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Heavy Metal Accumulation in Plant Species Indigenous to a Contaminated Portuguese Site: Prospects for Phytoremediation

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Abstract Phytoremediation is a promising alternative to conventional soil clean-up methods; however, up to date, there is still not enough information on plant species suitable for application in this field of science. Therefore, plant screening on contaminated sites can lead to the identification of further species of interest. In the present study, pedological and botanical characteristics of an industrialised area known for its metal contamination, in special with Zn—Esteiro de Estarreja, in Portugal—were examined in a 1-year screening. Twenty-seven species were found, with a higher occurrence and variability in the summer/spring season. Zinc levels in the tissues of the collected plant samples ranged from 34 mg kg^{-1} in shoots to 2,440 mg kg^{-1} in roots of different species. Species as *Verbascum virgatum*, *Hypochoeris radicata*, *Phalaris arundinacea*, *Conyza bilbaoana*, *Paspalum urvillei* and *Aster squamatus* have shown high Zn shoot accumulation and bioconcentration factors ($BCF_{shoots}>1$) and high metal translocation factors ($TF>1$). Others, namely *Spergularia capillacea*, excluded Zn from the shoot tissues and stored the metal at the root zone ($BCF_{roots}>1$), behaving as tolerant plants. Plants were also screened for arbuscular mycorrhizal fungi colonisation, and only few species showed mycorrhizal presence, namely *C. bilbaoana*, *Hirschfeldia incana*, *Epilobium tetragonum*, *Conyza sumatrensis*, *Pteridium aquilinum*, *P. urvillei* and *A. squamatus*. The present work showed important indigenous species that can cope with installed harsh conditions and with potential for utilisation in phytoremediation strategies, either through metal removal to aerial parts or through its immobilisation in the root zone.

Keywords Soil · Phytoremediation · Arbuscular mycorrhizal fungi · Zn · Bioavailability · Survey

Capsule Twenty seven plant species were found in a one year round survey in a Zn contaminated site and several plants with potential application in phytoremediation strategies, either in metal removal or immobilisation, were found.

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1 Introduction

Pollution of the biosphere with toxic metals has accelerated dramatically since the beginning of the industrial revolution (Nriagu 1979). The primary sources of this pollution are the burning of fossil fuels, mining

and smelting of metalliferous ores, metallurgical industries, municipal wastes, fertilisers, pesticides and sewage. Soil, at the interface between the atmosphere and the earth's crust, is open to inputs of heavy metals from many of these sources (Alloway 1990). The danger of toxic metals' pollution is aggravated by their almost indefinite persistence in the environment (Garbisu and Alkorta 2001).

There is a considerable past legacy of poor reclamation practice of heavy metal contaminated sites (Berger 1990). This lack of restoration of degraded soils is often related to the high cost of remediation solutions and to problems of soil disturbance, which render sometimes the land useless as a medium for further activities, such as plant growth (Marques et al. 2010). Phytoremediation has become an important field of research as it is a safe and potentially cheaper restoration technology, especially when compared to the conventional physic and chemically based remediation methods (Salt et al. 1998). Nevertheless and despite the crescent efforts in order to investigate the potential of phytoremediation as an alternative and efficient soil clean-up technology, few plant species have been shown to be efficient for phytoremediation purposes. Heavy metals can cause severe toxicity and may act as a powerful force for the evolution of tolerant plant populations. The search for plants capable of survival, growth and reproduction under metal-stressful field conditions may be an adequate approach to find plant species with metal resistance capabilities and even with the capacity to accumulate metals at very high levels (Pitchel et al. 1999; Yoon et al. 2006). Therefore, any effort towards the identification of new species as a contribution to the increase of the database of plants with phytoremediation abilities seems of great importance. In this context, geobotanical surveys and plant screenings appear as an important tool as they can lead to the identification of such species with increased value for application in plant-based remediation techniques.

Several tools can be used to increase phytoremediation effectiveness as a soil rehabilitation method, and biotechnological instruments—such as soil microorganism which can influence plant development, especially in toxic environments—are of great importance. Arbuscular mycorrhizal fungi (AMF) are a group of soil organisms of great ecological importance that form symbiotic associations with the roots of the majority of land plants (Smith and Read 1997). AMF may contribute to overcome the problems caused by environmental stresses that hamper plant survival, growth and reproduction in disturbed ecosystems and can also influence plant toxicity and impact plant uptake of soil metals (Leyval et al. 1997)—some studies reported a metal exclusion strategy in plants (Huang et al. 2002), whilst others demonstrate enhanced metal accumulation in plants due to AMF colonisation (Marques et al. 2006, 2007a).

The present work was undertaken to acquire information from a highly polluted area in Portugal in the summer and winter seasons. The site has a long history of metal contamination due to the industrial activity in the surrounding area. Amongst the metals present at high levels in the area, Zn appears as the main and mostly spread contaminant, and it is known from previous studies that the contamination in the area is very heterogeneous (Marques et al. 2007b). The aim of the study was to assess the capacity of the predominant plant species growing in this polluted area to accumulate Zn, by determining the levels of the metal in the soil and its available fractions, in the plant shoot and root. Mycorrhizal colonisation in the field was also evaluated.

2 Material and Methods

2.1 Description of Site and Sampling Techniques

A large industrial complex, composed essentially by chemical facilities, is in the centre of the area considered in the present study. For many years, several of these industries have discharged their solid residues directly to the soil in the surrounding area and released its wastewaters into the nearby watercourses. Therefore, the levels of heavy metals in the area reach hazardous levels. Zinc appears as one of the main contaminants of the sediments, with the soils presenting levels of up to 3,620 mg Zn kg^{-1} (total Zn), whilst other metals are also present at high concentrations, with average levels of 835 mg Pb kg^{-1}, 66 mg Hg kg^{-1}, 26 mg Cr kg^{-1}, 37 mg Ni kg^{-1} and 16,800 mg Fe kg^{-1} (Oliveira et al. 2001). Despite this scenario, the vegetation remains prolific, yet heterogeneously distributed. Plant and soil sampling was made at four different locations surrounding the complex, as shown in Fig. 1. Location A refers to a former wastewater discharge point; location B is at the border of the

