



Assessment of metal accumulation capacity of *Dittrichia viscosa* (L.) Greuter in two different Italian mine areas for contaminated soils remediation



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ABSTRACT

The study aimed at assessing the capacity of *Dittrichia viscosa* (L.) Greuter to absorb and accumulate in its tissues some potentially harmful elements (PHEs), as Cd, Cu, Fe, Ni, Pb and Zn, in order to verify its possible use as phytoremediation in polluted mine soils. Plants from two different Italian mine areas, Montevecchio and Libiola, were considered and compared with plants from unpolluted areas. In each site ($n = 21$), both rhizospheric soil materials and *D. viscosa* plants were sampled, suitably prepared and analyzed. Soil samples were examined for both total composition (XRF) and bioavailable fractions (DTPA-extraction). *Dittrichia viscosa* roots, stems and leaves were analyzed separately to quantify the metal presence in the different plant parts. The root/soil ratio values calculated on the basis of the total soil concentrations, displayed a generally scarce capacity of *D. viscosa* to absorb soils PHEs, especially those more concentrated in the substrata. Concerning the root/soil ratio calculated on the basis of DTPA-extractable soil metals, values displayed instead a greater capacity of *D. viscosa* to absorb metals in the roots thus behaving as a potential phytostabilization plant in both mine areas.

Regarding the leaf/root ratio, this species also displayed a greater capacity, in sub alkaline mine areas, to shift PHEs from roots to aboveground tissues, along with Ca transfer, revealing interesting characteristics for phytoextraction. In sub acid mine areas, this tendency was instead weaker and PHEs tended to be accumulated in all plant parts. *Dittrichia viscosa* seems to be appropriate for mine soil stabilization in both sub acid and sub alkaline environments and also for phytoextraction in the latter.

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1. Introduction

A large number of human activities lead to environmental pollution. Among these, mine exploitation generates a great amount of abandoned tailings with high concentrations of Potentially Harmful Elements (PHEs) which persist for long periods of time and could be released in neighboring areas adversely affecting soil, water and wildlife (Barbafieri et al., 2011). Furthermore, these contaminated heaps provide unfavorable substrate for plant growth (Martínez-Sánchez et al., 2012) thus showing a lack of vegetation cover and therefore subjected to erosion, increasing the risk to human and animal health. In these cases, phytoremediation represents a useful approach that can provide a cost-effective, long-lasting and aesthetic solution for the remediation of contaminated sites. Phytoremediation is defined as the use of plants for containment, degradation or extraction of xenobiotics from water or soil substrates (USEPA, 2000). The plants used in this activity have

to face different kind of stress (multi-stress resistance) represented not only by the presence of contaminants but also by unfavorable climatic conditions like drought and heat, especially in Mediterranean areas (Poschenrieder et al., 2012). Plants eligible for phytoremediation are often native of the environment in which they are supposed to be used (Yoon et al., 2006) and have a high growth rate to absorb considerable quantities of toxic elements in their tissues (Mendez and Maier, 2008).

Plants useful for phytostabilization should accumulate trace elements in their roots and not transfer them to their shoots to avoid further transfer into the food chain (Mendez and Maier, 2008). Instead, an ideal plant for phytoextraction should possess the following features: i) metal tolerance; ii) fast growth and highly effective metals/metalloids accumulating biomass; iii) accumulation of trace elements in the above-ground parts; iv) easy to harvest.

We focused our attention on *D. viscosa* (L.) Greuter, a hemicryptophyte with remarkable pioneer characteristics, without clear microhabitat restriction. It is a common perennial herbaceous plant in Mediterranean regions, it grows rapidly and it is well adapted to a wide range of environmental stresses (Parolin et al., 2014). It has the ability to colonize highly contaminated soils and is comprised in the group of pseudometallophytes. *Dittrichia viscosa* has already been considered for

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bio-remediation purposes (e.g. Barbafieri et al., 2011; Marchiol et al., 2013) however there is still a lack of knowledge upon its behavior with respect to metals uptake and translocation in different environments. In this respect, the objectives of the study were to assess plant metals accumulation in unpolluted and polluted areas to highlight possible differences also with respect to major elements absorption and accumulation.

On this basis, we examined the destination of some metals inside *D. viscosa* plant parts (i.e. roots, stems and leaves) collected in two different Italian mining districts, Montecvecchio (MON) and Libiola (LIB), differing mainly in pH and PHEs content, and from unpolluted sites (UPS). Both mines closed their activities a few decades ago but uncontrolled mine-tailings still remain and the risk of pollution to both water and wind erosion is on going. The concerned tailings surfaces are so large that it is unthinkable to proceed with their remediation in a conventional way. Therefore, the use of *D. viscosa* could represent a useful tool to minimize mine waste erosion and PHEs dispersion.

In every one of the 21 sampling sites major soil parameters together with total and DTPA-extractable amounts of Cd, Cu, Fe, Ni, Pb and Zn were determined. The absorption of metals by *D. viscosa* was assessed through the calculation of the root/soil metal concentration ratio,

considering both total (Badr et al., 2012; Conesa et al., 2011; Martínez-Sánchez et al., 2012; Pérez-Sirvent et al., 2008; Wu et al., 2015) and DTPA-extractable (Mendoza et al., 2015; Nirmal Kumar et al., 2009; Pérez-Sirvent et al., 2008) soil metal concentrations.

The metal translocation to the aerial part of *D. viscosa* was estimated via the calculation of the leaf/root metal concentration ratio (Martínez-Sánchez et al., 2012; Nadimi-Goki et al., 2014).

Finally the presence of feasible synergistic or antagonistic effects with other soil elements (e.g. Ca and Mg) in the PHEs uptake and translocation inside the plants, were also evaluated.

