



Metal Tolerance Capability of *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo & Giusso: A Candidate for Phytostabilization in Abandoned Mine Sites

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Abstract

Sardinia was known as an important mine pole in Europe during his history. Still after decades from mine closure, 75.000.000 m³ of mine waste, rich in heavy metals, were left abandoned causing a huge environmental legacy on the mine district area. Consequently, cost effective remediation is required. In this frame, phytoremediation is considered a feasible candidate. This research was focused on *Helichrysum microphyllum* subsp. *tyrrhenicum*, which is pioneer in xeric soils with low-functions, like mine tailings. The aim of this study was to evaluate its ability to extract heavy metals from mine soils and accumulate them in plant tissues and its suitability for phytostabilization. Sundry samples of soil, roots and epigeal organ were collected through field sampling and analysed in order to obtain metals concentration and mineralogical characteristics. Our results indicate that this species tolerates high concentration of zinc, lead and cadmium, behaving as a species suitable for phytostabilization.

Keywords Heavy metals · Mine tailings · Mediterranean flora · Phytostabilization · Vascular plant

Sardinia was one of the most important mining poles in Europe until the fifties of twentieth century when it reached the most productive period of its history (Bacchetta et al. 2012; Jimènez et al. 2014). However, after the mines closure, only few remediation actions have been done. As a result, high quantities of polluted materials are left abandoned and exposed to weathering (Jimènez et al. 2011). This process combined to the low presence or the absence of plant canopy affect mobilization of pollutants like soluble salts of heavy

metals, which were recognized in high concentration in fresh waters, soils and plants (Bacchetta et al. 2015; Concas et al. 2015; De Giudici et al. 2017). Phytoremediation can be applied in these areas by using vascular plant species and their associated microbiota in combination with amendments and different kind of agronomic strategies in order to remove, limit or make contamination as harmless as possible (Raskin and Ensley 2000). Moreover, it can provide a cost efficient, long-lasting and visual impact solution for contaminated sites (Mulligan et al. 2001). In order to preserve local plant diversity, it is important to use autochthonous species because they do not interfere with floristic and vegetation dynamics (Cao et al. 2009; Sprocati et al. 2014; Concas et al. 2015; Lai et al. 2015), and are adapted to local climatic conditions. Lately, several studies on Sardinia mining sites have suggested different autochthonous plant species which present these properties, like *Dittrichia viscosa* L. (Greuter), *Cistus salviifolius* L. (Jimènez et al. 2005, 2014), *Pistacia lentiscus* L. and *Scrophularia canina* L. subsp. *bicolor* (Sibth.) Greuter (Jimènez et al. 2005, 2014; Bacchetta et al. 2012, 2015; De Giudici et al. 2015; Tamburini et al. 2016), *Euphorbia cupanii* Bertol ex Moris (Jimènez et al. 2005; Medas et al. 2015), *Phragmites australis* (Cav.) Trin. ex Steud and *Juncus acutus* L. (De Giudici et al. 2017;

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Medas et al. 2017). It has been shown that in *E. cupanii*, *P. lentiscus* and *P. australis*, there are bio-minerals of Zn, Si and/or Pb on roots epidermis different from soil's minerals (De Giudici et al. 2015, 2017; Medas et al. 2015), indicating that roots build bio-minerals as a survival strategy of the species (Caldelas et al. 2017).

Helichrysum microphyllum Cambess. subsp. *tyrrhenicum* Bacch., Brullo & Giusso (hereafter *H. tyrrhenicum*), is an endemic shrub of Sardinia and Corse. It is well adapted to the Mediterranean climatic conditions and grows on different kind of substrates, especially on sandy and muddy soils, and arid substrates with low organic matter content (Angiolini et al. 2005; Bacchetta et al. 2009). In Sardinia, it can grow from sea level up to 1500 m a.s.l. and it can be recognized as part of different vegetation assemblages of mine environment (Angiolini et al. 2005; Bacchetta et al. 2003, 2007). Specific studies involving the metal tolerance and the accumulation strategies of *H. tyrrhenicum* are at present fairly scarce (Cao et al. 2004). In this study, we collected specimens of *H. tyrrhenicum* spontaneously grown in heavy metals contaminated areas and their related soils and we evaluated the content of cadmium, zinc and lead in soil samples and their distribution in the plant's tissues, with the aim to perform a preliminary evaluation of the plant's metal tolerance and accumulation mechanisms, in the perspective of using it within phytoremediation applications.

