Use of Native Plants for the Remediation of Abandoned Mine Sites in Mediterranean Semiarid Environments

G. Bacchetta [1]

G. Cappai [2,*]

Phone +39 070 6755551
Email gcappai@unica.it

A. Carucci [2]

E. Tamburini [3]

[1] DISVA - Department of Life and Environmental Sciences, Centre for the Conservation of Biodiversity, University of Cagliari, V.le S. Ignazio da Laconi 11-13, 09123 Cagliari, Italy

[2] DICAAR - Department of Civil-Environmental Engineering and Architecture, University of Cagliari, Piazza d'Armi, 09123 Cagliari, Italy

[3] DiSB - Department of Biomedical Sciences, University of Cagliari, Cittadella Universitaria, 09042 Monserrato, CA, Italy

Abstract

Abandoned tailing dumps from mining industry represent important sources of metal contamination in the surrounding environments. This study evaluates the potential of two Mediterranean native plants, *Pistacia lentiscus* and *Phragmites australis*, for phytoremediation of two Sardinian contaminated mine sites. A 6 months study has been conducted at greenhouse-controlled conditions with the aim of investigating the plant capability to tolerate high metal concentrations and to extract or immobilize them within the roots. The possibility to mitigate stress on the plants and improve treatment efficiency by adding compost as amendment was also evaluated. Both species were able to restrict accumulation of Cd, Pb and Zn to the root tissues exhibiting a metal concentration ratio of plant roots to soil bioavailable fraction higher than two (four in the case of Zn). However, the two species showed different adaptation responses, being the survival of *P. australis* after 6 months in contaminated soil lower (25 %–58 %) than that observed for *P. lentiscus* (77 %–100 %). Compost addition resulted in a lower metal uptake in tissues of both plants and a higher survival of *P. australis,* whilst almost no effect was observed as regard the growth of both species. The two tested species appear to be promising candidates for phytostabilization, *P. lentiscus* exhibiting a greater adaptability to heavy metal contaminated matrices than *P. australis*.

Keywords

Heavy metals
Mine tailings
Phytoremediation
Sardinia
Phragmites australis
Pistacia lentiscus

Mine tailings are waste materials that result from the mineral separation process aimed at extracting the metals of interest from the mineral rocks. Due to the specificity and partial efficiency of the extraction process, mine tailings are mostly composed of silt or sand sized particles, which lack of nutrients and organic matter while containing high quantities of heavy metals, up to 50 g/kg (Mendez and Maier 2008a). The mine tailing dumps have been important sources of air, soil and water pollution where the closure of mining activities was not supported by an adequate implementation of pollution containment plans (Jiménez et al. 2014). The contamination impact is even higher in semiarid environments, where the long periods of drought and heat, the seasonal heavy rainfalls and the absence or scarcity of vegetation cover amplify the weathering processes responsible for the mobilization of the contaminants and the degradation of soils (Mendez and Maier 2008b).

Abandoned mine sites present a particular challenge for remediation. The generally wide size of the contaminated areas makes the application of conventional technologies (e.g. capping, chemical stabilization, soil washing, excavation) unsuitable due to the high implementation costs and the modification induced on landscape and soil properties. Chemical–physical treatments indeed adversely affect the physical structure and the biological activity of the treated medium (Mulligan et al. 2001; Dybowska et al. 2006). Conversely, phytoremediation has been recognized by the scientific community and gained acceptance in the last 20 years as an efficient and cost-effective option for in situ mine sites reclamation (Tordoff et al. 2000; Wong 2003; Pilon-Smits 2005; Mendez and Maier 2008a). It is based on the use of plants and their associated microbes to reduce human and wildlife exposure to heavy metals in mine tailings. This technology is on average tenfold cheaper than engineering-based remediation methods, since biological processes are basically solar-driven (Pilon-Smits 2005). Specifically, phytoremediation can be implemented as a phytoextraction process, involving the extraction of heavy metals from mine tailings and their accumulation in the above-ground plant biomass, or as a phytostabilization process, concerning the immobilization of metals within the plant rhizosphere, reducing the ecosystem exposure (Baker et al. 1994; Wong 2003). When phytoextraction is applied, plants are harvested and disposed of as hazardous waste or incinerated for metal recovery, whilst the phytostabilization approach is intended as a containment strategy wherein a vegetation cover is created for the long-term stabilization of tailings (Mendez and Maier 2008b).

