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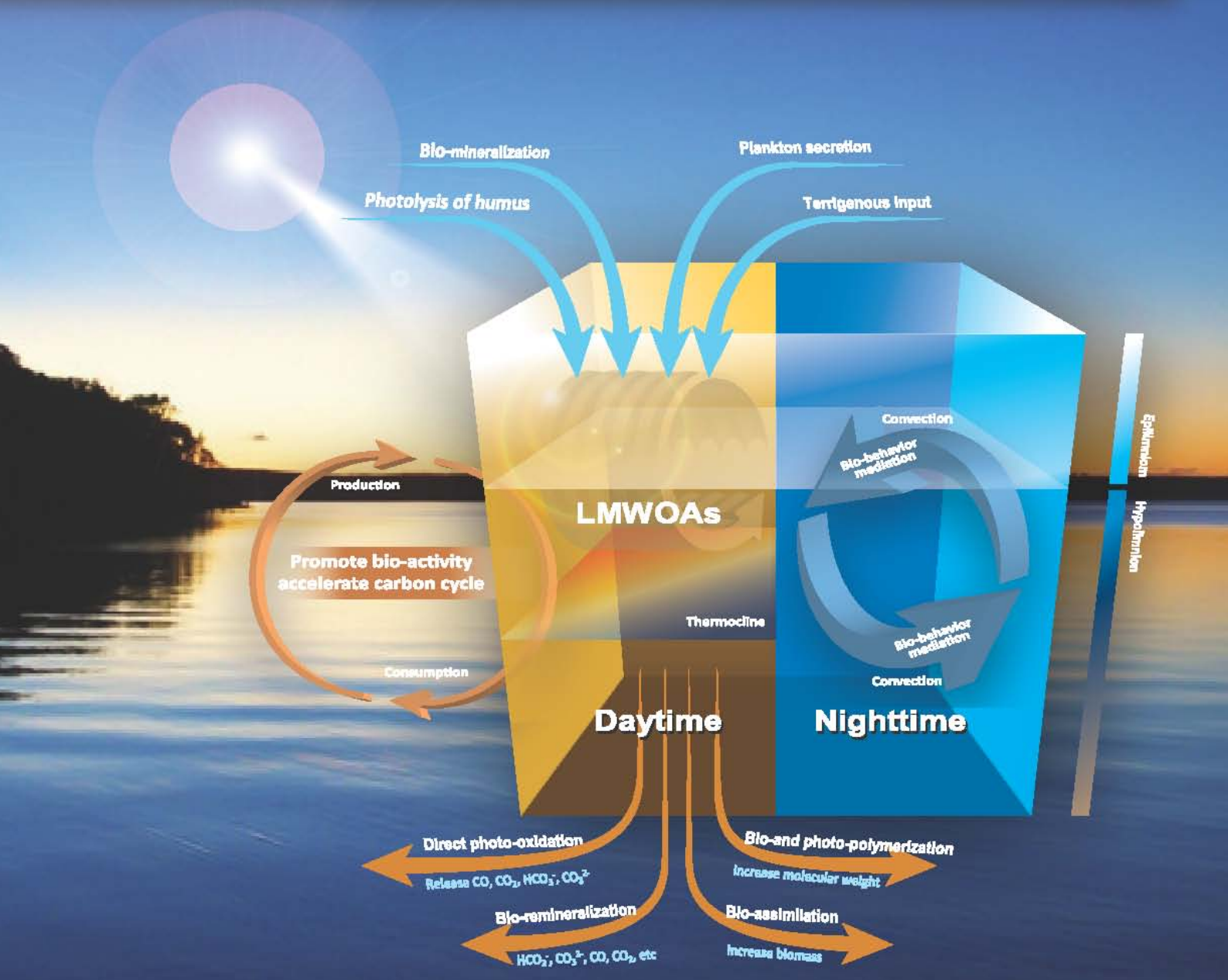

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Tolerance of *Chrysantemum maximum* to heavy metals: The potential for its use in the revegetation of tailings heaps

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Abstract

To find if ornamental plants are applicable to the remediation of metal-polluted areas, the tolerance of chrysanthemum plants (*Chysanthemum maximum*) var. Shasta to different metals under hydroponic conditions was studied. Their responses as influenced by the mycorrhizal fungus *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe BEG25 on substrates containing mine residues were also investigated. Our results showed that chrysanthemum is a metal-tolerant plant under hydroponic conditions, plants behaving as Pb-excluders, whereas Cd, Cu and Ni were accumulated in roots. Low accumulation in flowers was observed for Cd and Cu but it was concentration-dependent. Ni and Pb were not translocated to flowers. Shoot biomass was not significantly affected by the different rates of mine residue addition for both mycorrhizal and non-mycorrhizal plants. Mycorrhizal plants accumulated less Pb and Cu in both shoots and roots than non-mycorrhizal plants. Chysanthemum could be a prospective plant for revegetation of tailings and the use of inoculation may decrease plant metal accumulation in polluted soils.