2. Materials and methods

2.1. Site description

Three areas of study were considered: UPS, MON and LIB (Fig. 1). The unpolluted sites include several sampling locations ($n = 8$) in the eastern Emilia-Romagna region (Fig. 1a), which extends from the Apennine hills to the southeastern Po Plain. The soils developed on different types of sedimentary materials. The bedrock of the upstream sites is made up

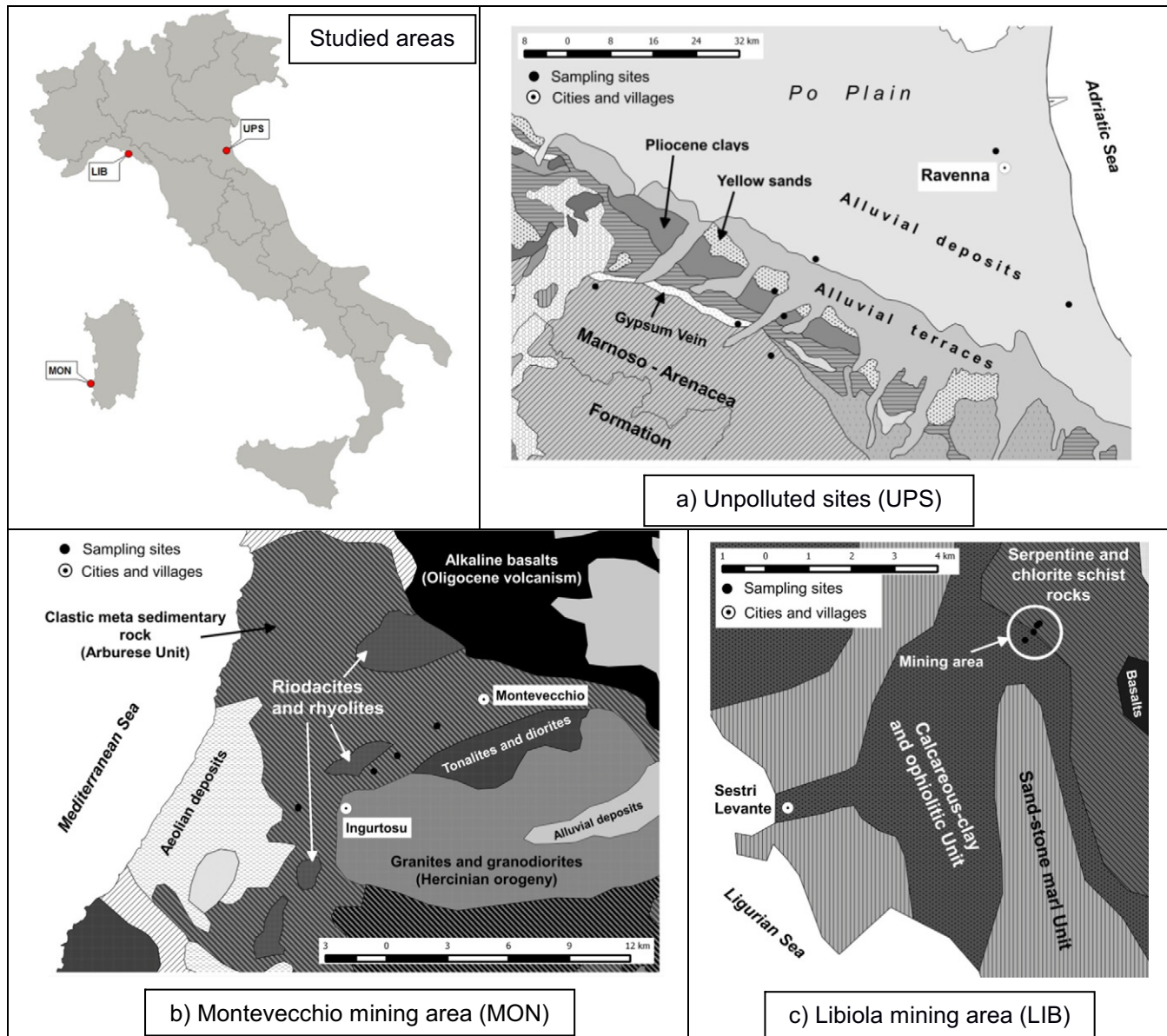


Fig. 1. Localization of the three studied areas and sampling sites with their main geological outlines.

of alternations of sandstones and marls followed by a thin band of evaporitic gypsum (Gypsum Vein), formed during the Messinian salinity crisis. Close to the plain there are Pliocene clays and Pleistocene yellow sands while, the plain itself consists of Pleistocene and Holocene alluvial sediments (Amorosi et al., 1999). Eight sampling sites were chosen: two on the Marnoso-Arenacea Formation, two on Pleistocene clays, one on yellow sands and three in the plain. All plants were found and collected in scarcely urbanized areas and roadsides.

Two mines were considered. The Montevecchio mining district is located in Southwestern Sardinia, close to Montevecchio and Ingurtosu villages (Fig. 1b). The bedrock consists of low-grade meta-sedimentary and meta-volcanic Cambrian-Ordovician rocks, intruded at the end of the Hercynian orogeny by the Arburese igneous complex. This igneous complex is made up of granodiorite and leucogranite but it is also characterized by radial fractures filled by acid and basic magmatic dykes, and by quartz and metalliferous hydrothermal deposits that formed the Montevecchio-Ingurtosu ores. The ore-veins are mainly composed of galena, sphalerite and quartz with a local occurrence of carbonate minerals. From 1848 to 1991, when mining activities ceased, approximately 3 Mt of Pb and Zn were exploited from the Montevecchio mining district. Nowadays several uncontrolled waste rock piles characterize the landscape and represent important sources of contamination (Caboï et al., 1993; Concas et al., 2006) due to the scarce vegetation cover and intense erosion. In this mining area, nine sampling sites were identified, grouped into four zones, corresponding to the major mine-tailings deposits, as shown in Fig. 1b. Samplings ($n = 9$) were gathered at each zone, at the top and at the bottom of the mine-tailings deposits to provide a better characterization of the site.

