Bull Environ Contam Toxicol (2007) 79:29–35
DOI 10.1007/s00128-007-9214-7

Arsenic Bioavailability in Polluted Mining Soils and Uptake by Tolerant Plants (El Cabaco mine, Spain)

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Received: 10 November 2006 / Accepted: 4 April 2007 / Published online: 7 July 2007

Arsenic contamination in soils, water and food chain is a global health concern due to its toxicity effect even at very low concentrations. The World Health Organisation (WHO) has set concentration limits for drinking water at 10 μg/L and for foodstuffs at 2 mg/kg on fresh weight basis (Robinson et al. 2003). About 1.4 million potentially contaminated sites have been identified within the European Community that are affected to various degree by trace metal/metalloid and/or organic pollutants (ETCS 1998). Arsenic uptake by plants has been shown to be associated with phosphate, where presumably arsenate is taken up as a phosphate analog. The study of As in plants is significant in two ways: (1) uptake of As by plants can indicate the fraction in the soil which is bioavailable (dependent on plant species) and the potential of a certain plant species for phytoremediation and (2) the residual As in the plant is then available to the next level in the food chain. Phytoremediation, that is a cost effective, promising and environment friendly technology to clean up the polluted soils with green plants, has recently attracted considerable attention. Phytostabilization may decrease the heavy metals flow, land erosion and spreading of pollutants from mining affected areas.

By random screening of plants, an intensive endeavor was made to get more and more information about plant communities growing in highly As contaminated abandoned El Cabaco mining sites, Salamanca province, Spain. The mining activities in the Salamanca province have led to contamination of the agricultural land, with top soil As values as high as 1,000–2,000 mg/kg due to weathering of arsenopyrite and dissolution of scorodite and Fe-oxyhydroxide minerals. However, As contents in non-contaminated soils of Salamanca are typically less than 10 mg/kg (Garcia-Sanchez et al. 1996). This study determined the extent of As contamination in soils, bioavailability of As and physico-chemical characteristics of soil influencing uptake of As by plants. Since the As hyperaccumulator plants reported at the present time are only fern species, this communication also investigated the uptake of As by the fern species *Pteridium aquilinum* (L.) grown in the soils affected by El Cabaco mining activities, and control area followed by conducting the greenhouse experiments to more closely observe the As accumulation capacity of *P. aquilinum* (L.). Therefore, the objectives of this study were (1) to determine As levels in plants and soils; (2) to know the bioavailable toxic As fractions of soil; (3) to estimate human and ecotoxicological risks posed by As levels in plants and soils; and (4) to investigate the potential of locally grown tolerant plant species for phytoremediation, especially, phytostabilization of polluted soils in El Cabaco mining area.

Materials and Methods

El Cabaco is an abandoned tungsten (major) and gold (minor) producing mine in the southern portion of the Salamanca province. The mining activities produced huge amounts of wastes composed of various barren rock types (mainly granites and schists), fine-grained ore minerals including arsenopyrite, and ore weathering products (Fe-oxyhydroxides and scorodite) that contained high As contents and polluted the top soils of the surrounding

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environment. The mineral paragenesis mainly consists of arsenopyrite, pyrite, wolframite, and minor chalcopyrite, bismuthinite and gold.

Plants and soil samples were collected from different locations at El Cabaco mine: site 1 (Cabaco-K72) is located around the main waste dump; site 2 (Cabaco-K5) is located near an ancient Roman little open pit 2 km to the east of site 1; site 3 (Calvarrines) corresponds to a minor open pit and waste dump situated 1 km to the south of site 1, and a control area 10 km away to the west. The sampling for both plants and soil was repeated four times in an area of 1 m^2 around each sampling point and the samples were mixed up to obtain a homogenous sample of plant and soil from each sampling point. For this study, a total of 10 soil samples were collected from the depth of 0–10 cm. A total of 55 plant samples belonging to 30 different species were collected from the different locations of study site 1, site 2, site 3, and control area. For plant samples, the aerial parts of the plant (stems, branches and leaves) were collected; and the roots were not included in the samples. 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 in a stainless-steel mill to obtain a homogeneous sample and prepared for analysis.

