

Article

Categorization of Mining Materials for Restoration Projects by Means of Pollution Indices and Bioassays

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Abstract: Sulfide mining wastes may lead to severe environmental and human health risks. This study aims to use geochemical and ecotoxicological indicators for the assessment of the ecological risks of potentially toxic elements (PTEs) in the San Quintín mining group to categorize wastes prior to mining restoration. Ecotoxicity was evaluated using crustacean (*Daphnia magna*, *Thamnocephalus platyurus*) and algae (*Raphidocelis subcapitata*) bioassays. The geochemical and mineralogical results suggested that the mining residues underwent intense weathering processes, with active processes of acidity generation and metal mobility. Total PTEs concentrations indicated that the mining materials were extremely polluted, with Pb, Zn and Cd geoaccumulation index (Igeo) values higher than 5 in more than 90% of the samples. The pollution load index (PLI) showed average values of 18.1, which classifies them as very highly polluted. The toxicity tests showed a higher toxicity for plants than crustaceans, being the highest values of toxicity related to toxic elements (Pb, Cd and Zn), electrical conductivity and to pH. This paper presents for the first time the combination of indices in the categorization of mining waste prior to its restoration. The combination of them has made it possible to categorize the waste and adapt the restoration and remediation procedures.

Keywords: tailings; dumps; potentially toxic elements; *Daphnia magna*; *Raphidocelis subcapitata*; *Thamnocephalus platyurus*; ecotoxicity; mining restoration

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1. Introduction

Potentially toxic elements (PTEs) are naturally occurring, ubiquitous substances in the human environment which typically originate from the weathering of parent materials. Nevertheless, due to a variety of human activities, PTEs have substantially accumulated in the global environment in recent years, particularly in soil and sediment environments. Potentially toxic elements include metals and non-metals such as arsenic (As) and selenium (Se) [1].

The presence of PTEs supposes a risk in areas affected by ancient mining activities [2,3]. These zones, such as the San Quintín Mining Group (SQMG), have large amounts of wastes with nonrecoverable sulfides that are dumped at disposal sites and exposed to weathering. Acid mine drainage (AMD) occurs when sulfides are exposed to atmospheric, hydrological or biological weathering (oxygen, water and bacteria), becoming oxidized products and producing low pH and high concentrations of PTEs and soluble sulfates in surficial water and groundwater. This environmental concern is especially relevant in areas with historical metallic mining activities, where the application of less efficient

mineral extraction and processing techniques and the lack of environmental awareness and controls have left huge environmental liabilities [4–6].

There are many derelict mining areas with these conditions that require the application of adapted restoration projects. A mining restoration project must seek to design restoration actions focused on minimizing erosive processes and preventing the generation of acidity and immobilizing dangerous PTEs contained in mining residue and soils affected by mining. Likewise, edaphic support must be provided to the roof for the revegetation of the restored materials. Restoration project design can be challenging in mining areas with a long history of exploitation that have dispersed mining materials (dumps and tailings) with very different metal loads and toxicities.

Traditionally, soil toxicity has been evaluated through quantifying total and/or extractable PTEs contents [7]. Once the PTEs contents are determined, one of the most used ways to assess the environmental risk in soils is the calculation of pollution indices based on the comparison of an element concentration in soil samples and the background level of this element in the study area. Among all indices, the geoaccumulation index (I_{geo}) and the pollution index (PI) are widely used as single indices and the pollution load index (PLI) is used among the integrated ones [8].

In SQMD, some studies have been carried out on the geochemical and mineralogical characterization of soils and waters [3–6]. Even if these procedures are sensitive, they fail to provide information about the bioavailability of contaminants and their synergistic or antagonistic interactions between pollutants. For that reason, and to estimate the environmental risk of contaminants, chemical analytical techniques need to be complemented with the use of bioassays [9].

Taking into account that the aquatic environment usually represents the destination of contaminants, organisms such as algae (i.e., *Raphidocelis subcapitata*) or crustaceans (i.e., *Daphnia magna* or *Thamnocephalus platyurus*) are very important to be included in the ecotoxicological evaluation procedure. Primary producers are the first link of aquatic systems, and adverse impacts on them may have important consequences for the health of the whole aquatic ecosystem. On the other hand, primary consumers are filter-feeding organisms and can be useful indicators of the bioavailability of particle-bound contaminants. *D. magna* and *R. subcapitata* have been commonly used for the assessment of the ecotoxicity of environmental samples such as water, soil leachates and sediments, but recently *T. platyurus* has gained interest as a potential reference organism for environmental monitoring [10]. The results of these tests (e.g., the effect concentration EC50 or the lethal concentration LC50) are often recalculated to toxic units (TU) in order to classify the potential toxicity. Persoone et al. [11] introduced the classification of the water and wastewater toxicity, becoming the most commonly used system in the last two decades [12].

The aim of the present research was to assess the polluting potential and geochemical behavior of PTEs in mine wastes accumulated by the SQMG (Ciudad Real, Spain), evaluating the mineralogical and geochemical composition of 18 mining waste samples. Additionally, we analyzed the ecotoxicity of PTEs using three environmentally relevant test species of different trophic levels and biological complexity: algae (*Raphidocelis subcapitata*) and crustaceans (*Thamnocephalus platyurus* and *Daphnia magna*). The suitability of those assays for the assessment of environmental risk in mine sites strongly polluted by PTEs is discussed and evaluated to determine if they could be applied for residue categorization prior to mining restoration.

2. Study Area

The SQMG is part of the so-called Alcudia Valley Mining District [13]. It is located within the so-called Meseta Sur (the Spanish southern mesa), which has a continental Mediterranean climate with contrasted seasonal variations in mean temperatures: 6–8 °C (winter) and 26–28 °C (summer). The rain concentrates in late autumn and early spring, with an annual total of 500–700 mm. The mining group is located within a region

morphologically characterized by WNW trending valleys and sierras, within a landscape ranging in altitude between 600–700 m above sea level [4].

