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ASSISTED AND NATURAL POTENTIAL OF HEAVY ACCUMULATION IN *PENNISETUM GLAUCUM* (L.) R. BR.

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Abstract

Pollution is the ultimate outcome of the rapid global development of mankind. Development and utilization of a safer remedial technology for degrading the pollutants, preventing future pollution and restoring the contaminated sites is the preferred research interest for the scientists. The current research investigate the potentials of *Pennisetum glaucum* (Pearl millet Var Bajra Super-1) for the three most commonly found and toxic heavy metals (Pb, Cr and Cd) in presence and absence of EDTA as chelating agent. The effect of these heavy metals at three different concentrations was studied on plant growth and heavy metal accumulation in the shoot tissue of *Pennisetum glaucum* (L.) R. Br. Significant differences were observed for shoot length when exposed to heavy metals. Control plants attained maximum shoot length (18.22 cm). Heavy metal produced no effect both on shoot fresh and dry weight. Plant growth response to EDTA was also not deleterious ($p>0.05$). Heavy metal accumulation levels increased with the increasing concentration of each of heavy metal. Maximum accumulation was noted for Pb (2.95mg kg^{-1}) at 200mgkg^{-1} eight weeks after sowing while minimum 0.22 mg kg^{-1} was observed noted for Cd concentration at 25 mg kg^{-1} six weeks after sowing. Moreover, EDTA application enhanced the metal uptake by plant and maintained maximum accumulation as compared to non EDTA treated plants, eight weeks after sowing. *Pennisetum glaucum* accumulated heavy metals in order as Pb>Cr>Cd.

Key Words: Heavy metals, EDTA, Millet, Cd, Cr, Pb, *Pennisetum glaucum*

Introduction

Mining, explosives, petroleum products and the use of various fertilizers, herbicides and pesticides are contaminating ground water, surface water as well as soil and air. Contaminated soil and water, however, proves to be seriously affecting humans and animal's health causing agricultural and environmental problems all over the world. The most prevalent organic contaminant radionuclide and metals especially heavy metals contaminants had increased since 1900 (Nriagu, 1979; Cunningham *et al.*, 1997; Prasad *et al.*, 2003). Usually the cleaning up of such contaminants involves their removal from polluted sites mainly by physical means like dumping excavation or stabilization etc. However, such practices are expensive and environmentally invasive causing negative effect on ecosystem (Ghosh & Singh, 2005)**.** Several plants can also accumulate heavy metals (Ahmad et al., 2014a, b; Khan *et al*., 2014, 2015; Ali *et al*., 2014; Colak *et al*., 2014; Azmat *et al*., 2015; Khan *et al*., 2015). However, certain high concentration and some specific combination of metals disrupt the plant physiology and morphology thus affecting plant biomass production and growth. However, some plant species have the indigenous ability to grow in metalliferous soils and accumulate metal ions in their specific parts. Such plants are a good source for cleaning of heavy metal polluted sites. Phytoremediation has been developed with the identification of some metal accumulation properties in plants (Evanko & Dzombak, 1991; Raskin & Ensley, 2000)**.** The major hindrance to the efficacy of plant remediation is the immobility of metals in soil and their less solubility and diffusion rate to the plants roots. Chelating agents such as Nitrilotriacetic acid [NTA], Ethylenediaminetetra acetic acid [EDTA], Pyridine-2-6-dicarboxylic acid [PDA], Nitric acid, Citric acid, Hydrochloric acid and Flourosilicic acid are added artificially to make the availability of metals to the plants **(**Romkens *et al.*, 2002**).** However, the most effective and commonly used chelating agent is the EDTA as it is a recoverable and strong relatively biostable chelating agent. EDTA proves to have potentials regarding soil remediation especially the calcium salt of EDTA instead of sodium salts. It is reported that EDTA increases the mobility of Zn and Ni in the soil and has specific affinity for Cd (Li & Shuman, 1996; Theodoratos *et al*., 2000).

