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Exposure and bioavailability of arsenic in contaminated soils from the La Parrilla mine, Spain

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Abstract Arsenic derived from mining activity may contaminate water, soil and plant ecosystems resulting in human health and ecotoxicological risks. In this study, exposure assessment of arsenic (As) in soil, spoil, pondwater and plants collected from the areas contaminated by mine tailings and spoils in and around the La Parrilla mine, Caceres province, Spain, was carried out using AAS method. Water solubility, bioavailability and soil–plant transfer coefficients of As and phytoremediation potential of plants were determined. Arsenic concentrations varied from 148 to 2,540 mg/kg in soils of site 1 and from 610 to 1,285 mg/kg in site 2 exceeding the guideline limit for agricultural soil (50 mg/kg). Arsenic concentrations in pond waters varied from 8.8 to 101.4 µg/l. High concentrations of water-soluble As in the soils that ranged from 0.10 to 4.71 mg/kg in site 1 and from 0.46 to 4.75 mg/kg in site 2 exceeded the maximum permitted level of water-soluble As (0.04 mg/kg) in agricultural soils. Arsenic concentrations varied from 0.8 to 149.5 mg/kg dry wt in the plants of site 1 and from 2.0 to 10.0 mg/kg in the plants of site 2. Arsenic concentrations in plants increased in the approximate

order: *Retama sphaerocarpa* < *Pteridium aquilinum* < *Erica australis* < *Juncus effusus* < *Phalaris caerulea* < *Spergula arvensis* in site 1. The soil–plant transfer coefficients for As ranged from 0.001 to 0.21 in site 1 and from 0.004 to 0.016 in site 2. The bioconcentration factor based on water-soluble As of soil varied from 3.2 to 593.9 in the plants of site 1 whereas it varied from 2.1 to 20.7 in the plants of site 2. To our knowledge, this is the first study in Europe to report that the fern species *P. aquilinum* accumulates extremely low contents of As in its fronds despite high As levels in the soils. Therefore, the *S. arvensis*, *P. caerulea* and *J. effusus* plant species grown in this area might be used to partly remove the bioavailable toxic As for the purpose of minimization of mining impacts until hypothetical hyperaccumulating and/or transgenic plants could be transplanted for the phytoremediation of As contaminated soils.

Keywords Mining activity · Bioavailability of arsenic · Food chain · Toxicity · As uptake by plant · *Pteridium aquilinum* (L.) Kuhn

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Introduction

Arsenic (As) is a highly toxic and carcinogenic agent, and its elevated levels in water and soil threaten the human health contaminating the drinking water and food chain. Arsenic has achieved great notoriety because of the toxicity of some of its compounds (Kesselring 1948; O'Neill 1995) and public concern may increase because the World Health Organisation (WHO) and the US Environmental Protection Agency have lowered the guideline value for As in drinking water from 50 to 10 $\mu\text{g}/\text{l}$ (WHO 1993; US EPA 2002). Mining activities may cause heavy local and regional As pollution of soils and waters, where mine tailings may contain As up to some hundreds of mg/kg , although its crustal average is only 2 mg/kg (Boyle and Jonasson 1973). Mining activities cause the degradation of cultivated, forest or grazing land with concomitant reduction in the production of biodiversity and economic wealth directly (Wong 2003).

Human beings and domestic animals are exposed to As via different pathway. Among them, drinking water is the main route of As intake in human body. Food chain and accidental intake of contaminated soil can also be significant routes of As uptake in human body, where people are consuming As contaminated crops, vegetables and animals. In the gastrointestinal tract, As in drinking water is more easily and highly absorbed into the cell (excluding the excretion of As through urine) whereas a few percentages of As in soil and food chain are absorbed into the human cell depending on the chemical form of As and associated matrices in which As exists in. The bioavailability of As is largely a function of the environmental matrices in which As occurs (Geffard et al. 2005). This varies from 100% for soluble salts in water to less than 10% in mine tailings. Thus, the bioavailability of As from soil is considerably lower than that from water, and is impacted by such factor as water solubility of As in soil. Sheppard (1992) and Smith et al. (1998) reported that the bioaccumulation of As in plants to hazardous concentrations for human and animal consumption seldom occurs, because phytotoxic effects limit transfer into the food chain. Arsenate is the dominant form of phytoavailable As in aerobic soils and is an analog of phosphate (Meharg and Macnair 1992). Speciation analyses by recent studies exhibit that inorganic forms of As (dominantly arsenite) are major fractions in plants (Koch et al. 1999, 2000). It has been reported that arsenate is taken up via the phosphate transporters in all plant species tested and reduced to arsenite (Meharg and Macnair 1992; Wang et al. 2002). Therefore, the contamination of food chain by As still remains a possible danger. On the basis of the literature review, Kloke et al. (1984) reported the typical soil-plant transfer coefficient range for As from 0.01 to 0.1.

This suggests that a soil with $>200 \text{ mg As}/\text{kg}$ and $\text{AsTC} = 0.1$ could produce a crop (95% water content) with more As than the statutory limit of 1 mg/kg in foods (Arsenic in Food Regulations 1959).

