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Growth of *Lygeum spartum* in acid mine tailings: response of plants developed from seedlings, rhizomes and at field conditions

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Lygeum spartum accumulated more metals when grown in the growth chamber from seeds than from rhizomes and much more than in the field.

Abstract

Lygeum spartum is a native species in semiarid Mediterranean areas that grows spontaneously on acid mine tailings. We aimed to study the suitability of this plant for phytostabilization. *L. spartum* was grown from both seeds and rhizomes in acid mine tailings with various fertilizer and lime treatments. Untreated soils had a solution pH of 2.9 with high concentrations of dissolved salts (Electrical Conductivity 25 dS m^{-1}) and Zn (3100 mg L^{-1}). Plants grown on untreated soil had high shoot metal concentrations (>4000 mg kg^{-1} Zn). Liming increased the solution pH to 5.5 and reduced the dissolved salts by more than 75%, resulting in lower shoot metal accumulation. Plants grown from rhizomes accumulated less metal than those grown from seeds. Plants collected in the field had metal concentrations an order of magnitude less than plants raised in the growth chamber. These differences may be due to the higher moisture content and homogeneous nature of the soils used in the pot experiment.

Keywords: Lygeum spartum; Acid soil; Mine tailings; Heavy metals; Phytostabilization

1. Introduction

Mining can deleteriously affect the environment due to the deposition of high volumes of waste. Mine tailings that contain mobile contaminants present an ecological and human health risk to surrounding areas. Merrington and Alloway (1994) considered them "the most environmentally important components of historic mine sites". Mine tailings often have geochemical properties that inhibit plant establishment. These include low pH, toxic heavy metal concentrations and low organic matter content. Mine tailings soils usually have an undeveloped soil structure resulting in a low water retention capacity. Most tailings have steep and unstable slopes (Henriques and Fernandes, 1991). This facilitates wind and water erosion and the spreading of tailing materials into surroundings areas. This is especially important when the tailings are located near agricultural land or human settlements, since there may be a high risk of human exposure to contaminants, either directly via dust, or indirectly through the consumption of agricultural produce from polluted land.

Removal of tailings or covering them with non-polluted materials reduces the environmental risk. However, theses techniques are expensive and in some cases impractical due to the large volumes of tailings and the need for an adequate disposal area. Revegetation of mine tailings is an inexpensive long-term option to immobilize contaminants and minimize the environmental risks presented by theses sites (Tordoff et al., 2000). Plants stabilize the soil by root growth and water uptake. This reduces water and wind erosion. A vegetative cover also improves the aesthetic value of degraded sites (Tordoff et al., 2000). It is not possible to decontaminate these sites by phytoextraction since the large amounts of metals in

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mine tailing soils would take an unacceptably long time to remove, and plant metal uptake may provide an exposure pathway to herbivores.

Phytostabilization, the use of plants to immobilize soil contaminations (Fitz and Wenzel, 2002), includes mechanical stabilization as well as chemical immobilization of pollutants, e.g. by precipitation. These processes reduce the risk of metal entry into the food chain (Wong, 2003). Plants also increase the nutrient levels of infertile soils (Cobb et al., 2000). A vegetative cover, once established, may be self-sustaining (Norland and Veith, 1995).

Successful revegetation of tailings in arid regions requires plants that are adapted to grow under dry conditions in low nutrient soils with high metal concentrations. Possible sources of such plants are old, abandoned tailings that tolerant species may have spontaneously colonized. Several authors have studied the naturally occurring vegetation that grows on and around mining zones in arid and semiarid regions (Henriques and Fernandes, 1991; Melendo et al., 2002; Flores-Tavizón et al., 2003; García et al., 2003; Alvarenga et al., 2004; Álvarez-Rogel et al., 2004; Conesa et al., in press).

One of these plant species is *Lygeum spartum* L. (*Poaceae*), a widespread rhizomatous grass in the Mediterranean zone. Previous studies in the field (Conesa et al., in press) found that this species accumulated less than 100 mg kg^{-1} Pb and Zn, while the acid (pH 3) mine tailing on which it grew contained 14 000 mg kg^{-1} Pb and 9500 mg kg^{-1} Zn. *Lygeum spartum* can tolerate extreme conditions of aridity, salinity and high temperatures (Diaz and Honrubia, 1993). It may thus be a suitable candidate for the phytostabilization of acid mine tailings in Southern Spain and other semiarid Mediterranean areas.

