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Effect of Chelator EDTA on Phyto-Remediation of Cadmium, Chromium and Lead and their Effect on Growth of Sunflower (*Helianthus annuus* L.)

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Abstract. The present study investigates the phyto-accumulation capacity of two cultivars of sunflower (*Helianthus annuus* L.) for heavy metals. Analysis of the data recorded ten weeks after sowing indicated that heavy metal application had significantly ($p<0.05$) affected all the parameters under study. Interaction of EDTA × cultivar and EDTA × cultivar × heavy metal had significantly ($p<0.05$) affected root fresh weight, root dry weight and heavy metal accumulation. EDTA application had significant ($p<0.05$) effect on heavy metal accumulation when data was noted ten weeks after sowing. The data also revealed that maximum plant height, number of leaves/plant, shoot fresh weight and dry weight, root fresh and dry weight was noted in control pots (0 mg/kg heavy metal). The suggested that maximum shoot fresh weight shoot dry weight, root fresh weight and root dry weight was noted in pots kept at control with San Sun-33 when applied with 5 mM EDTA. Similarly, maximum heavy metal accumulation was recorded in treatment sown of HiSun-33 applied with 5 mM EDTA and 50 mM chromium. Maximum root fresh weight was noted in control pots treated with 5 mM EDTA and planted with San Sun-33. Similarly, heavy metal accumulation was more in HiSun-33 treated with 50 mg/kg chromium and 5 mM EDTA. In terms of accumulation of heavy metals, HiSun-33 demonstrated better accumulation of the tested heavy metals then SanSun-33, anyhow the growth of SanSun-33 was better than HiSun-33 due to lesser accumulation of heavy metals.

Keywords: phytoremediation, heavy metals, EDTA, sunflower

Introduction

Heavy metals are potential threat to the environment and human health. Heavy metals present in soil which can be taken in by plants specially edible plants and can enter into food chain. Heavy metals if accumulated are very harmful to health in lesser concentrations even in micromoles if accumulated (Michael *et al*., 2008). Various techniques including *ex-situ* treatment with physio-chemical and *in-situ* immobilization of heavy metal have been carried out to develop techniques for the remediation of contaminated soils which are generally costly and often harmful to different soil properties. Phyto-extraction of contaminated soils is a low cost method and is also environment friendly (Salt *et al*., 1998; Raskin *et al*., 1994; McGrath *et al*., 1993). The estimated cost of remediation heavy metals contaminated soils using conventional excavation, land filling etc. are approximately $150-350/tonnes, while on the other hand, the estimated cost for phyto-remediation treatment of a lead polluted is $20-50/tonnes. Several different plant species have been studied for their potential application in removal of toxic wastes from the soils. Therefore, the use of plants to remove the contamination and clean up the hazardous waste is much better than the usual conventional methods.

Synthetic chelators are usually being used to increase the supply of micronutrients to plants in both soil and water. Similarly, use of chelator enhances phytoaccumulation by increasing heavy metals bioavailability thus enhancing uptake by plant and translocation of heavy metals from roots to the upper parts of the plants (Epstein *et al*., 1999). Ethylene diamine tetraacetic acid (EDTA) is one of the most effective agents to increase the accumulation of heavy metals in the aerial parts of plants (Hadi *et al*., 2014; Lipadzi *et al*., 2003).

Materials and Methods

The present study investigates the phyto-accomulation potential of sunflower cultivars (San Sun-33 and HiSun-33) for Pb, Cr and Cd uptake. Pots experiment using completely randomized (CR) design with three replications was used. Different heavy metals i.e. cadmium

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(10, 20 and 40 mg/kg), chromium (50, 100 and 150 mg/kg) and lead (100, 150 and 200 mg/kg) were applied in the form of nitrate to the pots containing soil before sowing. EDTA was applied (5 mg/kg) to each treatment six weeks after sowing. Soil samples were collected before and after sowing for the determination of heavy metals concentration. Data was collected ten weeks after sowing on shoot fresh and dry weight, root fresh and dry weight, plant height and tissue heavy metal concentration. Standard agronomic practices were carried out during the experiment.