Phytoremediation of metal and metalloid contaminated soil and water (Mkandawire et al. 2004) is a new advancement of biotechnology that is attracting enormous attention recently. Many toxic metals and metalloids are bioaccumulated by plants by default pathway along with essential elements. Phytoremediation can be accomplished by mainly phytostabilization and/or phytoextraction using hyperaccumulating plants to stabilize or decontaminate and render the soils environment-friendly. The plant species, which is capable of taking up abnormally large quantities of contaminants from soils and sequestering them in its aboveground biomass, is known as a hyperaccumulator (Brooks 1998). Plants species growing on abandoned As mine sites are tolerant to As, and they can accumulate or exclude As depending on the mineral composition of the soil and plant species (Pratas et al. 2005). Plant species that can grow fast producing high biomass and accumulate large amounts of As from the soil with degraded quality in the mine area can be the promising plant to phytoremediate the mine site lowering the threats to mankind.

La Parrilla mine situated in Caceres province, southwestern Spain, was one of the most tungsten producing areas in Spain; and is associated with elevated environmental levels of As, tin (Sn) and tungsten (W). This contamination originated in As-rich ores that were liberated both by the mining and smelting operations carried out here recently. Thus, there is considerable impetus in understanding the As biogeochemistry of this site. Therefore, it is very important to find out the appropriate plant species that can grow in the toxic metalliferous soils of mine area and can be applied for mine stabilization/remediation. Therefore, the objectives of this study were to (1) investigate the distribution pattern of As in soils and water, bioavailability and ecotoxicological risk to animals grazing on the mine area, (2) obtain more information about plant communities that are growing on As-enriched mine soils, and (3) determine the bioaccumulation of As in different plant species to assess their phytoextraction potential.

Materials and methods

Study area

“La Parrilla” deposit is located at the South of Caceres province (Spain) and this deposit is defined as a quartz-scheelite vein deposit with patterns of “stockwork” type. The mine area and open pit have a diameter of about 240 m. The tailings and spoil-affected areas cover some

hectares around the open pit. The mineral association mainly consists of scheelite, the most important economic mineral, arsenopyrite, cassiterite, wolframite, sphalerite, chalcopyrite, pyrite, pyrrhotine, quartz, muscovite and tourmaline (Gumiel and Pineda 1981), and weathering products like scorodite, tungstite, limonite, covellite and meymacite. Mining activity peaked in this area during the 1940–1987 period; however, today this mine is abandoned. Mining activities produced huge amounts of wastes composed of various barren rock types (mainly schists), fine-grained ore minerals including arsenopyrite, and ore weathering products (Fe-oxyhydroxides, sulfates and scorodite). Scorodite occurs mainly as green-white botryoidal forms on the surface of arsenopyrite-bearing rocks. The waste dumps have high As contents and continue to be a source of pollution of the surrounding environment. The volume of mine tailings is about 11 Mt with As contents ranging between 0.1 and 0.5%.