Successful revegetation of mine soils requires preliminary studies to determine the optimal conditions for plant growth. Soil amendments may be necessary to improve substrate fertility by increasing plant nutrients and organic matter content, and neutralizing acidity. Pot experiments may reveal the plant responses to various amendments. Treatments can be studied under identical growth conditions (light, humidity, water). However, it is important to relate these controlled conditions to those that occur in the field.

We aimed to determine the effect of fertilizer and lime treatments on the growth and metal uptake of *Lygeum spartum* L. growing on acidic mine tailings soil. We also sought to compare metal uptake in pot experiments in a growth chamber with metal uptake by wild plants growing in field conditions in order to know the potential of this plant species in favorable growing conditions. We also investigated how plant metal uptake changes when plants are grown from seeds or from rhizomes in order to assess the best option to effect further remediation actions.

2. Materials and methods

2.1. Sampling

The Cartagena-La Union Mining District (0–392 m, a.s.l.; 37°37′20″ N, 0°50′55″ W- 37°40′03″ N, 0°48′12″ W) is located on the Southeast of the Iberian Peninsula and covers an area of 50 km^2, mostly belonging to the Sierra of Cartagena-La Unión, also called Sierra Minera. This region has a semiarid Mediterranean climate with an annual rainfall of 250–300 mm, occurring mostly during spring and autumn. The annual average temperature is 18 °C.

Soil was taken at points homogeneously distributed from the upper 40 cm of the ‘‘Belleza’’ mine tailing, located in the La Unión’s town surroundings (U.T.M. X688 760 m; Y4165 700 m; Z190 m). The tailing had a length of 160–170 m, a width of 21 m, a height of 22 m, and a volume of 370 000 m^3 (I.G.M.E., 1999). The soil samples were mixed to give a single homogeneous sample. The soil was air dried, sieved to <2 mm and stored in plastic bags. The pH of the soil was 3 and it contained high total metal concentrations (Table 1), especially Pb, As and Zn (Conesa, 2005; Conesa et al., in press).

Five plant samples (roots and shoots) of *L. spartum* were taken from various locations on the mine tailing. The plants were washed with deionised water, and dried at 65 °C for 72 h. Shoots and roots were separated prior to grinding (Janke & Kunkel IKA® Labortechnik A-10). The resulting samples were analyzed for the total element contents by X-ray fluorescence (Spectro X-Lab 2000). Seeds and rhizomes were collected in December 2003 and 2004 respectively for pot studies.

2.2. Pot experiment

Plants were grown in plastic pots containing 1 kg of soil. Four treatments were used: untreated, fertilizer (80 N, 80 P, 110 K, 25 Mg, in mg kg^{-1} soil), lime (25 g $CaCO_3$/kg soil), and fertilizer and lime together. KNO_3, KH_2PO_4, NH_4NO_3, and $Mg(NO_3)_2$ $6H_2O$ were used like chemicals. The fertilizer was added with the irrigation water: 30% added in the first week, 20% in the second and 10% in each following week until 100% was reached.

The plants were grown for eight weeks in a growth chamber with a light cycle of 16 h light/8 h darkness, and controlled humidity (50–90%) and temperature (16/23 °C night/day). Seeds were germinated in sand and seedlings transferred to the pots. Rhizomes were maintained one week in sand after harvest from the field before being transplanted directly into the pots. Three seedlings or one rhizome were placed in each pot. Three replicates of all four treatments were made for the plants grown from seeds and four replicates for the two fertilizer treatments (with and without lime) in the plants grown from rhizomes.

2.3. Soil solution sampling and analysis

Soil solution samples were taken in the 4th week of the experiment using Rhizon Flex soil moisture samplers (Rhizosphere Research Products, Wageningen, Netherlands). Samples were stored in plastic tubes at 5 °C prior to analyses. We measured: pH using a 691 pH Meter (Metrohm), electrical conductivity using a conductivity meter WTW LF318/SET, anions (Cl^-, NO_3^-, SO_4^{2-}) using a IC20 Ion Chromatograph (Dionex), and cations using ICP-OES (Vista-MPX Varian) for Pb, Cu, Cd and As and Flame AAS (SpectraAA 220/FS, Varian) for Zn, Ca, Mg, Na and K.

