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Phytoextraction of Pb and Cd; the effect of Urea and EDTA on *Cannabis sativa* growth under metals stress

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Abstract

Contamination of soil with toxic heavy metals is a wide spread environmental problem. Heavy metals adversely affect plant growth, productivity and ultimately human health. A pot experiment was conducted to evaluate the effect of two different concentrations of urea (1500 and 2500 ppm) and single concentration of ethylenediaminetetra acetic acid (EDTA) (100 ppm) on growth and metal (Pb and Cd) Phytoextraction potential of *Cannabis sativa* in metals polluted soil. The T2 (Urea 2500 ppm) showed highly significant increase in plant growth and dry biomass, followed by T1 (Urea 1500 ppm) on metals contaminated soil as compared to control (C1). Similarly T4 (Urea 2500 ppm+ EDTA 100 ppm) showed higher plant growth, biomass and highly enhanced the uptake of Pb and Cd in different parts of the plant than T3 (Urea 1500 ppm + EDTA 100 ppm) in metal polluted soil, While T2 (Urea 2500 ppm) alone produced maximum plant growth and biomass than T1 (Urea 1500 ppm) when compared with C1.The application of EDTA alone (T5) (100 ppm EDTA) increased the Pb and Cd uptake but declined plant growth and biomass which subsequently reduced the total phytoextraction of Pb and Cd. Conclusively,Plant growth and biomass really reduced in metal polluted soil but was increased in metals combination with urea .Addition of EDTA considerably enhanced metals (Pb and Cd) phytoaccumulation.

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Introduction

Soil pollution with heavy metals is one of the most serious environmental problems. Lead and Cadmium are amongst the most toxic heavy metals for both plants and animals including human beings (Peralta-Videa*et al.*, 2009; Sekara*et al.*, 2005; Singh *et al.*, 2009). From soil these metals reach human bodies through food chain. Increased Cadmium levels beyond threshold values are mutagenic, teratogenic and carcinogenic in several animal species and also cause endocrine disruption (Degraeve, 1981; Awofolu, 2005). While on the other hand, frequent lead exposure leads to abnormal functioning of muscles and kidneys, production of sperm and its transport and also results in chronic neurological diseases primarily in children and fetus. In children lead poisoning cause neurological abnormality which ultimately defects memory (Padmavathiamma and Li, 2007). The remediation of heavy metals polluted soil has become a challenging problem for the scientific community. Therefore, cost effective and eco-friendly technologies for the safe restoration of contaminated soils are needed. Phytoremediation is a plant based technology that removes pollutants through phytoextraction and rhizofiltration (Jing *et al.*, 2007). Different techniques of phytoremediation include Phytoextraction, phytostabilization, phytovolatilization, rhizofiltration, and phytodegradation (Alkorta *et al.*, 2004; Chaney *et al.*, 1997).The efficiency of phytoremediation mainly focused on the potential of plants metal translocation and bioconcentration factors. However, the plants with high metal accumulating capacity (Hyper accumulators) produced small biomass and grow slowly on contaminated soils (Denton, 2007). The efficiency of phytoremediation could be improved by reducing the noxious effects of heavy metals on plants.

The problem encountered with phytoremediation is that heavy metals penetrate in food chain through animals pasturing on toxic metal polluted plants (Seaward & Richardson, 1993). Therefore, due to the inedible status of *Cannabis sativa* to herbivores, this weed plant was used in the current study in order to avoid food chain from the contamination of heavy metals. *Cannabis sativa* belongs to family Cannabaceae (Evans, 1989). It is a good tolerant and fast growing plant for Phytoextraction of contaminated soil because it has tall shoot with multiple branching and deep root system for the efficient accumulation and absorption of heavy metals, like lead, cadmium, chromium,nickle and zinc (Citterio *et al.,* 2003;Linger *et al.*, 2002; Kos and Lestan, 2003; Kos *et al.*, 2003).

