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Zn, Pb and Hg Contents of *Pistacia lentiscus* L. Grown on Heavy Metal-Rich Soils: Implications for Phytostabilization

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Abstract In this study, we determined the metal (Zn, Pb and Hg) contents in epigean and hypogean organs of *Pistacia lentiscus* L., a Mediterranean native plant grown on heavy metal-rich soils of Iglesiente (southwestern Sardinia, Italy), in view of its perspective use for revegetation and phytostabilization of mine waste piles. Plant samples were collected from four different areas in the district. Metal contents in the different plant tissues are roughly dependent on their total and mobile (diethylene triamine penta acetic acid (DTPA)-extractable) contents in soil and are shown in the following ranges: 48–628 mg kg^{-1} (Zn), 2–354 mg kg^{-1} (Pb) and 13–530 μg kg^{-1} (Hg) and usually decrease in the following order: roots>stems>leaves; the apparent exception for Hg, with an order of leaves>stems, is ascribed to foliar absorption of this element. The biological concentration factors are consistently low (≤0.05) for all metals and support the concept that the strategy of metal tolerance of *P. lentiscus* is based on exclusion. These results are consistent with most previous literature data, confirming that *P. lentiscus* is well suited for revegetation actions and could decrease metal mobility through the soil stabilization strategy.

Keywords *Pistacia lentiscus* · Zinc · Lead · Mercury · Iglesiente · Sardinia

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1 Introduction

Sardinia is one of the Italian regions where mining industry had a profound influence on history, economy, culture and landscape, leaving a substantial legacy on the environment that requires remediation and rehabilitation. Heavy metal soil contamination is one of the main issues (e.g. Vacca et al. 2012; Concas et al. 2015). Many technologies for contaminated soil remediation are costly and invasive or do not achieve a long-term solution (Mulligan et al. 2001; Cao et al. 2002). Phytoremediation (also called phytomanagement) can provide a cost-effective, long-lasting and aesthetic solution for remediation of contaminated sites (Cao et al. 2009). The most radical approach of phytoremediation for inorganic contaminants is phytoextraction, whereby metals are taken up and accumulated into plant shoots, which can then be harvested and removed from the site (e.g. Baker and Whiting 2002). However, whenever for practical reasons phytoextraction is not feasible (e.g. Mertens et al. 2004; Van Nevel et al. 2007), a convenient alternative can be phytostabilization, where plants are used to minimize metal mobility from contaminated soils (Mendez and Maier 2008a, b). A well-established plant cover would decrease the risk of wind and water erosion and thus reduce the exposure pathways of humans and animals to heavy metals at reclamation sites (Wong 2003). This aspect is particularly important in regions like Sardinia, typically swept by strong winds and subjected to sudden storms during the rainy season. Thus, soil stabilization would minimize a significant source of metal dispersion, represented by mechanical removal of contaminated material due to wind deflation and rain runoff. Moreover, a thick vegetal cover would slow the percolation of soluble metals from the contaminated horizons to the subjacent horizons, and eventually to the water table, and would reduce the development, during the dry season, of efflorescent salt crusts, which represent an important cause of metal mobilization at the beginning of the rainy season (washout effect; e.g. Canovas et al. 2010).

It is important to use native plants for phytoremediation (e.g. Whiting et al. 2004; Cao et al. 2009; Barbafieri et al. 2011), because these plants are usually not invasive or dangerous to regional plant diversity, and are often better, in terms of survival, growth and reproduction under environmental stress than exotic species as naturally selected (Jiménez et al. 2011). Between 2008 and 2010, a research group of Università di Cagliari set up a revegetation test of mine tailings at Campo Pisano, one of the main mining centers of Pb-Zn in the Iglesiente District, southwestern Sardinia (Bacchetta et al. 2012). One of the chosen species was *Pistacia lentiscus* L., a wild perennial shrub typical of maquis, widely occurring in Sardinia and in many other Mediterranean areas, with a good potential for revegetation (García-Fayos and Verdú 1998; Moreno-Jiménez et al. 2009; Bacchetta et al. 2012). On the other hand, specific studies of soil-plant systems involving *P. lentiscus* in heavy metal-contaminated areas are fairly scarce (see Section 3.2). In this study, we assess the contents of some heavy metals (Zn, Pb and Hg) in soil samples and their distribution in the different parts of *P. lentiscus* plants collected in various areas of the Iglesiente District to evaluate the biological coefficients, metal tolerance and accumulation strategies of this species. The study includes soils and plants collected in 2012 from one of the experimental plots established by Bacchetta et al. (2012); our data can thus document any intervened change(s) in this 2-year interval. The data presented in this study can be useful for long-term projection of possible evolution of revegetation actions based on *P. lentiscus* as a soil stabilizer cover on metal-contaminated substrates.

2 Materials and Methods

2.1 Study Sites

The study area of this work lies within the very important Pb-Zn mining center in the Iglesiente District (Fig. 1), where the metalliferous bodies are hosted by Cambrian limestones and dolomites (Bechstädt and Boni 1994). The deposits were exploited for centuries until final closure in 1997. According to the bioclimatic classification of Rivas-Martínez et al. (2002) and taking into account the thermo-pluviometric data processed by Bacchetta et al. (2009), the whole study 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.