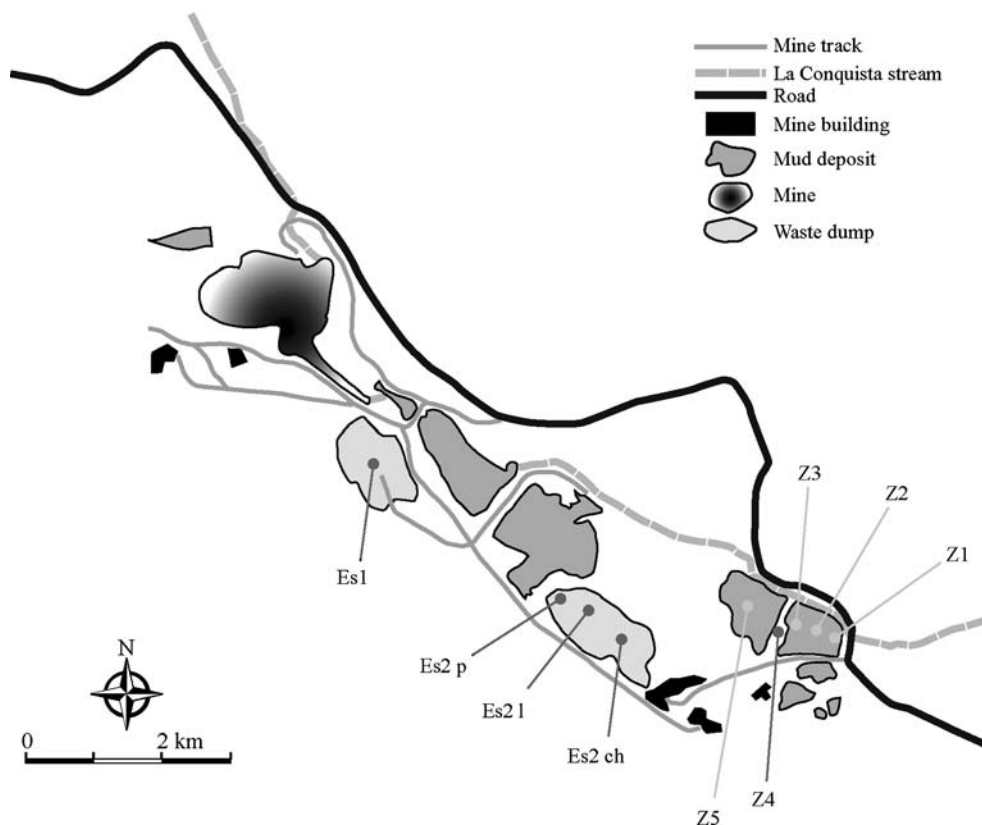
Sampling

The sampling points in this study area were designed in such a array that may represent the whole area. The parent materials of soil in mine sites are of two types; one is mud of host rocks and ore reject from grinding

and flotation processes and another one is coarse mining waste dumps. The area of mud sampling (site 1) was divided into five small zones such as Z1 (border of mud deposit), Z2 (center of mud deposit), Z3 (border of mud deposit), Z4 and Z5. The soils in zone Z1, Z2, Z3, and Z5 are muddy samples and in the zone of Z4 are mixture of mine spoil and mud. The waste dumps area (site 2) had one subdivision as Es1 and other three subdivisions as Es2 p (top surface of waste dump deposit), Es2 l (slope of waste dump deposit) and Es2 ch (foot of waste dump deposit). The sampling points according to characteristics of the soil are shown in the Fig. 1.

For this study a total of 19 top soil samples were collected. The soil samples in site 1 were collected to the depth of 0–10 cm. A few mineral samples of mining spoils (sample L-S-5, L-S-6, L-S-10a, L-S-13a and L-S-18) were collected to a depth of a few millimeters. Each soil sample consisted of a homogenate of four subsample points located in an imaginary circle about 2 m around the sampling point. After sampling, the soil and waste dump samples were kept in polyethylene bags and preserved in the refrigerator until analysis was done. A total of 20 plant samples were collected from the different species of plants grown on the mine and the areas surrounding the mine. For plant samples, the aerial parts of the plant (stems, branches and leaves) were collected and the roots were not included in the samples. Plants were

Fig. 1 A skeptical map showing the sampling points in the locality of La Parrilla mine



collected within the site 1 and 2 following the local herbarium and floras. Plant samples were cleaned with fresh-water, rinsed with deionized water and air-dried at room temperature for several days. The air-dried plant samples were powdered homogeneously and prepared for analysis. A total of five natural water samples were collected from the pond situated on the mine area. Water samples were filtered (0.45 m) on site into acid-washed polyethylene bottles and were acidified to 1% v/v HCl, avoiding Fe hydroxide precipitation.

Soil characteristics

The soil samples were dried at 50°C, mixed and homogenized and sieved through a 2-mm screen. The <2-mm fraction was used to determine the main soil properties: pH was determined potentiometrically in a soil paste saturated with water; organic matter was determined by dichromate oxidation using the Tiurin method; cation exchange capacity (CEC) was determined according to the ammonium acetate method and particle size distribution (sand, silt, and clay) was analyzed by the pipette method. Detailed descriptions of methods are written in Garcia-Sanchez and Alvarez-Ayuso (2003) and references therein.

Analysis of As in soil, spoil, pondwater and plant samples

The finely powdered and homogenized soil samples (0.1 g) were digested with 5 ml aqua regia + 1 ml HF using a CEM (MDS-2000) microwave oven; a pressure of 7 atm was applied for 30 min. Arsenic determinations were performed using a VARIAN spectra AA-220 and hydride generator VGA-76 Atomic Absorption Spectrophotometer (AAS). Analytical accuracy was checked with BCR reference material CRM-320 (river sediment) and US Geological Survey reference G-2 (granite). The precision of the method was assessed by performing the analysis ten times for a single sample. The relative standard deviation (RSD) was between 5 and 10%.

Samples of plants (0.5 g) were digested with water (2 ml), hydrogen peroxide (2 ml) and conc. HNO₃ (8 ml) using a CEM (MDS-2000) microwave oven at pressure of 9 atm (10 min) and at 12 atm (15 min). After cooling, the digests were passed through a pre-washed filter (Whatman no. 540), the digestion tubes were rinsed three times, passing washings through the filter and the digests were enriched with conc. H₂SO₄ (0.5 ml). The digests were then heated at 230°C for 2–3 h and concentrated by evaporation to approximately 0.5 ml. Finally 5 ml ascorbic acid (25%), 5 ml potassium iodide (25%) and hydrochloric acid (2 M) were added to the

digest up to the volume of 25 ml. Arsenic concentrations were measured in duplicate by using VARIAN spectra AA-220 and hydride generator VGA-76 AAS with a commercial stock standard (Panreac Quimica SA) and the calibration curve fit (at least five standard concentrations) was of $R^2 > 0.98$ in all cases. The method's recovery of As (0.79 ± 0.08 mg/kg) from a certified reference material (Maize leaves material FD8, Commission of the European Communities, Joint Research Centre ISPRA) was not significantly different from the certified value (0.77 ± 0.1 mg/kg). The mean As concentration in blank digests was 0.09 µg/l and the method detection limit for As in plant tissue was 0.07 µg/l.

