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Full Length Research Paper

Phyto-accumulation of heavy metals by sunflower (*Helianthus annuus* L.) grown on contaminated soil

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This study was carried out at the Institute of Biotechnology and Genetic Engineering, KPK Agricultural University, Peshawar, Pakistan to investigate the phyto-accumulation capacity of heavy metals [lead (Pb), chromium (Cr) and cadmium (Cd)] by two cultivars of sunflower (*Helianthus annuus* L.). The experiment was conducted in pots using completely randomized (CR) design with three replications. Three heavy metals, that is, Pb, Cr and Cd were applied to the pots containing soil before sowing. Statistical analysis of the data recorded eight weeks after sowing revealed that heavy metal and interaction between ethylene diamine tetraacetic acid (EDTA) x cultivar and EDTA x cultivar x heavy metal had a significant ($p<0.05$) effect on plant height, shoot fresh weight, shoot dry weight, root fresh weight and heavy metal accumulation. The lone effect of cultivars and EDTA was non significant ($p>0.05$) on all the parameters, except heavy metal accumulation. The data revealed that maximum plant height, number of leaves plant^{-1}, shoot fresh weight and dry weight, root fresh and dry weight was noted in the control pots (0 mg kg^{-1} heavy metal). The data shown eight weeks after sowing suggested that maximum shoot fresh weight, shoot dry weight, root fresh weight and root dry weight were noted in pots kept at control with San Sun-33 applied with 5 mM EDTA. Similarly, maximum heavy metal accumulation was recorded in treatment sown with Hisun-33 and applied with 5 mM EDTA and 50 mM chromium.

Key words: Phytoaccumulation, heavy metals, sunflower, ethylene diamine tetraacetic acid (EDTA).

INTRODUCTION

Hazardous pollutants consist of variety of organic compounds and heavy metals, which pose serious threat to environment, human and animal health (Toppi and Gabbrielli, 1999; Jabeen et al., 2009; Shao et al., 2010). Concern associated with the heavy metal contamination of soil is well documented (Brown et al., 1985). Heavy metals are of primary concern as they cannot be destroyed by degradation. The remediation of contaminated soils, surface and ground water requires the removal of toxic metals from contaminated areas. Different methods to clean heavy metals polluted soils consists mainly of excavation, stabilization of the soil with cement or similar materials, and dumping of hazardous wastes, which are very costly and invasive. It has been estimated that such practices have an average cost of one million US dollars per acre of cleaned soil. Various efforts have been carried out to develop technologies for the remediation of contaminated soils, that is, *ex-situ* washing with physio-chemical and *in-situ* immobilization of metal pollutants. These methods are generally very costly and often harmful to different soil properties e.g. soil texture, useful microorganisms and organic matter (Srivastava and Goyal, 2010). Phyto-extraction of contaminated soils is considered to be economical and environmental friendly (McGrath et al., 1993; Salt et al., 1998; Shah and Nongkynrih, 2007; Lone et al., 2010) as opposed to mechanical cleanup methods such as soil excavation or pumping polluted groundwater.

The contamination of soil by heavy metal enhances plants uptake causing their accumulation in different plant

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Table 1. Levels and sources of different heavy metals used in the experiment.

S/N	Name of heavy metal	Source	Concentration (mg kg^{-1})
1	Lead (Pb)	Lead nitrate	100, 150 and 200
2	Chromium(Cr)	Chromium nitrate	50, 100 and 150
3	Cadmium (Cd)	Cadmium nitrate	10, 20 and 40

tissues (Gimmler et al., 2002; Chaturvedi, 2004; Mathe-Gaspar et al., 2005). Phytoaccumulation (phytoremediation) has many advantages over the conventional processes due to the natural capacity of plants to take up nutrients (heavy metals) from the soil and the ability of the plant's cellular components to store metal ions (Raskin et al., 1994). Phytoremediation can be used in association with other remedial methods as a finishing step to the remedial process. Over the past 20 years, this technology has become increasingly popular and employed at sites with soils contaminated with lead, uranium, arsenic, chromium and other heavy metals. Some plants not only tolerate higher levels of metals but hyper-accumulate them.

