Research Article

Received: 30 November 2008 Revised: 16 February 2009 Accepted: 17 February 2009 Published online in Wiley Interscience: 6 April 2009

(www.interscience.wiley.com) DOI 10.1002/jctb.2179

Use of native species and biodegradable chelating agents in the phytoremediation of abandoned mining areas

Alessia Cao,[a] Alessandra Carucci,[a*] Tiziana Lai,[a] Gianluigi Bacchetta[b] and Mauro Casti[b]

Abstract

BACKGROUND: The application of phytostabilization and assisted phytoextraction to the remediation of abandoned mining areas can be a valuable method to reclaim these areas without modifying soil and landscape characteristics. An *in situ* application of a continuous phytoextraction technique was carried out in the area of Campo Pisano (Sardinia, Italy), followed by a laboratory assisted phytoextraction test using the biodegradable chelating agents methylglycine diacetic acid (MGDA) and iminodissuccinic acid (IDSA). The plants used were *Scrophularia canina* subsp. *bicolor*, *Cistus salviifolius* and *Teucrium flavum* subsp. *glaucum*.

RESULTS: The plant that accumulated more Pb was *T. glaucum* (353 mg kg^{-1}) while *C. salviifolius* demonstrated better ability to accumulate Zn (1560 mg kg^{-1}). *S. bicolor* showed a better tolerance to metals but accumulated 119 mg kg^{-1} of Pb. Accumulation of metals immediately after chelant application was up to 300 mg kg^{-1} of Pb and 3000 mg kg^{-1} of Zn which did not further increase during the assisted phytoextraction experiment.

CONCLUSION: The plant that demonstrated to be most suitable for phytoremediation application was *S. bicolor* due to its higher biomass production and tolerance to metals. The low cation exchange capacity and the high concentration of Ca and Mg in soil determined a low chelant effectiveness.

Keywords: phytoremediation; assisted phytoextraction; native species; abandoned mining areas; MGDA; IDSA

INTRODUCTION

Abandoned mining areas are a significant problem all over Europe. In Sardinia (Italy) the closure of mining activities has presented serious threats as a result of insufficient attention to the possible environmental impacts and inadequate definition of pollution containment plans. In particular, the area of Sulcis-Iglesiente was the most important metalliferous region in Italy, and hundreds of mining dumps exist,[1] with the volume of abandoned and contaminated materials estimated at more than 70 million m^3.

The tailing dam of Campo Pisano is a basin where the wastes of the flotation process were settled. Before flotation the materials extracted from the mine were ground, reducing them to muddy and sandy particles. A flotation process was carried out to separate sulfides (galena, blende and pyrite) from the gangue through the use of chemical reagents.

In these abandoned wastes, heavy metals concentration is high and toxic to many organisms and plants, as shown by the absence of vegetation over most of the basin surface. The extent of the area subjected to mining activities and thus prone to heavy metals contamination makes the application of traditional disruptive technologies inappropriate because of the high cost necessary for soil remediation and due to the potential impacts to the environment, with particular regard to the modification of landscape and of soil agronomical properties.

Phytoremediation can be considered a good solution for soil remediation in these areas because it can be used as an *in situ* low cost technique despite requiring a long remediation time. In particular, the application of phytostabilization (a phytotechnology that allows one to stabilize contaminants in soil) to mine sites has been accepted by the scientific community to reclaim mine waste *in situ*.[2,3] Vegetation can provide effective protection against wind and water erosion and can improve nutrient concentration and consequently soil agronomic properties.[4] The high concentration of metals at the Sardinian mine sites also suggests investigation of the phytoextraction technique and its optimization in order to lower reclamation times.