The soil samples were dried at 50°C, mixed and homogeneized 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 (Jackson 1960); cation exchange capacity was determined according to the ammonium acetate method by extracting with a 1.0 mol/L NH_4OAc solution (pH 7.0); and particle size distribution (sand, silt, and clay) was analysed by the pipette method (SCS 1972).

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. The nitric acid was removed by mixing 2 ml H_2O_2 (30% w/v) followed by heating in a water bath at 100°C. Arsenic determination was 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 10 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. HNO_3 (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 enriched with conc. H_2SO_4 (0.5 mL). The digests were then heated at 150°C for 2–3 hours 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 Atomic Absorption Spectrophotometer (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 sample was 0.07 μg/L.

Water soluble As in soil samples was measured as follows: Soil and Milli-Q water were mixed in 1:10 proportion, after which the suspension was shaken for 24 h, allowed to stand for 10 h and, finally, shaken for a further 2–3 h. The suspension was centrifuged at 3,000 rpm and then the supernatant was collected followed by filtration using 0.45 m filter. For acid–ammonium–oxalate extractable As determination, soil samples were weighed (1.0 g) into 250 mL polyethylene bottles and 100 mL of 0.2 M acid–oxalate solution (pH = 3) added. Solutions were then shaken in the dark for 2 h and centrifuged. Reproducibility of three samples extracted in duplicate in acid–oxalate solution was within 5%. To determine bioavailable As content in soils, Bray–Kurtz extractable As was determined by shaking 2 g dry soil with 14 mL of 0.03 M NH_4F and 0.025 M HCl for 1 min followed by filtration. Arsenic in the filtered solution was measured by above HG-AAS method.

The fern seedlings of *P. aquilinum* were collected from the fields. Arsenic was added to the mine soil at 0 (control, pot No 1), and 100 mg/kg (As was added to 9 pot soils) as Na_2HAsO_4. However, the mine soils (used as control) originally contained some As. Soils were mixed with the designated amounts of Hoagland solution (1.18 g/L of $Ca(NO_3)_2$, 0.49 g/L of $MgSO_4$, 0.29 g/L of KH_2PO_4 and 1.02 g/L of KNO_3) to supply with elemental nutrients. The soil was then brought to its field capacity with deionized water and incubated for one week. Following the incubation, one fern seedling with 3–4 fronds was transplanted to each pot containing As-spiked soil and a nutrient solution; and 10 pot experiments were conducted for a few weeks in the greenhouse. The nutrient solution was aerated continuously and renewed every 5 days. All reagents in the study were of analytical grade. The average temperature in the

greenhouse ranged from 14 (night) to 30°C (day), with an average photosynthetically active radiation (PAR) of 825 μmol/m/s. The soils were maintained at 70% of its water-holding capacity with de-ionized water during the plant growth. After growing for a few weeks, the fern plants were harvested and rinsed thoroughly with deionized water.

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 = $[As_{plant}]/[As_{H_2O}]$, where $[As_{plant}]$ is the As concentration (mg/kg) accumulated in the plant and $[As_{H_2O}]$ is the water soluble As concentration (mg/kg) in the soil. Data are presented as geometric mean, baseline and observed range.

Results and Discussion

The pH, organic matter (OM), cation exchange capacity (CEC), and soil texture at three sites studied are given in Table 1. The pH values of soils (4.6–5.4) showed an acidic nature. The CEC of the soils at site 1 (11.53–21.55) showed a higher buffering capacity to acid deposition, while those at sites 2 and 3 showed a low buffering capacity to acid deposition. Soil texture exhibits the higher proportions of coarse particles at site 1. The soils of site 2 also showed similar results (Table 1). However, the soils of site 3 exhibited higher proportions of fine particle (Table 1). The silt and clay contents of soils at site 1 indicate the higher proportions of clay contents than those of silt. The opposite characteristics were found in the soils of site 3 (Table 1). Arsenic contents in soils at site 1 varied from 440 to 1,540 mg/kg with the mean value of 956 mg/kg; and the concentrations were 128 and 130 mg/kg at site 3, and 50 mg/kg at site 2 (Table 1). The soils of site 1 contained the highest As concentrations and were affected to a great extent by mine tailings and spoils. However, the mean value of As content at all of three sites was higher compared to the average toxicity threshold of 40 mg/kg established for crop plant (Sheppard 1992), the reported world-wide As levels in soils (0.1–55.0 mg/kg, Boyle and Jonasson 1973; 0.1–40.0 mg/kg, Bowen 1979) and the background concentration of As in soils of Salamanca province, Spain (<10 mg/kg, Garcia-Sanchez et al. 1996). The fact that arsenopyrite and weathering products (scorodite), initial sources of As in mine soils, had been broken down to Fe-oxyhydroxides, and aqueous arsenate was adsorbed by Fe-oxyhydroxides at the pH (≅5) and Eh (≅300–400 mV) soil conditions found in soils of Salamanca province, is substantiated by the absence of arsenopyrite or scorodite in soils (not detected by XRD).

