Environmental Engineering and Management Journal October 2014, Vol.13, No. 10, 2583-2592
http://omicron.ch.tuiasi.ro/EEMJ/

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PHYTOACCUMULATION, COMPETITIVE ADSORPTION AND EVALUATION OF CHELATORS-METAL INTERACTION IN LETTUCE PLANT

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Abstract

Present study was envisaged to study chelators-metal interaction in phytoaccumulation of metals as a bioremediation technology to decontaminate metal pollution and adsorption of metals on soil. *Lactuca sativa* was used as model plant for phytoaccumulation to identify uptake of Cadmium (Cd) and Lead (Pb) and their subsequent accumulation in edible tissue of plant. Cadmium and lead were two selected metals for phytoaccumulation and copper was also studied for adsorption experiment. Green house experiments on Lettuce plant were conducted for 3 months and a significant difference of growth with metal chelator addition was observed. The whole study was divided into two experiments first enhanced phytoaccumulation with chelator addition in plants was conducted in green house and in second experiment batch studies were conducted to evaluate competitive adsorption of selected metals on agricultural soils in laboratory conditions. Three main trends were identified in the experiment. First, transfer of metals from root to shoot was increased after DTPA application as it helped to increase metal bioavailability. Second a negative trend was observed with increasing metal-chelator concentration and > 50 % reduction in plant dry biomass. Thirdly, water solubility of metals in soil was significant after 3 months of DTPA which shows low degradation and higher bioavailability. Bioconcentration factor and metal transportation index were calculated to find relation with metal uptake and plant growth. Phytoaccumulationan and adsorption of Cd is higher than Pb and copper.

Key words: cadmium, competitive adsorption, DTPA, lead, lettuce

Received: February, 2012; Revised final: June, 2012; Accepted: July, 2012

1. Introduction

Heavy metal concentration in upper horizon of soil is increasing with increased industrialization and urbanization. Vehicular emission is also a documented source of metals contamination in upper horizon (Minkina et al., 2014). Different chemical and biological treatment techniques are available; phytoremediation and phytoextraction with plants is one of the important techniques for in situ remediation of heavy metals present in soil (Kirkham, 2006; Malschi et al., 2013). Chemical amendment like addition of chelators is being used to improve the phytoextraction process (Muhammad et al., 2009; Schmidt, 2003). Chelator application increases the metal uptake by plants (Chen and Cutright, 2001; Engelen et al., 2007; Nascimento et al., 2006; Robinson et al., 2000) due to increased metal concentration in soil solution by increasing its bioavailability. However, reduced uptake is also reported, although metals solubility was enhanced after chelator application (Inaba and Takenaka, 2005; Liu et al., 2008; Nascimento et al., 2006). Inaba and Takenaka (2005) applied EDTA and DTPA for enhancing Cu uptake by lettuce but decrease in Cu concentration was observed. Toxicity of chelators to

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lettuce plants at sprouting stage was reported (Inaba and Takenaka, 2005) and lettuce plants might have different behavior in other growth stages. Plants suffer with the higher concentrations of chelators in soil with or without metals (Chen and Cutright, 2001; Nascimento et al., 2006).

Chelator (EDTA) has ability to increase the Cd concentration in a day (Lombi et al., 2001) or two (Luo et al., 2005) after application and thereafter metal availability remains constant or concentration decrease rather slowly (Lombi et al., 2001; Luo et al., 2006a). EDTA and DTPA both persist in soils (Meers et al., 2008). DTPA is photo degraded relatively earlier than EDTA (Evangelou et al., 2007; Metsärinne et al., 2004) particularly in shallow water but is very resistant against biodegradation (photodegadation is not possible in soil) (Metsärinne et al., 2004; Nörtemann, 2005). DTPA is persistent but not having very high Cd solubility in leachate as compare to most other synthetic chelators (Engelen et al., 2007). Water soluble Cd in leachate water was found only 2 times greater when treated with DTPA as compare to other chelators where Cd was 35 times greater with EDTA, 26 times greater with EGTA, and 8 times greater with CDTA, whereas, DTPA increased Cd concentration in *Brassica juncea* was most relative to solubility of Cd in runoff water (Engelen et al., 2007).

Chelator assisted phytoremediation has widely been used to decontaminated soil however, before application, metal chelator interaction is given conditions must be critically analyzed keeping in view this aspect objective of present study is to check Cd and Pb uptake by lettuce plants under the influence of DTPA, plant response to different metal and DTPA levels, and after 3 months DTPA enhanced metal water solubility in soil and adsorption potential of selected metals on agricultural soils.