Assisted phytoextraction is an optimization that, by increasing the metal bioavailable fraction in soil, enables enhanced metal transport into the aerial parts of plants, so reducing soil

* Correspondence to: Alessandra Carucci, DIGITA, Dept. of Geoengineering and Environmental Technologies, University of Cagliari, Piazza d'Armi, 09123 Cagliari, Italy. E-mail: carucci@unica.it

a DIGITA, Dept. of Geoengineering and Environmental Technologies, University of Cagliari, Italy

b Centre for Conservation of Biodiversity (CCB), Dept. of Botanical Sciences, University of Cagliari, Italy

remediation times. Recent research has been focused on the study of easily biodegradable chelating agents with low impact due to their short persistence in the environment. Previous experiments[5] demonstrated the capacity of methylglycinediacetic acid (MGDA) to mobilize both Pb and Zn in soil: in the days immediately after chelant addition Pb concentration in soil solution reached 5700 and 2000 mg L^{-1} in reactors treated with 8 mmol and 4 mmol kg^{-1} soil, respectively.

The utilization of Mediterranean native species allows acceleration of the development process towards mature plant communities reproducing the ecological conditions of the site before mining activities.

This study aims at investigating the possibility to identify Mediterranean native plant species for the application of phytostabilization and assisted phytoextraction in remediation projects for abandoned mining sites.

MATERIALS AND METHODS

Plant selection

Plant selection was based on previous field studies related to geobotanical aspects of SW Sardinia mining areas[6–8] and was aimed at selecting metal-tolerant plants that develop enough biomass to decontaminate soil in acceptable times. Since the vegetal species that usually grow on mining dumps are small plants, three small shrubs that differ in ecological features and in size were selected: *Cistus salviifolius* L., *Teucrium flavum* L. subsp. *glaucum* (Jordan and Fourr.) Ronn. and *Scrophularia canina* L. subsp. *bicolor* (Sibth. and Sm.) Greuter. *C. salviifolius* is a very common species in Sardinia that can develop both on poor and rocky soils or form secondary communities as a result of destruction of Mediterranean maquis or evergreen forests. In the first case it appears as a very small shrub but, when it colonizes rich soils, it can grow up to 2 m tall. *T. glaucum* is a chamaephyte that often grows on carbonatic substrata; on deeper soils, it generally develops in the glades of sclerophyllous formations of Mediterranean maquis belonging to the *Pistacio-Rhamnetalia alaterni* phytosociological order. *S. bicolor* is an endemic subspecies of the central Mediterranean basin, smaller than the other species, with xeromorfic characters and very well adapted to grow on poor and arid soils. It is a typical pioneer plant on moving and gravelly ground. Owing to this ecological feature this species is very common on Sardinia mine dumps, especially in the most pebbly and polluted ones. *C. salviifolius* and *T. glaucum*, in contrast, can colonize the mining waste only when the pedogenetic process has started and the roots can develop in substrata richer in nutrients than the original mine dumps.

Plant and soil analyses

Soil was chemically and physically characterized using Italian official analytical methods.[9] Metals concentration in soil was determined using the *aqua regia* extraction method.[9] Carbon and nitrogen total contents (CHN) were determined with an elemental analyser, while cation exchange capacity (CEC) was measured using the $BaCl_2$ method.[9] The bioavailable metal content was evaluated through the sequential extraction procedure.[10] This method divides the metal mobile fraction into three different pools: H_2O extractable (immediately soluble metals), KNO_3 extractable (exchangeable metal form), and ethylenediaminetetraacetic acid (EDTA) extractable (complexed or adsorbed metal forms), whose sum represents the metal bioavailable fraction.

In order to understand how soil characteristics can influence chelation capacity of selected chelants (EDTA and MGDA) some simulation tests of the chelant treatment were made. The soil used for the experiment was compared with a soil characterized by higher cation exchange capacity and lower Ca and Mg concentration used in a similar assisted phytoextraction experiment.[11] The experiments were performed by leaving 1 g of soil in contact with 40 mL of a chelant solution with a concentration 10 times higher than the metal (Pb + Zn) concentration in soil for 24 h.

The heavy metals accumulation capability was determined by measuring the concentration of Pb and Zn in leaves. Plant samples were washed with tap water and dried at 105 °C before analysis. The dried material was digested with *aqua regia*. All determinations were performed in triplicate.

