

CHANGE IN METALS AND ARSENIC DISTRIBUTION IN SOIL AND THEIR BIOAVAILABILITY BESIDE OLD TAILING PONDS

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Abstract

The objectives of this study were to determine the metals and arsenic transfer from mining ponds to agricultural and forest soils, and identify the dynamic of metal(loid)s in the soil-plant system for a native plant species (*Ballota hirsuta*) in two old mining districts: La Unión and Mazarrón (Spain). Soils and plants from mining ponds and natural and agricultural areas were collected and analysed for soil properties, and chemical partitioning of Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Zn and As. Results showed that mine, forest and agricultural soils were contaminated by As, Cd, Cu, Pb, and Zn. Chemical partitioning revealed higher mobility of metals in mining ponds than natural and agricultural soils except for Fe and As which were mostly bound to soil matrix due to the mineralogical compositions of soils. The accumulation of metal(loid)s in *B. hirsuta* in La Unión decreased as Fe>As>Cr>Ni>Cu>Zn>Cd>Mn>Co>Pb while in Mazarrón was As>Fe>Cr>Pb>Cu>Ni>Co>Mn>Zn>Cd, showing that *B. hirsuta* has high ability to bioaccumulate Fe, As, Cr, Cu and Ni; and Pb (in Mazarrón), transferring a significant concentration of these metal(loid)s, except Pb, to edible parts without exceeding the toxicity limits for animals. Therefore, *B. hirsuta* could be useful as phytoextractor species for Cr, Cu, As and Ni, while it can be used as phytostabilizer species for Zn, Co, Pb and Cd.

Keywords: bioaccumulation, chemical partitioning, mining pond, *Ballota hirsuta*

1. Introduction

Mining activities have a negative impact on the environment by causing changes in landscapes, destruction of habitats, contamination of soil and water, and degradation of land resources (European Environmental Bureau, 2000). Even after mine closure, huge unrestored tailing ponds remain in the mining areas with the consequent environmental risks, such as metals aerial dispersion, leaching of metals and groundwater contamination, runoff of tailing, runoff of acid mine drainage etc.

In semiarid regions, lack of vegetation and scarce but torrential precipitation favour erosion of abandoned and unreclaimed mining ponds, promoting dust spread and acid mining drainage generation, which affects adjacent natural and agricultural lands (Li et al, 2006; Liao et al, 2016). Once the metal(loid)s reach these areas, native and crop plants can uptake metal(loid)s and accumulate them on their tissues (Chopra and Pathak, 2012; Gitet et al, 2016; Moreno-Jimenez et al, 2016) which causes a high environmental, ecological and human health risk. The accumulation of metal(loid)s by consumables plants can cause toxicity for wild fauna, cattle and human. In order to reduce the risk of toxicity for human, some countries have developed regulations which establish allowed concentrations of metals in foods (McGrath and Zhao, 2015). However, the metal exposure for wild fauna by ingestion mainly come from accumulated metals in edible parts of plants used as feed (Martínez- López et al, 2014; Gall et al, 2015). For humans the metals exposure occurs mainly through directly plants consumption, such as cereals and vegetables which can accumulate metals in grains and leaves (Li et al, 2006; Alvarenga et al, 2014). In addition, contamination of drinking water sources by mining operations is other important route of human exposure (Northey et al, 2016, Mhlongo et al, 2018, Rakotondrabe et al, 2018).

La Unión and Mazarrón municipalities (Murcia Region, southeast Spain) have been highly exposed to the negative effect of mining and ore operation during decades. Although the mining activity was abandoned twenty five years ago, more than eighty tailing ponds remain in the area, most of them unrestored. These mining ponds are composed by high amounts of Fe-oxyhydroxides, sulphates, and potentially leachable metal(loid)s due to extreme acidic conditions. For a long time, these mining residues have been transported downstream during periods of high rainfall and atmospherically dispersed, negatively affecting natural, agricultural and populated areas (Zornoza et al,

2012; Alcolea et al, 2015, Sánchez et al, 2017). However, the dynamic of metal(loids) in soil-plant systems from natural lands has been little investigated. In fact, some common plant species growing in natural soils around mine areas has not yet been studied, such is the case of *Ballota hirsuta*, a native species which grows in natural soils from both mining districts. This plant protects the soil against wind and water erosion, being adapted to both semiarid climatic condition and high metal(loids) content in soils. Consequently, we expect that *B. hirsuta* would be able to good species for using in phytoremediation techniques.

Therefore, the objectives of this study were: (1) to determine the metals and arsenic transfer from mining ponds to near agricultural and forest soils; (2) to evaluate the behaviour of metals and arsenic in the soil-plant system for a predominant endemic species (*B. hirsuta*); and (3) to study the ability to accumulate and transfer metal(loids) of *B. hirsuta* in order to determine its potential use as phytoremediation plant.

The results of this study can be used by the public administration and private companies to design low-cost restoration strategies, using native plants species, which will prevent the transfer of metal(loids) from these areas to nearby agricultural areas with the consequent benefits for farmers.

2. Materials and methods

2.1. Study area and sampling collection

Sampling areas were located in two different mining districts: Cartagena-La Unión and Mazarrón (southeast of Spain) (Fig.1). Wastes from both mining districts comes from the extractive activity of Pb, Zn and Fe sulphides which started in the times of the Romans and has continued until 1991 and 1960 in La Unión and Mazarrón, respectively. The climate is semiarid Mediterranean characterized by an annual average temperature of 18°C and an annual precipitation of 290 mm (AEMET, 2016). The mining districts of Cartagena-La Unión and Mazarrón, and other mining districts around the world, are composed by a succession of areas which include a mine, tailing ponds, natural areas, and sometimes agricultural or urban areas. For this study, one of these areas in each mining district was selected, including a mining pond, a natural area, and an agricultural area (Fig.1). La Unión pond (30S X684915, Y4165525) has an extension approximately of 15500 m² and Mazarrón pond (30S X647316, Y4162776) of 6100 m². The sampling in the surface of each tailing pond was carried out following a random procedure in La

Union and linear pattern in Mazarrón. In agricultural areas the sampling follows the slope, the top of the slope corresponds with the area near to the pond NE to SW in Mazarrón, while the top of the slope corresponds with the natural area NE-SW in La Unión . In natural soils, the sampling was also random trying to find plants with the same size.