2. Methods

2.1. Apparatus and Reagents

pH meter provided with a combined glass-calomel electrode an thermal probe was used for pH. Metal determinations were carried out by (model) Atomic absorption spectrophotometer. The calibrations were always performed with standard solutions prepared in aliquots of sample blanks. High purity water (HPW) from Millipore Q system was used throughout. Standard metal solutions were prepared from concentrated stock solutions. All the reagents used were of analytical grade.

2.2. Sample preparation and soil characterization

For phytoaccumulation experiment surface soil (0-15cm) was collected from Arid Agriculture University Rawalpindi farm area and for sorption on agriculture soil. Soil was collected from peri urban agricultural soils of Gujranwala and Shahdara two agricultural cities of Punjab Pakistan. The soil of first experiment was silt loam and represents Rawalpindi series classified as a Typic Calciustept. While soil of Gujranwala was clay loam and Shahdara was silt loam. The soil samples were air-dried, crushed passed through 2mm sieve and filled in experimental pots. The soil was analyzed for texture by Bouyoucos hydrometer method (Sheldrick and Wang 1993), soil pH by 1:1 soil to 1 N KCl suspension (by volume) (Rhoades, 1982), electrical conductivity (ECe) of the 1:1 suspension extract by a conductivity meter (Rhoades, 1982) total organic carbon (TOC) by Walkly Black method (FAO, 2007) and dissolve organic carbon (DOC) in soil by extracting soluble OC in K_2SO_4, digestion in $K_2Cr_2O_7$ and concentrated H_2SO_4 and measurement of excess $K_2Cr_2O_7$ with $Fe(NH_4)_2$ $(SO_4)_2$ (Nelson and Sommers, 1982). Native bioavailable soil Pb and Cd were extracted by Ammonium Bicarbonate-DTPA and measured by an atomic absorption spectrophotometer (Soltanpour, 1985). Saturation percentage of soil was measured by making soil saturated paste, remained overnight in Buckner funnel and leave for 24 h in oven at 105°C.

2.3. Experimental Protocol

Phytoaccumulation of selected metals in Lettuce plant was carried out in following steps

2.3.1. Raising Metals Level in Soil

Cadmium nitrate was applied to the potted soil at the rate of 0, 5, 10, 20, 30 mg Cd kg^{-1} soil and $Pb(NO_3)_2$ at the rate of 0, 100, 500, 1000 and 1500 mg Pb kg^{-1} soil separately. The salts were applied by making solutions and were applied according to saturation percentage of soil.

2.3.2. Growth Experiment

Preliminary analysis indicated less than 1 percent organic matter in soil used for the pot experiment. The soil metal equilibrium was established in green house over 45 days by assuming that the time period is considerably adequate as soil with low organic matter content has minimum dynamic activity that is associated with colloidal nature of organic matter (Manouchehri et al., 2006) and maximum chelator effect for metal recovery (Hong et al., 2002). For first 15 days soil was spiked with respective metal concentration and then soil of each pot was thoroughly mixed to get uniform distribution. Lettuce (*Lactuca sativa*) nursery was raised in amended soil and 4 months old seedlings were planted in each pot. After one week plants were thinned to 3 plants per pot.

One month after transplanting, DTPA solutions were applied at the rate of 0, 500, 1000 and 2000 mg DTPA kg^{-1} soil to Cd contaminated soil and 0, 500, 1000 and 1500 mg DTPA kg^{-1} soil to Pb contaminated soil. Due to insolubility of DTPA, it was titrated with $Ca(OH)_2$ to make it water soluble. Independently, $Ca(OH)_2$ is not water soluble so different ratios of DTPA and $Ca(OH)_2$ in solid forms were tried and minimum titrateable quantity of $Ca(OH)_2$ was calculated. About 250 mg was able to titrate and completely dissolved 1g of DTPA. Further titrations were done by following the above mention ratios of two chemicals.

Plant leave tissue (above ground part or shoot of single plant) were collected before DTPA application, dried and analyzed. After DTPA application, plants were allowed to grow for 45 days with distilled water irrigation and shoots and roots were separately harvested. There were 20 combinations each for Cd-DTPA and Pb-DTPA with three replicates the total number of pots were 120.

2.3.3. Cd and Pb concentration in plant tissue

Plant tissues of both harvestings were washed in tap water, then with distilled water dried at 65 °C for 48 hrs and weighed to determine biomass. All the plant samples were ground using grinder and digested in 1:2 $HClO_4$ + HNO_3 acidic mixture. Cadmium and Lead concentrations in the digested samples were determined by Atomic Absorption Spectrophotometer (AAS) (Issac and Johnson, 1975).

2.3.4. Cd and Pb concentration in soil

For water soluble metals suspensions were made by mixing 10 g soil and 50 ml DI water (1:5) in 100 ml conical flask, then this suspension was shaked on orbital shaker at 85 rpm for 16 hr (Santos et al., 2006). Samples for water soluble Cd were shaken for 90 days of DTPA application whereas samples for water soluble Pb after 60 days. After shaking, samples were centrifuge, filtered through 0.45 µm membrane filter and analyzed by AAS.

