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Full Length Research Paper

Effect of heavy metal and EDTA application on heavy metal uptake and gene expression in different *Brassica* species

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The present study investigates the effect of different concentration of heavy metals (Cd, Cr and Pb) and ethylenediaminetetraacetic acid (EDTA) application on two Brassica species (*Brassica carinata* and *Brassica juncea*). EDTA application had significant ($p<0.05$) effect on shoot length, shoot fresh weight, shoot dry weight, root length, root fresh weight, root dry weight and accumulation of heavy metals in both species. Species also produced significant ($p<0.05$) effect on all parameters except shoot length of the plant. The effect of heavy metals on shoot length, shoot fresh weight, root fresh weight and accumulation of heavy metals was also reported to be significant **($p<0.05$). Interaction between heavy metals × species showed a significant ($p<0.05$) effect on shoot fresh weight, shoot dry weight, root length and accumulation of heavy metal in both Brassica species. The data reveal that maximum shoot length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight was achieved by control plants. In addition, maximum heavy metals (142.88 mg kg^{-1}) were observed for *B. juncea* that were grown under 150 mg kg^{-1} Pb and 0 mM EDTA stress. Exposure of Brassica species to heavy metals and EDTA resulted in the expression of newly synthesized and abundantly expressed polypeptides, which may play a role in phytoremediation.**

Key words**:** *Brassica*, phytoextraction, heavy metals, EDTA, gene expression.

INTRODUCTION

Industrialization and modern lifestyle have led to increased pollution of air, water and soil (Siegel, 2002). A major cause of contamination of soil is the dispersal of industrial and urban wastes generated by anthropogenic activities. Agricultural soils are being contaminated pollutants from the contaminated sites as dust or leachate. Both controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils etc. are the main causes of contamination of our ecosystem (Alloway, 1990). A variety of organic and inorganic pollutants exist (Prasad and Freitas, 1999; Alcantara et al., 2000; Glass, 1999, 2000a, b; Raskin and Ensley, 2000; Watanabe, 1997), amongst which heavy metals, combustible and putrescible substances, hazardous wastes, explosives and petroleum products are of major concern. (Alloway, 1990).

Enhanced uptake of heavy metals by crops means excessive metals in human nutrition that can be toxic and cause acute and chronic diseases (Geldmacher, 1984). (Prasad and Freitas, 2003). Cadmium, lead and chromium are the major toxic pollutants even at very low concentrations. They enter the water streams and other components of ecosystem through various indu-strial operations. The potential sources of chromium wastes are effluents from metallurgy, electroplating, leather tanning, textile dyeing, paint, ink, and aluminium

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manufacturing industries (Bhattacharyya and Gupta, 2006; Verma et al., 2006). Lead is used as industrial raw material in the manufacturing of storage batteries, pigments, leaded glass, fuels, photographic materials, solder and steel products (Nadeem et al., 2006). The presence of Pb, even in very low concentrations, causes anemia, hepatitis and nephritic syndrome (Zulkali et al., 2006). Moderate Pb poisoning leads to severe damage to kidney, nervous system, reproductive system, liver and brain (Ozer, 2007; Chen et al., 2007).

Different chemical, physical and biological techniques can be employed to remedy soil polluted by metal. In phytoremediation naturally occurring or genetically engineered plants are used for cleaning contaminated environments (Flathman and Lanza, 1998). Phytoremediative technologies is low-cost, efficient and environmental-friendly (Ensley, 2000). (Ebbs et al., 1997). Plants may behave like metal excluders, metal indicators or metal hyperaccumulators (Raskin et al., 1994). Phyto-remediation may consider different strategies like rhizofiltration, phytostabilization, phytovolatilization, phytodegradation and phytoextraction individually or in combination; (Raskin and Ensley, 2000; Berti and Cunningham, 2000; Henry, 2000; Bañuelos, 2000; Dushenkov, 2003). Phyto-extraction is the best approach for removing pollutants primarily from soil without damaging soil structure and fertility. It is also referred as phytoaccumulation. (Rulkens et al., 1998).

Two basic strategies for phytoextraction of heavy metals include natural or continuous phytoextraction and chelate assisted phytoextraction. Chelating agent increases the uptake of heavy metals and various other ions by plants from soil or water. Synthetic chelates are used to increase the supply of micronutrients to plants in both soil and water. These chelating agents can also be used for phytoaccumulation by increasing heavy metals bioavailability and translocation of heavy metals from roots to upper parts of the plants (Epstein et al., 1999). Among these, ethylenediaminetetraacetic acid (EDTA) is found to be the most effective agent in enhancing the accumulation of heavy metals in plants (Blaylock et al., 1997; Madrid et al., 2004; Turgut et al., 2004; Nowack et al., 2006; Liphadzi and Kirkham, 2006; Wahla and Kirkham, 2008). Natural phytoaccumulation uses the natural ability of the plant to remediate metal polluted sites. In this method, only the number of plant growth repetitions is controlled (Salt et al., 1997). While in chelate induced phytoextraction, artificial chelates are added to increase the uptake of metal contaminants (Salt et al., 1997; Rafi et al., 2011)). In order to make this technology feasible, the plants must, extract large concentrations of heavy metals into their roots and translocate the heavy metals to surface biomass, (Brooks,1983; Brooks et al., 1998; Chen et al., 2004).

