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Lupinus versicolor Response in Soils Contaminated with Heavy Metals From a Petroleum Extraction Field

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Abstract: Phytoremediation uses the survival capacity of certain plant species in environments contaminated with heavy metals to extract such metals. The objective of this study was to evaluate the response of Lupinus versicolor as a biological agent in soils contaminated with Cu, Pb, Cd, Ni and Zn. The present study was conducted in a greenhouse. The experimental design was a completely randomized block with 6 replications. Results showed that the plants accumulated as much as 0.12 mg kg^{-1} of Cu within foliage, 0.94 mg kg^{-1} of Pb within stems, 0.31 mg kg^{-1} of Cd within stems, 1.03 mg kg^{-1} of Ni within stems and 2.46 mg kg^{-1} of Zn within the leaves, being these values higher than those found by other researchers. In this experiment, favorable results were obtained for the accumulation of Cd in the leaves, Ni in the stems and Cu in the roots. Therefore, based on the results obtained, *L. versicolor* is a plant that can be considered as a good alternative for removing heavy metals in contaminated soils and for the phytostabilization and revegetation of contaminated soils.

Key words: *Lupinus*, soils, heavy metals, phytoremediation

INTRODUCTION

The wide spread heavy metal contamination poses long-term risks to environmental quality and sustainable food production (Weckx and Clijsters, 1996). Oil extraction processes and spills contaminate air surrounding wells, causing severe damages to the atmosphere and producing ground contamination. This is a problem that frequently occurs in Mexico, causing physical alterations and changes in the structure of the soil and contamination by heavy metals since most of the components of petroleum are relatively insoluble, accumulating within the ground (Assmuth and Strandberg, 1993). Contamination with petroleum is characterized by its persistence in the ecosystem, in spite of natural and/or anthropogenic degradation processes to which it may be subjected (Nadal *et al*., 2004). In the same way, it produces a series of harmful wastes which are soil contaminants. Among these are toxic components like lead, mercury, arsenic, selenium, cadmium, nickel, copper and zinc (Bizily *et al*., 2000).

The excess of heavy metals and metalloids released from mine tailings may cause severe damage to ecosystems including plants, animals, micro-organisms and human health. Uncontrolled mining activities can generate a large amount of particulate emissions and waste containing heavy metals and metalloids that can contaminate the surroundings-soil, water and air. Such effects may be particularly serious and may pose a severe ecological and human health risk when mining activities are located in the vicinity of urban environments. Therefore, it is necessary to minimize or mitigate the impacts of resource utilization to the extent reasonably feasible (Nazmul *et al*., 2009).

Remediation of contaminated soil is possible by amending such soils with agricultural materials, as soil metals are adsorbed onto the amendment materials, immobilizing such metals and thus, reducing their soil and plant availability (Clemente *et al*., 2005).

Bioremediation is one of the most economic alternatives for soil and aquifer restoration (Schwartz *et al*., 2003; Zhao *et al*., 2003). This technique includes phytoremediation which uses the capacity of certain plant species to survive in environments contaminated with heavy metals and organic substances while extracting, accumulating and immobilizing or transforming the contaminants (Clemente *et al*., 2005; Menzies *et al*., 2007) by altering its molecular structure (Keller *et al*., 2003). It is for this reason that phytoremediation is a natural, clean and economic

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alternative treatment to other physical and chemical processes which are more invasive to soil recuperation (Wenzel *et al*., 2003). Thus, phytoremediation, technique using plant 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 (Eshan e*t al*., 2009; Luo *et al*., 2008).

One of the plant groups used in phytoremediation includes legumes, which fix nitrogen, improve soil fertility and help other plant species colonize the site (Vazquez *et al*., 2006). The importance of legumes is not only deals with the biological fertilization it carries out, but it goes way beyond. In fact, legumes comprise the most used plant group by man, only exceeded by grains (Pastor *et al*., 2003).

In this study, the potential of *Lupinus versicolor* to improve degraded soils was analyzed provided that it grows along roadsides and in degraded forests. Since, the biological processes that are frequently applied to the treatment of soils contaminated with hydrocarbons is under investigation, the capacity of *Lupinus versicolor* as a biological agent for phytoremediation of soil contaminated by hydrocarbons and its capacity to extract heavy metals were explored.

MATERIALS AND METHODS

The present study was carried out under greenhouse conditions from February to April of 2008. The temperature in the greenhouse ranged from 33-44°C during daytime and from 12-19°C during nighttime. The contaminated soil used was collected at a petroleum field in Tabasco State, Mexico. *Lupinus versicolor* seeds were obtained from the Tláloc Mountain, in the Nevada Mountain Range, within the municipality of Texcoco, State of Mexico, at 3016 m.a.s.l., on slopes of 25-45%, 19° 26' 21.8'' N and 98° 45' 43'' W (Alderete *et al*., 2008).

