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THE ASSESSMENT OF TOLERANCE TO HEAVY METALS (Cd,Pb and Zn) AND THEIR ACCUMULATION IN THREE WEED SPECIES

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Abstract

Phytoextraction is gaining great attention as an alternative technique for remediation of heavy metal contaminated soils. A greenhouse study was conducted to assess heavy metal (Cd, Pb, and Zn) accumulation in three weed species viz., *Avena sterils, Isatis tinctoria* and *Xanthium strumarium*. A range of phytoavailable Cd, Pb and Zn concentrations in soils was created by applications of five different levels of Cd (0, 25, 75, 150 and 300 mg kg^{-1}), Pb (0, 100, 200, 400 and 800 mg kg^{-1}) and Zn (0, 150, 300, 600 and 1200 mg kg^{-1}). Data indicated that in terms of relative yield, the most sensitive weed among the species used in this study was *A. sterils*. On the other hand, the most tolerant one was *I. tinctoria*. Increasing metal concentrations in soils increased shoot metal concentrations. Shoot Cd concentrations ranged from 2 to 93 mg kg $^{-1}$ for *I. tinctoria*, <0.1 to 77 mg kg $^{-1}$ for *A. sterils* and <0.1 to 6 mg kg $^{-1}$ for *X. strumarium.* The highest shoot Pb concentration was in *I. tinctoria* having no significant relative yield decrease, whereas *A. sterils* and *X. strumarium* had a closer Pb accumulation in shoots. *A. sterils* had significantly higher shoot Zn concentrations compared to *I. tinctoria* and *X. strumarium*. Shoot Zn concentrations varied from 24 to 264 mg kg $^{-1}$ for *A. sterils*, 10 to 101 mg kg $^{-1}$ for *I. tinctoria* and 28 to 48 mg kg $^{-1}$ for *X. strumarium*. *I. tinctoria* extracted the highest metals among the three weed species used in this study.

Introduction

Contaminated soils with heavy metals can potentially lead to the uptake and accumulation of these metals in the edible plant parts causing risk to human and animal health (Gupta & Gupta, 1998; Jarup, 2003; Ghosh & Singh, 2005; Gisbert *et al.*, 2006). Agricultural practices such as fertilization and pesticides application often result in some heavy metal deposition in soils (Kaplan *et al.*, 2005).

Most of the plant species including crops and weeds cannot survive on polluted sites due to toxic effects of heavy metals (Wong, 2003). Thus, it is urgently necessary to remediate heavy metal contaminated soils (Wei *et al.*, 2005). There are several remediation methods such as soil dressing, soil washing and replacement of polluted soils, but most of them are too expensive and time consuming and also require huge amount of water and unpolluted soil (Abe *et al.*, 2006). Therefore, phytoremediation has attracted great attention as a new and inexpensive technology (Salt *et al.*, 1998).

Some weed species have hyperaccumulator properties, and they can survive in highly polluted soils and exclude metals from the soil. Compared with crops, weeds often possess stress resistant properties and can maintain their growth under adverse water and fertilizer conditions as well as heavy metal polluted soils (Wei *et al.*, 2005). There are 400 taxa of terrestrial plants which have been identified as hyperaccumulator of various heavy metals (Zhao *et al.*, 1998). Plants can be classified into three categories in relation to their ability to absorb, accumulate and tolerate heavy metals within their tissues as hyperaccumulator, indicators and excluders (Wagner & Yeargen, 1986; Ghosh & Singh, 2005). Hyperaccumulator plants more often produce low biomass. Indicator plants have lower metal bioaccumulation in comparison with the hyperaccumulators but have at least 10 times more biomass production, so that the actual amount of extraction is relatively higher (Ghosh & Singh, 2005). Excluders produce rather high biomass but accumulate lower amount of metals. They produce biomass up to 30 tones per hectare (Robinson *et al.*, 2000).

In the present study, three weed species were selected, and two of them viz., *A. sterils* and *I. tinctoria* are common in winter crops such as wheat, barley, lentil. The other one *X. strumarium* commonly grows in summer season crops such as cotton, corn, soybean, sesame and peanut. Abe *et al.*, (2006) reported that *X. strumarium* is more tolerant and produces huge biomass per unit area and *Avena fatua* can be classified as tolerant to Cd.

