Use of the native vascular flora for risk assessment and management of an industrial contaminated soil

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Abstract

This study was carried out in an industrial site contaminated by potentially toxic elements (PTEs) to assess the relationship between spontaneous vegetation and pollution levels, the potential risks for biological communities and ecosystems, and the potential of native plant species for phytoremediation. PTE concentrations had negative effects on plant biodiversity, as determined through changes in the Shannon index, Pielou evenness index and species richness. *Poaceae* and *Asteraceae* were moderately affected by soil contamination, while PTE levels had a negative effect on the other species groups. Cadmium had the greatest effect on plant species diversity, followed by zinc and then lead. The ecological risk index showed a mean value of 4924, corresponding to a very high risk in most plants. Target PTEs for phytoremediation were Cd (3813 on average) followed by Pb (937 on average) contributing to the ecological risk index, respectively from 42 to 81% and from 11 to 24%, in spite of the much higher concentrations of Pb.

The most frequent species were *Holcus lanatus* subsp. *lanatus* and *Silene latifolia* that showed good adaptability to contamination, growing in very high-risk areas. *S. latifolia* reported high concentrations of Tl both in shoot and in roots, at levels typical of hyperaccumulator species. High values of bioaccumulation (BAC_S, BAC_R) and translocation factors (TF) confirmed that this species may be considered a hyperaccumulator of Tl. *Holcus lanatus* and *Silene latifolia* proved the most suitable species respectively for Cd and Pb phytostabilization and can be used in association for soil cover during the summer when soil resuspension is generally more intense and for protecting groundwater from pollutant leaching.

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Key words: Plant biodiversity; bioaccumulation coefficient; ecological risk index; potentially toxic elements; spontaneous flora.

Italian Journal of Agronomy 2018; 13(s1):23-33

Introduction

The most hazardous potentially toxic elements (PTEs) in urban and industrial areas are widely acknowledged to be lead and cadmium. Lead (Pb) is the second most hazardous element for human health, causing a decline in mental and cognitive capacities (ATSDR 2015). Pb content in soils can have a geological origin, but vehicle emissions, mining, smelting and battery recycling can release the element into the environment through atmospheric fallout and waste disposal. By contrast, cadmium (Cd) is often used as a doping agent in batteries or in some paints. It is a non-essential element for plants and animals, and exposure even at low concentrations could have carcinogenic effects (Goering *et al.,* 1994).

PTEs can also affect normal plant functions and metabolic processes like photosynthesis, respiration and enzymatic activities (Furini, 2012). They stimulate defence mechanisms to oxidative damages based on antioxidant systems that can differ among plant species (Meharg, 2005; Sytar *et al.,* 2013; Antoniadis *et al.,* 2017). Therefore, composition of native flora in contaminated environments can be affected, causing selection pressure that permits tolerant, bioaccumulator or excluder species to proliferate, eliminating the most sensitive populations (Chowdhury *et al.,* 2016). Moreover, analysis of native vascular flora may also help to select the most suitable species in phytoremediation projects (Barbafieri *et al.*, 2011).

Phytoremediation is a technique for removing contaminants from soils or for interrupting exposure pathways that can be viewed as belonging to the general class of bioremediation systems (Vidali, 2001). It is a promising technique thanks to its cost-effectiveness and the positive effects on local landscapes and soil ecosystem services. Phytoextraction is based on uptake of toxic substances by plant roots and their subsequent accumulation in shoots (Raskin and Ensley, 1999).

An alternative approach, namely phytostabilization, entails the use of vegetation to reduce the mobility of contaminants toward other environmental compartments such as air and groundwater. Plant growth and contaminant uptake, as well as their bioavailability, can be improved by assisting phytoremediation with fertilisers and biostimulants, thereby improving the efficiency of this technique and reducing the restoration time required (Fiorentino *et al.*, 2013, 2017).

The effectiveness of phytoremediation requires selected plants for uptaking or immobilizing PTEs. Furthermore, it is important to find plant species not only tolerant to contaminants, but also adapted to the site-specific conditions, which are often limiting for plant growth, such as soil physical degradation due to compaction and aggregate destruction caused by the weight of waste or by the transit of heavy vehicles (Fiorentino *et al.*, 2018).

The aim of the present study was threefold: i) to assess the risks for biological communities and ecosystems due to PTE pollution; ii) identify target PTEs for phytoremediation; and iii) evaluate the potential for phytoremediation of native species growing *in situ*.

Materials and methods

Site description

The study site is located in the industrial area of the municipality of Marcianise (Campania, southern Italy: 41° 00' 48.9" N - 14° 17' 49.7" E) in the western part of the Campanian Plain at about 24 m a.s.l. It has a typical Mediterranean climate with precipitation mostly occurring in the autumn and winter and a long summer drought.

The area in question is a 3.5-ha plot near an industrial plant for recycling automotive electric batteries classified by the regional authorities as potentially contaminated by Cd and Pb due to past storage of waste from the industrial plant itself. The site was thus classified as contaminated, since risk analysis showed that there was a serious potential risk for workers due to inhalation or dermal contact with contaminated soil particles. A phytoremediation project was approved, using the ECOREMED protocol (2017) based on ecological systems (*i.e.* a poplar stand and permanent meadows) to prevent soil particles becoming air-borne, thereby interrupting exposure pathways to contaminants. The study site presents the typical scenario of a disused industrial area, where the recolonization of natural vegetation has allowed the formation of herbaceous and shrubby secondary plant communities.