Statistical analysis. Data were analyzed statistically for analysis of variance (Gomez and Gomaz, 1984). MSTATC computer software was used for statistical analysis (Russel and Eisensmith, 1983). The significance of differences among means was determined by Least Significant Difference test (Steel and Torrie, 1997).

Results and Discussion

Plant growth and development. Heavy metal application had significantly ($p<0.05$) affected plant height, while the lone effect of cultivars and EDTA application and their interactions were non-significant ($p>0.05$). Taller plants were recorded by control treatments (0 mg/kg heavy metals) when compared with growth of the plant treated with cadmium, chromium and lead. Among the three heavy metals used, lead had more adverse effects on the growth of the plant than cadmium and chromium, when the plants were treated with 40 mg/kg cadmium, chromium and lead. The least adverse effect on plant height was demonstrated by plants treated with chromium. In case of cultivar × EDTA application, maximum plant height was noted in San Sun-33 treated with 0 mM EDTA and minimum was observed in HiSun-33 when applied with 5 mM EDTA. Among interaction between cultivars, heavy metals and EDTA application, maximum plant height was observed in treatments of San Sun-33 with 150 mg/kg chromium and 0 mM EDTA (Table 1). Minimum plant height was revealed by Hi sun-33 when treated with lead in the concentration of 150 mg/kg. Babu *et al*. (2003) reported that the growth of *L. gibba* was significantly affected by the application of heavy metals.

Table 1. Plant height (cm) of sunflower cultivars as affected by heavy metals and EDTA application ten weeks after sowing

Varieties	EDTA	Heavy metals	Level-1	Level-2	Level-3	Variety × EDTA × Heavy metals
HiSun-33	0 mM	Lead	31.17	28.50	28.23	29.30
	0 mM	Chromium	25.46	25.97	34.33	28.58
	0 mM	Cadmium	34.73	36.23	31.83	34.26
	5 mM	Lead	30.31	23.33	22.91	25.51
	5 mM	Chromium	29.00	27.63	24.26	26.96
	5 mM	Cadmium	25.01	32.17	28.87	28.68
SanSun-33	0 mM	Lead	53.67	48.50	54.00	2.05
	0 mM	Chromium	51.33	49.33	47.50	49.38
	0 mM	Cadmium	44.90	41.38	38.86	41.71
	5 mM	Lead	44.38	48.73	48.46	47.19
	5 mM	Chromium	50.97	43.5	38.97	44.48
	5 mM	Cadmium	47.70	42.5	42.20	44.13
Variety × level						
HiSun-33	--	--	29.28	28.97	28.40	28.88
SanSun-33	--	--	48.82	45.65	44.99	46.49
EDTA × levels						
--	0 mM	--	40.21	38.31	39.125	39.22
--	5 mM	--	37.89	36.31	34.27	36.16
Heavy metals × levels						
--	--	Lead	39.88	37.26	38.40	38.51
--	--	Chromium	39.19	36.60	36.26	37.35
--	--	Cadmium	38.08	38.07	35.44	37.19
Control	--	--	57.50	57.50	57.50	57.50
Rest (treatments)	--	--	39.05	37.32	36.71	37.69

Heavy metals had a significant ($p<0.05$) effect on number of leaves and the lone effect of cultivars and EDTA application and their interactions were non-significant. Regarding the production of leaves, more number of leaves/plant were recorded by plants kept at control (0 mg/kg heavy metals) and minimum leaves production was noted in treatments of 150 mg/kg lead compared with other treatments. Number of leaves/plants was highest in treatment receiving 5 mM EDTA. San Sun-33 recorded more leaves/plants applied with 5 mM EDTA in case of interaction between cultivars and EDTA. Leaves/plant were more in control pots of San Sun-33 with or without EDTA and less in HiSun-33 treated with EDTA without the application of heavy metals (Table 2). Minimum number of leaves were counted in San Sun-33 when treated with lead in concentration of 150 mg/kg of soil and 5mM of EDTA, chromium and cadmium had moderate effects as compared to lead. These results are in agreement with Vivek *et al*. (2000) who reported that Cd significantly decreased the growth and biomass of pea.

