Phytostabilization of cadmium contaminated soils by *Lupinus uncinatus* Schldl.

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Abstract

Phytoremediation offers the benefits of being *in situ*, low cost and environmentally sustainable. *Lupinus* species is starting to generate interest for phytoremediation of soils showing intermediate metal pollution. The aim of this study was to explore the accumulating behavior and tolerance of *Lupinus uncinatus* Schldl. towards increasing Cd concentrations in soil. For this purpose the effects of different Cd treatments on plant growth, survival, metal tolerance, Cd accumulation and distribution in various plant organs were investigated. An 18 week pot trial was performed under greenhouse conditions. Cd was added as $CdCl_2.2_{1/2}H_2O$ at the rate of 0, 3, 6 and 9 mg Cd kg^{-1} soil at three different occasions (after 4th, 12th and 15th week of plant growth) with four replicates. The Cd treatments applied, thus, were 9, 18 and 27 mg kg^{-1}. Cd inhibited plant height and number of leaves and induced a significant change in dry matter yield of roots, stems and leaves. Metal tolerance indices of 88, 82 and 49% were obtained for 9, 18 and 27 mg Cd kg^{-1} treatments. The maximal shoot Cd concentration (stem+leaves) of 540 mg Cd kg^{-1} dry matter was found at 27 mg Cd kg^{-1} treatment. The poor translocation of Cd from roots to shoot was evident from shoot:root ratios <1. The present work is the first report about the growth performance of *L. uncinatus* under Cd stress, its degree of tolerance and pattern of Cd accumulation in response to varying Cd treatments in soil suggesting the use of *L. uncinatus* for phytostabilization and revegetation of Cd polluted soils.

Additional key words: heavy metals, lupin plants, metal tolerance, phytoremediation, soil contamination.

Resumen

Fitoestabilización de los suelos contaminados con cadmio mediante el uso de *Lupinus uncinatus* Schldl.

La fitorremediación tiene las ventajas de ser *in situ,* de bajo costo y sustentable para el ambiente. El objetivo del presente trabajo fue explorar la acumulación y tolerancia de Cd por *Lupinus uncinatus* en suelos contaminados con Cd. Se investigó el efecto de diferentes tratamientos de Cd sobre el crecimiento y supervivencia de la planta, tolerancia al metal, así como acumulación y distribución del Cd en diferentes órganos de la planta. El experimento se llevó a cabo en macetas bajo condiciones de invernadero durante 18 semanas. Se agregó $CdCl_2.2_{1/2}H_2O$ después de 4, 12, y 15 semanas del crecimiento en las siguientes concentraciones (0, 3, 6, 9 mg Cd kg^{-1} suelo) con cuatro repeticiones. El Cd inhibió altura de la planta y número de hojas e indujo un cambio significativo en el rendimiento de la materia seca de las raíces, hojas y tallos. El índice de tolerancia al metal fue 88, 82 y 49% para los tratamientos 9, 18 y 27 mg Cd kg^{-1} respectivamente. Se detectó una concentración máxima en parte aérea (540 mg Cd kg^{-1} materia seca) en el tratamiento 27 mg Cd kg^{-1}. La translocación desde la raíz hacia el tallo fue pobre y la proporción del Cd en raíz:tallo fue <1. El presente trabajo es el primer reporte sobre el crecimiento de *L. uncinatus* bajo estrés de Cd, su grado de tolerancia al Cd y su acumulación en la planta en respuesta a diferentes tratamientos del Cd en el suelo, sugiriendo su uso en la fitoestabilización y revegetación de los suelos contaminados con Cd.

Palabras clave adicionales: contaminación del suelo, fitorremediación, lupino, metales pesados, tolerancia.

Abbreviations used: IT (index of tolerance).

Introduction

The wide spread heavy metal contamination poses long-term risks to environmental quality and sustainable food production (Weckx and Clijsters, 1996). Techniques currently employed to control soil contamination include excavation and burial of toxic metal-contaminated soil, or microbial or chemical treatment of organic polluted soil. Most of them are expensive and labor intensive, especially for the cleanup of metal-contaminated soil. Thus, phytoremediation, a technique using plants to remove contamination from soil, has become a topical research field in the last decade as it is safe and potentially cheap compared to traditional remediation techniques (Chaney *et al.,* 1997).

