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Phytoextraction of Metal Contaminants by *Typha angustifolia*: Interaction of Lead and Cadmium in Soil-Water Microcosms

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ABSTRACT

A greenhouse study was conducted on phytoextraction and accumulation of lead (*Pb*) *and cadmium* (*Cd*) *from contaminated soil – water microcosms by the narrow-leaved cattail*, *Typha angustifolia. The plants were grown in sandy loam soil containing* 1,666 *and* 38.5 *mg/L of* $Pb(NO_3)_2$ *and* $Cd(NO_3)_2$ *respectively. The trends of lead and cadmium by T. angustifolia for all soil – water microcosms suggested interaction effects as decreased soil lead concentrations and increased water cadmium concentrations over time. T. angustifolia expressed trends as increased biomass in all contaminated shoots and roots examined. Cadmium uptake in shoot and root biomass slightly decreased when lead was initially added to the soil but cadmium uptake in root biomass increased after* 30 *days. Data suggested an interaction between lead and cadmium and that lead uptake was inhibited when cadmium was present.*

Keywords: *Phytoextraction*, *Contaminant Interaction*, *Lead*, *Cadmium*, *Microcosm*, *Typha angustifolia*

1. Introduction

Lead and cadmium are elements that are highly toxic to plants [1]. Both heavy metals are capable of interacting in biological systems and are persistent contaminants that can change their speciation, but do not biodegrade. Cadmium is the heavy metal of greatest concern in most agricultural soils. It is loosely held by soil constituents and is readily available to plant roots and transported through the xylem to the vegetative and reproductive organs, negatively affecting the crops [2]. In general, broadleaf plants such as lettuce and swiss chard accumulate more cadmium than grasses. Plant leaves and stems can also accumulate more than seeds. Nevertheless, lead and cadmium can be toxic and inhibit DNA synthesis, mitosis, cell division and germination [3]. Iqbal *et al.* [3] also investigated the effect of lead and cadmium individually and in combination on the germination and seedling growth of *Leucaena leucocephala* and *Delonix regia.* They found reduced germination and seedling growth, and that both species showed more tolerance to lead than cadmium. Kastori *et al.* [4] found that the treatment of sunflower plants with lead diminished the concentrations of chlorophylls and carotenes, while Larsson *et al.* [5] observed a reduction in the chlorophyll concentrations of plants exposed to cadmium. Mohan and Hosetti [6] suggested that both cadmium and lead drastically depressed catalase activity but stimulated peroxidase activity. They reported that the interaction between lead and cadmium on growth of other plants e.g., the root of *Juncus acustus,* was strongly inhibited by lead nitrate. Iqbal *et al.* [3] reported that the toxicity of lead and cadmium to young trees of *Fagus silvatica* is higher when both are combined. Other reports indicated that these two metals can bind to the cell wall, thus weakening their toxic effects to plants [7].

Phytoremediation is the use of plants to remove contaminants from soil, water and air. One promising phytoremediation process is the phytoextraction of heavy metal contaminants from soil. *Typha* spp has been studied for their ability to phytoextract metals from soil, but most research to date reports the phytoextraction of individual heavy metal contaminants rather than mixtures of metals that commonly occur at contaminated sites. This

study examined the interaction of lead and cadmium during metal phytoextraction from soil-water microcosms by *Typha angustifolia*.

2. Materials and Methods

2.1. Plant Microcosm

T. angustifolia were obtained from New England Wetland Plant Company and planted in plastic bucket microcosms (50 cm height, 30 cm diameter) containing 10 kg of sandy loam soil, collected from Orchard Hill, Massachusetts, USA. Sixteen buckets of microcosms were prepared, each with 2 plants in 10 kg of soil with a 5 liter layer of surface water. The microcosms were maintained in a greenhouse at a temperature of 24 ± 1℃ under a full spectrum of 400 W light source providing a 16 hour per day photoperiod. The plants in microcosms were allowed to grow for 30 days until the plants reached an average height of 30 cm and then received the heavy metal treatments.