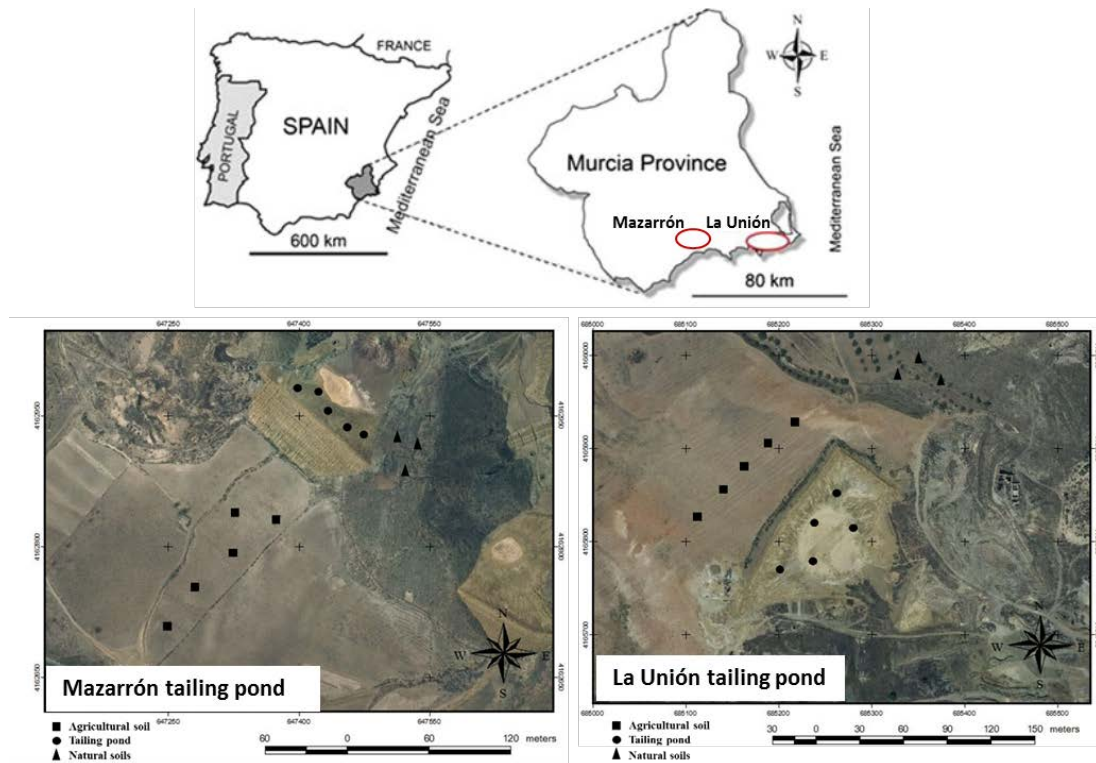


Fig. 1. Sampling points in La Unión (right) and Mazarrón (left).

In order to achieve the objectives, 10 waste samples from the mining ponds (5 replicate samples from each pond), 12 soil samples from natural soils (6 samples from rhizospheric soil of *B. hirsuta* and 6 samples from non-rhizospheric soils, 3 replicate samples of each mining district) and 10 soil samples from cropland adjacent to the ponds (5 replicate samples in each area) were collected in La Unión and Mazarrón mining districts. Each sample was composed by 5 sub-samples from 0-15 cm of depth.

In addition, six whole plants of *B. hirsuta* from natural areas were collected in Mazarrón and La Unión districts (3 plants in each district). All sampled plants showed the same size, without symptoms of diseases and pests.

2.2. Analytical methods

Soil and waste samples were dried at 40°C for 48 h, and passed through a 2 mm sieve. A subsample of each sample was ground using an agate mortar (RetschRM 100). The pH was measured in a 1:1 water/soil solution (Soil Survey Staff, 2004) while the electrical conductivity (EC) was measured in a 1:5 soil/water suspension (Andrades, 1996). The equivalent calcium carbonate was determined using the Bernard's calcimeter and the organic carbon by dichromate oxidation method (Soil Survey Staff, 2004).

The total metal(loid)s content (As, Cd, Co, Cr, Cu, Fe, Ni, Mn, Pb, and Zn,) in soil and waste were determined by acid digestion using a nitric-perchloric solution at 210°C during 90 min. After cooling, 0.1 N HCl was added to fill a 100-mL volumetric flask (Risser and Baker, 1990). Bioavailable metals and arsenic were extracted for neutral soils in 1:2 soil/diethylenetriaminepentaacetic acid (DTPA) ratio and 1:5 soil/ethylenediaminetetraacetic acid (EDTA) ratio for acid wastes (Lindsay and Norvell, 1978; Kabas et al, 2014). Soluble water metals and arsenic were measured from a 1:5 soil/water solution according to Buurman et al (1996) and Frau (2000) method.

Chemical speciation of metals in soil and waste was determined by a sequential digestion procedure adapted from Tessier et al (1979) and modify by Li et al (1995). The following fractions were obtained: exchangeable; bound to carbonates; reducible phase (bound to Fe/Mn-oxides); oxidizable phase (bound to organic matter and sulphide) and residual phase. Certified reference material (BAM-U110) from the Federal Institute for Materials Research and Testing (F.I.M.R.T., 2010) and reagent blanks were used as quality control samples during the analyses. We obtained recoveries (the sum of the five fractions to the total element concentration of BAM-U110) of 94–106% for Cd, 97–108% for Pb, 95-101 % for Co, 99-102% for Cr, 91-102 % Ni, 94-105 % for As, 98-106 % for Fe, 92-102 % for Mn, 95–99% for Cu, and 95–101% for Zn.

For plants analyses, each plant was separated in roots, stems and leaves. In order to eliminate all dust from the surface of the plants, and soil adhered to the roots, each part was carefully washed with tap water and deionized water, finally was dried at 50 °C for 72h. The dried material was ground using a mechanical mill, 0.7 g of each sample was calcined at 450°C prior to metal redilution in 0.6 N nitric acid (Alvarenga et al, 2014; Moreno-Barriga et al, 2017). We used the certificate referent material ERM-CD281 (European Commission, Institute for Reference Materials and Measurements) as quality control sample, obtaining recoveries of 92-100% for Cd, 93-98% for Pb, 97-105% for Cr,

96-99 % Ni, 98-106 % for As, 93-108 % for Mn, 94-103% for Cu, and 96–102% for Zn. All chemicals used were supplied by PANREAC.