Site description and soil characterization

The area of Iglesiente (south-western Sardinia, Italy) is an independent biogeographic subsector in Sardinia[12] characterized by exclusive endemic species. Owing to its geological features it has been a very important mining area for centuries. The Gonnesa Group, known as the 'Metalliferous Ring',[13] was one of the richest deposits of argentiferous lead and zinc. In this area about 40 mines were distributed over approximately 150 km^2, and exploitation has been documented since the Punic and Roman period.[14] The cessation of mining activity left large quantities of mine wastes on dumps and flotation tailings estimated at about 45 million m^3 for the whole area; this was followed by groundwater problems, especially in the Monteponi area.[14] The area that is the object of study is a tailing dam of the mine 'Campo Pisano', located near the more important mine of 'Monteponi', within the municipality of Iglesias. The amount of mine residues accumulated in this area is about 8 million m^3. The tailings are characterized by high concentrations of heavy metals and low organic carbon and nitrogen concentrations, while the cation exchange capacity is usually low. This has made the area bare and unvegetated. The increasing concentration of metals with the progress of flooding is due to the interaction of water with the ore deposit, together with mine waste accumulated in the pits, and represents the most important long-term hazard for groundwater quality.[15] Studies on tailings from the area of a mine in Spain,[16] demonstrated that most Pb was associated with non-residual fractions, mainly in reducible form, in all the collected samples. This geochemical form of Pb may be released upon decomposition of iron and manganese oxides under reduced conditions. Zn appeared mainly associated with the acid-extractable form in mine tailing samples, while the residual form was the predominant form in samples belonging to surrounding areas. An analogous result,[17] showing the similarity of mine tailings in the Sulcis-Iglesiente area, demonstrated that Pb was associated mainly with Fe and Mg oxides while Zn was related to the soluble and exchangeable fraction.

The simultaneous presence of Ca and Mg and metals makes the treatment of this type of tailings particularly difficult. It has been demonstrated[18] that the presence of these cations determines a reduction in EDTA and EDDS chelation efficiency towards metals.

The characterization of a representative sample of Campo Pisano soil used for the experiments is presented in Table 1. The soil is characterized by a high content of sand and a limited content of both silt and clay, and a low cation exchange capacity. The Ca and Mg concentration was higher than 6 and 4%, respectively, and heavy metal contamination level was considerable (3200 mg kg^{-1} of Pb and 12 000 mg kg^{-1} of Zn). Pb and Zn extractability in H_2O and KNO_3 was different and in particular Pb was not detectable. It is known that Pb has a limited solubility in soil water and

Table 1. Campo Pisano soil characterization

	Physical characterization			pH				Total [mg kg^{-1}]		H_2O [mg kg^{-1}]		KNO_3 [mg kg^{-1}]		EDTA [mg kg^{-1}]	
CEC [cmol kg^{-1}]	Sand [%]	Silt [%]	Clay [%]	H_2O	KCl	N [%]	C [%]	Pb	Zn	Pb	Zn	Pb	Zn	Pb	Zn
10	70	13	17	7.3	7.2	0.01	6.28	3258	12 038	DL*	776	DL*	50	612	3504

* Value lower than instrument detection limit (inductively coupled plasma-optical emission spectrometer, ICP-OES), which is equal to 0.0015 and 0.0002 mg L^{-1} for Pb and Zn, respectively.

a low availability for plant uptake.[19] The soil was characterized by a low fertility: the concentration of carbon and nitrogen were respectively about 6% and 0.01%. The low concentration of nutrients and the high content of sand in the soil determines the low value of the cation exchange capacity.

Organization of the experiments

The experiment was divided into two phases. During the first phase a continuous phytoextraction field test was performed in the Campo Pisano mining area. In the second phase the plant that demonstrated the best tolerance to high heavy metals concentrations, specifically *S. bicolor*, was extracted from the site and assisted phytoextraction tests were made in the laboratory under controlled conditions using easily biodegradable chelants.

The continuous phytoextraction experiment

In order to produce plants for the phytoextraction experiment a germination test was undertaken. The selected species were seeded in the same polluted soil used for the experiments in a plant growth chamber at a constant temperature of 20 °C and a 12 h photoperiod.

