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Ecotoxicity assessment of primary producers in metal mining areas: biological indicators for ecosystem restoration

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ABSTRACT

Abandoned mines represent a considerable risk to ecosystems surrounding former exploitation sites. In metal mining, the exposure of waste significantly increases the mobility and bioavailability of potentially toxic elements (PTEs), affecting adjacent soils and organisms. Assessing the toxicity of mining waste involves challenges related to selecting appropriate bioassays and those recommended by current environmental regulations. Considering the expected increase in metal and metalloid extraction to supply critical raw materials, improving our understanding of the advantages and limitations of specific bioassays is essential for accurate risk assessment. Three representative abandoned metal mining sites in the Iberian Peninsula were selected to apply and compare different bioassays for a robust ecotoxicological assessment of mining waste. Total and soluble PTEs concentrations at all sites significantly exceeded geochemical threshold values (GTVs) and water quality standards. Bioassays using *Lepidium satuvium, Spirodela polyrhiza* and *Raphidocelis subcapitata* revealed that acidic conditions combined with elevated PTEs concentration (e.g., Cd, Pb), are highly toxic to primary producers. Conversely, root growth measurements suggested that low soluble concentrations of metalloids (As, Sb) may stimulate root development. Overall, the results indicated that Sb was not a major contributor to observed toxicity in the bioassays under the studied conditions. The Zucconi test showed low sensitivity and reliability, limiting its suitability for risk assessment. Moreover, stimulation effects observed in the algal bioassay question its effectiveness for delineating contaminated areas, as they may lead to false negatives. Therefore, combined bioassay approaches are recommended to avoid underestimation of toxicity. Tailings and dumps from the three mines were classified at least as moderately toxic, particularly in areas affected by acid mine drainage (AMD), identified as the main factor increasing toxicity.

1. Introduction

The pollution caused by PTEs in mining environments poses a significant hazard to ecosystems and the human population. Prolonged exposure to certain PTEs, such as As, Cd or Pb, is related to the appearance of negative effects on health and the development of carcinogenic diseases (Cheng et al., 2023). This risk is increased when mobility and availability are high, which are conditioned by soil characteristics like pH, organic matter content (OM), texture, electrical conductivity (EC), redox potential, cation exchange capacity (CEC), microorganisms, etc.

In the European legislative framework, the recent Soil Monitoring Directive (EU 2025/2360) (European Commission, 2025) establishes common criteria for identifying contaminated soils across member states. These criteria include the identification of contaminants, evaluation of exposure and assessment of potential toxicity. Similarly, other international frameworks such as the Soil Screening Guidance, carried out by the Environmental Protection Agency (U.S. Environmental Protection Agency, 1996), are primarily based on total element content and do not define toxicity-based control values for soils. However, total content does not necessarily reflect bioavailability or mobility of PTEs (Bori et al., 2016), two critical factors for assessing toxicity in organisms. Previous studies have provided valuable insights into environmental assessment through chemical indices (Wang et al., 2025, Yüksel and Ustaoğlu, 2025). Nevertheless, these studies do not directly assess bioavailability or biological response. In this sense, bioassays provide a

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complementary approach for assessing adverse consequences to organisms (Paniagua-López et al., 2023). Within this framework, in Spain, the Royal Decree 9/2005 (BOE, 2005), introduced bioassays as a recommended and complementary tool to characterize potentially contaminated soils.

To complement chemical analysis, the use of primary producers, such as unicellular green algae, for impact assessment and environmental monitoring is a widespread practice (Andreu-Sánchez et al., 2022). Their study is particularly relevant because primary producers constitute the first link in the food chain and represent a major pathway for the entry of toxic substances (Soliman et al., 2022), potentially posing risks to higher trophic levels, including humans, due to biomagnification processes (Martínez-Colón et al., 2021; Yazman et al., 2025).

For primary producers, certain elements can act as essential trace elements, nonetheless, prolonged exposure to elevated concentrations may cause chronic effects or even mortality (Collin et al., 2022), making then useful bioindicators in ecotoxicological studies. Conversely, various plant species are capable of accumulating high levels of PTEs in tissues without exhibiting damage (Rascio and Navari-Izzo, 2011), which confers a considerable ecological advantage in contaminated environments.

In this context, numerous locations, affected by historical mining activities, such as the Iberian Pyrite belt, Sierra Morena or Sierra Minera mining district, retain mining liabilities from former exploitations that pose environmental risks due to elevated concentrations of PTEs. The lack of treatment increases the hazard to surrounding areas. This dispersion is aggravated in semi-arid climates, where the limited vegetation cover and episodic torrential rains favour the transport of PTEs-enriched sediments and waters.

Although several geochemical soil and water analyses have been carried out in these mining districts (Murciego et al., 2007; Rodríguez et al., 2009, 2022; García-Lorenzo et al., 2009; Garrido et al., 2021; Álvarez-Ayuso et al., 2022) the evaluation of ecosystem impacts remains limited. Regarding ecotoxicological studies, previous works have been focused on assessing the effects of mining waste on the development and accumulation of PTEs in tissues of spontaneously growing species (Murciego et al., 2007; Martínez-Sánchez et al., 2012; Álvarez-Ayuso et al., 2013; Higueras et al., 2017; Pérez-Sirvent et al., 2012; Garrido et al., 2021). Other works have evaluated toxicity towards seed germination, primary consumers, microbial diversity or the luminescence inhibition produced in marine bacteria (García-Lorenzo et al., 2009, 2019; Bes et al., 2014; Peco et al., 2020; Gallego et al., 2021; Ferri-Moreno et al., 2023).

The aim of this study is to evaluate the application of different bioassays that allow an accurate characterisation of the ecotoxicological risk posed by mining liabilities to primary producers in metal mining operations. To achieve this overall objective, three areas of study have been selected: San Quintín (As-Pb-Zn mine), San Francisco Javier (Pb-Zn mine) and San Antonio (W-Sb). The comparative study aims to determine the most efficient combination of bioassays for the evaluation of toxicity of the mining sites, as well as the principal factors involved in the increase in toxicity, especially physicochemical parameters. The specific objectives are as follows:

I) Geochemical and mineralogical characterisation of the waste to determine the total content of PTEs and the degree of pollution in comparison to the surrounding areas.
II) Determination of available PTEs through leachates, to provide the potential toxic effect of the studied materials.
III) Determination of growth inhibition in aquatic plants (*Spirodela polyrhiza*), freshwater algae (*Raphidocelis subcapitata*), and cress (*Lepidium sativum*) germination and root development.
IV) Sample categorisation based on the total content of PTEs and their ecotoxicological risk.

The generation of new knowledge will contribute to the identification of suitable bioindicators for environmental monitoring and support decision-making in waste management and pollution mitigation strategies. Although several studies have addressed the effects of critical raw materials like Sb on vegetation and plant physiological mechanisms, the application of standardized bioassays to Sb-enriched samples remains limited. In this context, the evaluation of its contribution to toxicity in standardized bioassays would provide new knowledge referred to toxic responses. Given the increasing exploitation of Sb due to its economic importance, understanding its actual contribution to toxicity in soils is imperative. The obtained results may be helpful improving ecological risk assessment and management strategies, in line with in the One Health framework (WHO, n.d.) and Sustainable Development Goals (SDGs).

2. Materials and methods

2.1. Study area and sampling design

The study areas were selected because of their AMD generation capacity, the presence of high contents of toxic elements like As or critical raw materials like Sb. The sites present diverse mineral paragenesis with variable total content of PTEs (As, Cd, Pb, Sb and Zn), but similar climate context governing their bioavailability.

The San Quintín mine (Mining district of the Alcudia Valley, Ciudad Real) shows a mineral paragenesis composed of sulphides (such asgalena (PbS) and sphalerite (ZnS)) (Reguilón Bragado et al., 1992). Its exploitation was primarily focused on the extraction of Cu, Fe, Pb and Zn (García-Lorenzo et al., 2019). The last mining operations were the re-work of former tailings as well as the processing of cinnabar from the Almadén mining area (Gallego et al., 2021), when Hg-enriched residues were produced.

A major environmental problem in the area is the generation of AMD (pH $<$ 4) which favours the dispersions of PTEs and has a detrimental effect in watercourses and water bodies. The Arroyo de la Mina (tributary of the Tirteafuera river) flows in the vicinity of the S. Quintín and is influenced by the occasional inflow of water from the contaminated area, conditioned by the continental semi-arid Mediterranean climate (average annual rainfall of 500-700 mm) (García-Lorenzo et al., 2019).

The San Francisco Javier mine (Sierra Minera mining district, Murcia) shows a main mineral paragenesis dominated by silicates and sulphides (as pyrite (FeS_2), sphalerite and galena) (Pérez-Sirvent et al., 2012). The area exhibits similar geochemical characteristics as described in S. Quintín, with elevated load presence of As. The high AMD generation is conditioned by the arid Mediterranean climate (average rainfall less than 400 mm per year) (García-García et al., 2004), characterised by torrential rainfall with high capacity of transport.

The San Antonio mine (Badajoz) is characterised by a mineral paragenesis of scheelite ($CaWO_4$) and stibnite (SbS) (Álvarez-Ayuso et al., 2013). The exploitation (W-Sb) worked during the last century until its abandonment. The low generation of AMD in this area is constrained by the presence of carbonates (Ferri-Moreno et al., 2025), which prevents acidity, and the precipitations (average annual rainfall of more than 600 mm (Garrido et al., 2021)).

The mines of S. Quintín and S. Antonio are located close to rural areas, where agricultural and livestock activities currently represent the primary economic drivers. In contrast, S. Francisco J. is located in a mining-affected area where tourism is the main economic activity. Dispersal in these abandoned mining works is both physical and chemical (Fig. 1) and can contribute PTEs to nearby soils through particle transport (aeolian or runoff) or through the arrival of leachates.

