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Phytoextraction potential of cadmium and lead contamination using *Melia azedarach* and *Populus alba* seedlings

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Vegetative growth, biomass, chemical content and uptake of cadmium (Cd) and lead (Pb) in *Melia azedarach* L. (chinaberry) and *Populus alba* L. (white poplar) seedlings were investigated using a 2-year pot experiment. The results indicated that *P. alba* and *M. azedarach* are tolerant to contaminated soil by Cd or Pb without any toxicity symptoms. Vegetative growth and chemical properties of *M. azedarach* are negatively affected by Cd more than Pb whereas, biomasses are negatively affected by Pb little than Cd. Likewise, vegetative growth and chemical properties are negatively affected by Cd more than Pb however, biomasses are negatively affected by Cd and Pb with the same significant level. Both species accumulate more concentrations of Cd and Pb in their roots than in leaves and stem. As a result, *P. alba* and *M. azedarach* are considered suitable phytoremediators for contaminated soils by Cd or Pb.

Key words: Phytoremediation, contamination, heavy metals, cadmium, lead, *Populus alba*, *Melia azedarach*.

INTRODUCTION

Pollution of soil and agricultural land is a complex and serious phenomenon that in recent decades has increased its negative effects on the environment. Transfer of toxic elements to human food chain is a concrete danger that has to be faced, taking into account the possibility for plants to accumulate and translocate contaminants to edible and harvested parts (Puschenreiter et al., 2005). Elevated Cd levels in agricultural soils are becoming a major environmental problem due to the high toxicity of Cd and its mobility from soil to plants and therefore into the food chain (Zhu et al., 2012). Lead accumulation in plant tissue impairs various morphological, physiological, and biochemical functions in plants, either directly or indirectly, and induces a range of deleterious effects. It causes phytotoxicity by changing cell membrane permeability, by reacting with active groups of different enzymes involved in plant metabolism (Pourrut et al., 2011). Heavy metals cannot be metabolized, therefore the only possible strategy to apply is their extraction from contaminated soil and transfer to the smaller volume of harvestable plants for their disposal (Padmavathiamma and Li, 2007) biomass can also be used in producing

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Table 1. Physical and chemical analysis of the used soil.

Parameter practical size distribution	Mean	Parameter soluble anions (meq/L)	Mean
Sand (%)	70.00	CO_3^{--}	0
Silt (%)	20.00	HCO_3^-	2.60
Clay (%)	10.00	Cl^-	20.05
Soil texture	Sandy loam	SO_4^{--}	8.62
pH	8.22	Available N (ppm)	2.17
E.C (ds/m)	3.38	Available P (ppm)	3.52
$CaCO_3$ (%)	33.36	Available K (ppm)	2.15
Organic matter (%)	0.28		
Soluble Cations (meq/L)		**Total heavy metals (ppm)**	
Ca^{++}	10.40	Cd	0
Mg^{++}	1.71	Pb	0
Na^+	22.27	Fe	4.15
K^+	44.00		

energy and, if economically profitable, metals can be eventually recovered (Zacchini et al., 2009). However, on a large scale, metal uptake by trees can be more effective, mainly because of a deeper root system and a greater yield of biomass (Fischerová et al., 2006). High productivity and elevated uptake and translocation of pollutants to the harvestable biomass are the basis for efficient *in situ* restoration by means of vascular plants (Chaney et al., 2007). There is an active effort to develop new, more cost-effective methods to remediate contamination of polluted soils, hence attention is now focusing on innovative biological technologies such as phytoremediation, based on the use of plants to extract, sequester and/or detoxify pollutants (Salt et al., 1993). Many woody species are now considered of interest to this aim where they are fast growing, have deep roots, produce abundant biomass, are easy to harvest, and several species revealed some capacity to tolerate and accumulate heavy metals. Chinaberry trees have been recommended for planting at landfills in developing countries to offset environmental problems caused by landfills (Kim and Lee, 2005). Poplars are particularly suitable for remediation purposes (Schnoor, 2000) also, salicaceae family are reported to grow even in severe soil conditions and to accumulate heavy metals (Berndes et al., 2004). Many studies have thus been focused on the use of willows and poplars in phytoextraction (Jensen et al., 2009). The objective of this study is exploring the effects of different levels of Cd and Pb on vegetative growth, biomas sand mineral content of *Populus alba* and *Melia azedarach* seedlings to evaluate the suitability of both tree species in phytoremediation.

