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ORIGINAL PAPER

Zinc and cadmium accumulation by *Lupinus uncinatus* Schldl. grown in nutrient solution

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Abstract The effects of zinc and cadmium on the growth, mineral composition (P, K, Ca, Mg, Fe, Zn, Mn, and Cu), and metal accumulation by lupine (bluebonnet, *Lupinus uncinatus* Schldl.) were investigated in a hydroponics experiment. Plants were exposed to increasing concentrations of Zn (0, 30, 40, and 50 μM) and Cd (0, 3, 4, and 5 μM) for 1 week. The species showed different patterns of metal accumulation and distribution in the plant parts, suggesting different mechanisms of metal tolerance for each metal. At the highest doses of Zn and Cd, the amounts of metals accumulated in roots, stems, and leaves were 1,289, 1,918, and 1,132 mg kg^{-1} dry matter and 2,467, 227, and 164 mg kg^{-1} dry matter, respectively. The shoot:root Zn ratios obtained for 50, 40, and 30 μM treatments were 2.36:1, 2.28:1, and 2.32:1, respectively, whereas the ratio in case of Cd remained <1 for the three Cd treatments. No significant effect on plant dry biomass was observed in either of the cases. Significant changes in plant mineral composition occurred, however, concentrations were generally above the deficiency levels. This suggests that this species may tolerate Cd without its hyperaccumulation and shows exclusion mechanism of Cd tolerance.

Keywords Heavy metals · Hydroponics · Lupin plants · Phytoextraction

Introduction

Heavy metals and metalloids represent a series of environmental hazards worldwide. Some industrial activities and agricultural practices increase their level in the substrate, and the possible introduction of these elements in the food chain is an increasing human health concern (Cakmak et al. 2000; Jabeen et al. 2009). Metals transport in surface waters and leaching through the soil profile may result in the contamination of consumable water through mining catchment as it flows into the native river (Ashraf et al. 2011). Unlike organic compounds, metals cannot be degraded, and the cleanup usually requires their removal. However, this energy-intensive approach can be prohibitively expensive. Conventional technologies suitable for soil and water remediation used in situ and ex situ are (1) soil flushing, (2) pneumatic fracturing, (3) solidification/stabilization (4) vitrification, (5) electrokinetics, (6) chemical reduction/oxidation, (7) soil washing, and (8) excavation, retrieval, and off-site disposal. These technologies are cost prohibitive and processes often generate secondary waste (Prasad 2003). In addition, the metal removing process often employs stringent physicochemical agents that can dramatically inhibit soil fertility with subsequent negative impacts on the ecosystem.

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Phytoremediation has been proposed as a cost-effective, environmental-friendly alternative technology (Lasat 2002).

Heavy-metal-tolerant plants belonging to the genera *Alyssum*, *Thalspi*, or *Silene* have been identified (Brooks 1998; Dede et al. 2012; Shah and Nongrynrih 2007), and their use for phytoextraction purpose has been recommended (Schwartz et al. 2003; Zhao et al. 2003). Some of the metal-tolerant plants are also hyperaccumulators, as a hyperaccumulator has been defined as a plant that can accumulate cadmium >100 mg/kg or zinc >10,000 mg/kg in their shoot dry matter, and the metal concentrations in shoots are invariably greater than that in roots, showing a special ability of the plant to absorb and transport metals and store them in their above-ground part (Baker et al. 1994). Most hyperaccumulators, however, are difficult to manage and have a shallow root system, and their use is, therefore, limited in the case of deep contamination (Keller et al. 2003). The accumulation of heavy metals in plants is related to the concentration and chemical fractions of the metals in soils (Ehsan et al. 2007).

The use of deep rooting *Lupinus* species is of particular interest in this context. These plants have shown a relatively high tolerance to various environmental stresses, nitrate excess, low root temperature, detopping (De Lorenzo et al. 1993; Iannetta et al. 1993), lime excess (Tang and Robson 1993), and salinity (Fernández-Pascual et al. 1996). Some *Lupinus* species are able to accumulate Zn (Pastor et al. 2003), Cd (Brennan and Bolland 2003), Mg and Al (Reay and Waugh 1981), Hg (Vera et al. 2002; Esteban et al. 2008), and Pb and Cr (Ximenez-Embun et al. 2001). Moreover, white lupin (*Lupinus albus* L.) tolerance to Cd and As with few toxic visual symptoms has been reported (Zornoza et al. 2002; Vazquez et al. 2005), as well as its ability for phosphate-assisted phytoextraction of As (Tassi et al. 2004) and for phytostabilization of acidified multi-contaminated soils (Vazquez et al. 2006). Ximenez-Embun et al. (2001) reported that *L. albus, L. luteus, L. angustifolius*, and *L. hispanicus* were able to grow under extreme conditions (wastewater, pH lower than 2) and to remove 98 % of the initial amount of toxic metals present in the sample. In a pot study, we also found that *L. uncinatus* could be used for phytostabilization of Cd-contaminated soils (Ehsan et al. 2009). However, to the best of our knowledge, no information is available concerning metal accumulation in *L. uncinatus* in nutrient solution.