Metals and arsenic concentrations in soils and plant tissues were measured by Inductively Coupled Plasma-Mass Spectrometer (ICP-MS) (Agilent 7500CE) following the method 6010D (USEPA, 2014), measuring three replicas of each sample. The samples solution were aspirated continuously at 5 ml min⁻¹ into an inductively coupled, argon plasma discharge, where metals and arsenic were converted to excited-state, gas-phase ions, emitting energy in the form of light at wavelengths that are characteristic of each specific element. The intensity of the energy emitted at the chosen wavelength is proportional to the concentration of that element in the analyzed sample. Reference standards of each metal and arsenic (1000 mg L⁻¹) were used.

2.3. Data treatment

2.3.1. Statistical analysis

To ensure the fitting of the data to a normal distribution the Kolmogorov-Smirnov test was applied. Some data do not followed normal distribution and log transformation was done. ANOVA test was used to identify differences among groups of variables using a Tukey's post hoc with a significance $p < 0.05$. Data that failed on normality, even log-transforming, were submitted to non-parametric test such as Kruskal-Wallis test and Mann-Whitney U test at $p < 0.05$ to assess the differences between variables.

The relationship among metal(loid)s content in soil and metal uptake by plants were study by Spearman correlations. All statistical analysis was performed using the statistics software SPSS 23 (IBM).

2.3.2. Bioaccumulation and translocation factors

The plant ability to accumulate metals from soils was measured with the Bioaccumulation Factor (BAF), that is defined as the ratio of the metal concentration in roots, stem and leaves and the metal concentration in soil (Yoon et al, 2006; Mendez and Maier, 2008; Zornoza et al, 2016). For the calculation of BAF, the DTPA extractable metal concentration on rhizospheric soil was used since it is taking account the interaction among metals and soil properties which controls the availability of metals (Li et al, 2006).

The Translocation Factor (TF) describes the ability of plants to transfer metal(loid)s from the roots to other part of the plants such as stems and leaves. TF was calculated as the

ratio of the metal concentration in stem/leaf and the metal concentration in the roots (Yoon et al, 2006; Mendez and Maier, 2008; Liao et al, 2016).

3. Results and discussion

3.1. Effect of mining ponds in soil properties from natural and agricultural soils

La Unión mining pond showed an extreme acidity, while the agricultural had a neutral pH due to a higher carbonate content (Table 1), since they were developed from carbonated quaternary sediments. This high percentage of carbonates in agricultural soils could neutralize the acidity generated by acid mine drainage from mining ponds. The neutral pH also indicates that the natural soil are not strongly affected by mining activities. Agricultural and natural soils were classified as non-saline, while mining ponds were categorized as slightly saline. The organic carbon (OC) content is very low and statistically similar in agricultural soils and mining waste, showing that agricultural practices (ploughing, removed natural vegetation, etc.) promote the mineralization of organic matter and reduce the OC content in soils. Contrarily the natural areas showed the highest percentage of OC, especially in rhizospheric soil of *B. hirsuta* (Table 1).

In Mazarrón mining district, mining pond showed an extremely acid pH, while natural and agricultural soils were categorized as moderately acid and slightly alkaline respectively, which is in accordance to their carbonates content (Table 1). Salinity was statistically higher in natural soils than in agricultural soils, indicating that irrigation water used in croplands mobilises soluble salts to deeper part of the soil profile, while very low rainfall and high evaporation rate promote the accumulation of soluble salt in topsoil in natural lands. In contrast, low OC contents were reported in natural and agricultural soils, with not statistically significant difference among them. However, OC content was statistically higher in rhizospheric soil of *B. hirsuta* than natural and agricultural soils. Kabas et al. (2014) reported an increment of organic carbon from 6.6 to 19.5 g kg⁻¹ in rhizospheric soil of *Zygophyllum fabago* L. growing in a tailing pond.

Table 1. Soil properties and metals/arsenic concentration (mg kg^{-1}), mean \pm standard deviation, in mining wastes, agricultural soils and natural soils

