



## 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



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### ABSTRACT

This work is aimed to the establishment of some clues concerning metal uptake by the holm oak (*Quercus ilex* L.) in abandoned base metals (Pb-Zn) mining sites from central Spain. The study is based on total contents of potentially harmful elements (PHEs), including Pb, Zn, Cu, As, Sb, Cd and Hg, in leaves from holm-oak trees as well as in the corresponding soils, in three different sites included in the Alcudia Valley Mining District. Besides, we present other analytical data for the corresponding soils: pH; electrical conductivity; organic matter; and selective extractions: i) Modified Geological Survey Field Leach Test (using deionized water); extraction with ammonium acetate solution at two different pH values; EPA Method 1312 (using a H<sub>2</sub>SO<sub>4</sub>:HNO<sub>3</sub> solution); and extraction with an EDTA solution. This is aimed to search for relationships between PHE concentrations in the leaves and the different parameters measured in the corresponding soil, in order to determine bioavailability of PHE in this type of environments. The results show areas impacted by different degrees of PHE; in the most polluted area Pb concentrations reach 300 times the regional baseline and background values indicated by World Health Organization. Also, detected concentrations of the elements in leaves show that the holm-oak tree is a species with a very low bioaccumulation capacity, as shown by a selective uptake of PHE, favouring Zn, Cd and Cu with respect to Pb and As, and with Hg as a peculiar case, influenced by foliar uptake. However, metal concentrations in leaves reach levels above published toxicity levels thresholds, in particular for Pb and Zn. After this study, the soil to plant transfer capacity depends on different parameters for each element, being extremely difficult to generalise a common scenario favouring or preventing this process. Instead, we have found a complex case, conditioned by the soil properties (reactivity, salt contents and presence and concentration of organic matter) and to lesser extent, by PHE speciation in the soils.

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### 1. Introduction

Mining and smelting activities of metal sulfide deposits usually result in contamination of water, air and soil, due to generation of tailings, waste materials and acid mine drainage (AMD) with high concentrations of heavy metals and metalloids considered as potentially harmful elements (PHEs) (Lillo et al., 2015). In turn, some forms of these elements can be transferred to biota through the food chain and induce toxic effects.

The Alcudia Valley Mining District (AVMD) (Ciudad Real, Spain; Fig. 1) is an important case of abandonment of base metals (Pb-Zn) sulfide

exploitations, with relatively high contents of other PHE such as As, Cu and Sb (Higuera et al., 2012a). Most of the land affected by these abandoned mining sites has not undergone reclamation. One of the worst cases is the San Quintín Mining Complex (SQMC) where the area affected by mining activities extends for about 600,000 m<sup>2</sup> (Ruiz, 2008; Ruiz et al., 2009; Rodríguez et al., 2009; López García et al., 2010). The complex hosts tailings deposits from two historical periods and these have high to extremely high concentrations of Pb, Zn and associated elements (As, Cd, etc.) (Martín-Crespo et al., 2015). A failed experiment to concentrate cinnabar (HgS) from the nearby Almadén mines via froth flotation in 1990 left additional mercury-rich residues in the area.

Heavy metal contamination from smelting and mining activities has been widely studied in several environmental compartments: air (Higuera et al., 2005; Llanos et al., 2011), water (Gray et al., 2004;

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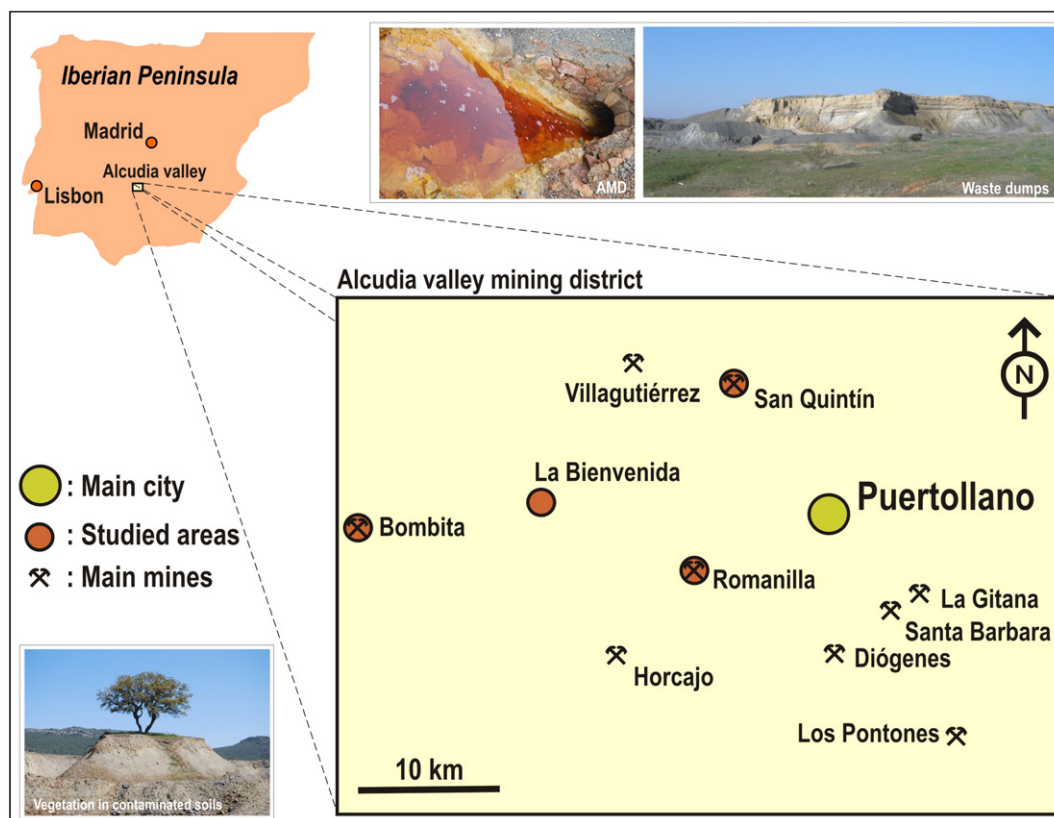


Fig. 1. Location of studied areas and main mines of Valle de Alcudia.

Nelson and Lamothe, 1993), soil (Adamo et al., 1996; Higuera et al., 2004) and biota (Molina et al., 2006; Bech et al., 2012; Higuera et al., 2015; Jung and Thornton, 1996; Pareja-Carrera et al., 2014; Rodríguez-Estival et al., 2014). Published works focused on soil contamination of AVMD and soil-plant transfer are relatively scarce: Reglero et al. (2008) studied PHE pollution of soils and the transfer to several species of plants in two mining areas (*Minas de Horcajo* and *Navalmartina*) located in the Alcudia Valley. Ruiz et al. (2009) and Rodríguez et al. (2009) characterized the Pb, Zn and Cd contamination of the soils in the surroundings of the San Quintín Mining Complex (SQMC). Higuera et al. (2012a) characterized soils and tailings from several mining areas in the Alcudia Valley District from a geochemical point of view.

Toxicity and dispersion of chemical elements in soils and other environmental matrices depend not only on total concentrations, but also on their specific chemical form, the type and strength of the chemical bonds, the physical and mineralogical properties of the solid phase, the solutions in contact with the sample, environmental factors such as climate, and last but not least, the bioavailability of the chemical form (Hasselriis and Licata, 1996; Nyamangara, 1998; Wang et al., 1999; Oyarzun et al., 2011a; among others).

The bioavailable fraction represents the concentration of a pollutant present in an environmental compartment that is available to participate in biological processes, being able to be absorbed by microorganisms or plants (e.g., Ruiz et al., 2009). This fraction includes the water soluble forms (ions, complex ions) present in the soil solution, the exchangeable species that are weakly adsorbed on particles, and the solid reactive phases that can easily undergo hydrolysis in soil-plant system (e.g., Anjos et al., 2012).

Selective extraction procedures (SEPs) by means of specific chemical extracts are widely applied to assess the geochemical fractionation and potential mobility of the elements in waste materials, sediments and soils, among many other substrates. Metal phytoavailability is a complex term, dependent of the plant species, the type of exposure and

the chemical speciation of the metals, among others. For this reason, many different procedures have been proposed and applied to assess this bioavailable fraction, even some of them are included in legislation, with different extractant agents: 1 M  $\text{NH}_4\text{NO}_3$  in Germany; 0.1 M  $\text{NaNO}_3$  in Switzerland; or 0.1 M  $\text{NH}_4\text{OAc}$  in France (Meers et al., 2007a). Many reagents have been used to extract water-soluble, exchangeable and organically bound fractions, finding significant correlations with metal uptake by vegetation (Ure, 1996a, 1996b). In this work, water-soluble fraction has been studied with ammonium acetate ( $\text{NH}_4\text{OAc}$ ) (Basar, 2009) and water (Hageman, 2007), while exchangeable and organically bound fraction was assessed with  $\text{CaCl}_2$  (Pueyo et al., 2004) and ethylene diamine tetra-acetic acid (EDTA) (Zhang et al., 2010).

Kirkby (2012) sets a list of 17 major and trace elements that are considered as essential for plant nutrition (C, O, H, N, Mg, S, P, Ca, K, Fe, Mn, Zn, Cu, Mo, among others). Some of these are necessary only for certain species, meanwhile others have stimulating effects for plant growth, although their functions have not yet been identified. Other elements, such as Cd, Co, Hg, Pb, As and also probably Be and Sn, appear as unneeded for plant life (Opik and Rolfe, 2005). On the other hand, and as it happens for human nutrition, many elements, essential or not, may be toxic for plants if present in the soil in excessive concentrations (Rout et al., 2001; He et al., 2005; Clemens, 2006; Smolders et al., 2009; White and Brown, 2010); these elements are referred as potentially harmful elements (PHE).

Information about the element translocation in the plant is provided by the ratios between elemental concentrations in leaves and soils (Mench et al., 1997). Leaf element concentration depends on their bioavailable concentrations in soils, which represent only a small fraction of the total concentration that can be absorbed by plants through roots and transferred to the leaves through the xylem (De Nicola et al., 2003). In a lower proportion and only some elements that deposit with dust on leaf surfaces or are present in the form of vapor in the atmosphere can be directly absorbed by the stomas (Reimann et al., 2001).