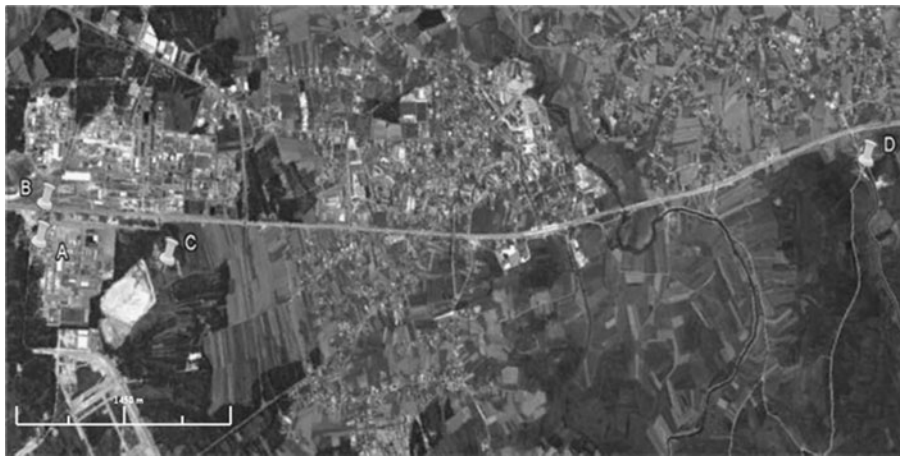

Fig. 1 General view of the area of Estarreja, with the collection sites *A*, *B*, *C* and *D* marked

industrial complex; location C is adjacent to a former 10-ha dry sedimentation pond deposit resulting from the production of polyvinylchloride over a 25-year period and is a periodically flooded soil with a small stream; location D is "Esteiro de Estarreja", a nearby stream which has received industrial wastewaters transforming it in a small and almost stagnated watercourse (Marques et al. 2007b). The area near the former exit of contaminated wastewaters is the most polluted one and is mainly occurring in the top 20-cm layer of the soil (Atkins 1999; Marques et al. 2007b). The banks of the stream (with a slope of ca. 45°), with ca. 2 m width, are periodically flooded with rainwater, from late October to late February, and the ditch of the stream (with ca. 1.5 m depth) remains almost dry during the remaining months.

The collection spots at each location were selected according to the existing plants. At each sampling spot, 0.5 m sided squares delimited the spot from which plant samples were collected. In each square, three plants were collected randomly—none of the collected plants presented visual toxicity signs. A soil sample from each plant rooting zone (0 to 20 cm depth) was also collected. Sampling was made in the cold (autumn/winter) and flowering (spring/summer) season (Garcia et al. 2002).

2.2 Soil Analysis

The following methods were based on Houba et al. (1995). Soil samples were oven dried at 40°C for 48 h and passed through a 2-mm sieve. The soil pH was measured using a 1:2.5 soil water ratio in a CRISON GLP® pH meter (Barcelona, Spain). Water content was determined by drying pre-oven dried (40°C) soil at 105°C until constant mass was achieved. Organic matter content was determined by loss on ignition. Samples for total phosphorous and nitrogen were digested at high temperatures (up to 330°C) with a selenium and salicylic and sulphuric acids mixture. For total nitrogen colorimetric determination, two reagents were added to the digests: reagent 1, consisting of a mixture of a 5×10^{-2}-M disodium hydrogen phosphate buffer (pH=12.3) and a 4% bleach solution and reagent 2 consisting of a mixture of a 1-M sodium salicylate solution, a 1×10^{-3}-M sodium nitroprusside solution and a 3×10^{-3}-M ethylenediaminetetraacetic acid (EDTA) solution. For total phosphorous colorimetric determination, two different reagents were added to the digests: reagent 1, consisting of a 3×10^{-2}-M ascorbic acid solution and reagent 2 consisting of a mixture of a 6×10^{-3}-M antimonyl tartrate solution, a 5×10^{-3}-M ammonium molybdate solution, 0.7 M sulphuric acid and an anticoagulation agent (Wetting aerosol 22, Cytek, NJ, USA). The elements concentration on the resulting preparations was determined on an UNICAM HELIOS® spectrophotometer (Waltham, USA), at 660 nm for nitrogen and 880 nm for phosphorous. For total Zn determination, soil samples were digested at high temperatures (up to 140°C) with concentrated nitric and hydrochloric acids (1:1). The water (De Koe 1994), exchangeable (Thomas 1982)—EDTA extractable—and available (Thomas 1982)—ammonium acetate (NH_4-Ac) extractable—Zn fractions were determined using, respectively, 1:5 soil water (De Koe 1994), 1:10 soil 0.05 M EDTA (Houba et al. 1995) and 1:5 soil 1 M NH_4-Ac (De Koe 1994) ratios. The resulting

solutions were incubated for 2 h at 20°C, after which they were filtered through a 0.45-µm cellulose acetate filter. The Zn content of the resulting digests and extracts was determined using flame atomic absorption spectroscopy (FA-AAS) in an UNICAM 960® spectrophotometer (Waltham, USA; Houba et al. 1995). BCR (Community Bureau of Reference) reference sample CRM 141 R (calcareous loam soil) was analysed through the above described total Zn determination analytical method. The value obtained by FA-AAS (282±2 mg Zn kg^{-1} sample) confirmed the accuracy and precision of the method by comparison with the certified value (283±5 mg Zn kg^{-1} sample).