Heavy metal application had a significant ($p<0.05$) effect on shoot fresh weight, while the sole effect of cultivars and EDTA application and their possible interactions were non-significant. Shoot fresh weight was highest in pots treated with 0 mg/kg heavy metals (control) and minimum in pots treated with cadmium (40 mg/kg). Shoot fresh weight was more in treatment of 0 mM EDTA. San Sun-33 recorded maximum shoot fresh weight applied with 0 mM EDTA. Maximum shoot fresh weight was produced in control pots of San Sun-33 with or without EDTA and minimum in San Sun-33 treated with 5 mM EDTA and 150 mg/kg chromium (Table 3). These results are in agreement with Kastori *et al*. (1992) who reported that the accumulation of heavy metals had a significant effect on shoots and roots of the sunflower. Heavy metal

Table 2. Number of leaves/plant of sunflower cultivars as affected by heavy metals and EDTA application ten weeks after sowing

Varieties	EDTA	Heavy metals	Level-1	Level-2	Level-3	Variety × EDTA × heavy metals
HiSun-33	0 mM	Lead	8.67	9.00	7.33	8.33
	0 mM	Chromium	10.00	8.33	11.00	9.77
	0 mM	Cadmium	10.00	10.67	10.00	10.2
	5 mM	Lead	9.00	8.67	9.00	8.89
	5 mM	Chromium	8.67	10.00	9.33	9.33
	5 mM	Cadmium	9.33	10.33	9.00	9.55
SanSun-33	0 mM	Lead	11.33	10.33	10.67	10.77
	0 mM	Chromium	11.33	9.67	11.67	10.89
	0 mM	Cadmium	10.33	10.33	9.00	9.88
	5 mM	Lead	9.33	11.67	10.67	10.55
	5 mM	Chromium	11.67	12.00	10.00	11.22
	5 mM	Cadmium	11.67	10.67	9.33	10.55
Variety × level						
HiSun-33	--	--	9.27	9.50	9.27	9.35
SanSun-33	--	--	10.94	10.77	10.22	10.64
EDTA × levels						
--	0 mM	--	10.27	9.72	9.95	9.98
--	5 mM	--	9.94	10.55	9.55	10.01
Heavy metals × levels						
--	--	Lead	9.58	9.91	9.42	9.63
--	--	Chromium	10.41	10.00	10.50	10.30
--	--	Cadmium	10.33	10.50	9.33	10.05
Control	--	--	23.00	23.00	23.00	23.00
Rest (treatments)	--	--	10.11	10.14	9.75	10

Table 3. Shoot fresh weight (g) of sunflower cultivars as affected by heavy metals and EDTA application ten weeks after sowing

Varieties	EDTA	Heavy metals	Level-1	Level-2	Level-3	Variety × EDTA × heavy metals
HiSun-33	0 mM	Lead	19.17	17.67	13.30	16.71
	0 mM	Chromium	14.71	15.54	17.07	15.77
	0 mM	Cadmium	18.34	16.23	14.66	16.41
	5 mM	Lead	17.97	10.65	14.24	14.28
	5 mM	Chromium	10.87	14.08	13.89	12.94
	5 mM	Cadmium	10.38	13.25	11.96	11.86
SanSun-33	0 mM	Lead	15.28	17.64	12.99	15.30
	0 mM	Chromium	15.72	16.39	14.48	15.53
	0 mM	Cadmium	10.65	15.15	10.19	11.99
	5 mM	Lead	14.77	14.79	12.91	14.15
	5 mM	Chromium	13.99	16.62	9.87	13.49
	5 mM	Cadmium	16.10	12.36	12.44	13.63
Variety × level						
HiSun-33	--	--	16.21	14.57	14.18	14.98
SanSun-33	--	--	13.84	15.49	12.14	13.82
EDTA × levels						
--	0 mM	--	15.20	16.43	13.78	15.14
--	5 mM	--	14.68	13.625	12.55	13.62
Heavy Metals × levels						
--	--	Lead	15.57	15.18	13.36	14.70
--	--	Chromium	13.82	15.65	13.82	14.43
--	--	Cadmium	13.99	14.25	12.31	13.51
Control	--	--	30.01	30.01	30.01	30.01
Rest (treatments)	--	--	14.83	15.03	13.17	14.34

