



Geochemical study of a mining-metallurgy site polluted with As and Hg and the transfer of these contaminants to *Equisetum* sp



N. Matanzas^a, M.J. Sierra^c, E. Afif^b, T.E. Díaz^b, J.R. Gallego^{a,*}, R. Millán^c

^a INDUROT, University of Oviedo, C/Gonzalo Gutiérrez Quirós s/n, 33600 Mieres, (Asturias), Spain

^b Department of Organisms and Systems Biology, University of Oviedo, Asturias, Spain

^c CIEMAT, Environment Department, Unidad de Conservación y Recuperación de Suelos, Madrid, Spain

ARTICLE INFO

Keywords:

Equisetum
Bioavailability
Mining waste
Mercury
Arsenic
Phytoremediation

ABSTRACT

Mining-metallurgy waste in uncontrolled disposal sites causes soil pollution. In this context, former mining sites have a complex legacy of contamination. Here we studied the paradigmatic site of La Soterraña (Asturias, NW Spain) after > 40 years of abandonment and weathering. First, a multivariate study of the geochemistry of the soil-waste system revealed average concentrations of thousands of ppm for Hg, and tens of thousands for As, whereas other elements of concern were also well-above soil threshold levels. Remarkably, and in spite of the potential toxicity of the waste mixed in the soil, the incipient soil overlaying the spoil heap has been colonised by pioneer plants that are tolerant to high metal(loid) content. Of note, we observed the predominance of *Equisetum* clusters in a random-like distribution and the preference of these plants for Al- and K-rich soils irrespective of As and Hg pollution levels. Interestingly, *Equisetum* showed low efficiency for As accumulation (excluder) but high efficiency for Hg accumulation (bioaccumulation factors clearly above 1) in its tissues. On the basis of these results, we propose that the *Equisetum*-soil system deserves further attention in the context of the phytoremediation of Hg-polluted soils.

1. Introduction

The abandonment of mining-metallurgy sites, together with long-term weathering, leads to the dramatic degradation of environmental compartments. In particular, mercury (Hg) mining and processing activities are commonly of environmental concern because of the abundance of this mineral and other toxic trace elements, such as arsenic (As), in the ores exploited (Millán et al., 2006; Gallego et al., 2015). Hg participates in a number of complex environmental cycles and, once in the environment, ionic Hg can be converted into organo-mercury compounds, which are highly toxic to most organisms (Davis et al., 1997). Moreover, As toxicity has caused environmental problems worldwide, in particular in relation to groundwater (Duker et al., 2005). Other metalloids frequently linked to Hg and/or As mining, such as Sb and Se, are also a matter of concern (Cao et al., 2014).

Mining has been one the main economic activities in the region of Asturias (NW Spain) since Roman times. In this context, Hg production in Asturias peaked in the twentieth century (Loredo et al., 1998). Within the main Hg mining areas, the municipality of Lena included several mines, among them La Soterraña, where Hg deposits were linked to As-rich minerals. Both As- and Hg-sulphides were treated in a

metallurgy plant at La Soterraña. During this treatment, the mineral was milled and roasted to obtain Hg vapour, which was then condensed in a process that produced a number of hazardous waste products (Gallego et al., 2015). The extraction and processing activities at La Soterraña finished abruptly in 1974 (Loredo et al., 1999).

Emissions of polluting steam and particles and the dumping of mining and smelting waste significantly affected about 80,000 m² of the area around La Soterraña (Loredo et al., 2006), with the subsequent mobilisation of Hg, As and other elements into the surrounding soils, waters and biota, as usually occurs in these sorts of site (Moore and Luoma, 1990). Pollutant distribution is currently determined mainly by mechanical dispersion of the spoil heap, together with the oxidation and lixiviation of heterogeneous As and Hg mine and metallurgy waste (Sierra et al., 2011). Although La Soterraña site has been studied previously (Loredo et al., 2006), the government of the Principality of Asturias has recently published Risk-Based Soil Screening Levels (RBSSLs), thereby providing a legal instrument through which to establish contamination levels (BOPA, 2014). The spoil heaps are currently covered by incipient soil development colonised by pioneer plants, dense vegetation at some points, and even pasture for cattle.

In this context and given the recognised toxicity of some trace

* Corresponding author.

E-mail address: jgallego@uniovi.es (J.R. Gallego).

elements, it is important to determine their uptake, transfer and accumulation processes in plants (Skinner et al., 2007). In fact, surveying natural vegetation is an efficient approach to identify two types of plant, namely those with the ability to (hyper)-accumulate and/or tolerate potentially harmful trace elements in their shoots and those that are highly efficient metal excluders (Van der Ent et al., 2013). Native plant species that grow on soils with high concentrations of pollutants are also tolerant to low nutrient status and show a high rate of growth and biomass. They are therefore of particular interest given their potential value in phytoremediation and as bioindicators of contamination (Pilon-Smits, 2005; Millán et al., 2006, 2012, 2014; Pratas et al., 2005; Yang et al., 2010; Pratas et al., 2013). *Equisetum* tolerates high concentrations of metal(oids) and has been reported to be a metal accumulator (Cannon et al., 1968), specifically for Hg (Siegel and Siegel, 1982). Indeed, this plant has partially colonised the entire study area (see below) and thus emerges as a species that is resistant to high concentrations of pollutants. Furthermore, it is of additional interest since it is sometimes used in herbal medicine.

Given the preceding considerations, here we sought to: i) study the current abundance and multivariate geochemical associations between heavy metal(oid)s in the incipient soil developed on the spoil heaps and establish a comparison with RBSSLs; ii) describe the geochemical and edaphological conditions in which *Equisetum* colonises wide areas of the incipient soil; and iii) evaluate the mobility of contaminants in soils and conduct a preliminary evaluation of the uptake and distribution of Hg and As through *Equisetum*.

2. Materials and methods

2.1. Site description and sampling

The site of La Soterraña (Fig. 1) was used simultaneously for two purposes, namely the exploitation of an underground Hg mine and the metallurgical processing of Hg ores from that mine and others in the surroundings. Therefore, the spoil heaps comprise a mixture of mining and metallurgy waste.

Regarding ore geology, mining was performed through a low-temperature hydrothermal ore composed of cinnabar (HgS), orpiment (As₂S₃), realgar and pararealgar (AsS), As-enriched pyrite, and arsenopyrite (FeAsS), hosted by highly fractured limestones with dispersion in the contiguous sandstones and lutites of Carboniferous age (Loredo et al., 1998). Regarding metallurgical waste, the nature of As and Hg solid residues has been recently described in a similar Hg and As-polluted site in the same Hg-mining district (Gallego et al., 2015). Made up predominantly of relatively small remnants, the spoil heaps initially had (by the 70s) an enormous amount of surface area of exposed sulphide/oxide minerals susceptible to reaction with water and/or air. The abundance and distribution of acid-producing and acid-buffering minerals must have varied extensively between the different parts of the spoil heap, depending on the type of materials stored. Subsequent rainfall provided ideal conditions for the dissolution of secondary weathering products and thus the transport of leached metal (loid)s in solution.

In parallel to these processes, the area has been slowly colonised by pioneer plants. Currently, the presence of vegetation is evident (Fig. 1), and the adaptation of some species to the polluted environment has recently been discussed (Fernández et al., 2017). However, our own observations point to the wide distribution of a specific plant, *Equisetum*, whose individuals are irregularly distributed in clusters over the spoil heap, initially without any recognisable pattern.