The current study was carried out to find out the effect of different concentrations of urea and EDTA either alone or in combination on plant growth and Phytoextraction of Lead and Cadmium.

Materials and methods

Soil and plant material preparation

A pot experiment was conducted in green house,of the University of Malakand, Pakistan.Seeds of *Cannabis sativa* were obtained from local market. The soil for the experiment was collected from the fields (0-25 cm depth) near the Malakand University. The soil was dried in sunlight for three days and then grounded into powdered form. The soil was then artificially polluted with Pb and Cd at 350mg per kg soil of each. Pb was added in the form of $Pb(NO_3)_2$ while Cd was added in the form of $CdCl_2$. The polluted soil was mixed well and 2 kg soil was transferred in each plastic pot (10 cm x 18 cm). The pH of the soil was measured as 7.8 (n=5).All pots were watered 24 hours before sowing the seeds of *Cannabis sativa* according to water holding capacity.

Five seeds were sown in each pot. For each treatment three replicate pots were used and arranged in a completely randomized factorial design. After germination, onehealthy plant per pot was allowed to grow while the rest were removed. All the plants were maintained in the green house at 30/15 ^{o}C temperature in natural sun light and the same amount of water was added to each pot twice a week.

Treatments used during the experiment

The following treatments were used during the whole experiment.

Table 1. Treatments used.

Treatment	Denoted as:	Treatment	Denoted as:
Control without Pb and Cd	C	Urea (1500 ppm) + 100 mg EDTA + Pb+Cd	T3
Control with Pb+Cd	C1	Urea (2500ppm)+100 mg EDTA +Pb+Cd	T4
Urea (1500ppm) +Pb+Cd	T1	100 mg EDTA+Pb+Cd	T5
Urea (2500ppm) +Pb+Cd	T2		

Note: Pb and Cd were added at concentration of 350 mg/kg soil (ppm) each.

Two different solutions (3000 and 5000 mg/L) of urea were prepared and from these solution 500 ml were poured into their respective pots (i.e. the pots received 1500 and 2500 mg/kg of urea respectively). EDTA was added to the treatments at the concentration of 100 mg/kg respectively. All the treatments were applied only once before sowing the seeds.

Plant growth and dry biomass

Sixty days old plants were harvested and thoroughly washed with tap water. Plant height was measured with a centimeter ruler from the base of the stem to the top of the apical leaf and root length was measured from the base to the top of the main root.Number of leaves and internode length were also measured for the plant. Plants were washed with a solution of 5 m mol L^{-1}Tris HCl (pH = 6.0) and 5 m mol L^{-1} EDTA and then rinsed three times with distilled water in order to remove surface bound metal ions (Genrich *et al*., 2000). Each plant was separated into three parts (roots, stem and leaves), weighed and then packed into separate paper envelops. Then dry biomass of each plant part was measured using analytical balance.

Metals analysis

The samples were oven dried for 48 h at 80° C and then grinded into powdered form. The powdered samples were acid digested using method of Allen (1974). A 0.25 g from a sample was taken in 50ml flask and 6.5 ml mixed acid solution of Nitric acid, Sulfuric acid and Perchloric acid (5:1:0.5 ratios respectively) were added to it and heated on electric hot plates till complete digestion. After cooling each digested sample was filtered (through a Whitman's filter paper) into 50 ml plastic bottles and the total volume was raised upto 50 ml by distilled water. . Samples were analysed for the concentration of Pb and Cd using atomic absorption spectrophotometer (ASS 700 Perkin Elmer, USA).

Determination of Bioconcentration and Translocation Factors

Bioconcentration factor (BCF) is the ratio of heavy metal concentration in plant tissues (root, stem or leaves) to that in soil and is calculated as given below (Zhuang *et al*., 2007).

BCF= [Metal] harvested tissue/ [Metal] soil

Translocation factor (TF) is the ratio of the concentration of the heavy metal in shoot (Stem and leaves) to that in the roots. It is calculated by the following equation (Padmavathiamma and Li, 2007; Adesodun *et al*., 2010).

