Environ Sci Pollut Res (2017) 24:7809–7820
DOI 10.1007/s11356-017-8488-5

RESEARCH ARTICLE

Response of spontaneous plants from an ex-mining site of Elba island (Tuscany, Italy) to metal(loid) contamination

Laura Pistelli[1] · **Francesca D'Angiolillo**[1] · **Elisabetta Morelli**[2] · **Barbara Basso**[2] · **Irene Rosellini**[3] · **Mauro Posarelli**[4] · **Meri Barbafieri**[3]

Received: 3 August 2016 / Accepted: 19 January 2017 / Published online: 27 January 2017

Abstract The release of large amounts of toxic metals in the neighboring sites of abandoned mine areas represents an important environmental risk for the ecosystem, because it adversely affects soil, water, and plant growth. The aim of the present study was to investigate the metal(loid) (As, Cr, Cu, Ni, Pb, and Zn) contents of native Mediterranean plants grown on the ex-mining area of Elba island (Italy), with the prospective of its recovery by further phytoremediation technology. Soil samples were collected and characterized for metal(loid) content in total and potentially available (EDTA-extractable) fractions. Arsenic was particularly high, being 338 and 2.1 mg kg^{-1} as total and available fractions, respectively. Predominant native species, namely *Dittrichia viscosa* L. Greuter, *Cistus salviifolius* L., *Lavandula stoechas* L., and *Bituminaria bituminosa* L., were analyzed for metal content in the different plant organs. *D. viscosa* exhibited the highest metal(loid) content in the leaves and the singular behavior of translocating arsenic to the leaves (transfer factor about 2.06 and mean bioconcentration factor about 12.48). To assess the healthy status of *D. viscosa* plants, the leaves were investigated further. The activities of the main antioxidant enzymes and the levels of secondary metabolites linked to oxidative stress in plants from the ex-mining area were not significantly different from those of control plants, except for a lower content of carotenoids, indicating that native plants were adapted to grow in these polluted soils. These results indicate that *D. viscosa* can be suitable for the revegetation of highly metal-contaminated areas.

Keywords Ex-mining area · Arsenic · Heavy metals · Plant metal(loid) uptake · *Dittrichia viscosa* · Oxidative stress

Responsible editor: Roberto Terzano

✉ Laura Pistelli
laura.pistelli@unipi.it

[1] Dipartimento di Scienze Agrarie, Alimentari e Agro-Ambientali, Università di Pisa, via del Borghetto 80, 56124 Pisa, Italy

[2] Istituto di Biofisica, CNR, via Moruzzi 1, 56124 Pisa, Italy

[3] Istituto per lo Studio degli Ecosistemi, CNR, via Moruzzi 1, 56124 Pisa, Italy

[4] Dipartimento Tecnologie Energetiche, ENEA, via Moruzzi 1, 56124 Pisa, Italy

Introduction

Elba island is the largest island of Tuscan archipelago, and it has been one of the most important iron mining areas in Italy since Etruscan times (Tanelli et al. 2001). Extraction activity ceased in 1981 and left many abandoned ore mines and waste dumps, mineral deposits, and distributions of heavy metals and metalloids related to iron extraction and processing, such as arsenic (As), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), nickel (Ni), and zinc (Zn) (ARPAT 2002). Nowadays, the abandoned ex-mining sites constitute the Elba Island Mineralogical and Mining Park located in the Rio Marina district. In a detailed geochemical study, Servida et al. (2009) reported that this area is characterized by hematite and pyrite ore association, including carbonate and siliceous bedrocks, together with large amounts of waste rock dumps derived from the mining activity. These sites now represent a potential source of metal(loid) pollutants (Benvenuti et al. 1999; Mascaro et al. 2001; Tanelli et al. 2001; Servida et al. 2009). Moreover, the intense open pit mining activity carried out in these sites produced strong environmental pollution, as demonstrated by contamination of water and soil samples, which showed pH values ranging from 2.8 to 3.4 and high concentrations of some heavy metals (like Cu, Pb, and Zn), exceeding the limits allowed by Italian Legislation (D. Lgs. 152/2006, GURI 2006; Servida et al. 2009). Metals such as Cu and Zn are essential for plant growth, but there are also many reports of the deleterious effects of high concentrations of these metals on plant growth and development (Emamverdian et al. 2015). Toxic levels of heavy metals hinder normal plant functions and metabolic processes affecting vital events, such as photosynthesis, respiration, and enzymatic activities (Furini 2012). Moreover, it is widely reported that heavy metals are associated with the generation of reactive oxygen species (ROS), which causes oxidative stress and alters the oxidative homeostasis in plant cells (Sytar et al. 2013). In addition to heavy metals, arsenic, a non-essential metalloid, is considered to be one of the most toxic elements for living organisms. Once As is inside the cell, it causes morphological, physiological, and biochemical alterations such as inhibition of seed germination and ROS generation leading to oxidative stress (Gupta et al. 2009; Finnegan and Chen 2012; Islam et al. 2015).

Plant cells possess several defense mechanisms to circumvent metal(loid) stress, such as enzymatic and non-enzymatic antioxidant systems, which maintain ROS levels under strict control (Sytar et al. 2013; Hernandez et al. 2015). The most important antioxidant enzymes involved in ROS scavenger activity are superoxide dismutase (SOD), catalase (CAT), peroxidases such as ascorbate peroxidase (APX), and glutathione reductase (GR). The main non-enzymatic antioxidants are glutathione, polyphenols, flavonoids, carotenoids, ascorbate, and organic acids. Polyphenols play an important role in the antioxidant defense since they can chelate metal ions and also react with ROS (Pereira et al. 2009), while glutathione protects cells from oxidative stress and is a precursor of phytochelatins (PCs) (Meharg 2005). Moreover, plant cells also synthesize different metabolites able to sequester intracellular metal(loid) ions into non-toxic stable complexes. An important group of ligands consists of Cys-rich peptides, like PCs synthesized from glutathione (Grill et al. 1985; Cobbett 2000), or metallothioneins (MTs), which are transcriptionally regulated (Meharg 2005). The ability of plants to implement defense mechanisms and tolerance of exposure to metal(loid)s can be exploited to develop phytoremediation technologies, aimed at alleviating the metal(loid) pollution of terrestrial ecosystems (Meharg 2005).

Phytoremediation is considered a novel approach to manage polluted soils, based on the ability of plants to extract and/or to stabilize the metal(loid) pollutants from these soils. Plants suitable for phytostabilization show tolerance and develop an extensive root system able to immobilize contaminant metal(loid)s in the rhizosphere (Kramer 2005). On the other hand, plants that translocate high levels of metal(loid)s to the aerial parts are suitable for phytoextraction. A variety of metal(loid)-accumulating plants have been described, especially those growing in mining areas (Baker and Brooks 1989; Pratas et al. 2013). However, phytoremediation technology for highly polluted soils requires identification of species not only able to tolerate such pollutants but also adapted to grow in the specific environmental conditions of the area of interest (Favas et al. 2014). Therefore, the selection of plant species suitable for phytoremediation needs to be carried out with site-specific studies.

The vegetation present in different Italian mining sites (Sardinia and South Tuscany) has already been studied by other authors, and their metal content has been determined (Baroni et al. 2004; Barbafieri et al. 2011; Bacchetta et al. 2015). The predominant species found in contaminated mining soils of Sardinia Island were *Cistus salviifolius* L., *Helichrysum italicum* (Roth) G. Don, *Arundo donax L.*, *Dittrichia viscosa* L. Greuter, and *Euphorbia dendroides L.*

However, specific studies of soil-plant systems related to the mining sites of Elba island are fairly scarce. The vegetation of Elba island is influenced by the Mediterranean climate and by the insularity and is thus adapted to grow on rocky soils, tolerating both drought and salt stress (Rinaldi 2000). The vegetation is mainly characterized by the "Mediterranean Maquis," constituted by a consortium of different spontaneous species from shrubs (low bush) to more strictly arboreal plants (high bush). Typical plants of this low bush are *Cystus* sp., *Cytisus scoparius* L., *Myrtus communis* L., *Rosmarinus officinalis* L., *Lavandula stoechas* L., and *H. italicum* (Rinaldi 2000). On the contrary, in the Mineral Park of Elba island, only a few species of small shrubs are present, including *D. viscosa*, *C. salviifolius*, *L. stoechas*, and *Bituminaria bituminosa* (L) C.H. Stirton.