MATERIALS AND METHODS

Pot experiment was carried out at the Nursery of Timber Trees Research Department of Sabaheia, Horticultural Research Station at Alexandria, Egypt. The study persisted from 22th April 2010 to 1st November 2012 to investigate the effect of Cd and Pb heavy metals on the vegetative growth, biomass and chemical composition of *P. alba*, L. and *M. azedarach*. L. transplants after two growing seasons (from 22th April 2010 to 1st November 2012) as well as, the effect of these tree species on soil properties at the end of plantation period.

Tree species

One year- old *M. azedarach* L. transplants of averaged 90 cm in height and 5 mm in diameter (from the soil surface) as well as, three months old *P. alba* L. transplants of averaged of 35 cm in height and 4 mm in diameter (from the soil surface) were used in this study. All transplants were homogenous and brought from the nursery of Timber Trees Research Department of Sabaheia, Horticultural Research Station.

Pollutant treatments

The pollutants were cadmium at the rates of 10, 20, 40 (as cadmium chloride $CdCl_2.H_2O$) and lead at the rate of 200, 400, 800 ppm (as lead acetate trihydrate$Pb(C_2H_3O_2)2.3H_2O$) in addition to non-pollutant treatment as control. The seedlings were transplanted on April 2010 in polyethylene bags (75 cm in depth and 52.5 cm in diameter), filled with 25 Kg of sandy loam soil which their physical and chemical properties are showed in Table 1 then, irrigated with pollutant solution to field capacity. After 2 weeks, each bag was planted with one seedling and irrigated with tap water to field capacity.

Experimental design

The experiment was laid out in complete randomized design (CRD) as described by Snedecor and Cochran (1989) that consisted of seven treatments replicated three times (21 seedlings per species). The treatments were conducted as follows: Control (without pollutants), Cd-10 ppm, Cd-20 ppm, Cd-40 ppm, Pb-200 ppm, Pb-400 ppm, Pb-800 ppm. Data generated from the experiment were analyzed using one-way ANOVA tests with Duncan's multiple range tests to separate means and data were processed by using SAS procedures.

Table 2. Response of vegetative growth of *M. azedarach to* different levels of cadmium and lead pollutants after two consecutive seasons

Treatment	Height increment (%)	Diameter increment (%)	Branch number (branch)	Leaf area (cm^2)	Length of the longest root (cm)	Green colour intensity (SPAD)
Control	178.03^{a}	252.72^{a}	8.00^{a}	42.67^{a}	94.70^{a}	53.57^{a}
Cd -10	165.60^{bc}	250.17^{ab}	4.33^{bc}	31.20^{bc}	92.68^{ab}	51.25^{a}
Cd -20	157.92^{cd}	245.28^{ab}	4.00^{bcd}	29.63^{bc}	84.00^{abc}	43.04^{c}
Cd -40	152.19^{d}	234.61^{c}	3.00^{d}	25.10^{c}	73.33^{c}	42.61^{c}
Pb -200	174.05^{ab}	249.14^{ab}	5.00^{b}	34.87^{ab}	94.02^{ab}	47.48^{b}
Pb -400	159.10^{cd}	249.30^{ab}	4.33^{bc}	26.64^{bc}	88.68^{ab}	45.32^{bc}
Pb -800	154.75^{d}	243.51^{b}	3.33^{cd}	22.34^{c}	79.68^{bc}	44.13^{bc}

Means followed by a similar letter within a column are not significantly different at the probability level of 0.05 using Duncan's Multiple Range Test.