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 *et al.*, 1999; Al-Khedhairy *et al.*, 2001). Cadmium contamination in agricultural soils is unlikely to affect plant growth, however, as Cd is easily transferred to human food chain from the soils, Cd contamination is a great threat to human health. These effects limit the marketing of agricultural products and reduce the profitability of the agricultural industry. Cadmium is residual in soil for thousands of years (Alloway, 1995).

Besides mining, smelting, industrial production and traffic, the agricultural soils could be contaminated with Cd through utilization of sewage sludge, phosphate fertilizers and liming materials (Alloway, 1995; Fergusson, 1990; Kabata-Pendias and Pendias, 1992; Adriano, 2001). Cadmium is one of the most mobile trace elements in soils and is often significantly taken up by plants. Its bioavailability in soils is high compared to other metals as a result of its high solubility and the predominance of low energy bonds to the soil solid phase (Alloway, 1995; Gérard *et al.*, 2000; McLaughlin, 2000).

Lupinus uncinatus is an annual, biannual or perennial plant, more than 1 m tall, herbaceous, of hollow stems, 5 to 8 leaflets, slightly sharp apex, widespread deciduous flowers with feathery apex of dark purple colour. The fruits are dehiscent pods containing 9 to 49 pods per branch and 1 to 7 mature seeds per pod, flowering starts in March and the first mature seeds (whitish to ash grey) appear in the middle of May. It is found in soils having pH around 6.3, with more than 3% soil organic matter (Alderete-Chavez *et al.*, 2008).

Some reports about Cd tolerance of *Lupinus albus* can be found in literature (Zornoza *et al.*, 2002; Ximenez-Embun *et al.*, 2002; Page *et al.*, 2006b). How-

ever no information about the response of *L. uncinatus* to metal stress is available. The aim of this study was, therefore, to determine the growth performance of Mexican native. *L. uncinatus* under Cd stress, their degree of tolerance to varying Cd treatments in soil and their pattern of metal accumulation. These results would thus establish the potential of this species for the phytoremediation of Cd contaminated soils.

Material and methods

A pot experiment was conducted in greenhouse to study the Cd phytoremediation potential of *L. uncinatus.* Soil was taken from the top 25 cm layer of a crop field at San Pablo Ixayoc, State of Mexico, Mexico, air dried, weighed and analyzed for its total Cd content and other physical and chemical properties (Table 1).

Plastic pots 15 cm in diameter were filled with 5 kg of soil. Seeds of *L. uncinatus* collected from the same site as soil were sown on 27th June, 2005. Germination occurred after 5-7 days. One week after germination plants were thinned to 3 plants per pot. After four weeks of growth (4th August, 2005) the plants were treated with cadmium in the form of cadmium chloride ($CdCl_2. 2._{1/2} H_2O$) at the rate of 0, 3, 6, and 9 mg kg^{-1} dissolved in 15 mL of deionized water.

The pots were placed in a randomized design in greenhouse under normal conditions of temperature and humidity. The average day and night temperatures ranged between 25-29°C and 8-11°C respectively. The pots were re-randomized three times in the duration of the experiment. Four replicates were prepared per treatment (0, 3, 6 and 9 mg kg^{-1}). The pots were watered with distilled water every 2-3 days to maintain 70% of field capacity. The plants were supplied with two additional and successive Cd doses at different intervals (Table 2). After the different Cd doses applied the treatments are so called: 0 (Control, no Cd added), 9 (three doses of 3 mg kg^{-1})), 18 (three doses of 6 mg kg^{-1})), 27 (three

Table 1. Selected physical and chemical properties of pot soil

pH	6.29
Electrical conductivity	0.16 dS m^{-1}
Organic matter	1.19%
Soil texture	Sand (34%), Silt (32%), Clay (34%)
Bulk density	1.05 g cm^{-3}
Total Cd content	0.16 mg kg^{-1}

Table 2. Different intervals of cadmium addition to pot soil (2005)

	Cadmium dose (mg kg^{-1})		
4th Aug	29th Sept	20th Oct	Total Cd concentration
0	0	0	0
3	3	3	9
6	6	6	18
9	9	9	27

doses of 9 mg kg^{-1}). Plant growth was measured in terms of plant height and number of leaves at each Cd level, and dry weight as well as the content and distribution of Cd in the roots, stem and leaves were determined at the conclusion of the experiment.