In the continuous phytoextraction experiment at field scale the species *T. glaucum, S. bicolor, C. salviifolius* were used. The total number of seedlings was 71 for *S. bicolor*, 31 for *C. salviifolius* species and 9 for *T. glaucum*; the number of seedlings was limited by the number of plants obtained from the germination tests. In particular *T. glaucum* showed a low percentage of germination in the selected soil. The plants were placed in the field in November after 6 months from germination. Autumn in the Mediterranean region is the best season for the acclimation of plants in the field, indeed the summer drought induces a total or partial dormancy in these species, whereas in autumn temperatures are lower and rain provides soil with the necessary water, allowing the life cycle and biomass production to start again. All seedlings had a height from 5 to 8 cm and were planted at a distance of 60 cm from each other covering a total area of about 28 m^2. The experiment lasted 11 months. During the field test the tolerance of the plants to high concentrations of metals was evaluated by regular counting of the vital plants. Plant accumulation capacity was also evaluated by periodic sampling of leaves. Sampling was made more frequently at the beginning of spring and summer because of the faster biomass production of the plants during these seasons.

The assisted phytoextraction experiments

S. bicolor plants drawn from the contaminated site with their clods were placed in 5 L pots and transported to the laboratory where they were maintained at a temperature of 20–25 °C and a 12 h photoperiod.

Two types of easily biodegradable chelating agents were used: IDSA (iminodisuccinate acid, Bayer, Leverkusen, Germany) and MGDA (BASF, Ludwigshafen, Germany). They were selected on the basis of both biodegradability and the capacity to mobilize metals in soil. MGDA has a far higher biodegradability with respect to EDTA: 89–100% of MGDA can be degraded in 14 days (data sheet, BASF) while no EDTA was degraded in 30 days. The OECD 301 E test on IDSA showed a 79% decrease in dissolved organic carbon (DOC) after 28 days (data sheet, Bayer). Chelants were applied by adding 100 mL of water, with a dose of 4 mmol kg^{-1} of soil. Untreated reactors were used as control. The experiment was performed in triplicate and lasted 2 months.

In order to evaluate the potential bioavailable metal fraction and the potential risks of chelant application, metals concentrations in soil solution and leachate were measured during the experiment. In each pot a Rhizon soil moisture sampler was installed (Eijkellkamp, Agrisearch equipment, the Netherlands). Every pot was equipped with a leachate sampling device. Soil solution, leachate and plants were sampled 1 day before and after chelant treatment, and monthly until the end of the experiment. At the end of the experiment the plants were extracted from the growing media and washed carefully, then roots and shoots were separated for analysis.

RESULTS AND DISCUSSION

The continuous phytoextraction experiment

During the continuous phytoextraction test plant tolerance to metals and metal accumulation capacity were evaluated and the performances of the different plants species compared.

Plant growth

During the first 30 days the plant species showed signs of suffering due to the extreme environmental conditions and the high heavy metals concentrations in the soil.

T. glaucum showed a high tolerance in the early months of observation, but only a small percentage of plants (11%) was alive at the end of the experiment because most of the plants were not able to begin a new vegetative cycle. Biomass production and plant growth was low (maximum height 11 cm). *C. salviifolius* suffered the most in field conditions and after 30 days live plants were reduced to 52%, dropping to 10% by the end of the observation period. Live plants at the end of the experiment reached a maximum height of 30 cm but did not show high biomass production.

The plant species that best tolerated the field extreme conditions (maximum height 24 cm and the highest biomass production) was *S. bicolor*. The percentage of live plants at the end of the test was 68% (Fig. 1).

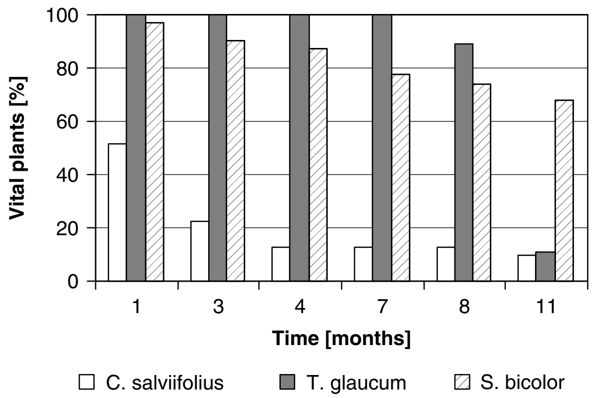

Figure 1. Percentage of live (vital) plants during the experiment.