Materials and Methods

The studied area was the mine dump of Campo Pisano (Iglesias, South-West Sardinia) which is hosted in the Paleozoic carbonate platform, in particular on the Gonnese's Formation and the Metalliferous belt (Bechstädt and Boni 1994). The metalliferous bodies are hosted in Cambrian limestones and dolomites (Bechstädt and Boni 1994). The activity at Campo Pisano ceased in 1997 after centuries of mine exploitation. The exploited ore minerals were mainly sphalerite and galena, plus some Zn calamina minerals like smithsonite, hydrozincite (and hemimorphite). The dump is made of fine material (< 100 µm), residue of flotation treatment for metal extraction, characterized by high levels of metal contamination. It is noteworthy that soils of this area are naturally enriched with heavy metals due to the geochemical

background, and Zn, Pb and Cd being the main pollutants (Bacchetta et al. 2012, 2015; Concas et al. 2015). The whole area is characterized by a Mediterranean pluviseasonal bioclimate, with thermotypes ranging between the upper thermo-Mediterranean and the lower meso-Mediterranean and ombrotypes between the upper dry and the lower sub-humid (Bacchetta et al. 2009).

For the aim of this study, samples of spontaneously growing plants of *H. tyrrhenicum* and related soils were collected in 2016 in four sampling sites: two inside the mining area (CP and PLOT), one outside the mine and with a similar mineralogical background (OCP), and one far away from the mine site and without mine impact (SS, Capoterra, South-West Sardinia) (see Table 1 for details about codes, and Fig. 1 for details about localisation). The PLOT sampling site was chosen within an area where a field phytoremediation experiment using native plants such as *Pistacia lentiscus* L. and *Scrophularia canina* L. subsp. *bicolor* (Sibth et Sm.) Greuter has been previously carried out (Bacchetta et al. 2012).

The number of specimens collected at each site was chosen according to the plant's availability at the different selected locations. In similar works the number of collected samples varied between 3 and 5 (Li et al. 2011; Jimenez et al. 2014; Concas et al. 2015; Erdemir et al. 2017) and these numbers were considered statistically adequate for inter- and intra-population variations (Concas et al. 2015).

Plants and soils were jointly sampled and immediately processed after harvesting. Plants of similar dimensions were collected, in order to have homogenous samples. The roots were shaken to remove the bulk soil (S), i.e. all the particles that were not tightly adherent, while the rizosphere soil (RZ), i.e. the particles more closely associated, was collected by putting the roots into a bag and shaking them vigorously. S and RZ samples were air dried for a week.

Zn, Pb and Cd content in S (D < 2 mm) and RZ were determined by a microwave assisted acid digestion (Start D, Milestone), adding to 0.50 g of matrix (dried at 40°C), 9 mL HNO₃ 65% and 4 mL HF (EPA method 3052). A reference material (GSS-4, limy-yellow soil) and blank solutions were used during analysis in order to guarantee trustworthy results. The bioavailable metal concentrations (BF) were determined by a single extraction suitable for not-acidic soils as proposed by Italian official analytical methods for

Table 1 Sampling sites and codes, geographical coordinates and number of collected samples

Sampling sites	Code	Coordinates (WGS 84)	N°
Campo Pisano mine dump	CP	39°17'45.2"N, 8°32'15.1"E	5
Phytoremediation plot in Campo Pisano mine dump	PLOT	39°17'47.9"N, 8°31'53.9"E	3
Out of Campo Pisano mine dump	OCP	39°17'32.3"N, 8°32'34.9"E	5
Su Spantu	SS	39°06'17.4"N, 8°56'09.3"E	3