D. viscosa is a ruderal shrub of the *Asteraceae* (*Inuleae* tribe, also known as *Inula viscosa* L.) and is considered to be an invasive plant in all the northwestern Mediterranean basin, due to its adaptation to different soils (ranging from siliceous to calcareous). This plant is a pioneer species due to its ability to survive under stressed environmental conditions, such as large excursions of humidity and salinity (Curadi et al. 2005; Batista et al. 2013). In particular, the adaptation of young plants to Cd treatment has been observed (Fernandez et al. 2013). Conesa et al. (2011) indicated *D. viscosa* as a species accumulating large quantities of metal(loid)s from sand dunes polluted by mining wastes in Spain. Moreover, *D. viscosa* was one of the species with the highest concentration of trace elements found in the aboveground biomass of Sardinia ex-mining soil, suggesting that this plant can be considered a good phytoextractor (Barbafieri et al. 2011).

The *Cistus* spp. (Cistaceae family) generally shows a great plasticity, and it is considered a pioneer plant after fire events. It is able to grow both in poor and/or contaminated soils, and it can tolerate important concentrations of metal(loid)s (Murciego et al. 2007; Abreu et al. 2012). Various studies confirmed that this plant can contribute to the rehabilitation of mining areas with semi-arid characteristics. In particular, the species *C. salviifolius* shows suitable behavior for phytostabilization and it can be considered a good indicator of contamination (Barbafieri et al. 2011; Abreu et al. 2012).

B. bituminosa (syn. *Psoralea bituminosa* L., Fabaceae, Pitch trefoil) is a perennial species widely distributed in the Mediterranean basin and the Canary Islands and is a common plant on Elba Island (Sternberg et al. 2006; Pecetti et al. 2016). It is easily recognizable by the marked "bitumen" smell of the foliage, the origin of which is still unclear (Bertoli et al. 2004). *B. bituminosa* is used to provide hay and forage for livestock (Sternberg et al. 2006), but it is also employed in industry because of its content of secondary metabolites such as phenolics, furocoumarins, and pterocarpans (D'Angiolillo et al. 2014). The plant adapts to abiotic stress conditions such as drought (Walker et al. 2010) and heavy metal-polluted sites, so that it can be considered for the phytostabilization of contaminated or degraded soils (Martínez-Fernández et al. 2011).

L. stoechas is a small shrub typically growing in low marquis and in acidophilus garrigues (frequently associated with *Cistus* spp.), occurring in Mediterranean regions from sea level up to 600 m a.s.l. (Pignatti 1982). *L. stoechas* is nowadays commercially cultivated around the world for several uses, including ornamental and/or soil stabilization purposes, or cosmetic uses (Carrasco et al. 2015). *L. stoechas* has been found in serpentinized rocks, accumulating Mn and Co (Freitas et al. 2004). Moreover, some attention has been given to the ability of *L. stoechas* to take up mercury in greenhouse experiments, in order to evaluate their use in Almadén mercury mining sites (Sierra et al. 2009).

The aim of the present study was to investigate the ability of autochthonous plants to survive on the Puppaio area, an ex-mining site included in the Mineral Park of Elba Island. We measured the total and potentially available fraction of metal(-loid)s in Puppaio soils. Metal(loid) concentration in different organs of the most representative plant species was also determined, in order to establish their metal uptake and translocation ability. Furthermore, we assessed the physiological adaptation of a selected plant species, *D. viscosa*, by measuring the antioxidant enzymatic activities, secondary metabolites, and photosynthetic pigments of the leaves.

Materials and methods

Description of the studied area

The studied area is located on Elba island, inside the Mining Park of Rio Marina, in an ex-mining site named Puppaio (SP, 42°50″18.7″N 10°26″10.2″E) (Fig. 1a). After the iron extraction period, at the end of the 1980s, the area was abandoned and, so far, no environmental remediation project has been undertaken. The Puppaio area is structured like a small basin constituted by a large amount of mining wastes, with a central seasonal pond characterized by extremely acidic water (pH = 1.7) (ARPAT 2002). The pond is surrounded by a mostly barren ring area with small shrubs and herbaceous plants (Fig. 1b, c). Externally to the basin, a small hill with a typical Mediterranean vegetation delimitates the area. The Mining Park of Rio Marina is characterized by Mediterranean climate with dry summer and wet autumn. The average annual temperature is 16.9 °C, and the annual precipitation ranges from 387 to 842 mm (Servida et al. 2009).

Collection of soil and plant samples

Two sampling campaigns were carried out in spring of 2013 and 2014. In the first campaign, soil and plant samples were collected in four sampling points near to the pond of the Puppaio area (SP, Fig. 1). A control site (SC, Fig. 1a) was chosen on Elba island, near Portoferraio (42°48″04.3″N 10°19″27.3″E), far from any mining activity. Three soil subsamples at each sampling point were taken at the 0–20-cm depth, put in a plastic bag, and brought to the laboratory. Thereafter, samples were dried at 50 °C until constant weight, ground, sieved through a 2-mm sieve, and stored in sealed plastic boxes until analysis.

Plant samples of *Dittrichia viscosa* L. Greuter, *Cistus salviifolius* L., *Lavandula stoechas* L., and *Bituminaria bituminosa* L. were collected near the barren area around the pond of the SP area. For each plant species, a variable number of samples were harvested within a 3-m radius of each soil sampling point and analyzed for metal(loid) content. In the second sampling campaign, samples of *D. viscosa* leaves were collected in the same sampling points for biochemical analyses. The leaves were washed in situ with deionized water to remove dust and wiped with filter paper. Sub-samples were immediately frozen in dry ice, brought to the laboratory, and stored at −70 °C.

Soil analysis

The main chemical and physical properties of soil (pH, E.C., C.E.C., clay, silt, and sand content) and some parameters of agronomic importance were determined according to procedures described in *Methods of Soil Analysis* (SSSA 1996). Briefly, soil pH was determined using a glass electrode at a soil/water ratio of 1:2.5, cation exchange capacity (CEC) using barium chloride (pH = 8.1), texture (sand, silt, and clay) by the pipette method, P by digestion with perchloric acid, and K by the ammonium acetate method. N and organic and inorganic C were determined using a CHNS analyzer Carlo Erba NA 1500 (Milano, Italy).