This mining district is formed by a large number of mines distributed along the southwest area of Ciudad Real province. In particular, SQMG represents the largest one and comprises two mining zones (Figure 1) delimited and separated by 800 m: San Quintín East (affecting an area of 12 Ha) and San Quintín West (affecting an area of 40 Ha) [13].

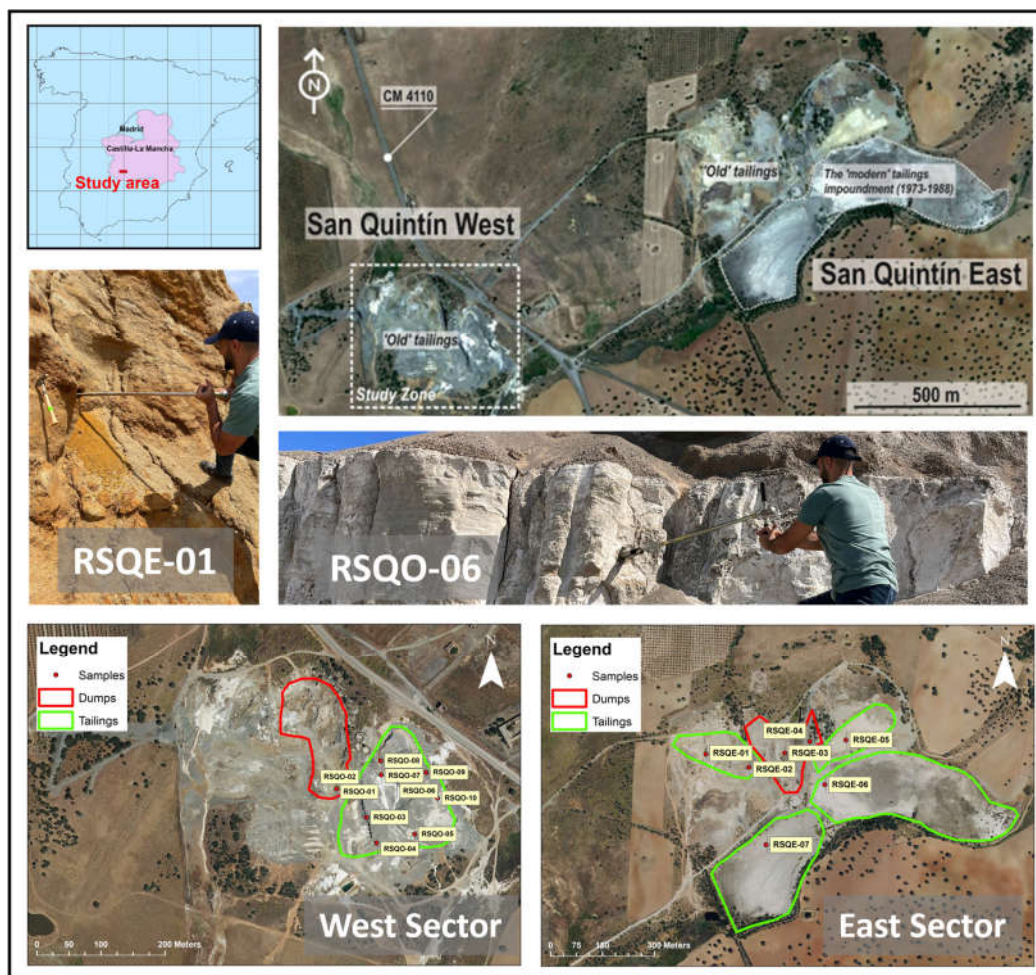


Figure 1. Situation map and sample location.

The main soils are entisols, with localized development of anthrosols. Other local soil-like materials include spolic technosols related to abandoned tailings deposits.

The SQMG (East and West) (Figure 1) was active between 1888 and 1923, when about 515,000 tons of galena concentrates were produced (via gravity concentration with the aid of primitive jigs). Subsequently, between 1973 and 1988, the *Sociedad Minero-Metalúrgica de Peñarroya España* carried out a second mineral recovery operation of the old tailings (this time using froth flotation), which was estimated at around 3 million tons and showed high Zn content—a metal with hardly any interest at the time of the older exploitation. However, this operation did not affect the tailings left at San Quintín West. From the older period, there are many waste dumps of barren rock whose environmental risk is low; thus, the main hazard relates to the tailings, which in this case are constituted by residues of mineral concentration via gravity, a process that was inefficient at the time and left behind residues very rich in galena (PbS) and sphalerite (ZnS). These old and chaotic tailing

deposits are a separate case and present a significant environmental hazard in relation to the heavy metals (Pb, Zn-Cd) and metalloids (As). These tailings not only contain high concentrations of Pb and Zn (up to 48,600 mg kg⁻¹ Pb and 34,000 mg kg⁻¹ Zn), but also are a source of AMD. Additionally, the lack of vegetation cover on these unstable wastes reveals other hazards associated with this type of infrastructure: slope instability, gully production, collapses and great sediment mobilization [13].

3. Materials and Methods

3.1. Sampling Design

The main criterion for the selection of the sampling points has been the access to the interior of the sludge pools for the sampling of humid material, neither aerated nor in an active oxidation process. To achieve this, the vertical profiles of the sludge ponds have been drilled with an Eijkkamp sampler until access to the fine materials was processed in the corresponding sink. In the large ponds in the East, samples have been taken from the upper deck to avoid taking samples from the containment dikes.

The selection of sampling points has been made to try to ensure the representativity of mining wastes in the studied area (Figure 1). A total of 18 samples were collected: 7 in San Quintín East and 11 in San Quintín West.

