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Ecotoxicological Risks of the Abandoned F-Ba-Pb-Zn Mining Area of Osor (Spain)

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Abstract

Due to its potential toxic properties, metal mobilization is of major concern in areas surrounding Pb-Zn mines. In the present study, metal contents and toxicity of soils, water extracts from soils, and mine drainage waters from an abandoned F-Ba-Pb-Zn mining area in Osor (Girona, NE Spain) were evaluated through chemical extractions and ecotoxicity bioassays. Toxicity assessment in the terrestrial compartment included lethal and sublethal endpoints on earthworms *Eisenia foetida*, arthropods *Folsomia candida* and several plant species whereas aquatic tests involved bacteria *Vibrio fischeri*, microalgae *Raphidocelis subcapitata* and crustaceans *Daphnia magna*. Metal quantifications revealed high concentrations of Ba (250-5110 mg kg^{-1}), Pb (940->5000 mg kg^{-1}) and Zn (2370-11300 mg kg^{-1}) that exceeded intervention values to protect human health. Risks for the aquatic compartment were identified through the release of drainage waters and by leaching and run-offs from metal-contaminated soils. Cd (1.98-9.15 µg L^{-1}), Pb (2.11-326 µg L^{-1}) and Zn (280-2900 µg L^{-1}) in water samples surpassed international values of aquatic life criteria. Terrestrial ecotoxicity tests were in accordance with metal quantifications and identified the most polluted soil as the most toxic. Avoidance and reproduction tests with earthworms showed the highest sensitivity to metal contamination. Aquatic bioassays with extracts from soils confirmed the results from terrestrial tests and detected severe toxic effects caused by the mine drainage waters. Algal growth inhibition was the most sensitive aquatic endpoint. In view of the results, the application of a containment or remediative procedure in the area is encouraged.

Keywords: mine wastes, mine drainage water, Osor, ecotoxicity, soil contamination

1. Introduction

Once released into the environment, most metals cannot be degraded and are distributed through the different environmental compartments according to their mobility and bioavailability (Misra et al. 1994; Jung et al. 2002; Liu et al. 2003). Soils are considered major sinks for heavy metals, whose release is associated with the anthropogenic application of fertilizers, animal manures, sewage sludge or pesticides, and with inadequate disposal of mine wastes among others (Khan et al. 2008; Zhang et al. 2010). The impact of mining activities on a given site is ultimately controlled by climate, mining methods, geological conditions, and whether the mine is active or abandoned (Bell et al. 2001). However, some common procedures like the accumulation of large volumes of tailings (residues formed during the processing of the mineral ore) in steep stock piles can increase the environmental risk posed by a mining area. Under these storage conditions, those residues are prone to erosion (Henriques and Fernandes 1991) and might be dispersed to soils, surface and ground waters, and stream sediments of the surrounding area through atmospheric emissions, mechanical dispersion or water-leaching (Johnson et al. 1994; Adriano 2001).

The abandoned Osor mining district lies some 35 km SE of Girona, in the La Selva basin and Montseny-Guilleries massif, which forms part of the Catalonian Coastal Range (CCR) in the NE section of the Iberian Peninsula. In this area, the exploitation of F-Ba-Pb-Zn ores until 1980 generated important amounts of mine-waste impoundments with high contents of cadmium, lead, zinc and other metals. Due to the lack of containment of the mining wastes prior to the closure of the mine, metal contamination is affecting surface waters, groundwater, sediments and soils located in the vicinity (Navarro et al. 2011; Navarro et al. 2015). The generation of neutral mine drainage waters within the area is also a major cause of concern due to their potential to mobilize metalloids such as As, Sb, Se and metals such as Cd, Pb, and Zn (Heikkinen et al. 2009; Jang and Kwon 2011; Plante et al. 2011). High Fe, Mn, Ni, Pb and Zn concentrations were already reported by Navarro et al. (2015) in the main dewatering system of the Osor area, which discharges its waters directly into a natural creek.

The environmental risks of metal-contaminated sites were traditionally assessed by chemical extractions. However, it was concluded that this approach did not provide enough information about the bioavailability of metals and was not able to reflect the toxicity of all substances in soil, the synergic and antagonistic effects of contaminants and their interactions with the soil matrix and test organisms (Gruiz 2005). In this context, the application of batteries of terrestrial ecotoxicity tests gained special relevance as complementary tools able to report realistic, non-overestimated effects of contaminated sites to soil organisms (Alvarenga et al. 2012; Bes et al. 2014; Bori and Riva 2015, Bori et al. 2015). At the same time, aquatic bioassays traditionally applied for the toxicity determination of aquatic pollutants (Lopez-Roldan et al. 2012) or industrial effluents (Riva et al. 1993; Riva and Valles 1994; Riva et al. 2007) were incorporated to assess the impacts of soil composition and runoffs on receiving waters (Loureiro et al. 2005a; Rocha et al. 2011).

With this in mind, the aim of this study was to help in the assessment of the environmental risks posed by the abandoned mining site of Osor. To do so, metal quantifications and ecotoxicological bioassays were applied to soils from the area and to their water extracts as well as to waters from the mine drainage system. Terrestrial tests studied the mortality, the inhibition of reproduction and the avoidance response of *Eisenia foetida*, the avoidance response of *Folsomia candida* and the germination and growth rates of different plant species. Impacts on the aquatic compartment were measured through the luminescence inhibition of bacteria *Vibrio fischeri*, the growth inhibition of microalgae *Raphidocelis subcapitata* and the mortality of crustacean Daphnia magna.