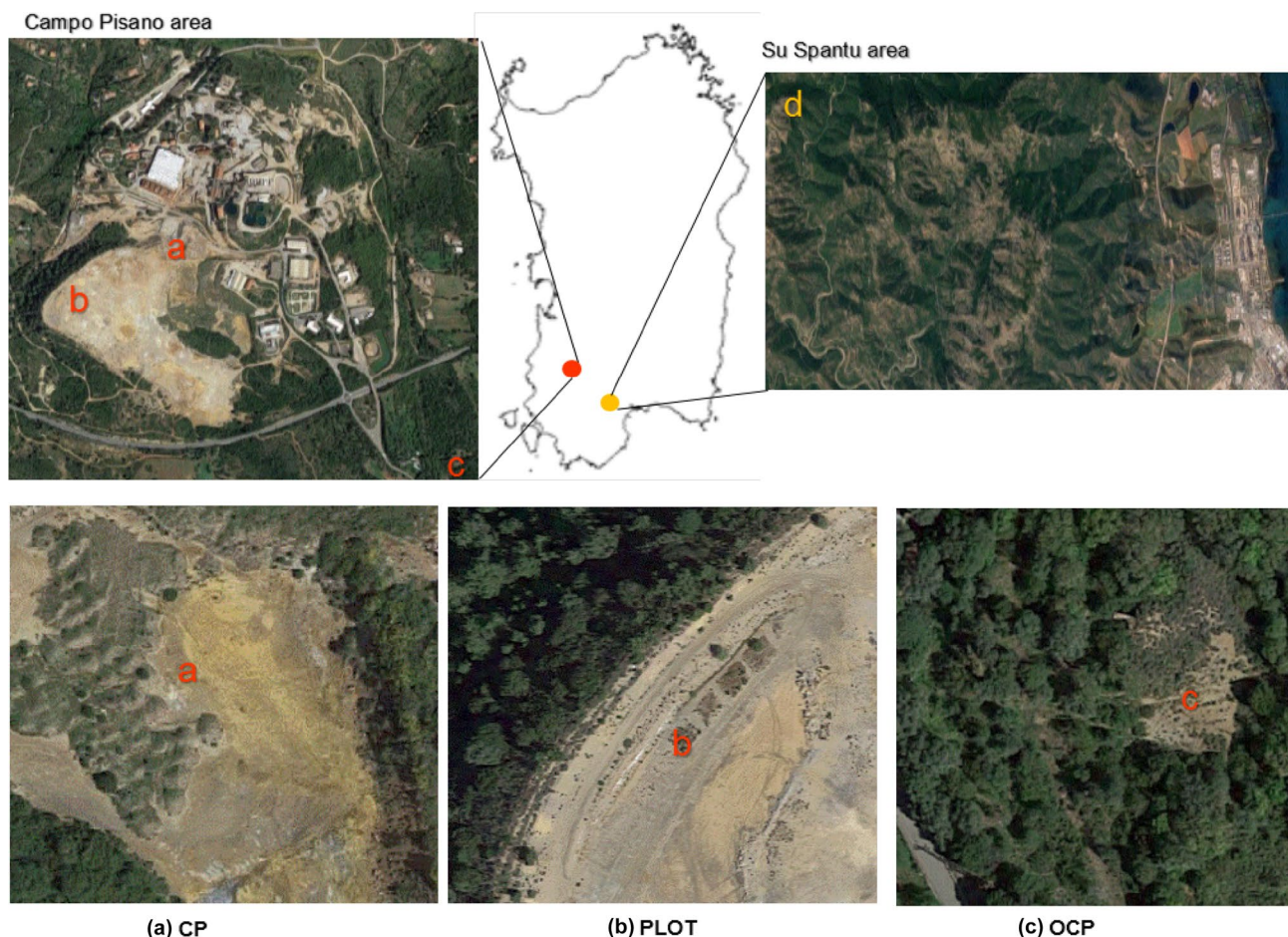


Fig. 1 Sketch of the sampling points. Upper part: localisation of Campo pisano area (red spot) and su Spantu area (yellow spot) and related sampling points (a, b, c, d); Lower part: detail on the Campo