Pennisetum glaucum belongs to genus *Pennistum*, is a cereal grain. It was previously named as *P. typhoides* (Burm), *P. typhoideum* (L), *P.americanum* (L). It is commonly called Pearl millet, cattail millet or bulrush millet. In semiarid land of Africa and Asia it is grown on about 26 million ha of land. It is stated that Pearl millet is grown on about 40 million ha of land worldwide for food purposes (Anon., 1986; Andrews & Kumar, 1992). Pearl millet is the first choice of farmers in the regions where there is relatively low rain fall and the soil is nutrient poor (Menezes *et al.*, 1997; Ali, 2010). The present study is conducted to evaluate both natural and EDTA assisted potential of *Pennisetum glaucum* for heavy metal uptake.

Materials and Methods

The phyto-accumulation capacity of *Pennisetum glaucum* Var. BAJRA SUPER-1 was studied under greenhouse condition. Three different concentrations of heavy metals including Lead (Pb), Cadmium (Cd) and Chromium (Cr) were added to Pots ($25 \times 18.5 \times 76$ cm^3) containing 5 kg of soil that were obtained from "Malakandher Research Farms of The Agriculture University Peshawar" (Table 1). The experiment was repeated 3 times using Complete Randomized (CRD) design. Heavy metals were applied in their nitrate forms (Table 2). Seeds of Pearl millet (Var. BAJRA SUPER-1) were sown at 7cm depth and after thinning 10 plants pot^{-1} was maintained. Four weeks after sowing, 5mM EDTA was applied to half sets of pots. Samples for different growth and heavy metal analysis parameters were recorded 6 and 8 weeks after sowing. Three pots for control plants were kept per treatment to which no heavy metal was added. Before sowing of seeds and addition of heavy metals to soil, the soil sample was analyzed for its heavy metal content. Throughout the experiment standard agronomic practices were observed.

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)	100	150	200
2.	Chromium (Cr)	50	100	150
3.	Cadmium (Cd)	25	50	100

Table 2. Sources of heavy metals used in the experiment.

S.No	Heavy metal	Source	Molecular formula	Molar mass (g mol^{-1})
1.	Lead (Pb)	Lead Nitrate	$Pb(NO_3)_2$	331.21
2.	Chromium (Cr)	Chromium Nitrate	$Cr(NO_3)_3.9H_2O$	400.15
3.	Cadmium (Cd)	Cadmium Nitrate	$Cd(NO_3)_2.4H_2O$	308.47

Growth measurement procedures: For the determination of shoot and root length, three plant from each treatment were randomly selected. The average shoot and root length (in centimeter) was measured with the help of ruler. Further the average weight of fresh shoot and root of these plants was weighed using electronic balance. Plants were dried at 80^0C for 48 hours, to investigate dry shoot and root weights. The same process of data collection adopted twice i.e. at 4 and 6 weeks interval.

Procedures for Analysis of heavy metals: Heavy metal analysis was carried out as described by Rafi *et al.,* (2011) and Madiha *et al*., (2012). Plant samples (shoot) were dried in oven at 80 ^{0}C and grinded by electric grinder to make fine powder. One gram dried powdered shoots were prepared to analyze on atomic absorption spectrophotometer. The prepared samples were digested using high concentration of fifteen ml HNO_3. After overnight digestion using a sand bath the prepared samples were heated in a fume hood at 250 ^{o}C till the appearance of white color fumes and when the remaining solution was decreased to 2.5 ml heating process was stopped. All solid particles were removed through filtration after cooling of the samples to 25^oC. The samples were diluted with distilled water to obtain a final volume of 25 ml. Atomic absorption spectrophotometer was utilized to determine the concentration of the targeted heavy metals.

Soil sample analysis: Fifteen g of dried soil sample was digested with 30ml of 1N solution of AB-DPTA (Ammonium bicarbonate and Diethylenetriamine penta acetic acid) (Rafi *et al.,* (2011). The mixture was shacked for 15 minutes and filtered through Whatman No.1 filter paper and analyzed for heavy metals by atomic absorption spectrophotometer**.** The results of **s**oil analysis before sowing indicated the concentration of 1.94mg kg^{-1} of Cd, 59.25mg kg^{-1} of Pb and 28.75mgkg^{-1} of Cr.

