600–5300	100
Microalgae EC_{50} μg L^{-1}	690 96 h	452 72 h	nr	31–1195 72 h	392 72 h	1983–4539 <96 h	36–49 72 h

Quotients of the averages of the elements between the values calculated for freshwater sediment TEC and PEC (MacDonald et al. 2000). The LC_{50} calculated for *D. magna* (μg L^{-1}) and *S. capricornutum* in water (EC_{50} in μg L^{-1})

[a] This study

[b] *TEC* threshold effect concentration and *PEC* probable effect concentration in fresh water sediment (MacDonald et al. 2000)

[c] Average for the Earth's crust (Wedepohl 1995); LC_{50} of *D. magna* and EC of *S. capricornutum* (ECOTOX Database 2004); *LC* lethal concentration for 50 % of test organisms, *EC* effective concentration for 50 % of test organisms

S. capricornutum showed different sensitivities in the stations close to the tailings, decreasing along the 49 km of the arroyo, with the lowest inhibition of diluting toward the discharge of the arroyo; however, an accumulation is reflecting in the arroyo mouth indicating a risk in the sediments. The ratio between *S. capricornutum* with PTEs indicate that antagonism and synergy processes are involved. Those non-predictable behaviors indicate that the bioassays tests in this study are suitable for determination of toxicity of the natural sediments influenced by mine waste.

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