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A field experiment on the use of *Pistacia lentiscus* L. and *Scrophularia canina* L. subsp. *bicolor* (Sibth. et Sm.) Greuter for the phytoremediation of abandoned mining areas

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Abstract
A two-year study has been conducted in an abandoned Pb/Zn mining site, with the aim of investigating the feasibility of phytoremediation using two native Mediterranean plants (*Pistacia lentiscus* and *Scrophularia bicolor*) and of assessing the performance of amendments able to reduce the toxic effects of heavy metals. The amendments used were compost, chemical fertilizer, and zeolites, used singly or in combination. Depending on the amendments applied, the two species showed different mortality rates in the different plots, but all produced an increase in *P. lentiscus* survival, while *S. bicolor* survival improved only when amended with zeolite or zeolite + fertilizer. *Scrophularia bicolor* proved to be a more efficient accumulator than *P. lentiscus*, especially for Pb uptake. *Pistacia lentiscus* accumulated metals mostly in the roots. The effect of amendments was to generally reduce the bioavailable metal fraction, especially lead, in the plots amended with compost. *Pistacia lentiscus* proved to be the most suitable species for phytostabilization and environmental restoration, both for its resistance to metals and high phytomass production. The experiments demonstrate that the use of compost not only encourages this kind of revegetation in degraded areas, but is also an economical option that uses a by-product of solid municipal waste treatment.

Keywords: Heavy metals, mining areas, phytoremediation, *Pistacia lentiscus*, *Scrophularia canina* subsp. *bicolor*, soil amendments

Introduction
Poor post-closure management of mines has led to the dispersion of significant amounts of contaminants by wind and water erosion over extensive areas in Sardinia (Italy). In particular, in the Sulcis-Iglesiente area (SW Sardinia), once the most important metal mining region in Italy, hundreds of mine dumps exist, from which a variety of heavy metals are dispersed into the environment (Boni et al. 1999; Fanfani et al. 2000; Cidu et al. 2001). These elements have been affecting the terrestrial and aquatic ecosystems in surrounding areas (Caredda et al. 1999; Baroli et al. 2001; Mandas 2009). The extent of the areas affected by mining activities and heavy metal contamination makes the application of traditional disruptive technologies inappropriate, due to the high costs associated with soil remediation and to the potential impact on the environment, especially alteration of the landscape and soil agronomic properties. The extensiveness of the area and the shallow contaminated soils make phytoremediation appropriate, since despite requiring long remediation times, it is a low cost, *in situ* technique. Its applicability depends on the possibility of identifying plants able to tolerate high concentrations of available heavy metals in the soil and either to accumulate them in their above-ground part (phytoextraction) (Baker et al. 1994) or immobilize...
contaminants at the soil-roots interface (phytostabilization), thereby reducing possible groundwater contamination. In particular, phytostabilization consists in the reduction of contaminants mobility through the release of chemical compounds by the roots (exudates) or through the use of soil amendments that induce the formation of less soluble forms of metals (Berti & Cunningham 2000). Phytostabilization in mine sites is now a practice recognized by the scientific community for in situ mine waste reclamation (Tordoff et al. 2000; Wong 2003; Mendez & Maier 2008; Tel-Or & Forni 2011). Vegetation can provide effective protection against wind and water erosion and improve soil agronomic properties (Norland & Veith 1995). The use of native Mediterranean plants accelerates the development process to restore pre-mining ecological conditions in the site. Plant growth can be enhanced by the addition of amendments, such as natural zeolites or compost, which improve soil chemical, biological, and physical properties increasing biomass production and favoring metal immobilization by reducing their bioavailability (Chen et al. 2000; Marques et al. 2008; Liu & Zhou 2009; Abbasi et al. 2011). In particular, the decrease in Pb and Zn bioavailability produced by compost was demonstrated by Rizzi et al. (2004) using Sardinian soils, though with different physical-chemical characteristics than those in the area under study.