3.2. Geochemical Analysis of Samples

Samples were air-dried and sieved through a 2-mm mesh. The pH and electrical conductivity (EC) were measured in a 1:5 (*w/v*) suspension of an aliquot of the sample in deionized water.

Organic matter (OM) was determined following the Standard Test Methods for the Moisture, Ash, and OM of Peat and Organic Soils [14]. In order to apply the step specifications of the standard method, the samples were powdered by an agate mortar and were sieved through a 53- μ m mesh. Then, they were dried in a laboratory oven for 24 h and cooled down in a desiccator. Afterwards, the samples were heated up in a furnace following temperature intervals determined by the ASTM [14].

Elemental concentration data in samples were achieved by means of Energy Dispersive X-ray Fluorescence Spectrometry (EDXRF) using a Bruker, S2 Ranger spectrometer with a Pd detector at the Unidad de Técnicas Geológicas, Universidad Complutense de Madrid. Samples were quartered and an aliquot of 10g was ground to fine powder using an agate ball mill and passed through a 100- μ m sieve prior to analysis. Sieved samples were placed into cylindrical sample holders with a 3.6- μ m Mylar filter and then introduced in the EDXRF spectrometer. An extended analysis time (23 min) was used to achieve the minimum standard deviation of each data. From the total list of major, minor and trace elements analyzed, As, Cd, Fe, Pb and Zn were specially chosen for this study because of their abundance in these types of mine wastes and because most of them are included in the priority contaminant list of environmental protection agencies [15]. To ensure the quality of the analyses, duplicate samples were analyzed to check precision and a certified reference material (SRM 2711) was analyzed for accuracy. The precision results of the studied elements were Zn (87.3%), As (82.9%), Pb (93.1%) and Fe₂O₃ (88.4%). The recovery percentages for the certified elements in SRM 2711 were Fe (97.7–98.0), Zn (98.8–99.9), As (79.1–83.9) and Pb (111.0–112.7).

3.3. Mineralogical Analysis of Samples

Main mineral phases were determined by means of X-ray diffraction (XRD) using a Bruker D8 Advance® diffractometer equipped with a Cu anticathode at CAI-Unidad de Técnicas Geológicas, Universidad Complutense de Madrid. An aliquot of each sample was grinded in an automatic agate mortar and manually until all the grain size was less than 53 μ m. Work conditions of the diffractogram obtention were: 2 θ angles (2°–65°), 0.02

stepping intervals and 1 s per step. The semiquantitative analysis was carried out according to the Chung method [16–18] using EVA from Bruker software.

3.4. Samples Leaching Procedure and Soluble PTE Content

The leaching process was carried out for each sample to obtain the aqueous extract used on bioassays with aquatic organisms. The method followed was in accordance with the Spanish legislation of contaminated soils, the Royal Decree (RD) 9/2005, which establishes a list of potentially polluting soil activities and the criteria and standards for the declaration of contaminated soils. The method proposed by the RD is the DIN 38414-S4 [19]. It specifies to use 100 g of dry soil sample (with a grain size <10 mm), mix it with 1000 mL of deionized water (leaching mass relation: 10 Liquid/Solid) and submit it to an upside-down agitation, in a Reax20 rotary agitator (Heidolph®), during 24 h at room temperature. The solid and liquid phases are separated via sedimentation at 4 °C overnight. Liquid one was filtered with a 0.45-µm pore size fiber membrane (Pall®). To avoid the growth of microorganisms during the storage, the samples were stored at 4 °C in darkness until assayed.

The Pb, Cu, Fe and Cd contents in leachates were determined via electrothermal atomic absorption spectrometry (ETAAS) using an ICE 3300 spectrometer (Thermo Fisher, USA). The reliability of the results was verified by means of certified reference material (EnviroMAT SS-2 Soil Standard), obtaining recoveries of Pb (101.5%), Fe (108.4%) and Cd (87.9%).

3.5. Bioassays

3.5.1. Immobilization Test with *Daphnia magna*

The assay with *Daphnia magna* (DM) was carried under the OCDE Technical Guideline 202 [20]. To determine the 48 h EC50 (sample dilution required to immobilize 50% of *Daphnia magna* population after 48 h exposure), *D. magna* organisms aged less than 24 h were obtained from dormant eggs (ephippia) supplied in a commercial kit, Daphtoxkit F™ (Microbiotests Inc., Ghent, Belgium). Ehippia were hatched after 72 h of incubation in culturing media at 21 ± 1 °C and 4000 lux of continuous illumination in a climatic chamber. Afterwards, twenty neonates (five neonates per four replicates) were exposed to different tested concentrations and a blank control [21]. The mortality rate in *Daphnia magna* was 0% in the controls. Concentrations were obtained via dilution of the leachates with the standard culture medium supplied with the Daphtoxkit™. The concentrations, expressed as percentages of the sample in the final dilution, were 50%, 25%, 12.5%, 6.25% and 3.12% (v/v). The assays were conducted in multiwell plates (30 wells/15 mL) and incubated in a climatic chamber at 21 ± 1 °C in darkness. After 48 h of incubation, immobility was checked in order to determine the mortality of the exposed population. Daphnids were considered immobilized if they were not swimming within the observation period of 15 s [22]. The toxicity was expressed as the EC50 (%; v/v), causing lethality on 50% of the exposed organisms.

