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Phytoremediation Potential of Native Herbaceous Plant Species Growing on a Paradigmatic Brownfield Site

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Abstract Phytomanagement techniques using native species allow the recovery of contaminated soils at low cost and circumvent the ecological risks associated with the use of non-native species. In this context, a paradigmatic brownfield megasite highly contaminated by As and Pb was sampled in order to analyze soil–plant interactions and identify plant species with phytoremediation potential. A survey was first carried out in a 20-ha area to obtain an inventory of species growing spontaneously throughout the site. We then performed another survey in the most polluted subarea (1 ha) within the site. Pseudototal concentrations of contaminants in the soil, aerial parts of the plants, and roots were measured by ICP-MS. A detailed habitat classification was done, and a specific index of coverage was applied by means of a 1-year quadrat study in various sampling stations. Results converged in the selection of six herbaceous species (*Dysphania botrys*, *Lotus corniculatus*, *Lotus hispidus*, *Plantago lanceolata*, *Trifolium repens*, *Medicago lupulina*). All of these plants are fast-growing, thereby making them suitable for use in phytostabilization strategies. Furthermore, they are all easy to grow and propagate and are generally self-sustaining. All six plants showed accumulation factors below 1, thus revealing them as pseudomethallophytes and excluders. However, *L. hispidus* and *M. lupulina* showed translocation capacity and are considered worthy of further study.

Keywords Phytoremediation · Herbaceous plants · Soil pollution · Brownfield · Phytoavailability

Abbreviations

PTEs	Potentially Toxic Elements

1 Introduction

Recent decades have witnessed the closure of many industrial and mining activities. These brownfields pollute soil and (ground)water, causing environmental and health threats, as well as economic and social costs (Schädler et al., 2011; Zanchi et al., 2021). In particular, soil contamination by potentially toxic elements (PTEs) is common in these types of abandoned areas (Gallego et al., 2015). Soil is the basis of agriculture and livestock, and the abandonment of these industrial activities can lead to bioaccumulation and biomagnification over time (Antonkiewicz et al.,

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2018; Fränzle et al., 2007; Iqbal, 2016; Khan et al., 2014; Mani et al., 2016; Padúa et al., 2021).

Traditional physicochemical methods for soil remediation (confinement, thermal desorption, soil washing, etc.) are often expensive and result in irreversible damage to the structure, biology, and fertility of the soil (Alkorta et al., 2010). Phytomanagement approaches emerge as potential alternatives as plants and trees play key roles in the biogeochemical cycling of nutrients and pollutants and can therefore be considered ecosystem engineers (Jones et al., 1994; Robinson et al., 2009). In this context, phytoremediation is a low-cost alternative to the physico-chemical treatments referred above. Phytoremediation is based on the ability of some plants (tolerant plants) and the associated microorganisms, which can grow in the presence of PTEs levels that are toxic for non-adapted plants, to degrade, extract, hold, or immobilize PTEs from soils (Khan et al., 2004; Lee, 2013; Leung et al., 2013; Souza et al., 2021; Wei et al., 2021). Although not plant micronutrients, PTEs can be removed or immobilized by plants by various processes in function of their metabolic requirements. In this context, phytostabilization is used to reduce the mobility of toxic elements from contaminated soil to the environment, whereas phytoextraction uses the plants' ability to absorb and remove PTEs from the soil and take them up into shoots and leaves (Awa & Hadibarata., 2020; Oyuela et al., 2017).

Plant species vary in their capacity to accumulate or tolerate PTEs in their vegetative aerial structures (leaves and stems) and roots. This capacity is determined by the level of pollution present in the soil, the physiological features of the species, and their selectivity for PTEs (Domínguez et al., 2012; Kashin & Ubugunov, 2012; Kashin, 2014; Mandzhieva et al., 2016; Tapia et al., 2020). In this regard, surveying the spontaneous vegetation growing in polluted sites is an efficient approach for identifying plants that may be useful both for phytostabilization and phytoextraction purposes (Bech et al., 2002; Chapman et al., 2019; Conesa et al., 2006; Freitas & Pacheco, 2004; Ginocchio & Baker, 2004; Liu et al., 2014; Monaci et al., 2020; Moreno-Jiménez et al., 2009; Poschenrieder et al., 2001; Pratas et al., 2005; Salt et al., 1995).

In fact, spontaneous vegetation is the result of strong environmental pressure for the selection of tolerance mechanisms that allow these plants to grow under the stressful conditions prevailing at polluted sites. Several studies have demonstrated that species and ecotypes from contaminated areas have either higher resistance—by more efficient exclusion—or higher accumulation and tolerance to potentially harmful concentrations of PTEs than those from non-contaminated sites (Macnair, 1993; Schat et al., 2000). Examples of plants with remarkable tolerance of polluted sites include *Noccaea caerulescens* (Mohtadi et al., 2012), *Silene vulgaris* (Pradas del Real et al., 2014), *Silene armeria* (Llugany et al., 2003), *Thlaspi arvense* (Martin et al., 2012), *Biscutella laevigata* (Pošćić et al., 2015), *Agrostis canina* (Bech et al., 2012a, 2012b), and *Agrostis capillaris* (Teodoro et al., 2020). Within these families, herbaceous species are usually predominant.