Statistical analysis: Data were analyzed as described by Gomez & Gomez (1986) for analysis of variance (ANOVA). MSTATC computer software was used to carry out statistical analysis (Russel & Eisensmith, 1983). Least Significant Difference (LSD) test was employed upon obtaining significant differences (Steel & Torrie, 1997).

Results and Discussion

Effect of heavy metal on plant growth and development: Statistical analysis of the data revealed that shoot lengths recorded six and eight weeks after sowing was significantly ($p<0.05$) affected by heavy metal and EDTA application (Tables 3 and 7). When the plants were exposed to 150mg kg^{-1} concentration of Pb, it reduced the shoot length as compared to the non-exposed control plants. Similar results were also reported for many vascular plant species in which Lead (Pb) had shown considerably less phytotoxic effects as compared to other heavy metals (Anna-Maj, 1989)**.** Similar reduction in shoot length was observed with 100 mg kg^{-1} of Cadmium concentration. This means that Cadmium is having similar adverse effects on plant growth as that of plumbum. Similar results were also noted in *Brassica juncea* cultivar when subjected to Cd (0.0-2.0 mM) where a reduction in its length was noted (Qadir *et al.,* 2004)**.** The data also showed that maximum shoot length was noted for plants treated with 5mM of EDTA as compared to 0mM EDTA treated plants. This is perhaps due to the chelating effect of EDTA which made the otherwise insoluble essential growth minerals available to plants.

Table 3. Shoot length (cm) of *Pennisetum glaucum* as affected by heavy metals and EDTA application (six weeks after sowing).

Heavy Metals ($mg\ kg^{-1}$)	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	12.91	13.03	12.98b
Pb 150	13.00	13.70	13.36b
Pb 200	11.90	12.66	12.29bc
Cr 50	12.80	13.25	12.67b
Cr 100	13.25	13.00	13.13b
Cr 150	12.30	12.50	12.42bc
Cd 25	11.08	12.83	11.96bc
Cd 50	11.30	11.60	11.49bc
Cd 100	9.70	11.10	10.47c
Control	16.70	19.73	18.22a
	12.49 a	13.34 b	

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

Observing the plants after eight weeks of sowing, the control treatment presented maximum shoot length as compared to the plant exposed to 100mg kg^{-1} of Plumbum concentration. In contrast plants exposed to 100 mg kg^{-1} concentration (Table 7) of Cadmium presented the lowest growth. Our results agree with a report which showed decline in the growth of *L. gibba* subjected to application of copper (Cu) (Babu *et al.,* 2003). Opeolu *et al.,* (2010) reported that *Lycopersicon esculentum* subjected to increased concentration of Pb revealed a gradual decline in their growth. Cr and Cd application also produced similar adverse effects on plants growth with increasing concentrations. A decrease in shoot growth of *Phaseolus vulgaris* L. was also observed when plants were subjected to chromium stress (Barcelo *et al.,* 1986). Similarly, in tomato seedlings and Chinese grass (*Bechmeria nivea* L.) growth inhibition by increased Cadmium concentrations has been reported (Mediouni *et al.,* 2006; Liu *et al.,* 2007). Application of 5 mM EDTA promoted shoot length as compared to 0 mM EDTA treated plants. These findings are contradictory to a report where significant reduction was observed in growth and photosynthesis of corn and sunflower in response to enhanced heavy metal uptake (Quartacci *et al.,* 2007).

Non-significantly ($p>0.05$) effects due heavy metal application were noted for shoot fresh weight six and eight weeks after sowing (Tables 4 and 8). The data also suggested shoot fresh weight was significantly ($p<0.05$) affected by EDTA application weeks after sowing (Table 8). Heavier shoots were noted in by control plants and minimum by plants exposed to 100 mg kg^{-1} of Cd. The data clearly showed that with increase in concentration of heavy metal, fresh weights of plants were non-significantly ($p>0.05$) decreased. For Pb treated plants non-significant ($p>0.05$) decline in fresh weight values were noted when applied with 100 to 200 mg $kg^{-1}$of metal concentrations. These findings are similar to a study where 9 different plant species were investigated for accumulation of As, Cd, Cu, Pb, and Zn. Among them, *Brassica carinata* showed highest accumulation without showing biomass reduction (Quartacci *et al.,* 2007). In contrast, biomass reduction when exposed to Cd and Pb was reported in *Brassica juncea* and *Thlaspi caerulescens* (Lombi *et al.,* 2001; Babu *et al.,* 2003).