In the Libiola mining district (Fig. 1c), located 8 km north of Sestri Levante in the eastern Liguria region, Cu and Fe ores were extracted. The mineralization is associated to Jurassic ophiolites that consist of an ultramafic/gabbroic basement overlaid by a volcano-sedimentary sequence. In the Libiola area tectonic and sedimentary-ophiolitic breccias, pillow basalts, and cherts can be recognized. The ore consists of pyrite and chalcopyrite, with minor sphalerite and pyrrhotite, typical of a strata bound volcanic-associated massive sulphide deposit. Non-valuable sulphide stockwork-veins, stringer ores and disseminated mineralizations are also present in the pillow basalts and serpentinised ultramafites. The Libiola mining site was exploited from 1864 until 1962 and produced over 1 Mt of Fe-Cu sulphides with an average grade ranging from 7 to 14 wt% Cu (Marescotti et al., 2008). Non-mineralized rocks (mainly basalts and serpentinites), non-valuable mineralizations (low-grade chalcopyrite and pyrite mineralizations) and tailings deriving from mechanical grinding, milling and handpicking were dumped in five open-air waste-rock dumps and in several smaller bodies close to the main mine adits. Acid Mine Drainage is present in the area and controls the precipitation of several secondary minerals, mainly Fe-oxides, Fe-oxyhydroxides and sulphate salts (Carbone et al., 2013). In this area, four sampling sites were chosen on the main tailings.

2.2. Sampling and analytical procedures

At each of the 21 sampling sites, entire *D. viscosa* plants and rhizospheric soils, sampled at a depth ranging between 5 and 30 cm, were collected. Both plants and soils were conserved in plastic bags. Soil material was air-dried at room temperature for two weeks, crushed and sieved through a 2 mm screen, and this fraction was retained for analysis. Soil reaction (pH) and electrical conductivity (EC) were determined in a 1:2.5 (*m/V*) soil-water suspension. The total limestone was determined by volumetric method according to ISO 10693 method (ISO, 1995). The cation exchange capacity (CEC) was determined by extraction with BaCl₂ solution buffered at pH 8.2 according to ISO 11260 method (ISO, 1996). From BaCl₂ extracts, the exchangeable cations (Ca, Mg, K and Na) were analyzed. The extractable metals concentrations (Cd, Cu, Fe, Ni, Pb and Zn) were obtained by extraction with a DTPA solution, according to Lindsay and Norvell (1978) as reported in

ISO 14870 method (ISO, 2001). This methodology is widely applied in metal extraction in non acid soils and in our case it fitted perfectly for UPS and MON sites, whereas could be more critical at LIB since the sub-acid reaction of the soil. However, in order to obtain comparable results, DTPA extraction was adopted for all soil samples. Major elements and trace metals total concentrations were determined by X-Ray Fluorescence (XRF) on pressed powder pellets using a Panalytical Axios 4000 spectrometer, following Franzini et al. (1972) and Leoni and Saitta (1976) for matrix correction methods. Volatile content was determined by Loss of weight On Ignition (LOI) after overnight heating at 950 °C (Heiri et al., 2001). The plant samples were separated into roots, stems and leaves, placed in an ultrasonic bath to remove soil particles, washed with deionized water, dried in a stove at 40 °C and minced. For major elements and trace metals total content determination, 250 mg of each plant part was digested with a mixture of 2 ml H₂O₂ 30% and 6 ml HNO₃ 65% using a microwave Milestone mls 1200 Mega. The digested solutions were filtered and stored in 50 ml volumetric flasks. The soils' exchangeable cations and plants' major elements (Ca, Mg) were analyzed by Flame Atomic Absorption Spectrometry (FAAS) using a Perkin-Elmer AAnalyst 100. Metals in the DTPA solutions and in the plants were analyzed by Graphite Atomic Absorption Spectrometry (GFAAS) using a Perkin-Elmer HGS-800.

2.3. Data quality control and statistical analysis

Reagent blanks and certified reference materials were used to ensure accuracy and precision in metal analysis. Quality control of soils DTPA-extractable metals was performed analyzing the NCSDC85102a certified reference material, obtaining the following recoveries (mean value \pm standard deviation in %): Cd 86 ± 1 , Cu 81 ± 2 , Fe 90 ± 1 , Ni 87 ± 2 , Pb 62 ± 3 , Zn 76 ± 1 . Quality control of plants total metals concentrations was performed analyzing the IAEA - 359 (Cabbage - Trace elements) certified reference material with the following recoveries (mean value \pm standard deviation in %): Cd 153 ± 2 , Cu 97 ± 2 , Ni 126 ± 9 , Zn 93 ± 10 . The analyses of the two certified materials were replicated four times. To verify the normal distribution of the data, the Shapiro Wilk test (Shapiro and Wilk, 1965) was adopted. For the evaluation of the statistical significance of the difference between the medians, a Mann-Whitney non parametric test was performed both between the different sampling sites and the different plant tissues. To evaluate the correlation between the variables, the Spearman rank correlation coefficient (Spearman, 1904) was used, assessing the significance of the correlations obtained with the critical values of the correlation coefficient, related to p -value < 0.05 and < 0.01 . The statistics processing was performed using the R software version 2.12.2.

3. Results and discussion

3.1. Soils

The main characteristics of the soils are reported in Table 1. Unpolluted sites showed neutral-alkaline pH values, similar to those of the Montevecchio mining district. Whereas, the Libiola mining district rhizospheric samples displayed lower pH values likely due to the presence of acid generating phases and the absence of minerals able to neutralize soil acidity (Marescotti et al., 2008). Electrical conductivity values were very low in all sites while total lime was significant in UPS and negligible in both MON and LIB areas. Cation exchange capacity was very low in the MON area. Whereas similar but greater values were found in the UPS and LIB areas. Between these last two areas, there was, however, a great difference in the metals present. In fact, while in UPS Ca and secondly Mg were the most represented exchangeable cations, in LIB, the most abundant exchangeable cations were likely represented by H, followed by Mg and Ca.

In UPS the major elements such as Si, Al, Ca and the LOI had the highest values, reflecting the siliceous and calcareous composition of

Table 1
Soils main characteristics in sampling areas. Median, minimum and maximum values of selected parameters. Unpolluted area (UPS) ($n = 8$); Montevecchio mining area (MON) ($n = 9$); Libiola mining area (LIB) ($n = 4$).