Ideal plants for phytoremediation are autochthonous species, as they are likely to have greater resistance to the extreme conditions of such an environment and preserve plant diversity in the site.

The aim of this study is to test different cultivation protocols for two Mediterranean plants commonly found in the mining districts of Sardinia, specifically Pistacia lentiscus L. and Scrophularia canina L. subsp. bicolor (Sibth. et Sm.) Greuter, with a view to using them for revegetation and phytoremediation projects in abandoned mining sites.

A few studies have investigated the use of S. caninas, L. and P. lentiscus in phytoremediation, the latter prevailing for its importance in the restoration of woody Mediterranean communities (Boularbah et al. 2006; Fuentes et al. 2007; Domínguez et al. 2008). Scrophularia bicolor was selected for its phytotoxic extractability, as already demonstrated by Cao et al. (2009).

Materials and methods

Study area and species

A field experiment was carried out in a contaminated area near Iglesias (Sardinia, Italy) on the tailings dump at the abandoned Campo Pisano mine, the most important ore processing facility up until the 1990s. The selected area is a settling pond for flotation tailings containing Pb and Zn as the most abundant toxic metals. The choice of this site was based on actual pollution, the availability of a flat area and the possibility of fencing it off to prevent entry of wild animals.

Concerning the two native Sardinian species used, S. bicolor is a half shrub (chamaephyte) endemic to Sardinia, Corsica and Sicily, that usually colonizes pebbly substrata, growing on both natural gravel and gravel derived from mining activities, where it forms a typical component of pioneer plant communities (Angiolini et al. 2005; Bacchetta et al. 2007). It can form garigues on unstable mine dumps as dominant or incidental taxon. Pistacia lentiscus is a shrub that grows up to 6 m tall (phanerophyte), typical component of the Mediterranean sclerophyllous shrubland, occasionally found growing on mine waste. Scrophularia bicolor forms communities with sparse cover and for this reason was compared with P. lentiscus which, on the contrary, is a high phytomass producing shrub. Navarro Cerrillo and Blanco Oyonarte (2006) showed that, among different Mediterranean shrubland ecosystems, the highest phytomass values were obtained for maquis dominated by P. lentiscus, with 1966 g m⁻².

Experimental design and chemical analyses

The site was prepared in different phases. First, the soil was homogenized and leveled with a bulldozer, forming an area of 300 m² and a channel was then created for surface water drainage. The site was fenced in and finally 10, 6 m × 5 m experimental plots were prepared in which different soil amendments were applied to the two plant species. Two of these plots were left untreated and used as controls for the two species. The amendments used were compost, chemical fertilizer and zeolites, used singly or in combination, as described in Table I. Five plots were planted with 80 plants each of P. lentiscus (A) and five plots with 120 plants of S. bicolor (B). The planted specimens were propagated from seeds collected in the surrounding areas and grown in a nursery.

Compost came from a yard waste treatment plant located in Quartu S. Elena (Cagliari), while the zeolites used were from Bonorva (Sassari), where a deposit of significant interest has been studied, containing about 50% clinoptilolite zeolite (Morbidelli et al. 1999).

Earlier ion exchange capacity tests on samples from the Bonorva site had shown the effectiveness of zeolites in heavy metals reduction (Lonis et al. 2002). Chemical characteristics of the Campo Pisano soil and of the two amendments are summarized in Table II.

After transplanting, the plots were regularly watered every other day for the first 4 months, and thereafter only received seasonal rainfall.
The plots were monitored for a period of two years (May 2008–May 2010) in order to assess both the behavior of the two species and the effects of the different amendments tested.

Plant resistance to heavy metals and poor soil was determined through monthly counts of live or suffering plants in the different plots.

Phytoremediation performance of the two plant species using the different amendments was assessed through the periodical determination of metal concentrations in the soil and in the different parts of the plant (roots and leaves).

The first 30 cm of soil in each plot was sampled every three months from five different (randomly selected) points using an Edelman hand auger. The five samples were then mixed to obtain a composite sample.