Soil analysis

Soil samples both from plots and from the rhizosphere of the most representative species were dried at 50°C until constant weight, homogenized and sieved at 2 mm. The <2 mm fraction was characterized for the following: texture (normalized methods for soil analysis, ISS, 1985), pH-H_2O (1:2.5 soil:water solution ratio), electrical conductivity (1:5 soil:water solution ratio - Conductimeter basic 30, Crison), organic carbon (Walkley and Black method, 1934), nitrogen (Kjeldahl method), carbonate content (Dietrich-Frühling calcimeter method, Loeppert and Suarez, 1996) and PTE concentrations (acid digestion with aqua regia followed by ICP-MS).

The potentially bioavailable fraction of PTEs was estimated by a single extraction with a 0.005 M diethylene triamine pentaacetic acid (DTPA) + 0.01 M triethanolamine (TEA) + 0.01 M calcium chloride ($CaCl_2$) solution adjusted to pH 7.3 (Lindsay and Norwell, 1978). PTE concentration in the solution was determined by inductively coupled plasma-atomic emission spectrometry (Perkin Elmer ICP-AES Optima 7300DV).

Vegetation analysis

On 9 June 2015 the analysis of the spontaneous vegetation was carried out by using nine square plots (3x3 m) selected according to the various vegetation types it presented. In each plot the presence/absence of the plant species, their abundance (expressed as per cent cover) and overall vegetation cover was detected. The plant specimens were directly identified in the field except for dubious cases, which were later identified at the Herbarium Porticense (PORUN) according to Pignatti (1982), Pignatti *et al.* (2018) and Tutin *et al.* (1964-1980, 1993). The nomenclature follows Bartolucci *et al.* (2018) and Galasso *et al.* (2018).

With the data collected, the Shannon-Weiner index, Pielou evenness index and species frequency were calculated for each plot. The Shannon-Weiner diversity index (H') is usually based on the number of species and the abundance of individuals within each species (Magurran, 1988), but in this survey the mean soil cover of the species was used, as suggested by Stefanska-Krzaczek (2012):

$$(H') = -\sum_{i=1}^{S} \frac{ci}{C} \; x \; log_e \frac{ci}{C} \qquad (1)$$

where S is the number of species, ci is the mean cover of i species on a plot and C is the sum of mean covers for all species.

The Pielou evenness index (J') (Grall and Coic, 2006) is used to measure the distribution of species within a site, regardless of species richness. Its value varies from 0 (dominance of one species) to 1 (equitable distribution of species). It is calculated as follows:

$$(J') = \frac{H'}{H'max} \qquad (2)$$

where H' = the Shannon-Weiner index and H'max = log_e of the total number of species S.

Frequency (F), *i.e.* the distribution or dispersion of individual species (Mukhopadhyay *et al.*, 2017), was estimated as percentage of occurrence calculated for the most representative plant species of each plot:

$$(F) = \frac{Number\ of\ areas\ in\ which\ a\ species\ occurred}{Total\ number\ of\ areas\ studied} \; x \; 100 \qquad (3)$$

Characterization of the most representative species

To analyse the behaviour of the single species detected in the site, plant species of the nine plots showing the highest soil coverage were identified and a variable number of plant samples (>3 when possible) were collected together with their respective rhizo-soils. Samples of each species were separated in shoots and roots and then washed with tap water followed by distilled water, oven-dried at 60°C until constant weight and finely ground.

Composite samples of roots and shoots of each selected species were then analysed (acid digestion with *aqua regia* followed by ICP-MS) for PTE content. PTE concentrations were compared to PTE thresholds in forage, considering the native species as a potential pasture (EU Reg. 1275/2013). For metals not considered by this Regulation the mean values found in grasses grown on polluted sites (Kabata-Pendias, 2011) were used as reference.

Potential ecological risk assessment

With the soil contamination data, both in the nine plots and in rhizo-soil of the most representative species, the potential ecological risk index (ERI) was calculated. ERI represents the sensitivity of the biological community to toxic substances and shows the potential ecological risk caused by the overall contamination (Zhao and Li, 2013). The index is also useful to evaluate the adaptability of plant species to soil contamination, given that species growing in high-risk areas might be adapted to contamination. The equation used for calculating ERI (Hakanson, 1980) is:

$$\mathrm{ERI} = \sum_{i=1}^{n} E_r^i = \sum_{i=1}^{n} T_r^i \times C_f^i = \sum_{i=1}^{n} \left(T_r^i \times \frac{C^i}{C_n^i} \right) \qquad (4)$$

where: E_r^i is the monomial potential ecological risk index of the PTE i; T_r^i is the toxic response factor for a specific PTE i (*e.g.* As=10, Cd=30, Cr=2, Cu=5, Pb=5, Tl=10 and Zn=1); C_f^i is the contamination factor of PTE i; C^i is the content of PTE i in the samples (mg kg^{-1}), and C_n^i is the background value of PTE i in the study area (mg kg^{-1}). The toxic response factor for Tl is reported by Liu *et al.* (2018).

In this study, soil background values (BV) of the area from Cicchella *et al.* (2008) were used (As: 10.50; Cd: 0.45; Cr: 10; Cu: 28.50; Pb: 46.50; Zn: 78.0; Tl: 1.00 mg kg^{-1}). The contamination degrees and the potential ecological risk of a single PTE (E_r^i) were classified as low (E_r^i<40), moderate (40 ≤ E_r^i<80), considerable (80 ≤ E_r^i<160), high (160 ≤ E_r^i<320) and very high (E_r^i≥ 320). The overall ecological risk (ERI) was classified as low (ERI <95), moderate (95 ≤ERI <190), high (190 ≤ERI <380) and very high (ERI ≥380) (Rehman *et al.,* 2018).