Pisano area sampling points. **a** CP (Campo Pisano); **b** PLOT (Area of a field phytoremediation experiment); **c** OCP (Out of Campo Pisano); **d** SS (Su Spantu)

soils (D.M. 13/09/1999). This method is based on the study of Lindsay and Norvell (1978) and Barbaferi et al. (1996) and it has been used in other similar phytoremediation studies (Gupta and Sinha 2007; Zhu et al. 2012; Li and Zhjang 2013). In this method, a solution of 0.5 M DTPA (Diethylene Triamine Penta acetic Acid), 0.01 M CaCl_2 (Calcium Chloride) and 0.1 M TEA (Tri Ethanol Ammine) buffered at the pH 7.3, is put in contact with soil (solid liquid ratio 1:2) for 2 hours. After the supernatant was filtered and finally analysed. DTPA extraction procedure was described by Lindsay and Norvell (1978) as the most thermodynamically efficient and is able to prevent the carbonates dissolution and the consequent release of bounded metals (Feng et al. 2005). The total and bioavailable metal concentrations in filtered extracts were analysed through Inductively Coupled Plasma Spectrometry (ICP-OES, Perkin Elmer Optima DV 7000). The operative wavelengths (nm) and detection limits (mg/L) were: Zn 213.857 (0.005), Pb 220.353 (0.02) and Cd 228.802 (0.02). The pH of soils was determined using

the potentiometric method proposed by GURI (1999) using a solution of CaCl_2 as eluent. This method gives realistic values of the reaction potential of field sample (Conyers and Davey 1988); total carbon (TC) and nitrogen (TN) were obtained by CHN analyzer (LECO, CHN 1000). A reference material (Ore Tailings) was used to calibrate CHN analyzer.

Plants were divided in roots (R) and epigeal organs (EO), washed in deionized water, dried at 40°C for a week and finely ground through electric mill. Zn, Pb and Cd contents in plant samples were determined by a microwave assisted acid digestion using 0.50 g of the matrix and a 9 mL HNO_3 and 0.5 mL HF solution (EPA method 3052) as described above for soil matrices. The metal concentrations in filtered extract were determined by ICP-OES. The operative wavelengths and detection limits were the same of S, RZ and BF. Two reference materials were used in order to guarantee trustworthy result's method (GSV-2 bush twigs and leaves and INCT-PVLT-6 Polish Virginia Tobacco leaves) and blank solutions were prepared and analysed.

Two biological indexes, namely the Biological Concentration Factor (BCF) and the Translocation Factor (TF) were calculated in order to evaluate the capability of *H. tyrrhenicum* to uptake Zn, Pb and Cd in roots and eventually translocate them to the epigeal organs. The BCF gives information about the uptake from soil to roots and it is defined as the ratio between the metal content in roots and soil (Fellet et al. 2007). In this work, this parameter was calculated with reference to both the total content (BCF) and the bioavailable fraction (BCF *bf*) in soils. The TF indicates the rate of heavy metals translocated from roots into epigeal organ (Brooks 1998).

S, RZ and roots samples were finely ground in an agate mortar and investigated by XRD analyzer in order to obtain mineralogical composition. XRD analysis were performed by a θ -2 θ conventional diffractometer (PANalytical X'PERT MPD) with Cu K α radiation (1.5418 Å). Samples were lightly ground in agate mortar and packed into the sample holder for X-ray diffraction analysis. Peaks for the mineral were attributed according to the Powder Diffraction Cards by using X'Pert Highscore plus software. Roots surface and chemical composition were investigated using a SEM coupled with an EDAX analyzer.