Wherever habitat degradation has led to the presence of tolerant species, mature specimens of such species could find application in remediation strategies. In fact, herbaceous species are fast-growing plants that produce a high biomass, thus making them suitable for use in phytoremediation. In addition, they are easy to grow and propagate and are, in general, self-sustaining, and many of them are perennial. Furthermore, they have been reported to grow in areas with high levels of pollution (Cunningham & Ow, 1996; Ligenfelter & Hartwig, 2007), although studies performed on multi-polluted industrial areas are scarce (Brunetti et al., 2009; Yoon et al., 2006).

Here, we addressed a paradigmatic brownfield site (mainly affected by As and Pb pollution) in which local ecotypes spontaneously colonized the soils.

This spontaneous flora could have the potential to be used for different phytoremediation strategies to rehabilitate these contaminated areas. The objectives of this study are as follows: (i) provide a systematic geobotanical description of the species growing in the highly polluted soils; and (ii) identification and evaluation of the most suitable herbaceous species for phytomanagement strategies.

2 Material and Methods

2.1 The Study Area

For decades, Nitrastur was one of the main fertilizer plants in Spain. It is located in La Felguera (Fig. 1), a district of Langreo (Asturias, 43° 17′ 41″ Lat. N, 5° 41′ 0″ Long. W. 211 m. asl. Spain), 22 km from Oviedo (capital city). In a year, the precipitation is

1293 mm. The average annual temperature in Langreo is 11.3 °C. Langreo has been one of the most important industrial areas in Spain since the nineteenth century, hosting activities such as coal mining and coal-fired power stations, steel and chemical industries (Martínez et al., 2014; Martínez-Santos et al., 2010).

Between 1950 and 1954, the Iberian Nitrogen Society, later renamed Asturian Nitrates (Nitrastur), built a fertilizer plant in a 20-ha plot. The foundations of the buildings were constructed on fillers mixed with natural soils resting on alluvial quaternary materials. The factory lost importance and reduced production in the late 1980s and was finally abandoned in 1997.

Nitrastur is currently in an advanced state of abandonment. It is one of the largest brownfields in Spain and was included in the national inventory of polluted areas in 2001. A detailed study (Gallego et al., 2016) revealed pyrite ashes, resulting from the roasting of pyrites for sulfuric acid production, as the main source of pollution. Now mixed with natural soil, this waste comprises mainly iron oxides and hydroxides and PTEs (Fedje et al., 2017). An assessment of site-specific human health risks has demonstrated the paradigmatic characteristics of this site and the need for remediation (Wcislo, 2016).

2.2 Selection of Plants

Despite the high concentrations of PTEs in the study area, various pollution-tolerant plant species have grown spontaneously throughout. A comprehensive sampling and identification campaign of the species was performed in three subsequent steps:

- First, the diversity of species with a significant presence in the study site were identified and the predominant plants were characterized.
- Second, a subgroup of the first set of plants was sampled in a 1-ha area located approximately in the center of the site. This subarea was reported in previous studies (Gallego et al., 2016; Wcislo et al., 2016) as the range showing the highest levels of surface pollution, corresponding to high As and Pb concentrations (Fig. 2a).

- Within this second set of plants, plant population frequency was studied monthly for 1 year by the quadrat method (1 m × 1 m) at 12 sites located in this 1-ha area (Fig. 2b). The importance and predominance of each plant was categorized by means of a coating index (CoI), ranging from levels 1 to 4 on the basis of the density, frequency, and surface covered by each species (Curtis, 1959; Finol, 1971; Matteucci & Colma, 1982; Mueller-Dombois & Ellenberg, 1974). Level 1 was attributed to abundant individuals but weak coverage (1 to 10% of the surface), level 2 to coverage between 10 and 25%, level 3 to coverage between 25 and 50%, and level 4 to coverage between 50% and almost full coverage.

2.3 Plant Classification

The geobotanical description and identification of the species was carried out "in situ," following the methodology and traditional techniques used in Plant Taxonomy; when needed, some specimens were herborized. The nomenclature of the taxa used followed that adopted in *Flora Ibérica* (Castroviejo, 1986) or in *Flora Europea* (Tutin et al., 1964) and, failing that, it followed the criteria established in Fernández Prieto et al. (2014), except in the case of the botanical families of Grasses (*Poaceae*) and the *Betula genus*, for which the criteria proposed by Hubbard (1985) and Ashburner and Mc Allister (2013) were used, respectively. The plants were also classified by strata in vegetation-covered areas (Arboreal (> 7 m), arborescent (3–7), shrubby (1–3), sub-arbustive (0.5–1), herbaceous (< 0.5), and bryophytes (mosses, lichens, and fungi)).