2. Materials and Methods

2.1 Study area and sampling sites

The Osor vein deposit is located 4 km SE of Anglès town (NE Spain) and includes several geologically similar and thick (1–4 m) fluorite-barite-sphalerite-galena veins exploited to a depth of 300 m. Gangue minerals included quartz, barite, calcite, pyrite, and silicates (mainly muscovite, albite and biotite). The exploitation of these veins concluded in 1980, after reaching yearly productions of 20000–30000 t of fluorite, 2000 t of Pb concentrates, and 3000 t of Zn concentrates. The Osor flotation tailings are homogeneous in grain size and composition and occupy an area of 3150 m² with a mean thickness of 15 m. Mine drainage is performed through the Coral adit, which drains the Osor vein system with an estimated discharge into the Osor creek between 300 and 1100 m³/day of metal-contaminated, near-neutral mine waters (Navarro et al. 2015). In addition, episodic discharges of contaminated sediments and draining waters from the Osor tailings area also occur.

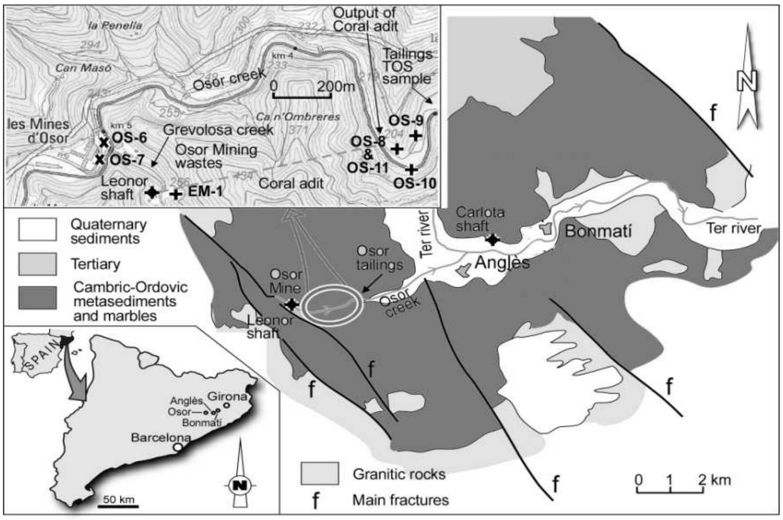

Figure 1. Location map and synthetic geology of the study area. Adapted from Navarro et al. (2015).

Three samples were collected from different sites within the Osor mining area: a sample of soil from a mine waste dump (sample EM-1) located close to the main extraction area (Leonor shaft), a sample of flotation tailings (TOS sample), and a sample of alluvial soil (sample OS-6) collected near Osor creek (Figure 1). Each sample was composed of 4 sub-samples collected within the same site and thoroughly mixed.

2.2 Soils and mine wastes collection and analysis

Soil samples were collected from a depth of 0-0.25 m, crushed, homogenized and subjected to physical and chemical characterization. The following physical-chemical characteristics were evaluated: pH (KCl, 1 mol L^{-1}), electrical conductivity (EC)(1:5 soil:water suspension), soil organic matter (SOM)(by loss on ignition at 550ºC for 2 hours) and texture (by the Pipette method). Samples for ecotoxicity testing were sieved through a 4 mm mesh and kept refrigerated at 4ºC until use.

Identification and analysis of mineral phases from selected samples were performed in the laboratories of the University of Barcelona (UB) by X-ray diffraction (XRD). Once the materials had been dried and ground, their geochemical compositions were analyzed using instrumental neutron activation analysis (INAA) by Actlabs (Ontario, Canada). The following elements were studied: Au, Ag, As, Ba, Br, Ca, Co, Cr, Cs, Fe, Hf, Hg, Ir, Mo, Na, Ni, Rb, Sb, Sc, Se, Sn, Sr, Ta, Th, U, W, Zn, La, Ce, Nd, Sm, Eu, Tb, Yb, and Lu. In addition, the concentrations of the following elements were determined by acid digestion and subsequent analysis by inductively coupled plasma atomic emission spectrometry (ICP-AES): Ag, Cd, Cu, Mn, Mo, Ni, Pb, Zn, Al, Be, Bi, Ca, K, Mg, P, Sr, Ti, V, Y, and S.

2.3 Water samples collection and analysis

Water extracts from the sampled soils were obtained according to the British Standard EN 12457-2 (2002). Samples were incorporated into 2-L glass vessels at a ratio of 1 kg/10 L, corresponding to 0.1 kg of soil per liter of deionized water. Vessels were placed at a rotating apparatus and mixed during 24 hours at a temperature of 20±2ºC. After a settling period of 15 minutes, samples were centrifuged (2000g, 10 minutes) and filtered through a 1µm pore size membrane filter. Additionally, a water sample from the output of the Coral adit (CA sample) was collected in a high-density polypropylene bottle, sealed with a double cap and stored in a refrigerator until analysis. Samples for metal analysis were filtered through a 0.45 µm pore size cellulose nitrate membrane, acidified and sent to Actlabs (Ontario, Canada). The following elements were analyzed by ICP-MS: Li, Na, Mg, Al, Si, K, Ca, Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ge, As, Se, Br, Rb, Sr, Y, Zr, Ag, Cd, Sn, Sb, Te, I, Cs, Ba, Hg, Pb, and REE. These determinations were compared to the reference sample NIST 1640 to confirm accuracy. The pH, electrical conductivity (EC) and total organic carbon (TOC) of the water samples were determined with a pH-meter, a conductivity meter and a Total Organic Carbon Analyzer TOC-VCSH (SHIMADZU, Japan) respectively.