Areas		UPS		MON		LIB		
		Median	Min–max	Median	Min–max	Median	Min–max	
Reaction in H ₂ O	pH	8.08	7.94–8.44	8.03	7.12–8.9	5.06	4.57–7.00	
EC	dS·m ⁻¹	0.26	0.14–0.63	0.26	0.11–0.87	0.17	0.11–0.26	
Total lime	g·kg ⁻¹	200	92.9–368	0	0.00–46.9	0	0.00–0.00	
Exchangeable complex	CEC	18.3	10.04–22.9	3.61	2.46–4.88	19.4	16.6–23.2	
	Ca	13.95	10.25–17.7	1.42	0.68–2.13	2.21	1.15–5.67	
	Mg	2.51	1.10–6.11	0.95	0.51–2.89	3.78	1.22–5.51	
	Na	0.41	0.20–1.00	0.26	0.03–0.33	0.08	0.00–0.26	
	K	0.78	0.73–1.03	0.19	0.11–0.24	0.07	0.05–0.28	
Major elements and LOI	Si	196,896	163,519–244,064	280,199	270,289–321,756	154,708	141,782–169,877	
	Ti	3446	2697–3836	4615	2158–6233	7702	6773–9290	
	Al	67,609	45,514–78,749	102,247	47,683–130,349	64,089	55,463–71,552	
	Fe	32,872	24,269–36,579	36,648	23,500–53,434	144,741	124,143–159,393	
	Mn	1007	774–1239	1549	1317–2711	1046	697–1471	
	Mg	22,295	19,416–27,316	9708	7432–11,449	85,430	57,511–107,323	
	Ca	95,269	46,955–173,599	6003	1930–24,657	8612	4574–11,006	
	Na	6250	5059–9003	8333	4315–18,154	4092	2604–4762	
	K	17,558	12,618–20,837	38,104	15,690–49,560	3238	1411–4068	
	P	676	567–873	567	393–655	785	567–1309	
	LOI	%	20.33	13.49–22.72	4.99	1.71–5.67	17.45	14.75–22.46
	Total metals	Cu	32	22–63	70	18–282	1875	1370–3100
		Ni	64	52–80	41	27–49	338	244–455
		Pb	21	17–36	500	95–3500	46	15–82
Zn		78	55–95	5800	4500–10,500	316	145–510	
DTPA-extractable metals		Cd	0.08	0.04–0.20	4.97	0.82–6.65	0.17	0.06–0.36
	Cu	2.5	0.6–7.9	5.3	0.5–11.1	72.9	61.2–179	
	Fe	9.5	5.1–22.6	5.93	1.2–18.4	52.9	26.3–78	
	Ni	0.4	0.2–1.1	0.4	0.2–0.9	4.7	3.8–11.6	
	Pb	1.2	0.75–3.04	16.6	6.1–247	1.6	0.49–6.3	
	Zn	1.2	0.14–2.41	516	185–628	6.9	1.1–22.6	

LOI: Loss of weight on ignition. Overnight heating at 950 °C to determine the loss of weight corresponding to volatile content (Heiri et al., 2001).

the substrate. In MON Si, Al, Fe and K prevailed testifying to the abundance of sheet silicates. Whereas LIB was characterized by high concentrations of Fe and Mg as well as Si and Al, related to the abundance of secondary Fe-rich mineral phases, serpentines and basalts rock fragments (Dinelli et al., 2001). The high LOI content was related to the Fe-oxyhydroxides water content and to serpentine minerals.

The UPS samples had a generally low median total concentration of metals, in line with those reported in the GEMAS project (Reimann et al., 2014). The MON sites were characterized by high Zn and Pb contents comparable to those previously described in the area (Barbafieri et al., 2011) while the LIB sites were characterized by high Cu, Ni and Zn concentrations similar to those reported by Maescotti et al. (2008).

Regarding DTPA-extractable metals concentrations, generally low values (except Fe) were observed in UPS. The MON area was characterized by high DTPA-extractable Zn, Pb and to a lesser extent Fe and Cd. While at LIB, high DTPA-extractable Cu, Fe and secondly Zn were observed. These results clearly reflected the peculiarities of each contaminated site, in line with what has been reported by other authors (Marchiol et al., 2013).

Table 2 reports the DTPA-extractable/total metals ratios, expressed as a percentage, used as a rapid tool to evaluate the metals' behavior. The percentages were generally low, especially in regards to Fe, although in some samples they reached a maximum of up to 42% as observed for Pb at the LIB areas. The differences between the three areas were mostly limited and statistically significant only for Zn in MON compared to the other two areas and for Ni in LIB compared to UPS. No systematic order in metal availability was observed, however, Pb and Cu seemed to be slightly more bioavailable independently from the soil conditions in the three areas.

3.2. Plants

Table 3 presents a statistical summary for the investigated elements in the different plant parts of *D. viscosa* collected in the three study

areas. Generally, plants in the unpolluted sites showed the lowest metals content with the exception of Ca, Cu and Ni. Plants collected in MON had a significantly different metals content than the one sampled in both UPS and LIB areas mainly for Cd, Pb and Zn concentrations. While plants collected at LIB differed from the other sites mainly for Cu, Fe, Ni and Mg content.

In the majority of the cases the leaf concentrations were higher in comparison to the other plant parts, although with some exceptions. While stem concentrations were, in general, the lowest with statistically significant differences only for Cu in UPS and Fe in LIB area.

In UPS sites, leaf metals concentrations (except Pb) were higher in comparison to both roots and stems contents. Calcium, Mg, Fe and Zn leaf concentrations were statistically different from stem and root content. Whereas for Cd, Cu and Ni the differences were not significant. These results proved the natural tendency of the plant to concentrate metals in the leaves. In regards to Pb, Fischerová et al. (2006) underlined the weak mobility of Pb in plants and its tendency to accumulate in roots rather than in the aerial tissues, a behavior also observed by Marchiol et al. (2013).

Table 2

Median, minimum and maximum DTPA-extractable/total metal ratios in soils, expressed in percentage (%). Unpolluted area (UPS) ($n = 8$); Montevecchio mining area (MON) ($n = 9$); Libiola mining area (LIB) ($n = 4$). Letters identify the areas (U for Unpolluted, M for Montevecchio and L for Libiola). Letters indicate the presence of differences, statistically significant at the 0.05 level, between percentages calculated for each element in each area according to the Mann-Whitney test. No letters means absence of statistically significant differences between areas.