2.4 Plant Sampling and Analyses

From the final selection of plants obtained, and based on the CoI described above (see results), three replicates of each plant were taken and placed in plastic bags. Samples were sorted by hand to separate plant structures (leaves and root samples). These were then thoroughly washed with tap water several times followed by distilled water, then cleaned using an ultrasonic bath to remove external contamination, and subsequently dried at room temperature for 2 weeks.

Samples were ground in a universal rotor and variable speed Ultra Centrifugal Mill ZM 200 (Retsch) (from 6.000 to 20.000 rpm). The milled samples were collected in stainless steel containers, homogenized, and screened to a size less of than 50 µm.

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 (Multiwave3000, Anton Paar) 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 an Inductive Coupled Plasma Mass Spectrometer (ICP-MS 7700, Agilent Technologies) and IDA (Isotopic Dilution Analysis); Standard Reference Material 1515 Apple leaves from NIST (National Institute of Standards and Technology) was used.

2.5 Soil Sampling and Analyses

In the sampling stations of the 1-ha zone, composite soil samples (0 to 30 cm depth) were collected using a Dutch auger, with three replicates for each sampling point. Soils were homogenized and dried at room temperature, then sieved to 2 mm, and the fraction less than 2 mm crushed (400 rpm, RS100 Retsch mill) to approximately 150 µm. Representative subsamples were leached by means of an "aqua regia" digestion ($HCl+HNO_3$) in an Anton Paar 3000 microwave. The samples were then diluted and filtered. Elements were quantified by IDA-ICP-MS as referred above. Reference materials ERM-CC141 and ERM-CC018 were used.

To determine the distribution of PTEs in the different soil fractions, a simplified sequential extraction procedure based on the first two fractions (exchangeable and carbonate-bound) of the Tessier method (Tessier et al., 1979) was carried out. Both extracts were passed through 0.45-µm PTFE filters and diluted 1:10 prior to analysis by ICPMS.

In addition, representative soil subsamples (before grinding) were analyzed to determine edaphic properties: soil texture (pipette method) was determined after particle dispersion with sodium hexametaphosphate and sodium carbonate (Gee & Bauder, 1996); pH was measured in a 1:2.5 suspension of soil and distilled water with a glass electrode (Thomas, 1996), and electrical conductivity (EC) was measured in a 1:5 suspension of soil and water using a conductivity meter; organic matter was measured by weight loss at 450 °C (loss on ignition method, LOI) (Schulte & Hopkins, 1996); total N was determined by Kjeldahl digestion (Klute, 1996), whereas available P was determined using the Mehlich 3 reagent (Mehlich, 1995); finally, exchangeable cations (Ca^{2+}, K^+, Mg^{2+}, and Na^+) were extracted with 1 M NH_4Cl, and exchangeable Al^{3+} was extracted with 1 M KCl and both were then analyzed by atomic absorption spectrophotometry (AAS) in an AA200 Perkin Elmer system (Pansu & Gautheyrou, 2006).

2.6 Accumulation of Metal(loid)s in Plant Tissues

To study the behavior of the PTEs in the soil–plant system, various factors were examined (Mesa et al., 2017). The bioconcentration factor (BCF) was calculated as the ratio of PTE concentration in roots to that in soil ($BCF = C_{root}/C_{soil}$), with BCF values > 1 indicating the accumulation of a particular PTE in roots. The mobility ratio (MR) was calculated as the ratio of PTE in vegetative aerial structures (leaves and stems) to those in soil ($MR = C_{above\ ground}/C_{soil}$), with MR values > 1 indicating enrichment of the plant structures (accumulators). Finally, the translocation factor (TF) was calculated as the ratio of PTE concentration in vegetative aerial structures (leaves and stems) to those in roots ($TF = C_{above\ ground}/C_{root}$), with TF values > 1 indicating that the plants translocate metals effectively from roots to aerial parts. Plants with a BCF and TF above 1 are appropriate for phytoextraction purposes, whereas when only the BCF exceeds 1 they show phytostabilization potential (Nazli et al., 2020; Pandey et al., 2021; Wahsha et al., 2012).

3 Results and Discussion

3.1 Geobotanical Study

In the first phase of this study, covering the total 20 ha of the site, 60 taxa belonging to 23 botanical families were identified. Of these taxa, 26 were perennial herbaceous plants (43.3%), 24 annual herbaceous plants (40%), 5 shrubs and bushes (8.3%), 4 trees (6.7%), and 1 lichen (1.7%) (Supplementary Material, Table SM1). These results revealed the predominance of herbaceous species.