2.4 Terrestrial ecotoxicity tests

Direct toxicity bioassays were performed using whole soils. When dilution was needed, test soils were mixed with an artificial soil (69% quartz sand, 20% caolinite clay, 10% finely ground sphagnum peat, 1% calcium carbonate and pH adjusted to 6.0 ± 0.5)(ISO 17512 2011) that also acted as control. In order to obtain different percentages of effect that allowed the calculation of effective and lethal median values (EC50 and LC50), test concentrations ranged from 0 to 100% of sampled soils mixed with artificial soil. All soil bioassays were carried out at 40-60% of their water holding capacity. When possible, EC50 and LC50 values were expressed as the percentage of sampled soil mixed with artificial soil (w/w) that reduced by 50% the endpoint measured.

Earthworms from the species *Eisenia foetida* and collembolans from the species *Folsomia candida* were obtained from synchronized cultures maintained at the Centre for Research and Innovation in Toxicology of the Technical University of Catalonia in Terrassa. Earthworms were cultured in 30-liters breeding boxes and a 1:1 mixture of horse manure and peat. Only clitellate adults between 300 and 600 mg of weight were selected for the performance of the tests. Earthworms were acclimated in control soil during 24 to 48 hours prior to the beginning of the tests. Collembolans were cultured in vessels filled with a substrate of plaster of Paris and charcoal (8:1, w/w) at 20±2ºC. Individuals were fed twice a week with granulated dry yeast added in small amounts to avoid spoilage by fungi. Organisms between 10 and 20 days old were used for toxicity testing.

2.4.1 E. foetida acute toxicity test

Acute toxicity tests with earthworms were adapted from the OECD 207 (1984) guideline. Ten organisms were placed in plastic containers (140x140x80 mm) containing 500 g (dry weight (dw)) of test soil. Test containers were kept under constant light (400-800 lux) at a temperature of 20±2ºC. Survival was determined after 7 and 14 days of exposure. Each test ran with 4 concentrations (12.5-25-50-100%) plus a control and three replicates per treatment. When two consecutive concentrations resulted in 0 and 100% mortality, those values were considered sufficient to indicate the range within which the LC50 fell (OECD 207 1984).

2.4.2 E. foetida reproduction test

Effects on the reproduction of earthworms were studied by means of the OECD 222 (2004) guideline. Ten earthworms were placed in 1-L plastic containers filled with 500 grams of soil (dw). Test vessels were incubated in a controlled chamber at 20±2ºC and a 16:8 h light:dark cycle. Animals were fed weekly with 2 grams of moistened bread during 4 weeks. Surviving earthworms were sorted by hand after 28 days. Juvenile production was recorded after 56 days of exposure to test soils. Tests ran with 5 concentrations (1-2.56-6.4-16-40% for samples EM-1 and TOS and 1.28-3.2-8-20-50% for OS-6) and three replicates per treatment. Six replicates with artificial control soil were tested.

2.4.3 Avoidance tests with E. foetida and F. candida

Avoidance tests with *E. foetida* and *F. candida* were adapted from ISO 17512 (2008) and ISO 17512 (2011) standards respectively. Rectangular plastic containers (220x140x50 mm) were used in tests with earthworms while cylindrical vessels (diameter 8 cm; depth 8 cm) were selected for tests with collembolans. Test containers were divided into two equal sections by a vertically introduced plastic card. Each section (control and test) of the test containers was filled with 250 g dw (test with earthworms) or 30 g (wet weight)(test with collembolans) of the corresponding soil and the divider was removed. Ten adult earthworms or twenty adult collembolans were carefully placed on the line separating both soils. Test containers were covered with a transparent plastic lid and incubated for 48 hours in an environmental chamber at 20±2 ºC and under a 16:8h light:dark photoperiod. At the end of the test period the plastic card was reinserted and the number of individuals at each section was counted. In tests with collembolans, the soil from each section was carefully emptied into two different vessels and flooded with water. After gentle stirring, the animals floating on the water surface were counted. Avoidance tests with both species ran with 4 concentrations plus a control and three replicates per treatment. All the assays with collembolans as well as the assay with earthworms in OS-6 were performed in 30-45-67-100% of test soil mixed with artificial soil whereas tests with earthworms in EM-1 and TOS required lower test concentrations (7.5-15-30-40%). Results were expressed as percentage of individuals in the control section at the end of the test.

2.4.4 Seedling emergence and growth tests

Bioassays with plants were adapted from the OECD 208 (2006) guideline. The species *Brassica rapa*, *Trifolium pratense* and *Lolium perenne* were selected as test organisms. Plastic containers with 100 grams (wet weight) of test soil (without dilution) were prepared. Twenty seeds of each plant were sown in each test soil and in the control artificial soil (four replicates per treatment i.e. 5 seeds per test container). Tests were performed in an environmental chamber at 24±2ºC and under a 16:8 hours light:dark photoperiod. The moisture content and the number of sprouts were checked daily. Fourteen days after 50% of emergence in the controls, plants were harvested and weighed. Results were expressed as percentage of seed emergence and fresh biomass.

