environments

MDPI

Article

Screening of Pioneer Metallophyte Plant Species with Phytoremediation Potential at a Severely Contaminated Hg and As Mining Site

Nora Matanzas [1], Elías Afif [2], Tomás Emilio Díaz [2] and José Luis R. Gallego [1,*]

[1] INDUROT & Environmental Biogeochemistry and Raw Materials Group, University of Oviedo, C/Gonzalo Gutiérrez Quirós s/n, 33600 Mieres, Asturias, Spain; noramatanzasvaltuille@gmail.com
[2] Department of Organisms and Systems Biology, University of Oviedo, Calle Cat. José María Serrano, 10, 33006 Oviedo, Asturias, Spain; elias@uniovi.es (E.A.); tediaz@uniovi.es (T.E.D.)
* Correspondence: jgallego@uniovi.es

Abstract: Phytoremediation of mine soils contaminated by potentially toxic elements (PTEs) requires the use of tolerant plants given the specific conditions of toxicity in the altered soil ecosystems. In this sense, a survey was conducted in an ancient Hg-mining area named "El Terronal" (Asturias, Spain) which is severely affected by PTE contamination (As, Hg, Pb) to obtain an inventory of the spontaneous natural vegetation. A detailed habitat classification was performed and a specific index of coverage was applied after a one-year quadrat study in various sampling stations; seven species were finally selected *(Agrostis tenuis*, *Betula celtiberica*, *Calluna vulgaris*, *Dactylis glomerata*, *Plantago lanceolata*, *Salix atrocinerea* and *Trifolium repens)*. A total of 21 samples (3 per plant) of the soil–plant system were collected and analyzed for the available and total concentrations of contaminants in soil and plants (roots and aerial parts). Most of the studied plant species were classified as non-accumulating plants, with particular exceptions as *Calluna vulgaris* for Pb and *Dactylis glomerata* for As. Overall, the results revealed interest for phytoremediation treatments, especially phytostabilization, as most of the plants studied were classified as excluder metallophytes.

Keywords: phytoremediation; soil contamination; potentially toxic elements; native plants; phytostabilization

check for updates

Citation: Matanzas, N.; Afif, E.; Díaz, T.E.; Gallego, J.L.R. Screening of Pioneer Metallophyte Plant Species with Phytoremediation Potential at a Severely Contaminated Hg and As Mining Site. *Environments* **2021**, *8*, 63. https://doi.org/10.3390/environments8070063

Academic Editors: Vernon Hodge and Gianniantonio Petruzzelli

Received: 13 May 2021
Accepted: 4 July 2021
Published: 5 July 2021

1. Introduction

Contamination derived of mining activities has been increasing dramatically since the beginning of the industrial revolution. Mining operations have produced many environmental problems, including soil contamination and ecosystem degradation [1,2], specially wherever potentially toxic elements (PTEs) were included within the ores exploited [3,4]. Furthermore, uncontrolled tailing disposal implies an important environmental impact especially on soils (losses of biological activity, structure, and fertility) and other environmental compartments affected by wind dispersion, water erosion, leaching, etc. [5,6].

Studies have shown that PTEs are persistent and widely dispersed in the environment; they interact with different natural components and pose threats to human health and the environment [7,8]. PTEs from air emissions can deposit directly on soils, and may be accumulated through rainwater transport [9,10]. Within usual PTEs found in mining areas As, Hg, and Pb are well-known toxics in low concentrations usually mobilized and adsorbed by animals and plants, and are also toxic by ingestion or inhalation for humans. Specifically, As toxicity has caused environmental problems in relation to groundwater and human illnesses [11–13]. Pb is potentially toxic to earthworms, but also for predators and detritovores in terrestrial food webs [14]; in humans an excessive intake of Pb may damage neurologic, vascular, endocrine, and immune systems [15]. Hg participates in a number of complex environmental cycles and, once in the environment, can be converted into organomercury compounds which are highly toxic to most organisms [16], causing neurological diseases, genotoxicity, a disruption to endocrine systems or sensory disturbance, among other effects [17].

Different approaches have been employed to remediate contaminated sites, and specifically former mining areas. In this sense, conventional engineering methods are usually expensive and non-sustainable [18], whereas phytoremediation is a possible effective and ecologically friendly alternative [19]. Phytoremediation can be defined as the use of plant species (shrubs, trees, aquatic plants, and grasses) and associated microorganisms, together with agronomic techniques, for the elimination, degradation, or separation of contaminated sites in an environment [19,20]. It also improves soil quality and structure, and can be applied in a variety of approaches such as phytoextraction, phytodegradation, rhizofiltration, phytostabilization, and phytovolatilization [21,22]. Phytoextraction requires plants able to take up, translocate, and accumulate high concentration of contaminants [23,24]. In this context, metallophytes can be classified into three major categories: excluders, accumulators and indicators [25]. In turn, phytostabilization is based on the ability of plants to fix the soil and immobilize metal(loid)s within the rhizosphere [26,27].

The use of vegetation covers in PTEs-contaminated mining areas is a viable alternative to reduce wind dispersion and water erosion, and also to immobilize or mobilize pollutants by plants. The efficacy of these processes depends on the tolerant plants. In this regard, the identification of natural vegetation growing in contaminated areas and the subsequent selection of specific metal-tolerant plants with potential value in phytoremediation are critical steps to select phytoextraction or phytostabilization techniques.

Following the preceding considerations, the main objectives of this study are:

1. To identify and describe species growing in a paradigmatic mining area affected by As, Hg, and Pb contamination.
2. To determine PTEs contents (in soils, roots, and aerial parts) and behavior of most representative plant species.
3. To assess the selection of the most suitable combination of plant species to design phytoremediation strategies.

2. Material and Methods

2.1. Site Description

The study site is an abandoned Hg mine and metallurgy area called “El Terronal”, located in a geographical radius of 10 km around of Mieres (20 km of Oviedo), in Asturias, NW Spain (Figure 1). With abundant Hg ore deposits, Asturias, from the 1950’s until the 1970’s, was an important mercury producer on a world scale, with an average annual production of 15,000 flasks (1 flask = 34.5 kg) [28].

The study area was exploited from the Roman occupation of the Iberian Peninsula in the first and second century A.D. [29]. In the late 1960’s–early 1970’s, production peaked, nevertheless, in 1974, the mine and the metallurgy ceased activity. The legacy remained for decades in the form of abandoned industrial installations and, heaped on a hillside, a large volume of waste contained heavy metals and arsenic disposed along the San Tirso River valley.