Water-soluble arsenic

Water-soluble As in soil samples was measured as follows: Soil and Milli-Q water were mixed in 1:10 proportion and the mixed solution was shaken for 24 h using a rotary shaker. The solution was centrifuged at 3,000 rpm; and then the supernatant was collected and filtered using 0.45 µm filter. The As concentration in the supernatant solutions was determined using the above AAS method.

Data analysis

The soil–plant transfer coefficients for As (AsTC) is the ratio of As in plant to that in soil. It was estimated by: $AsTC = [As_{plant}]/[As_{soil}]$, where $[As_{plant}]$ is the As concentration (mg/kg) accumulated in the plant and $[As_{soil}]$ is the As concentration (mg/kg) in the soil. The bioconcentration factor, BFW based on water-soluble As was estimated by: $BFW = [As_{plant}]/[AsH_2O]$, where $[As_{plant}]$ is the As concentration (mg/kg) accumulated in the plant and $[AsH_2O]$ is the water-soluble As concentration (mg/kg) in the soil.

Results and discussion

Soil characteristics

The pH, organic matter, CEC and soil texture have been measured for a few selected soils and weathering products from study site 1 and 2 that are presented in the Tables 1 and 2. The pH values varied from 2.85 to 4.58 with the mean value of 3.53 indicating the acidic nature of soils and waste dump in site 1 and site 2 (Table 1). The contents of organic matter ranged from 0.01 to 1.97 wt% with the mean value of 0.39% and the CEC varied from 2.72 to 11.73 meq/100 g with the mean value of 7.24 meq/100 g in the soils of site 1 and site 2 (Table 1).

Table 1 Total and WS As (mg/kg) and physico-chemical characteristics of soil

Study site	Sample ID	As in soil	WS As	WS As (%)	PH	OM	Sand	Silt	Clay	CEC	
Site 1	L-S-1	1,280	1.24	0.10	3.16	0.67	37.03	40.46	22.47	8.94	
	L-S-2	998	4.71	0.47	ND	ND	ND	ND	ND	ND	
	L-S-3	720	0.76	0.11	3.45	0.05	56.04	22.1	21.89	9.4	
	L-S-4	445	0.13	0.03	3.31	0.87	32.06	37.99	29.94	11.73	
	L-S-7	730	1.45	0.20	ND	ND	ND	ND	ND	ND	
	L-S-8	1,610	0.31	0.02	3.58	0.31	28.44	49.6	21.95	8.16	
	L-S-9	712	0.64	0.09	3.54	0.05	55.85	25.2	18.9	7.58	
	L-S-10b	620	0.27	0.04	3.60	0.06	71.06	20.74	8.2	4.66	
	L-S-12	995	0.59	0.06	3.50	0.38	62.55	17.9	19.4	7.95	
	L-S-13b	1,090	0.31	0.03	3.58	0.01	76.87	14.4	8.7	4.66	
	L-S-14	2,540	0.16	0.006	2.85	1.97	43.88	35.5	20.6	9.39	
	L-S-15	1,305	0.23	0.02	2.97	0.15	70.2	20.8	8.88	5.29	
	L-S-17	148	0.10	0.07	3.82	0.25	74.83	12.07	12.97	2.72	
	Site 2	E-S-1	610	0.46	0.07	3.91	0.06	77.8	13.8	8.42	5.96
		E-S-2	1,280	0.46	0.04	3.52	0.25	47.4	34.7	18.14	8.66
		E-S-5	1,285	4.75	0.37	4.58	0.35	44.24	37.8	17.9	6.24

Unit of OM, sand, silt and clay is in % and CEC in meq/100 g WS water soluble, ND values not determined, OM organic matter

The soil texture contained sand, silt and clay materials in different proportions from sample to sample.

Minerals in soils

The constituent minerals in soil and spoil samples measured by XRD were illite, kaolinite, muscovite, plagioclase, and quartz. Although not abundant, Fe oxide coatings on soil minerals were very evident in the natural state because of their yellowish red appearance. Analysis by conventional XRD methods provided little information about Fe oxides. The most common minerals resulting from the oxidation of pyrite in the mine tailings and spoils are goethite, ferrihydrite, jarosite and other sulfate minerals. The XRD study showed the presence of very weak peaks of goethite, ferrihydrite and jarosite in soil and waste dump samples.