The first step to assess the potential interest of a plant species for phytoextraction is to quantify, in a controlled environment, the mean level of toxic metal accumulation in relation to the growth rate. The present study, carried out during June–July 2006, at Colegio de Postgraduados, Mexico, deals with Zn and Cd, two elements that share numerous similar chemical properties and are often present concomitantly in polluted areas. Cadmium is a highly toxic metal and has been ranked number 7 among the top 20 toxins mainly due to its negative influence on the enzymatic systems of cells (Sanita and Gabbrielli 1999; Al-Khedhairy et al. 2001). Accumulation of these elements was quantified in *L. uncinatus*. Plants were exposed to a nutrient solution with varying treatments of Zn or Cd to determine the specific toxicity of these elements in relation to their level of accumulation. In addition, the concentration and distribution of plant nutrients and Zn and Cd in various plant parts were studied.

Materials and methods

Plant material and growth conditions

A nutrient solution study was conducted to define patterns of Zn and Cd uptake by *L. uncinatus* at varying solution concentrations of Zn and Cd. Seeds were collected from the plants growing naturally on the mountainside in San Francisco, State of Mexico, Mexico. The study was conducted in a greenhouse where the average day and night temperatures were 33 and 10 °C, respectively.

Seeds were placed in plastic pots where they germinated after 4–6 days. The plants were allowed to grow in pots for 6 weeks before they were transplanted to each 2-L plastic buckets. The transplanted plants had an average height of 8 cm having on average 22 leaves plant^{-1}. The buckets were filled with 1.5 L of the nutrient solution having the following composition (Table 1).

Plants were maintained in the growth medium for 1 week before treatments were initiated. Four plants were placed into each of the 2-L plastic buckets for the experiment. The buckets were aerated continuously with an air pump and placed in a completely randomized designed with five replications. Buckets were filled with the previously described (Table 1) solution minus Zn and Cd. Four treatments each of Zn and Cd were then applied separately

Table 1 Basic nutrient solution used for growing *L. uncinatus* in this experiment

Compound	Concentration
KH_2PO_4, mM	3.24
KNO_3, mM	0.64
NH_4NO_3, mM	6.87
$CaCl_2 \cdot 2H_2O$, mM	3.75
$MgSO_4 \cdot 7H_2O$, mM	2.07
H_3BO_3, μM	10
$MnCl_2 \cdot 4H_2O$, μM	3
$ZnSO_4 \cdot 7H_2O$, μM	1
$CuSO_4 \cdot 5H_2O$, μM	0.3
FeEDTA (10 % Fe), mg/l	50

as $ZnCl_2$ and $CdCl_2 \cdot 2.5H_2O$, respectively. Zinc and Cd treatments were 0, 30, 40, and 50 μM Zn and 0, 3, 4, and 5 μM Cd, respectively. The treatments with 0 μM Zn and Cd were the control treatments, where no Cd or Zn was added to the nutrient solution. Normal concentrations of Zn for nutrient solutions range between 0.75 and 1 μM (Brown et al. 1995; Lutts et al. 2004). The Zn treatments were thus based on this criterion of basal Zn concentration, while those of the Cd were assigned keeping in view Zn treatments as reference. In most uncontaminated soils, water-soluble Zn and Cd are <I μM (Brown et al. 1995). Solution pH was maintained near 6.0. In all the treatments, the number of plants used was the same. Deionized water was added daily to buckets to maintain solution volume. The solution was replaced completely every week.

Harvest

Eight days after application of treatments, all the plants were harvested. At harvest, roots, stems, and leaves of each plant were separated. Roots were rinsed in deionized water and then gently blotted dry. Plant parts were dried for 5 days in an oven at 70 °C for their subsequent grinding.

Sample analysis

For each sample, digestion of dry matter (approximately 50 mg) was accomplished at 80 °C with nitric acid. The concentrations of P and K were determined by flame photometry following the methods described by Garcia-Gomez et al. (2002) and Isaac and Kerber (1971), respectively.

Ca, Mg, Zn, Fe, Cu, Mn, and Cd were determined by atomic absorption spectrophotometry (Lucho-Constantino et al. 2005). With Zn treatments, due to insufficient dry matter of plant organs, and with Cd treatments for roots, three replicates were analyzed for elemental composition.

Statistical evaluation

Data obtained from each type of organ were analyzed by a two-way analysis of variance performed with the model procedure of SAS version 9.1 (SAS 2000). If the F value indicated significant differences ($P < 0.05$), mean differences were compared according to the Tukey studentized range (HSD) test.

Results and discussion

Plant survival and visual toxicity symptoms

Two days after the initiation of treatments, the lower leaves of the plants receiving 50 μM Zn or 5 μM Cd treatments started exhibiting leaf wilting. The leaves also had necrotic spots. The most conspicuous and widespread symptom of toxicity was bronzing of affected leaves accompanied by tip necrosis and inward curling, starting with the older leaves but progressing up the plant with time.