Measurements

At first at November 2012 for each treatment of the two species, the total heights were measured from ground level to the seedling apex to the nearest 0.5 cm. Also, stem diameter was measured at ground level to the nearest 1.0 mm. The measurements were done to calculate the increment of height and diameter growth (%). Number of the branches per seedling was counted for each treatment. The three seedlings from each treatment were harvested, and then 15 leaves from each were harvested from different locations along the seedling level (top, middle and bottom). As well leaf area in cm^2 was measured by using Auto Cad software. A SPAD-502 chlorophyll meter (based on light transmittance through leaves) was used as a non-destructive tool for estimating leaf chlorophyll (Markwell et al., 1995). Two readings per leaf were taken midway between the leaf mid vein and margin and then averaged. On the other hand, a half gram of the ground material of different plant parts was digested by sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) mixture on hot plate until a clear digest was obtained. The solution was left to cool then, filtered and diluted to 50 ml with distilled water (Evenhuis and DeWaard, 1980). The digested samples were prepared for measuring nitrogen, phosphorus, potassium, cadmium and lead. Nitrogen and phosphorus were measured colormetrically as determined by Evenhuis (1976) and Murphy and Riley (1962). Potassium was measured using a flame photometer (Page et al., 1982). Cadmium and lead was measured by using Perkin Elmer, 3300 Atomic Absorption Spectrophotometer. Contents of cadmium and lead were expressed as mg/kg dry weight then uptake (expressed as mg) was calculated as follows: Cd or Pb content X dry weight (leaves, stem, roots) / 1000. Total Cd or Pb uptake = leaves uptake + stem uptake + roots uptake. Available N of the soil was determined using Kjeldahl method (Bremner and Mnlvaney, 1982) and available P was determined according to Olsen and Sommers (1982). Soil micronutrients were extracted by 0.05 M DTPA solution (Lindsay and Norvell,1978). Also, the concentrations of Cd and Pb were quantified through Atomic absorption spectrophotometer (AAS).

RESULTS AND DISCUSSION

Vegetative growth

Table 2 reveals that both stem height increment and stem diameter increment (%) of *M. azedarach* were affected by all rates of Pb and Cd, therefore, the highest rates of Cd (40 ppm) and Pb (800 ppm) significantly decreased both parameters. On the other hand, the number of branches was extremely affected by contamination of Cd and Pb, therefore, after the two growing seasons, the decreases in numbers of branches were maximized up to 62.5 and 58.4% for Cd 40 ppm and Pb 800 ppm, respectively. Likewise, the leaf area responded to different rates of Cd and Pb where Cd 40 ppm and Pb 800 ppm decreased the leaf area by 41.2 and 47.7%, respectively. Also, the length of the longest root in the soils contaminated by Cd 40 and Pb 800 ppm were decreased by 22.6 and 15.9%, respectively; less than the non-contaminated (Table 2). However, both Cd 40 and 20 ppm declined the green colour intensity (GCI) by 20.5 and 19.7%, respectively; less than non-contaminated leaves. Likewise, Pb 800 and 400 ppm declined (GCI) of *M. azedarach* leaves by 17.6 and 15.4%, respectively, less than the non-contaminated. Table 3 shows that the high rates of cadmium (40 ppm) and lead (800 ppm) slightly decreased both stem height increment and stem diameter increment (%) compared to the control. On the other hand, branch number of white poplar was varied significantly by increasing the rates of Cd and Pb therefore, Cd 40 ppm and Pb 800 ppm were intensely minimized the branch numbers by 49.2 and 36.1%, respectively, fewer than non-contaminated seedlings. Also, leaf area of *P. alba* seedlings that planted in contaminated soil with Pb 800 ppm and Cd 40 ppm reduced the leaf area by 34.1 and 32.9%, respectively, comparing to non-contaminated seedlings. Moreover, the seedlings in contaminated soil with Cd 40 ppm and Pb 800 ppm reduced the length of the longest root by 37.4 and 30.3%, respectively, comparing with non-contaminated seedlings. The data in Table 3 presented that the GCI were declined by 16.7 then 16.0% less than control when contaminated by Cd 40 and Pb 800 ppm, respectively.

These results are in agreement with those of Cosio et al. (2006) on *Salix viminalis*. Pb is considered as a general protoplasmic poison, which is accumulative, slow acting and subtle. Also, Kabata-Pendias and Pendias (1992) mentioned that when Pb is presented in soluble forms in nutrients solutions, plant roots are able to take up great amounts of this metal which leads to an inhibitory effect on plant metabolism. As well as, Hall

Table 3. Response of vegetative growth of *Populus alba* to different levels of cadmium and lead pollutants after two consecutive seasons.