The roots of *Brassica juncea* are effective in the removal of Cd, Cr, Cu, Ni, Pb, and Zn (Prasad and Freitas, 2003). *Brassica carinata* is known for its phyto-extraction potential (Quartacci et al., 2007; Purakayastha et al., 2008 and Panwar et al., 2005). *B. carinata* is also known for its oil containing seeds but suffers from limitations like low oil quality characterized by high level of erucic acid (Velasco et al., 1998) and unacceptable level of meal glucosinolates (Getinet et al., 1997). This could make it an attractive plant species for phytoremediation. The present study was initiated to investigate the effect of different concentration of heavy metals (Cd, Cr and Pb) and EDTA application on the growth and heavy metal accumulation on two *Brassica* species.

MATERIALS AND METHODS

The present study was conducted at the Institute of Biotechnology and Genetic Engineering, KPK Agricultural University Peshawar Pakistan. The aim of the study was to investigate the response of two species of *Brassica* (*B. carinata* and *B. juncea)* towards heavy metals and EDTA application and the phytoaccumulation capacity of both *Brassica* species for different heavy metals (Cd, Cr and Pb) at different metal concentrations. For this purpose a pot experiment was conducted under greenhouse conditions using completely randomized design (CRD) with three replications. Seeds of two *Brassica* species (*B. carinata* and *B. juncea)* were grown for 30 days on artificially contaminated soil with different concentration of heavy metals (Table 1). 30 days after sowing, 5 mM EDTA was added and the plants were allowed to grow for additional ten days. Forty days after sowing, samples were collected for different growth parameters, that is, shoot length, shoot fresh weight, shoot dry weight, root fresh weight and root dry weight. Samples were also collected for the analysis of heavy metal concentrations of Cd, Cr and Pb and protein analysis by SDS-PAGE. Before sowing, a composite soil sample was collected for heavy metal concentration. Standard agronomic practices were observed throughout the experiment.

Procedures for heavy metal analyses

Samples were dried at 80°C for 48 h and then finely grinded by electric grinder. One gram (1 g) of dried and crushed sample was prepared for atomic absorption spectrophotometer analysis. For this purpose samples were acid digested with 15 ml of concentrated HNO_3 overnight. Digested samples were then heated to 250°C until white fumes appeared. They were then heated for another one hour. The samples were then cooled down to room temperature and diluted to 25 ml with distilled water and then filtered. The concentrations of CD, Cr and Pb were determined by atomic absorption spectrophotometer at wavelengths of 228, 357 and 283 nm, respectively. Analysis of the soil before sowing revealed that the concentrations of Cd, Cr and Pb were 1.94, 28.75, 59.25 mg kg^{-1}.

Protein analysis

For sodium dodecyl sulfate–polyacrylamide gel electrophresis (SDS-PAGE), young leaves of the plants were collected from each treatment. Leaves were washed with distilled water and were stored at -80°C until used. One hundred millgram (100 mg) of leaf tissues was first homogenized with 1 ml protein extraction buffer (50 mM

Table 1. Different levels and sources of heavy metals used in the experiment.

S/N	Heavy metal	Source	Molecular formula	Molar mass (g mol^{-1})	Concentration (mg kg^{-1})
1	Cadmium (Cd)	Cadmium nitrate	$Cd(NO_3)_2.4H_2O$	308.47	10, 20, 40
2	Chromium (Cr)	Chromium nitrate	$Cr(NO_3)_3.9H_2O$	400.15	50, 100, 150
3	Lead (Pb)	Lead nitrate	$Pb(NO_3)_2$	331.21	100, 150, 200

Table 2. Shoot length (cm) of *Brassica* species as affected by heavy metals and EDTA application.