Key words: mine residues; ornamental plants; phytostabilization; urban remediation

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Introduction

Amongst the soil contaminants, metals are the most prevalent forms in the environment, and their remediation in soils and sediments is a difficult task (Cunningham et al., 1997). Mining activities leave behind huge amounts of waste and tailings which are often very unstable and become sources of environmental pollution (Wong, 2003). These residues contain a wide range of pollutants, depending on the mineral composition of the ores mined (Müller et al., 2009) and the extraction and processing procedures used during mining activities. Due to a high content of heavy metals and low nutrient concentrations in most abandoned tailings heaps, edaphic conditions are stressful and hence tailings are almost vegetation-free. In order to avoid dispersion and environmental health risks to different organisms, remediation must be followed at sites and for surrounding soils. Phytoremediation, a biological approach, has received substantial attention in recent years and has been shown to be cost-effective and a 'greener' technique than conventional physico-chemical alternatives to remediate metal-polluted soils. Due to the increased contamination of urban areas, more and more attention has also been paid to the role of ornamental plants in phytoremediation (Liu et al., 2008; Panizza et al., 2011). They can be grown in polluted areas, tolerate metals toxicity and so reduce their dispersion. In addition, they will visually embellish the environment of metal-impacted areas (Rodríguez-Elizalde et al., 2010) and, as many of them are not edible plants, the risk of entering metals into the human food chain is reduced. There are several reports showing that ornamental plants are able to grow in polluted areas (Prasad and Freitas, 2003; Díaz-Garduño et al., 2005; González and González-Chávez, 2006; González-Chávez et al., 2009) which provides a substantial base for suggesting their use in phytoremediation. However, there is still little research on the practical use of ornamental plants in the remediation of metal-polluted areas.

Chrysanthemum maximum is a very abundant ornamental resource; it is also an important ornamental plant in the world. This plant was therefore selected in this study. Moreover, chrysanthemum grows in polluted areas surrounding cities (Saxena et al., 1999) and close relatives, such as *C. coronarium* or *C. sagetum* have been reported as plants able to grow in polluted soils (de Haro et al., 2000). This plant possesses key traits that make it very attractive

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to use in phytoremediation of metal-polluted sites, e.g., ease of cultivation, rapid growth, abundant flowering, large foliar cover, and a short life cycle (Hamrick, 2003).

Arbuscular mycorrhizal (AM) fungi are commonly colonizing more than 80% of the roots of plant species in all soil systems, conferring multiple benefits (Smith and Read, 2008). In heavy-metal-polluted soils many plant species adapted to grow on these sites are colonized by AM fungi, possibly playing a central role in alleviating the plant toxicity of these pollutants (González-Chávez et al., 2009, 2011; Weissenhorn and Leyval, 1995; Hildebrandt et al., 2007; Ortega-Larrocea et al., 2010). Some authors have suggested that AM fungi are the key factor in soil development and successful plant establishment in these contaminated sites (González-Chávez et al., 2009; Khan et al., 2000; Shetty et al., 1994). Hence, an understanding of the participation of AM fungi in tolerance to extreme soil conditions is basic for improving the management of mycorrhizal plants in soils polluted with metal-mine residues. With this perspective, the aims of this present research were: (1) to study tolerance of a suitable chrysanthemum cultivar to heavy metals; (2) to test its ability to grow in soil mixtures containing different proportions of metal-mine residues; (3) to evaluate the effect of an AM fungal strain in metal-polluted soil conditions.

1 Materials and methods

Two experiments were conducted using *Chrysanthemum maximum* var. Shasta. In the first, plant metal tolerance under hydroponic conditions was studied. In the second, the effect of inoculation with the AM fungus *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe) BEG25 on the amelioration of heavy metal contamination was investigated using different amounts of mine residues in the substrate.

1.1 Metal tolerance in chrysanthemum plants

Chrysanthemum plants were grown in perlite and exposed to different concentrations of several heavy metals (Cd, Cu, Ni and Pb). Identically-sized (5 cm height) chrysanthemum plants were used. Plants were grown in 1 L pots containing 450 g perlite as the growth medium. A single plant was transplanted into each pot. Plants were grown for 15 days receiving an addition of Hoagland′s nutrient solution (Millner and Kitt, 1992) ca. 80% of the field capacity. After this time and until the end of the experiment at 60 days, plants were watered with Hoagland′s nutrient solution containing one of the four metal concentrations tested: Cd, Cu and Ni (0.5, 1, 2.5, 5, 10 mg/L) and Pb (6, 12.5, 25, 50, 100 mg/L). Control plants were established under the same conditions receiving only Hoagland′s nutrient solution. The experiment was carried out in a glasshouse (average daily temperature 25°C and photoperiod 12 hr).

1.2 Metal tolerance index and translocation factor

A metal tolerance index was calculated as the quotient of the dry weight of plants grown under metal-enriched conditions divided by the dry weight of plants grown under non-metal condition (control), according to Murphy and Tayz (1995). The translocation factor for metals within a plant was calculated by the quotient of metal concentration in shoot tissue/metal concentration in root tissue, according to Stoltz and Greger (2002). Translocation factors < 0.99 show that a metal is retained in roots, whilst translocation factors > 0.99 indicate that a metal is accumulated in shoots.