Arsenic in soils

Arsenic concentrations varied from 148 to 2,540 mg/kg with the mean of 1,015 mg/kg (Table 1) in the soils influenced by mine tailings to the eastern side of mine (site 1). These concentrations substantially exceeded the guideline limit of 50 mg/kg for agricultural soil (MAFF 1993). At the southern site of mine, the concentrations of As in dump samples ranged from 610 to 1,285 mg/kg (Table 1) that are also high or very high in relation to the guideline limit of 50 mg/kg. The soils of site 1 and waste dump samples of site 2 had high concentrations of As that is presumed due to the presence of ore weathering products (Fe-oxyhydroxides, sulfates and scorodite) and ore primary mineral, fine-grained ore minerals including arsenopyrite and pyrite. Accidental ingestion

of As-rich soils during grazing may occur in livestock. There was high within-site variability of As contents. Around the mine, the distributions of As and its high degree of contamination in different soil types were mainly influenced by the nature of soil parent rocks influenced by mine tailings and spoils (Tables 1, 2). The worldwide As levels in soils have been reported to range between 0.1 and 55 mg/kg (Boyle and Jonasson 1973), and 0.1 and 40 mg/kg (Bowen 1979); the background concentration of As is less than 10 mg/kg in soils of Salamanca province of Spain (Garcia-Sanchez et al. 1996). Arsenic contents in all of the soil and dump samples of this study are much higher than the above ranges of worldwide As levels and the background concentration in soil. Literature reviews (Azcue et al. 1995; Davies 1980) have shown that As concentrations up to some hundreds of mg/kg are found in mine tailings-influenced soils in other parts of the world and support the results found in this study. The soil and ore weathering product samples of L-S-4, L-S-10a and L-S-13a, which had the higher CEC than the rest of the soils studied, contained the much lower As concentrations than the other soil samples (Tables 1, 2). This is attrib-

Table 2 Total and WS As (mg/kg) and physico-chemical characteristics of WP in site 1

Sample ID	As in WP	WS As	WS As (%)	pH	OM	Sand	Silt	Clay	CEC
L-S-5	1,996	0.11	0.01	ND	ND	ND	ND	ND	ND
L-S-6	152	24.32	16.00	ND	ND	ND	ND	ND	ND
L-S-10a	201	0.003	0.001	3.52	1.06	8.95	41.7	49.3	11.57
L-S-13a	276	0.19	0.07	3.35	0.93	8.36	41.1	50.6	11.59
L-S-18	61	60.56	99.28	ND	ND	ND	ND	ND	ND

Unit of OM, sand, silt and clay is in % and CEC in meq/100 g WP weathering product, WS water soluble, ND values not determined, OM organic matter

uted to the more affinity of soil samples with high CEC for cations and less affinity for arsenate that is predominantly present as anions sorbed specifically on oxyhydroxide minerals. The soil samples of L-S-1, L-S-8 and L-S-14, which had higher concentrations of As, and contained medium to higher content of organic matter, were of dominantly silty to sandy soils (Table 1). The soil samples of L-S-13b, L-S-15, E-S-2 and E-S-5, which had high contents of As and comparatively low to medium concentrations of organic matter, were dominantly sandy soils (Table 1).

Ore weathering product

Arsenic levels ranged from 61 to 1,996 mg/kg with the mean value of 537.2 mg/kg in ore weathering products (Table 2). The samples L-S-5, L-S-10a and L-S-13a (a few mm layer of bright orange or yellowish red color), which had As contents of 1,996, 201 and 276 mg/kg, respectively, were quite distinct from the rest of the samples due to high contents of ore weathering products (Fe-oxyhydroxides and scorodite). The samples L-S-5, L-S-10a and L-S-13a had very low water-soluble As concentration that is presumed due to the presence of elevated Fe-oxyhydroxides having strong adsorption affinity for As, and very insoluble scorodite mineral. The sample L-S-10a had water-soluble As below the critical level of 0.04 mg/kg and the remaining samples (Table 2) exceeded the maximum permitted level of 0.04 mg/kg for water-soluble As in soils (Bohn et al. 1985). The samples L-S-6 and L-S-18 were white efflorescent mineral material, which had relatively low As contents (152 and 61 mg/kg) (Table 2). The XRD, IR and DTA analyses showed the presence of gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), basaluminite ($\text{Al}_4\text{SO}_4(\text{OH})_{10} \cdot 5\text{H}_2\text{O}$), syngenite ($\text{K}_2\text{Ca}(\text{SO}_4)_2 \cdot \text{H}_2\text{O}$) and jarosite ($\text{KFe}(\text{SO}_4)_2(\text{OH})_6$) in these weathering products. The samples L-S-6 and L-S-18 had very high water-soluble As concentration (24–60.56 mg/kg and accounted for between 16 and 99.28% of the total As content) due to the easy dissolution of sulfate mineral (Table 2). The pH value (3.35–3.52) indicates the acidic nature of ore weathering products of L-S-10a and L-S-13a that had lower contents of sand and higher contents of silt and clay. In contrast to the soil and waste dump samples of site 1 and site 2 (Table 1), the samples of L-S-10a and L-S-13a had higher (11.57–11.59) CEC (Table 2).

