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PHYTOREMEDIATION: ASSESSING TOLERANCE OF TREE SPECIES AGAINST HEAVY METAL (PB AND CD) TOXICITY

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Abstract

The toxicity effects of heavy metals (lead and cadmium) on germination, root length and dry biomass of tree species viz., *Thespesia populneoides*, *Leucaena leucocephala* and *Delonex regia* were evaluated. Cadmium was found to be more toxic than lead. The results further showed that dose response of heavy metals were inversely proportional to germination, root growth and dry biomass. The percentage germination and higher % DFC values showed that the seeds of *L. leucocephala* were least affected by lead and cadmium toxicity while scoring the best germination response among the three tree species. Exposure to high concentration (125ppm) of Cd decreased the root size of *L. leucocephala, T. populneoides* and *D. regia* by 89.79, 71.8 and 62.26% respectively. However, at the same concentration, lead inhibited the root growth (39.62%) in case of *D. regia* while much higher reduction was observed for *L. leucocephala* and *T. populneoides* around 62.7%. Furthermore, the % phytotoxicity and tolerance index confirmed that *D. regia* appeared to be the most tolerant species whereas, *T. populneoides* and *L. leucocephala* were moderately tolerant and less tolerant species respectively against the Pb and Cd treatment. This study gives an insight to the possible mechanism of hypertolerance, signifying that trees can be successfully used for phytoremediation.

Introduction

Heavy metals are natural constituents of the earth's crust but human activities have drastically altered their geo-chemical cycles and biochemical balance consequently, large areas of land, all over the world are contaminated with heavy metals derived from urban activities, agricultural operations, industrial processing and automobiles (Sesbastiani *et al.,* 2004). Metal concentrations in soil ranges from less than 1 mg / kg to as high as 100,000mg / kg, whether due to the geological origin of the soil or as a result of human activity (Baylock & Huang, 2000). Plants show considerable amount of heavy metals like Pb and Cd in their tissues near busy roads because of highly increasing vehicles (Iqbal & Shazia, 2004). It is observed by Zhang (2012), that roadside trees plays a vital role in controlling heavy metal pollution in the area and in the soils of tree planted areas, concentration of these metals is statisticaly lower than the places without tree.

Excess of metals may produce some common effects of individual metals such as Cd, Cr, Cu, Ni, Zn, Fe, Pb etc., on different plants (i.e. both macro and micro flora). The biota may require some of these elements like Fe, Zn and Cu in trace quantities but at higher concentrations there may be toxicity problems because they cause oxidative stress by formation of free radicals (Ghosh & Singh, 2005; Rout & Das, 2003). Plants are sometimes exposed to non-essential heavy metals, including Pb and Cd that are present in soil and water naturally or as contaminants due to human activities (Li *et al.,* 2005). These heavy metals in higher doses inhibits seedling growth in terms of shoot and root length, number of leaves, biomass and chlorophyll contents (Shah *et al.,* 2008), it may also cause metabolic disorders like interference with the biological processes relating to photosynthetic activities, mitochondrial respiration and stomatal clogging of plants and growth inhibition for most of the plants (Peralta *et al*., 2001; Ahmed & Qadir, 1975). Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their functions. Houshmandfar & Moraghebi (2011), reported that elevated levels of heavy metals in contaminated soils are widely spread and concerns have been raised over the potential risks to humans, animals and agricultural crops. Thus metals tends to render the land unstable for plant growth and destroy the biodiversity (Ghosh & Singh, 2005, Shafiq, 2011)). Despite the fact that heavy metals cause detrimental effect on plants and other organisms at the same time it is also found that plants have the ability to grow in sites where soil contains greater than usual amounts of heavy metals or other toxic compounds. To exploit this characteristic of plants the term phytoremediation was coined which is the use of plants to remove pollutants or to render them harmless as plant's constitutive and or adoptive mechanisms for accumulation and tolerance in the rhizosphere to clean up metals from soil (Salt *et al*., 1998; Khan *et al*., 2000, Zhang, 2012). In fact, with phytoremediation the soil biological properties and physical structure are maintained and the fertility and biodiversity can be improved (Cunningham *et al*., 1995; Watanabe, 1997)