2.5 Aquatic toxicity tests

Water extracts from test soils and the water sample from the Coral adit were tested through indirect toxicity bioassays. When required, dilutions were prepared mixing water samples with the correspondent test medium. Toxicity results were expressed as the percentage of water sample in the test medium (V/V) reducing by 50% the endpoint measured (EC50 or LC50). Organisms from the species *R. subcapitata* and *D. magna* were cultured in the Centre for Research and Innovation in Toxicology of the Technical University of Catalonia in Terrassa.

2.5.1 Bacteria luminescence inhibition test

Acute toxicity to the bioluminescent bacteria *V. fischeri* was assessed in accordance with the ISO 11348 (2007) standard. Organisms were exposed to 4 concentrations of water extracts (5.63-11.25-22.5-45%) and the luminescence emitted was measured after 15 minutes with a Microtox® 500 system (Microbics©). Three replicates per treatment were analyzed.

2.5.2 Algal growth inhibition test

Effects on the growth of microalgae were assessed according to the OECD 201 (2011) guideline. Cultures of *R. subcapitata* were kept under constant illumination (4000-5000 lux) at a temperature of 20±2ºC. Only populations in the exponential phase were used in tests. Assays were carried out in tubes containing 9 mL of test solution and 1 mL of algal inoculums of known concentration that were placed in a controlled room at 20±2 ºC and under constant illumination (4000-5000 lux) and agitation. After 72 hours of incubation, the absorbance of each replicate was measured at 665 nm with a CECIL CE9200 spectrophotometer. Tests ran with three replicates per treatment and 7 concentrations (0.1-0.32-1-3.2-10-32-90%) plus a control that consisted in algae culture medium. In order to avoid interferences in the spectrometric measure of the water extracts at the end of the test, one extra tube was prepared with 9 mL of extract, 1 mL of culture medium and no algae. Results were expressed as percentage of algal growth inhibition.

2.5.3 Daphnia magna acute immobilization test

The acute toxicity test with *D. magna* was carried out according to the OECD 202 (2004) guideline. Bulk cultures of 15 daphnids were kept in 2.5 liters of ASTM hard synthetic water (ASTM 1988). Culture medium was changed three times per week and an organic extract and a concentrate of *Chlorella vulgaris* were added as food. Neonates were removed daily. Cultures were maintained at 20±2ºC in a 16:8h light:dark cycle. Only neonates with less than 24 hours old were used for toxicity testing. Assays were performed in glass tubes containing 10 mL of test medium and 5 daphnids. Test vessels were kept in an incubator at 21±2ºC and in the dark. Immobilization was visually recorded after 24 and 48 hours of exposure. Daphnids were exposed to 7 dilutions of water-extracts (1-2.2-4.8-10-22-48-100%) plus a control in four replicates per treatment. Mortality at the end of the test was expressed as a percentage.

2.6 Statistical analysis

Statistical analysis was performed using SPSS software (SPSS 15.0 for Windows; SPSS Inc., Chicago, IL, USA). Data were checked for their homogeneity of variances and normality. Differences between means were tested with one-way ANOVA. Whenever significant differences were found ($P < 0.05$), Tukey post-hoc test was applied to further elucidate differences. Non-normal data were transformed and when the assumption of normality was not reached, non-parametric Kruskall-Wallis tests alongside with Mann-Whitney post hoc tests were performed. Relationships between physicochemical properties, metal concentrations and ecotoxicological parameters were studied through the Pearson's correlation coefficient.

Data from avoidance tests were analyzed using the Fisher Exact test (Zar 1998), which compares the distribution of organisms between test sections with an expected distribution with no avoidance. A two-tailed test was used to check the homogeneous distribution of the organisms in dual-control tests whereas a one-tailed test was used in avoidance assays with the sampled soils.

Median effective, lethal and inhibitory concentrations (EC50, LC50 and IC50 respectively) and their 95% confidence intervals were calculated by Probit regression. A normal or logistic distribution was assumed depending on results from normality tests. Estimated values were compared using the Confidence Interval Ratio Test recommended by Wheeler et al. (2006).

3. Results and Discussion

3.1 Physicochemical characteristics and geochemistry of soils and mine wastes

All the studied soils presented similar physicochemical parameters. The pH was slightly acid in OS-6 (6.12), neutral in EM-1 (7.22) and moderately alkaline in TOS (8.12). The electrical conductivity was low in all the studied sites, with values of 278.5 µS cm^{-1}, 338.5 µS cm-^{1} and 439.5 µS cm-^{1} in OS-6, EM-1 and TOS respectively. Organic matter contents remained below 10% in all sites (3.40% in EM-1, 3.69% in TOS and 3.76% in OS-6), thus classifying the studied samples as mineral soils. Sand was the main component of all samples, which presented a loamy sand texture.