Treatment	Height Increment (%)	Diameter increment (%)	Branch number (branch)	Leaf area (cm^2)	Length of the longest root (cm)	Green colour Intensity (SPAD)
Control	535.30^{a}	247.10^{a}	18.3^{a}	11.76a	74.60^{a}	52.00^{a}
Cd -10	532.22^{a}	244.37^{bc}	15.7^{bc}	11.01^{ab}	68.70^{ab}	50.57^{b}
Cd -20	529.34^{a}	241.18^{d}	12.0^{d}	9.65^{c}	65.36^{b}	48.36^{c}
Cd -40	500.93^{b}	200.97^{f}	9.3^{e}	7.89^{d}	46.67^{c}	41.76^{e}
Pb -200	532.93^{a}	245.11^{ab}	17.7^{ab}	10.29^{bc}	71.67^{ab}	50.34^{b}
Pb -400	530.69^{a}	242.52^{cd}	15.0^{c}	9.36^{c}	64.00^{b}	48.72^{c}
Pb -800	505.27^{b}	222.22^{e}	11.7^{d}	7.75^{d}	52.00^{c}	43.67^{d}

Means followed by a similar letter within a column are not significantly different at the probability level of 0.05 using Duncan's Multiple Range Test.

Table 4. Response of biomasses of *Melia azedarach* and *Populus alba* to different levels of cadmium and lead pollutants after two consecutive seasons.

Treatment	*Meliaazedarach*			*Populus alba*		
	Leaves dry weight (g $plant^{-1}$)	Stem dry weight (g $plant^{-1}$)	Roots dry weight (g $plant^{-1}$)	Leaves dry weight (g $plant^{-1}$)	Stem dry weight (g $plant^{-1}$)	Roots dry weight (g $plant^{-1}$)
Control	18.39^{a}	94.27^{a}	57.28^{a}	15.66^{a}	58.32^{a}	55.21^{a}
Cd -10	14.41^{b}	93.83^{a}	56.21^{ab}	12.27^{b}	55.90^{ab}	50.19^{abc}
Cd -20	13.61^{b}	87.06^{ab}	51.02^{c}	10.96^{bc}	53.07^{c}	48.89^{abc}
Cd -40	10.47^{c}	77.64^{bc}	43.38^{d}	9.96^{c}	47.22^{d}	43.76^{c}
Pb -200	14.43^{b}	92.67^{a}	54.70^{ab}	15.90^{a}	56.73^{ab}	50.41^{ab}
Pb -400	13.44^{b}	88.07^{ab}	52.80^{bc}	15.15^{a}	55.34^{bc}	48.65^{bc}
Pb -800	11.72^{bc}	73.44^{c}	45.17^{d}	10.04^{c}	49.14^{d}	36.75^{d}

Means followed by a similar letter within a column are not significantly different at the probability level 0.05 using Duncan's Multiple Range Test.

(2002) indicated that Cd and Pb can result in growth inhibition and toxicity symptoms. Bindhu and Bera (2001) studied the effect of different concentrations of $CdSO_4$ on leaf area in mungbean seedlings. Leaf area decreased with an increase in the concentration of $CdSO_4$ in comparison to untreated control. Total chlorophyll decreased thereafter with an increase in the concentration of Cd^{2+}. The toxic effects of cadmium on the photosynthetic system cause several structural and functional disorders. The main targets are the photosynthetic pigments biosynthesis pathways though cadmium reduces chlorophyll production by the inhibition of proto-chlorophyllide reductase. Also, it can interfere with the photosynthetic pigments by substituting Mg^{2+}ions with Cd^{2+}ions in chlorophyll molecules. These substituted molecules have much lower fluorescence quantum yields compared to magnesium chlorophylls. These two toxic effects reduce the production of chlorophyll and consequently photosynthesis, which can then lead to senescence and cell death (Santos et al., 2010). Also, positive correlations between the photosynthetic rate and N content of the plant have been reported by Keulen and Stol (1991) and Makino et al. (1994). Lead accumulation in plant tissue is changing cell membrane permeability, by reacting with active groups of different enzymes involved in plant metabolism and by reacting with the phosphate groups of ADP or ATP, and by replacing essential ions. Lead toxicity causes inhibition of ATP production, lipid peroxidation, and DNA damage by over production of ROS. In addition, lead strongly inhibits root elongation, seedling development, plant growth, transpiration and chlorophyll production. The negative effects that lead has on plant vegetative growth mainly result from the following factors: distortion of chloroplast ultrastructure, obstructed electron transport, inhibition of Calvin cycle enzymes, impaired uptake of essential elements, such as Mg and Fe, and induced deficiency of CO_2 resulting from stomatal closure (Pourrut et al., 2011).