Chelating agent increases the uptake of heavy metals and various other ions by plants from soil or water. Synthetic chelates are mostly being used to increase the supply of micronutrients to plants in both soil and water. These chelating agents can also be used for phyto-accumulation by increasing heavy metals bioavailability, thus enhancing uptake by plant and translocation of heavy metals from roots to upper parts of the plants (Epstein et al., 1999). Among these, ethylene diamine tetraacetic acid (EDTA) is found to be the most effective agent in enhancing the accumulation of heavy metals in the aerial parts of plants (Blaylock et al., 1997). Sunflower can accumulate a significant amount of Pb when induced in combination with EDTA (Huang and Cunningham, 1996; Blaylock et al., 1997). Nehnevajova et al. (2005) reported that sunflower can be used for the remediation of metal-contaminated soils. Its high biomass production makes this plant specie interesting for phytoextraction and using sunflower oil for a technical purpose may improve the economic balance of phyto-remediation. Lipadzi et al. (2003) reported that leaves of sunflower accumulated more heavy metals with EDTA than the leaves of sunflower without EDTA. Keeping in view the role of plants in general and sunflower in particular in phytoremediation, this study was initiated to investigate the phytoaccumulation capacity of sunflower for cadmium, lead and chromium with or with out the application of EDTA.

MATERIALS AND METHODS

This study was carried out at the Institute of Biotechnology and Genetic Engineering, KPK Agricultural University, Peshawar, Pakistan to study the phytoaccomulation capacity of heavy metals (Pb, Cr and Cd) by two cultivars of sunflower (*Helianthus annuus* L.). The experiment was conducted in pots using completely randomized (CR) design with three replications. Three heavy metals, that is, Pb, Cr and Cd were applied to the pots containing soil before sowing. Different levels of heavy metals and their sources are shown in Table 1.

Six weeks after sowing, EDTA was applied at the rate of 5 mg kg^{-1} to each treatment. Two cultivars of sunflower (Hi sun-33 and Samsun-33) were used during the course of the study. Before and after sowing, soil samples were collected for the determination of heavy metals concentration. Data on shoot fresh and dry weight, root fresh and dry weight, plant height and tissue heavy metal concentration was collected eight weeks after sowing. Standard agronomic procedures (fertilizers, irrigation, weeding, etc.) were carried out during the entire course of the experiment.

For heavy metal analysis, plant samples were oven-dried for 48 h at 80°C. 1 g each of the dried samples was digested with 15 ml of concentrated nitric acid (HNO_3) overnight. Digested samples were heated up to 250°C until white fumes were produced and heating was continued for another half hour, and allowed to cool down to room temperature. 25 µl of distilled water was added to each digested sample. Pb, Cd and Cr concentrations were determined by atomic spectrophotometer at wavelengths of 283, 228 and 357 nm, respectively. Analysis of the soil before sowing revealed that the concentration of Pb, Cr and Cd was 51.50, 220 and 120 mg kg^{-1} respectively.

Statistical analysis

All data are presented as mean values of three replicates. Data were analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez and Gomez (1984). MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983). The significance of differences among means was compared by using least significant difference (LSD) test (Steel and Torrie, 1997).

RESULTS AND DISCUSSION

Plant growth and development

Statistical analysis of the data revealed that heavy metal application had a significant ($p<0.05$) effect on plant height, while the sole effect of cultivars and EDTA application was non significant ($p>0.05$; Table 2). Maximum plant height (37.33 cm) was attained by control treatments (0 mg kg^{-1} heavy metals) when compared with other treatments. Similarly, minimum plant height (29.79 cm) was observed in treatments applied 20 mg kg^{-1} cadmium. In the case of cultivar x EDTA interaction, maximum plant height of 38.39 cm was noted in San Sun-33 when treated with 5 mM EDTA and minimum (25.27 cm) was observed in HiSun-33 treated with the

Table 2. Plant height (cm) of sunflower cultivars as affected by heavy metals and EDTA application eight weeks after sowing.