A stratified sampling was carried out, taking a total of 23 samples (Fig. 2) of tailings (n = 6), dumps (n = 6), contaminated soils (n = 6) and reference soils (n = 5). A shovel or an Eijkelkamp driller (for fine-grained loose materials) were used. Three subsamples were taken as composite samples (2-25 cm), after discarding the litterfall and

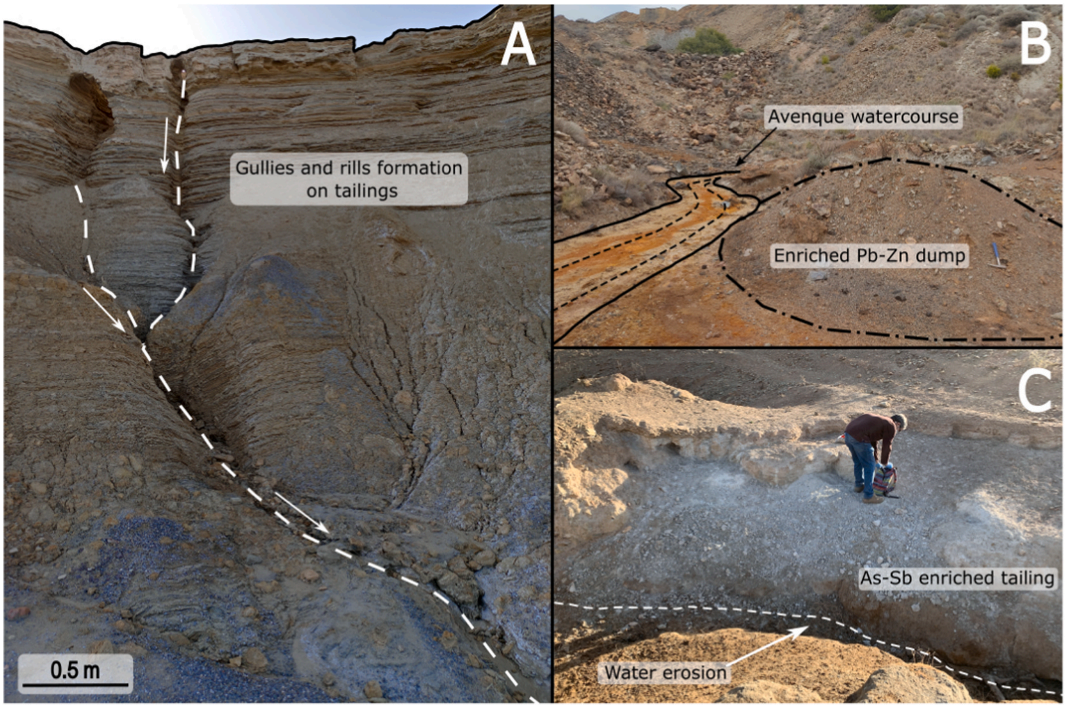


Fig. 1. Representative photographic panorama of the studied mining areas, highlighting environmental concerns. A) Water erosion in tailings from S. Quintín mine, showing runoff pathways toward the Arroyo de la Mina stream. B) The Avenque watercourse crossing through waste materials in S. Francisco J. mine. C) An As-Sb enriched tailing near the mine entrance at S. Antonio area.

vegetation cover. Materials were air-dried in the laboratory and then sieved through 2 mm mesh.

2.2. Mineralogical and geochemical characterisation

The mineralogical semi-quantification was performed on 1 g (<53 μm) using a Bruker D8 Advance® diffractometer equipped with a Cu anticathode. Diffractograms were obtained under the following conditions: 2θ angles (2° - 65°), 0.02 stepping interval and 1 s. The crystalline phases were determined following the Chung method (Chung, 1974a, 1974b, 1975).

For total content of PTE, aliquots of each sample were analysed by Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF) using a Bruker, S2 Ranger spectrometer with a Pd detector. The pH and EC were measured in 1:5 suspensions (w/v) (AENOR, 2019) (UNE-EN ISO 21268-4:2019) using a HANNA HI 9811-5 multiparametric system. The organic matter (OM) content was measured on 5 g dried aliquots based on the ASTM D 2974.

2.3. Leaching procedure

Leachates 10:1 (water:solid) from each sample were obtained according to DIN, 1984 and poured into 1 L PET sealed bottles and agitated for 24 h at room temperature in a laboratory Rotary Shaker at 20 rpm (Heidolph™). The leachates were allowed to settle for 2 h at 4 °C and then the supernatant was collected. For the algae bioassay, leachate aliquots were centrifugated (3500 rpm for 15 min) followed by filtration through a 0.45 μm nitrocellulose syringe filters (Millipore) to achieve the necessary transparency.

Soluble contents in leachates were determined using an ICE 3300 spectrometer (Thermo Fisher, USA) with flame atomic absorption spectrometry (FAAS) for Cd, Fe, Pb and Zn and hydride generation atomic fluorescence spectrometry (HG-AFS) (PSA Millennium Excalibur 10.055) for Sb and As. Quality control and assurance included multipoint calibrate curves ($R^2 > 0.995$), triplicate measurements for each sample and method blanks.

2.4. Ecotoxicity methodology

2.4.1. Seed ecotoxicity bioassay

The seed plate-based germination bioassay was carried out according to Boluda et al. (2011). Fifty g of sample were moistened with deionised water to achieve 70 % of its water holding capacity (WHC) and placed in Petri dishes. Ten cress seeds (*Lepidium sativum*) were added per dish and incubated at 25 °C in a plant growth chamber (Sanyo® model MLR351) for 3 days in darkness. All assays were conducted in triplicated including the blank controls with a reference soil.

After incubation, germinated seeds were counted and the relative seed germination (RSG %) (Equation (1)) was calculated based on the control (100 %).

$$RSG\ (\%) = \frac{germinated\ seeds}{germinated\ seeds\ in\ control} x\ 100 \qquad \text{[Eq.1]}$$

The Zucconi test (1981) was performed to assess phytotoxicity of soluble PTEs on seeds. Ten seeds of *Lepidium sativum* were placed on filter paper in Petri dishes and completely moistened with 4 mL of leachate. Controls were prepared using deionised water. All dishes incubated at 25 °C in darkness for 3 days in the plant growth chamber. Then, the number of germinated seeds was counted, and the root length was measured. Relative seed germination (RSG) [Eq. (1)], relative radicle growth (RRG) [Eq. (2)] and the germination index (GI) were determined [Eq. (3)]. The germination of the control was the 100 %.

$$RRRG\ (\%) = \frac{mean\ radicle\ lenght\ in\ contaminated\ extract}{mean\ radicle\ lenght\ in\ distiller\ water} x\ 100 \qquad \text{[Eq. 2]}$$

$$GI\ (\%) = \frac{RSG\ x\ RRG}{100} \qquad \text{[Eq. 3]}$$

2.4.2. Growth inhibition test with Spirodela polyrhiza

The bioassay was performed using the commercial Duckweed

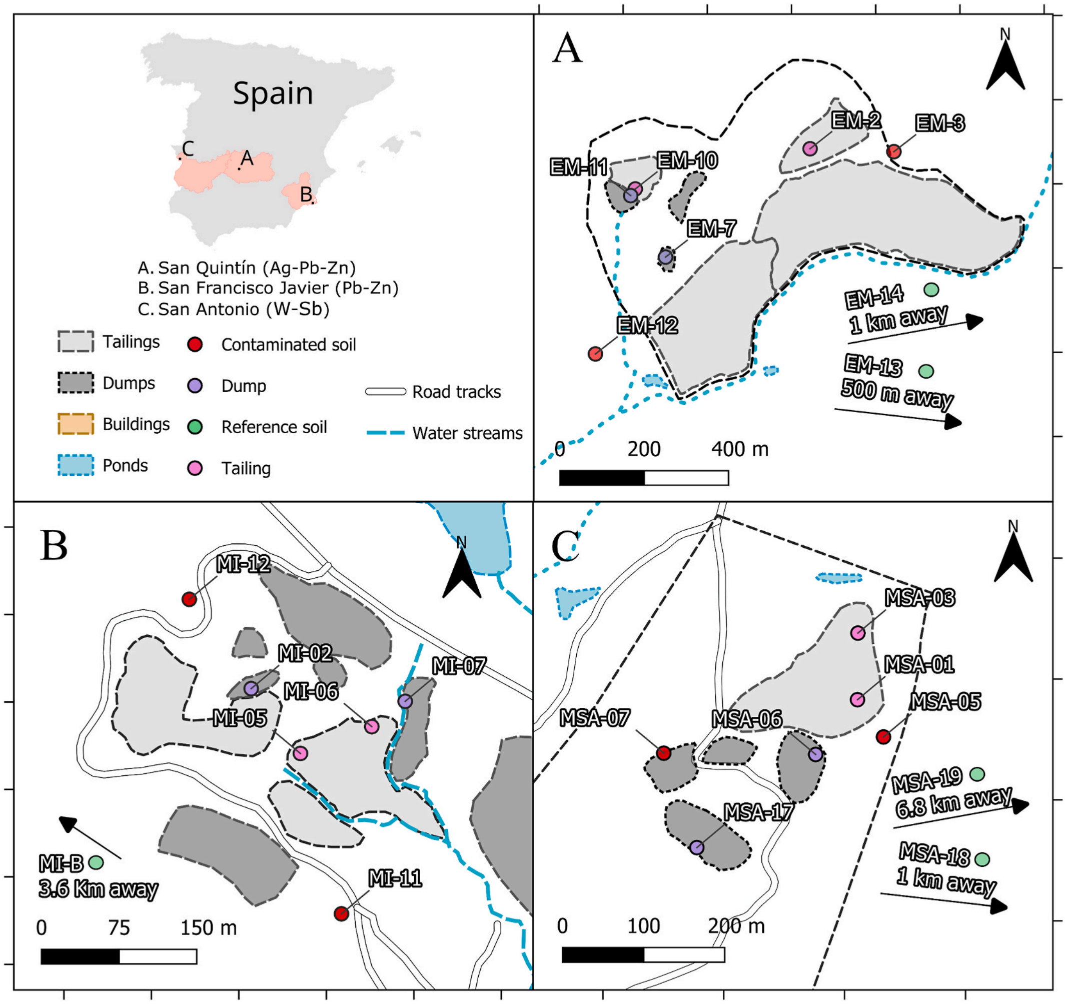


Fig. 2. Sample locations in the study areas. Contaminated and reference soil samples were collected from the surroundings of the mines. A) S. Quintín mine (October 2022). B) S. Francisco J. mine (January 2024). C) S. Antonio mine (August 2024).

Toxkit™ (Microbiotest Inc., Ghent, Belgium) following ISO 20227:2017 (AENOR, 2018). *S. polyrhiza* turions were germinated in Petri dishes in *Steinberg* growth medium at 25 °C under continuous illumination (6000 lux) for 3 days in an ARTI-180L climatic chamber (Microbiotest Inc, Ghent, Belgium). Germinated turions were transferred into 48-well test plates (Corning™) containing serial leachate dilutions (50 %, 25 %, 12.5 %, 6.25 %, 3.12 %) and *Steinberg* growth medium as a control. An initial photograph with a scale reference was taken. Plates were incubated under the same conditions and periodically rotated to ensure uniform light exposure. After 72 h, a final photograph was taken to estimate duckweed growth by measuring the size of the first frond and comparing it to the control.