TF = [Metal] shoot / [Metal] root

Statistical Analysis

Results were shown as mean ± standard deviation**.**Data were statistically analyzed by one way analysis of variance (ANOVA) and mean values were compared by using Tukey's comparison test at $p < 0.05$. The graphpadprism 2012 Statistical software was used for the analysis.

Results and discussion

Effect of treatments on leaf number, inter nodal distance, plant height and root length

The Pb and Cd significantly reduced leaf number (Fig. 1a), inter nodal distance (Fig 1b), plant height (Fig. 1c) and root length (Fig. 1d) when control C (without Pband Cd) was compared with C1 (with Pb+Cd). Heavy metals generally reduce plant growth (Hadi *et*

al., 2010; Hadi and Bano, 2009; Dudka *et al.*,1996 and Leita *et al.,* 1993). Control C1 was compared with the treatments to determine their effects on leaf number, inter nodal distance, plant height and root length. Increased leaf number, intermodal distance, plant height and root length was observed in 2500 ppm urea (T2) (1a, 1b, 1c and 1d), followed by 1500 ppm urea (T1), when compared with C1.These findings can be correlated with the results of Boroujerdnia and Ansari,(2007), who reported that a nitrogen fertilizer stimulates vegetative growth by increasing the number of leaves. Similarly Demir *et al.* (1996) reported that increase in nitrogen fertilizer stimulated increase in leaf area, stem length and yield of spinach. Our results showed a comparatively high number of leaves, intermodal distance, plant height and root length in T4 (2500 ppm urea+ 100 mg EDTA) as compared to T3 (1500 ppm urea + 100 mg EDTA) (Fig. 1 a, b, c and d). Addition of 100 mg EDTA alone (T5) highly reduced the leaf number, intermodal distance, plant height and root length (Fig. 1a, b, c and d). This might be due to the reason that EDTA generally increase the bioavailability of metals in soil and thus their uptake into plant roots and to aerial parts, causing plant stress and phytotoxicity (Lou *et al.*,2007; Reinhard *et al.*,2007).

