Phytoremediation of Sardinian abandoned mine site: a preliminary study on the use of *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo & Giusso

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Abstract

Sardinia was an important mine pole in Europe during the 19th and 20th century. Mine waste, rich in heavy metals, were left abandoned in tailing dumps, causing a relevant impact on the quality of water bodies and soils in nearby areas. Consequently, appropriate remediation activities are required in order to reduce the environmental contamination.

Phytoremediation can be applied in these contexts because some plants are able to catch metals in roots or in epigean organs, offering plant coverage, improving soil characteristics and re-launching vegetation dynamics.

This research is focused on *Helichrysum microphyllum* Cambess. subsp. *tyrrhenicum* Bacch., Brullo & Giusso, which is an endemic shrub of Sardinia, Corsica and Balearic Islands and it can grow in different edaphic condition, including mine's environments. The aim of this study is to evaluate the plant's ability to extract heavy metals from mine soils and accumulate them in the plant tissues. Sundry samples of soil, roots and epigean organ were collected in Campo Pisano mine dump and analyzed in order to obtain metals concentration and mineralogical characteristics.

Our preliminary result indicates that *H. microphyllum* subsp. *tyrrhenicum* tolerates high concentration of them, decreasing from roots to epigean organs, thus behaving as a species suitable for phytostabilization.

Keywords: Heavy metals, Phytoremediation, *Helichrysum microphyllum* subsp. *tyrrhenicum*, Mine waste, tolerance

Introduction

Sardinia was one of the most important mine poles in Europe until the fifties of 20th century. From the end of the mine activities to nowadays, there have been only few actions promoting remediation of these areas. As a result, high quantities of polluted materials are left abandoned and exposed to weathering (Jimenez et al., 2010). This process and the absence of plant coverage result in a mobilization of pollutants like heavy metals, which have been recognized in high concentration in fresh water, soils and plants (Cidu et al., 2005; Concas et al., 2015; Bacchetta et al., 2015; De Giudici et al., 2017). Phytoremediation can be used in these areas in order to rehabilitate contaminated soils. This is possible using 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 & Ensley, 2000). Moreover, it can provide a cost efficient, long-lasting and visual impact solution for contaminated sites (Mulligan et al., 2001). It is important to use autochthonous species because they do not interfere with floristic and vegetational dynamics and they are not harmful to local biodiversity (Concas et al., 2015; Cao et al., 2009). Lately, much studies on Sardinia mining sites have shown different autochthonous species with these properties, like: *Dittrichia viscosa* L. (Greuter), *Cistus salviifolius* L. (Jimenez et al., 2005), *Pistacia lentiscus* L. *Scrophularia canina* L. subsp. *bicolor* (Sibth) Greuter (Bacchetta et al., 2012; Bacchetta et al., 2015; Jimenez et al. 2005; Tamburini et al., 2016), *Euphorbia cupanii* Bertol ex Moris (Medas et al., 2015). It has been shown that in *E. cupanii*, *P. lentiscus* and *Phragmites australis* (Cav.) Trin. ex Steud. there are bio-minerals of Zn, Si and/or Pb on roots epidermis different from soil's minerals (De Giudici et al., 2015; Medas et al., 2015; De Giudici et al. 2017), 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, is an endemic shrub of Sardinia, Corsica and Balearic Island and it grows up to 60 cm. It is well adapted to the hardest Mediterranean ecologic conditions and on different kind of substratum, especially on sandy and muddy soils (Angiolini et al., 2005). In Sardinia, it grows up from the sea level up to 1800 m and it can be recognized as part of different vegetation formations of mine environment (Bacchetta et al., 2013). The aim of this study is to evaluate the ability of *H. microphyllum* subsp. *tyrrhenicum* to extract heavy metals from soils contaminated by Zn, Cd and Pb and to investigate the uptake in the plant's tissues.

Methods and materials

Study area

The study area is the mine dump of Campo Pisano (South-West Sardinia) which is hosted in the Paleozoic carbonatic platform, in particular on the Gonnesa's Formation and the Metalliferous Ring. Campo Pisano was exploited for centuries but the mine activity ended in 1997. The main extracted minerals were Blende and Galena and some Zn ores called "calamine" like Smithsonite, Hydrozincite (and Hemimorphite). The dump is made of fine materials, like sand and mud, which are by products of grinding and flotation treatments. Soils of this area are naturally enriched in Zn, Pb and Cd, because of the geochemical background (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 soils and plants were collected in spring and autumn 2016 in two places within the Campo Pisano mine site, one site outside the mine, and two additional sites far away from Campo Pisano without anthropic mine impact, as listed: Campo Pisano mine dump (CP 39°17'45.2"N, 8°32'15.1"E): 5 samples; experimental plots of a previous phytoremediation study at Campo Pisano mine dump (PLOT 39°17'47.9"N, 8°31'53.9"E): 3 samples; area outside the mine site (OCP 39°17'32.3"N, 8°32'34.9"E): 5 samples; areas far away from the mine site "Su Spantu" (SS 39°06'17.4"N, 8°56'09.3"E): 3 samples and "Is Molas" (ISM 38°59'31.7"N 8°56'04.4"E): 1 sample.