The toxicity symptoms became more severe with increasing Zn and Cd levels and exposure time. The 50 μM Zn treatment and the 5 μM Cd treatment were the first to show symptoms where the leaf wilting and fall of leaves were severe followed by plant death. At the end of 1 week exposure to different metal doses, all of the plants died in 50 μM Zn or 5 μM Cd, whereas in 40 μM Zn or 4 μM Cd the plants survived but showed signs of toxicity. In the lowest dose of either Zn or Cd, the toxicity symptoms were minimum with some purpling of older leaves.

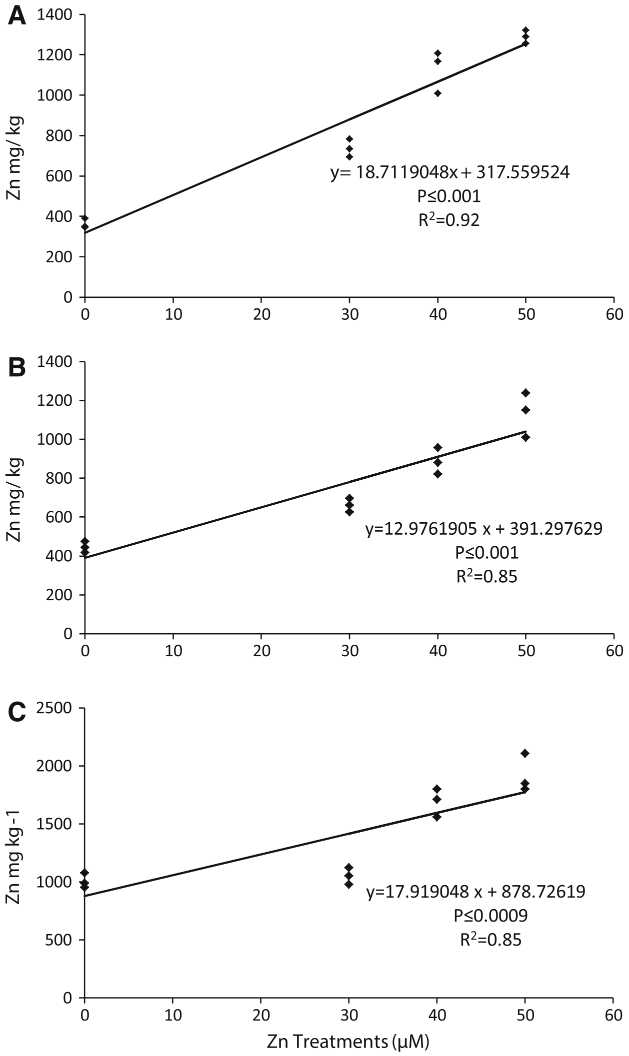

Fig. 1 Zinc concentration in **a** root, **b** leaf, and **c** stem tissues of *L. uncinatus* grown in nutrient solution for 1 week with varying Zn concentrations. In the regression models, $y =$ plant tissue Zn concentration, and $x =$ Zn concentration in nutrient solution

Concentration of zinc and cadmium in plant tissues

Concentrations of Zn accumulated in different plant organs of *L. uncinatus* after 1 week of exposure to various doses of Zn are given in Fig. 1. Generally, significantly higher concentrations accumulated in various Zn treatments than with no Zn added to the solution ($P \leq 0.001$). Although a considerable amount of Zn was accumulated in roots as shown in Fig. 1, accumulation of this element was significantly higher in aerial parts (stem + leaves). For plants grown for 1 week in the presence of 50 μM Zn, Zn constituted 1,289, 1,918, and 1,132 mg kg^{-1} dry matter in roots, stems, and leaves, respectively. For the 40 μM Zn treatment amounts of Zn accumulated in roots, stems, and leaves were 1,128, 1,690, and 885 mg kg^{-1} dry matter, respectively. With the lowest Zn treatment (30 μM Zn), the concentrations were 737, 1,051, and 661 mg Zn kg^{-1} dry matter in roots, stems, and leaves, respectively. Although the tissue Zn concentration increased with the Zn content of the growth medium, no significant difference occurred between 50 μM Zn and 40 μM Zn treatments.