The second phase of the study was performed in the 1-ha zone with the highest soil PTE concentrations (the species identified in this specific area are shown in Table 1). Interestingly, plants showed one of the three following distribution patterns: (i) areas with disperse vegetation at a low soil-cover rate (plants grew isolated from each other); (ii) areas with high density patches of "fertility islands," comprising individuals of a range of plant species; and (iii) average levels of soil-cover rate (Fig. 3). For all of these plants, the quadrat methodology and the subsequent calculation of the CoI revealed six herbaceous plants as the most interesting for the purpose of this study (CoI = 4, as shown in Table 1).

3.2 Edaphological Characterization

The results of the analyses of the soil properties correspond with the range of values characteristic of humid temperate climate zones (Trueba et al., 1998). The edaphological characteristics of the soil showed notable heterogeneity (Table 2). Soil ranged from extremely acidic to

Table 1 Distribution of vegetation taking into account coating index (abundance, coverage, density, and frequency)

Identified species	Botanical family	Coating index (CoI)
Dysphania botrys (L.) Mosyakin & Clemants	Amaranthaceae	**4**
Lotus corniculatus L	Fabaceae	**4**
Lotus hispidus Desf. ex DC	Fabaceae	**4**
Medicago lupulina L	Fabaceae	**4**
Trifolium repens L	Fabaceae	**4**
Plantago lanceolata L	Plantaginaceae	**4**
Daucus carota L	Apiaceae	3
Hirschfeldia incana (L.) Lagr.-Foss	Brassicaceae	3
Mentha suaveolens Ehrh	Lamiaceae	3
Pastinaca sativa L. subsp. *sylvestris* (Mill.) Rouy y Camus	Apiaceae	3
Sonchus asper (L.) Hill	Asteraceae	3
Sonchus oleraceus L	Asteraceae	3
Agrostis capillaris L	Poaceae	2
Betula celtiberica Rothm. & Vasc	Betulaceae	2
Cirsium arvense (L.) Scop	Asteraceae	2
Conyza canadensis (L.) Cronquist	Asteraceae	2
Dactylis glomerata L	Poaceae	2
Holcus lanatus L	Poaceae	2
Hypericum pulchrum L	Hypericaceae	2
Hypochaeris glabra L	Asteraceae	2
Lolium perenne L	Poaceae	2
Poa annua L	Poaceae	2
Prunella vulgaris L	Lamiaceae	2
Pteridium aquilinum (L.) Kuhn	Dennstaedtiaceae	2
Rubus gr. *fruticosus* L	Rosaceae	2
Stellaria media L	Caryophyllaceae	2
Trifolium dubium Sibth	Fabaceae	2
Trifolium pretense L	Fabaceae	2
Verbena officinalis L	*Verbenaceae*	2
Verbascum pulverulentum Vill	Scrophulariaceae	2
Betula pubescens Ehrh	Betulaceae	1
Blechnum spicant (L.) Roth	Blechnaceae	1
Chenopodium polyspermum L	Amaranthaceae	1
Rubus ulmifolius Schott	Rosaceae	1
Verbascum virgatum Stokes	Scrophulariaceae	1
Vulpia bromoides (L.) Gray	Poaceae	1

Table 2 Soil parameters in the 1-ha study area

Parameter	Units	Average	Typical deviation
pH		6.46 ± 0.16	0.82
C.E[1]	dS m^{-1}	0.01 ± 0.0001	0.01
Sand	%	64.61 ± 2.91	15.13
Silt	%	21.15 ± 2.81	14.60
Clay	%	14.24 ± 1.06	5.52
D.A[2]	g cm^{-3}	1.52 ± 0.013	0.07
O.M[3]	%	11.00 ± 0.41	2.34
F.C[4]	%	9.36 ± 0.5	2.13
C (SOC)	%	5.43 ± 0.23	1.24
N (TOTAL)	%	0.43 ± 0.05	0.27
C/N[5]		18.36 ± 2.33	12.13
Fe	g kg^{-1}	9.00 ± 0.87	4.56
PM3[6]	mg kg^{-1}	3.82 ± 0.52	2.69
Ex Ca	$\text{cmol}_+\ \text{kg}^{-1}$	15.01 ± 0.85	4.44
Ex Mg	$\text{cmol}_+\ \text{kg}^{-1}$	0.56 ± 0.01	0.08
Ex K	$\text{cmol}_+\ \text{kg}^{-1}$	0.57 ± 0.05	0.3
Ex Na	$\text{cmol}_+\ \text{kg}^{-1}$	1.85 ± 0.06	0.3
C.E.C[7]	$\text{cmol}_+\ \text{kg}^{-1}$	17.99 ± 0.88	4.61
Available As[8]	%	4.03 ± 0.42	1.91
Available Pb[9]	%	11.01 ± 0.95	4.26

[1] *C.E*, electrical conductivity; [2] *D.A*, bulk density; [3] *O.M*, organic matter; [4] *F.C*, field capacity; [5] *C/N*, carbon vs. nitrogen ratio; [6] *PM3*, P by Mehlich method; [7] *C.E.C*, cation exchange capacity (bases); [8,9] Total As and Pb are 761 and 2817 mg kg^{-1} as indicated in Fig. 2

neutral, with an average value that was slightly acidic. Soils did not show salinity, and organic matter content was normal while total nitrogen content and the average values of C/N relations were also normal (Nicolardot et al., 2001). In general terms, soil showed an excess of calcium and deficiency of phosphorus, magnesium, and sodium, and normal values of potassium (Tomasic et al., 2013). Exchangeable aluminum was not detected. On the basis of the particle size distribution data, samples were classified mostly as highly heterogeneous sandy loam soils with a high degree of compaction.