Mineralogical studies of El Terronal spoil heaps showed that iron sulphides are very abundant (pyrite, marcasite and pyrrhotite), and, in general, weathering has altered them to hematite and iron hydroxides [30]. Mercury generally appears in the form of cinnabar, irregularly distributed in a brecciated conglomerate and disseminated in the matrix, and arsenic appears as realgar and As-rich pyrite [31].

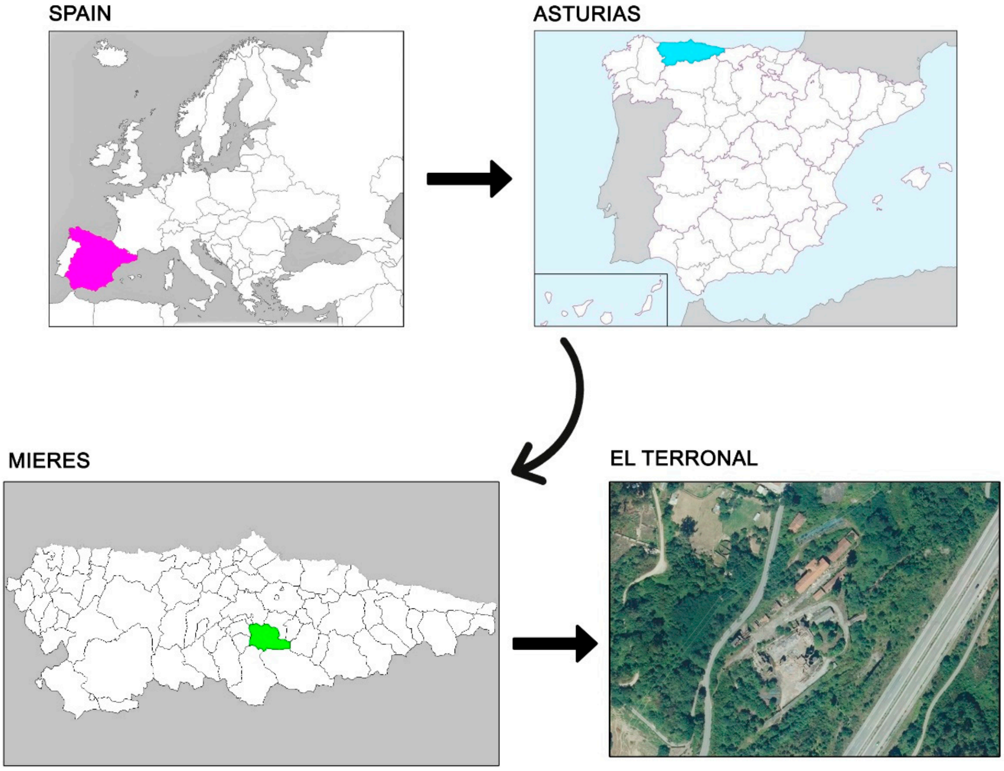

Figure 1. "El Terronal" study area location and an aerial view of the site.

2.2. Plant Classification

The diversity of species with a significant presence in the study site was identified and the predominant plant species were characterized. The identification took place initially "in situ" in zones of the site where the PTEs contents were high as determined in previous studies [4,32], and the conflicting specimens were herborized for identification, according to the methodology and traditional methods of plant taxonomy.

The nomenclature of the taxons mentioned in the text and the tables is in accordance with *Flora Ibérica* [33], or *European Flora* [34], otherwise, the criteria established in Fernández Prieto et al. (2014) [35] are followed. Subsequently, data were also classified by strata (Arboreal (>7), arborescent (3–7), shrubby (1–3), subshrub (i.e., short woody plant) (0.5–1), herbaceous (<0.5), and muscinal (mosses, lichens, and fungi).

For the nomenclature and the general characteristics of the plants used, the criteria of Castroviejo (1986–2012) [33] was followed, except in the case of the botanical families of the Grasses (Poaceae) and Betula genus, in which Hubbard (1985) and Ashburner & Mc Allister (2013) criteria were respectively followed [36,37].

2.3. Soil and Plant Sampling

As indicated above, previous studies were useful to identify zones of the site with high contamination levels [31,32], whereas another general study of plants potentially useful for phytoremediation [2] was also taken into account.

All things considered, samples of the soil–plant system in the area "El Terronal" were selected according to the density, frequency, and surface covered in the most contaminated areas of the site. The importance and predominance of each plant was categorized from level 1 to 4 (Table 1) by means of a coating index (CoI) [38–41]. This plant population frequency study was carried out monthly for one year by the quadrat method (1 m × 1 m) at 7 sampling areas selected after an initial screening of contaminated soils (i.e., areas selected presented the highest contamination levels). In these areas, 39 plant species were identified (see results); finally, 7 were selected and a total of 21 plant samples (7 different species with 3 replicas each) were taken.

Table 1. Coating Index classification.

Coating Index Categories	Description
1	Quite abundant individuals but of weak coverage. Covering from 1% to 10% (Medium coating = 5%)
2	Very abundant individuals that cover at least 1/20 of the surface. Covering from 10% to 25% (Medium coating = 17.5)
3	Individuals of variable number, but who cover from $\frac{1}{4}$ to $\frac{1}{2}$ of the surface. Covering from 25% to 50%. (Medium coating = 37.5%)
4	Individuals of variable number, but that cover of $\frac{1}{2}$ to $\frac{3}{4}$ of the surface. Coating from 50% to 75%. (Medium coating = 62.5%)

After plant sampling, individuals were sorted by hand to separate plant structures (aerial parts and root samples). These were then thoroughly washed with tap water several times followed by distilled water, and then cleaned using an ultrasonic bath to remove external contamination, and subsequently dried at room temperature for two weeks. Samples were ground in a universal rotor and variable speed Ultra Centrifugal Mill ZM 200 (Retsch, Haan, Germany) (from 6.000 rpm to 20.000 rpm). The milled samples were collected in stainless steel containers, homogenized, and screened to a size of less than 50 µM.

Representative soil samples (21) were taken in the tilled depth (0–25 cm) in the same sampling stations that the plants were sampled. Sampling was carried out using a soil auger and individual plastic bags were used for storage. All of the soil samples were dried to a constant weight at room temperature over a period of 20 days. Samples were then sieved through 2 mm, ground below 150 µM 400 rpm, (RS100 Retsch vibratory disc mill), homogenized, and quartered by means of an aluminum riffler (cleaned between samples using ethanol, distilled water, and compressed air) to provide a representative subsample of approximately 500 g.