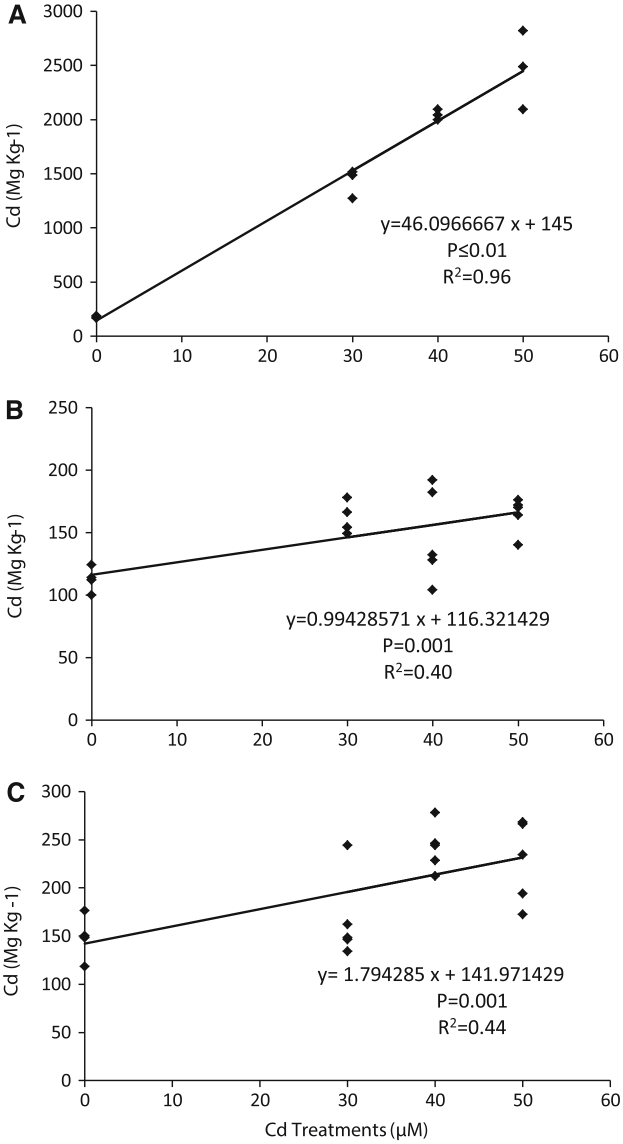

Fig. 2 Cadmium concentration in **a** root, **b** leaf, and **c** stem tissues of *L. uncinatus* grown in nutrient solution for 1 week with varying cadmium concentrations. In the regression models y = plant tissue cadmium concentration and x = cadmium concentration in nutrient solution

Cadmium concentrations in plants followed a different pattern than Zn concentration (Fig. 2). The major portion of Cd accumulated was retained by roots. Root Cd concentrations were 175, 1,424, 2,045, and 2,467 mg kg^{-1} for 0, 3, 4, and 5 μM Cd, respectively. All the treatments showed significantly higher Cd accumulation in various organs of the plant as compared to the control ($P \leq 0.01$). In view of the results obtained in this study, Cd accumulation by the lupin plants was a function of the Cd concentration in the growth medium. A consensus exists that over a certain range, there is a positive, almost curvilinear, correlation between the levels of Cd in the medium and the resulting Cd concentration in the plant tissues (Adriano 2001). In other experiments too, a similar increase in metal content in the growth medium (soil) has been accompanied by higher accumulation in plants. For example, Grytsyuk et al. (2006) found high values of Cd and Zn correlation coefficients ($R^2 \geq 0.74$) between the metal content in two forage crops (clover and perennial cereal grasses) and the content of this metals in the soil in which the plants were growing.

On the other hand, Yanqun et al. 2005 found 145.2 mg Cd kg^{-1} and 329.8 mg Cd kg^{-1} in plant shoot growing in a non-polluted soil in *Picris hieracioides L.* subsp. Japonica and *Corydalis petrophila Franch.* Similarly, they reported 231.4 mg Cd kg^{-1} and 354.5 mg Cd kg^{-1} in the roots of *Plantage erosa* Wall. and *P. hieracioides L.* subsp. J. krylv. However, in polluted soil, these plants accumulated 145 times higher amount of metal than in non-polluted soil, so they concluded the use of these plants as potential phytoremediators. In this work, amount of Cd in roots of L. *uncinatus* growing in the highest Cd treatment in nutrient solution was 2,467 mg kg^{-1}. This plant response is similar to that found by the above-mentioned authors. For this reason, we propose the use of *Lupinus* as a candidate for future research to explore its potential for phytoremediation of Cd- and Zn-contaminated soils.

Zn and Cd translocated

Total Zn and Cd translocated to shoot (stem + leaves) tissue (concentration of metal in dry matter × dry weight) was calculated as reported by Dechamps et al. (2005). The highest amount of Cd translocated to shoot,

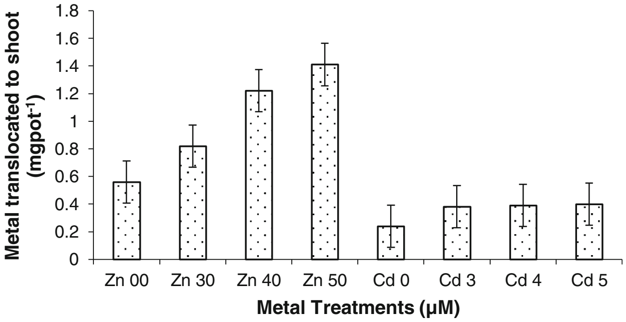

Fig. 3 Cadmium and Zn translocated to harvestable biomass by *L. uncinatus* grown in nutrient solution at various levels for 1 week. *Bars* represent standard deviations of values averaged across treatments, $n = 5$

0.4 mg Cd pot^{-1} (Fig. 3), by *L. uncinatus* was found in case of the 5 µM Cd treatment which was 60 % higher the treatment with no Cd added ($P = 0.05$). However, all the treatments with added cadmium had no significant difference among them, while, to the contrast; they proved to be significantly different as compared to the control treatment. The literature reveals that cadmium is translocated readily throughout the plant following its uptake by the roots. Distribution between roots and shoots differs with plant species, rooting medium, and time of treatment (Adriano 2001).