Areas	Cu	Fe	Ni	Pb	Zn
UPS	7.75	0.03	0.71	6.03	1.96
	1.74–12.6	0.01–0.07	0.25–1.52	3.80–12.6	0.18–2.63
MON	6.66	0.01	1.03	5.51	7.07 L-U
	1.28–9.20	0.003–0.05	0.48–1.79	1.20–23.3	3.87–10.82
LIB	5.12	0.03	1.77 U	10.92	2.66
	2.68–5.78	0.02–0.06	0.99–2.55	0.94–42.6	0.79–4.42

Table 3

Median, minimum and maximum total concentration values (mg·kg⁻¹) of selected metals, in *D. viscosa* plants portions. Unpolluted area (UPS) (*n* = 8); Montevecchio mining area (MON) (*n* = 9); Libiola mining area (LIB) (*n* = 4). The presence of the letters r, s, l (respectively roots, stems and leaves) indicates differences statistically significant at the 0.05 level, between the analyzed plant tissues within each area, according to the Mann–Whitney test. The presence of the letters U, M, L (respectively Unpolluted, Montevecchio and Libiola areas) indicates differences statistically significant at the 0.05 level, in regards to the same plant tissue in between the areas, according to the Mann–Whitney test. No letters means absence of statistically significant differences.

Metals	Areas	Roots	Stems	Leaves	Metals	Areas	Roots	Stems	Leaves
Cd	UPS	0.15 0.05–0.42	0.15 0.04–0.32	0.21 0.06–0.55	Pb	UPS	0.41 0.04–0.70	0.21 0.04–1.68	0.25 0.04–0.71
	MON	7.32 L-U 2.33–11.82	8.19 L-U 2.69–13.54	13.01 r-s L-U 5.53–17.13		MON	18.61 U 2.66–53.12	8.06 L-U 1.62–31.17	91.38 r-s L-U 6.39–185.1
	LIB	0.86 U 0.28–2.78	1.05 U 0.25–2.62	1.37 0.19–2.62		LIB	4.97 U 1.64–51.89	0.66 0.39–2.04	1.74 U 1.40–15.58
Cu	UPS	11.28 8.34–13.03	7.25 r-l 4.89–9.85	13.93 9.12–15.65	Zn	UPS	15.3 6.72–39.3	15.3 6.82–34.9	42 r-s 26.8–85.4
	MON	7.46 s 5.23–16.04	5.35 3.53–9.99	16.47 r-s 9.90–40.30		MON	207.6 U 95.4–417.1	191 L-U 86.7–264.1	1032 r-s L-U 552–1777
	LIB	156.39 s-l M-U 135.2–398	32.42 M-U 13.58–36.07	91.27 s M-U 70.44–113		LIB	60.9 U 37.4–232	44.9 U 35.3–131	95.5 46.8–209
Fe	UPS	153 s 46.8–283	27.6 10.8–57.6	217 r-s 159–456	Ca	UPS	1434 775–3008	1552 1028–2363	13,217 r-s 7555–17,296
	MON	224 41.1–542	100 U 13.2–293	2030 r-s U 431–6668		MON	1383 879–3042	1761 1113–3689	12,310 r-s 7143–14,531
	LIB	7208 M-U 5610–10,240	1129 r-l M-U 192–1296	7114 M-U 3047–14,116		LIB	2592 1439–5367	2525 1458–3276	7881 r-s 6159–12,468
Ni	UPS	2.21 1.16–7.41	1.56 0.37–2.70	2.29 1.01–4.01	Mg	UPS	1086 400–1640	791 532–1531	2476 r-s 1644–2974
	MON	1.58 0.20–2.75	0.9 0.27–2.40	4.63 r-s U 2.41–7.75		MON	1112 545–2080	1207 U 749–3421	3785 r-s U 1144–5633
	LIB	28.55 M-U 12.60–93.35	7.95 M 1.72–27.54	35.52 M-U 7.73–69.29		LIB	5006 M-U 2207–5959	2424 U 1249–3686	7603 2567–14,108

In MON sites, all the considered metals, without distinction, were concentrated in the leaves and the differences with root and stem contents were always statistically significant. In this polluted environment, the plant enhanced its natural tendency to transfer metals to the leaves as a likely survival mechanism. As well as Ca, Mg, Fe and Zn, also Cd, Cu, Ni and especially Pb, extremely present in this mine area, were all accumulated in the leaves. Lead leaf accumulation is somehow in contrast with what has been observed in UPS as also reported by Barbaferi et al. (2011) in the same area and also by Martínez-Sánchez et al. (2012) and Pérez-Sirvent et al. (2008).

In LIB, major leaf concentrations were observed for Ca, Cd, Mg, Ni and Zn but only for Ca the difference with both roots and stems content resulted statistically significant. Copper and iron, the most bioavailable metals in this mine area, and also Pb, preferentially concentrated in the roots, but only iron had a statistically significant difference in respect to both stem and leaf content. In fact, Fe tended to be highly concentrated also in the stems and in the leaves. The tendency of *D. viscosa* to concentrate Cu in the roots was also reported by Marchiol et al. (2013) and Martínez-Sánchez et al. (2012). In these cases, Cu was likely partitioned preferentially in the roots, as a plant mechanism to improve metal tolerance (Kabata-Pendias, 2010; Remon et al., 2007).

Attention has to be dedicated to the distribution of both Ca and Mg concentration inside the plants. For Ca plant concentrations, the differences between the three areas were limited, while on the contrary, generally statistically significant leaf accumulations were detected. Plant parts concentrations in UPS and MON were similar but soil Ca content in UPS was one order of magnitude greater than in MON, with respect to both total and exchangeable fractions (Table 1). As stated by Kabata-Pendias (2010), Ca exerts an antagonistic effect on a variety of metals, which include Cd, Cu, Fe, Ni, Pb and Zn and it is likely that, in contaminated areas like MON, *D. viscosa* enhanced Ca uptake and leaf transfer occurs in order to combat the absorption of other toxic elements.