Biomass

Table 4 presented that the higher rate of cadmium (40 ppm) and lead (800 ppm) were the most harmful

Table 5. Mean content and uptake of cadmium in the leaves, stem and roots and total uptake of *Melia azedarach* and *Populus alba* responded to different levels of cadmium pollutant at the end of second season.

Treatment	Content (mg/kg)			Uptake (mg)			Total uptake (mg/plant)
	Leaves	Stem	Roots	Leaves	Stem	Roots	
Melia azedarach							
Control	-	-	-	-	-	-	-
Cd -10	2	3.6	13.5	0.03	0.34	0.76	1.13
Cd -20	2.5	5.2	16.5	0.22	0.45	0.84	1.51
Cd -40	2.5	5.9	17.5	0.19	0.46	0.76	1.41
Populus alba							
Control	-	-	-	-	-	-	-
Cd -10	4.5	3.5	8.5	0.06	0.19	0.43	0.68
Cd -20	7.5	4	17	0.08	0.21	0.83	1.12
Cd -40	14.5	11.5	17.5	0.14	0.54	0.77	1.45

Each value represented the average of three replicates.

treatments that decreased the leaves dry weight (LDW) of chinaberry seedlings by 43.1 and 36.3%, respectively, less than the non-contaminated. Also, roots dry weight (RDW) had a same manner, therefore, it was significantly shorter by 24.3 and 21.1%, respectively, less than non-contaminated seedlings. On the other hand, Pb 800 and Cd 40 ppm decreased the stem dry weight (SDW) of Chinaberry by 22.1 and 17.6%, respectively, less than non-contaminated seedlings. Table 4 shown that Cd was more negatively affect leaves and stem biomass of white poplar seedlings than Pb though, both Cd 40 and Pb 800 ppm decreased (LDW) by 36.4 and 35.9%, and (SDW) by 19.0 and 15.7%, respectively, less than non-contaminated seedlings. In contrast, the biomass of the roots was negatively affected by Pb more than Cd contamination, therefore, Pb 800 followed by Cd 40 ppm significantly decreased (RDW) of white poplar by 33.4 and 20.7%, respectively, less than non-contaminated seedlings. The above mentioned results are in parallel with Chiraz et al. (2004) that they observed inhibition of photosynthesis, and as a consequence, a decrease in dry weights. Also, the roots from Cd-stressed plants were shorter than those from controls. The decrease in biomass as a result of contamination by Cd and Pb may be due to the decrease in photosynthesis, carbohydrates metabolism as well as, production of reactive oxygen, therefore, Santos et al. (2010) concluded that the effects of cadmium in the carbohydrate metabolism are mostly due to the inhibition of enzymes such as RuBisCO. Also, the exposure of plants to metals such as cadmium can also stimulate the production of reactive oxygen species that cause the oxidation of proteins, lipids and nucleic acids, membrane damage, mutagenesis and the inactivation of enzymes.

Mineral content

Table 5 indicated that cadmium content and uptake of stem and roots of *M. azedarach* increased with increasing the soil contamination level from 10 to 40 ppmtherefore, Cd contents of leaves, stem and roots rose up to 25.0, 63.9 and 29.6%, respectively, also, Cd uptake of stem and roots rose up to 35.3 and 10.5%, respectively, whilst, the uptake of leaves of Cd-40 treatment rose up to 533.0% more than Cd-10 treatment. The highest content and uptake of Cd was recorded for roots, stem then leaves in decreasing order. Likewise, content and uptake of leaves, stem and roots increased by about 1.0 to 1.6-fold when contamination level in the soil increased from Pb 800 to Pb 200 ppm and the most content and uptake recorded for roots followed by stem. In addition, the total uptake of Pb increased by 20.3% when contamination level of Pb increased from 200 to 800 ppm (Table 6). Also, Table 5 demonstrated that Cd content of leaves and stem of *P. alba* contaminated by Cd 40 rose approximately 3-fold more than Cd 10 ppm and Cd uptake rose 2.3 to 2.8-fold more than Cd 10 ppm whilst, the increase in content and uptake of roots were slight. The highest uptake of Cd was recorded for roots, stem then leaves in decreasing order. The increase of Pb uptake for leaves, stem and roots ranged 1.3 to 1.5 fold when the contamination level in the soil increased from Pb 200 to Pb 800 ppm (Table 6).