Four different sampling areas were selected. One of these is the Campo Pisano mine, where, as previously mentioned, Bacchetta et al. (2012) set up, in 2008, a revegetation experiment on tailing piles; a number of different soil amendments (zeolites, chemical fertilizer and compost) were applied, and two Mediterranean species (*P. lentiscus* and *Scrophularia canina* L. subsp. *bicolor* (Sibth. and Sm.) Greuter) were planted. In May 2012, when plants for this study were sampled, only four (out of the initial 80) dead *P. lentiscus* plants (with growth and size lower than those of the other plots) were counted in the untreated control plot whereas, in the amended plots, several individuals survived. The best success was recorded for the plot treated with compost (55 individuals were alive out of 80). Although no specific test of physiological functionality was carried out, these plants were apparently in good shape, with no visible signs of stress. Therefore, the plot was selected for this study and is described here as P3 (Fig. 1). A vertical profile was opened in the plot, showing a distinct visual zone. According to the standard soil nomenclature (see Section 2.2), we describe each visually distinct layer as a horizon, although these substrates are not natural soils; hereafter, the terms soil and horizon are used for ease of description.

Soil and plant samples were also collected in the same period (May 2012) at two natural sites (P6 and P7), where the Metalliferous Ring (calcareous rocks hosting the mineralization) crops out (Fig. 1); therefore, these sites should represent the local geochemical

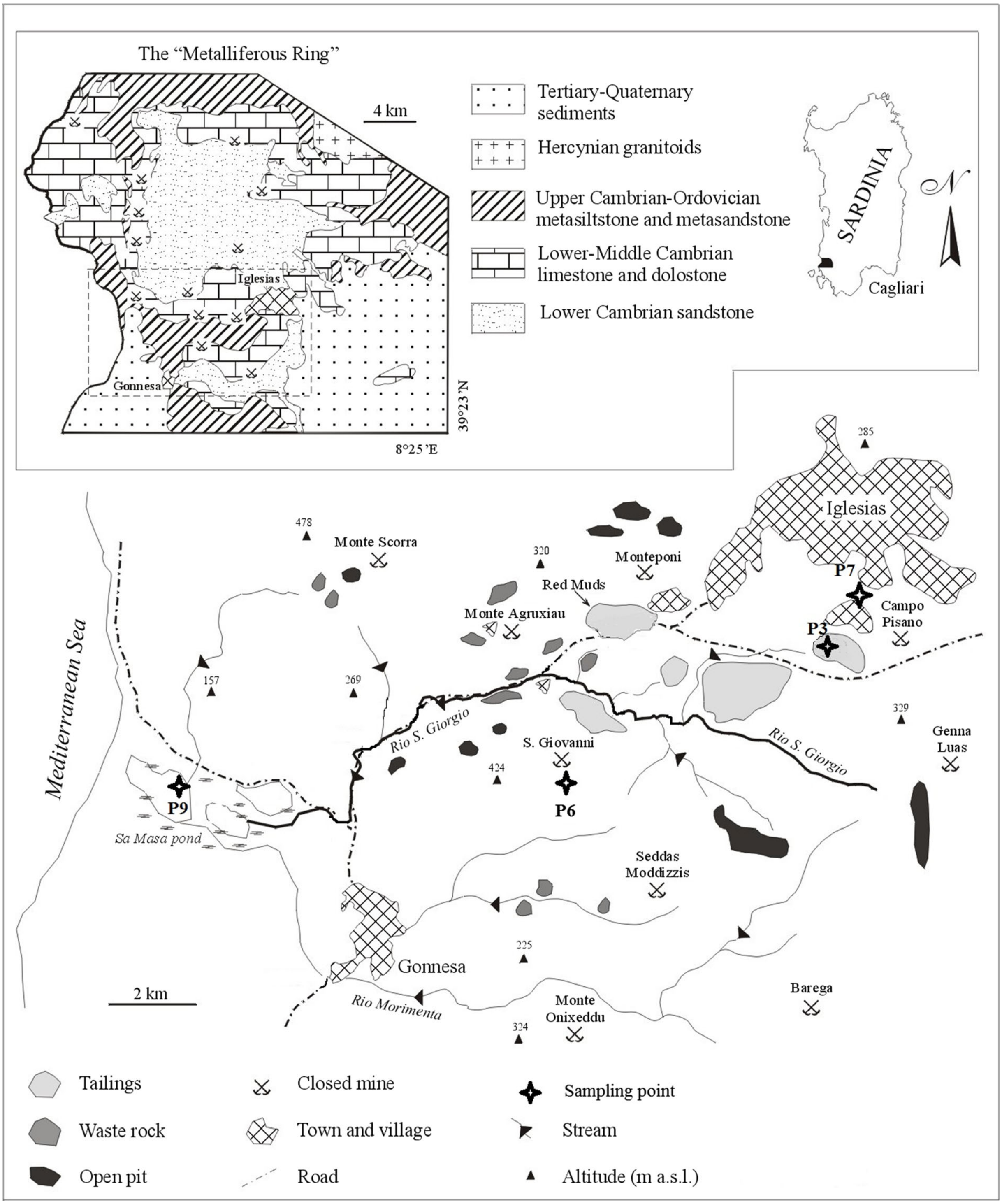

Fig. 1 Geological sketch of the study area, with indication of sampling points (modified after Cidu et al. 2005)

background (see, however, below). Finally, another sampling site, P9, was inside the 'Sa Masa' swamp, the final collector of waters and sediments of the San Giorgio River, draining the mining area. In this locality, an anthropogenic metal contamination is superimposed on the natural anomaly (e.g. Boi 2013).