	LA UNIÓN				MAZARRÓN			
	Mining pond	Agricultural	Natural	Natural Rhizosphere	Mining pond	Agricultural	Natural	Natural Rhizosphere
pH	3.4 \pm 0.1B ¹	7.4 \pm 0.1C	7.2 \pm 0.2C	6.4 \pm 0.1C	2.4 \pm 0.1A	7.7 \pm 0.0C	5.9 \pm 0.9C	6.9 \pm 0.2C
EC *(dS/m)	4.1 \pm 0.9C	1.5 \pm 0.5B	0.6 \pm 0.2A	1.0 \pm 0.2AB	5.9 \pm 0.6C	0.3 \pm 0.0A	1.5 \pm 0.6AB	1.0 \pm 0.1A ¹
O. C.* (%)	0.4 \pm 0.08A	0.4 \pm 0.01A	2.3 \pm 0.32B	6.8 \pm 0.23C	0.1 \pm 0.0A	1.5 \pm 0.1B	1.6 \pm 0.5B	6.3 \pm 1.4C
CaCO ₃ (%)	0.6 \pm 0.08AB	15 \pm 1.52C	1.4 \pm 0.15B	1.3 \pm 0.17B	0.1 \pm 0.0A	10 \pm 1.8C	0.4 \pm 0.1AB	0.6 \pm 0.0B
Total Pb	4199 \pm 846C	860 \pm 180A	2582 \pm 100B	2857 \pm 224B	1257 \pm 148AB	1785 \pm 317AB	6208 \pm 1473C	4576 \pm 1428
Total Zn	2473 \pm 440B	1660 \pm 362AB	1893 \pm 101AB	1969 \pm 147AB	5436 \pm 565C	1005 \pm 140A	1668 \pm 194AB	1754 \pm 38A
Total Cu	95 \pm 14C	50 \pm 7AB	51 \pm 11AB	59 \pm 5AB	61 \pm 6AB	32 \pm 2A	60 \pm 4AB	58 \pm 6AB
Total Cd	6 \pm 1A	6 \pm 1A	7 \pm 1A	8 \pm 1A	8 \pm 2A	5 \pm 1A	7 \pm 1A	9 \pm 1A
Total Cr	31 \pm 2AB	26 \pm 3AB	37 \pm 8AB	26 \pm 6AB	19 \pm 2A	58 \pm 2C	45 \pm 5BC	47 \pm 11BC
Total Co	5 \pm 0A	8 \pm 0B	13 \pm 1C	11 \pm 1C	6 \pm 0AB	7 \pm 0AB	5 \pm 0A	5 \pm 1A
Total Ni	17 \pm 1A	19 \pm 0A	80 \pm 5C	25 \pm 2C	13 \pm 1B	17 \pm 0A	13 \pm 1BC	13 \pm 1BC
Total Fe	102762 \pm 2523B	49809 \pm 4423A	57092 \pm 2060A	53093 \pm 625A	142919 \pm 15591BC	55077 \pm 4179A	169048 \pm 36891BC	136265 \pm 2673
Total Mn	1052 \pm 123B	1130 \pm 77B	1861 \pm 176C	1743 \pm 121C	882 \pm 94B	316 \pm 17A	319 \pm 54A	377 \pm 105 ²
Total As	190 \pm 14B	98 \pm 19A	79 \pm 14A	65 \pm 8.1A	436 \pm 51C	149 \pm 12AB	706 \pm 155Cd	513 \pm 112C
Bioav.*Pb	77 \pm 59AB	37 \pm 2.6A	457 \pm 29C	391 \pm 137BC	20 \pm 1A	165 \pm 50B	791 \pm 519C	31 \pm 21A
Bioav. Zn	868 \pm 276C	113 \pm 41AB	144 \pm 27AB	208 \pm 87AB	2525 \pm 687C	45 \pm 12A	165 \pm 89AB	308 \pm 96BC
Bioav. Cu	4.2 \pm 0.9AB	1.4 \pm 0.1A	4.0 \pm 0.4AB	3.5 \pm 0.7A	16.8 \pm 3.9C	2.6 \pm 0.3A	2.9 \pm 0.8A	3.0 \pm 0.3A
Bioav. Cd	3.8 \pm 1.2A	1.1 \pm 0.3A	2.4 \pm 0.2A	2.7 \pm 1.2A	6.7 \pm 1.6A	0.8 \pm 0.3A	2.1 \pm 0.3A	4.1 \pm 0.3A
Bioav. Cr	0.2 \pm 0.0A	0.2 \pm 0.0A	0.2 \pm 0.0A	0.2 \pm 0.0A	0.8 \pm 0.1B	0.2 \pm 0.0A	0.2 \pm 0.0A	0.2 \pm 0.0A
Bioav. Co	0.9 \pm 0.3AB	0.2 \pm 0.0A	0.4 \pm 0.1AB	0.4 \pm 0.1AB	1.4 \pm 0.3B	0.3 \pm 0.0AB	0.4 \pm 0.2AB	0.3 \pm 0.1A ¹
Bioav. Ni	1.8 \pm 0.6BC	0.2 \pm 0.0A	0.4 \pm 0.1A	0.8 \pm 0.4AB	3.5 \pm 0.6C	0.3 \pm 0.0A	0.6 \pm 0.2AB	0.8 \pm 0.3A ¹
Bioav. Fe	267 \pm 85C	13 \pm 1B	19 \pm 4B	28 \pm 11B	870 \pm 133d	8 \pm 1A	57 \pm 51B	17 \pm 1B
Bioav. Mn	365 \pm 124B	32 \pm 0.9A	75 \pm 7A	93 \pm 41AB	499 \pm 109B	26 \pm 2A	59 \pm 35AB	76 \pm 7B
Bioav. As	0.04 \pm 0A	0.02 \pm 0A	0.11 \pm 0.02AB	0.14 \pm 0.04AB	0.07 \pm 0.01A	0.17 \pm 0.02AB	0.10 \pm 0.04AB	0.25 \pm 0.07
Sol.* Pb	2.2 \pm 1.9B	0.06 \pm 0.01A	2.1 \pm 0.9B	2.9 \pm 0.7B	0.1 \pm 0.0A	0.9 \pm 0.24AB	4.7 \pm 3.3BC	0.7 \pm 0.3A
Sol. Zn	777 \pm 285B	2 \pm 1A	2 \pm 1A	8 \pm 2A	2377 \pm 662C	0.6 \pm 0.1A	49 \pm 46A	5 \pm 1A
Sol. Cu	2.3 \pm 0.7B	0.3 \pm 0.0A	0.5 \pm 0.0A	0.7 \pm 0.2A	11 \pm 2.4C	0.4 \pm 0.0A	0.3 \pm 0.0A	0.6 \pm 0.1A
Sol. Cd	3.5 \pm 1.3B	0.01 \pm 0.0AB	0.01 \pm 0.0A	0.04 \pm 0.01A	6.5 \pm 1.6B	0.01 \pm 0.0A	0.33 \pm 0.24AB	0.03 \pm 0.0 ²
Sol. Cr	0.14 \pm 0.05B	0.01 \pm 0.0A	0.01 \pm 0.0A	0.02 \pm 0.01A	0.62 \pm 0.1C	0.01 \pm 0.0A	0.06 \pm 0.04AB	0.02 \pm 0.0 ²
Sol. Co	0.86 \pm 0.3B	0.01 \pm 0.0A	0.04 \pm 0.01A	0.05 \pm 0.02A	1.3 \pm 0.3B	0.01 \pm 0.0A	0.15 \pm 0.12A	0.03 \pm 0.0 ²
Sol. Ni	1.7 \pm 0.6B	0.08 \pm 0.02A	0.18 \pm 0.08A	0.19 \pm 0.05A	3.2 \pm 0.7B	0.06 \pm 0.0A	0.23 \pm 0.16A	0.13 \pm 0.02
Sol. Fe	9 \pm 4A	1 \pm 0A	7 \pm 3A	6 \pm 1A	227 \pm 103B	3 \pm 0A	2 \pm 1A	8 \pm 4A
Sol. Mn	334 \pm 119C	0.3 \pm 0.1A	3 \pm 1AB	10 \pm 3B	469 \pm 105C	0.3 \pm 0.0A	36 \pm 29B	5 \pm 2B
Sol. As	0.02 \pm 0.0A	0.01 \pm 0.0A	0.08 \pm 0.03AB	0.11 \pm 0.03B	0.05 \pm 0.01AB	0.13 \pm 0.01B	0.06 \pm 0.04AB	0.16 \pm 0.05

*CE: electrical conductivity; O.C.: organic carbon; Bioav.: bioavailable metals; Sol.: soluble metals. All metals are expressed in mg kg^{-1} .