The aim of this study was to determine heavy metal tolerance and accumulation of three weed species that commonly grow in the fields of some potential crops.

Materials and Methods

The taxonomic classification of the soil used was clay, mixed, Thermic, Typic Udifluvent. The soil had clay texture with a pH value of 7.8, containing 0.5 % organic matter and 25% of $CaCO_3$. Total metal concentrations of the soil were 2 mg Cd kg^{-1}, 0.5 mg Pb kg^{-1}, and 31 mg Zn kg^{-1}. Soil was air dried and sieved through a stainless steel 2-mm screen. The soils were amended with $Cd(NO_3)_2$, $Pb(CH_3COO)_2$, and $ZnSO_4$ to create different levels of Cd, Pb and Zn phytoavailability. The treatments included three weed species viz., *A. sterils, I. tinctoria* and *X. strumarium* and five different levels of Cd (0, 25, 75, 150 and 300 mg kg^{-1}), Pb (0, 100, 200, 400 and 800 mg kg^{-1}) and Zn (0, 150, 300, 600 and 1200 mg kg^{-1}). Air dried and sieved soils were mixed well with desired concentrations of metals and left to seven days for incubation. At the end of the incubation, initial soil samples were obtained for analysis.

A greenhouse study was conducted. To avoid free drainage, pots were lined with a cellophane bag and 1 kg of incubated soils were then added and mixed again in the pots. Deionized water was used to adjust the gravimetric moisture content of each pot to 20%. The gravimetric moisture content was kept constant during the seven days of incubation and each time after addition of the water soils were mixed. After seven days of incubation, three weed species were planted. The experimental design was a randomized complete block design with three replications.

Each pot was watered daily with distilled water from emergence to harvest and 100 ml Hoagland nutrient solution (Rafi & Epstein, 1999) were added to pots twice per week. Approximately two weeks after seeding, plant populations were reduced to 2 plants/pot for *X. strumarium*, 4 for *I. tinctoria* and 10 for *A. sterils*. After 60 days of growth under greenhouse conditions, weeds were harvested. Shoot and root materials were washed with deionized water to remove adhering soil particles. However, roots could not be taken properly due to heavy clay soils and were not analyzed. Shoots were then oven dried at 55°C, and ground. Plant material (0.25g) was digested in concentrated sulphuric acid/ 30% hydrogen peroxide. Digested solutions were analyzed for Cd, Zn, Fe, Cu and Mn by using inductively coupled plasma atomic emission spectrophotometer (ICP-AES) and for Pb using Varian graphite tube atomizer connected to a atomic absorption spectrometer (AASP).

Table 1. Shoot Cd, Pb, and Zn concentrations of weed species grown at different concentrations of heavy metals.

Treatment	*Avena sterils*			*Isatis tinctoria*			*Xanthium strumarium*		
	Cd	**Pb**	**Zn**	**Cd**	**Pb**	**Zn**	**Cd**	**Pb**	**Zn**
	------------------ **mg kg^{-1} DW** ------------------								
Control	<0.1 D[a]	<0.1 B	24 D	2 E	<0.1 C	10 D	<0.1 D	<0.1 B	**28 C**
Trt.# 1	8 D	<0.1 B	78 C	15 D	1 C	39 C	1 CD	<0.1 B	**41 AB**
Trt.# 2	20 C	1 B	96 C	29 C	5 B	64 B	2 BC	<0.1 B	**39 B**
Trt.# 3	47 B	4 A	162 D	74 B	10 A	94 A	3 B	<0.1 B	**45 AB**
Trt.# 4	77 A	3 A	264 A	93 A	11 A	101 A	6 A	1 A	**48 A**

[a]Means with the same letter within a column are not significantly different using least significant differences and p = 0.05. Control: no additions of metals, Trt. # 1: 25, 100, and 150 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 2: 75, 200, and 300 mg kg^{-1} Cd, Pb and Zn, respectively. Trt.#3: 150, 400, and 600 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 4: 300, 800, and 1200 mg kg^{-1} Cd, Pb and Zn, respectively.

