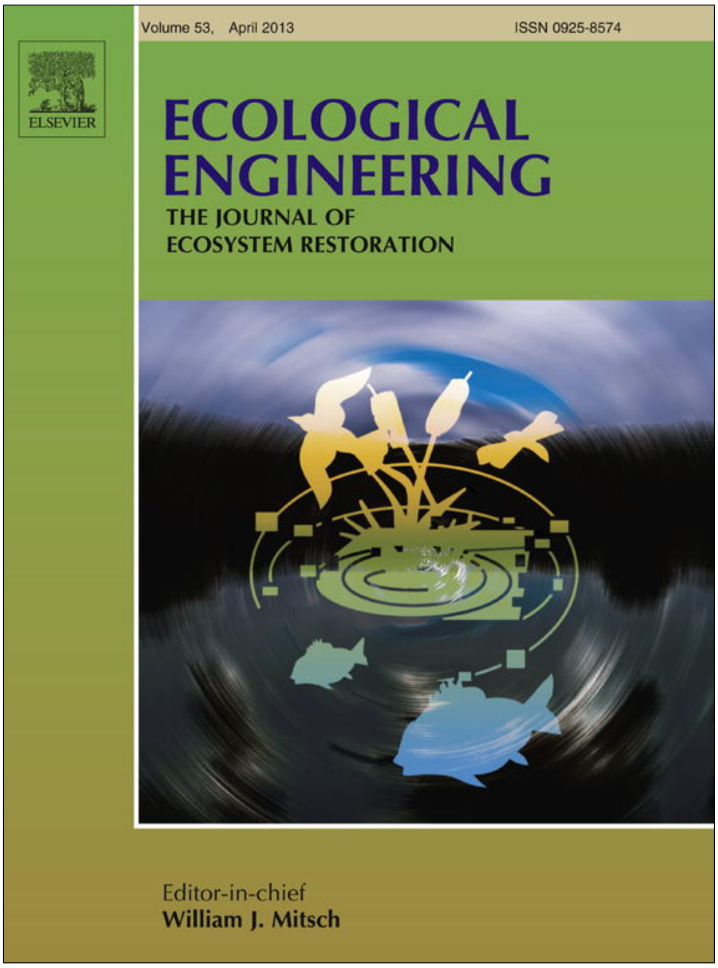
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Gentle remediation at the former “Pertusola Sud” zinc smelter: Evaluation of native species for phytoremediation purposes

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ABSTRACT

The master plan for a soil clean-up of the former zinc smelter “Pertusola Sud” (Crotone, Italy) considered gentle remediation options for a specific area where both by-products and industrial wastes had been disposed in the past. Although the soil is severely contaminated by metals (Cd, Cu, Ge, Hg, In, Pb, Tl and Zn) and metalloids (As and Sb), several plant species grow spontaneously in this area. Plants and soil samples were collected and analysed for trace element concentrations.

In the shoots of *Dittrichia viscosa* the Cd concentration (112 mg kg^{-1}) exceeded the hyperaccumulation threshold. *Phragmites australis* and *Silene bellidifolia* concentrated about 30 and 40 mg kg^{-1} of Tl in their shoots, respectively. Sb accumulation in leaves of *Acacia saligna*, *Eucalyptus camaldulensis* and *P. australis* was more than 20 times higher than herbs. The highest Zn concentration in shoots was recorded in *D. viscosa* (1172 mg kg^{-1}). The phytoremediation potential of plants was evaluated considering the concentration of metals and metalloids in the plant tissues and also the bioconcentration factor (BF) and translocation factor (TF). The plant requirements for Sb phytoextraction were verified for *P. australis* (1.66 BF, TF 9.02), and for *E. camaldulensis* (BF 1.11, TF 1.71) and *Galactites elegans* (BF 2.30, TF 1.37) for Tl. *Scirpoides holoschoenus* could be considered for phytostabilization and recommended to restore a green cover on bare soils at the Pertusola Sud site.

1. Introduction

Poorly regulated metal processing industries and inefficient industrial processes resulted in sites contaminated with metals and metalloids (Clemente et al., 2008). The traditional soil clean-up treatments are based on chemical and physical approaches (*e.g.* solidification and stabilization, soil washing, electrokinetics, redox reactions). Such treatments are usually costly, power consuming and may negatively impact soil structure and functions (Wu et al., 2010).

A number of alternative techniques utilize an *in situ*, low-invasive approach where plants are used for reducing contaminant transfer to the environment by direct extraction of pollutants (clean up) or their stabilization (inactivation). Collectively, these techniques may be referred to as “gentle remediation” (GR) options (Onwubuya et al., 2009).

A gentle remediation approach comprises two main options (ITRC, 2009). (i) Phytoextraction, typically used to tackle metals, metalloids and radionuclides, involves the use of plants to remove contaminants from soil. The metals accumulated in the aerial parts can be removed by harvesting and burning the biomass to recover metals. (ii) Phytostabilization is a mechanism that immobilizes pollutants – mainly metals – within the root zone, by adsorption, chelation and precipitation of metal ions, thus preventing migration of contaminants by erosion, leaching and runoff.

Phytostabilization might be often considered as a provisional solution until other techniques become affordable. However, for large contaminated sites (*e.g.* related to mining or industrial activities), phytostabilization is probably the only reasonable option to restore ecosystems (Schwitzguébel et al., 2009).

Gentle remediation options are ultimately based on soil-plant relationships (Chaney et al., 2007). Thus, both soil characterization and knowledge of plant traits are fundamental elements to evaluate the efficiency of a soil clean-up project *a priori*. With regard to plant species, it should always be considered that the ecological pressure due to soil pollution must be at least counteracted by using metal tolerant species adapted to the local conditions. For this reason, the

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phytoremediation potential of native plants colonizing the polluted site must be evaluated (Bidar et al., 2007; Nedunuri et al., 2009; Jiménez et al., 2011) prior to choosing other species to be used in GR. Such plants are able to perform better than other species in terms of survival, growth and reproduction as they are already adapted to the local climate and polluted soils (Yoon et al., 2006).

From 1930 onwards, "Pertusola Sud" carried out electrolytic zinc smelting by processing concentrated raw material (*i.e.* sphalerite, ZnS). The industrial process produced refined Zn, Cd, Ge and In. Production ceased in 1999. The soil within the whole area of the industrial plant is heavily polluted by several trace elements. At present, the industrial site is included within the perimeter of the polluted area named 'Crotone-Cassano e Cerchiara' identified as a site of national interest by the Italian Ministerial Decree 468/2001 'National Programme for Environmental Restoration of Polluted Sites'. The company has drawn up a master plan for the clean-up of the whole polluted site. GR options were considered for a specific area where both by-products and wastes had been disposed of in the past. A gentle remediation approach was chosen mainly to reduce the cost of soil remediation. However there is another important reason. The area is rich in archaeological finds. A campaign of archaeological investigations conducted in the mid-1970s demonstrated that a part of the industrial area lies on the site of the northern district of *Kroton*, an ancient city of *Magna Graecia* that was founded around the year 710 BC (Cavagnaro Vanoni and Linington, 1977).

A polluted industrial site in which ancient settlements are hidden underground appeared to be a paradigmatic scenario that necessarily requires GR for soil clean up.

With the ultimate goal of designing a GR project, some preliminary investigations were done on the contaminated site. The overall objectives of this research were: (i) to determine the concentrations of trace elements in plants spontaneously growing on a contaminated site, (ii) to compare metal concentrations found in the aboveground biomass to those in roots and in soils, and (iii) to assess the feasibility of using these plants for phytoremediation purposes.

2. Materials and methods

2.1. Site description and sample collection

This study was conducted in Crotone, S Italy (39°05′09″N, 17°07′06″E and 6 m above sea level) within the former Zn smelter "Pertusola Sud" property. The full site is about 50 ha divided in different working sectors. Our investigation was carried out at "Area Sottoprodotti" (By-products Area). This site is about 5 ha and was used in the past for the disposal of industrial wastes. The climate is Mediterranean, with a mean annual temperature of 17.4 °C and an average annual rainfall of 681 mm (Bellecci et al., 2003).

Before collecting plants, we verified that there were different ecological conditions in the site. Five ecologically homogeneous areas were identified. Area A is an open scrub vegetation of shallow damp sandy soil. Area B is a wet zone, whereas C and D host a commixture of annual and perennial communities. Bare soil is the distinctive trait of area E.