Total concentrations of metals and metalloids for which the Waste Agency of Catalonia has established General Reference Levels to protect human health (WAC 2015) are shown in Table 1. The results from chemical extractions revealed that, in some sites, Ba, Pb, Sb and Zn contents exceeded up to one order of magnitude the intervention values for soils under industrial use. The mine waste sample from the Osor sector (EM-1) was the most heavily polluted. The high amounts of Pb (>5000 mg kg^{-1}) in this site may be linked to the presence of argentiferous galena whereas Sb (56.5 mg kg^{-1}) contents might be linked to galena or undetected sulfosalts (Navarro et al. 2015). High concentrations of Zn (11300 mg kg^{-1}) and Cd (24.9 mg kg^{-1}) were also detected and associated with sphalerite. The Osor flotation tailings sample (TOS) contained lower amounts of Pb (940 mg kg^{-1}) and Zn (2370 mg kg^{-1}) although both metals markedly exceeded their intervention values (550 mg kg^{-1} and 1000 mg kg^{-1} respectively). On the other hand, TOS showed the highest Ba content of the area probably due to the concentration of gangue material in the flotation processes. Concentrations of Ba (2200 mg kg^{-1}), Pb (>5000 mg kg^{-1}), and Zn (2730 mg kg^{-1}) in the alluvial soil (OS-6) also exceeded the intervention values, which was unexpected due to its distance from the main mining areas.

Table 1. Total metal concentrations (mg kg^{-1} for all metals with the exception of Ti and Sn, which are expressed as %) in sampled soils. CAL: Catalonia intervention values for soils under industrial use.

	As	Ba	Be	Cd	Co	Cr	Cu	Hg	Ni	Pb	Sb	Se	Ti	V	Zn	Sn

EM-1	15.2	250	<1	24.9	6	<2	11	3	3	>5000	56.5	<0.1	0.03	4	11300	<0.01
TOS	12.5	5110	2	7.6	14	39	47	<1	18	940	1	<0.1	0.24	45	2370	0.02
OS-6	5.7	2200	3	11.5	9	44	24	<1	19	>5000	6.1	<3	0.21	48	2730	<0.01
CAL	30	1000	90	55	90	1000	1000	30	1000	550	30	70		1000	1000	

3.2 Physicochemical characteristics and hydrochemistry of aquatic samples

The water samples obtained from the Osor mining area presented similarities in all physicochemical parameters except for the electrical conductivity. All samples presented neutral pH, with values of 7.56, 7.77, 7.83 and 8.08 for EM-1, TOS, OS-6 and CA respectively. Total contents of organic carbon were low in the extracts from EM-1 and TOS (1.41% and 1.87% respectively) and increased in CA (2.96%) and in the extract from OS-6 (8.71%). Electrical conductivity was significantly lower in the extracts from test soils (63.50 µS cm^{-1}, 125.55 µS cm^{-1} and 156.45 µS cm^{-1} for OS, TOS and EM-1 respectively) than in the sample from the Coral adit (958 µS cm^{-1}).

Table 2. Total metal concentrations (µg L^{-1}) in water extracts from test soils and in the Coral adit (CA). US EPA: Aquatic Life Criteria for acute exposures by the United States Environmental Protection Agency (in µg L^{-1}).

	As	Ba	Be	Cd	Co	Cr	Cu	Hg	Ni	Pb	Sb	Se	Ti	V	Zn	Sn
EM-1	0.76	340	<0.1	9.15	0.4	<0.5	3.8	1.1	0.3	183	1.7	<0.2	5.1	0.2	498	<0.1
TOS	0.24	450	<0.1	3.64	0.36	<0.5	3.7	0.8	<0.3	3.33	0.52	<0.2	<0.1	<0.1	280	<0.1
OS-6	1.4	306	<0.1	2.34	2.03	1.7	18.4	2	3	326	1.52	1	15.6	3.6	901	<0.1
CA	1.59	34.1	-	1.98	19.1	-	7.3	-	17.9	2.11	-	4.4	-	-	2900	-
US EPA	340	-	-	2		570	-	-	-	65	-	-	-	-	120	-

Metal concentrations in water samples are shown in Table 2. Total contents of most metals in water extracts represented less than 1% of their soil contents, thus revealing their relatively immobility in soils. Even so, Ba, Pb and Zn presented high concentrations in water that were attributed to their soil contents. Ba concentrations were similar between water extracts and ranged from 306 µg L^{-1} (OS-6) to 450 µg L^{-1} (TOS). Despite its tendency to be adsorbed by Fe and Mn oxyhidroxides and the low solubility of Pb sulfate and hydroxycarbonate, Pb contents in EM-1 and TOS reached values of 183 µg L^{-1} and 326 µg L^{-1} respectively. Zn presented the highest concentrations in water extracts (280 to 901 µg L^{-1}), which was in accordance with its abundance in soils. As seen in soils, the TOS sample presented markedly lower Pb and Zn concentrations (3.33 µg L^{-1} and 280 µg L^{-1} respectively) than EM-1 (183 µg L^{-1} and 498 µg L^{-1} respectively). In contrast, the highest Pb and Zn concentrations (326 µg L^{-1} and 901 µg L^{-1} respectively) were detected in the extract from the alluvial soil (OS-6), suggesting a higher risk of metal leaching from this site. Such risk was associated to the lower acidity and higher contents of sand in the site, which can facilitate metal solubilization and leaching (Navarro Flores and Martínez Sola 2010). The sample collected from the mine dewatering system (CA) presented metal contents that fell within the rank quantified in water extracts. The only exceptions were Ba and Zn, whose concentrations in CA (34.1 µg L^{-1} and 2900 µg L^{-1} respectively) were one order of magnitude lower (Ba) and higher (Zn) than their contents in the extracts from contaminated soils. The low concentration of barium in the water from the Coral adit was attributed to its sedimentation throughout the mine drainage system whereas the high concentration of Zn was associated to its precipitation as a secondary phase (carbonate, hydroxide, etc). In view of the results from metal quantifications, Cd, Zn and Pb were considered the contaminants of greatest environmental concern because they exceeded the aquatic life criteria of the US Environmental Protection Agency (2016) in most water samples. These criteria establish the highest concentration of specific pollutants that are not expected to pose a significant risk to the majority of species in a given aquatic environment.