Water-soluble As, that is a good guide to assess the bioavailable As and risk level of contamination in soils, is more phytotoxic than other firmly bound forms. Water soluble As in the soils of site 1 (1.18–5.89 mg/kg and accounted for between 0.14% and 0.38% of total As in soils) was higher than those at site 2 (0.08 mg/kg) and site 3 (0.09–0.21 mg/kg, Table 1). The higher water solubility and bioavailability of As in the soils of site 1 compared to site 2 and site 3 were reflected in higher As accumulation in the plants of site 1 (Tables 1, 2). Out of 10, four soil samples had relatively elevated water-soluble As concentrations (0.34%, 0.35%, 0.36% and 0.38% of total As content in soils) than other samples and those reported by the study of Kavanagh et al. (1997) in agricultural soils and mine wastes in the Tamar Valley, north Cornwall, UK. However, Xu and Thornton (1985) and Cao and Ma (2004) found the maximum water-soluble As of 2.78% in garden

Table 1 Physico-chemical characteristics and different forms of As (mg/kg) of El Cabaco mine soils

SN	SL	pH	OM (wt%)	CEC meq/100	Coarse sand (%)	Fine sand (%)	Silt (%)	Clay (%)	As_{water}	Total As	As_{water} (%)	As_{ox}	As_{BK}
1	Site 1	4.91	3.87	12.67	42.0	20.5	13.6	20.8	2.98	890	0.34	503	16.8
2	Site 1	4.84	9.82	21.55	40.0	18.0	12.7	17.5	5.89	1540	0.38	703	16
3	Site 1	5.13	3.75	12.75	45.5	19.5	11.6	19.3	2.27	920	0.25	670	18.2
4	Site 1	5.37	3.75	12.92	40.5	18.0	14.3	23.6	1.18	440	0.27	305	12.2
5	Site 1	5.20	2.79	13.25	31.5	20.5	14.8	28.2	3.53	1007	0.35	354	22.5
6	Site 1	5.22	5.86	15.15	34.5	21.0	14.0	24.7	2.47	685	0.36	490	20.6
7	Site 1	5.25	3.00	11.53	47.0	20.5	11.0	19.1	1.65	1208	0.14	738	26.6
8	Site 2	5.34	1.80	4.29	43.7	26.2	12.1	12.3	0.08	50	0.16	9.8	0.5
9	Site 3	4.63	3.96	6.18	23.1	24.5	22.8	17.0	0.21	128	0.17	20.1	0.6
10	Site 3	4.98	1.57	3.78	22.0	29.2	20.4	18.2	0.09	130	0.07	13.8	0.3

OM Organic matter, *CEC* cation exchange capacity, As_{water} water soluble As, As_{ox} As extractable by oxalate, As_{BK} As extractable by Bray–Kurtz reagent, *SN* sample no., *SL* sample location

Table 2 Arsenic contents in plants (mg/kg)