2.3 Plant Analysis

Plant roots were washed free of soil with deionised water. For assessing AMF colonisation, an adequate amount of fresh fine roots was collected from the plants sampled at each selected spot. Roots and shoots were separated and washed again with tap water, followed by washing with HCl 0.1 M and with de-mineralised water, oven dried at 70°C for 48 h, grinded and sieved to <1 mm. For assessing AMF colonisation, freshly cut fine root samples were divided into approximately 1 cm pieces, heated in a pressure pan at 120 C in 10% KOH and stained using an adaptation of the Phillips and Hayman (1970) protocol including a longer incubation in 2% HCl (Oliveira et al. 2001). Stained root samples were examined microscopically to assess the percentage of mycorrhizal colonisation using the grid-line intersect method (Giovannetti and Mosse 1980). The resulting samples were digested at high temperature (up to 205°C) with a mixture of concentrated nitric, perchloric and sulphuric acids (40:4:1). Zinc content was determined using FA-AAS of the digests (Wallinga et al. 1989). BCR (Community Bureau of Reference) reference sample CRM 279 (sea lettuce) was analysed using the above described total zinc determination analytical method (three replicates were tested). The value obtained by FA-AAS (51.9± 0.9 mg kg^{-1}) confirmed the accuracy and precision of the method by comparison with the certified value (51.3±1.2 mg kg^{-1}).

2.4 Statistical Analysis

Statistical analysis was performed using the SPSS program (SPSS Inc., Chicago, IL, USA, Version 15.0). Three independent replicates were used for each treatment group. The variance homogeneity and the normality of the distribution were verified in each group. The data concerning Zn levels vs. plant tissue was analysed through one-way ANOVA, and the Tukey test was performed to detect the statistical significance of differences ($P<0.05$) between means in this analysis. Correlations were performed with different variables and Spearman's correlation coefficients were determined.

2.5 Chemicals

The chemicals used were analytical grade and were obtained from Pronalab (liquid reagents), Merck (solid reagents) and Sigma (Trypan blue stain).

3 Results

3.1 Plants

3.1.1 Zn Levels in the Root and Shoot

At the sampling sites, the vegetation cover was not uniform and at each location different species appeared as dominant. Additionally, in different seasons, the plant diversity and abundance were not similar, with a clear higher abundance in the summer/spring season—24 species were found in the summer vs. 12 species in the winter. Zinc levels in the tissues of the collected plant samples ranged from 34 mg kg^{-1} for *Convolvulus* sp. shoots to 2,440 mg kg^{-1} for *Spergularia capillacea* roots in the winter and from 35 mg kg^{-1} for *Cyperus eragrostis* shoots to 1,503 mg kg^{-1} for *Atriplex prostrata* shoots in the summer (Table 1). Clearly, *S. capillacea*, *Holcus lanatus* and *Solanum nigrum* appeared as the main Zn accumulators in the root, uptaking significantly ($P<0.05$) higher levels of the metal—respectively 2,440, 1,300 and 1,079 mg Zn kg^{-1}. In the shoot tissue, *A. prostrata*, *Verbascum virgatum*, *Hypochoeris radicata* and *Aster squamatus* presented the significantly ($P<0.05$) highest Zn accumulation, registering levels of 1,503, 707, 589 and 525 mg Zn kg^{-1}. There was not a clear trend in Zn accumulation in plants collected, with some species showing no significant ($P<0.05$) differences between Zn levels in their tissues in both seasons, whereas others showed different

Table 1 Zn levels in shoots and roots of the collected plant species (milligrams of Zn per kilogram)

Plants collected (family)	Family	Life cycle	Life form	Spring/summer		Autumn/winter	
				Shoots	Roots	Shoots	Roots
Site A							
Agrostis stolonifera L.	Poaceae	PE	HE	120±48 ^{nopq}	147±52 ^{fgh}	135±39 ^{mnopq}	81±33 ^{h}
Calluna vulgaris (L.) Hull	Erciaceae	PE	SH	126±23 ^{mnopq}	127±11 ^{fgh}	n.f.	n.f.
Conyza bilbaoana J. Rémy	Asteraceae	AN	HE	295±76 ^{fghijk}	56±18 ^{*h}	n.f.	n.f.
Conyza bonariensis (L.) Cronq.	Asteraceae	AN	HE	206±55 ^{mnopq}	178±60 ^{fgh}	n.f.	n.f.
Conyza sumatrensis (Retz.) E. H. Walker	Asteraceae	AN	HE	149±47 ^{ijklmn}	256±102 ^{fgh}	462±69 ^{de}	323±74 ^{ef}
Cyperus eragrostis Lam.	Cyperaceae	PE	HE	157±39 ^{lmnopq}	160±36 ^{fgh}	n.f.	n.f.
Digitalis purpurea L.	Scrophulariaceae	BI/PE	HE	182±34 ^{jklmnop}	183±45 ^{fgh}	202±42 ^{ijklmno}	200±10 ^{fgh}
Epilobium tetragonum L.	Onagraceae	PE	HE	255±106 ^{hijklmn}	195±35 ^{fgh}	n.f.	n.f.
Hirschfeldia incana (L.) Lagr.-Foss.	Brassicaceae	AN/PE	HE	243±72 ^{hijklmn}	230±76 ^{fgh}	414±24 ^{def}	211±74 ^{*fgh}
Holcus lanatus L.	Poaceae	PE	HE	383±117 ^{efg}	1,300±205 ^{*b}	n.f.	n.f.
Hypochaeris radicata L.	Asteraceae	AN/PE	HE	589±90 ^{c}	112±9 ^{*fgh}	n.f.	n.f.
Lycopus europaeus L.	Labiatae	PE	HE	299±105 ^{fghij}	199±79 ^{fgh}	n.f.	n.f.
Paspalum urvillei Steud.	Poaceae	PE	HE	128±12 ^{mnopq}	697±180 ^{*de}	n.f.	n.f.
Spergularia capillacea (Kindb.) Willk.	Caryophyllaceae	AN/PE	HE	n.f.	n.f.	354±29 ^{efgh}	2,440±557 ^{*a}
Verbascum virgatum Stokes	Scrophulariaceae	PE	HE	707±109 ^{b}	85±15 ^{*gh}	n.f.	n.f.
Site B							
Agrostis castellana Boiss. et Reut.	Poaceae	PE	HE	163±71 ^{lmnopq}	320±61 ^{*efg}	n.f.	n.f.
Atriplex prostrata Boucher ex DC.	Chenopodiaceae	AN	BU	1,503±219 ^{a}	889±46 ^{*cd}	n.f.	n.f.
Cyperus eragrostis Lam.	Cyperaceae	PE	HE	35±17 ^{q}	128±17 ^{*fgh}	n.f.	n.f.
Juncus effusus L.	Juncaceae	PE	HE	168±27 ^{klmnop}	740±202 ^{*d}	315±96 ^{fghi}	281±72 ^{fgh}
Phalaris arundinacea L.	Poaceae	PE	HE	227±62 ^{ijklmn}	86±33 ^{gh}	n.f.	n.f.
Site C							
Apium nodiflorum (L.) Lag.	Umbelliferae	BI/PE	HE	n.f.	n.f.	212±63 ^{ijklmn}	215±13 ^{fgh}
Aster squamatus (Spreng.) Hieron.	Asteraceae	PE	HE	525±35 ^{cd}	262±20 ^{*fgh}	n.f.	n.f.
Salix atrocinerea Brot.	Salicaceae	PE	SH/TR	n.f.	n.f.	198±67 ^{ijklmn}	163±5 ^{fgh}
Site D							
Convolvulus sp. L.	Convolvulaceae	PE	HE	n.f.	n.f.	34±3 ^{q}	324±10 ^{*fg}
Phragmites australis (Cav.) Trin. ex Steud.	Poaceae	PE	HE	54±2 ^{pq}	85±2 ^{*gh}	57±1 ^{pq}	63±1 ^{*h}
Pteridium aquilinum (L.) Kuhn	Dennstaedtiaceae	PE	HE	126±22 ^{mnopq}	206±29 ^{*fgh}	n.f.	n.f.
Rubus ulmifolius Schott	Rosaceae	PE	SH	37±2 ^{q}	537±43 ^{*c}	72±3 ^{opq}	514±9 ^{*ef}
Solanum nigrum L.	Solanaceae	AN	HE	280±16 ^{ghijkl}	1,079±27 ^{*c}	128±7 ^{mnopq}	1,079±27 ^{*c}

