Pak. J. Bot., 47(5): 1679-1684, 2015.

EFFECT OF HEAVY METAL AND EDTA APPLICATION ON PLANT GROWTH AND PHYTO-EXTRACTION POTENTIAL OF SORGHUM (*SORGHUM BICOLOR*)

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Abstract

Pot experiment was conducted to evaluate the phyto-extraction capacity of heavy metals by Sorghum. *Sorghum bicolor* was grown in soil artificially contaminated with different concentrations of lead (300, 350 and 400 mg/kg), chromium (50, 100 and 150 mg/kg) and cadmium (100, 150 and 200 mg/kg). Five mM EDTA was applied, as chelating agent to the plants after 4 weeks of sowing. Plants were grown for a total of two months and fresh weight and dry weight of shoot and heavy metal accumulation were analyzed at six and eight weeks after sowing. The results revealed that application of cadmium, chromium and lead and EDTA adversely affected shoot length, fresh weight and dry weight of *S. bicolor* at both time intervals. Heavy metals uptake increased with the increment of heavy metal by *S. bicolor* species. Application of 5mM EDTA enhanced the uptake of heavy metal.

Key words: Heavy metal, EDTA, Cd, Pb, Cr, Phyto-extraction, *Sorghum biocolor*.

Introduction

Soil pollution by heavy metals is one of the most serious problems demanding immediate attention**.** attention (Chen & Shen, 2004; Soltan *et al*., 2011; Ali *et al*., 2014; Azmat *et al*., 2015; Khan *et al*., 2015). It is of great concern due to its effects on the eco-system and human health (Marchiol *et al.*, 2007; Fellet & Marchiol, 2011)**.** Heavy metal pollution usually results from various human activities such as industrialization, use of fertilizers, energy generation, fuel production, mining and waste disposal etc (Ping *et al.*, 2009). Weathering and soil erosion can also be one of the reasons through which the heavy metals are released into the environment (Anamika *et al.*, 2009). Heavy metals have toxic effects on human health when it finds ways through food chain such as higher uptake by plant. Some of the plant species have the potential to survive on soils heavily contaminated with heavy metals (Zaimoglu and Atilla, 2012; Haung *et al*., 2012; Majid *et al*., 2013). The plant species are classified as pseudometallophytes and the absolute metallophytes each one of them have their own strategies for survival in metal contaminated soils. The former follow heavy metals avoiding strategy by restricting the metal transport to the shoots (Cunningham, 1995; Abou-Shanab *et al.*, 2007) while the later adopts the accumulation strategy in which there is a high uptake of metal and its storage in vacuoles preventing the metal toxicity (Abou-Shanab *et al.*, 2007)**.** Accumulator plants have leaf to root metal concentration ratio greater than 1 as they have the potential of translocating metals from roots to the above ground parts while excluder plants have a lesser leaf to root metal concentration ratio (Baker *et al.*, 2000)**.** About 400 plant species have been reported to have tendencies for accumulating heavy metals (Baker & Brook, 1989)**.** For a successful phytoremediation, a suitable plant species has to be selected that must be able to hyperaccumulate heavy metals and should have a large biomass (Begonia *et al.*, 1998).