3.3 PTEs in the plant/soil system

As shown in Table 3, as concentration in aerial parts of the plants ranged from 33 mg kg^{-1} to 303 mg kg^{-1} (*Lotus hispidus* > *Medicago lupulina* > *Plantago lanceolata* > *Dysphania botrys* > *Trifolium repens* > *Lotus corniculatus*), whereas the internal structure of the roots revealed significantly higher contents of this pollutant, ranging from 152 to 247 mg·kg^{-1}. Similar results (Table 3) were obtained for Pb concentrations for various plant parts; i.e., the concentration of Pb in leaves varied between 105 and 719 mg·kg^{-1} and in roots between 582 and 1805 mg·kg^{-1}. In general, despite the very high concentrations found for As and Pb, plant samples did not show any signs of toxicity.

The phytoremediation potential of the six species of interest was studied by analyzing the concentration of As and Pb in soils vs. the plant concentrations and the accumulation factors (Table 3).

All the plant species sampled can be classified as pseudometallophytes since BCF values were below 1

Table 3 Average soil content (3 samples per plant) and accumulation factors obtained for the six selected species

Species	Element	Soil concentrations (mg kg^{-1}Distribution Center, 1–28. Retrieved July)	Aerial part concentrations (mg kg^{-1})	Root concentrations (mg kg^{-1})	BCF	TF	MR
Dysphania botrys	**As**	1505.4	81.9	191.1	0.13	0.43	*0.05*
	Pb	3738.1	354.8	726.8	0.19	0.49	*0.09*
Lotus corniculatus	**As**	811.7	32.7	198.8	0.25	0.16	*0.04*
	Pb	2713.5	105.2	904.2	0.33	0.12	*0.04*
Lotus hispidus	**As**	8552.7	303.1	209.5	0.02	**1.45**	*0.04*
	Pb	12,239.5	479.86	581.7	0.05	**0.82**	*0.04*
Medicago lupulina	**As**	4600.5	226.1	151.5	0.03	**1.49**	*0.05*
	Pb	11,973.2	719.2	793.7	0.07	**0.91**	*0.06*
Plantago lanceolata	**As**	1098	95.3	246.8	0.22	0.39	*0.09*
	Pb	7071.4	419	1804.8	0.26	0.23	*0.06*
Trifolium repens	**As**	634.4	73.4	195.1	0.31	0.38	*0.12*
	Pb	2776.2	270.4	878.6	0.32	0.31	*0.10*