¹Different letters indicate significant differences ($p < 0.05$) between means after an ANOVA test.

3.2. Effect of mining ponds in s metal(loid)s content from natural and agricultural soils

The highest concentrations in mining, agricultural and natural soils were found for Fe, Pb, Zn, Mn and As (Table 1) in both mining districts, which is in accordance with results obtained in other studies (Martínez-Pagán et al, 2011; Acosta et al, 2011; Conesa and Faz, 2011). Concentrations of As, Cd, Cu, Pb, and Zn exceeded the limits to consider a soil as contaminated (Table 2). High levels of Fe, Zn and Pb were linked to the nature of the parent materials enriched on pyrite, sphalerite and galena, respectively.

Table 2. Guidelines maximum allowed concentration and local background of metals and arsenic in soil (mg kg^{-1})

	Pb	Zn	Cu	Cd	Cr	Co	Ni	As
Spain ^a	300	450	210	3	150	-	112	-
Netherland ^b	400	1000	500	5	-	190	100	76
Finland ^c	60	200	100	1	100	20	50	5
Background zone 3a ^d	9.3	41.4	12.6	0,32	40.4	9.3	21.7	7
Background zone 4 ^d	9.8	55.2	18.7	0,12	44.6	7.7	16.8	8.1

^aReal Decreto 1310/1990; ^bSRC (2009); ^cMEF (2007); ^dMartínez and Pérez (2007). Zone 3a corresponds to La Union and zone 4 to Mazarrón.

In the agricultural areas the concentrations of Cd were twenty and forty-fold higher than background values in La Unión and Mazarrón respectively (Martinez and Perez, 2007) (Table 2). High Cd concentrations are associated to the exploitation of some zinc sulphide minerals such as sphalerite (Alvarez-Ayuso et al, 2013).

The As concentrations found in agricultural soils from La Unión and Mazarrón were 98 mg kg⁻¹ and 149 mg kg⁻¹ respectively, while in natural soils were 79 mg kg⁻¹ and 706 mg kg⁻¹ respectively (Table 1), in all cases being much higher than the regional background levels (7 and 8 mg kg⁻¹ respectively) (Martinez and Perez, 2007). The presence of high concentrations of As can be associated to ferrous minerals as arsenopyrite (Esteve et al, 2003), common on the studied area. In fact, soils originating from metal processing can reach As concentrations up to 300-2000 mg kg⁻¹ (Kabata Pendias and Pendias, 1992).

No statistically significant differences on the total concentration of Cd and Zn in the agricultural and natural soils and mining wastes from La Unión and Cd in Mazarrón were found, whose concentrations were higher than background levels (Table 1 and 2), indicating that Cd and Zn in natural lands likely come from both mining activities and parent material, while Cd and Zn found in agricultural areas likely come from tailing ponds and natural areas nearby. Contrarily, higher concentrations of Cu, Fe and As were reported in mining waste than natural and agricultural soils of La Unión (Table 1) with no statistically significant differences between natural and agricultural lands, indicating that a low transfer of these metal(loid)s is taking place from mining to agricultural/natural soils. However, the high concentration of Pb and As in agricultural soils, 860 and 98 mg kg⁻¹ respectively, suggests an enrichment of these metal(loid)s from mining waste in La Unión. According to Martinez and Perez (2007) the background concentrations of Pb and As in soils in the study area are 9.3 and 7 mg kg⁻¹, respectively.

Concentrations of Mn were statistically lower in mining wastes than natural soils, while they were statistically similar in agricultural soil and mining pond. Co showed statistically differences among agricultural and natural soils and the mining wastes of La Unión, with the highest concentration on natural soils (13 mg kg⁻¹). Similarly, Ni concentrations in mining and agricultural soils were lower (19 and 17 mg kg⁻¹ respectively) than concentrations in natural soils (80 mg kg⁻¹). According to Martinez and Perez (2007) the background concentrations of Co and Ni in the study area are 9.3 and 21.7 mg kg⁻¹,

respectively. Because of the concentration of these metals in natural soils is higher than in mining pond, and there are not any anthropogenic activity releasing these metals in the study area, the geological origin is the most probable source of those metals.

No statistically significant differences in the concentration of Pb between agricultural soils and mining wastes from Mazarrón was found (Table 1), indicating an important transfer of Pb from mining pond to croplands. Also, the high concentration of Pb in natural soils suggests that parent material can be a secondary source of Pb in agricultural soil. In contrast, statistically higher total concentrations of Zn, Fe, Mn and As were reported in mining waste than agricultural soils, suggesting a less transfer of these metal(loid)s from mining area to croplands. However, due to Fe and As showed similar concentrations in the mining and natural areas, also parent material can be a secondary source of these elements in agricultural area. Likely minerals such as arsenopyrite, pyrite and sphalerite are aerially transported from natural soils and ponds to near agricultural areas, increasing the concentrations of As, Fe and Zn, respectively (Sánchez et al, 2017). Concentrations of Cr were statistically higher in natural/agricultural soils than mining waste, suggesting a geological origin of this metal. Similarity, concentrations of Co were lower than the background level (Table 2) and statistically similar among uses, indicating a natural origin of this metal in natural and agricultural lands.

3.3. Chemical partitioning of metals and arsenic in soil/waste

Residual phase was the predominant for all metals in mining wastes from La Unión, except for Cd where the exchangeable phase was the dominant (Figure 2). In contrast, in agricultural and natural soils, Cd was mainly bound to carbonates and Fe and Mn oxides. Carbonates has been identified as a sink of metals (e.g. Pb, Zn, Cd) (Acosta et al, 2014), where metals reach with CO_3^{-2} to form metals-carbonates complex/minerals on the surface of calcitic or dolomitic crystals (Acosta et al, 2009). High percentages of Co, Ni, Mn, Pb and Zn bound to Mn/Fe oxides (reducible phase) were reported in natural and agricultural soils from La Unión, revealing a redox process on the oxidized forms of metals that was not observed in the mining pond. In addition, higher percentage of metals bound to organic matter (oxidizable fraction) in the natural area than in agricultural soils was reported, which is due to an higher amount of organic matter in natural soils, promoting the organometallic complexation (Table 1).