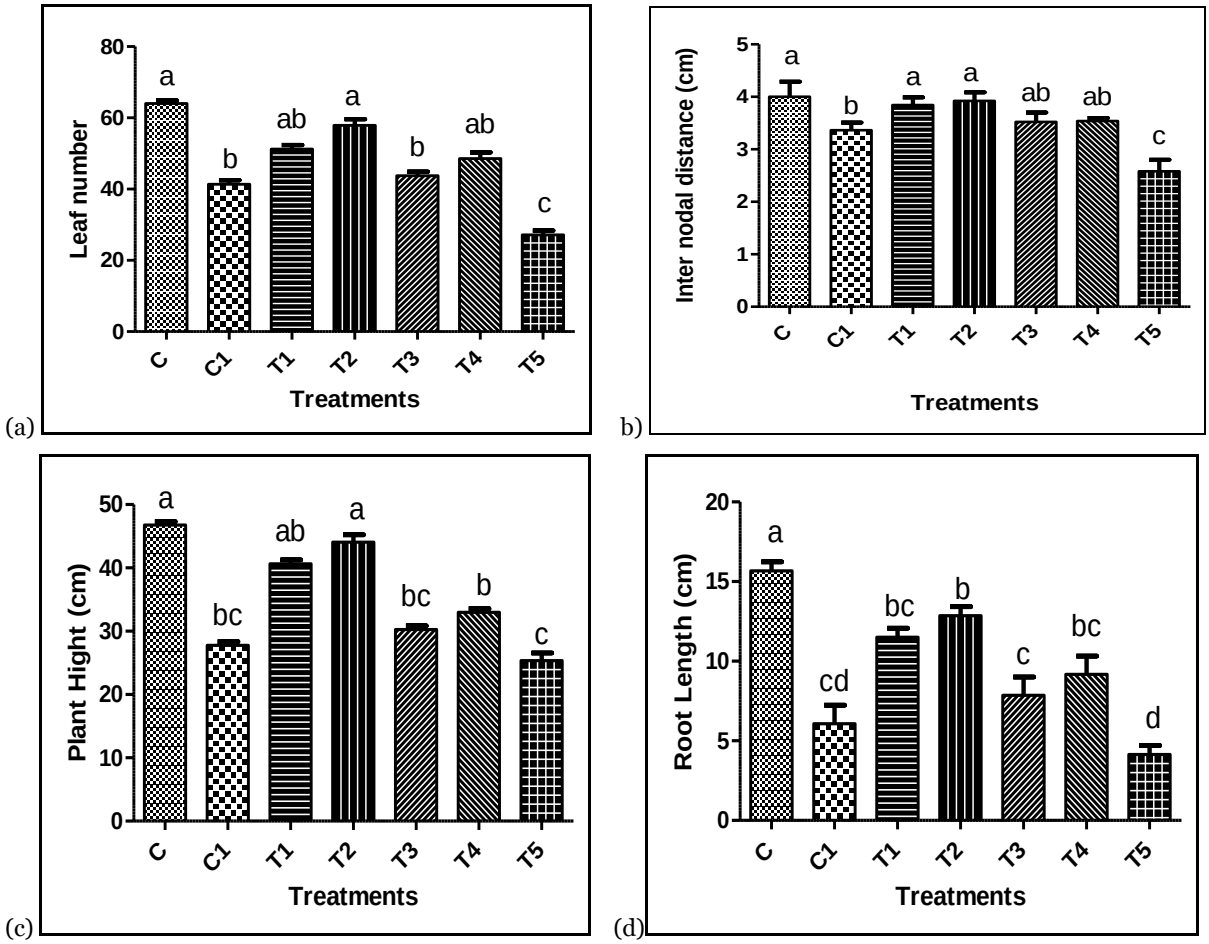

Fig. 1. Effect of treatments on plant growth (a) leaf number, (b) Inter nodal distance, (c) Plant height(d) Root length. Bars indicate standard deviation. Control without Pb and Cd (C), with Pb+Cd only (C1), 1500ppm Urea with Pb+Cd (T1), 2500 ppm Urea with Pb+Cd (T2), 1500ppm Urea with Pb+Cd+100 mg EDTA (T3), 2500ppm Urea with Pb+Cd+100 mg EDTA (T4), 100 mg EDTA+Pb+Cd (T5).

Effect of treatments on root, stem, leaf, and entire plant dry biomass

The effects of different treatmentson dry biomass (DBM) of plant root, stem, leaves and entire plant is shown in fig. 2.The fig. showed that the metals (Pb

and Cd) reduced biomass of all parts of the plant (compare C with C1). The reduction in dry biomass is one of the common symptom of heavy metal stress on plants and several scientists have reported such effect of heavy metals on biomass (John *et al.*, 2009 and Hadi *et al.,* 2010). The results showed that 2500 ppm urea (T2) showed highest biomass in all parts of the plant. Similarly Mahmood (2005) reported that increasing fertilizer concentration in soil have significant effect on yield and biomass of lettuce plant. Treatment T4 (2500 ppm urea along with 100 mg of EDTA) produced comparatively higher biomass, than T3 (1500 ppm urea + 100 mg EDTA) as mentioned in fig. 2.Dry biomass of the of the plants treated with urea only showed higher dry biomass compared to the plants treated with combination of Urea and EDTA. The dry biomass of root, stem, leaves and entire plant of the T5 (100 mg EDTA alone) significantly ($P<0.05$) reduced compared to C1 on Pb and Cd added soil, it is concluded that it might be due the presence of EDTA, which generally reduce the plant growth and dry biomass by mobilizing heavy metals in the soil,causing plant stress and phytotoxicity (Lou *et al.,* 2007; Reinhard *et al.*, 2007).Similarly Sun *et al.* (2009) reported that the addition of EDTA inhibited the growth of plants and the dry biomass yields of roots, stems, leaves and shoots.