EDTA (mM)	Variety	Heavy metal (mg kg^{-1})										Mean
		Lead			Chromium			Cadmium			Control	
		50	100	150	50	100	150	10	20	40		
0	HiSun-33	25.76	24.13	24.13	22.28	24.72	28.64	24.55	26.33	26.84	35.51	26.29
	SanSun-33	35.56	34.28	41.06	40.64	40.13	39.91	37.65	35.14	32.17	38.84	37.54
5	HiSun-33	24.73	21.91	23.46	22.56	27.60	25.82	22.86	25.08	22.86	35.81	25.27
	SanSun-33	36.65	40.22	38.10	42.33	41.91	34.79	38.52	32.59	39.69	39.15	38.39
	Mean	30.68^a	30.13^a	31.69^a	31.95^a	33.59^a	32.29^a	30.89^a	29.79^a	30.39^a	37.33^b	
	EDTA (0 mM)						31.91					
	EDTA (5 mM)						31.83					

Means followed by different letters are statistically different at $p<0.05$.

Table 3. Number of leaves $plant^{-1}$ of sunflower cultivars as affected by heavy metals and EDTA application eight weeks after sowing.

EDTA (mM)	Variety	Heavy metal (mg kg^{-1})										Mean
		Lead			Chromium			Cadmium			Control	
		50	100	150	50	100	150	10	20	40		
0	HiSun-33	10.00	8.00	7.00	9.00	10.67	11.67	10.00	8.33	9.33	19.33	10.33
	SanSun-33	10.67	10.00	9.67	9.33	10.00	8.33	7.33	8.33	8.67	23.00	10.53
5	HiSun-33	9.33	6.67	9.33	8.00	9.67	8.67	8.67	11.00	10.33	19.00	10.07
	SanSun-33	9.67	9.00	9.33	11.33	10.33	7.00	8.00	8.33	8.67	24.33	10.60
	Mean	9.92^b	8.42^b	8.83^b	9.42^b	10.17^b	8.92^b	8.50^b	9.00^b	9.25^b	21.42^a	
	EDTA (0 mM)						10.43					
	EDTA (5 mM)						10.34					

Means followed by different letters are statistically different at $p<0.05$.

same amount of EDTA. Among interactions between cultivars, heavy metals and EDTA application, maximum plant height (41.06 cm) was observed in treatment sown San Sun-33 and treated with 150 mg kg^{-1} lead and 0 mM EDTA. These results are in agreement with Babu et al. (2003). Similarly, Carlson et al. (1975) and Hamid et al. (2010) studied the effect of heavy metals on growth of corn, sunflower and mung bean and found that application of heavy metals was toxic to growth and photosynthesis. Table 3 shows data concerning number of leaves $plant^{-1}$ recorded eight weeks after sowing as affected by heavy metal and EDTA application. Number of leaves was significantly ($p<0.05$) affected by heavy metal application, while the sole effect of cultivars and EDTA application was non-significant ($p>0.05$). Maximum number of leaves $plant^{-1}$ (21.42) was produced by plants treated with 0 mg kg^{-1} heavy metals (control), while minimum leaves $plant^{-1}$ (8.42) was found in treatments of 100 mg kg^{-1} lead when compared with other treatments. In the case of EDTA application, number of leaves $plants^{-1}$ was more (10.34) in pots treated with 5 mM EDTA. Interaction between cultivars and EDTA revealed that San Sun-33 produced more leaves $plants^{-1}$ when treated with 5 mM EDTA, while minimum number of leaves $plants^{-1}$ was produced by HiSun-33 when treated with the same amount of EDTA (5 mM). Interaction among cultivars, heavy metals and EDTA application suggested that number of leaves $plant^{-1}$ was more (24.33) in control pots of San Sun-33 with or without EDTA and minimum leaves $plant^{-1}$ (7.00) were recorded in HiSun-33 when treated with 0 mM EDTA and 150 mg kg^{-1} lead. Vivek et al. (2000) studied the effect of cadmium on roots and leaves of pea and reported that Cd significantly decreased the growth of leaves and production of its biomass. Similarly, Yahua et al. (2004) reported that EDTA increased the uptake of heavy metals but showed no effect on leaves biomass production.

Table 4 presents data concerning shoot fresh weight noted eight weeks after sowing as affected by heavy metal EDTA application. Statistical analysis of the data indicated that shoot fresh weight was significantly ($p<0.05$) affected by heavy metal application, cultivar x

Table 4. Shoot fresh weight (g) of sunflower cultivars as affected by heavy metals and EDTA application eight weeks after sowing.