1.3 Metal concentrations in plants

Plants were harvested and carefully rinsed with tap water, then with deionized water. The tissues were oven dried for 72 hr at 45°C, then ground to a fine powder. Plant biomass dry weights were recorded. Inflorescences, shoots and roots were separated at this time and analyzed. All plant material was wet digested using hydrogen peroxide and a mixture of sulfuric and perchloric acids (4:1, *V/V*) (Walinga et al., 1995). Metal concentrations were determined by atomic absorption spectrometry (3110 Perkin Elmer, USA).

1.4 Plant – *Glomus mosseae* BEG25 interaction

To allow the stabilization of adsorption-desorption reactions, the mixtures soil/tailings were incubated for one month at 80% of field water capacity before planting chrysanthemum. Tailings and soil were characterized (pH, organic matter, available P, particle size) following the procedures described in Rowell (1994) and total concentrations of metals (Cd, Cu, Ni, Mn, Pb and Zn) were analyzed after *aqua regia* digestion (British Standard, 1995) (**Table 1**). In addition, "extractable" metal concentrations (**Table 2**) were measured after one month of incubation, using the procedure proposed by Lindsay and Norvell (1978).

Table 1 Physico-chemical properties and total metal concentrations of soil and mine residues

	Soil	Tailing
pH water	6.85 ± 0.1	6.25 ± 0.8
pH $CaCl_2$	6.90 ± 0.1	6.43 ± 0.7
OM (%)	0.75 ± 0.12	0.38 ± 0.1
Clay (< 2 μm) (%)	24 ± 1	1 ± 1
Silt (2–200 μm) (%)	38 ± 1	7 ± 1
Sand (> 200 μm) (%)	38 ± 1	92 ± 1
P (Olsen) (mg/kg)	272 ± 12	1221 ± 846
Total metal (mg/kg)		
Cd	5.7 ± 1	24 ± 11
Ni	53 ± 4	22 ± 6
Pb	102 ± 10	1217 ± 465
Mn	306 ± 15	1078 ± 287
Cu	62 ± 1	172 ± 168
Zn	54 ± 5	1513 ± 586

Values are mean ± standard deviation, $n = 3$.

Table 2 Extractable metals with DTPA-$CaCl_2$-TEA solution from soil, tailing and soil-tailing mixtures

Element	Soil	Tailing	Soil-tailing mixtures with different percentage of mining wastes			
			5%	10%	20%	30%
Cd (mg/kg)	0.17 ± 0.01	3.6 ± 2.4	0.21 ± 0.06	0.43 ± 0.16	0.70 ± 0.02	1.40 ± 0.07
Ni (mg/kg)	0.87 ± 0.05	0.6 ± 0.2	0.29 ± 0.19	0.22 ± 0.15	1.05 ± 0.32	2.96 ± 0.36
Pb (mg/kg)	6.00 ± 0.70	143 ± 79	3.39 ± 0.3	4.23 ± 0.48	6.78 ± 1.50	4.09 ± 0.49
Mn (mg/kg)	3.75 ± 4.0	18 ± 2.5	3.72 ± 1.22	6.34 ± 1.22	12.00 ± 2.73	35.06 ± 10.89
Cu (mg/kg)	0.30 ± 0.2	13 ± 15	1.64 ± 0.40	1.76 ± 0.24	3.63 ± 0.41	3.98 ± 0.31
Zn (mg/kg)	1.30 ± 0.1	60 ± 63	3.73 ± 1.22	6.34 ± 1.22	20.08 ± 1.84	42.60 ± 3.20

Values are mean ± standard deviation, $n = 3$.

Identically-sized plants chrysanthemum (5 cm high) were transplanted into pots containing 250 g of a mixture of soil with mine residue containing high concentrations of different heavy metals, in proportions of 0%, 5%, 10%, 20% and 30%. In order to establish the mycorrhizal treatments, 30 g of inoculum of *Glomus mosseae* BEG25 was added into the hole where plants were transplanted. It contained >100 spores and sorghum roots with 80% of fungal colonization. Non-mycorrhizal treatments received the same amount of sterilized inoculum and were added in the same way.

Plants were grown for two months under glasshouse conditions. Plants were watered daily at ca. 80% of field capacity with water and once a week with Hoagland′s nutrient solution. Plant biomass dry weights, height, foliar area, leaf number per plant and fungal root colonization were recorded. Foliar area was quantified by image analysis (Image Tool for Windows version 3.0) after leaf digitalization (Scanner HP Scanjet G4050, resolution 300 dpi) and segmentation (GIMP program version 2.7.1 for Linux) according to Wilcox et al. (2002).

For fungal root colonization evaluation, a root subsample of each treatment and replicate were carefully washed with distilled water, cut into 1 cm segments and processed as follows: roots were cleared and stained with 0.05% Trypan Blue in 50% aqueous glycerol solution, according to Koske and Gemma (1989). One hundred stained root segments were mounted on slides and examined under a compound microscope. The frequency of colonization was estimated by rating the presence or absence of fungal structures in the stained root segments and expressed as a percentage. Plant tissue metal concentrations were determined as described earlier.