3.5.2. Immobilization Test with *Thamnocephalus platyurus*

The *Thamnocephalus platyurus* (TP) test was performed using the Thamnotoxkit F™ (Microbiotests Inc., Ghent, Belgium) following the standard operational procedure provided in the kit [18], with some modifications [23]. The test follows the ISO 14380:2011 standard [24]. Dormant eggs of *T. platyurus* were incubated 24 h prior to the start of the toxicity test under continuous illumination (4000 lux) at 24 ± 1 °C in a climatic chamber (model ARTI-150L, Microbiotests Inc, Gent, Belgium). The assays were performed in 24-well plates (10 organisms per well in triplicate). Five dilutions (100%, 50, 25, 12.5, 6.25 and 3.12%; v/v) were prepared from each leachate by using the standard fresh water (U.S. EPA moderately hard reconstituted water) supplied in the Toxkit™. Ten milliliters of each dilution were added to each well; the fresh medium was used as a control. The number of

dead organisms was used as an endpoint, and the EC50 (%; *v/v*) causing lethality on 50% of the exposed organisms was calculated. Exposed organisms were considered immobilized (dead) if they were not swimming within the observation period of 10 s [22]. The mortality rate in *Thamnocephalus platyurus* was 0% in the controls.

3.5.3. Freshwater Algae—Growth Rate Inhibition Test with *Raphidocelis subcapitata*

Raphidocelis subcapitata (RS) (formerly *Selenastrum capricornutum*) cells from the commercial Algaltoxkit F™ (Microbiotests Inc., Ghent, Belgium) were used. This kit test conformed with ISO 8692:2012 and OECD TG 201 [25]. The alginate beads containing algae were disaggregated according to OECD guidelines (Annex 3, TG 201, 2011). The cells were exposed to undiluted samples and five serial dilutions (50%, 25%, 13.5%, 6.25% and 3.12%, *v/v*) and one negative control (100% of growth). Sample dilutions were conducted with an ISO algal culturing medium. Each sample was run in triplicate. The algae growth assays were carried out in 100-mm long spectrophotometric cuvettes. Initially, the initial biomass concentration was 10^4 cells mL⁻¹. The cuvettes were incubated at 25 ± 1 °C for 72 h under continuous illumination (4000 lux) in a climatic chamber with model ARTI-150L (Microbiotests Inc., Ghent, Belgium), equipped with four white light led lamps. Growth inhibition rates relative to negative controls were determined using measurements of optical density (OD₆₇₀) in a spectrophotometer (model Auris 2021, CECIL Instruments™, Cambridge, UK) equipped with a holder for 10-cm path-length cells. The inhibition growth (% I) in the tested dilutions versus the control growth is based on the determination of the average growth rates (μ) after transformation of the OD₆₇₀ values into cell numbers according to the manufacturer protocol [26]. The toxicity was expressed as a 72 h ErC50, the concentration (%; *v/v*) causing a 50% on the growth rate inhibition.

3.6. Assessment of Potential Environmental Risk

The potential environmental risk in SQMD was assessed by using the geoaccumulation index (I_{geo}), the pollution index (PI) and the pollution load index (PLI).

I_{geo} index [27] is calculated by conforming to Equation (1):

$$I_{geo} = \log_2 \left(\frac{C_i}{1.5B_i} \right) \quad (1)$$

where C_i the total concentration of the metal i in the sample and B_i the background concentration of this metal in the San Quintín mine. Those regional background values have been previously reported by Gallego et al. [28]. Based on the values obtained for I_{geo} , samples can be classified as [29]: <0, practically unpolluted; 0–1, unpolluted-to-moderately polluted; 1–2, moderately polluted; 2–3, moderately–strongly polluted; 3–4, strongly polluted; 4–5, strongly–extremely polluted and >5, extremely polluted.

PI is an index single that evaluates the pollution of soils and sediments. Its equation of calculation is defined using Equation (2):

$$PI = \frac{C_i}{B_i} \quad (2)$$

where PLI is the pollution load index and n is the number of metals evaluated. PI is the pollution index of each metal.

Finally, the PLI is calculated using the n th root of the multiplication of the contamination factors of the investigated elements, as in the following expression (3):

$$PLI = (PI_1 \times PI_2 \times PI_n \times \dots)^{1/n} \quad (3)$$

where PI_1 , PI_2 and PI_n are the pollution indexes of the elements 1, 2 and n , respectively. This index classifies the soil or water into four categories, which are: $PLI < 2$ (unpolluted-to-moderately polluted); $2 \leq PLI \leq 4$ (moderately polluted); $4 \leq PLI < 6$ (highly polluted) and $PLI > 6$ (very highly polluted) [30].

3.7. Statistical Analysis

A multivariate analysis was applied for the geochemical and mineralogical variables, including a correlation matrix and principal component and factor analysis, using Statgraphics 19 software package (Statgraphics Technologies, The Plains, VA, USA) [31]. The multivariate analysis was used to determine potential relationships between the PTE total contents and the mineralogical composition of the original samples and between the PTE contents in the leachates and ecotoxicological results. Factorial analysis permits a statistical approximation for analyzing interrelations between a large number of variables. The factorial analysis was carried out via the principal component extraction method, using a varimax normalized rotation of the factors. Toxicity was expressed as the percentage of effect as the median effective concentration (EC50) and the 10% effect concentration (EC10), as an alternative to the No Observed Effect Concentration (NOEC) [32]. Whenever possible, the 95% confidence limit values were set. ECx was calculated by means of Probit regression [33] in EPA Probit software (v1.5).

4. Results

4.1. Geochemistry and Mineralogy of Samples

The pH values ranged from 2.0 to 6.5, with a mean value of 4.7 in SQ east and 5.1 in the west area. Samples RSQO-01, RSQE-01 and RSQE-05 are the most acidic, with pH values of 2.0, 2.3 and 2.8, respectively (Table S1). As regards the EC values, SQ east showed similar values (average of 2200 $\mu\text{S cm}^{-1}$) to the west area, with average values of 2380 $\mu\text{S cm}^{-1}$. The highest EC values were found in RSQE-01 and RSQO-01, which showed 5470 and 4100 $\mu\text{S cm}^{-1}$, respectively. The obtained results suggested that samples with lower pH values correspond to samples with the highest of EC.