After three weeks of the last Cd dose (10th Nov, 2005, a total of 18 weeks of plant growth), the plants were collected, separating them into roots, stems and leaves and were rapidly and profusely but gently washed with water and rinsed in several recipients containing deionized water. This was followed by drying in an oven at 70°C for 72 hours to estimate dry weight. The dried material was ground and 500 mg of these plant parts were treated with nitroperchloric acid. Concentrations of Cd in roots, stem and leaves were determined by atomic absorption spectrophotometry. Data were subjected to one-way ANOVA analysis followed by a Tukey test for multiple comparison of means (significance level $P < 0.05$). All the statistical tests were performed using SAS version 9.1 (SAS, 2000).

Calculation of metal tolerance

It was calculated as the mean weight of a plant grown in the presence of a metal divided by the mean weight of a control plant, expressed as percentage. An index of tolerance of 50%, which means 50% of optimum growth, is considered to be the minimum desired biomass production for plants growing in a metal contaminated site (Ximenez-Embun *et al.*, 2002).

Results

Plant height, number of leaves and dry matter yield as affected by Cd treatments

Effect of different Cd treatments on plant height and number of leaves is shown in Table 3. A reduction in plant height was observed with increasing Cd treatments which was although, statistically significant only for 27 mg Cd kg^{-1} treatment when compared with control. The other two Cd treatments (9 mg Cd kg^{-1} and 18 mg Cd kg^{-1}) did not affect significantly plant height as compared to the control.

A significant decrease in number of leaves was observed among various Cd treatments. This decrease was progressive with the rising treatments of Cd. After 14 weeks of cadmium stress the reduction in number of leaves as compared to control in 27 mg Cd kg^{-1} treatment was 31%.

Dry matter yield of roots, stem and leaves of *L. uncinatus* exposed to different Cd treatments is given in Table 3. A significant reduction in root, stem and leaf dry matter was observed in response to various Cd treatments imposed. The reduction in plant growth (plant height and number of leaves) observed in our experiment coincided with the appearance of visible toxicity symptoms in 27 mg Cd kg^{-1} treatment (69% leaf loss with respect to the control) and the magnitude increased with the increasing Cd treatments. The symptoms included bronzing of leaves and inward curling resulting in leaf drop in some cases (Figure 1).

Table 3. Effect of different Cd treatments on plant height, number of leaves, dry matter yield and shoot:root Cd ratio of *L. uncinatus*

Cd treatments (mg kg^{-1})	Plant height (cm)	Number of leaves	Dry matter yield (g pot^{-1})				Shoot:Root Cd ratio
			Root	Stem	Leaf	Plant	
0	66.25a	32a	2.12a	4.13a	2.70a	8.95a	1.2
9	64.50a	18ab	1.97ab	3.29b	2.08ab	7.34b	0.72
18	60.25a	18ab	1.45b	2.66c	1.56bc	5.67c	0.71
27	29.42b	10b	0.74c	1.57d	0.98c	3.29d	0.75

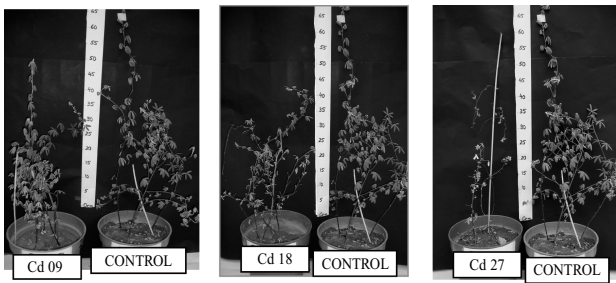Numbers in the same column followed by different letters differ significantly (P< 0.05) according to Tukey test.