Generalized Linear Models (GLMs) were used to evaluate the differences in concentration of Zn, Pb and Cd in bulk soil (Tot S), rhizosphere (Tot RZ) and in bioavailable fraction (BF S) of bulk soil (mg/kg), as well as in the plant tissues (e.g., R and EO). GLMs were used also for the biological indexes (BCF, BCF *bf* and TF) calculated for the three metals. Significant differences highlighted by GLM (with a log link function and quasi poisson error structure) were then analysed by a post hoc pairwise comparisons t-test (with Bonferroni adjustment). Quasi poisson error structure and F test with an empirical scale parameter instead of chi-squared on the subsequent ANOVA were used in order to overcome residual overdispersion (Crawley 2007). Statistical analyses were carried out using R version v. 3.0.3 (R Development Core Team 2014).

Results and Discussion

The main chemical characteristics of bulk soils were reported in Table 2. The pH of both Campo Pisano's waste surface samples (CP, PLOT) and those outside the mine area (OCP) was classified as neutral or slightly alkaline on the basis of the USDA classification (1998), while SS ones showed an acidic pH which is ascribable to granitic lithology. The content of TC was similar for all the sites influenced by mine activity, and low content of TN was measured in all sites inside and outside the mine district. The pH values and TC of the mine district are consistent with the dominant carbonate lithology as reported by Bacchetta

Table 2 pH, TC (g/kg) and TN (g/kg) in the bulk soils of sampling sites (mean values \pm SD)

	pH	TC	TN
CP	7.2 \pm 0.2	45 \pm 22	0.3 \pm 0.2
PLOT	7.2 \pm 0.1	69 \pm 8	0.7 \pm 0.2
OCP	7.3 \pm 0.3	74 \pm 6	0.2 \pm 0.1
SS	6.3 \pm 0.3	11 \pm 5	0.2 \pm 0

et al. (2015), where carbon was present mainly in an inorganic form such as calcite (48.0 \pm 4.4 g/kg) and dolomite (430.0 \pm 10.1 g/kg). These data are also consistent with XRD analysis conducted in this study on bulk soils and rhizosphere (see in the Results chapter, Mineralogical analysis paragraph). Moreover, the poor agronomic properties are typical for soils of mining areas and this has been recognized as one of the critical issues in applying phytoremediation actions (Nicoara et al. 2014; Sprocati et al. 2014).

Table 3 reports the total metal concentrations in S and RZ and the BF in bulk soils. Metal concentrations in S and RZ appeared very similar: Zn was always the most concentrated element, followed by Pb and Cd. As reported in other similar studies (Bacchetta et al. 2012, 2015; Concas et al. 2015), samples at the mine dump (CP and PLOT) contained very high quantities of metals, well above the threshold contamination levels established by the Italian law (Dlgs 152/2006) for an industrial use of soil (1500, 1000, 15 mg/kg for Zn, Pb and Cd, respectively). The high variation in total concentrations measured in CP and PLOT samples can be explained by the heterogeneity of mine waste deposited in the Campo Pisano dump. Metal concentration values assessed in PLOT soils are consistent with the study of Bacchetta et al. (2012). It is noteworthy that also the OCP soils samples were highly contaminated, confirming a very high contaminant dispersion in the areas surrounding the mine site due to the wind dispersion of fine soil particles. The differences between metals content measured at OCP, nearby the mine area, and those inside the mine area (CP / PLOT) were in fact, in most cases, not statistically significant ($p < 0.05$). In the case of Zn measured in rhizosphere soils, concentration in OCP was found to be even higher than that measured in PLOT.

As expected, SS did not exceed the above mentioned threshold levels, and this indicates the absence of either an anthropogenic impact or an anomalous geochemical background. The BF S values were a minimal percentage of the total content (Zn and Pb values $< 1\%$ and Cd around 3%) and this indicates that only a small quantity of metals is linked to the soluble fraction and to mineral and organic phases and thus available for plant species. The differences between metals content measured at the sampling sites inside and nearby the mine area and those measured in the site not influenced by the mining activity were statistically

Table 3 Zn, Cd and Pb total content (*Tot S*) and bioavailable fraction in bulk soil (*BF S*) and rizosphere (*Tot RZ*) (mg/kg); mean \pm SD (n=5 for CP and OCP; n=3 for PLOT and SS); n.d.: not detected; Different letters indicate significant differences between sampling sites at $p < 0.05$