From a geomorphological point of view, the heap can be divided into two main areas: one with a notable incline (slope area), and the other, closer to the road surrounding the area, with a lower incline (base area). In this context, the soil sampling campaign consisted of taking 14 randomly distributed samples from each of these two areas. Sampling points of the slope area were labelled L1 to L14, and in the

base area B1 to B14. This scheme was modified slightly to include the presence of *Equisetum* in 4 of the 14 sampling points of each area (L1 to L4, and B1 to B4) (Fig. 1). Two additional clusters were selected in nearby uphill locations where *Equisetum* was also present: sampling station T, in a minor spoil heap separated from the main one; and sampling station C (Control), in a natural soil previously characterized and not affected by heap waste and thus considered a limit of the distribution of the colonizing species.

Five soil subsamples were taken from the surface up to 20 cm of depth from each sampling station (L1 to L14, B1 to B14, C and T) using a Dutch auger. These subsamples were combined to make composite samples (2.5 kg approximately) and then stored in polyethylene bags at 4 °C. Additionally, regarding *Equisetum*, composite samples comprising five plants each were collected in each of the 10 selected sampling stations mentioned above (L1 to L4, B1 to B4, C and T). Plants were collected on the basis of primary vegetative growth, i.e., individuals of comparable age, with a similar number of knots.

2.2. Soil analyses

2.2.1. General physico-chemical characterisation

Composite soil samples were dried for eight days at room temperature in order to minimise the loss of volatile contaminants. They were then weighed, disaggregated, and sieved through a 2-mm screen. The final dried fraction below 2 mm was mechanically split (riffle) to obtain a set of 50-g representative replicates.

Multi-element analysis was performed on the 30 composite soil samples taken. Subsamples were milled to a particle size of < 100 µm in an Agatha mortar grinder (90 rpm for 2 min). All utensils were thoroughly cleaned with ethanol between samples. 0.250-g representative subsamples were leached with 'Aqua regia' digestion (HCl + HNO₃) in an Anton Paar (Graz, Austria) 3000 microwave at 800 W for 15 min. The samples were diluted up to 50 mL with ultrapure water and passed through 0.45-µm PTFE filters prior to analysis. Twenty-eight trace and major elements were quantified by Inductively Coupled Plasma Mass Spectrometry (ICP-MS 7700, Agilent Technologies, California, USA) using IDA (Isotopic Dilution Analysis) with a spike solution from ISC Science, Spain. High-purity standards (Charleston, SC, USA) for calibration and a Certified Reference Material (CRM) (soil, ERM-CC018) were used.

In addition, edaphological properties were determined only for the composite soil samples taken in the 10 sampling stations linked to the *Equisetum* clusters. These were analysed following standard procedures (2 determinations per sample): pH was measured in a suspension of soil and water (1: 2.5) with a glass electrode, and electrical conductivity was determined in the same extract (diluted 1:5). Organic matter was measured by weight loss at 450 °C (ignition method) (Schulte and Hopkins, 1996). Total N was determined by Kjeldahl digestion (Klute, 1996). Mehlich 3, considered to be the most suitable extractant for a wide range of soils (Monterroso et al., 1999), was used for the colorimetric determination of available P. Particle-size distribution was determined by the pipette method, after particle dispersion with sodium hexametaphosphate and sodium carbonate (Gee and Bauder, 1996).

2.2.2. Sequential extraction

The distribution of metal(oid)s in the different soil fractions was determined by a simplified sequential extraction procedure for the speciation of particulate trace metals, following Tessier et al. (1979). This method is also frequently used to characterise mine waste (Gallego et al., 2015 and references therein) and it is a widely accepted approach given the absence of clear criteria to select the best sequential extraction method (Larios et al., 2012). Three individual fractions, namely exchangeable, bound to carbonates, and residual were obtained and compared with total trace metal concentrations (Table 1). Pollutant concentrations present in the first two fractions (exchangeable and bound to carbonates) were considered as phyto-available or

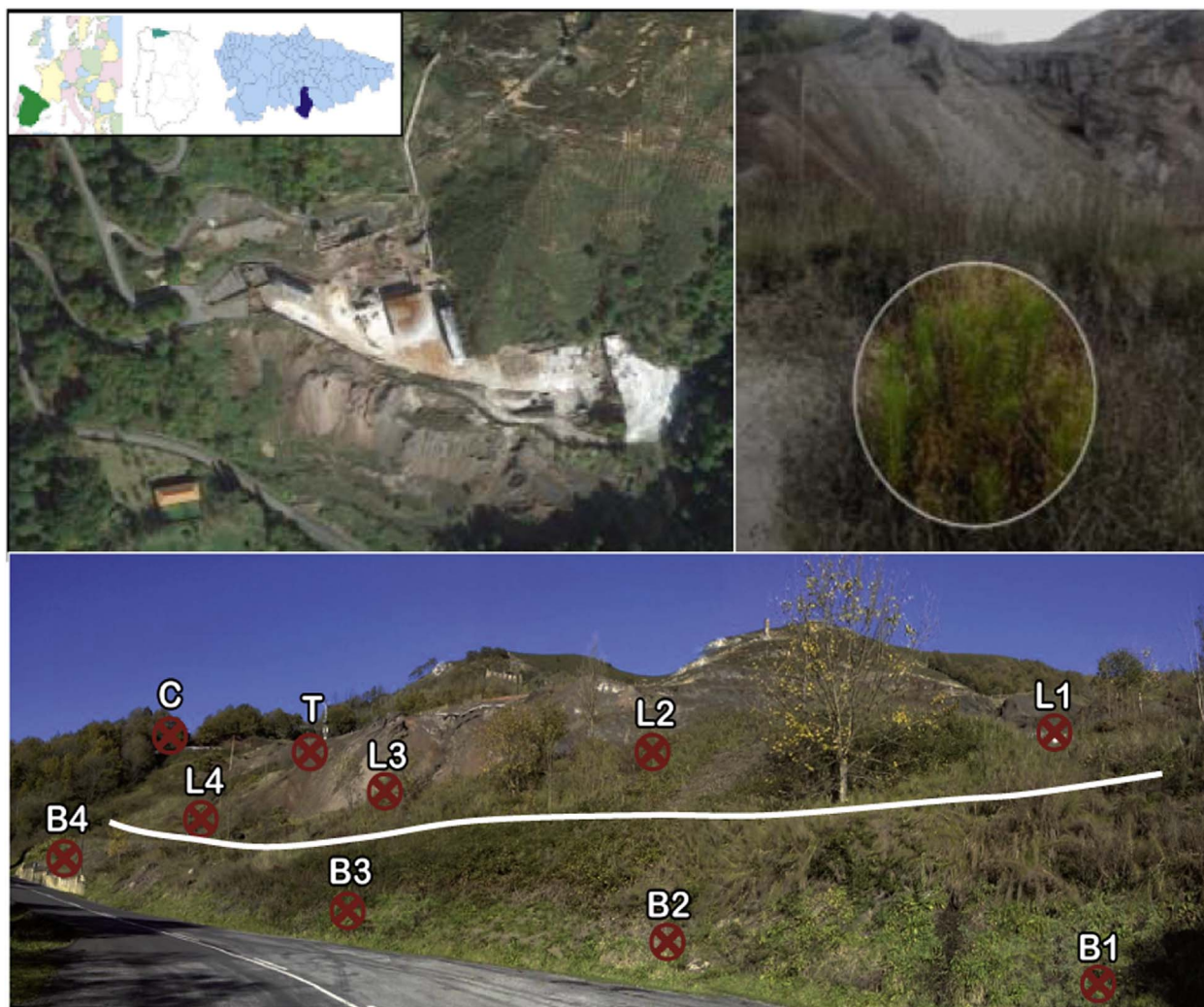


Fig. 1. (Top left) Geographical location and aerial view of La Soterraña site (old buildings and main spoil-heap in the middle of the image). (Top right) Equisetum cluster found within the spoil-heap.(Bottom) Sampling stations for Equisetum clusters: B1 to B4, basal area; L1 to L4 slope area; C, scarcely affected control area surrounding the spoil-heap; T, Equisetum cluster in a secondary spoil-heap; an approximated border between basal and slope area is indicated in white.