2.4. Soil Analyses

A physicochemical characterization of the composite soil samples were carried out according to standard procedures (3 determinations per sample), pH was measured in a suspension of soil and distilled water (1:2.5) with a glass electrode [42], and electrical conductivity (EC) was determined in a 1:5 suspension of soil and wate, using a conductivity meter. Organic matter was measured by weight loss at 450 °C (loss-on-ignition method) [43]. Total N was determined by Kjeldahl digestion [44]. Mehlich 3 reagent [45] was used to colorimetrically determine available P. Exchangeable Al was extracted with 1 M KCl, and exchangeable cations (Ca, Mg, K and Na) with 1 M NH_4Cl; both were then analyzed by atomic absorption spectrophotometry [46] in a AA200 Perkin Elmer system (Massachusetts, USA). The effective cation exchange capacity (ECEC) was estimated as the sum of exchangeable Al and exchangeable cations. Particle-size distribution was determined by the pipette method, after particle dispersion with sodium hexametaphosphate and sodium carbonate [47].

Representative soil subsamples were leached by means of an 'Aqua regia' digestion ($HCl + HNO_3$, 1:1) (250 mg of sample for 8 mL of 1aqua regia) in an Anton Paar 3000 microwave (Graz, Austria) operated for 25 min at 1000 W. The samples were diluted and filtered. Major elements were quantified by an inductively coupled plasma mass spectrometer (ICP-MS 7700, Agilent Technologies, Santa Clara, CA, USA) using isotopic dilution analysis (IDA). Reference materials, ERM-CC141 and ERM-CC018, were used. Detection limits for As, Hg, and Pb were 0.1 $\mu g.L^{-1}$, with RSD > 5% (reproducibility), and the percent of recovery was above 95%.

PTEs soil phytoavailability was estimated by a sequential extraction procedure based on the first two fractions (exchangeable and carbonate-bound) of the Tessier method [48].

Both extracts were passed through 0.45-µM PTFE filters and diluted 1:10 prior to analysis by ICP-MS, as referred above.

2.5. Plant Analyses

In order to determine the concentration of PTEs in the different plant organs, 0.2 g of powdered samples was digested with 8 mL of 50% nitric acid using a microwave at 800 W (Multiwave 3000, Anton Paar, Graz, Austria) for 15 min. The solutions were diluted to 50 mL with ultrapure water and passed through 0.45-µM PTFE filters before analysis. The elements of interest were measured using the same ICP-MS device, as described above. Standard reference material apple leaves NIST® SRM® 1515 were used with a percent of recovery above 95%.

2.6. Data Analysis

The correlations between different variables were evaluated using Pearson's coefficient. All statistical analysis (multiples regression) was performed using IBM (Armonk, NY, USA) SPSS Statistics software 22.0.

Accumulation Factors

To evaluate the Pb, As, and Hg accumulation efficiency in the plants, various factors were examined [49–51].

The biological concentration factor (BCF) was calculated as the metal concentration ratio of plant roots to soil (BCF = C root/C soil); values of BCF > 1 indicate the accumulation of a particular trace metal in the roots.

The translocation factor (TF) indicates the ratio of trace metals in the aboveground plant parts (shoot, branches, or leaves) to those in the plant root (TF = C above ground part/C root); TF > 1 indicates that plant translocate metals effectively from root to shoot.

Finally, mobility radio (MR), also known as the biological-accumulation coefficient (BAC), was calculated as the ratio of heavy metal in the aboveground plant parts (shoots, branches, or leaves) to those in the soil (MR = C above ground part/C soil). A mobility ratio of >1 indicates that the plant is enriched with metals (i.e., the accumulator, which can tolerate high tissue concentrations of trace metals), a mobility radio of =1 indicates an indifferent behavior of the plant toward metals (i.e., the indicator, which is characterized by metal uptake proportional to concentrations of trace metals in soil), and a mobility radio of <1 indicates that the plant excludes metals from the uptake (i.e., the excluder, which has a low rate of uptake or actively excludes trace metals).

3. Results and Discussion

3.1. Description of the Identified Plant Species

According to Díaz and Fernández (2007) [52], phytogeographically, the site is framed as the Eurosiberian Region, the European Atlantic Province, the Cantabrian-Atlantic Subprovince, the Ovetense Litoral District, the Galaico-Asturian Sector, the Ovetense Subsector. Bioclimatically, according to the cartography of Rivas-Martínez et al. (2004) [53], the site is included within the sub-Mediterranean oceanic temperate macroclimate.

The study habitat revealed the presence of many tolerant species in their mature state which spontaneously grew on the contaminated soil. Herbaceous species were predominant (81%, from which 46% corresponds to perennial herbaceous), 5% were arboreal, 12% shrubby, and 2% lichenic and muscinal (for details, see Supplementary Material, Table S1). In this sense, it is well-known that the herbaceous can tolerate areas with notable contamination [54,55]. In addition, herbaceous have a lot of advantages, as they are easy to cultivate and propagate. In general, they are self-sustainable, and many of them are perennial species, which have advantages for extracting or immobilizing the contaminants.

Once identified, the species that develop naturally in the site, and in order to select those species of greatest interest, the quadrat methodology and the subsequent calculation of the CoI revealed 7 plant species with the highest CoI (level 4, as shown in Table 2):

Agrostis tenuis; *Betula celtiberica*; *Calluna vulgaris*; *Dactylis glomerata*; *Plantago lanceolata*; *Salix atrocinerea,* and *Trifolium repens*.

Table 2. Distribution of vegetation taking into account Coating index (abundance, coverage, density, and frequency). The botanical and ecological characteristics of the selected 7 species with CoI = 4 are described in the Supplementary Materials, Table S2.