Phytoremediation is a simple technique with no negative effects on the environment and is cost effective It is suitable for the treatment of large areas with surface contamination where other methods may prove costly. No expensive equipments or highly specialized personnel are required to implement this process (Anon., 2000). Generally, phytoremediation is also favored by both public and private sectors because in this process the natural ability of the environment is exploited to restore itself. Phytoremediation is also an aesthetically pleasing remediation technique. The plant samples used in phytoremediation can also serve as the indicators of the extent of contamination. Moreover, it is also possible to grow various phytoremediator plant species together on the same site to simultaneously remediate more than one contaminant. The phyto-extraction capacity of plants not only depends on plant factors like metal tolerance, the accumulation and translocation, but it also depends on the metal mobility and most importantly the soil metal phyto-availability (Hernandez-allica *et al.*, 2008)**.** There are some chelating agents that are reported to enhance the metal uptake capability of plants, by increasing the metals bioavailability (Barren & Tahira, 2010)**.** Some of the important chelating agents are ethylenediaminetetraacetic acid EDTA, Hydroxyethylene-diaminetriacetic acid (HEDTA), Nitrilotriacetic acid (NTA) Nitric acid, Citric acid, and Hydrochloric acid (Romkens *et al.*, 2002; Garba *et al.*, 2012)**.** The chelates actually make complexes with the metals in the soil and/or they lower the pH of the soil solution, this increases the bioavailability and thus facilitating the translocation of metals from root to shoot (Blaylock *et al.*, 1997; Huang *et al.*, 1997; Cooper *et al.*, 1999; Hsu & Cunningham, 1999; Ockert, 2006). Many evidences show the efficacy of EDTA and confirm it to be the most effective chelating agent**.** EDTA is more biostable than the other chelating agents and in addition to lead, it is also proved to be effective against other heavy metals like Cd, Ni and Zn (Li & Shuman, 1996; Theodoratos *et al.*, 2000).

Sorghum bicolor is the fifth major cereal crop grown in the world. It belongs to the family poaceae. Its seeds are used in flour making and as cattle feed. The high biomass of *Sorghum bicolor* makes it advantageous for phytoremediation over the other plants like sunflower and maize. The present study was conducted to investigate the effect of heavy metal and EDTA application on plant growth and phyto-remediation potential of sorghum.

Materials and Methods

The present study was conducted at the Institute of Biotechnology and Genetic Engineering, University of Agricultural Peshawar Pakistan. Aim of the study was to investigate the response of *Sorghum bicolor* towards heavy metals and EDTA application and the phytoaccumulation capacity of *Sorghum bicolor* for Lead (Pb), Chromim (Cr) and Cadmium (Cd) at three different concentrations (Table 1). For this purpose a pot experiment was conducted under greenhouse conditions using completely randomized design (CRD) with three replications. *Sorghum bicolor* was grown in pots (21.5 cm width 17.5 cm depth and 76cm circumference) filled with 5 kg surface soil collected from Malakandhair farm of University of Agriculture Peshawar artificially contaminated with heavy metals by applying the heavy metals in solution of different concentration. Heavy metals were applied in the form of their nitrates as in this form these metals have high solubility (Table 1). The plants were allowed to grow for eight weeks. After germination, thinning was done and 5 plants pot^{-1} (having 5 kg of soil) were maintained. Same set of experiment was carried out simultaneously, to which 5mM EDTA with and without heavy metals was added at four weeks after sowing. Plants were harvested after six and eight weeks after sowing to analyze different growth parameters i.e. shoot length, shoot fresh weight, and shoot dry weight. Samples were also collected for the analysis of heavy metal accumulation in shoots by Atomic absorption spectrophotometer (Ullah *et al.*, 2011; Madiha *et al.*, 2012). Before sowing a composite soil sample was collected for heavy metal concentration. Standard agronomic practices were observed throughout the experiment. Write the procedure for determining the metals in soil and plant.

Procedures for heavy metal analysis in plants and soil: Harvested plant samples were chopped into small pieces, packed in paper bags and dried in oven at 80°C for 48 hrs. After complete drying, the samples were finely grinded into powdered using an electric grinder. One gram each of the dried samples was digested with 15 ml of concentrated nitric acid (HNO_3) overnight. Digested samples were then heated up to 250ºC until white fumes were produced and heating was continued for another thirty minutes, allowed to cool down to room temperature. Twenty five ml of distilled water was added to each digested sample. The concentrations of Cd, Cr and Pb were detected in the samples via Atomic Absorption Spectrophotometer (Hitachi Z-8100, Japan) at their respective wavelengths. For soil samples, one gram dry soil sample was weighed and digested in 15 ml of concentrated nitric acid overnight followed by acid digestion carried out in a fume hood till the appearance of reddish brown flames. The digested soil samples were allowed to cool down at room temperature and then diluted with 25 ml distilled water and subsequent filtration with filter paper. The concentrations of Cd, Cr and Pb were detected in the samples via atomic absorption spectrophotometer at their respective wavelengths as described earlier (Ullah *et al.*, 2011; Madiha *et al*., 2012).