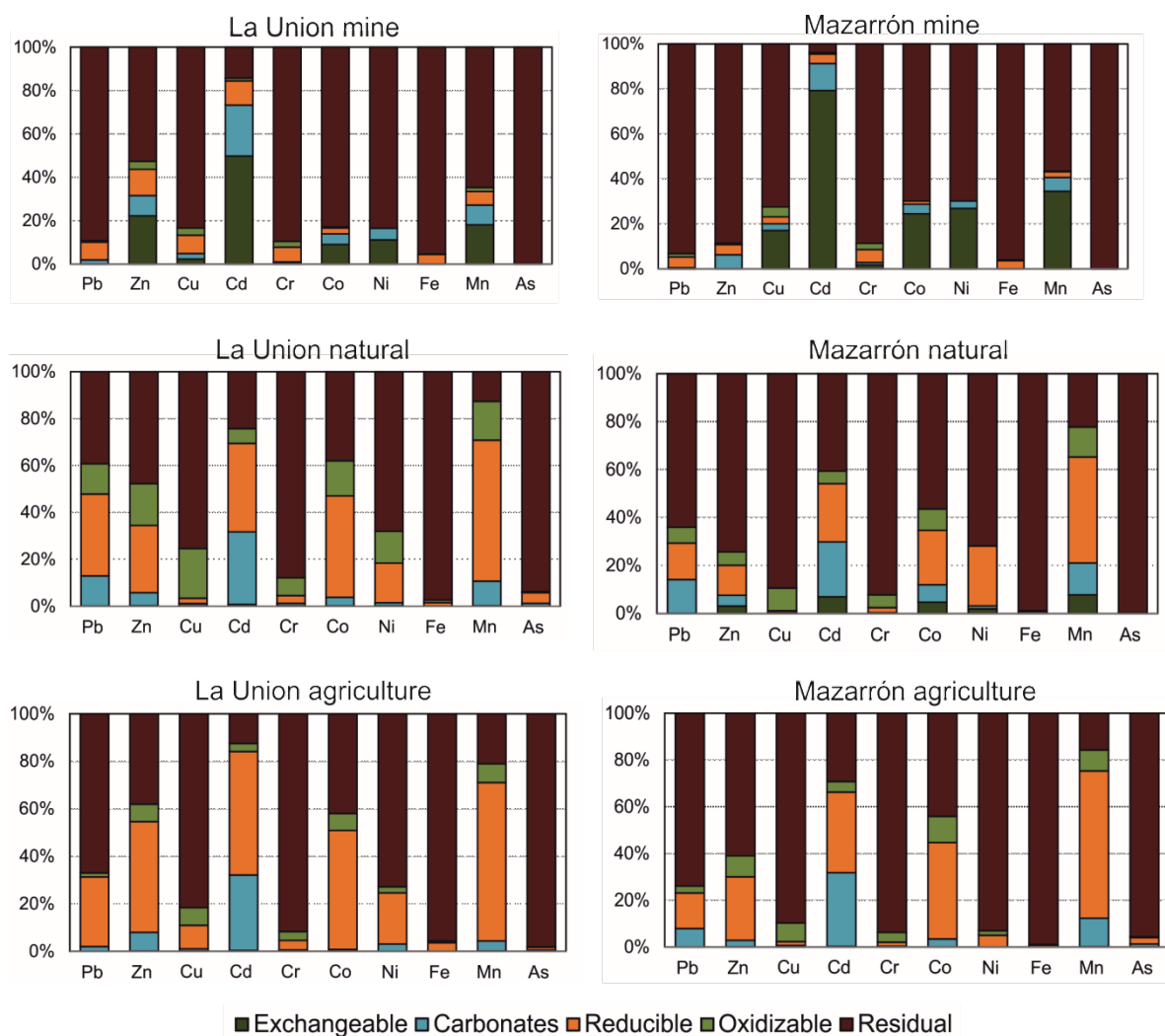


Fig.2. Chemical partitioning of metals and arsenic. The chemical phases were: exchangeable, bound to carbonates, reducible phase (bound to Fe/Mn-oxides), oxidizable phase (bound to organic matter and sulphide) and residual phase.

The chemical distribution of metals in Mazarrón mining pond was similar to La Unión. The exchangeable fraction of all metal(loid)s was higher in mining wastes than natural and agricultural soils, which can be related to the lower pH in mining pond, promoting metals mobility. In contrast, a neutral pH and a high content of carbonates in natural and agricultural soils (Table 1) contribute to metal precipitation, decreasing the concentration of metal(loid)s in the exchangeable fraction. In addition, water-soluble concentrations of Cd, Co, Cr, Cu, Fe, Mn, Ni and Zn were higher in the mining pond than agricultural and natural soils. These observations indicate that those metals are being mobilized due to the low pH and the high amount of sulphurs in mining ponds, which can generate acid mine

drainage (AMD) by the accelerated dissolution of sulphide-minerals (Cruz-Hernandez et al, 2016).

The percentages of Pb and Zn bound to residual phase in the mining wastes, agricultural and natural soils from Mazarrón were higher than those in La Unión, indicating a higher risk of Pb and Zn mobility in La Unión than Mazarrón mining district. Oppositely, higher percentage of Cd was associated to exchangeable phase in mining pond in Mazarrón than in La Unión. Finally, As was mainly bound to the soil matrix (residual phase) in both studied mining districts, suggesting a low risk of mobility for this metalloid.

3.4. Metals and arsenic concentrations in *Ballota hirsuta*

Plants can uptake the fractions of metal(loid)s that are dissolved in soil solutions. The ability to absorb trace elements by roots varies greatly depending on the metal and the specie (Kabata-Pendias and Pendias, 1992).

No statistically significant differences were found among metal(loid)s in plant tissues from both mining districts (Table 3), suggesting a similar behaviour of metal(loid)s in *B. hirsuta* from both natural areas, except for Pb where differences among roots from both mining districts were reported. Fe, Zn, Pb and Mn showed the highest concentrations followed by As, Ni, Cr, Cd and Cu. Statistically significant differences were observed for Mn and Pb among roots and stems in La Unión and Mazarrón; and for Fe, Mn and As in stem and leaves in Mazarrón mining district (Table 3), showing a different accumulation of these metal(loid)s in the different parts of *B. hirsuta*.

Table 3. Metals and arsenic concentrations (mg kg⁻¹) in *Ballota Hirsuta*, mean±standard deviation.