Calculation of PTE accumulation indices

For each of the most representative species, the following indices were calculated for assessing the ability of plants to accumulate and translocate PTEs: bioaccumulation coefficient in shoots (BACs), bioaccumulation coefficient in roots (BAC_R) and translocation factor (TF). The BACs and BAC_R were calculated as the ratio between the concentration of PTEs in shoots and roots, respectively, and the concentration of PTEs in the rhizospheric soils (Putwattana *et al.,* 2015). The translocation factor was calculated as the ratio between the concentration of PTEs in shoots and that in roots (Baker and Brooks, 1989):

$$BAC_S = \frac{PTE\ concentration\ in\ shoots\ (mg\ kg^{-1})}{PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \quad (5)$$

$$BAC_R = \frac{PTE\ concentration\ in\ roots\ (mg\ kg^{-1})}{PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \quad (6)$$

$$TF = \frac{PTE\ concentration\ in\ shoots\ (mg\ kg^{-1})}{PTE\ concentration\ in\ roots\ (mg\ kg^{-1})} \quad (7)$$

To determine the capacity of plants to accumulate the bioavailable fractions of contaminants, a modified bioaccumulation coefficient (mBAC) was calculated for shoots and roots (Hamon and McLaughlin, 1999; Barbafieri *et al.,* 2011; Petruzzelli *et al.,* 2011) as follows:

$$mBAC_S = \frac{PTE\ concentration\ in\ shoots\ (mg\ kg^{-1})}{bioavailable\ PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \quad (8)$$

$$mBAC_R = \frac{PTE\ concentration\ in\ roots\ (mg\ kg^{-1})}{bioavailable\ PTE\ concentration\ in\ soil\ (mg\ kg^{-1})} \quad (9)$$

To evaluate the presence of hyperaccumulator plants we also compared PTE concentrations in shoots with reference values given by Van der Ent *et al.* (2013).

Statistical analysis

The statistical analyses were all carried out by using Ms Excel 2007 and SPSS 21 (SPSS Inc. Chicago, USA). Pearson correlation analyses were made to investigate the relationships between ERI, soil factors (pH, electric conductivity, organic carbon, total nitrogen, total and bioavailable PTEs) and ecological parameters (H', J', plant species richness and total plant cover) of each plot. Statistical significance in this analysis was defined at P<0.05 and P<0.01.

Results and discussion

Soil features of the nine plots

All the soils showed a good organic carbon content and a subalkaline pH, while EC and $CaCO_3$ were low for all rhizo-soils (Table 1). Soil texture was sandy loam (USDA) without differences among the plots with 15% of clay, 23% of silt and 62% of sand on average (data not shown). The soils of all nine plots showed good fertility, and thus cannot be considered limiting for plant growth.

Soil Pb concentrations ranged from 409 to over 100,000 mg kg^{-1} and exceeded the Italian screening values (SV) for industrial sites (DL 152/06: 1000 mg kg^{-1}) in 89% of cases (Table 2). Soil Cd concentration ranged from 1.6 to 298 mg kg^{-1} and was above SV (15 mg kg^{-1}) in 67% of plots. Soil As concentration ranged from 16 to 861 mg kg^{-1} and exceeded SV (50 mg kg^{-1}) in 56 % of cases.

Soil Cu, Cr, Tl and Zn concentrations were lower than SVs for industrial sites (Cu: 600 mg kg^{-1}; Cr: 800 mg kg^{-1}; Tl: 10 mg kg^{-1}; Zn: 1500 mg kg^{-1}) in all the plots. Due to the very high concentra-

Table 1. Selected soil physico-chemical properties in the plots monitored.

Plots	pH	Electrical conductivity (dS m^{-1})	$CaCO_3$ (g kg^{-1})	Organic carbon (g kg^{-1})	Total nitrogen (g kg^{-1})
1	7.2	197.0	15.0	26.9	2.5
2	7.2	174.8	21.4	42.4	6.3
3	7.1	231.0	13.1	42.5	4.4
4	7.0	472.0	87.5	68.3	9.7
5	6.9	524.4	51.9	55.7	4.0
6	7.2	311.5	46.4	48.9	5.5
7	7.4	398.5	41.0	55.9	1.3
8	7.4	238.5	64.1	31.4	0.5
9	7.5	500.9	81.1	16.9	0.6
Average	7.2	338.7	46.8	43.2	3.9
St. err	0.07	45.9	9.1	5.4	1.0

tion of some PTEs (mainly Pb and Cd), the ERI of the nine plots proved very high in 89% of cases, with values up to 26,556 (plot 5), 70 times higher than the maximum threshold reported by Rehman *et al.* (2018). These values were higher than other Pb contaminated sites (Ogunkunle and Fatoba, 2013; Jiang *et al.*, 2014; Kaddour *et al.*, 2017).

Plant community characteristics of the nine plots

In all, 34 species and 17 families were recorded in the nine plots (Table 2). The most abundant families were *Poaceae* and *Asteraceae*. The highest species diversity was reported in the plots with lower ecological risk and PTE content (plots 1, 3 and 4), while the lowest values were found in the plots with highest risk (plots 5 and 9), as also reported by Vidic *et al.* (2006) who reported that the highest biodiversity and evenness of spontaneous plant species were found in the plots with the lowest concentration of Cd, Pb and Zn furthest away from a lead smelter chimney. In particular, plot 5 had a very high concentration of PTEs, with invasive alien species such as *Artemisia annua* L. and *Erigeron sumatrensis* Retz. *Poaceae* were present in all nine plots, also with only one species in the most contaminated plots (5 and 9), *Asteraceae* were present in only 56% of plots, while *Fabaceae* were present with only one species in the least contaminated plot, thus suggesting a decreasing tolerance to soil contamination from *Poaceae* to *Asteraceae* to *Fabaceae,* confirming the results obtained by many authors in other countries (Wang *et al.*, 2004; Shu *et al.*, 2005; Weiersbye *et al.,* 2006; Gawronski and Gawronska, 2007; Mansfield *et al.,* 2014; Salas-Luevano *et al.,* 2017; Nguemte *et al.,* 2018). The overall soil cover of nine plots was always close to 100%, even in the most contaminated plots, thus confirming the findings of Bes *et al.* (2010) who found that elimination of the most PTE-sensitive species allows the more tolerant species to proliferate, which thus occupy the space left by the former.