One important aspect in applying phytoremediation technologies is the selection of the most suitable plant species (Jiménez et al. 2011). It is well established that autochthonous plant species are better adapted to the local climate with respect to non-native ones. In this frame, it is challenging to identify, among the plant species colonizing mine tailings, potential candidates for phytoremediation, by evaluating their phytoremediation potential (Mendez and Maier 2008a). Since each mine tailings site possesses specific physico-chemical characteristics (e.g. metal content, pH, cation exchange capacity, electrical conductivity) influencing plant communities that have naturally established on it, the identification of the ideal candidate for phytoremediation must be tailored on the individual mine site (Mendez and Maier 2008a). Although proved to be a promising technology, phytoremediation needs consequently further optimization in order to lower reclamation times and increase process efficiency and durability. The process can be enhanced by adding amendments, which could improve the soil chemical, biological, and physical properties, assist the plant growth and conveniently modify metal bioavailability (Chen et al. 2000; Kumpiene et al. 2008; Liu and Zhou 2009; Bacchetta et al. 2012).

In the experimental study here described, the phytoremediation technology was applied at greenhouse-controlled conditions to contaminated soils from the area of Sulcis-Iglesiente (South–West Sardinia, Italy), which has been one of the most important European mining district for centuries (Bacchetta et al. 2012). The cessation of mining activity, over the 1970–1990 period, has left in this area 113 abandoned mine sites and large quantities of mine wastes in dumps and flotation tailings. Estimated at about 70 million m^3 (RAS 2003, 2008), mine wastes are subjected to water erosion and wind dispersion. Two different mine sites were investigated in this study: the flotation tailings dump of Campo Pisano and the contaminated marshy area of Sa Masa. Two native plants were selected for the phytoremediation tests, namely *Pistacia lentiscus* L. and *Phragmites australis* (Cav.) Trin. *ex* Steudel. Several studies have demonstrated the ability of *P. australis* to grow as native species in mine tailings, despite their high metal content and the adverse environmental conditions (Ye et al. 1997; Chiu et al. 2006; Conesa et al. 2006; Wang et al. 2008). On the contrary, only few studies have currently evaluated the applicability of *P. lentiscus* for the revegetation and phytostabilization of heavy metal contaminated sites (Fuentes et al. 2007; Domìnguez et al. 2008; Moreno-Jiménez et al. 2009; Domìnguez et al. 2011; Bacchetta et al. 2012) indicating its suitable properties, such as high levels of metal tolerance, metal retention into roots, and phytomass production. The aim of the present study was to evaluate: (1) the capability of the two selected species to tolerate high metal concentrations and to extract or immobilize them within the roots; (2) the possibility to stimulate plant growth and mitigate stress on the plants by adding an organic amendment (compost).

Materials and Methods

The area of interest is the Rio San Giorgio valley (South West Sardinia). The transport of large quantities of fine solids from mine sites into the S. Giorgio and Sa Masa rivulets, caused by surface runoff as well as wind diffusion, has resulted in high levels of water and soil contamination by heavy metals (particularly Zn, Pb and Cd) in a wide area downstream former mines and tailing dumps, such that they are not always the more polluted areas. The Rio San Giorgio valley is included in the Sulcis-Iglesiente-Guspinese site of national interest (SIN), hence requiring restoration actions. The area is characterized by a thermomediterranean dry bioclimate (Bacchetta et al. 2009), with long-term mean precipitation of 600 mm/y concentrating in 50 mean rainy days and long summer drought period (>90 days). The average annual temperature is 17°C, evapotranspiration and runoff around 57 % and