Experimental design

Plants (P), soils (S) and rhizosphere materials (RZ) were simultaneously sampled and immediately processed after harvesting. Plants of similar dimensions were collected, in order to have homogenous samples.

- *Soil analysis*

Soil was separated from roots and air dried for a week. Zn, Pb and Cd content in soil samples (< 2mm) were determined by a microwave assisted acid digestion dissolving 0.50 g in an acid mixture of 9 ml of HNO_3 and 4 ml of HF (EPA method 3052). The metal concentration in filtered solutions was evaluated ICP-OES (Perkin Elmer Optima DV 7000). A reference material (GSS-4, limy-yellow soil) was used during analysis in order to guarantee trustworthy results. The pH of soils was determined using the GURI method (1999); total carbon (TC) and nitrogen (N) were obtained by CHN analyzer (LECO, CHN 1000). A reference material (Ore Tailings) was used to calibrate CHN analyzer.

- *Plant analysis*

Plants were divided in roots (R) and epigean organs (EO), washed in deionized water, dried at 40°C for a week, finely ground and stored in closed jars. Zn, Pb and Cd content in plant samples were determined by a microwave assisted acid digestion (Start D, Milestone) dissolving 0.50 g in an acid mixture of 9 ml of HNO_3 and 0.5 ml of HF (EPA method 3052). The resultant solutions were filtered at 2 mm and metal concentration was evaluated by Inductively Coupled Plasma spectrometry (ICP-OES, Perkin Elmer Optima DV 7000). Wavelengths (nm) and detection limits (mg/l) are: Zn 213.857 (0.005), Pb 220.353 (0.02) and Cd 228.802 (0.02). 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). During analysis, blank solutions were prepared and analyzed.

- *Mineralogical analysis*

S < 2mm, RZ and roots 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-2θ conventional diffractometer (PANalytical X'PERT MPD) with Cu Kα 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.

Results and discussions

- *Soils data*

Table 2 reports the content of Zn, Pb, Cd, pH, total carbon and nitrogen evaluated in soil samples. The most concentrated element in studied samples is Zn, followed by Pb and Cd, as reported in other similar studies (Bacchetta et al., 2015; Concas et al., 2015). As expected, soils sampled at the mine dump contains very high quantities of metals. The highest concentration of Cd, Pb and Zn is measured in CP, followed by OCP and PLOT samples. SS and ISM show the lowest concentration, even if ISM presents higher level of these metals than SS. However, in other studies in the same area (Concas et al., 2015, Bacchetta et al., 2015) lower concentration of Zn in CP have been recorded, while Pb and Cd are similar only for samples collected during spring.

pH of Campo Pisano's area can be classified as neutral or slightly alkaline (pH equal to 7.3) on the basis of the USDA classification (1998), while SS and ISM samples show an acid pH due to their lithology as ascribable to granitic rocks. Total C and N values in CP soil are low (respectively 3-4 and 0.2-0.5%) and this indicates poor agronomic characteristics.

Table 2. Zn, Cd and Pb content (mg/kg) in soils (n=2) Total C and N (%) (a = autumn); <dl = under detection limit

	Zn	Pb	Cd	pH	Tot C	Tot. N
CP1	21979	4927	82.20	7.2	3.18	0.18
CP2	25131	5033	99.80	7.0	4.64	0.24
CP3	27462	5020	105	7.3	4.66	0.57
CP4a	34006	5335	119	7.4	3.66	0.26
CP5a	15832	5025	71.80	7.3	6.25	0.08
PLOT1	18781	1837	45.62	7.1	6.00	0.08
PLOT2	13044	1701	50.34	7.3	7.40	0.09
PLOT3	10927	1439	56.97	7.3	7.43	0.05
OCP1	26797	1438	165	7.5	6.96	0.24
OCP2	46820	1162	640	7.2	10.42	0.23
OCP3	24545	1124	217	7.7	6.89	0.09
OCP4a	15548	2317	130	7.0	5.95	0.20
OCP5a	11455	3493	114	7.4	6.85	0.25
SS1	37.40	31.44	0.44	6.6	1.10	0.12
SS2	56.45	29,16	<dl	6.1	1.61	0.13
SS3	26.86	26.86	<dl	6.1	0.54	0.06
ISMa	1936	1433	<dl	5.6	0.76	0.09