EDTA (mM)	Variety	Heavy metal (mg kg^{-1})									Mean	
		Lead			Chromium			Cadmium			Control	
		50	100	150	50	100	150	10	20	40		
0	HiSun-33	13.79^{e-h}	9.27^{h}	14.25^{e-h}	9.69^{h}	11.49^{gh}	21.79^{a-g}	23.91^{a-e}	16.97^{b-h}	11.98^{fgh}	10.81^{gh}	14.39^{a-h}
	SanSun-33	10.97^{gh}	15.51^{b-h}	18.46^{b-h}	12.81^{h}	13.43^{e-h}	23.02^{a-f}	11.19^{gh}	16.38^{b-h}	14.65^{d-h}	15.20^{c-h}	15.16^{a-h}
5	HiSun-33	14.61^{d-h}	17.80^{b-h}	25.84^{a-d}	9.44^{h}	11.98^{fgh}	11.67^{gh}	14.25^{e-h}	26.67^{ah}	16.19^{b-h}	13.57^{e-h}	16.02a-h
	SanSun-33	15.80^{b-h}	14.99^{d-h}	7.71^{h}	12.92^{e-h}	26.33^{abc}	21.38^{a-g}	14.91^{d-h}	12.29^{fgh}	10.99^{gh}	31.68^{a}	16.89a-h
	Mean	13.79^{b-h}	14.39^{b-h}	16.56^{a-h}	11.21^{e-h}	15.81^{a-h}	19.46^{a-h}	16.06^{a-h}	18.08^{a-h}	13.45^{b-h}	17.79^{a-h}	
	EDTA (0 mM)						14.78					
	EDTA (5 mM)						16.46					

Means followed by different letters are statistically different at p<0.05.

EDTA application and cultivar x EDTA x heavy metal application, while the lone effect of cultivars and EDTA application was non-significant (p>0.05). Maximum shoot fresh weight (17.79 g) was produced by plants treated with 0 mg kg^{-1} heavy metals (control) and minimum (11.21 g) was recorded in chromium (50 mg kg^{-1}) treated plants when compared with other treatments. In the case of EDTA application, shoot fresh weight was more (16.46 g) in pots treated with 5 mM EDTA. Interaction between cultivars x EDTA application revealed that San Sun-33 produced more shoot fresh weight (16.89 g) when treated with 5 mM EDTA. Interaction among cultivars x heavy metals x EDTA application indicated that shoot fresh weight was more in control pots of San Sun-33 when treated with 5 mM EDTA and minimum was recorded in San Sun-33 treated with 5 mM EDTA and 150 mg kg^{-1} lead. Shoot dry weight was significantly (p<0.05) affected by heavy metal application and interaction between cultivars and EDTA and cultivars x EDTA x heavy metals application, while the sole effect of cultivars and EDTA application was non-significant (p>0.05; Table 5). The data suggest that maximum shoot dry weight (2.60 g) was produced by plants treated with 0 mg kg^{-1} heavy metals (control) and the minimum (1.67 g) was recorded in chromium (150 mg kg^{-1}) treated plants when compared with the other treatments. Shoot dry weight was more (2.08 g) in plants treated with 5 mM EDTA. Interaction between cultivars and EDTA application indicated that San Sun-33 produced more shoot dry weight (2.36 g) when treated with 5 mM EDTA. Cultivars x heavy metals x EDTA application suggested that shoot dry weight was more (4.49 g) in control pots of San Sun-33 when treated with 5 mM EDTA, while minimum shoot dry weight (0.98 g) was recorded in San Sun-33 treated with 0 mM EDTA and 20 mg kg^{-1} cadmium. Kastori et al. (1992) reported that heavy metals had significant effect on shoots and roots of sunflower. Root fresh weight was significantly (p<0.05) affected by heavy metal application and interaction between cultivars and EDTA and cultivars x EDTA x heavy metals application, while the sole effect of cultivars and EDTA application was non-significant (p>0.05). Maximum root with 0 mg kg^{-1} heavy metals (control) and minimum root fresh weight (1.04 g) was recorded in cadmium (40 mg kg^{-1}) treated plants when compared with other treatments. Root fresh weight was more (2.08 g) in 5 mM EDTA treated plants. Interaction between cultivars x EDTA application indicated that San Sun-33 produced more root fresh weight (2.11 g) when treated with 5 mM EDTA. Interaction among cultivars x heavy metals x EDTA application revealed that root fresh weight was more (3.63 g) in control pots of San Sun-33 treated with 5 mM EDTA and minimum root fresh weight (0.60 g) was recorded in San Sun-33 treated with 5 mM EDTA and 40 mg kg^{-1} cadmium (Table 6). Analysis of the data also indicate that root dry weight was significantly (p<0.05) affected by heavy metal application, while the sole effect of