24 %, respectively (Cidu et al. 2005). Two different sites from this area were selected on account of the high levels of heavy metals contamination and with the aim of testing both arid and humid local habitats: the Campo Pisano flotation tailing dump (N39° 17.746′, E8° 31.909′) and the Sa Masa marsh (N39° 16.558′, E8° 27.394′). The tailing dump of the abandoned Campo Pisano mine (hereafter CP) is a basin where the mine wastes from the flotation process were settled. Sa Masa pond (hereafter SM), a coastal marshy area at about 10 km downstream of the mine sites, is the ending point of most of the drainage of the whole Iglesias valley and presents metal concentrations in soils well above the intervention thresholds established by the Italian Ministry of Environment (Boni et al. 1999). The selection of the native species was made on the basis of a geobotanical study carried out in the Rio San Giorgio valley (Angiolini et al. 2005). *P. lentiscus* is a shrub that grows up to 6 m tall, typical of the Mediterranean sclerophyllous shrubland, frequently found growing on mine waste. *P. australis* is a helophyte that may reach 5–6 m tall, massively present in the Sa Masa pond. These naturally growing species are thus ecologically adapted to the local climate and are likely to have greater tolerance to the extreme conditions of such environments; their use allows also preserving plant diversity in the site. Seeds of the two selected species were collected in the San Giorgio catchment basin.

Soil sampled from CP and SM sites were air-dried and 2 mm sieved. On the undersize fraction the particle size distribution was evaluated by Sedigraph Method (Sedigraph 5100, Micromeritics). Compost from selected municipal solid waste organic fractions was provided by SECIT Spa (Ozieri, SS).

Phytoremediation tests were performed in a greenhouse. Temperature was set at 25°C, humidity at 65 %–70 % with a 12-h photoperiod. Experiments were carried out using 1 L-polyethylene pots (10 × 10 × 12 cm) filled with 0.8 L of soil and wetted. Untreated matrices (CPU, SMU) and matrices amended with 10 % w/w compost (CPC, SMC) were used. Control tests were performed with unpolluted reference soil (hereafter RS) consisting of 45 % peat, 45 % sand, and 10 % commercial soil. Ten replicates were used for each treatment.

Triplicate matrix composite samples were chemically characterized using Italian official analytical methods for soils (D.M. 13/09/99). Cation exchange capacity (CEC) was measured using the $BaCl_2$ method. Nitrogen, total and organic carbon content were determined by dry combustion method using LECO CHN 1000 analyser. Calcite and dolomite contents were evaluated in triplicate by calcimetry analysis. Total metals (Cd, Pb and Zn) and phosphorus concentrations were evaluated by digestion with *aqua regia*. The bioavailable metal concentrations were assessed through the sequential extraction procedure proposed by Barbafieri et al. (1996). All the extracted metals and phosphorus concentrations were finally analysed by Inductively Coupled Plasma spectrometry (ICP-OES, Perkin Elmer Optima DV 7000).

Preliminary tests showed a low germination efficiency of *P. australis* on soil, which could be ascribed to the reproduction method of this species by vegetation from underground plant tissues. On the contrary, seedling of *P. lentiscus* are strongly compromised by transplantation. Consequently, two different protocols were implemented: in the experiments with *P. lentiscus*, nine seeds were sawn in each pot, while in tests with *P. australis* three seedlings, previously germinated from seeds and grown in phytotron for 15 days, were planted in each pot. Consistently with their respective presence in the two different habitats, *P. lentiscus* was studied on both CP and SM soils, whilst *P. australis* was exclusively tested on SM soil. Pots were watered with 20 mL distilled water aliquots, differentiating the watering frequency between the two soils: 3-time/week for CP pots and 5-time/week for RS or SM pots. As for *P. lentiscus*, the percentage of germination was calculated by comparing the total number of seedlings after 2 months with the initial number of seeds. Survival was calculated by comparing the number of plants after 3 months and at the end of the experimental period with the number of germinated seeds. In case of *P. australis*, survival was calculated by comparing the total number of plants surveyed for each treatment with the initial number of seedlings.