Water solubility and bioavailability of As in relation to soil and source

Water-soluble As in the soils ranged from 0.10 to 4.71 mg/kg with the mean value of 0.84 mg/kg and accounted for between 0.006 and 0.47% with the mean

value of 0.10% of total As in the samples of site 1 (Table 1). However, in the case of waste dump samples (site 2), the water-soluble As concentrations ranged from 0.46 to 4.75 mg/kg with the mean value of 1.89 mg/kg (three samples) and accounted for between 0.04 and 0.37% with the mean value of 0.16% of the total As (Table 1). All of the soil and dump samples that exceeded the maximum permitted level of 0.04 mg/kg for water-soluble As in soils (Bohn et al. 1985) in site 1 and site 2 (Table 1) had elevated water-soluble fraction of As, indicating the enriched bioavailability of As in soil and dump samples for food chain (crops, vegetables and edible plants) and pose environmental hazards. Warren et al. (2003) reported that the solubility of As in the soils contaminated by mining, deposition and waste disposal is lower than those in the soils contaminated by As-based pesticides and herbicides. This study also found the similar results that are presumed due to the specific soil characteristics, chemistry and mineral composition of the soil in mine tailings and spoils. Our results are for soils where contamination was, in general, approximately 20–50 years old and significant bioavailability of As may exist there. Arsenic solubility in soil is irrespective of the soil total As content (Tables 1, 2) but depends on the soil texture and mineral composition of the soil. The soil and dump samples of L-S-1, L-S-3, L-S-9, L-S-12, E-S-1, E-S-2 and E-S-5, which had the high content of sand, low content of clay and medium concentrations of CEC, had higher water-soluble As concentrations than those in other samples (Table 1). These results support the findings of Woolson (1973), O'Neill (1995) and Warren et al. (2003): the order of increasing bioavailability is found in the soil as the order for increasing sand content and decreasing clay content. A review of phytotoxic levels of soil As found that inorganic As is five times more toxic in sandy and loamy soils than in clay soils (Sheppard 1992). Adsorption of As on soil mineral surfaces may be favored when the hydroxyl groups are abundant on the surface of soil minerals such as oxyhydroxides and clay minerals at low pH of this study area. However, the results may also be interpreted in terms of the source of contamination. Warren et al. (2003) reported that the high bioavailability of As may be a result of the chemical form and fine particulate nature of the weathering products. However, the soil samples of L-S-13b, L-S-14, L-S-15, and L-S-17 (Table 1), which were enriched in As content, and had the higher content of sand, lower content of clay and lower to medium concentrations of CEC, had lower water-soluble As concentrations than those in other samples. It can be concluded that if the ore minerals in the deposition of mine spoils are weathered little, then the solubility and bioavailability of As will be reduced drastically. The soil samples of L-S-4, L-S-10a and L-S-13a, which had the higher CEC and clay content than those in rest of the soil samples studied,

contained the much lower total and water-soluble As concentrations (Tables 1, 2).

Arsenic in pondwater of mine

Although a few water samples were collected from the pond situated in the abandoned mine, La Parrilla, they represent all the surface waters in and around the mine. Arsenic concentrations in pond waters were 8.8, 27.0, 40.2, 53.7 and 101.4 $\mu\text{g/l}$ with the mean value of 46.22 $\mu\text{g/l}$. Out of the five, two samples contained As concentrations above 50 $\mu\text{g/l}$ (the formerly recommended permitted limit for drinking water by WHO) and four samples contained As concentrations above 10 $\mu\text{g/l}$, the present maximum permitted limit for drinking water (WHO 1993; USEPA 2002). Similarly, the higher concentrations of As are reported in surface waters situated around the mines of Salamanca province, Spain (Garcia-Sanchez and Alvarez-Ayuso 2003) and in stream waters adjacent to tailing deposits from different mine types of other regions of the world (Azcue et al. 1995; Williams et al. 1996) as well. The cattle, which drink this As-contaminated water in the La Parrilla mine area, may suffer from disruption of essential metal homeostasis in the body tissue resulting in toxicity and may endanger the food chain.

Soil–plant relationship

High levels of As in the mine tailings (on average, 812.6 mg/kg) were not reflected in plants (on average, 47.2 mg/kg) due to the physico-chemical nature of the tailings which mitigate against plant uptake (Milton and Johnson 1999). The speciation and water solubility of As in soil, which may govern the bioavailability and phytotoxicity of inorganic and organic As species in plants, can be influenced by various factors including total or extractable contents of As in soil, source of contamination, soil texture, soil minerals, alteration of the soil, soil pH, Eh, organic matter content, plant species, plant part and age and climatic condition. Although the extent of uptake by plants varies depending upon various factors, it is generally agreed that concentrations of As in plants increase with increasing those in soils (Li and Thornton 1993). The hyperaccumulating plants have the ability to solubilize metals and metalloids from the soil matrix by alteration of rhizosphere pH or redox potential and releasing metal chelating compounds into the rhizosphere, efficiently absorb them into the root and translocate them to the shoot (Pollard et al. 2002; Ma and Nomoto 1996). Although As toxicity effects on plants increase under more acidic conditions, when As-binding species become more soluble, the uptake of As by plants may be increased on higher pH soils (O'Neill

1990). However, an individually significant factor affecting the availability of As in plants was not found in this study. Thus, it can be concluded that the uptake of As by the plants can be controlled by the combination of various factors.