2.2 Soil Analysis

At the sampling points, soil profiles were opened and described, making a distinction in pedogenetic horizons according to standard procedures of soil description (Schoeneberger et al. 2002). Samples (about 2 kg each) were taken from each soil horizon, air-dried at room temperature for 4 or 5 weeks, sieved using a 2-mm stainless steel sieve and carefully mixed. Physical and chemical analyses were carried out following the internationally recommended procedures and the Italian official methods (GURI 1999). Prior to pedogeochemical analysis, the samples were ground to powders using an agate electric grinder. About 0.25 g of each sample was digested in an HNO_3/HF/$HClO_4$ (2.5:1:0.5 *v*/*v*) mixture in a microwave oven (Milestone Ethos 1), using the EPA Method 3051a procedure, and finished in a hot plate with the addition of $HClO_4$ (1 ml) and HNO_3 (2 ml). The solutions obtained were analyzed for total metal concentrations using a PerkinElmer Optima 2000 DV inductively coupled plasma optical emission spectrometer (ICP-OES). A reagent blank and a reference material (NIST Montana Soil 2710) were also analyzed. Analysis of Hg was made with the AMA 254 automatic solid/liquid mercury analyzer, without acid digestion, directly on the dried and powdered samples; in this case, the reference material was PACS-1 (National Research Council Canada). To our knowledge, there were no previous data of Hg contents of Iglesiente soils. This element occurs in non-negligible amounts in the ores, and it has been the cause of some environmental concern (e.g. Cidu et al. 2001). The mobile fraction of metals in soils was determined by extraction in diethylene triamine penta acetic acid (DTPA) solution (Barbafieri et al. 1996; GURI 1999). Random duplicates were run to assess reproducibility. We estimate the overall precision and accuracy at ±10 % or better.

2.3 Plant Analysis

Five young (3- or 4-year-old) plants of *P. lentiscus* were collected from each site. This number is deemed adequate to statistically account for intraspecific variations. Each plant was divided into roots, stems and leaves and washed accurately with deionized water and finally with Milli-Q water. After washing, the plant samples were oven dried at 60 °C for 3 weeks and then ground to powders using a Retsch steel electric grinder. About 0.5 g of these powders was dissolved with a microwave oven (Milestone Ethos 1) using the EPA Method 3051a procedure, in an HNO_3/H_2O_2 acid mixture (3:1 *v*/*v*). The resulting solution was analyzed for total metal concentration using a PerkinElmer Optima 2000 DV ICP-OES. A reagent blank and a reference material (Metals in Soil, SQC 001) were also analyzed. We specifically sought Zn and Pb that are the two most abundant heavy metals in the area (Boni et al. 1999) and other elements of environmental significance (As, Cd, Cr, Cu and Ni). Specific analyses of Hg were made with an AMA 254 automatic solid/liquid mercury analyzer, without acid digestion, directly on the dried and powdered samples. The analytical quality was checked with a reference material (NIST 1573a; tomato leaves). Random duplicates were run to assess reproducibility. We estimate the overall precision and accuracy at ±15 % or better for most elements; accuracy for Hg is slightly worse (about 20 %).

The metal uptake ability by plants can be expressed through the use of biological coefficients (Fellet et al. 2007). Here, we adopted the following parameters: biological accumulation coefficient (BAC), biological concentration factor (BCF) and translocation factor (TF), defined as follows:

$$BAC=[\mathbf{M}_{ep}]/[\mathbf{M}_s]$$ (Marchiol et al. 2013)

where

[$\mathbf{M}_{ep}$] Metal concentration in the epigean parts
[$\mathbf{M}_s$] Metal concentration in the soil

$$BCF=[\mathbf{M}_r]/[\mathbf{M}_s]$$ (Fellet et al. 2007)

where

[$\mathbf{M}_r$] Metal concentration in the roots
[$\mathbf{M}_s$] Metal concentration in the soil

$$TF=[\mathbf{M}_{ep}]/[\mathbf{M}_r]$$ (Brunetti et al. 2009)

where

[$\mathbf{M}_{ep}$] Metal concentration in the epigean parts
[$\mathbf{M}_r$] Metal concentration in the roots

2.4 Statistical Analysis

Statistical analysis was performed using Statistica version 6.0 (StatSoft). One-way ANOVA was used to analyze the treatment effects, and a *post hoc* analysis of variance was used to evaluate differences among means using the least significant difference at 5 % level.

3 Results and Discussion

3.1 Soil Data

Table 1 reports some soil characteristics that usually influence metal mobility (e.g. Brunetti et al. 2009). A full description of soil data is reported by Concas (2014). Using the USDA texture classification triangle, the texture of soil samples ranges from loam to sandy. The organic carbon (OC) content in natural soil horizons ranged from 5 g kg^{-1} at the P9 site to 38 g kg^{-1} at the P7 site; higher values, ranging from 61 to 66 g kg^{-1}, were found in the soil horizons at the P3 site, where compost was added during the experiment of Bacchetta et al. (2012). The pH ranged from neutral to moderately alkaline (7.1 to 8.1) for all soils, which is normal for soils developed onto a carbonate substrate and usually corresponds to conditions of limited mobility of metals. The total carbonate ranged from 199 to 773 g kg^{-1}, except at the P6 site where it was in trace. At the Campo Pisano (P3) site, pH and cation-exchange capacity (CEC) values can be compared with those determined by Bacchetta et al. (2012) on the same soil in 2008, before starting the vegetation experiment: pH values are similar (7.6 vs. 7.2), whereas the CEC reported here (54 cmol kg^{-1}) is higher than that in 2008 (16 cmol kg^{-1}), probably because of the addition of compost during the experiment.