Metal accumulation in plants

During the experiment the capability of the plant species to accumulate Pb and Zn in the aerial part was evaluated (Fig. 2). In the period immediately after planting *C. salviifolius* showed an anomalous accumulation of metals that decreased and remained constant for the following period, with a slight increase in the next vegetative cycle. At the end of the observation period plant species showing the greatest ability to accumulate Pb in the aerial part were *T. glaucum* (353 mg kg^{-1}) and *C. salviifolius* (185 mg kg^{-1}). The accumulation of Pb in the aerial part for *S. bicolor* was 119 mg kg^{-1}. The concentration of Zn was high for all species: 1560 mg kg^{-1} for *C. salviifolius*, 1190 mg kg^{-1} for *S. bicolor* and 840 mg kg^{-1} for *T. glaucum*. The difference between Pb and Zn accumulation in plants can be due to the fact that Zn is an essential element for plant health and consequently its absorption is related to the quantity necessary for this.[20]

The results of this experiment for both plant growth and metal accumulation suggest that *T. glaucum* and *C. salviifolius* probably accumulate a high quantity of toxic elements because of their limited ability in the selection of metallic ions, which affected their survival. In contrast, *S. bicolor,* probably due to a higher capacity to limit the absorption of toxic elements, has shown greater biomass production and greater ability to tolerate high concentrations of both metals compared with other plant species.

The assisted phytoextraction experiments

During the assisted phytoextraction test the capacity of the chelating agents to mobilize heavy metals and to enhance metal accumulation in plants was evaluated. Chelant treatment resulted in signs of clorosys after 7 days and plant death after 2 months from treatment.

Metal mobilization

Chelant capacity to mobilize heavy metals was evaluated through the determination of heavy metal concentration in soil solution. Both chelants demonstrated enhanced mobilization of Zn in soil solution and to a less extent that of Pb. Zn concentration in soil solution reached a maximum value of 600 mg L^{-1} in the presence of MGDA and 320 mg L^{-1} in the presence of IDSA, while Pb concentration reached 1.5 mg L^{-1} using MGDA and 0.4 mg L^{-1} using IDSA (Fig. 3). The low heavy metals mobilization could be a consequence of soil characteristics and in particular of its low cation exchange capacity and high Ca and Mg concentrations. It has been demonstrated[21] that soil properties, and in particular cation exchange capacity, can affect soil washing processes. This statement can be confirmed by the results of a similar assisted phytoextraction experiment using the same plant species and the chelant MGDA[11] with a soil from the *Corti baccas* site near Montevecchio, characterized by a high cation exchange capacity (18 cmol kg^{-1}), low Ca (0.6%) and Mg (0,8%) contents, and concentrations of heavy metals of 4200 mg kg^{-1} for Zn and 8900 mg kg^{-1} for Pb. In this case MGDA was able to mobilize far higher quantities of metals, up to 360 mg L^{-1} of Pb and 1350 mg L^{-1} of Zn.

The simulation experiments made by comparing the soil from *Corti baccas* with the soil used for the experiment showed that the chelation capacity of MGDA is far lower in the Campo Pisano soil. In fact MGDA was able to chelate up to 44% of Pb and 57% of Zn in the soil with low concentrations of Ca and Mg, while its chelation capacity in the soil used for the experiment was 2% for Pb and 11% for Zn. Similar results were obtained using EDTA: the chelation capacity reached 68% and 59% for Pb and Zn, respectively, in the soil with low concentrations of Ca and Mg, and was 23% for Pb and 44% for Zn in the soil used for the experiment.

The low capacity of the chelating agents to mobilize metals in the selected soil was confirmed by the concentration of metals in leachate. Zn concentration in leachate immediately after chelant treatment was higher than Pb concentration and MGDA showed a higher mobilization capacity than IDSA (Fig. 4).