2.3.5. Relative indices

Transportation Index (Ti) for Cd and Pb was calculated using the following equations (Gosh and Singh, 2005) (Eq. 1).

$$Ti = \frac{Cd\ or\ Pb\ content\ of\ the\ leaves\ mg\ kg^{-1}}{Cd\ or\ Pb\ content\ of\ root\ mg\ kg^{-1}} \times 100 \qquad (1)$$

Bioconcentration Factor (BCF) was calculated as (Gosh and Singh, 2005) (Eq. 2):

$$BCF = \frac{Average Cd\ or\ Pb\ conc. in\ the\ plant tissue (mg\ kg^{-1})}{Spiked Cd\ or\ Pb in\ soil (mg\ kg^{-1})} \qquad (2)$$

2.3.6. Sorption experiment

To analyze sorption potential of heavy metals second study was planned selecting three metals on agricultural soils collected from two different cities Gujranwala and Shahdara. Three gram soil sample was taken into centrifuge tube equilibrated with 30 ml 0.0 M Ca $(NO)_3$ solution containing graded levels of heavy metals for Cd and Pb i.e 0, 20, 40, 80, 120, 160, 200, 240 and 280 mg Cd/L using as $Cd(NO_3)_3$ the shaking of centrifuge tubes was carried out for 16 hours to complete the reaction of metals with soil solid phase. Similarly another set was equilibrated with 30 ml 0.01 M $Ca(No_3)$ solution containing graded levels of Cu i.e 0, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 and 2000 mg Cu/L using $Cu(No_3)_3$. Each sorption set for Cu, Pb and Cd was replicated thrice. After shaking suspension was centrifuged at the speed of 5000 rpm for 5 minutes and supernatant was filtered and collected for the determination of sorbed concentration of cadmium, copper and Lead in two types soil samples using atomic absorption spectrophotometer (Adhikari and Singh, 2000). Langmuir isotherm was applied to observe adsorption potential. Adsorption values were reported as average of three. The adsorbed concentration was determined from standard calibration curve.

3. Results

The summary of soil characteristics for phytoaccumulation experiment is presented in Table 1 and sorption experiment is presented in Table 2.

3.1. Metals accumulation and translocation

Metals content was measured in roots and shoots in the lettuce (Fig. 1 a-d). Root and shoot Cd and Pb contents increases linearly in response to increasing concentrations. Lead content in roots displayed a different pattern than shoots, applied Pb concentration of 1000 mg kg^{-1} of soil showed more accumulation in roots than 1500 mg Pb kg^{-1} of soil. In the control treatment with no DTPA applied to soil (Fig. 1 d), the root Pb concentration did not changed much till 500 mg kg^{-1} Pb application.

However, there was a gradual rise in root Pb contents but remained significantly lower than all DTPA treatments mainly because low organic matter content in soil did not formed Pb-OM complexes and hence high dose application of soil Pb (beyond 500 mg kg^{-1} soil) promoted the naturally available soil solution Pb that was readily available for root uptake. Cd translocation from roots to shoots was more than Pb translocation Pb uptake in roots was higher than shoots which shows poor translocation and more uptakes as evident from values of Table 3.

3.2. Metal effect on lettuce growth

In order to analyze the effects of metal chelator complex on lettuce growth, 15 day old lettuce plantlets were exposed over 75 days different concentrations of Pb and Cd and effect on root shoot growth was observed. All the plants displayed similar overall responses with respect to metal toxicity symptoms. Exposure to increasing concentrations of Cd and Pb resulted in significant (Cd, $P \leq 0.05$) and (Pb, $P \leq 0.005$) shoot growth reduction. A decrease in shoot and root dry weight was observed Fig. 2 (a) and (b) with application of 30 mg kg^{-1} Cd in soil, 38% decrease in biomass of shoot was observed in control plants and 62% dry weight decrease was observed with 2000 mg DTPA kg^{-1}application. While 41% and 55% decrease in dry root weight per plant was observed in control and 2000 mg DTPA kg^{-1} applied plants. Application of 1500 mg kg^{-1} of Pb caused 40% and 25% reduction in shoot dry weight of control and 1500 mg DTPA kg^{-1} while 35% and 37% respectively reduction was observed in root dry weight Fig. 2 (c) and (d).