The holm oak (*Quercus ilex* L.) is a sclerophyllous evergreen tree species that is typically and widely distributed throughout the Mediterranean region of Europe. Approximately 60% of *Q. ilex* forest is located in Spain (a total coverage of about 3 Mha; Corcuera et al., 2004), and almost 44% of these forests are coppice stands (the so called *dehesa*, a traditional type of woodland management) (Rivas-Martínez and Sáenz, 1991). *Q. ilex* is a drought-tolerant species that grows on a wide variety of soil and physiographic settings (Martínez-Vilalta et al., 2002). The holm oak trees provide refuge for livestock, breeding sites for vertebrates and firewood from pruning. Their fruits are used to feed livestock and in the past they were also used for human consumption. The holm oak shows a high capacity to retain Cd in the roots (Domínguez et al., 2009), enhancing its potential for the stabilization of polluted sites. Most of the study area is covered by the *dehesa*-type landscape, in which the initial forest is reduced to isolated groups of trees in a landscape dominated by perennial and annual grasses.

The aims of this research are: *i*) to evaluate the extent of PHE contamination of soils and holm oak in the Alcudia Valley Mining District, especially in the San Quintín Mining Complex; *ii*) to determine fractionation and mobility of selected PHEs in soil samples through selective extraction procedures; *iii*) to evaluate the soil-plant transfer rate of PHE; and *iv*) to establish the selective extraction procedure that better relates the soil extracted concentration with the absorbed concentration by the plant (bioavailable concentration).

## 2. The Alcudia Valley decommissioned mines

The Alcudia Valley is located in the Ciudad Real province (Castilla-La Mancha region), in South-Central part of the Iberian Peninsula, approximately 250 km South of Madrid (Fig. 1). The climate is Mediterranean sub-humid with marked seasons. The Alcudia Valley was one of the most important mining districts of Spain, and is located within a broad metallogenic province known as *Sierra Morena*, which was the main lead producer in Spain during the second half of the 19th century (Palero and Martín-Izard, 2005). The Pb-Zn deposits have been classified into different types based on their morphology, host rock relations, structural setting, mineral assemblages and geochemical data. Galena (PbS) was the most abundant ore mineral, whereas sphalerite (ZnS), chalcopyrite (CuFeS<sub>2</sub>), pyrite (FeS<sub>2</sub>) and marcasite (FeS<sub>2</sub>) were of lesser importance (Palero, 1991; Palero et al., 1992). Mining started at the beginning of the 1st Century (A.D.) (Reglero et al., 2009). During the late 19th and early 20th century, the Alcudia Valley District was an important Pb-Zn-Ag producer (Palero and Martín-Izard, 2005), but from the 1930s onwards, production fell, and, in 1988, the last mine was definitely closed (Hevia, 2005). A total of 484 old prospects and mines are located in an area of about 2500 km<sup>2</sup>, the majority of which targeted Pb-Zn veins (Palero et al., 1992). Ag, As, Bi, Cu, Sb, Sn and W, among other metals, were also obtained in the AVMD. The most important producer was the SQMC (500,000 Mt of Pb metal) (Palero and Martín-Izard, 2005). The SQMC (Fig. 1) had several mines and is divided into the so called San Quintín East and San Quintín West sectors. The veins were exploited up to 2500 m in length and to >700 m in depth (Gómez-Ortiz et al., 2010), and the main ore minerals were galena and sphalerite. Other local minerals that pose additional environmental concerns are boumonite (PbCuSbS<sub>3</sub>), boulangerite (Pb<sub>5</sub>Sb<sub>4</sub>S<sub>11</sub>) and tetrahedrite Cu<sub>6</sub>[Cu<sub>4</sub>(Fe,Zn)<sub>2</sub>]Sb<sub>4</sub>S<sub>13</sub> (Palero and Martín-Izard, 2005).

The first mining works references are from 1559 and activity ended in 1934. Later (from 1973 to 1988), a froth flotation plant was installed for the recovery of about three million tons of low-grade Pb, Zn and Ag minerals from the tailing (Palero, 1991; Palero et al., 1992). Additionally, during the early 1990s an unknown number of tons of cinnabar from Almadén Mining District were partially processed at San Quintín. At present, the mining complex is abandoned and tailings with extremely high contents in Pb and Zn deposits are recognized throughout the area (López García et al., 2010).

## 3. Materials and methods

### 3.1. Sampling

The holm oak (*Q. ilex* L. subsp. *ballota*) was selected as main biotic receptor of the PHEs contained in the soils because it is the most widely distributed species in the *dehesa* landscape of the Alcudia Valley. Given that our aim was to evaluate the soil-plant transfer rate of PHE, soil samples were taken in the areas where *Q. ilex* specimens grow. Soil samples and the corresponding *Q. ilex* leaf samples (50 leaves per tree) were collected in three different areas affected by Pb-Zn mining and their surroundings: *i*) the San Quintín Mining Complex (SQ samples, *n* = 7 trees); *ii*) the Romanilla Mining Group (*Veredillas* and *Romanilla* mines; RM samples, *n* = 8 trees); and *iii*) the Bombita Mine (BM samples, *n* = 9 trees) (Fig. 1). According to other authors that studied the *Q. ilex* species (Prasad and Freitas, 2000; De Nicola et al., 2008; Ugolini et al., 2013), only old leaf samples (older than one year; so, taken away from the extreme of branches) were taken from the branches far apart to the buds. Leaves were collected by hand, using gloves, from the lower to medium part of the canopies, in order to obtain representative samples. All holm oak trees were aged (trunk diameter ≥ 15–20 cm).

Since the topsoil is the most important part of the soil profile for degradation control (Spaargaren and Nachtergaele, 1998), and the best option to assess PHE incorporation to soil profiles, the soil samples (2.5 kg) were collected at 0–25 cm from the surface, using a peak and a spade, and removing the organic debris. In addition, we took soil and *Q. ilex* leaf samples away from the studied mines at *La Bienvenida* (Fig. 1) to define baseline concentrations for the different elements (BA samples; *n* = 37 trees). All samples were stored in labeled polyethylene bags for their transport to the Almadén School of Mines (ASM) laboratory.

### 3.2. Sample preparation and analysis

#### 3.2.1. Sample preparation

Soil samples were dried at room temperature, with relative humidity lower than 60%, until the mass loss of the sample was <5% in 24 h (approximately 72 h in total). Plant debris and the largest pebbles were manually removed from the soil samples, and then these were disaggregated, homogenized and splitted to obtain three representative subsamples. One of these subsamples was used for the pH, electric conductivity and organic matter measurements; a second one was labeled and stored (bulk sample); and the third was crushed with a wood roller to pass a 2-mm sieve. A small fraction of the <2-mm soil particles were further ground with an agate mortar to 0.10–0.15 mm.

The *Q. ilex* leaf samples were cleaned several times to eliminate adhered dust particles, dried at room temperature, grounded in a blades electric mill and homogenized before chemical analysis.

#### 3.2.2. Soil and leaf chemical analysis

For soil total concentration analysis, approximately 500 mg of each sample were microwave (Telstar Cryodos) assisted digested at 200 °C ± 5 °C, for 15 min, using a hydrochloric acid (37%): nitric acid (69%) mixture in a 3:1 proportion (Melaku et al., 2005). 400–500 mg of *Q. ilex* leaves were microwave assisted digested at 180 ± 5 °C, for 9.5 min, using 9 ml of nitric acid (69%) and 3 ml of hydrochloric acid (37%) according to the EPA Method 3052 (USEPA, 2007).

Accuracy was obtained by using certified standards: NIST2710A (Montana Soil), for the soil microwave assisted digestion, and CRM482 (lichen) and LGC7162 (strawberry leaves), with the leaf microwave assisted digestion. The relative standard deviation in % (RSD) for three replicates of each certified standards and element was <10% and recovery values were inside the certified range for each certified standard. Also, the quality control at the ASM laboratory was done by analyzing duplicate samples and blanks to check precision.

**Table 1**  
Soil properties of the studied areas. Aver.: Average; SD: Standard Deviation.

Subarea	pH	Electrical conductivity ( $\mu\text{s cm}^{-1}$ )	Organic matter content (%)
Blank area (BA samples, n = 5)			
Aver. $\pm$ SD	6.17 $\pm$ 0.57	76.2 $\pm$ 56.6	4.65 $\pm$ 1.09
Range	5.57–6.79	28.2–160.8	3.53–6.36
San Quintín mining complex (sq samples, n = 7)			
Aver. $\pm$ SD	5.69 $\pm$ 1.27	273.2 $\pm$ 298.4	1.84 $\pm$ 1.04
Range	4.09–7.84	28.2–792.0	0.78–3.75
Bombita mine (bm samples, n = 9)			
Aver. $\pm$ SD	6.29 $\pm$ 0.62	48.5 $\pm$ 37.9	2.05 $\pm$ 2.10
Range	5.41–7.25	18.0–126.5	0.57–7.32
Romanilla mining group (rm samples, n = 8)			
Aver. $\pm$ SD	6.82 $\pm$ 0.83	136.8 $\pm$ 91.6	3.83 $\pm$ 1.62
Range	5.79–8.37	32.1–281.0	1.70–6.58

The contents of seven elements (Pb, Zn, Cu, As, Sb, Cd and Hg) on the acid digest solutions (soils and *Q. ilex* leaves; baseline and mining areas) were determined by inductively coupled plasma-mass spectroscopy (ICP-MS, Thermo Electron, XSeriesII) at the laboratories of the Faculty of Environmental Sciences and Biochemistry/Institute of Environmental Sciences (ICAM-UCLM). Detection limits for the analyzed elements are: Pb (0.21 mg kg<sup>-1</sup>), Zn (0.23 mg kg<sup>-1</sup>), Cu (0.05 mg kg<sup>-1</sup>), As (0.005 mg kg<sup>-1</sup>), Cd (0.01 mg kg<sup>-1</sup>) and Hg (0.01 mg kg<sup>-1</sup>). All the leaves samples had Sb contents below detection limit, and so this element has not been considered for the soil to plant transfer part of this study.