These results were matched with Nylund (2003) that the uptake of Cd in tree seedlings was proportional to the concentration of Cd in soil. Also, Zhivotovskya (2011) on various tree species determined that Cd and Pb were mainly accumulated in roots higher than leaves and stem. Plant roots are able to release into the rhizosphere chelating agents with binding ability for metals (Salt et al., 1993). These metal chelators or other molecules within plant cells that have a high affinity for metals can help in the metal sequestering (Fulekar et al., 2009). Consequently, most of the Cd uptake occurs in the epidermis of the root tips (Landberg and Greger, 1996). Root tips lack the casparian band, and Cd is therefore transported apoplastically through cell walls directly to the xylem.

Table 6. Mean content and uptake of lead in the leaves, stem and roots and total uptake of *Melia azedarach* and *Populus alba* responded to different levels of lead pollutant at the end of second season.

Treatment	Content (mg/kg)			Uptake (mg)			Total uptake (mg/plant)
	Leaves	Stem	Roots	Leaves	Stem	Roots	
Melia azedarach							
Control	-	-	-	-	-	-	-
Pb -200	12	10.3	36.2	0.17	0.95	1.98	3.1
Pb -400	12.1	11.4	45.5	0.16	1	2.4	3.56
Pb -800	17	16.3	51.5	0.2	1.2	2.33	3.73
Populus alba							
Control	-	-	-	-	-	-	-
Pb -200	25	21.6	48.6	0.4	1.22	2.45	4.07
Pb -400	40.6	30.2	41.5	0.62	1.67	2.09	4.38
Pb -800	60.8	31.1	68.5	0.61	1.53	2.52	4.66

Each value represented the average of three replicates.

Table 7. Mean soil Extractable Cd and Pb as affected by Cd and Pb contamination at the end of second season.

DTPA extractable	Control	Cd 10 ppm	Cd 20 ppm	Cd 40 ppm	Pb 200 ppm	Pb 400 ppm	Pb 800 ppm
Melia azederach							
Cd	0.00	03.50	11.60	13.90	0.00	0.00	0.00
Pb	0.00	0.00	0.00	0.00	121.14	217.50	367.18
Populus alba							
Cd	0.00	1.30	1.80	2.45	0.00	0.00	0.00
Pb	0.00	0.00	0.00	0.00	114.50	200.17	247.50

Each value represented the average of three replicates.

Cations in the xylem move upwards in the negative walls of the xylem, but most (70 to 90%) remain in the root tissue. The reason for this may possibly that Cd is adsorbed to negative charges on cell walls and macromolecules in cells, or is taken up by the root cell and accumulates in the cytoplasm and vacuoles. Also, Pourrut et al. (2011) mentioned that under lead stress, plants possess several defense strategies as reduced uptake into the cell, sequestration of lead into vacuoles by the formation of complexes and binding of lead. In addition, activation of various antioxidants to combat increased production of lead-induced ROS constitutes a secondary defense system.

Soil extractable

Table 7 demonstrated that, extractable Cd and Pb were increased with increasing the contamination rate of each pollutant from lower to higher rates for both tree species.

Recommendation

M. azedarach and *P. alba* could be employed in phyto-remediation of soils contaminated with cadmium (up to 40 ppm) or lead (up to 800 ppm) whereas, they grow reasonably in these soils without any toxicity symptoms. In addition, autumn litter fall of both species do not create a risk of cadmium and lead input into the soil.

Conflict of Interests

Authors have not declared any conflict(s) of interests.

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