Phytoremediation has great potential and many experiments have already been performed to set up methodologies, but it is still in the developmental phase. As asserted by Van der Lelie *et al*., (2001) there is an urgent need for research aimed at fundamental understanding of mechanisms involved in soil and plant compartments and demonstration projects to optimize phytoremediation processes. In this regard some plant species endemic to metalliferous soils that can tolerate greater than usual amounts of heavy metals or other toxic compounds have been discovered (Baylock & Herang, 2000; Raskins & Ensley, 2000). Several studies have been conducted in order to evaluate the effects of different heavy metals concentrations on live plants and most of these studies have been performed using seedling or adult plants while in few cases studies, the seeds have been exposed to the contaminants (Peralta *et al*., 2001; Oncel *et al*., 2000; Chatterjee & Chatterjee, 2000; Flores *et al*., 1999; Thompson *et al*., 1997; Xiong *et al*., 1998; Reeves & Baker, 2000; Claire *et al*., 1991).

During plant's life cycle, seed is the only stage that is well protected against various stresses but soon after imbibition and subsequent vegetative development processes, it becomes stress sensitive. Therefore, seeds require careful monitoring against external parameters so as to maintain the protective state until external conditions become favourable for developmental processes (Li *et al*., 2005). The present study was designed to understand how heavy metals affect the ability of seed germination whereby the toxicity to non-essential metal ions i.e., Pb and Cd was examined during germination and seedling growth of three trees viz., *T. populneoides, L. leucocephala* and *D. regia*.

Materials and Methods

The seeds of *Delonex regia, Leucaena leucocephala* and *Thespesia populneoides* were collected from the Karachi University campus. The seeds of *D. regia* and *L. leucocephala* were rubbed with sand paper near the testa and the soft tips of *T. populneoides* were cut down by scissors. Later on, seeds were sterilized by soaking for 3-5 minutes in 50% Sodium hypochlorite solution. Afterwards, sterilized seeds were washed thrice with double distilled water. The seeds were placed on double layered filter papers wetted with double distilled water (control) or test solutions (treatments) in the Petri dishes. Twenty five seeds were placed in each Petri dish with three replicates.

Metal treatments of Pb and Cd were prepared with lead acetate and Cadmium chloride and were applied at 25, 50, 75, 100, 125ppm concentration. Two ml of respective treatment were applied to each Petri dish. Petri dishes were sealed and incubated for14 days under continuous white light at 25±2^{o}C.

Seeds were considered germinated when the breakage of seed coat was visible. Seedling development was regarded as being inhibited 6 days after imbibition if the seed coat was visibly broken (germination), but the embryo did not grow further (Lie *et al*., 2005). Petri dishes were checked daily. Response to the treatments was measured by counting the number of germinated seeds for 10 days. Germination was scored as protrusion of the radicle through the testa. The seeds that showed further growth, their root lengths were also measured after two weeks period. At the end, the dry weight of each replicate was taken. To obtain dry weight, seedlings were oven dried at 80^{o}C till constant weight was achieved. The completely randomized designed (C.R.D.) was applied in this experiment and data was analyzed by ANOVA (Analysis of Variance) and Duncan's multiple range test.

Mean indices of tolerance (TI) were calculated as percentage for each treatment (Turner & Marshal, 1972).

$$\text{Tolerance Index (TI)} = \frac{\text{Mean length of root in test solution}}{\text{Mean length of root in control}} \times 100$$

To understand the germination response % difference from control (%DFC) or % germination was measured (Mhatre & Chaphekar, 1982).

$$\%\ \text{DFC} = \frac{\%\ \text{Germination of control} - \%\ \text{Germination of test solution}}{\%\ \text{Germination of control}} \times 100$$

% Phytotoxicity for root length of 14 days old seedlings was determined by the following formula (Chou *et al.,* 1978).