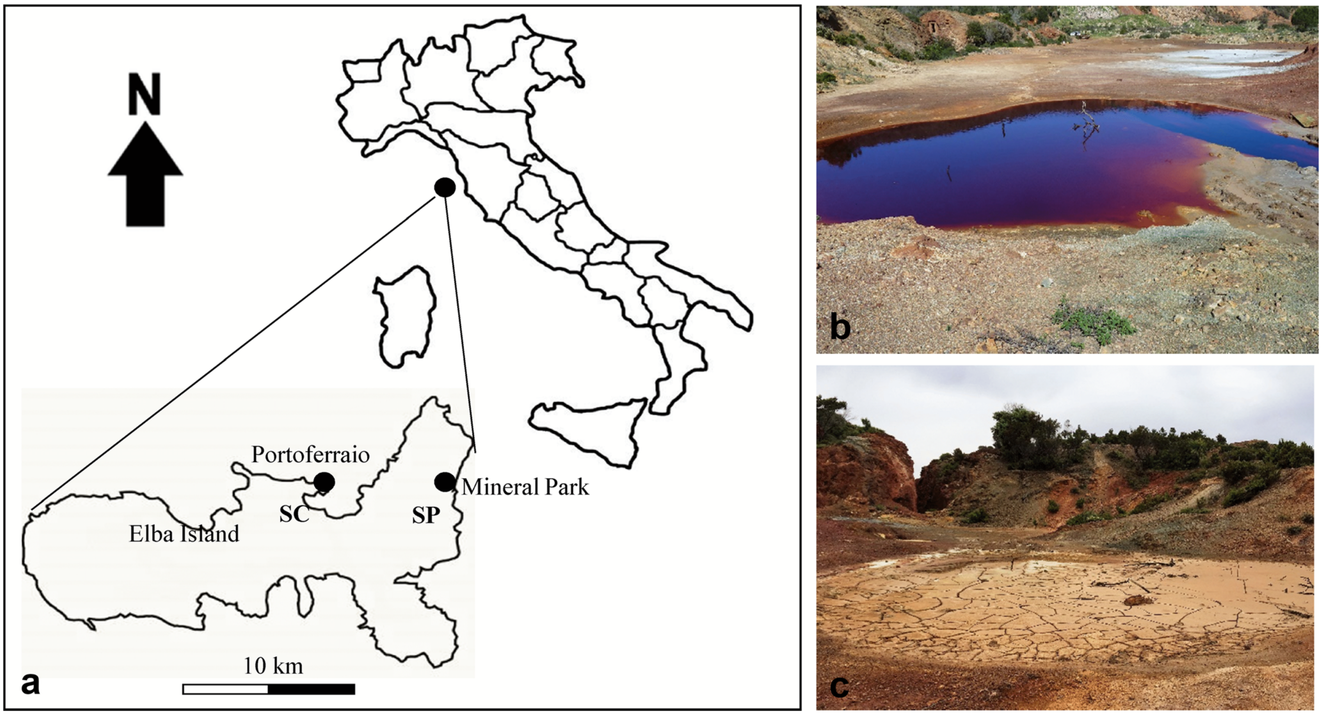

Fig. 1 **a** Geographical location of the study sites in Elba Island (Tuscan, Italy). The Puppaio site in the Mineral Park and the control site in Portoferraio are indicated with *SP* and *SC*, respectively. **b**, **c** Pictures of the sampling area around the seasonal pond of the Puppaio site

Metal(loid) analysis

The soil samples were digested with an HNO_3/H_2O_2 solution (2.5:1 ratio) in a microwave oven system (Milestone 1200, Bergamo, Italy) for (pseudo) total metal(loid) analysis. The available fraction of metal(loid)s in soils was determined by extracting soils for 2 h at room temperature with 0.05 M ethylendiaminetetraacetic acid (EDTA), using 1:10 ratio as described elsewhere (Barbafieri et al. 1996; Italian Official Gazette 1999; Marques et al. 2009). Plant samples for metal(loid) analysis, separated into their organs (leaves, stems, and roots), were carefully washed with deionized water, oven-dried at 50 °C until constant weight, and finely powdered. As regards roots, we added a washing step using an ultrasonic bath to remove adsorbed soil particles. Successively, the samples were digested with an HNO_3/H_2O_2 solution (2.5:1 ratio) in a microwave oven and analyzed for metal(loid) content in the different organs.

All the samples were analyzed by a Liberty Axial Varian (Torino, Italy) inductively coupled plasma optical emission spectrometer (ICP-OES). Quality assurance and quality control were performed by testing a standard solution (multi-elements standard 504305 and 504303, Carlo Erba, Milano, Italy) every 10 samples. A certified reference material was used both for soil (SQC 001) and plants (SRM 1573a) to control the quality of the analytical procedure. A reagent blank was also analyzed. The detection limits ($\mu g\ L^{-1}$) of Cr, Cu, Zn, Pb, Ni, and As were 1, 1, 2, 5, 8, and 5, respectively.

The metal(loid) uptake and distribution in collected plants were expressed using biological coefficients. The biological concentration factor (BCF) for each species was calculated to evaluate the capacity for metal(loid) transfer from the matrices to the plant, accumulating in different organs. It is defined as the ratio of plant metal(loid) content to soil metal(loid) content (Baker et al. 1994). Here, the BCF was calculated using the available metal(loid) fraction (Geebelen et al. 2003; Barbafieri et al. 2011):

$$\mathrm{BCF} = [\mathrm{M_{leaves}}]/[\mathrm{M_{sAv}}]$$

where

$[\mathrm{M_{leaves}}]$ metal(loid) concentration in leaves
$[\mathrm{M_{sAv}}]$ metal(loid) concentration in the soil, available fraction

Another interesting parameter is the translocation factor (TF) indicating the rate of metal(loid) translocated from roots to shoots (Concas et al. 2015):

$$\mathrm{TF} = [\mathrm{M_{leaves}}]/[\mathrm{M_{roots}}]$$

where

$[\mathrm{M_{roots}}]$ metal(loid) concentration in roots

The standard error (SE) of the mean for both the TF and BCF parameters was calculated by using the formula of the propagation of the error:

$$\text{SE for TF} = \left(SE_{[Mleaves]} \times [M_{roots}] + SE_{[Mroots]} \times [M_{leaves}]\right)/[M_{roots}]^2$$

$$\text{and SE for BCF} = \left(SE_{[Mleaves]} \times [M_{sAv}] + SE_{[MsAv]} \times [M_{leaves}]\right)/[M_{sAv}]^2$$

where

$SE_{[Mleaves]}$, $SE_{[Mroots]}$, and $SE_{[MsAv]}$ are the SEs calculated on $[M_{leaves}]$, $[M_{roots}]$, and $[M_{sAv}]$, respectively.

Pigment analysis

The concentration of pigments in 100 mg of fresh leaves of *D. viscosa* was determined, following the method described by Lichtenthaler (1987). The chlorophyll and carotenoids were extracted overnight in 5 mL of pure methanol at 4 °C in the dark. The absorbance of the extracts at 665, 652, and 470 nm was measured using a UV-Vis spectrophotometer (Cintra 101, GBC scientific instruments, Melbourne, Australia), and the content of total chlorophyll and carotenoids was expressed as milligrams per gram of fresh weight. The data presented are the mean of three independent replicates.

Total soluble phenol compounds

One hundred milligrams of fresh *D. viscosa* leaves was pulverized and homogenized in a mortar with 70% (*v*/*v*) methanol to facilitate the extraction. After 30 min of incubation on ice, the extracts were centrifuged at 10000×*g* for 10 min, and the supernatants were utilized for further analyses. Total soluble phenol compounds were assayed using Folin-Ciocalteau's phenol protocol (Singleton and Rossi 1965) with minor modification (Bretzel et al. 2014). Different methanol extracts were added and mixed with 0.5 mL Folin-Ciocalteau's reagent and 0.45 mL of 7.5% (*w*/*v*) solution of saturated sodium carbonate. The samples were incubated at room temperature for 2 h. Absorbance was measured at 765 nm, and the results were expressed as milligrams of chlorogenic acid per gram of fresh weight (mg g^{-1} fresh weight). Quantification data were in triplicate.

DPPH radical scavenging activity

The free radical scavenging activity of *D. viscosa* methanol extracts was measured with the stable radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) method (Brand-Williams et al. 1995). One milliliter of methanol extract at different concentrations (0.10, 0.25, 0.50, mg mL^{-1}) was added to 0.5 mL of a 0.25-mM (*w*/*v*) DPPH solution and incubated at room temperature in the dark for 30 min. The activity was measured as the decrease of absorbance at 517 nm using a UV-Vis spectrophotometer (Cintra 101). The percent inhibition of the DPPH radical by the samples was calculated according to the formula: % inhibition = $(A_{blank} - A_{sample} / A_{blank}) \times 100$, where A_{blank} is the absorbance of the DPPH radical without the antioxidant and A_{sample} is the absorbance of the samples. The concentration (μg mL^{-1}) of the extract providing 50% of antioxidant activities (IC_{50}) was calculated by plotting a graph of the inhibition percentage against extract concentration. All determinations were performed in triplicate.

Determination of non-protein thiols

Glutathione and PCs were measured by a methodology employing pre-column derivatization with monobromobimane and quantification by reverse-phase high-performance liquid chromatography, following a procedure reported elsewhere (Giansoldati et al. 2012).