Table 4. Shoot fresh weight (g) of *Pennisetum glaucum* as affected by heavy metals and EDTA application (six weeks after sowing).

Heavy Metals ($mg\ kg^{-1}$)	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	5.20	5.10	5.18
Pb 150	4.70	4.60	4.65
Pb 200	3.30	4.40	3.90
Cr 50	6.10	5.80	5.98
Cr 100	5.00	4.90	4.99
Cr 150	3.70	4.70	4.23
Cd 25	5.70	6.60	6.20
Cd 50	6.20	4.70	5.51
Cd 100	3.30	4.40	3.38
Control	6.00	7.2	6.63
	4.32	5.24	

Table 5. Shoot dry weight (g) of *Pennisetum glaucum* as affected by heavy metals and EDTA application (six weeks after sowing).

Heavy Metals ($mg\ kg^{-1}$)	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	0.46	0.61	0.54
Pb 150	0.52	0.54	0.53
Pb 200	0.34	0.43	0.39
Cr 50	0.48	0.58	0.53
Cr 100	0.54	0.57	0.55
Cr 150	0.41	0.41	0.41
Cd 25	0.55	0.88	0.71
Cd 50	0.60	0.53	0.56
Cd 100	0.47	0.54	0.50
Control	0.71	0.80	0.75
	0.51	0.50	

Data regarding shoot fresh weight eight weeks after sowing that EDTA treatment had significantly ($p<0.05$) increased shoot fresh weight of Pearl millet as compared to 0 mM EDTA exposed plants (Table 8). Similarly, shoot fresh weight was maximum in controls followed by plants exposed to 50 mg kg^{-1} of Cd. Minimum shoot fresh was noted in treatment exposed to 150 mg kg^{-1} of Chromium. In Pb applied treatments, maximum shoot fresh weight was noted for plants treated with 100 mg kg^{-1} of lead while minimum value was noted for 200 mg kg^{-1} of Pb. In case of Chromium, shoot fresh weight decreased with increase in its concentration. From these result it is clear that shoot fresh weight was non-significantly ($p>0.05$) affected by Pb, Cd, and Cr application even after eight weeks of sowing. Probably, pearl millet had shown some resistance to the adverse effects of heavy metals as its biomass was not reduced even after exposure of the plant to heavy metals. These findings are contradictory to a report where reduced biomass of *Brassica juncea* and *Thlaspi caerulescens* response to Cd and Pb was noted (Lombi *et al.*, 2001; Qadir *et al.*, 2004).

Heavy metal, EDTA and their interaction had no significant ($p>0.05$) effect on shoot dry weight six and eight weeks after sowing (Tables 5 and 9). However, maximum shoot dry weight was observed in control plants while minimum shoot dry weight was noted in plants treated with 200 mg kg^{-1} of Pb. For Pb and Cd exposed plants, shoot dry weight decreased as the concentration of these heavy metal increased. Maximum shoot dry weight was noted for Pb at 100mg kg^{-1} while minimum shoot dry weight was noted for 200 mg kg^{-1} Pb. Plants showed resistance to adverse effects of heavy metals up to certain concentration. Significant decrease in root dry mass and total biomass production has been observed in Brassica species and other vascular plants due to heavy metal accumulation (Anna-Maj, 1989; Ebbs & Kochian, 1997). The data also indicated that maximum shoot dry weight was noted for 5mM EDTA exposed plants. These findings are contradictory to toxicity effects of EDTA noted in red clover and soil fungi (Grman *et al.*, 2011). After eight weeks of sowing we observed maximum shoot dry weight for control plants. On the other hand, those plants which were exposed to 150 mg kg^{-1} of Chromium were on second position. 100mg kg^{-1} of Pumblum and 150 mg kg^{-1} of Chromium presented the adverse effect on shoot dry weight. In contrast, 5mM Ethylene diamine tetraacetic Acid (EDTA) maximized the shoot dry weight. It is also reported that *Brassica carinata* accumulates high concentrations of heavy metals without showing biomass reduction (Quartacci *et al.*, 2007).