Toxic effects of DTPA on plants were immediately observed after its application and severity of toxicity was increased with level of concentration. Plants showed symptoms of leave chlorosis and become yellowish with higher dosage of Cd and Pb spiked levels. In Cd added soil with 2000 mg DTPA kg^{-1} soil 2 plants per pot in 4 pots while 1 plant per pot in 3 pots died while in 1000 mg DTPA kg^{-1} soil 1 plant per pot died in 3 pots and in 500 mg DTPA kg^{-1} no plant died. All dead plants were harvested as they were seen dried. A similar trend was observed with DTPA application in Pb added soil except at 500 mg DTPA kg^{-1} 1 pot per plant in 2 pots was died which shows more toxicity in Pb soils at low concentration of DTPA.

Table 1. Basic characteristics of soil used for experiment 1

	Soil used for Cd spiking	*Soil used for Pb spiking*
pH	7.25	7.21
EC	3.16	0.94
O.M (%)	0.67	0.89
DOC (mg kg^{-1})	107.3	148.5
Saturation percentage	25.4	23.2
Texture	Silt loam	Silt loam
Sand (%)	29	31
Silt (%)	51	55
Clay (%)	20	14
Cd (mg kg^{-1})	–	–
Pb (mg kg^{-1})	–	–

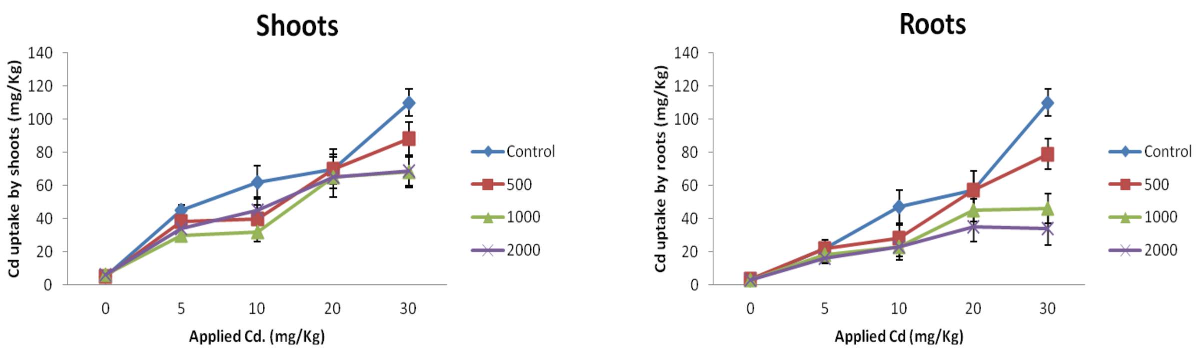
Table 2. Basic characteristics of soil used for experiment 2

	Gujranwala Soil	*Shahdara Soil*
pH	8.21	7.21
EC	0.71	0.94
O.M (%)	0.58	0.89
$CaCO_3$ (%)	5.5	3.5
Texture	Clay loam	Silt loam
CEC (meq) 100/g soil	11.51	6.35
Cd (mg kg^{-1})	–	–
Pb (mg kg^{-1})	–	–

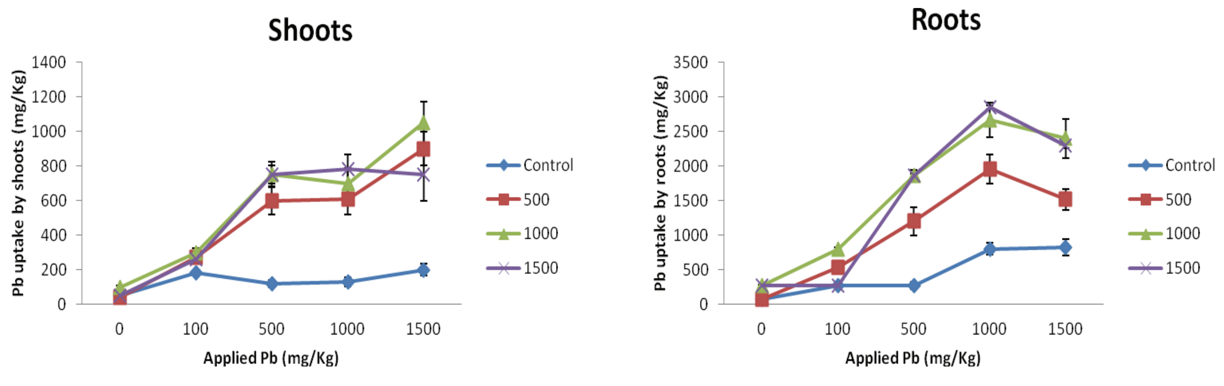
Table 3. Comparison between shoot and root mean concentration for Cd and Pb under different DTPA treatments in soil

DTPA treatment	*Shoot vs Root for Cd*		*Shoot vs Root for Pb*	
	Difference	*P-value*	*Difference*	*P-value*
Control	+10.6	0.068	–311.2	0.087
500 mg kg^{-1}	+10.3	0.013*	–573.6	0.061
1000 mg kg^{-1}	+13.2	0.020*	–1019.2	0.032*
2000 mg kg^{-1}	+21.6	0.017*	–993.2	0.064