For each treatment, half of the pots, selected randomly, were analysed after 3 months by harvesting the related plants, while the remaining reactors were analysed after 6 months. The phytoremediation potential of the two plant species was assessed through evaluating plant survival, plant growth (dry weight and length) as well as metal concentrations in soils (total content and bioavailable fraction) and in the different plant fractions (epigeal and hypogeal parts). Plants were gently washed with tap water in order to remove soil particles, rinsed with distilled water, air-dried and splitted into epigeal and hypogeal fractions, which were measured in length, subsequently dried at 105°C and weighed. Cd, Pb and Zn concentrations on the different dried and ground tissues were finally evaluated by digestion with *aqua regia* and subsequent analysis with ICP-OES. Due to the low plant biomass production, a single composite sample for each treatment was produced. For quality control, the following standard reference materials (Institute of geophysical Exploration, Langfan, Hebei, P.R. of China) were analised: bush leaves (GBW07603) and poplar leaves (GBW07604). Germination and growth data were submitted to analysis of variance (ANOVA one way) and the Tukey test ($p < 0.05$) was used for comparison of means as implemented in the software PAST 1.42 (Hammer et al. 2001).

Results and Discussion

Table 1 reports the results of the preliminary characterization of the different soil matrices used during the phytoremediation tests. Both CP and SM soils appeared highly contaminated, being total Cd, Pb and Zn concentrations well above the threshold contamination levels established by the Italian law (D.Lgs. 152/2006) for an industrial use of soil (15, 1,000, 1,500 mg/kg for Cd, Pb and Zn, respectively). Conversely, the concentrations measured for other potentially dangerous elements such as As, Be, Cr, Sb, Se and Tl were well below the above mentioned limits (data not shown in Table 1). The higher metal contamination for both soils was found for Zn, followed by Pb and Cd, being metal concentrations significantly higher ($p < 0.05$) in SM than in CP. High levels of metal bioavailability were also observed, especially for Pb in SM soil, whose available fraction accounts for the 35 % of the total metal content. The two tested soils were slightly alkaline and presented low CEC values. Moreover, a low content of nitrogen and organic carbon was observed for both matrices, coherently with the origin of the two substrates. Scarcity of both nutrients and organic matter is indeed one of the critical aspects in the remediation of mine tailing disposal sites. Carbon was mainly present in the form of calcite and dolomite. Calcite concentrations were 48.0 ± 4.4 and 162.5 ± 9.6 g/kg in CP and SM, respectively, while in the same two matrices dolomite presence accounted for 430.0 ± 10.1 and 230.1 ± 9.6 g/kg respectively. It can be stressed that both soils consisted of fine-grained particles, with 86.2 % and 100 % of particles <425 μm for CP and SM, respectively. Specifically, the particle size distribution indicates for CP a lime (<50 μm) and silt (<2 μm) fraction of 27 % and 3 %, respectively, while for SM, lime and silt content accounted for 68 % and 6 %, respectively. This represents an unfavourable property with regard to plant establishment, since fine particles favour compaction and cementation processes, reducing roots penetration and soil ventilation (Hauser 2009). Compost addition improved the soil properties of mine matrices by slightly increasing organic carbon, CEC, and total N. Furthermore, lower metal contents (both total and bioavailable fractions) were found almost corresponding to the dilution operated by compost addition (10 % w/w).