- *Plants data*

As shown in Table 3, Zn is the most abundant heavy metal in roots and epigean organs, followed by Pb and Cd. The highest concentration of metals in roots are recognized in CP samples followed by PLOT and OCP ones whilst much lower values were found in SS and ISM. The amount of these metals in epigean organs decrease in the order CP> PLOT> OCP> ISM> SS. In general, all the samples show the following order for every metal tested: roots > epigean organs. In other works carried on *P. lentiscus*, *C. salviifolius*, *S. canina* subsp. *bicolor* (Bacchetta et al., 2015, Concas et al., 2015, Cao et al., 2009) growing in this area, the concentration of Zn, Cd and Pb in roots and aerial organs are lower with respect to 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).

Table 4 shows the values of two different biological accumulation parameters: Biological Accumulation Coefficient (BAC) calculated as root-soil concentrations ratio, and Translocation Factor (TF) as ratio by epigean organs and roots concentrations. BAC values for Zn related to CP are very low (0.02- 0.2) and the TF values are near 1 (0.85 - 1.65). This behavior is similar for Pb, even if there are some exception in TF values (>1.5). TF for Cd are low (0.1 - 0.6), except for some sample where is near 1. TF indicates that there is some translocation of Zn and Pb from roots to aerial organs. However, this phenomenon is weak for Cd, as suggest by data. BAC and TF values for Zn and Pb are higher than values measured on *P. lentiscus* by Concas et al. (2015). In the study of Lai et al. (2015), *C. salviifolius* showed TF values for Pb and Zn (2 and 2.2, respectively) higher than *H. microphylllum* subsp. *tyrrhenicum*, while *S. canina* subsp. *bicolor* presented a lower TF for Pb and TF for Zn is similar to our results.

This indicates that *H. microphyllum* subsp. *tyrrhenicum* has better root's catching and translocating capability for these two metals if compared to *P. lentiscus* or *S. canina* subsp. *bicolor* (at least for Pb). These values indicate that *H. microphyllum* subsp. *tyrrhenicum* behaves like a tolerant and indicator plant for the heavy metals under study, potentially suitable for phytostabilization projects.

Table 3. Total concentration (mg/kg) of Zn, Pb, Cd in plant (n=2) (a = autumn) (E.o= Epigean organs); <dl = under detection limit

	Roots			E.o.		
	Zn	Pb	Cd	Zn	Pb	Cd
CP1	4012	835	32.81	3426	1160	18.78
CP2	2857	647	25.65	2874	950	14.59
CP3	3015	546	15.10	2931	954	13.27
CP4 a	1043	235	12.79	4352	1434	29.53
CP5 a	2239	442	13.69	3687	676	19.09
PLOT1	871	56.21	7.24	752	135	4.04
PLOT2	1501	214	13.91	1374	288	9.17
PLOT3	4229	459	18.56	2912	636	20.65
OCP1	403	21.39	8.43	481	10.02	3.12
OCP2	1762	62.82	61.94	786	35.29	15.57
OCP3	2087	108	23.95	1413	101	20.90
OCP4a	1561	216	22.81	409	51.89	4.04
OCP5a	2652	295	48.30	439	32.11	5.01
SS1	44.49	3.04	<dl	41.42	2.50	<dl
SS2	35.82	2.17	0.29	45.84	2.26	<dl
SS3	16.77	1.89	<dl	44.84	1.70	<dl
ISMa	114	15.22	0.64	77.61	5.72	0.59

Table 4. BAC (r/S= root/soil) and TF (eo/r=epigean/soil); / = undeterminated value

	BAC Zn	TF Zn	BAC Pb	TF Pb	BAC Cd	TF Cd
CP1	0.18	0.85	0.17	1.39	0.40	0.57
CP2	0.11	1.01	0.13	1.47	0.26	0.57
CP3	0.11	0.97	0.11	1.75	0.14	0.91
CP4a	0.03	4.15	0.04	6.10	0.11	2.31
CP5a	0.14	1.65	0.09	1.53	0.19	1.39
PLOT1	0.05	0.86	0.03	2.42	0.16	0.56
PLOT2	0.12	0.92	0.13	1.35	0.28	0.66
PLOT3	0.39	0.69	0.32	1.39	0.33	1.11
OCP1	0.02	1.19	0.01	0.47	0.05	0.37
OCP2	0.04	0.45	0.05	0.56	0.10	0.25
OCP3	0.09	0.68	0,10	0,93	0.11	0.87
OCP4a	0.10	0.26	0.09	0.24	0.17	0.18
OCP5a	0.23	0.16	0.07	0.11	0.42	0.10
SS1	1.19	0.93	0.10	0.82	/	/
SS2	0.63	1.28	0.07	1.04	/	/
SS3	0.74	2.27	0.07	0.90	/	/
ISMa	0.06	0.68	0.01	0.38	/	0.92

- *Mineralogical data*

CP soil samples are mainly made of Quartz, Dolomite and Pyrite, according to previous mineralogical studies of this area (De Giudici et al., 2015). Plot samples are similar to CP, but they contain also clay minerals such as Alloysite. Rizosphere of CP and PLOT has a similar composition to soil with the addiction of some minerals like Ankerite, Jarosite, Goethite and Pb Oxide. Root's powders show the presence of amorphous cellulose and quartz, also observed by Medas (2015).