Cd and Pb reported the highest contribution to the ecological risk index (Table 3). Cd contributed from 54 to 81 % of the ERI, while Pb contributed from 12 to 21% of the ERI, confirming that these elements must be considered the target PTEs of this site. Furthermore, these PTEs are considered the most hazardous also for human health. Indeed, the reference doses of oral soil ingestion

Table 2. PTE levels, ERI, biodiversity and plant cover for the plant communities of the plots.

Plots descriptors	1	2	3	4	5	6	7	8	9
As (mg kg^{-1})	14	34	19	16	861	87	92	94	97
Cd (mg kg^{-1})	1.6	41.9	13.9	5.3	298	91.7	97.6	63.1	153.2
Cr (mg kg^{-1})	44	48	47	48	69	61	62	44	55
Cu (mg kg^{-1})	145	218	339	133	609	606	252	181	340
Pb (mg kg^{-1})	409	10,505	2998	1567	100,000	26,538	26,600	17,508	39,263
Tl (mg kg^{-1})	1.8	1.7	2.2	1.6	1.6	8.6	5.7	2.1	3.8
Zn (mg kg^{-1})	217	251	209	178	450	401	250	172	429
ERI	198	3491	1209	512	26,556	7931	8226	5356	12651
Soil cover (%)	100	95	90	95	100	95	90	100	100
Shannon-W index (H')	1.76	1.10	1.64	2.14	0.06	0.97	1.44	0.90	0.08
Pielou index (J')	0.76	0.69	0.70	0.79	0.08	0.54	0.58	0.43	0.10
Species number	11	5	11	15	2	6	12	8	2
Poaceae	2	2	3	3	1	3	4	5	1
Fabaceae	1	0	0	0	0	0	0	0	0
Asteraceae	2	1	2	6	0	0	2	0	0
Miscellaneous species	6	2	6	6	1	3	6	3	1

Table 3. Mean values of ecological risk factor (E_r^i) and potential ecological risk index (ERI) in the different plots.

	E_r^i							ERI
Plots	As	Cd	Cr	Cu	Pb	Tl	Zn	
1	13	107	8.9	26	23	18.3	2.8	199
2	32	*2793*	9.6	38	*597*	17.6	3.2	*3491*
3	18	*927*	9.4	60	170	22.7	2.7	*1209*
4	15	*357*	9.7	23	89	16.1	2.3	*512*
5	*820*	*19907*	13.8	112	*5682*	16.6	5.8	*26,557*
6	83	*6113*	12.2	124	*1508*	86.4	5.1	*7932*
7	88	*6510*	12.5	44	*1511*	57.3	3.2	*8227*
8	90	*4207*	8.8	32	*995*	21.8	2.2	*5356*
9	92	*10,213*	11.0	60	*2231*	38.3	5.5	*12,651*
Average	139	*5681*	10.7	58	*1423*	32.8	3.6	*7348*
St. Err.	86	2096	0.6	12	589	8.1	0.5	2770

Very high risk is reported in italics.

(RfD in mg d^{-1} kg^{-1} body weight) given by USEPA (1989) are very low: 0.0010 for Cd and 0.0035 mg for Pb. It should be noted that Cd had the highest ecological risk, even though the Pb concentrations in soil were much higher. This was due to the higher toxicity of Cd for the biosphere even at low concentrations (Duri *et al.,* 2018; Zhao *et al.,* 2018). The degree of risk due to the other PTEs was lower, excepted for As in plot 5 (very high risk).

Correlation analysis (Table 4) revealed that the ecological risk index has the greatest deleterious effect on plant diversity and evenness ($P<0.01$), but also on the total number of species, as also reported by Andreucci *et al.* (2006) This effect was more evident in the miscellaneous group ($P<0.05$), confirming the findings of Koptsik *et al.* (2003). As regards the single elements, the total soil content of Cd, followed by Pb and Zn, affected the plant community in question, showing a significant negative correlation with diversity, evenness and species richness. The reduction in plant species diversity by these three elements has also been reported by others in industrial areas elsewhere (Vangronsveld *et al.,* 1996; Vidic *et al.,* 2006). This effect was more evident in the miscellaneous group that seemed to be the best indicators of soil PTE pollution while the other groups were unaffected by soil pollution, according to the results of Hernandez and Pastor (2008). The better adaption to soil contamination of *Poaceae* and *Asteraceae* has already been reported extensively elsewhere (Wang *et al.*, 2004; Shu *et al.*, 2005; Gawronski and Gawronska, 2007; Nguemte *et al.,* 2018) and confirmed that such families appear more resistant than other families to soil contamination.

Behaviour of the most representative species

An inventory of the 12 most representative species identified in the nine plots is shown in Table 5. *Artemisia annua* (native to East Europe and Asia) and *Sorghum halepense* (native to tropical areas of Africa and Asia) are considered invasive aliens in Italy. The most frequent families were *Poaceae* and *Asteraceae*. The most frequent species were *Holcus lanatus* subsp. *lanatus* (78% of plots) and *Silene latifolia* (56% of plots). Most of the inventoried species of *Asteraceae* family are common for areas contaminated by industrial waste like *Artemisia vulgaris* (Wojcik *et al.,* 2014), *Cirsium arvense* (Desjardins *et al.,* 2014) and *Poaceae* such as *Elymus repens* subsp. *repens* and *Dactylis glomerata* subsp. *glomerata* (Dygus, 2013). *Rubus ulmifolius* was also reported by Massa *et al.* (2010) in an industrial area in northern Italy.

