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EFFECT OF LEAD AND CADMIUM ON GROWTH OF *MEDICAGO SATIVA* L. AND THEIR TRANSFER TO FOOD CHAIN

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ABSTRACT

Lead and cadmium are considered potential hazards for being most common pollutants in our environments. Current study was conducted at Government College University Botanic Garden, Lahore to examine the effects of Pb and Cd on different growth parameters of a major fodder crop *Medicago sativa*, and their transfer to a herbivore (rabbit). Plants were grown under field conditions with different treatments prepared as, control (T_0), Pb 200 µg/g soil (T_1), Pb 400 µg/g soil (T_2), Cd 4 µg/g soil (T_3), Cd 8 µg/g soil (T_4), Pb 200 µg/g soil + Cd 4 µg/g soil (T_5) and Pb 400 µg/g soil + Cd 8 µg/g soil (T_6), each in triplicate and in a completely randomized design. After harvest, rabbits (*Oryctolagus cuniculus*) were fed with the plant tissues of all treatments for ten days. Significant ($P < 0.05$) reduction was observed in growth parameters of the plants with increase in metal levels both in single and combined treatments. The maximum accumulation of Pb (96%) and Cd (89%) was observed in roots while the highest translocation of Pb and Cd to shoots was 38 and 37%, respectively. Faeces of rabbits showed much higher values of Pb and Cd than blood samples as about 65% Pb and 38% Cd was excreted via faeces. Overall, results of the study show that *M. sativa* has the ability to retain maximum concentrations of Pb and Cd in roots and restricts their translocation to shoot and leaves. So the risk of transfer of toxic amounts of Pb and Cd to human beings through this route seems low.

Key words: Lead, cadmium, soil, alfalfa, rabbit, food chain.

INTRODUCTION

The concentrations of different heavy metals and their compounds have increased into the soil-water environment due to various anthropogenic activities. As a result, these metals bio-accumulate in the plants and other living organisms, as these can not be degraded (Singh *et al.*, 2009). Heavy metals are usually present in combination of more than one metal in soil under natural conditions and their combined effects are different from individual metal effects due to certain interactions between these metals (Zhou *et al*., 2006). These combined metals have been shown to cause more toxic effects on plants as compared to individual metals (Guo and Zhou, 2003). The presence of these metals in soil has a great potential to modify some vital metabolic processes in plants (Pederson *et al*., 2002; Veselov *et al*., 2003).

Soil metals have always been investigated thoroughly due to their adverse effects on soil system; nutrient cycling and primary production (Smolders *et al*., 2009). Some metals are integral part of bio molecules and are also necessary for the catalytic activity of many enzymes. Where as metals like cadmium, lead and mercury have no biological function instead these disturb the existing biological balance of the food chains, when released and accumulated in higher amounts in natural ecosystems (Beiglböck *et al*., 2002).There is a tremendous increase in levels of toxic elements in aquatic and terrestrial ecosystems in the last century due to rapid industrial and agrarian development. This has resulted in accumulation of metals in soil where these may interfere with bio-chemical processes in soil and get entry into food chains (He *et al*., 2005). Lead (Pb) and cadmium (Cd), the most common pollutants in agricultural soils of Punjab, are cumulative toxins. The potential sources of these metals in our soils are mostly automobile exhausts, industrial effluents, irrigation with sewage water and fertilization practices (Ahmad *et al*., 2011; Lone *et al*., 2003). Although these metals are still not present at toxicity levels in our agricultural soils but the very long resident life of these metals can raise their concentrations up to toxic levels (Khan *et al*., 2012; Khan *et al*., 2013). These can enter human body by uptake of food and fodder crops via herbivores (Ahmad and Bibi, 2010; Farid *et al*., 2013). Therefore, information about Pb & Cd uptake can be important in designing various experiments for the passage of these metals into food chain. Both metals are known to affect each other's uptake, when exist together in soil (Madyiwa *et al*., 2004). However, the interaction between both the metals depends upon the edaphic and environmental conditions, as well as the plant type (Zhi *et al*., 2007).