2.4.3. Algal growth inhibition test

The freshwater algal growth inhibition test was conducted following OECD TG 201 (OECD, 2011) and ISO 8692 (ISO, 2012), using the Algaltoxkit F™ (Microbiotest Inc., Ghent, Belgium). *Raphidocelis subcapitata* alginate beads were de-immobilised in growth medium. Algal density was measured at 670 nm using an Aurius™ 2021 spectrophotometer (CECIL Instruments, Cambridge, UK). Four serial dilutions (50 %, 25 %, 12.5 %, 6.25 %) were prepared and triplicate 100 mL Erlenmeyer flasks were filled with 75 mL of each dilution and subsequently inoculated with the algal suspension (a 1:100 ratio) to reach 10^4 cell mL^{-1}. The flasks were incubated at 25 °C, 4000 lux for 72 h in a climatic chamber, with periodic agitation and random repositioning. Algal density was measured every 24 h to calculate growth rate. Aliquots of coloured leachates were used as a background reference in the spectrophotometer measurements.

2.5. Data treatment

The effective concentration (EC_{50}) for each test was calculated from inhibition values across the serial dilutions. Calculations were performed using a Probit regression model, as recommended by ISO and OECD guidelines (OECD, 2011). To facilitate interpretation, toxic units (TU) were calculated for each bioassay using the following equation:

$$TU = \frac{100}{EC_{50}} \qquad \text{[Eq. 4]}$$

To complete the ecotoxicological risk assessment, the samples were classified according to Lopes et al. (2025). The detailed classification procedure is explained in the Supplementary Material.

To provide a more comprehensive and globally comparable assessment, toxicity values for aquatic organisms were classified according to Persoone et al. (2003), a widely used framework. This classification facilitates their comparison with other studies. The classes are defined as follows: TU < 0.4 = no acute toxicity (I); 0.4 < TU < 1 = slight acute toxicity (II); 1 < TU < 10 = acute toxicity (III); 10 < TU < 100 = high acute toxicity (IV); TU > 100 = very high acute toxicity (V).

A complete statistical analysis was performed using Statgraphics 19 software package (Statgraphics Technologies, The Plains, VA, USA). Correlation analysis ($p < 0.05$), principal component analysis (PCA), and factor analysis were performed to identify latent relationships among variables. After verifying data normality, either ANOVA or Kruskal-Wallis tests were applied as appropriate.

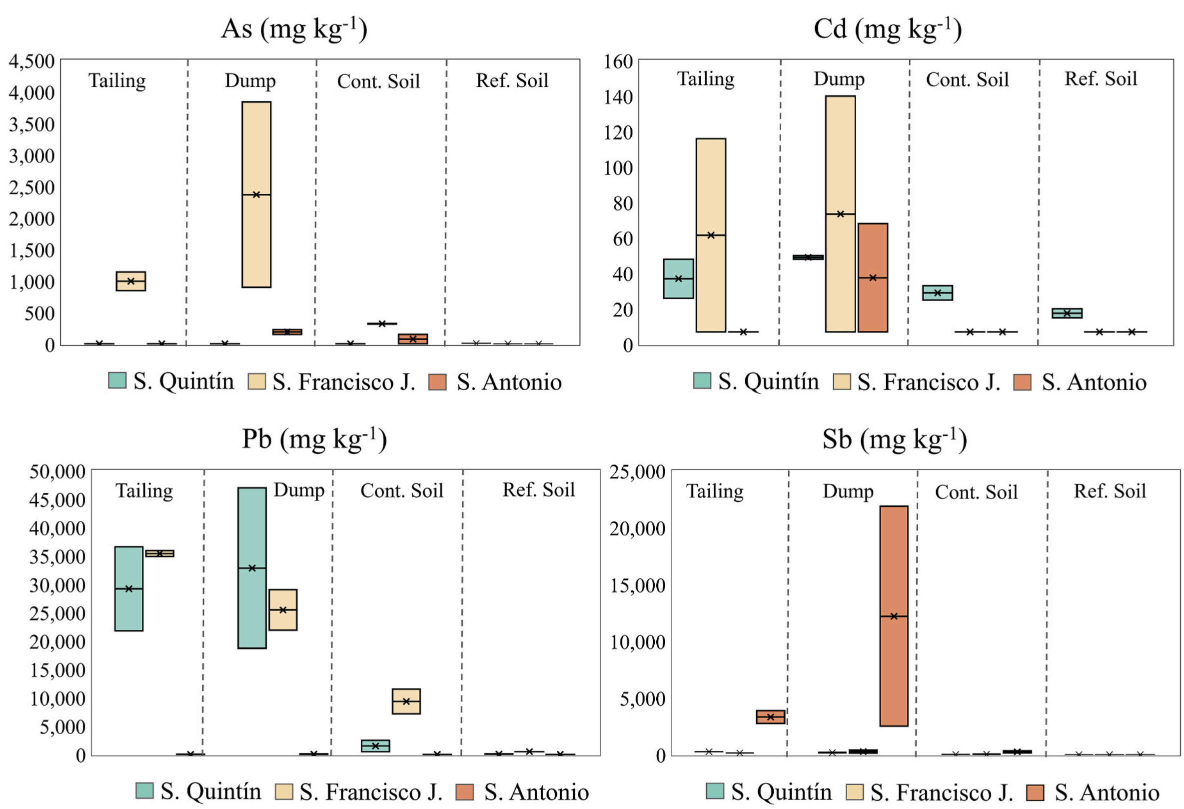
3. Results and discussion

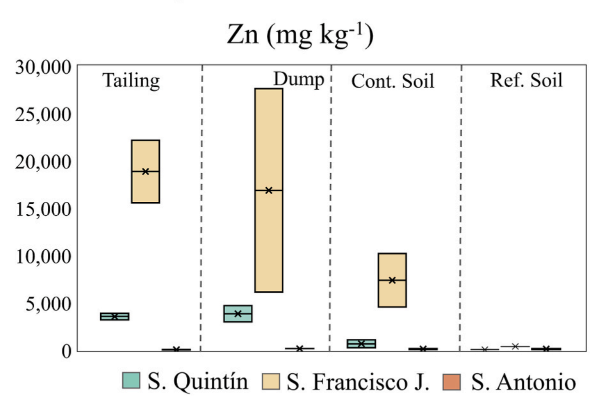
3.1. Mineralogical and geochemical characterisation of samples

The mineral phases (Table S2) commonly present in all three locations are quartz, felspar, plagioclase and clay minerals. In S. Quintín and S. Francisco J. small quantities of secondary sulphate phases, are present in tailing and dumps samples. The appearance of these minerals is in concordance with high contents of PTEs. In S. Quintín, tailings reached 29,200 mg kg^{-1} Pb, 3550 mg kg^{-1} Zn and 37 mg kg^{-1} of Cd, while dumps displayed levels of 32,850 mg kg^{-1} Pb, 3850 mg kg^{-1} Zn and 49 mg kg^{-1} Cd (Fig. 3). By contrast, contaminated soils showed average concentrations of 1491 mg kg^{-1} Pb and 679 mg kg^{-1} Zn, whilst reference soils exhibited values below 75 mg kg^{-1} in both cases. In S. Francisco J., tailings and dumps reached extremely high concentrations of Pb (35,415 and 25,482 mg kg^{-1}), Zn (18,880 and 16,887 mg kg^{-1}) and As (996 and 2370 mg kg^{-1}).

No W-Sb primary phases were detected from S. Antonio. However, carbonate and dolomite phases were described by XRD in tailing and dump samples. This explains the attenuation of AMD at S. Antonio by providing a buffering capacity. In this site, As reached 153 mg kg^{-1} in dumps, while Sb reached 3316 mg kg^{-1} in tailings and 12,196 mg kg^{-1} in dumps. Contaminated soils showed 117 mg kg^{-1} As and 250 mg kg^{-1} Sb, while reference soils were below detection limits.

The total contents determined in S. Quintín, S. Francisco J. and S. Antonio, exceed the Geochemical Threshold Values defined for Castilla La Mancha (defined by Jiménez Ballesta et al., 2010), Murcia Region (Sánchez and Sirvent, 2007), and those defined for Extremadura (Decree 49/2015, DOE, 2015), respectively (Fig. 3). This confirms the severe degree of contamination in the areas.





Reference	Geochemical threshold values (mg kg^{-1})				
	As	Cd	Pb	Sb	Zn
Jiménez Ballesta et al., 2010	16.1	4.4	44.2	5.7	86.5
Sánchez & Sirvent, 2007	16	0.5	57	2	90
Junta de Extremadura, 2015	61	0.35	48	1	281

Fig. 3. Total content of PTEs in the three areas. The reduced variability of the lowest values due to analytical constraints compresses graphical data. The application of stratified sampling enables the visualisation of the overall trends in the load of PTEs. The GTVs defined for the regions where mines are located are included. Cont. Soil = contaminated soils; Ref. Soil = reference soils.

3.2. Natural mobility and leachate characteristics

Table 1 presents the pH, EC, and soluble content of leachates. S. Quintín and S. Francisco J. samples exhibited acidic conditions (especially in tailings and dumps) with a pH average of 3.2 and 3.5 in tailings, respectively. The acidity of is consistent with the presence of minerals such as anglesite ($PbS0_4$), jarosite ($KFe_3^{3+}(SO_4)_3(OH)_6$) and kintoreite ($PbFe_3(PO_4)$ (PO_3OH) $(OH)_6$), identified by XRD. The mineral association is typically present in areas exhibiting acidification and oxidation processes (Parbhakar-Fox, 2016; Hong et al., 2024). By contrast, S. Antonio samples were classified as slightly acid (average pH of 6.2), attributable to the carbonate buffering. The average EC in S. Quintín and S. Francisco J. tailings (3460 and 6340 μS cm^{-1}) is remarkably higher than those observed in S. Antonio (860 μS cm^{-1}), indicating greater ionic load.

Concentrations in samples showed a clear trend, with tailings and dumps exhibiting higher soluble contents than soils in general. The low solubility in soils may be related to the presence of organic compounds, Fe oxyhydroxides and clay minerals, acting as immobilising agents. Concentrations of soluble Zn were the highest, especially in S. Francisco J. (average of 1560 mg L^{-1} in tailings) and S. Quintín (161 mg L^{-1}) followed by Cd and Pb. Their mobility was probably favoured by the acidic environment. In both areas As showed concerning soluble concentrations (average of 21.5 and 3.75 μg L^{-1} in tailings, respectively), whereas in S. Antonio, Sb soluble concentration reached 791.5 μg L^{-1} in tailings and 914.7 μg L^{-1} in dumps.

All the analysed values exceeded the environmental quality standards according to Royal Decree (817/2015) (BOE, 2015), which sets out the criteria for monitoring and evaluating surface water and environmental quality standards in Spain. Specifically, the maximum permissible concentration for Cd (0.45 and 1.5 μg L^{-1} for weakly and high mineralised water, respectively) and Pb (14 μg L^{-1}) are exceeded in leachates from all three mines, and for Zn (30 and 500 μg L^{-1} for weakly and high mineralised, respectively) in waste materials from S. Quintín and S. Francisco J. The values established for As (50 μg L^{-1}) are not exceeded in any case. These results suggest the existence of a high risk to ecosystems. However, the leaching procedure applied (DIN, 1984) may overestimate the natural conditions of PTEs bioavailability due to its conservatism. Therefore, the implementation of different approaches may change the potential toxicity of the leachates.