Results are shown as means±SD (n=3). When no plants were available of a particular species in the collection season, the indication n.f is presented. Results of the comparison between the roots and shoots accumulation of each species are shown. One-way ANOVA was performed for each plant section, and the test results are 48.18 (P<0.001) and 47.70 (P<0.001) for the roots and shoots, respectively. Means with different letters in each plant section are significantly different from each other (P<0.05) according to the Duncan test

PE perennial, *AN* annual, *BI* biennial, *HE* herbaceous, *SH* shrub, *TR* tree, *BU* bush, *n.f.* not found

*P<0.05 (significantly different from that of the shoots according to the t test)

patterns in root or shoot accumulation from one season to the other.

3.1.2 AMF Colonisation

Only the species *Conyza bilbaoana* (27±3), *Hirschfeldia incana* (34±5), *Epilobium tetragonum* (22±6), *Conyza sumatrensis* (16±11 in the summer and 22±3 in the winter), *Digitalis purpurea* (37±22 in the summer and 31±3 in the winter), *Pteridium aquilinum* (39±1), *Paspalum urvillei* (47±4) and *A. squamatus* (69±1) showed mycorrhizal colonisation (values are shown as mean percentages±standard deviation).

3.1.3 Translocation Factors

Translocation factors were determined as the ratio of Zn concentration in the shoot tissue over Zn concentration in the root tissues (Fig. 2). Several species presented TF values above 1. Nevertheless, four species appeared with significantly ($P<0.5$) higher factors, ranging from 3.25 to 8.33, in the following order: *V. virgatum*>*H. radicata*=*C. bilbaoana*.

3.2 Soil

Levels of Zn in the soil are presented in Table 2 and metal levels ranging from 38 to 7,023, 102 to 2,139, 27 to 3,796 and 298 to 992 mg Zn kg^{-1} were found for locations A, B, C and D, respectively; available (EDTA extractable) Zn in the soil ranged from 5 to 678, 12 to 129, 6.3 to 75.1 and 20 to 179 mg kg^{-1}, and exchangeable (NH_4-Ac extractable) Zn in the soil ranged from 0.2 to 64.1, 2 to 43.4, 2.2 to 3.5 and 1.9 to 69 mg kg^{-1}, for locations A, B, C and D, respectively. Spearman's correlation analysis of the levels of total Zn in the soils versus the available (NH_4-Ac extractable) and exchangeable (EDTA extractable) Zn fractions was performed, and strong positive ($P<0.01$) correlations were found—0.574 and 0.621, respectively, for the relation with EDTA and NH_4-Ac.

With the exception of location D, levels of N and P were generally low (Table 3) and ranged from 15 to 244 mg Nkg^{-1} and 14 to 710 mg Pkg^{-1} for location A, 27 to 142 mg Nkg^{-1} and 34 to 446 mg Pkg^{-1} for location B, 12 to 37 mg Nkg^{-1} and 29 to 42 mg P kg^{-1} for location C and 2,233 to 3,959 mg Nkg^{-1} and 9 to 259 mg Pkg^{-1} for location D; pH was generally acidic to neutral in all samples, with average values of 6.35±1.09, 6.03±0.71, 7.15±0.51 and 6.62±0.98 for locations A, B, C and D, respectively (Table 3). Humidity and organic matter contents were also low, ranging from minimum values of 0.05 and 0.9% (in location C) to 4 and 43% (in locations C and A), respectively (Table 3).