Medicag sativa (alfalfa), an important fodder crop of family *Fabaceae*, is grown widely throughout Punjab. It is a perennial plant and can be one of the potential routes of heavy metal transfer from soil to

human being via herbivores. So there was a need to determine the potential of this crop to accumulate and transfer metals from soil to food chain. The present study was conducted to assess the effects of Pb and Cd on growth of *M. sativa* and their transport in soil–plant–animal continuum. The information about metal uptake by crop plants therefore, is important in predicting the transfer of metals into the food chain which ultimately affects human beings present at the end of the food chain.

MATERIALS AND METHODS

Experimental set-up: The experiment was conducted at Government College University Botanic Garden Lahore (lat 31°32´59″ long 74°20´37″ elevation 217 m) during November 2009 - March 2010. Average minimum and maximum temperatures were (6 & 22°C) while mean rainfall was 13 mm during the study period. Separate plots (each of 2 m^2) were prepared for each treatment in a completely randomized design (CRD). All the treatments were replicated thrice. No crop was previously grown in the plots. Soil of the plots was clay loam in texture having pH (7.2), N (0.1%), P (0.09%), K (0.1%), organic matter (4.9%), Pb (1.45 µg/g) and Cd (0.46 µg/g). Seeds of *Medicago sativa* were obtained from Rice Research Institute Kala Shah Kakoo, Sheikhupura and sterilized with 10% ethanol for 30 seconds. Sterilized seeds were soaked in 0.1% Mercuric Chloride solution for 5 minutes and washed with distilled water to avoid any fungal growth (Singh *et al*., 2009). Concentrations of Pb 200 µg/g soil (T_1), Pb 400 µg/g soil (T_2), Cd 4 µg/g soil (T_3), Cd 8 µg/g soil (T_4), Pb 200 µg/g soil + Cd 4 µg/g soil (T_5) and Pb 400 µg/g soil + Cd 8 µg/g soil (T_6) were prepared by calculating the amounts of Pb and Cd from $Pb(NO_3)_2.4H_2O$ and $Cd(NO_3).4H_2O$, respectively. Soil layer up to 0 - 20 cm (plough layer) was mixed thoroughly with Pb and Cd salts to make homogenous treatment concentrations. No metal was added to the control (T_0). Plots were fertilized with super phosphate at the rate of 60 kg/hectare. In each plot 75 seeds were sown at a depth of 1/4 inch during November. Plants were irrigated twice a week. Crop was harvested after 120 days at the appearance of floral buds. Roots were taken out very carefully with the help of spade and separated from shoots. After harvest root lengths and shoot lengths were measured. Plant biomass was determined on the basis of oven dried weights of roots, shoots and leaves. Oven dried soil and plant tissue samples from each treatment were taken for determination of heavy metals. Six months old male *Oryctolagus cuniculus* (rabbits) with an average weight of 2.5 kg were chosen for the experiment. Three rabbits under each treatment were kept in a separate chamber so there were a total of 21 rabbits in seven treatment cages that were placed in a completely randomized design. The rabbits were allowed to feed with treated crop plants for ten days. No other food supplement was given to the animals during the experimental period. After that period, their faeces and blood samples were collected.

Sample Preparation: Soil samples were air dried and passed through a 1 mm sieve to free from waste materials. Soil pH was determined with pH meter (pH 210 Micro Processor). Pb & Cd were determined by Atomic Absorption Spectrophotometer (UNICAM 969 UK) by preparing the samples with Diethylene triamine penta acetic acid (DTPA) extractant solution (FAO, 1982). Root and shoot lengths were measured with the help of meter rod. Roots, shoots, leaves and faeces were washed with tap water and air-dried. The samples were oven dried at 80°C for 24 hours and dry weights were measured. Samples were then ground to make a homogenous powder. About 2.0 g of dried sample was taken and incinerated to remove moisture. Dry ashing was done at 480°C in muffle furnace for 4-8 hours. Samples were digested by 6 N HCl and filtered through filter paper (Whatman No.1). Final volume of the filtrate was made up to 50 ml. The solution was run through Atomic Absorption Spectrophotometer (AAS) for Pb and Cd detection (AOAC, 1984).