3.3. Ecotoxicity assessment

3.3.1. Phytotoxicity to cress seeds (Lepidium sativum)

The Zucconi test showed higher seed germination percentages (mostly 90 % to 100 %) than the plate-based test (Table S3). This suggests that either insufficient phytotoxic substances are present (Aguerre and Gavazzo, 2012; Zucconi, 1981), or that their mobility from the solid matrix is restricted. This low bioavailability may be limited by the adsorption of PTEs onto clays, organic matter or Fe-Mn oxyhydroxides.

The measure of radicle growth exhibited stimulation processes (Table S3 and Fig. S1) (GI > 100%) that were not bound to a specific material. This complicated the establishment of a direct toxic-effect relationship between the soluble PTE concentrations and radicle elongation. The stimulation could be due to the presence of certain toxic elements, that can induce stimulatory effects at low doses (Baderna et al., 2015; Bożym and Rybak, 2024), or be influenced by factors that cannot be excluded by the applied methodology, such as nutrient availability, salinity and other physicochemical factors (Belz and Cedergreen, 2010). The positive correlations ($p < 0.05$) between As and GI (Table S4) suggest that the low concentration of the element has a stimulating effect on *L. sativum* seeds. Conversely, only 19 % of the samples from the areas with AMD generation capacity, exhibited some degree of toxicity (GI < 100 %). In S. Antonio only one contaminated soil showed low phytotoxicity (GI = 67%). The Pearson correlation ($p < 0.05$) showed a negative relationship between GI and Cd, Zn, Fe and EC, and a positive relationship with pH, indicating an inhibitory effect of leachates with high salt and PTE concentrations and acidic character. Sb was not significantly related to any parameter. However, the effect of the combination of multiple PTEs is not straightforward, as mixtures can exhibit additive, synergistic, or antagonistic interactions, potentially reducing or enhancing the toxicity (Baderna et al., 2015; Šestinova et al., 2015).

On the other hand, the plate-based test revealed a different toxicity

Table 1
Soluble content (As, Cd, Fe, Sb, Pb, Zn), pH and EC of leachates used in algae, Zucconi test and duckweed bioassay. LoQ = limit of quantification; Cont. Soil = Contaminated soil; Ref. Soil = Reference soil.

Sample	Type	As (μg L^{-1})	Soluble content					pH	EC (mS cm^{-1})
			Cd (μg L^{-1})	Fe (μg L^{-1})	Sb (μg L^{-1})	Pb (μg L^{-1})	Zn (μg L^{-1})		
	San Quintín mine								
EM-02	*Tailing*	<LoQ	1.2E+03	5.2E+01	2.5E-01	2.7E+03	1.9E+05	3.9	2.45
EM-10		4.4E-01	9.2E+02	1.8E+05	1.1E+00	2.0E+03	1.4E+05	2.5	4.47
EM-07	*Dump*	<LoQ	2.4E+03	1.8E+02	6.1E+00	2.5E+03	2.1E+05	4.5	2.16
EM-11		<LoQ	1.0E+03	2.4E+02	<LoQ	2.4E+03	1.0E+05	4.2	2.37
EM-03	*Cont. Soil*	1.5E-02	4.6E+01	2.5E+03	8.6E-01	4.2E+02	4.1E+02	5.1	0.04
EM-12		5.6E-01	<LoQ	3.5E+03	7.8E-01	<LoQ	<LoQ	5.2	0.11
EM-13	*Ref. Soil*	<LoQ	<LoQ	2.6E+02	<LoQ	2.5E+02	<LoQ	5.6	0.14
EM-14		2.8E-01	<LoQ	4.4E+03	<LoQ	2.4E+02	<LoQ	5.6	0.03
	San Francisco Javier mine								
MI-05	*Tailing*	3.0E+00	5.0E+03	4.2E+02	<LoQ	2.2E+03	1.4E+06	3.4	6.03
MI-06		4.5E+00	7.4E+03	1.9E+02	<LoQ	2.2E+03	1.7E+06	3.5	6.65
MI-02	*Dump*	2.1E+01	9.9E+02	1.2E+02	3.9E+00	1.2E+03	1.6E+05	4.8	3.15
MI-07		1.2E+01	4.3E+02	2.6E+03	<LoQ	1.2E+03	8.5E+04	2.9	3.27
MI-11	*Cont. Soil*	9.8E+00	4.8E+00	1.5E+02	1.0E+00	1.2E+03	<LoQ	5.5	0.22
MI-12		1.0E+0.1	7.2E+00	2.2E+02	7.9E+00	6.2E+02	1.1E+02	5.7	0.36
MI-B	*Ref. Soil*	4.6E-01	<LoQ	1.7E+02	<LoQ	3.9E+02	<LoQ	6.0	0.16
	San Antonio mine								
MSA-01	*Tailing*	2.9E+01	1.8E+01	1.3E+02	7.9E+02	5.9E+02	<LoQ	5.8	0.27
MSA-03		1.4E+01	2.7E+00	8.0E+01	3.2E+02	9.3E+02	<LoQ	5.8	1.45
MSA-06	*Dump*	1.7E+00	<LoQ	9.0E+02	9.2E+02	<LoQ	<LoQ	6.1	0.15
MSA-17		3.1E+00	4.9E+00	1.4E+03	4.9E+01	3.4E+02	<LoQ	6.1	0.18
MSA-05	*Cont. Soil*	6.0E-01	2.0E+00	1.5E+03	3.1E+02	<LoQ	<LoQ	6.4	0.00
MSA-07		5.0E-01	1.0E+00	2.2E+02	3.5E+00	2.9E+02	<LoQ	6.4	0.04
MSA-18	*Ref. Soil*	<LoQ	<LoQ	1.5E+03	1.1E+00	2.9E+02	<LoQ	6.3	0.04
MSA-19		2.0E-01	6.0E+00	2.2E+03	<LoQ	<LoQ	<LoQ	6.3	0.01

profile, with several samples showing 100 % inhibition during the germination stage. The statistical analysis reflects a significant negative relationship between PTEs (excluding As and Sb), EC and RSG, whereas pH was positively correlated with RSG. The inhibition is more evident in tailing and dump samples from mines with AMD generation (which showed 0 % RSG) compared to S. Antonio, where only one tailing sample showed complete inhibition. Other authors have reported that factors such as texture and soil structure can inhibit seedling development (Alvarenga et al., 2008) and should be considered in risk assessment.

The results obtained demonstrated that, during the initial phase of plant growth, the seeds can germinate despite the impact of various factors such as PTEs content. This phenomenon has also been observed in research involving lettuce (*Lactuca sativa L.*) and cauliflower (*Brassica cretica*) seeds (You et al., 2019). The overall results from Zucconi test suggest that the methodology is not reliable for screening acute toxicity in seeds. This limitation is clearly reflected in Table S3, where most samples exhibit a RSG value of 100 % or near to it, but never falling below 70 %. Alternative options, such as the plate-based bioassay, must be considered.

3.3.2. Toxicity to aquatic plants (Spirodela polyrhiza)

The TU values for *S. polyrhiza* and the classification proposed by Persoone et al. (2003) are illustrated in Fig. 4. The results demonstrate a clear distinction in hazard level, with S. Quintín and S. Francisco J. exhibiting significantly greater toxicity than S. Antonio.

Based on the TU values (Table S5) and the classification, tailings and dumps from S. Quintín and S. Francisco J. are classified as extreme hazard (Class IV), where TU $>$ 30. Conversely, S. Antonio mine presents a generally lower risk profile, contaminated soils, tailings and dumps fall into the medium hazard (Class III) category, and the reference samples are categorised as low hazard (Class I). The statistical analysis revealed (Table S4) a highly negative correlation with pH and a strong positive correlation with EC, indicating the clear influence of these parameters on the growth of the aquatic plants, as noted by Paquet et al. (2019). Regarding the soluble PTE content, Pb, Cd and Zn showed a positive correlation with the toxicity, supporting a negative influence on growth. On the other hand, the limited mobility of elements in S. Antonio does not produce adverse effects on the growth of *S. polyrhiza* leaves. No statistical correlation was observed between toxicity and metalloids.

Comparing our results with those obtained in other studies, it was observed that only Zn exceeded the EC_{50} value defined by Baudo et al. (2015) (Zn = 2.29 mg L^{-1}). For Cd (0.31 mg L^{-1}) and Pb (6.7 mg L^{-1} for *L. minor*), none of the samples exceeded the values defined by Baudo et al. (2015) and Dirilgen (2011) respectively. Arsenic is an exception, given that other studies report higher tolerance thresholds for this element (0.18 mg L^{-1} according to Zhang et al., 2011). In fact, numerous studies consider *S. polyrhiza* and other aquatic plants to be suitable for the remediation of water contaminated by As (Rahman et al., 2007; Mishra et al., 2008; Zhang et al., 2011; Singh et al., 2016; Rai, 2019) because of its tolerance (Rahman et al., 2007; Rai, 2019). Conversely, for Sb, there are currently no published studies defining EC_{50} values for *S. polyrhiza*, thus comparison is limited.

During the bioassay, visual signs of toxicity, specifically wilting and chlorosis in the fronds, were observed at the end of the test in tailings, dumps and contaminated soils (Fig. S2). Some authors have linked these morphological alterations to high concentrations of certain metals such as Pb (Goswami et al., 2019; Spriņģe et al., 2024). Specifically, the samples exhibiting these disturbances yielded the highest soluble values of Cd, Pb, and Zn.

The ANOVA-Kruskal Wallis statistical analysis revealed a differentiation in toxicity among the material types based on the mean group values ($p < 0.05$). Consequently, the lowest toxicity values correspond to reference soil samples, while the highest toxicity is found in dumps and followed closely by tailings. This finding underscore the importance of stratified sampling, as this approach groups toxicity by sample type, facilitating the accurate identification and hazard classification of the most problematic areas.

3.3.3. Toxicity to green algae (Raphidocelis subcapitata)

In the case of *R. subcapitata* (Fig. 4), the toxicity produced by the leachates does not appear to be as evident as for *S. polyrhiza* ($EC_{50} > 50$ %). The greatest toxicity (TU = 16) is observed in dumps with the highest soluble PTE content. The highest hazard level (Class IV) in S. Quintín is observed in two dumps and one tailing sample, while in S. Francisco J. and S. Antonio is associated with dumps and contaminated soils.