Plants may grow in hostile environment (metal and metalloid-contaminated and nutrient-deficient mine soils) by specific adaptation capability that plants develop under environmental stress. Arsenic tolerance must be an evolved character under genetic control (Rocovich and West 1975). A grass species, *Andropogon scoparius Michx.* collected on an As mine exhibited a wide range of tolerance to As in solution, whereas plants of the same species growing in uncontaminated soil showed no tolerance (Rocovich and West 1975). Chen et al. (2002) reported that As can be easily accumulated in Chinese brake in ordinary As contaminated soil (50–4,030 mg As/kg) with bioaccumulation coefficients greater than 1 while the values of bioaccumulation coefficients are lower than 1 in plants growing directly on mine tailings with highly elevated As contents (23,400 mg/kg). The similar results are also found in this study indicating the poor bioavailability of As for phytoremediation in mine tailings. The low break down of the As minerals induces lower bioavailability, thus resulting in a relatively higher concentration of unaltered As containing minerals in the mine soils (Pratas et al. 2005).

Arsenic in plants

Arsenic concentrations ranged from 0.8 to 149.5 mg/kg with the mean value of 47.2 mg/kg in the plants (Table 3) grown in the soils that are influenced by tailings to the eastern site of the mine (site 1) and its concentrations varied from 2.0 to 10.0 mg/kg with the mean value of 5.6 mg/kg in the plants grown in the waste dump to the south-western site of mine (site 2) (Table 4). The plant species of *Spergula arvensis* (L.), *Phalaris caerulea* Desf. and *Juncus effusus* accumulated more As in their biomass than the other plant species. Some of the *J. effusus* (9.9 mg/kg) and *P. caerulea* Desf. (0.8 mg/kg) plants accumulated very low concentrations of As, although they were grown in the soils enriched with high As contents. The ranges of As concentrations in the plants of *S. arvensis* (L.), *P. caerulea* Desf. and *J. effusus* were 80.8–149.5, 0.8–133.0, and 9.9–58.7 mg/kg DM (dry matter), respectively. Arsenic concentrations in plants increased in the approximate order: *Retama sphaerocarpa* < *Pteridium aquilinum* < *Erica australis* < *J. effusus* < *P. caerulea* Desf. < *S. arvensis* (L.) in site 1 (Table 3). The plant species of *Rumex conglomeratus* Murray (one out of four) and *R. sphaerocarpa* accumulated a little elevated concentrations of As than the other plant

Table 3 Total As concentrations, AsTC and BFW of As in different plant species from site 1

Plant species	As (mg/kg)	AsTC	BFW
<i>Juncus effusus</i> (L.)	9.9–58.7 (<i>n</i> = 4)	0.03–0.082	38.5–100.7
<i>Spergula arvensis</i> (L.)	80.8–149.5 (<i>n</i> = 2)	0.197–0.21	235.2–593.9
<i>Phalaris caerulescens</i> Desf.	0.8–133 (<i>n</i> = 2)	0.001–0.083	3.2–428.8
<i>Pteridium aquilinum</i> (L.) Kuhn	2.7–3.8 (<i>n</i> = 3)	0.0025–0.003	12–16.8
<i>Retama sphaerocarpa</i> Bois	3.3	ND	ND
<i>Erica australis</i> (L.)	18.9	0.128	186.6

n number of samples, *ND* not detectable, *AsTC* soil–plant transfer coefficient of As, *BFW* bioconcentration factor of As based on water-soluble As in soil

species in site 2. Although the soils of site 1 and waste dump of site 2 had high concentrations of As, the plants grown in site 2 had less As concentrations in the plant biomass. Arsenic levels in most of the plants studied here are higher than the general background concentrations in plants [nondetectable to 3 mg/kg dry weight (Koch et al. (2000))]. Dietary exposure of cattle to As was well above the WHO limit for foodstuffs (2 mg/kg on a fresh weight basis) in this area (Mkandawire et al. 2004). Therefore, there appears to be little ecotoxicological risk associated with As residues at the mine site investigated.

The AsTC in this study ranged from 0.001 to 0.210 with the mean value of 0.086 in site 1 (Table 3) and from 0.004 to 0.016 with the mean value of 0.009 in site 2 (Table 4). The AsTC in site 2 are lower indicating the lower solubility and bioavailability of As in this site compared to site 1. Kloke et al. (1984) reported that the soil–plant transfer coefficients for As (AsTC) were typically in the range of 0.01–0.1 for agricultural crops; and Warren et al. (2003) found the lower AsTC in the range of 0.0007–0.032 for crops and vegetables in their work. The soil–plant AsTCs in only three plant samples of this study (Table 3) are higher than that reported by Kloke et al. (1984) suggesting the relatively elevated water solubility and bioavailability of As in those soils around the La Parrilla mine; however, the soil–plant AsTCs in rest of the samples lie within the typical range of

0.01–0.1 for agricultural crops (Kloke et al. (1984)) and higher than that reported by Warren et al. (2003).