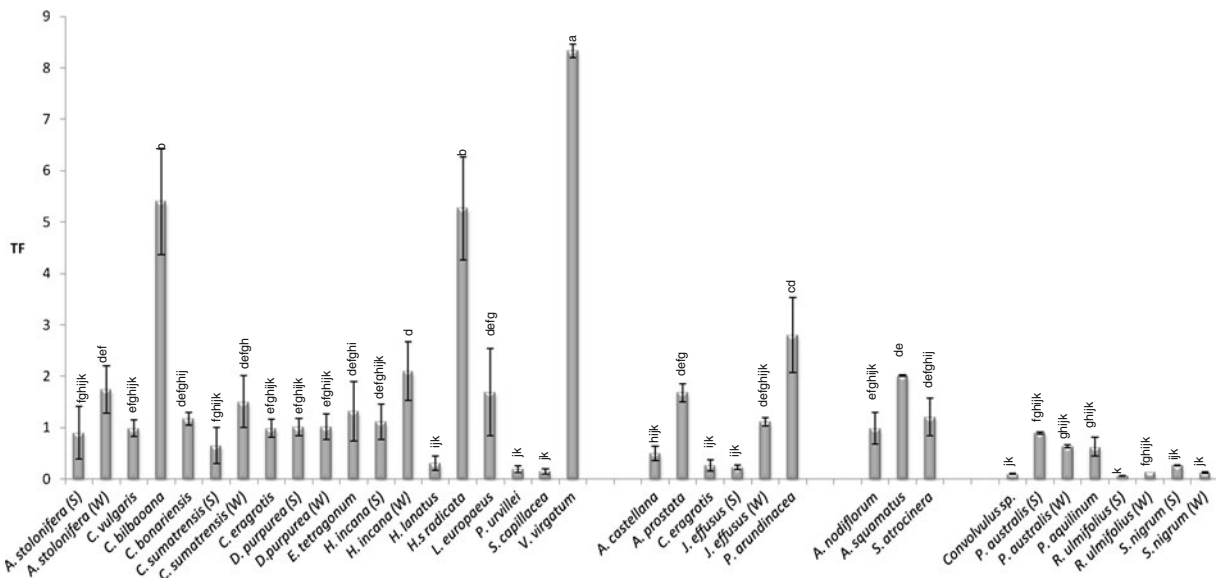

Fig. 2 Translocation factors (*TF*) of the collected plant species. Results are expressed as means±SD ($n=3$). One-way ANOVA was performed and the test results are 28.91 ($P<0.001$). Means with *different letters* are significantly different from each other ($P<0.05$) according to the Duncan test

Table 2 Total, available and extractable Zn concentrations in soils from different sampling locations

Soil adjacent to the plant	Zn (mg/kg dry soil) in adjacent soil		
	Total	EDTA	NH_4-Ac
Site A			
Agrostis stolonifera	2,296±596	90.6±38.3	2.3±1.5
Agrostis stolonifera	498±327	8.5±2.4	0.5±0.4
Calluna vulgaris	38±5	8.4±0.2	3.6±0.3
Conyza bilbaoana	1,158±169	490.4±67.8	2.7±1.2
Conyza bonariensis	1,053±441	45.3±20.1	1.3±0.4
Conyza sumatrensis (S)	1,577±287	56.0±22.8	4.1±1.9
Conyza sumatrensis (W)	541±540	41.9±30.2	2.0±0.3
Cyperus eragrostis	2,179±12	24.5±0.2	1.6±0.6
Digitalis purpurea (S)	3,577±21	678.1±4.1	64.1±0.2
Digitalis purpurea (W)	2,805±201	84.8±53.1	7.3±4.4
Epilobium tetragonum	5,896±11	298.4±9.5	6.5±0.2
Hirschfeldia incana (S)	1,295±154	59.4±32.3	1.9±1.7
Hirschfeldia incana (W)	848±259	314.0±69.2	9.3±8.6
Holcus lanatus	3,183±179	49.7±73.1	5.4±2.8
Hypochoeris radicata	340±187	4.8±1.9	0.2±0.2
Lycopus europaeus	737±39	86.6±44.1	5.1±2.9
Paspalum urvillei	38±5	8.4±0.2	3.6±0.3
Spergularia capillacea	7,023±249	311.2±109.9	32.1±18.3
Verbascum virgatum	135±10	16.3±0.2	1.7±0.8
Site B			
Agrostis castellana	801±270	58.5±0.2	43.4±0.2
Atriplex prostrata	2,139±352	129.4±18.9	8.6±6.5
Cyperus eragrostis	1,417±111	99.7±4.7	6.2±0.5
Juncus effusus (S)	146±113	19.4±2.4	6.0±10.6
Juncus effusus (W)	1,601±39	95.3±0.2	7.0±0.0
Phalaris arundinacea	102±60	11.7±7.6	2.0±1.3
Site C			
Apium nodiflorum	3,796±21	75.1±0.4	2.3±0.2
Aster squamatus	27±1	6.3±0.7	2.2±0.3
Salix atrocinerea	1,973±21	64.2±0.5	3.5±0.2
Site D			
Convolvulus sp.	299±43	21.0±7.0	1.91±0.1
Phragmites australis (S)	452±24	113.1±3.2	20.1±1.9
Phragmites australis (W)	328±26	91.3±1.5	14.9±0.4
Pteridium aquilinum	298±42	20.0±7.2	1.9±0.1
Rubus ulmifolius (S)	992±30	87.1±2.3	4.1±0,2
Rubus ulmifolius (W)	957±74	102.3±21.6	12.2±1.4
Solanum nigrum (S)	426±13	121.5±3.2	15.3±0.7
Solanum nigrum (W)	433±13	179.2±20.2	69.3±42.4

Results are expressed as mean±SD (n=3). When samples of the same species were collected in both seasons, the following indication is given: *W* results for soils adjacent to plants collected in the winter season, *S* results for soils adjacent to plants collected in the summer season

3.3 Soil–Plant Relations

Spearman's correlation analyses between the levels of Zn in plants roots and shoots versus the available metal concentrations in the soil were performed. Significant (P<0.05) correlations were only found between available (NH_4-Ac extractable) levels of Zn in the soil and the accumulation of the metal in the root tissues, with a positive correlation coefficient of 0.309 (P<0.01). As to the remaining values, the correlations were not significant.

Bioconcentration factors (BCF) in the root and shoot tissues, expressed as the ratio between the metal concentration in the plant section and in soil, were determined; values ranged from 0.033 (for *E. tetragonum* in location A) to 20.97 (for *P. urvillei* in location A) for the roots and 0.023 (for *Agrostis castellana* in location B9) to 19.68 (for *A. squamatus* in location B) for the shoots (Fig. 3). The significantly (P<0.05) highest BCF in the roots were registered for *P. urvillei*, but values for *A. squamatus* were also amongst those significantly (P<0.05) higher; additionally, values higher than 1 were observed for many other species (Fig. 3a). For the shoots, again *A. squamatus* and *P. urvillei* appeared amongst the species with significantly (P<0.05) higher values of BCF, but species such as *Calluna vulgaris* and *V. virgatum* also appeared in this group (Fig. 3b).