Table 5. Shoot dry weight (g) of sunflower cultivars as affected by heavy metals and EDTA application eight weeks after sowing.

EDTA(mM)	Variety	Heavy metal (mg kg^{-1})										Mean
		Lead			Chromium			Cadmium			Control	
		50	100	150	50	100	150	10	20	40		
0	HiSun-33	2.33^{bcd}	2.29^{bcd}	1.90^{bcd}	1.38^{bcd}	1.78^{bcd}	1.98^{bcd}	1.65^{bcd}	0.98^{d}	1.62^{bcd}	1.96^{bcd}	1.79^{bcd}
	SanSun-33	1.78^{bcd}	1.97^{bcd}	1.90^{bcd}	2.42^{bc}	1.43^{bcd}	1.0^{8cd}	1.62^{bcd}	1.52^{bcd}	2.16^{bcd}	2.10^{bcd}	2.32^{bcd}
5	HiSun-33	1.57^{bcd}	2.34^{bcd}	4.13^{A}	1.22^{bcd}	2.46^{b}	2.10^{bcd}	2.25^{bcd}	4.00^{a}	1.47^{bcd}	1.65^{bcd}	1.80^{abcd}
	SanSun-33	2.18^{bcd}	1.84^{bcd}	1.62^{bcd}	1.38^{bcd}	4.56^{a}	1.51^{bcd}	2.02^{bcd}	1.83^{bcd}	2.00^{bcd}	4.69^{a}	2.36^{abcd}
	Mean	1.97^{bcd}	2.11^{bcd}	2.39^{abcd}	1.60^{bcd}	2.57^{abcd}	1.67^{bcd}	1.88^{bcd}	2.08^{abcd}	1.80^{bcd}	2.60^{abcd}	
	EDTA (0 mM)						2.05					
	EDTA (5 mM)						2.08					

Means followed by different letters are statistically different at $p<0.05$.

Table 6. Root fresh weight (g) of sunflower cultivars as affected by heavy metals and EDTA application eight weeks after sowing.

EDTA(mM)	Variety	Heavy metal (mg kg^{-1})										
		Lead			Chromium			Cadmium			Control	Mean
		50	100	150	50	100	150	10	20	40		
0	HiSun-33	1.80^{c-g}	1.30^{c-g}	1.85^{c-g}	1.16^{d-g}	2.23^{b-f}	1.8^{c-g}	2.12^{c-f}	1.52^{c-g}	1.22^{c-g}	1.18^{c-g}	1.62^{c-g}
	SanSun-33	1.35^{c-g}	2.02^{c-g}	2.61^{bc}	1.84^{c-g}	1.8^{c-g}	1.82^{c-g}	0.85^{fg}	1.45^{c-g}	1.06^{d-g}	1.27^{c-g}	1.61^{b-g}
5	HiSun-33	1.38^{c-g}	2.34^{b-e}	4.96^{a}	1.22^{c-g}	2.06^{c-f}	0.98^{efg}	1.48^{c-g}	4.08^{a}	1.30^{c-g}	1.29^{c-g}	2.11^{a-g}
	SanSun-33	2.43^{bcd}	1.94^{c-g}	0.95^{efg}	2.12^{c-f}	4.8^{a}	1.88^{c-g}	1.12^{d-g}	1.02^{d-g}	0.60^{g}	3.63^{ab}	2.05^{a-g}
	Mean	1.74^{a-g}	1.90^{c-g}	2.59^{a-g}	1.58	2.73^{a-g}	1.62^{c-g}	1.39^{c-g}	2.08^{a-g}	1.04^{c-g}	1.84^{a-g}	
	EDTA (0 mM)						1.615					
	EDTA (5 mM)						2.08					