2.4. Plant analyses

Plant chlorosis was estimated visually by measuring the chlorotic part of the leaves compared to their total length since this plant species has long and narrow leaves. The plants were harvested in the 8th week of the experiment, washed with deionised water, and dried at 65 °C. Shoots and roots were separated prior to grinding (RETSCH Schieritz und Hauenstein AG). Dry weights were determined for individual plants (three in pots with seedlings and one in pots with rhizomes). For the metal content analyses, individual plant samples were bulked and mixed for each pot.

Samples were digested with 2 mL H_2O_2 (30%), 5 mL HNO_3 (65%) and 2 mL H_2O using a microwave digester (MLS-1200 MEGA ETHOS). The digests were diluted to 25 mL with water. Copper, Zn, As, Pb and Cd were measured by ICP-OES (Vista-MPX Varian). Quality control was carried out using the Certified Reference Material of the Community Bureau of Reference BCR No 62 (*Olea europaea*). We obtained recoveries of 85% for Pb, 100% for Zn and 80% for Cu.

Table 1
Soil properties of "Belleza" tailing according to Conesa (2005) and Conesa et al. (in press): pH; Electrical Conductivity of the saturated extract (ECe); Cation Exchange Capacity (C.E.C.); % Organic Carbon (O.C.); % Total Nitrogen (T.N.); % of clay, silt and sand; total and soluble metal concentrations

pH	ECe	C.E.C.	O.C.	T.N.	Clay	Silt	Sand		Metals				
	$dS\ m^{-1}$	$cmol(+)\ kg^{-1}$	%	%		%			Cu	Zn	Cd	Pb	As
									$mg\ kg^{-1}$				
3	18	16	0.5	0.035	12	33	55	*total*	380	5400	9	7000	1900
								soluble	7	990	5	12	na

$n = 30$. na: not available.

All the statistical analyses (ANOVA and LSD test for comparison of means) were carried out with SPSS 14.0 (SPSS, 2005) with log transformed data. Differences at $P < 0.05$ level were considered significant.

3. Results

3.1. Soil solution

Lime addition significantly increased pH and significantly decreased the electrical conductivity of the soil solution (Table 2). In the non-limed soils, high SO_4^{2-} concentrations represented over 95% of total anions. The change of the pH in the lime-amended pots decreased SO_4^{2-} to 3000 mg L^{-1}. NO_3^- only occurred in high concentrations in the fertilized soils. It was the only anion that was significantly higher in the fertilized treatments compared to the non-fertilized treatments. NO_3^- in the non-fertilized soils was low, a finding consistent with the low fertility of mining soils (Tordoff et al., 2000).

Soil solution Cl^- concentrations were unaffected by the treatments.

Potassium was significantly and positively correlated to NO_3^- ($r = 0.74$, $P < 0.01$). All other cations except Ca and Na were significantly and positively correlated with SO_4^{2-} ($r > 0.965$, $P < 0.01$).

As expected, Ca reached high levels when lime was added (400–500 mg L^{-1}). Elevated K concentrations were due to the addition of fertilizer. The unfertilized soil had a dissolved K concentration below 2 mg L^{-1}. Like Cl^-, Na concentrations did not significantly differ ($P > 0.05$) between treatments.

Dissolved metal concentrations were significantly higher in the untreated than in the limed soil. Dissolved Zn decreased by over 99% after addition of lime. For As, Cd, Pb and Cu the values in the limed soil were below or near the detection limit (0.1 mg L^{-1}).

3.2. Plants grown from seeds

Growth of *Lygeum spartum* was better on the limed soils. There were twice as many leaves per stalk on the limed soil as compared to the untreated pots (Table 3). The addition of nutrients did not cause significant increase in biomass (expressed as weigh per plant). In the treatments with lime, the fertilized plants had significantly less leaves per stalk (2.6) compared to non-fertilized plants (3.2), but the fertilized plants showed the highest percentage of leaves (77%) with less than 5% chlorosis. In the limed and non-fertilized pots this percentage was only 35%, but in both limed treatments the percentages of leaves with more than 95% chlorosis were similar with about 25%. In the treatments without lime, the