Soil samples were collected from each pot after harvest. Samples of the soil (before seeding and after harvest) were air-dried and sieved through a stainless steel 2-mm screen and analyzed for pH in 1:1 soil:deionized water with a combination pH electrode. Soil samples were extracted by 4 M HNO_3 digestion and analyzed for Cd, Pb and Zn by using ICP-AES.

Statistical analyses were performed with SAS for Windows version 9 (SAS Inc., Cary, NC, USA). For comparison of mean values, Least Significant Difference (LSD) values were used.

Results and Discussion

Relative yield: Three weed species significantly differed in relative yield (p<0.05). There was no decline in relative yields of *I. tinctoria*. In fact, relative yields in Trt. 1 and 4 of *I. tinctoria* increased compared to control (Fig. 1). For *X. strumarium*, relative yield only significantly decreased in Trt. 4 and rest of the treatments did not differ from control. Relative yield of *A. sterils* gave significant response to treatments (Fig. 1). Increasing metal concentrations decreased relative yields. The highest reduction (61%) was with the Trt. 4 compared to the control (Fig. 1). Data indicated that in terms of relative yield, the most sensitive weed species was *A. sterils*. On the other hand, the most tolerant was *I. tinctoria*.

Ghosh & Singh (2005) studied Cd phytoextraction by weed species (*Brassica juncea, Ipomoea carnea, Dhatura innoxia, Phragmites karka, Cassia tora* and *Lantana camara*). They reported that stem biomass was the greatest in *I. carnea*. *I. carnea* produced more than 5 times more biomass compared to *B. juncea*, which is a Cd accumulator. If whole plant or above ground biomass is harvested, *I. carnea* followed by *D. innoxia*, *P. karka* were the most suitable species for phytoextraction of Cd in soils.

Shoot Cd concentrations: Increasing Cd concentrations in soils increased shoot Cd concentrations. Shoot Cd concentrations ranged from 2 to 93 mg kg $^{-1}$ for *I. tinctoria*, <0.1 to 77 mg kg $^{-1}$ for *A. sterils* and <0.1 to 6 mg kg $^{-1}$ for *X. strumarium* (Table 1). *I. tinctoria* had significantly higher shoot Cd concentrations compared to *A. sterils* and *X. strumarium* (Fig. 2). Although *X. strumarium* is a known Cd accumulator, *I. tinctoria* and *A. sterils* accumulated more shoot Cd. Our literature review shows that there was no published data for these weed species regarding Cd uptake. *I. tinctoria* and *A. sterils* had more shoot Cd concentrations compared to *X. strumarium*. Of these two weed species, *I. tinctoria* showed more tolerance to higher shoot Cd concentrations compared with *A. sterils*. Although *I. tinctoria* had higher shoot Cd, its relative yield was not declined by the increasing metal concentrations. *A. sterils* also had a high Cd concentrations up to 77 mg kg $^{-1}$. However, its relative yields decreased to 61% compared to control.

Qian *et al.,* (1999) studied metal uptakes and accumulations by wetland plants and reported that parrot's feather (*Myriophyllum brasielense* Camb.) attained high root Cd (1426 mg Cd kg^{-1}) and greatest shoot Cd accumulation among 10 plants was attained by water zinnia (*Wedelia trilobata* Hitchc.) (148 mg Cd kg^{-1}). Smartweed (*Polygonum hydropiperoides* Michx.) attained the second highest concentrations of Cd in both roots (1300 mg Cd kg^{-1}) and shoots (90 mg Cd kg^{-1}). They also reported that smooth cordgrass (*Spartina alterniflora* Loisel) had the lowest shoot Cd concentration (5 mg Cd kg^{-1}) whereas water lettuce (*Pistia stratiotes* L.) had the lowest root Cd concentration (193 mg Cd kg^{-1}). Zayed *et al.,* (1998) and Zhu *et al.,* (1999) found that the floating plants water hyacinth (*Eichhornia crassipes* Mart.) and duckweed (*Lemna polyrhiza* L.) were very good accumulators of Cd. They accumulated 6 and 13 mg Cd kg^{-1}. Ghosh & Singh (2005) compared the phytoextraction ability of 6 weed species. They reported that *I. carnea* was the most effective in extracting Cd among the weed species used in their study.