Soil Ca concentrations at MON and LIB areas were comparable, although greater at LIB. In the former, elements concentrated notably (almost double) in the plants' leaves while, in the latter, Ca was more distributed in the whole plant displaying, nonetheless, a statistically significant leaf concentration. Although the acid soil reaction in LIB, which

favors Ca availability (Table 1), Ca accumulation in the plant was less than at MON. These findings were likely linked to soil Mg concentrations and destination inside the plants.

Statistically significant Mg leaf accumulation was detected at both the UPS and MON areas, with major concentrations in the latter. However at UPS, Mg soil concentrations were two and a half times greater than at MON. As for Ca, also Mg exerts an antagonistic action on Zn, Ni, Cu and Fe absorption (Kabata-Pendias, 2010) and tended to be concentrated in the plants' leaves at the MON area, to inhibit metal absorption and translocation. It should be remembered that plants collected at MON had statistically significant leaf concentration for all metals.

Also at LIB, Mg leaf concentration was detected, but not in a statistically significant way, being more uniformly distributed inside the plant. In this case, in fact, the great Mg soil concentration, which was one order of magnitude greater than at MON, led to a significant Mg presence not only in the leaves but also in the roots and in the stems. At LIB, the contrasting effect of heavy metals absorption and translocation was performed equally by both Ca and Mg while at MON, it was preferentially performed by Ca.

The Ca/Mg ratios in soils were progressively in decreasing order at UPS > MON > LIB, the latter displaying very low values and thus major Mg availability, both in respect to total and exchangeable amounts (Table 1).

It has to be said that the metals concentration reached in *D. viscosa* leaves were always below the thresholds in order to consider a species as an hyperaccumulator according to Baker and Brooks (1989), modified by van der Ent et al. (2013). However, *D. viscosa* could be an interesting species for phytoextraction at MON, with respect to all the considered metals, and for phytostabilization at LIB, with respect primarily to Cu and secondly to Fe and Pb.

3.3. Soil-plant relationship

Correlation coefficients and statistical *p*-values between soil metal concentrations (total and exchangeable fractions) and plant concentrations (roots and leaves) are reported in Table 4. Total soil metal concentrations correlated significantly with Fe, Pb and Zn concentrations in both the plants' roots and leaves. Copper soil content correlated only

Table 4

Correlations among total and bioavailable metals concentrations in soils and in *D. viscosa* roots and leaves, in the three investigated areas. Unpolluted area (UPS) ($n = 8$); Montevocchio mining area (MON) ($n = 9$); Libiola mining area (LIB) ($n = 4$).

Fraction	Metals	Roots		Leaves	
		SRCC ^a	<i>p</i> value	SRCC ^a	<i>p</i> value
Total	Ca	0.074	ns	0.127	ns
	Cu	0.442	ns	0.935	**
	Fe	0.742	**	0.666	**
	Mg	0.437	ns	0.125	ns
	Ni	0.748	**	0.051	ns
	Pb	0.592	**	0.741	**
	Zn	0.905	**	0.870	**
Bioavailable	Ca	0.148	ns	-0.016	ns
	Cd	0.911	**	0.927	**
	Cu	0.586	**	0.897	**
	Fe	0.285	ns	0.118	ns
	Mg	0.412	ns	0.129	ns
	Ni	0.555	*	0.396	ns
	Pb	0.734	**	0.717	**
	Zn	0.877	**	0.858	**

Bioavailable: i.e. exchangeable Ca and Mg; DTPA-extractable Cd, Cu, Fe, Ni, Pb and Zn.
ns: not significant.

^a Spearman Rank's correlation coefficient.

* : $p < 0.05$.

** : $p < 0.01$.

with leaf content whereas Ni soil content correlated only with root concentrations. With respect to bioavailable soil metals concentrations, significantly positive correlations emerged for Cd, Cu, Pb and Zn in both roots and leaves. Whereas for Ni, it was noted only in root concentrations. Calcium and Mg displayed no correlation between soil and plant concentrations.

It is still not clear, among total and bioavailable soils metals' forms, which correlate better with plant contents. In fact, available literature data are differing. Marchiol et al. (2013) found that the plant tissue metals concentrations correlate with total metals concentrations in soils, better than the bioavailable metals. In contrast, Robinson et al. (1998) found that the plant metal content was strongly correlated with the bioavailable fraction in the soils.

According to Lombini et al. (1998), the positive correlations between plants metal content and soil bioavailable forms, as verified in our study, with regards to Cd, Cu, Pb, Zn and partially for Ni, should provide evidence for the usefulness of the chemical extractions in evaluating the metal availability for plant uptake. As reported later, comparisons made with soils bioavailable metals concentrations permitted a better distinction between plants' behavior in the three studied areas, but not always.

3.4. Root/soil metal concentration ratio

In order to evaluate the ability of *D. viscosa* to absorb elements in its tissues, thus behaving as a useful plant for phytostabilization or phytoextraction purposes, metals concentrations in both roots and soils were compared, applying the root/soil ratio (Table 5). This ratio was widely adopted by researchers and reported in literature with different acronyms (Badr et al., 2012; Conesa et al., 2011; Martínez-Sánchez et al., 2012; Pérez-Sirvent et al., 2008; Wu et al., 2015). In the ratio calculation, both total and bioavailable soil metal concentrations were considered because, lately, more and more authors tended to use EDTA or DTPA-extractable soil metals concentrations in the computation of plant/soil (e.g.: Khaokaew and Landrot, 2014), leaf/soil (e.g.: Poschenrieder et al., 2012) stem/soil (e.g.: Barbaferi et al., 2011) or root/soil (e.g.: Mendoza et al., 2015; Nirmal Kumar et al., 2009; Pérez-Sirvent et al., 2008) ratios.

Root/soil ratio median values calculated with respect to total soil metal concentrations were all negative and, sometimes, very low (Table 5).

At UPS, the greatest median values corresponded to Cu and Zn while the other elements displayed values one (Ca, Mg, Ni and Pb) or two (Fe) orders of magnitude lower. At the MON area, which is particularly poor in lime, *D. viscosa* showed a preferential Ca and Mg uptake, while it showed smaller ratio values for Pb and Zn, significantly abundant in this area. At LIB, Ca, Zn and Pb, scarcely present in the substrate, were concentrated in the roots compared to other more abundant elements in the soils such as Ni, Cu, Mg and Fe. These low values generally emerged from a high soil total metal content, with respect to a relatively modest concentration in the roots.