Metal accumulation in plants

Pb accumulation in plants was increased by chelant treatment and reached 300 mg kg^{-1} in the pots treated with IDSA immediately after chelant application, while no significant difference could be noticed between control and MGDA treatment. One month from chelants treatment the differences among the reactors were not significant. Zn concentration increased, reaching a maximum value of 2700 mg kg^{-1} in pots treated with IDSA and 2200 mg kg^{-1} in

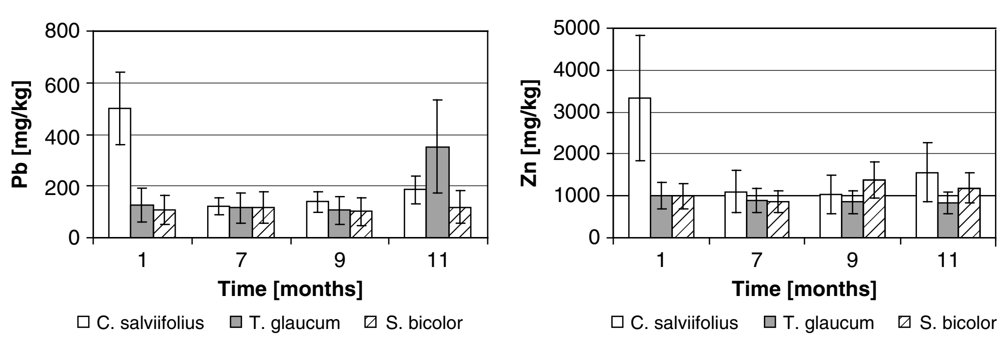

Figure 2. Average Pb and Zn accumulation (standard deviation) in the aerial part of the plants during the continuous phytoextraction experiment.

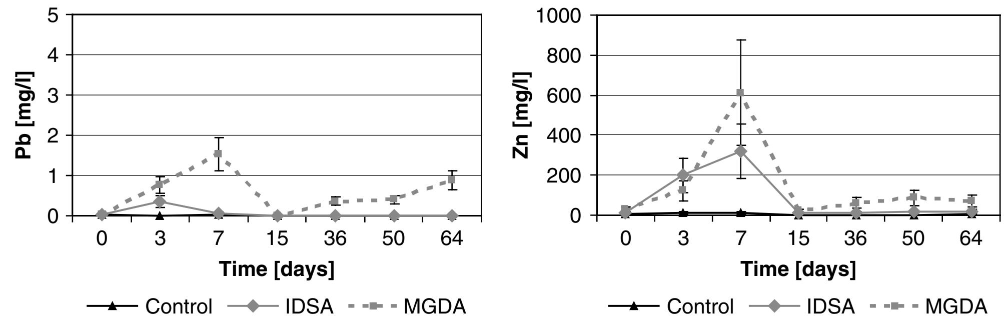

Figure 3. Pb and Zn average concentration (standard deviation) in soil solution during the experiment.

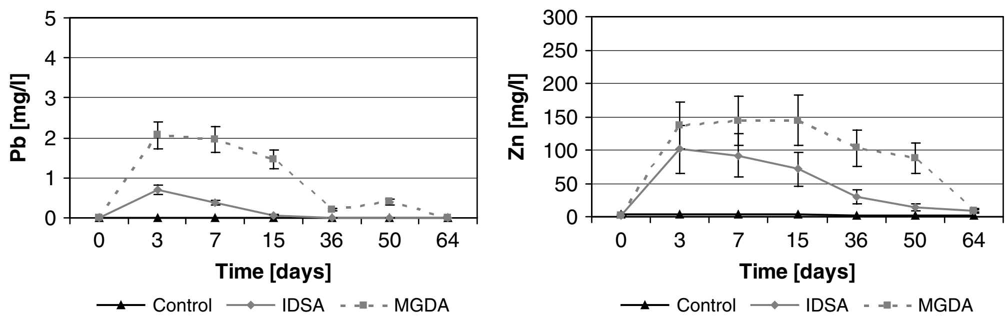

Figure 4. Pb and Zn average concentration (standard deviation) in leachate during the experiment.