All samples showed a low OM content, with an average value of 1.65% in the west sector and 1.62% in the east sector. The highest OM value was 7.8% and was found in the west zone.

Total and soluble PTE contents are summarized in Tables S2 and S3. Average values for target elements were 130 mg kg^{-1} for Cd, 42,900 mg kg^{-1} for Pb, 15,100 mg kg^{-1} for Zn, 63,600 mg kg^{-1} for Fe and 47 mg kg^{-1} for As. Except for Fe, determined total PTE contents are higher in the west area (Table S2).

Compared with background values in the study region (Table 1), all samples exceed the average of the local background levels established by Gallego et al. [28] for the mining area. The mean concentrations are 85 times higher than the background for Cd and 268 times for Pb. The average values are also higher or similar than other studies, such as [4,13,28,34]. The results expose the need for treatment of the tailings and the restoration of the mining area. The determination of the potential risk of the pollutants is required to determine their influence on human life and associated ecosystems.

Table 1. Main PTE content in samples from the San Quintín mine, local background levels established in the San Quintín area [24] and other values of PTEs in other studies of the area [4,13,28,34]. All values are expressed as mg kg^{-1} .

	Cd	Pb	Zn	Fe	As	References
Mean SQMD	130	42,900	15,100	63,600	47	This work
Mean value east zone	58	32,400	6,700	71,300	7	
Mean value west zone	170	49,600	20,400	58,700	72	
Minimum	25	1800	600	36,300	0.05	
Maximum	440	144,900	47,700	202,600	549	
Background levels San Quintín mining area						
Local Background	1.5	160	87	28,963	8	[28]
Other studies in San Quintín mining area						
Mean	38	18,036	8825	42,597	88	[4]

Mean	-	21,892	11,242	54,573	-	[13]
Mean	-	11,260	8549	42,170	-	[34]

PTE content in leachates (Table S3) showed an average value of 9.7 mg kg⁻¹ for Cd, 20 mg kg⁻¹ for Pb, 580 mg kg⁻¹ for Zn and 230 mg kg⁻¹ for Fe. Considering both subzones, in the SQ west zone the mean values are much higher than those determined in the SQ east zone.

The semi-quantitative mineralogical composition is summarized in Table S4. In most cases, samples showed similar mineral composition, mainly composed of quartz (23%–51%), phyllosilicates (in some cases with chamosite of note contain) (15%–44%), feldspars (2%–8%), plagioclase (3%–10%), gypsum (3%–19%) and, to a lesser extent, iron oxides such as goethite, magnetite or hematite; sulfates and/or hydroxysulfates such as jarosite, baryte or anglesite and also alunite, sphalerite, siderite, pyrite, cerussite and dolomite/an-kerite, all as minor phases.

The mineralogy observed is coherent with the geological and mining context. The higher Pb concentrations are explained with the greatest amount of minerals phases such as jarosite or anglesite. In some cases, the content of these minerals reached 22% (RSQO-01). The presence of galena, sphalerite, and pyrite in some of the waste samples should be highlighted, even though they appeared in a low content percentage in most of the cases. Nevertheless, there are samples where the quantity of these ore, such as with FeS₂ and ZnS, rise to 6% (RSQO-6A) and 9% (RSQO-6B), respectively. The presence of pyrite is especially important in the context of mining restoration since it implies a very high capacity to generate acidity from mining waste. In this sense, the results suggest that the tailings in the western area, which were concentrated in old mineralurgical facilities with inefficient technologies, are more reactive and will need more aggressive amendments during restoration work. This oxidation process is evidently active in some areas of the western zone, where the presence of jarosite reaches concentrations of 22%, although it appears to be active in a low proportion in both areas and in almost all tailings, regardless of the efficiency of the oxidation concentration technology used.

4.2. Bioassays

The results of the ecotoxicity bioassays (48 h EC₅₀ and 48 h EC₁₀) are shown in Table S5; the toxicity values are shown by the percentage of the water extract accompanied by the confidence interval and the toxic units (T.U.). There is also a classification of samples based in R.D. 9/2005 and in [11].

For *Daphnia magna* organisms, the toxicity levels (EC₅₀) are between 0.2% and 14.9% of dilution. In three of the samples, the toxicity values have not been determined because the levels are over 50% of dilution, so they are not considered assails that are toxic toward *D. magna* (RSQE-03, RSQE-07 and RSQO-03). The higher mortality is attributed to RSQO-6A, which showed 458.7 toxic units. On the other hand, EC₁₀ presents values between 0.1% and 6.5% of dilution (Table S6).

According to the hazard classification system for wastes discharged into the aquatic environment carried out by Persoone et al. [11], 27.7% of the samples showed very high acute toxicity. Based on R.D. 9/2005, 28% (EC₅₀) of the samples are contaminated, corresponding to RSQE-01, RSQE-05, RSQO-02, RSQO-05 and RSQO-6A in both cases.

The toxic effect toward *Thamnocephalus platyurus* showed the greater mortality in RSQE-01, RSQE-04, RSQE-06, RSQO-05, RSQO-06A and RSQO-08. RSQE-01 showed the most mortal levels, reaching 2500 toxic units.

For *T. platyurus* organisms, 33.3% of the samples presented high acute toxicity, slightly greater than the *D. magna* tests [11]. In addition, 33% of the samples were contaminated, 5% higher than in the *D. magna* test. In the case of EC₁₀, 44% of the samples exceed levels (R.D. 2005) (Table S6).