2.2. Soil Characterization

One to three gram samples of soil were dried overnight in an oven at 105℃. Soil samples were sieved through 2mm and 0.5 mm screen, thoroughly mixed and ground in a mortar and pestle to obtain a uniform texture. Soil subsamples of 0.1-0.3 g were digested in 3 ml of concentrated HNO_3 using microwave digestion (CEM model MDS – 2100) for approximately 30 minutes. Samples were then evaporated to near dryness, filtered, and adjusted to a volume of 25 ml in distilled water. Soil samples were analyzed for lead and cadmium concentration before and after the addition of lead and cadmium mixtures using Flame Atomic Absorption Spectrophotometer (FAAS), Graphite Furnace Atomic Absorption Spectrophotometer (GFAAS), and Hydride Generation (HG).

2.3. Preparation of Stock Solutions and Application of Lead and Cadmium

Lead as $Pb(NO_3)_2$ and cadmium as $Cd(NO_3)_2$ were dissolved in distilled water to prepare a stock solutions for treatment groups at concentrations of 10,000 mg/L and 1,000 mg/L, respectively. One liter of the lead stock solution was added to 5 L of distilled water to obtain a concentration of 1,666 mg/L for application to each microcosm. Two hundred ml of the cadmium stock solution was added into 5 L to obtain a concentration of 38.5 mg/L. The sixteen microcosms were randomly divided into 4 treatment groups, each group with four replicates as follows:

Group 1 served as the controls (no additions of lead and cadmium),

Group 2 received cadmium solutions at a concentration of 38.5 mg/L,

Group 3 received lead solutions at a concentration of 1,666 mg/L,

Group 4 received a mixture of lead at 1,666 mg/L and cadmium at 38.5 mg/L.

2.4. Plant Analysis

Plant samples were harvested every fifteen days after heavy metal treatment. The whole plant was washed thoroughly with running tap water, divided into shoots and roots, and weighed, dried in an oven at 80℃ for two days and weighed again. Dried plant tissues were cut into small pieces, and homogenized. Approximately 0.1-0.3 g of shoot and root material were digested in a 3 ml concentration of HNO_3 and microwave digestion (CEM model MDS – 2100) for 30 minutes. Samples were evaporated to near dryness, filtered, and adjusted to a volume of 25 ml distilled water. The sample solutions were collected in polypropylene bottles and measured using FAAS, GFAAS, and HG.

2.5. Water Analysis

After fifteen days of treatment, 5 ml water samples were collected from the control and the treatment groups. The water samples were adjusted to a volume of 25 ml, collected in polypropylene bottles, and analyzed for lead and cadmium using FAAS, GFAAS, and HG.

2.6. Data Analysis

Data were analyzed using Excel analysis of variance (ANOVA) for significant differences ($P \leq 0.05$). Duncan's New Multiple Range Test was used to determine significant differences ($P \leq 0.05$).

3. Results

3.1. Soil and Water Characteristics

The basic characteristics of the soil used in the soil-water microcosms are presented in **Table 1**. The results indicate that the soil is a sandy loam typical of many ecosystems. At the end of the experiment, the soil and water were examined in each microcosm for total lead and cadmium.

Table 2 and **Figures 1** and **2** summarized the lead and cadmium concentrations for all soil – water microcosms after 15 days. The results indicated that soil lead levels of Group 4 microcosms were higher than Group 3 microcosms (1061 ± 476.6 as compared to 841.7 ± 39.82 mg/kg), but the water lead levels were slightly different,

Table 1. Physical and chemical characteristics of microcosm soils.