In case of Zn (Fig. 3), the amount translocated to shoots biomass was much greater with Zn added than without Zn addition. For example, the plants exposed to 50 µM Zn translocated 252 % more Zn to the shoots than plants not exposed to supplemental Zn. The magnitude of Zn translocation increased with the increasing Zn solution concentration. These results suggest that this species can be a candidate for Zn phytoextraction.

Shoot/root partitioning of zinc and cadmium

Shoot:root ratios of metals are <1 when non-tolerant or indicator species are grown in contaminated soils (Peterson 1983; Baker and Walker 1990). In this study, with regard to shoot:root Zn ratio, *L. uncinatus* exhibited ratios >1 in all of the treatments. The results of this experiment indicate that with increasing Zn concentration in solution, the ability of the plant to translocate Zn to its aerial parts decreased significantly compared with no Zn added ($P = 0.05$). However, the fact that it remained >1 shows hyperaccumulation potential of this species for Zn (Table 2).

Table 2 Shoot:root Zn concentration ratios of *L. uncinatus* after 1 week exposure to various Zn concentrations in nutrient solution

Zn treatment (µM)	Shoot:root Zn ratio
0	4.00 a
30	2.32 b
40	2.28 b
50	2.36 b

Means followed by the same letter are not significantly different at ($P = 0.05$)

Table 3 Shoot:root Cd concentration ratios of *L. uncinatus* after 1 week exposure to various Cd concentrations in nutrient solution

Cd treatment (µM)	Shoot:root Cd ratio
0	1.48 a
3	0.23 b
4	0.18 b
5	0.15 b

Means followed by the same letter are not significantly different at ($P = 0.05$)

This pattern of accumulation was not followed by *L. uncinatus* in case of Cd (Table 3). The shoot:root Cd ratios remained <1 in all the treatments where Cd was added to the nutrient solution. However, with both of the metals, the partitioning between shoot and root with Zn or Cd added was significantly ($P = 0.05$) lower than with the treatment without these metals added, suggesting that exposure to heavy metal concentrations tends to decrease the ability of the plant to transport the same to its aerial parts. In case of Cd, lower accumulation in shoot may also be a mechanism to avoid Cd toxicity as was concluded by Zornoza et al (2002). In general, roots contain at least twice the Cd concentration of shoots (Koeppe 1977). Chino and Baba (1981), while studying Cd accumulation by rice (*Oriza sativa* L.) observed that environmental factors such as Cd concentration in the medium, ambient temperature, and light intensity can affect the distribution of the metal between the roots and the shoots. The findings from the present study also point toward the fact that the specialized mechanisms responsible for Zn accumulation in *L. uncinatus* do not control Cd accumulation. The specificity of metal uptake mechanism has also been reported by Brown et al. (1995) for *Thlaspi caerulescens*. Although the present experiment did not examine the accumulation of Zn and Cd when supplied concomitantly, different accumulation patterns for the two metals from solution to root cell plasma may suggest that different mechanisms of transport are involved for each metal.

Zn and Cd distribution in various plant parts

Separation of the plant tissue of *L. uncinatus* into leaves, stems, and roots and subsequent chemical analysis showed significantly higher concentrations of Zn accumulated in stem tissues than leaf or root irrespective of the treatment (Fig. 4). It appears that stem accumulation of Zn is one of

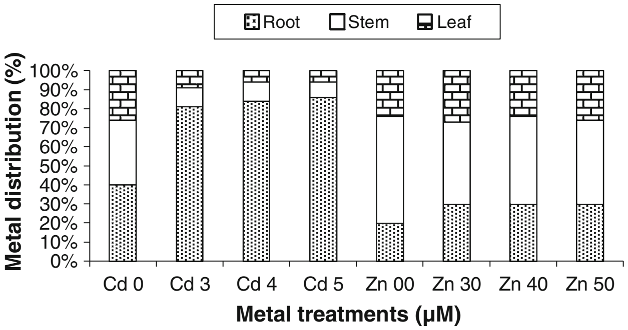

Fig. 4 Cd and Zn distribution in various plant organs of *L. uncinatus* after 1 week of growth in nutrient solution at different Cd and Zn levels

the plant response mechanisms to imposed Zn stress as we also observed enhanced stem Zn concentration in another experiment with the same species (unpublished results). Changed partitioning of metals in various organs of the plant as a response to metal stress has been reported by other authors as well (Brown et al. 1995; Pastor et al. 2003).