The EC_{50} values obtained exceeded those proposed by other authors for Cd (4.5 μg L^{-1}), Pb (162 μg L^{-1}), and especially for Zn (3000 μg L^{-1}) (Alho et al., 2019; Al-Hasawi et al., 2020), but however, algae growth seemed to be stimulated. The hormetic effect could be related to antagonistic processes or strategies used by green algae cells to reduce toxicity. The strategies include exclusion, adsorption, chelation, and antioxidant systems (Miazek et al., 2015; Nowicka, 2022; Xiao et al., 2023) and decrease in membrane permeability because of low pH values (Lavoie et al., 2012). This stimulation is also associated with the presence of specific substances (macro- or micronutrients) that promote algal proliferation (Afione Di Cristofano et al., 2021; Andreu-Sánchez et al., 2022; Zhang et al., 2024; Lopes et al., 2025), which are not dismissed by the methodology.

The Pearson correlation analysis revealed no significant relationship ($p < 0.05$) between algal population growth, physicochemical characteristics and leachable PTEs. Although no statistical differences between the samples and controls have been observed, the data showed a

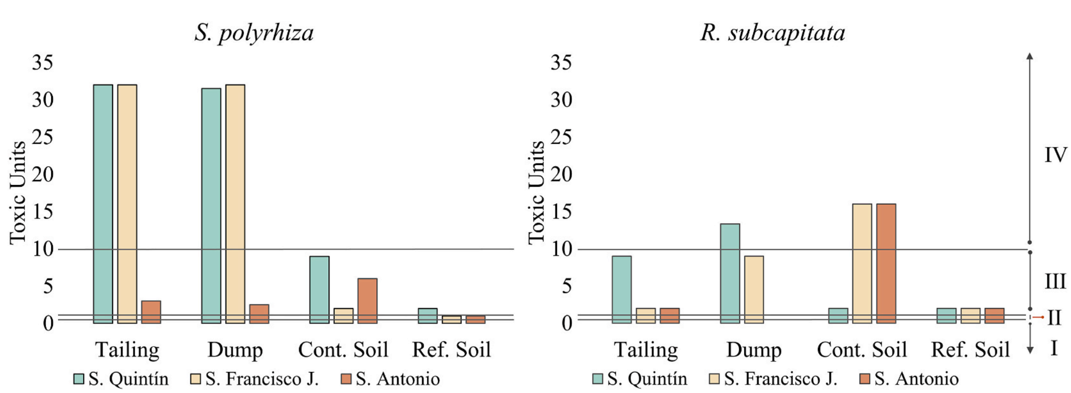


Fig. 4. *S. polyrhiza* and *R. subcapitata* toxicity in each area studied. Horizontal lines represent Persoone et al. (2003) classification. Cont. Soil = contaminated soil; Ref. Soil = reference soil. TU $<$ 0.4 = no acute toxicity (I); 0.4 $<$ TU $<$ 1 = slight acute toxicity (II); 1 $<$ TU $<$ 10 = acute toxicity (III); 10 $<$ TU $<$ 100 = high acute toxicity (IV); TU $>$ 100 = very high acute toxicity (V).

tendency toward stimulation.

The results contrast with a previous study in S. Quintín (Ferri-Moreno et al., 2023), where higher toxicity values were obtained for *R. subcapitata* (up to 2080 TU). These differences likely stem from the high heterogeneity of contamination within the waste materials. This disparity highlights the need for in-depth studies involving a larger and more representative number of samples to ensure the accuracy of ecological hazard assessments. The limited number of samples in this study represent a constraint that should be addressed in future research to differentiate the most hazardous areas. Furthermore, this reveals that bioassays carried out only with this type of algae could yield false negatives, leading to misinterpretations and complications in subsequent treatment proposals, as well as unnecessary cost overruns in restoration projects.

3.4. Multivariate analysis and hazard classification

Two distinct clusters emerged from the correlation (Fig. 5A). In the first group, the close clustering of Pb and *S. polyrhiza* toxicity is notable, suggesting a strong statistical association between Pb concentrations a growth inhibition. The second group includes metalloids (Sb and As), pH, soluble Fe, organic matter and *R. subcapitata* toxicity. The observed statistical relationship between bioavailable Fe and *R. subcapitata* toxicity could indicate possible stimulation of growth induced by this

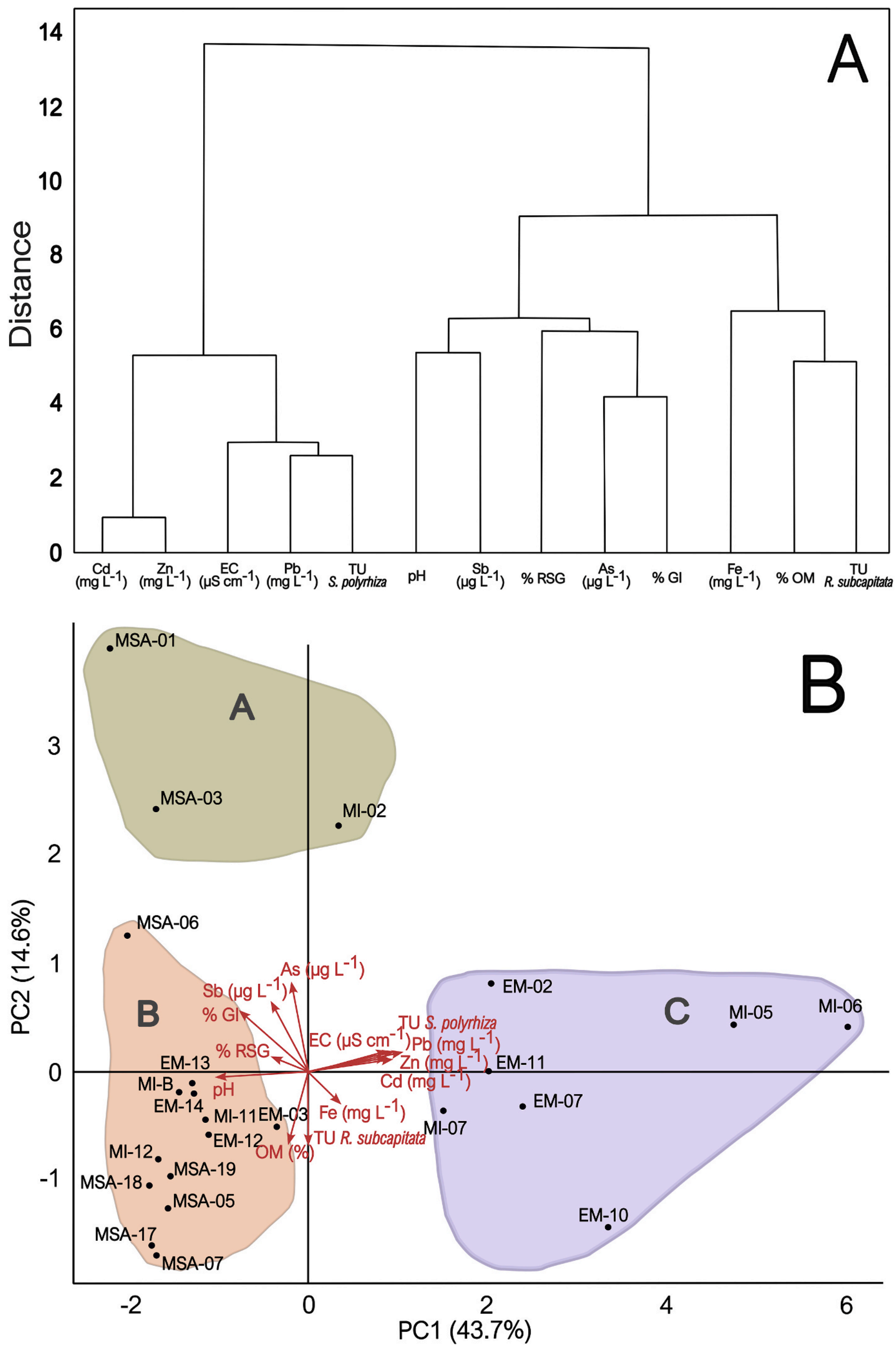


Fig. 5. Multivariate analysis of soluble geochemical properties and ecotoxicological results. A) Cluster analysis of the soluble contents, pH, EC and toxicity results. B) PCA for soluble elements, pH, EC and toxicity results.

element. Similarly, the proximity between the RSG and GI and soluble As content may indicate a potential stimulatory effect at low doses. Even though organic matter is known as a key factor in PTEs bioavailability (Karimi et al., 2020), statistical analysis did not reflect any relationship with other parameters.

The PCA identified three main groups of samples, associated with the overall toxicity profile across the tested organisms (Fig. 5B). The first two components accounted for a total of 58.3 % of the variance (PC1 43.7 % and PC2 14.6 %).

Group A includes S. Antonio tailings (e.g. MSA-01, MSA-03) and one dump sample from S. Francisco J. (MI-02). These samples are characterised by low toxicity and a hormetic effect, particularly with *L. sativum*. Group B (formed by contaminated and reference soils) is characterised by low overall toxicity, occupying the left side of the plot, and strongly associated with high pH and low concentrations of PTEs. However, exceptions like MSA-06 and MSA-17 (dumps) fall into this low-toxicity region, probably due to their soluble PTE content and high pH which favoured germination. Conversely, Group C (includes dumps and tailings from S. Quintín and S. Francisco J.) is positioned on the right side of the plot and exhibits a greater negative effect, particularly with *S. polyrhiza*. This cluster is strongly associated with the vectors of high EC, Cd, Pb, Zn and *S. polyrhiza* toxicity, and it is clearly located in the opposite orientation to the pH vector, suggesting acidity environment. Tailings from S. Francisco J. (MI-05 and MI-06) comprise the most acutely toxic samples situated in the furthest right position in the plot.

Fig. 6 provides a spatial representation of the ecotoxicological classification for the collected samples. In general, tailings and contaminated soils from the three mines exhibit elevated levels of toxicity. The results obtained from the S. Quintín, and S. Francisco J. mines indicate significant toxicity, with TV values exceeding 50 ('Toxic') in some cases for both matrices.

Almost 63% (n = 5) of the leachates from S. Quintín were found to be toxic. Three are classified as 'slightly toxic' (dump, tailing and contaminated soil), while two are categorised as 'moderately toxic' (dump and tailing). Samples EM-10 (tailing) and EM-11 (dump) are positioned near a rain-fed drainage system whose course leads directly to the Arroyo de la Mina stream, identifying a clear pathway for contaminant dispersion. The nearby soils studied are classified as non-hazardous materials. Regarding the solid-phase toxicity, only dumps and one tailing sample were classified as 'toxic'.

At S. Francisco J. mine, all samples exhibit some degree of toxicity (except from reference soils). The toxicity of the solid matrix of two tailings and one dump samples has been classified as 'highly toxic'. A tailing sample (MI-06) represents the riskiest, as high toxicity was observed in tests conducted in both matrices. The environmental hazard is heightened by the continuous weathering of these materials and their proximity to the Avenque watercourse, which contributes to the PTEs dispersion.