Table 1
Reagents used for the sequential extraction procedure used (modified Tessier).

Fraction	Extracting reagent	Extraction procedure
F1: Exchangeable	MgCl ₂ 1 M pH 7 (25 mL)	Sediment was extracted at room temperature, shaken for 1 h and centrifuged at 12000 rpm.
F2: Carbonates	Buffer CH ₃ COONa/ CH ₃ COOH 1 M pH 5 (25 mL)	Leached at room temperature. Adjusted to pH 5.0. Agitation for 6 h and centrifuged.
F3: Residual	HNO ₃ (69% purity) HCl (37% purity)	Residue digested in a microwave reaction system.

bioavailable, as suggested in the references cited above. The usual third and fourth fractions of the Tessier method (associated to Fe-Mn oxides and to organic matter respectively) were not extracted as their bioavailability is considered not significant when compared with that of the first two fractions.

The liquid fractions of the sequential extraction were passed through 0.45-µm PTFE filters and diluted 1:10 prior to analysis by ICP-MS (see Section 2.2.1. for standards and CRM).

2.2.3. As and Hg speciation

The methods followed for As and Hg speciation are described in

Garcia-Manyes et al., 2002 (As) and Chen et al., 2005 (Hg) respectively. In brief, for measuring As species, 0.1 g of soil and an extracting agent (1 M H₃PO₄ + 0.1 M ascorbic acid) were placed in a microwave vessel (60 W, 10 min). The extracts were diluted and filtered. The As species were separated in an 4.6 × 250 mm As-separation column (Agilent Technologies) with a mobile phase of 2 M PBS (Phosphate Buffer Saline)/0.2 M EDTA (Ethylenediaminetetraacetic acid) (pH = 6.0) by means of a 1260 Infinity HPLC coupled to a 7700 ICPMS (Agilent Technologies, California, USA). Standards used for calibration were supplied by Alfa Aesar (Dimethyl arsenic, As(III) and As (V) and Supelco (Methyl arsenic).

Hg species were extracted using a solution of 7.6% HCl and 10% 2-mercaptoethanol in an ultrasonic bath. The blend was then centrifuged and the supernatant diluted. Measurements were done in the HPLC-ICPMS described above, using a ZORBAX Eclipse XDB C18 (2.1 mm i.d. × 50 mm, 5 µm) column and 0.06 M ammonium acetate, 5% methanol and 0.1% 2-mercaptoethanol (pH = 6.8) as mobile phase. Standards used for calibration were supplied by Sigma Aldrich (Methyl mercury), Supelco (Ethyl mercury), and High-Purity Standards (inorganic species).

2.3. Plant analyses

Each plant sample was vigorously rinsed with tap water. Samples

Table 2

Univariate statistics of the 28 elements analysed in the 30 samples studied. RBSSLs for natural soil are indicated (elements with at least one sample above RBSSLs are shown in bold).

Element	Average \pm Std. Dev. (mg kg ⁻¹)	RSD (%)	RBSSL (mg kg ⁻¹)	Number of samples above RBSSL
Ag	0.18 \pm 0.10	53.8	20	0
Al	35,656.51 \pm 15,097.27	42.3	–	–
As	17,193.85 \pm 9172.32	53.4	40	30
B	22.92 \pm 10.97	47.8	–	–
Ba	265.17 \pm 128.92	48.6	1540	0
Ca	55,551.66 \pm 46,085.01	83.0	–	–
Cd	0.56 \pm 0.24	42.5	2	0
Co	13.45 \pm 3.93	29.2	25	0
Cr	67.24 \pm 45.84	68.2	10,000	0
Cu	41.66 \pm 14.16	34.0	55	4
Fe	39,047.44 \pm 10,666.39	27.3	–	0
Hg	1950.10 \pm 2191.47	112.4	1	30
K	5522.03 \pm 3719.78	67.4	–	–
Mg	2581.61 \pm 987.84	38.3	–	–
Mn	409.40 \pm 238.92	58.4	2135	0
Mo	3.07 \pm 1.39	110.7	6	0
Na	702.20 \pm 353.10	50.3	–	–
Ni	39.51 \pm 7.61	19.3	65	0
Pb	70.85 \pm 30.24	42.7	70	10
Sb	114.54 \pm 70.08	61.2	5	29
Se	4.43 \pm 1.57	35.5	25	0
Sn	3.25 \pm 0.96	29.7	10,000	0
Sr	335.22 \pm 96.98	28.9	–	–
Ti	420.14 \pm 233.37	55.6	–	–
Tl	0.43 \pm 0.30	70.2	1	2
U	1.49 \pm 0.26	17.4	–	–
V	71.26 \pm 19.95	28.0	50	23
Zn	137.24 \pm 59.63	43.5	4550	0

were then divided into aboveground mass and roots. Thereafter, each part was cleaned with distilled water using an ultrasonic bath (Ultrasons-H Selecta) to remove any external contamination remaining (up to 8 cycles of 10 min). Finally, they were air-dried for 7 days to ensure constant weight and afterwards ground using an electric mill (A 11, IKA), to obtain a particle size of < 420 μ m.

Hg concentration in all plant samples was measured using an atomic absorption spectrophotometer (AMA254, LECO Company, Michigan, USA) specifically designed for direct Hg determination in solid and liquid samples without prior chemical pre-treatment of samples. This equipment uses an Hg vapour generation technique that attains exceptionally high sensitivity levels independently of the sample matrix. Certified reference materials (CRMs) were used to determine the accuracy and precision of the measurements and to validate the methods applied. These reference materials were ZC-73018 (Citrus leaves, 0.150 \pm 0.020 mg kg) and CRM049-50G (Sandy clay soil, 17.8 \pm 0.9 mg kg). The repeatability was $S_r \leq 15\%$ and the relative uncertainty associated with the method ($k = 2$) was $\pm 20\%$.

Regarding As content, plant samples were digested in a microwave vessel (1600 W, 30 min) using H₂O, HNO₃ and H₂O₂ (10:3:2). Element analyses were performed by ICP-MS as described in Section 2.2.1.

2.4. Statistics

For the multivariate statistical study of soil geochemistry, most of the variables showed behaviour adapted to a log-normal distribution, as usually happens in geochemical studies (Yongming et al., 2006; Sierra et al., 2014). We then performed a log-transformation. Factor analysis (principal components) and cluster analysis were performed using SPSS v.24. The principal components method was used to extract the factors, whereas Varimax rotation was applied because it minimises the number of variables that have a high loading on each factor (Gallego et al., 2013 and references therein). In turn, cluster analysis was undertaken

Table 3

Factor loadings (absolute values lower than 0.5 omitted), communalities, and explained variance in PCA. Elements above RBSSLs (see Table 2) are indicated in bold.

Variables	Factors					Communalities
	1	2	3	4	5	
Ag	0.538					0.633
Al		0.839				0.884
As	0.879					0.884
B		0.831				0.766
Ba				0.616		0.625
Ca			0.919			0.877
Cd	0.655					0.529
Co			– 0.695			0.864
Cr				– 0.843		0.763
Cu	0.827					0.778
Fe			– 0.698			0.922
Hg	0.834					0.711
K		0.882				0.900
Mg			0.742			0.790
Mn		0.696				0.644
Mo				– 0.816		0.786
Na			0.590			0.555
Ni						0.374
Pb	0.741					0.872
Sb	0.900					0.870
Se	0.803					0.833
Sn					0.670	0.666
Sr				0.595		0.515
Ti	0.509			0.586		0.743
V		0.739				0.912
Zn	0.535			0.693		0.842
Tl					0.695	0.618
U					0.688	0.513
Variance explained (%)	22.869	16.166	13.925	12.299	8.562	
Cumulative variance (%)	22.869	39.035	52.960	65.260	73.821	

following the Ward algorithmic method, which maximises the variance between groups and minimises it between members of the same group. The measurement used was the Squared Euclidean distance.