Cadmium concentrations in leaf and stem tissue were significantly lower than in roots for all of the Cd treatments (Fig. 4). The pattern of Cd distribution between root, stem, and leaves remained consistent over the range of treatments where Cd was applied to the growth medium since major portion of the metal (>80 % of the total metal) accumulated in the roots. However, untreated plants showed different trend of Cd distribution in various plant organs where the roots accumulated 40 % of the total metal accumulated in the plant. This may suggest that the observed pattern of Cd partitioning is a plant strategy to counter toxicity as also suggested by Zornoza et al. (2002). The Cd uptake in the lupin roots serves to offer the benefit of metal phytostabilization (Ehsan et al. 2009).

Plant species differ with respect to the pattern of metal accumulation in the plant parts. Angelova et al. (2004) reported that the accumulation of Pb, Cu, Zn, and Cd in flax followed the order: roots > stems > leaves > flowers. For hemp, the order was: flowers > roots > stems > leaves, whereas for cotton the distribution of Zn and Pb (leaves > roots > flowers > stem) was different from Cu (leaves > roots > stems > flowers). The abovementioned authors, however, suffered lack of data for explaining physiological mechanisms of heavy metal uptake, transport, and accumulation in these species. Also, the heavy metal accumulation in plant parts of lavender was different for each metal analyzed (Cd, Cu, Mn, Zn, and Fe), with the concentrations always in leaves and frequently in roots being higher than in inflorescences (Zheljazkov and Nielsen 1996).

This suggests that *L. uncinatus* may tolerate Cd without its hyperaccumulation and shows exclusion mechanism of Cd tolerance. Overall, the results support the hypothesis that *L. uncinatus* could be used in the combined strategies to recover Zn- and Cd-contaminated soils. The use of *L. uncinatus* could associate the advantages of using leguminous plants and the efficiency in phytostabilization (Ehsan et al. 2009) as also demonstrated for other trace elements in the case of white lupin (Vazquez et al. 2006; Esteban et al. 2008) and also in case of other plants such as *Viguiera dentata*, *Parthenium bipinnatifidum*, *Flaveria angustifolia*, *F. trinervia*, and *Sporobolus indicus* which could be used to vegetate soils with large amounts of As, Cu, Pb, and Zn (Franco-Hernández et al. 2010). Its efficiency in accumulating Cd in the roots, with very low translocation rate to the shoots, could make it a good choice in phytostabilization, preventing leaking of Cd from contaminated soils. It must be emphasized here that although the hydroponic technique can provide a useful investigative tool, it does not accurately reflect the growth or patterns of metal uptake that would occur in the complex physicochemical and biological environment of soils. Further research, therefore, in the field conditions is necessary to assess the trends under other environmental conditions obtained in this study.

Heavy metal accumulation and mineral nutrition

Zn treatments

No significant effect on P concentrations was recorded in response to various Zn doses irrespective of the plant part (Table 4). Phosphorus concentrations in plant aerial parts ranged from 0.3 to 0.5 % of dry matter, which is the P requirement for optimal growth during vegetative stages according to Marschner (1995). These results, thus, indicate that the P nutrition requirements of the plants were met adequately. The exposure of *L. uncinatus* to 30, 40, or 50 μM Zn did not significantly affect stem and leaf K concentrations. A significant reduction in root K, however, was observed with the supplemental Zn treatments than with no Zn addition causing a significant increase in shoot:root accumulation ratios varying from 1.7:1 (no Zn), 4.9:1 (30 μM Zn), 4.3:1 (40 μM Zn) to 4.9:1 (50 μM Zn). These results are in agreement with the findings of Kidd et al. (2004) who reported an increase in transport of K to shoot tissue of gum rockrose (*Cistus ladanifer* L.) when grown in a nutrient solution in presence of various heavy metals such as Cd, Cr, Mn, or Pb. They attributed this increase to the maintenance of a charge balance between organic acid production and metal complexation in the leaf cells.

As also reported by Bernal and McGrath (1994), Zn treatments in this experiment significantly increased shoot Ca, Mg, and Mn compared with the roots showing transport of these elements from the root system to the aerial parts.

Table 4 Mineral nutrient distribution within leaf, stem, and root tissues of *L. uncinatus* grown in nutrient solution with varying Zn concentrations

Treatment Zn (μM)	Plant organ	P gkg^{-1}	K	Ca	Mg	Fe mgkg^{-1}	Mn	Cu
00	Leaf	3.8 a	34.58 a	13.12 a	1.87 a	4,366.3 a	2,135.3 a	103.3 b
	Stem	5.78 a	27.30 a	5.77 b	5.92 b	ND	431.7 a	593.7 a
	Root	5.35 a	36.20 a	8.61 a	7.10 b	ND	4,104 a	ND
30	Leaf	3.25 a	35.87 a	11.81 a	2.54 a	3,827.3 a	2,085.0 a	135.0 a
	Stem	5.33 a	35.23 a	11.26 a	8.86 a	ND	630.0 a	308.3 b
	Root	3.93 a	14.31 b	6.75 a	5.56 c	ND	650.0 d	ND
40	Leaf	3.08 a	34.43 a	12.40 a	2.58 a	3,424.3 a	2,165.0 a	121.6 ab
	Stem	3.91 a	42.30 a	10.60 a	8.36 a	ND	562.3 a	338.3 ab
	Root	4.17 a	17.74 b	6.21 a	8.24 a	ND	1,071.0 c	ND
50	Leaf	2.80 a	35.83 a	13.76 a	2.82 a	3,561.3 a	2,287.0 a	115.6 ab
	Stem	4.07 a	41.44 a	10.94 a	7.53 ab	ND	637.7 a	581.7 a
	Root	4.96 a	15.93 a	7.48 a	7.71 ab	ND	1,435.0 b	ND