3.3 Ecotoxicological evaluation

Terrestrial and aquatic ecotoxicological bioassays were successfully applied. Marked differences in sensitivity were appreciated depending on test endpoints and organisms. Within the bioassays with earthworms, lethality tests were not sensitive enough to estimate LC50 values for any of the studied soils whereas reproduction and avoidance tests detected toxicity in all samples (Table 3). The exposure of *E. foetida* to EM-1 and TOS in acute tests caused 40% of mortality when the sample was not diluted, with 69.76% and 74.31% average decrease in the body mass of test organisms respectively. Mortality was not appreciated when earthworms were exposed to OS-6. In contrast, reproduction tests with earthworms presented an extreme sensitivity and estimated EC50 values of 1.05%, 1.48% and 1.09% for EM-1, TOS and OS-6 respectively. The avoidance behavior of earthworms was a slightly less sensitive endpoint than reproduction and estimated EC50 values of 2.75%, 7.99% and 31.32% for EM-1, TOS and OS-6 respectively. Even so, avoidance tests proved to be a very valuable tool for the risk assessment of metal-contaminated soils because they were able to reduce the duration of the assay to 2 days. Additionally, the studied samples were considered to have a limited habitat function because more than 80% of the test organisms preferred the control soil instead of the test soils at the end of the assay (Hund-Rinke and Wiechering 2001). Results from terrestrial bioassays with earthworms were in agreement with previous studies that highlighted the higher sensitivity of sub-lethal endpoints in the ecotoxicological evaluation of contaminated soils (Hund-Rinke et al. 2002; Davies et al. 2003; Bori et al. 2016). On the other hand, avoidance tests with the soil arthropod *F. candida* were not able to detect toxicity in any of the studied samples. The lower sensitivity of collembolans in comparison with earthworms was already reported by Natal da Luz et al. (2012), Hentati et al. (2013), and Bori et al. (2016) after studying avoidance responses in soils contaminated with pesticides, petroleum compounds and metals respectively. Even more, collembolans showed a significant attraction towards the contaminated section of the containers that increased while increasing test concentrations. Such response was previously reported after exposure to the pesticide Dimethoate although it was attributed to the incapability of collembolans to escape from the contaminated soil (due to the effects of the pesticide in their nervous system) rather than an attraction towards it (Pereira et al. 2013). Since the presence of metals is not reported to have such effects on the nervous system of collembolans, we concluded that the observed response was associated to the lower sensitivity of arthropods.

Table 3. EC50 values (95% confidence intervals) of terrestrial ecotoxicity tests with *E. foetida* expressed as percentage of soil sample mixed with ISO artificial soil (w/w).

	EM-1	TOS	OS-6
Reproduction inhibition	1.05 (0.48-1.56)	1.48 (0.57-2.52)	1.09 (0.11-1.94)
Avoidance response	2.75 (1.61-7.12)	7.99 (4.84-11.15)	31.32 (24.66-37.9)

Percentages of seedling germination and growth of the selected plants in undiluted test soils are depicted in Figure 2. Seedling emergences were high in controls (95% to 100%) and were not completely inhibited in any of the tested soils. Even so, statistically significant differences ($P < 0.05$) in germination rates were detected between species and soils. The emergence of *B. rapa* and *T. pratense* was statistically inhibited in EM-1 (germination rates of 60% and 20% respectively) and TOS (60% and 40% respectively) whereas only *T. pratense* was inhibited in OS-6 (60%). The germination of *L. perenne* was not inhibited in any site. No statistical differences in emergence rates were detected between EM-1 and TOS while germination of *B. rapa* and *T. pratense* was significantly higher in OS-6 than in EM-1. The observed inhibition of plant emergence was associated to As contents, which showed a negative and statistically significant correlation with germination rates ($r = -0.999$; $P < 0.01$). Despite remaining below the intervention value established by the Waste Agency of Catalonia, As concentrations in the studied sites were in accordance with EC10 and EC50 ranks (1.95-568.12 mg kg^{-1} and 14.86-795 mg kg^{-1} respectively) derived from experiments conducted with different plant species in As-contaminated soils (Sun et al. 2012). Between species, *T. pratense* was most sensitive to the contaminated soils, followed by *B. rapa* and *L. perenne*. The same sensitivity ranking was reported by Ramírez et al. (2008) and Bori et al. (2016) after exposing the same species to sewage sludge and metal-contaminated soils respectively. Regarding plant growth, all species were significantly inhibited in EM-1 and TOS whereas only *L. perenne* growth was inhibited in OS-6. No statistical differences in the average fresh biomass of *B. rapa* and *T. pratense* were detected between sites EM-1(28.45 mg and 1.95 mg respectively) and TOS (35.91 mg and 5.93 mg respectively) whereas the growth of *L. perenne* was significantly higher in TOS (17.86 mg) than in EM-1 (10.93 mg). Similarly to seedling emergence, seedling growth in OS-6 was statistically higher than in EM-1 and TOS. Among the studied species, *B. rapa* showed the highest growth in all sites although *T. pretense* was most sensitive to the presence of contamination.