Classic phytosociological *relevés* (Braun-Blanquet, 1964) aimed to identify the predominant species in each area were carried out. The collected data were interpreted in terms of vegetation classification, based on cover and floristic affinities, following the Zurich-Montpellier approach and the International Code of Phytosociological Nomenclature (Weber et al., 2000), including local vegetation classification contributions (Brullo et al., 2011). Flora nomenclature followed Conti et al. (2005) and Pignatti (1982).

Table 1
Family, scientific name, life form and life cycle (Pignatti, 1982) of the collected species.

Species	Code name	Family	Ecology
Area A			
Dittrichia viscosa L.	Dit	*Asteraceae*	H[a], Pe[b]
Scirpoides holoschoenus (L.) Rchb.	Sci	*Cyperaceae*	G, Pe
Silene bellidifolia Juss	Sil	*Caryophyllaceae*	T, An
Area B			
Phragmites australis Trin.	Phr	*Poaceae*	G, Pe
Area C			
Daucus carota L.	Dau	*Apiaceae*	H, Bi
Galactites elegans Moench	Gal	*Asteraceae*	H, Bi
Piptatherum miliaceum (L.) Coss.	Pip	*Poaceae*	H, Pe
Area D			
Lathyrus odoratus L.	Lat	*Fabaceae*	T, An
Sulla coronaria (L.) Medik.	Sul	*Fabaceae*	H, Pe
Area E			
Acacia saligna (labill.) H.L. Wendl.	Aca	*Fabaceae*	P, Pe
Eucalyptus camaldulensis Dehnh.	Euc	*Myrtaceae*	P, Pe

[a] H, hemicryptophytes; T, therophytes; G, geophytes; P, phanerophytes.
[b] An, annual; Bi, biennial; Pe, perennial.

In the middle of May 2011, six specimens of each of the following species *Acacia saligna* (Labill.) H.KL. Wendl, *Daucus carota* L., *Dittrichia viscosa* L., *Eucalyptus camaldulensis* Dehnh, *Galactites elegans* (All.) Soldano, *Lathyrus odoratus* L., *Phragmites australis* (Cav) Trin. Ex Steud, *Piptatherum miliaceum* (L.) Cos, *Scirpoides holoschoenus* (L.) Rchb., *Silene bellidifolia* Juss and *Sulla coronaria* (L.) Medik, were randomly collected. Table 1 reports the life forms and life cycle of the species.

The collected plant specimens were then divided into two fractions: root apparatus and aboveground biomass. The aboveground biomass of *A. saligna*, *E. camaldulensis* and *P. australis* was in turn divided into stems and leaves. A bulk soil sample ($n = 66$) from each plant rooting zone (0 to 25 cm depth) was also collected.

2.2. Soil and plant chemical analysis

Soil samples were dried at room temperature for two days, sieved to <2 mm and their particle size measured by the Bouyoucos hydrometer method (ASTM, 1972). The pH of each sample was measured in a soil/water slurry at a 1/2.5 ratio. The electrical conductivity (EC) was measured in a soil/water slurry at a 1/2 ratio. The soil samples were oven-dried at 40 °C for 24 h and then ground to a powder; up to 10 mg of powder were weighed and hydrochloric acid (18%) was added to each sample to remove the carbonate content. The organic carbon (OC) and nitrogen (N) content were determined following Nelson and Sommers (1996). After drying overnight, each silver cup was placed in a tin cup and wrapped for CHN-analysis (Carlo Erba CHN 1500).

Plant fractions were rinsed with abundant tap water to remove dust or adhering soil and carefully washed with deionized water.

The soil and plant samples were oven-dried at 105 °C for 24 h and acid-digested in a microwave oven (CEM, MARSXpress) according to the USEPA 3051 and 3052 methods, respectively (USEPA, 1995a,b). After mineralization, both the soil and plant extracts were filtered (0.45 μm PTFE), diluted and analysed. Total content of As, Cd, Cu, Ge, Hg, In, Pb, Sb, Tl, and Zn in soils and plant extracts were determined by an ICP-OES (Varian Inc., Vista MPX).

The bioavailable fraction of elements in soil was determined following: (i) Hudson-Edwards et al. (2004) for As (NH_4OAcetate extraction), (ii) Martens and Lindsay (1990) for Cd, Cu, Pb and Zn (DTPA extraction) and (iii) Al-Najar et al. (2005) for Tl (NH_4OAcetate extraction).

2.3. Quality control

The accuracy of the analytical procedure adopted for ICP-OES analysis was checked by running standard solutions every 20 samples. Yttrium was used as the internal standard. A reagent blank and certified reference material (NIST SRM® 2710 a Montana soil and NIST SRM® 1573 tomato leaves, for soils and plants, respectively) were included for quality control of analysis.

2.4. Bioconcentration and translocation factors

For all collected plants the bioconcentration factor (BF) was calculated for each element. BF is defined as the ratio of metal concentration in shoots to that in the soil (Brooks, 1998). The translocation factor (TF) – the effectiveness of a plant in metal translocation – is defined as the ratio of element concentrations in the shoots to that in the roots (Brooks, 1998).

2.5. Data analysis

The Dixon's test was used to test the presence of outliers in the dataset. A test of normal distribution and homogeneity of variance was performed in order to choose the proper statistical tools. The assumption of normality underlying ANOVA was violated by several variables so non-parametric tests were used.

Differences between groups were first tested using non-parametric analysis of variance (Kruskal–Wallis). The Mann–Whitney *U* test with a Bonferroni correction was applied. Non parametric bivariate correlations coefficients (Spearman's test) were used when calculating correlations between metal concentrations in the soils and in the plant roots and shoots. Statistical analysis was performed using the SPSS program (SPSS Inc., Chicago, IL, USA, ver. 17). Graphics were produced using CoPlot (CoHort ver. 6.204, Monterey, CA, USA).

3. Results and discussion

3.1. Ecology of native species

The whole surveyed area consists of an environmental mosaic of artificial tree plantation and pioneer communities. The herbaceous species sampling areas (A–D in Table 1) are ascribable to four sub-nitrophilous plant communities characterized by different water availability and dynamical stage. Area A is an open scrub vegetation of shallow damp sandy loam soil. The herbaceous stand is discontinuous with high cover of tall hygro-nitrophilous perennial species such as *S. holoschoenus* and *P. australis*. The vegetation complex also includes dry zones where annual species such as *Bartsia trixago* and *S. bellidifolia*, become more frequent.

Area B is a wet zone dominated by *P. australis*. Dense cover of this species (close to 100% of the surface) and ecological factors limit the possibility of colonization for other species. The few additional species found in this area are, with low cover values, *S. holoschoenus*, *Carex otrubae* and *Arundo plinii*.

The main cover recorded in areas C and D is of *P. miliaceum* and *D. viscosa*, typical species of perennial nitrophilous communities, locally included in the *Bromo-Oryzopsion miliaceae* alliance of *Lygeo-Stipetea* phytosociological class. Annual species, such as *G. elegans*, *S. coronaria* and *Trifolium stellatum*, characteristic of therophytic sub-nitrophilous vegetation (*Echio plantaginei–Galactition tomentosae* alliance) are also common. This type of vegetation develops on secondary dry soils and can be considered a "repairing" community in abandoned fields, uncultivated ruderal areas or roadsides in the Mediterranean area. The presence of both annual and perennial plants proves the existence of a natural restoration trend in the area. Young specimens of *A. saligna* and *E. camaldulensis* were collected in a bare soil area (E) without herbaceous vegetation.

3.2. Soil characterization and trace elements

The soil samples were analysed for the soil parameters that usually influence metal mobility (Adriano, 2001) (Table 2). All soils were classified as sandy loam. No differences were recorded in either pH or CEC values between zones A–E. The pH-H_2O was slightly alkaline and varied from 7.97 to 8.26 which are usually recognized as conditions for less mobility of metals (Adriano, 2001). Although industrial soils may be severely impacted by pollutants, they may have a reserve of nutrients and organic matter. In our case the OC and total N percentages recorded in A–E soils were within the following ranges: 1.04–1.72% and 0.05–0.11%, respectively (Table 2). These values are not very high, so the soils were not particularly fertile.