The toxicity profile from S. Antonio samples is generally low: all leachates from tailings, dumps and contaminated soils are classified as

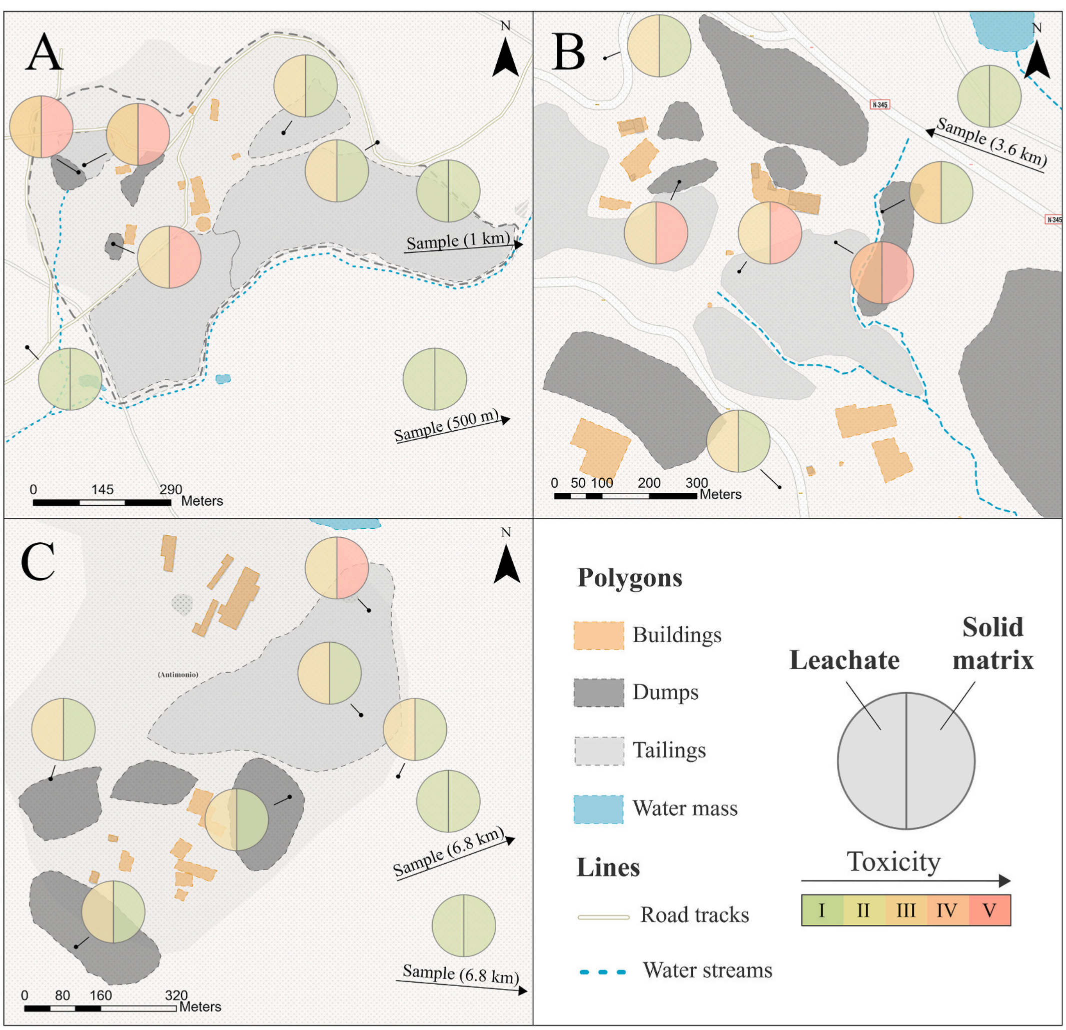


Fig. 6. Ecotoxicological classification of samples. A) S. Quintín mine; B) S. Francisco J. mine; C) S. Antonio mine. Classification: non-toxic (I); slightly toxic (II); moderately toxic (III); toxic (IV); highly toxic (V).

'slightly toxic' (except from one tailing). The bioassays results suggest that the absence of AMD has resulted in reduced toxicity values compared to the other sites. This finding raises the possibility of considering a less intensive, and therefore less costly, intervention for waste remediation. However, a comprehensive analysis of all materials and groundwater remains imperative considering that neutral pH and anoxic conditions, are optimal for increasing the mobility of As and Sb. Moreover, it is fundamental that the long-term risk to ecosystems and human health are comprehensively identified in order to dismiss any potential deleterious effect caused by long periods of exposure. While a previous study has addressed dermal exposure (Ferri-Moreno et al., 2025), a complete risk assessment requires the inclusion of the ingestion and inhalation exposure pathways to define the potential hazard to the nearby population. In fact, the results exhibited by Ferri-Moreno et al. (2025) showed non-carcinogenic risks and a potentially carcinogenic risk. This highlights the importance of integrated studies that collect toxicity values on different targets to gain a comprehensive and realistic understanding of the consequences of pollution.

The findings of the plate-based bioassay (with *L. sativum* seeds) substantiated the deleterious impact of mining waste on soil quality, particularly in S. Quintín and S. Francisco J. mines. The vegetal cover loss due to the acidic conditions could favour the dispersion of PTEs-enriched sediments, especially during cut-off low events common in semi-arid regions like Murcia.

The categorisation of samples based on their hazard can be decisive and fundamental for future restoration projects that can be carried out in the selected areas. The delineation of higher-risk areas is strategic for the development of effective restoration plans. Such identification is essential to achieving environmental objectives and enabling environmental improvements.

4. Conclusions

The stockpiling of large quantities of mining waste in the study areas represents a major source of contamination, the hazard of which is potentially exacerbated by its continuous degradation. The geochemical analysis demonstrates that the total PTEs content exceeds the GTVs established in Castilla-La Mancha, Extremadura and Murcia regions, confirming the potential risk posed by waste. As a direct result of these findings, the materials and soils examined should be considered contaminated as defined in Royal Decree 9/2005 (BOE, 2005). Consequently, the competent authorities must assume responsibility identifying polluted soils and delimiting areas, requiring the implementation of management and treatment strategies to reduce their environmental impacts.

The assorted results from the tests highlighted the importance of conducting series of bioassays with diverse exposed organisms. Obtaining false negatives is counterproductive for determining risk areas in restoration project context, as technical and scientific errors can be made due to an underestimation of the results. It is therefore necessary to delve deeper into the organisms tested to obtain the most accurate results in terms of toxicity in primary producers. This study found that the Zucconi test and the algal bioassay did not demonstrate comparable results with the plate-based bioassay and the aquatic plants, which exhibited greater conservatism. The germination test conducted using Zucconi methodology showed low sensitivity, with high values of germination. This finding indicates that seeds pose the capacity to germinate despite the presence of leachates with elevated concentrations of soluble PTEs. On the other hand, in the case of the algal bioassay, the stimulatory effects provoked on algae population by leachates masked the toxicity assessment results, hindering the process of risk evaluation. In order to enhance the reliability of the results obtained with the Zucconi test and the algal bioassay, it is imperative to combine different target organisms for acquiring more robust and reliable results.

This study provides an overview of the toxicity that the tailings, dumps and soils from derelict metallic mines represent for the surrounding ecosystems, identifying a significant risk to biodiversity in the different regions. Even though the total content of PTEs was a relevant agent, other parameters such as pH had higher influence in the toxic process. Concerning this, the AMD capacity generation of the materials seemed to be the most determinant factor that might be considered when assessing the toxicity towards primary producers. Beyond evaluating direct ecosystem impacts, it is necessary to develop complementary risk assessments for higher trophic links in the food chain and for human health. The integration of these results will enable the precise mapping of areas with the greatest toxicological risk, ensuring that remedial action and management methodologies are geochemically optimised. This targeted strategy is crucial for reducing project costs and guaranteeing efficient waste management.

CRediT authorship contribution statement

Inmaculada Ferri-Moreno: Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Iker Martínez-del-Pozo:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **José María Esbrí:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Mari Luz García-Lorenzo:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization. **Oscar Andreu-Sánchez:** Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix ASupplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envres.2026.123954.

Data availability

Data will be made available on request.

References

Afione Di Cristofano, C., Juárez, Á.B., Moretton, J., Magdaleno, A., 2021. Efectos de metales pesados, glifosato y sus mezclas binarias sobre el crecimiento de algas verdes. Ecol. Austral 31 (1), 53–64. https://doi.org/10.25260/ea.21.31.1.0.1146.
Aguerre, Y.S., Gavazzo, G.B., 2012. Fitotoxicidad de papel reciclado. Ensayospreliminares. In: Proceedings of the ABTCP 2012. VII CIADICYP.The 45th ABTCP International Pulp and Paper Congress and VII Ibero American Congress on Pulp and Paper Research.