** showing significant difference at P<0.05*

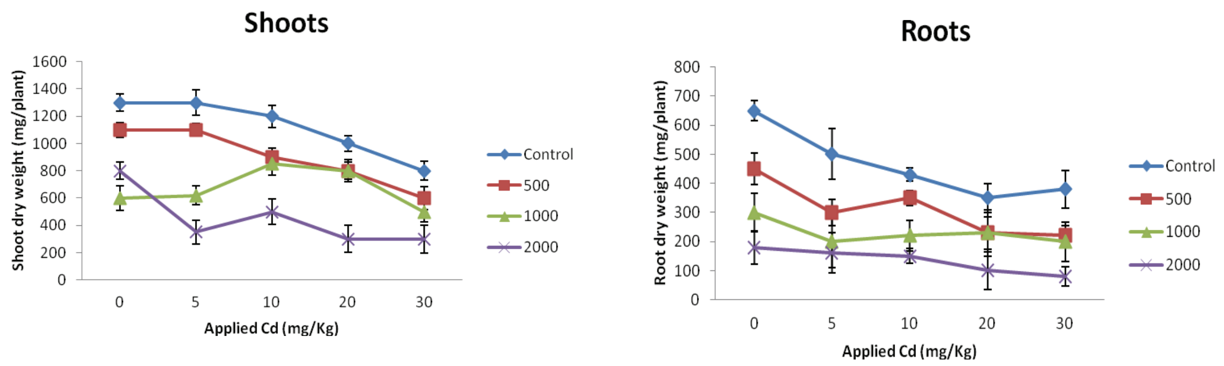


a) b)



c) d)

Fig. 1. Cd uptake a) by shoots and b) by roots under different applied Cd and DTPA (mg kg^{-1}) applications; Pb uptake by c) shoots and d) by roots under different applied Pb and DTPA (mg kg^{-1}) applications



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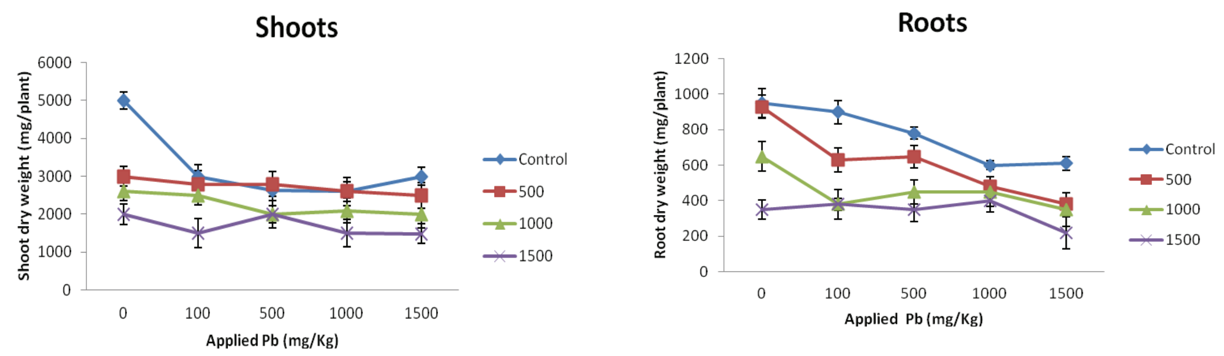


c) d)

Fig. 2. a) Shoot dry weight and b) Root dry weight under different applied Cd and DTPA (mg kg^{-1}) applications; c) Shoot dry weight and d) Root dry weight under different applied Pb and DTPA (mg kg^{-1}) applications

3.3. Metal transportation Index

Transportation index is ability of plant to translocate metals from root to shoot and it was observed above 100% in all combinations exposed to Cd. Transportation index remained unchanged with constant DTPA and increasing spiked Cd levels but increase in applied DTPA significantly increased Cd from root to shoot.

While in Pb transportation index was below 100% and a positive effect was manifested with DTPA applications in the form of increased transportation index.

3.4. Relationship between Metal accumulation and bioconcentration factor

Metal bioconcentration factor BCF was calculated and a decreasing trend was observed with increasing metal concentration. Highest Cd BCF, 9.5 in root was observed at 5 mg spiked Cd kg^{-1} soil and 8.3 at 100 mg spiked Pb kg^{-1} of soil was observed. Maximum BCF in root and shoot was observed at lowest levels of Cd and increased DTPA and it was reduced as concentrations were increased. Role of DTPA was clear in enhancing BCF when compared with DTPA control.

BCF in roots was higher than shoots and decrease in dry weight was also following the similar pattern.