Statistical analysis: Replicated data was subjected to analysis of variance (ANOVA) according to CR design (Gomez & Gomez, 1984). MSTATC computer software was used to carry out statistical analysis (Russell & Eisensmith, 1983). The significance differences among means were determined by using Least Significant Difference (LSD) test (Steel *et al.*, 1997)**.**

Results and Discussion

Heavy metal accumulation and plant development six weeks after sowing: Heavy metals application had a significant ($p<0.05$) effect on shoot length of *Sorghum bicolor* (Fig. 1). Maximum shoot length was attained by control pots followed by plants treated with 50 mg kg^{-1} of Cr. It has been previously reported that low concentrations of Cr had no effects on plant growth (Cataldo & Wildung, 1978). Minimum shoot length was noted in plants treated with 200 mg kg^{-1} Cd. In the Pb treated plants, maximum shoot length was measured in lowest concentration of Pb and shoot length decreased with the increase in its concentration. It is reported that the growth of *Hordeum vulgare* was significantly reduced in the presence of 10 mg Cd kg^{-1} (Hernandez-allica *et al.*, 2008). Similarly, deleterious effects of Cd on plant growth have also been reported (Shamima & Sugiyama, 2008). *Brassica juncea* also showed a reduction in its length when subjected to 0.1-0.2 mM cadmium (Qadir *et al.*, 2004). In case of EDTA application, maximum shoot length was recorded in those treatments which were applied with 5 mM EDTA (Fig. 1). Among interaction between heavy metal x EDTA, maximum shoot length was observed in control plants treated with 5 mM EDTA while minimum shoot length was observed in 200 mg kg^{-1}Cd and 0 mM EDTA. It is reported that 5 mM EDTA was the most suitable concentration for mobilizing heavy metal like Cr (Bareen & Tahira, 2010).

Data concerning shoot fresh weight of *Sorghum bicolor* harvested after six weeks of sowing is shown in Fig. 2. Heavy metal and EDTA had a significant ($p<0.05$) effect on shoot fresh weight of *Sorghum bicolor* while the effect of interaction of heavy metal x EDTA was non-significant ($p>0.05$). Maximum shoot fresh weight was noted in control plants followed by plants treated with 100 mg kg^{-1} of Cd. Minimum shoot fresh weight was recorded for plants exposed to 400 mg kg^{-1} of Pb. Pb and Cd considerably decreased plant fresh weight with their increased concentrations. Maximum shoot fresh weight was observed in plants treated with 100 mg kg^{-1} of Cd (5 mM EDTA). Minimum shoot fresh weight was recorded in 400 mg kg^{-1} of Pb (0 mM EDTA). These results are in line with a study who revealed biomass reduction in plants exposed to Cd and Pb (Qadir *et al.*, 2004; Lombi *et al.*, 2001). In contrast, *Brassica carinata* showed a high accumulation of heavy metals when exposed to As, Cd, Cu, Pb and Zn without showing biomass reduction (Quartacci *et al*., 2007).

Table 1. Different concentrations of the heavy metals used in the experiment.

S. No.	Heavy metal	Concentration 1 (mg kg^{-1} of soil)	Concentration 2 (mg kg^{-1} of soil)	Concentration 3 (mg kg^{-1} of soil)
1.	Lead (Pb)	300	350	400
2.	Chromium (Cr)	50	100	150
3.	Cadmium (Cd)	100	150	200

Table 2. Heavy metals (mg kg^{-1}) accumulation by *Sorghum bicolor* species as affected by heavy metals and EDTA application (six weeks after sowing).