Blood samples: Blood samples (3- 4 ml) were drawn from marginal ear vein/artery of rabbits with the help of 5 ml pyrogen free syringes having 2-3 drops of Heparin (anti coagulant). Blood and Heparin were mixed instantly to avoid clotting. Samples were centrifuged at 2500 rpm for 15 minutes. Separated plasma was poured into bottles and refrigerated. Plasma was digested with conc. HNO_3 and heated to obtain a transparent solution and diluted up to 5 ml using double distilled water and analyzed on AAS (Bokhari *et al*., 2005).

Statistical analyses: One-way ANOVA was applied to see the effect of Pb and Cd on plant growth and biomass parameters. Accumulation of Pb and Cd in soil, plant tissues and rabbit samples (blood and faeces) were also compared using One-way ANOVA. Multiple Comparisons were made using Tukey HSD. Bioaccumulation factor (BAF) of Pb and Cd in roots and shoots was calculated with the help of following formula (Luan *et al*., 2008)

$$\text{BAF in root/shoot} = \frac{\text{Mean Pb/Cd contents in root/shoot}}{\text{Mean Pb/Cd contents in soil}}$$

RESULTS AND DISCUSSION

Effect of Pb and Cd on vegetative growth Parameters: All plant growth and biomass parameters showed significant ($P < 0.05$) reduction when treated with Pb and Cd single and combined treatments except leaf biomass, where this reduction was not statistically significant (Table 1). Maximum reduction in all the studied

parameters was observed in T_6 and minimum in T_3 as compared to control. Pb single treatments showed more reduction as compared to Cd single treatments in all the studied parameters (Table 1). Maximum root and shoot lengths were attained by control plants (8.2 and 32.9 cm, respectively) and their minimum values were shown by T_6 plants (2.9 and 15.2 cm, respectively). Similarly maximum root, shoot and leaf biomass were attained by control plants (10.62, 14.14 and 5.36 g, respectively) while both Pb and Cd treated plants (T_6) had minimum values of the three parameters (4.1, 6.38 and 1.71 g, respectively). Root lengths and shoot lengths were reduced up to 65% and 54% respectively in T_6. Where as in T_3 this reduction in root and shoot lengths was only up to 10% and 5%, respectively. Plant biomass was reduced up to 58% in T_6 and 13.7% in T_3. The effect of Pb on growth parameters was more pronounced than that of Cd. Although Cd is considered more toxic than the Pb, but in lower concentrations mobility of Cd is greatly reduced and only a small amount is translocated to shoots as also described by Walley (2005). The results of the current study are also supported by Wani *et al.* (2008) who described that single Cd had least effect on growth parameters of pea plants. It seems that both metals have little effect on each other at low concentrations resulting in less growth reduction while at higher concentrations some strong interactions between both metals existed. In combined treatments the metal toxicity may be dependent on their ionic combinations. Luan *et al*. (2008) have also suggested that different interactions might be present between metals. In our study, Pb and Cd in combined treatments showed synergistic response in roots while the interaction was additive in shoots (Peralta-Videa *et al*., 2003). Metals like Pb and Cd can reduce shoot growth by reducing the activity of photo system I which results in chlorophyll content reduction (Houshmandfar and Moraghebi, 2010).

Metal in soil, plant tissues and animal samples: According to results of One-way ANOVA, there were significant ($P < 0.05$) differences between the treatments in Pb and Cd concentrations in soil, plant tissues (root, shoot & leaf) and blood and faeces of rabbits (Table 2). Mean Pb and Cd contents in all samples increased with the increase in concentration levels both in single and combined treatments (Table 3). Pb and Cd contents of treated soils were significantly higher ($P < 0.01$) as compared to control (Table 3). Mean soil Pb ranged from 1.45 – 265.3 µg/g while mean soil Cd contents ranged from 0.46 – 6.45 µg/g. The interaction of Pb and Cd in soil and other edaphic factors are reported to affect the mobility of both metals in the soil (Chimbria and Moyo, 2009). Soils have the potential to accumulate large amounts of Pb and Cd if exposed for a longer period. Soil pH greatly influences metal mobility. High pH restricts metal movement while its low values facilitate the movement of metals not only in soil but towards different plant parts (Celechovska *et al*., 2008). In our study the soil pH (7.2) does not favour the movement of Pb and Cd in soil-plant system.