For given elements means followed by the same letter across treatments and within a plant part are not significantly different at ($P = 0.05$)

ND not determined

Table 5 Shoot:root concentration ratios of some nutrients as affected by 1 week exposure of *L. uncinatus* to various Zn concentrations in nutrient solution

Zn treatment (μM)	K	Ca	Mg	Mn
0	1.7 b	2.2 b	1.1 b	0.62 c
30	4.9 a	3.4 a	2.1 a	4.17 a
40	4.3 a	3.7 a	1.32 b	2.54 b
50	4.9 a	3.3 a	1.34 b	2.03 b

Means of a given element with the same letter are not significantly different ($P = 0.05$)

This phenomenon is also clear from the higher shoot:root ratios for both of the elements observed in this experiment (Table 4). Tolra et al. (1996) reported the same pattern of enhanced Ca and Mg shoot:root ratios for alpine pennycress (*T. caerulescens J. Presl.* and *C. Presl)* when grown in nutrient solution at various solution Zn concentrations ranging from 100 to 1,500 μM. The ability of a plant to maintain the concentrations of essential elements within the range considered adequate for growth in normal plants, has been termed as an accompanied trait along with phytoextraction of metals (Tolra et al. 1996).

Iron concentrations of content of leaves were not affected significantly by Zn or Cd treatments (Table 4). Lutts et al. (2004) reported reduced Fe concentrations in stem of saltbush (*Atriplex halimus* L.) in response to 0.1 mM Zn in solution culture. However, they did not note any inhibition in Fe accumulation in spite of a reduced translocation from roots to shoots. Tolra et al. (1996) reported no effect on shoot Fe in *T. caerulescens* plants exposed to 750, 1,000, or 1,500 μM Zn in nutrient solution. In this experiment, zinc doses significantly increased leaf Cu concentration while the stem Cu concentrations were reduced as compared to plants where no Zn was added (Table 5).

Cd treatments

Cadmium treatments did not produce any significant effect on P, Ca, or Mn concentrations irrespective of the plant part (Table 6). Shoot P concentrations in this study were in the range of 0.75–0.8 % of dry matter, suggesting that *L. uncinatus* did not suffer from P deficiency (Marschner 1995).

In some non-tolerant species, exposure to heavy metals, such as Cu, results in disruption of the root plasma membranes (Ernst et al. 2000). After only a short period, this disruption can lead to K^+ leakage and a reduction in root K concentration. In the present research, the root concentration of K was significantly reduced after exposure to 4 or 5 μM Cd which could have resulted due to K^+ leakage from the disrupted root plasma membrane. Shoot:root K increased from 5.79:1 (no Cd) to 6.53:1 (4 μM Cd) and 9.79:1 (5 μM Cd) (Table 7), suggesting that *L. uncinatus* maintained or increased relative K transport to shoot at Cd levels imposed in this study. Potassium is a dominant cation in ionic charge balance in cells. Plants can produce increased concentrations of organic acids to complex foliar metals such as Zn and Cd (Marschner 1995), and the increased transport of K to the shoots observed in this investigation could be to maintain a charge balance between acid production and metal complexation in the leaf cells (Kidd et al. 2004).

Table 6 Mineral nutrient distribution within leaf, stem, and root tissues of *L. uncinatus* grown in nutrient solution with varying Cd concentrations

Treatment Cd (μM)	Plant organ	P gkg^{-1}	K	Ca	Mg	Zn mgkg^{-1}	Fe	Mn	Cu
0	Leaf	3.48 a	34.26 a	12.70 a	2.64 b	405.2 b	2,940.6 a	1,574 a	163.6 a
	Stem	4.59 a	40.84 a	8.00 a	6.76 a	725.6 a	1,537.4 a	438.4 a	488.0 a
	Root	5.72 a	12.97 ab	7.15 a	5.14 ab	814.0 c	1,735.7 c	1,143.3 a	977.3 ab
3	Leaf	3.30 a	34.32 a	12.12 a	4.91 a	682.8 a	2,640.6 a	1,718 a	197.2 a
	Stem	4.43 a	44.18 a	7.92 a	6.11 a	411.2 b	1,285.4 a	586.8 a	343.8 bc
	Root	4.64 a	14.25 a	9.42 a	6.03 a	1,250 b	2,818.7 b	1,202.7 a	635.0 b
4	Leaf	3.08 a	33.38 a	12.95 a	4.0 ab	599.2 ab	2,967.8 a	1,840 a	223.4 a
	Stem	4.60 a	38.50 a	6.86 a	5.13 a	543.2 ab	1,594.2 a	502.8 a	282.2 c
	Root	4.55 a	11.00 ab	10.09 a	5.04 ab	1,282 b	3,181 ab	1,383.3 a	1,086.0 a
5	Leaf	3.31 a	35.09 a	11.46 a	4.96 a	667.6 a	2,703.6 a	1,924 a	152.4 a
	Stem	4.24 a	39.18 a	7.05 a	5.27 a	570.8 ab	1,408.4 a	465.2 a	472.8 ab
	Root	4.74 a	7.58 b	7.46 a	4.62 b	1,533 a	3,532.0 a	1,299.3 a	945.0 ab