Table 2 shows the contents of heavy metals (zinc, lead and mercury) measured in each soil horizon. The contents of the other analyzed elements in plant organs were below instrumental detection limits (mg kg^{-1}: Cu 0.05, Cr 0.2, Ni 0.25, As 2.5). For comparison of these soil data with heavy metal concentrations in plant samples, the weighted average was used for each site, because the sampled roots extend across all soil horizons. Accordingly, heavy metal values found in plants are the result of uptake from the total soil depth. In all the sites, the most abundant metal is zinc; metal ratios are roughly of the same magnitude order as observed in primary mineralization (Boni et al. 1999). In general, the total contents in soil samples are much higher than the limits imposed by Italian laws (GURI 2006, D.lgs. 152) for sites for commercial and industrial use (Zn = 1500 mg kg^{-1}, Pb = 1000 mg kg^{-1}, and Hg = 5 mg kg^{-1}). Actually, these limits exceeded also at the two natural sites, P6 and P7. This suggests an anomalous geochemical background, in agreement with the presence of mineralized bodies in this area. It should be noted that Pb and Zn values at these sites are, in fact, higher than median values of stream sediments in the district (Boni et al. 1999) that can be taken as an

Table 1 Main soil characteristics

Profile	Horizon	Depth (cm)	Fine earth fraction (g kg^{-1})					pH (H_2O)	CEC (cmol kg^{-1})	Total CO_3 (g kg^{-1})	OC (g kg^{-1})
			Coarse sand	Fine sand	Coarse silt	Fine silt	Clay				
P3	^Ap1	0–1.8	199	539	144	104	14	7.2	30	402	61
	^Ap2	1.8–17	333	446	177	36	8	7.1	54	322	66
P6	A	0–10	286	155	216	222	121	7.1	29	Trace	37
	Bw	10–37/50	175	140	188	224	273	7.5	22	Trace	18
P7	A1	0–4	151	349	270	190	40	7.9	19	448	38
	A2	4–20/25	228	329	254	146	43	7.9	17	472	24
P9	A	0–8	340	132	438	60	30	7.5	18	199	25
	2C1	8–12	36	148	338	424	54	7.8	5	629	9
	3C2	12–25	84	652	200	46	18	7.8	9	701	6
	4C3	25–40	42	488	337	122	11	7.8	26	733	5
	5C4	40–59	6	335	372	264	23	8.0	21	773	6
	6C5	59–120	261	608	101	20	10	8.1	5	693	5

Table 2 Total and mobile content of main metals (for each horizon and weighted average)

Profile	Horizon	Depth cm	Total Zn mg kg^{-1}	Mobile Zn DTPA mg kg^{-1}	Weighted average±std. dev. Zn mg kg^{-1}		Total Pb mg kg^{-1}	Mobile Pb DTPA mg kg^{-1}	Weighted average± std. dev. Pb mg kg^{-1}		Total Hg mg kg^{-1}	Weighted average Hg mg kg^{-1}
					Total	Mobile			Total	Mobile		
P3	^Ap1	0–1.8	9360	491	8436±318	456±12	1760	52	1587±60	34±6	8	10±0.6
	^Ap2	1.8–17	8327	452			1566	32			10	
P6	A	0–10	4680	467			1360	88			2	
	Bw	10–37/50	5244	465	5131±226	465±1	1453	75	1434±37	78±5	3	3±0.4
P7	A1	0–4	9225	390			4054	506			6	
	A2	4–20/25	8659	364	8750±208	368±10	4050	502	4051±2	503±2	5	5±0.4
P9[a]	A	0–8	(220,946)	147			37,616	13			16	
	2C1	8–12	22,400	692			4300	83			24	
	3C2	12–25	18,805	624	(39,828) 26,891±9211	(522) 549±45	3401	238	(6827)	(292)	21	(20)
	4C3	25–40	13,792	491			3398	462	4628±1481	312±75	21	29±8
	5C4	40–59	17,122	563			2335	282			18	
	6C5	59–120	35,173	534			5927	315			37	
Limits D.lgs 152/2006			1500				1000				5	
Local background values (Boni et al. 1999)			1208				954				Not reported	

[a] For this profile, the A horizon is much different from the others; the weighted averages in parenthesis include this horizon, whereas the other numbers shown do not include this horizon

Table 3 Zn, Pb and Hg contents of plant organs compared with soil contents

		Zn (mg kg^{-1})			Zn in soil (mg kg^{-1})		Pb (mg kg^{-1})			Pb in soil (mg kg^{-1})		Hg (μg kg^{-1})			Hg in soil (μg kg^{-1})
		Roots	Stems	Leaves	Total	Mobile	Roots	Stems	Leaves	Total	Mobile	Roots[a]	Stems[a]	Leaves[a]	Total
P3 2012	Median±MAD	449±46	364±39	152±31			29±12	25±3	10±1			91	45	242	
	Weighted average				8436	456				1587	34				10,000
P3 2009[b]		340		181	10,744	1738	53		13	2632	210				
P6	Median±MAD	79±23	61±4	48±3			16±8	7±2	6±1			16	13	62	
	Weighted average				5132	465				1434	77				3000
P7	Median±MAD	102±5	90±6	34±4			33±3	12±2	2±1			63	23	68	
	Weighted average				8750	368				4051	503				5000
P9	Median±MAD	628±117	293±41	147±21			354±58	26±6	12±3			530	26	97	
	Weighted average				26,891[c]	549[c]				4628[c]	312[c]				20,000

Other metals determined are below the instrumental detection limits (mg kg^{-1}): Cu 0.05, Cr 0.2, Ni 0.25 and As 2.5

MAD median absolute deviation

[a] Composite samples of five individuals of *P. lentiscus* sampled in each site

[b] Bacchetta et al. (2012)

[c] See footnote of Table 2

indication of the local post-mining geochemical baseline. Concas (2014) reports that at both sites, the rock substrates (R layer) show appreciably lower metal contents, suggesting that either the sites are, in some way, contaminated by anthropic activities (e.g. by wind transport from nearby mine sites) and/or pedogenetic processes led to a (passive) enrichment in some heavy metals. This issue is beyond the purpose of this paper, and it will not be further discussed. On the other hand, at the P9 site, the even higher levels of metals, especially Zn and Hg, represent the superposition of anthropogenic impact onto this natural anomaly.