In the case of physico-chemical data and data related to the soil-plant system, non-parametric tests were carried out to evaluate significant differences. The Wilcoxon, Kruskal-Wallis and Mann-Whitney tests were applied in function of the case. Moreover, in order to identify relationships between Hg and As in plant organs and Hg and As in soil, Kendall's Tau-b test was performed; a probability of 0.05 or lower was considered significant. In the Mann-Whitney test, significant level was calculated as follows: 0.05/k, where k is the number of pair combinations to compare.

3. Results and discussion

3.1. Geochemistry of metal(loid)s in soil

Univariate statistics of the results obtained after the multi-element analyses of the 30 composite soil samples are shown in Table 2. Taking into account the maximum values measured and also the average values, the main soil contaminants found were As and Hg (higher dispersion of values for Hg). In fact, all samples presented anomalous values for these elements, i.e. much higher than the respective RBSSLs, thereby indicating that a large area was contaminated. In this regard, the extremely high concentrations of As and Hg detected pointed to a blend of natural soil aggregates with some of the abovementioned mining-metallurgy waste. In addition, other elements such as Sb, V and Pb (Tl and Cu to a lesser extent) showed average or maximum concentrations in excess of the RBSSLs used in the Principality of Asturias.

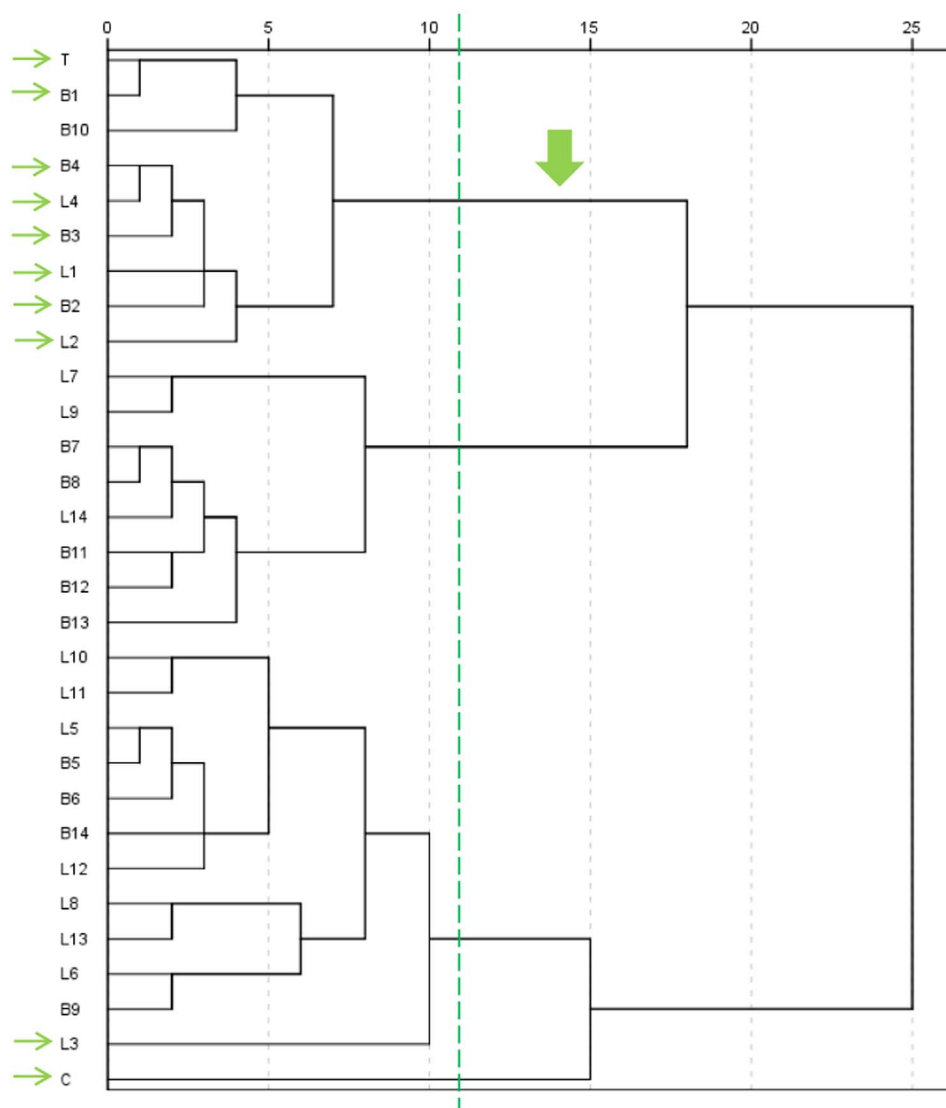


Fig. 2. Dendrogram obtained (Ward's algorithm) for cluster analysis of samples. The cluster grouping most of soil samples linked to *Equisetum* is marked in green (as shown only L3 and C samples are not included). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Following the methodology indicated in Section 2.2.2, factor analysis by means of the principal components and Varimax rotation was performed with the chemical elements (variables) analysed in the 30 composite soil samples (cases). Five distinct factors explained approximately 80% of the total variance (Table 3). The number of factors was initially determined by the Kaiser/Guttman criterion (only eigenvalues larger than 1 were considered). However, on the basis of the amount of total variance explained (close to 75%) and the communalities of the elements, only five factors were finally selected.

Factor 1 presented the highest loads for heavy metal(loid)s and included five of the seven elements of concern defined in Table 2 (As, Cu, Hg, Pb and Sb); in addition, other chalcophile elements such as Ag and Se were well-represented in this factor. Taking into account the ore mineralogy and the type of waste previously described, this factor was clearly associated with the As- and Hg-rich waste. In contrast, the second and third factors were related to gangue minerals or bed-rocks in the area; i.e. Factor 2 (high load in Al, K, B and also V) can be attributed to Al- and K-rich minerals included in natural soil aggregates, whereas Factor 3 (high load in Ca and Mg) appeared to be associated with the presence of carbonates (limestone and dolomite). Implicitly, the presence of iron oxy-hydroxides was also reflected by the negative load of Fe in Factor 3 (carbonatic factor). Finally, Factors 4 and 5 (lower variance explained) were not so relevant and probably reflect mixed behaviours of minor elements such as Cr, Mo and Tl.

Regarding the spatial distribution of the elements, the dendrogram shown in Fig. 2 revealed a random-like distribution of the samples, irrespective of whether they were from the base or slope zone. Remarkably, eight of the ten soil samples corresponding to *Equisetum* clusters were clearly grouped (only L3 and C samples were not included, Fig. 2). This observation points to the presence of specific geochemical features that favour *Equisetum* growth (Table 4). Particularly, As and Hg contents showed very low variation between samples associated and not associated with *Equisetum* presence, thus inferring that the occurrence of *Equisetum* is not linked to degree of pollution. On the other hand, elements such as Al, K and other minor elements with high loads of Factor 2 (see factor loadings in Table 3) revealed a significantly higher amount in soils colonised by *Equisetum*, thereby suggesting the preference of this plant for soils with minerals enriched in Al and K (for example, potassium feldspar or muscovite) in the study area.

3.2. Soil properties

As described above, soil properties were determined for the 10 soil samples linked to the *Equisetum* clusters (Table 5).

Soils were classified as sandy loam (weakly compact), revealing slightly alkaline pH and low CE. Organic matter and the percentages of C, and N, and P were notably higher in the basal zone of the study site (zone B), possibly as a result of the high slope of the spoil heap and

Table 4

Difference between average concentrations in samples taken where *Equisetum* clusters (excluded L3 and C) appear and the rest of the samples. Values above 50% are marked in bold.