Figure 1. Toxicity symptoms exhibited by *L. uncinatus* in response to various Cd treatments imposed. (Cd 00=control, Cd 09=9 mg Cd kg^{-1}, Cd 18=18 mg Cd kg^{-1}, Cd 27=27 mg Cd kg^{-1}).

Metal tolerance

After 14 weeks of plant growth in soil contaminated progressively with varied Cd treatments, the ability of *L. uncinatus*. to tolerate Cd stress was assessed using index of tolerance (IT). Figure 2 depicts the tolerance of *L. uncinatus* grown in Cd contaminated soil.

Cd accumulation and transport in plant organs

The Cd concentration in the roots, stems and leaves of *L. uncinatus* supplied with varied Cd treatments have been shown in Figure 3. Cd concentration in all the plant organs was significantly higher than the control treatment. A linear increase in Cd accumulation, irrespective of the plant organ, was found with the increasing treatments of Cd in the growing medium. After 14 weeks of exposure to different Cd treatments, Cd constituted 109,
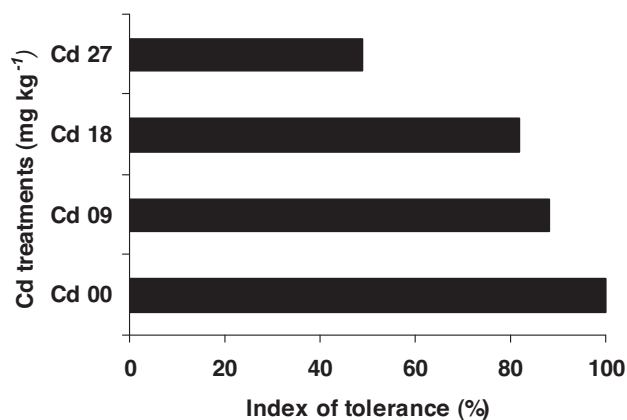

Figure 2. Tolerance index for *L. uncinatus* after a 14-week growing period in a soil contaminated progressively with Cd
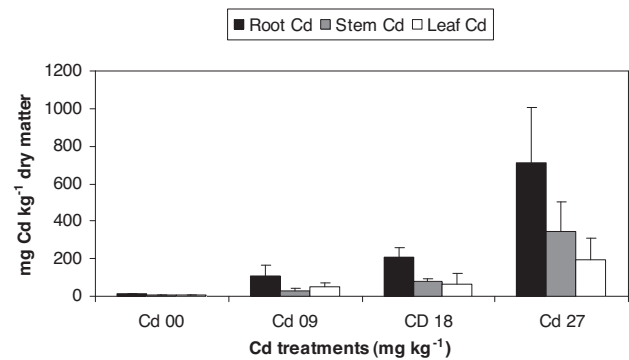

Figure 3. Cd uptake by roots, stem and leaves of *L. uncinatus* exposed to different Cd treatments for 14 weeks. Vertical bars represent ±SE (*n=4*).

29 and 50 mg kg^{-1} dry matter in roots, stem and leaves respectively for 9 mg kg^{-1} treatment. The Cd accumulation for 18 mg kg^{-1} treatment was 208, 81 and 68 mg kg^{-1} dry matter in roots, stem and leaves respectively. The range of values for Cd concentration in roots, stem and leaves of *L. uncinatus* for the highest Cd treatment (27 mg kg^{-1}) was 713, 343 and 197 mg kg^{-1} dry matter respectively. The shoots (stem+leaves) of *L. uncinatus* accumulated 79, 149 and 540 mg Cd kg^{-1} for the lowest, medium and the highest Cd treatment respectively.

The shoot:root Cd concentration ratios were calculated (Table 3). The said ratio in non-Cd treated plants was >1, while in plants treated with varied Cd treatments the ratio remained < 1. The ratio was <1 irrespective of the Cd treatment, being the lowest (0.71) for 18 mg Cd kg^{-1}.