**Table 2**

Total heavy metal concentrations in the studied soils. SD: Standard Deviation; CLM: mean values for soils from Castilla-La Mancha region (Jiménez-Ballesta et al., 2010); CLM-RL: reference levels for soil calculated according to the Spanish Royal Decree 9/2005 (BOE., 2005) (using the CLM-RL data from Jiménez-Ballesta et al., 2010); WHO: Background levels for total metal concentrations in soil, established by World Health Organization (Tembo et al., 2006); MAC: maximum allowable concentration in agricultural soils (Chen, 1999; Kabata-Pendias and Sadurski, 2004); n.d.: not data available. All values in  $\mu\text{g g}^{-1}$  (dry wt.).

Subarea	Pb	Zn	Cu	As	Sb	Cd	Hg
SQMC (SQ samples, n = 7)							
Average $\pm$ SD	10,023.68 $\pm$ 15,779.21	2360.34 $\pm$ 1503.56	375.36 $\pm$ 424.37	110.41 $\pm$ 220.79	137.18 $\pm$ 223.05	8.84 $\pm$ 5.83	228.75 $\pm$ 473.02
Range	133.36–45,015.97	53.88–4450.48	6.61–1115.50	7.12–609.77	2.47–634.03	0.27–18.23	1.33–1289.53
RPI	290.88	23.63	16.98	5.94	59.13	63.14	357.42
RPLI	50.01–51.22 con Sb						
Bombita Mine (BM samples, n = 9)							
Average $\pm$ SD	2154.75 $\pm$ 3332.25	796.98 $\pm$ 1030.49	95.12 $\pm$ 157.54	17.75 $\pm$ 5.94	7.45 $\pm$ 6.37	1.29 $\pm$ 1.93	2.08 $\pm$ 1.44
Range	41.40–9572.85	63.24–3212.77	14.89–504.35	11.08–25.90	0.64–21.80	0.10–5.93	0.45–4.28
RPI	62.53	7.98	4.30	0.95	3.21	9.21	3.25
RPLI	6.28–5.71 con Sb						
Romanilla Mining Group (RM samples, n = 8)							
Average $\pm$ SD	4054.19 $\pm$ 4632.05	644.18 $\pm$ 872.71	63.98 $\pm$ 64.77	21.56 $\pm$ 5.98	24.05 $\pm$ 23.36	3.77 $\pm$ 5.54	1.03 $\pm$ 0.80
Range	46.95–14,243.75	40.64–2233.43	14.73–205.24	10.55–28.34	2.34–68.35	0.07–13.40	0.43–2.55
RPI	117.65	6.45	2.89	1.16	10.37	26.93	1.61
RPLI	6.93–7.35 con Sb						
Blank area (BA samples, n = 5) <sup>a</sup>							
Average $\pm$ SD	34.46 $\pm$ 7.98	99.89 $\pm$ 13.90	22.11 $\pm$ 2.42	18.59 $\pm$ 2.38	2.32 $\pm$ 0.69	0.14 $\pm$ 0.02	0.64 $\pm$ 0.34
Range	26.40–47.84	81.20–117.24	18.70–24.96	14.81–20.54	1.46–3.35	0.11–0.16	0.38–1.17
Reference values							
CLM	19.3	35.7	10.3	7.4	2.7	3.9	n.d.
CLM-RL	44.2	86.5	27.0	16.1	5.7	4.4	n.d.
WHO	<35	90	30	n.d.	n.d.	0.35	n.d.
MAC	20–300	100–300	60–150	15–20	<10	1–5	0.5–5

<sup>a</sup> Values obtained in this study.

### 3.2.3. Potentially harmful elements fractionation study (selective extraction procedures)

The following single selective extraction procedures have been carried out in the soil samples:

1. Modified Geological Survey Field Leach Test (Hageman, 2007). It consisted in putting 5 g of soil (<2 mm) in contact with 100 ml of de-ionized water and shaking at 50 rpm, room temperature, for 2 h in a shaking water bath. The supernatant was filtered through 0.45  $\mu\text{m}$  polytetrafluoroethylene (PTFE) filters. After this procedure, the elements removed are those being water soluble.
2. A quantity of 5 g of soil (<2 mm) was put in contact with 100 ml of a 1 M ammonium acetate solution at two different pH values. At pH = 7, samples were shaken at 50 rpm, room temperature, for 2 h in a shaking water bath. The supernatant was filtered through 0.45  $\mu\text{m}$  PTFE filters, and the filtered liquid can be considered as those which are exchangeable (Iyengar et al., 1981; Soon and Bates, 1982). Besides, extraction with ammonium acetate solution 1 M pH 4.5 was performed at the same conditions of the aforementioned step. Elements content in the filtered extract can be considered as those which are related to oxidizable mineralogical species (e.g. sulfide and organic matter bound fraction, etc.) (Sahuquillo et al., 1999).
3. EPA Method 1312, Synthetic Precipitation Leaching Procedure (USEPA, 1994). It consisted in putting 5 g of soil (<2 mm) in contact with 100 ml of a 60:40 (wt%) H<sub>2</sub>SO<sub>4</sub>:HNO<sub>3</sub> solution (pH = 4.2  $\pm$  0.05), shaking at 30 rpm, room temperature, for 18 h in a shaking water bath. After decantation, the supernatant was filtered through 0.70  $\mu\text{m}$  fiberglass syringe filters. After this procedure, the elements removed are those soluble in acid media.
4. A quantity of 5 g of soil (<2 mm) was put in contact with 100 ml of a 0.05 M EDTA solution. It was shaken at 50 rpm, room temperature, for 2 h in a shaking water bath. The supernatant was filtered through 0.45  $\mu\text{m}$  PTFE filters. Among the chelating agents, EDTA has been widely used to extract PHEs from soils because of its strong chelating ability (Sun et al., 2001). The extraction with EDTA allows the



**Table 3**  
Mean, standard deviation (SD), range (minimum–maximum) values and percentages of the total concentration (in brackets) of the extracted Cu, Zn, As, Cd, Hg and Pb concentrations ( $\mu\text{g g}^{-1}$ , dry wt.) by selective extraction procedures in the SQMC. b.d.l.: below the detection limit.

	Extracting solution	Deionized H <sub>2</sub> O	NH <sub>4</sub> Ac (pH = 4.75)	NH <sub>4</sub> Ac (pH = 7)	H <sub>2</sub> SO <sub>4</sub> :HNO <sub>3</sub> mixture	EDTA solution	
San Quintín Mining Complex	Cu	Average (%) $\pm$ SD	0.48 (0.43) $\pm$ 0.54	5.89 (3.38) $\pm$ 9.91	1.00 (0.54) $\pm$ 1.64	0.51 (0.46) $\pm$ 0.62	18.57 (12.22) $\pm$ 24.25
		Minimum-maximum (%)	0.08–1.62 (1.26)	0.16–28.02 (9.28)	0.04–4.64 (1.14)	0.09–1.83 (1.35)	1.24–71.85 (23.03)
	Zn	Average (%) $\pm$ SD	64.77 (2.88) $\pm$ 78.31	506.17 (21.31) $\pm$ 512.80	145.00 (6.27) $\pm$ 121.11	83.45 (3.60) $\pm$ 97.82	540.54 (23.24) $\pm$ 485.35
		Minimum-maximum (%)	0.33–200.00 (12.37)	5.37–1343.20 (43.59)	1.22–294.20 (12.59)	0.47–239.60 (14.82)	7.27–1196.20 (43.88)
	As	Average (%) $\pm$ SD	0.07 (0.35) $\pm$ 0.05	0.11 (0.45) $\pm$ 0.07	0.05 (0.28) $\pm$ 0.04	0.05 (0.28) $\pm$ 0.04	0.23 (0.92) $\pm$ 0.21
		Minimum-maximum (%)	0.02–0.15 (1.30)	0.03–0.20 (1.24)	0.02–0.14 (1.05)	0.01–0.12 (0.98)	0.03–0.68 (2.79)
	Cd	Average (%) $\pm$ SD	0.43 (7.31) $\pm$ 0.54	2.71 (28.05) $\pm$ 2.68	1.71 (19.45) $\pm$ 1.58	0.44 (7.40) $\pm$ 0.55	2.14 (27.73) $\pm$ 2.01
		Minimum-maximum (%)	0.01–1.41 (25.40)	0.13–7.80 (49.69)	0.07–4.61 (36.42)	0.01–1.37 (24.72)	0.15–5.85 (55.02)
	Hg	Average (%) $\pm$ SD	b.d.l.	0.08 (0.2) $\pm$ 0.06 <sup>a</sup>	b.d.l.	b.d.l.	b.d.l.
		Minimum-maximum (%)	b.d.l.	b.d.l.–0.15 (0.3)	b.d.l.	b.d.l.	b.d.l.
	Pb	Average (%) $\pm$ SD	36.68 (0.68) $\pm$ 44.95	4036.28 (40.90) $\pm$ 5887.92	2151.44 (13.89) $\pm$ 3879.99	36.45 (0.63) $\pm$ 45.05	1940.11 (37.68) $\pm$ 1807.87
		Minimum-maximum (%)	2.16–125.54 (1.62)	45.95–16,606.00 (63.03)	8.49–10,692.00 (26.19)	2.00–124.82 (1.50)	91.74–5234.00 (68.79)

<sup>a</sup> Mean for values above the detection limit.

determination of the mobilizable metals and may be well correlated with plant contents of Pb, Zn, Cd, Cu and Ni (Sahuquillo et al., 2003).

All the analysis has been performed in acid-washed pre-cleaned centrifuged tubes. Quality control at the ASM laboratory was accomplished by analyzing replicate samples to check precision. Blank extractions were carried out. The contents of the elements in the extracts were measured by inductively coupled plasma-mass spectroscopy (ICP-MS, Thermo Electron, XSeriesII). Detection limits for the extraction procedures are summarized on Table S1.