Element	Soil samples <i>Equisetum</i> clusters (average in mg kg ⁻¹)	Other soil samples (average in mg kg ⁻¹)	Difference (%)
Ag	0.25	0.13	92.31
Al	48,591.29	26,705.00	81.96
As	16,374.44	19,037.50	- 13.99
B	28.69	17.30	65.84
Ba	339.14	212.95	59.26
Ca	65,388.90	50,820.00	28.67
Cd	0.61	0.54	13.45
Co	12.58	13.55	- 7.15
Cr	45.32	117.45	- 61.41
Cu	34.06	45.55	- 25.22
Fe	38,600.83	38,025.00	1.51
Hg	1778.10	1779.70	- 0.09
K	8621.25	3110.00	177.21
Mg	3070.29	2305.00	33.20
Mn	499.64	333.45	49.84
Mo	1.24	6.95	- 82.19
Na	695.51	674.50	3.12
Ni	35.83	41.40	- 13.45
Pb	62.71	71.75	- 12.59
Sb	109.54	122.50	- 10.58
Se	3.60	5.08	- 29.10
Sn	3.08	3.29	- 6.30
Sr	327.41	344.40	- 4.93
Ti	539.01	315.00	71.11
Tl	0.44	0.39	12.04
U	1.48	1.46	1.52
V	78.83	65.70	19.99
Zn	171.92	114.00	50.81

water run-off. This basal zone was also revealed to be more clayey, although all samples, irrespective of their position, were classified as sandy loam. In general terms, the total N content was within the normal range in all the soil samples while the C/N ratios were low; on the other hand, all samples showed low levels of available P, which was consistently below the critical level of 30 mg P kg⁻¹ (Monterroso et al., 1999). All this considered, the study soils revealed a short development, conditioned mainly by the slope and the mixture of aggregates of mining and metallurgy waste present. This conclusion is complementary to the levels observed in Table 4, which indicated the preference of *Equisetum* for soils with minerals enriched in Al and K. Interestingly, the soil sampled in the cluster labelled as Control (low As and Hg contents) showed edaphic parameters similar to those of the others. This suggests the adaptation of *Equisetum* to the general soil properties within the study area, irrespective of the higher or lower level of toxic trace elements in a specific point, as discussed below in Section 3.4.

Table 5

Average soil parameter results and ranges for the 10 soil samples corresponding to the *Equisetum* clusters (analytical errors below 10%).

Parameter/units	Zone B (Clusters B1, B2, B3, B4)			Zone L (L1, L2, L3, L4)			Cluster T	Cluster control
	Av. ± Std. Dev.	Max	Min	Av. ± St. Dev.	Max	Min		
pH (1:2.5)	7.51 ± 0.25	7.84	7.27	7.62 ± 0.42	7.99	7.02	6.99	6.94
EC (1:5) (dS m ⁻¹)	0.12 ± 0.01	0.14	0.11	0.13 ± 0.01	0.14	0.11	0.14	0.14
Organic matter (%)	3.46 ± 0.69	4.16	2.85	1.73 ± 0.06	1.81	1.67	1.55	2.56
C (%)	2.01 ± 0.40	2.42	1.65	1.01 ± 0.03	1.05	0.97	0.90	1.49
N (%)	0.30 ± 0.15	0.52	0.19	0.23 ± 0.07	0.32	0.15	0.19	0.22
C/N	6.70 ± 1.85	9.14	4.61	4.34 ± 1.78	7.17	3.12	4.80	6.91
P (mg kg ⁻¹)	20.76 ± 3.83	26.44	17.97	10.32 ± 3.14	15.18	7.98	16.52	14.93
Sand (%)	67.8 ± 16.9	84.2	50.1	64.5 ± 20.5	85.7	42.9	75.9	58.5
Clay (%)	15 ± 12.7	35.3	8.2	14.1 ± 10.5	29.2	7.2	11.9	21.3
Silt (%)	11.5 ± 5.7	19.6	7.6	13.9 ± 10	27.9	7.1	12	20.2

3.3. Mobility and speciation of contaminants

As and Hg content surpassed their respective RBSSLs in all samples (Table 2). Accordingly, given the high toxicity of As and Hg and their abundance in the ores and waste linked to the study site, we addressed the behaviour of these elements in the soil-*Equisetum* system.

The results of the sequential extraction (simplified Tessier method) are shown in Table 6. From the three fractions initially obtained, data were grouped into two main fractions, namely the bioavailable fraction (subdivided in exchangeable and carbonate-bound fractions) and the residual fraction.

In general terms, As showed moderate bioavailability, being only slightly higher than 10% in the basal area, whereas Hg data reflected its low mobility (values consistently below 5%, although with notable variability). These observations imply that, although the total concentrations of As and Hg were very high, only a minor fraction was readily available for plants. However, given that the total contents were extremely high, the amount of As and Hg, even in the exchangeable fraction, was well-above the RBSSLs (for example, exchangeable As contents in zones B and L were around 200 ppm while carbonate-bound As surpassed 1000 ppm in some samples).

In a complementary approach, we measured Hg and As speciation. We did not detect any substantial difference within the sampling points studied. Regarding Hg, no traces of methyl-Hg or ethyl-Hg were found in any of the samples. We thus conclude that most of the Hg present in the samples was in inorganic forms, i.e. Hg(0), Hg(I), and Hg(II), which were probably bound to organic matter or found as a sulphide in cinnabar or metacinnabar minerals (Rumayor et al., 2017). With respect to As, As (V) was found to be prevalent, which is consistent with the origin of the waste present in the spoil heap (oxidising roasting; Gallego et al., 2015); concretely, in the 10 samples studied, As (V) was well above 95% of total As, whereas As (III) ranged between 0.5 and 3.8% of the total As content, and no organo-arsenic compounds were detected in any case.

3.4. Soil-plant system. Bioaccumulation and translocation factors

The species colonizing the study area is *Equisetum telmateia* Ehrh. (*Equisetum maximum* auct.), which belongs to the family of Equisetaceae. Giant horsetail is an herbaceous perennial that can reach between 150 and 240 cm in height. Regarding distribution, this plant shows a preference for slopes, edges of streams and wet meadows, preferably on calcareous clay substrates. In Asturias, it is found in damp and shady sites so it is not uncommon in the shade of hazel, ash or alder trees and it endures submersion at the edges of streams and ponds (Díaz González et al., 1994).

Fig. 3 summarises the As and Hg concentration in soil and in *Equisetum* clusters. The graphs reveal a similar accumulation trend for As and Hg in roots, and As and Hg availability in soils of the different zones. Nevertheless, according to the statistical criteria mentioned in

Table 6
Fractionation and bioavailability of As and Hg (measured in the 10 soil samples corresponding to Equisetum clusters).

Element	Cluster	Fractions (mg kg ⁻¹)						Bioavailability* (%)	
		Exchangeable		Carbonates		Residues		Average	Range
		Average	Range	Average	Range	Average	Range		
As	B	223.8	48.1–440.4	1597.3	954.7–3115.6	14,933.4	7303.6–21,688.4	11.1	6.7–16.8
	L	195.5	127.3–316.3	1252.9	825–2320	17,854.1	5841.4–23,795.8	8.5	4.7–14.0
	T	10.3	–	748.6	–	12,439.6	–	5.7	–
	C	0.1	–	1.5	–	164.4	–	0.9	–
Hg	B	19.6	0.1–77.4	7.1	0.1–28.4	1824.8	847.9–4245.2	0.6	0.1–2.4
	L	161.9	0.3–560.2	59.5	0.1–188.9	2658.3	392.4–4947.8	4.3	0.1–14.4
	T	0.1	–	0.1	–	383.1	383.1	0.1	–
	C	–	–	–	–	12.8	12.8	0.2	–

* Exchangeable + carbonate-bound divided by residue.