Table 1

Physico-chemical properties of the soils used in the phytoremediation tests (mean ± SD;

	Sa Masa (SMU)	Sa Masa + compost (SMC)	Campo Pisano (CPU)	Campo Pisa compost (CP
Cd (mg/kg)	126.5 ± 3.4^{a}	113.4 ± 0.6^{b}	77.2 ± 1.6^{c}	73.6 ± 1.5^{c}
Pb (mg/kg)	5,220.2 ± 17.5^{a}	4,339.7 ± 166.9^{b}	3,710.8 ± 27.0^{c}	3,390.9 ± 46.
Zn (mg/kg)	2~~,~~1,077.5 ± 249.7^{a}	18,391.8 ± 465.0^{b}	13,893.8 ± 185.7^{c}	13,780.7 ± 34
Cd$_{BAF}$ (mg/kg)	19.1 ± 2.7	17.5 ± 4.0	13.7 ± 0.4	11.8 ± 2.0
Pb$_{BAF}$ (mg/kg)	1,834.2 ± 347.6	1,318.8 ± 162.8	695.6 ± 49. 9	549.5 ± 75.0
Zn$_{BAF}$ (mg/kg)	2,984.2 ± 522.6	1,863.3 ± 376.9	1,271.2 ± 108.3	1,050.8 ± 142
C$_{tot}$ (%)	8.20 ± 0.07	9.10 ± 0.07	6.70 ± 0.12	8.40 ± 0.07
C$_{org}$ (%)	1.98 ± 0.00	3.81 ± 0.00	1.84 ± 0.00	3.10 ± 0.00
N$_{tot}$ (%)	0.02 ± 0.01	0.09 ± 0.00	0.00 ± 0.00	0.04 ± 0.02
P$_{tot}$ (mg/kg)	304.6	804.5	357.9	932.7
pH (−)	7.76	8.00	7.88	8.05
CECcmol (+)/kg	3.86	6.39	4.27	5.01
BAF bioavailable fraction				
Different letters indicate significant difference between treatments at $p < 0.05$				

The phytoremediation potential of the two plant species in terms of plant survival is shown in Table 2. Data related to plant growth (dry weights and lengths of epigeal and hypogeal parts) are shown in Fig. 1.

Table 2

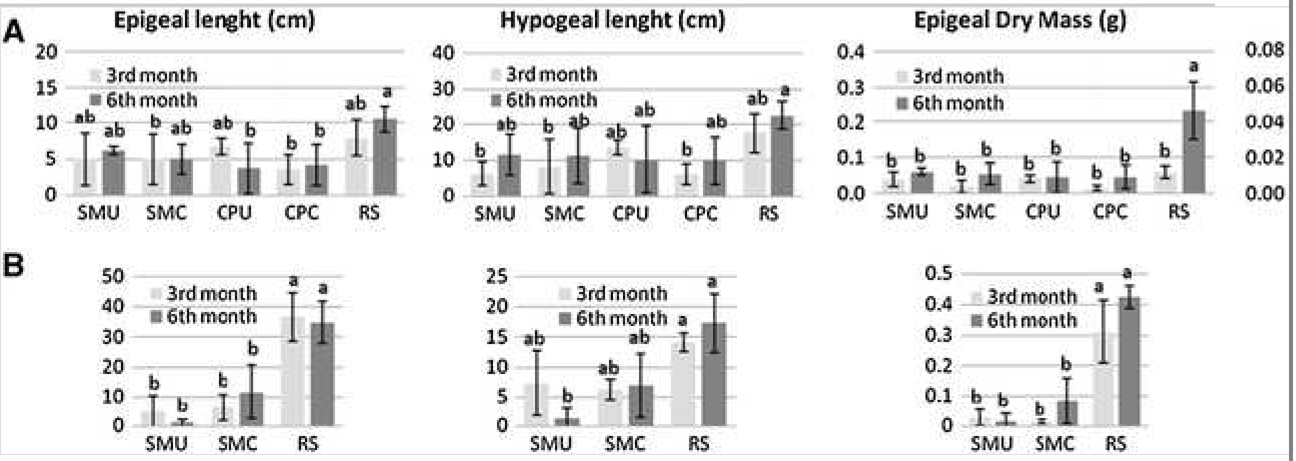
Survival of *P. lentiscus* and *P. australis* in the phytoremediation tests (mean; n = 10)

Matrix	**Survival (%)**			
	P. lentiscus		***P. australis***	
	3 months	**6 months**	**3 months**	**6 months**
RS	100	98	100	88
SMU	100	100	58	25
SMC	90	90	83	58
CPU	80	77	–	–
CPC	89	89	–	–