Table 2
Anions and cations in soil solution from the pot experiment

Parameter		No fertilizer	Fertilizer	No fertilizer	Fertilizer
		No $CaCO_3$ $n = 5$	No $CaCO_3$ $n = 5$	$CaCO_3$ $n = 3$	$CaCO_3$ $n = 5$
pH		2.9 (<0.1)a	2.9 (0.1)a	5.6 (0.2)b	5.4 (0.4)b
E.C. ($dS\ m^{-1}$)		25 (3)a	22 (2)a	4 (1)b	5 (1)b
Anions ($mg\ L^{-1}$)	Cl^-	130 (23)a	120 (37)a	140 (88)a	120 (54)a
	NO_3^-	51 (14)b	1200 (560)a	33 (41)b	690 (240)a
	SO_4^{2-}	36 000 (6600)a	30 000 (5900)a	3100 (770)b	3500 (680)b
Metals ($mg\ L^{-1}$)	Na	7.5 (1.5)a	7.1 (1.7)a	9.0 (3.0)a	8.0 (3.1)a
	Mg	3800 (850)a	3300 (770)a	600 (460)b	660 (230)b
	K	0.5 (0.3)a	28 (8.9)b	1.5 (1.0)c	3.8 (1.5)d
	Ca	71 (45)b	170 (14)c	460 (40)a	500 (33)a
	Cu	16 (1.1)a	13 (2.0)b	<0.1	<0.1
	Zn	3100 (1200)a	2500 (640)a	1.1 (0.6)b	4.6 (4.0)c
	Cd	17 (3.0)a	13 (2.2)b	<0.1	<0.1
	Pb	4.5 (0.2)a	4.3 (0.3)a	<0.1	<0.1
Metalloid ($mg\ L^{-1}$)	As	5.0 (0.8)a	4.3 (0.7)a	<0.1	0.1 (<0.1)b

E.C. is Electrical Conductivity of soil solution. Numbers in brackets are standard deviation. Different letters indicates significant differences between different treatments ($P < 0.05$) after LSD test.

Table 3
Biomass and growth parameters of plants grown from seeds and rhizomes in the growth chamber and from plants sampled in the field

Plants from	Treatment	Weight per plant (g)	Leaves/stalk
Seeds	No fertilizer No $CaCO_3$	0.09 (0.02)	1.4 (0.6)a
	Fertilizer No $CaCO_3$	0.11 (0.02)	1.4 (0.7)a
	No fertilizer $CaCO_3$	0.19 (0.04)	3.2 (0.4)b
	Fertilizer $CaCO_3$	0.26 (0.05)	2.6 (1.1)c
Rhizomes	Fertilizer No $CaCO_3$	1.4 (0.63)	–
	Fertilizer $CaCO_3$	1.8 (0.54)	–
Field	–	47 (15)	–

Values in brackets are standard deviation. Blank cells mean not available data. Different letters indicates significant differences between different treatments ($P < 0.05$) after LSD test.

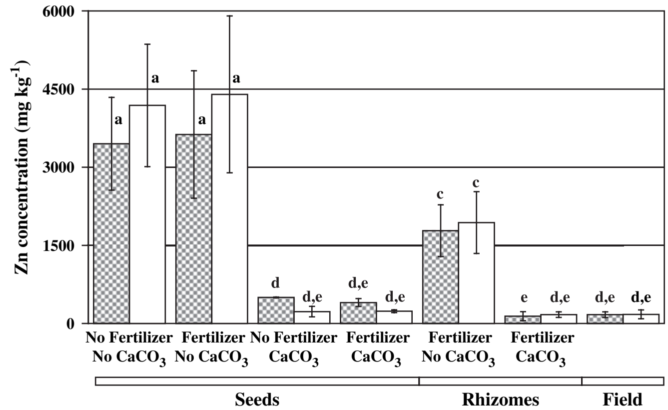

Fig. 2. Zn accumulation in roots (filled columns) and shoots (white columns) of plants grown from seeds ($n = 3$) or rhizomes ($n = 4$) under different treatments and of plants collected in the field ($n = 5$). Black bars on columns are standard deviation. Different letters indicates significant differences ($P < 0.05$) after LSD test.

number of leaves per stalk was the same for fertilized and non-fertilized soils. Chlorosis affected more than 75% of the leaves of the plants in both these treatments and the leaves with less than 5% chlorosis represented less than 10%.

Except for Pb and As, the lime treatment significantly decreased the metal accumulation of shoots and roots (Figs. 1–5). Zn concentrations (Fig. 2) reached 4200 mg kg^{-1} in shoots and 3500–3600 mg kg^{-1} in roots in treatments without lime addition. When lime was added, the concentrations decreased by more than 80% in roots and by 90% in shoots.