Alho, L. de O.G., Gebara, R.C., Paina, K. de A., Sarmento, H., Melão, M. da G.G., 2019. Responses of Raphidocelis subcapitata exposed to Cd and Pb: mechanisms of toxicity assessed by multiple endpoints. Ecotoxicol. Environ. Saf. 169, 950–959. https://doi.org/10.1016/j.ecoenv.2018.11.087.
Alvarenga, P., Palma, P., Gonçalves, A.P., Fernandes, R.M., de Varennes, A., Vallini, G., Duarte, E., Cunha-Queda, A.C., 2008. Evaluation of tests to assess the quality of mine-contaminated soils. Environ. Geochem. Health 30 (2), 95–99. https://doi.org/10.1007/s10653-008-9147-z.
Álvarez-Ayuso, E., Otones, V., Murciego, A., García-Sánchez, A., Regina, I.S., 2013. Mobility and phytoavailability of antimony in an area impacted by a former stibnite mine exploitation. Sci. Total Environ. 449, 260–268. https://doi.org/10.1016/j.scitotenv.2013.01.071.
Álvarez-Ayuso, E., Murciego, A., Rodríguez, M.A., Fernández-Pozo, L., Cabezas, J., Naranjo-Gómez, J.M., Mosser-Ruck, R., 2022. Antimony distribution and mobility in different types of waste derived from the exploitation of stibnite ore deposits. Sci. Total Environ. 816. https://doi.org/10.1016/j.scitotenv.2021.151566.
Al-Hasawi, Z.M., Abdel-Hamid, M.I., Almutairi, A.W., Touliabah, H.E., 2020. Response of Pseudokirchneriella subcapitata in free and alginate immobilized cells to heavy metals toxicity. Molecules 25 (12). https://doi.org/10.3390/molecules25122847.
Andreu-Sánchez, O., Moratalla-López, J., Rodríguez-Martín, J.A., Roca-Pérez, L., 2022. Application of an ecotoxicological battery test to the paddy field soils of the albufera natural park. Toxics 10 (7). https://doi.org/10.3390/toxics10070375.
Asociación Española de Normalización, 2018. UNE-EN ISO 20227:2018. Calidad del agua. Determinación de los efectos de inhibición de las aguas residuales, aguas naturales y productos químicos sobre el crecimiento de la lenteja de agua Spirodela polyrhiza. Método utilizando Un Ensayo Biológico Miniaturizado Independiente del Cultivo Madre. *ISO 20227:2017)*.
Asociación Española de Normalización (AENOR), 2019. UNE-EN ISO 21268-4:2019. Soil Quality — Leaching Procedures for Subsequent Chemical and Ecotoxicological Testing of Soil and Soil Materials — Part 4: Influence of pH on Leaching with Initial acid/base Addition.
Baderna, D., Lomazzi, E., Pogliaghi, A., Ciaccia, G., Lodi, M., Benfenati, E., 2015. Acute phytotoxicity of seven metals alone and in mixture: are Italian soil threshold concentrations suitable for plant protection? Environ. Res. 140, 102–111. https://doi.org/10.1016/j.envres.2015.03.023.
Baudo, R., Foudoulakis, M., Arapis, G., Perdaen, K., Lanneau, W., Paxinou, A.C.M., Kouvdou, S., Persoone, G., 2015. History and sensitivity comparison of the Spirodela polyrhiza microbiotest and Lemna toxicity tests. Knowl. Manage. Aquat. Syst. (416). https://doi.org/10.1051/kmae/2015019. EDP Sciences.
Belz, R.G., Cedergreen, N., 2010. Parthenin hormesis in plants depends on growth conditions. Environ.l Exp. Bot. 69 (3), 293–301. https://doi.org/10.1016/j.envexpbot.2010.04.010.
Bes, C.M., Pardo, T., Bernal, M.P., Clemente, R., 2014. Assessment of the environmental risks associated with two mine tailing soils from the La Unión-Cartagena (Spain) mining district. J. Geochem. Explor. 147 (PB), 98–106. https://doi.org/10.1016/j.gexplo.2014.05.020.
Boluda, R., Roca-Pérez, L., Marimón, L., 2011. Soil plate bioassay: an effective method to determine ecotoxicological risks. Chemosphere 84 (1), 1–8. https://doi.org/10.1016/j.chemosphere.2011.02.013. Elsevier Ltd.
Bori, J., Vallès, B., Navarro, A., Riva, M.C., 2016. Geochemistry and environmental threats of soils surrounding an abandoned mercury mine. Environ. Sci. Pollut. Res. 23 (13), 12941–12953. https://doi.org/10.1007/s11356-016-6463-1.
Bożym, M., Rybak, J., 2024. In vitro chronic phytotoxicity of heavy metals and metalloids to Lepidium sativum (garden cress). Ecotoxicology 33 (1), 94–103. https://doi.org/10.1007/s10646-024-02729-z.
Cheng, B., Wang, Z., Yan, X., Yu, Y., Liu, L., Gao, Y., Zhang, H., Yang, X., 2023. Characteristics and pollution risks of Cu, Ni, Cd, Pb, Hg and as in farmland soil near coal mines. Soil Environ. Health 1 (3). https://doi.org/10.1016/j.seh.2023.100035.
Chung, F.H., 1974a. Quantitative interpretation of X-ray diffraction patterns of mixtures. I. Matrix-flushing method for quantitative multicomponent analysis. J. Appl. Crystallogr. 7 (6). https://doi.org/10.1107/s0021889874010375.
Chung, F.H., 1974b. Quantitative interpretation of X-ray diffraction patterns of mixtures. II. Adiabatic principle of X-ray diffraction analysis of mixtures. J. Appl. Crystallogr. 7 (6). https://doi.org/10.1107/s0021889874010387.
Chung, F.H., 1975. Quantitative interpretation of X-ray diffraction patterns of mixtures. III. Simultaneous determination of a set of reference intensities. J. Appl. Crystallogr. 8 (1). https://doi.org/10.1107/s0021889875009454.
Collin, S., Baskar, A., Geevarghese, D.M., Ali, M.N.V.S., Bahubali, P., Choudhary, R., Lvov, V., Tovar, G.I., Senatov, F., Koppala, S., Swamiappan, S., 2022. Bioaccumulation of lead (Pb) and its effects in plants: a review. J. Hazard. Mater. Lett. 3. https://doi.org/10.1016/j.hazl.2022.100064.
Deustsches Institute für Normung, 1984. *DIN 38414-S4: 1984. German Standard Methods for the Examination of Water, Wastewater and Sludge – Sludge and Sediments (Group S) – Determination of Leachability by Water (S 4)*.
Diario Oficial de Extremadura, 2015. *Decreto 49/2015, de 30 de marzo, por el que se regula el régimen jurídico de los suelos contaminados en la Comunidad Autónoma de Extremadura*. https://doe.juntaex.es/pdfs/doe/2015/630O/15020031.pdf.
Dirilgen, N., 2011. Mercury and lead: assessing the toxic effects on growth and metal accumulation by Lemna minor. Ecotoxicol. Environ. Saf. 74 (1), 48–54. https://doi.org/10.1016/j.ecoenv.2010.09.014.
European Commission, 2025. *Directive (EU) 2025/2360 on soil monitoring*. Off. J. Eur. Union. https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32025L2360.
Ferri-Moreno, I., Barquero-Peralbo, J.I., Andreu-Sánchez, O., Higueras, P., Roca-Pérez, L., García-Lorenzo, M.L., Esbrí, J.M., 2023. Categorization of mining materials for restoration projects by means of pollution indices and bioassays. Minerals 13 (4). https://doi.org/10.3390/min13040492.
Ferri-Moreno, I., Martínez-del-Pozo, I., Huertas, P., Higueras, P., Barquero-Peralbo, J.I., Esbrí, J.M., García-Lorenzo, M.L., 2025. Dermal bioaccessibility and health risk assessment associated with mining production of metals (Pb-Zn-Ag) and critical raw materials (Sb-W) in the Iberian Peninsula. Environ. Chem. Ecotoxicol. 7, 506–515. https://doi.org/10.1016/j.enceco.2025.02.011.
Gallego, S., Esbrí, J.M., Campos, J.A., Peco, J.D., Martin-Laurent, F., Higueras, P., 2021. Microbial diversity and activity assessment in a 100-year-old lead mine. J. Hazard. Mater. 410. https://doi.org/10.1016/j.jhazmat.2020.124618.
García-García, C., Robles-Arenas, V.M., Peñas Castejón, J.M., Rodríguez Pacheco, R.L., 2004. El riesgo ambiental de residuos minero-metalúrgicos de la Sierra Minera de Cartagena-La Unión (Murcia, España). In: III Congreso de la Naturaleza de la Región de Murcia: Gestión y conservación de ecosistemas, flora y fauna (21–24 de octubre de 2004), Sangonera La Verde, Murcia, España.
García-Lorenzo, M.L., Martínez-Sánchez, M.J., Pérez-Sirvent, C., Molina, J., 2009. Ecotoxicological evaluation for the screening of areas polluted by mining activities. Ecotoxicology 18 (8), 1077–1086. https://doi.org/10.1007/s10646-009-0362-x.
García-Lorenzo, M.L., Crespo-Feo, E., Esbrí, J.M., Higueras, P., Grau, P., Crespo, I., Sánchez-Donoso, R., 2019. Assessment of potentially toxic elements in technosols by tailings derived from Pb–Zn–Ag mining activities at San Quintín (Ciudad real, Spain): some insights into the importance of integral studies to evaluate metal contamination pollution hazards. Minerals 9 (6). https://doi.org/10.3390/min9060346.
Garrido, I., Ortega, A., Hernández, M., Fernández-Pozo, L., Cabezas, J., Espinosa, F., 2021. Effect of antimony in soils of an Sb mine on the photosynthetic pigments and antioxidant system of Dittrichia viscosa leaves. Environ. Geochem. Health 43 (4). https://doi.org/10.1007/s10653-020-00616-0.
Goswami, C., Bandyopadhyay, K., Majumder, A., 2019. Spirodela polyrhiza: a potential accumulator of pb from contaminated water. Lect. Civ. Eng., 21 LNCE 16–21. https://doi.org/10.1007/978-3-030-02707-0_3.
Higueras, P., Esbrí, J.M., García-Ordiales, E., González-Corrochano, B., López-Berdonces, M.A., García-Noguero, E.M., Alonso-Azcárate, J., Martínez-Coronado, A., 2017. Potentially harmful elements in soils and holm-oak trees (Quercus ilex L.) growing in mining sites at the Valle de Alcudia Pb-Zn district (Spain)–Some clues on plant metal uptake. J. Geochem. Explor. 182, 166–179. https://doi.org/10.1016/j.gexplo.2016.07.017.
International Organization for Standardization, 2012. ISO 8692:2012. Water Quality — Fresh Water Algal Growth Inhibition Test with Unicellular Green Algae.
Jiménez Ballesta, R., Bueno, P., Rubí, J., Giménez, R., 2010. Pedo-geochemical baseline content levels and soil quality reference values of trace elements in soils from the Mediterranean (Castilla la Mancha, Spain). Cent. Eur. J. Geosci. 2 (4), 441–454. https://doi.org/10.2478/v10085-010-0028-1.
Karimi, F., Rahimi, G., Kolahchi, Z., et al., 2020. Using industrial sewage sludge-derived biochar to immobilize selected heavy metals in a contaminated calcareous soil. Waste Biomass Valoriz. 11, 2825–2836. https://doi-org.bucm.idm.oclc.org/10.1007/s12649-018-00563-z.
Lavoie, M., Le Faucheur, S., Boullemant, A., Fortin, C., Campbell, P.G.C., 2012. The influence of pH on algal cell membrane permeability and its implications for the uptake of lipophilic metal complexes. J. Phycol. 48 (2), 293–302. https://doi.org/10.1111/j.1529-8817.2012.01126.x.
Lopes, T.O.M., Silveira, C. R. da, Araujo da Silva, J., Guedes, T., Tavella, R.A., Rola, R.C., Marques, J.A., Vieira, C.E.D., Bianchini, A., Martins, C., de, M.G., 2025. A six-year ecotoxicological assessment of the doce river and coastal marine areas impacted by the Fundão tailings dam failure, Brazil. Environ. Pollut. 371. https://doi.org/10.1016/j.envpol.2025.125897.
Martínez-Colón, M., Alegría, H., Huber, A., Kubra-Gul, H., Kurt-Karakus, P., 2021. Bioaccumulation and biomagnification of potential toxic elements (PTEs): an Avicennia germinans–Uca rapax trophic transfer story from Jobos Bay, Puerto Rico. Ecol. Indic. 121. https://doi.org/10.1016/j.ecolind.2020.107038.
Martínez-Sánchez, M.J., García-Lorenzo, M.L., Pérez-Sirvent, C., Bech, J., 2012. Trace element accumulation in plants from an aridic area affected by mining activities. J. Geochem. Explor. 123, 8–12. https://doi.org/10.1016/J.GEXPLO.2012.01.007.
Miazek, K., Iwanek, W., Remacle, C., Richel, A., Goffin, D., 2015. Effect of metals, metalloids and metallic nanoparticles on microalgae growth and industrial product biosynthesis: a review. Int. J. Mol. Sci. 16 (10), 23929–23969. https://doi.org/10.3390/ijms161023929. MDPI AG.
Mishra, V.K., Upadhyay, A.R., Pathak, V., Tripathi, B.D., 2008. Phytoremediation of mercury and arsenic from tropical opencast coalmine effluent through naturally occurring aquatic macrophytes. Water, Air, Soil Pollut. 192, 303–314. https://doi.org/10.1007/s11270-008-9657-4.
Murciego, A.M., Sánchez, A.G., González, M.A.R., Gil, E.P., Gordillo, C.T., Fernández, J. C., Triguero, T.B., 2007. Antimony distribution and mobility in topsoils and plants (Cytisus striatus, Cistus ladanifer and Dittrichia viscosa) from polluted Sb-mining areas in Extremadura (Spain). Environ. Pollut. 145 (1). https://doi.org/10.1016/j.envpol.2006.04.004.
Nowicka, B., 2022. Heavy metal–induced stress in eukaryotic algae—mechanisms of heavy metal toxicity and tolerance with particular emphasis on oxidative stress in exposed cells and the role of antioxidant response. Environ. Sci. Pollut. Res. 29 (12), 16860–16911. https://doi.org/10.1007/s11356-021-18419-w. Springer Science and Business Media Deutschland GmbH.
Organisation for Economic Co-operation and Development, 2011. Development Co-operation Report 2011. OECD. https://doi.org/10.1787/dcr-2011-en.
Paniagua-López, M., Aguilar-Garrido, A., Contero-Hurtado, J., García-Romera, I., Sierra-Aragón, M., Romero-Freire, A., 2023. Ecotoxicological assessment of polluted soils