Table 6. Heavy metal accumulation ($mg\ kg^{-1}$) of *Pennisetum glaucum* as affected by different concentration of heavy metals and EDTA application (six weeks after sowing)

Heavy Metals ($mg\ kg^{-1}$)	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	1.39	1.39	1.39b
Pb 150	1.71	3.01	2.36a
Pb 200	2.24	2.36	2.30a
Pb(Control)	0.04	0.21	0.129d
Cr 50	0.50	0.77	0.63cd
Cr 100	0.99	0.93	0.96bc
Cr 150	1.07	1.85	1.46b
Cr(Control)	0.04	0.21	0.130d
Cd 25	0.11	0.33	0.22d
Cd 50	0.23	0.67	0.45cd
Cd 100	0.38	0.99	0.68cd
Cd (Control)	0.13	0.04	0.08d
	0.73	1.06	

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

Table 7. Shoot length (cm) of *Pennisetum glaucum* as affected by different concentration of heavy metals and EDTA application (eight weeks after sowing).

Heavy Metals (mg kg^{-1})	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	16.75	20.10	18.46b
Pb 150	15.90	19.40	17.67b
Pb 200	14.75	19.00	16.91bcd
Cr 50	14.20	19.60	16.97bcd
Cr 100	14.20	17.50	15.87bcde
Cr 150	13.70	15.50	14.64cde
Cd 25	15.50	19.30	17.42bc
Cd 50	13.00	15.90	14.50de
Cd 100	12.60	14.50	13.63e
Control	20.10	23.10	21.63a
	15.11a	18.43b	

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

Effect on heavy metal uptake

It is evident from the data that heavy metal accumulation was significantly affected while EDTA and interaction between heavy metal x EDTA did not significantly affect ($p>0.05$) plant heavy metal accumulation six weeks after sowing (Table 6). Heavy metal accumulation increased with increase in heavy metal concentrations. The highest accumulation level was noted for Pb when applied at the rate of 200 mg kg^{-1}.Control plants showed the lowest level of Pb accumulation levels. Similarly, plants treated with Cr (150 mg kg^{-1}) accumulated maximum heavy metal. EDTA increases the availability of heavy metal to plants and therefore enhances its accumulation in their shoots (Grman *et al.,* 2001). Heavy metal uptake by pearl millet eight weeks after sowing showed that heavy metal accumulation was significantly ($p<0.05$) affected by heavy metal and EDTA application (Table 10). Maximum accumulation was achieved by plants treated with 200 mg kg^{-1} of Pb concentration, while cadmium uptake by the plants was at the minimum range. With increasing concentration heavy metal uptake was also increased. Moreover, EDTA application had significantly ($p<0.05$) increased the uptake of heavy metals when compared to 0 mM EDTA plants. EDTA being a chelating agent enhances the accumulation of metals by plants (Grman *et al.,* 2001). A significant increase in accumulation of lead in Indian mustard when subjected to EDTA application has been reported (Andrew *et al.,* 1998). Studies on enhanced accumulation of lead by applied synthetic chelates and role of chelating agents in removing heavy metals also supports the chelating action of EDTA in case of heavy metal accumulations (Jianwei *et al.,* 1997; Liphadzi *et al.,* 2003; Michael *et al.,* 2008).

Table 8. Shoot fresh weight (g) of *Pennisetum glaucum* as affected by different concentration of heavy metals and EDTA application (eight weeks after sowing).