### 3.2.4. Statistical analysis of data

Analytical data were treated statistically in order to assess variations in elemental contents, as well as to establish relationships between contents in the leaves and total contents as well as those obtained from the extractions. Statistical treatment included the following steps:

- Basic statistics of single parameters (average, standard deviation, range);
- Calculation of a “Regional Pollution Index” (RPI), as the ratio between the average concentration of each analyzed element in our soil samples and a reference element concentration (Lee et al., 2006), in this case the average concentration for the baseline area;...
- Calculation of a “Regional Integrated Pollution Load Index” (RPLI), using  $(RPI_1 \times RPI_2 \times \dots \times RPI_n)/n$ , being  $n$  the number of PHEs (6 in our case excluding the Sb) (modified after Lee et al., 2006);
- Calculation of Biological Accumulation Coefficient (BAC) (Alloway et al., 1988), as the ratio between elemental concentrations in plant and soil;
- Correlation matrix of analytical data plus BAC calculated values, for each one of the studied areas and for each element plus edaphic parameters;
- Cluster analysis of analytical data plus BAC calculated values; link

**Table 4**  
Average, standard deviation (SD), range (minimum–maximum) values and percentages of the total concentration (in brackets) of the extracted Cu, Zn, As, Cd, Hg and Pb concentrations ( $\mu\text{g g}^{-1}$ , dry wt.) by selective extraction procedures in the Bombita Mine. b.d.l.: below the detection limit.

	Extracting solution	Deionized H <sub>2</sub> O	NH <sub>4</sub> Ac (pH = 4.75)	NH <sub>4</sub> Ac (pH = 7)	H <sub>2</sub> SO <sub>4</sub> :HNO <sub>3</sub> mixture	EDTA solution	
Bombita Mine	Cu	Average (%) $\pm$ SD	0.35 (0.76) $\pm$ 0.27	2.53(2.89) $\pm$ 3.41	0.63 (0.73) $\pm$ 0.87	0.37 (0.64) $\pm$ 0.39	10.04 (16.48) $\pm$ 10.10
		Minimum-maximum (%)	0.09–0.89 (1.87)	0.19–9.84 (10.40)	0.03–2.30 (3.00)	0.09–1.32 (1.07)	1.77–30.71 (35.91)
	Zn	Average (%) $\pm$ SD	6.95 (0.75) $\pm$ 10.36	175.48 (11.54) $\pm$ 282.14	83.26 (5.41) $\pm$ 126.76	17.04 (1.23) $\pm$ 26.31	189.14 (13.78) $\pm$ 289.86
		Minimum-maximum (%)	0.14–31.26 (2.94)	1.40–824.80 (27.74)	0.10–298.20 (19.42)	0.06–61.78 (5.73)	2.12–849.20 (29.66)
	As	Average (%) $\pm$ SD	0.09 (0.53) $\pm$ 0.10	0.10 (0.52) $\pm$ 0.09	0.08 (0.48) $\pm$ 0.05	0.07 (0.39) $\pm$ 0.05	0.20 (1.01) $\pm$ 0.18
		Minimum-maximum (%)	0.03–0.31 (1.34)	0.02–0.31 (1.20)	0.02–0.17 (1.15)	0.02–0.19 (0.83)	0.03–0.62 (2.52)
	Cd	Average (%) $\pm$ SD	0.03 (2.15) $\pm$ 0.04 <sup>a</sup>	0.59 (29.84) $\pm$ 0.85	0.47 (26.99) $\pm$ 0.68	0.06 (4.15) $\pm$ 0.07 <sup>a</sup>	0.46 (24.51) $\pm$ 0.56
		Minimum-maximum (%)	b.d.l.–0.10 (5.11)	0.02–2.50 (29.84)	0.003–1.91 (37.53)	b.d.l.–0.16 (7.96)	0.02–1.53 (41.61)
	Hg	Average (%) $\pm$ SD	b.d.l.	0.02 (1.63) $\pm$ 0.03 <sup>a</sup>	b.d.l.	b.d.l.	b.d.l.
		Minimum-maximum (%)	b.d.l.	b.d.l.–0.08 (9.62)	b.d.l.	b.d.l.	b.d.l.
	Pb	Average (%) $\pm$ SD	13.13 (1.64) $\pm$ 11.79	1388.13 (45.93) $\pm$ 2386.57	440.31 (10.03) $\pm$ 770.89	22.17 (1.20) $\pm$ 36.26	970.33 (50.34) $\pm$ 1325.10
		Minimum-maximum (%)	0.71–31.26 (3.18)	2.57–7264.00 (83.70)	0.13–2246.00 (25.17)	0.42–113.22 (1.78)	6.76–3848.00 (69.96)

<sup>a</sup> Mean value of above the detection limit data.

**Table 5**

Average, standard deviation (SD), range (minimum-maximum) values and percentages of the total concentration (in brackets) of the extracted Cu, Zn, As, Cd, Hg and Pb concentrations ( $\mu\text{g g}^{-1}$ , dry wt.) by selective extraction procedures in the Romanilla Mining Group. **b.d.l.:** below the detection limit.<sup>a</sup>

	Extracting solution	Deionized H <sub>2</sub> O	NH <sub>4</sub> Ac (pH = 4.75)	NH <sub>4</sub> Ac (pH = 7)	H <sub>2</sub> SO <sub>4</sub> :HNO <sub>3</sub> mixture	EDTA solution	
Romanilla Mining Group	Cu	Average (%) $\pm$ SD Minimum-maximum (%)	1.05 (1.14 $\pm$ 1.91) 0.11–5.72 (2.79)	2.92 (2.87) $\pm$ 5.18 0.17–15.12 (7.37)	0.69 (0.70) $\pm$ 1.32 0.04–3.92 (1.91)	0.72 (0.91) $\pm$ 1.17 0.10–3.56 (1.74)	13.06 (20.27) $\pm$ 13.70 1.92–43.13 (28.86)
	Zn	Average (%) $\pm$ SD Minimum-maximum (%)	13.89 (1.09) $\pm$ 28.52 0.10–82.26 (3.68)	16.57 (15.38) $\pm$ 283.94 2.14–778.20 (34.84)	86.44 (6.10) $\pm$ 194.61 0.24–562.20 (25.17)	15.65 (1.15) $\pm$ 34.70 0.05–100.36 (4.49)	171.79 (22.81) $\pm$ 250.44 5.48–628.20 (33.88)
	As	Average (%) $\pm$ SD Minimum-maximum (%)	0.16 (0.69) $\pm$ 0.10 0.04–0.29 (1.22)	0.24 (1.13) $\pm$ 0.21 0.04–0.63 (3.57)	0.16 (0.73) $\pm$ 0.17 0.03–0.50 (2.87)	0.12 (0.51) $\pm$ 0.06 0.03–0.21 (0.95)	0.60 (2.27) $\pm$ 0.49 0.11–1.59 (4.89)
	Cd	Average (%) $\pm$ SD Minimum-maximum (%)	0.13 (1.91) $\pm$ 0.29 0.001–0.84 (6.26)	1.75 (35.51) $\pm$ 3.07 0.02–8.88 (42.34)	1.36 (25.64) $\pm$ 2.81 0.004–8.18 (61.01)	0.13 (2.50) $\pm$ 0.28 <sup>a</sup> b.d.l.–0.77 (5.71)	1.32 (42.65) $\pm$ 1.78 0.03–4.35 (66.29)
	Hg	Average (%) $\pm$ SD Minimum-maximum (%)	b.d.l. b.d.l.	b.d.l. b.d.l.	b.d.l. b.d.l.	b.d.l. b.d.l.	b.d.l. b.d.l.
	Pb	Average (%) $\pm$ SD Minimum-maximum (%)	62.06 (1.05) $\pm$ 114.24 0.49–341.20 (2.40)	1553.29 (24.99) $\pm$ 2267.61 1.90–6868.00 (48.22)	686.57 (7.89) $\pm$ 1431.12 0.05–4202.00 (29.50)	40.18 (0.74) $\pm$ 69.59 0.29–209.80 (1.47)	1657.13 (43.31) $\pm$ 1550.53 19.80–4572.00 (57.24)

<sup>a</sup> Mean value of above the detection limit data.

method used was Ward, and a dendrogram was obtained for each element and each area, showing the squared Euclidean distance for each of the parameters. This methodology has been widely used in this type of studies (Girvan et al., 2003; Meers et al., 2007b; Singh et al., 2016, among others).

Software used in this part of the study was Minitab 15® statistical software.

### 3.2.5. Hazards assessment

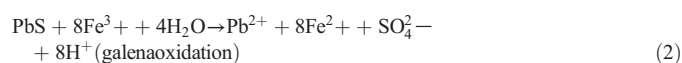
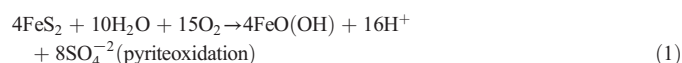
In order to assess the hazards related to the presence of metals and metalloids in the soils, we have selected the Bombita Mine area. We considered this site to be a good example, due to the lesser affection of soils by metal pollution and salts, and the higher homogeneity of the edaphic conditions. Thus, to assess the hazards related with the presence of Pb and Zn, we have calculated regression equations between metal content in plants and total and EDTA metal content in soils. On the basis of the values estimated by means of these equations, geographic metal distribution was studied via variogram modelization and point linear kriging (using the best fit model) with Surfer 13® (Golden Software Inc.). The 2D spatial variability of data and the resulting grid file were

geostatistically conditioned by the variogram model. The interpolation weights are therefore direct functions of the variogram model.