Soil was chemically characterized using official Italian analytical methods (D.M. 13/09/99). Both total and bioavailable soil metal concentrations were evaluated in order to determine the influence of the different treatments. The bioavailable metal content was evaluated through the sequential extraction procedure proposed by Barbafieri et al. (1996).

Total carbon and nitrogen contents were determined with an elemental analyzer (Leko CHN-1000), while cation exchange capacity (CEC) was measured using the BaCl2 method.

Leaves were sampled every month, whilst roots were analyzed every three months by harvesting a plant from each plot. Leaf and root samples were washed with tap water and dried at 105°C before analysis. The dried material was digested with aqua regia.

Metals concentration in the extracted solutions was measured by inductively coupled plasma spectrometry (ICP-OES, Varian 710 ES).

**Statistical analysis**

The behavior of the two plant species and the effect of the different amendments were analyzed, adopting parametric and non-parametric approaches. The use of classical statistical tests (i.e. analysis of variance, ANOVA), for analyzing differences among data sets, requires some strong assumptions that are not always verified: the assumptions of normality, graphically checked by quantile-quantile plots and tested by means of the Shapiro–Wilk test (Shapiro & Wilk 1965), and of homoscedasticity, graphically checked by boxplots and tested using Levene’s test (Levene 1960). When these two fundamental assumptions were satisfied a classical ANOVA was used, otherwise the non-parametric Friedman’s test. Furthermore, when significant statistical results were obtained, the so called “post hoc analysis” was performed (Conover 1980, Bortz et al. 2000), which is interesting for identifying, among a set of variables, those most affecting the results.

**Results and discussion**

**Initial characterization**

Chemical characterization of the soil in the different plots at the beginning of the experiment is shown in Table III.

The table shows the effect of the different amendments on the soils' chemical properties. In particular, the addition of compost resulted in a considerable decrease in the bioavailable Pb, due to the formation of complexes and precipitates (which reduce the mobile metal fraction, Padmavathiam & Li 2010) and in a general increase in C.E.C. The pH
values remained within the range 7.4–7.8, with no significant effects due to the amendments.

**Plant survival**

The two species showed different mortality rates depending on the amendments applied. Results were always better for *P. lentiscus* indicating a greater adaptability of this species with respect to *S. bicolor* (Figure 1).

The most similar profiles were obtained for the untreated soil, where *P. lentiscus* showed, however, greater survival rates in the first months.

All the amendments produced an increase in *P. lentiscus* survival, while *S. bicolor* survival improved only when amended with zeolite or zeolite + fertilizer.

Compost addition (alone or in combination with zeolite) actually caused a rapid reduction in the number of *S. bicolor* plants right from the early months of the experiment, probably due to the on-going curing processes during summertime which affected this species more than *P. lentiscus*. One possible explanation is the different phenology of the two species, since *S. bicolor* tends to accumulate reserve substances before leaf desiccation through an intense metabolic activity at the beginning of summer. On the contrary, *P. lentiscus* preserves the leaves produced during springtime and suffers less from possible stress factors associated with soil properties. The adaptive strategy of Mediterranean maquis species is to reduce photosynthetic activities during drought, in particular, the metabolism of *P. lentiscus* only slows down slightly in the summer (Gratani & Varone 2004). Consequently, following the first period of reduced growth after transplanting, this species benefited from the improved soil properties achieved through compost addition. The highest survival rate was in fact obtained in the plots containing compost.

As for the other amendments, they all favored the survival of both species with better results for *P. lentiscus*.

Due to violation of the classical assumptions required for the use of ANOVA, the Friedman’s test...
(Friedman 1937) was used to statistically verify the above observations, as well as the post hoc analysis. The results for both *P. lentiscus* and *S. bicolor* are shown in Table IV. For this kind of statistical test, the null hypothesis assumes that no differences exist among distributions. A high value of the Friedman test, together with a low *p*-value, indicates that this assumption cannot be accepted either for *P. lentiscus* or for *S. bicolor*. The post hoc analysis provides a more detailed account and identifies the most significant pairs, and therefore the most different survival rates. By ranking all pairs in decreasing order, a list of the differences is obtained, ordered by importance, i.e. (in Table IV) the lowest *p*-value corresponds to the greatest differences in the survival rate distributions (Control vs. Compost for both *P. lentiscus* and *S. bicolor*).