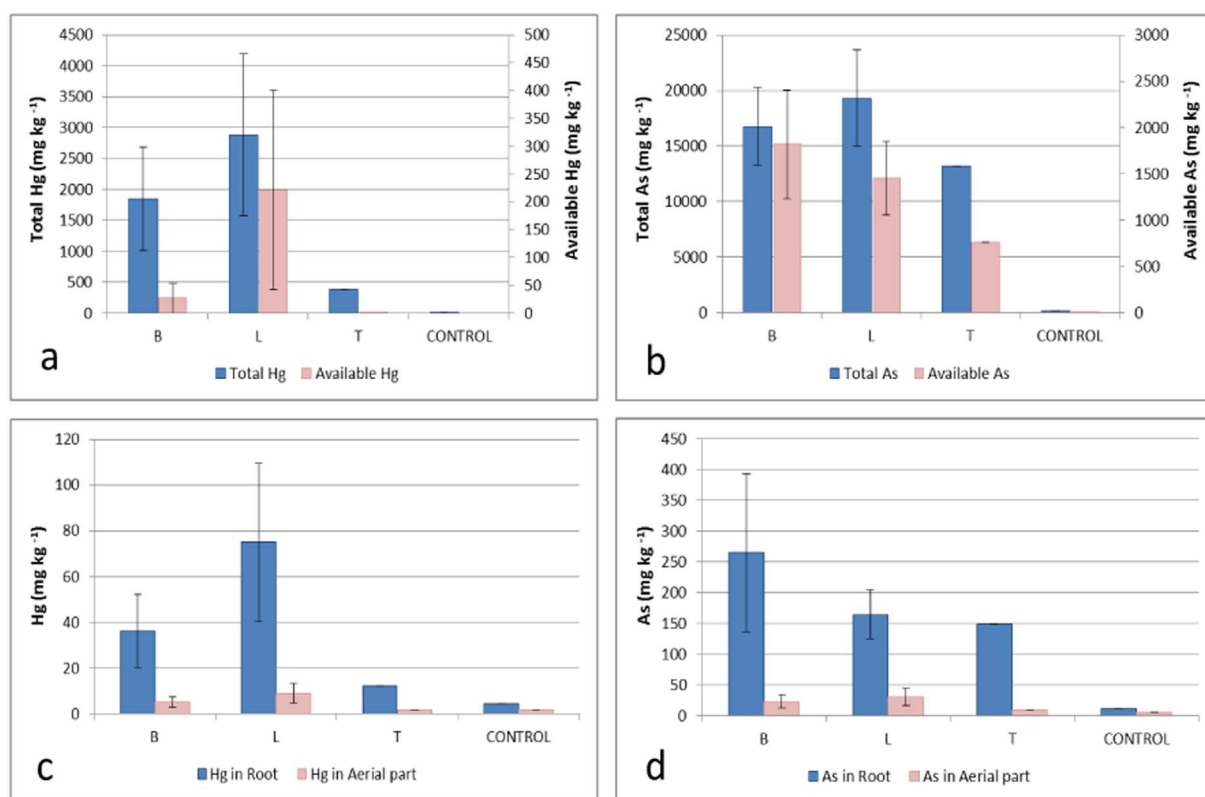


Fig. 3. (Top) Total and available Hg (a) and As (b) concentrations in soils from the four areas (B, L, T and control). (Bottom) Hg (c) and As (d) concentration in the root and shoot of Equisetum species. The plants were collected from four areas (B, L, T and control area). Error bars indicate \pm S.E.

Table 7
Translocation factors and bioaccumulation factors in the Equisetum clusters sampled.

Clusters	Translocation (Aerial/Root Ratio)		Aerial part BAF*		Root BAF*	
	Hg	As	Hg	As	Hg	As
B	0.18 \pm 0.05	0.11 \pm 0.06	6.6 \pm 3.60	0.01 \pm 0.01	44.9 \pm 22.3	0.19 \pm 0.11
L	0.11 \pm 0.02	0.16 \pm 0.04	2.6 \pm 1.70	0.02 \pm 0.00	24.6 \pm 13.9	0.12 \pm 0.01
T	0.15 \pm 0.00	0.07 \pm 0.00	21.7 \pm 0.00	0.01 \pm 0.00	142 \pm 0.00	0.20 \pm 0.00
C	0.43 \pm 0.00	0.48 \pm 0.00	1932 \pm 0.00	3.50 \pm 0.00	4481 \pm 0.00	7.2 \pm 0.00

* Bioaccumulation Factor.

Section 2.4, the distinct zones showed no significant differences for any of the contaminant concentrations determined (Hg_{Root} ; Hg_{Aerial_Part} ; total and available Hg in soil; As_{Root} ; As_{Aerial_Part} ; total and available As in soil).

Hg concentrations in the plants varied between 1.1 and 21 mg kg⁻¹ in aerial parts and 3.6 and 167 mg kg⁻¹ in roots (average values shown in Fig. 3c). In all cases, Hg concentration was significantly higher in roots than in aerial parts ($z = -2.94$, $p < 0.05$). The higher values

are comparable to those found in other plant species like *Crupina vulgaris*, also studied in La Soterraña (Fernández-Martínez et al., 2015), and *Marrubium vulgare*, *Cynoglossum cheirifolium* and *Tamarix africana*, which were examined by Millán et al. (2006) in the historic Hg mining district of Almadén (Ciudad Real, Spain). These species were shown to have great capacity to remove Hg from soil. Moreover, As concentrations ranged between 5.3 and 68.9 mg kg⁻¹ in aerial parts and 11 and 646 mg kg⁻¹ in roots of *Equisetum* (average values shown in Fig. 3d). These values are also similar to those reported in other plant species in other Hg mining sites in the same region as this study site (Larios et al., 2012).

Although the Hg threshold concentration of phytotoxicity varies from 1 to 3 mg kg⁻¹ (Massa et al., 2010) and the plants studied surpassed these limits in some cases, no visual toxic effects were observed. Similarly, concentrations of As in plants grown in uncontaminated soils usually vary from 0.5 to 80 µg kg⁻¹ and the threshold concentration of phytotoxicity between 5 and 100 mg kg⁻¹ dry weight (Kabata-Pendias, 2011)—similar to those found for the aerial parts of the plants and without visual symptoms of toxicity. These observations may indicate that an effective defence mechanism exerted a homeostatic response even when the plants were growing in areas affected by mining waste and in those in undisturbed habitats.

As previously described, contaminant accumulation in plants depends on the species, concentration and phytoavailability of the contaminant in soil, among other factors. Therefore it is necessary to study the particular behaviour of *Equisetum* in order to establish its suitability for phytoremediation techniques in sites contaminated by As and/or Hg. In this regard, Table 7 shows the translocation and bioaccumulation factors for the *Equisetum* clusters sampled.

The translocation factor (ratio pollutant in aerial part/pollutant in root) values were lower in highly polluted zones (B, L and T) than in control (C) area, thereby suggesting that *Equisetum* tends to sequester Hg and As into roots. As a consequence, this species should be considered an excluder for these contaminants.

However, the bioaccumulation factor (BAF) of *Equisetum* differed for each contaminant. Given that bioavailability is the key factor that defines the extent and magnitude of biological responses in dose-response relationships in terrestrial ecosystems (Adriano, 2001), the BAF has been estimated as the relation between the element concentration in plant tissue and forms easily available to plants (Millán et al., 2006).