	CP	PLOT	OCP	SS
Zn				
<i>Tot S</i>	24,823 \pm 6713 ^a	14,251 \pm 4064 ^{ab}	25,043 \pm 13702 ^a	40 \pm 14 ^b
<i>BF S</i>	124 \pm 9 ^a	93 \pm 26 ^a	135 \pm 34 ^a	0.8 \pm 0.7 ^b
<i>Tot RZ</i>	25,218 \pm 5366 ^a	8296 \pm 2819 ^b	23,209 \pm 9180 ^a	46 \pm 14 ^b
Pb				
<i>Tot S</i>	5082 \pm 183.55 ^a	1659 \pm 202.59 ^b	1380 \pm 562 ^b	29 \pm 2 ^b
<i>BF S</i>	21 \pm 7 ^a	5 \pm 1 ^b	13 \pm 5 ^{ab}	0.31 \pm 0.27 ^b
<i>Tot RZ</i>	4540 \pm 1189 ^a	1316 \pm 583 ^b	1499 \pm 513 ^b	21 \pm 0 ^b
Cd				
<i>Tot S</i>	95 \pm 19 ^a	51 \pm 5 ^a	253 \pm 219 ^a	n.d
<i>BF S</i>	2.1 \pm 0.3 ^a	1.6 \pm 0.6 ^a	8 \pm 6 ^a	n.d
<i>Tot RZ</i>	158 \pm 29 ^{ab}	68 \pm 20 ^{ab}	222 \pm 112 ^a	4.14 \pm 0.20 ^b

Table 4 Concentration (mg/kg) of Zn, Pb, Cd in plant tissue (mean \pm SD; n=5 CP; n=3 PLOT; n=5 OCP; n=3 SS); R and EO correspond to roots and epigeal organ respectively; n.d.: not detected; different letters indicate significant difference between sampling sites at $p < 0.05$

	CP	PLOT	OCP	SS
<i>Zn R</i>	2634 \pm 1091 ^a	2200 \pm 1785 ^{ab}	1700 \pm 839 ^{ab}	33 \pm 12 ^b
<i>Zn EO</i>	3466 \pm 620 ^a	1679 \pm 1229 ^b	718 \pm 413 ^c	44 \pm 2 ^c
<i>Pb R</i>	541 \pm 224 ^a	243 \pm 203 ^{ab}	140 \pm 113 ^{ab}	2.37 \pm 0.60 ^b
<i>Pb EO</i>	1035 \pm 28 ^a	353 \pm 313 ^b	46 \pm 34 ^b	2.08 \pm 0.32 ^b
<i>Cd R</i>	20 \pm 8 ^a	13 \pm 5 ^a	33 \pm 21 ^a	n.d
<i>Cd EO</i>	19 \pm 6 ^a	11 \pm 8 ^a	9.73 \pm 8.22 ^a	n.d

significant ($p < 0.05$). In some cases, significant differences were found also among the sampling site of the mine district CP and PLOT.

The plant samples of *H. tyrrhenicum* collected at the different sites exhibited different metal concentrations and metal distributions in the plant tissues. As shown in Table 4, Zn was the most abundant heavy metal in roots and epigeal tissues, followed by Pb and Cd, consistently with the abundance order observed in bulk soils and rizosphere. The highest concentrations of metals in R were recognized in CP samples followed by PLOT and OCP ones, and as expected much lower values were found in SS, coherently with the lower metal content measured in the bulk and rizosphere soils at SS site. The amount of these metals in EO decreased in the order CP > PLOT > OCP >> SS and generally the concentrations measured in roots were comparable with that assessed in epigeal organs for every metal tested. Studies carried out for other pioneer plants investigated in this area, for instance *P. lentiscus*, *C. salviifolius*, *S. canina* subsp. *bicolor* (Cao et al. 2009; Bacchetta et al. 2015; Concas et al. 2015) report concentrations of Zn, Cd and Pb in roots and aerial organs lower in comparison to the results found in this study (around 500 and 200 mg/kg of Pb and around 3500

and 1000 mg/kg of Zn in *C. salviifolius* and *S. canina* subsp. *bicolor*, respectively).