$$\%\ \text{Phytotoxicity} = \frac{\text{Root length of control} - \text{Root length of treatment}}{\text{Root length of control}} \times 100$$

Results and Discussion

The toxicity of selected heavy metals (Pb and Cd) on seed germination, root length and dry biomass was examined in three tree species viz., *T. populneoides, L. leucocephala* and *D. regia*. Overall germination was not affected by induced Pb and Cd toxicity but reduction was observed with the increase of metal concentration (Tables 1 and 2). This is consistent with the previous reports by Shah *et al*., (2008), Li *et al*., (2005) Yogeetha *et al*., (2004), and Peralta *et al*., (2000). Sherbateskoy *et al*., (1986) explained the similar phenomenon that growth is least affected by acidity or metals like Pb and Cd because it depends on the imbibition whereas selection for metal tolerance occurs at the establishment stage in seedling development. *T. populneoides* showed 50% reduction in germination at 100ppm of Cd toxicity, but in case of *D. regia* and *L. leucocephala* less than 50% reduction was observed even at 125ppm of Cd and Pb. It is proved statistically that there is non-significant difference in the germination of the species. The percentage germination showed that seeds of *L. leucocephala* were least affected by the Pb and Cd toxicity than *T. populneoides* and *D. regia*. Its germination exceeds 90% in lead acetate and 70-80% in Cadmium chloride. Germination decreased with the increase of metal concentration.

Table 1. Effect of Cd on % germination, root length and dry weight of three tree species.

Treatment ppm	% Germination			Root length			Dry weight		
	T. populneoides	*L. leucocephela*	*D. regia*	*T. populneoides*	*L. leucocephela*	*D. regia*	*T. populneoides*	*L. leucocephela*	*D. regia*
0	52.00 ± 2.30	96.00 ± 2.31	92.00 ± 1.09	3.29 ± 0.51	8.13 ± 0.38^{ag}	0.53 ± 0.03^{ag}	0.60 ± 0.06	0.93 ± 0.11	2.55 ± 0.13
25	49.30 ± 5.81	82.66 ± 9.61	70.66 ± 14.11	1.52 ± 0.57	1.91 ± 0.37^{bg}	0.43 ± 0.52^{bfg}	0.43 ± 0.03	0.44 ± 0.07	0.31 ± 0.10
50	42.60 ± 8.74	76.00 ± 1051	68.00 ± 12.87	1.50 ± 0.56	1.66 ± 0.28^{cg}	0.40 ± 0.52^{cf}	0.29 ± 0.03	0.45 ± 0.05	0.40 ± 0.13
75	24.30 ± 4.35	73.30 ± 7.14	65.33± 10.91	1.46 ± 0.67	1.13 ± 0.09^{dg}	0.30 ± 0.52^{df}	0.27 ± 0.03	0.45 ± 0.04	0.39 ± 0.05
100	25.30 ± 5.81	65.33 ± 9.33	57.00 ± 4.61	1.10 ± 0.44	1.05 ± 0.11^{cg}	0.25 ± 0.026^{e}	0.26 ± 0.06	0.43 ± 0.11	0.37 ± 0.05
125	14.60 ± 1.33	65.33 ± 11.85	54.6 ± 14.4	0.91± 0.03	0.83 ± 0.03^{f}	0.20 ± 0.41^{e}	0.08 ± 0.19	0.40 ± 0.09	0.32 ± 0.05

Mean values followed by same letters in the columns do not significantly differ ($p<0.05$)

Table 2. Effect of Pb on germination, root length and dry weight of three tree species.