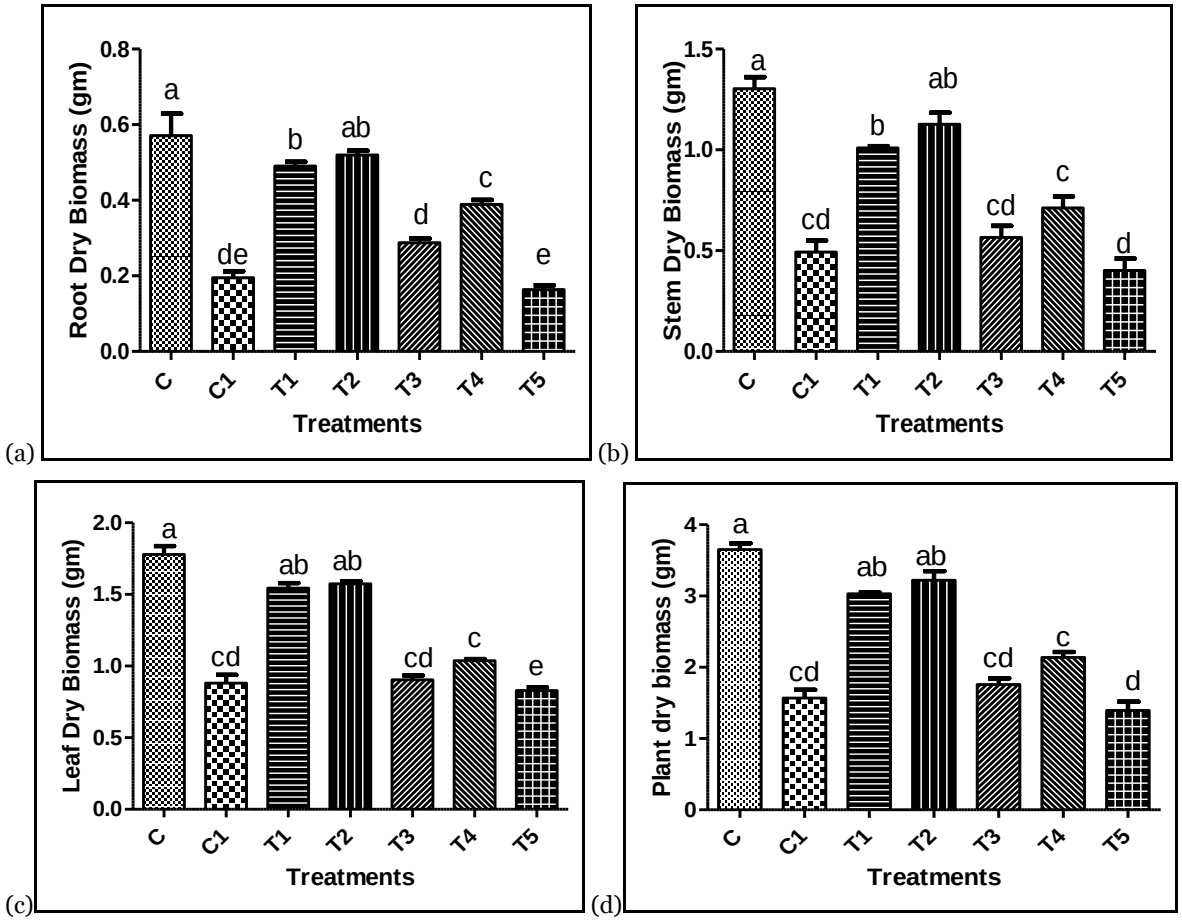

Fig. 2. Effect of treatments on dry biomass (a) Root dry biomass, (b) Stem dry biomass, (c) Leaf dry biomass. (d) Entire plant dry biomass. Bars indicate standard deviation. Control without Pb and Cd (C), with Pb+Cd only (C1), 1500ppm Urea with Pb+Cd (T1), 2500 ppm Urea with Pb+Cd (T2), 1500ppm Urea with Pb+Cd+100 mg EDTA (T3), 2500ppm Urea with Pb+Cd+100 mg EDTA (T4), 100 mg EDTA+Pb+Cd (T5).