## 4. Results and discussion

### 4.1. Soil parameters

The studied mining soils have mild acid average pH values (Table 1): SQ = 5.69  $\pm$  1.27; BM = 6.29  $\pm$  0.62; RM = 6.82  $\pm$  0.83; having those from San Quintín the lowest measured pH (4.09). The relatively low pH values are related to oxidation-leaching of sulfides (particularly pyrite; see reactions 1–3) eventually resulting in Acid Mine Drainage (AMD) present in the area (Oyarzun et al., 2011b):

**Table 6**

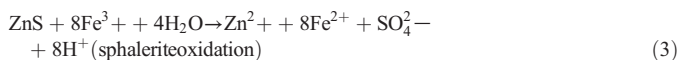
Total heavy metal concentrations in the holm-oak tree leaves. Aver.: Average; SD: Standard Deviation; E-T: excessive-toxic concentrations in mature leaf tissue generalized for various species (compiled by Kabata-Pendias, 2001); b.d.l.: below detection limit. All values in  $\mu\text{g g}^{-1}$  (dry wt.).

Subarea	Cu	Zn	As	Cd	Hg	Pb
Blank area (BA samples, n = 5)						
Aver. $\pm$ SD	4.63 $\pm$ 0.53	20.62 $\pm$ 6.23	b.d.l.	0.05 $\pm$ 0.03	0.03 $\pm$ 0.06 <sup>a</sup>	1.54 $\pm$ 1.14
Range	4.12–5.46	13.44–30.66	b.d.l.	0.02–0.10	b.d.l.–0.15	0.43–2.89
San Quintín Mining Complex (SQ samples, n = 7)						
Aver. $\pm$ SD	5.19 $\pm$ 2.96	164.98 $\pm$ 86.13	0.56 $\pm$ 0.62 <sup>a</sup>	0.59 $\pm$ 0.22	19.71 $\pm$ 33.09	130.65 $\pm$ 138.12
Range	2.59–9.71	27.37–276.92	b.d.l.–1.58	0.20–0.85	0.54–92.25	22.02–396.42
Bombita Mine (BM samples, n = 9)						
Aver. $\pm$ SD	2.62 $\pm$ 0.47	65.15 $\pm$ 58.06	0.19 $\pm$ 0.04 <sup>a</sup>	0.13 $\pm$ 0.09 <sup>a</sup>	1.72 $\pm$ 2.17 <sup>a</sup>	9.75 $\pm$ 11.52 <sup>a</sup>
Range	1.84–3.39	12.40–192.11	b.d.l.–0.22	b.d.l.–0.31	b.d.l.–3.26	b.d.l.–27.11
Romanilla Mining Group (RM samples, n = 8)						
Aver. $\pm$ SD	4.51 $\pm$ 0.71	53.28 $\pm$ 40.01	0.32 $\pm$ 0.23	0.22 $\pm$ 0.09 <sup>a</sup>	0.36 $\pm$ 0.23 <sup>a</sup>	70.30 $\pm$ 45.04
Range	3.17–5.50	13.88–132.75	0.06–0.78	b.d.l.–0.31	b.d.l.–0.69	3.88–136.62
E-T	20–100	100–400	5–20	5–30	1–3	30–300

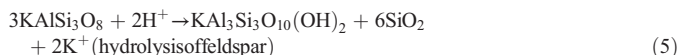
<sup>a</sup> Mean value of above the detection limit data.

**Table 7**  
Biological Accumulation Coefficient (BAC) for the different elements and the different studied areas. Aver.: Average; SD: Standard Deviation.

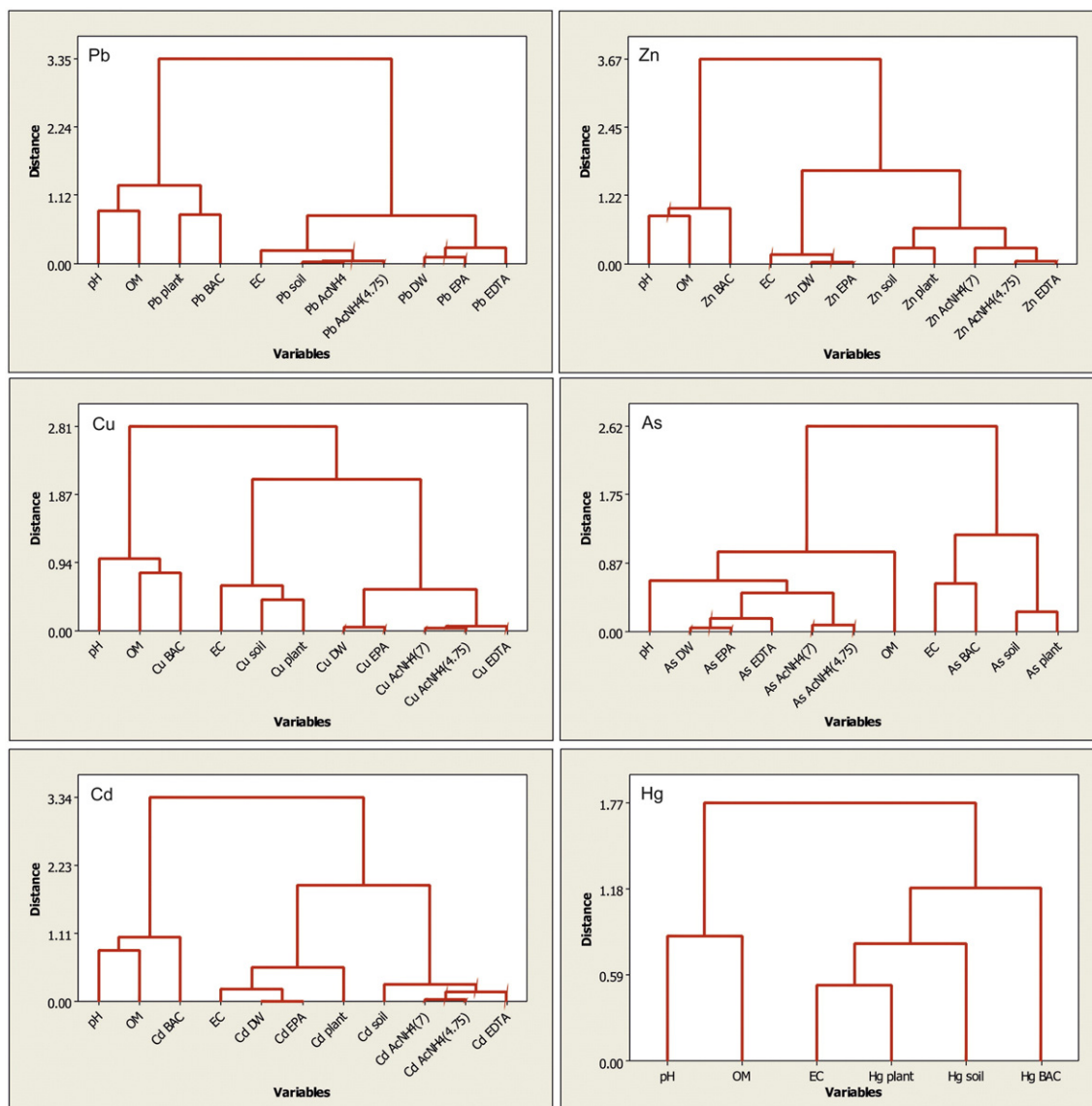
Subarea	Cu	Zn	As	Cd	Hg	Pb
Blank area (BA samples, n = 5)						
Aver. ±	0.211 ±	0.208 ±	0.000 ±	0.408 ±	0.038 ±	0.042 ±
SD	0.031	0.067	0.000	0.252	0.086	0.028
San Quintín Mining Complex (SQ samples, n = 7)						
Aver. ±	0.110 ±	0.144 ±	0.012 ±	0.176 ±	0.897 ±	0.048 ±
SD	0.198	0.168	0.015	0.260	1.522	0.060
Bombita Mine (BM samples, n = 9)						
Aver. ±	0.089 ±	0.145 ±	0.002 ±	0.161 ±	0.064 ±	0.006 ±
SD	0.089	0.145	0.002	0.161	0.064	0.006
Romanilla Mining Group (RM samples, n = 8)						
Aver. ±	0.136 ±	0.204 ±	0.014 ±	0.112 ±	0.337 ±	0.062 ±
SD	0.089	0.157	0.008	0.136	0.514	0.071
All data (n = 24)						
Aver. ±	0.106 ±	0.155 ±	0.008 ±	0.141 ±	0.351 ±	0.029 ±
SD	0.121	0.135	0.011	0.226	0.902	0.056



The acidity detected in the soils is not higher because: 1) the acid solutions become neutralised through reactions with carbonates and feldspars present in the lithotypes (see reactions 5–5) (e.g., Lillo et al., 2015); and 2) The relatively dry environment prevents formation of a thick organic layer in the soils (e.g., Oyarzún and Oyarzun, 2011) thus impeding large generation of organic acids.



The AMD process involves generation of extremely acid leachates with high contents of sulfate ions and heavy metals (e.g., Banks et al.,



**Fig. 2.** Dendrogram of the data (clusters by complete-link Ward method) for all data sets considered on this study. Abbreviations: BAC: Bioaccumulation coefficient; OM: Organic matter; EC: Electrical conductivity.

1997; Parker and Robertson, 1999). In our case, the oxidized metallic sulfides are galena (PbS) (see reaction 2) and sphalerite (ZnS) (see reaction 3), which are still present in substantial quantities in the abandoned mining wastes (López García et al., 2010). The main leached PHEs are Pb and Zn. Besides, other accompanying PHEs such as Ag, As, Sb, Hg, etc., can be also present both in the residual phases and in the mobilized, leachate fractions.

The soils from SQMC displayed a wide range of electrical conductivities (EC) (28.2–792.0  $\mu\text{S cm}^{-1}$ ), being the highest average of all obtained values (273.2  $\pm$  298.4  $\mu\text{S cm}^{-1}$ ). BM and RM soils have average EC values (48.5  $\pm$  37.9  $\mu\text{S cm}^{-1}$  and 136.8  $\pm$  91.6  $\mu\text{S cm}^{-1}$ , respectively) closer to those of BL soils (76.2  $\pm$  56.6  $\mu\text{S cm}^{-1}$ ) (Table 1). These data can be considered as good indicators of the intensity of the leaching process: the EC represents the proportion of salts in the soil, which in turn is a consequence of the leaching of sulfides in mining areas (e.g. Oyarzun et al., 2011b).