Treatment ppm	% Germination			Root length			Dry weight		
	T. populneoides	*L. leucocephela*	*D. regia*	*T. populneoides*	*L. leucocephela*	*D. regia*	*T. populneoides*	*L. leucocephela*	*D. regia*
0	52.00 ± 2.30	96.00 ± 2.31	92.00 ± 1.09	3.29 ± 0.51	8.13 ± 0.38^{ag}	0.53 ± 0.03^{ag}	0.60 ± 0.09^{af}	0.93 ± 0.11^{ad}	2.55 ± 0.13
25	45.30 ± 9.61	96.00 ± 1.89	78.60 ± 1.76	2.19 ± 0.50	7.38 ± 0.53^{bg}	0.45 ± 0.04^{bg}	0.58 ± 0.06^{be}	0.83 ± 0.01^{acd}	2.50 ± 0.26
50	46.60 ± 9.61	95.00 ± 4.02	77.33 ± 1.53	2.15 ± 0.64	5.18 ± 0.04^{cg}	0.37 ± 0.03^{cg}	0.57 ± 0.05^{ce}	0.60 ± 0.02^{bc}	2.11 ± 0.06
75	45.50 ± 3.53	92.00 ± 4.12	77.3 ± 1.23	2.18 ± 0.83	3.26 ± 0.42^{d}	0.35 ± 0.27^{dg}	0.52 ± 0.09^{de}	0.55 ± 0.01^{b}	2.09 ± 0.27
100	45.30 ± 3.35	92.00 ± 4.00	60.00 ± 1.23	2.00 ± 0.93	3.13 ± 0.10^{e}	0.34 ± 0.02^{e}	0.52 ± 0.05^{de}	0.54 ± 0.12^{b}	2.04 ± 0.12
125	38.60 ± 70.5	81.00 ± 3.30	57.33 ± 2.33	1.24 ± 0.36	3.00 ± 0.79^{f}	0.32 ± 0.03^{e}	0.50 ± 0.05^{d}	0.54 ± 0.02^{b}	1.61 ± 0.02

Mean values followed by same letters in the columns do not significantly differ ($p<0.05$)

Furthermore, DFC values present the comparative picture and reveal the relative inhibitory effects of Pb and Cd on germination of seeds (Table 3). Thus these values increased with the increase in concentration of metals, although Cd has more toxic effect than Pb in this regard. Similar result were also observed by Farooq *et al.*, (2009) and Kabir *et al.*, (2008).The higher values of % DFC in *D. regia* than *T. populneoides* and *L. leucocephala* suggest that its seeds pose relatively greater susceptibility to these heavy metals.

The effect of the concentration using Pb and Cd on root elongation or root length was inhibitory (Tables 1 and 2). Exposure to 25ppm dose of Cd reduced the root length of *L. leucocephala*, *T. populneoides* and *D. regia* by 76.50, 53.7 and 18.86% respectively, compared with the root length of control. The high concentration (125ppm) of Cd caused marked decrease in the root size of *L. leucocephala*, *T. populneoides* and *D. regia* by 89.79, 71.8% and 62.26% respectively.

Results obtained also showed that Pb has less toxic effects than Cd on the root length as well. Pb at the concentration of 125ppm reduced the root size by 62.31 and 63.09% of *T. populneoides* and *L. leucocephala*. In contrast at the same concentration only 39.62% reduction of root size of *D. regia* was observed. Furthermore, these metals at the concentration of 100 and 125ppm lead to the death of most of the seedlings by the end of the second week. This could be due to the reduction of chlorophyll a & b (Oncel *et al.*, 2000, Shah *et al.*, 2008, Bavi *et al.*, 2011), water potential and Fe (II) concentration (Chatterjee & Chatterjee, 2000).

The values of % phytotoxicity of Pb and Cd further validate the above results revealing the differential effects of these metals on root growth (Table 4; Figs. 1 & 2). Statistical analysis showed significant difference in case of root length for *L. leucocephala* and *D. regia* for both metal treatments. Although Cd was more phytotoxic than Pb in stages of root development, their higher concentration obviously produced greater effects. The lower % phytotoxicity values indicate that *D. regia* was the most tolerant species against Pb and Cd concentration which is also supported by the data of Tolerance Index (TI), a measure of relative tolerance of plants to metal toxicity which decreased significantly in all of the three tree species (Table 5; Figs. 3 & 4). *T. populneoides* appeared to be moderately tolerant and *L. leucocephala* showed less tolerance according to % phytotoxicity and TI values. Another study reported that Pb and Cd individually and in combination produce adverse significant effect on all the growth phases particularly on root growth (Iqbal *et al.*, 2001).