Plant species	Arsenic	AsTC	BFw
At study site 1 (CAB-K72)			
Teesdalia nudicaulis	13.69	0.014	4.80
Sarothamnus scoparius (n = 3)	0.26–0.87	0.0003–0.001	0.09–0.30
Arenaria montana	2.56	0.003	0.90
Festuca arundinacea	0.56	0.001	0.20
Quercus pyrenaica (n = 2)	0.87–4.42	0.001–0.005	0.30–1.55
Pteridium aquilinum (n = 4)	0.23–0.39	0.0002–0.0004	0.08–0.14
Erica lusitanica	0.13	0.0001	0.05
Halimium alyssoides	1.5	0.002	0.54
Avena sulcata	0.68	0.001	0.24
Lotus corniculatus	3.98	0.004	1.40
Leontodon crispus	9.93	0.010	3.48
Nardurus patens	0.74	0.001	0.26
Rumex crispus	4.66	0.005	1.63
Crucianella angustifolia	0.74	0.001	0.26
Anthoxanthum odoratum	0.38	0.0004	0.13
Avena elatior	1.67	0.002	0.59
Hippocrepis comosa	4.47	0.005	1.57
Cistus monspeliensis	18.53	0.019	6.50
At study site 2 (CAB-K5)			
Erica australis	0.08	0.002	1.04
Erica arborea	0.035	0.001	0.44
Anthoxantum odoratum (n = 2)	0.24–0.53	0.005–0.011	2.94–6.61
Pteridium aquilinum (n = 2)	0.003–0.028	0.0001–0.001	0.04–0.35
Quercus pyrenaica	0.024	0.0005	0.30
Genista anglica	0.156	0.003	1.95
Cistus albidus (n = 2)	0.29–0.30	0.006	3.59–3.79
At study site 3 (Calvarrines)			
Quercus pyrenaica	0.14	0.001	0.91
Rumex acetosella	0.97	0.008	6.49
Chamaespartium tridentatum	0.67	0.005	4.47
Vulpia bromoides	1.08	0.008	7.19
Secale cereale	0.77	0.006	5.16
Cistus hirsutus	0.18	0.001	1.19
Holcus lanatus	0.73	0.006	4.87
Cistus salicifolium	0.30	0.002	1.98
Erica arborea	0.64	0.005	4.29
Erica australis	0.20	0.002	1.35
Senecio jacobaea	1.15	0.009	7.67
Festuca arundinacea	0.17	0.001	1.13

AsTC Soil–plant transfer coefficient of As, *n* number of samples, *BFw* bioconcentration factor of As in plant based on water soluble As

soils of Cornwall and 3.02%–13.6% in the CCA contaminated soils, USA, respectively that far exceed the results of the present and other studies. Therefore, the low water soluble As in soils for a heavily contaminated site like this study, suggest that the major fraction of As is predominantly bound to the Fe–Mn oxide (FM-As) phase and immobilized; and very low contents of As are present in water-soluble, exchangeable and carbonate fraction (WEC-As). The As–OH group was observed by IR (2,215 cm^{-1}) (Raade et al. 1984) in a sample of Fe-oxyhydroxide from site 1, identified as lepidocrocite. The water solubility of As in soils was irrespective of the total As content (Table 1). The contents of organic matter correlated with water soluble As in soils ($R^2 = 0.63$, $p < 0.01$) due to the fact that humic or fulvic acids blocking adsorption sites of amorphous soil colloids may reduce arsenate fixation and enhance As solubility. The relatively higher water solubility of As in soils of sample 1, 2, 5, and 6, that have

higher fine particle than large particle, higher clay content and cation-exchange capacity, could be due to the preferential desorption of As by the negative electric charges in soil, thereby maintaining bioavailability for soil organisms and plants.

Acid ammonium oxalate extractable As (As_{ox}) contents (9.76–738 mg/kg and accounted for between 10.65% and 61.09% of total As in soils, Table 1), that is associated with amorphous Fe oxides correlated strongly with total As ($R^2 = 0.93$, $p < 0.01$) and showed positive correlation with those of water soluble As ($R^2 = 0.57$, $p < 0.05$) and Bray–Kurtz extractable As ($R^2 = 0.77$, $p < 0.02$) in the soils, suggesting that the amorphous Fe oxides are the source of the more mobile and bioavailable As in soils. The Bray-Kurtz extractable As contents in the soils (0.26–26.64 mg/kg) correlated well with oxalate extractable As_{ox} concentrations ($R^2 = 0.77$, $p < 0.01$) and correlated weakly with water soluble As ($R^2 = 0.41$, $p < 0.01$), indicating that the Bray–Kurtz extractable As could be also a suitable indicator of soluble and bioavailable As in soils.