The studied contaminated mining soils have much lower organic matter (OM) contents (SQ = 1.84  $\pm$  1.04%; BM = 2.05  $\pm$  2.10%; RM = 3.83  $\pm$  1.62%) than the BA soils (4.65  $\pm$  1.09%) not impacted by mine activities. This fact is common in not reclaimed mining environments, as the studied, where the waste materials composition acts as

limiting factor for the vegetation growth, because only select species may live at these extreme environments. In consequence the input of degraded vegetal debris is very low producing low organic matter contents. Between the studied mine areas, the lowest OM contents were measured in samples from the SQMC samples (Table 1), which are in a similar range to the previous reported by Rodríguez et al. (2009) at the same area, and show this area as the most degraded of all studied.

#### 4.2. Total PHEs concentrations in soils

Table 2 shows the analytical results for total PHE concentrations in the analyzed soils along with: i) average values, standard deviation and range of concentrations obtained in the baseline area (BA samples); ii) average concentrations for Castilla-La Mancha (CLM) region (data from Jiménez-Ballesta et al., 2010); iii) reference levels for these elements (CLM-RLs), calculated according to the Spanish Royal Decree 9/2005 (BOE, 2005); iv) the levels for total metal concentrations in soil, established by the World Health Organization (WHO; Tembo et al., 2006); and v) the maximum allowable concentration (MAC) in agricultural soils most commonly reported in the literature (compiled from Chen, 1999, and Kabata-Pendias and Sadurski, 2004). RLs can be defined

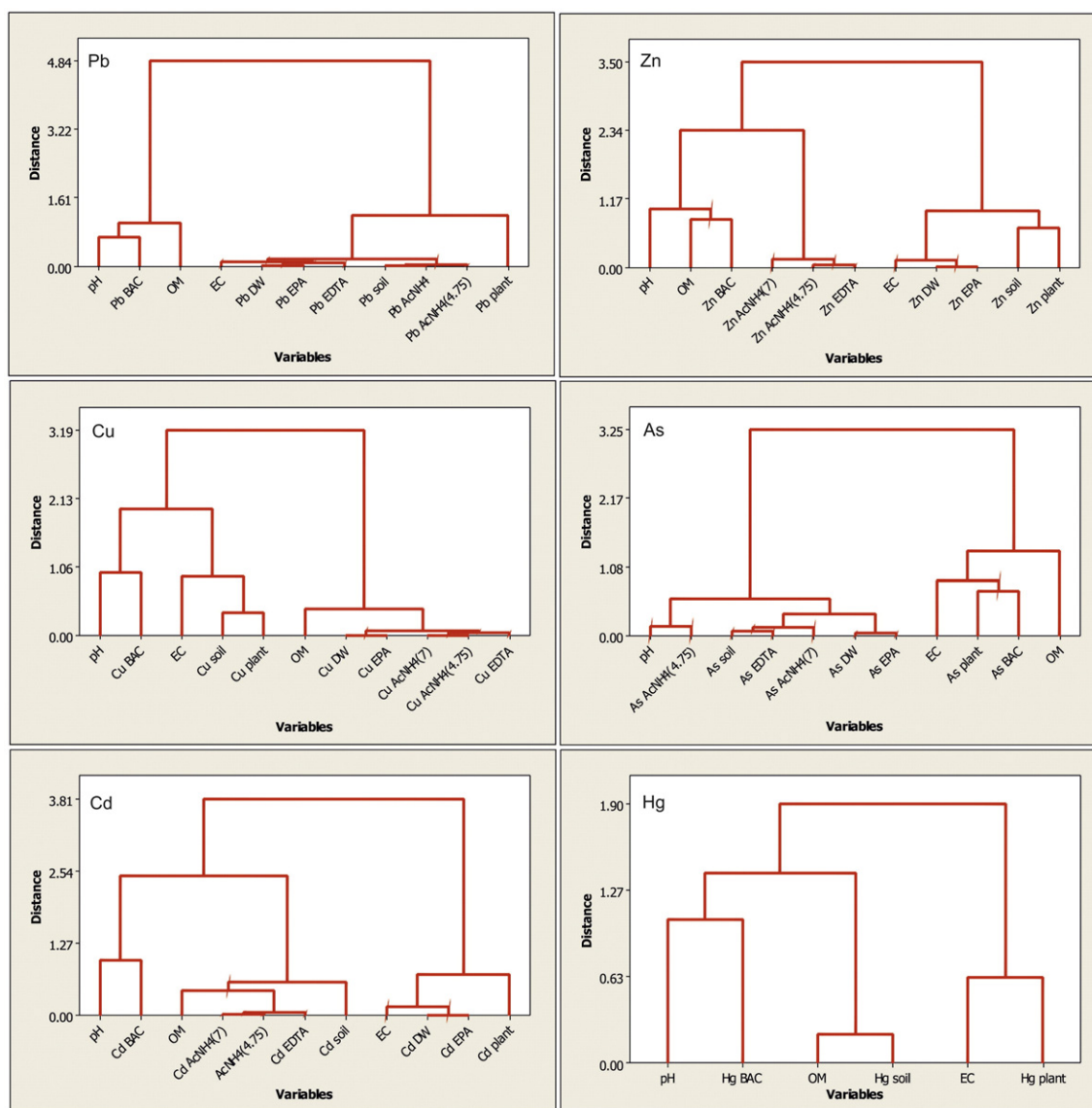


Fig. 3. Dendrogram of the data (clusters by complete-link Ward method) for San Quintín (SQ) data set. Abbreviations: BAC: Bioaccumulation coefficient; OM: Organic matter; EC: Electrical conductivity.



as the upper limit for the background values and/or the concentration of an element in the soil that carries a risk to the human health or ecosystems higher than the maximum acceptable, so they provide a guide to assess soil quality (BOE, 2005). Besides, we have calculated and expressed in the same Table 2 a “Regional Pollution Index” (RPI) as well as a “Regional Integrated Pollution Load Index” (RPLI) (Lee et al., 2006, modified as expressed in 3.2.4), which allows an adequate comparison between each studied areas.

The RPLI allows to classify the areas as “regional background” (RPLI = 0); “regionally unpolluted” ( $0 < \text{RPLI} \leq 1$ ), “regionally unpolluted to moderately polluted” ( $1 < \text{RPLI} \leq 2$ ), “regionally moderately polluted” ( $2 < \text{RPLI} \leq 3$ ), “regionally moderately to highly polluted” ( $3 < \text{RPLI} \leq 4$ ), “regionally highly polluted” ( $4 < \text{RPLI} \leq 5$ ), or “regionally very highly polluted” (RPLI > 5) (modified after Zhang et al., 2011).

The first question arising from the analysis of Table 2 is why the concentrations obtained in the baseline area are higher than those established by Jiménez-Ballesta et al. (2010) and Reglero et al. (2008) for the whole region and other uncontaminated sites in the same province respectively. However, these values are close to those described by Tembo et al. (2006) as background values as established by World Health Organization. Given that no mining activity has taken place in

the baseline area we can only conclude that this sector is naturally enriched in metals. Besides, the amount of samples ( $n = 37$  soils) and the extension of the sampled area ( $6.25 \text{ km}^2$ ) are large enough to discard outlier concentrations induced by the existence of an old mine (or residues) not recognized during our survey.

The SQMC has the highest average elemental concentrations, the highest RPI values for all studied elements, and also the highest RPLI (50.01), which allows classification of San Quintín as “regionally very highly polluted”. The metals with the highest average concentration are Pb ( $10,023.68 \text{ mg kg}^{-1}$ ) and Zn ( $2360.34 \text{ mg kg}^{-1}$ ). The range of values obtained for these two elements were:  $133.36\text{--}45,015.97 \text{ mg kg}^{-1}$  for Pb, and  $53.88\text{--}4450.48 \text{ mg kg}^{-1}$  for Zn. Average total concentrations of all studied elements at *San Quintín* exceed the four reference values presented in Table 2. Besides, the elements with the higher RPI are Hg (357.42) and Pb (290.88); RPI for the rest of analyzed elements is much lower comparing with the aforementioned, although relatively high for Cd (63.14), Zn (23.63) and Cu (16.98), with As showing the lowest value (5.94). Mercury pollution is not directly related to the ores that were mined and processed at *San Quintín*, but its presence derived from cinnabar, brought to this area from Almadén much after the closure of the local mines in order to test concentration