Plant roots showed higher Pb contents in combined treatments as compared to single treatments. It seemed that Cd enhanced Pb accumulation in roots. That is why T_6 showed maximum Pb accumulation (96%) as compared to T_1 which showed minimum accumulation (87%) in roots. Roots are the main accumulation sites of Pb and Cd and provide primary route for metal ion penetration. Plants have the ability to inactivate metal toxicity by binding these metals with certain substances forming complexes (phytochelatins) which allow them to accumulate larger amounts of metals in their roots reducing transport to their aerial parts (Hussain *et al*., 2007). However, Pb contents in the shoots and leaves of alfalfa plants exhibited a different pattern. Plants treated with Pb alone showed significant increase in Pb contents in their shoots and leaves as compared to combine treatments (Table 3). Maximum translocation of Pb in shoots was shown in T_2 (35%) while minimum translocation was exhibited by shoots treated with T_5 (18%).

According to results, roots of alfalfa plants had higher Cd contents in single treatments (3.15 & 5.96 µg/g for T_3 & T_4 respectively) as compared to combined treatments (2.53 & 5.35 µg/g for T_5 & T_6 respectively) which are contrary to Pb accumulation in roots. Over all, Cd accumulation percentage was less in roots as compared to Pb percentage both in single and combined treatments. Roots and shoots of the alfalfa plants did not exhibit a uniform pattern for Cd contents. As regards the Cd contents in shoots and leaves of the alfalfa, its values are significantly higher in combined treatments than single treatments. Faeces of the rabbits had much higher Pb and Cd contents as compared to their blood samples (Table 3). The result is supported by Berse'nyi (2003) who described that about 65% Pb can be excreted via faeces, 1% via urine and 34% is retained by animal body in the form of tissue deposition and blood. About 62% Cd is retained and 38% excreted from the body exclusively via faeces. Cd can be more toxic than Pb due to its high deposition percentage in different tissues and organs of the herbivores. On the contrary, only little amounts of Pb and Cd are deposited in muscles (Berse'nyi, 2003). Bioaccumulation factor (BAF) was calculated to analyze the mobility of both metals (Pb & Cd) in the soil → plant system (Fig.1 & 2). BAF of roots were much higher than shoots for both Pb and Cd. Similarly Pb showed higher BAF values than Cd in both roots and shoots. All BAF values were well below 1 which means that both metals are accumulated mainly in roots and transported a small percentage to aerial parts (Wang and Shen, 2001).

Pb affected Cd accumulation in roots but the translocation of Cd to shoots and leaves was very little

Table 1. Effect of different treatments of Pb and Cd alone and in combination on some growth parameters of *Medicago sativa*

Treatment	Root length (cm)	Shoot length (cm)	Plant biomass(g)	Root biomass(g)	Shoot biomass(g)	Leaf biomass(g)
T_0	8.2 ± 1.15 a	32.9 ± 2.87 a	26.77 ± 3.34 a	10.62 ± 0.57 a	14.14 ± 1.28 a	5.36 ± 1.34
T_1	5.8 ± 0.40 b	25.2 ± 2.05 ab	18.08 ±1.98 c	6.86 ± 1.32 cd	9.46 ± 1.77 bc	3.50 ± 0.80
T_2	4.0 ± 1.06 bc	19.0 ± 0.72 cd	13.3 ± 1.38 cd	4.9 ± 0.73 d	7.78 ± 0.50 bc	2.11 ± 0.57
T_3	7.4 ± 0.87 ab	31.4 ± 0.87 ab	23.10 ± 1.14 ab	9.42 ± 0.70 ab	12.29 ± 1.02 ab	4.48 ±1.10
T_4	6.9 ±0.43 ab	26.9 ± 2.25 ab	21.30 ± 1.72 ab	8.12 ± 0.35 ab	11.0 ± 0.66 ab	3.96 ± 1.04
T_5	4.9 ±0.95 bc	21.5 ± 2.60 cd	15.43 ± 0.95 cd	6.68 ± 0.63 cd	8.49 ± 0.62 bc	2.49 ± 0.88
T_6	2.9 ± 0.55 c	15.2 ± 2.20 d	11.15 ± 1.77 d	4.1 ± 0.90 d	6.38 ± 0.95 c	1.71 ± 0.511