Characterization of rhizo-soils of the more representative species

Soil PTE concentrations of the 12 rhizo-soils are shown in Table 6 along with PTE content in plants. Also the rhizo-soils showed very high PTE contents, reflecting average values found in the nine plots (see Table 2).

The ecological risk index calculated in the rhizo-soils of the 12 most representative species showed very high risk in all habitats except for *Artemisia vulgaris*, *Dittrichia viscosa* subsp. *viscosa* and *Epilobium tetragonum* subsp. *tetragonum*. The rhizosphere of *Rubus ulmifolius* and *Silene latifolia* showed the highest ERI values, thus suggesting a particular adaptability of the latter two species to PTE contamination, in agreement with Moreira *et al.* (2011) who reported the same tolerance in a site contaminated by Pb, As and Ni.

The Cu concentration in shoots ranged from 9 to 56 mg kg^{-1} with the highest value in *S. latifolia*, that showed concentrations above values recorded by Kabata-Pendias (2011) (21 mg kg^{-1}) along with *D. glomerata*, *C. arvense*, *E. repens* and *B. nigra*. Total

Table 4. Correlations between biodiversity markers for plant communities, ERI, pseudototal, bioavailability and other soil properties.

	Shannon index	Evenness index	Species number	Poaceae	Fabaceae	Asteraeae	Miscellaneous species	Plant cover
ERI	–0.84**	–0.88**	–0.71*	–0.47	–0.33	–0.54	–0.68*	0.37
Cu (mg kg^{-1})	–0.58	–0.53	–0.58	–0.33	–0.33	–0.55	–0.47	0.04
Cu DTPA (mg kg^{-1})	+0.20	+0.21	+0.14	–0.01	–0.22	0.07	0.27	–0.54
Pb (mg kg^{-1})	–0.80**	–0.84**	–0.68*	–0.46	–0.30	–0.51	–0.65	0.37
Pb DTPA (mg kg^{-1})	–0.41	–0.46	–0.18	+0.48	–0.48	–0.49	–0.16	–0.05
Zn (mg kg^{-1})	–0.82**	–0.79*	–0.81**	–0.66	–0.23	–0.59	–0.73*	0.32
Zn DTPA (mg kg^{-1})	+0.47	+0.39	0.46	–0.03	–0.35	0.73	0.39	–0.47
As (mg kg^{-1})	–0.63	–0.68	–0.54	–0.43	–0.18	–0.36	–0.53	0.37
As DTPA (mg kg^{-1})	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Cd (mg kg^{-1})	–0.85**	–0.89**	–0.72*	–0.47	–0.33	–0.55	–0.69*	0.37
Cd DTPA (mg kg^{-1})	–0.22	–0.23	–0.07	0.29	–0.33	–0.32	0.01	–0.32
Cr (mg kg^{-1})	–0.55	–0.59	–0.42	–0.32	–0.37	–0.33	–0.35	–0.06
Cr DTPA (mg kg^{-1})	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Tl (mg kg^{-1})	–0.13	–0.07	–0.09	0.21	–0.55	–0.31	0.00	–0.31
Tl DTPA (mg kg^{-1})	–0.59	–0.60	–0.49	–0.24	–0.05	–0.53	–0.39	0.16
Ph-H_2O	–0.50	–0.48	–0.43	–0.06	–0.04	–0.55	–0.36	0.22
EC (μS cm^{-1})	–0.42	–0.62	–0.20	–0.36	–0.39	0.12	–0.24	0.17
Carbonates (g kg^{-1})	–0.50	–0.61	–0.35	–0.29	–0.31	–0.08	–0.43	0.41
OC (%)	+0.38	+0.30	+0.46	0.20	–0.38	0.56	0.39	–0.50
Total N (%)	+0.48	+0.50	+0.35	–0.12	–0.17	0.65	0.25	–0.29

**Significant at the 0.01 level; *significant at the 0.05 level; n.d., not detectable.

Cu concentrations in roots ranged from 15 to 70 mg kg^{-1} with the highest accumulation in *S. latifolia*.

Zn shoot concentrations ranged from 23 to 69 mg kg^{-1}, with the maximum in *D. glomerata*. Zn shoot concentrations above the value of 31.5 mg kg^{-1}, recorded by Kabata-Pendias (2011) in plants grown in polluted soils, were reported for all the species except for *A. vulgaris*, *E. tetragonum*, *R. ulmifolius* and *B. nigra.* Zn concentration in the roots ranged from 13 to 97 mg kg^{-1}, with the maximum concentration observed for *E. tetragonum*.

Shoot Cd concentrations ranged from 0.2 to 9.4 mg kg^{-1}, with the maximum concentration in *A. annua*. All plant species except *A. vulgaris, D. viscosa and E. tetragonum* accumulated Cd above the threshold of 1.0 mg kg^{-1} for forage (EU Reg. 1275/2013), suggesting a potential pollutant transfer to food chains in nearby areas by soil resuspension. Cd concentrations in roots ranged from 0.3 to 41.2 mg kg^{-1}, the maximum value occurring in *S. latifolia*.

Pb shoot concentrations ranged from 32 to 326 mg kg^{-1}, reaching a maximum in *D. glomerata*. All plant species accumulated Pb above legal PTE thresholds in plants (EU REG 1275/2013 30 mg

Table 5. Botanical characteristics of the species collected from the site, analysis of frequency and abundance.