Referring to root/soil ratio values, always < 1 when considering total soil concentrations, *D. viscosa* could not be considered useful for phytostabilization, on the basis of what is stated, among others, by Fitz and Wenzel (2002) and Yoon et al. (2006).

In this respect, one consideration has to be made: other than considering a plant useful for phytoremediation on the basis of the root/soil threshold value of one, it seems that the ratio itself doesn't always furnish a reliable result because it doesn't consider metal destination in the plant. At the MON area, ratio values were low because Pb and Zn were actively transferred to the leaves. Likewise in LIB, Cu and Fe ratio values were low because of their high total amount in soil. However, in both area, a significant metal accumulation was performed by *D. viscosa* with respect to these pollutants. According to Li et al. (2007), *D. viscosa* could be considered as an accumulator species because it contains higher quantities of metals when found growing in contaminated areas.

Root/DTPA-extractable soil metals concentration ratios in *D. viscosa* were also calculated and the results appear in Table 5. The ratios' median values were in the majority of cases higher than the value of one which means that *D. viscosa* was able to accumulate significant amounts of bioavailable metals in its roots. At UPS, the median values were all > 1 (except Ca and Pb) and were particularly high in respect to Fe and Zn. Also at MON, the ratio median values were generally > 1 , particularly high for Fe and Mg while strangely negative for Pb and Zn, the most significant PHEs in this area (Table 1). The low ratio values found in the study are due on the one hand to the considerable soil metal concentrations and on the other hand to the active metal transfer to the leaves. Notwithstanding, in this mining district, *D. viscosa* accumulated two orders of magnitude more Pb and one order of magnitude more Zn and Cd in the roots, with respect to UPS (Table 3). At LIB, the root/soil ratio median values were > 1 for all the elements, reaching extremely high values for Fe and very high values for Mg and Zn. Most likely, this occurred for two main reasons: i) major soils elements concentrations and bioavailability are reached in this environment because of the soils' low pH; ii) an insignificant metal transfer to the leaves and, as a result, high root concentrations. In this mining district, *D. viscosa* accumulated one order of magnitude more Cu, Fe and Ni in the roots, in respect to UPS (Table 3).

In trying to estimate the plant potential for phytoremediation purposes, in these areas, it seemed more appropriate to consider the effective soil metal amounts available for plant uptake. In fact, as seen in Table 4, a major number of metals displayed positive soil to root correlations when considering soils' bioavailable fractions. Furthermore, it seems that a comparison between the plant's behavior in polluted and non polluted areas, instead of the simple BCF calculations, could furnish a more reliable plant evaluation.

3.5. Leaf/root metal concentration ratio

In order to evaluate the capacity of *D. viscosa* to transfer metals from the roots to its aboveground tissues and hence to be suitable for phytoextraction purposes, the leaf/root metal concentration ratios were calculated (Table 6). This ratio was extensively applied by several authors (Martínez-Sánchez et al., 2012; Nadimi-Goki et al., 2014), assuming different names such as Translocation factor, Transfer factor or Transportation index.

Table 5

Median, minimum and maximum root/soil metal concentration ratios and median, minimum and maximum root/DTPA-extracted soil metal concentration ratios of selected metals in *D. viscosa*. Unpolluted area (UPS) ($n = 8$); Montevecchio mining area (MON) ($n = 9$); Libiola mining area (LIB) ($n = 4$).

Ratio	Areas	Ca	Cd	Cu	Fe	Mg	Ni	Pb	Zn
Root/total soil	UPS	0.014	–	0.360	0.005	0.049	0.035	0.013	0.181
		0.007–0.039	–	0.140–0.584	0.002–0.009	0.023–0.082	0.015–0.108	0.002–0.038	0.084–0.428
	MON	0.275	–	0.105	0.006	0.113	0.033	0.024	0.036
		0.123–0.891	–	0.047–0.378	0.002–0.012	0.078–0.280	0.005–0.067	0.004–0.241	0.017–0.051
	LIB	0.338	–	0.089	0.052	0.056	0.097	0.175	0.255
		0.250–0.488	–	0.049–0.271	0.038–0.072	0.043–0.085	0.044–0.205	0.020–3.530	0.173–0.455
Root/DTPA-extracted soil	UPS	0.54	2.23	5.08	17.10	3.20	4.03	0.19	9.88
		0.23–0.90	0.68–3.72	1.12–14.65	5.05–52.76	1.69–6.04	2.06–23.50	0.03–0.65	3.57–108.94
	MON	6.33	1.48	1.53	24.81	9.10	3.08	0.38	0.49
		2.96–11.60	1.30–4.30	0.70–11.44	5.33–393.04	1.55–25.45	0.54–11.22	0.10–3.14	0.27–0.90
	LIB	6.07	6.16	2.37	124.48	12.40	5.81	3.71	10.17
		2.50–10.91	4.07–7.69	0.84–4.84	91.03–389.02	7.19–15.07	3.31–8.05	2.89–8.29	6.46–35.37

Leaf/root median values were often positive indicating that *D. viscosa* tended to transfer several elements from the roots to the leaves. This was always true, at all sites, with respect to Ca, Mg and Zn content. This is likely related to the synergistic effect exerted by Zn on Ca and Mg (Kabata-Pendias, 2010). At UPS, leaf/root median values were all positive except for Ni. This means that in non polluted soils, *D. viscosa* actively transfers metals from roots to leaves concentrating them in aboveground tissues. This ability was particularly evident in regards to Ca and, and to a lesser extent, all the other elements. At MON, the Cd, Pb and Zn contaminated site, these metals displayed the largest median ratio values among all the sites, and were thus actively transferred to the leaves. Therefore, this capacity could be useful as a phytoextraction strategy for soil remediation. This significant leaf metal accumulation seems to be also linked to a significant Ca and Mg increase in the leaves, since it is known that Ca exerts, in plants, an antagonistic effect with respect to many other elements (Kabata-Pendias, 2010). At LIB, apart from Ca, Mg and Zn, all the other considered elements had leaf/root ratios values <1. This means that the elements with the highest soils' concentrations (Cu, Fe and Ni) were transferred less to the aboveground tissues, but remained concentrated in the roots. As reported by Kabata-Pendias (2010) Cu, Fe and Pb are strongly bound in roots cells and this could explain the findings. In this highly polluted area, *D. viscosa* could thus be useful for phytostabilization interventions.