T_0 = control, T_1 = Pb 200 µg/g soil, T_2 = Pb 400 µg/g soil, T_3 = Cd 4 µg/g soil, T_4 = Cd 8 µg/g soil, T_5 = Pb 200 µg/g soil + Cd 4 µg/g soil and T_6 = Pb 400 µg/g soil + Cd 8 µg/g soil. Means are presented with ± S.E. Means followed by different letters in a column for each treatment are statistically different at $P < 0.05$ by the Tukey HSD test.

Table 2. One way ANOVA for Pb and Cd concentrations in different samples ($P < 0.05$)

Sample	Sum of squares	df	Mean squares	F	Sig
Pb					
Soil	134365.05	4	33591.262	1294.232**	0.000
Root	123838.838	4	30959.710	970.754**	0.000
Shoot	16142.505	4	4035.626	319.212**	0.000
Leaf	173.520	4	43.380	49.717**	0.000
Faeces	73.252	4	18.313	9.437**	0.002
Blood	0.192	4	0.048	22.900**	0.000
Cd					
Soil	77.956	4	19.489	16.711**	0.000
Root	61.840	4	15.460	9.432**	0.002
Shoot	8.517	4	2.129	18.589**	0.000
Leaf	0.283	4	0.071	9.723**	0.002
Faeces	0.038	4	0.010	14.425**	0.000
Blood	0.001	4	0.000	14.438**	0.000

** Highly significant ($P < 0.01$)

Table 3. Mean contents of Pb and Cd in soil and different tissues of *Medicago sativa* along with blood and faecesof rabbits exposed to different treatments

Treatment	Soil	Root	Shoot	Leaves	Blood	Faeces
Pb single treatments						
T_0	1.45 ± 0.23^a	1.32 ± 0.032^a	0.36 ± 0.02^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
T_1	112.0 ± 2.43^b	97.4 ± 3.30^b	27.2 ± 0.51^b	5.95 ± 0.95^{bc}	0.20 ± 0.035^b	3.86 ± 0.73^b
T_2	237.4 ± 2.79^d	220.78 ± 4.65^d	83.89 ±0.40^c	9.85 ± 0.32^d	0.33 ± 0.020^c	6.40 ± 1.12^b
Cd single treatments						
T_0	0.46 ± 0.034^a	0.33 ± 0.02^a	0.07 ± 0.02^a	0.00 ± 0.00^a	0.00 ± 0.00^a	0.00 ± 0.00^a
T_3	3.85 ± 1.06^b	3.15 ± 0.42^{ab}	0.50 ± 0.25^a	0.06 ± 0.017^{ab}	0.004 ± 0.001^a	0.02 ± 0.003^{ab}
T_4	6.72 ± 0.47^c	5.96 ± 1.26^b	1.79 ± 0.19^b	0.27 ± 0.09^{bc}	0.016 ± 0.001 ^{bc}	0.10 ± 0.020^{cd}
Pb mixed treatments						
T_5	131.41 ± 4.63^c	115.6 ± 3.34^c	24.27 ± 0.83^b	4.45 ± 0.34^b	0.17 ± 0.023^b	2.89 ± 0.67^{ab}
T_6	265.3 ± 2.81^e	254.6 ± 3.05^e	78.92 ± 4.45^c	8.25 ± 0.56^{cd}	0.28 ± 0.036 ^{bc}	5.36 ± 0.98^b
Cd mixed treatments						
T_5	3.52 ± 0.68 ^{bc}	2.53 ± 0.72^{ab}	0.60 ± 0.19^a	0.19 ± 0.035^{abc}	0.011 ± 0.003^{ab}	0.07 ± 0.020^{bc}
T_6	6.45 ± 0.32^c	5.35 ± 0.64^b	1.97 ± 0.22 ^{b}	0.38 ± 0.040^c	0.023 ± 0.003^c	0.14 ± 0.015^d