The concentrations of the trace elements in soils are presented in Table 3. A very high spatial variability was encountered in the soil total concentration data. It is likely that this is the result of handling and burial of waste materials coming from different industrial processes. On comparing the trace elements concentration of the soil samples, area A had the highest average levels of As, Cd, Ge, Hg, Pb, Sb and Tl, whereas areas B and E showed the highest average values of Zn and Cu respectively (Table 3).

The mean concentrations of As, Cu, Hg, Pb, Sb, Tl in soil were always higher than the permissible threshold values for industrial soils by factors of 4.8, 2.6, 6.4, 5.8, 1.8 and 2.2 respectively (Table 3). The average values of Cd and Zn concentrations were much higher, being 33 and 29 times higher respectively than the threshold allowed for industrial soils.

Apart the administrative permission values, the concentrations of pollutants in our site are very high. On the other hand, smelting activities typically resulted in the emission of large quantities of trace elements in the environment. One of the most documented cases of smelter contamination is represented by Palmerton zinc Superfund Site (PA, USA). Surface soil samples collected on Blue Mountain indicated the presence of concentrations of Cd from 363 to 1300 mg kg^{-1}, Pb from 1200 to 6400 mg kg^{-1} and Zn from 13,000 to 35,000 mg kg^{-1} (EPA, 2007). In our case the range of the same elements in the soil were the following: Cd 19.4–2778 mg kg^{-1}, Pb 49–25,890 mg kg^{-1} and Zn 1390–250,080 mg kg^{-1}.

Ge and In are somewhat rare elements that are associated with sulphide ores containing Cu/Pb/Zn in various combinations (chalcopyrite, $CuFeS_2$; galena, PbS; sphalerite, ZnS). Zn recovery and refinery processes represent the largest single source of both Ge and In (Alfatazi and Moskalyk, 2003; Moskalyk, 2004) and for this reason they were considered in this study. Data of Ge content of soils is very limited. A range of 0.1–50 mg Ge kg^{-1}, with a median content of 1.0 mg Ge kg^{-1} has been reported by Bowen (1979). Our average Ge concentration (1.47 mg kg^{-1}) was coherent with this reference.

With regard to In, the median content of this element in European topsoils is 0.05 mg kg^{-1}, with a range varying from <0.01 to 0.41 mg kg^{-1}(Salminen et al., 2005). In enrichment in topsoil may be related to fly ash from coal power plants and also from smelting of Pb and Zn ores (Smith et al., 1978). According to this reference, we recorded an average concentration of 18.7 mg kg^{-1} of In.

It is well known that the mobility of heavy metals in soil is strongly influenced by several factors, *e.g.*, pH, redox potential, organic matter content and water content. Bioavailability, in turn, is a function of the mobility and solubility of the elements (Brümmer et al., 1986). Therefore, it may be influenced by the same factors. Table 4 reports the bioavailability of As, Cd, Cu, Pb, Tl and Zn.

Table 2
Physical and chemical characteristics of the collected soil samples. Data are mean values and standard deviation (in brackets).

Area	Sand (%)	Clay (%)	Silt (%)	pH	EC (mS/cm)	CEC (mmol$_c$ kg^{-1})	OC (%)	Total N (%)
A (*n* = 18)	64	17.3	18.7	7.97 (0.28)	2.26 (0.96)	9.26 (3.28)	1.04 (0.40)	0.05 (0.03)
B (*n* = 6)	73.7	9.67	16.7	8.08 (0.28	1.05 (1.04)	10.8 (3.26)	1.53 (0.41)	0.07 (0.03)
C (*n* = 18)	67	16.3	16.7	8.26 (0.29)	0.66 (1.04)	14.6 3.41)	1.21 (0.38)	0.06 (0.03)
D (*n* = 12)	71	16	13	8.15 (0.21)	1.23 (1.02)	13.3 (3.56)	1.72 (0.34)	0.11 (0.02)
E (*n* = 12)	62	19.3	18.7	8 (0.2)	1.34 (1)	14.4 (3.62)	1.46 (0.33)	0.10 (0.02)

Table 3
Range, mean values and standard deviation (SD) of total concentration of trace elements in the collected soil samples. Means with different letters are significantly different from each other according to *post hoc* Mann–Whitney *U* tests with a Bonferroni correction ($P < 0.05$).

Area	As (mg kg^{-1})	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Ge (mg kg^{-1})	Hg (mg kg^{-1})	In (mg kg^{-1})	Pb (mg kg^{-1})	Sb (mg kg^{-1})	Tl (mg kg^{-1})	Zn (mg kg^{-1})
A	109–793[a]	37–1748	1015–2834	n.d.[c]–22.5	3.92–138	12.7–40.7	1145–15,605	37.6–116	11.9–70.7	15,833–48,451
	419 a	731 a	1910 a	4.98 a	65.2 a	24.5 a	8314 a	87.9 a	37.3 a	38,912 b
	148[b]	472	527	8.45	41.5	7.42	3655	25.2	15.5	7939
B	134–553	85.5–761	714–2844	–	5.37–125	5.96–31.9	2624–7371	9.03–125	11.3–34.1	11,582–250,080
	256 bc	407 a	1788 a	n.d.	33.9 ab	19.3 a	5137 ab	61.3 ab	19.9 abc	92,371 a
	168	218	805	–	46.1	9.19	1790	34.5	9	84,142
C	23.6–210	23.4–2778	127–1675	n.d.–13.1	1.44–93.5	n.d.–21.3	352–25,890	2.23–56.8	0.49–24.8	2049–28,664
	119 c	710 a	657 b	1.10 a	18.3 b	5.81 b	3443 b	19 c	7.35 c	11,819 c
	53.6	919	442	3.38	22.5	6.81	5814	14.1	9.03	7982
D	3.32–632	52.8–557	286–3493	n.d.–8.62	2.75–44.1	n.d.–41.2	1340–14,766	2.49–116	0.51–81.6	6703–55,947
	265 b	256 a	1501 a	0.72 a	17.4 b	26.5 a	6757 ab	63.2 ab	18.3 bc	27,353 bc
	192	180	966	2.49	12.9	15.3	4916	39.6	24.3	14,777
E	5.22–432	19.4–797	31.6–7233	n.d.–6.42	2.04–114	n.d.–55.1	49–13,502	3.85–107	0.50–56.1	1329–133,874
	152 bc	388 a	2540 a	0.53 a	24.5 b	17.2 a	5359 ab	47.3 b	26.3 ab	49,788 b
	130	263	1878	1.85	32.4	16.6	3701	33.9	16.6	37,722
Average A–E	242	498	1535	1.47	31.8	18.7	5802	55.7	21.8	44,048
DLgs 152/06[d]	50	15	600	–	5	–	1000	30	10	1500

[a] Range.
[b] SD.
[c] Not detected.
[d] Permissible threshold values in industrial soils.

Statistically significant differences between the investigated areas were only recorded for bioavailable concentrations of Pb and Zn (Table 4).

The correlations between the total concentrations and the extractable fractions of the elements were studied. They were found significant with the only exceptions of As and Tl. A high positive correlation was observed for Cd ($r = 0.726$), whereas for Cu ($r = 0.375$), Pb ($r = 0.466$) and Zn ($r = 0.582$) the positive correlation was moderate.

The bioavailable content as a percentage of the total metal content was generally not very high. The relative availability and consequently the comparative mobility of the elements followed the order Tl (12.5%) > Cd (11.8%) > Pb (5.95%) > Cu (5.44) > Zn (2%) > As (0.26%). However, it should be underlined that the strong

Table 4
Range, mean values and standard deviation (SD) of bioavailable elements. Means with different letters are significantly different from each other according to *post hoc* Mann–Whitney *U* tests with a Bonferroni correction ($P < 0.05$).