RS reference soil, *SMU* Sa Masa untreated, *SMC* Sa Masa + compost, *CPU* Campo Pisano untreated, *CPC* Campo Pisano + compost

Fig. 1

Dry weight and length of the epigeal and hypogeal tissues assessed at 3 and 6 months for *P. lentiscus* (~~a~~A) and for *P. australis* (~~b~~B). Significant differences ($p < 0.05$) are represented by *different lower-case letter* labels



As for *P. lentiscus*, germination evaluated after 2 months was low for all treatments ranging between 19 % (SMC) and 34 % (RS); moreover, differences between treatments were not statistically significant (data not shown). Table 2 shows the survival of *P. lentiscus* after 3 and 6 months. In all treatments, the majority of seedlings was able to survive after 6 months in tested soils. These results suggest that both germination and plant survival of *P. lentiscus* are not good indicators of matrix toxicity. Moreover, overall survival data confirm the high adaptability of this plant species to environmental stress.

It's noteworthy that plants grown on contaminated soils displayed biomass not significantly different compared to values registered on the reference soil after 3 months ($p > 0.05$), indicating a great adaptability of the plants on contaminated soils (Fig. 1 ~~a~~A). However, the biomass data in the reference soil were significantly higher ($p < 0.05$) at the end of the trial compared to the intermediate sacrifice, while plants grown on contaminated soils did show neither a significant biomass increase during time ($p > 0.05$) nor a difference between treatments ($p > 0.05$). Similar results were also obtained for length parameters. Thus, overall data demonstrated that *P. lentiscus* exhibited the same growth pattern irrespectively of both the soil matrix (marshy soil vs. mine tailings) and compost addition. The growth interruption after 3 months on contaminated soils could be attributed to both metal toxicity and nutrient lack suppressing biological development. In a field experiment on the mining area of Campo Pisano reported by Bacchetta et al. (2012), *P. lentiscus* demonstrated a great adaptability to the high metal concentrations, expecially when organic amendments were used. After 2 years, the assessed plant survival was still around 95 % in the amended plots, whilst it dropped to 10 % in the untreated one. In the study of Fuentes et al. (2007), where *P. lentiscus* was grown in nutrient solutions containing a range of Cu, Ni and Zn concentrations, the assessed overall growth was low when the lowest metal doses were applied, whilst biomass production was promoted at intermediate doses. Even if seedlings may respond to heavy metal enrichment differently in hydroponic cultures than in solid substrates, this would indicate a great adaptability of the plants to metal contaminated substrates.

As to *P. australis*, plant survival in contaminated soil was remarkably lower than in the reference soil and compared to *P. lentiscus* (Table 2). The addition of compost increased the plant survival compared to that observed on untreated Sa Masa soil. Moreover, the exposure to stress conditions on contaminated matrices induced an increasing mortality over time. Length and dry weight values (Fig. 1 ~~b~~B) were found to be significantly higher in plants grown on reference soil than in those on contaminated matrices both at 3 and 6 months. Thus, differently from *P. lentiscus*, this plant species exhibited a reduced growth in contaminated soils. Average values were always higher in soil treated with compost than in untreated soil even though differences were not statistically significant ($p > 0.05$) due to high standard deviations of data.