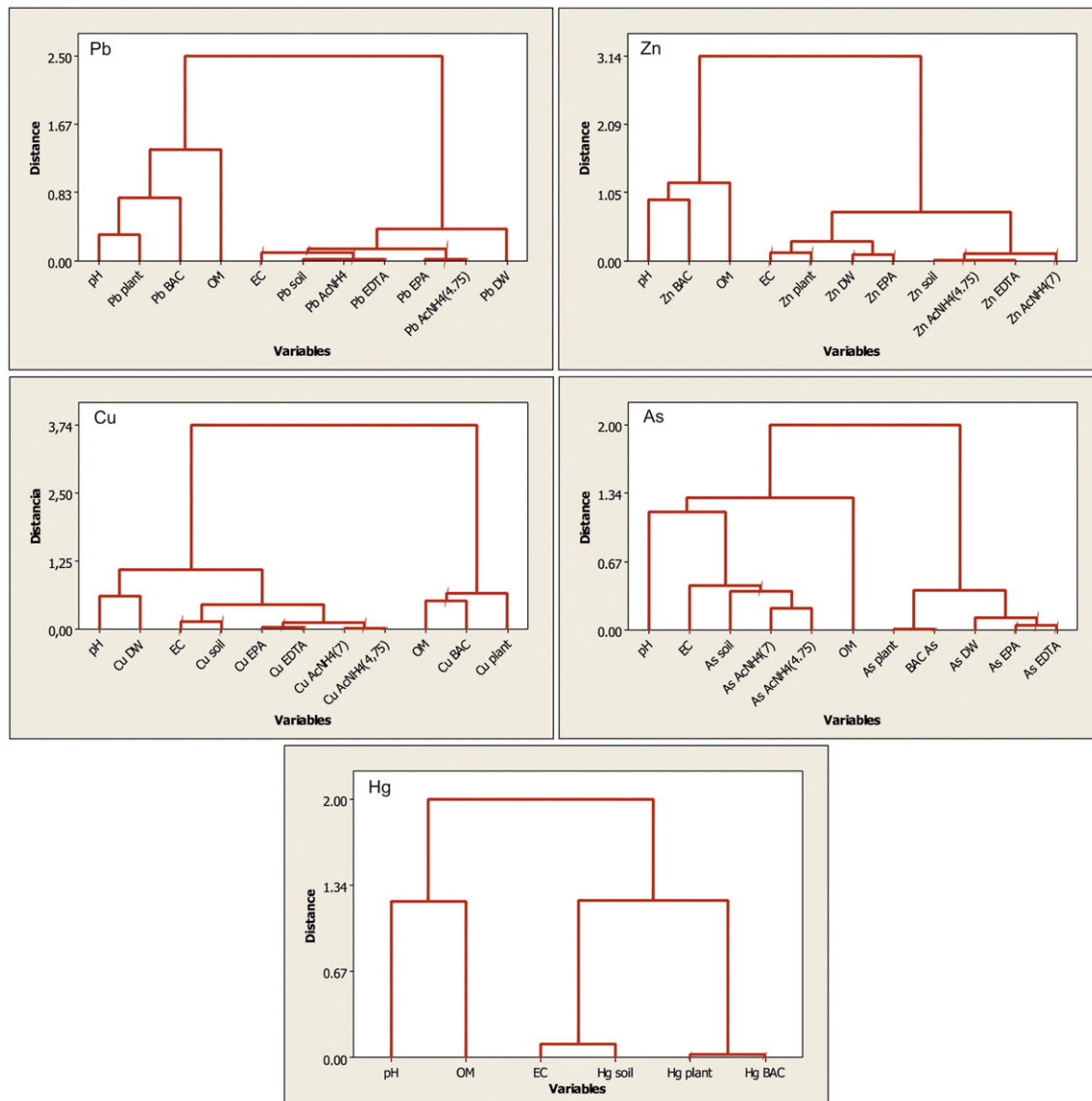


Fig. 4. Dendrogram of the data (clusters by complete-link Ward method) for Bombita mine (BM) data set. Abbreviations: BAC: Bioaccumulation coefficient; OM: Organic matter; EC: Electrical conductivity.

procedures. These were unsuccessful trials that only left behind a large pile of untreated cinnabar ore. As expressed before, *San Quintín* represents the most advanced stage of affection in response to the leaching processes. This is essentially due to the size (larger than the other two sites) and therefore, the higher volume of accumulated tailings deposits.

In the rest of studied areas, the RPLI index allows to classify the *Bombita* Mine (with RPLI = 6.28) and the *Romanilla* Mining Group (with RPLI = 6.93) as “regionally very highly polluted”. Lead is the metal with the highest average concentrations at *Bombita* (2154.75 mg kg<sup>-1</sup>) and *Romanilla* (4054.19 mg kg<sup>-1</sup>), followed by Zn, which also has high concentrations at the same areas (796.98 mg kg<sup>-1</sup> and 644.18 mg kg<sup>-1</sup> respectively). These concentrations are higher than all the reference values shown in Table 2. In the *Bombita* Mine the average concentrations of the other studied elements (Cu, As, Cd and Hg) exceed in some cases the reference values, although none of them is higher than the maximum allowable concentration in agricultural soils (MAC) (Chen, 1999; Kabata-Pendias and Sadurski, 2004). However, As presents an average total concentration higher than this MAC in the *Romanilla* Mining Group. Besides, both As concentrations (*Bombita* and *Romanilla*) have RPI values close to unit, indicating that they have a regional, geogenic origin.

4.3. Potentially harmful elements fractionation assessment in soils by selective extraction procedures

The results obtained from the five single extraction procedures are shown in Tables 3, 4 and 5. Results show that very small amounts (comparing with the total contents) of a heavy metal in the soils from the three studied sites are in the exchangeable ‘pool’. The extractability follows the sequence (in particular for EDTA): Pb > Cd > Zn > Cu ≫ As ~ Hg, which corresponds to the sequence of mobility and in consequence to their potential bioavailability for plants.

Taking into account the different extraction processes applied to the soils, and based on the percentages of recovery, the strength of the different methods follows the common order: EDTA > ammonium acetate at pH = 4.75 > ammonium acetate at pH = 7 ≫ deionized water ~ H<sub>2</sub>SO<sub>4</sub>:HNO<sub>3</sub> mixture for the three studied sites. The aforementioned order for the different extractant agents is related to the release of metal ions weakly bound electrostatically promoted by ion exchange with cations such as ammonium and others (Ure, 1996a, 1996b). Within the studied extractant agents, EDTA is the most powerful, as consequence of its chelating capacity to forms strong complexes with numerous heavy metals in different geochemical forms. For example, it

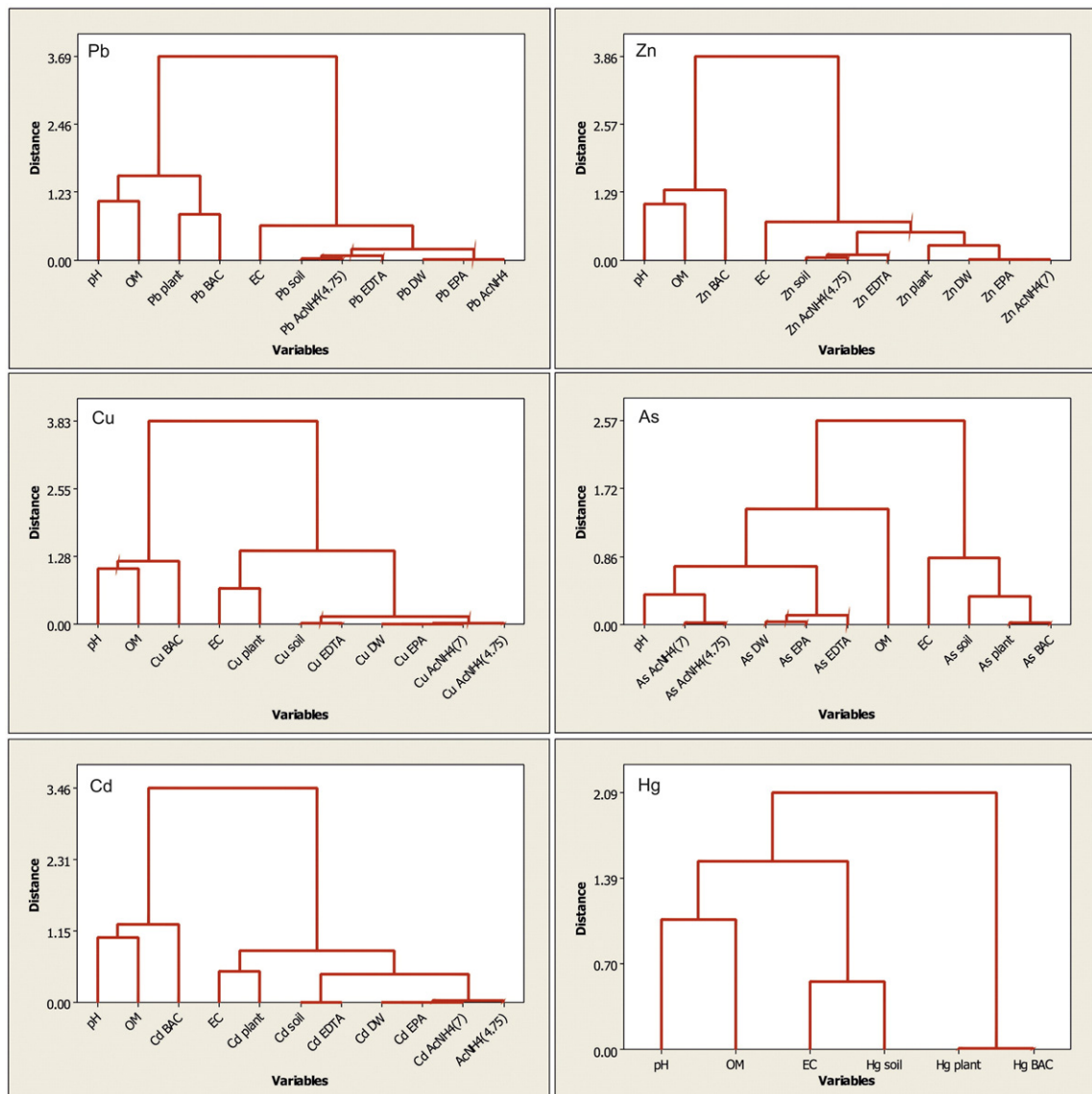


Fig. 5. Dendrogram of the data (clusters by complete-link Ward method) for *Romanilla* Mining Group (RM) data set. Abbreviations: BAC: Bioaccumulation coefficient; OM: Organic matter; EC: Electrical conductivity.

**Table 8**

Toxicity thresholds (TT) for plants (after Kabata-Pendias, 2001) and calculated toxicity thresholds (CTT) for soils for Pb and Zn in *Bombita* mine area. All data in  $\text{mg kg}^{-1}$ .

	Pb	Zn
TTplant	30	100
CTTsoil (total)	267	1620
CTTsoil (EDTA)	132	394

releases heavy metals from soil exchangeable (Kabata-Pendias, 2010) and organically complexed 'pools' (Singh et al., 1998; Podlesáková et al., 2001) and also, it has an influence on heavy metals bound in carbonates and hydroxides of iron (Beckett, 1989). In consequence, the wide potential species that may be extracted by EDTA produced the major recovery of this method. Between the Ammonium Acetate at pH 4.75 and pH 7 extractions, recovery percentages showed that pH has a considerable influence on extractable heavy metals. Ammonium acetate (pH 4.75) releases not only exchangeable but also carbonate minerals (Ure, 1996a, 1996b). Therefore, this reagent extracts much bigger contents of heavy metals than does ammonium acetate (pH 7). Finally, deionized water  $\sim \text{H}_2\text{SO}_4:\text{HNO}_3$  mixture was the less powerful extraction method. Extraction under acid media may extract heavy metals from many geochemical soil fractions e.g., exchangeable, organically complexed and secondary minerals (carbonates, oxides and hydroxides of iron and manganese), but in this case the low chelating capacity of this mixture in comparing with the previous methods, produced the low recovery percentages for the different elements.