3.5. Effect of DTPA on post harvest metal solubility in soil

Water soluble metals represent readily bioavailable proportion (Fig. 3 a, b). After three months application of contaminants metals were extracted and still high concentration was found which shows constant bioavailability and low degradation. There was about 120 times more soluble Cd in soil and 8 times more Pb with highest spiked concentrations Cd and DTPA as compared to control of both were present after three months spiking of metals chelator complex. Both the metals applied to soil have responded differently in the presence of DTPA concentrations for their removal from soil by plants.

The interaction between Cd and DTPA has reduced the phytoaccumulation of this metal by lettuce owing to greater affinity of Cd to make complexes with chelating agent. The Pb-DTPA interaction has resulted in enhanced uptake and tissue Pb accumulation which in turn reflects an increased tendency of this metal to become bioavailable in soil solution with increasing dose of DPTA applied to soil.

3.6. Metal sorption on soil

To analyze sorption potential of heavy metals on two selected soils of Punjab batch experiments were conducted on ambient conditions in lab. The sorption curves were very close to the theoretical 100% sorption curve represented by a square line. Initially, there was a rapid increase in Cd sorption followed by a slow rise of isotherm associated with diffusion into clay minerals.

Adsorption trend of Gujranwala soil was higher than Shahdara soil. Cd and Cu shows similar trends of increasing adsorption with increasing concentration in Gujranwala soils and a continuous rise shows still more can be adsorbed and equilibrium is not established yet in 24 hours batch while Pb shows very less adsorption and establishment of equilibrium Fig. 4 a, b.

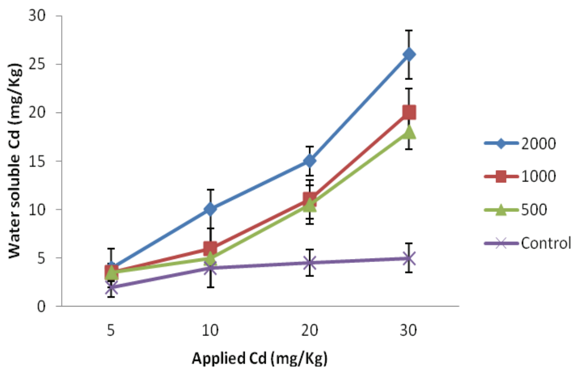
Soils collected from Shahdara shows similar trends of lower adsorption and equilibrium establishment by application of Pb and Cu While Cd still shows a rising trend. Shahdara soils shows less affinity for Cu adsorption than Gujranwala soils. It was also observed that at initial concentrations trends were similar and at 2 mg/L applied metal concentration sharp rise and difference of trends started appearing it means 2 mg/L is a trigger point and beyond it rising slope of adsorption increases with applied concentration.

4. Discussion

In the present study, we aimed to identify trends linking different characters related to metal chelator response and examined lettuce plants for its ability to remove metals from soil. Growth of lettuce plants was restricted due to presence of Cd and Pb in the soil and ultimately reduction in dry weights occurred with the toxicity of those metals.

Plant biomass reduction due to heavy metal toxicity was reported in many studies (Grčman et al., 2001). Plant dry weights were decreased at various applied Pb levels except at lowest level of 100 mg Pb kg^{-1} soil. Michalska and Asp (2001) and Xiong (1998) agreed with increasing plants dry weight at lower levels of Pb. At lower concentrations $Pb(NO_3)$ gives stimulating effect and higher concentrations stimulates inhibiting effect. The combined effect of metals and DTPA application were even severe than independent effect of chelators or metals that might exceed the capacity of plants to activate defense system (Sun et al., 2009).

A measureable amount of Pb and Cd was extracted by the lettuce from soil. Cadmium removal from soil was more than applied levels of Cd and about 50 mg Cd kg^{-1} dry weight of lettuce was extracted with 20 mg spiked Cd kg^{-1} soil, which was nearer to the reported values of Podar and Ramsey (2005), they got up to 40 mg Cd kg^{-1} dry biomass with similar spiked level. Addition of DTPA increased Cd uptake by almost 3.5 folds in *Brassica juncea* observed by Engelen et al. (2007).