Pots with a capacity of 4 kg were filled with the soils, including their respective controls. Soil samples were dried and sifted through a wire 2 mm mesh and in the laboratory the following properties were determined: bulk density by the test tube method, soil texture by Boyoucus, pH using a potentiometer in a soil-water ratio of 1:2. Subsequently, the total content of Cu, Zn, Pb, Ni and Cd was determined using the DTPA extractor solution 0.005 M-TEA 0.1 M-$CaCl_2$ 0.01 M along with the physical and chemical properties (Table 1). Under greenhouse conditions ten seeds of *L. versicolor* were placed in each pot. Pots were irrigated every other day with tap water. The experimental design used was a complete randomized blocks one with six replications.

Table 1: Physicochemical characteristics of the control and contaminated soil

Characteristics	Control	Contaminated
Sand (%)	43	45
Loam (%)	36	40
Clay (%)	21	15
Textural class	F	F
Gravimetric moisture	6.6	5.5
Field capacity (%)	35.6	34.6
Permanent wilting point (pwp)	16.7	16.6
Electrical (C. Sm^{-1})	2.50	3.95
Hydraulic (C. Cm h^{-1})	0.79	1.76
Saturation P (%)	66.7	71.1
Bulk density (g cm^{-3})	1.15	1.14
Real density (g cm^{-3})	2.28	2.36
1:2 pH	6.16	6.29
Cu (mg kg^{-1})	480	660
Zn (mg kg^{-1})	1400	2200
Pb (mg kg^{-1})	300	1560
Cd (mg kg^{-1})	120	140
Ni (mg kg^{-1})	580	680

The experiment lasted 80 days following the emergence of the first plants. At the end of the experiment, plants were uprooted and washed, firstly with tap water and then with distilled water. Thereafter, plants were separated into roots, stems and leaves to determine fresh weight. Then, plant parts were placed in paper bags and dried in a heat cabinet at 70°C for 72 h to determine the dry weights. Plant tissues were then sent to the laboratory for chemical analyses.

The determination of Cu, Zn, Cd, Pb and Ni from roots, stem and leave extracts of *L. versicolor*, was done by the wet digestion method (Ussiri and Rattan, 2008). Readings were made using an automatic absorption spectrophotometer Pekín-Elmer, 3110 and element concentrations were given on dry matter basis.

The experimental design used was completely randomized blocks with six replications. The SAS (2003) program for microcomputers, was employed to conduct the ANOVA test (Proc Mixed) and mean comparisons (significant minimum difference) also, with the SAS program. The treatments were considered as fixed effects, the blocks as random effects.

RESULTS

The plants grown in contaminated soils extracted Cu in dry matter at concentration of 0.12 mg kg^{-1} in leaves, followed by stems with 0.08 mg kg^{-1} and roots, with 0.05 mg kg^{-1}, respectively. In the control plants, Cu extracted in the leaves 0.04 mg kg^{-1}, followed by the stems with a concentration of 0.03 mg kg^{-1} and the roots with 0.01 mg kg^{-1} (Fig. 1).

In the case of Zn extraction, the highest accumulation in the plants under treatment was in the leaves with 2.46 mg kg^{-1}, followed by the roots with 0.81 and 0.7 mg kg^{-1} in the stem. In the control plants, the

Table 2: ANOVA heavy metals in roots, stems and leaves of *L. versicolor* growing in contaminated soil

Variables	SS effect	df effect	MS effect	SS error	df error	MS error	F-value	p-value
Metal	18.603	4	4.6507	24.875	175	0.1421	32.718	0.000
Plant parts	1.2224	2	0.6112	42.256	177	0.2387	2.5602	0.080
Soil	5.1928	1	5.1928	38.286	178	0.2150	24.142	0.000

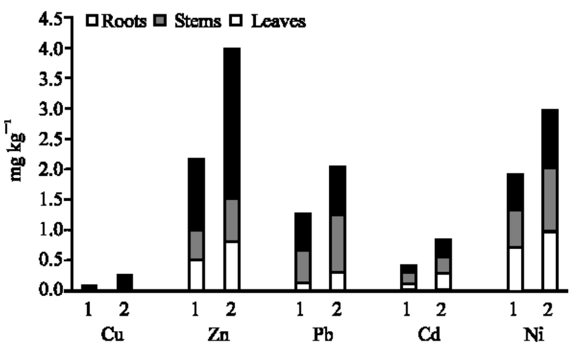

Fig. 1: Concentrations of heavy metals in *L. versicolor* tissues growing in contaminated soil compared with control. 1: control plants, 2: treatment

highest extraction of Zn was 1.18 mg kg^{-1} occurring within the leaves, followed by 0.48 mg kg^{-1} in the stem and 0.05 mg kg^{-1} in the roots (Fig. 1).