At the Campo Pisano (P3) site, the average total contents of Pb and Zn are of the same order of magnitude as in the study of Bacchetta et al. (2012) (see their Table 3); the moderate variations most probably reflect random heterogeneities of the sampled material. When comparing the metal mobility, we must emphasize that the analytical methods employed in the two studies are different. In fact, the data reported by Bacchetta et al. (2012) result from a more drastic three-step sequential extraction (G. Cappai, personal communication) than the single extraction in DTPA solution applied in this study. Therefore, the values

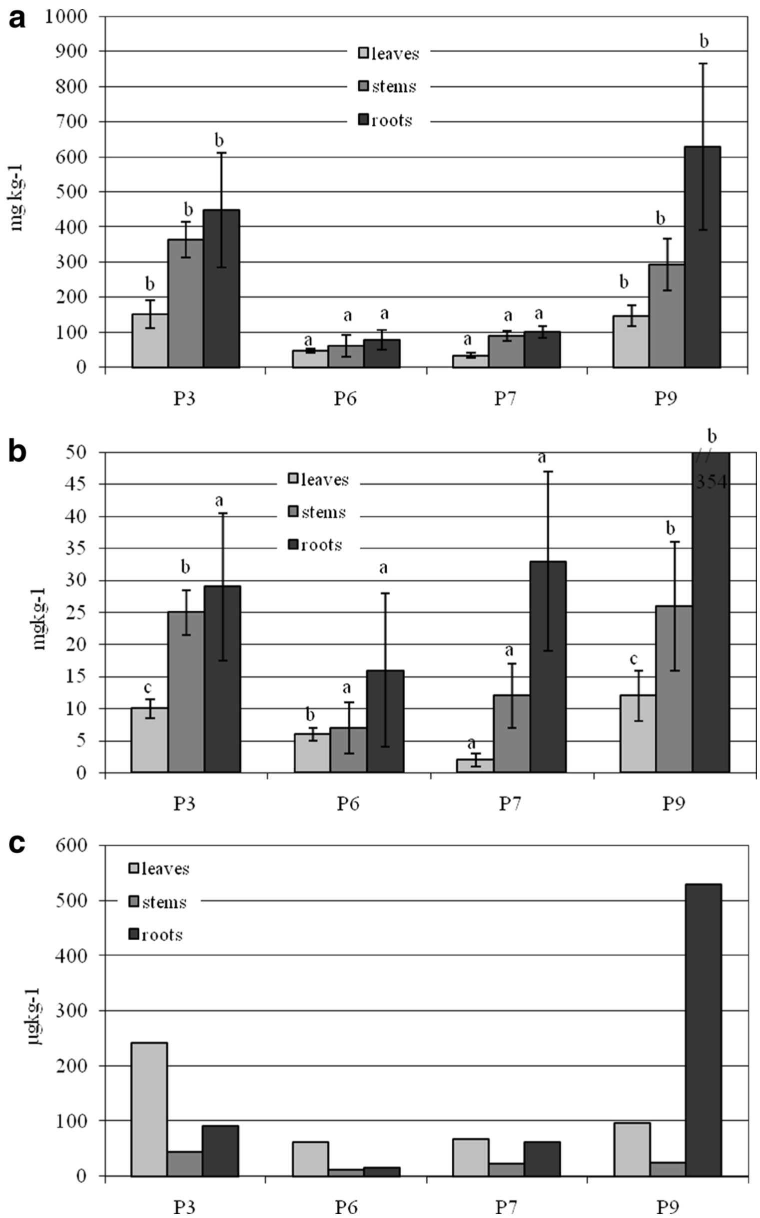

Fig. 2 Bar diagrams showing metal contents in different organs of *P. lentiscus* at the studied sites: **a** Zn, **b** Pb and **c** Hg. For **a**, **b**, the data are the average of independent analyses on the five plants; the *line bars* indicate the standard deviation, and *different letters* indicate statistically different values (p<0.05) among the values of the same plant tissues in the different sampling points. Data for Hg (**c**) are single analyses of composite samples of the five plants (see Section 2)

reported here are about four to six times lower than those of Bacchetta et al. (2012).

3.2 Plant Data

The metal contents of plants are reported in Table 3, compared with total and mobile contents in soils, and displayed graphically in Fig. 2. In general, all the plants show for Zn (Fig. 2a) and Pb (Fig. 2b) the following commonly observed order for metal contents: roots> stems>leaves. A significant exception is an individual plant at the P3 site (data not shown), where both metals show an inverse order of stems>roots, because of the anomalously low contents in the roots. For mercury (Fig. 2c), leaves are systematically enriched with respect to stems and, in the three cases out of four, also with respect to roots (see below). For Zn and Pb, where the analyses of single plants are available, a statistical treatment of data is possible (graphically displayed in Fig. 2a, b). Considering the statistically significant ($p<0.05$) differences for each specific organ, a distinction between two groups—natural sites (P6 and P7) and sites with a marked anthropogenic influence (P3 and P9)—appears. This distinction is especially evident for Zn (all organs consistently show statistically equivalent values within each group and statistically different values between one group and the other). For Pb, root values at the P3 site are statistically similar to those at P6 and P7 sites, rather than to the P9 site; leaf values at the P6 site are statistically different from those at the P7 site.