1.5 Experimental design and statistical analysis

The first experiment was established under a completely randomized design (with 6 replicates for each treatment) using four metals (Pb, Cd, Cu and Ni) at five concentrations added to the nutrient solution. The second experiment was also a completely randomized design with four replicates for each treatment, where inoculation (+ or –) and level of mine residue in the substrate were the treatments (0%, 10%, 15%, 20% or 30% of mine residue was used in the growth substrate).

All statistical analyses were performed using ANOVA analysis to identify significant treatment effects. When differences were observed, a Tukey's test was performed ($p < 0.05$). Linear regression was used to examine relationships between variables ($p < 0.05$).

2 Results

2.1 Metal tolerance in chrysanthemum plants

Metals had no effect on chrysanthemum plant survival under hydroponic conditions and all plants were alive at the end of the experiment. No plant showed damage caused by metal toxicity with any of the metals tested. Plant biomass production was increased by all metals; Pb increased 47%, Cd 52%, Cu 58% and Ni 60% (**Table 3**). Tolerance indices were thus in the range of 1.30–1.75, showing this stimulatory effect of the metals. All plants bloomed and no effect by metal concentrations was observed.

Table 3 Aerial biomass, tolerance index and translocation factor in Chrysanthemum plants grown in perlite and exposed to four different heavy metals

Treatment	Concentration (mg/L)	Dry weight* (g)	Tolerance Index	Translocation factor
Control		1.8 ± 0.3		
Pb	6	2.8 ± 0.3	1.54	0.23
	12.5	2.4 ± 0.5	1.30	0.22
	25	2.7 ± 0.7	1.48	0.16
	50	3.1 ± 0.7	1.70	0.19
	100	2.5 ± 0.6	1.37	0.20
Cd	0.5	2.8 ± 0.6	1.56	0.54
	1	2.5 ± 0.3	1.40	0.46
	2.5	3.0 ± 0.7	1.65	0.18
	5	2.8 ± 0.4	1.53	0.25
	10	2.7 ± 1.0	1.47	0.20
Cu	0.5	2.7 ± 0.3	1.51	0.18
	1	3.2 ± 0.7	1.75	0.16
	2.5	2.7 ± 0.8	1.49	0.11
	5	3.0 ± 0.5	1.67	0.12
	10	2.8 ± 0.6	1.53	0.23
Ni	0.5	3.2 ± 0.3	1.73	1.08
	1	2.9 ± 0.5	1.60	0.86
	2.5	2.9 ± 0.4	1.61	0.59
	5	2.6 ± 0.4	1.40	0.51
	10	3.1 ± 0.9	1.69	0.21

* Values are means and standard deviations ($n = 6$).

2.2 Metal translocation factors

In general, plants with increased concentrations of Ni presented lower translocation factors. However, no clear trend was observed at different concentrations of Pb, where translocation factors were in the range 0.16–0.23 (**Table 3**); plants tended to translocate lower Pb to their leaves. translocation factors for Cu decreased when plants grew at higher concentrations of this element, except at 10 mg/L, confirming that plants accumulated more Cu in roots than in leaves. The range of translocation factors for Cd was 0.18–0.54. The higher translocation factors for Cd (0.54 and 0.46) were observed at the lowest Cd concentrations (0.5 and 1 mg/L, respectively). However, translocation factors decreased when Cd concentrations increased, showing that at higher concentrations plants restricted more shoot Cd accumulation.

2.3 Plant metal concentrations under hydroponic conditions

Metals were accumulated in the order roots > shoots ≫ flowers. Chrysanthemum behaved as a Pb excluder plant (**Fig. 1A**), as concentrations in either shoots or roots were very similar in all treatments (16–22 and 84–93 mg/kg, respectively). Roots had 4.2–5.9 folds higher Pb accumulation than shoots. Pb was not translocated to flowers.

Cadmium accumulation depended on Cd concentrations in the nutrient solution. Higher Cd accumulation was observed in roots (1.9–5.7 folds) than in shoots (**Fig. 1B**). Cd shoot and root concentrations significantly increased as Cd increased in the nutrient solution ($p < 0.001$), especially at the two highest Cd concentrations tested (5 and 10 mg/L). The highest Cd concentrations in shoots and roots were 64 and 320 mg/kg dry weight, respectively. Cd was translocated to flowers, the extent of translocation depended on Cd concentration in the nutrient solution ($p < 0.001$). The maximum Cd accumulation occurred at 5 and 10 mg/L Cd (2.8–3 mg/kg dry flower weight), whereas it was between 1.2–1.5 mg/kg dry weight when plants grown at 1–2.5 mg/L of Cd. At 0.5 mg/L, Cd flower concentration was 0.3 mg/kg dry weight.

Roots had 4.3–9 folds higher Cu accumulation than shoots. Cu concentration in shoots and roots increased as Cu was raised in the nutrient solution (**Fig. 1C**). At the highest level of Cu in the nutrient solution (10 mg/L), the maximum concentration of Cu in shoots was 74 mg/kg dry weight ($p < 0.001$) and in roots 324 mg/kg dry weight ($p < 0.001$). Cu was not strongly translocated to flowers (7.3–8.5 mg/kg dry weight). Cu translocation to flowers was similar over all the range of the Cu tested concentrations.