	La Unión			Mazarrón		
	Root	Stem	Leaf	Root	Stem	Leaf
Pb	66±14B*	35±3A	38±7A	178±70C	55±14AB	96±23BC
Zn	184±44AB	84±11AB	95±17AB	161±42AB	78±29A	137±40AB
Cu	12±1.1C	9.8±0.6BC	15±1.6C	8.6±0.6ABC	11±2.0C	10±0.3BC
Cd	1.3±0.5BC	1.1±0.3BC	0.84±0.2BC	1.5±0.36BC	0.49±0.08AB	0.46±0.06AB
Cr	3.3±0.5AB	2.2±0.2AB	4.4±1.1B	4.9±0.3BC	2.2±0.1AB	3.6±0.4AB
Co	0.33±0.04A	0.20±0.02A	0.24±0.03A	0.22±0.01A	0.10±0.02A	0.21±0.03A
Ni	1.7±0.3C	1.2±0.1ABC	1.8±0.4C	1.2±0.1ABC	1.0±0.01ABC	1.6±0.2BC
Fe	328±62ABC	387±52ABC	645±91C	512±60BC	239±33AB	653±116C
Mn	64±8.4C	28±2.7AB	55±12ABC	43±4ABC	23±3.3A	59±14BC
As	2.3±0.5AB	1.3±0.2A	2.1±0.3AB	12±3C	2.4±0.4B	6.6±1.2C

*Different letters indicate significant statistical differences (p<0.05) between means concentrations for all plant tissues and both locations after an ANOVA test.

A key factor in the human and ecological exposure risk is the accumulation of metal(loid)s in plants (Li et al, 2006; Clemens, 2006, McGrath and Zhao, 2015) which can be quantified using bioaccumulation factors (BAF). Figure 3 shows the BAF values for *B. hirsuta*. The bioaccumulation of metal(loid)s in La Unión decreases as Fe>As>Cr>Ni>Cu>Zn>Cd>Mn>Co>Pb while in Mazarrón did as As>Fe>Cr>Pb>Cu>Ni>Co>Mn>Zn>Cd, where the concentration of metals and arsenic in plant did not exceed the soil-plant toxicity levels (Table 4) ensuring the metal tolerance for plants (Mendez and Maier, 2008).

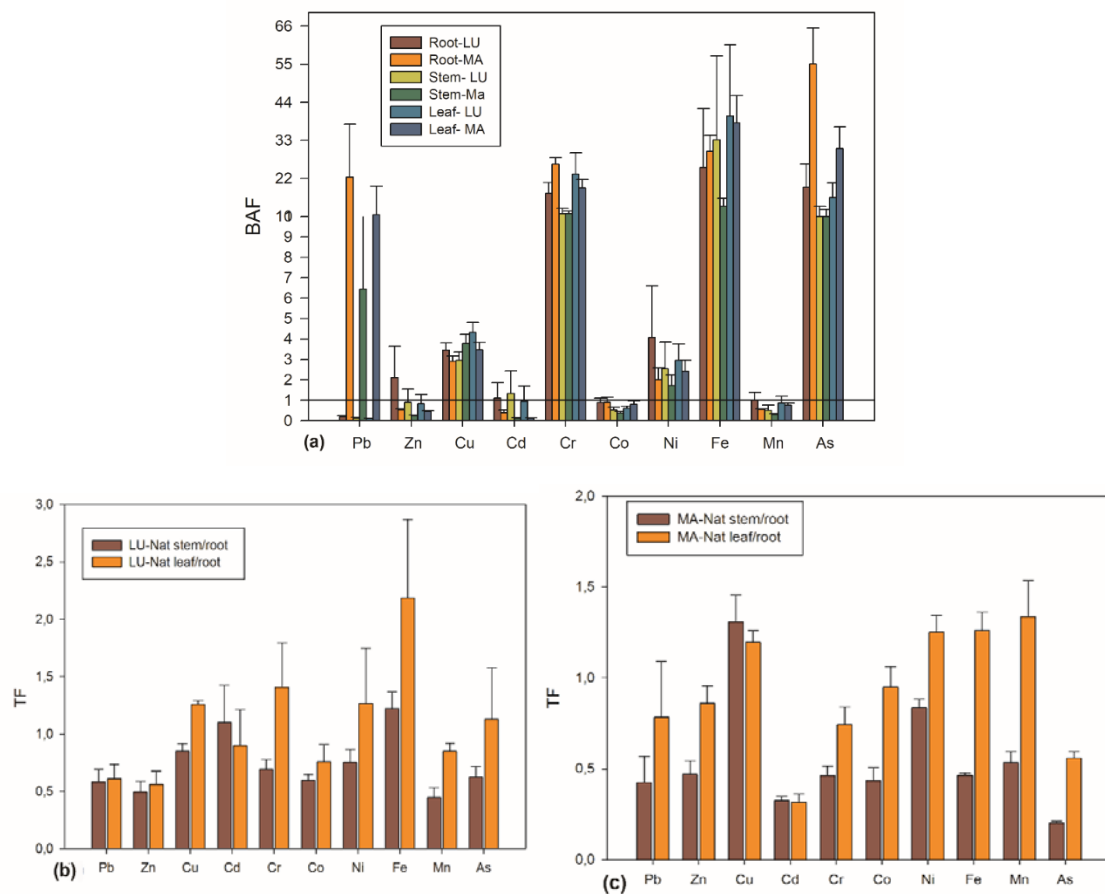


Fig.3 (a) Bioaccumulation factor of *Ballota hirsuta* in La Unión (LU) and Mazarrón (MA) mining district (plant parts differentiation), BAF higher than 1 represents metal bioaccumulation; (b) Translocation Factor of *B. hirsuta* in La Unión and (c) Mazarrón mining district. Columns represent mean and lines standard deviation (n=3)

BAF values for Cd (1.11-0.39), Co (0.88-0.90) and Mn (1.01-0.57) in roots from La Unión and Mazarrón, respectively, Pb (0.19) in La Unión and Zn (0.54) in Mazarrón were close to one, indicating that *B. hirsuta* did not accumulate those metals. However, a high

bioaccumulation of As, Cr and Fe was reported in both mining districts and for Pb in Mazarrón (Figure 3a). Especially remarkable is the bioaccumulation of As and Pb due to the high toxicity of these elements (OSHA, 2016), with values higher than 10, which can be due to a low concentration of available P in mining soils. Arsenate is a chemical analog of phosphate and shares the uptake pathway, therefore if the soils have a high concentration of available phosphorus the arsenic uptake can be reduced (Lee et al, 2016), contrary soils with poor concentration of phosphate promote arsenic uptake.