In the case of Hg, the BAF was always higher than 1, thereby revealing a positive flux of this element from the soil to the plant. Significant correlations were detected between total and available Hg and Hg in roots ($\tau = 0.67$; $p < 0.01$, $\tau = 0.78$; $p < 0.01$; respectively). However, no similar significant correlations for Hg were observed in the aerial parts. In addition to root uptake, Hg could enter the aerial parts of plants via the stomata and be associated with atmospheric deposition (Iverfeldt, 1991; Munthe et al., 1995; Erickson et al., 2003). Fernández-Martínez et al. (2015) estimated that Hg from atmospheric deposition in the area of La Soterraña varied between 4.2 and 11.7% of the total Hg measured in the aerial parts. Although the maximum value of percentage (11.7) was considered for atmospheric deposition, the Hg concentrations in the aerial part absorbed by root would be close, or even surpassed, the hyperaccumulating threshold for Hg established at 10 mg kg⁻¹ (Lasat, 2000; Prasad, 2004; Anning et al., 2013). As a consequence, our results reveal that *Equisetum* has high Hg accumulation capacity and that this capacity is not reflected in visual symptoms of toxicity.

In the case of As, in all cases except the control cluster (sample C), the BAF was lower than 1 (0.01–0.02 in case of aerial parts, and 0.12–0.20 in the case of roots). This results are comparable to those obtained by Larios et al. (2012) at La Soterraña with total concentration of As three times lower than those of the present work but similar available concentrations. On the contrary, these values are far from those reported by Del Río et al. (2002) for aerial parts of *Amaranthus blitoides* (1.74) and *Cynodon dactylon* (0.33) grown in soils in

Aznalcóllar (Seville, Southern Spain) contaminated by a pyrite slurry spill; of *Amaranthus hybridus* (0.47) reported by Jonnalagadda and Nenzou (1997) in As mine dumps in Zimbabwe; and of *Dirtrichia viscosa* (12.48) reported by Pistelli et al. (2017) for plants with a singular capabilities for translocating As in a former mining area on Elba Island (Italy). Furthermore, significant correlations were detected between total and available As vs. As in roots ($\tau = 0.70$, $p < 0.01$; $\tau = 0.52$, $p < 0.05$; respectively); and total and available As vs. As in aerial parts ($\tau = 0.70$, $p < 0.01$; $\tau = 0.59$, $p < 0.05$; respectively). Thus, *Equisetum* shows low As absorption capacity.

4. Conclusions

Hg mining and processing are frequent causes of environmental concern because of the abundance of this compound and other toxic trace elements, such as As, in the ores exploited. In the abandoned and paradigmatic site of La Soterraña, we addressed multicomponent pollution of heavy metal(loid)s by performing a multivariate statistical geochemical study. In spite of the extremely high combined Hg-As contamination, the complex geochemistry of the site also revealed high concentrations of Sb or Pb, which deserve attention in further studies. In addition, an incipient soil has developed on the spoil heap abandoned > 40 years ago and has been colonised by pioneer plants. Remarkably, *Equisetum telmateia* clusters were irregularly distributed, irrespective of the As and Hg soil pollution levels, whereas they showed a preference for higher soil concentrations of K and Al.

The behaviour of contaminants in the soil-plant system depends not only on total and available concentrations of the contaminant but also on physical, chemical and biological properties of the soil and plant species. Therefore specific studies should be performed at each contaminated site in order to achieve a wide scientific data base containing an ample spectrum of contamination scenarios. In the present study, the *Equisetum* individuals examined showed their capacity to grow on incipient soils with low nutrient content and high metal(loid)s concentrations, and a low efficiency for As accumulation but high efficiency for Hg accumulation in tissues (bioaccumulation factor above 1), without symptoms of toxicity. These properties could be advantageous for the revegetation of mine tailings. Furthermore, *Equisetum* has high phytostabilisation or phytoextraction potential for Hg and is thus suitable for the remediation of soil contaminated with high contents of this metal. However, phytostabilisation strategies do not generally involve the use accumulator plants; therefore this purpose should require further attention. Additional studies focusing on the behaviour of Hg in the *Equisetum*-soil system are being carried out to evaluate *Equisetum* potential in phytoremediation strategies for the treatment of soils contaminated with Hg.

Acknowledgment

This research was partially funded by the LIFE I+DARTS (LIFE11ENV/ES/000547). We would also like to thank the Environmental Assay Unit of the Scientific and Technical Services of the University of Oviedo for technical support.

References

- Adriano, D.C., 2001. Mercury, in *Trace Elements in the Terrestrial Environments*. Springer.
- Anning, A.K., Korsah, P.E., Addo-Fordjour, P., 2013. Phytoremediation of wastewater with *Limncharis flava*, *Thalia geniculata* and *Typha latifolia* in constructed wetlands. *Int. J. Phytoremediation* 15, 452–464.
- BOPA, Boletín Oficial del Principado de Asturias, 2014. Generic reference levels for heavy metals in soils from Principality of Asturias, Spain. <http://sede.asturias.es/bopa/2014/04/21/2014-06617.pdf>, Accessed date: March 2017.
- Cannon, H.L., Shacklette, H.T., Bastron, H., 1968. Metal Absorption by *Equisetum* (Horsetail). *United States Geological Survey Bulletin* (1278-A).
- Cao, S., Duan, X., Zhao, X., Ma, J., Dong, T., Huang, N., Wei, F., 2014. Health risks from the exposure of children to As, Se, Pb and other heavy metals near the largest coking