Tests based on *Raphidocelis subcapitata* showed the largest mortality data against PTE content, where 50% of the samples were under 1% of dilution for EC₅₀ values and 78%

for EC10 (Table S6). For that reason, 50% is considered as a very high acute toxic sample [11] and also as a contaminated sample, which manifests the sensibility of these organisms to PTEs with respect to *D. magna* and *T. platyurus* results. The most toxic sample was RSQO-05. The bioassays reveal that a major part of the samples presented at least acute toxicity [11] for *D. magna*, *T. platyurus* and *R. subcapitata*, in particular the samples whose higher element concentrations were Pb and Zn. This relationship between mortality and PTE contents is shown in Table 2. There is also a relation between the EC, pH and the bioassay results; EC especially seems to be an important factor for life development. However, the higher mortality in all the cases was not attributed to the same samples, therefore the effect of the characteristics of the leachates was different for each organism.

The sensitivity of *D. magna* against these parameters is confirmed in other works [9,35,36] that also show a correlation between Pb and Zn content.

Table 2. Pearson's correlation coefficients between leachates, PTE content, general geochemical parameters and bioassay's results (n = 18). * values are $p < 0.05$.

	Cd	Pb	Zn	Fe	pH	EC	EC50 <i>D.m.</i>	EC50 <i>T.p.</i>
Pb	0.3							
Zn	0.3	0.1						
Fe	−0.2	0.1	0.0					
pH	−0.4	−0.3	−0.7 *	−0.6 *				
EC	0.5	0.3	0.5 *	0.5	−0.8 *			
EC50 <i>D.m.</i>	−0.5 *	−0.7 *	−0.4	−0.2	0.5 *	−0.7 *		
EC50 <i>T.p.</i>	−0.5 *	−0.6 *	−0.4	−0.1	0.4	−0.5 *	0.8 *	
EC50 <i>R.s.</i>	−0.4	−0.8 *	−0.4	−0.2	0.5	−0.6 *	0.9 *	0.8 *

A cluster analysis has differentiated very clearly the group of Fe and OM from the rest of the variables, suggesting an absolute absence of influence of these factors on the toxicity of mining materials (Figure 2A). However, the relationship between the other variables appears to be very high, although two subgroups can be distinguished: the subgroup that includes pH with toxicity in crustaceans and algae, and the subgroup that includes PTEs (Cd, Pb and Zn) together with EC. The factorial analysis (Figure 2B) helped to discriminate between the samples that did not present acute toxicity—the B subgroup (RSQE-03; RSQE-07 and RSQO-03)—from the rest of the samples with acute toxicities in at least one of the tests carried out. In this group, the A1 subgroup (RSQE-02, RSQE-05, RSQO-05 and RSQO-06A) is related to high EC and Cd contents, the A2 subgroup (RSQO-04, RSQO-6B, RSQO-07, RSQE-04 and RSQE-06) shows low Cd concentrations and the last, the A3 subgroup (RSQO-02, RSQO-08, RSQO-09 and RSQO-10), presents slightly acute-to-acute toxicities and medium concentrations of Cd, Pb and Zn.

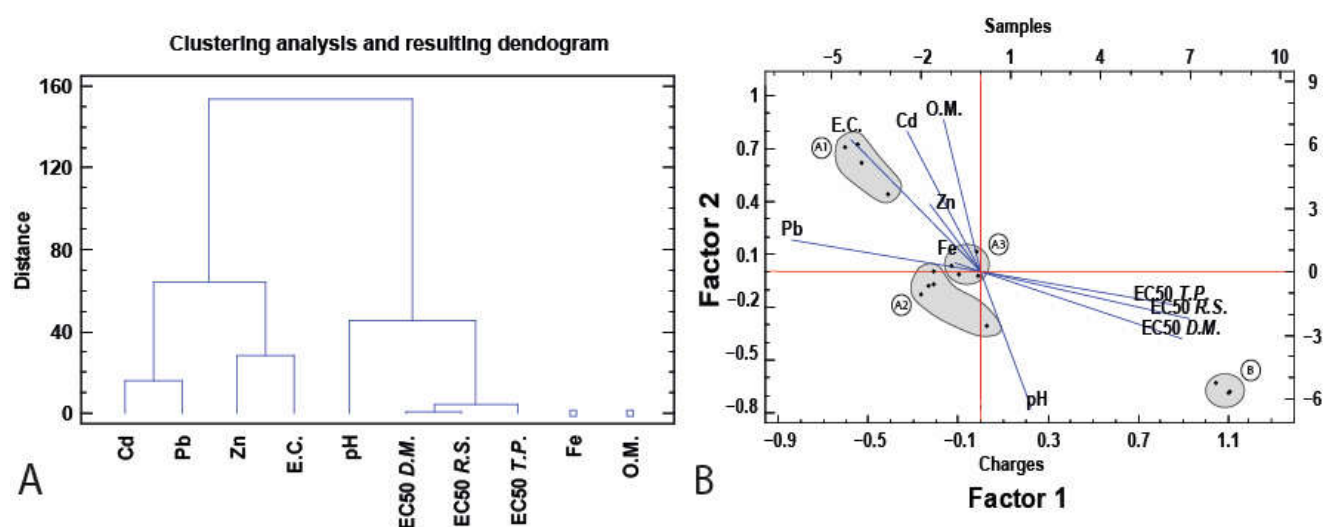


Figure 2. (A): Cluster and factor analysis for the studied samples. The lines correspond to the punctuations for the first and second factors of the considered values. (B): Principal component analysis (PCA) for the PTE, bioassays values, pH, EC and OM. Groups in the factor analysis graph were obtained in the cluster analysis.

4.3. Assessment of Potential Environmental Risk

Cd and Pb I_{geo} results are high in most of the samples, showing a high degree of contamination that is interpreted as being from strongly to extremely polluted. The highest one for Cd corresponds to RSQO-6B, whereas lead showed the greatest value in the RSQO-05 sample. As Cd I_{geo} and Pb I_{geo} values, Zn I_{geo} results are elevated; nevertheless, two samples (RSQE-03 and RSQO-01) showed slightly lower values than the rest; thus, they are considered as strongly polluted. For Fe and As, the results are quite different. Most of the samples were moderately polluted according to Fe I_{geo} . Arsenic values were the lowest ones, indicating unpolluted mining materials; however, there are highlighted samples that exceed the low values determined for the rest: RSQE-02, RSQO-05 and RSQO-6B. The I_{geo} values reveal the potential risk that the area poses to the surroundings as well to the human and environmental life.