For Zn and Pb concentrations in roots and leaves at the P3 site, a comparison is possible with previous data of Bacchetta et al. (2012). These authors from 2008 to 2010 periodically sampled roots of a single plant and leaves (randomly from different plants). Very large variations of metal contents were observed (e.g. Zn in leaves from 27 to 1300 mg kg^{-1}), depending on the specific plot and on rainfall. The highest metal contents were typically observed in the control plot, whereas Zn

Table 4 Biological coefficients calculated for *P. lentiscus* from the data in Table 3

Biological coefficients	Profile	BAC		BCF	TF	
		Leaf/soil	Stem/soil	Root/soil	Leaf/root	Stem/root
Zn	P3 2012	0.0180	0.0431	0.0532	0.3385	0.8107
	P3 2009	0.0168	n.d.	0.0316	0.5324	n.d.
	P6	0.0094	0.0119	0.0154	0.6076	0.7722
	P7	0.0039	0.0103	0.0117	0.3333	0.8824
	P9	0.0005	0.0011	0.0023	0.2341	0.4666
	Average	0.0097	0.0166	0.0229	0.4092	0.7329
	SD	0.0077	0.0183	0.0200	0.1549	0.1834
Pb	P3 2012	0.0063	0.0158	0.0183	0.3448	0.8621
	P3 2009	0.0049	n.d.	0.0201	0.2453	n.d.
	P6	0.0042	0.0049	0.0112	0.3750	0.4375
	P7	0.0005	0.0030	0.0081	0.0606	0.3636
	P9	0.0003	0.0006	0.0076	0.0339	0.0734
	Average	0.0032	0.0060	0.0131	0.2119	0.4342
	SD	0.0027	0.0067	0.0058	0.1581	0.3257
Hg	P3 2012	0.0242	0.0045	0.0091	2.6593	0.4945
	P3 2009	n.d	n.d.	n.d.	n.d.	n.d.
	P6	0.0207	0.0043	0.0053	3.8750	0.8125
	P7	0.0136	0.0046	0.0126	1.0794	0.3651
	P9	0.0049	0.0013	0.0265	0.1830	0.0491
	Average	0.0158	0.0037	0.0134	1.9492	0.4303
	SD	0.0085	0.0016	0.0092	1.6421	0.3161

n.d. not determined, *SD* standard deviation content, in general, increased following rainy periods. Therefore, we find our data (referred to May 2012) appropriate to compare with those of a comparable period of the year in the same plot (described here as the P3 site). Root and leaf data are available for June 2009. The comparison (Table 3) indicates for both metals, both in roots and leaves, contents of the same order of magnitude in 2009 and 2012.

The values of the three biological accumulation parameters (BAC, BCF and TF) are shown in Table 4 and graphically displayed in Fig. 3. BAC and BCF values are consistently very low, with no statistically significant difference among the different metals; this fact suggests that the tolerance of *P. lentiscus* to these heavy metals is based on exclusion. Translocation to leaves, as expressed by TF values, is moderate (TF <1), with the apparent exception of Hg. However, this behaviour most probably reflects the foliar uptake of this metal volatilized from the soil (e.g. Millhollen et al. 2006). It is believed that the bioavailable fraction of mercury in soil is generally negligible, and therefore, it is not significantly taken up through plant roots (e.g. Boszke et al. 2008; García-Sánchez et al. 2009).

Correlation analyses were conducted to examine how metals in soil (total and available contents) were related to metal phytoaccumulation in the various plant tissues of *P. lentiscus* (Table 5). There is a certain degree of positive correlation, especially for Zn, between metals in soils and plant organs, but Pearson coefficients are not statistically significant ($p>0.05$). Therefore, a certain indicator character of *P. lentiscus* can be hypothesized but is not conclusively proven by the present data (see further discussion).

These data can be complemented by those reported by previous studies on metal contents in *P. lentiscus* grown on contaminated soils. These studies include, beside that by Bacchetta et al. (2012), those by Leita et al. (1989), Kadem et al. (2004), Molina et al. (2006), Domínguez et al. (2008) and Belabed et al. (2014). We also mention the recent contribution by Bacchetta et al. (2015), which is, in fact, a greenhouse germination

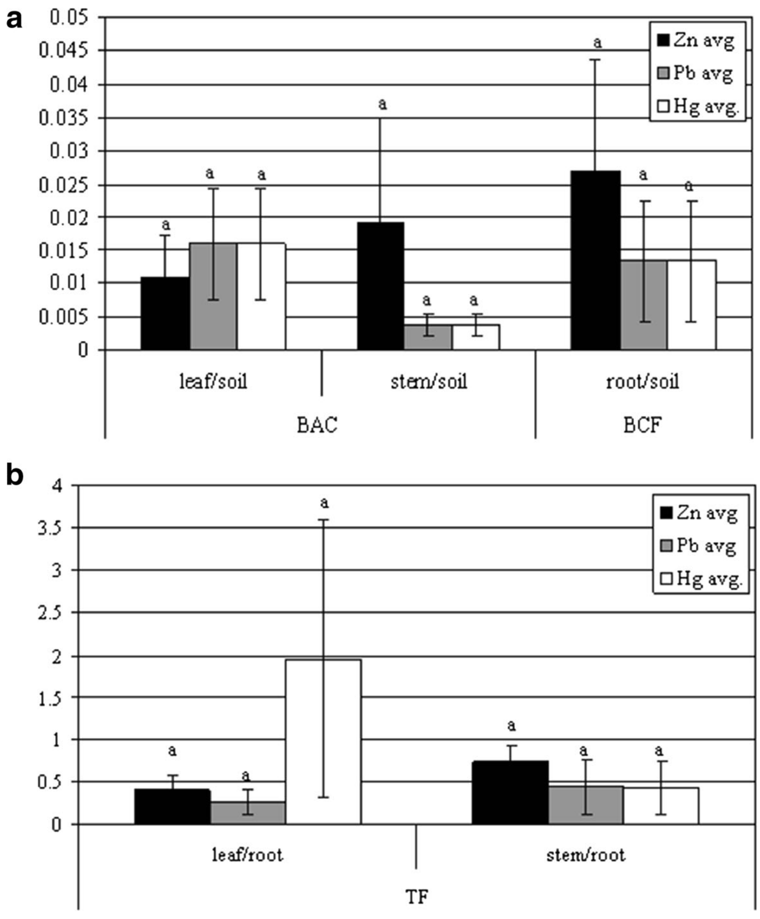

Fig. 3 **a**, **b** Bar diagrams showing the biological coefficients (as defined in the text) calculated for plants of this study. The *bars* indicate the standard deviation