The bioconcentration factor based on water-soluble As (BFW) of the soil ranged from 3.2 to 593.9 with the mean value of 186.81 in the plants of site 1 (Table 3) whereas it varied from 2.1 to 20.7 with the mean value of 11.2 in the plants of site 2 (Table 4). As expected, the BFW of the soil more accurately reflects the plant accumulation of As from the soil than that based on total soil As (Table. 3, 4), as only a portion of total soil As is readily taken up by plant roots. High levels of BFW for As in plants indicate that relatively significant bioavailability of As exists for agricultural crops and vegetables in As contaminated soils of La Parrilla mine. However, in contrast with high assimilation of total As in soils, the plant species accumulated very low contents of As. Wang et al. (2002) reported that the transfer of As from soil to plant is generally low for most plant species that are caused due to the (a) low bioavailability of As in soil, (b) restricted uptake by plant roots, and (c) limited translocation of As from roots to shoots, and (d) As phytotoxicity at relatively low concentrations in plant tissues. With respect to the biogeochemical anomaly, *S. arvensis* (L.) is the species that shows better information for bioaccumulation of As.

In order to clean up the As and metal contaminated soils by phytoremediation biotechnology, it is crucial to select drought-resistant fast-growing plants or fodder with high biomass which can grow in metal-contaminated and nutrient-deficient soils dominantly prevailing in low quality mine soils. The soils of La Parrilla mine area are so heavily contaminated that cleaning the soils up using plants grown in these areas would take an unrealistic amount of time. Therefore, the plants grown in these soils can be used to partly remove the bioavailable toxic As, and then, based on the aforementioned results, the plant species to be cultivated for the purpose of mine restoration and minimization of mining impacts in La Parrilla mine area are the following: *S. arvensis* (L.), *P. caerulescens* Desf. and *J. effusus*. In this study area, no As hyperaccumulating plant was found; and however, it was observed that the plants grown in the La Parrilla mine area are well adapted to the As toxicity.

Table 4 Total As concentrations, AsTC and BFW of As in different plant species from site 2

Plant species	As (mg/kg)	AsTC	BFW
<i>Rumex conglomeratus</i> Murray	2.4–9.5 (<i>n</i> = 4)	ND–0.016	ND–20.7
<i>Retama sphaerocarpa</i> Bois	6.2–10.0 (<i>n</i> = 2)	ND–0.008	ND–2.1
<i>Phalaris caerulescens</i> Desf	2.0	ND	ND

n number of samples, *ND* not detectable, *AsTC* soil–plant transfer coefficient of As, *BFW* bioconcentration factor of As based on water-soluble As in waste dump

Arsenic accumulation in fern species

Chen and Wei (2000), Ma et al. (2001) and Chen et al. (2002) have discovered that Chinese brake (*Pteris vittata* L.) fern, which acts as an As hyperaccumulator, can accumulate up to 23 g As/kg when grown on an As-spiked soil and shows considerable promise as a phytoremediator of As contaminated sites. Francesconi et al. (2002) and Visoottiviset et al. (2002) have found another fern [*Pityrogramma calomelanos* (L.) which can hyperaccumulate As up to 8,350 mg/kg dry mass from soil containing 135 mg As/kg. Zhao et al. (2002) identified that, in addition to *P. vittata*, three new species such as *P. cretica*, *P. longifolia*, and *P. umbrosa* in the *Pteris* genus also hyperaccumulate As to a similar extent. They suggested that As hyperaccumulation is a constitutive property in *P. vittata*. By investigating a range of 45 fern species and their allies, Meharg (2003) identified two fern species (*Pteris straminea* and *tremula*) belonging to the *Pteris* genus as non-hyperaccumulator for As and also pointed out that the appearance of As hyperaccumulation traits arrived relatively late in terms of fern evolution, as this character was not exhibited by primitive ferns or their allies. This communication investigated a fern species, *P. aquilinum* (L.) that is widely grown in soils of La Parrilla mine. The results of this study exhibit that the fern species, *P. aquilinum* accumulates extremely low contents of As (max. 3.8 mg/kg) in spite of the high As concentrations in the soils (1,305 mg/kg). To our knowledge, this is the first report in Europe to identify that the fern species *P. aquilinum* does not hyperaccumulate As.

Recommendations from this study

A few As hyperaccumulating fern species have been discovered in some of the tropical countries which are usually not grown /or found in cold/temperate countries. Practical application of phytoremediation biotechnology in the fields using hyperaccumulating fern species to decontaminate the As-polluted soils in these countries faces difficulty. Therefore, the development of transgenic hyperaccumulating plant and its commercial production in the field for phytoremediation is very essential. Dhankher et al. (2002) developed transgenic *Arabidopsis* plants using a genetics-based phytoremediation strategy by transfer of *Escherichia coli* ars C and γ -ECS genes. These plants accumulated 4- to 17-fold greater fresh shoot weight and accumulated 2- to 3-fold more As per gram of tissue than wild type or plants expressing γ -ECS or ArsC alone. However, currently no transgenic plant has so far been commercially used for phytoremediation. Therefore, deeper insights into the genetics and biochemical processes involved in hyperaccumulation of As and metals by hyperaccumulating plant is necessary for the development of transgenic plants with improved phytoremediation capability.

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