The role of different treatments on Pb and Cd Phytoextraction

Lead and cadmium concentrations ($\mu g g^{-1}$dry weight) in different parts of *Cannabis sativa* plant is shown in Fig. 3.The highest Pb and Cd accumulation in root (Fig. 3a), stem(Fig. 3b), leaf (Fig. 3c) and entire plant (Fig. 3d) was observed in the treatment T4 (2500 ppm urea+100 ppm EDTA).This might be due to the

increasing effect of urea on plant growth and biomass while that of EDTA on mobilization of metals in soil and metal translocation within plant tissues. The Pb and Cd accumulation was comparatively higher for T2 (2500 ppm urea) for root, stem, leaves, than 1500 ppm urea (T1) as shown in fig. 3. The EDTA alone (T5) resulted significantly higher uptake of Pb and Cd in different parts of the plant, when compared to the control with Pb and Cd (C1) as shown in fig. 3a-c.These results are correlated with the findings of Hadi *et al.*, 2010; Wei *et al.,* 2010. In the current experiment, the bioaccumulation of lead by *Canabis sativa* was found significantly higher than that of cadmium concentration(Fig. 3).These findings are confirmed by Ali *et al.,* 2012, who reported in their experiment higher phytoextraction of lead by *Trifolium alexandrinum* than that of cadmium.