Table 5 Pearson correlation coefficients for Pb and Zn in soils and in plant organs

	Leaves	Stems	Roots
Pb			
TS	0.296	0.017	0.719
MS	0.479	0.107	0.272
Zn			
TS	0.586	0.477	0.816
MS	0.699	0.514	0.766

TS total content of metal in soils, *MS* mobile (DTPA extraction) content of metal in soils

experiment, because it was conducted with soils obtained from the localities of Campo Pisano and Sa Masa. Other data for this area can be found in Leita et al. (1989), who determined Pb and Zn contents in *P. lentiscus* leaves in two sites of Iglesiente with extremely high metal contents in soils (Table 6). Their results show similar contents for Zn to our study, although the corresponding BAC values are slightly lower; on the contrary, they report Pb values in leaves quite higher (up to 30 times) than those of our study and also higher than all other literature data. The BAC values are, however, just slightly higher than those in this study and comparable to that calculated from data of Domínguez et al. (2008) for plants grown in southern Spain. The high Pb values in leaves reported by Leita et al. (1989) may be related to the extremely high mobile (EDTA-extractable) fractions of Pb in the corresponding soils.

Comparison with the data obtained by Bacchetta et al. (2015) is also useful. As previously noted, these data refer to a greenhouse germination study using soils from Campo Pisano and Sa Masa; the analyzed shoots were 6 months old maximum; therefore, no distinction was made between stems and leaves, but the whole epigean parts were considered as single samples. BAC values calculated for epigean parts are, both for Pb and Zn, two to three times higher than those calculated for

Table 6 Compilation of literature data for Pb and Zn in *P. lentiscus*

Reference		Total Zn in soil	Mobile Zn in soil[a]	Zn in leaves	BAC Zn in leaves/soil	Total Pb in soil	Mobile Pb in soil[a]	Pb in leaves	BAC Pb in leaves/soil
Leita et al. (1989)	Onixeddu (site 2)	15,100	6300	124[b]	0.0082	71,000	28,000	402[b]	0.0057
	Seddas M. (site 5)	243,240	19,455	218[b]	0.0009	34,500	13,880	285[b]	0.0083
Dominguez et al. (2008)		427[c]	n.r.	14.9±0.4	0.0351	198[c]	n.r.	1.18±0.39	0.0059
Kadem et al. (2004)		29.2	n.r.	16.64[d]±1.29	0.5899	9.80	n.r.	1.26[d]±1.49	0.1286
Belabed et al. (2014)		1008	n.r.	9169[e]	–	104	n.r.	19[e]	–
Bacchetta et al. (2012)	Control plot	11,949	1639	519[f]	0.0434	3176	533	86[f]	0.0271
	Compost plot[g]	10,744	1738	248[f]	0.0231	2632	210	30[f]	0.0114
Bacchetta et al. (2015)		21,077	2984	890[h]	0.0422	5222	1834	233[h]	0.0446
		18,391	1863	585[h]	0.0318	4330	1319	175[h]	0.0404
		13,893	1271	483[h]	0.0348	3711	696	104[h]	0.0280
		13,780	1051	515[h]	0.0374	3391	550	144[h]	0.0425

All data are expressed in milligrams per kilogram

n.r. not reported

[a] EDTA extraction for Leita et al. (1989) and three-step extraction for Bacchetta et al. (2012, 2015)

[b] Corrected for the water-soluble fraction

[c] Weighted average of their data

[d] Mixed with *Olea europaea*

[e] Leaves+roots

[f] Average of their data in the time span May 2008–May 2010

[g] Corresponding to site P3 in this study

[h] Shoots (6 months old)

leaves in this study. Similar to other plants (e.g. Dinh et al. 2015, and references therein), *P. lentiscus* appears to accumulate more metals in the early stages of growth.

The data by Kadem et al. (2004) and Belabed et al. (2014) present some difficulties for a full comparison. Kadem et al. (2004) report a single value, with no distinction, for leaves of *P. lentiscus* and *Olea europaea*; the corresponding BAC values are much higher than those found in most other studies, including ours. Belabed et al. (2014) report a single value, with no distinction, for *plant tissues* apparently including both roots and leaves of *P. lentiscus*; for this reason, we cannot calculate a BAC value to be compared with our and other studies, but from their data, it appears that *P. lentiscus* should behave as an accumulator for zinc; this finding is totally at variance with all the other literature. Specifically, the low accumulation and translocation observed in this study are in agreement with the results of Leita et al. (1989), Fuentes et al. (2007), Domínguez et al. (2008), Moreno-Jiménez et al. (2009) and Bacchetta et al. (2012, 2015), indicating lower metal translocations for *P. lentiscus* in comparison to most species grown under the same conditions. As opposed to phytoextraction, for plant species to be used in phytostabilization, a limited metal translocation is a positive factor, because it minimizes the chances of metal transfer to the trophic chain (e.g. Mertens et al. 2004).