Cadmium distribution in different plant organs

The distribution of Cd in the leaves, stems and roots of *L. uncinatus* subjected to different Cd treatments is presented in Figure 4. No change in pattern of Cd distribution in different plant organs was noted in response to varied Cd treatments with respect to control. The results indicate that most of the Cd taken up by the plant was retained in roots (root Cd made up 58% of the total Cd uptake by the plant, irrespective of the Cd treatment). Cd concentration decreased in the order: roots>stems> leaves, however no significant difference was found in the Cd content of stems and leaves. The gradient of Cd concentration across the plant indicates poor Cd translocation from roots to shoots.
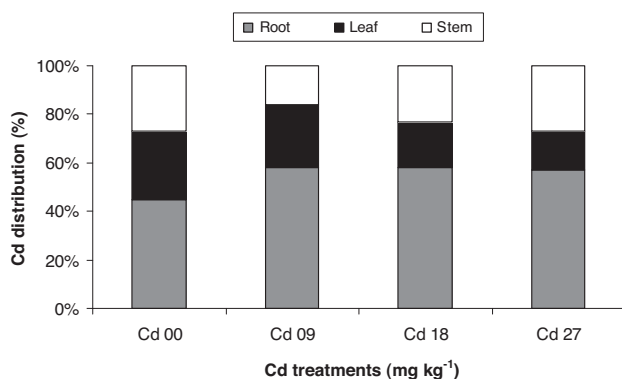
Figure 4. Cd distribution in various plant organs of *L. uncinatus* after 14 weeks of growth in presence of different Cd treatments.

Discussion

Plant height, number of leaves and dry matter yield

A similar reduction in plant height and leaf production as compared to control plants was reported by Pastor *et al*. (2003) in case of *Lupinus albus* L. grown in an acid soil in the presence of varied Zn supply levels.

It is noteworthy that the symptoms appeared after the application of third Cd dose which implies that the plant managed to tolerate the preceding Cd treatments. Other heavy metals have also been reported to affect the plant growth. Kidd *et al.* (2004) found that increment in plant height and leaf production was completely arrested in five populations of *Cistus ladanifer* in response to 250 μM Ni and 500 μM Co.

The decrease in dry matter (Table 3) may be attributed to reduced plant height and leaf loss. Zornoza *et al.* (2002) while growing *L. albus* cv Multolupa hydroponically in the presence of 0, 18 and 45 μM Cd noted a 38 and 15% reduction in shoot and root dry weights respectively for 45 μM Cd treatment as compared to the control. The decrease in yield may also be due to the toxicity caused by high plant available fraction of Cd in soil as we observed in an earlier soil incubation study using the same soil (Ehsan *et al.,* 2007).

Metal tolerance

The presence of 9 and 18 mg Cd kg^{-1} treatments did not affect considerably the metal tolerance of the plant with indices of 88% and 82% respectively, while a con-

siderable decrease in metal tolerance can be noticed in case of 27 mg Cd kg^{-1} treatment. The highest treatment affected the plant growth of *L. uncinatus* as also evident from significant reduction in other growth parameters like plant height and number of leaves (Table 3), as well as appearance of visible toxicity symptoms (Figure 1). Ximenez-Embun *et al.* (2002) reported a metal tolerance index close to 100% for *L. albus* when grown for 4 weeks in sand contaminated with 50 mg L^{-1} of Cd (NO$_3$)$_2$ solution. However this difference in plant response may be attributed to difference in metal exposure period, plant genotype, metal species or growing medium.

Cd accumulation and transport in plant organs

Cd hyperaccumulation is defined as plant species capable of accumulating more than 100 mg Cd kg^{-1} dry matter in the shoot dry weight (Baker *et al.,* 2000). In most plant species, Cd concentration is generally lower than 3 mg Cd kg^{-1}, but may reach 20 mg kg^{-1} or more in the Cd enriched soils. A plant concentration of >100 mg kg^{-1} may be regarded as exceptional, even on a Cd-contaminated soil (Reeves and Baker, 2000). A Cd accumulation of 4900 mg kg^{-1} in the shoots of *L. albus* was reported by Ximenez-Embun *et al.* (2002) when grown in sand contaminated with 50 mg L^{-1} of Cd (NO$_3$)$_2$ solution.