Area	As (mg kg^{-1})	Cd (mg kg^{-1})	Cu (mg kg^{-1})	Pb (mg kg^{-1})	Tl (mg kg^{-1})	Zn (mg kg^{-1})
A	0.15–4.08[a]	5.70–104	4.84–85.9	43.3–214	n.d.[c]–10.2	139–693
	0.47 a	57.5 a	49.6 a	93.4 b	2.76 a	546 b
	0.90[b]	24.7	21.3	51.8	3.07	117
B	0.41–3.20	10.9–42.1	15.7–122	10.3–1183	n.d.–14.4	448–1883
	1.17 a	24.3 a	78.9 a	215 ab	2.48 a	1083 a
	1.11	11.6	39.3	474	5.87	530
C	0.15–1.23	4.75–550	15–203	71.1–3760	n.d.–7.26	54.5–1292
	0.46 a	106 a	74.2 a	642 a	3.80 a	579 b
	0.29	129	55.1	948	2.66	424
D	0.14–0.98	17.2–126	26.2–574	45.9–1075	n.d.–7.90	147–1697
	0.59 a	53.5 a	121 a	574 ab	2.62 a	972 a
	0.18	28.8	148	376	2.63	484
E	0.08–0.6	8.49–89.6	7.46–345	13.9–1366	n.d.–11.7	224–1801
	0.40 a	52.6 a	134 a	201 ab	3 a	1235 a
	0.25	25.1	103	372	3.60	506

[a] Range.
[b] SD.
[c] Not detected.

soil metal pollution of the studied site makes even low percentage values potentially harmful for the environment. Bioavailable Zn is only 2% of total Zn but this corresponds to almost 900 mg kg^{-1} bioavailable Zn. At last, we verified a positive correlation between organic carbon and bioavailable forms of respectively Cd (r = 0.292), Cu (r = 0.424) and Zn (r = 0.434) confirming the results provided by Vega et al. (2004).

3.3. Trace elements in plants

Metals (Cd, Cu, Hg, Pb, Tl and Zn) and metalloids (As and Sb) concentrations in plant tissues collected from the polluted area (shoots and roots, separately) are shown in Figs. 1 and 2. Analysis of variance and the *post hoc* test showed some significant differences between the species in terms of accumulation of trace elements in the plant fractions.

Despite the high concentrations in soil, very low As contents were found in plant species. The concentrations of As varied from 0.46 to 8.20 mg kg^{-1} (mean 2.83 mg kg^{-1}) and from 0.22 to 2.66 mg kg^{-1} (mean 0.96 mg kg^{-1}) for plant roots and aboveground biomass, respectively (Fig. 1). *S. coronaria* was clearly the main As accumulator in the roots and shoots, taking up significantly (P < 0.05) higher levels of the element. *E. camaldulensis* resulted as the most efficient As excluder species, having the significantly lowest As average concentrations in the roots (Fig. 1).

The Cd concentration in roots ranged between 9.05 mg kg^{-1} (*P. australis*) and 417 mg kg^{-1} (*P. miliaceum*) (Fig. 1). This element was translocated to the plant shoots at different intensity in the species (P < 0.05). In fact, while *D. viscosa* showed a similar Cd concentration in roots and shoots (106 and 122 mg kg^{-1}, respectively), significantly lower values than in roots were detected in the shoots of other species. The average Cd concentration observed in the aerial plant fraction of *G. elegans*, *Daucus carota*, *S. bellidifolia* and *S. holoschoenus* was 45.3 mg kg^{-1}; mean Cd concentration in *L. odoratus*, *S. coronaria*, *P. miliaceum* and *P. australis* was 7.76 mg kg^{-1} (Fig. 1). The leaves of *E. camaldulensis* accumulated a significantly higher amount of Cd than *A. saligna* (15.7 *vs.* 6.90 mg kg^{-1}) (Fig. 1).

Cu has a low mobility in plants in comparison to other elements; therefore, according to Kabata-Pendias and Pendias (2001), the majority of this metal is retained in the roots (Fig. 1). Higher mean root Cu concentration was recorded for *S. holoschoenus* (232 mg kg^{-1}), whereas for other species data ranged from 4.49 mg kg^{-1} for *G. elegans* to 724 mg kg^{-1} for *P. miliaceum* (Fig. 1). Nevertheless, the lowest Cu accumulations in shoots were recorded in *D. carota* and *P. miliaceum* (5.28 and 5.22 mg kg^{-1}, respectively). Significantly higher Cu average values (P < 0.05) in the shoots of the species were recorded in *D. viscosa*, *S. holoschoenus* and *S. bellidifolia* (55.9, 44.7 and 29.5 mg kg^{-1}, respectively).

The behaviour of plants in the accumulation of Hg was not clear (Fig. 1). Hg content in plant tissues varied from n.d. to 12.7 mg kg^{-1} (detected in the roots of *P. miliaceum*). With regard to the trees, *A. saligna* accumulated a significantly lower Hg concentration in the leaves than *E. camaldulensis*. Although grown in a polluted soil, the Hg concentrations recorded in the plant fractions were not very high. The average value of Hg concentration recorded in plant roots (3.44 mg kg^{-1}) was about 10% of the total soil concentration of this element.

The highest average Pb root concentrations were recorded in *S. holoschoenus* and *D. viscosa* (930 and 569 mg kg^{-1}, respectively). Moreover, these species translocated a similar amount of this metal to the aerial parts: 162 and 173 mg kg^{-1} Pb (Fig. 2). Lower concentrations were recorded for other species.

Antimony is a metalloid that shares some chemical properties with As. These elements are often associated in polluted soils (Feng et al., 2011). In the roots of *A. saligna*, *E. camaldulensis* and the shrub *D. viscosa* the concentrations were about 105, 74 and 25 mg kg^{-1} of Sb, respectively (Fig. 2). As a reference, the Sb content in higher

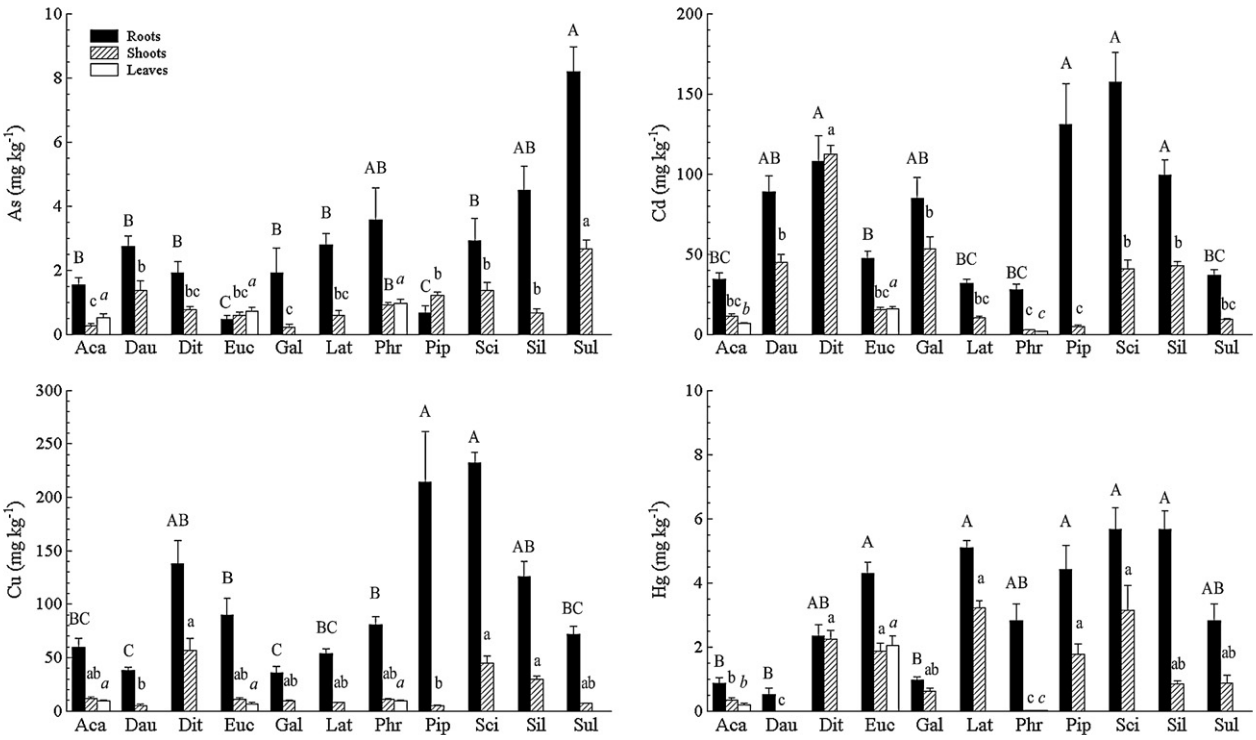

Fig. 1. Concentration of As, Cd, Cu and Hg in roots and aboveground biomass of *Acacia saligna* (Aca), *Daucus carota* (Dau), *Dittrichia viscosa* (Dit), *Eucalyptus camaldulensis* (Euc), *Galactites elegans* (Gal), *Lathyrus odoratus* (Lat), *Phragmites australis* (Phr), *Piptatherum miliaceum* (Pip), *Scirpoides holoschoenus* (Sci), *Silene bellidifolia* (Sil) and *Sulla coronaria* (Sul). Error bars on columns are standard errors (n = 6). Error bars with different letters indicate significant differences among plant species at P < 0.05.