Table 3. Effect of Pb and Cd on % DFC of three different tree species.

Treatment	*T. populneoides*		*L. leucocephala*		*D. regia*	
	Cd	Pb	Cd	Pb	Cd	Pb
25ppm	5.19	3.84	13.89	1.04	23.19	14.58
50ppm	18.07	20.83	20.83	3.1	26.08	16.3
75ppm	53.26	16.73	23.64	4.16	29.98	16.3
100ppm	51.34	22.5	31.94	4.16	38.04	34.78
125ppm	71.92	25.76	31.94	15.62	40.65	38.04

Table 4. Effect of Pb and Cd on % phytotoxicity of three different tree species.

Treatment	*T. populneoides*		*L. leucocephala*		*D. regia*	
	Cd	Pb	Cd	Pb	Cd	Pb
25ppm	53.79	33.43	76.5	9.22	18.86	9.22
50ppm	54.4	34.65	79.58	36.28	24.52	36.28
75ppm	55.62	33.73	86.1	59.9	43.39	59.9
100ppm	66.56	39.2	87.08	61.5	52.83	61.5
125ppm	72.34	62.31	89.79	63.09	62.26	63.09

Table 5. Effect of Pb and Cd on Tolerance Index (TI) of three different tree species.

Treatment	*T. populneoides*		*L. leucocephala*		*D. regia*	
	Cd	Pb	Cd	Pb	Cd	Pb
25ppm	44.25	66.56	24.11	90.77	81.13	84.9
50ppm	44.37	65.34	20.95	63.71	75.47	69.81
75ppm	43.19	66.26	14.26	40.09	56.6	66.03
100ppm	32.54	60.79	13.25	38.49	47.16	64.41
125ppm	26.92	37.69	10.47	36.9	37.73	60.37
Average TI	38.39	59.32	16.6	53.99	53.38	69.1

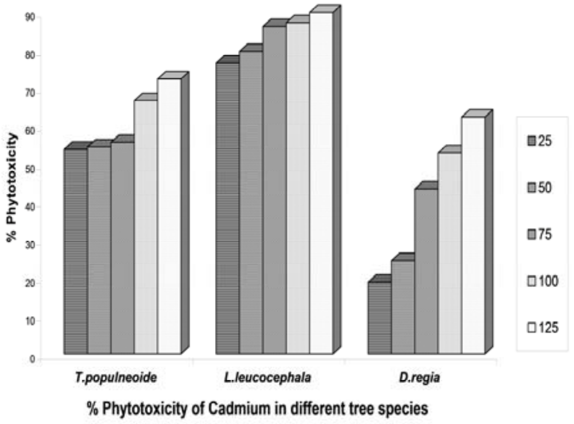

Fig. 1. % Phytotoxicity of Cadmium in different tree species.

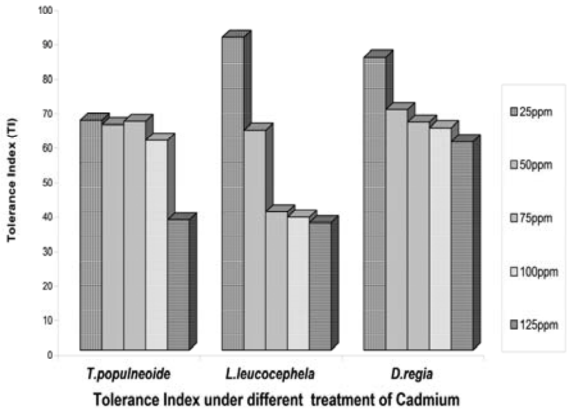

Fig. 3. Tolerance index under different treatment of Cadmium.