Heavy Metals (mg kg^{-1})	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	7.60	11.9	9.81
Pb 150	7.00	9.60	8.31
Pb 200	6.70	9.10	7.93
Cr 50	9.50	9.40	9.60
Cr 100	7.16	8.40	7.80
Cr 150	4.83	7.20	6.05
Cd 25	7.83	11.70	9.80
Cd 50	9.73	11.60	10.70
Cd 100	8.46	10.20	9.40
Control	10.23	11.70	10.96
	7.92b	10.13a	

Table 9. Shoot dry weight (g) of *Pennisetum glaucum* as affected by different concentration of heavy metals and EDTA application (eight weeks after sowing).

Heavy Metals ($mg\ kg^{-1}$)	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	0.41	1.02	0.71
Pb 150	0.84	0.93	0.88
Pb 200	0.48	1.24	0.86
Cr 50	1.05	1.08	1.07
Cr 100	0.92	0.92	0.92
Cr 150	0.65	0.77	0.71
Cd 25	0.89	1.10	0.99
Cd 50	0.80	0.67	0.73
Cd 100	0.69	1.00	0.85
Control	1.09	1.11	1.10
	0.78	0.98	

Table 10. Heavy metal accumulation ($mg\ kg^{-1}$) of *Pennisetum glaucum* as affected by different concentration of heavy metals and EDTA application (eight weeks after sowing)

Heavy Metals ($mg\ kg^{-1}$)	*Pennisetum glaucum*		Mean
	EDTA (0 m M)	EDTA (5 m M)	
Pb 100	1.77	2.10	1.93bc
Pb 150	2.06	2.69	2.37cxe
Pb 200	2.77	3.13	2.95a
Pb (Control)	0.06	0.17	0.12
Cr 50	0.98	1.32	1.15de
Cr 100	1.39	1.98	1.68cd
Cr 150	1.80	2.55	2.18bc
Cr(Control)	0.07	0.23	0.15fg
Cd 25	0.28	0.78	0.53fg
Cd 50	0.46	1.02	0.74ef
Cd 100	0.72	1.86	1.29de
Cd(Control)	0.04	0.06	0.05g
	1.03a	1.49b	

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

Conclusions

From our results Shoot lengths of the plant were significantly affected by heavy metal (Pb, Cr and Cd). Application of heavy metal concentration did not significantly affected the biomass production of *Pennisetum glaucum* as no significant effects are being noted in shoot fresh weights and dry weights. Thus *Pennisetum glaucum* is rather resistant to adverse effects of the studied heavy metals. *Pennisetum glaucum* accumulates heavy metals in order of Pb>Cr>Cd. Phyto accumulation rate of heavy metal had significantly increased with increasing dose of the heavy metal. EDTA treatment had significantly enhanced the metal accumulation rate after eight weeks of sowing.

References

Ahmad, K., Z.I. Khan, A. Ashfaq, M. Ashraf and S. Yasmin. 2014a. Assessment of heavy metal and metalloid levels in spinach (*Spinacia oleracea* l.) grown in wastewater irrigated agricultural soil of sargodha, Pakistan. *Pak. J. Bot.*, 46(5): 1805-1810.

Ahmad, K., Z.I. Khan, S. Yasmin, M. Ashraf and A. Ishfaq. 2014b. Accumulation of metals and metalloids in turnip (*Brassica rapa* l.) Irrigated with domestic wastewater in the peri-urban areas of khushab city, Pakistan. *Pak. J. Bot.*, 46(2): 511-514.

Ali, J., Y.C. Najma and A. Faheem. 2014. *In vitro* development of chromium (VI) affected adventitious roots of *Solanum tubersum* L with GA3 and IAA application. *Pak. J. Bot.*, 46(2): 687-692.

Andrew, D.V., Y. Kapulnik, I. Raskin and D.E. Salt. 1998. The role of EDTA in lead transport and accumulation by Indian mustard. *Plant Physiol.,* 117: 447-453.

Andrews, D.J. and K.A. Kumar. 1992. Pearl millet for food, feed, and forage. *Adv. Agron.,* 48: 89-139.