Differences in the absorption of Pb (Figure 3) for the same plant species in soil with similar properties (Table 1) suggest that the bioavailable and water soluble Pb concentrations are the main responsible of the differential absorption of Pb by *B. hirsuta* (Table 1).

Translocation factors (TFs) for leaves were higher than those for stems in *B. hirsuta*, indicating that metals preferably are accumulated in leaves (Figure 3b and 3c). For Cr, Ni, Fe, Mn and As, TFs higher than one were reported (Figure 3b and 3c), indicating that *B. hirsuta* accumulates those metal(loid)s in its aerial parts, specifically in leaves.

The accumulation of metals in plants and its subsequent toxicity can limit their colonisation and development, which is assessed with the plant leaf tissue toxicity limits (Table 4), for *B. hirsuta* no metal exceeded this limit. Similarly, no metals in aerial part of *B. hirsuta* exceeded the toxicity limits for domestic animals (NRC, 2005) (Table 4).

Table 4. Toxicity levels of metals and arsenic in plants (mg kg⁻¹)

	Pb	Zn	Cu	Cd	Cr	Co	Ni	Mn	As
Soil-plant toxicity level ¹	100-500	400	200	3	1-10	-	90	3000	15
Leaf tissue toxicity limit ¹	30-100	100-400	20-100	5-30	-	-	10-300	400-1000	5-20
Domestic animal toxicity limit ²	100	500	40	10	-	-	100	2000	30
Permissible limit in aliments ³	0.3	99.4	73	0.2	2.3	50	67.9	500	-

¹Kabata-Pendias and Pendias, (1992); Mendez and Maier (2008). ²NRC (2005). ³FAO/WHO (2001)

3.5. Metals and arsenic dynamic in soil-plant system of *Ballota hirsuta*

Bioaccumulation factor of Cu had a positive correlation with the most mobile fractions of Cu in soils (F1 r =0.81 p< 0.01; F2 r =0.87 p< 0.01 and F3 r =0.941 p< 0.05) and with its transfer factor from roots to leaves (r =0.94, p<0.05). These results indicate that the absorption of Cu is mainly affected by its concentration in the labile fractions, and once absorbed is immediately translocated to *B. hirsuta* leaves. This behaviour has been also observed in cabbage-plants (Bian et al, 2016).

Behaviour of Cr in *B. hirsuta* from both natural areas was similar, with highest contents of Cr in roots and leaves, as showed by the translocation factor (TF) (Figure 3b and 3c) which indicates that the Cr transfer to stem is just an intermediate step in the transfer from roots to leaves as shows the positive correlation among Cr concentration in stems and leaves ($r = 0.87$, $p < 0.01$).

Nickel concentration in *B. hirsuta* leaves is correlated with its bioavailable concentration in soil ($r = 0.94$, $p < 0.05$), indicating that an increase of bioavailable Ni in soil will promote its accumulation in *B. hirsuta*. Accumulation and translocation factors (Figure 3) showed that Ni is a mobile element and it is accumulated in *B. hirsuta* leaves, which depends on plants metabolism (Kabata-Pendias and Pendias, 1992).

Although high total concentrations of Fe and Mn were reported in natural soils, their bioavailable concentrations were low (Table 1). However, a positive correlation among Fe and Mn in roots and their concentrations in the residual fraction were found ($r = 0.83$, $p < 0.01$). In addition, the highest values of TF were found for Fe and Mn in leaves of *B. hirsuta*. Mn concentration in plants is preferentially transported to meristematic tissues, thus its concentration is mostly observed in young tissues because it has an important role in the synthesis of chlorophyll and in nitrogen metabolism (Bravo et al, 2017).

Arsenic was likely bound to Fe-minerals, and therefore presented in more insoluble and less labile forms (Signes-Pastor et al, 2016), however As bound to Fe-minerals can be liberated by hydrolysis processes (Obeidy et al, 2016). In this study, a positive correlation among concentration of As in roots and As bound to residual fraction of soil was reported ($r = 0.88$, $p < 0.01$), indicating that As bound to the mineral fraction can be mobilized and absorbed by *B. hirsuta*. Obeidy et al, (2016) concluded that the major part of As mobilized from mineral fraction can be likely re-distributed on the less available fractions on the soil solid-phase.

Cd in plant is very mobile and is easily adsorbed by roots and transferred and accumulated in stem and leaves (Sghayar et al, 2015) such is showed by the positive correlation of stem and leaf ($r = 0.88$, $p < 0.01$) and the bioavailable fraction in soil ($r = 0.83$, $p < 0.01$). The bioaccumulation factor of Cd for *B. hirsuta* showed a low level of accumulation but the little amount accumulated is transferred to edible parts (Figure 3).

4. Conclusions

In general, the concentrations of metal(loid)s in mining, agricultural and natural in both mining districts followed the order: Fe>Pb>Zn>Mn>As>Cu>Cr>Ni>Cd>Co. However,

the concentration of Cd, Cu, Pb, Zn, and As exceeded the limit to consider contaminated soil. An enrichment of the agricultural soils by As, Cd, Fe, Pb and Zn from the mining wastes was observed, while the geological origin is the main source of Cr, Co, Mn and Ni. The chemical partitioning revealed higher concentration of metals in the most labile fraction from mining ponds than natural and agricultural soils, where metals were mainly bound to residual fraction follow by Mn/Fe oxides (reducible phase) and carbonates. Cr, Ni, Fe, As and Cu were mostly bound to the soil matrix due to the mineralogical compositions of soils in both mining districts.

B. hirsuta showed a higher ability to bio-accumulate Fe, As, and Cr transferring a large concentration of them to the edible parts, especially leaves, without exceeding the toxicity limits for animals. Therefore *B. hirsuta* could be useful in phytoremediation of these elements in mining areas under semiarid climate. Differences observed on Cu and As transfer and accumulation in both mining districts can be explained by the different concentration of these metals in the most labile fractions.

In accordance with the results, future reclamation action of mining ponds should be based on the reduction of metal(loid)s transfer from mine areas to agricultural lands by means of low-cost restoration strategies, such as phytoremediation and promoting the development of vegetation in the tailing ponds.

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