Chrysanthemum plants did not present Ni toxicity symptoms and behaved as a Ni-excluder; concentrations in shoots were not > 38 mg/kg dry weight and were independent of the concentration of Ni in the nutrient solution (**Fig. 1D**). Roots had ≤ 5 folds higher Ni accumulation

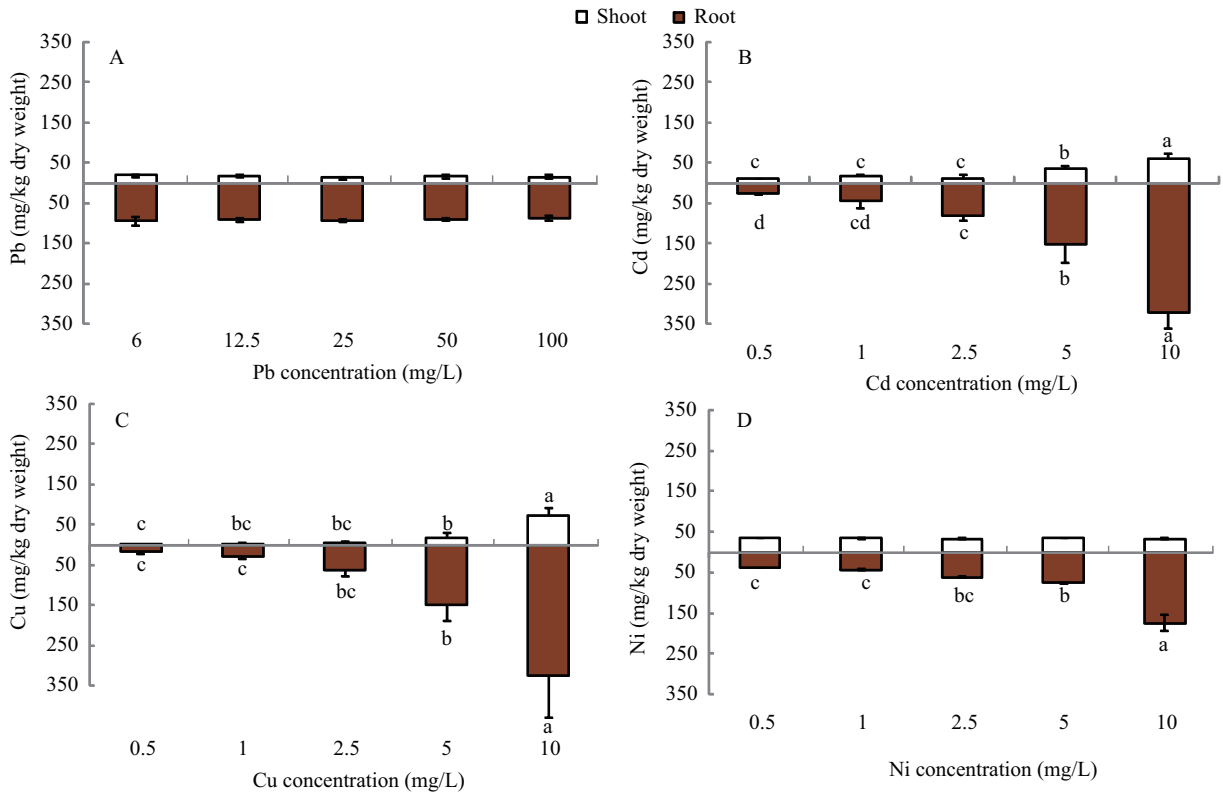

Fig. 1 Concentrations of Pb (A), Cd (B), Cu (C) and Ni (D) in shoots and roots of plants grown at five concentrations added to the nutrient solution. The bars indicate the standard error ($n = 6$). Different letters indicate different metal concentrations in dry biomass ($p < 0.001$) in each concentration tested according to a metal tested. Where no letters are indicated, no significant differences were observed.

than shoots. Significant differences ($p < 0.001$) in Ni root accumulation were due to Ni concentration in the growth medium. The maximum Ni root accumulation (172 mg/kg dry weight) occurred at the highest Ni concentration (10 mg/L).

2.4 Plant-*Glomus mosseae* BEG25 interaction

Shoot biomass was not significantly affected by the different levels of mine residue in mycorrhizal and non-mycorrhizal plant treatments (**Fig. 2**), but root biomass was negatively affected by mine residue addition ($p < 0.001$). The highest root biomass was observed with no addition of mine residue either in mycorrhizal or non-mycorrhizal plants, and the lowest (3 folds less) was obtained in non-mycorrhizal plants growing with 30% of mine residues.

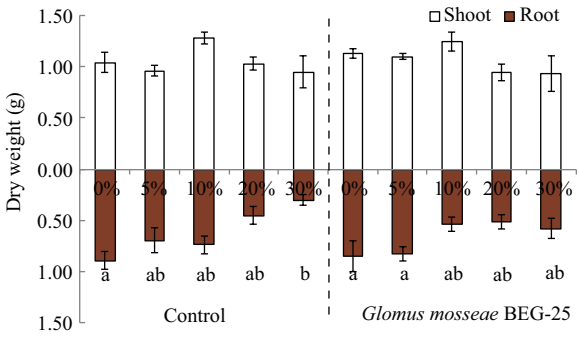

Fig. 2 Dry weight of mycorrhizal and non-mycorrhizal plants exposed to substrates containing different concentrations of mine residues (0%–30%). The bars indicate the standard error ($n = 4$). Letters indicate significant difference in dry weight ($p < 0.001$). Where no letters are indicated, no significant differences were observed.