Enzymatic activities

Frozen *D. viscosa* leaves (0.2 g) were ground under liquid nitrogen and homogenized in 2 mL of extraction buffer, consisting of 50 mM sodium phosphate buffer (pH 7.0), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, and 2% (*w*/*v*) insoluble polyvinylpolypyrrolidone. The homogenate was centrifuged at 20000×*g* for 30 min at 4 °C and the supernatant used for the enzymatic activity. Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed by measuring its ability to inhibit the photoreduction of nitro blue tetrazolium (NBT) according to the method of Beyer and Fridovich (1987). In this assay, one unit of SOD is defined as the amount required to inhibit the photoreduction of NBT by 50%. Catalase (CAT, EC 1.11.1.6) activity was measured according to Aebi (1974), by monitoring the decomposition of H_2O_2. The enzyme activity was calculated by measuring the decrease of absorbance at 240 nm in 1 min (ε = 0.04 mM^{-1} cm^{-1}). Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined by following the decrease of absorbance at 290 nm (ε = 2.7 mM^{-1} cm^{-1}) due to ascorbate oxidation (Nakano and Asada 1981). Glutathione reductase (GR, EC 1.6.4.2) activity was determined by following the decrease of absorbance at 340 nm (ε = 6.2 mM^{-1} cm^{-1}) due to the glutathione-dependent NADPH oxidation, according to the modified method of Foyer and Halliwell (1976). One unit of activity was defined as the amount of enzyme that can oxidize 1 μmol of substrate per minute, in the case of CAT, APX, and GR. All enzymatic activities were calculated per milligram of protein. The soluble protein content was determined according to Bradford (1976) using bovine serum albumin as standard. Enzymatic assays were carried out at 25 °C using a double-beam Jasco UV/Visible Spectrometer (Model V-550, Lecco, Italy).

Statistical analysis on biochemical parameters

One-way ANOVA test and post hoc analysis of variance (Tukey test) were conducted using OriginPro 9.0 software (OriginLab Corporation, MA, USA). Evaluation of normality was carried out using the Shapiro-Wilk test, and a normal distribution of the data was verified at the 0.05 level. The homogeneity of the variance was tested by using Levene's test. At the 0.05 level, the population variances were not significantly different.

Results and discussion

Soil characteristics

The main soil characteristics of the Puppaio ex-mining area (SP) and of the uncontaminated control soil (SC) are reported in Table 1. The SC and SP samples showed similar physical characteristics. According to the USDA classification, the soil texture was sandy loam, the content of sand being very high. The pH values of the SP soil was close to 4.0, with the samples having acidic pH values ranging from 3.96 to 4.38, while that of the SC soil was higher (pH = 5.5). The SP soil samples showed a lower organic carbon and plant nutrient content, but with higher EC in comparison to the SC sample. These chemical characteristics are typical of exploited ex-mining soils (Wong 2003).

The concentration of metal(loid)s (As, Cr, Cu, Ni, Pb, and Zn) was determined in SP and SC soils (Table 2). The choice of such elements was based on previous data regarding the main potentially polluting elements found in the Rio Marina district (ARPAT 2002; Servida et al. 2009). The amount of metal(loid)s detected in the Puppaio area is in agreement with the values reported by ARPAT (2002), showing metal(loid) concentrations typical of this polluted area. The distribution of metal(loid) concentration within the SP area showed a high heterogeneity and variability. The highest metal(loid) concentrations, compared to baseline values measured in SC soil, were found for As, Cu, Pb, and Zn, with mean values of 338, 552, 152, and 271 mg kg^{-1}, respectively. In particular, As and Cu in the SP area were very high, being about 30 times higher than in the control soil. In general, the total content of metal(loid)s in the SP soil samples was much higher than the limits imposed by the Italian legislation (GURI 2006, D. lgs. 152/2006) for public green sites (As = 20 mg kg^{-1}, Cu = 120 mg kg^{-1}, Pb = 100 mg kg^{-1}, Zn = 150 mg kg^{-1}). These limits were exceeded by 17 times for As, 4 times for Cu, 1.5 times for Pb, and 2 times for Zn.

In Table 2, data on the metal(loid) potentially available fraction (M_{sAv}), evaluated by EDTA extraction, are also reported. It exhibited a maximum value for Zn (27%) and a minimum value for As (0.6%), while for Cu and Pb, it was 17 and 10%, respectively. These values were much lower than those reported by other authors (Monterroso et al. 2014) in a NW Spain mining soil (Pb 61%, Cu and Zn 31–39%), while data for As are in agreement with similar low values observed in other mining areas (Abreu et al. 2012; Pérez-Sirvent et al. 2012). As far as Cr and Ni are concerned, these heavy metals did not show specific differences among the SP and SC sites, both for M_{tot} and M_{sAv}.

Metal(loid)s in plants

The plant species collected at the ex-mining site (SP) exhibited different metal(loid) concentrations (Table 3) with a variable distribution, depending on the metal(loid) and plant species. Generally, *D. viscosa* showed the highest metal(loid) accumulation in leaves, while *B. bituminosa* showed the highest metal(loid) accumulation in stems and roots.

Arsenic was detected only in *D. viscosa* while no other plant was able to absorb it. *D. viscosa* organs showed a significant As uptake at root level (12.7 mg kg^{-1}) and translocation to the aerial system according to the following accumulation pattern: leaves > stems > roots. Baroni et al. (2004) investigated the same species in a contaminated area in southern Tuscany (mainland Italy) and reported a similar accumulation pattern, with the highest As values in leaves (about 3 mg kg^{-1}). However, this value was nine times lower than that found in the SP area (about 25 mg kg^{-1}). On the other hand, in a study conducted on a Pb/Zn mining soil (Sierra Minera, Spain), Pérez-Sirvent et al. (2012) reported a limited

Table 1 Physical and chemical characteristics of the soil samples collected in the Puppaio ex-mining area (SP) and in the control site (SC)

	pH	EC	mg kg^{-1}			CEC	%			$C_{organic}$	$C_{inorganic}$
		µS cm^{-1}	K	P	N	Cmol^{+}kg^{-1}	Clay	Silt	Sand	%	%
SP	4.1 ± 0.2	1934 ± 1524	184 ± 46	n.d.	1283 ± 370	20.4 ± 2.7	14.5 ± 5.1	24.7 ± 4.0	60.8 ± 6.3	0.22 ± 0.19	0.0046 ± 0.0002
SC	5.5 ± 0.5	716 ± 341	825 ± 79	180 ± 28	1290 ± 285	25.5 ± 4.2	25.7 ± 7.3	26.5 ± 5.5	47.8 ± 3.5	1.42 ± 0.56	0.01550 ± 0.0035

Data are mean values ± standard deviation (*n* = 4)

n.d. not detectable

Table 2 Total (M_{tot}) and available (M_{sAv}) metal(loid) concentration in the soil samples collected in the Puppaio ex-mining area (SP, n = 12) and in control sites (SC, n = 3)

Soil		mg kg^{-1} DW					
		As	Cr	Cu	Ni	Pb	Zn
SP	M_{tot}	338.4 ± 333.8 (28–928)	35.5 ± 16.7 (25–81)	552.6 ± 380.5 (122–1337)	44.9 ± 36.6 (9–129)	152.4 ± 98.2 (18–377)	271.6 ± 206.3 (63–573)
	M_{sAv}	2.1 ± 0.9 (0.8–2.7)	n.d.	93.1 ± 83.0 (36.4–240.0)	14.1 ± 10.3 (7.5–32.4)	14.8 ± 14.4 (1.5–34.4)	73.2 ± 35.3 (39.4–126.0)
SC	M_{tot}	10.2 ± 8.9 (5–21)	26.7 ± 15.4 (11–42)	16.7 ± 13.3 (7–32)	40.1 ± 27.4 (10–63)	33.9 ± 31.9 (10–70)	80.6 ± 22.6 (65–107)
	M_{sAv}	n.d.	n.d.	2.6 ± 0.5 (1.8–3.5)	7.9 ± 2.3 (5.2–9.7)	n.d.	12.5 ± 3.6 (10.7–16.3)

Data are expressed as milligrams per kilogram of dry weight. Mean values ± standard deviation and ranges (min and max values in brackets)

n.d. not detectable

ability of *D. viscosa* plants to translocate As from roots to leaves. These contrasting results seem to suggest a site-specific response of plant adaptation to As contamination.

In general, in the SP plants, the accumulation of Zn was the highest followed by that of Cu, as previously observed by Jiménez et al. (2011). Moreover, these elements showed a similar accumulation pattern: leaves > stems > roots in all the studied plants, except for *B. bituminosa*, which showed the highest Zn and Cu concentrations in stems. Among the plant species, *D. viscosa* exhibited the highest content of Cu and Zn in the leaves, with mean values of 62 and 133 mg kg^{-1}, respectively.