- plant in China. *Sci. Total Environ.* 472, 1001–1009.
- Chen, D., Jing, M., Wang, X., 2005. Determination of methyl mercury in water and soil by HPLC-ICP-MS. Application note 5989-3572EN. Agilent Technologies, USA http://www.cffet.net/ait/A3_Hg_Agilent.pdf, Accessed date: July 2017.
- Davis, A., Bloom, N.S., Que Hee, S.S., 1997. The environmental geochemistry and bioaccessibility of mercury in soils and sediments: a review. *Risk Anal.* 17, 557–569.
- Del Río, M., Font, R., Almela, C., Vélez, D., Montoro, R., De Haro Bailón, A., 2002. Heavy metals and arsenic uptake by wild vegetation in the Guadamar river area after the toxic spill of the Aznalcóllar mine. *J. Biotechnol.* 98, 125–137.
- Díaz González, T.E., Fernández Prieto, J.A., Nava, H.S., Fernández Casado, M.A., 1994. Catálogo de la Flora Vascular de Asturias. *Itinera Geobotánica.* 8, pp. 529–600.
- Duker, A.A., Carranza, E.J., Hale, M., 2005. Arsenic geochemistry and health. *Environ. Int.* 31, 631–641.
- Ericksen, J.A., Gustin, M.S., Schorran, D.E., 2003. Accumulation of atmospheric mercury in forest foliage. *Atmos. Environ.* 37, 1613–1622.
- Fernández, S., Poschenrieder, C., Marcenó, C., Gallego, J.R., Jiménez-Gómez, D., Bueno, A., Afif, E., 2017. Phytoremediation capability of native plant species living on Pb-Zn and Hg-As mining wastes in the Cantabrian range, north of Spain. *J. Geochem. Explor.* 174, 10–20.
- Fernández-Martínez, R., Larios, R., Gómez-Pinilla, I., Gómez-Mancebo, B., López-Andrés, S., Loredó, J., Ordóñez, A., Rucandio, I., 2015. Mercury accumulation and speciation in plants and soils from abandoned cinnabar mines. *Geoderma* 253–254, 30–38.
- Gallego, J.R., Ortiz, J.E., Sierra, C., Torres, T., Llamas, J.F., 2013. Multivariate study of trace element distribution in the geological record of Roñanzas peat bog (Asturias, N. Spain), paleoenvironmental evolution and human activities over the last 8000 calyr BP. *Sci. Total Environ.* 454–455, 16–29.
- Gallego, J.R., Esquinas, N., Rodríguez-Valdés, E., Menéndez-Aguado, J.M., Sierra, C., 2015. Comprehensive waste characterization and organic pollution co-occurrence in a Hg and As mining and metallurgy brownfield. *J. Hazard. Mater.* 300, 561–571.
- García-Manyes, S., Jiménez, G., Padró, T., Rubio, R., Rauret, G., 2002. Arsenic speciation in contaminated soils. *Talanta* 58, 97–109.
- Gee, G.W., Bauder, J.W., 1996. Particle size analysis. In: Klute, A. (Ed.), *Methods of Soil Analysis*. American Society of Agronomy, Madison, WI, pp. 383–411.
- Iverfeldt, A., 1991. Mercury in forest canopy through fall water and its relation to atmospheric deposition. *Water Air Soil Pollut.* 56, 553–564.
- Jonnalagadda, S.B., Nenzou, G., 1997. Studies on arsenic rich mine dumps. II. The heavy element uptake by vegetation. *J. Environ. Sci. Health A* 32, 455–464.
- Kabata-Pendias, 2011. *Trace Elements in Soils and Plants*, fourth edition. CRS Press, Taylor and Francis Group, Boca Ratón, London, New York, pp. 534.
- Klute, A., 1996. Nitrogen-total. In: Klute, A. (Ed.), *Methods of Soil Analyses*. American Society of Agronomy, Madison, WI, pp. 595–624.
- Larios, R., Fernández-Martínez, R., LeHecho, I., Rucandio, I., 2012. A methodological approach to evaluate arsenic speciation and bioaccumulation in different plant species from two highly polluted mining areas. *Sci. Total Environ.* 414, 600–607.
- Lasat, M.M., 2000. Phytoextraction of metals from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. *J. Hazard. Subst. Res.* 2, 1–25.
- Loredó, J., Luque, C., García-Iglesias, J., 1998. Conditions of formation of Hg deposits from the Cantabrian zone (Spain). *Bull. Mineral.* 111, 393–400.
- Loredó, J., Ordóñez, A., Gallego, J.R., Baldo, C., García-Iglesias, J., 1999. Geochemical characterisation of mercury mining spoil heaps in the area of Mieres (Asturias, northern Spain). *J. Geochem. Explor.* 67, 377–390.
- Loredó, J., Ordóñez, A., Álvarez, R., 2006. Environmental impact of toxic metals and metalloids from the Muñón Cimero mercury-mining area (Asturias, Spain). *J. Hazard. Mater.* 136, 455–467.
- Massa, N., Andreucci, F., Poli, M., Aceto, M., Barbato, R., Berta, G., 2010. Screening for heavy metal accumulators amongst autochthonous plants in a polluted site in Italy. *Ecotoxicol. Environ. Saf.* 73, 1988–1997.
- Millán, R., Gamarra, R., Schmid, T., Sierra, M.J., Quejido, A.J., Sánchez, D.M., Cardona, A.I., Fernández, M., Vera, R., 2006. Mercury content in vegetation and soils of the Almadén mining area (Spain). *Sci. Total Environ.* 368, 79–87.
- Millán, R., Lominchar, M.A., López-Tejedor, I., Rodríguez-Alonso, J., Schmid, T.F., Sierra, M.J., 2012. Behaviour of mercury in soils from the Valdeazogues riverbank and transfer to Nerium oleander L. *J. Geochem. Explor.* 123, 136–142.
- Millán, R., Lominchar, M.A., Rodríguez-Alonso, J., Schmid, T., Sierra, M.J., 2014. Riparian vegetation role in mercury uptake (Valdeazogues River, Almadén, Spain). *J. Geochem. Explor.* 140, 104–110.
- Monterroso, C., Álvarez, E., Fernández-Marcos, M.L., 1999. Evaluation of Mehlich 3 reagent as a multielement extractant in mine soils. *Land Degrad. Dev.* 10, 35–47.
- Moore, J.N., Luoma, S.N., 1990. Hazardous wastes from large-scale metal extraction. A case study. *Environ. Sci. Technol.* 24, 1278–1285.
- Munthe, J., Hultberg, H., Iverfeldt, A., 1995. Mechanisms of deposition of methylmercury and mercury to coniferous forests. *Water Air Soil Pollut.* 80, 363–371.
- Pilon-Smits, E., 2005. Phytoremediation. *Annu. Rev. Plant Biol.* 56, 15–39.
- Pistelli, L., D'Angiolillo, F., Morelli, E., Basso, B., Rosellini, I., Posarelli, M., Barbaferri, M., 2017. Response of spontaneous plants from an ex-mining site of Elba island (Tuscany, Italy) to metal (loid) contamination. *Environ. Sci. Pollut. Res. Int.* 24, 7809–7820.
- Prasad, M.N.V., 2004. *Heavy Metal Stress in Plants: From Biomolecules to Ecosystems*. Springer-Verlag, Berlin Heidelberg, New York.
- Pratas, J., Prasad, M.N.V., Freitas, H., Conde, L., 2005. Plants growing in abandoned mines of Portugal are useful for biogeochemical exploration of arsenic, antimony, tungsten and mine reclamation. *J. Geochem. Explor.* 85, 99–107.
- Pratas, J., Favas, P.J., D'Souza, R., Varun, M., Paul, M.S., 2013. Phytoremediation assessment of flora tolerant to heavy metals in the contaminated soils of an abandoned Pb mine in Central Portugal. *Chemosphere* 90, 2216–2225.
- Rumayor, M., Gallego, J.R., Rodríguez-Valdés, E., Díaz-Somoano, M., 2017. An assessment of the environmental fate of mercury species in highly polluted brownfields by means of thermal desorption. *J. Hazard. Mater.* 325, 1–7.
- Schulte, E.E., Hopkins, B.G., 1996. Estimation of soil organic matter by weight loss-on-ignition. In: Magdoff, F.R., Tabatabaib, M.A., Hanlon, E.A. (Eds.), *Organic Matter: Analysis and Interpretation*. Soil Science Society of America, Madison, WI, pp. 21–31.
- Siegel, B.Z., Siegel, S.M., 1982. Mercury content of *Equisetum* plants around Mount St. Helens one year after the major eruption. *Science* 216, 292–293.
- Sierra, C., Menéndez-Aguado, J.M., Afif, E., Carrero, M., Gallego, J.R., 2011. Feasibility study on the use of soil washing to remediate the As-Hg contamination at an ancient mining and metallurgy area. *J. Hazard. Mater.* 196, 93–100.
- Sierra, C., Boado, C., Saavedra, A., Ordóñez, C., Gallego, J.R., 2014. Origin, patterns and anthropogenic accumulation of potentially toxic elements (PTEs) in surface sediments of the Avilés estuary (Asturias, northern Spain). *Mar. Pollut. Bull.* 86, 530–538.
- Skinner, K., Wright, N., Porter-Goff, E., 2007. Mercury uptake and accumulation by four species of aquatic plants. *Environ. Pollut.* 145, 234–237.
- Tessier, A., Campbell, P.G., Bisson, M., 1979. Sequential extraction procedure for speciation of particulate trace metals. *Anal. Chem.* 51, 844–850.
- Van der Ent, A., Baker, A.J.M., Reeves, R.D., Pollard, A.J., Schat, H., 2013. Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. *Plant Soil* 362, 319–334.
- Yang, S.-X., Liao, B., Li, J.-T., Guo, T., Shu, W.-S., 2010. Acidification, heavy metal mobility and nutrient accumulation in the soil-plant system of a revegetated acid mine wasteland. *Chemosphere* 80, 852–859.
- Yongming, H., Peixuan, D., Junji, C., Posmentier, E.S., 2006. Multivariate analysis of heavy metal contamination in urban dusts of Xi'an, Central China. *Sci. Total Environ.* 355, 176–186.