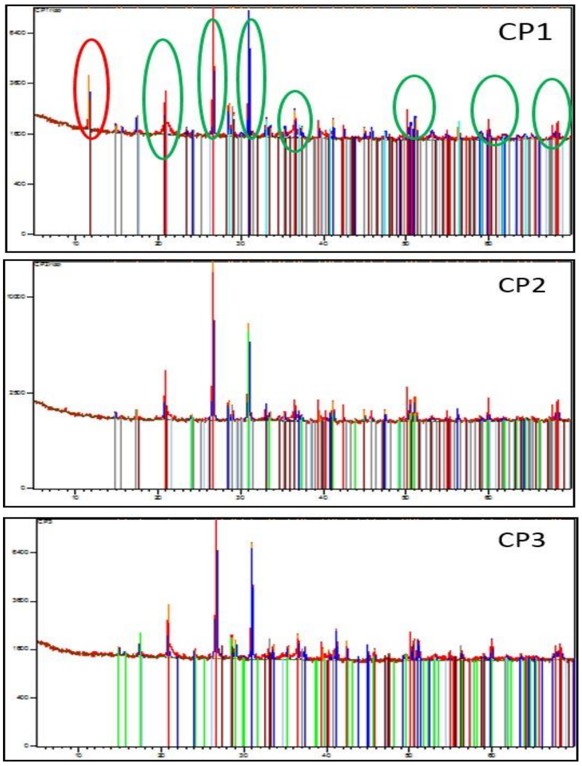

Figure 1. XRD patterns of CP sample

SEM investigation on CP root samples shows (bio)mineral layers and isolated grains coating the surface of the roots. These layers are 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 have been observed (Fig.1). Furthermore, trace of Si and Cl are recognized on all the samples, also out of the mine area. On the surface of PLOT and OUT roots, there are grains and coats, with Zn, Fe and Ti traces. Minerals coat of Zn and Si have been observed also in other studies carried on *E. cupanii* (Medas et al., 2015), *P. lentiscus* and *P. australis* (De Giudici et al., 2015, 2017) grown on Campo Pisano's area and they have been considered a survival strategy of these species. (Bio) mineral layer coats of Fe or Zn have not been observed on the surface of SS roots, even if there were single grains of Ca and C ascribable to Ca Oxalate which is used by plants like a mechanism of depuration from toxic agents.

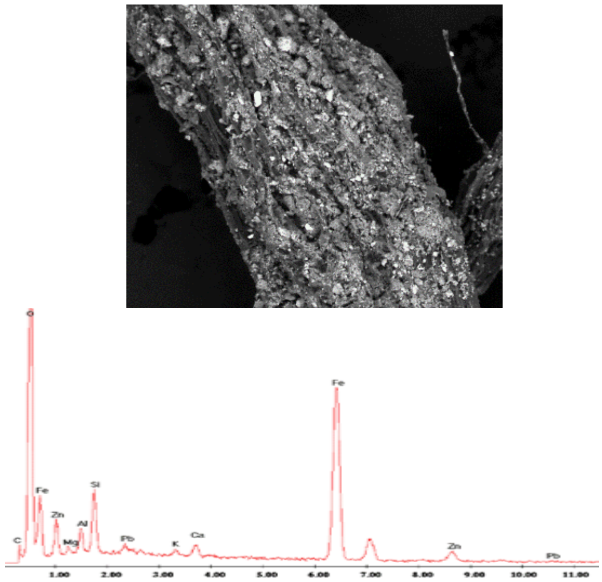

Figure 2. SEM imaging on roots surface and chemical composition analysis on EDAX

Conclusion

In this study, the behavior of *H. microphyllum* subsp. *tyrrhenicum* grown on mine waste towards Zn, Pb and Cd was studied, with the aim of evaluating its suitability for phytoremediation activities. This species shows a good tolerance to these metals and according to the biological indexes (BAC and TF), we can consider it as tolerant plant with a discreet translocation to aerial organs. The plant is also able to influence the minerals in the rizosphere, that could be part of its pioneering strategy. These reasons, along with the capability to spontaneously colonize mining sites, allow us to consider this plant species for phytostabilization projects.

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