As far as Pb is concerned, its uptake was very low in the studied plant species, with the exception of *D. viscosa* where a high value (25 mg kg^{-1}) was observed in the leaves, but not in stems or roots. Also, Barbafieri et al. (2011) in a study carried out in a mining site of Sardinia observed a higher Pb content in *D. viscosa* leaves (mean value 420 mg kg^{-1}) with respect to roots and stems.

Cr was not detected in any collected plant species, and Ni was present at very low concentrations or undetectable in *D. viscosa*. This result reflects the low content of these metals measured in the SP soil, as reported in Table 2; therefore, these data will not be discussed further.

The biological concentration factor (BCF) and translocation factor (TF) for each species were calculated to evaluate the capacity to transfer metal(loid)s (As, Cu, Pb, and Zn) from the matrices to the plants and their translocation from the roots to the leaves (Table 4). The standard error values showed a limited variability, ranging from 1 to 22% and from 14 to 37% for TF and BCF, respectively. BCF and TF have been often used as indicators to estimate the phytoremediation ability of plants (Baker et al. 1994). Generally, values of BCF >1 and TF >1 are required for phytoextraction processes, while lower values are preferred for phytostabilization processes (Concas et al. 2015).

According to the BCF and TF values listed in Table 4, *D. viscosa* showed a marked capability to transfer the potentially bioavailable fraction of all the considered metal(loid)s (As, Cu, Pb, Zn) to the leaves. In particular, this plant exhibited the unique ability to translocate As from roots to the aerial parts, with a high TF (2.06) and a very high BCF (12.48). *L. stoechas* and *C. salviifolius* showed TF >1 for Cu, Pb, and Zn, but the BCF value was >1 only for Zn. However, the BCF values of these two plants were always lower than those of *D. viscosa*. Moreover, the TF values of *L. stoechas* and *C. salviifolius* were lower for Pb and Zn but higher for Cu, with respect to those calculated for *D. viscosa*. In agreement with our data, Jiménez et al. (2011) and Abreu et al. (2012) reported low accumulation values of trace elements in *Cistus* species. *B. bituminosa* showed the lowest TF and BCF values compared to the other plants, for all the measured metal(loid)s. These results are in agreement with those reported by

Table 3 Metal (loid) concentration in organs of plants collected in the Puppaio ex-mining area (SP), during the sampling campaign of 2013

Plant species	mg kg^{-1} DW					
	As	Cr	Cu	Ni	Pb	Zn
D. viscosa (*n* = 6)						
Leaves	26.2 ± 8.6	n.d.	61.7 ± 10.8	n.d.	25.0 ± 1.4	133.2 ± 36.0
Stems	18.5 ± 8.7	n.d.	37.0 ± 7.5	n.d.	7.3 ± 2.8	27.4 ± 1.5
Roots	12.7 ± 2.6	n.d.	55.4 ± 5.4	n.d.	4.5 ± 1.3	20.3 ± 1.7
L. stoechas (*n* = 5)						
Leaves	n.d.	n.d.	17.0 ± 0.5	5.5 ± 0.6	4.6 ± 0.5	82.7 ± 1.2
Stems	n.d.	n.d.	7.8 ± 1.0	1.5 ± 0.3	2.0 ± 0.6	29.1 ± 5.0
Roots	n.d.	n.d.	7.9 ± 1.1	3.0 ± 0.3	1.2 ± 0.1	25.8 ± 0.2
C. salviifolius (*n* = 5)						
Leaves	n.d.	n.d.	17.9 ± 0.7	2.0 ± 0.2	3.1 ± 0.9	113.4 ± 2.2
Stems	n.d.	n.d.	14.8 ± 0.4	3.5 ± 1.3	2.1 ± 0.8	58.6 ± 8.1
Roots	n.d.	n.d.	12.4 ± 4.7	2.7 ± 0.5	1.7 ± 0.3	26.0 ± 8.2
B. bituminosa (*n* = 3)						
Leaves	n.d.	n.d.	12.7 ± 1.0	2.9 ± 0.3	4.0 ± 0.6	70.4 ± 7.0
Stems	n.d.	n.d.	35.6 ± 3.6	5.2 ± 0.3	16.7 ± 0.6	107.0 ± 7.2
Roots	n.d.	n.d.	33.6 ± 6.7	7.8 ± 0.4	4.7 ± 0.7	62.9 ± 1.3

Data are expressed as milligrams per kilogram of dry weight. Mean values ± s.d. ($n \geq 3$)

n.d. not detectable

Martínez-Fernández et al. (2011) and Walker et al. (2007), which confirmed a scarce ability of this plant to translocate heavy metals (Zn and Pb) to the upper parts.

Native flora of ex-mining sites from southern Europe are actually studied for their phytoremediation potentiality (Abreu et al. 2012; Pratas et al. 2013; Favas et al. 2014). These plant species are adapted to grow in metal-polluted sites depending on the pedoclimatic characteristics and the metal abundance (Favas et al. 2014). *Cistus* spp. and *D. viscosa* are often found in these polluted sites, and their metal accumulation and translocation are peculiar to the metal(loid) concentration and the type of soil.

In the present study, all collected plant species were able to survive under the pedoclimate conditions of the metal(loid)-contaminated SP area and showed different patterns of metal(loid) uptake and distribution among different plant tissues. In particular, our data showed that *D. viscosa* exhibited the highest ability to accumulate As and other metals (Cu, Pb, and Zn), although it cannot be considered a hyper-accumulator as defined by Baker et al. (1994).

Biochemical analysis of *D. viscosa* leaves

Plant defense mechanisms towards metal(loid) stress involve different strategies, ranging from the activation of the antioxidant machinery, including enzymatic and non-enzymatic defense systems, to the modulation of the photosynthetic pigments (Sytar et al. 2013; Hernandez et al. 2015). We tested a battery of biochemical parameters to evaluate the physiological adaptation of *D. viscosa* of the SP ex-mining site, because of its greater ability to transfer metal(loid)s to the leaves in comparison with other plants (Table 4). In the second

Table 4 Translocation factor (TF) as a ratio Me_{leaves}/Me_{roots} and bioconcentration factor (BCF) as a ratio Me_{leaves}/M_{sAv} for As, Cu, Pb, and Zn calculated in the sampled plants

Plant species	As		Cu		Pb		Zn	
	TF	BCF	TF	BCF	TF	BCF	TF	BCF
D. viscosa	2.06 (0.45)	12.48 (3.22)	1.11 (0.12)	0.66 (0.22)	5.56 (0.78)	1.69 (0.51)	6.56 (0.62)	1.82 (0.27)
L. stoechas			2.15 (0.16)	0.18 (0.05)	3.83 (0.34)	0.31 (0.10)	3.21 (0.03)	1.13 (0.16)
C. salviifolius			1.44 (0.27)	0.19 (0.05)	1.82 (0.37)	0.21 (0.09)	4.36 (0.65)	1.55 (0.23)
B. bituminosa			0.38 (0.06)	0.14 (0.04)	0.85 (0.15)	0.27 (0.10)	1.10 (0.08)	0.96 (0.19)

Mean values and standard error (between brackets) are reported

Table 5 Metal (loid) concentration in leaves of *D. viscosa* plants collected in the SP site of the Puppaio ex-mining area and in the control site near Portoferraio (SC), during the sampling campaign of 2014

	mg kg^{-1} DW					
	As	Cr	Cu	Ni	Pb	Zn
SP leaves	18.7 ± 9.2	n.d.	43.5 ± 5.4	n.d.	11.1 ± 7.0	174.9 ± 48.4
SC leaves	n.d	n.d	4.0 ± 1.7	n.d	n.d	42.5 ± 2.4

Data are expressed as milligrams per kilogram of dry weight. Mean values ± s.d. (n = 6)

sampling campaign, leaves of *D. viscosa* were collected in both SP and SC sites and characterized for their metal(loid) content (Table 5). Data showed metal(loid) concentrations in SP samples similar to those measured in the first sampling campaign and reported in Table 3. Values obtained for the SC control site showed lower concentrations of the essential metals (Cu and Zn) and undetectable values for the other metal(loid)s.