T_0 = control, T_1 = Pb 200 µg/g soil, T_2 = Pb 400 µg/g soil, T_3 = Cd 4 µg/g soil, T_4 = Cd 8 µg/g soil, T_5 = Pb 200 µg/g soil + Cd 4 µg/g soil and T_6 = Pb 400 µg/g soil + Cd 8 µg/g soil

Means are presented with ± *S.E.* Means followed by different letters in a column for each treatment are statistically different at $P < 0.05$ by the Tukey HSD test.

affected by the presence of Pb (Table 3). The results are in accordance with the findings of Kadukova *et al*. (2006) and Yuebing *et al*. (2007). Although Cd is more mobile than Pb but its ions are mostly retained in roots and only small amounts are transported to shoots and leaves because metal ions have to cross plasma membrane of root endodermal cells which acts as a barrier against metal transport (Singh *et al*., 2009).The reason of less Cd transport in single metal treated plants may be due to the immobility of Cd on root cell walls at low levels of contamination (Walley, 1995). The interaction between Pb and Cd depends on level of contamination and tolerance of different tissues (Hussain *et al*., 2007; Wani *et al.,* 2008).

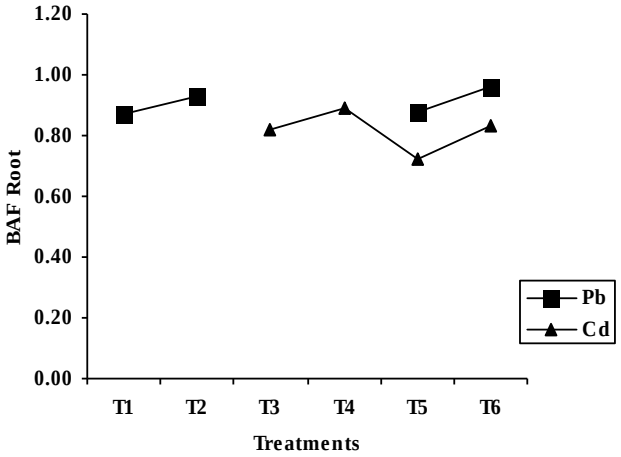

Fig.1 Bioaccumulation Factor (BAF) of root of *M. sativa* exposed to different (Pb & Cd) concentrations in the soil.

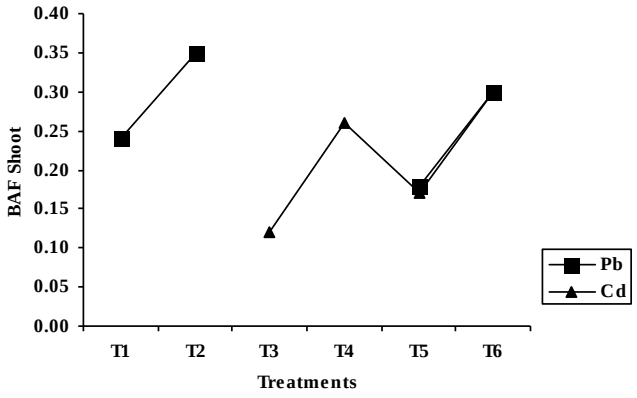

Fig.2 Bioaccumulation Factor (BAF) of shoot of *M. sativa* exposed to different (Pb &Cd) concentrations in the soil.

Conclusion: From the results it is concluded that *M. sativa* can retain large amounts of Pb & Cd in its roots and transport lesser amounts to aerial parts. Lead translocation to shoots and leaves was affected by the presence of Cd, on the other hand Pb had little effect on Cd translocation to aerial parts. There were not any detrimental effects of metals on plant morphology suggesting that alfalfa may be a metal tolerant plant and can be a good candidate for growing on metal/metals polluted soils. It is further concluded that only a small proportion of metal is accessible to herbivores by ingestion and this soil–plant–herbivore food chain doesn't seem to be an efficient pathway of metal transfer to human being.

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