Identified Species	Botanical Family	Coating Index (CoI)
Agrostis tenuis **L.**	Poaceae	**4**
Betula celtiberica **Rothm. & Vasc.**	Betulaceae	**4**
Calluna vulgaris **L. Hull**	Ericaceae	**4**
Dactylis glomerata **L.**	Poaceae	**4**
Plantago lanceolata **L.**	Plantaginaceae	**4**
Salix atrocinerea **Brot.**	Salicaceae	**4**
Trifolium repens **L.**	Fabaceae	**4**
Agrostis capillaris **L.**	Poaceae	3
Cornus sanguinea **L.**	Cornaceae	3
Lolium perenne **L.**	Poaceae	3
Lotus hispidus Desf. ex DC.	Fabaceae	3
Medicago lupulina **L.**	Fabaceae	3
Pastinaca sativa **L. subsp.** ***sylvestris*** **(Mill.) Rouy & Camus**	Apiaceae	3
Piptatherum miliaceum **L. Coss.**	Poaceae	3
Sonchus asper **L. Hill**	Asteraceae	3
Sonchus oleraceus **L.**	Asteraceae	3
Holcus lanatus **L.**	Poaceae	3
Hypericum pulchrum **L.**	Hypericaceae	3
Cirsium vulgare **L. Scop.**	Asteraceae	2
Conyza canadensis **L. Cronquist**	Asteraceae	2
Desmazeria rigida **L. Tutin (=** ***Catapodium rigidum*****)**	Poaceae	2
Lolium perenne **L.**	Poaceae	2
Lotus corniculatus **L.**	Fabaceae	2
Poa annua **L.**	Poaceae	2
Prunella vulgaris **L.**	Lamiaceae	2
Pteridium aquilinum **L. Kuhn**	Dennstaedtiaceae	2
Rubus **gr.** ***fruticosus*** **L.**	Rosaceae	2
Sagina apetala **Ard.**	Caryophyllaceae	2
Stellaria media **L.**	Caryophyllaceae	2
Trifolium dubium **Sibth.**	Fabaceae	2
Verbena officinalis **L.**	*Verbenaceae*	2
Arabis glabra **L. Bernh.**	Brassicaceae	1
Blechnum spicant **L. Roth**	Blechnaceae	1
Festuca nigrescens Lam.	Poaceae	1
Hedera Helix **L.**	Araliaceae	1
Melilotus albus **Medik.**	*Fabaceae*	1
Rubus ulmifolius **Schott**	Rosaceae	1
Verbascum virgatum **Stokes**	Scrophulariaceae	1
Vulpia bromoides **L. Gray**	Poaceae	1

3.2. Physicochemical Characterization of Soil Samples

The main edaphological soil parameters of "El Terronal" samples that were taken in areas of maximum CoI are shown in Table 3. Overall, results revealed a moderate homogeneity, pH values generally had slightly low alkaline levels, and soils did not show salinity, whereas organic matter levels were high (above 6%). On the basis of the particle size distribution data, samples were classified as sandy soils with normal contents of total N, a very high C/N relation, and P and Mg deficiencies.

Table 3. Physicochemical characterization of the 21 soil samples studied.

Soil Parameter	Units	Average	Std. Deviation
pH	1:2.5 H_2O	7.67	0.82
C.E [1]	dS m^{-1}	0.01	0.001
Sand	%	89.49	4.50
Silt	%	6.36	3.99
Clay	%	5.45	1.66
O.M [2]	%	6.25	0.78
C	%	9.43	1.03
N (total)	%	0.17	0.07
C/N [3]	-	58.30	14.11
Fe	mg kg^{-1}	8.33	3.01
PM3 [4]	mg kg^{-1}	1.70	0.61
Ex Ca	$cmol_{(+)}kg^{-1}$	17.07	0.65
Ex Mg	$cmol_{(+)}kg^{-1}$	1.55	0.07
Ex K	$cmol_{(+)}kg^{-1}$	1.83	0.16
Ex Na	$cmol_{(+)}kg^{-1}$	1.60	0.21
E.C.E.C [5]	$cmol_{(+)}kg^{-1}$	22.35	1.60

[1] C.E: electrical conductivity; [2] O.M: organic matter; [3] C/N: carbon and nitrogen ratio; [4] PM3: Phosporous (Melhrich method); [5] E.C.E.C: effective cation exchange capacity.

3.3. PTEs in Soil–Plant System

Total As, Hg, and Pb concentrations in soils and plants (aerial parts and roots) corresponding with the areas with maximum CoI (Figure 2) are shown in Table 4.

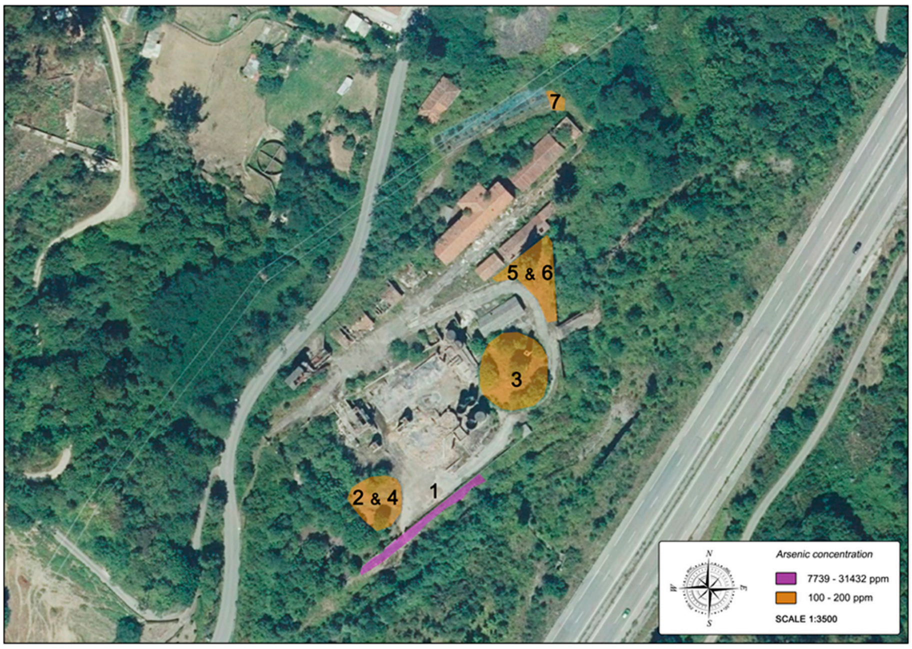

Figure 2. Distribution of sampling areas in which the highest values of CoI were found: 1 (*Calluna vulgaris*), 2 (*Betula celtiberica*), 3 (*Salix atrocinerea*), 4 (*Dactylis glomerata*), 5 (*Agrostis tenuis*), 6 (*Plantago lanceolata*) and 7 (*Trifolium repens*).