4 Discussion

In the present study, four different sites of a polluted area were analysed in order to understand the botanical diversity and Zn accumulation in the prevailing species. The analysed soils were generally characterized by a low nutrient level. Low levels of organic matter (OM) and nutrients are usual to metal disturbed sites, as indicated by the reports of other surveys for mining and industrial sites (Buondonno et al. 1998; Venditti et al. 2000). The lack of N and P may be due to poor humification of the OM probably due to disturbed soil microbial activity resulting from the presence of metal contaminants (Remon et al. 2005). This low activity is also indicated in the present study by the low coverage of mycorrhization in the selected plants, as only 26% (seven of the 27 species) of the surveyed species showed mycorrhizal colonisation—number at a significant distance from the 90% to 95% of all land plants that form some type of mycorrhizal

Table 3 Soil properties of samples collected in different sampling locations

Soil adjacent to the plant	N (mg/kg dry soil)	P (mg/kg dry soil)	pH	Water (%)	OM (%)
Site A					
Agrostis stolonifera (S)	21±36	116±60	7.31±0.32	0.40±0.02	39.1±2.7
Agrostis stolonifera (W)	24±20	21±6	5.03±1.05	0.71±0.08	43.2±1.1
Calluna vulgaris	43±12	65±3	3.70±0.40	0.40±0.20	4.6±2.6
Conyza bilbaoana	15±6	57±6	6.53±0.31	0.29±0.02	20.5±2.0
Conyza bonariensis	167±20	15±1	7.13±0.61	0.20±0.06	40.8±3.0
Conyza sumatrensis (S)	64±75	31±11	6.62±0.63	0.37±0.20	3.6±0.2
Conyza sumatrensis (W)	151±38	14±5	6.63±0.39	0.27±0.06	3.3±0.4
Cyperus eragrostis (S)	34±2	83±1	7.28±0.00	0.25±0.00	7.8±0.0
Digitalis purpurea (S)	20±0	159±0	7.25±0.00	0.90±0.10	7.9±0.7
Digitalis purpurea (W)	234±90	33±26	6.98±0.39	1.17±0.15	14.1±2.3
Epilobium tetragonum	40±1	127±1	7.44±0.00	1.07±0.02	8.5±0.4
Hirschfeldia incana (S)	184±88	26±12	6.74±0.55	0.29±0.13	38.6±6.3
Hirschfeldia incana (W)	111±45	89±125	6.87±0.16	0.38±0.03	41.9±1.7
Holcus lanatus	45±15	172±107	7.55±0.01	0.19±0.00	6.08±0.0
Hypochoeris radicata	61±47	61±87	5.21±0.52	0.34±0.16	2.22±0.0
Lycopus europaeus	63±17	96±69	6.09±0.18	0.79±0.01	9.1±0.8
Paspalum urvillei	27±11	68±12	3.70±0.40	0.40±0.20	4.6±2.6
Spergularia capillacea	113±24	710±126	5.55±0.43	1.15±0.00	20.0±1.8
Verbascum virgatum	244±16	25±2	6.68±0.00	0.24±0.00	42.3±0.9
Site B					
Agrostis castellana	142±9	446±1	5.97±0.00	3.59±0.58	1.9±0.6
Atriplex prostrata	87±87	402±426	6.32±0.67	1.22±1.02	26.0±2.2
Cyperus eragrostis	80±4	34±1	7.96±0.00	0.12±0.01	40.2±1.5
Juncus effusus (S)	29±16	43±13	5.25±0.92	0.37±0.10	27.6±2.4
Juncus effusus (W)	49±2	291±2	6.54±0.00	0.82±0.16	22.6±1.6
Phalaris arundinacea	27±11	56±14	4.70±0.18	0.50±0.04	26.0±2.2
Site C					
Apium nodiflorum	14±2	29±20	7.68±0.01	0.39±0.04	38.9±8.8
Aster squamatus	12±1	31±4	5.90±0.40	0.05±0.03	0.9±0.4
Salix atrocinerea	37±2	42±0	7.86±0.00	1.11±0.09	21.5±1.2
Site D					
Convolvulus sp.	2,233±45	198±27	7.60±0.70	0.17±0.07	15.4±1.8
Phragmites australis (S)	3,848±9	242±2	6.20±0.05	1.46±0.09	10.1±0.2
Phragmites australis (W)	3,626±4	9±3	5.61±0.07	1.12±0.06	8.5±0.1
Pteridium aquilinum	2,572±124	126±12	7.60±0.70	0.17±0.07	15.0±18.0
Rubus ulmifolius (S)	3,513±4	201±8	7.12±0.23	1.51±0.09	10.1±0.3
Rubus ulmifolius (W)	2,706±8	214±4	7.51±0.21	1.18±0.06	8.5±0.2
Solanum nigrum (S)	3,959±128	179±16	6.24±0.03	1.63±0.09	12.1±0.1
Solanum nigrum (W)	2,345±123	259±3	5.07±0.05	1.11±0.09	8.2±0.1

Results are expressed as mean±SD (n=3). When samples of the same species were collected in both seasons, the following indication is given: *W* results for soils adjacent to plants collected in the winter season, *S* results for soils adjacent to plants collected in the summer season