In this study, results from terrestrial ecotoxicity bioassays were in accordance with metal quantification by chemical analysis and linked metal concentration with toxicity to terrestrial organisms. EM-1 was the most heavily polluted and toxic soil although all sites were considered to have a limited habitat function and posed a risk to terrestrial organisms. The toxicity revealed by soil bioassays was mainly attributed to Pb and Zn contents throughout the area. Pb levels in the studied sites markedly surpassed (EM-1 and OS-6) or were close (TOS) to the EC50 for reproduction tests with earthworms (1068 mg Pb kg^{-1}) established by Savard et al. (2007) in natural soils artificially contaminated with $Pb(NO_3)_2$. Similarly, Zn concentrations were markedly higher than the rank of EC50 values established by Scheffczyk et al. (2014) for avoidance and reproduction tests with earthworms in several natural soils artificially contaminated with zinc nitrate-tetrahydrate (46.77-362.42 mg Zn kg^{-1} dry soil and 216.10-475.97 mg Zn kg^{-1} dry soil for avoidance and reproduction tests respectively). Even so, toxicity comparisons between soils freshly spiked with metals and those historically contaminated must be performed with caution since aging is known to reduce the bioavailability of metals in soils (Lock and Janssen 2003). Between sites, the higher toxicity of EM-1 was associated to the significantly higher presence of metals in the site. Besides Pb and Zn, Sb content in EM-1 was also high and close to the EC50 for reproduction tests (70 mg Sb kg^{-1}) established by Kuperman et al (2006) after contaminating a natural soil with antimony sulfate. The toxicity of tailings (TOS sample) to terrestrial organisms was lower than expected despite the accumulation of metals that occurred during the concentration of gangue material in flotation processes. The low toxicity of tailings was mainly attributed to the lower contents of Pb and Zn, which were previously separated and extracted from the gangue material. The concentration of Ba, on the other hand, clearly surpassed the EC50 for reproduction tests with earthworms (664 mg Ba kg^{-1}) established by Kuperman et al (2006). The authors consider that the lack of a higher response of earthworms to TOS due to its Ba content was attributed to the low bioavailability of Ba, which might have been influenced by decades of aging while stored in stock piles. Moreover, germination and growth rates of plants in TOS were surprisingly high according to the low fertility associated to tailings, which are known to lack several parameters needed for the proper development of flora (Dudka and Adriano 1997). On the contrary, the alluvial soil (OS-6) presented higher concentrations of metals and consequently higher toxicity than expected according to the location of the sampling site, which was farther from the main extraction and waste storage sites. Metal concentrations and toxicity values in this site suggest that the sample might have been collected above and old mine waste impoundment, thus highlighting the lack of control on the management of mining wastes and the risk of contaminating the nearby creek.

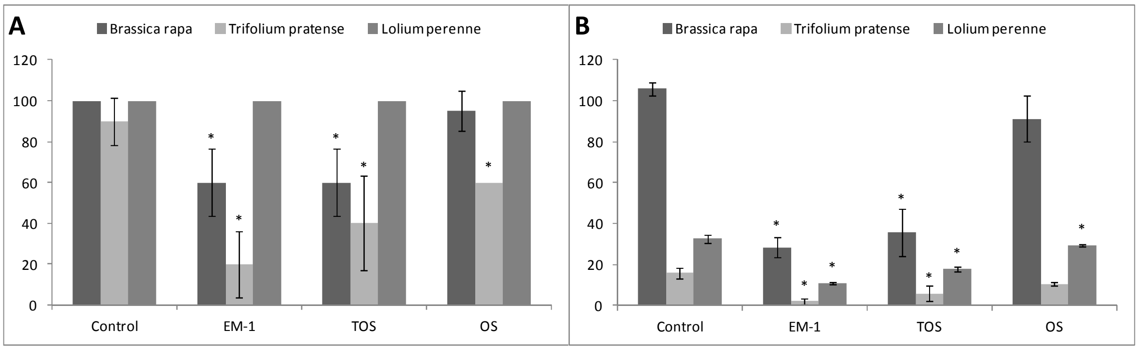

Figure 2. *Brassica rapa, Trifolium pratense* and *Lolium perenne* seedling emergence (in %)(A) and fresh biomass (in mg)(B). Means and standard deviations from four replicates. .'*' means statistically different from control ($P < 0.05$).

The toxicity of water samples to aquatic organisms is shown in Table 4. The bioluminescence inhibition of *V. fischeri* was the least sensitive endpoint and showed no detrimental response to any of the water samples. The lesser sensitivity of *V. fischeri* luminescence towards leachates from mine soils was previously documented (Alvarenga et al. 2008; Teodorovic et al. 2009; Maisto et al 2011; Bori et al. 2016) and might be related with the pH correction of the leachate suggested by standard methods (Alvarenga et al. 2013). As previously reported by several authors (Maisto et al. 2011; de Paiva Magalhães et al. 2014; Bori et al. 2016), algal growth inhibition showed the highest sensitivity towards metal-contaminated water samples. Half maximal inhibitory concentrations (IC50) were estimated for all samples and were significantly higher (P<0.05)(i.e less toxicity detected) in water extracts from TOS and OS-6 sites (13.2% and 14.1% respectively) and in waters from the Coral adit (20%) than in the extract from EM-1 (3.7%). The toxicity of the different samples to *R. subcapitata* was explained by their Cd content, which showed a positive and statistically significant correlation (r=0.99; P<0.05) with algal growth inhibition. *D. magna* showed moderate sensitivity to metal contamination and estimated similar LC50s for samples EM-1 and CA (67% and 57% respectively). The exposure of daphnids to water extracts from TOS and OS-6 caused no mortality to test organisms.