one year after the application of different soil remediation techniques. Toxics 11 (4). https://doi.org/10.3390/toxics11040298.

Paquet, N., Indiketi, N., Dalencourt, C., Larivière, D., Roberge, S., Gruyer, N., Triffault-Bouchet, G., Fortin, C., 2019. Toxicity of tailing leachates from a niobium mine toward three aquatic organisms. Ecotoxicol. Environ. Saf. 176, 355–363. https://doi.org/10.1016/j.ecoenv.2019.03.065.

Parbhakar-Fox, A., 2016. Geoenvironmental characterisation of heap leach materials at abandoned mines: Croydon Au-Mines, QLD, Australia. Minerals 6 (2), 52. https://doi.org/10.3390/min6020052.

Peco, J.D., Higueras, P., Campos, J.A., Olmedilla, A., Romero-Puertas, M.C., Sandalio, L. M., 2020. Deciphering lead tolerance mechanisms in a population of the plant species Biscutella auriculata L. from a mining area: accumulation strategies and antioxidant defenses. Chemosphere 261. https://doi.org/10.1016/j.chemosphere.2020.127721.

Pérez-Sirvent, C., Martínez-Sánchez, M.J., Martínez-López, S., Bech, J., Bolan, N., 2012. Distribution and bioaccumulation of arsenic and antimony in Dittrichia viscosa growing in mining-affected semiarid soils in southeast Spain. J. Geochem. Explor. 123, 128–135. https://doi.org/10.1016/j.gexplo.2012.08.002.

Persoone, G., Marsalek, B., Blinova, I., Törökne, A., Zarina, D., Manusadzianas, L., Nalecz-Jawecki, G., Tofan, L., Stepanova, N., Tothova, L., Kolar, B., 2003. A practical and user-friendly toxicity classification system with microbiotests for natural waters and wastewaters. Environ. Toxicol. 18 (6), 395–402. https://doi.org/10.1002/tox.10141.

Rahman, M.A., Hasegawa, H., Ueda, K., Maki, T., Okumura, C., Rahman, M.M., 2007. Arsenic accumulation in duckweed (Spirodela polyrhiza L.): a good option for phytoremediation. Chemosphere 69 (3), 493–499. https://doi.org/10.1016/j.chemosphere.2007.04.019.

Rai, P.K., 2019. Heavy metals/metalloids remediation from wastewater using free flotating macrophytes of a natural wetland. Environ. Technol. Innov. 15. https://doi.org/10.1016/j.eti.2019.100393.

Rascio, N., Navari-Izzo, F., 2011. Heavy metal hyperaccumulating plants: how and why do they do it? And what makes them so interesting? Plant Sci. 180 (2), 169–181. https://doi.org/10.1016/j.plantsci.2010.08.016.

Reguilón Bragado, R.M., Martín Izard, A., Palero, J., Mangas Viñuela, J., Both, R.A., 1992. Metalogénesis de los yacimientos de Pb-Zn de la región del Valle de Alcudia (Sierra Morena Occidental). In: Recursos Minerales de España. Consejo Superior de Investigaciones Científicas (CSIC), pp. 1027–1067. https://accedacris.ulpgc.es/handle/10553/57157.

Rodríguez, L., Ruiz, E., Alonso-Azcárate, J., Rincón, J., 2009. Heavy metal distribution and chemical speciation in tailings and soils around a Pb-Zn mine in Spain. J. Environ. Manag. 90 (2), 1106–1116. https://doi.org/10.1016/j.jenvman.2008.04.007.

Rodríguez, L., González-Corrochano, B., Medina-Díaz, H.L., López-Bellido, F.J., Fernández-Morales, F.J., Alonso-Azcárate, J., 2022. Does environmental risk really change in abandoned mining areas in the medium term when no control measures are taken? Chemosphere 291. https://doi.org/10.1016/j.chemosphere.2021.133129.

Sánchez, M.J.M., Sirvent, C.P., 2007. Niveles de Fondo y Niveles Genéricos de Referencia de Metales Pesados en Suelos de la Región de Murcia.

Šestinova, O., Findoráková, L., Hančuľák, J., Šestinová, L., 2015. Study of metal mobility and phytotoxicity in bottom sediments that have been influenced by former mining activities in Eastern Slovakia. Environ. Earth Sci. 74 (7), 6017–6025. https://doi.org/10.1007/s12665-015-4625-y.

Singh, N.K., Raghubanshi, A.S., Upadhyay, A.K., Rai, U.N., 2016. Arsenic and other heavy metal accumulation in plants and algae growing naturally in contaminated area of West Bengal, India. Ecotoxicol. Environ. Saf. 130, 224–233. https://doi.org/10.1016/j.ecoenv.2016.04.024.

Soliman, M.M., Hesselberg, T., Mohamed, A.A., Renault, D., 2022. Trophic transfer of heavy metals along a pollution gradient in a terrestrial agro-industrial food web. Geoderma 413, 115748. https://doi.org/10.1016/J.GEODERMA.2022.115748.

Spriņģe, G., Grīne, I., Melece, I., Melecis, V., Purmalis, O., Valters, K., 2024. Heavy metal pollution and phytotoxicity of small urban stream sediments. Sustain. Water Resour. Manage. 10 (3). https://doi.org/10.1007/s40899-024-01096-1.

U.S. Environmental Protection Agency, 1996. Soil screening guidance: user's guide (EPA540/R-96/018). https://www.epa.gov/superfund/superfund-soil-screening-guidance.

Wang, X., Deng, R., Wang, C., Xu, R., Hou, B., Zhou, S., et al., 2025. Pollution characteristics, risk assessment and source comparative analysis of potentially toxic elements in soils of three superlarge antimony ore areas in the Zijiang River, Hunan, China. Environ. Pollut. 382, 126701. https://doi.org/10.1016/j.envpol.2025.126701.

WorldHealthOrganization. (n.d.). One Health — European Region Initiatives.

Xiao, X., Li, W., Jin, M., Zhang, L., Qin, L., Geng, W., 2023. Responses and tolerance mechanisms of microalgae to heavy metal stress: a review. Mar. Environ. Res. 183. https://doi.org/10.1016/j.marenvres.2022.105805. Elsevier Ltd.

Yazman, M.M., Ustaoğlu, F., Yüksel, B., 2025. Nutritional profiling of Oncorhynchus mykiss from çamlıgöze dam, Türkiye: health risk assessment through Monte Carlo simulation and elemental source attribution using positive matrix factorization. Process Saf. Environ. Prot. 198, 107172. https://doi.org/10.1016/j.psep.2025.107172.

You, R., Domínguez, C., Matamoros, V., Bayona, J.M., Díez, S., 2019. Chemical characterization and phytotoxicity assessment of peri-urban soils using seed germination and root elongation tests. Environ. Sci. Pollut. Res. 26 (33), 34401–34411. https://doi.org/10.1007/s11356-019-06574-0.

Yüksel, B., Ustaoğlu, F., 2025. Pollution analysis of metals in the sediments of lagoon lakes in Türkiye: toxicological risk assessment and source insights. Process Saf. Environ. Prot. 193, 665–682. https://doi.org/10.1016/j.psep.2024.11.085.

Zhang, X., Hu, Y., Liu, Y., Chen, B., 2011. Arsenic uptake, accumulation and phytofiltration by duckweed (Spirodela polyrhiza L.). J. Environ. Sci. 23 (4), 601–606. https://doi.org/10.1016/S1001-0742(10)60454-8.

Zhang, Y., Sun, D., Gao, W., Zhang, X., Ye, W., Zhang, Z., 2024. The metabolic mechanisms of Cd-induced hormesis in photosynthetic microalgae, Chromochloris zofingiensis. Sci. Total Environ. 912. https://doi.org/10.1016/j.scitotenv.2023.168966.

Zucconi, F., 1981. Evaluating toxicity of immature compost. Biocycle 22, 54–55.

Boletín Oficial del Estado (2005). *Real Decreto 9/2005, de 14 de enero, por el que se establece la relación de actividades potencialmente contaminantes del suelo y los criterios y estándares para la declaración de suelos contaminados.* https://www.boe.es/eli/es/rd/2005/01/14/9.

Maoxin Hong, Jun Wang, Baojun Yang, Yang Liu, Xin Sun, Laishun Li, Shichao Yu, Shitong Liu, Yang Kang, Wei Wang, Guanzhou Qiu, Inhibition of pyrite oxidation through forming biogenic K-jarosite coatings to prevent acid mine drainage production, Water Research, Volume 252, 2024, 121221, ISSN 0043-1354, https://doi.org/10.1016/j.watres.2024.121221.

Boletín Oficial del Estado (2015). *Real Decreto 817/2015, de 11 de septiembre, por el que se establecen los criterios de seguimiento y evaluación del estado de las aguas superficiales y las normas de calidad ambiental.* https://www.boe.es/eli/es/rd/2015/09/11/817/con.