**Pb and Zn in soils**

Table V gives total Pb and Zn contents in the different plots at the beginning and end of the experiment that exhibit a general decreasing trend. The decrease in Pb content varied between 33 and 51%, except for the control (8% and 15% for *P. lentiscus* and *S. bicolor*, respectively) and for *S. bicolor* in the compost plot (6%), while for Zn it varied between 52 and 67%, with values of 50% in the control plots. Note that metal content reduction can be partially attributed to rainwater leaching, as can be deduced from the control plots, particularly the plot with *S. bicolor*, where plant survival was very limited overtime. However, differences with respect to the controls clearly emerge, due both to the amendments and plant species used. Indeed the greatest reductions in metal content were observed in the *P. lentiscus* plots amended with compost and compost + zeolite where survival rate was very high, whilst the plots with the lowest survival rate (*S. bicolor* in control and compost plots) showed the smallest reduction.

However, the results varied widely due to the difficulties in obtaining homogeneous soil samples.

The different metal content reductions for *P. lentiscus* and *S. bicolor* were tested using the classical ANOVA. The only case in which the differences among plots were statistically significant concerned total Pb contents in the soil for *P. lentiscus* (*F*-statistics = 2.612; *p*-value = 0.049).

Conversely, the bioavailable fraction did not show any significant variations for the same period (Figure 2).

Lead bioavailability seems to be affected by the amendments, particularly by compost. The graphs in Figure 2(a) and (c) show a decrease of the bioavailable metal fraction even in the plots amended with zeolite and zeolite + fertilizer. However, it appears that in the plots with compost + zeolite, the effects of the two amendments are not cumulative, since the reduction in bioavailability with respect to the control is less evident than that obtained with compost alone.

As for Zn, the bioavailability reduction does not appear to be significantly affected by the amendments, even though the values for the compost plots (particularly *S. bicolor*) are also lower.

The bioavailability vs. time profiles show differences mainly related to the plant species. In the plots with *P. lentiscus* some seasonal variations can be observed, with an increase in bioavailability in the rainier and colder months, particularly for Zn. The same trend was not observed for *S. bicolor*. This may be explained by the different phenology of the species: *S. bicolor* – starts the vegetative period at the end of winter, while photosynthetic activity in *P. lentiscus* increases consistently after summer drought.

**Pb and Zn uptake by plants**

Figure 3 shows both the different metal accumulation of the two species and the differences in Pb and Zn concentrations in the leaves. *P. lentiscus* shows

<table>
<thead>
<tr>
<th>Pairs</th>
<th><em>P. lentiscus</em></th>
<th><em>S. bicolor</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Friedman statistic = 3.6007, <em>p</em>-value = 0.003</td>
<td>Friedman statistic = 3.6312, <em>p</em>-value = 0.002</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Pairs</th>
<th>Post hoc analysis, <em>p</em>-value</th>
<th>Post hoc analysis, <em>p</em>-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compost + Zeolite – Compost</td>
<td>0.976</td>
<td>0.988</td>
</tr>
<tr>
<td>Control – Compost</td>
<td>0.002</td>
<td>0.003</td>
</tr>
<tr>
<td>Zeolite – Compost</td>
<td>0.010</td>
<td>0.132</td>
</tr>
<tr>
<td>Zeolite + Fertilizer – Compost</td>
<td>0.262</td>
<td>0.346</td>
</tr>
<tr>
<td>Control – Compost + Zeolite</td>
<td>0.021</td>
<td>0.015</td>
</tr>
<tr>
<td>Zeolite – Compost + Zeolite</td>
<td>0.060</td>
<td>0.347</td>
</tr>
<tr>
<td>Zeolite + Fertilizer – Compost + Zeolite</td>
<td>0.616</td>
<td>0.657</td>
</tr>
<tr>
<td>Zeolite – Control</td>
<td>0.996</td>
<td>0.695</td>
</tr>
<tr>
<td>Zeolite + Fertilizer – Control</td>
<td>0.501</td>
<td>0.382</td>
</tr>
<tr>
<td>Zeolite + Fertilizer – Zeolite</td>
<td>0.728</td>
<td>0.988</td>
</tr>
</tbody>
</table>
lower values than S. bicolor, already recognized by Cao et al. (2009) as a species capable of accumulating high concentrations of the two metals. Pb bioaccumulation levels were always lower than Zn, in keeping with both the higher Zn soil concentration and the toxicity of Pb, which is not usually accumulated in large amounts by plants. On the contrary, Zn is an essential element for plant health and consequently its absorption is necessary and usually enhanced by specific root exudates or mycorrhizal symbiosis in natural soils (Sequi 1989; Marschner 1995).