According to the PLI calculated for the selected elements, only RSQE-03 and RSQE-07 can be categorized as moderately polluted–unpolluted and moderately polluted, respectively, whereas the rest are highly polluted-to-very highly polluted. The maximum PLI value is 100.16 and corresponds to RSQO-05, located in one of the ponds of San Quintín West. Seventy-eight percent of the samples are classified as very highly polluted ($PLI > 6$).

In relation to the I_{geo} index and PI and PLI results, we could make a comparison with other works in the San Quintín mine [4,37]. In case of the I_{geo} [37], the results concerning Pb and Zn are quite similar, manifesting extremely contaminated wastes. For Cd values, results show differences, moderately contaminated for [37] and extremely contaminated in our case. The same phenomena appear in the comparison of [4] the PLI results. The data from this work estimate larger values, except for As and Fe, that are similar to those determined by García-Lorenzo et al. [4]. Thus, the PLI is higher in our case. In any case, all the works interpret the result as being a very highly polluted residue. Differences may be due to the background values selected.

5. Discussion

In the characterization of the risk produced by mining waste from the San Quintín mine, data on basic pedological parameters (pH, EC, OM) as well as mineralogical and multielemental data have been obtained. Based on the latter, both the I_{geo} of the most important PTEs in the area have been obtained, as well as the PLI of a combination of these, to evaluate the synergies that can be established between them. Finally, the toxicity of the residues has been evaluated by means of tests with crustaceans (*Daphnia magna* and *Thamnocephalus platyurus*) and algae (*Raphidocelis subcapitata*), obtaining a categorization of the wastes, both from the multi-elemental point of view (I_{geo} and PLI) and from a toxicological point of view (based on the EC50 of the three biotests) (Table 3).

Table 3. Categorization of bioassays, I_{geo} values and PLI results, according to [11,29,30], respectively. The color scale is shown for each classification. DM: *Daphnia magna*; TP: *Thamnocephalus platyurus*; RS: *Raphidocelis subcapitata*. Toxicity results are shown as TU. Toxicity values less than 2 are indicated without color in the cell because they cannot be assigned to a single class.

Sample	DM TU	TP TU	RS TU	I_{geo} Cd	I_{geo} Pb	I_{geo} Zn	I_{geo} Fe	I_{geo} As	PLI
RSQE-01	100	2500	430	5.9	8.9	6.9	3.1	−6.1	9.0
RSQE-02	76	68	490	6.1	9.7	6.8	1.9	3.3	32
RSQE-03	<2	20	<2	4.6	4.1	3.4	1.1	−6.0	1.8
RSQE-04	7	145	<2	6.5	7.7	8.3	1.3	−6.0	7.9
RSQE-05	320	<2	4760	6.3	8.4	6.4	1.9	−6.0	7.0
RSQE-06	15	1560	250	5.9	7.7	7.0	1.5	−6.0	6.2
RSQE-07	<2	<2	<2	5.0	5.0	5.1	1.2	−6.0	2.8
RSQO-01	38	10	59	5.1	7.9	3.5	3.4	−6.0	4.6
RSQO-02	180	<2	2080	6.8	8.5	8.6	1.6	−6.0	10
RSQO-03	<2	<2	<2	6.0	6.5	7.8	0.9	−6.0	5.6
RSQO-04	66	19	90	7.3	9.0	8.3	0.9	−6.0	10
RSQO-05	270	660	190	8.3	10	9.2	1.5	6.8	100
RSQO-06A	460	240	130	7.4	10	7.9	1.4	−6.0	12
RSQO-6B	38	44	110	8.8	10	9.7	1.2	5.5	84
RSQO-07	90	66	150	6.9	7.7	8.1	0.9	−6.0	7.7
RSQO-08	48	110	28	7.4	7.7	8.2	1.2	−6.0	8.8
RSQO-09	54	50	8	6.8	7.3	8.7	1.0	−6.0	7.9
RSQO-10	26	55	49	7.5	7.5	8.4	1.0	−6.0	8.6

The values obtained from I_{geo} that categorize a greater number of samples as extremely polluted correspond to Pb, Zn and Cd, the elements present in the mineral phases of paragenesis (galena and sphalerite). Other PTEs related with pyrite or arsenopyrite (Fe, As) appear to be less pollutant in terms of I_{geo} , surely due to the presence of Fe oxides in the local lithologies. Three samples with low I_{geo} values have been found for almost all PTEs, a sample from an old mineralurgical facility (RSQE-03), another from a modern tailing of coarse (sandy) material (RSQE-07) and a sample from a waste material from the western zone with abundant signs of acidity generation and high proportions of OM (RSQO-01). When we consider the PTEs as a whole through the PLI, all the samples are categorized as very highly polluted except for the three aforementioned samples, which coincide with the Cd, Pb and Zn I_{geo} . [32] have applied a similar approach to categorize the evolution of the risk of contaminated soils in the San Quintín area through the use of I_{geo} and PLI. Their findings are in accordance with our I_{geo} data for Pb and Zn, with differences in relation to Cd and Cu.

From a toxicological point of view, mining waste has shown a great variety of categories according to [11]. Some of the samples have shown toxicities typical of classes I, II or III (<2 TU) for the three tested organisms and low toxicity values determined in samples

of processed materials (tailings) from the two areas of the mining group. A remarkable fact is the values of the RSQE-05 sample, which has shown low toxicity values (<2 TU) for *Thamnocephalus platyurus*, but high values for the other two organisms tested.