Table 4. IC50 and LC50 (95% confidence intervals) of aquatic ecotoxicity tests expressed as percentage of water extract in test medium (V/V). "-": non-applicable.

	Water Extracts			
	EM-1	TOS	OS-6	CA
Algal Growth Inhibition (IC50)	3.7 (2.7 - 5.2)	13.2 (10.0 - 17.9)	14.1 (7.6-28.1)	20.4 (9.2-46.5)
Daphnia Immobilization (LC50)	67 (49 - 101)	-	-	57 (39.3-101)

The ecotoxicological evaluation of water extracts from sampled soils confirmed the results from terrestrial tests and identified the sample EM-1 as the most toxic. Among the metals of greatest environmental concern (Cd, Pb, and Zn), Cd was considered to have a major role in the toxicity exerted by water extracts due to its concentration (surpassing the US EPA criterion in all samples) and the significant correlation found with the toxicity to algae. However, cadmium alone was not sufficient to explain the observed toxicity since Cd contents in the extracts were markedly lower than IC50 and LC50 values estimated for *R. subcapitata* (67 µg L^{-1}) and *D. magna* (101.17 µg L^{-1}) by Rodgher et al. (2012) and Shaw et al. (2006) respectively. Nonetheless, previous studies by Biesinger et al. (1986) and Barata et al. (2002) reported additive effects in Cd-Zn mixtures that led to a toxicity increase, which could better explain the toxicity of the extracts. Additionally, Pb contents might have contributed to algal growth inhibition in TOS and OS-6 extracts, where Pb concentrations were one order of magnitude higher than the IC50 (83.9 µg L^{-1}) reported by De Schamphelaere et al. (2014). On the other hand, the influence of Pb in the lethality *D. magna* was considered negligible since Pb concentrations were markedly lower than the LC50 established by Fagašová A (1994)(19498 µg Pb L^{-1}). The toxicity associated to the water from the mine dewatering system (CA sample) was attributed to Zn, whose concentration widely exceeded the IC50 (100 µg L^{-1}) and LC50 (819.99 µg L^{-1}) estimated for *R. subcapitata* and *D. magna* by Kasemets et al. (2003) and Shaw et al. (2006) respectively.

4. Conclusions

The application of chemical extractions and ecotoxicity tests to soils from the Osor mining area revealed that this abandoned mine site poses an important risk to the surrounding environment due to its high contents of metals. Metal contamination derived from past mining activities was high in those sites where mine wastes (EM-1) and flotation tailings (TOS) were abandoned although threatening contamination levels were also reached in sites where no mining-related activities were expected (OS-6). Ba (250-5110 mg kg^{-1}), Pb (940->5000 mg kg^{-1}) and Zn (2370-11300 mg kg^{-1}) concentrations in soils were of greatest environmental concern because they all exceeded the General Reference Levels to protect human health established by the Waste Agency of Catalonia. Besides soils, metal contamination also affected or is likely to affect the aquatic compartment either through the leaching of metals towards the mine dewatering system or through run-off from metal-contaminated soils. The studied draining waters and water extracts from contaminated soils presented patterns of metal contamination similar to soils, with concentrations of Cd (1.98-9.15 µg L^{-1}), Pb (2.11-326 µg L^{-1}) and Zn (280-2900 µg L^{-1}) that surpassed international values of aquatic life criteria.

The application of terrestrial ecotoxicity tests confirmed the results from chemical extractions and linked metal concentrations with toxicity to soil organisms. All the studied soils caused detrimental effects to earthworms although toxicity was mainly attributed to Pb and Zn contents. Additionally, As contents had a negative impact in the development of plants. Among terrestrial ecotoxicity tests, sublethal endpoints with *E. foetida* and emergence and growth of plant species were the most sensitive endpoints and should be prioritized when aiming to directly assess the toxicity of metal-contaminated soil samples. Aquatic bioassays were in accordance with terrestrial tests and identified the sample from the main waste dump (EM-1) as the most toxic. Toxicity in the aquatic compartment was again related with Pb and Zn contents although Cd content was found responsible of algal growth inhibition. The water sample collected from the drainage system was heavily polluted by Zn (2900 µg L^{-1}) and toxic to aquatic organisms. The liberation of contaminated mine waters through the adit is of special concern because they reach the Osor creek and can further spread metal contamination downstream. Among the applied aquatic bioassays, the growth inhibition of *R. subcapitata* was the most sensitive endpoint whereas the luminescence inhibition of *V. fischeri* showed no responses and is not recommended in further analysis of metal-contaminated water samples. In view of the results from our study, the abandoned mining area of Osor is considered to pose an important environmental threat and the application of a containment or remediative procedure in the area is encouraged.

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Compliance with ethical standards

The authors declare that they have no conflict of interest.

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