Biochemical parameters are reported in Table 6. Leaves of *D. viscosa* collected in SP showed total chlorophyll concentrations not significantly different from that of the control samples (Table 6). Conversely, the content of carotenoids in SP plants was significantly lower than that measured in control plants. Some authors report that the chlorophyll level can be inhibited by the increase of As concentration, due to the peroxidative breakdown of pigments and chloroplast membrane lipids by ROS (Duman et al. 2010). However, the stability of chlorophyll and the weak alteration of carotenoids found in our samples may suggest that the photosynthetic pigments of *D. viscosa* growing on this ex-mining site were not much affected by the soil contamination. Similarly, Martins et al. (2011) did not observe a breakdown of pigments in mature leaves of *N. tabacum* plants under metal stress (Cd), even if negative effects on physiological parameters were clearly observed in young leaves. Actually, oxidative stress responses have been reported to occur in young seedlings grown under controlled conditions (Malar et al. 2014; Fernandez et al. 2013), whereas few data are available for leaves picked up from adult native plants growing in polluted areas.

The antioxidant power, total polyphenol content, and glutathione concentration in leaves collected in the SP site were not significantly different from those of control plants (Table 6). Since As, Cu, and Zn are strong inductors of PCs (Cobbett 2000), the presence of PCs in the leaves of *D. viscosa* could be expected. However, PCs were not detected (data not shown) in our samples, and this is in agreement with the similar level of glutathione found in SP and SC plants (about 5 nmol g^{-1} FW), suggesting that the tolerance towards metal(loid)s exhibited by these plants does not seem to be related to the level of non-protein thiols. The lack of alteration of either the level of secondary metabolites or the antioxidant power indicates that the amount of metal(loid)s taken up by the plant was not sufficient to activate the non-enzymatic defense mechanisms against ROS, also suggesting that *D. viscosa* was well adapted to grow in such conditions.

The antioxidant enzymatic defense system was also examined, by measuring the activities of SOD, CAT, APX, and GR. The results (Table 6) showed that the enzymatic activities in SP plants were comparable to those measured in SC plants. It is well known that these enzymes are implied in ROS-scavenging activities, so the general stability of these activities confirmed the absence of an oxidative stress, as above reported for antioxidant non-enzymatic activities (Gill and Tuteja 2010). Different levels of antioxidant enzymes have been reported in two populations of metallicolous and non-

Table 6 Biochemical parameters measured in leaves of *D. viscosa* plants collected in the Puppaio site (SP) and in the control site (SC). Photosynthetic pigments, secondary metabolites, antioxidant power, and antioxidant enzymatic activity (SOD, CAT, APX, GR) are reported

Biochemical parameters	Control leaves (SC)	Puppaio leaves (SP)
Total chlorophyll (mg g^{-1} FW)	1.01 ± 0.08a	0.82 ± 0.09a
Carotenoids (mg g^{-1} FW)	69.79 ± 1.66a	52.90 ± 7.98b
Total polyphenols (mg g^{-1} FW)	1.65 ± 0.37a	1.89 ± 0.51a
Total glutathione (nmol g^{-1} FW)	5.0 ± 0.2a	5.2 ± 0.8a
Antioxidant power IC$_{50}$ (mg FW mL^{-1})	5.71 ± 2.86a	4.29 ± 1.96a
SOD activity (U mg protein^{-1})	412 ± 115a	385 ± 110a
CAT activity (U mg protein^{-1})	10.9 ± 0.8a	12.8 ± 2.9a
APX activity (U mg protein^{-1})	0.29 ± 0.06a	0.30 ± 0.07a
GR activity (U mg protein^{-1})	0.040 ± 0.011a	0.035 ± 0.014a

Mean values (± s.d.) were obtained from six independent replicates. Numbers followed by different letters in the same row are statistically different for $P \leq 0.05$

metallicolous *D. viscosa* plants tolerant to Cd (Fernandez et al. 2013), even though these data referred to *ex vitro* young plants exposed to Cd treatment for 10 days. In the literature, relevant data are reported for the modulation of antioxidant activities in response to heavy metal pollution, but the trials have been carried out using young plantlets and artificial substrates (e.g., hydroponic conditions) (Shri et al. 2009; Ahmad and Gupta 2013; Fernandez et al. 2013; Malar et al. 2014; Mirza et al. 2016). To our knowledge, few data about the antioxidant activity in spontaneous plants collected in mining sites have been reported so far (Marquez-Garcia and Cordoba 2009; Boojar and Goodarzi 2007). The spontaneous plants grown in metal(loid)-polluted soils exhibited variable antioxidant enzyme activities, depending on several factors such as plant species, metal ion concentration, and bioavailability. In fact, Boojar and Goodarzi (2007) reported that *Datura stramonium* L. and *Chenopodium ambrosioides* L. increased SOD, CAT, and GPX activities in response to Cu toxicity. On the other hand, *Erica australis* L. grown in pyrite mine wastes (Iberian Pyritic Belt) did not exhibit significant changes of antioxidant enzyme activities (Marquez-Garcia and Cordoba 2009).

The overall view of the biochemical parameters indicated a generally healthy status of *D. viscosa* plants grown in the polluted site, as confirmed by visual observation of the plants in situ, despite the higher levels of As and heavy metals in soil. These results confirmed previous studies on micropropagated *D. viscosa* explants collected in SP, which showed very good ability to grow in culture media with added As, Zn, and Cu at low pH (Pistelli et al. 2017). Otherwise, the explants from the SC site could not survive in such conditions, suggesting that a selection of *D. viscosa* capable of surviving in acidic and metal(loid)-polluted media has occurred.

Conclusions

Ex-iron mining areas of Elba island represent a potential source of metal(loid) pollutants. In fact, the soil from the ex-mining site Puppaio is still highly contaminated by As, Cu, Pb, and Zn, decades after the closure of the mine. Nevertheless, some native species (*C. salviifolius*, *L. stoechas*, *B. bituminosa*, and *D. viscosa*) can grow in these very stressful conditions, showing peculiar characteristics of metal(loid) accumulation. The use of these shrub plants in the revegetation of the area could stabilize the soil by their root system and, at the same time, could act as a barrier to soil degradation (rain and wind erosion). Only *D. viscosa* was able to take up As, with high TF and BCF values. In addition, *D. viscosa* grown in this area showed no significant alteration in the pigment and foliar metabolites, as well as in the antioxidant enzymatic activities, with respect to that collected in SC. These results suggest that a new ecotype of *D. viscosa* could have been selected in the ex-mining sites in Elba. Further experiments should be conducted to elucidate the adaptation of *D. viscosa* for survival in these stressful conditions, and additional studies are needed to evaluate the biomass production, contaminant uptake, and the real efficiency of the plant in phytoextraction processes. Once these parameters will be measured, these plants could become very interesting for future in situ phytoremediation projects aiming at restoring the ecological and touristic value of this area.

Acknowledgements The authors are grateful to the Municipality of Rio Marina and the Mineral Park of Elba Island for providing site data and assistance in the selection of areas to be investigated. The authors acknowledge Manuele Scatena for the valuable technical assistance. The research laboratory is supported by the University of Pisa and the Italian National Research Council.

References

Abreu M, Santos ES, Magalhães MCF, Fernandes E (2012) Trace elements tolerance, accumulation and translocation in *Cistus populifolius*, *Cistus salviifolius* and their hybrid growing in polymetallic contaminated mine areas. J Geochem Expl 123:52–60. doi:10.1016/j.gexplo.2012.05.001

Aebi H (1974) Catalase. In: Bergmeyer HU (ed) Methods of enzymatic analysis, 2. Academic Press, NY, pp 673–684

Ahmad MA, Gupta M (2013) Exposure of *Brassica juncea* (L) to arsenic species in hydroponic medium: comparative analysis in accumulation and biochemical and transcriptional alterations. Environ Sci Pollut Res 20:8141–8150. doi:10.1007/s11356-013-1632-y

ARPAT- Agenzia Regionale per la Protezione Ambientale della Toscana (2002) Indagine ambientale sulle aree ex-minerarie dell'Isola d'Elba, 133 p

Bacchetta G, Cappai G, Carucci A, Tamburini E (2015) Use of native plants for the remediation of abandoned mine sites in Mediterranean semiarid environments. Bull Environ Contam Toxicol 94:326–333. doi:10.1007/s00128-015-1467-y

Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements. A review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126

Baker AJM, Reeves RD, Haiar ASM (1994) Heavy metal accumulation and tolerance in British populations of the metallophyte *Thlaspi caerulescens* J.&C. Presl. (Brassicaceae). New Phytol 127:61–68. doi:10.1111/j.1469-8137.1994.tb04259.x