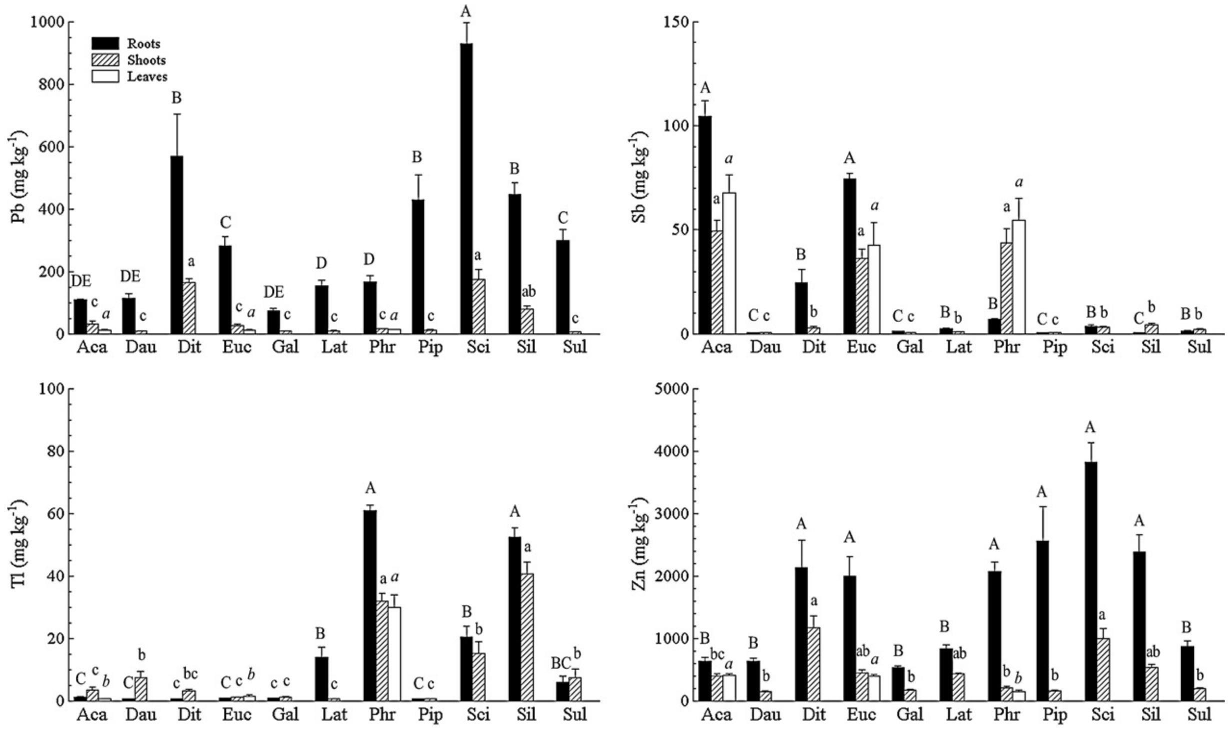

Fig. 2. Concentration of Pb, Sb, Tl and Zn in roots and aboveground biomass of *Acacia saligna* (Aca), *Daucus carota* (Dau), *Dittrichia viscosa* (Dit), *Eucalyptus camaldulensis* (Euc), *Galactites elegans* (Gal), *Lathyrus odoratus* (Lat), *Phragmites australis* (Phr), *Piptatherum miliaceum* (Pip), *Scirpoides holoschoenus* (Sci), *Silene bellidifolia* (Sil) and *Sulla coronaria* (Sul). Error bars on columns are standard errors ($n = 6$). Error bars with different letters indicate significant differences among plant species at $P < 0.05$.

plants was found to range from 0.2 to 50 mg kg^{-1} (Brooks, 1972). In the aboveground biomass of the trees, the Sb content was substantially half that in the roots. Finally, *P. australis* showed an interesting ability in root to shoot Sb translocation, being the average concentration of this element in the stem/leaves about six times higher than that detected in roots (Fig. 2).

The highest concentrations of Tl in plant roots were found in *P. australis* (60.9 mg kg^{-1}) and *S. bellidifolia* (52.4 mg kg^{-1}). These values are about three times higher than the Tl toxicity thresholds reported by Krämer (2010). Significantly lower values were recorded for the other species in which the Tl range was between 0.55 and 20.3 mg kg^{-1}, recorded in *D. viscosa* and *S. holoschoenus*, respectively (Fig. 2). Focusing on Tl translocation, *P. australis* and *L. odoratus* showed that they were not particularly able to mobilize Tl to the plant aboveground biomass. Conversely, in *A. saligna*, *D. carota* and *D. viscosa* most of the Tl was accumulated in the aerial parts. It should be noted that *P. australis* and *S. bellidifolia*, on average, concentrated about 30 and 45 mg kg^{-1} of Tl in their shoots, respectively (Fig. 2).

Zinc content in plant fractions varied from 185 to 8870 mg kg^{-1}, 67.4 to 3327 mg kg^{-1} and 54.2 to 627 mg kg^{-1} in roots, stems/trunks and leaves, respectively (Fig. 2). As occurred for Cd, Cu and Pb in the roots the maximum average value was recorded in *S. holoschoenus* (3825 mg kg^{-1} Zn). In *A. saligna*, *D. carota*, *G. elegans*, *S. coronaria* and *L. odoratus* root Zn contents below 1000 mg kg^{-1} were recorded. The highest Zn concentration in shoots was recorded in *D. viscosa* (1172 mg kg^{-1}).

Correlation coefficients and statistical *P*-values between metal concentration in soil (total and bioavailable fraction) and in plant fractions (roots and shoots) of studied species are given in Table 5. Usually, metal concentrations in plant tissues correlate poorly with the total metal concentration in the soil (Kabata-Pendias and Pendias, 2001). In contrast, this is mostly correlated with the extractable metal content of the soils (Robinson et al., 1998; Bose and Bhattacharyya, 2008) however significant correlations of plant metal concentrations with total soil metal concentrations are not unusual in soils with a large range of metal concentrations (Steinbörn and Breen, 1999; García-Salgado et al., 2012).

In our case contradictory results were observed. A significant positive correlation was observed for soil total concentrations of As ($r = 0.342$, P 0.0049), Cd ($r = 0.604$, P 0.0000), Cu ($r = 0.362$, P 0.0028), Hg ($r = 0.288$, P 0.0192), Pb ($r = 0.658$, P 0.000) and Zn ($r = 0.464$, P 0.0001) and those in plant roots (Table 5). Significant correlations between total metal concentration and plants shoots were observed for Cd ($r = 0.406$, P 0.0023), Cu ($r = 0.445$, P 0.0007), Pb

Table 5
Correlation among total and bioavailable trace element concentrations in soil and in plant fractions.

Element	Element	Roots		Shoots	
		SRCC[a]	*P* value	SRCC	*P* value
Total	As	0.342	**	−0.079	ns
	Cd	0.604	****	0.406	**
	Cu	0.362	**	0.445	***
	Hg	0.288	*	0.004	ns
	Pb	0.658	***	0.467	***
	Sb	0.081	ns	−0.063	ns
	Tl	0.080	ns	0.274	*
	Zn	0.464	***	0.472	***
Bioavailable	As	0.13	ns	0.020	ns
	Cd	0.541	***	0.422	**
	Cu	0.188	ns	−0.193	ns
	Pb	−0.047	ns	−0.370	**
	Tl	−0.038	ns	−0.223	ns
	Zn	0.202	ns	0.087	ns

[a] Spearman Rank's correlation coefficient. ns, not significant.
* $P < 0.05$.
** $P < 0.01$.
*** $P < 0.001$.