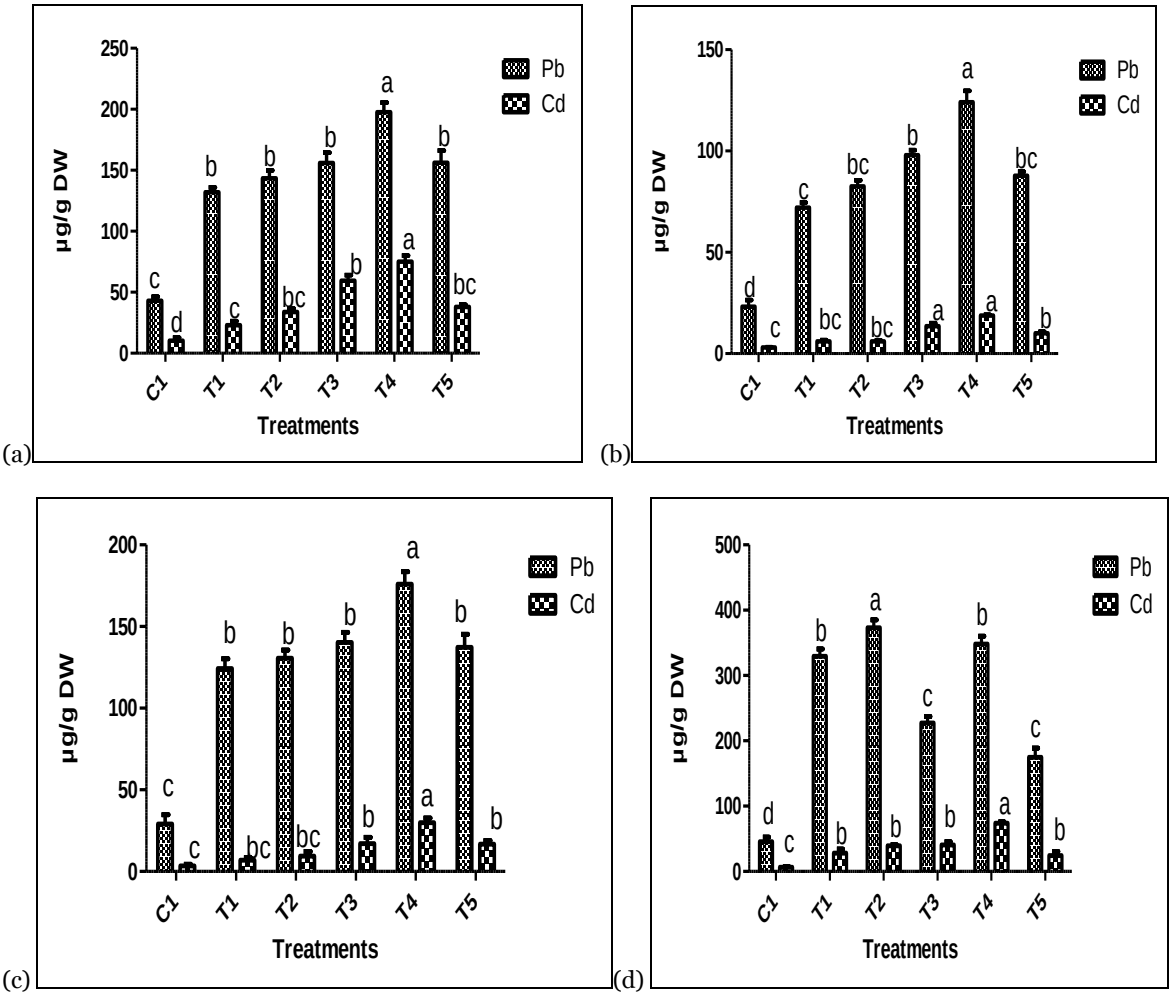

Fig. 3. Accumulation of Pb and Cd in plant (a) Pb and Cd in Root (b) Pb and Cd in Stem (c) Pb and Cd in Leaf (d) Pb and Cd in entire plant. Bars indicate standard deviation. Control with Pb+Cd only (C1), 1500ppm Urea with Pb+Cd (T1), 2500 ppm Urea with Pb+Cd (T2), 1500ppm Urea with Pb+Cd+100 mg EDTA (T3), 2500ppm Urea with Pb+Cd+100 mg EDTA (T4), 100 mg EDTA+Pb+Cd (T5).

Distribution of Pb and Cd in entire plant

The accumulation of Pb and Cd in the whole plant is shown in fig. 3d. The maximum Pb uptake was observed in T2 (2500 ppm urea alone), followed by T4 (2500 ppm urea+100 mg EDTA), T1(1500 ppm urea) and lowest in T3(1500 ppm Urea+100 mg EDTA), when compared to control C1 (Pb and Cd only) as shown in fig. 3d.While the highest Cd uptake was found in T4 (2500 ppm urea+100 mg EDTA) followed by T3 (1500 ppm Urea+100 mg EDTA), T2 (2500 ppm urea alone) and T1 (1500 ppm urea) when compared to C1. The EDTA alone T5 (100 mg EDTA) increased Pb and Cd concentration in different parts, but declined plant growth and dry biomass, ultimately

reduced Pb and Cd accumulation in entire plant, when compared to C1 (Pb and Cd only) as shown in fig. 3d.Similar results were reported by Hadi *et al.*, 2010.

Bioconcentration factor of Cannabis sativa for Pb and Cd

The Pb and Cd bioconcentration factors (BCF) of *cannabis sativa* are shown in table 2. The highest BCF was found for Pb and Cd in roots, stems, leaves and entire plant in T4 (2500 ppm Urea+100 ppm EDTA) followed by T3 (1500 ppm Urea + 100 mg EDTA), T2 (2500 ppm Urea) and lowest in T1 (1500 ppm urea) as mentioned in table 2. Similarly EDTA alone T5 (100 mg EDTA) also increased BCF in root, stem, leaves and whole plant (Table 2).Similar results were also found by Wei *et al.*, 2010, who reported that with addition of urea, the phytoextraction potential (root, stem, and leaves) could be increased. The BCF for Pb was higher than Cd for all treatments applied and different plant parts (root, stem and leaves) and whole plant, which is in accordance with the findings of Ali *et al.*, 2012.