Cadmium behaved similarly to Zn (Fig. 3), except that the concentrations were much lower. The plants from unlimed treatments accumulated around 20–25 mg kg^{-1} in shoots and roots. These values decreased by more than 80% with the lime amendment.

The treatment effects on Pb (Fig. 4) and As (Fig. 5) accumulation by roots were different from those on Cu, Zn, and Cd. Liming increased Pb concentrations in roots from 175 mg kg^{-1} in untreated pots to 273 mg kg^{-1} but these differences were not significant. Fertilization alone and liming in combination with fertilization had no significant effect. The increase in Pb accumulation by roots upon addition of lime did not correspond with an increase in the Pb concentration in soil solution, which remained below 0.1 mg L^{-1}. In contrast to the effect on Pb accumulation by roots, shoot uptake of Pb responded in the same way to liming as in the case of the other metals.

The behavior of As was similar to Pb, but the concentrations were lower. Shoot concentrations were around 6–8 mg kg^{-1} of plants that grew in the absence of lime. When lime was added, the concentration decreased to 2 mg kg^{-1}. In roots, the concentration was 26 mg kg^{-1} for the untreated pots with and without fertilizer. However, adding lime increased the As in roots to 32 mg kg^{-1} in the presence of fertilizer and to 48 mg kg^{-1} in absence of fertilizer.

3.3. Plants grown from rhizomes

In general, Zn, Cu, and Cd concentrations in plants grown from rhizomes were lower than in the plants that were grown

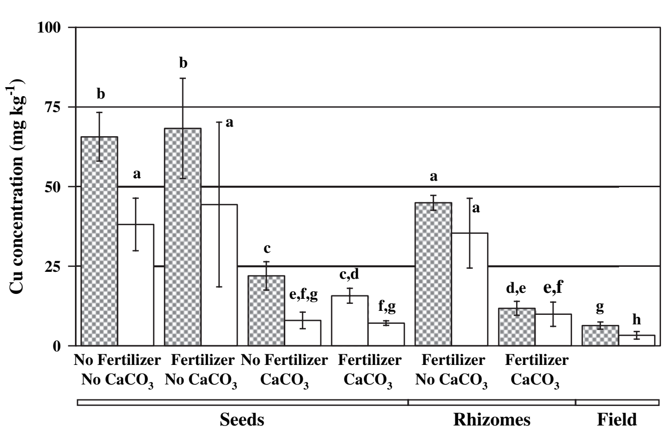

Fig. 1. Cu accumulation in roots (filled columns) and shoots (white columns) of plants grown from seeds ($n = 3$) or rhizomes ($n = 4$) under different treatments and of plants collected in the field ($n = 5$). Black bars on columns are standard deviation. Different letters indicates significant differences ($P < 0.05$) after LSD test.

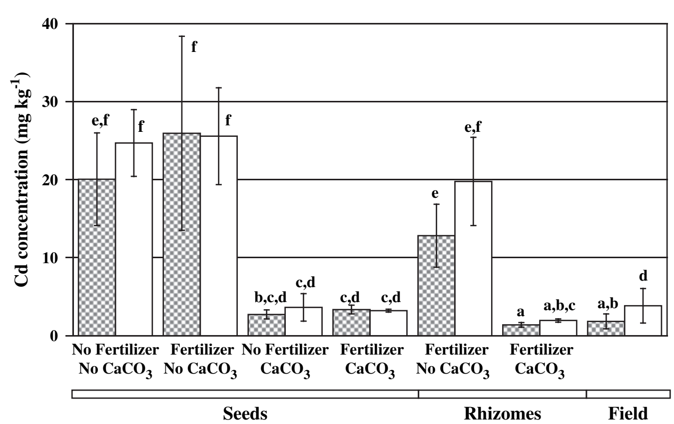

Fig. 3. Cd accumulation in roots (filled columns) and shoots (white columns) of plants grown from seeds ($n = 3$) or rhizomes ($n = 4$) under different treatments and of plants collected in the field ($n = 5$). Black bars on columns are standard deviation. Different letters indicates significant differences ($P < 0.05$) after LSD test.