Foliar area was significantly affected by the level of mine residues in the plant growth substratum. The highest foliar area was observed with 10% of mine residues, whilst the lowest in treatments with 30% of these residues. No significant differences were detected between mycorrhizal and non-mycorrhizal plants (data not shown). Leaf number was higher in mycorrhizal plants than non-mycorrhizal plants in the control treatment. However, there was no difference in other treatments (data not shown). There was a strong correlation between shoot weight and foliar area ($r = 0.73$).

2.5 Metal accumulation in plants growing in mine residues

Metal concentrations in plants followed the trend of the concentration of the available metals in the system which increased as the percentage of tailings in the mixture increased (**Tables 2** and **3**). Copper accumulated more in chrysanthemum roots than in shoots. However, Cu accumulated less in these organs of mycorrhizal plants ($p < 0.001$) than the non-mycorrhizal (**Fig. 3A**). In mycorrhizal

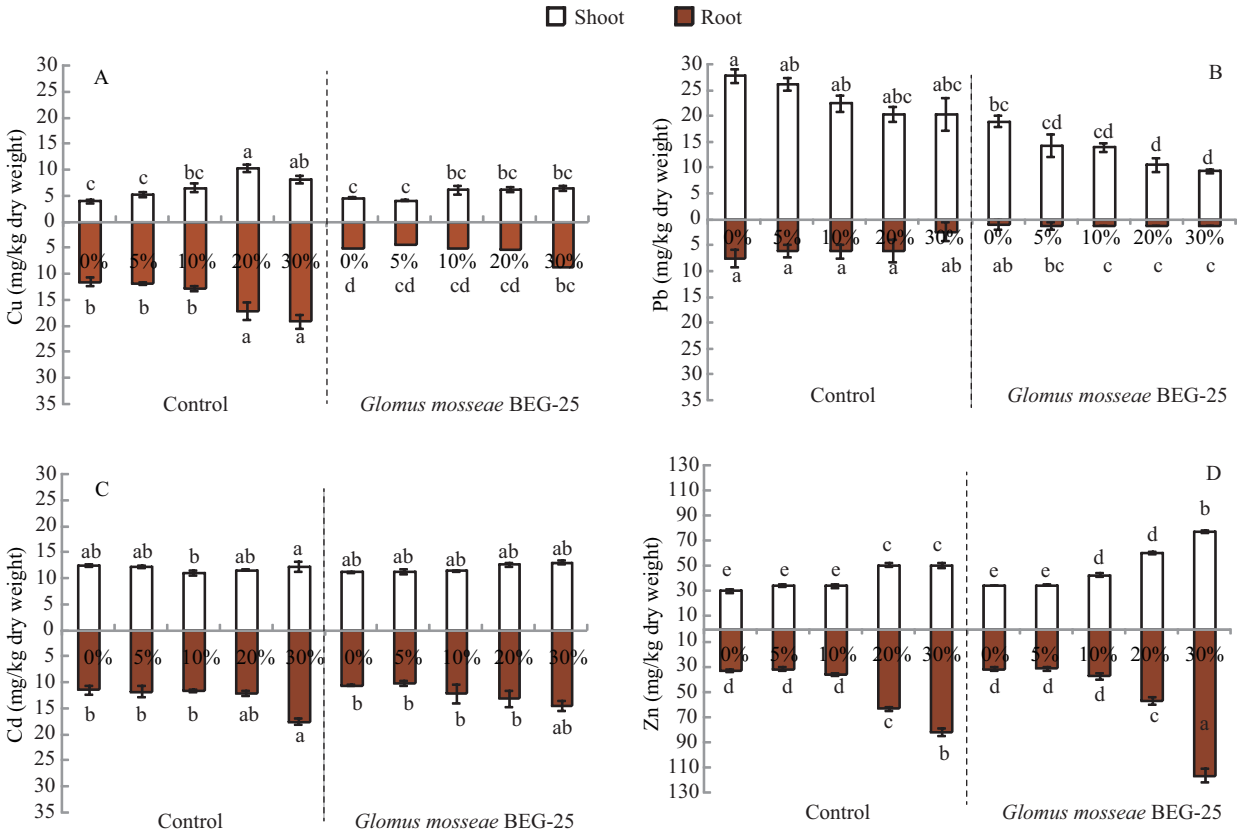

Fig. 3 Concentrations of Cu (A), Pb (B), Cd (C) and Zn (D) in shoots and roots of mycorrhizal and non-mycorrhizal plants exposed to substrates containing different concentrations of mine residues (0%–30%). The bars indicate the standard error ($n = 4$). Letters indicate significant differences ($p < 0.001$).

plants, growing in 20% and 30% mine residues, shoot Cu concentrations were 40%–20% lower than these of non-mycorrhizal plants, whilst between 39%–45% less Cu accumulated in roots of mycorrhizal plants.