Barbafieri M, Lubrano L, Petruzzelli G (1996) Characterization of pollution in sites contaminated by heavy metals: a proposal. Ann Chim 86:585–594

Barbafieri M, Dadea C, Tassi E, Bretzel F, Fanfani L (2011) Uptake of heavy metals by native species growing in a mining area in Sardinia, Italy: discovery native flora for phytoremediation. Int J Phytorem 13:985–997. doi:10.1080/15226514.2010.549858

Baroni F, Boscagli A, Di Lella LA, Protano G, Riccobono F (2004) Arsenic in soil and vegetation of contaminated areas in southern Tuscany (Italy). J Geochem Expl 81:1–14. doi:10.1016/S0375-6742(03)00208-5

Batista MJ, Gonzalez-Fernandez O, Abreu MM, Carvalho L, Queralt I (2013) Pioneer plant species contributing to phytostabilization of contaminated soils in mine areas. Geophys Res Abst 15: EGU2013–EGU5916

Benvenuti M, Mascaro I, Corsini F, Costagliola P, Parrini P, Lattanzi P, Tanelli G (1999) Environmental problems related to sulfide mining in Tuscany. Chron Rech Min 534:29–45

Bertoli A, Menichini F, Noccioli C, Morelli I, Pistelli L (2004) Volatile constituents of different organs of *Psoralea bituminosa* L. Flavour Frag J 19:166–171. doi:10.1002/ffj.1315

Beyer WF Jr, Fridovich I (1987) Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. Anal Biochem 161:559–566. doi:10.1016/0003-2697(87)90489-1

Boojar MMA, Goodarzi F (2007) The copper tolerance strategies and the role of antioxidative enzymes in three plant species grown on copper mine. Chemosphere 67:2138–2147. doi:10.1016/j.chemosphere.2006.12.071

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Ann Biochem 72:248–259. doi:10.1016/0003-2697(76)90527-3

Brand-Williams W, Cuvelier ME, Berset C (1995) Use of free radical method to evaluate antioxidant activity. Lebensm-Wiss U-Technol 28:25–30. doi:10.1016/S0023-6438(95)80008-5

Bretzel F, Benvenuti S, Pistelli L (2014) Metal contamination in urban street sediment in Pisa (Italy) can affect the production of antioxidant metabolites in *Taraxacum officinale* Weber. Environ Sci Pollut Res 21:2325–2333. doi:10.1007/s11356-013-2147-2

Carrasco A, Ortiz-Ruiza V, Martinez-Gutierrez R, Tomas V, Tudela J (2015) *Lavandula stoechas* essential oil from Spain: aromatic profile determined by gas chromatography-mass spectrometry, antioxidant and lipoxygenase inhibitory bioactivities. Ind Crop Prod 73:16–27. doi:10.1016/j.indcrop.2015.03.088

Cobbett CS (2000) Phytochelatins and their roles in heavy metal detoxification. Plant Physiol 123:825–832. doi:10.1104/pp.123.3.825

Concas S, Lattanzi P, Bacchetta G, Barbafieri M, Vacca A (2015) Zn, Pb and Hg contents of *Pistacia lentiscus* l. grown on heavy metal-rich soils: implications for phytostabilization. Water Air Soil Pollut 226:340. doi:10.1007/s11270-015-2609-x

Conesa HM, Marìa-Cervates A, Alvarez-Roguel J, Gonzalez-Alcaraz MN (2011) Influence of soil properties on trace element availability and plant accumulation in a Mediterranean salt marsh polluted by mining wastes: implication for phytomanagement. Sci Total Environ 409:4470–4479. doi:10.1016/j.scitotenv.2011.07.049

Curadi M, Graifenberg A, Magnani G, Giustiniani L (2005) Growth and element allocation in tissues of *Inula viscosa* in sodic-saline conditions: a candidate for programs of desertification control. Arid Land Res Manag 7:257–265. doi:10.1080/15324980590951423

D'Angiolillo F, Pistelli L, Noccioli C, Ruffoni B, Piaggi S, Scarpato R, Pistelli L (2014) In vitro cultures of *Bituminaria bituminosa*: pterocarpan, furanocoumarin and isoflavone production and cytotoxic activity evaluation. Nat Prod Commun 9:477–480

Duman F, Oztruk F, Aydin Z (2010) Biological response of duckweed (*Lemna minor* L.) exposed to the inorganic arsenic species As(III) and As(V): effects of concentration and exposure time of exposure. Ecotoxicol 19:983–993. doi:10.1007/s10646-010-0480-5

Emamverdian A, Ding Y, Mokhberdoran F and Xie Y (2015) Heavy metal stress and some mechanisms of plant defense response. Scient World J ID 756120. doi:10.1155/2015/756120

Favas PJC, Pratas J, Varun M, D'Souza R, Paul MS (2014) Phytoremediation of soils contaminated with metals and metalloids at mining areas: potential of native flora. In: Soriano MCH (ed) Environmental risk assessment of soil contamination, pp 485–517 InTech. ISBN: 978-953-51-1235-8

Fernandez R, Bertrand A, Reis R, Mourato MP, Martins LL, Gonzales A (2013) Growth and physiological responses to cadmium, stress of two population of *Dittrichia viscosa* (L) Greuter. J Haz Mat 244-245:555–562. doi:10.1016/j.jhazmat.2012.10.044

Finnegan PM, Chen W (2012) Arsenic toxicity: the effects on plant metabolism. Front Physiol 3:1–18. doi:10.3389/fphys.2012.00182

Foyer CH, Halliwell B (1976) The presence of glutathione and glutathione reductase in chloroplast: a proposed role in ascorbic acid metabolism. Planta 133:21–25. doi:10.1007/BF00386001

Freitas H, Prasad MNV, Pratas J (2004) Analysis of serpentinophytes from north–east of Portugal for trace metal accumulation—relevance to the management of mine environment. Chemosphere 54:1625–1642. doi:10.1016/j.chemosphere.2003.09.045

Furini A (2012) Plants and heavy metals. Springer Briefs in Molecular Science, Berlin

Geebelen W, Adriano DC, Van der Lelie D, Mench M, Carleer R, Clijsters H, Vangronsveld J (2003) Selected bioavailability assays to evaluate the efficacy of amendment induced immobilization of lead in contaminated soils. Plant Soil 249:217–228. doi:10.1023/A:1022534524063

Giansoldati V, Tassi E, Morelli E, Gabellieri E, Pedron F, Barbafieri M (2012) Nitrogen fertilizer improves boron phytoextraction by *Brassica juncea* grown in contaminated sediments and alleviates plant stress. Chemosphere 87:1119–1125. doi:10.1016/j.chemosphere.2012.02.005

Gill SS, Tuteja N (2010) Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiol Biochem 48:909–930. doi:10.1016/j.plaphy.2010.08.016

Grill E, Winnacker EL, Zenk MH (1985) Phytochelatins: the principal heavy-metal complexing peptides of higher plants. Science 230:674–676. doi:10.1126/science.230.4726.674

Gupta M, Sharma P, Sarin NB, Sinha AK (2009) Differential response of arsenic stress in two varieties of *Brassica juncea* L. Chemosphere 74:1201–1208. doi:10.1016/j.chemosphere.2008.11.023

GURI (Gazzetta Ufficiale Repubblica Italiana), 2006. Norme in materia ambientale, Decreto Legislativo 3 aprile 2006, n. 152, Supplemento Ordinario n.96, alla Gazzetta Ufficiale n. 88, del 14 aprile 2006 (in Italian)

Hernandez LE, Sobrino-Plata J, Montero-Palmero MB, Carrasco-Gil S, Flores-Caceres ML, Ortega-Villasante C, Escobar C (2015) Contribution of glutathione to the control of cellular redox homeostasis under toxic metal and metalloid stress. J Exp Bot 66:2901–2911. doi:10.1093/jxb/erv063

Islam E, Khan MT, Irem S (2015) Biochemical mechanisms of signaling: perspectives in plants under arsenic stress. Ecotox Environ Safety 114:126–133. doi:10.1016/j.ecoenv.2015.01.017

Italian Official Gazette. 1999. n.248, 21/10/1999–Decreto Ministeriale 13/9/1999, n.185 Metodi Ufficiali di Analisi del Suolo; [cited January 2010]. Available from: http://www.guritel.it/free-sum/ARTI/1999/10/21/sommario.html.