3.6. Ca/Mg molar ratio

The Ca/Mg molar ratio of the soil's exchangeable fractions and the molar ratios in each plant part is reported in Table 7. The ratio of these two macro elements is of importance for the plants nutritional balance, being Ca and Mg competitors, both in uptake and in translocation processes.

Due to the high soil exchangeable Ca detected at UPS, Ca/Mg ratios were higher in this area with respect to MON and LIB. Comparing soils Ca/Mg molar ratios with those in plant parts, at UPS the leaf ratio halves while, at MON, it nearly doubles. These results are evidence of a significant plant Ca accumulation at MON, likely related to metals transfer to the leaves. On the contrary, at LIB, the Ca/Mg ratio slightly decreased passing from soil to leaves, likely due to the greater soil Mg content (Table 1), the relevant root's uptake (Table 5) and the positive

translocation to the leaves (Table 6). Furthermore, at LIB, in the plants, the Ca/Mg ratios increased slightly from roots to leaves, signifying greater Mg leaf enrichment, with respect to the other two areas.

4. Conclusions

When trying to estimate the capacity of *D. viscosa* to absorb metals from the soils, it seems preferable to consider soil bioavailable fractions, which furnishes a more reliable evaluation of the plant, even if the percentage of DTPA-extractable forms varied widely in the studied areas. From our experience, it seems that the root/soil ratio itself doesn't always furnish reliable results because it doesn't consider metal destination in the plant which greatly alters the results. Instead of the simple BCF calculations, a comparison between the plant's behavior in polluted and non polluted areas, could furnish more reliable plant evaluations. *Dittrichia viscosa* could be considered as an accumulator species because it contains higher quantities of metals when found growing in contaminated areas. In this context, results from naturally unpolluted sites, revealed a considerable capacity of *D. viscosa* to absorb elements (except Ca and Pb) from the soil when considering DTPA-extractable fractions. Furthermore, leaf/root ratios were always greater than one (except for Ni), revealing a natural aptitude of this plant to transfer metals, once absorbed into the roots, to its aboveground tissues, thus being of interest for phytoextraction purposes. In non polluted environments, rich in lime, the Ca/Mg ratio halves in the leaves with respect to soils. At MON, a Cd, Pb and Zn contaminated mine area, characterized by neutral soil pH, root accumulations were significant for all metals (except for Pb and Zn which are greatly transferred to the leaves) when considering soil DTPA-extractable fractions. All considered elements were actively and greatly accumulated in the leaves and some of them (Cd, Pb and Zn) to such an extent which is considered harmful for plants; toxicity is primarily mitigated by a significant amount of Ca and, to a lesser extent, Mg leaf accumulation. In this environment *D. viscosa* could represent an interesting species for phytoextraction with respect to all the main pollutants. Results obtained at LIB, a Cu, Fe, and Ni contaminated mine area, characterized by a very strong acid to neutral soil reaction, a positive root metal enrichment was observed with respect to all DTPA-extractable soil elements, likely due to the greater solubility and availability of the elements.

Table 6

Median, minimum and maximum leaf/root total metal concentration values of selected metals in *D. viscosa*. Unpolluted area (UPS) ($n = 8$); Montevecchio mining area (MON) ($n = 9$); Libiola mining area (LIB) ($n = 4$).

Areas	Ca	Cd	Cu	Fe	Mg	Ni	Pb	Zn
UPS	10.10	1.36	1.22	1.26	2.35	0.95	1.01	3.40
	3.95–14.90	0.98–2.75	0.85–1.64	0.71–8.99	1.65–4.11	0.33–1.72	0.06–9.17	1.64–6.23
MON	8.81	1.86	2.06	10.07	3.18	4.25	3.97	4.97
	2.35–13.55	1.14–2.42	1.43–4.90	3.76–49.35	0.55–6.55	1.59–33.34	1.06–27.86	1.71–15.29
LIB	3.07	0.84	0.46	0.92	1.61	0.91	0.45	1.35
	1.15–8.66	0.69–2.82	0.28–0.84	0.47–1.99	1.02–2.37	0.53–1.75	0.23–1.07	0.90–1.77

Table 7

Ca/Mg exchangeable molar ratio in soils and Ca/Mg molar ratio in plants' parts. Unpolluted area (UPS) ($n = 8$); Montevocchio mining area (MON) ($n = 9$); Libiola mining area (LIB) ($n = 4$).

Areas	Ca/Mg exchangeable	Ca/Mg roots	Ca/Mg stems	Ca/Mg leaves
UPS	6.70	1.05	1.20	3.20
	2.02–13.96	0.60–1.84	0.41–2.02	1.57–6.38
MON	1.17	0.84	0.79	2.09
	0.42–3.43	0.38–1.78	0.44–1.31	0.77–7.70
LIB	0.85	0.36	0.56	0.69
	0.36–1.32	0.29–0.55	0.31–1.59	0.26–2.95

In the plant, some metals (Ca, Mg, and Zn) were positively transferred to the leaves, some (Cd, Fe and Ni) had a high content in both leaves and roots and some others (Cu and Pb) were preferentially stored in the roots. In this area, particularly rich in Mg, significant Ca leaf accumulation was observed coupled with a relevant increase of Mg content in the whole plant. In this mining area *D. viscosa* could profitably be used in phytostabilization interventions. Despite the significant presence of PHEs in the different mine soils, *D. viscosa* was one of the few species which could survive therefore displaying interesting characteristics for environmental engineering interventions for the stabilization of polluted mine spoils and in offsetting the dispersion of contaminants in the surrounding environment due to wind and rainfall.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at <http://dx.doi.org/10.1016/j.gexplo.2016.10.001>. These data include the google map of the most important areas described in this article.

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