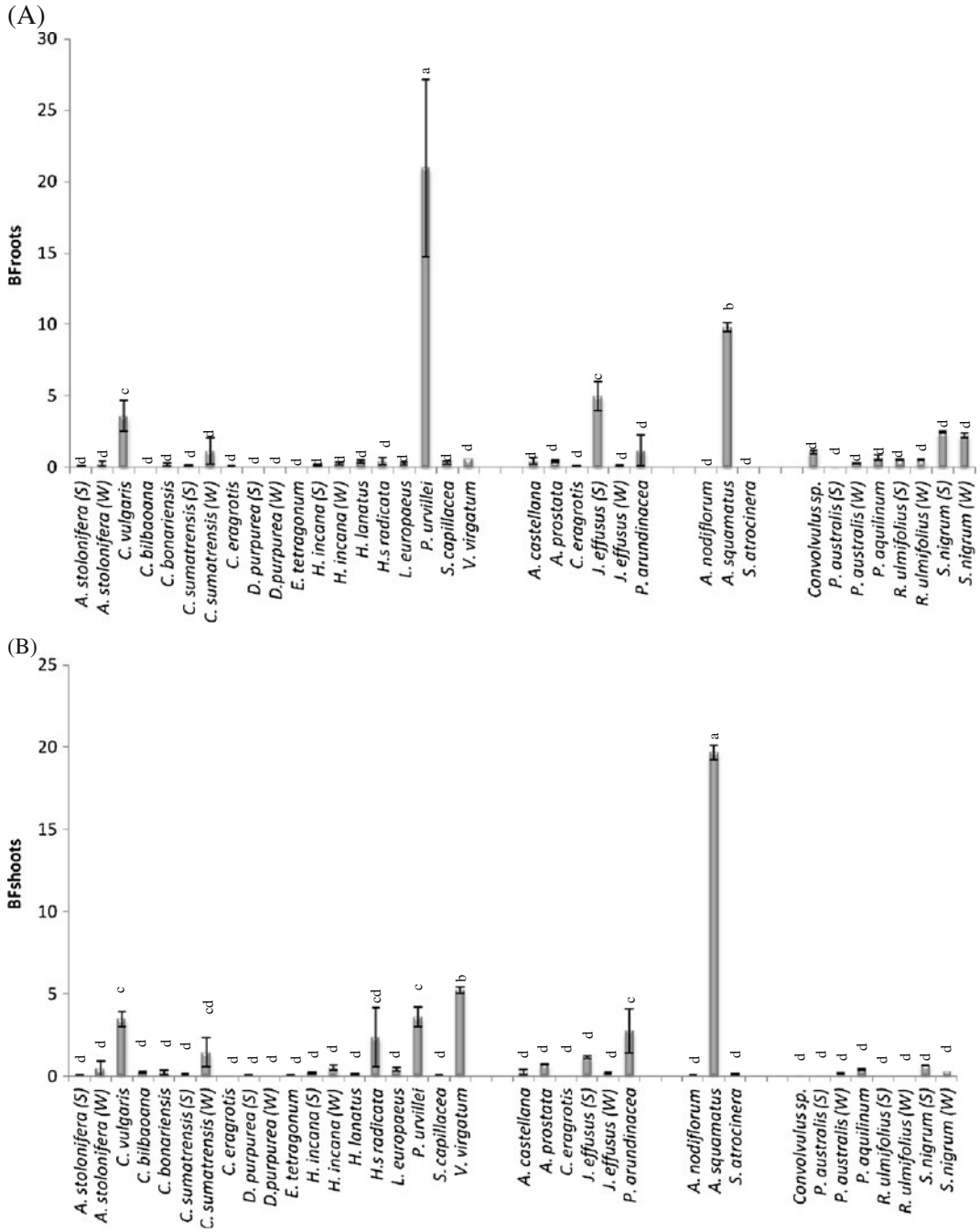

Fig. 3 Bioconcentration factors (*BF*) of roots (**a**) and shoots (**b**) of the collected plant species—adjacent soils. Results are expressed as means±SD (n=3). One-way ANOVA was performed for the TF of each plant section, and the test results are 4.52 (P<0.001) and 64.32 (P<0.001) for the roots and shoots respectively. Means with *different letters* in each section are significantly different from each other (P<0.05) according to the Duncan test

associations (Bago et al. 2000; Entry et al. 2002), with the arbuscular mycorrhizas associations between AMF and the roots of terrestrial plant species being the most widespread (Smith and Read 1997).

The choice of these four different locations in the studied area allowed for the coverage of a diverse range of soil metal concentration, from 27 to 5,896 mg Zn kg^{-1}. In general, Zn levels are much

higher than those proposed as normal in soils, as a total fraction of 70 to 400 mg kg^{-1} Zn in the soil would already be considered as toxic to plants (Kabata Pendias and Pendias 1992). However, metal uptake by plants depends mainly upon metal bioavailability in the soil and on the supply from less plant-available fractions, namely exchangeable metals. Availability to plants is led by dynamic equilibria involving these fractions, rather than the total metal content (Diez Lázaro et al. 2006). The metals considered readily available for plant uptake represent often only a portion of the total metal content of the soil (Blaylock and Huang 2000). In fact, the present study showed levels of EDTA extractable Zn ranging from 1.1% to 42% and levels of NH4-Ac extractable Zn ranging from 0.61% to 74% of the total metal content in soils. Although no relation between soil total Zn levels and plant accumulation was observed, the importance of bioavailability was again confirmed, as positive significant correlations were found between the available (NH4-Ac extractable) Zn levels in soils and the concentrations in the plants. According to the reports found in the literature, a relatively clear pattern of increasing plant accumulation with increasing soil concentration is usually found for Zn (Cardwell et al. 2002; Marques et al. 2007b). The existing plants may modify rhizosphere conditions through processes such as production of metal solubilising root exudates or alteration of pH (Adriano et al. 2004). In fact, soil pH is an important factor controlling metal availability and generally lower pH increases metal mobility and consequently their bioavailability (Kabata Pendias and Pendias 1992); thus, the slightly acidic pH observed in the generality of soil samples favours bioavailability.

The patterns of metal accumulation in plants were influenced by the species and plant section. In general, the collected plants presented Zn accumulation levels in its tissues above those considered as normal levels of Zn in plant tissues—10 to 100 mg kg^{-1}, according to Frisberg et al. (1986). Additionally, some plant species showed levels of Zn placed amongst the reported phytotoxic levels—500 to 1,500 mg kg^{-1}, according to Chaney (1989), with emphasis to the high Zn root levels found in *S. capillacea*, *H. lanatus* and *S. nigrum* and Zn shoot levels in *A. prostrata*, *H. radicata*, *A. squamatus* and *V. virgatum*. *A. squamatus* and *V. virgatum* also showed high bioconcentration factors in the shoot, and *V. virgatum* had the significantly highest translocation factor of the species in the study. These characteristics are important when assessing the phytoextraction abilities of a particular plant species.