Species	Family	Frequency (%)
Holcus lanatus L. subsp. *lanatus*	*Poaceae*	77.8
Silene latifolia Poir.	*Caryophyllaceae*	55.6
Elymus repens (L.) Gould subsp. *repens*	*Poaceae*	44.4
Epilobium tetragonum L. subsp. *tetragonum*	*Onagraceae*	44.4
Dactylis glomerata L. subsp. *glomerata*	*Poaceae*	33.3
Dittrichia viscosa (L.) Greuter subsp. *viscosa*	*Asteraceae*	33.3
Artemisia annua L.	*Asteraceae*	22.2
Artemisia vulgaris L.	*Asteraceae*	22.2
Cirsium arvense (L.) Scop.	*Asteraceae*	22.2
Rubus ulmifolius Schott	*Rosaceae*	22.2
Ballota nigra L. subsp. *meridionalis* (Bég.) Bég.	*Lamiaceae*	11.1
Sorghum halepense (L.) Pers.	*Poaceae*	11.1

Table 6. PTE concentrations (mg kg^{-1} d.w.) in plants and rhizo-soils of the native species.

Species		As	Cd	Cr	Cu	Pb	Tl	Zn	ERI
Artemisia annua	Soils	17.0	4.9	51.0	107	1428	1.70	148	472
	Shoots	0.4	9.4	2.1	14	106	0.10	40	
	Roots	2.1	8.5	3.9	33	321	0.60	38	
Artemisia vulgaris	Soils	15.0	0.7	37.0	67	208	1.70	121	111
	Shoots	0.3	0.4	1.6	9	33	0.04	23	
	Roots	0.4	0.4	1.8	15	17	0.30	18	
Ballota nigra subsp. *meridionalis*	Soils	86.0	55	65	187	21135	5.90	184	5057
	Shoots	0.6	5.9	2.5	35	176	1.70	31	
	Roots	0.1	20.0	1.7	28	671	4.10	34	
Cirsium arvense	Soils	22.0	21.9	47.0	572	4663	2.10	281	1880
	Shoots	0.1	3.8	3.8	24	213	0.20	54	
	Roots	0.3	7.6	1.8	39	197	0.60	47	
Dactylis glomerata subsp. *glomerata*	Soils	54.5	50.7	47.0	155	11795	2.30	164	4167
	Shoots	0.3	3.8	7.0	44	323	0.30	69	
	Roots	2.6	17.9	3.1	47	590	0.90	53	
Dittrichia viscosa subsp. *viscosa*	Soils	13.0	2.5	52.0	224	609	1.90	313	286
	Shoots	0.1	0.8	2.0	15	47	0.10	33	
	Roots	0.9	0.3	10.2	20	16	0.40	13	
Elymus repens subsp. *repens*	Soils	70.0	59.7	52.0	477	16085	5.20	278	5102
	Shoots	0.2	5.2	2.7	28	283	1.00	44	
	Roots	1.8	26.2	2.3	38	1407	1.80	67	
Epilobium tetragonum tetragonum	Soils	14.0	2.1	49.0	160	561	1.70	235	224
	Shoots	0.1	0.2	1.6	12	32	0.03	28	
	Roots	0.1	1.8	2.5	50	101	0.20	97	
Holcus lanatus subsp. *lanatus*	Soils	15.0	5.8	46.0	159	1707	1.50	208	553
	Shoots	0.3	1.3	2.5	11	70	0.10	33	
	Roots	1.0	4.9	3.3	46	358	0.40	71	
Rubus ulmifolius	Soils	479.0	225	62.0	490	69631	2.70	439	19,604
	Shoots	0.1	2.0	2.5	14	106	0.20	29	
	Roots	0.2	3.6	2.6	32	174	0.60	48	
Silene latifolia	Soils	159.0	175	67.0	460	49647	9.40	437	14,873
	Shoots	0.9	7.7	2.9	56	217	102.50	41	
	Roots	20.7	41.2	3.1	70	3404	44.00	81	
Sorghum halepense	Soils	54.0	81.7	47.0	276	20449	1.80	267	6739
	Shoots	0.4	1.0	2.4	16	74	0.20	50	
	Roots	0.1	2.2	2.8	36	76	0.30	72	

kg^{-1}). This behaviour suggests, as for Cd, a potential Pb transfer to the food chain by dust lift. Root Pb concentration ranged from 16 to as high as 3404 mg kg^{-1}. The maximum root PTE content was reported in *S. latifolia*.

As concentrations in shoots ranged from 0.1 to 0.9 mg kg^{-1}. Despite the high concentrations of As in soils, no plant species accumulated As above the threshold for forage of 2.0 mg kg^{-1} (EU Reg 1275/2013). Concentrations of As in plant roots ranged from 0.05 to 20.70 mg kg^{-1}. The highest concentration in shoots and roots was reported in *S. latifolia.*

Cr shoot concentrations ranged from 1.6 to 7.0 mg kg^{-1}, with a maximum concentration in *D. glomerata* while Cr root concentrations ranged from 1.65 to as high as 10.20 mg kg^{-1}, the maximum being found in the roots of *D. viscosa*.

Tl concentrations ranged from 0.03 to 102.54 mg kg^{-1}, with maximum concentrations observed in *S. latifolia*. Only *S. latifolia*, *E. repens* and *B. nigra* accumulated Tl above the concentrations of terrestrial plants according to Kabata-Pendias (2011) (0.51 mg kg^{-1}). Tl concentrations in the roots ranged from 0.21 to 43.99 mg kg^{-1}, the maximum occurring in *S. latifolia*.