Plant species differ greatly in their ability to take up and transport Cd within the plant. Difference in Cd accumulation capacity and localization appear to be major factors in determining plant tolerance to Cd exposure (Obata and Umebayashi, 1993). The different mobility of metals through the plant can be related to the shoot/root ratio (Shahandeh and Hossner, 2000).

The results indicate that the majority of Cd was accumulated in the roots, particularly for the highest Cd treatment, suggesting a strong Cd retention during its long-distance transport from roots to shoots which might be a plant mechanism to tolerate the metal stress (Zornoza *et al.*, 2002). The same results have been reported for *L. albus* (Ximenez-Embun *et al.*, 2002; Zornoza *et al.*, 2002) and other crops e.g *Betula pendula* Roth.(Gussarsson*,* 1994), *Phaseolus vulgaris* L.*, Oryza sativa* L., *Brassica oleracea* L. and *Zea mays* L. (Guo and Marschner, 1995). The restricted Cd transport from roots to shoots reduces the Cd concentration in grains much more than in leaves, and consequently lupin seeds should contain only very low levels of Cd, which is an advantage for seed consumption (Page *et al.*, 2006a).

In view of the results obtained in this study, *L. uncinatus* can be used to remediate polluted soils by mechanisms other than phytoextraction. The prompt restoration of a dense vegetation cover is the most useful and widespread method to stabilise contaminated areas like mine wastes and to reduce effects of metal pollution (Bargagli, 1998). The amounts of Cd extracted by *L. uncinatus* are insufficient to use this species for phytoextraction. The excluder behaviour of *L. uncinatus* for Cd lead to higher concentration of this element in roots. These results suggest that this species can be utilized for the phytostabilization and revegetation of the Cd contaminated soils.

Cadmium distribution in different plant organs

The strong retention of Cd observed in the roots of *L. uncinatus* is in agreement with the previous results obtained while observing the response of lupin plants to heavy metal stress (Römer *et al*., 2000, 2002; Ximenez-Embun *et al.,* 2002; Zornoza *et al.*, 2002; Page *et al.,* 2006a,b; Vazquez *et al.,* 2006). Cadmium being a non-essential and pollutant heavy metal found in different concentrations in soil (Sauvé *et al.,* 2000; Lugon-Moulin *et al.,* 2004) is recognized as a toxic compound at the root level and, as it is not needed in the shoot, the plant sequesters it in the roots to avoid damage to the shoot. Moreover distribution between roots and shoots differs with plant species, rooting medium and period of exposure. Some environmental factors such as Cd concentration in the medium, ambient temperature, and light-intensity can affect the distribution of the metal between the shoots and roots (Chino and Baba, 1981).

The treatment of Cd-polluted soils is a priority keeping in view the high mobility and ecotoxicity of this trace element. However, few techniques combine efficiency, soil preservation and low cost. Phytoremediation may be an effective and efficient solution to these requirements. In the present study the period of plant growth under metal stress was 14 weeks and toxicity symptoms were only seen after the third metal dose in the last three weeks (after a total of 11 weeks Cd exposure) of the plant growth. Metal tolerance indices obtained indicate tolerance of the plant species for Cd treatments of 9 mg kg^{-1} and 18 mg kg^{-1}. The roots of *L. uncinatus* absorbed substantial quantities of Cd especially in case of the highest treatment (27 mg Cd kg^{-1}). Keeping in view all these considerations, it may be concluded that this species can be used in the phytostabi-

lization of Cd by immobilizing it, mainly in roots during each growing season. The Cd tolerance coupled with the ability of *L. uncinatus* to grow under various environmental stresses, nitrate excess, low root temperature, detopping, lime excess and salinity make this species an excellent candidate to form an important part of plant material for phytoremediation research.

The present investigation was the first attempt to determine the growth performance of Mexican native *L. uncinatus* under Cd stress, their degree of tolerance to varying Cd treatments in soil and their pattern of metal accumulation. Further research, however, is vital for comprehensive understanding of the mechanisms involved in response functions of the *L. uncinatus* to Cd stress.

Acknowledgements

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