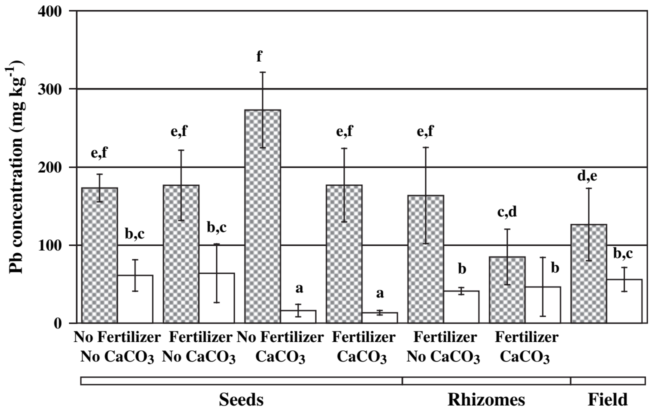

Fig. 4. Pb accumulation in roots (filled columns) and shoots (white columns) of plants grown from seeds ($n = 3$) or rhizomes ($n = 4$) under different treatments and of plants collected in the field ($n = 5$). Black bars on columns are standard deviation. Different letters indicates significant differences ($P < 0.05$) after LSD test.

from seeds for the same treatments (Figs. 1–3), although in most cases these differences were not significant. For Zn the decrease was approximately 50% and significant for the fertilizer and non-limed treatments. For Pb (Fig. 4) and As (Fig. 5) this effect only occurred in roots with the lime treatment. In contrast, shoot uptake of Pb and As was higher in rhizome than in seed-grown plants for the limed pots. Without liming, Pb and As contents were similar in relation to the same treatments in seed-grown plants.

3.4. Plants collected in the field

Plant samples from the field had metal concentrations in the same range as the plants grown from rhizomes and seeds in soils treated with lime, despite the fact that the wild plants were growing on untreated soil. Cadmium concentrations were higher in shoots of wild plants than in the roots. The Zn concentration averaged 174 mg kg^{-1} in the shoots. Cu,

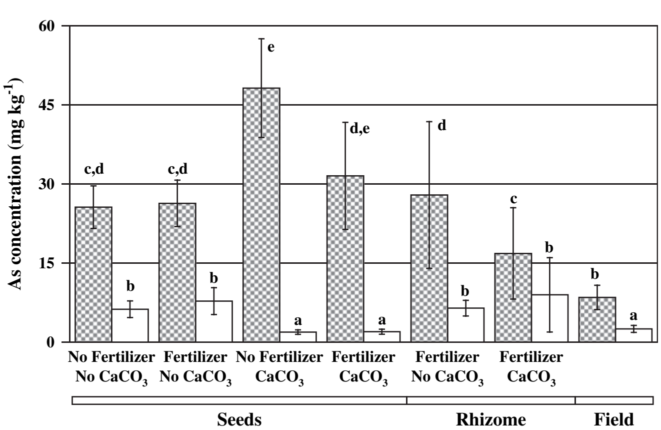

Fig. 5. As accumulation in roots (filled columns) and shoots (white columns) of plants grown from seeds ($n = 3$) or rhizomes ($n = 4$) under different treatments and of plants collected in the field ($n = 5$). Black bars on columns are standard deviation. Different letters indicates significant differences ($P < 0.05$) after LSD test.

Table 4
Ratios between shoot metal concentrations in plants from pots and from the field

	Treatment	Cu	Zn	As	Cd	Pb
Seeds	No fertilizer No $CaCO_3$	12	24	2.5	13	1.1
	Fertilizer No $CaCO_3$	14	25	3.1	13	1.1
	No fertilizer $CaCO_3$	2.4	1.3	0.8	1.9	0.3
	Fertilizer $CaCO_3$	2.2	1.3	0.8	1.6	0.2
Rhizomes	Fertilizer No $CaCO_3$	11	11	2.6	10	0.7
	Fertilizer $CaCO_3$	3.0	1.0	3.6	1.0	0.8

Values larger than 1 indicate higher metal uptake in pots compared to the field.

Pb and As were accumulated mainly in the roots. Arsenic and Cu levels were both below 10 mg kg^{-1}. Pb concentrations were around 100 mg kg^{-1} in roots.