Means followed by different letters are statistically different at $p<0.05$.

cultivars and EDTA application was non-significant ($p>0.05$). Maximum root dry weight (1.64 g) was produced by plants treated with 0 mg kg^{-1} heavy metals (control) and the minimum (0.16g) was found in plants treated with 40 mg kg^{-1} cadmium when compared with other treatments. Similarly, root dry weight was (0.35 g) in plants treated with 0 mM EDTA. Interaction between cultivars and EDTA application showed that San

Table 7. Root dry weight (g) of sunflower cultivars as affected by heavy metals and EDTA application eight weeks after sowing.

EDTA (mM)	Variety	Heavy metal (mg kg^{-1})										Mean
		Lead			Chromium			Cadmium			Control	
		50	100	150	50	100	150	10	20	40		
0	HiSun-33	0.22	0.23	0.39	0.48	0.19	0.24	0.36	0.21	0.18	1.00	0.35
	SanSun-33	0.21	0.25	0.15	0.33	0.34	0.22	0.12	0.14	0.14	1.61	0.35
5	HiSun-33	0.36	0.09	0.41	0.16	0.21	0.21	0.36	0.19	0.13	1.43	0.36
	SanSun-33	0.36	0.21	0.23	0.22	0.23	0.13	0.21	0.16	0.19	2.49	0.44
	Mean	0.29^b	0.19^b	0.29^b	0.29^b	0.24^b	0.19^b	0.26^b	0.18^b	0.16^b	1.64^a	
	EDTA (0 mM)						0.35					
	EDTA (5 mM)						0.40					

Means followed by different letters are statistically different at p<0.05.

Sun-33 produced more root dry weight (0.44 g) when treated with 5 mM EDTA. Cultivars x heavy metals x EDTA application indicated that root dry weight was more (2.49 g) in the control pots sown with San Sun-33 when treated with 5 mM EDTA. Wong and Bradshaw (2006) reported that application of heavy metals significantly affected the growth of roots in rye grass (*Lolium perenne*) and was toxic for the roots growth (Table 7).

Heavy metals accumulation

Data regarding heavy metals accumulation by two sunflower cultivars when recorded eight weeks after sowing as affected by heavy metal EDTA application is shown in Table 8. Heavy metals accumulation was significantly ($p<0.05$) affected by heavy metal application and interaction between cultivars x EDTA and cultivars x EDTA x heavy metals application, while the effect of cultivars and EDTA application alone was non-significant ($p>0.05$). The data showed that with increasing levels of heavy metal application, accumulation was also increased. Maximum heavy metal accumulation (164.56 mg kg^{-1}) was observed in plants treated with 150 mg kg^{-1} chromium, while minimum heavy metals accumulation (26.79 mg kg^{-1}) was observed in 10 mg kg^{-1} cadmium treated plants. Heavy metals accumulation was more (95.06 mg kg^{-1}) in plants treated with 5 mM EDTA. Interaction between cultivars and EDTA application showed that San Sun-33 accumulated maximum heavy metals (120.33 mg kg^{-1}) when treated with 5 mM EDTA and minimum (39.95 mg kg^{-1}) was accumulated by San Sun-33 treated with 0 mM EDTA. Interaction among cultivars, heavy metals and EDTA application revealed that heavy metal accumulation was maximum (235.67 mg kg^{-1}) in HiSun-33 treated with 5 mM EDTA and 50 mg kg^{-1} chromium, while minimum (3.30 mg kg^{-1}) was accumulated by San Sun-33 when treated with 0 mM EDTA and 150 mg kg^{-1} chromium. These results are in agreement with Grˇcman et al. (2001), who reported that application of EDTA enhanced the accumulation of heavy metals by plants and acted as chelating agents. Similar results were also shown by Jianwei et al. (1997), Andrew et al. (1998), Shahandeh and Hossener (2002), Liphadzi and Kirkham (2006) and Michael et al. (2008).

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