Table 4. Average content of metal(loid)s in soil and plant samples for the seven selected species analyzed (n = 3, uncertainties below 10% for all determinations). Data for elements with soil contents below 100 mg·kg^{-1} (= ppm) are not indicated and were not considered for the calculation of accumulation factors (see Table 5).

Specie	**Element**	**Concentration (mg·kg^{-1} = ppm)**		
		Soil	**Aerial Parts**	**Roots**
Agrostis tenuis	As	197	3	23
	Hg	131	5	18
Betula celtiberica	As	107	9	15
	Hg	238	2	8
Calluna vulgaris	As	24,600	571	1270
	Pb	105	11	10
Dactylis glomerata	As	180	28	11
	Hg	260	5	9
Plantago lanceolata	As	177	12	26
	Hg	132	9	16
Trifolium repens	As	142	6	15
	Hg	222	3	8
Salix atrocinerea	As	112	6	12
	Hg	229	2	5

Table 5. Accumulation factors for the plant species studied (BCF: Bioaccumulation Factor. TF: Translocation Factor. MR: Mobility Ratio).

Specie	**BCF**			**TF**			**MR**		
	As	**Hg**	**Pb**	**As**	**Hg**	**Pb**	**As**	**Hg**	**Pb**
A. tenuis	0.12	0.14	-	0.13	0.27		0.01	0.04	
B. celtiberica	0.14	0.03	-	0.59	0.27		0.08	0.01	
C. vulgaris	0.05	-	0.09	0.45	-	**1.13**	0.02	-	0.11
D. glomerata	0.06	0.03	-	**2.54**	0.54	-	0.15	0.02	-
P. lanceolata	0.15	0.12	-	0.45	0.55	-	0.07	0.06	-
T. repens	0.10	0.04	-	0.45	0.38	-	0.05	0.01	
S. atrocinerea	0.11	0.02		0.45	0.38	-	0.05	0.01	

With regards to soil, when compared with the soil screening levels established for PTEs in the Asturias region [56], the average values are notably above the levels in force, especially for As and Hg. Although PTEs contents are therefore very high, it should be noted that average phytoavailability levels found using sequential extraction were low (2.17% for As, 1.5% for Hg, and 0.2% for Pb).

The plant species studied notably exceed As concentrations usual for plants growing on uncontaminated soils which range between 0.009 and 1.5 mg/kg [57]. In fact, all of the species studied (except *A. tenuis*) revealed concentrations of As in the aerial parts surpassing 5–10 mg·kg^{-1}, i.e., levels that are usually considered to be toxic [58]. Similar effects are observed with Hg contents, as sub-lethal damage to vital functions occur at 1 mg·kg^{-1}; depending upon the species, values that are exceeded in all the species with the exception *C. vulgaris* that on the contrary revealed high levels of Pb in the leaves (the normal range of metal concentrations in plants for Pb are 0.1–10 mg kg^{-1}).

Accumulation factors presented in Table 5 indicate that most of the plant species studied showed low values (below 1) for the three parameters. This may suggest that uptake of PTEs was very low even considering the high PTE contents in soil, thus plant species under study were classified as non-accumulating plants, with two particular exceptions: *C. vulgaris* for Pb translocation factor and *D. glomerata* for As translocation factor. In accordance, only these two plant species could be of interest for phtoextraction treatments whereas the other five could be useful in phytostabilization approaches. The distribution of trace elements in selected species at the mature stages is different for all of them.

Previous studies support the different behaviors observed in our study. In fact, *C. vulgaris* has been identified as an As-tolerant species [59–61] and the same occurred with *D. glomerata* [62]. In turn, *Plantago lanceolata* is considered as a good As bioindicator, although, in contaminated soils, it recovered much less As than hyperaccumulator plants [63]. Other studies have reported low efficiency As extraction and translocation of *T. repens* [64] and *S. atrocinerea* [65]. Nevertheless, and disagreeing with our study, *Agrostis tenuis* was defined as hyperaccumulator of As [66,67] and *T. repens* showed strong Hg enrichment ability [68]. At any case, the efficiency and potential use of species in phytoremediation strategies is limited by factors such as the rate of growth, the development of roots, and the production of biomass [69].

The correlation between PTEs and accumulation factors, expressed as Pearson correlation coefficients ($p < 0.05$), did not reveal significant values. This can be explained as a result of the different mechanisms of assimilation and translocation factors for different elements in these plant species. However, significant correlations were found between contents of, for instance, As and Pb in soil and aerial parts ($r = 0.972$ and $r = 0.905$, respectively), and between soil contents and roots (0.940 for As and 0.896 for Hg). These facts show that plants contain information about the quality of the soil.

In a somehow different approach, we also found estimations of accumulation factors by obtaining a multiple linear regression equations as follows:

$$\text{BCF-As} = -0.055 + 0.721\ \text{BCF-Pb} + 1.216\ \text{MR-As}\ (R^2 = 0.958)$$

$$\text{BCF-Hg} = 0.109 - 0.001\ \text{Soil-Hg} + 0.005\ \text{Roots-Hg}\ (R^2 = 0.991)$$

$$\text{TF-As} = 0.352 + 1.286\ \text{TF-Pb} - 1.298\ \text{BCF-Pb}\ (R^2 = 0.901)$$

These equations could be useful to estimate the global behavior of this group of species in other areas of the study site.

4. Conclusions

The examination of plant species growing in a former abandoned Hg-mining area revealed the presence of a diverse flora tolerant to the main toxic PTEs in the site (mainly As, Hg, and secondarily Pb). Remarkably, herbaceous species were predominant in the most contaminated areas of the site, as demonstrated in a one-year study of plant coverage.

The predominant species identified (*Agrostis tenuis*; *Betula celtiberica; Calluna vulgaris*; *Dactylis glomerata*; *Plantago lanceolata*; *Salix atrocinerea*, and *Trifolium repens)* revealed a methallophyte behavior consistent with a potential forthcoming use for phytostabilization. Therefore, regarding site remediation, future studies should focus on the application of phytostabilization as a first option. However, some of the species identified (*Calluna vulgaris* and *Dactylis glomerata*) are also of specific interest because of their ability to translocate Pb and As, respectively, thus pointing out to their potential as bio-indicators or even in phytoextraction.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/environments8070063/s1. Table S1: Plants identified in the geobotanical study. SM2: *Agrostis tenuis*; *Betula celtiberica; Calluna vulgaris*; *Dactylis glomerata*; *Plantago lanceolata*; *Salix atrocinerea*, and *Trifolium repens* description.