Almost all collected species showed higher than normal PTE concentrations. These results indicate that the species were tolerant to such metals in varying degrees. In particular, *S. latifolia* accumulated the highest concentration of As, Cd, Cu, Tl and Pb in its roots. This behaviour had already been observed in the *Silene* genus (Chaabani *et al.,* 2017; Wojcic *et al.,* 2017; Yildirim *et al.,* 2017) and some species ((*Silene vulgaris* (Moench) Garcke)) are perennial facultative metallophytes with high tolerance to multi-element polluted soils (Schat *et al.*, 1996). Such characteristics, along with a wide range of adaptation (Sloan *et al.,* 2012), make this plant genus of great interest for phytoremediation purposes (Garcia-Gonzalo *et al.*, 2017). *D. glomerata* accumulated the highest concentration of Zn, Pb and Cr in its shoots, confirming the results of Swiercz *et al.* (2015) who reported a high concentration of the three elements in a two-cycle pot experiment.

None of the species showed metal concentrations that allow them to be defined as hyperaccumulators according to the concentration criteria (100 mg kg^{-1} for Cd, Se and Tl; 300 mg kg^{-1} for Co, Cu and Cr; 1000 mg kg^{-1} for Ni, Pb and As; 3000 mg kg^{-1} for Zn; and 10,000 mg kg^{-1} for Mn) of Van der Ent *et al.* (2013) except for *Silene latifolia* which accumulated in its shoots Tl concentrations above 100 mg kg^{-1}. This result finds agreement with Escarrè *et al.* (2011). BAC_S, BAC_R and TF values are shown in Table 7. Data pertaining to the element As are not shown because none of the

Table 7. BAC_S, BAC_R and TF of native plant species.

Plant species		Cd	Cr	Cu	Pb	Tl	Zn
Artemisia annua	TF	*1.10*	0.54	0.42	0.33	0.23	*1.04*
	BAC_S	*1.92*	0.04	0.13	0.07	0.08	0.27
	BAC_R	*1.74*	0.08	0.30	0.22	0.36	0.26
Artemisia vulgaris	TF	0.89	0.89	0.59	*1.95*	0.12	*1.28*
	BAC_S	0.57	0.04	0.14	0.16	0.02	0.19
	BAC_R	0.64	0.05	0.23	0.08	0.19	0.15
Ballota nigra subsp. *meridionalis*	TF	0.30	*1.47*	*1.24*	0.26	0.41	0.91
	BAC_S	0.11	0.04	0.19	0.01	0.28	0.17
	BAC_R	0.36	0.03	0.15	0.03	0.69	0.19
Cirsium arvense	TF	0.50	*2.11*	0.62	*1.08*	0.33	*1.16*
	BAC_S	0.17	0.08	0.04	0.05	0.09	0.19
	BAC_R	0.35	0.04	0.07	0.04	0.28	0.17
Dactylis glomerata subsp. *glomerata*	TF	0.21	*2.26*	0.95	0.55	0.29	*1.30*
	BAC_S	0.07	0.15	0.29	0.03	0.12	0.42
	BAC_R	0.35	0.07	0.30	0.05	0.42	0.32
Dittrichia viscosa subsp. *viscosa*	TF	*2.52*	0.20	0.72	*2.84*	0.15	2.52
	BAC_S	0.33	0.04	0.07	0.08	0.03	0.11
	BAC_R	0.13	0.20	0.09	0.03	0.22	0.04
Elymus repens subsp. *repens*	TF	0.20	*1.14*	0.73	0.20	0.54	0.65
	BAC_S	0.09	0.05	0.06	0.02	0.19	0.16
	BAC_R	0.44	0.05	0.08	0.09	0.35	0.24
Epilobium tetragonum tetragonum	TF	0.10	0.64	0.24	0.31	0.14	0.29
	BAC_S	0.08	0.03	0.07	0.06	0.02	0.12
	BAC_R	0.84	0.05	0.31	0.18	0.12	0.41
Holcus lanatus subsp. *lanatus*	TF	0.26	0.76	0.23	0.20	0.24	0.47
	BAC_S	0.22	0.05	0.07	0.04	0.07	0.16
	BAC_R	0.85	0.07	0.29	0.21	0.29	0.34
Rubus ulmifolius	TF	0.56	0.98	0.42	0.61	0.40	0.61
	BAC_S	0.01	0.04	0.03	0.00	0.08	0.07
	BAC_R	0.02	0.04	0.07	0.00	0.21	0.11
Silene latifolia	TF	0.19	0.94	0.80	0.06	*2.33*	0.50
	BAC_S	0.04	0.04	0.12	0.00	*10.90*	0.09
	BAC_R	0.23	0.05	0.15	0.07	*4.68*	0.19
Sorghum halepense	TF	0.47	0.86	0.44	0.97	0.65	0.70
	BAC_S	0.01	0.05	0.06	0.00	0.12	0.19
	BAC_R	0.03	0.06	0.13	0.00	0.19	0.27

bioaccumulation coefficients reported values higher than one. As regards Cd, *A. annua* showed BAC_S, BAC_R and TF higher than 1, with a high concentration in the shoots. For Tl *S. latifolia* reported BAC_S, BAC_R and TF higher than 1, confirming the hyperaccumulator hypothesis of this plant species. According to our results, *S. latifolia* and *A. annua* have the potential to be used for phytoextraction of Cd and Tl, respectively. The modified bioaccumulation coefficient for the shoots ($mBAC_S$) and the modified bioaccumulation coefficient for the roots ($mBAC_R$) for each species were also calculated for Cd and Pb, the most hazardous elements (Table 8).

According to the values listed in Table 8, *D. viscosa* and *A. annua* for Cd, *A. vulgaris*, *D. viscosa* and *E. tetragonum* for Pb, reported the highest ability to accumulate the potential bioavailable fraction to their shoots ($mBAC_S$>1). Nevertheless, their use in phytoextraction is not recommended because of their low biomass produced. Indeed, for maximising the amount of PTE uptake per unit area, high biomass-yielding crops (such as *Arundo donax* L.) are preferred even if they show lower PTE concentrations (Fiorentino *et al.,* 2013; 2017).