Shoot Pb concentrations: Soot Pb concentrations followed similar trends to shoot Cd. Shoot Pb concentrations gave response to the applications of Pb to the soils. Increasing Pb concentrations in soil led high accumulations of Pb in shoots of weeds (Table 1). The highest shoot Pb concentration was with *I. tinctoria* having no significant relative yield decrease, whereas *A. sterils* and *X. strumarium* had closer Pb accumulations in their shoots (Fig. 3).

In most soils, Pb has low geochemical mobility and low bioavailability. Furthermore, transport of Pb to aboveground is minimal due to its retention in plant roots by sorption and precipitation (Brennan & Shelley, 1999). In our study, shoot Pb concentrations compared to shoot Cd and Zn were low as expected.

Qian et *al.,* (1999) have reported that the highest Pb concentrations in both shoot (64 mg kg^{-1}) and roots (1882 mg kg^{-1}) were attained by smartweed. Parrot's feather, umbrella plant (*Cyperus alternifolius* L.), fuzzy water clover (*Marsilea drummondii*) accumulated Pb levels ranging from 30 to 45 mg kg^{-1} in shoots and 1000 to 1200 mg kg^{-1} in roots. They indicated that lead uptakes of other plants viz., sedge (*Cyperus pseudovegetus*), smooth cordgrass, monkey flower (*Mimulus guttatus* Fisch.), mare's tail (*Hippuris vulgaris* L.) and iris-leaved rush (*Juncus xiphioides* E. Mey.) did not exceed 18 mg kg^{-1} in shoots and 200 mg kg^{-1} in roots. Muramoto & Oki (1983) found that water hyacinth accumulated 25800 mg Pb kg^{-1} when it was treated with 8 mg Pb L^{-1}.

Shoot of Indian mustard grown on contaminated soils (total Pb 31000 mg kg^{-1}) treated with160 mmol S kg^{-1} and EDTA had 7100 mg Pb kg^{-1}, whereas wheat accumulated 1095 mg Pb kg^{-1} at the same treatment (Cui *et al.*, 2004).

Shoot Zn concentrations: *A. sterils* had significantly higher shoot Zn concentrations compared to *I. tinctoria* and *X. strumarium* (Fig. 4). Shoot Zn concentrations varied from 24 to 264 mg kg $^{-1}$ for *A. sterils*, 10 to 101 mg kg $^{-1}$ for *I. tinctoria* and 28 to 48 mg kg $^{-1}$ for *X. strumarium* (Table 1).