pots treated with MGDA (Fig. 5). In contrast metal concentration in roots decreased considerably in the treated reactors. Pb concentration in control reactors was around 120 mg kg^{-1} while its concentration was 30 and 80 mg kg^{-1} in reactors treated with IDSA and MGDA, respectively. The concentration of metals in roots is slightly lower in the reactors treated with the chelating agents showing an increased transport into the aerial part of the plant. This difference demonstrates the modification of plant reaction to metals subsequent to the application of chelating agents.[22]

Previous experiments[11] performed using the same plant species and MGDA but in a soil characterized by a higher cation exchange capacity showed higher metal accumulation in plants (1800 mg kg^{-1} for Pb and 10 900 mg kg^{-1} for Zn).

CONCLUSIONS

The results of the field experiment allowed one to evaluate the metal accumulation capability of the native plant species selected, as well as their tolerance to high heavy metals concentrations.

The species *C. salviifolius* and *T. glaucum* showed clear signs of suffering, confirmed by their low survival and biomass production, so that they can be considered less tolerant to heavily polluted soils. At the end of the observation period, the plant species that showed the greatest ability to accumulate Pb was *T. glaucum* while *C. salviifolius* accumulated the greatest amount of Zn. These species were probably able to absorb a high quantity of toxic elements because of their limited ability in the selection of metallic ions. In contrast, *S. bicolor,* which normally grows on contaminated soils, has shown higher biomass production (not much lower than that usually produced by this species in natural conditions) and an ability to tolerate high concentrations of Pb and Zn compared with the other plant species; this was probably due to a greater capacity to limit the absorption of toxic elements. Therefore *S. bicolor* seems the more suitable for application to phytoremediation treatment in mining areas.

The application of chelating agents to the soil did not appreciably increase metal mobility and metal accumulation in plants, probably because of the low cation exchange capacity and the high concentration of Ca and Mg in soil, as was demonstrated

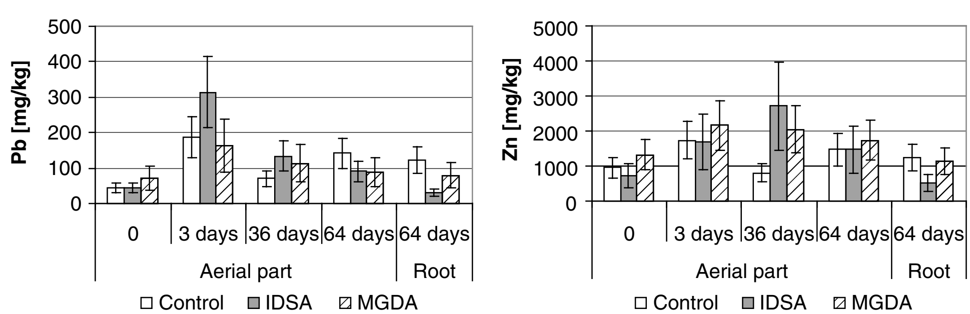

Figure 5. Average Pb and Zn accumulation in the aerial part of the plants (standard deviation) during the assisted phytoextraction experiment and in roots at the end of the experiment.

in parallel tests carried out with the same chelating agents and a different soil.

The application of assisted phytoextration to this type of soil needs to be optimized, for instance by the addition to the soil of materials with high cation exchange capacity such as zeolites or compost. Also the use of different chelants can be investigated.

ACKNOWLEDGEMENTS

The authors wish to thank Francesca Tolu for part of the experimental work and Orietta Masala for supporting in the analytical work.