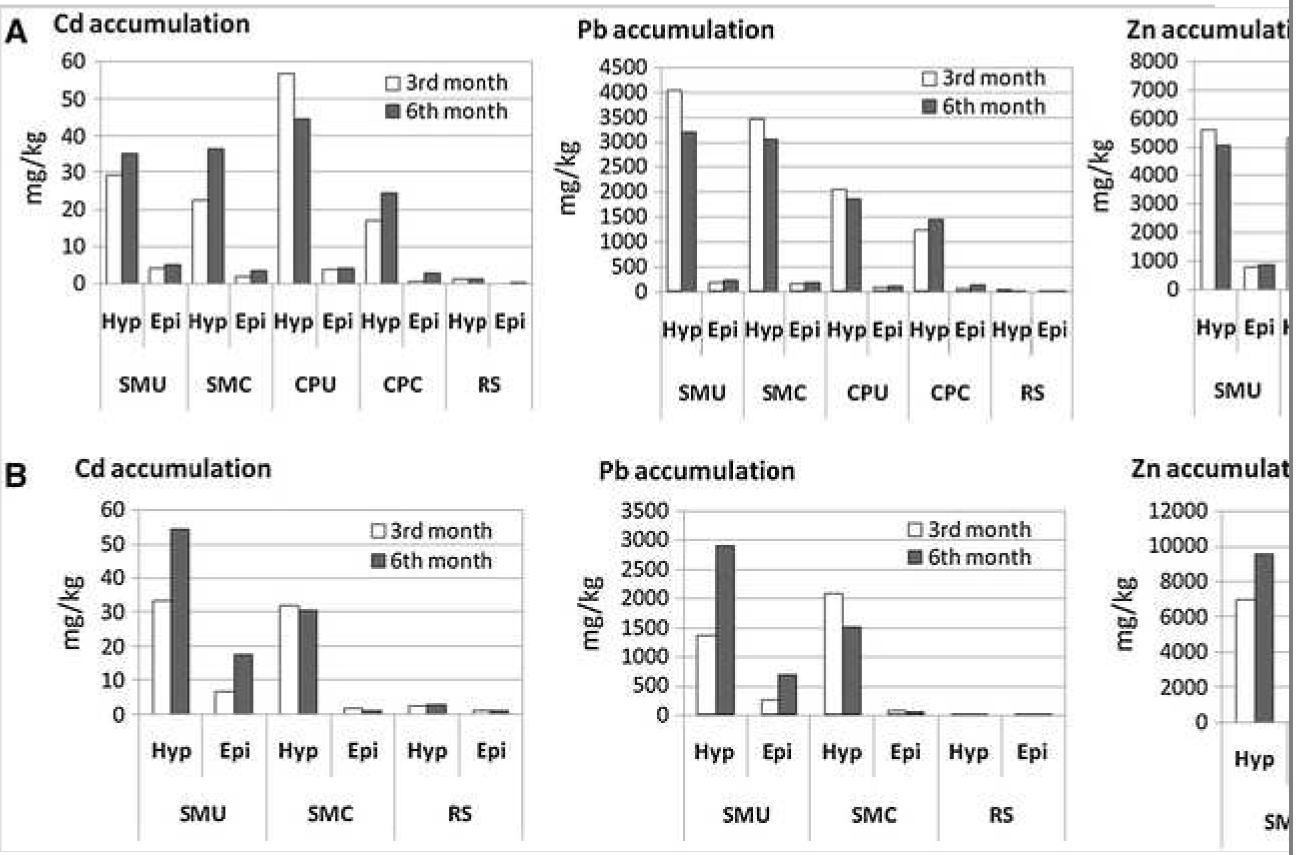
Pioneer herbaceous and rhizomatous plants such as *P. australis* have been reported to possess high metal tolerance, fast growth rate and high coverage (Bragato et al. 2006; Wang et al. 2008), which are desirable properties denoting the species aptitude for phytoremediation. The low survival and reduced growth assessed for *P. australis* in this study in contaminated soil with respect to reference one could be ascribed also to the poor soil physical structure, that could be a very important constraint to plant establishment. The very fine grained particles composing mine tailings expose roots to compaction and cementation processes. This in turn means scarce root penetration and ventilation, which is fatal to most plants (Wang et al. 2008). Conesa et al. (2006) and Chiu et al. (2006) point out that organic amendments could be able to improve physical properties of tailings (water retention, bulk density). This effect may explain the higher survival obtained in this study by compost addition suggesting to more deeply investigate the optimal amendment quantity.