Heavy metals (mg kg-1)	*Sorghum bicolor*	
	EDTA (0 m M)	EDTA (5 m M)
Cd 100	16.50	16.75
Cd 150	22.25	23.75
Cd 200	31.75	46.25
Control	0.78	0.88
Cr 50	15.00	16.25
Cr 100	20.56	24.25
Cr 150	29.75	47.25
Control	0.10	4.00
Pb 300	67.00	68.07
Pb 350	69.00	70.75
Pb 400	73.00	75.25
Control	1.85	11.00
	28.96 a	33.64 b

Means of the similar categories followed by different letters are statistically different at $p<0.05$

Table 3. Heavy metals (mg kg-1) accumulation by *Sorghum bicolor* as affected by heavy metals and EDTA application (Eight weeks after sowing).

Heavy metals (mg kg-1)	*Sorghum bicolor*	
	EDTA (0 m M)	EDTA (5 m M)
Cd 100	25.25	28.25
Cd 150	24.25	30.50
Cd 200	33.25	49.25
Control	1.20	1.66
Cr 50	23.00	25.25
Cr 100	24.50	32.00
Cr 150	41.25	50.50
Control	1.83	5.91
Pb 300	120.75	125.25
Pb 350	124.25	132.00
Pb 400	127.00	147.75
Control	1.66	4.33
	45.68 a	52.72 b

Means of the similar categories followed by different letters are statistically different at $p<0.05$

Statistical analysis of the data showed that heavy metals and EDTA had a significant ($p<0.05$) effect on shoot dry weight (Fig. 3). Maximum shoot dry weight was observed in control plants followed by plants grown in the lowest concentration of Cd (100 mg kg^{-1}) applied. Minimum shoot dry weight data was recorded for plants under 400 mg kg^{-1}Pb stresses. Plants showed reduction in growth with increase in Cr concentration. These findings are similar to toxicity level for zinc and copper in Brassica species and many other vascular plants which showed significant decrease in root dry mass and total biomass production because of heavy metal accumulation (Ebbs & Kochian, 1997). Maximum shoot dry weight was noted in plants grown exposed to 5 mM EDTA. For the interaction between heavy metal x EDTA, maximum shoot dry weight was found for control plants (5 mM EDTA), while minimum was recorded for plants treated with 400 mg kg^{-1} of Pb (5 mM EDTA). This showed that EDTA application did not affect dry biomass of *Sorghum bicolor*. These findings are contradictory to the toxic effects of EDTA on red clover and fungi (Grcman *et al.*, 2001).

Contents of Table 2 indicates heavy metal accumulation levels in the shoots of *Sorghum bicolor* species as affected by heavy metals and EDTA application six weeks after sowing. The data showed that heavy metal, EDTA and interaction of heavy metal x EDTA had a significant ($p<0.05$) effect on heavy metal accumulation by *Sorghum bicolor*. Maximum accumulation of heavy metals was achieved by plants exposed to 400 mg kg^{-1} of Pb followed by plants grown on 350 mg kg^{-1}Pb concentration. Minimum accumulation was noticed in control plants for Cd. In case of Cr, heavy metal accumulation in shoots increased with increase in concentration of chromium in the soil and control plants exposed to chromium had the minimum accumulation of Cr. Concentration of Cd accumulation in plant shoots was also directly proportional to its concentration in soil. Accumulation of Cd was highest in plants treated with the maximum concentration of Cd. When subjected to EDTA, maximum accumulation occurred in plants when treated with 5 mM EDTA. For interaction between heavy metal x EDTA, maximum accumulation was observed in those plants that were grown under 400 mg kg^{-1} Pb stress (5 mM EDTA) while minimum accumulation was noted in control plants grown under 0 mM EDTA concentration. Addition of EDTA in appropriate amounts always favored the accumulation of heavy metals in plant shoots. Addition of 10 mmol EDTA enhanced the uptake of Pb, Zn and Cd by 104.6, 3.2 and 2.3 times respectively as compared with untreated EDTA plants (Grcman *et al.*, 2001).