Table 8. Soil DTPA, extracted Pb and Cd, mBACS and mBACR of native plant species.

Plant species		Cd	Pb
Artemisia annua	DTPA	3.6 (mg kg^{-1})	304.60 (mg kg^{-1})
	$mBAC_S$	*2.61*	0.35
	$mBAC_R$	*2.36*	*1.05*
Artemisia vulgaris	DTPA	0.6 (mg kg^{-1})	23.60 (mg kg^{-1})
	$mBAC_S$	0.63	*1.42*
	$mBAC_R$	0.71	0.73
Ballota nigra subsp. *meridionalis*	DTPA	60.8 (mg kg^{-1})	1660.00 (mg kg^{-1})
	$mBAC_S$	0.10	0.11
	$mBAC_R$	0.33	0.40
Cirsium arvense	DTPA	13.0 (mg kg^{-1})	852.00 (mg kg^{-1})
	$mBAC_S$	0.29	0.25
	$mBAC_R$	0.59	0.23
Dactylis glomerata subsp. *glomerata*	DTPA	25.5 (mg kg^{-1})	3103.40 (mg kg^{-1})
	$mBAC_S$	0.15	0.10
	$mBAC_R$	0.70	0.19
Dittrichia viscosa subsp. *viscosa*	DTPA	0.3 (mg kg^{-1})	31.46 (mg kg^{-1})
	$mBAC_S$	*3.03*	*1.50*
	$mBAC_R$	*1.20*	0.53
Elymus repens subsp. *repens*	DTPA	143.2 (mg kg^{-1})	6598.00 (mg kg^{-1})
	$mBAC_S$	0.04	0.04
	$mBAC_R$	0.18	0.21
Epilobium tetragonum tetragonum	DTPA	1.2 (mg kg^{-1})	26.60 (mg kg^{-1})
	$mBAC_S$	0.14	*1.19*
	$mBAC_R$	*1.41*	*3.79*
Holcus lanatus subsp. *lanatus*	DTPA	3.1 (mg kg^{-1})	382.20 (mg kg^{-1})
	$mBAC_S$	0.40	0.18
	$mBAC_R$	*1.56*	0.94
Rubus ulmifolius	DTPA	42.6 (mg kg^{-1})	2576.00 (mg kg^{-1})
	$mBAC_S$	0.05	0.04
	$mBAC_R$	*0.08*	*0.07*
Silene latifolia	DTPA	46.8 (mg kg^{-1})	1754.00 (mg kg^{-1})
	$mBAC_S$	0.16	0.12
	$mBAC_R$	0.88	*1.94*
Sorghum halepense	DTPA	1.2 (mg kg^{-1})	117.60 (mg kg^{-1})
	$mBAC_S$	0.88	0.63
	$mBAC_R$	*1.88*	0.65

D. viscosa, E. tetragonum, S. halepense, A. annua and H. lanatus were efficient at accumulating bioavailable Cd in roots ($mBAC_R$>1), while *E. tetragonum*, *A. annua* and *S. latifolia* accumulated Pb efficiently in roots ($mBAC_R$>1). The high accumulation efficiency of Cd on the part of *D. viscosa* was also reported by Barbafieri *et al.* (2011) in a mining area. Instead, *E. tetragonum* was also native of an industrial area (Moreira *et al.,* 2011) where showed a high Zn accumulation. *H. lanatus* displayed the potential for phytoremediation of a mining area (Favas and Pratas, 2015) while *S. halepense* was studied previously in a battery recycling site (Salazar *et al.* 2014) where it showed good Pb phytostabilization capacity. *S. latifolia* was only found spontaneously growing in a mining area by Escarrè *et al.* (2011) with a high accumulation of Tl in its shoots. Thus the above species may be considered suitable for Cd and Pb phytostabilization protocols aimed at revegetating the area, stabilizing the soil with their root systems. In addition, they can act as a barrier limiting wind erosion and the consequent dispersion of contaminated soil particles in the environment.

Conclusions

The composition of native vegetation can give interesting information about the distribution of contamination and of the consequent ecological risks in contaminated industrial sites. Our findings indicate that plant species diversity is negatively affected by PTE contents. *Poaceae* and *Asteraceae* species were more tolerant to contamination, while *Fabaceae* and other families were strongly affected by the very high levels of PTEs. Calculation of the ecological risk index afforded insights into the impact of contaminants on ecosystems, highlighting the fact that cadmium is more hazardous than lead despite its lower concentrations due to its higher toxicity for the biosphere.

Comparing PTE contents in plant biomass with thresholds fixed for forage crops can provide useful indications on potential PTE transfer in the food chain. Analyses of Cd and Pb accumulation in plants, above the threshold for forage, confirmed their hazardousness, suggesting that there might be a potential transfer of pollutants to the food chain through the dispersion and fallout of contaminated soil particles in surrounding farmland, thus reinforcing the need of a barrier to reduce contaminant mobility.

In addition, both analysis of PTE concentrations in plants and calculation of bioaccumulation and translocation factors allowed species suitability for PTE phytoextraction or phytostabilization to be determined. *Silene latifolia* was identified as a hyperaccumulator of Tl. *Holcus lanatus* subsp. *lanatus* and *S. latifolia* were the most frequent species on the site and also proved well adapted to the site-specific conditions, growing in areas with the highest ERI. The above two species also proved the most suitable for phytostabilization respectively of Cd and Pb, accumulating the two elements in their roots. They could therefore be used in association to increase soil cover during the summer in order to avoid generally more intense wind erosion during the dry season.

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