Table 2. Bioconcentration factor of *Cannabis sativa* for Pb and Cd.

Treatment	BCF in root		BCF in stem		BCF in leaves		BCF in whole plant	
	Pb	Cd	Pb	Cd	Pb	Cd	Pb	Cd
C1	0.121 ± 0.009	0.029 ± 0.007	0.065 ± 0.009	0.008 ± 0.001	0.082 ± 0.016	0.010 ± 0.003	0.267 ± 0.029	0.047 ± 0.004
T1	0.370 ± 0.013	0.066 ± 0.009	0.204 ± 0.007	0.017 ± 0.002	0.348 ± 0.017	0.019 ± 0.004	0.922 ± 0.020	0.102 ± 0.012
T2	0.408 ± 0.018	0.095 ± 0.010	0.231 ± 0.010	0.018 ± 0.002	0.367 ± 0.015	0.027 ± 0.008	0.999 ± 0.019	0.139 ± 0.017
T3	0.439 ± 0.024	0.167 ± 0.013	0.274 ± 0.007	0.038 ± 0.004	0.393 ± 0.018	0.048 ± 0.011	1.106 ± 0.029	0.252 ± 0.011
T4	0.553 ± 0.022	0.211 ± 0.014	0.350 ± 0.016	0.053 ± 0.001	0.493 ± 0.021	0.084 ± 0.008	1.396 ± 0.054	0.348 ± 0.023
T5	0.437 ± 0.028	0.106 ± 0.007	0.246 ± 0.007	0.031 ± 0.002	0.384 ± 0.023	0.047 ± 0.006	1.068 ± 0.056	0.184 ± 0.006

Translocation factorof Cannabis sativafor Pb and Cd

The translocation factor (TF) of *Cannabis sativa* for the selected heavy metals Pb and Cd is shown in table 3.The translocation factor was determined for stem and leaves for the respective metals Pb and Cd. All the treatments (T1, T2, T3,T4 and T5) increased Translocation of Pb for both stem and leaves when compared to C1. While the translocation of Cd declined in all the treatments in stem and leaves except for T4 and T5, where the translocation factor increased in leaves in comparison to C1 (Table 3).Values of translocation factor is higher for Pb than Cd and less than 1. These results were confirmed by Turan and Esringu (2007),who reported that the big difference between root and shoot concentrations shows an important restriction of the internal transport of Pb and Cd from roots to shoots resulting in higher root concentrations instead of translocation to shoots (stem and leaves) as shown in table 3.

Table 3. Translocation factors of *Canabis sativa* for Pb and Cd.

Treatment	Translocation factor of shoots			
	TF stem		TF leaves	
	Pb	Cd	Pb	Cd
C1	0.535 ± 0.036	0.308 ± 0.096	0.675 ± 0.116	0.368 ± 0.196
T1	0.551 ± 0.022	0.259 ± 0.022	0.943 ± 0.073	0.294 ± 0.070
T2	0.577 ± 0.049	0.194 ± 0.034	0.911 ± 0.039	0.276 ± 0.054
T3	0.627 ± 0.049	0.230 ± 0.040	0.896 ± 0.050	0.289 ± 0.079
T4	0.632 ± 0.023	0.251 ± 0.016	0.891 ± 0.034	0.397 ± 0.011
T5	0.563 ± 0.030	0.290 ± 0.040	0.879 ± 0.004	0.442 ± 0.082

Conclusions

It is concluded that the heavy metals uptake is increased by using the EDTA along with urea in combination while EDTA alone at higher concentration enhanced Pb and Cd but declined plant growth and biomass subsequently result in reduced accumulation of heavy metals by the plant. These findings suggest that EDTA at lower concentration along with higher concentration of urea lead maximum accumulation of these selected heavy metals.

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Abbereviation

EDTA	(Ethylenediaminetetra-acetic acid)
Pb	(Lead)
Cd	(Cadmium)
ppm	(Part per million)
T	(Treatment)

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