Author Contributions: Conceptualization, J.L.R.G. and N.M.; Methodology, J.L.R.G., E.A. and N.M.; Validation, J.L.R.G. and T.E.D.; Formal Analysis and Investigation, T.E.D., J.L.R.G. and N.M.; Resources, J.L.R.G., E.A. and N.M.; Data Curation, N.M.; Writing—Original Draft Preparation, N.M.; Writing—Review & Editing, T.E.D. and J.L.R.G.; Visualization, N.M.; Supervision, T.E.D. and J.L.R.G.; Project Administration and funding acquisition: J.L.R.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the European Commission project LIFE I+DARTS (LIFE11ENV/ES/000547).

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Available upon request.

Acknowledgments: We would also like to thank the Environmental Assay Unit of the Scientific and Technical Services of the University of Oviedo for technical support.

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

CoI	Coating Index
PTEs	Potentially Toxic Elements
BCF	Bioaccumulation Factor
TF	Translocation Factor
MR	Mobility Ratio

References

1. Héctor, C.M.; Rainer, S. The Cartagena—La Union mining district (SE Spain): A review of environmental problems and emerging phytoremediation solutions after fifteen years research. *J. Environ. Monit.* **2010**, *12*, 1225–1233. [CrossRef]
2. Fernández, S.; Poschenrieder, C.; Marcenò, C.; Gallego, J.R.; Jiménez-Gámez, D.; Bueno, A.; Afif, E. Phytoremediation capability of native plant species living on Pb-Zn and Hg-As mining wastes in the cantabrian range, north of spain. *J. Geochem. Explor.* **2017**, *174*, 10–20. [CrossRef]
3. Millán, R.; Gamarra, R.; Schmid, T.; Sierra, M.J.; Quejido, A.J.; Sánchez, D.M.; Cardona, A.I.; Fernández, M.; Vera, R. Mercury content in vegetation and soils of the Almadén mining area (Spain). *Sci. Total Environ.* **2006**, *368*, 79–87. [CrossRef] [PubMed]
4. Gallego, J.R.; Esquinas, N.; Rodríguez-Valdés, E.; Menéndez-Aguado, J.M.; Sierra, C. Comprehensive waste characterization and organic contamination co-occurrence in a Hg and As mining and metallurgy brownfield. *J. Hazard. Mater.* **2015**, *300*, 561–571. [CrossRef]
5. Mouron, S.A.; Golijow, C.D.; Dulout, F.N. DNA damage by cadmium and arsenic salts assessed by the single cell gel electrophoresis assay. *Mutat. Res.* **2001**, *498*, 47–55. [CrossRef]
6. Gall, J.E.; Boyd, R.S.; Rajakaruna, N. Transfer of heavy metals through terrestrial food webs: A review. *Environ. Monit. Assess.* **2015**, *187*, 201. [CrossRef]
7. Park, K.; Dam, H. Characterization of metal aerosols in PM10 from urban, industrial, and Asian dust sources. *Environ. Monit. Assess.* **2010**, *160*, 289–300. [CrossRef]
8. Pavlik, M.; Pavlikova, D.; Zemanova, V.; Hnilicka, F.; Urbanova, V.; Szakova, J. Trace elements present in airborne particulate matter—Stressors of plant metabolism. *Ecotoxicol. Environ. Saf.* **2012**, *79*, 101–107. [CrossRef] [PubMed]
9. Gunawardena, J.; Egodawatta, P.; Ayoko, G.A.; Goonetilleke, A. Atmospheric deposition as a source of heavy metals in urban storm water. *Atmos. Environ.* **2013**, *68*, 235–242. [CrossRef]
10. Huang, T.C.C.; Lo, K.F.A. Effects of Land Use Change on Sediment and Water Yields in Yang Ming Shan National Park, Taiwan. *Environments* **2015**, *2*, 32–42. [CrossRef]
11. Rasheed, H.; Kay, P.; Slack, R.; Gong, Y.Y. Arsenic species in wheat, raw and cooked rice: Exposure and associated health implications. *Sci. Total Environ.* **2018**, *634*, 366–373. [CrossRef]
12. Gress, J.; de Oliveira, L.M.; da Silva, E.B.; Lessl, J.M.; Wilson, P.C.; Townsend, T.; Ma, L.Q. Cleaning-induced arsenic mobilization and chromium oxidation from CCA-wood deck: Potential risk to children. *Environ. Int.* **2015**, *82*, 35–40. [CrossRef]
13. World Health Organization. *Preventing Disease through Healthy Environments: Exposure to Arsenic: A Major Public Health Concern*; World Health Organization: Geneva, Switzerland, 2010.
14. Richardson, J.B.; Görres, J.H.; Sizmur, T. Synthesis of earthworm trace metal uptake and bioaccumulation data: Role of soil concentration, earthworm ecophysiology and experimental design. *Environ. Pollut.* **2020**, *262*, 114–126. [CrossRef]
15. Zhang, X.; Yang, L.; Li, Y.; Li, H.; Wang, W.; Ye, B. Impacts of lead/zinc mining and smelting on the environment and human health in China. *Environ. Monit. Assess.* **2012**, *184*, 2261–2273. [CrossRef] [PubMed]