Accumulation of Pb was higher in shoots than in roots of chrysanthemum plants, but it was much more restricted in mycorrhizal plants compared with non-mycorrhizal plants ($p < 0.001$) (**Fig. 3B**). Roots and shoots of mycorrhizal plants accumulated on average 80% and 30%, respectively, less Pb than non-mycorrhizal plants. Our results showed that Pb was in high concentrations in the soil used to dilute the mine residues. For this reason, Pb concentrations are high in plant tissues, but clearly show that mycorrhizal treatment depletes Pb accumulation by a factor of two in both shoots and roots.

Cadmium was similarly accumulated in shoots and roots of chrysanthemum plants (**Fig. 3C**). Significant differences in shoot and root Cd accumulation were observed between treatments (**Fig. 3C**). The highest root Cd concentrations were observed in plants grown in 30% mine residues. No differences were observed between mycorrhizal and non-mycorrhizal plants.

Zn accumulation was similar between shoots and roots of chrysanthemum plants, except at the highest concentration of mine residues (30%). Roots of mycorrhizal and non-mycorrhizal plants accumulated 49% and 60%, respectively, more Zn than the shoots of these plants. In contrast to the other metals, mycorrhizal plants grown at 30% mine residues accumulated higher Zn concentrations in shoots (53%) and roots (42%) than non-mycorrhizal plants (**Fig. 3D**).

No relationship was observed between shoot dry weight and concentrations of metals in shoots or roots. A negative correlation was only found between shoot dry weight vs. Cd root concentrations ($r = –0.41$). Lineal regressions showed that the chrysanthemum plant growth variables were negatively correlated with metal-extractable concentrations except for Pb-extractable very weak relationship was observed in the substrate (**Table 4**).

2.6 Mycorrhizal colonization

Frequency of mycorrhizal colonization in *G. mosseae* BEG25-inoculated treatments ranged from 30% to 42% and was not significantly influenced by percentage of mine residue added to the substrate, except at 30% mine residues, where mycorrhizal colonization was reduced to 8%. In non-inoculated treatments, mycorrhizal colonization was between16%–23% at the lower mine residue rates added (0% and 5%) but increased to 40% and 65% of colonization at the highest levels of residues (10% and 30%, respectively). As plant substrates were not sterile, it appears that the last higher expressed colonization is due to AM fungi native in soil or mine residues used in the plant substrates, rather than the inoculated *G. mosseae* BEG25. However, unlike the fungi present in the soil or mine residues, *G. mosseae* BEG25 was able to significantly decrease shoot metals (Pb and Cu) translocation and therefore shoot metal accumulation, which was not observed by the native AM fungi.

3 Discussion

Chrysanthemum plants were metal tolerant under hydroponic conditions. The different metals did not negatively affect plant survival, biomass production or flowering, on the contrary, metals improved growth. Copper and Ni are trace nutrients and so could enhance plant growth in small amounts, whereas Pb and Cd are toxic. High tolerance to Cd and plant growth promotion was also observed in *Calendula officinalis* (Liu et al., 2008). However, other studies show that this does not always happen. For example, flowering was highly metal sensitive in *Pelargonium hortorum* (Orroño and Lavado, 2009). In general, plants had low metal translocation factors under hydroponic conditions and these values were in a narrower range to those reported by Leung et al. (2007) for Pb, Ni and Cu, but were similar for Cd. The narrower translocation factors range the lower metal translocation, then more restriction to Cu shoot translocation was observed than for Ni or Cd.

Several times higher metal concentrations were detected in roots than in shoots; which accords with the data of Cataldo et al. (1978), who reported, for instance, >50% of Ni absorbed by plants was retained in roots. Copper concentrations in shoots and roots increased as Cu concentration were increased in the nutrient solution. At 5 and 10 mg Cu/L shoots and roots accumulated more Cu; which is toxic for some plants (10–20 mg/kg) (Vamerali et al., 2010). In relation to Cd, in all concentrations tested, Cd shoot and root concentrations were greater than critical plant concentrations (5–10 mg/kg) (Vamerali et al., 2010),

Table 4 Linear regressions (coefficient r, $p < 0.05$) between plant growth variables and DTPA-$CaCl_2$-TE-extractable soil metal concentrations in the soil-tailing mixtures

Plant variable	Treatment	Extractable metal concentrations			
		Cu	Pb	Cd	Zn
Shoot dry weight	Control	–0.30	0.02	–0.31	–0.41
	G. mosseae BEG25	–0.78	–0.29	–0.71	–0.78
Root dry weight	Control	–0.98	0.03	–0.92	–0.94
	G. mosseae BEG25	–0.75	–0.17	–0.62	–0.56
Foliar area	Control	–0.66	0.12	–0.55	–0.64
	G. mosseae BEG25	–0.57	0.17	–0.63	–0.71

but not toxic symptoms were observed.

Lead shoot concentrations were above the toxicity threshold in plant tissues (10–20 mg/kg) (Vamerali et al., 2010), but concentrations did not increase as available Pb augmented in the nutrient solution. Therefore, chrysanthemum may be considered a Pb excluder plant. The same condition may be applied for Ni, shoot concentrations were <50 mg/kg; which can be toxic in Ni-sensitive and moderately tolerant species, respectively (Kozlow, 2005; Chen et al., 2009).