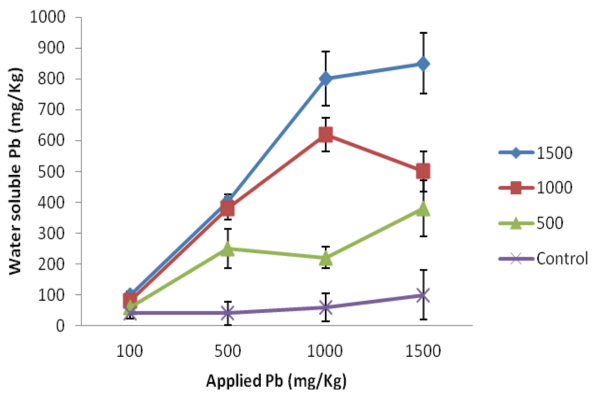


Fig. 3. Metal ions concentration in soil after three months application of DTPA (mg kg^{-1}): a) Cadmium; b) Lead

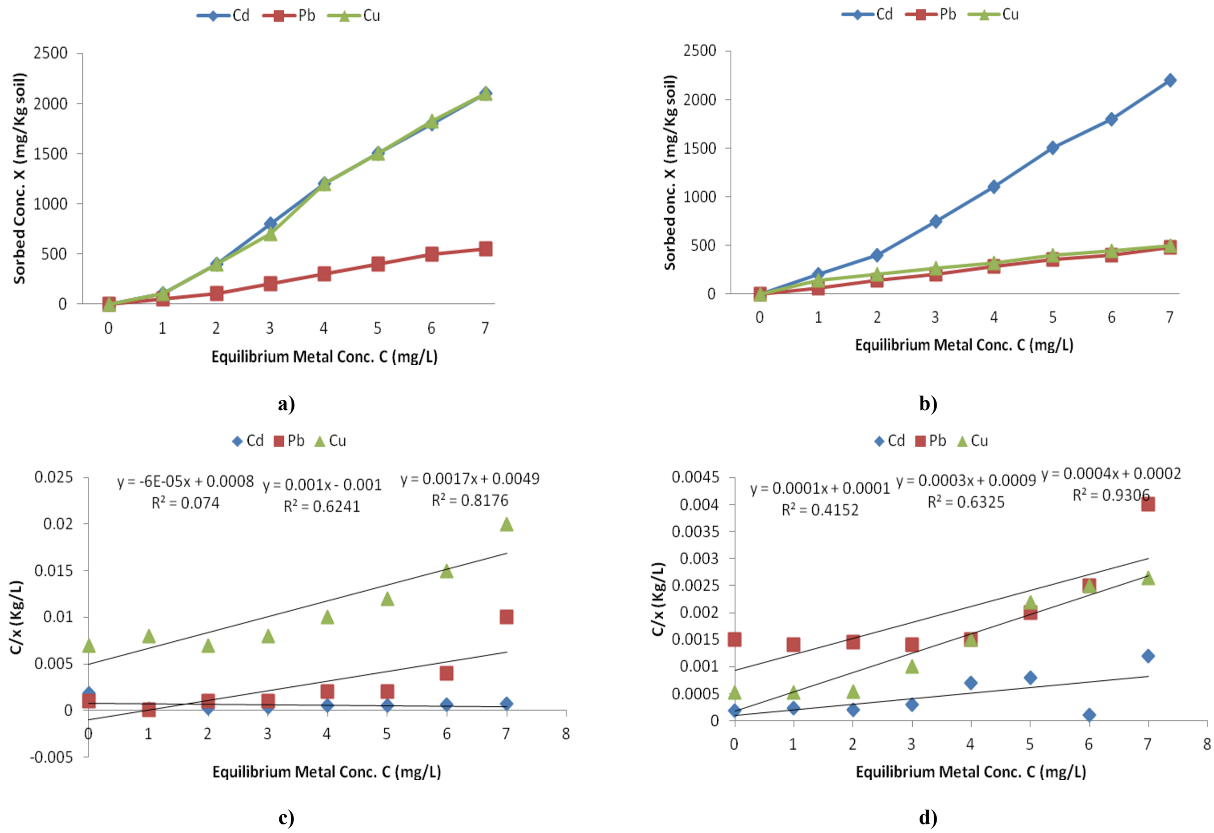


Fig. 4. a) Sorbed conc. of metals on Gujranawala soil; b) Sorbed conc. of metals on Shahdara soil; c) Langmuir isotherm of metals on Gujranawala soil; d) Langmuir isotherm of metals on Shahdara soil

But in the present study, Cd concentration in lettuce was decreased with increasing concentration of DTPA in soil. The results were in line with Inaba and Takenaka (2005), they reported opposite effect of DTPA in Cu extraction by lettuce. Exact mechanisms involved in phytoaccumulation of heavy metals is not yet clear. Several studies suggest that metals damage the membranes which normally act as barriers (Kumar et al., 1995; Marschner, 1995; Salt et al., 1995) while addition of chelators removes Ca and Zn from plasma membranes changing physiology of membranes. The extent of assimilation of heavy metals from soil depends on whether they are present in a form that can be absorbed by plants. For example, Pb can be strongly absorbed by soil particles and, thus, it is scarcely translocated to plants, while Cd ions are relatively mobile in soil and can be more easily absorbed by vegetation.