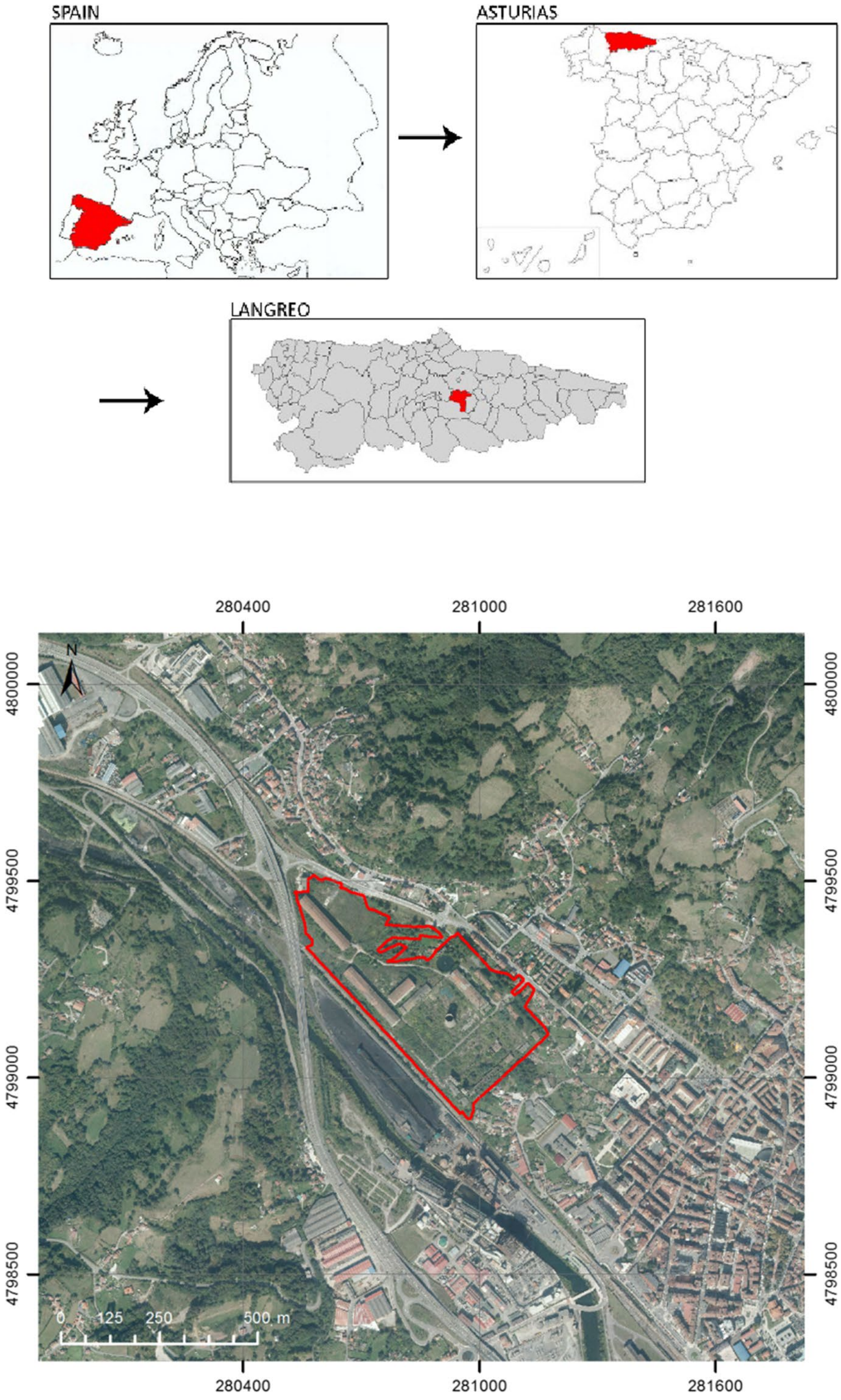
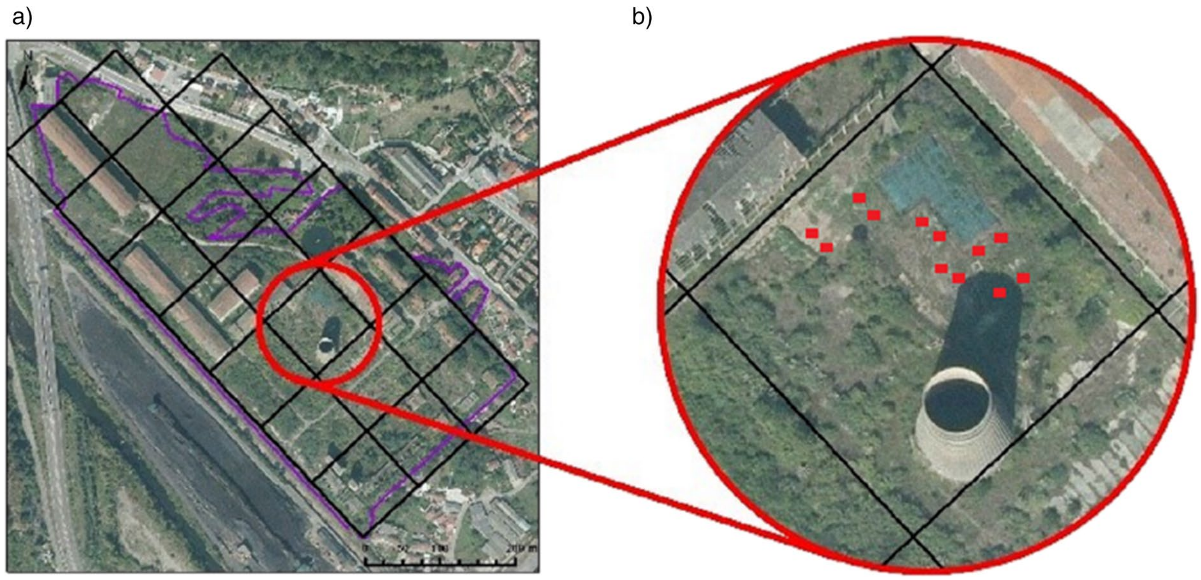


Fig. 1 Location of study area (top) and aerial view (bottom) of the site (red contour)

in all cases, probably conditioned by the low bioavailability of the PTEs (Fedje et al., 2016; Mesa et al., 2017), and none of them registered an accumulation factor exceeding the limits established for hyperaccumulative plants (1000 mg kg^{-1} for As) (Kabata-Pendias, 2011; Sun et al., 2008). These plants showed



Fi.2 **a** Location of the 1-ha zone in which the second set of plants were sampled within the whole study site. The soil of this zone showed an average of 761 mg kg^{-1} of As and 2817 mg kg^{-1} of Pb, well-above of the soil screening levels for industrial uses in the region of Asturias (200 and 800 mg kg^{-1} respectively). **b** Location of the sampling stations for the quadrat method.

some differences with respect to the exclusion strategy. In this regard, *Lotus corniculatus* and *Lotus hispidus* were those with the highest ability to reduce As and Pb transport (very low MR) from roots to the aerial parts.