Comparison of the different plots shows that accumulation in the aerial part is always highest in the untreated plots, for both species and both metals. As far as P. lentiscus is concerned, the amendments promoting the greatest bioavailability reduction (compost and compost + zeolite) also produced the greatest decrease in the amount of accumulated metals.

In order to confirm this direct relationship, a statistical analysis was performed on the ratios between accumulated metal and bioavailability. In particular, the ANOVA test demonstrated the same behavior of the ratios in the different plots, i.e. no statistically significant differences have been detected.

Figure 4 shows Pb and Zn concentrations in the roots. In this case, the highest values for P. lentiscus were not observed in the control plots but generally in those that were not treated with compost, particularly the plot amended with zeolite alone, with 235 mg · kg$^{-1}$ Pb and 1732 mg · kg$^{-1}$ Zn. The ratio between metal concentrations in the leaves and roots (translocation factor) is usually lower than 1 for P. lentiscus (on average between 0.56 and 0.88 for Pb and between 0.54 and 0.74 for Zn in the different plots), even though higher values were measured in certain cases.

Unlike the leaves, in this case no clear differences were found between the two species, even though some slightly higher values were occasionally detected for P. lentiscus. Despite containing root metal concentrations similar to those of P. lentiscus, S. bicolor showed better phytoremediation, as demonstrated by the higher translocation factor, usually more than 1 (values higher than three were attained for Pb and higher than two for Zn in zeolite and zeolite + fertilizer plots, where plant survival was highest). This confirms the behavior of S. bicolor, already reported by Cao et al. (2009).

The comparison of plots planted with S. bicolor does not show any clear influence of the different amendments on root accumulation, particularly because of the impossibility of sampling all the plots throughout the entire study period. The reason for this was its high mortality rate caused by the considerable amount of metals accumulated during

<table>
<thead>
<tr>
<th>Metal</th>
<th>Plant</th>
<th>Initial (mg kg$^{-1}$)</th>
<th>Final (mg kg$^{-1}$)</th>
<th>% red.</th>
<th>Initial (mg kg$^{-1}$)</th>
<th>Final (mg kg$^{-1}$)</th>
<th>% red.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb</td>
<td>P. lentiscus</td>
<td>3176</td>
<td>2118</td>
<td>35</td>
<td>11,472</td>
<td>1375</td>
<td>81</td>
</tr>
<tr>
<td></td>
<td>S. bicolor</td>
<td>2833</td>
<td>1716</td>
<td>32</td>
<td>11,747</td>
<td>4458</td>
<td>64</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3247</td>
<td>2318</td>
<td>46</td>
<td>11,599</td>
<td>4519</td>
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<td>1213</td>
<td>49</td>
<td>10,983</td>
<td>4303</td>
<td>66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3068</td>
<td>1839</td>
<td>46</td>
<td>11,386</td>
<td>4974</td>
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<td>3704</td>
<td>2767</td>
<td>46</td>
<td>10,520</td>
<td>5066</td>
<td>52</td>
</tr>
</tbody>
</table>

Table V. Initial and final total metal contents in the different plots with percentage reductions.
Figure 2. Bioavailability of Pb and Zn in the different plots for the two plants. (a) *P. lentiscus*, Pb; (b) *P. lentiscus*, Zn; (c) *S. bicolor*, Pb; and (d) *S. bicolor*, Zn.