Soil properties	
Clay (%)	6-18
Moist bulk density (g/cm^3)	1.3-1.6
Permeability (h)	0.6-6.0
Available water capacity (in)	0.1-0.16
Soil pH	3.6-6.0
Organic matter (%)	2-6
Total soil Pb (mg/kg)	8.71
Total soil Cd (mg/kg)	0.38
Total water Pb (μg/L)	9.96
Total water Cd (mg/L)	ND

ND: Not detected

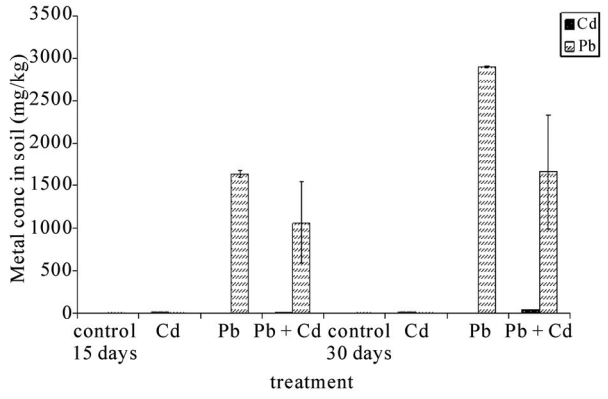

Figure 1. Cadmium and lead concentrations in soil microcosms (mg/kg).

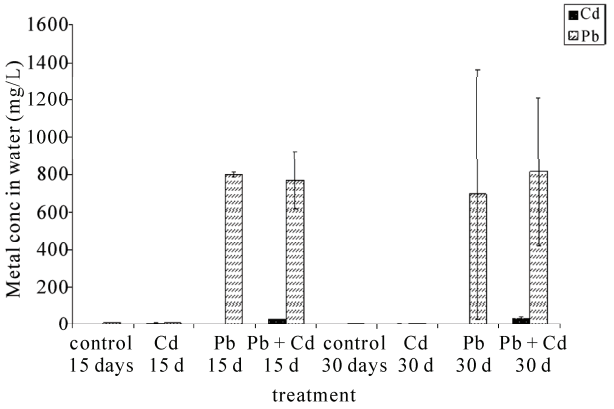

Figure 2. Cadmium and lead concentrations in water microcosm (mg/L).

thus there was a significant difference in mean lead in water ($P \leq 0.05$). Group 2 microcosms soil cadmium levels were approximately 13 ± 0.5 mg/kg as compared to 39.8 ± 2 mg/kg in Group 4 microcosms indicating that soil cadmium level increased 3 folds. There was a significant difference in mean lead in soil ($P \leq 0.05$). The water cadmium level in Group 4 microcosms (24.2 ± 2.6) was higher than Group 2 microcosms (4.8 ± 1.1). There was a significant difference in mean cadmium in water ($P \leq 0.05$).

After 30 days, Group 4 microcosms had soil lead levels of approximately 1,658 ± 663 mg/kg as compared to 998 ± 11 mg/kg in Group 3 microcosms and the soil lead level in Group 4 microcosms was higher than Group 3 microcosms. The water lead level in Group 4 microcosms (814.8 ± 396.2) was higher than Group 3 microcosms (697.4 ± 668.9). Cadmium accumulation in soil decreased when lead was added to the soil (14.3 ± 1.3 as compared to 6.5 ± 0.6 mg/kg). There was a significant difference in mean cadmium in soil ($P \leq 0.05$). Group 4 microcosm water cadmium levels were 28.6 ± 11.8 mg/L as compared to 1.3 ± 0.6 in Group 2 microcosm water. The statistical trends suggested interaction effects between the two metals as increased soil lead concentrations and decreased soil cadmium concentrations.

3.2. *T. Angustifolia* Growth Characteristics

The growth patterns of *T. angustifolia* expressed as fresh and dry weights are summarized in **Table 3** and **Figures 3** and **4**. Statistical trends indicated increased biomass over time in all contaminated and control soil microcosm. The lead and cadmium nitrate salts used as contaminants most likely explain the differences in control and experimental biomass. However, mean fresh and dry weights were not significantly different between treatment groups ($P > 0.05$) over the 30 day exposure period (**Figures 3** and **4**). Interestingly, in Group 3 and 4 the fresh and dry weight of shoot biomass increased while those of root biomass seem to unchanged (**Figures 3** and **4**). Group 2 plants had trends with increased fresh and dry weight shoot and root biomass.