Anna-Maj, B.P. 1989. Toxicity of heavy metals (Zn, Cu, Cd, Pb) to vascular plants. *Water, Air & Soil Pollut.,* 47: 287-319.

Anonymous. 1986. Production Year Book. 1986. Vol. 40. FAO. United Nations, Rome.

Azmat, R., Q. Noshab, N.B. Hajira, N. Raheela, D. Fahim and K. Mustafa. 2015. Aluminum induced enzymatic disorder as animportant eco-biomarker in seedlings of *Lens culinaris* Medic. *Pak. J. Bot.,* 47(1): 89-93.

Babu, T.S., T.A. Akhtar, M.A. Lampi, S. Tripuranthakam, D.G. Dixon and B.M. Greengerg. 2003. Similar stress response elicited by copper and ultraviolet radiation in the aquatic plant *Lemna gibba*: Implication of reactive oxygen species as common signals. *Plant Cell Environ.,* 44: 1320-1329.

Barcelo, J., C. Poschenrieder and B. Gunse. 1986. Water relations of chromium VI trwated bush bean plants (*Phaseolus vulgaris L.* ev. Contender) under both normal and water stress conditions. *J. Exp. Bot.,* 37: 178-187.

Colak, G., M. Celalettin, B.R. Gurler, E. Catak and N. Caner. 2014. Investigation of the effects of aluminum stress on some macro and micro-nutrient contents of the seedlings of *lycopersicon esculentum* mill. By using scanning electron microscope. *Pak. J. Bot*.,. 46(1): 147-160.

Cunningham, S.D., J.R. Shann, D.E. Crowley and T.A. Anderson. 1997. In: *Phytoremediation of soil and water contaminants*. (Eds.): E.L. Krueger, T.A. Anderson and J.P. Coats. ACS symposium series 664. Amer. Chem. Soc. Washington, D.C. pp. 2-19.

E.A. 2010. Grain yield and nitrogen use efficiency of pearl millet as affected plant density, nitrogen rate and splitting in sandy soil. *Am-Eur. J. Agric. Environ. Sci.,* 7: 327-335.

Ebbs, S.D. and L.V. Kochian. 1997. Toxicity of Zinc and copper to brassica species: implications for phytoremediation. *J. Environ. Qual.,* 26: 776-781.

Evanko, C.R. and D.A. Dzombak. 1991. Remediation of metals-contaminated soils and groundwater. Technology Evaluation report, Ground water remediation Technologies Analysis Center.

Ghosh, M. and S.P. Singh. 2005. A review on phytoremediation of heavy metals and utilization of its byproducts. *Appl. Ecol. Environ.* Res., 3: 1-18.

Gomez, K.A. and A.A. Gomez. 1986. Statistical procedures in agricultural research. *Exp. Agric.,* 22: 313.

Grman, H., B. Velikonja, D. Vodnik, B. Kos and D. Letan. 2001. EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. *Plant and Soil,* 235: 105-114.

Jianwei, W.H., J. Chen, W.R. Berti and S.D. 1997. Cunninghum. Phytoremediation of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. *Environ. Sci. Technol.,* 31: 800-805.

Khan, M.A., K. B. Marwat, B. Gul, F. Wahid, H. Khan and S. Hashim. 2014. *Pistia stratiotes* L. (Araceae): Phytochemistry, use in medicines, phytoremediation, biogas and management options. *Pak. J. Bot.,* 46(3): 851-860.

Khan, Z.I., K. Ahmad, M. Ashraf, R. Parveen, I. Mustafa, A. Khan, Z. Bibi and N.A. Akram. Bio-accumualtion of heavy metals and metalloids in Luffa (*Luffa cylindrical* L.) irrigated domestic water in Jhang, Pakistan: A prospect humannutrition. *Pak. J. Bot.,* 47(1): 217-224.

Li, Z.B. and L.M. Shuman. 1996. Extractability of zinc, cadmium, and nickel in soils amended with EDTA. *Soil Sci.,* 161: 226-232.

Liphadzi, M.S., M.B. Kirkham, M.B. Mankin and K.R. Paulsen. 2003. Role of chelating agents in removal of heavy metals. *Plant and Soil,* 257: 171-182.