Statistical analysis on Zn and Pb content measured in R and EO (Table 4) highlighted statistically significant differences among sampling sites ($p < 0.05$), while no statistical differences were observed for Cd ($p > 0.05$). In details, Zn and Pb content in R measured at CP site were significantly higher than those measured in SS ($p < 0.05$), while metal uptake in roots assessed at PLOT and OCP did not show significant differences ($p > 0.05$) with respect to CP and SS. As far as Zn content in EO was considered, significant differences were found among the different sampling sites ($p < 0.05$) with the exception of OCP and SS, which showed similar values to each other. Pb content in EO measured in CP site was significantly higher than the other sites ($p < 0.05$).

Table 5 shows the values of BCF, BCF *bf* and TF. Bioaccumulation and translocation data assessed at each sampling site reflect the variability already found for the concentration of metals in soil and plant tissues. Despite this considerable variability, one can generally find the same accumulation pattern irrespectively of both sampling site and metal. The BCF values were always lower than 1, but as far as BCF *bf* values are considered, a significant attitude to accumulate metals in roots tissue can be revealed. As far as metals distribution among plant tissues is concerned, *H. tyrrhenicum* showed the capability to transfer the metals from roots to leaves. The BCF *bf* and TF values measured for the different sites and the different metals indicate the attitude to behave as a species suitable for phytostabilization (Baker et al. 1994). Moreover, the root's concentrations of every metal tested were above the reported phytotoxic levels (Kabata-Pendias and Pendias 1992), indicating the high metal tolerance of the species. In the study of Cao et al. (2004), *H. tyrrhenicum* showed a high tolerance to high concentration of both Zn and Pb, behaving as accumulator for Zn and as excluder for Pb. Taking into account the values measured on

Table 5 BCF, BCF *bf* and TF mean values; / = undeterminable value; different letters indicate significant differences between sampling sites at $p < 0.05$

	CP	PLOT	OCP	SS
Zn				
<i>BCF</i>	0.1 ± 0.1 ^a	0.2 ± 0.2 ^a	0.1 ± 0.1 ^a	0.9 ± 0.3 ^b
<i>BCF bf</i>	21 ± 9 ^a	28 ± 29 ^a	15 ± 6 ^a	53 ± 29 ^a
<i>TF</i>	1.7 ± 1.4 ^a	0.8 ± 0.1 ^a	0.5 ± 0.4 ^a	1.5 ± 0.7 ^a
Pb				
<i>BCF</i>	0.11 ± 0.05 ^a	0.2 ± 0.1 ^a	0.07 ± 0.13 ^a	0.10 ± 0.01 ^a
<i>BCF bf</i>	29 ± 19 ^a	42 ± 30 ^a	18 ± 14 ^a	13 ± 8 ^a
<i>TF</i>	2.45 ± 2.05 ^a	1.7 ± 0.6 ^a	0.5 ± 0.3 ^a	0.9 ± 0.1 ^a
Cd				
<i>BCF</i>	0.2 ± 0.1 ^a	0.3 ± 0.1 ^a	0.2 ± 0.1 ^a	/
<i>BCF bf</i>	9.6 ± 4 ^a	11 ± 10 ^a	6.6 ± 4.2 ^a	/
<i>TF</i>	1.1 ± 0.7 ^a	0.8 ± 0.3 ^a	0.4 ± 0.3 ^a	/

other pioneering species, *H. tyrrhenicum* has BCF and TF higher than *P. lentiscus* (Bacchetta et al. 2015; Concas et al. 2015), and lower than *C. salviifolius* (TF values 2.2 and 2 for Zn and Pb respectively), whilst *S. canina* subsp. *bicolor* presented a lower TF for Pb and a TF similar to our results for Zn (Lai et al. 2015). The statistical analysis showed no significant differences among the different sampling sites ($p < 0.05$) in BCF, BCF *bf* and TF (Table 5) with the exception of the BCF for Zn measured far away to the mine district (SS), that was significantly higher if compared to sampling sites inside the mine district (CP, PLOT and OCP).