3.3. Lead and Cadmium Accumulation in Plant Biomass

Concentrations of lead and cadmium in *T. angustifolia* shoot and root biomass are summarized in **Table 4** and **Figures 5** and **6**. The results on both 15 and 30 day treatments indicated that there was a lead and cadmium interaction which resulted in a marked reduction in lead uptake and a slight decrease in cadmium uptake by *T. angustifolia*.

Lead levels in shoot and root biomass in the combined

Table 2. Lead and cadmium concentrations in soil – water microcosms.

Time	Treatment (group)	Soil (mg/kg)[a]		Water (mg/L)[a]	
		Cd	Pb	Cd	Pb
15 days	1 (control)	[b]0.1 ± 0	[h]6.3 ± 0.7	[l]ND	[p,*]8 ± 0.7
	2 (Cd)	[c]13 ± 0.5	[h]10.3 ± 0	[l]4.8 ± 1.1	[p,*]7.5 ± 0.5
	3 (Pb)	[b]0.1 ± 0.1	[h]841.7 ± 39.8	[l,*]0.3 ± 0.1	[q]800 ± 12.5
	4 (Pb + Cd)	[d]39.8 ± 2	[i]1061.3 ± 476.6	[m]24.2 ± 2.6	[q]770 ± 149.2
30 days	1 (control)	[e]0.1 ± 0	[j]5.2 ± 0.1	[n,*]0.4 ± 0.2	[r,*]4.6 ± 0.3
	2 (Cd)	[f]14.3 ± 1.3	[j]5.1 ± 0.1	[n]1.3 ± 0.6	[r,*]2.5 ± 0.5
	3 (Pb)	[e]0.1 ± 0.1	[j]997.6 ± 11.4	[n,*]0.3 ± 0.1	[r]697.4 ± 668.9
	4 (Pb + Cd)	[g]6.5 ± 0.6	[k]1657.6 ± 663.2	[o]28.6 ± 11.8	[r]814.8 ± 396.2

*μg/L; [a]values are means from 2 replications with standard error (n = 2); [b,c,d]homogeneous subsets of soil cadmium in 15 days; [e,f,g]homogeneous subsets of soil cadmium in 30 days; [h,i]homogeneous subsets of soil lead in 15 days; [j,k]homogeneous subsets of soil lead in 30 days; [l,m]homogeneous subsets of water cadmium in 15 days; [n,o]homogeneous subsets of water cadmium in 30 days; [p,q]homogeneous subsets of water cadmium in 15 days; [r]homogeneous subsets of water cadmium in 30 days.

Table 3. Fresh and dry weight in shoot and root biomass of *T. angustifolia* in contaminated soil-water microcosms.

Time	Treatment (group)	Fresh weight (g)[a]		Dry weight (g)[a]	
		Shoot	Root	Shoot	Root
15 days	1 (control)	23.9 ± 2.2	22.3 ± 1	2.2 ± 0.2	2.3 ± 0
	2 (Cd)	26.7 ± 11.4	12 ± 5.3	2.5 ± 1.2	1.6 ± 0.4
	3 (Pb)	35.7 ± 19.9	11.1 ± 5.1	3.2 ± 1.8	1.6 ± 0.3
	4 (Pb + Cd)	48.4 ± 14.3	22.5 ± 2	5 ± 1.4	2.3 ± 0.1
30 days	1 (control)	37.3 ± 11.3	25.7 ± 4.7	4.1 ± 1.3	1.8 ± 0.2
	2 (Cd)	36.5 ± 5.3	19.5 ± 7.3	4.1 ± 0	1.6 ± 0.4
	3 (Pb)	77.6 ± 26	17.5 ± 2.1	10.7 ± 3.5	1.9 ± 0.3
	4 (Pb + Cd)	75.5 ± 22.7	24.2 ± 7.5	10.6 ± 4.4	2.1 ± 0.8

[a]values are means from 2 replications with standard error (n = 2).