application especially by lead had significantly ($p<0.05$) affected shoot dry weight and the lone effect of cultivars, EDTA application and interaction between cultivars × EDTA × heavy metals application was non-significant. Shoot dry weight was maximum in pots treated with 0 mg/kg heavy metals (control) and minimum by treatments of lead (100 mg/kg). In case of EDTA application, shoot dry weight was more in pots treated with 5 mM EDTA. Interaction between cultivars and EDTA application indicated that San Sun-33 produced more shoot dry weight when applied with 5 mM EDTA. Shoot dry weight was more in control pots of San Sun-33 when applied with 5 mM EDTA and minimum in San Sun-33 treated with 5 mM EDTA and 100 mg/kg lead (Table 4). These results are in agreement with Stephen and Kochain (1998) who revealed that heavy metals accumulation reduced the root dry weights.

Heavy metal application and interaction between cultivars and EDTA had a significant ($p<0.05$) effect on root fresh weight and cultivars × EDTA × heavy metals application, while the sole effect of cultivars and EDTA application was non-significant. Root fresh weight was maximum in plants treated with 0 mg/kg heavy metals (control) and minimum in pots treated with chromium (50 mg/kg). These results are in agreement with Kastori *et al*. (1992) who reported that heavy metals application had more adverse effects on roots than shoots. Root fresh weight was highest in treatments of 5 mM EDTA. San Sun-33 produced maximum root fresh weight applied with 5 mM EDTA. Root fresh weight was more in control pots of San Sun-33 when applied with 5 mM EDTA and minimum was recorded in San Sun-33 treated with 5 mM EDTA and 150 mg/kg lead in case of interaction among cultivars, heavy metals and EDTA application (Table 5). These results agree with Wong and Bradshaw (2006) who reported that application of heavy metals significantly affect the growth of roots in rye grass (*Lolium perenne*) and was toxic for the roots growth. Heavy metal application had a significant ($p<0.05$) on root dry weight and the lone effect of cultivars and EDTA application and their all possible interactions were non-significant. Root dry weight was highest in pots treated with 0 mg/kg heavy metals (control) and minimum in pots treated with 20 mg/kg cadmium. Root dry weight was highest in treatments of 0 mM EDTA. San Sun-33 recorded more root dry weight when applied with 0 mM EDTA. Root dry weight was highest in control pots of San Sun-33

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

Varieties	EDTA	Heavy metals	Level-1	Level-2	Level-3	Variety × EDTA × heavy metals
HiSun-33	0 mM	Lead	1.97	2.07	1.54	1.86
	0 mM	Chromium	1.95	1.71	2.47	2.04
	0 mM	Cadmium	2.00	1.97	2.78	2.25
	5 mM	Lead	2.33	0.98	1.03	1.44
	5 mM	Chromium	1.54	1.58	2.81	1.97
	5 mM	Cadmium	2.08	1.87	1.99	1.98
SanSun-33	0 mM	Lead	3.29	2.22	2.67	2.72
	0 mM	Chromium	2.82	3.49	1.72	2.67
	0 mM	Cadmium	1.79	2.27	1.57	1.87
	5 mM	Lead	2.15	2.02	2.15	2.13
	5 mM	Chromium	3.03	2.43	2.71	2.72
	5 mM	Cadmium	1.61	2.59	1.29	1.83
Variety × level						
HiSun-33			1.97	1.69	2.10	1.92
SanSun-33			2.44	2.50	2.01	2.32
EDTA × levels						
0 mM			2.30	2.28	2.15	2.23
5 mM			2.12	1.91	1.99	2.01
Heavy metals × levels						
		Lead	2.43	1.82	1.84	2.03
		Chromium	2.33	2.30	2.42	2.35
		Cadmium	1.87	2.17	1.90	1.98
Control			4.66	4.66	4.66	4.66
Rest (treatments)			2.21	2.10	2.06	2.12