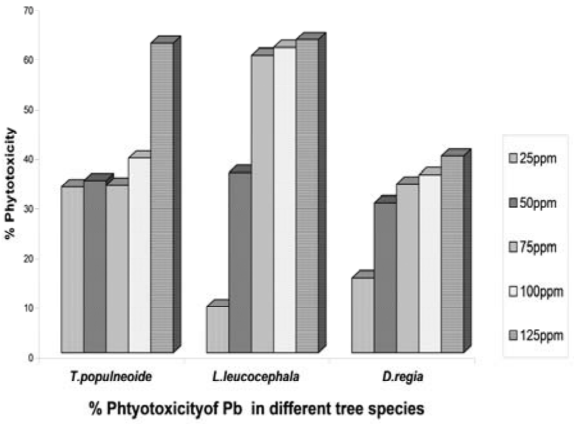

Fig. 2. % Phytotoxicity of Pb in different tree species.

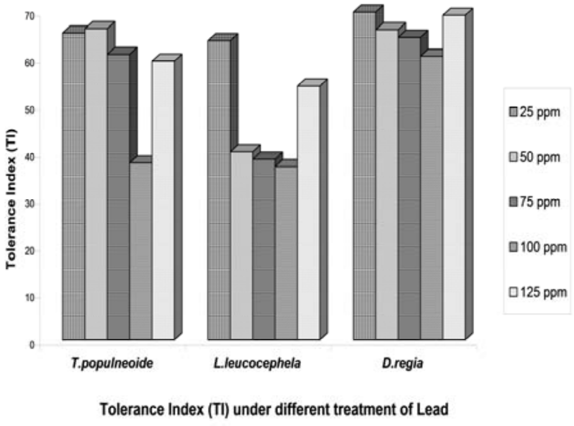

Fig. 4. Tolerance index (TI) under different treatment of Lead.

The decrease in dry mass was evident under different treatments of Pb and Cd (Tables 1 and 2). Significant difference was proved in dry biomass for *T. populneoides* and *L. leucocephala* for lead treatment statistically. Shafiq *et al*., 2011, also indicated that heavy metals toxicity destroys individual cells and reduces the ability to produce biomass. The inhibition of root growth is also evident by the dry mass that could also provide another relative indicator of phytotoxic effects of the heavy metals and also the relative sensitivity of the tree species towards them.

Plants react to heavy metal stress differently. Their resistance and tolerance dropped on the single species ability to activate molecular mechanisms such as metal sequestration in the cell wall and or in vacuoles usually by the binding with appropriate ligands (Chaney *et al.*, 1997).

It is noteworthy that *D. regia* was susceptible to the heavy metal contamination at germination stage but emerged as the most tolerant tree species at later developmental stage and *vice versa* for *L. leucocephala*. Selection pressure during seedling establishment, eliminate individuals less able to tolerate in the presence of metals (Abouguendia *et al.*, 1979). It is also revealed from the present study that *D. regia* came out to be tolerant to Cd and Pb and possessed multiple metal tolerance, this employs more than one mechanism of resistance but the adaptation of any one or any combination impose important physiological and ecological constraints. However, in plants usually co-tolerance (cross-resistance) to Cd and Pb takes place (Cox & Hutchinson, 1980). Antonovics (1966) found that the evolution of tolerance is not necessarily a long term process but it can occur rapidly. Metal tolerant individuals can be selected in a few or even within one generation.

Since hypertolerance is a key property that makes hyperaccumulation later on possible. Therefore, it is necessary to consider tolerance of different tree species like *D. regia*, *T. populneoides* and *L. leucocephala* to heavy metal concentrations. This pertinent study provides a significant approach in the understanding of natural mechanism of tolerance in trees. It will open avenues for the researchers all over the world to exploit trees for phytoremediation, which is an inevitable technology in the present era, as our planet is facing the continuously increasing exposure to heavy metal pollution that is hazardous for life.

It could be concluded from the present study that the *D. regia* appeared to be the most tolerant tree species among *T. populneoides* and *L. leucocephala* and could be used as a tool for phytoremediation against Pb and Cd toxicity. Hence further detail studies are required to find out its strategies of tolerance mechanism.

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