3.5. Comparison metal accumulation field-growth chamber

The ratio of the metal concentration in the plants from the growth chamber experiment compared to those collected in the field (Table 4) enables us to observe the effects of the controlled growth conditions on metal uptake. Ratios near one indicate that there was no effect of experimental conditions on metal uptake. The highest ratios occurred for Zn and plants grown from seeds in the untreated soils accumulated 20 times more Zn than those collected from the field. Cadmium and Cu uptake was significantly higher in the non-limed treatments (ratios around 10–14) than in the limed pots (0.8–3.0). Lead and As had the lowest ratios (between 0.2–3.6).

4. Discussion

4.1. Soil solution

The soil solution is in direct contact to the roots and therefore one of the most important soil compartments to investigate. SO_4^{2-} concentrations in soil solution were higher than 30 meq L^{-1} (1440 mg L^{-1}), a value considered detrimental to plant growth (Alarcón-Vera, 2004); however Cl^- values were lower than 5 meq L^{-1} (177 mg L^{-1}), a value considered unlikely to affect plant growth. The high Zn concentrations in the unlimed soils (>2000 mg L^{-1}) are clearly phytotoxic to most plant species. Based on these results we expected phytotoxicity on the unlimed soils, due primarily to the high concentrations of Zn and SO_4^{2-}, and little or no phytotoxicity in the treatments with addition of lime.

Although the tailings had low calcium content, the dissolution of the added $CaCO_3$ increased the Ca^{2+} content of the solution, and gypsum was largely oversaturated under these conditions. SO_4^{2-} was therefore precipitated in the limed treatments and the phytotoxicity was reduced.

The K: Mg and Ca: Mg ratios are important indicators for plant growth. Ratios of available Ca: Mg below 1 meq meq^{-1} and K: Mg below 0.2 meq meq^{-1} inhibit plant growth (Alarcón-Vera, 2004). This was the case in all the pots in our experiment. The Ca:Mg ratio was an order of magnitude lower in the un-limed compared to limed soil. The K:Mg ratios were below 0.2 meq meq^{-1} in all treatments.

The beneficial effect of lime addition was therefore due to: i) the increased pH resulting in a decrease in the solubility of metals; and ii) the precipitation of SO_4^{2-} as gypsum. The addition of Ca also increased the Ca: Mg ratio. However, this ratio was still below one, far from the ideal ratio of 5 (Alarcón-Vera, 2004).

4.2. Plant growth

The toxicity symptoms in the untreated soils without lime resulted from the combination of low pH, high SO_4^{2-} and metal concentrations, especially Zn. All three parameters affect the plant growth directly or indirectly. The effect of protons (H^+) is mainly indirect by increasing salinity and the release of toxic metals (Salisbury and Ross, 1992). SO_4^{2-} limits Ca uptake by plants more than having a direct toxic effect. It increases the uptake of Na and K and reduces the water potential of the soil solution (Alarcón-Vera, 2004). Cl^- and NO_3^- weakly sorbed to the soil matrix, and the retention of SO_4^{2-} is also limited. These anions are readily available for roots uptake. High concentrations of metals in soil solution reduce plant growth by a variety of phytotoxic effects (Tordoff et al., 2000; Ye et al., 2000; Shu et al., 2001; Wong, 2003).

Fertilizer did not improve the establishment of *Lygeum spartum* in this soil. This indicates that the availability of the applied nutrients was not a limiting factor for this plant during our experiment. Nevertheless, fertilization may be beneficial for vigorous growth over longer periods.

Lygeum spartum is special compared to most other plants because its growth was only reduced in the untreated soils whereas most plant species would not be able to survive such harsh conditions of acidity, salinity and metal content. It is therefore a good candidate for phytostabilization of acid mine tailings, even without soil amendments.

4.3. Comparison of metal accumulation between field and growth chamber

The chemical characteristics of the field soil were close to the soil properties in the untreated soil (Conesa et al., in press). Nonetheless, *Lygeum spartum* accumulated significantly more metals in the growth chamber than in the field. The bioavailability of the metals was therefore higher in the pots than in the field. Air-drying of soil is known to modify the distribution of metal fractions (Nowack et al., 2004) resulting in an increase in the water soluble and exchangeable fractions (Wang et al., 2002). In the growth chamber the plants experienced optimal growing conditions. Mine tailings usually consist of fine-grained materials (<2 mm) (Tordoff et al., 2000), are dense and have compact layers. This results in poor water retention but also in restricted access of roots to the soil matrix (Ernst, 1996). The soil used in the pots was sieved and coarse fragments were broken up. The homogeneous mixture resulting from this preparation may have increased the contact between roots, soil water and the soil matrix. The breaking of aggregates also increased the reactive surface, enhancing oxidation processes that can facilitate the acidification of the soil.