Therefore, and consistent with the findings of other studies, PTE accumulation occurred more frequently in roots than in the aerial parts of herbaceous plants under study (Bidar et al., 2007; Marques et al., 2009). However, *Lotus hispidus* and *Medicago lupulina*, classified as As and Pb excluders on the basis of a very low BCF ratio (Table 3), showed a TF higher than 1 for As and very close to 1 for Pb. The concentrations of As in *Lotus hispidus* biomass were higher than in those of *Medicago lupulina*, in agreement with As concentrations in soils. However, according to the BCF and TF, both species showed similar ability with regard to As translocation and uptake. On the contrary, Pb concentration in *Medicago lupulina* was higher than in those of *Lotus hispidus*, regardless of the soil concentration of this element. Based on our observations, these two plants emerge as potentially useful as bioindicators of As and/or Pb pollution because of their ability to translocate these pollutants. Furthermore, they might show accumulation potential in their vegetative aerial structures (leaves and stems) in different conditions to those of the study site (higher PTE availability would be required).

Overall, the main interest of these herbaceous species could be their application in phytostabilization techniques. The phytostabilization capacity of the herbaceous plants studied herein has been reported and discussed elsewhere and the results of those studies also support the conclusions of this work.

For instance, plant species belonging to the Leguminosae family, such as *Medicago lupulina*, *Lotus corniculatus*, and *Trifolium repens*, were reported to make major contribution to phytostabilization (Amer et al., 2013; Bert et al., 2000; Bidar et al., 2007), whereas only *Medicago lupulina* showed some Pb translocation ability to aerial parts (Amer et al., 2013). The tolerance of *Trifolium repens* to contaminated sites should also be noted, with the roots being the main organ of Pb accumulation (Bidar et al., 2007; Oleńska et al., 2020). In another study, *Lotus corniculatus* showed the bioaccumulation of PTEs not exceeding current legal limits for Cd, Cr, and Pb. On the basis of this finding, this plant was proposed for use in the phytostabilization of a soil contaminated with organic and inorganic