Table 4. Concentration of lead and cadmium in shoot and root biomass of *T. angustifolia.*

Time	Treatment (group)	Pb (mg/kg)[a]		Cd (mg/kg)[a]	
		Shoot	Root	Shoot	Root
15 days	1 (control)	[b]2.8 ± 1.8	[f]6.6 ± 2.3	[j]ND	[m]0.6 ± 0.1
	2 (Cd)	[b]3 ± 0.4	[f]6.1 ± 0.1	[j]42.3 ± 14.9	[n]378.3 ± 141.5
	3 (Pb)	[c]1,875.9 ± 663.6	[g]22,462 ± 2,804.6	[j]0.7 ± 0.4	[m]0.6 ± 0.2
	4 (Pb + Cd)	[b]529.3 ± 57.8	[g]16,555 ± 2,854.5	[j]33.5 ± 24.7	[m,n]223.3 ± 18.7
30 days	1 (control)	[d]1.2 ± 0.2	[h]3.4 ± 0.2	[k]0.1 ± 0	[o]0.8 ± 0.1
	2 (Cd)	[d]1.1 ± 0.5	[h]4 ± 0	[l]20.3 ± 2.6	[o,p]241 ± 119.5
	3 (Pb)	[e]354.9 ± 22.3	[i]20,173.6 ± 2,165.6	[k]0.4 ± 0.2	[o]1.5 ± 0.7
	4 (Pb + Cd)	[e]404.3 ±2 1.1	[i]13,675 ± 3,925	[l]20.5 ± 1.9	[p]369.2 ± 82.3

[a]values are means from 2 replications with standard error (n = 2); [b,c]homogeneous subsets of lead in shoot in 15 days; [d,e]homogeneous subsets of lead in shoot in 30 days; [f,g] homogeneous subsets of lead in root in 15 days; [h,i]homogeneous subsets of lead in root in 30 days; [j]homogeneous subsets of cadmium in shoot in 15 days; [k,l]homogeneous subsets of cadmium in shoot in 30 days; [m,n]homogeneous subsets of cadmium in root in 15 days; [o,p]homogeneous subsets of cadmium in root in 30 days.

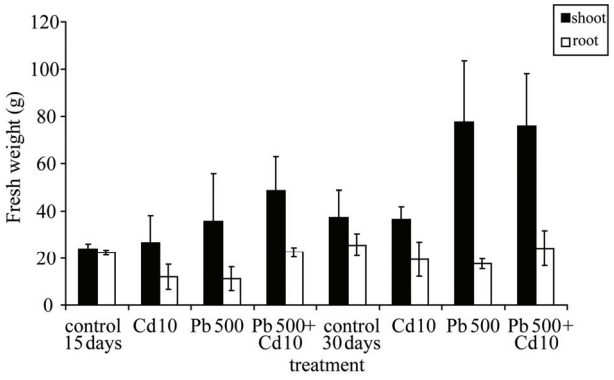

Figure 3. Biomass fresh weight (shoots and roots) (g).

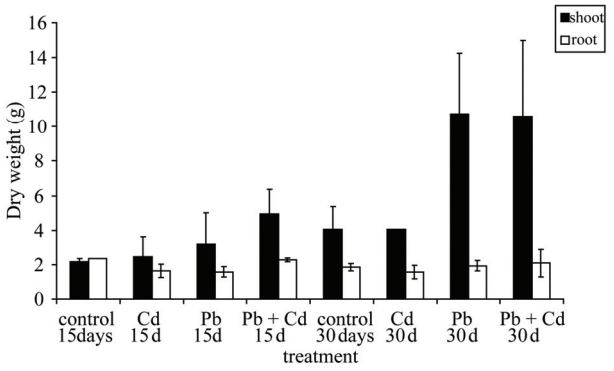

Figure 4. Biomass dry weight (shoots and roots) (g).