Jiménez MN, Bacchetta G, Casti M, Navarro FB, Lallena AM, Fernandez-Ondoño E (2011) Potential use in phytoremediation of three plant species growing on contaminated mine-tailing soils in Sardinia. Ecol Eng 37:392–398. doi:10.1016/j.ecoleng.2010.11.030

Kramer U (2005) Phytoremediation: novel approaches to cleaning up polluted soils. Curr Opin Biotech 16:133–141. doi:10.1016/j.copbio.2005.02.006

Lichtenthaler HK (1987) Chlorophylls and carotenoids, pigments of photosynthetic biomembranes. Met Enzymol 147:350–382. doi:10.1016/0076-6879(87)48036-1

Malar S, Manikandan R, Favas PJC, Sahi SV, Venkatachalam P (2014) Effect of lead on phytotoxicity, growth, biochemical alterations and its role on genomic template stability in *Sesbania grandiflora*: a potential plant for phytoremediation. Ecotox Environ Safety 108:249–257. doi:10.1016/j.ecoenv.2014.05.018

Marquez-Garcia B, Cordoba F (2009) Antioxidative system and oxidative stress markers in wild populations of *Erica australis* L. differentially exposed to pyrite mining activities. Environ Res 109:968–974. doi:10.1016/j.envres.2009.08.001

Marques AP, Moreira H, Rangel AO, Castro PM (2009) Arsenic, lead and nickel accumulation in *Rubus ulmifolius* growing in contaminated soil in Portugal. J Hazard Mater 165:174–179. doi:10.1016/j.jhazmat.2008.09.102

Martínez-Fernández D, Walker DJ, Romero-Espinar P, Flores P, del Río JA (2011) Physiological responses of *Bituminaria bituminosa* to

heavy metals. J Plant Physiol 168:2206–2211. doi:10.1016/j.jplph.2011.08.008
Martins LL, Mourato MP, Cardoso AI, Pinto AP, Mota AM, Gonçalves MLS, de Varennes A (2011) Oxidative stress induced by cadmium in *Nicotiana tabacum* L.: effects on growth parameters, oxidative damage and antioxidant responses in different plant parts. Acta Physiol Plant 33:1375–1383. doi:10.1007/s11738-010-0671-y
Mascaro I, Benvenuti M, Corsini F, Costagliola P, Lattanzi P, Parrini P, Tanelli G (2001) Minewastes at the polymetallic deposit of Fenice Capanne (southern Tuscany, Italy). Mineralogy, geochemistry and environmental impact. Environ Geol 41:417–429. doi:10.1007/s002540100408
Meharg A (2005) Mechanisms of plant resistance to metal and metalloid ions and potential biotechnological applications. Plant Soil 274:163–174. doi:10.1007/s11104-004-0262-z
Mirza N, Mubarak H, Chai LY, Yang ZH, Yong W, Tang CJ, Mahmood Q, Pervez A, Farooq U, Fahad S, Nasim W, Siddique KHM (2016) Biochemical and metabolic changes in arsenic contaminated *Boehmeria nivea* L. BioMed Res Int ID1423828. doi:10.1155/2016/1423828
Monterroso C, Rodríguez F, Chaves R, Diez J, Becerra-Castro C, Kidd PS, Macías F (2014) Heavy metal distribution in mine-soils and plants growing in a Pb/Zn-mining area in NW Spain. Appl Geochem 44:3–11. doi:10.1016/j.apgeochem.2013.09.001
Murciego AM, Garcia-Sanchez A, Rodriguez Gonzalez MA, Pinilla Gill E, Toro Gordillo C, Cabezas Fernandez J, Buyolo Triguero T (2007) Antimonium distribution and mobility in topsoils and plants (*Cistus striatus, Cistus ladanifer and Dittrichia viscosa*) from polluted Sb-mining area in Estremadura (Spain). Environ Pollut 145:15–21. doi:10.1016/j.envpol.2006.04.004
Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplast. Plant Cell Physiol 22:867–880
Pecetti L, Mella M, Tava A (2016) Variation in herbage biochemical composition among pitch trefoil (*Bituminaria bituminosa*) populations from elba island, Italy. J Agric Food Chem 64:195–203. doi:10.1021/acs.jafc.5b05050
Pérez-Sirvent C, Martínez-Sánchez MJ, Martínez-López S, Bech J, Bolan N (2012) Distribution and bioaccumulation of arsenic and antimony in *Dittrichia viscosa* growing in mining-affected semiarid soils in southeast Spain. J Chem Expl 123:128–135. doi:10.1016/j.gexplo.2012.08.002
Pignatti S (1982) Flora d'Italia, vol 2. Edagricole, Bologna, pp 500–501
Pistelli L, D'Angiolillo F, Bortolazzo M, Morelli E, Basso B (2017) Establishment of in vitro plants selected from heavy metals contaminated soils for further phytoremediation use. Acta Horticul (in press)
Pereira DM, Valentão P, Pereira JA, Andrade PB (2009) Phenolics: from chemistry to biology. Molecules 14:2202–2211. doi:10.3390/molecules14062202
Pratas J, Favas PJC, D'Souza R, Varun M, Paul MS (2013) Phytoremedial assessment of flora tolerant to heavy metals in the contaminated soils of an abandoned Pb mine in Central Portugal. Chemosphere 90:2216–2225. doi:10.1016/j.chemosphere.2012.09.079
Rinaldi G (2000) In Flora dell'Arcipelago Toscano. Edizioni Archipelagos, Portoferraio (Livorno)
Servida D, Grieco G, De Capitani L (2009) Geochemical hazard evaluation of sulphide-rich iron mines : the Rio Marina district (Elba Island, Italy). J Geochem Expl 100:75–89. doi:10.1016/j.gexplo.2008.03.005
Shri M, Kumar S, Chakrabarty D, Trivedi PK, Mallick S, Misra P, Shukla D, Mishra S, Srivastava S, Tripathi RD, Tuli R (2009) Effect of arsenic on growth, oxidative stress, and antioxidant system in rice seedlings. Ecotox Environ Safety 72:1102–1110. doi:10.1016/j.ecoenv.2008.09.022
Sierra MJ, Millán R, Esteban E (2009) Mercury uptake and distribution in *Lavandula stoechas* plants grown in soil from Almadén mining district (Spain). Food Chem Toxicol 47:2761–2767. doi:10.1016/j.fct.2009.08.008
Singleton VL, Rossi JAJ (1965) Colorimetry of total phenolics with phosphomolybdic–phosphotungstic acid reagent. Amer J Enol Viticol 16:144–158
Soil Science Society of America Book series -SSSA (1996) Methods of soil analysis—part 3. Chemical Methods, Inc, Madison (WI)
Sternberg M, Gishri N, Mabjeesh SJ (2006) Effects of grazing on *Bituminaria bituminosa* (L.) Stirton: a potential forage crop in Mediterranean grasslands. J Agron Crop Sci 192:399–407. doi:10.1111/j.1439-037X.2006.00227.x
Sytar O, Kumar A, Latowski D, Kuczynska P, Strzałka K, Prasad MNV (2013) Heavy metal-induced oxidative damage, defense reactions, and detoxification mechanisms in plants. Acta Physiol Plant 35:985–999. doi:10.1007/s11738-012-1169-6
Tanelli G, Benvenuti M, Costagliola P, Dini A, Lattanzi P, Maineri C, Mascaro I, Ruggieri G (2001) The iron mineral deposits of Elba Island: state of the art. Ofioliti 26:239–248
Walker DJ, Romero P, Correal E (2010) Cold tolerance, water relations and accumulation of osmolytes in *Bituminaria bituminosa* L. Biol Plantarum 54:293–298. doi:10.1007/s10535-010-0051-x
Walker DJ, Bernal MP, Correal E (2007) The influence of heavy metals and mineral nutrient supply on *Bituminaria bituminosa*. Water Air Soil Pollut 184:335–345. doi:10.1007/s11270-007-9422-0
Wong MH (2003) Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. Chemosphere 50:775–780. doi:10.1016/S0045-6535(02)00232-1