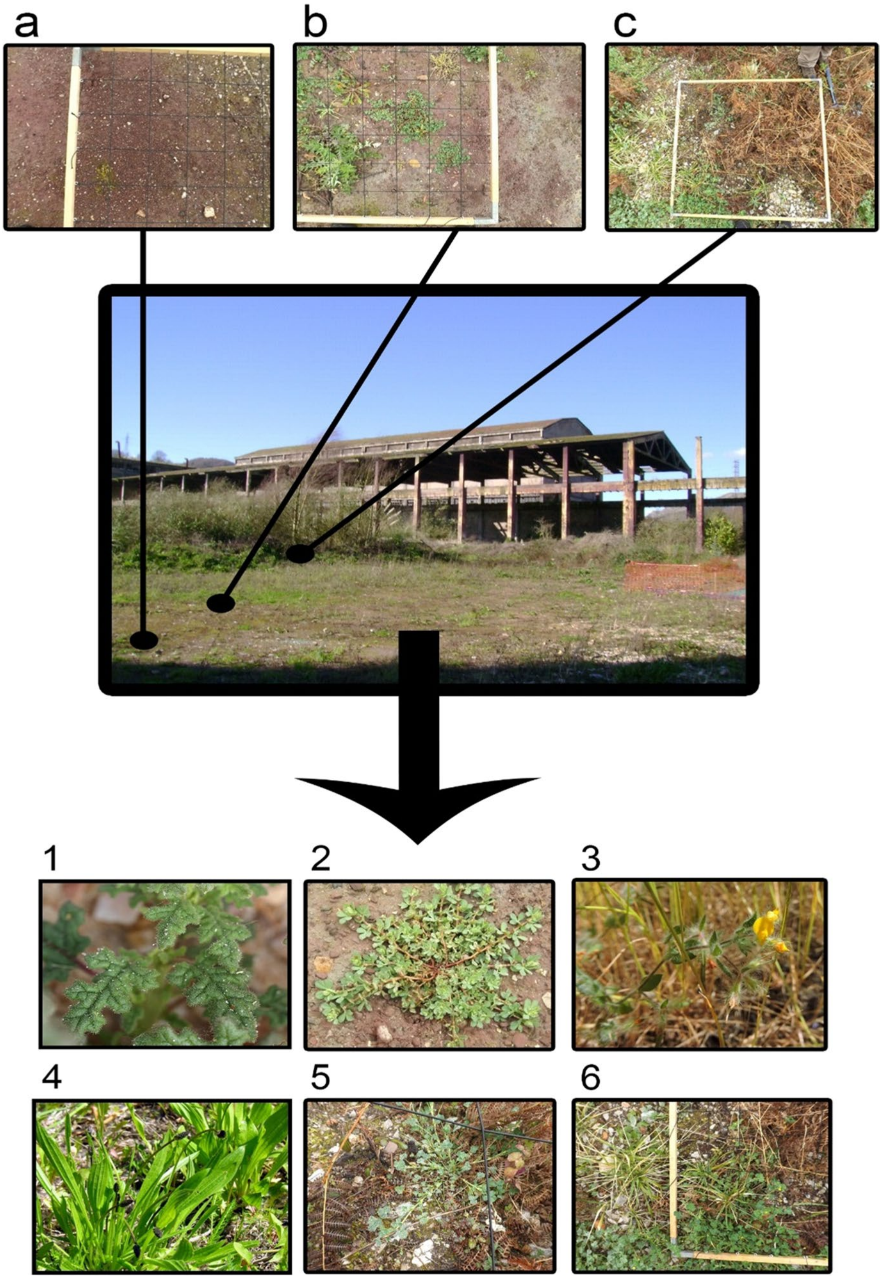


Fig. 3 (Center) "Nitrastur" subarea with the highest levels of surface pollution. (Top) Quadrats established in areas with low plant coverage (**a**), areas with medium coverage (**b**), and areas with high coverage (**c**). (Bottom) Illustrative images of the herbaceous plants with the highest CoI: (1) Sticky goosefoot, *Dysphania botrys* (L.) Mosyakin & Clemants (*Dysphaniabotrys* L.), (2) *Bird's foot trefoil* (*Lotus corniculatus* L.), (3) Hairy bird's foot trefoil (*Lotus hispidus* Desf. ex DC.), (4) Ribwort plantain (*Plantago lanceolata* L.), (5) White clover (*Trifolium repens* L.), (6) Black medick (*Medicago lupulina* L.)

pollutants (Obeidy et al., 2016; Souto et al., 2015). Regarding *Lotus hispidus*, to the best of our knowledge, no specific studies related to its potential for phytoremediation have been conducted.

The capacity of *Dysphania botrys* to accumulate PTEs was studied (Cheraghi et al., 2011), obtaining low values of the coefficient of bioaccumulation for Fe, Zn, and Cu, thereby pointing to its ineffectiveness in the phytoextraction of PTEs, with the only exception of Mn. Nouri et al. (2009) also indicated that the root tissues of this plant accumulate higher concentrations of metals than shoots.

Plantago lanceolata has provided good model plant for toxicity bioassays (Dimitrova & Yurukova, 2005; Laffont-Schwob et al., 2020; Turnau et al., 2005). This plant occurs in diverse habitats and is resistant to a wide range of stress factors, including high levels of As (Baroni et al., 2000; Meharg &

Hartley-Whitaker, 2002; Pollard, 1980; Schwanitz & Hahn, 1954; Wu & Antonovics, 1976). Mycorrhizal colonization of this plant was proposed as a useful tool for addressing soil quality and effectiveness of restoration processes (Orlowska et al., 2002; Turnau et al., 2005).

Trifolium repens is able to reduce the mobility and the availability of contaminants through their fixation in roots (Bidar et al., 2007; Lambrechts et al., 2014). PTE pollution appears to have dramatic effects on the physiology of this specie (Bidar et al., 2008). More recently, Lopareva-Pòhu et al. (2011) studied the influence of different amendments on the mobility and phytoavailability of the metals, as well as oxidative damage in *Trifolium repens*.

4 Conclusions

The study of a paradigmatic brownfield affected by high concentrations of PTEs (especially As and Pb) revealed the presence of a diverse flora tolerant to the PTEs present. The site was active from the 1950s until its abandonment more than 20 years ago, thus explaining the presence of rich spontaneous vegetation.

Notably, herbaceous species were predominant in the most polluted area of the site, as demonstrated in a 1-year study of plant coverage. The most predominant herbaceous species identified (*Lotus hispidus*, *Medicago lupulina*, *Plantago lanceolata*, *Dysphania botrys*, *Trifolium repens*, and *Lotus corniculatus*) presented a methallophyte behavior consistent with their potential use for phytostabilization.

According to the results, and regarding site remediation, future studies should focus on the application of phytostabilization as a first option. However, some of the species identified (*Lotus hispidus* and *Medicago lupulina*) are also of particular interest because of their ability to translocate As and Pb. This capacity indicates that they could potentially also be used as bioindicators. Furthermore, they might even serve as phytoextractors accumulators in different conditions (higher availability of the PTEs in soil) to those of the study site.

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Data Availability The authors declare that all data supporting the findings of this study are available within the article (and its supplementary information files).

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