16. Davis, A.; Bloom, N.S.; Que Hee, S.S. The environmental geochemistry and bioaccessibility of mercury in soils and sediments: A review. *Risk Anal.* **1997**, *17*, 557–569. [CrossRef]
17. Yang, L.; Zhang, Y.; Wang, F.; Luo, Z.; Guo, S.; Strähle, U. Toxicity of mercury: Molecular evidence. *Chemosphere* **2020**, *245*, 125586. [CrossRef] [PubMed]
18. Salt, D.E.; Blaylock, M.; Kumar, P.B.; Dushenkov, V.; Enslev, B.D.; Chet, I.; Raskin, I. Phytoremediation: A novel strategy for the removal of toxic metals from the environment using plants. *Biotechnology* **1995**, *13*, 468–475. [CrossRef] [PubMed]
19. Asante-Badu, B.; Kgorutla, L.E.; Li, S.S.; Danso, P.O.; Xue, Z.; Qiang, G. Phytoremediation of organic and inorganic compounds in a natural and an agricultural environment: A review. *Appl. Ecol. Environ. Res.* **2020**, *18*, 6875–6904. [CrossRef]
20. Del Río, M.; Font, R.; Almela, C.; Vélez, D.; Montoro, R.; De Haro Bailón, A. Heavy metals and arsenic uptake by wild vegetation in the guadiamar river area after the toxic spill of the aznalcóllar mine. *J. Biotechnol.* **2002**, *98*, 125–137. [CrossRef]
21. Franchi, E.; Cosmina, P.; Pedron, F.; Rosellini, I.; Barbafieri, M.; Petruzzelli, G.; Vocciante, M. Improved arsenic phytoextraction by combined use of mobilizing chemicals and autochthonous soil bacteria. *Sci. Total. Environ.* **2019**, *655*, 328–336. [CrossRef] [PubMed]
22. Park, S.; Kim, K.S.; Kim, J.T.; Kang, D.; Sung, K. Effects of humic acid on phytodegradation of petroleum hydrocarbons in soil simultaneously contaminated with heavy metals. *J. Environ. Sci.* **2011**, *23*, 2034–2041. [CrossRef]
23. Van Der Ent, A.; Baker, A.J.; Reeves, R.D.; Pollard, A.J.; Schat, H. Hyperaccumulators of metal and metalloid trace elements: Facts and fiction. *Plant Soil* **2013**, *362*, 319–334. [CrossRef]
24. Reeves, R.D.; Baker, A.J.; Jaffré, T.; Erskine, P.D.; Echevarria, G.; Van Der Ent, A. A Global database for plants that hyperaccumulate metal and metalloid trace elements. *New Phytol.* **2018**, *218*, 407–411. [CrossRef] [PubMed]
25. Baker, A.J.M.; McGrath, S.P.; Reeves, R.D.; Smith, J.A.C. Metal hyperaccumulator plants: A review of the ecology and physiology of a biological resource for phytoremediation of metal contaminated soils. In *Phytoremediation of Contaminated Soils and Waters*, 1st ed.; Terry, N., Bañuelos, G., Eds.; CRC Press: Boca Raton, FL, USA, 2000; pp. 85–107.
26. Eskander, S.; Saleh, H. Phytoremediation: An overview. In *Environmental Science and Engineering, Soil Contamination and Phytoremediation*, 1st ed.; Abrol, Y.P., Gurjar, B.R., Eds.; Studium Press: Devon, UK, 2017; Volume 11, pp. 124–161.
27. Lebrun, M.; Miard, F.; Hattab-Hambli, N.; Bourgerie, S.; Morabito, D. Assisted phytoremediation of a multi-contaminated industrial soil using biochar and garden soil amendments associated with Salix alba or Salix viminalis: Abilities to stabilize As, Pb, and Cu. *Water Air Soil Pollut.* **2018**, *229*, 163. [CrossRef]
28. Gutiérrez-Claverol, M.; Luque, C. *Recursos del Subsuelo de Asturias*, 1st ed.; Servicio de Publicaciones: Oviedo, Spain, 1993; 374p.
29. Dory, A. Le mercure dans les Asturies. *Rev. Univ. Mines Metall.* **1984**, *32*, 145–210.
30. Loredo, J.; Luque, C.; García Iglesias, J. Conditions of formation of mercury deposits from the Cantabrian Zone (Spain). *Bull. Mineral.* **1988**, *111*, 393–400. [CrossRef]
31. Loredo, J.; Ordóñez, A.; Gallego, J.R.; Baldo, C.; García-Iglesias, C. Geochemical characterization of mercury mining spoil heaps in the area of Mieres (Asturias, northern Spain). *J. Geochem. Explor.* **1999**, *67*, 377–390. [CrossRef]
32. González-Fernández, B.; Rodríguez-Valdés, E.; Boente, C.; Menéndez-Casares, E.; Fernández-Braña, A.; Gallego, J.R. Long-term ongoing impact of arsenic contamination on the environmental compartments of a former mining-metallurgy area. *Sci. Total Environ.* **2018**, *610–611*, 820–830. [CrossRef] [PubMed]
33. Castroviejo, S. *Flora Ibérica*, 1st ed.; Real Jardín Botánico, CSIC: Madrid, Spain, 1986; pp. 1–8.
34. Tutin, T.G.; Heywood, V.H.; Burges, D.M.; Moore, D.H.; Valentine, S.M. *Flora Europaea*; Cambridge University Press: Cambridge, UK, 1964; pp. 1–5.
35. Fernández Prieto, J.A.; Cires Rodríguez, E.; Bueno Sánchez, A.; Vázquez, V.M.; Nava Fernández, H.S. *Catálogo de las Plantas Vasculares de Asturias*; Jardín Botánico Atlántico: Gijón, Spain, 2014; Volume 11, pp. 7–267.
36. Hubbard, C.E. *A Guide to Their Structure, Identification, Uses and Distribution in the Bristish Isles*; New Edition Penguin Books: London, UK, 1985; 476p.
37. Ashburner, K.; Mc Allister, H.A. *The Genus Betula. A Taxonomic Revision of Birches*; Royal Botanical Gardens: Kew, UK, 2013; 431p.
38. Curtis, J. *The Vegetation of Wisconsin. An Ordination of Plant Communities*; University of Wisconsin Press: Madison, WI, USA, 1959; 657p.
39. Finol, H. Nuevos parámetros a considerarse en el análisis estructural de las selvas vírgenes tropicales. *Rev. For. Ven.* **1971**, *13*, 29–42.
40. Mueller-Dumbois, D.; Ellenberg, H. *Aims and Methods of Vegetation Ecology*; John Wiley and Sons: New York, NY, USA, 1974; 547p.
41. Matteucci, S.D.; Colma, A. *Metodología Para el Estudio de la Vegetación. Secretaría General de la Organización de Estados Americanos*; Programa Regional de Desarrollo Científico y Tecnológico: Washington, DC, USA, 1982; 72p.
42. Thomas, G.W. Soil pH and soil acidity. In *Methods of Soil Analysis: Part 3 Chemical Methods*; Soil Science Society of America: Madison, WI, USA, 1996; pp. 475–490.
43. Schulte, E.E.; Hopkins, B.G. Estimation of Soil Organic Matter by Weight Loss-On-Ignition. In *Soil Organic Matter: Analysis and Interpretation*; Magdoff, F.R., Tabatabai, M.A., Hanlon, E.A., Jr., Eds.; SSA Special Publications: Madison, WI, USA, 1996; Volume 46, pp. 21–31.
44. Klute, A. Nitrogen-Total. In *Methods of Soil Analyses*; Klute, A., Ed.; American Society of Agronomy: Madison, WI, USA, 1996; pp. 595–624.