($r = 0.467$, P 0.0004), Tl ($r = 0.274$, P 0.0446) and Zn ($r = 0.472$, P 0.0003). With regard to bioavailable metals, a significant positive correlation was noted in the case of Cd for both roots ($r = 0.541$, P 0.0000) and shoots ($r = 0.422$, P 0.0014), whereas a negative correlation was observed for Pb and plan shoots ($r = -0.369$, P 0.0059) (Table 5).

Plants absorb a number of elements from the soil; some of them are referred to as essentials, certain are known as micronutrients (Cu, Fe, Mn, Mo, Ni and Zn) because they are required by plants in small quantity. Other elements (Ag, Al, Au and Co), albeit not essential, have proven to have a stimulatory effect on plant growth. The "standard reference plant" has the following elemental concentrations (mg kg^{-1}): As 0.1, Cd 0.05, Co 0.2, Cu 10, Hg 0.02, Ni 1.5, Pb 1, Sb 0.1, Tl 0.02 and Zn 50 (Dunn, 2007).

Growing in moderately polluted soils plants can also absorb elements which have no known biological function and are toxic at low concentrations (As, Cd, Cr, Hg, Tl and Pb). When absorbed above certain threshold values even micronutrients become toxic for plants (Peralta-Videa et al., 2009). Critical toxicity levels of trace elements in plants are (mg kg^{-1}): As 2–80, Cd 6–10, Cu 20–30, Hg 8, Pb 30–300, Sb < 2, Tl 20, Zn 100–300 (Adriano, 2001; Kabata-Pendias and Pendias, 2001; Krämer, 2010).

Most of the collected plant species showed concentrations above the toxic levels for Cd, Cu, Sb, Tl and Zn. In addition, although grown in a soil also contaminated by As, Hg and Pb (Table 3) they did not show toxic concentrations of these elements in their fractions.

With regard to the metal uptake, Baker (1987) classified plants into three categories: (i) excluders, that grow in metal-contaminated soil and maintain the shoot concentration at low level avoiding root-to-shoot transport; (ii) indicators, that are able to regulate metal uptake and transport to the shoot so that internal concentration reflects soil levels, at least until toxicity occurs; (iii) accumulators, that concentrate metals in the aerial parts.

The extreme level of metal tolerance in higher plants is represented by hyperaccumulators, a restricted group of plants that contain >100 mg kg^{-1} of Cd, >1000 mg kg^{-1} of Ni, Co, Cr, Cu, Pb and Tl, >10,000 mg kg^{-1} of Zn (Baker and Brooks, 1989; Krämer, 2010) in their tissues. Recently van der Ent et al. (2012) critically revised the threshold values for hyperaccumulation previously proposed by Baker and Brooks (1989). It was proposed to lower the threshold for hyperaccumulation of Tl to 100 mg kg^{-1} in dried plant leaves, the thresholds for hyperaccumulation of Co, Cr and Cu to be lowered to 300 mg kg^{-1} in dried plant leaves, and the one for hyperaccumulation of Zn to 3000 mg kg^{-1}. Although further laboratory tests are necessary for confirming hyperaccumulation, we report that *D. viscosa* – with an average shoot Cd concentration of 112 mg kg^{-1} – exceeds that threshold (Fig. 1).

Metal tolerance refers to specific individuals or populations (genotypes) of a species that are able to withstand greater amounts of toxicity than their immediate relatives on normal soil (Antonovics et al., 1971; McNair et al., 1999). Although none of the species considered in this study was reported in the literature as hyperaccumulator, having colonized a strongly metal polluted soil, they are assumed to have a potentially high level of metal tolerance.

Metal accumulations in some of the plant species included in this work have been studied by other authors. Freitas et al. (2004) reported As concentration of about 0.6 mg kg^{-1} in leaves and twigs of *E. camaldulensis*, respectively. Baroni et al. (2004) reported As concentration of 4.40 and 2.97 and 3.71 mg kg^{-1} in leaves of *G. elegans*, *D. viscosa* and *P. australis*, respectively. Relatively higher As levels in several plants – not As hyperaccumulators – were found in mine wastes in the UK (6640 mg kg^{-1}; Porter and Peterson, 1975), northeast Portugal (60–300 mg kg^{-1}; de Koe, 1994), and geothermal area of New Zealand (1766 mg kg^{-1}; Robinson et al., 2003). In general, As uptake by plants is largely dependent on the source, chemical speciation and pedological factors affecting As-bioavailability.

Our data on effective Cd translocation observed in *D. viscosa* is consistent with recent literature data. Conesa et al. (2006) found respectively 1.06 and 5.66 mg kg^{-1} of Cd in roots and shoots of specimens collected in a Mediterranean salt marsh polluted by mining wastes, whereas Barbafieri et al. (2011) reported Cd concentration ranges of 5–10, 5–17 and 10–44 in roots, stems and leaves, respectively.

With regard to Cu our data on *D. viscosa* are in contrast with those provided by Conesa et al. (2006) who reported a higher Cu accumulation in shoots than in roots (19 *vs.* 5.60 mg kg^{-1}). Freitas et al. (2004) reported Cu concentrations (mg kg^{-1}) of 23.8 and 22.7 respectively, in leaves and twigs of *E. camaldulensis* growing in a mining site. Kabas et al. (2012) reported a Cu accumulation of 16.1 and 19.7 mg kg^{-1} in roots and shoots of plants of *P. miliaceum* grown in amended mine tailings. Other data on Cu accumulation by native species in polluted sites were provided by Bech et al. (2012) and Ha et al. (2011).

Mercury is one of the most toxic heavy metals and does not have any known biological function. In general the availability of Hg in soil to plants is low, and there is a general tendency to Hg accumulation in the roots, indicating that roots serve as a barrier to Hg uptake (Beauford et al., 1977; Patra and Sharma, 2000) whereas the Hg content in above-ground parts of plants is largely dependent on foliar uptake of Hg^0 from the atmosphere (Millhollen et al., 2006). As most plants, our species stored the metal mainly in the roots; this was particularly evident in *D. carota* and *S. bellidifolia* (Fig. 1). The results on *P. australis* confirmed the findings provided by Afrous et al. (2011) and Anjum et al. (2012). The only exception was represented by *D. viscosa*. However, Hg concentration in our plants was under the toxicity limits for plants (Adriano, 2001).

Thresholds for Pb hyperaccumulation were set at 1000 mg kg^{-1} Pb in shoots (Baker and Brooks, 1989). We did not find any value exceeding this limit. In our species Pb was poorly accumulated in the aerial parts and, to a lesser extent, in the leaves of *A. saligna* and *E. camaldulensis*. However Pyatt (2001) reported that some *Eucalyptus* species were tolerant to Pb concentration of 500 mg kg^{-1}. Moreover, it was verified that the interaction between arbuscular mycorrhizal and saprobe fungi with *Eutetramorus globosus* promotes plant growth in polluted soils and supports Pb hyperaccumulation by increasing Pb storage in stems where the expected harmful effects on plant metabolism are minor (Arriagada et al., 2005). Kabas et al. (2012) reported concentrations (mg kg^{-1}) of Pb of 350 and 20.4 respectively in roots and shoots of *P. miliaceum* growing in tailing ponds. Our data on *P. miliaceum* confirmed the findings of Conesa et al. (2006) but are contrasting with those provided by Melendo et al. (2002) that reported higher lead concentration in the shoots of *P. miliaceum* (981 mg kg^{-1}).

Elevated levels of Sb in plant tissues have been reported in contaminated areas. Contents from 110 to 900 mg kg^{-1} in grasses growing around Pb and Sb smelters were reported by Ainsworth et al. (1991). Murciego Murciego et al. (2007) reported Sb concentration of 22.4–1136 in plants of *D. viscosa* collected in a mine site. More recently, Pérez-Sirvent et al. (2012) reported Sb concentration not exceeding 12 mg kg^{-1} in leaves of plants *D. viscosa* growing on mining soils. Robinson et al. (2008) and Tschan et al. (2009) demonstrated that atmospheric deposition of Sb onto plant surfaces may be a dominating pathway in the soil-to-plant transfer of Sb under field conditions, so this could explain in part the high variability of literature data. According to Anawar et al. (2011) and Bech et al. (2012), among the studied species, herbaceous plants have been shown to possess a Sb-excluding mechanism; the average Sb concentration recorded in the roots ranged from 0.64 to 10.3 mg kg^{-1}, which, however, is five times higher than the toxicity threshold (2 mg kg^{-1}; Krämer, 2010). While herbs seem to exclude Sb, *E. camaldulensis*, *A. saligna* and *P. australis* accumulated respectively 49.2, 36.3 and 43.5 mg Sb kg^{-1} in the aerials fraction. Since we are comparing herbs with trees a possible explanation could be that the trees accumulate much more Sb because they are able to explore a greater volume of soil. If this were true, we should observe the same result also for other elements. However, this never occurred.