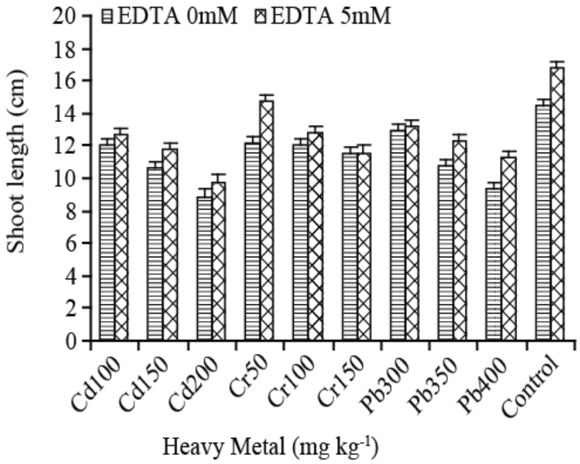

Fig. 1. Effect of heavy metal and EDTA application on shoot length of *Sorghum biocolor* six weeks after sowing (Bar shows LSD at p<0.05).

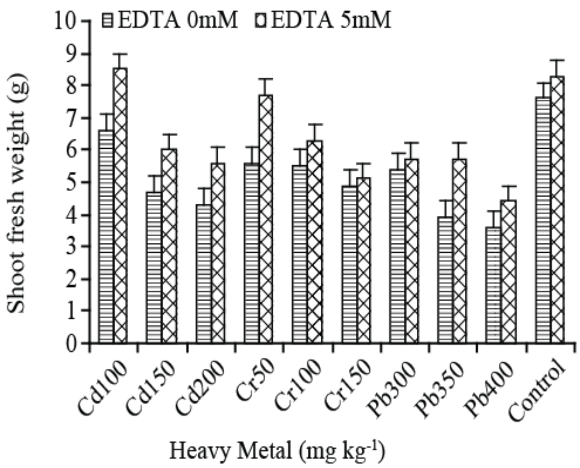

Fig. 2. Effect of heavy metal and EDTA application on shoot fresh weight of *Sorghum biocolor* six weeks after sowing (Bar shows LSD at p<0.05).

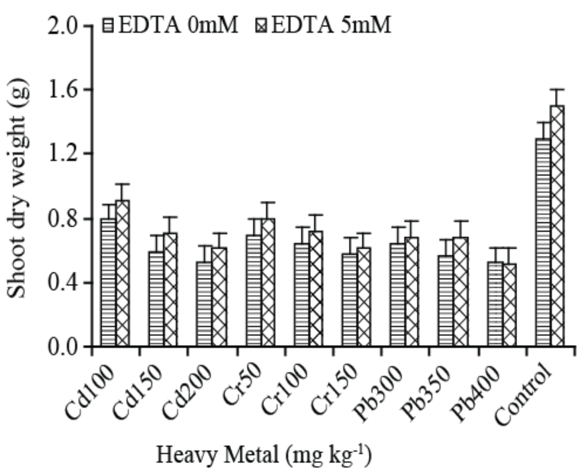

Fig. 3. Effect of heavy metal and EDTA application on shoot dry weight of *Sorghum biocolor* six weeks after sowing (Bar shows LSD at p<0.05).