Differences can be observed between crustaceans and algae, with a higher number of samples with acute toxicity in the case of algae, which suggests that the phytotoxicity of these mining residues could be of concern. These findings are in accordance with García-Lorenzo et al. (2019) [4], who described an inhibition of the germination of *Sorghum saccharatum* (Sorgho), *Lepidium sativum* (Garden cress) and *Sinapis alba* (mustard) for samples with high contents of Pb, As and Cd, in addition to root inhibition in the samples with high Pb contents in the San Quintín area. However, the non-toxicity for the TS of two samples that have acute toxicity for DM and RS does not seem to be explained by the data obtained in the present study. It could be a synergistic effect between inorganic contaminants (PTEs) and major elements, such as those described for Cd-Zn-Cu in some plant species [38].

The combination of “inorganic” indices is often used in the characterization of risks in mining areas, such as gold mines in Ghana [39] or Brazil [40], or coal mines in Australia [41] and India [42], but the combination with toxicological risk categorization using bioassays does not have many published references. For instance, Varga et al. [43] describes the differences in sensitivity of different plant species in the categorization of risk for sediment and pore water in a polluted channel in Serbia.

This paper presents for the first time the combination of these indices in the categorization of mining waste prior to its restoration, taking into account not only the presence of PTEs in high concentrations compared to local geochemical backgrounds, but also their toxicity through tests with organisms that live in an aquatic environment (crustaceans and algae). In the case of the San Quintín area, the combination of these indices shows that practically all wastes can be considered as polluted (moderately–extremely) based on their Pb, Zn and Cd content, but that the risk derived from the presence of these PTEs does not imply acute toxicity in a group of five samples from tailings and dumps nor a risk of slight acute toxicity in a large group of samples from tailings in the western area of the mining group. It has also been possible to establish that phytotoxicity is higher than the toxicity to small organisms (crustaceans), in accordance with data described by García-Lorenzo et al. [4], which could have implications for the management of these wastes during restoration. The fact that the toxicity of the mining materials is less than their contaminated levels offers the possibility of not completely confining these residues or allowing the access of the roots of trees and shrubs to the residues through the technosol designed to cover the mining materials. Some recent studies suggest that Pb and Zn are less toxic for local plants than Cd [44,45]; therefore, it would be possible to categorize wastes with non-acute toxicity risk and a low Cd content (samples RSQE-03 and RSQE-07) as susceptible to lighter restoration treatments and thinner technosol covers, which would produce an improvement in the optimization of restoration treatments. This optimization will be especially interesting in the case of the RSQE-07 sample, which represents tailings with a large volume of materials and a thickness of 8–12 m. It would be excessively expensive to apply a limestone amendment to prevent the generation of acidity, so the possibility of applying the amendment to the first meter of waste and covering it with a 50-cm technosol makes it possible to restore this large volume of waste.

6. Conclusions

This work reveals that the San Quintín mine area is highly polluted by PTEs, especially the western sector, where the mineral concentration treatment was not effective enough. The geochemical and mineralogical characterization shows high contents of Pb, Cd, Zn, Fe and also As. The most elevated ones are Pb, Zn and Fe, reaching values of 202,600 mg kg⁻¹, 144,900 mg kg⁻¹ and 47,700 mg kg⁻¹, respectively, all of them located in the western area. The mineralogical phases are constituted principally by quartz, phyllosilicates, oxides, sulfates, hydroxysulfate and others in less percentage. The presence of

ores such as pyrite and sphalerite in the west area of San Quintin has to be highlighted; indeed, this has to be considered in future restorations and also in acid drainage amendments.

Bioassays reveal that algae organisms (*R. subcapitata*) are more sensitive to PTE contents; thus, their toxicity values are higher than the results for the crustaceans (*D. magna* and *T. platyurus*). The lowest EC50 data from *R. subcapitata*, *D. magna* and *T. platyurus* were 0.20, 0.22 and 0.04, respectively. Statistical information disclose that the highest values of toxicity are related to PTEs (Pb, Cd and Zn), EC and especially to pH.

The joint use of biological, geochemical and mineralogical techniques can provide useful information for environmental management and also to improve the restoration procedure in terms of cost–benefit.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/min13040492/s1>, Table S1: pH, EC ($\mu\text{S cm}^{-1}$) and OM values (%). Table S2: Total PTEs values determined for target elements (mg kg^{-1}); Table S3: PTEs content in leachates (mg kg^{-1}); Table S4: Mineralogical composition (%); Table S5: EC50 values calculated (Probit regression) and their respective confidence intervals (95%) for *Daphnia magna*, *Thamnocephalus platyurus* and *Raphidocelis subcapitata*. The values are expressed as a percentage of sample in the final dilution. * TU: toxic units, 100/EC50. ** R.D. 9/2005 (Annex III); <1% C: contaminated; >1% NC: not contaminated. *** [11] Hazard classification system (HC); <0.4: Class I, no acute toxicity; 0.4 < TU < 1: Class II, slight acute toxicity; 1 < TU < 10: Class III, acute toxicity; 10 < TU < 100: Class IV, high acute toxicity; TU > 100: Class V; very high acute toxicity. ND: undetermined, HC between I–III. Table S6: EC50 and EC10 values calculated and their respective confidence interval (95%) for *Daphnia magna*, *Thamnocephalus platyurus* and *Raphidocelis subcapitata*. The values are expressed in percentage of dilution used in the bioassay. * TU: toxic units, 100/EC50. ** R.D. 9/2005 (Annex III); <1% C: contaminated; >1% NC: not contaminated. *** Persoone et al., 2003 Hazard classification system (HC); <0.4: Class I, no acute toxicity; 0.4 < TU < 1: Class II, slight acute toxicity; 1 < TU < 10: Class III, acute toxicity; 10 < TU < 100: Class IV, high acute toxicity; TU > 100: Class V; very high acute toxicity.

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