Liu, Y.G., X. Wang, G.M. Zeng, D. Qu, J.J. Gu, M Zhou and L.Y. Chal. 2007. cadmium-induced stress and response of the ascorbate-glutathione cycle in *Bechmeria nivea* (L.) Gaud. *Chemosph.,* 69: 99-107.

Lombi, E., F.J. Zhao, S.J. Dunham and S.P. McGrath. 2001. Phytoremediation of heavy metal-contaminated soils natural hyperaccumulation versus chemically enhanced phytoextraction. *J. Environ. Qual.,* 30: 1919-1926.

Madiha, I., J. Bakht, M. Shafi and R. Ullah. 2012. Effect of heavy metal and EDTA application on heavy metal uptake and gene expression in different Brassica species. *Afri. J. Biotechnol*., 11: 7649-7658.

Mediouni, C., O. Benzarti, B. Tray, M.H. Ghorbel and F. Jemal. 2006. Cadmium and copper toxicity for tomato seedlings. *Agron. Sustain. Develop.,* 26: 227-232.

Menezes, R.S.C., G.J. Gascho, W.W. Hanna, M.L. Cabrera and J.E. Hook. 1997. Subsoil nitrate uptake by grain pearl millet. *Agron.,* 89: 189-194.

Michael, J.B., D.E. Salt, S. Dushenkov, O. Zakharova, C. Gussman, Y. Kapulnik, B.D. Ensley and I. Raskin. 2008. Enhanced accumulation of Pb in Indian mustard by soil applied chelating agents. *Environ., Sci. Technol.,* 31: 860-865.

Nriagu, J.O. 1979. Global inventory of natural and anthropogenic emissions of trace metals to the atmosphere. *Nature,* 279: 409-411.

Opeolu, B.O., O.O. Adenuga, P.A. Ndakidemi and O.O. Olujimi. 2010. Assessment of phytotoxicity potential of lead on tomato (*Lycopersicon esculentum* L.) planted on contaminated soils. *Intl. J. Phys. Sci.,* 5: 68-73.

Prasad, M.N.V. and H.M.O. Freitas. 2003. Metal hyperaccumulation in plants Biodiversity prospecting for phytoremediation technology. *Electron. J. Biotechnol.,* 6: 275-321.

Qadir, S., M.I. Qureshi, S. Javed and M.Z. Abdin. 2004. Genotypic variation in phytoremediation potential of *Brassica juncea* cultivars exposed to Cd stress. *Plant Sci.,* 167: 1171-1181.

Quartacci, M.F., B. Irtelli, A.J.M. Baker and F. Navari-Izzo. 2007. The use of NTA and EDDS for enhanced phytoextraction of metals from a multiply contaminated soil by *Brassica Carinata*. *Chemosh.,* 68: 1920-1928.

Rafi, U., J. Bakht, M. Shafi, M. IqbalJehan, A. Khan and S. Mohammad. 2011. Phytoaccumulation of heavy metals by sunflower (*Helianthus annus* L.) grown on contaminated soil. *Afr. J. Biotechnol.,* 10: 17192-17198.

Raskin, I. and B.D. Ensley. 2000. Phytoremediation of toxic metals: using plants to clean up the environment. New York: John Wiley.

Romkens, P., L. Bouwman, J. Japenga and C. Draaisma. 2002. Potentials and drawbacks of chelate-enhanced phytoremediation of soils', *Environ. Pollut.,* 116: 109-121.

Russel, D.F. and S.P. Eisensmith. 1983. MSTAT-C. Crop Soil Science Department, Michigan State University USA.

Steel, R.G.D. and J.H. Torrie. 1997. Principles and procedures of statistics: A biometrical approach. 3rd ed.; McGraw-Hill, New York.

Theodoratos, P., N. Papassiopi, T. Georgoudis and A. Kontopoulos. 2000. A. Selective removal of lead from calcareous polluted soils using the Ca-EDTA salt. *Water Air and Soil Pollut.,* 122: 351-368.

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