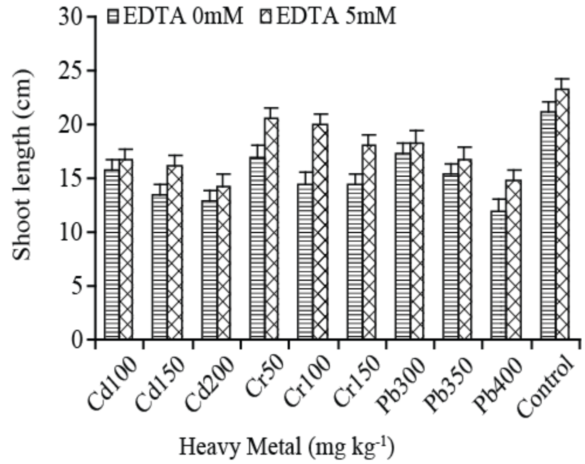

Fig. 4. Effect of heavy metal and EDTA application on shoot length of *Sorghum biocolor* eight weeks after sowing (Bar shows LSD at p<0.05).

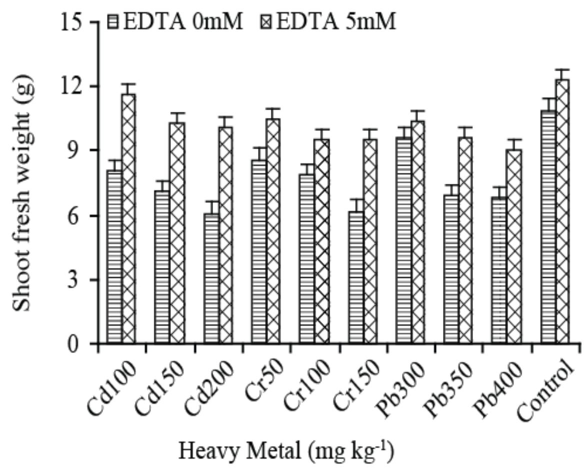

Fig. 5. Effect of heavy metal and EDTA application on shoot fresh weight of *Sorghum biocolor* eight weeks after sowing (Bar shows LSD at p<0.05).

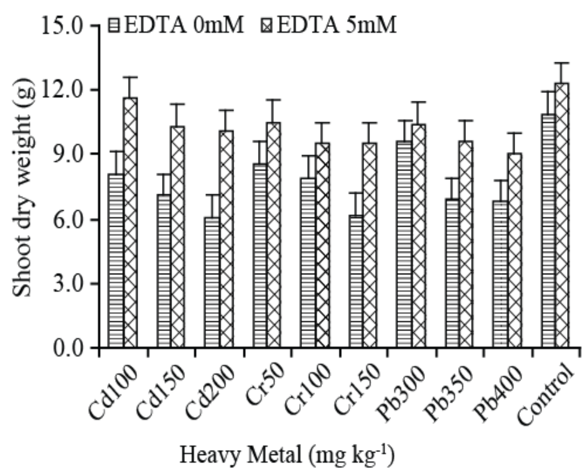

Fig. 6. Effect of heavy metal and EDTA application on shoot dry weight of *Sorghum biocolor* eight weeks after sowing (Bar shows LSD at p<0.05).

Development of plant and accumulation of heavy metals eight weeks after sowing: Data concerning shoot length collected and analyzed eight weeks after sowing indicated that heavy metal and EDTA had a significant ($p<0.05$) effect on shoot length of *Sorghum bicolor*. Maximum shoot length was noted in control plants followed treatment with 50 mg kg^{-1} of Cr (Fig. 4). Minimum shoot length was recorded for plants exposed to 400 mg kg^{-1} of Pb. A gradual decline in the growth of *Lycopersicon esculentum* was observed when subjected to increased concentration of Pb (Opeolu *et al*., 2010)**.** The data also indicated that the effects of Pb on shoot length was intensified with the increase in its concentrations. These findings are in agreement with a report which showed a reduction in growth of *L. gibba* treated with high concentrations of Cu (Babu *et al*., 2003). Similar trend was also observed for Cr or Cd. *Phaseolus vulgaris* L. showed reduced growth when treated with high concentrations of Cr (Barcelo *et al*., 1986). Similarly, growth of tomato seedlings and Chinese grass (*Bechmeria nivea* L.) showed decrease in their growth with increasing concentrations of Cd (Mediouni *et al*., 2006; Liu *et al*., 2007). Plants produced maximum shoot length when treated with 5 mM EDTA. When interaction between heavy metal x EDTA was considered, maximum shoot length was observed in control plants (5 mM EDTA) (Fig. 4).