REFERENCES

1 Regione Autonoma della Sardegna, Piano regionale di gestione dei rifiuti – Piano di bonifica siti inquinati della Sardegna (2003).
2 Tordoff GM, Baker AJM and Willis AJ, Current approaches to the revegetation and reclamation of metalliferous wastes. *Chemosphere* **41**:219–228 (2000).
3 Wong MH, Ecological restoration of mine degraded soils, with emphasis on metal contaminated soils. *Chemosphere* **50**:775–780 (2003).
4 Norland MR and Veith DL, Revegetation of coarse taconite iron ore tailing using municipal waste compost. *J Hazard Mater* **41**:123–134 (1995).
5 Cao A, Carucci A, Lai T, La Colla P and Tamburini E, Effect of biodegradable chelating agents on heavy metals phytoextraction with *Mirabilis jalapa* and on its associated bacteria. *Eur J Soil Biol* **43**:200–206 (2007).
6 Angiolini C, Bacchetta G, Brullo S, Casti M, Giusso del Galdo G and Guarino R, The vegetation of the mining dumps in SW-Sardinia. *Feddes Repertorium* **116**:243–276 (2005).
7 Bacchetta G, Casti M, Mossa L and Piras L, La flora del distretto minerario di Montevecchio (Sardegna sud-occidentale). *Webbia* **62**:27–52 (2007).
8 Bacchetta G, Casti M and Zavattero L, Analisi della vegetazione del distretto minerario di Montevecchio (Sardegna sud-occidentale). *Fitosociologia* **44**:83–107 (2007).
9 Decreto Ministeriale 13/09/1999, Metodi ufficiali di analisi chimica del suolo. *Gazzetta Ufficiale della Repubblica Italiana* S.O. 248 21/10/1999.
10 Barbafieri M, Lubrano L and Petruzzelli G, Characterization of pollution in sites contaminated by heavy metals: a proposal. *Ann Chim* **86**:585–594 (1996).
11 Lai T, Fitodecontaminazione assistita di suoli contaminati da metalli pesanti PhD thesis, University of Cagliari (2008).
12 Bacchetta G and Pontecorvo C, Contribution to the knowledge of the endemic vascular flora of Iglesiente (SW Sardinia -Italy). *Candollea* **60**:481–501 (2005).
13 Carmignani L, Oggiano G, Barca S, Conti P, Eltrudis A, and Funedda A etal, Note illustrative della Carta Geologica della Sardegna in scala 1: 200.000. *Memorie descrittive della Carta Geologica d'Italia*, Istituto Poligrafico e Zecca dello Stato, Roma (2001).
14 Cidu R, Biagini C, Fanfani L, La Ruffa G and Marras I, Mine closure at Monteponi (Italy): effect of the cessation of dewatering on the quality of shallow groundwater. *Appl Geochem* **16**:489–502 (2001).
15 Caboi R, Cidu R, Fanfani L, Zuddas P and Zanzari AR, Geochemistry of the high-PCO2 waters in Logudoro, Sardinia, Italy. *App Geochem* **8**:153–160 (1993).
16 Rodriguez L, Ruiz E, Alonso-Azcarate J and Rincon J, Heavy metal distribution and chemical speciation in tailings and soils around a Pb–Zn mine in Spain. *J Environ Manage* **90**:1106–1116 (2009).
17 Ardau C, Chessa A, Collu M, Cucca L, Da Pelo S, Dessí R, Fanfani L, Ullu F and Zuddas P, Caratterizzazione geochimica e mineralogica dei fanghi di flottazione e dei suoli contaminati da metalli pesanti. *Atti della Facoltà di Ingegneria* (1999).
18 Nowack B, Schulin R and Robinson BH, Critical assessment of chelant-enhanced metal phytoextraction. *Environ Sci Technol* **40**:5225–5232 (2006).
19 Lasat MM, Phytoextraction of toxic metals: a review of biological mechanisms. *J Environ Qual* **31**:109–120 (2002).
20 Sequi P, *Chimica del suolo*, ed by Patron, Bologna (1989).
21 Zou Z, Qiu R, Zhang W, Dong H, Zhao Z and Zhang T, The study of operating variables in soil washing with EDTA. *Environ Pollut* **157**(1):229–236 (2008).
22 Vassil DA, Kapulnik Y, Raskin I and Salt ED, The role of EDTA in lead transport and accumulation by Indian Mustard. *Plant Physiol* **117**:447–453 (1998).