The highest extraction of Pb in plants from contaminated soil occurred within stems with 0.94 mg kg^{-1}, followed by the leaves with 0.78 mg kg^{-1} and the roots with 0.31 mg kg^{-1}. In this case in the control plants the leaves had the highest Pb accumulation with 0.61 mg kg^{-1}, followed by the stem with 0.53 mg kg^{-1} and the root with 0.13 mg kg^{-1} (Fig. 1).

In plants under treatment, the highest Cd extraction of 0.31 mg kg^{-1} occurred in the stems, followed by the roots with 0.28 mg kg^{-1} and the leaves with 0.25 mg kg^{-1}. In the control plants the leaves showed the highest extraction reaching 0.12 mg kg^{-1}, while the stems had 0.17 mg kg^{-1} and the roots 0.13 mg kg^{-1} which represent 28.6, 40.5 and 30.9%, respectively (Fig. 1).

In the case of Ni, the highest extraction was found in the stem with 1.03 mg kg^{-1}, followed by the roots with 0.98 mg kg^{-1} and the leaves with 0.96 mg kg^{-1} in plants from accumulated soil. In the control plants Ni was accumulated mostly in the roots, with 0.7 mg kg^{-1}, followed by the stem with 0.63 and 0.56 mg kg^{-1} in the leaves (Fig. 1). According to the analysis of variance for these heavy metals significative differences were found $p<0.05$ (Table 2).

DISCUSSION

Results similar to the ones found in this study were reported for *Pergularia tormentosa* by Al-Farraj and Al-Wabel (2007). This indicates that this specie accumulates high concentrations of Cd, Cu, Pb and Zn in its tissues when grown in natural conditions in contaminated soils. This is due to the fact that these elements are moderately transposable and they are absorbed only if available (Alonso *et al*., 2002).

Chaney *et al*. (2004) reported Pb, Cd and Zn concentrations in leaves of vegetables cultivated in contaminated soils, which indicated that when grown in contaminated soils, the ecotype Lanestosa of the hyper accumulative species *Thlaspi caerulescens* accumulated Zn in the stem and not in the leaves given that this element is easily transposable to the plant (McGrath *et al*., 2006).

Martínez *et al*. (2006) reported that species of the genus *Thlaspi* and *Amaranthus retroflexus* have the capacity to accumulate Pb in their vegetative structures roots. According to Grill *et al*. (1985), this may be due to the low mobility of Pb, when it is found in low quantities in the soil, given that the root systems prevents the migration of Pb towards the above ground part of the plant and it only reaches this part if it is found in high concentrations.

Ehsan *et al*. (2007, 2009) reported the accumulation of Cd in *Lupinus uncinatus* under hydroponic conditions. According to Chaney *et al*. (2004), this element is easily absorbed by the roots and can be easily transposed to the entire plant. This also, depends on the age and origin of the plant.

Ghaderian *et al*. (2007) reported that *Alyssum bracteatum* has the capacity to accumulate Ni in certain parts of the plant. Li *et al*. (2003) obtained similar results in two selected species of *Alyssum* sp. and found that they accumulate Ni in the leaves bud and not in the stems. This is because Ni is rapidly absorbed by the roots and can be accumulated in high concentrations in different parts of the plant.

According to the results obtained in this study, the metals Cu, Pb and Zn were found in concentrations within the acceptable ranges (10.9±0.0, 3.5±0.20, 26.9±1.2, respectively). The Cd and Ni surpassed the permissible limits (0.175±0.027 and 2.88±0.38, respectively) which contradicts the findings of Ghaderian *et al*. (2007).

CONCLUSION

Based on the results of this study *Lupinus versicolor* is a plant which can be considered for extracting heavy metals and can be utilized for the phytostabilization and revegetation of contaminated soils, specifically with Cd, Ni and Cu. In this experiment, favorable results were obtained for the accumulation of Cd in the leaves and Ni in the stem. Cu extracted within the roots was significantly higher with respect to the control plant, surpassing the permissible critical limits. However, further research is vital for comprehensive understanding of the mechanisms involved in the response functions of the *L. versicolor* to heavy metals stress.

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