Figure 2 shows metal accumulations assessed in the plant tissues. The accumulation levels evaluated in the epigeal and hypogeal tissues do not seem to differ comparing the two plant species. Both *P. australis* and *P. lentiscus* concentrated metals preferentially in roots, exhibiting a translocation factor (TF, metal concentration in the aerial part divided by that in root) invariably lower than 0.1. Accumulation of the different metals in both species followed the order Zn > Pb > Cd, consistently with the different levels of metal bioavailability in soils; moreover, Zn is an essential element for plant growth, whilst Pb and Cd are highly toxic and not usually accumulated in large amounts by plants. All the metal concentrations measured in the hypogeal fraction were higher than the bioavailable fraction in soils and well above the metal contents considered phytotoxic (Vamerali et al. 2010; Kabata-Pendias and Pendias 1992), thus indicating a metal tolerance of both plants, which may have developed an internal detoxification mechanism (Wang et al. 2008). The metal concentration ratio of plant roots to soil bioavailable fraction was higher than two, reaching a value of four in the case of Zn. Uptake data suggested that both species applied an exclusion strategy of Zn, Pb, and Cd by their immobilization in root tissues (Baker 1981). As for *P. lentiscus,* results are in agreement with previous studies reporting higher heavy metal accumulation in roots than in shoots (Fuentes et al. 2007; Domìnguez et al. 2008, 2011; Bacchetta et al. 2012). The same mechanism of metal tolerance and immobilization in root tissues was also observed in *P. australis* by various authors (Ye et al. 1997; Chiu et al. 2006; Conesa et al. 2006; Wang et al.

2008). For both plant species, metal uptake in the plant tissues was generally lower in soils amended with compost than in untreated ones, consistently with the lower metal bioavailability measured in the soil-compost mixture (Table 1). In agreement with the present study, Bacchetta et al. (2012) have demonstrated the reduced metal uptake of *P. lentiscus* when organic amendments were added. After 2 years, the assessed plant survival was still around 95 % in the amended plots, whilst it dropped to 10 % in the untreated one.

Fig. 2

Cd, Pb and Zn accumulation in plant tissues assessed at 3 and 6 months for *P. lentiscus* (~~a~~A) and *P. australis* (~~b~~B). *Hyp.* Hypogeal tissue, *Epi*. Epigeal tissue



As far as the evolution over time of metal accumulation is concerned, it must be pointed out that a clear trend could not be identified. The concentrations of metals in the different plant tissues in some cases appears to be reduced after 6 months when compared to that assessed after 3 months (Fig. 2) even if differences are not statistically supported. Being the plant biomass not significantly increased after 6 months as compared to 3 months, data cannot be interpreted as an effect of a decreased accumulation efficiency.

Based upon the presented data, it can be concluded that the native species *P.*

lentiscus and *P. australis* possess relevant characteristics for phytostabilization projects: they are both tolerant to high levels of metal contamination and able to restrict metal accumulation to root tissues, which, in turn, limits the risk of metals entering the food chain. In addition, they are known to have fast growth rate and soil coverage, that are additional desired properties in phytoremediation systems (Gonzalez and Gonzalez-Chavez 2006; Mendez and Maier 2008b). However, the overall survival and growth data indicate a higher adaptability to the tested soils of *P. lentiscus* if compared to *P. australis*. Indeed, *P. lentiscus* displayed in the short-term a survival and biomass production in contaminated soils comparable to those measured in uncontaminated soil, whilst *P. australis* had higher mortality and lower growth. The use of compost as amendment was able to improve soil properties, such as nutrient content and CEC, and to mitigate the initial toxicity of the heavy metal contaminated matrices, resulting in a lower metal uptake in the plant tissues, which in turn can increase the plant establishment on mine sites and limit the migration of heavy metals in the surrounding environment. The addition of the optimal quantities of compost could also contribute to enhance the physical structure of mine soils; their texture indeed does not support biological growth, which is, besides the high contamination levels, another important limiting factor for plant survival and growth.

Acknowledgments

This research has been funded by the Regional Sardinian Government, in the framework of “L.R. 7/2007, Promotion of scientific research and technological innovation in Sardinia”.

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