Data regarding shoot fresh weights eight weeks after sowing presented in Fig. 5 revealed that only EDTA had a significant ($p<0.05$) effect on shoot fresh weight of *Sorghum bicolor*. Maximum shoot fresh weight was noted in control plants followed by 300 mg kg^{-1} of Pb. Minimum shoot fresh weight was recorded for plants treated with 150 mg kg^{-1} of Cr. With the increase in concentrations of Pb and Cr, shoot fresh weights were also reduced. In the case of Cd, plants treated with 100 mg kg^{-1} had the highest fresh weight and plants exposed to 200 mg kg^{-1} showed minimum weights. Plants produced maximum shoot fresh weight when treated with 5 mM EDTA. Control plants exposed to EDTA (5 mM EDTA) produced maximum shoot fresh weight and minimum in plants treated with 200 mg kg^{-1} of Cd (0 mM EDTA). Maximum shoot fresh weight was observed in plants exposed to 300 mg kg^{-1} Pb and minimum in 400 mg kg^{-1} Pb treatments. Chromium applied plants also showed a decrease in plant fresh weight with increase in its concentration. These results are parallel with our data obtained at six weeks after sowing. These results clearly suggested that *Sorghum bicolor* shoot fresh weight was not affected by Cd, Cr and Pb even when they were applied for longer times. The probable reason could be that *Sorghum bicolor* plants might have mechanism for detoxifying heavy metals and, therefore, no significant effect on plant growth even after such a long time was observed.

Heavy metals, EDTA and interaction of heavy metal x EDTA did not significantly ($p>0.05$) affect shoot dry weight of *Sorghum bicolor* eight weeks after sowing (Fig. 6). However, maximum shoot dry weight was observed in control plants followed 300 mg kg^{-1} of Pb treatment. Minimum shoot dry weight data was recorded for plants under 150 mg kg^{-1} Cr stress. In the case of Cd application, maximum shoot dry weight was observed in plants treated with 100 mg kg^{-1} Cd and the plants with 200 mg kg^{-1} showed the least dry weight. Maximum shoot dry weight was noted in plants grown at 5 mM EDTA. For the interaction among heavy metal x EDTA, maximum shoot dry weight was found for control plants (5 mM EDTA), while minimum shoot dry weight was recorded 200 mg kg^{-1} of Cd (0 mM EDTA). The data of Table 3 showed that heavy metal, EDTA and interaction of heavy metal x EDTA had a significant ($p<0.05$) effect on heavy metal accumulation of *Sorghum bicolor*. Maximum accumulation of heavy metals was achieved by plants exposed to 400 mg kg^{-1} of Pb, followed 350 mg kg^{-1} Pb treatments. Minimum accumulation was noticed in control plants for Cd. Cr accumulation also increased with increase in its concentration in the soil. Cr accumulation was lowest in control for Cr and highest in plants treated with Cr 150 mg kg^{-1} . The accumulation of Cd was also highest in plants treated with the maximum concentration of Cd (200 mg kg^{-1}). EDTA application showed maximum accumulation in plants exposed to 5 mM EDTA. For interaction between heavy metal x EDTA, maximum accumulation was observed for those plants that were grown under 400 mg kg^{-1} Pb stress (5 mM EDTA) while minimum accumulation was noted in control plants for Cd grown in 0 mM EDTA concentration. It has been reported that EDTA enhances the availability of heavy metal to plants and thus increases the accumulation in their shoots (Grcman *et al*., 2001).

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(Received for publication 8 October 2014)