lead and cadmium microcosms decreased approximately two and one fold after 15 days of treatment Group 4 (529.3 ± 57.8) as compared to Group 3 (1,875.9 ± 663.6)). There was a significant difference ($P \leq 0.05$) in mean shoot and root lead accumulation of *T. angustifolia* at day 15. After 30 days, lead accumulation in shoot biomass of Group 4 was slightly higher than that of Group 3 (404.3 ± 21.1 as compared to 354.9 ± 22.3 mg/kg). In root biomass, lead concentration was lower in Group 4 as compared to Group 3 (13,675 ± 3,925 vs 20,173.6 ± 2,165.6 mg/kg). There was a significant difference ($P \leq 0.05$) in mean shoot and root lead accumulation of *T. angustifolia* in 30 days (**Figure 5**).

Cadmium uptake in shoot biomass decreased when lead was added to the soil in 15 days (**Table 4)** (Group 4 compared to Group 2). There was no significant difference ($P > 0.05$) in mean shoot but there was a significant difference in mean root cadmium accumulation of *T. angustifolia* in 15 days. After 30 days, cadmium uptake in root biomass increased when lead was added to the soil (Group 4 compared to Group 2). There was a significant difference ($P \leq 0.05$) in the mean shoot but there was no significant difference in mean root cadmium accumulation of *T. angustifolia* biomass in 30 days (**Figure 6**). The statistical trends suggested interaction effects with cadmium inhibiting lead uptake in both roots and shoots.

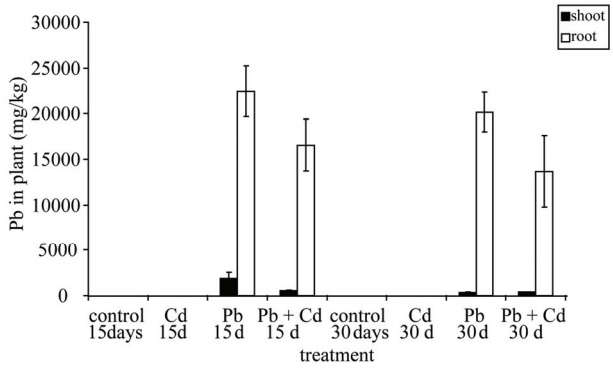

Figure 5. Concentration of lead in shoot and root biomass (mg/kg).

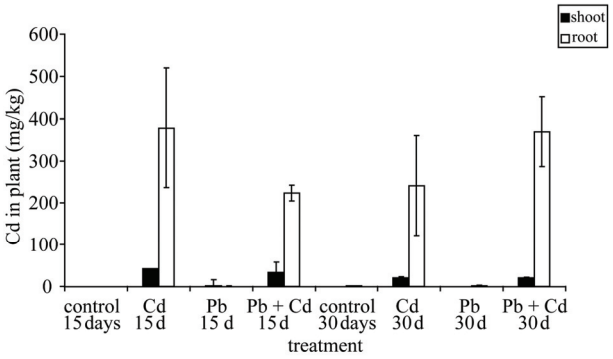

Figure 6. Concentration of cadmium in shoot and root biomass (mg/kg).

4. Discussion

In this study, lead treatments did not affect fresh or dry weights of *T. angustifolia*. Plants growing in microcosms with lead contaminated soils grew as well or better than plants in control microcosms. Cadmium treatment plants had fresh weight of shoot biomass similar to controls. It is possible that cadmium could be taken up by the roots and transported by the xylem to the vegetative and reproductive organs, negatively affecting plant health [8]. Plants could also accumulate high quantities of cadmium without suffering adverse effects on growth [9].

In the present study, increasing cadmium input to soil microcosm highly increased cadmium accumulation in shoot biomass and hence inhibited cadmium accumulation in root biomass. This is in agreement with the finding by Cunningham *et al.* [10] Plants grew well in the lead and cadmium contaminated soils in this study, however accumulated lower concentrations of lead a

30-day growth period.