Figure 3. Metal accumulation in the aerial part of the plants in the different plots. (a) *P. lentiscus*, Pb; (b) *P. lentiscus*, Zn; (c) *S. bicolor*, Pb; and (d) *S. bicolor*, Zn.
the first summer, which reduced the overall biomass to be sampled in the different experimental phases.

Influence of climate and phenology on metal accumulation

Figure 3 shows, besides the absolute levels of bioaccumulation in the leaves, the time profiles, which exhibit a clear cyclic behavior. Except for the first acclimation period (summer 2008), bioaccumulation values in leaves were higher during wintertime. This clearly emerges for *P. lentiscus* throughout the whole observation period in all the plots, while, as already pointed out, a clear time profile for *S. bicolor* could not be obtained, even though it too seems to show an increase of bioaccumulation in wintertime.

In order to confirm this observation, Figure 5 shows Zn concentration profiles in leaves for *P. lentiscus*, represented using the same style for all the plots, superimposed on monthly rainfall for the same period. The same comparison is also possible for Pb concentrations.

The similar profile of the curves can be clearly seen, except for the very initial phase (summer 2008), due to artificial irrigation and to the fact that the plants from the nursery had no initial accumulation.

In the first months of 2009, a slight decrease in rainfall was observed while Zn accumulation was found to be higher. Note that though scarce, rainfall in
that period occurred on many, often consecutive days. This fact, together with the low temperatures during that period, provided plentiful water for the plants.

These results are also in agreement with the previous observation concerning bioavailability which showed an increasing trend during the wetter months, especially for *P. lentiscus*.

The temporal variations in metal uptake appear to be related to the phenology of *P. lentiscus*. Net photosynthesis and stomatal conductance in this species are high in winter and spring dropping to a minimum between August and October (Flexas et al. 2001). At the same time, at the end of the summer the plants have low metal contents, but after the first autumn rains they sprout new leaves that progressively accumulate metals in their cells until complete development, peaking in winter, when photosynthetic activity is high.

**Conclusions**

The experimental tests demonstrated the feasibility of *P. lentiscus* and *S. bicolor* for restoring plant cover for reclamation of the tested site by reducing metal concentrations in the soil and contaminant transport through wind and water erosion.

The two plant species behaved differently: *S. bicolor* is a more efficient accumulator than *P. lentiscus*, especially for Pb uptake. *P. lentiscus* accumulated metals mostly in the roots. However, *S. bicolor* showed a high mortality rate owing to the considerable amount of metals accumulated.

The effect of amendments was to generally reduce the bioavailable metal fraction, particularly of Pb, in the plots amended with compost.

*P. lentiscus* appears to be the more suitable species for phytostabilization and revegetation, both for its resistance to metals and high phytomass production. Compost proved to be the best amendment in the long-term for plant growth. Indeed, *P. lentiscus* is a species requiring a nutrient-rich soil and after two years the plots amended with compost and planted with *P. lentiscus* produced a stable population of vital plants. In the nutrient-poor soils root development was probably inhibited, due to their tendency to grow towards enriched soil patches, avoiding poor quality soils (Barlow 2010).

On the other hand, *S. bicolor*, a species that normally grows on poor and/or contaminated soils, suffered from spring transplanting owing to its phenology. Nevertheless, after two years it steadily colonized the plots amended with zeolite and zeolite + fertilizer, even producing vital seeds and new plants.

The experiment has demonstrated that the use of compost alone may well be sufficient for promoting revegetation in degraded areas and is a low-cost option as it uses a by-product of solid municipal waste treatment.

**Acknowledgments**

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