The concentrations of As in the plants varied from 0.13 to 18.53 mg/kg with the mean value of 3.02 mg/kg (dw) at study site 1, and the maximum As concentration was found in *Cistus monspeliensis* (Table 2). However, other members of Cistaceae family including *Cistus albidus* L., *Cistus hirsutus* Lam., and *Cistus salvifolius* L. found at sites 2 and 3 accumulated extremely low concentrations of As (Table 2). Together with *Cistus monspeliensis*, the plant species *Teesdalia nudicaulis* and *Leontodon Crispus* accumulated relatively higher As concentrations in their shoots than other plant species at site 1 (Table 2). The higher As uptake by plants at site 1 compared to that of sites 2 and 3 was linked to the greater water solubility of As in the soils of site 1 than the other sites (Tables 1, 2). The As contents in plants were related to water soluble, oxalate extractable, Bray–Kurtz extractable and total As in soils. The better relationship (Fig. 1) was found between As contents in plants and oxalate extractable As in soils ($R^2 = 0.36$, $p < 0.001$). The other correlation coefficients were $R^2 = 0.18$ and $p < 0.003$ for water soluble As, $R^2 = 0.19$ and $p < 0.003$ for total As, and $R^2 = 0.21$ and $p < 0.001$ for Bray–Kurtz extractable As in soils. These data suggest that amorphous Fe oxides are presumed to be the source of the bioavailable As to soil pore water and phytoavailable pool. The As mobilization mechanisms may include dissolution by low molecular organic acids secreted in plant rhizosphere and/or Fe^{3+} dissolution by siderophores, which are produced by plant roots and some aerobic bacteria.

Literature survey shows that although the concentrations of As in crops, vegetables and herbage plants grown on mining and smelting affected soils are higher than controls, however, in general, the As levels are very low (0.01–1.15 and 0.04–0.85 mg/kg) when grown on contaminated or uncontaminated land (Bowen 1979; Xu and Thornton 1985). However, relatively higher As levels in several plants were found in mine wastes in the UK (6,640 mg/kg, Porter and Peterson 1975), northeast Portugal (60–300 mg/kg, de Koe 1994), and geothermal area of New Zealand (1,766 mg/kg, Robinson et al. 2003) but these plants are not strictly As hyperaccumulators due to the very low ratios of As contents in plant shoots to those in soils. In general, As uptake by plants is largely dependent on the source, chemical speciation, pedological factors (pH, Eh, organic matter and colloid contents, soil texture, minerals and drainage conditions), plant species, and age and part of plants.

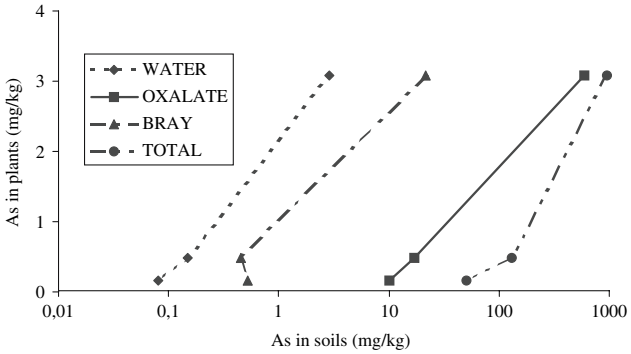

Fig. 1 Relationship of As in plants with water soluble, oxalate and Bray extractable and total As in soils (average concentrations of the three study sites)

The soil–plant transfer coefficients for As (AsTC) can be used to assess the As bioaccumulation of plants. The values of AsTC at site 1 (0.0001–0.019), site 2 (0.0001–0.011) and site 3 (0.001–0.009) are much lower (Table 2) than the reported value of Cao and Ma (2004) for carrot and letuce (0.10–1.61) grown on CCA-contaminated soils, but very similar to the results (0.0007–0.032) of Warren et al. (2003) for crops. As expected, a bioconcentration factor based on water-soluble As (BFw) reflects more accurately the plant accumulation of As than that based on total soil As, since only a small portion of total soil As is readily taken up by plant roots. The bioconcentration factors in plants (Table 2) of site 3 (0.91–7.67), which has fine particulate nature of soil, are higher than those in plants of site 1 (0.05–6.50) and site 2 (0.04–6.61).