To our knowledge, no data on Tl accumulation in either species have been published up to now. However, two species belonging to the genus *Silene* were reported to be Tl tolerant or accumulator. In specimens of *S. vulgaris* collected in an industrial area in southern Poland, Wierzbicka et al. (2004) recorded 14 and 7 mg kg^{-1} of Tl in roots and shoots, respectively. A paper by Escarré et al. (2011), describing the heavy metal content in native species of the Les Malines Mining District (Southern France), reported values of Tl concentrations of up to 1489 mg kg^{-1} in *S. latifolia*.

As for Zn, it is likely that in the conditions of the surveyed site the species studied use a Zn-exclusion strategy (Fig. 2). After all, it should be remembered that "Pertusola Sud" was formerly a Zn smelter and refinery that processed Zn sulphides as raw material. A very high Zn concentration in soil was therefore expected. Indeed, we found a mean concentration of bioavailable Zn of about 880 mg kg^{-1} Zn (Table 3), which is a very high value for plants (Kabata-Pendias and Pendias, 2001), although it represents less than 3% of actual total soil Zn (Table 3). Our data are consistent with those provided by similar studies. Jiménez et al. (2005) found more than 2000 mg kg^{-1} Zn in leaves of *D. viscosa* plants collected in the mining sites of Inglesiente (Sardinia, Italy). Barbafieri et al. (2011) reported 200–370, 210–430 and 770–2900 mg kg^{-1} Zn respectively in roots, stems and leaves of specimens sampled near the abandoned Ingurtosu mine (Sardinia, Italy). Conesa et al. (2006) reported 638 mg kg^{-1} Zn in the shoots of specimens of *D. viscosa* colonizing mine tailings (soil total Zn 5320 mg kg^{-1}; extractable EDTA Zn 834 mg kg^{-1}). Kabas et al. (2012) reported concentrations (mg kg^{-1}) of Zn of 662 and 520 in roots and shoot of plants *P. miliaceum* collected in mine tailings. In the leaves of the deciduous trees *A. saligna* and *E. camaldulensis* about the same Zn concentrations were recorded (respectively 403 and 390 mg kg^{-1}). These values were higher than those observed in the evergreen *P. australis*.

The role of Ge and In in plant physiology has been less studied than other trace elements. Although Ge is not known to have any biological function and is generally considered to have a low toxicity, Kabata-Pendias and Pendias (2001) reported Ge concentrations in plants ranging from 50 to 754 mg kg^{-1}. Smith et al. (1978) reported that In root-toxicity occurs in various plants at 1–2 mg kg^{-1} In concentration in culture solution. In content from vegetation collected in unpolluted soils ranged from 0.03 to 0.71 mg kg^{-1}, whereas in plants collected in industrial areas the values were 0.008–2.1 mg kg^{-1}. In our case, Ge concentration in plant fractions was always no detectable (d.l. 0.033 mg kg^{-1}). The high variability recorded in the soil concentration of In was reflected in an even more evident manner in plant fractions. In roots and shoots of plants the In concentration ranged between n.d.–11.9 mg kg^{-1} and n.d.–18.6 mg kg^{-1}, respectively.

3.4. Potential for phytoremediation of native species

Phytoremediation comprises technologies that use higher plants to clean up and revegetate contaminated sites (Cunningham and Berti, 1993). With regard to metals/metalloids the main technologies are phytoextraction and phytostabilization. Phytoextraction refers to the use of plants to remove metals/metalloids from contaminated site by translocating them to their aboveground matter. An ideal plant for phytoextraction should possess the following traits: (a) metal tolerance, (b) fast growth and highly effective metals/metalloids accumulating biomass, (c) accumulation of trace elements in the aboveground parts, and (d) easy to harvest (Vangronsveld et al., 2009). Phytostabilization is not a soil clean up technology as it focuses on sequestration of the metals within the roots and rhizosphere, but not into the aboveground plant tissues. The use of soil amendments strongly reduces the availability of the pollutants to plant uptake and thus limits eventual toxicity to plants, allowing revegetation of contaminated sites (Mendez and Maier, 2008).

The third aim of this work was to assess the feasibility of using these plants for phytoremediation purposes, therefore both the bioconcentration factor (BF) and translocation factor (TF) were assessed as they are strong indicators of a plant's potential for phytoremediation. Plants that efficiently take up and translocate metals and metalloids in the shoot biomass have BF and TF values greater than 1 and are used for phytoextraction (Fitz and Wenzel, 2002). In plants used for phytostabilization BF and TF values would be $\ll$1 but they should not exceed a ratio of 1 indicating that a given species is unable to extract large amounts of metal from the soil and translocate it to the shoots (Mendez and Maier, 2008).

A metal tolerant species can grow on soils with concentrations of a particular element that are toxic to most plant species (McNair et al., 1999). The species we studied grow in a heavily contaminated soil and therefore can be considered metal tolerant. Moreover, as they are easily found in unpolluted surrounding areas they can be comprised in the group of pseudometallophytes (Pollard et al., 2002).

As a general rule, a metal tolerant non hyperaccumulating plant retains most of the heavy metals taken up from the polluted soil in root tissues, minimizing the potential risks to its metabolism through metal-chelation or storage in vacuoles. Conversely, one of the typical traits of hyperaccumulators is the very high BF due to their active metal sequestration and concentration in the shoot *via* xylem pathway (Rascio and Navari, 2011; van der Ent et al., 2012). Although none of the species studied are known as hyperaccumulators, nor have concentrations of As, Sb and metals been detected above the hyperaccumulator thresholds – excluding Cd in *D. viscosa* – some data are rather interesting.

Table 6 reports the values of BF for each species. Among them, BF values were >1 for only two elements. For Sb, the values were: 1.48, 2.02 and 1.47 in *A. saligna*, *P. australis* and *P. miliaceum*, respectively. In the case of Tl BF values > 1 were recorded for *E. camaldulensis* (1.12), *G. elegans* (2.30), *P. australis* (1.85) and *S. bellidifolia* (1.21). For other elements, almost all species showed BF values <0.5; the only exception being BF 0.56 recorded for Cd in *D. viscosa*. In Table 7 are reported the TFs data. In general, the element root to shoot translocation varied in the following order (mean TF values in brackets): Tl (3.35), Sb (2.71), Hg (0.63), Zn (0.56), Cd (0.49), As (0.32), Cu (0.32) and Pb (0.28) (Table 6). A number of TF values >1 were recorded for these elements but a statistical significance of these data was not verified by ANOVA.

As regards the phytoremediation potential, as expected, none of the species were proved to be potentially useful for the accumulation of all the metals/metalloids in the soil. In fact, the potential for phytoextraction was verified only for *P. australis* for Sb (BF 1.66, TF 9.02), and for *E. camaldulensis* (BF 1.11, TF 1.71) and *G. elegans* (BF 2.30, TF 1.37) for Tl. But the soil at the Pertusola Sud is polluted by several elements, so according to our data, none of these species might be able alone to clean up the polluted soil. The fact that plants could be effective for some elements but not for others was neither surprising nor expected. On the other hand, also hyperaccumulators – the most efficient metal-accumulating plants – are specialized for a single element whereas only some examples of

Table 6
Bioconcentration factor (BF) for As, Cd, Cu, Ge, In, Hg, Pb, Sb, Tl and Zn in the studied species. Means with different letters are significantly different from each other according to *post hoc* Mann–Whitney *U* tests with a Bonferroni correction ($P < 0.05$). Values > 1 are in bold font.