The assessed biological indexes and the ability of this native species to withstand high concentrations of Zn, Pb and Cd in the soils of mining site indicate that *H. tyrrhenicum* can be potentially suitable for phytostabilization projects.

Table 6 shows the mineralogical composition of S, RZ and root’s powder obtained through XRD. CP soil samples were mainly made of quartz, dolomite, pyrite and blende according to previous mineralogical studies of this area (De Giudici et al. 2015). PLOT samples were similar to CP, but they contained also clay minerals such as alloysite. Rizosphere of CP, PLOT and OCP had a similar

composition to bulk soil with the addition of ankerite, jarosite, goethite. Root’s powders showed the presence of amorphous cellulose and quartz in all samples collected, also observed by Medas (2015). SEM investigation on CP root samples showed biomineral layers and isolated grains coating the surface of the roots. These layers were rich in Fe oxide, whereas the grains are constituted of barite, Fe and Ti oxides and Al silicate. On the uncoated root’s surface traces of Si, Cl, Zn and Pb were observed. Furthermore, trace of Si and Cl were recognized on all the samples, also out of the mine area. On the surface of PLOT and OUT roots, there were grains and coats, with Zn, Fe and Ti traces.

Mineral coating of Zn and Si were observed also in other studies carried on *E. cupanii* and *J. acutus* (Medas et al. 2015, 2017), *P. lentiscus* and *P. australis* (De Giudici et al. 2015, 2017) grown on Campo Pisano’s area. They were interpreted as a result of a survival strategy. Particularly, their physiological purpose is limiting the bioavailability of Zn and other ions (Caldelas and Weiss, 2017). Similarly to other biomineralization occurring in the area, amorphous Zn biomineralization could be present in *H. tyrrhenicum* roots, but they cannot be detected by XRD (Medas et al. 2014; Podda et al. 2014).

The aim of this study was evaluating the suitability of *H. tyrrhenicum* for remediation of contaminated sites. Samples of the plant grown on mine waste rich in Zn, Pb and Cd were studied. Different plant compartments and soil samples were analysed for their content in heavy metals. It was found that high content of pollutants in *H. tyrrhenicum* roots with respect to the bioavailable metals in the soils. Moreover, the calculated translocation factor indicated fair translocation to aerial organs, thus, *H. tyrrhenicum* can be considered as a tolerant plant. *H. tyrrhenicum* has also been found able to influence the minerals in the rizosphere and this could be part of its pioneering strategy. These reasons, along with the capability to spontaneously colonize mining sites, allowed us to consider this plant species for revegetating the mine areas and developing phytostabilization technology. Phytoremediation laboratory experiment and seed germination tests on *H. tyrrhenicum* under heavy metals stress are taking place

Table 6 Mineralogical composition of bulk soil, rizosphere and root powder in the different sampling sites

	Bulk Soil	Rizosphere	Root’s powder
CP	Quartz, dolomite, pyrite, jarosite, gypsum, blende	Quartz, dolomite, ankerite, jarosite, gypsum, pyrite, goethite	Amorphous cellulose; quartz
PLOT	Quartz, dolomite, pyrite, gypsum, goethite, alloysite	Quartz, dolomite, ankerite, gypsum, pyrite, goethite, cerussite, alloysite	Amorphous cellulose, quartz
OCP	Quartz, dolomite, pyrite, goethite, phyllosilicate	Quartz, dolomite, muscovite, smithsonite, kaolinite, anglesite	Amorphous cellulose, quartz
SS	Quartz, muscovite, albite, microcline	Quartz, muscovite, albite, microcline, illite, rutilo	Amorphous cellulose, quartz

with the aim of defining the best condition for future phytostabilization actions to be applied in this area and similar ones in other Mediterranean mine contexts.

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