We finally attempt using the data of Table 6, combined with those reported in this study, to explore possible correlations between Pb and Zn in soils and in leaves of *P. lentiscus* adult plants grown in (quasi)natural environments. For the above-outlined reasons, we excluded from this exercise the data of Belabed et al. (2014) but we included the data by Kadem et al. (2004); because of the lack of distinction by those authors between values for *P. lentiscus* and *O. europaea*, the true value for *P. lentiscus* could be different but we believe that this difference has a little influence on the overall trend. As shown by the graphs of Fig. 4, in spite of the (largely unknown) differences in soil properties (most notably, metal speciation) and specific climatic conditions, there is an excellent correlation between Pb in soils and that in the leaves of *P. lentiscus*; a less good but statistically significant ($p<0.01$) logarithmic correlation exists between Zn in soils and that in plant leaves. These graphs represent purely empirical evidence arising from a limited data set but lend support to the concept that *P. lentiscus* may have some character of indicator for these two metals in soils. This concept is further reinforced when considering the data of

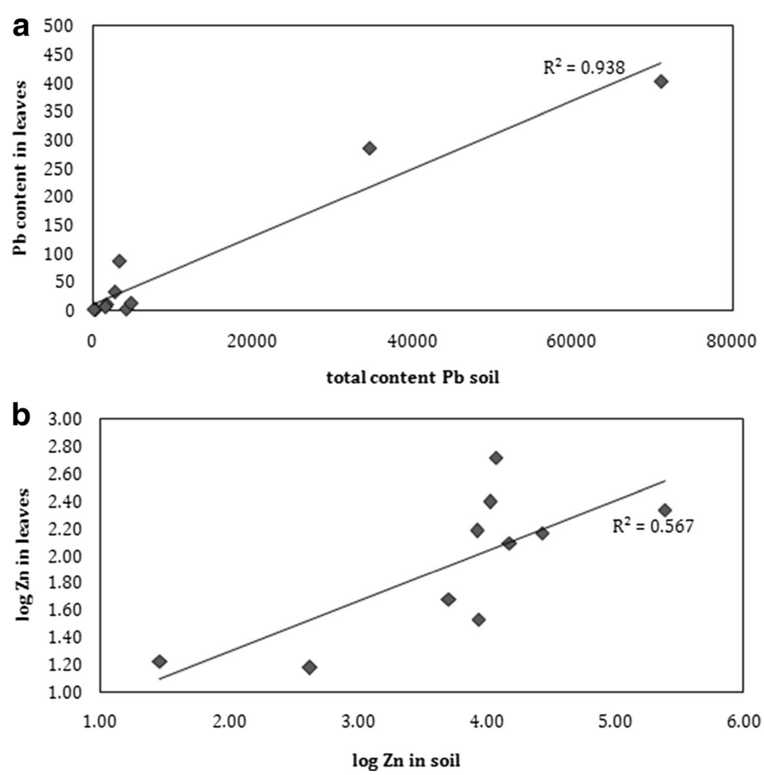

Fig. 4 Correlation between total metal (**a** Pb and **b** Zn) contents in soils and in leaves of *P. lentiscus* (graphs were compiled from data in Tables 3 and 6; see text for details)

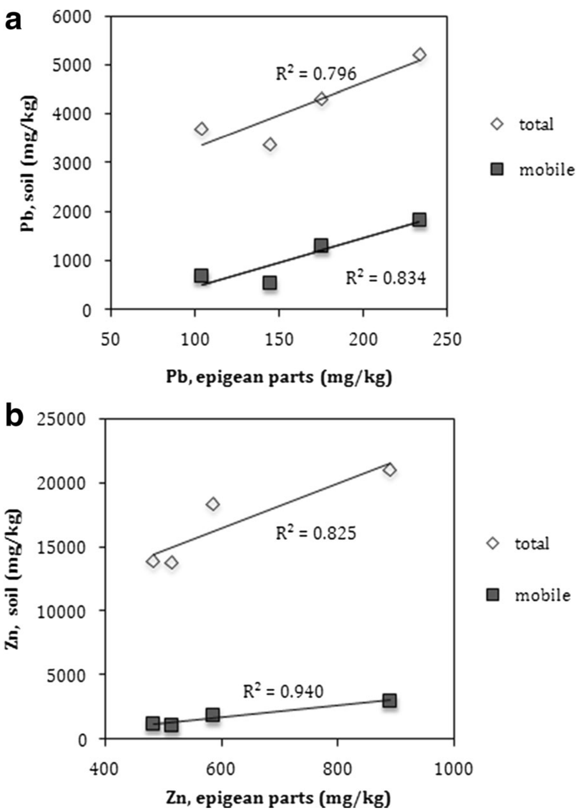

Fig. 5 **a**, **b** Correlation between metal contents of epigean parts of 6-month-old, greenhouse-grown *P. lentiscus* shoots and total and mobile metal contents in soils (data from Bacchetta et al. 2015, as reported here in Table 6)

Bacchetta et al. (2015) for the whole epigean part of greenhouse-grown shoots. The graphs of Fig. 5 show the correlation between total and mobile contents of Pb and Zn in soils and root and leave contents. Regarding Pb, the total content is not significantly correlated with the content in the epigean parts, while the mobile fraction is correlated at p=0.1. For Zn, the total content is correlated with p=0.1 and the mobile content shows a higher correlation, with p=0.05. Such data confirm not only that *P. lentiscus* is a good metal indicator but also that the metal mobile fraction is the most suitable to evaluate the metal transfer in this soil-plant system.

4 Conclusions

In this study, we examined the metal (Zn, Pb and Hg) contents in *P. lentiscus* grown in soils rich of these heavy metals, because of either a natural geochemical background or an increase due to the past mining activity. The *P. lentiscus* seems well tolerant to these highly anomalous metal concentrations. According to biological coefficients (BAC and BCF), the strategy of this plant is that of exclusion, with low translocation to the epigean parts, usually stems>leaves. The apparent exception observed for mercury contents (leaves>stems) is probably due to the contribution by foliar adsorption of this metal. Combining data of this study with literature data, we can suggest that metal contents of plant organs show a rough relationship to soil contents; therefore, this plant has character of being an indicator. All these data confirm that *P. lentiscus* is well suited for revegetation actions and could decrease metal mobility through the soil stabilization strategy.

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Conflict of Interest The authors declare that they have no competing interests.

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