45. Mehlich, A. Mehlich 3 soil test extractant: A modification of Mehlich 2 extractant. *Soil Sci. Plant Anal.* **1984**, *15*, 1409–1416. [CrossRef]
46. Pansu, M.; Gautheyrou, J. *Handbook of Soil Analysis: Mineralogical, Organic and Inorganic Methods*; Springer: Berlin/Heidelberg, Germany, 2006; 993p.
47. Gee, G.W.; Bauder, J.W. Particle size analysis. In *Methods of Soil Analysis*; Klute, A., Ed.; American Society of Agronomy: Madison, WI, USA, 1996; pp. 383–411.
48. Tessier, A.; Campbell, P.G.; Bisson, M. Sequential extraction procedure for speciation of particulate trace metals. *Anal. Chem.* **1979**, *51*, 844–850. [CrossRef]
49. Malik, R.N.; Husain, S.Z.; Nazir, I. Heavy metal contamination and accumulation in soil and wild plant species from industrial area of Islamabad, Pakistan. *Pak. J. Bot.* **2010**, *42*, 291–301.
50. Zhang, X.; Zhang, S.; Xu, X.; Li, T.; Gong, G.; Jia, Y. Tolerance and accumulation characteristics of cadmium in *Amaranthus hybridus* L. *J. Hazard. Mater.* **2010**, *180*, 303–308. [CrossRef] [PubMed]
51. Bech, J.; Duran, P.; Roca, N.; Poma, W.; Sánchez, I.; Roca-Pérez, L.; Boluda, R.; Barceló, J.; Poschenrieder, C. Accumulation of Pb and Zn in *Bidens triplinervia* and *Senecio* sp. Spontaneous species from mine spoils in Peru and their potential use in phytoremediation. *J. Geochem. Explor.* **2012**, *123*, 109–113. [CrossRef]
52. Díaz, T.E.; Fernández, J. Biogeografía de Asturias: Bases Para su Actualización. In Proceedings of the I Congreso de Estudios Asturianos, Oviedo, Spain, 10–13 May 2006.
53. Rivas-Martínez, S.; Penas, A.; Díaz, T.E. *Bioclimatic and Biogeographic Maps of Europe*; Universidad de León: Leon, Spain, 2004.
54. Cunningham, S.D.; Ow, D.W. Promises and prospects of phytoremediation. *Plant Physiol.* **1996**, *110*, 715–719. [CrossRef]
55. Ligenfelter, D.D.; Hartwig, N.L. *Introduction to Weeds and Herbicides*; Pennsylvania State University, Publication Distribution Center: State College, PA, USA, 2007.
56. BOPA Generic Reference Levels for Heavy Metals in Soils from Principality of Asturias, Spain. Available online: http://sede.612asturias.es/bopa/2014/04/21/2014e06617.pdf (accessed on 25 March 2021).
57. Kabata-Pendias, A.; Pendias, H. *Trace Elements in Soils and Plants*, 3rd ed.; CRC Press: Boca Raton, FL, USA, 2001; 413p.
58. Gisbert, C.; Almela, C.; Vélez, D.; López-Moya, J.R.; de Haro, A.; Serrano, R.; Montoro, R.; Navarro-Aviñó, J. Identification of As accumulation plant species growing on highly contaminated soils. *Int. J. Phytoremediat.* **2008**, *10*, 185–196. [CrossRef]
59. Sharples, J.M.; Meharg, A.A.; Chambers, S.M.; Cairney, J.W.G. Symbiotic solution to arsenic contamination. *Nature* **2000**, *404*, 951–952. [CrossRef]
60. Pichtel, J.; Salt, C.A. Vegetative growth and trace metal accumulation on metalliferous wastes. *J. Environ. Qual.* **1998**, *27*, 618–624. [CrossRef]
61. Pichtel, J.; Kuroiwa, K.; Sawyerr, H.T. Distribution of Pb, Cd and Ba in soils and plants of two contaminated sites. *Environ. Pollut.* **2000**, *110*, 171–178. [CrossRef]
62. Rasmussen, G.; Olsen, R.A. Sorption and biological removal of creosote-contaminants from groundwater in soil/sand vegetated with orchard grass (Dactylis glomerata). *Adv. Environ. Res.* **2004**, *8*, 313–327. [CrossRef]
63. Salas-Luévano, M.A.; Mauricio-Castillo, J.A.; González-Rivera, M.L.; Vega-Carrillo, H.R.; Salas-Muñoz, S. Accumulation and phytostabilization of As, Pb and Cd in plants growing inside mine tailings reforested in Zacatecas, Mexico. *Environ. Earth. Sci.* **2021**, *76*, 806. [CrossRef]
64. Bech, J.; Roca, N.; Tume, P.; Ramos-Miras, J.; Gil, C.; Boluda, R. Screening for new accumulator plants in potential hazards elements contaminated soil surrounding Peruvian mine tailings. *Catena* **2016**, *136*, 66–73. [CrossRef]
65. Otones, V.; Álvarez-Ayuso, E.; García-Sánchez, A.; Santa Regina, I.; Murciego, A. Arsenic distribution in soils and plants of an arsenic impacted former mining area. *Environ. Pollut.* **2011**, *159*, 2637–2647. [CrossRef]
66. Wang, S.; Mulligan, C.N. Natural attenuation for remediation of arsenic contaminated soils and groundwater. *J. Hazard. Mater.* **2006**, *138*, 459–470. [CrossRef]
67. Ma, L.Q.; Komat, K.M.; Tu, C.; Zhang, W.; Cai, Y. A fern that hyperaccumulates arsenic. *Nature* **2001**, *409*, 579. [CrossRef]
68. Liu, Z.; Wan, L. A plant species (Trifolium repens) with strong enrichment ability for mercury. *Ecol. Eng.* **2014**, *70*, 349–350. [CrossRef]
69. Brooks, R.R. Plants that hyperaccumulate heavy metals. In *Plants and The Chemical Elements: Biochemistry Uptake, Tolerance and Toxicity*, 1st ed.; Farago, M.E., Ed.; Wiley-VCH: Weinheim, Germany, 1994; pp. 88–105.