Plants accumulate heavy metals from soils through different mechanisms such as: absorption, ion exchange, redox reactions, and precipitation–dissolution. In addition to these accumulation mechanisms, the solubility of trace elements in soils depends on the minerals present in them (carbonates, oxides, hydroxides, etc.), on the level of soil organic matter (humic acids, fulvic acids, polysaccharides and organic acids), soil pH, redox potential, temperature and humidity (Tarradellas et al., 1996). Only the portions of elements which present availability are transferred into plants (Smical et al., 2008).

Metal transportation index of lettuce was decreased with increasing spiked metal concentration whereas increased with increasing chelator concentration. Liu et al. (2008) studied on *Sedum alfredi* and found similar trends of transportation index. Phytochelatin metal complex are considered to be involved in the long distance root to shoot transport (Gong et al., 2003). Other compounds like citrate-metal complexes present in xylem sap which have been reported by Senden et al. (1995) might be responsible in root to shoot translocation. In Pb these complexes might be weak or may be Pb shows weak affinity in making citrate-complexes its accumulation in roots is 60% more than shoots and translocate less in shoots.

Bioconcentration factor greatly depends upon translocation as the translocation decreases with increasing metal concentration similar way BCF decreases with higher applied concentrations. Niu et al. (2007) also reported that BCF of Alfalfa and Indian mustard decreased with increased in soil Cd or Pb concentrations. Here also, heavy metals extraction by the lettuce was increased with increase in soil heavy metals but BCF was reduced (Audet and Charest, 2007). Generally, BCF increased with application of chelators. But here Cd BCF was negatively affected with application or increased concentration of DTPA in soil for Cd metal. Whereas, lettuce BCF was significantly increased after DTPA application in Pb spiked experiment.

The effectiveness of phytoaccumulation was clearly manifested when both the metals were compared for their tissues sinks. A 2-fold increase in shoot Cd than root showed a marked effect on translocation of Cd from underground to above ground parts and reflects characteristic efficiency for phytoremediation of this metal by lettuce. Contrary to this, Pb was more exclusively found in root tissue and in fact its reduced translocation (low Ti and BCF shoot values) to shoot showed Pb phytoremediation inefficiency.

However, greater amount of Pb being retained in root tissues by lettuce is an indication of good phytoaccumulator species especially when DTPA application has gradually increased the soil phytoavailable pool of Pb which was successfully exploited by lettuce. Therefore, our results clearly distinguish Cd and Pb from lettuce-based remediation perspective where former being indifferent under the influence of DTPA but has significant translocation from root to shoot whereas the latter demonstrated positive response to chelator application and subsequent plant uptake which was confined to roots only.

Post harvest metal availability in Pb spiked soils is more than Cd spiked soils and verifies previous results that uptake of Cd chelator complex was more than Pb chelator complex. These findings validate the results of Zorrig et al. (2010). The Fig. 4 a, b revealed that Gujranwala soil absorbs more Cu & Cd than Shahdara soils as there was a linear increase in sorption with further increase in Cu & Cd concentrations. The sorption of Pb followed the similar trend in both soil types. The metal concentrations were found to be linear only at lower concentration while at higher level the plot shows a deviation from linearity and Langmuir exhibited a tendency to form a curvilinear trend.

The curve for all soils can be resolved into two portions as suggested by (Syers et al., 1973). Singh et al. 2001 also observed that cat ion exchange capacity CEC play a major role in sorption of heavy metals. These observations further sustained by positive correlations. Cadmium sorption on soil surface was highest at equilibrium in both types of soils which shows more Cd bioavailability of Cd plant soil ion exchange processes and higher phytoaccumulation of Cd also prove that Cd bioavailability is higher than Pb. Moreover relatively larger metal ion size of Pb may be a crucial factor for higher Pb confinement in root tissue.

Langmuir adsorption isotherm best fits in the data and higher values of intercept for Cu in Gujranwala soil shows good adsorption while in Shahdara soils intercept value of Pb is higher which shows Pb adsorb more than any other selected metal in soil. Overall all the metal sorption isotherms are near linear. The higher adsorption potential of soils reflects their good degradation ability (Anwar et al., 2010; Saba et al., 2011).

5. Conclusions

The current study identifies three main trends in phytoaccumulation of metals in lettuce plant. Increase in bioavailability increase root to shoot uptake which effects negatively plant biomass. Cd transportation from root to shoot is higher which shows its strong affinity forming complex with plant enzymes while Pb accumulation in roots are higher than shoots. Post harvest availability of metals after three months spiking shows persistence of metals and slow degradation.

Second experiments of adsorption of metals proves the results of first experiment that metal adsorb on soil surfaces are more bioavailable for plant uptake. Cd bioavailability and adsorption is higher than other metals. These trends help in better understanding of limiting growth effect of metals on plant while changes in plant physiology and cationic balance in soil needs analysis.

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