For given elements means followed by the same letter across treatments and within a plant part are not significantly different at ($P = 0.05$)

Table 7 Shoot:root concentration ratios of some nutrients as affected by 1 week exposure of *L. uncinatus* to various Cd concentrations in nutrient solution

Cd treatment (μM)	K	Mg	Zn	Fe	Cu
0	5.79 b	1.82 b	1.38 a	2.58 a	0.70 b
3	5.50 b	1.82 b	0.87 b	1.39 b	0.85 a
4	6.53 b	1.81 b	0.89 b	1.43 b	0.46 c
5	9.79 a	2.21 a	0.81 b	1.16 b	0.70 b

Means of a given element with the same letter are not significantly different ($P = 0.05$)

Shoot:root Zn decreased significantly ($P = 0.05$) from 1.38 (no Cd) to 0.87, 0.89, and 0.81 in 3, 4, and 5 μM Cd treatments, respectively (Table 7), suggesting an antagonistic effect of Cd on Zn uptake—transport processes, since both may be transported by a similar carrier mechanism (Bernal and McGrath 1994; Meharg 2005; Jabeen et al. 2009). A similar increase in root and leaf Zn as a response to 3-week exposure to 0.1 mM Cd in nutrient solution has been reported by Lutts et al. (2004) with *A. halimus.* Moreover, Cd and Zn appear to compete for certain organic ligands in vivo. Because Cd competes with Zn in forming protein complexes, a negative association between the two can be expected (Adriano 2001).

Leaf Mg concentrations increased significantly ($P = 0.05$) with the highest Cd treatment compared with the rest of Cd treatments, indicating that Cd increased Mg transport to the shoot. Root Cu decreased significantly at 3 μM Cd, but increased at 4 μM Cd, and no significant difference as compared to no Cd was observed at 5 μM Cd. Stem Cu decreased at 3 and 4 μM Cd, but increased at 5 μM Cd (Tables 6, 7).

High levels of Fe were retained in the roots, indicating that Fe transport to the shoots was reduced (Table 7). This reduction in Fe translocation to the shoot appears to be a common feature in plants after exposure to heavy metals. Bernal and McGrath (1994) reported a similar retention of Fe in the roots of *Alyssum murale,* a nickel hyperaccumulator when grown in a nutrient solution at 120 and 8.8 μM Zn and Cd respectively. Liu et al. (2000) also found that exposure of mung bean plants to 5 μM of Cd, Cu, and Ni produced the same effect on Fe transport. In another experiment by Ebbs and Kochian (1997), 100 μM Zn reduced shoot Fe in three *Brassica* species grown in hydroponic solution.

Fodor et al. (1996) demonstrated that in cucumber (*Cucumis sativus* L.), a strong inhibition of photosynthesis may result from Fe deficiency in Cd-treated plants. While 10 μM Cd reduced by more than 90 % the leaf Fe concentration of *Cucumber*, Lutts et al. (2004) found that 0.1 mM Cd had no effect at all on the leaf Fe concentration of *A. halimus*.

In the present study, the highest Cd concentration was found in roots (2467 mg kg^{-1}) in response to the highest Cd treatment (5 μM of Cd) added to the nutrient solution. This tendency has been shown by others plant of the same family, for example (Singh et al. 2009) found 4,120 mg Cd kg^{-1}, in the root dry matter of *Medicago sativa* treated with different concentrations of Cd in the growth medium. It does not necessarily indicate the higher capacity of *M. sativa* for Cd accumulation because the absorption of this metal is not only dependent on the nature of the species but also on other factors relative to experimental or environmental conditions.

Conclusion

A preliminary test has been conducted to evaluate the relative performance of *L. uncinatus*, their degree of tolerance to various concentrations of bioavailable Zn and Cd, and their patterns of metal accumulation. The hydroponics allows for these plant responses to be quantified in a rapid and simple manner. The shoot:root Zn ratios obtained in this experiment showed that *L. uncinatus* had the potential to hyperaccumulate Zn. The toxicity symptoms were only seen at the highest Zn treatment. However, in case of Cd, the behavior of the plant was different. Although it tolerated 3 and 4 µM Cd treatments, the bulk of the metal accumulated in the roots and the pattern of some essential nutrients such as K, Mg, Zn, Fe, and Cu was altered. Based on these findings, it can be concluded that *L. uncinatus* may be considered as a candidate in the future search of plant species suitable for phytoremediation of metal-contaminated sites.

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