Table 5. Root fresh weight (g) of sunflower cultivars as affected by heavy metals and EDTA application ten weeks after sowing

Varieties	EDTA	Heavy metals	Level-1	Level-2	Level-3	Variety × EDTA × heavy metals
HiSun-33	0 mM	Lead	1.99	2.31	1.85	2.05
	0 mM	Chromium	1.76	2.06	2.41	2.07
	0 mM	Cadmium	1.78	1.14	1.86	1.59
	5 mM	Lead	1.54	1.79	4.63	2.65
	5 mM	Chromium	1.43	1.74	2.53	1.90
	5 mM	Cadmium	2.61	4.43	1.87	2.97
SanSun-33	0 mM	Lead	2.02	2.32	2.21	2.18
	0 mM	Chromium	1.92	1.06	1.56	1.51
	0 mM	Cadmium	1.29	1.88	1.94	1.70
	5 mM	Lead	2.41	2.09	1.04	1.85
	5 mM	Chromium	1.25	4.33	1.82	2.46
	5 mM	Cadmium	1.98	2.31	2.43	2.24
Variety × level						
HiSun-33	--	--	1.85	2.24	2.52	2.21
SanSun-33	--	--	1.81	2.33	1.83	1.99
EDTA × levels						
0 mM	--	--	1.79	1.79	1.97	1.85
5 mM	--	--	1.87	2.78	2.38	2.34
Heavy metals × levels						
--	--	Lead	1.99	2.12	2.43	2.18
--	--	Chromium	1.59	2.29	2.08	1.98
--	--	Cadmium	1.91	2.44	2.02	2.12
Control	--	--	4.75	4.75	4.75	4.75
Rest (treatments)	--	--	1.83	2.28	2.17	2.09

Table 6. Root dry weight (g) of Sunflower cultivars as affected by heavy metals and EDTA application ten weeks after sowing

Varieties	EDTA	Heavy metals	Level-1	Level-2	Level-3	Variety × EDTA × heavy metals
HiSun-33	0 mM	Lead	0.17	0.43	0.39	0.33
	0 mM	Chromium	0.81	0.74	0.19	0.58
	0 mM	Cadmium	0.19	0.21	0.43	0.27
	5 mM	Lead	0.82	0.29	0.46	0.52
	5 mM	Chromium	0.42	0.39	0.72	0.51
	5 mM	Cadmium	0.45	0.12	0.16	0.24
SanSun-33	0 mM	Lead	0.22	0.14	0.18	0.18
	0 mM	Chromium	0.20	0.20	0.16	0.18
	0 mM	Cadmium	0.09	0.13	0.11	0.11
	5 mM	Lead	0.17	0.15	0.16	0.16
	5 mM	Chromium	0.36	0.19	0.14	0.23
	5 mM	Cadmium	0.12	0.16	0.18	0.15
Variety × level						
HiSun-33	--	--	0.47	0.36	0.39	0.41
SanSun-33	--	--	0.19	0.16	0.15	0.17
EDTA × levels						
--	0 mM	--	0.28	0.30	0.24	0.27
--	5 mM	--	0.39	0.21	0.30	0.30
Heavy metals × levels						
--	--	Lead	0.34	0.25	0.29	0.29
--	--	Chromium	0.44	0.38	0.30	0.37
--	--	Cadmium	0.21	0.15	0.22	0.19
Control	--	--	1.75	1.75	1.75	1.75
Rest (treatments)	--	--	0.33	0.26	0.27	0.29

with 5 mM EDTA (Table 6). These results are in conformity with Anna-Maj (1989). He reported that heavy metals applications was toxic to small roots, inhibited its growth and lessen its biomass production.