On the other hand, metal tolerance of chrysanthemum plants was also observed when they were grown in soil-tailing mixture containing high concentrations of available metals. Contrary to Orroño et al. (2009), shoot biomass was unaffected by metal presence. These authors observed that aerial biomass was more affected by heavy metals than roots of *C. morifolium* plants. However, these authors used cation-metal-spiked soil instead of soil polluted with metal mine residues, which may impede direct comparisons. The source of heavy metals may have strong influences on AM fungus and affect symbiosis. Cations are generally the most toxic inorganic metal form in any experimental application (Roane et al., 1996).

Mycorrhizal inoculation had positive influences on root biomass and leaf number, but in general, limited metal accumulation. Less shoot and root accumulation of Cu and Pb was observed in mycorrhizal plants than in non-mycorrhizal plants, especially at the higher concentrations of mine residues. All shoot Cu concentrations were below published phytotoxicity levels (15–20 mg/kg) (Vamerali et al., 2010), whereas only root Cu concentrations of non-mycorrhizal plants surpassed these values. Similarly, non-mycorrhizal treatments accumulated higher Pb concentrations, especially in shoots; which exceeded the range considered as phytotoxic (> 20 mg/kg) (Vamerali et al., 2010). In relation to Cd accumulation, Liu et al. (2008) reported that both Cd concentrations in shoots and roots increased with increasing concentrations of Cd in the soil, but Cd accumulation in shoots was lower than that in roots of *C. officinalis*. In contrast, in the present research, Cd concentrations in both shoots and roots were similar and these were independent of Cd concentration in the substratum and mycorrhizal conditions. The Cd plant concentrations were slightly above phtotoxicity levels (5–10 mg/kg) as suggested by Vamerali et al. (2010).

Apparently *G. mosseae* BEG25 is able to increase the accumulation of Zn in this plant but Zn concentrations are below published phytotoxicity levels (150–200 mg/kg) (Vamerali et al., 2010). In previous research the same fungus significantly increased Zn accumulation in roots (1100–1200 mg Zn/kg) of the metallophyte *Viola calaminaria* (Gingins.) Lej. when it was exposed to increased Zn concentrations (200–400 mg/L) under hydroponic conditions (Fernández-Fernández et al., 2008). Hence it seems that responses in Zn accumulation depend on fungus-plant interactions, levels of metal, type of metal and plant uptake strategy (excluder or accumulator) (George et al., 1994; Leyval et al., 1995).

Lineal regressions revealed that bioavailable metals in the growth substrate had a stronger negative effect on the plant aerial part (shoot dry weight and foliar area) of mycorrhizal plants than in their roots, because higher negative r values were observed in the inoculated treatments. In contrast, in the mycorrhizal treatments negative r values lower than in controls were obtained when metal concentrations vs. root dry weights were correlated. A protective effect of *G. mosseae* was more evident in the roots. Although there is still considerable speculation on the mechanisms involved in plant metal protection by AMF, it has suggested that hyphal complexes of mycorrhizal fungi bind metals, preventing translocation to shoots. In addition, high cation exchange capacity and elevated metal-sorption or -accumulation have been reported as a mechanism for *G. mosseae* BEG25 to deal with metals (González-Chávez, 2000), and other authors have confirmed these mechanisms in other AM fungi (Joner et al., 2000). Moreover, Cuellar-Sanchez et al. (2011) observed the capacity of this fungus to sequester metals, in the order Pb>Zn>Cd. These fungal properties represent metal immobilization in the rhizosphere of mycorrhizal plants, which may have relevant implications for metal plant tolerance and phytostabilization potential. Several authors suggested that tolerant behaviour of the fungi may be an important factor conferring plant tolerance. However, the influence on plant tolerance depends upon the compatibility of fungal isolate with the host plant, rather than fungal tolerance to metals (Weissenhorn and Leyval, 1995).

4 Conclusions

The present research investigated the tolerance of chrysanthemum plants to different metals, either under hydroponic conditions or metal mine residue supplemented substrates, and the participation of *G. mosseae* BEG25 in this last condition. No plant toxicity damage was observed with any of the metals tested. Furthermore, all plants exposed to metals flowered. Under hydroponic conditions, chrysanthemum behaved as a Pb- and Ni-excluder plant. High Cu and Cd concentrations in shoots, but more in roots, were observed only at the highest concentrations tested (10 mg/L). However, no significantly enhanced concentrations were detected in flowers.

In mine residue enriched substrates, plants proved to be tolerant to multiple metals present in the growth medium. Shoot dry weights were unaffected by the different levels of mine residues tested, but the root dry weights of non-mycorrhizal plants were reduced in the highest mine residue treatment (30%). Mycorrhizal plants accumulated less Cu and Pb in their shoots and roots, thus restricting these metals below toxic levels. It is confirmed that AM

fungi can act as a barrier system preventing metal plant accumulation in plants. Results from this research show that chrysanthemum is a metal tolerant ornamental plant, which may potentially be used in phytostabilization of polluted soils with mine residues, and that AM fungi may participate in this remediation alternative. More research is necessary about plant ability to grow on mine residues containing high metal concentrations in field conditions.

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