The plants in the field had a higher biomass and were older than the plants in the pot experiment. Plants may be more vulnerable to heavy metals in the early stages of their life cycle (Cheng, 2003). Lower metal concentrations in older plants can also be explained by the phytodilution process (Robinson et al., 1998). This effect may have been a reason for the lower metal accumulation observed in the plants grown from rhizomes. The starting biomass was higher in the plants grown from rhizomes, as they were already older than the plants grown from seeds, when the experiment began.

Differences in root exudates or other rhizosphere processes may have contributed to the lower accumulation in the field. Root exudates can modify the soil properties around the roots (Wang et al., 2002). Adult plants in the field do not only have a more developed root system and have more time to modify the rhizosphere, they can also invest more assimilates into exudation than rhizomes and seedlings.

Nevertheless, seedlings must be able to survive the hostile site conditions when they colonize the tailings. There are at least three possible ways: (i) seedlings may slowly modify soil properties by means root exudates; (ii) they gradually invade the soil from areas where soil conditions are less aggressive for initial plant establishment (better water retention, no compacted layers, etc…) and successively "ameliorate" the newly colonized soil; and (iii) the plant roots grow selectively into the soil, taking advantage of the high degree of heterogeneity in the distribution of metals, i.e. by avoiding the most contaminated parts of the mine tailings. In contrast, in the pot experiments there was only a short time available, seedlings had no "support" from already established plants and the soil was homogeneous.

Such long-term growth strategies limit the usefulness of pot experiments to determine optimal amendments and growth conditions for plants growing naturally on mine tailings in arid regions. In particular, liming may not have the same effect on plant growth and metal accumulation in the field. Liming is often considered necessary in these soils to supply better conditions to plant establishment. This is a common recommendation prior to the revegetation of acidic mine wastes (Monterroso et al., 1998; Tordoff et al., 2000; Shu et al., 2001).

4.4. Bioconcentration and bioaccumulation factor of *Lygeum spartum*

Plant metal accumulation is often expressed as a bioconcentration factor (BF). It is calculated as the dry plant-tissue/soil metal concentration quotient (Mattina et al., 2003). In our study, the BF for all metals was below 1 with the exception of the Cd in the untreated soils, where it had a value of 2. Apart from Cd, only Zn accumulation reached values higher than 0.2. McGrath and Zhao (2003) considered <0.2 normal for plants growing on polluted materials. In the field plants, the BF was 0.4 for Cd accumulation by shoots and in all other cases below 0.2. This is consistent with an exclusion strategy for metal tolerance (McGrath et al., 2001).

Analogous to the BF, the accumulation factor (AF) indicates the efficacy of metal translocation from roots into shoots (Fitz and Wenzel, 2002). Also called translocation factor (Mattina et al., 2003), it is the shoot/root metal concentration quotient. Excluder plants have AF values $\ll 1$, whereas for hyperaccumulators it is $\gg 1$. In our study, the accumulation factor for Zn and Cd reached values between 1 and 2 for plants from the field and pot plants in the untreated soils. In all other cases, the values of the accumulation factor were below one.

The reduction in soil solution concentration resulted in a significant decrease in shoot uptake of all metals and root uptake of Cu, Zn, and Cd. However, the soil solution concentration does not seem a good parameter to explain the increase of Pb and As uptake in roots in the limed soil since the levels of soluble metals extracted with suction cups are lower than in not limed pots. We did not distinguish between adsorption onto the root surface and uptake. Adsorption of Pb is strongly pH dependent and we would expect much more adsorption at pH 5.5 compared to pH 3.

5. Conclusions

Lygeum spartum took up inordinate amounts of metals when raised in the growth chamber under controlled conditions. Lime increased soil pH and decreased soluble metals. This significantly reduced metal uptake. Growth under controlled conditions resulted in significantly higher metal uptake compared to plants sampled in the field. This may be attributed to homogenization of the soil and the free availability of water for a plant that normally grows in a semi-arid climate. Both factors may increase the availability and accessibility of soil metals for uptake by roots.

Further studies could focus on elucidating survival strategies of these plants *in situ*. An investigation is warranted into how roots behave in mine tailings where the contaminants are heterogeneously distributed.

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