If the phytoavailability, AsTC and bioconcentration factor of As in plants for this study are compared with other available data from the literatures, it is distinctly noticeable that soil-plant transfer of As in this study is extremely low due to low bioavailability of As even when grown on mine soils with high As levels due to the low weathering degree of As-rich ore minerals (Warren et al. 2003). The soils investigated here are so heavily polluted that removal of As using plants grown here is unlikely in relation to time and

cost. Therefore, if the target is to partly remove the bioavailable toxic As and phytostabilization, then, based on the aforementioned results, the suitable plant species to be used are the following: *Cistus monspeliensis, Teesdalia nudicaulis*, and *Leontodon Crispus*. The As contaminated plants species can be discarded to ocean or abandoned remote areas. The plant species or their ashes can be composted in a closed pit where spread of contamination is not possible (security toxic wastes deposit).

Chaney (1989) suggests that a 'normal' inorganic As concentration in dry foliage might be 0.01–1 mg/kg and a phytotoxic level may be 3–10 mg/kg. A prescribed limit of < about 20 mg/kg (dw) total As in feedstuff containing plants for domestic livestock was stated in the literature (Eisler 1994). Out of 46, only 7 plant species grown in the soils of site 1 was found to contain >3 mg/kg As in upper plant parts; and no plants sampled in this study exceeded the limit for domestic livestock reflecting that these species appear to have no toxicological effects from consumption of the aboveground parts of these species. Nevertheless, accidental uptake of As-polluted soils and plant roots containing As-enriched iron plaque may cause poisoning in grazing livestock.

During recent years, the discovery of a few fern species [*Pteris vittata* (Ma 2001); *P. cretica, P. longifolia* and *P. umbrosa* (Zhao et al. 2002); *Pteris multifida* Poir (Du et al. 2005); *Pteris biaurita, Pteris quadriaurita* and *Pteris ryukyuensis* (Srivastava et al. 2006); *Pityrogramma calomelanos* L. (Francesconi et al. 2002)], excellent As hyperaccumulators, launched phytoremediation technology in progress. Meharg (2003) investigated a range of 45 fern species and their allies; and identified two fern species (*Pteris straminea* and tremula) belonging to the *Pteris* genus as non-hyperaccumulator for As. This study investigated As accumulation and phytoextraction potential of the fern species *P. aquilinum* from the site 1, site 2 and one control area. This species grown in soils of site 1, site 2 and control area accumulated very low As concentrations (0.228–0.385, 0.003–0.028 and 0.098 mg/kg of As) in spite of high As contents in soils (on average, 956, 50 and 10 mg/kg, respectively) reflecting that this species acts as above ground non-accumulator like other terrestrial plants. Arsenic content in above ground plant biomass corresponded to that in soils to some degree and a linear relationship was found between As contents in plants and those in soils ($R^2 = 0.86$).

Arsenic contents in pot soils of this greenhouse study varied from 280 to 800 mg/kg and the water soluble As ranged from 1.65 to 6.83 mg/kg. The *P. aquilinum* accumulated very low As content (1.88 mg/kg) in control and pot No. 4 and 5. However, in pots of 3, 6, 7, 8 and 10, As accumulation was relatively higher (4.3, 4.2, 3.8, 5.1 and 7.9 μg/g, respectively) over the control plant. The max. content of As (21.07 mg/kg) was found in pot 9. Some of the younger plants died after a few weeks of transplanting in the As-treated pot soils and they accumulated very low concentrations of As with the exception of one dead sample (pot No. 2, 8.03 mg As/kg). However, the older plants accumulated relatively higher As concentrations in the tissue compared to the plants died earlier. One week after transplanting, As toxicity was observed in fronds of some younger *P. aquilinum* plants in the 300–800 mg As/kg treatment. These fronds had dark brown coloration at the leaves, and plants were dead after 1 week. In the treatment of pot 3 with 280 mg As/kg, the symptoms of As toxicity were not observed in the old fronds of *P. aquilinum*, but the plants survived throughout the study. Given the phytotoxic effect of As, Sheppard (1992) concluded that the mean As toxicity threshold for plants is 40 and 200 mg As/kg in sandy and clayey soils, respectively.

Acknowledgments The authors would like to thank the Government of Castilla y Leon, Spain for supporting this study under the Project Grant No. CSI 13/03. The suggestions and technical comments of the anonymous reviewers are highly appreciated.

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