#### 4.4. Total PHE concentrations in *Quercus ilex* leaves and Biological Accumulation Coefficient (BAC)

The total contents of the studied PHE in the holm oak tree leaves are summarized in Table 6, together with reference values reported by Kabata-Pendias (2010). Values are systematically above the toxicity level threshold defined by these authors, and in particular, average values for Pb and Zn ( $130.65$  and  $164.98 \text{ mg kg}^{-1}$  in average respectively) from SQMC are the highest. Besides, together the aforementioned elements, Hg contents are extremely high in this area, with an average value ten times higher than toxicity threshold and extreme values 30 times higher than this limit ( $2.17$  and  $3.26 \text{ mg kg}^{-1}$  respectively). At *Bombita* area, some samples surpass the excessive threshold level for Zn as well as the toxicity threshold level for Hg. Additionally, in the *Romanilla* area some of the samples surpass the excessive level threshold for Zn and Pb.

On the basis of the PHEs contents in leaves and soil, we have estimated the Biological Accumulation Coefficient (BAC) (Alloway et al., 1988), in order to assess the plant uptake in relation to the total content of the

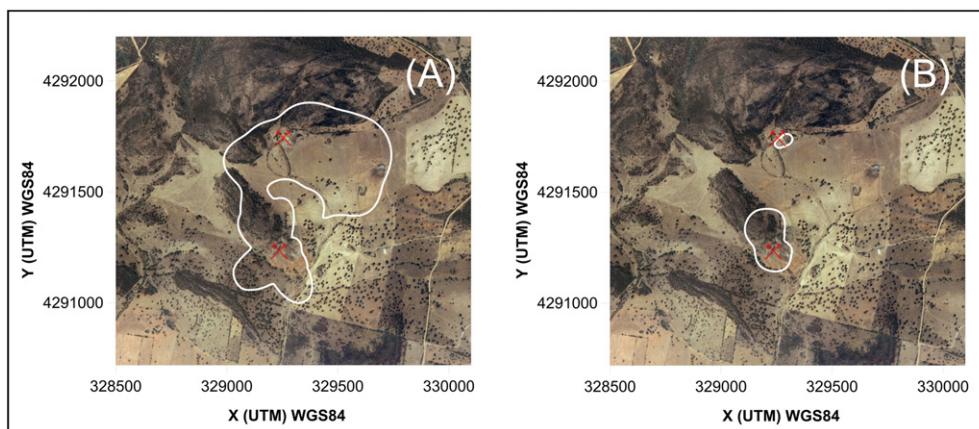
elements in the corresponding soils (Table 7). The obtained values are variable for the different elements and areas considered in this work, but all of them (except one for Hg, from the SQMC area) are below 1, indicating that *Q. ilex* is not a PHEs bioaccumulating plant. The BAC for these elements and for the conditions studied here is:  $\text{Hg} \geq \text{Zn} \geq \text{Cd} \geq \text{Cu} \gg \text{Pb} \gg \text{As}$ , indicating that the elements with minor uptake are Pb and As. The mercury case, which presents high BAC values, is peculiar. At *San Quintin* area, Hg presents the highest BAC, probably due to atmospheric uptake, as described for olive-trees by Higuera et al. (2012b, 2015). But Hg also presents the highest BAC for *Romanilla*, where no atmospheric Hg has been detected; in consequence, Hg contents in leaves only may come from soil uptake. The same happens at the *Bombita* area, where mercury BAC value only have an edaphologic origin and its value is comparable to Zn, Cd and Cu values. Finally, in the blank area, where Hg is very low in the soil and more than probably in the atmosphere, its BAC is very low, comparable to BAC values for Pb and As.

#### 4.5. Statistical correlation between the studied variables

We have studied statistical relationships between the following variables: soil total concentration (Soil), leaf total concentration (Leaf), Biological Accumulation Coefficient (BAC), water extractable concentration (DW),  $\text{H}_2\text{SO}_4:\text{HNO}_3$  extractable concentration (EPA), ammonium acetate extractable concentration at pH 7 ( $\text{NH}_4/7$ ), ammonium acetate extractable concentration at pH 4.75 ( $\text{NH}_4/4.75$ ), EDTA extractable concentration (EDTA), soil pH, soil electric conductivity (EC) and soil organic matter content (OM).

To establish these relationships, we generated clusters by group average; in this analysis the output is a dendrogram where the distance (Ward method) is plotted on Y axis, whereas the sample units (in this case the elements and some parameters such as pH) are shown on the X axis. The diagram depicts how analyzed variables are combined into clusters. The height of each branching point corresponding to the distance at which two clusters are joined (Figs. 2–5); the shorter the distance, the higher the relationship between the corresponding elements linked. The main results are the following:

- BAC-Pb shows a high relationship with Pb contents in the plant, with pH, and OM contents in the three studied areas (Figs. 3–5); only at SQMC the Pb content in plant shows a lower relationship (Fig. 3), probably because of the local importance of pH in this area, with high soil acidity because of formation of AMD (Ruiz et al., 2009).
- BAC-Zn appears linked to pH and OM for all the samples (Fig. 2) and in particular at *Bombita* and *Romanilla* areas (Figs. 4 and 5). At SQMC it also shows relationships with OM in soil and with the most aggressive extractants.



**Fig. 6.** Geographic distribution of soils overcoming calculated toxicity threshold of  $132 \text{ mg Pb kg}^{-1}$  (A) and  $394 \text{ mg Zn kg}^{-1}$  (B) after EDTA extractions in *Bombita* Mine area (see Table 8 for more details).



- BAC-Cu shows the highest relationship physical-chemical properties of soil: OM and pH for all the samples and for *Romanilla* area (Fig. 5), pH and EC in SQMC, OM and Cu in the soil in *Bombita* area (Fig. 4), meanwhile the lower relationship are in general with extractions.
- BAC-Cd shows a behavior very similar to that of Zn.
- BAC-As appears related to As contents in plant and in soil as well as to soil electrical conductivity (salts contents) for all the samples (Fig. 2). At SQMC, the contents of OM are also related to this parameter (Fig. 3), probably indicating a relationship with loss of vegetal cover in the areas with higher concentration of this element.
- BAC-Hg is linked to total Hg in the soil and in the plant at *Bombita* and *Romanilla* areas (Figs. 4 and 5), where Hg contents in the soil are low and probably related to contents in sphalerite; on the other hand, at SQMC (Fig. 3) BAC-Hg is correlated with Hg contents in the soil, to pH and to OM content, which is probably due to the presence of cinnabar in the area affected by AMD which affects pH and OM content.

These observations suggest that to properly assess the bioavailability of elements in the soils, no single method is valid for the whole set of variables. The most aggressive extractants (EDTA and ammonium acetate -based) have been good indicators for Cu, Cd, Pb and Zn, in particular at areas with high soil acidity, while As bioavailability was marked by leachable fractions. Mobility of first group of elements depend on the pH, adsorption by Fe-Mn hydroxides and clay minerals, organic matter, and the co-precipitation with Fe and Mn hydroxides, and in consequence, at the study areas chelating capacity of EDTA and ammonium acetate to form strong complexes seems to be the most effective method to estimate the bioavailable of Cu, Cd, Pb and Zn for the plants in the mining areas. However, mobility of As depends on the same mechanisms that the aforementioned elements, but in these particular cases, the acidic media increase its mobility (Reimann and de Caritat, 1998) and in consequence less aggressive extractants seems to be the most effective method to estimate the bioavailable of the element.

#### 4.6. Geographic distribution of pollution

Based on the equations relating metal content in plants and the total and EDTA fractions in soils, we have estimated the PHE concentrations in the soils needed to surpass the Kabata-Pendias (2010) toxicity thresholds in plants. These thresholds are summarized in Table 8, while areas with soil concentrations above those thresholds delimitate the higher hazard areas, as shown by maps elaborated by means of geostatistics (Fig. 6).

## 5. Conclusions

The main conclusions arising from this study are the following:

- The Alcuía Valley Mining District present areas with soils affected by severe pollution by PHE caused by decommissioned mining activity; in particular soils from the San Quintín mining area display the most severe impacts.
- Concentrations of the studied elements overcome all the international thresholds for soils (to a lower or higher extent) in the different areas, and in particular at SQMC, where Pb average concentrations surpass about 300 times the WHO levels.
- Bioaccumulation of the analysed PHE is in general very low for the studied plant species (the holm-oak tree), in particular for Pb and As, elements present in the area in extremely high and relatively low concentrations, respectively, However, in all the studied areas the PHE concentrations surpass the plant toxicity levels, in particular for Pb and Zn.
- Clear relations between the plant uptake and the bioavailable metals fraction present at soil by different methods were not detected and no apparent toxicity consequences were observed; these facts

suggest a potential selective uptake from the studied species to prevent its toxification.

- The strength of the different selective extraction procedures assessed in this study follows the order: EDTA > ammonium acetate at pH = 4.75 > ammonium acetate at pH = 7 >> deionized water ~ H<sub>2</sub>SO<sub>4</sub>:HNO<sub>3</sub> mixture for the three studied sites. However, there is not clear relationships among extraction fractions and Biological Accumulation Coefficient, indicating the need to define the “bioavailable fraction” for each area and element by means of local studies.
- The risk assessment studies for plant species living in contaminated sites must consider not only the total contents and extractable fractions, but also the local physical-chemical edaphologic characteristics of the area. In our case, the existence of Acid Mine Drainage promotes more aggressive extractants as better indicators of bio-availability.
- A simple way to assess toxicity produced by the presence of PHE in soils is proposed, considering the correlations between contents in the leaves surpassing known toxicity levels and total or extractable contents in the soil.

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gexplo.2016.07.017>.

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