Coughtrey and Martin [11] studied Midger plant (*Holcus lanatus*) uptake of cadmium, lead, and zinc in solution culture and showed interactions on lead concentrations in roots. Interestingly, the significant effects in shoots of this population were positive and tend towards increased metal concentrations. Miller *et al.* [12] reported positive interactions between lead and cadmium on lead and cadmium uptake in corn roots. Moshe *et al.* [13]. demonstrated that low levels (< 1 mg/L) of lead increased the toxicity of cadmium (0.1 mg/L) in phytoplankton. Antagonism occured when the concentration of lead exceeded that of cadmium but no synergistic effects were noted in *Chlorella* when cadmium, copper, chromium, and nickel were added to the culture media. Pretreatment of algae with nickel and mercury reduced cadmium toxicity; this may reflect competition among metals for cellular binding sites.

The results of this study provide evidence for phytoextraction interactions of lead and cadmium in soil-water microcosms. The statistical trends suggested that the interaction is complex and produced, decreased lead in root biomass and increased cadmium in shoot biomass although there were no significant variations in biomass production. Chukwuma [14] compared the accumulation of cadmium, lead, and zinc in cultivated and wild plant species in a derelict lead - zinc mine and found an overall reduction in the potential toxicity of cadmium by zinc through simple mass action effects specific for cultivated plants, they noted that other additional tolerant or adaptive mechanisms might be operative in the wild plants. McKena *et al.* [15] reported the interactions between zinc and cadmium in nutrient solution and their effects on the accumulation of both metals in plant roots and leaves and also reported higher cadmium concentrations in older compared to younger leaves of lettuce and spinach.

Many other studies involving several metals and nutrients have been reported. Lagerwerff and Biersdorf [16] reported that cadmium and zinc were competitive cations. Similarly, Robert *et al.* [17] showed that cadmium can functionally substitute for zinc. The changes in the iron and zinc concentrations induced by increased cadmium levels resulted in alterations of the iron/zinc ratio and the alteration was more pronounced in roots than shoots, with both tissues exhibiting increases in the iron/zinc ratio with increased concentrations of cadmium. On the contrary, the toxicity of cadmium has been linked to the fact that cadmium competes for similar active sites but does not functionally substitute for zinc [18].

Cadmium and lead are toxic heavy metals and zinc an essential element makes this association interesting as it raises the possibility that the toxic effects of cadmium may be preventable or treatable by zinc. Hinesly *et al.* [19] indicated that both cadmium and zinc uptake by plants were dependent on the pH of the growing media. Ravera [20] showed that cadmium had toxic effects in plants on photosynthesis and also indicated various changes in biological activities. Subsequent studies have confirmed these findings and extended the interaction to other toxic effects of cadmium like inhibition of cell proliferation, and cytotoxic action [21] and growth suppression in plants [22].

The biochemical mechanisms of cadmium - zinc interaction are unknown, but various cellular and subcellular processes like the ratio of the cadmium to zinc in the tissues, induction of synthesis of different types of metallothionein, alteration of absorption and tissue distribution of one metal by another, and competition at the level of zinc containing metalloenzymes are known to be involved in the interaction [23]. Minnie *et al.* [24] studied phytoextraction of soil cobalt using hyperaccumulator plants and found interaction and decreased uptake of nickel in the presence of cobalt. Homer *et al.* [25] also reported that the uptake of cobalt may suppress nickel uptake, indicating a possible synergistic or antagonistic relationship between the elements.

5. Conclusions

T. angustifolia exhibited very good potential for the phytoextraction of mixtures of lead and cadmium contaminants from soil-water microcosms. Although cadmium interaction appeared to reduce the uptake of lead in soil-water microcosms contaminated with both metals, uptake of lead into *T. angustifolia* roots was high, reaching levels of 13,675 ± 3,925 mg/kg after 30 days growth.

6. Acknowledgements

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