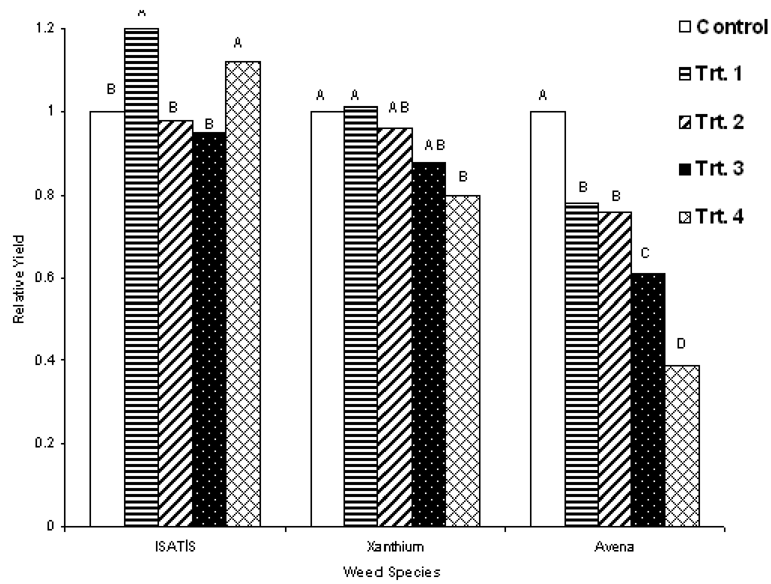

Fig. 1. Relative yields of weed species grown at different concentrations of heavy metals.
[a]Means with the same letters within a weed species are not significantly different using least significant differences and $p = 0.05$. Control: no additions of metals, Trt. # 1: 25, 100 and 150 mg kg^{-1}Cd, Pb and Zn, respectively. Trt. # 2: 75, 200 and 300 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 3: 150, 400 and 600 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 4: 300, 800, and 1200 mg kg^{-1} Cd, Pb and Zn, respectively.

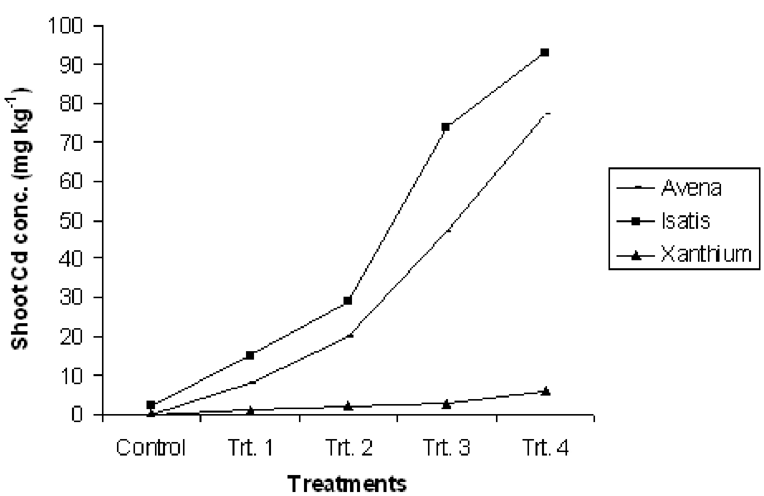

Fig. 2. Shoot Cd concentrations of three weed species grown at different concentrations of heavy metals. Control: No additions of metals, Trt. # 1: 25, 100 and 150 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 2: 75, 200 and 300 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 3: 150, 400 and 600 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 4: 300, 800 and 1200 mg kg^{-1} Cd, Pb and Zn, respectively.

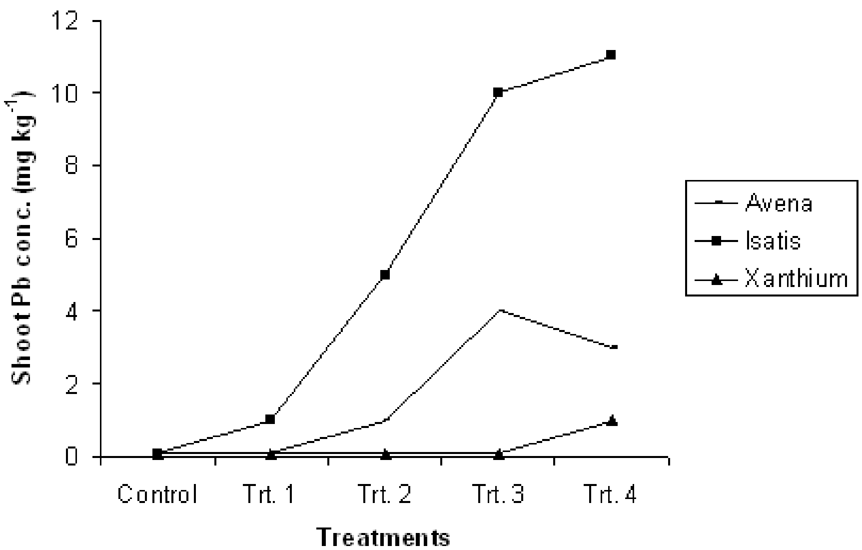

Fig. 3. Shoot Pb concentrations of three weed species grown at different concentrations of heavy metals. Control: No additions of metals, Trt. # 1: 25, 100 and 150 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 2: 75, 200 and 300 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 3: 150, 400 and 600 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 4: 300, 800 and 1200 mg kg^{-1} Cd, Pb and Zn, respectively.