	BF = $[\text{element}]_{\text{shoot}}/[\text{element}]_{\text{soil}}$							
Species	As	Cd	Cu	Hg	Pb	Sb	Tl	Zn
Acacia saligna	0.002 a	0.06 b	0.01 b	0.03 c	0.01 b	**1.48** a	0.09 c	0.02 b
Daucus carota	0.02 a	0.11 b	0.02 ab	0.000 d	0.01 b	0.18 a	0.73 b	0.01 b
Dittrichia viscosa	0.003 a	0.56 a	0.03 a	0.25 a	0.06 a	0.04 cd	0.71 b	0.05 a
Eucalyptus camaldulensis	0.01 a	0.10 b	0.01 b	0.34 a	0.03 a	0.83 b	**1.12** a	0.05 a
Galactites elegans	0.003 a	0.34 ab	0.03 a	0.12 b	0.01 b	0.04 a	**2.30** a	0.02 b
Lathyrus odoratus	0.004 a	0.05 b	0.02 ab	0.05 a	0.03 ab	0.27 bc	0.67 b	0.02 b
Phragmites australis	0.01 a	0.01 c	0.01 b	0.001 d	0.003 c	**2.02** a	**1.85** a	0.00 c
Piptatherum miliaceum	0.09 a	0.07 b	0.01 b	0.29 a	0.003 c	**1.47** a	0.57 b	0.02 b
Scirpoides holoschoenus	0.01 a	0.06 b	0.01 c	0.13 c	0.000 d	0.73 bc	0.50 b	0.01 a
Silene bellidifolia	0.001 a	0.07 b	0.02 ab	0.02 c	0.01 b	0.36 bc	**1.21** a	0.02 b
Sulla coronaria	0.01 a	0.03 bc	0.01 b	0.29 b	0.004 bc	0.03 cd	0.57 b	0.02 b

Table 7
Translocation factor (TF) for As, Cd, Cu, Ge, In, Hg, Pb, Sb, Tl and Zn in the studied species. Means with different letters are significantly different from each other according to *post hoc* Mann–Whitney *U* tests with a Bonferroni correction ($P < 0.05$). Values > 1 are in bold font.

	TF = $[\text{element}]_{\text{shoot}}/[\text{element}]_{\text{roots}}$							
Species	As	Cd	Cu	Hg	Pb	Sb	Tl	Zn
Acacia saligna	0.15 a	0.40 b	0.32 b	0.44 b	0.34 b	0.46 a	**1.59** b	0.73 b
Daucus carota	0.44 a	0.51 b	0.16 bc	n.c.	0.08 b	0.99 a	**11.4** a	0.27 b
Dittrichia viscosa	0.43 a	**1.80** a	**1.38** a	**1.77** a	**1.73** a	**4.01** a	**5.89** ab	**2.56** a
Eucalyptus camaldulensis	0.26 a	0.41 b	0.16 bc	0.50 b	0.13 b	0.49 a	**1.71** b	0.31 b
Galactites elegans	n.c.[a]	0.66 b	0.47 b	0.89 ab	0.11 b	0.61 a	**1.37** b	0.37 b
Lathyrus odoratus	0.53 a	0.28 bc	0.19 b	0.50 b	0.10 b	0.68 a	0.58 c	0.70 b
Phragmites australis	0.46 a	0.12 c	0.15 bc	0.01 c	0.13 b	**9.02** a	0.55 c	0.12 b
Piptatherum miliaceum	0.27 a	0.36 c	0.27 b	0.65 b	0.06 b	0.96 a	0.97 c	0.20 b
Scirpoides holoschoenus	0.35 a	0.29 bc	0.11 bc	0.25 b	0.19 b	**2.44** a	0.85 c	0.28 b
Silene bellidifolia	0.13 a	0.48 b	0.27 b	0.24 b	0.19 b	**7.10** a	0.71 c	0.29 b
Sulla coronaria	0.24 a	0.07 d	0.06 c	**1.25** a	0.02 b	**2.99** a	**1.12** a	0.31 b

[a] Not calculated.

metal co-accumulation (*i.e.* the ability of a plant to take up unusually high levels of two or three metals), were observed by Homer et al. (1991) and Reeves and Baker (2000).

As for the potential of phytostabilization, if we consider the experimental data separately for each element then we can verify that all species have the requisites for As phytostabilization. With the exception of *D. viscosa*, all other species have the potential for Cd, Cu, Pb and Zn phytostabilization. However, if we consider the actual conditions of the soil of Pertusola Sud, we verified that only *L. odoratus* has the potential for phytostabilization since BF and TF were <1 for each element.

While most of the species partitioned a major part of metals in their roots, *D. viscosa* showed a very interesting behaviour, especially taking into account the soil conditions of the Pertusola site. The TFs of *D. viscosa* were >1 for Cd (1.80), Cu (1.38), Hg (1.77), Pb (1.73), Sb (4.01), Tl (5.89) and Zn (2.56) (Table 7). A greater proportion of these elements tend to be allocated to the aboveground biomass after root-uptake. It is likely that this species is naturalized to the contaminated matrix in which it had germinated and grown and that its metabolism could tolerate the multi-metal contamination showing adaptation to such a disturbed environment. Although *D. viscosa* showed a particularly high ability to accumulate the pollutants (Cd in particular) in the aerial parts, its BFs were always lower than 1. Therefore, it cannot be categorized *sensu stricto* as a potentially suitable species for phytoextraction. However, it should be considered that BF strongly depends on the denominator term and this sometimes could produce misinterpretation of experimental data particularly for plants growing in soil with high metal concentrations (van der Ent et al., 2012). This is the case of Pertusola Sud, in fact *D. viscosa* was collected in a soil with 731 mg kg^{-1} and 57.5 mg kg^{-1} of respectively total and bioavailable Cd (area A, Tables 3 and 4) and we recorded about 112 mg kg^{-1} Cd in the shoots. For these reasons, we also considered *D. viscosa* as a potentially useful species for Cd phytoextraction.

However, for this species, and for all deciduous species potentially useful for phytoremediation it must be considered that leaf fall would return the metals to the soil, rendering the previous stages of metal uptake, transport and storage of contaminants within plant tissues useless. Moreover, it was proved that this process induces soil acidification that might give rise to metal mobilization and belowground dispersion of metals (Castiglione et al., 2009; Van Nevel et al., 2011; Evangelou et al., 2012). These findings indicate that a special attention has to be paid to the fate of trace elements in the litter fall.

Lastly, special consideration must be devoted to the grasses *P. miliaceum* and *S. holoschoenus*. Both species were able to tolerate high concentrations of several metals and were capable of avoiding soil-shoot transfer of pollutants. The BF calculated for *P. miliaceum* was <1 for all the elements but for Sb (BF 1.47) whereas TF was <1 for all the elements confirming the findings of Conesa et al. (2006). *S. holoschoenus* had both BF and TF < 1 for all the elements. The requirements for phytostabilization were always verified and, according to Otones et al. (2011) *S. holoschoenus* could be considered for phytostabilization and recommended to restore a green cover on bare soils at the Pertusola Sud site.

4. Conclusions

This study was conducted to screen plants growing on a heavily polluted industrial site to determine their potential for phytoremediation purposes. Multi-element contaminated soils contain several pollutants, so it is necessary to screen out plants that can accumulate different pollutants simultaneously.

According to the field investigation, despite the condition of severe soil multi-metal contamination some plants could vegetate and absorb a wide range of soil metals (Cd, Cu, Ge, Hg, In, Pb, Tl and Zn) and metalloids (As and Sb).

None of the species assessed in this investigation displayed high values in terms of the hyperaccumulation thresholds in aboveground biomass for different metals. However, it was shown that the metal uptake ranges are encouraging when compared with reported toxic concentrations in plants, making them potentially useful for future gentle remediation strategies.

The potential for phytoextraction was demonstrated in *P. australis* for Sb, and in *E. camaldulensis* and *G. elegans* for Tl. *S. holoschoenus* behaved as a potential phytostabilizer species. The performances and potential role of *D. viscosa* were discussed.

Due to their notable metal excluding capacity and low accumulation in aboveground parts, most of the other species can be considered adequate candidates for revegetation of barren areas of the polluted site.

In addition to the investigations on native plants, other trials – both in pot and field conditions – are currently underway in order to design the specific GR strategy at the former "Pertusola Sud" smelter site.

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