Heavy metals accumulation. Heavy metal and EDTA application had significantly ($p<0.05$) affected heavy metal accumulation and non-significant ($p>0.05$) effect of cultivars and interaction between cultivars and EDTA and cultivars × EDTA × heavy metals application was observed. The data suggested that heavy metal accumulation was highest in plants treated with 150 mg/kg chromium while minimum was noted in 10 mg/kg cadmium treated pots (Table 7). In case of EDTA application, heavy metals accumulation was increased when plants were applied with 5 mM EDTA, especially accumulation of chromium was recorded greater than cadmium and lead (Table 7). These results are in agreement with Vivek *et al*. (2000) and Wong *et al*. (2006), who in their individual studies reported that EDTA acted as chelating agent for the accumulation of heavy metals. EDTA also act as chelating agent for nutrients other than heavy metals like potassium and calcium etc. (Shahandeh *et al*., 2002) anyhow some studies suggested that EDTA did not have any effect on the accumulation of heavy metals by plants. Interaction between cultivars and EDTA application showed that HiSun-33 accumulated maximum heavy metals when treated with 5 mM EDTA and minimum by San Sun-33 treated with 0 mM EDTA. Heavy metal accumulation was more in HiSun-33 when applied with 5 mM EDTA and 150 mg/kg chromium while minimum heavy metals (10 mg/kg cadmium) were accumulated by Hi Sun-33 when treated with 0 mM EDTA (Table 7). These results are in agreement with Grcman *et al*. (2001), who reported that application of EDTA enhanced accumulation of heavy metals by plants and act as chelating agents. Andrew *et al*. (1998) suggested that EDTA significantly increases the accumulation of heavy metals in Indian mustard. These results also agree with (Michael *et al*., 2008; Liphadzi and Kirkhan, 2006; Shahandeh and Hossener, 2002; Jianwei *et al*., 1997), however, contradictory to Lipadzi *et al*. (2003) who reported that EDTA application did not affect the uptake of the applied heavy metals.

Table 7. Heavy metals (Pb, Cr and Cd) accumulation by sunflower cultivars as affected by heavy metals and EDTA application ten weeks after sowing

Varieties	EDTA	Heavy metals	Level-1	Level-2	Level-3	Variety × EDTA × heavy metals
HiSun-33	0 mM	Lead	33.25	36.25	143.3	70.91
	0 mM	Chromium	188.3	271.5	332.8	264.16
	0 mM	Cadmium	1.93	2.1	8.23	4.08
	5 mM	Lead	77.00	139.5	123.6	113.36
	5 mM	Chromium	270.5	266	364.8	300.41
	5 mM	Cadmium	3.43	26.98	27.4	19.27
SanSun-33	0 mM	Lead	37.88	56.13	69.88	54.63
	0 mM	Chromium	161.5	186	271.6	206.39
	0 mM	Cadmium	2.15	5.44	6.64	4.743
	5 mM	Lead	71.75	145	143.1	119.96
	5 mM	Chromium	248.5	188.5	319.4	252.12
	5 mM	Cadmium	6.40	14.86	18.06	13.10
Variety × level						
HiSun-33	--	--	95.73	123.7	166.7	128.70
SanSun-33	--	--	88.04	99.32	138.1	108.49
EDTA × levels						
--	0 mM	--	70.83	92.9	138.7	100.82
--	5 mM	--	112.9	130.1	166.1	136.37
Heavy Metals × levels						
--	--	Lead	54.97	94.22	120	89.72
--	--	Chromium	217.2	228	322.1	255.7
--	--	Cadmium	3.478	12.35	15.08	10.31
Control	--	--	0.04	0.04	0.04	0.04
Rest (treatments)	--	--	91.88	111.5	152.4	118.59

Conflict of Interest. The authors declare that there is no conflict of interest.

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