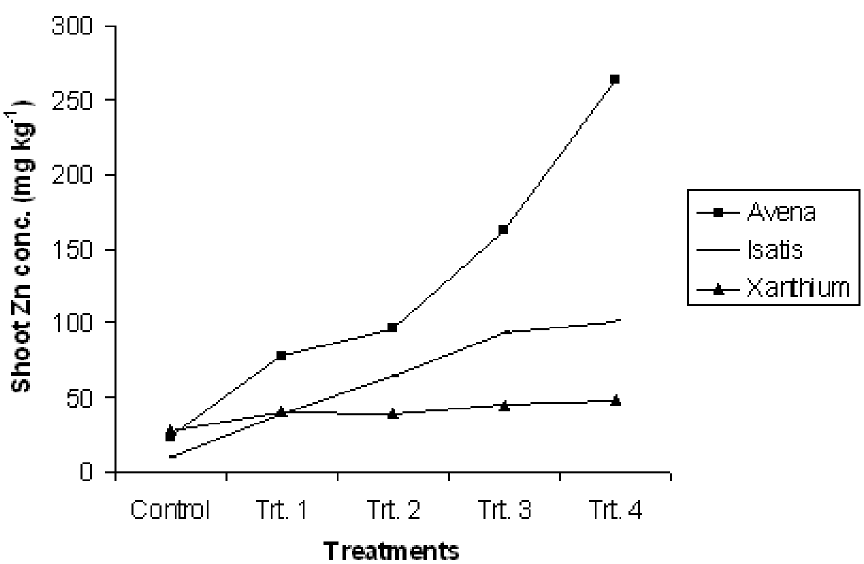

Fig. 4. Shoot Zn concentrations of three weed species grown at different concentrations of heavy metals. Control: no additions of metals, Trt. # 1: 25, 100 and 150 mg kg^{-1} Cd, Pb and Zn, respectively. Trt.# 2: 75, 200 and 300 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 3: 150, 400 and 600 mg kg^{-1} Cd, Pb and Zn, respectively. Trt. # 4: 300, 800 and 1200 mg kg^{-1} Cd, Pb and Zn, respectively.

Most economic crops suffer significant yield reduction when shoot Zn exceeds 500 mg kg $^{-1}$ on dry-weight basis (Chaney, 1993). In general, native species tolerate more foliar Zn compared to most economic crops, although there are limited data for them. Concentrations of Zn exceeded 1057 mg kg $^{-1}$ for shoots and 3244 mg kg $^{-1}$ for roots of sorghum-sudan grass grown in mine wastes (Sonmez & Pierzynski, 2005). Cui *et al*., (2004) reported that Indian mustard and wheat grown on contaminated soils (total Zn 480 mg kg^{-1}) accumulated 777 and 480 mg Zn kg $^{-1}$, in shoots and roots, respectively.

In literature, there are too many controversial results for accumulation of metals by plants. In fact, it is hard to compare plant species in their ability to remove various trace elements due to differences in their growth rate, size, propagation material, physiology and morphology. To minimize variations among plant species, all plants used in a study should be at the same growth stage and size (Qian *et al.*, 1999).

As mentioned earlier, there was not any published data comparing the phytoextraction ability of *I. tinctoria*, and *A. sterils.* We believe our study would be helpful to fill a gap in the literature.

Conclusion

Data indicated that in terms of relative yield, the most sensitive weed among the weed species used in this study was *A. sterils*. On the other hand, the most tolerant one was *I. tinctoria*. Increasing metal concentrations in soils increased shoot metal concentrations. *I. tinctoria* had the highest shoot Cd and Pb concentrations compared to *X. strumarium* and *A. sterils*. However, *A. sterils* had significantly higher shoot Zn concentrations compared to *I. tinctoria* and *X. strumarium*. *I. tinctoria* extracted highest amount of metals among the three weed species used in this study. However, its accumulation was much lower compared to the other metal hyperaccumulators.

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