Several plant species collected in the contaminated area were found to be present in other naturally or anthropologically contaminated soils: *H. radicata* and *V. virgatum* have been previously found in serpentine soils (Freitas et al. 2004) accumulating levels of Zn of up to 20 and 24 mg kg^{-1}, respectively, as well as in a copper smelter (Ginocchio 2000). *A. squamatus* was found growing in As-contaminated sites of southern Tuscany (Italy), with levels of up to 9.63 mg As kg^{-1} (Baroni et al. 2004). *H. lanatus* is also considered a serpentinophyte and is commonly found in metal rich soils in Portugal (Freitas et al. 2004). *A. prostrata* has been found growing in oil sands mining land (Trites and Bayley 2009). Several studies concerning metal accumulation, namely Zn, have been performed with *S. nigrum* (Marques et al. 2006, 2007a, 2008) pointing out this species as a good metal accumulator. No information was found concerning the occurrence of *S. capillacea* in contaminated areas or on the potential of the species in phytoremediation trials. However, none of the collected plants accumulated more than 10,000 mg kg^{-1} of Zn in the aboveground tissues (or even in the roots), the criteria indicated for Zn hyperaccumulators (Baker et al. 2000), although the levels found for the collected plants are above those found in the surveys of Diez Lázaro et al. (2006) or Remon et al. (2005). Only few of the species showed AMF colonisation, and there was no clear pattern between mycorrhization and pattern of metal accumulation. However, it was interesting to observe that mycorrhizal and non-mycorrhizal plants were co-existing at these different locations and the sole presence of heavy metal contamination does not explain this heterogeneity—some plant extracts secreted essentially by roots may contain biologically active substances such as hormones, antibiotics, etc., that inhibit microbial growth inside plant cells and thus restrain mycorrhizal proliferation (Thangaswamy et al. 2005).

Accumulation and exclusion are two basic strategies by which plants respond to elevated heavy metal soil levels (Vogel-Mikus et al. 2005), and accumulator and excluder plants can grow together in the same environment (Yanqun et al. 2005). Each plant species has a unique mechanism against heavy metals, and in

this work, a few species showed significantly higher accumulation in shoots (*C. bilbaoana*—295 mg kg^{-1}, *H. radicata*—589 mg kg^{-1}, *V. virgatum*—707 mg kg^{-1}, *A. squamatus*—525 mg kg^{-1} and *A. prostrata*—1,503 mg kg^{-1}), others in roots (*H. lanatus*—1,300 mg kg^{-1}, *P. urvillei*—697 mg kg^{-1}, *S. capillacea*—2,440 mg kg^{-1}, *Juncus effusus*—740 mg kg^{-1}, *Convolvulus* sp.—324 mg kg^{-1}, *Phragmites australis*—85 mg kg^{-1}, *Rubus ulmifolius*—537 mg kg^{-1} and *S. nigrum*—1,079 mg kg^{-1}) whereas some plants showed no differential accumulation from one section to the other. In metal accumulator species, a TF higher than one is common and desirable whereas in metal excluders the values are typically lower than 1 (Yanqun et al. 2005). Several species presented TF> 1; nevertheless, *V. virgatum*, *H. radicata* and *C. bilbaoana* (found in the site with average shoot dry weights of 0.8775, 0.5496 and, 1.2509 g, respectively, and root dry weights of 0.3691, 0.3848 and 1.2930 g, respectively) showed the significantly higher factors. Translocation factors higher than one indicate an efficient transport of Zn from roots to aboveground tissues, most likely due to efficient metal transport systems (Zhao et al. 2002). Additionally, an accumulator suitable for phytoextraction should accumulate the metal in its aboveground sections and show a higher BCF in the shoot tissues, and the three first above-mentioned species showed BCF values higher than 1. Other few species showed BCF>1, and the significantly highest BCF in the shoots were registered for *P. urvillei* (plants were found in the site with average dry weights of 0.1371 g for the roots and 0.5496 g for the shoots) and *A. squamatus* (plants were found in the site with average dry weights of 0.3721 and 0.8982 g in the roots and shoots, respectively (with the latter presenting also TF>1)), rendering these species with metal extracting potential. Plants which exhibited high BCF values in the roots and values lower than 1 in the aboveground tissues, as well as low TF, are unsuitable for phytoextraction, but their potential for phytostabilisation can be considered (Fitz and Wenzel 2002; Marques et al. 2007b). In this survey, species such as *S. capillacea*—with a root dry weight of 0.2311 g and shoot dry weight of 0.8494 g—excluded the metal from the aboveground sections, concentrating it at the root zone to very high levels, behaving thus as tolerant plants. Tolerant plants tend to restrict soil–root and root–shoot transfers, having much less accumulation in their aboveground biomass which, from a toxicological point of view, may be a desirable property, as Zn would not pass into the food chain via herbivores and metals accumulated in the roots are considered relatively stable concerning their release to other environmental compartments, such as water, thus avoiding further environmental risks and rendering them suitable for phytostabilisation purposes (Deng et al. 2004; Yoon et al. 2006).

5 Conclusions

The soils analysed in the present study have shown to be nutrient-deprived and with high levels of Zn in acidic conditions, and although strongly vegetated, plants showed low mycorrhizal colonisation. Metal uptake and accumulation varied amongst plant species and soil conditions. Amongst the 27 species found in the year round survey, *V. virgatum*, *H. radicata*, *C. bilbaoana*, *P. urvillei* and *A. squamatus* have shown higher Zn shoot accumulation and bioconcentration, as well as high metal translocation, seeming to fall in the category of accumulators suitable for phytoextraction. Others, namely *S. capillacea*, excluded the metal from the aboveground sections, concentrating it at the root zone, thus behaving as tolerant plants, suitable for phytostabilisation strategies. Surveys of this type can contribute to building powerful databases for plants applicable to metal phytoremediation strategies.

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