Heavy metal (mg kg^{-1})	EDTA (0 mM)		EDTA (5 mM)		Mean
	B. carinata	*B. juncea*	*B. carinata*	*B. juncea*	
Cd 10	20.92	17.44	28.58	22.57	22.38def
Cd 20	19.53	17.47	25.92	24.50	21.85ef
Cd 40	20.42	19.87	23.33	22.33	21.49f
Cr 50	22.08	29.33	31.17	26.08	27.17bc
Cr 100	17.92	27.00	28.50	28.20	25.40bcd
Cr 150	22.75	27.65	22.42	23.63	24.11cdef
Pb 100	25.50	22.33	33.27	28.92	27.50ab
Pb 150	24.92	19.32	29.42	29.52	25.79bc
Pb 200	23.33	21.02	31.33	24.70	25.10bcde
Control	31.50	32.50	32.33	26.50	30.71a
	23.14 a (25.76 a)		27.16 b (24.54 b)		

Means followed by different letters are statistically different at $p<0.05$. Means in parenthesis refers to species while the other refers to EDTA application.

Tris-HCl; pH 8.00; 25 mM DTT; 1, 4-dithio-DL-Threitol; 1% SDS and 1% β-mercaptoethanol) in a chilled mortar and pestle. After grinding, samples were mixed well by vortex. Samples were then centrifuged at 10,000 rpm for 10 min. Supernatant containing proteins were stored at -20°C for analysis. Samples for protein quantification were prepared by mixing 10 µL protein samples with 2 ml CBB solution (CBB powder G250-10%; 95% ethanol; 85% phosphoric acid). Samples were then analyzed for concentration of protein by UV absorption spectrophotometer. Spectrophotometric data was collected for the samples as well as standard protein solution of BSA (Bovine Serum Albumin). Fifty microgram (50 µg) samples were then run on 12% polyacrylamide gel containing 4% stacking gel. After electrophoration, protein gels were stained in staining solution (0.25 g CBB powder R250, 125 ml methanol, 25 ml glacial acetic acid and 100 ml distilled water) for 40 min followed by overnight destaining, in destaining solution (30% methanol; 10% acetic acid and 60% distilled water). The banding profile of the gels was recorded by gel documentation system.

Statistical analyses

All data are presented as mean values of three replicates. Data was analyzed statistically for analysis of variance (ANOVA) following the method described by Gomez and Gomaz (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

Statistical analysis of the data revealed that heavy metal, EDTA and interaction of heavy metal × EDTA and EDTA × species had a significant ($p<0.05$) effect on shoot length of *Brassica* species (Table 2). Interaction between heavy metal × EDTA × species did not significantly ($p>0.05$) affect shoot length. The data obtained indicate that maximum shoot length (30.71 cm) was attained by control plants followed by treatment of 100 mg kg^{-1} of Pb (27.50 cm). While minimum shoot length (21.49 cm) was noted in plants treated with 40 mg kg^{-1} Cd. In case of EDTA application, maximum shoot length (27.16 cm) was recorded in those treatments which were applied with 5 mM EDTA. Between species, maximum shoot length (25.76 cm) was observed in *B. carinata* when compared with *B. juncea* (24.54 cm). These results are in agreement with Qadir et al. (2004) who studied *B. juncea* cultivar for their phytoextraction efficiency and found a reduction in shoot length of *B. juncea* cultivar subjected to Cd (0.0–2.0 mM). Heavy metal, EDTA, species and interactions of heavy metal × EDTA, heavy metal × species, EDTA × species and heavy metal × EDTA × species had a significant ($p<0.05$) effect on shoot fresh weight of *Brassica* species (Table 3). The data indicated

Table 3. Shoot fresh weight (g) of *Brassica* species as affected by heavy metals and EDTA application.

Heavy metal (mg kg^{-1})	EDTA (0 m M)		EDTA (5 m M)		Mean
	B. carinata	*B. juncea*	*B. carinata*	*B. juncea*	
Cd 10	9.74	26.55	27.22	25.27	22.20de
Cd 20	8.73	25.80	17.39	35.24	21.79e
Cd 40	12.77	27.90	20.43	23.67	21.19e
Cr 50	10.53	42.29	24.15	52.14	32.28bc
Cr 100	18.37	33.65	15.76	50.96	29.68bc
Cr 150	16.53	32.23	12.21	52.79	28.44bcd
Pb 100	20.71	34.57	24.62	57.25	34.29b
Pb 150	16.43	22.96	24.47	50.62	28.62bc
Pb 200	16.78	21.32	26.45	44.62	27.29cde
Control	33.83	70.63	22.37	60.18	46.75a
	25.12 a (18.97 a)		33.39 b (39.53 b)		

Means followed by different letters are statistically different at $p<0.05$. Means in parenthesis refers to species while the other refers to EDTA application.

that maximum shoot fresh weight (46.75 g) was noted in control plants followed by plants treated with 100 mg kg^{-1} of Pb (34.29 g). Minimum shoot fresh weight (21.19 g) was recorded for 40 mg kg^{-1} of Cd treatment. Plants produced maximum shoot fresh weight (33.39 g) when treated with 5 mM EDTA. Similarly, maximum shoot fresh weight (39.53 g) was observed in *B. juncea* compared with *B. carinata* (18.97 g). When interaction between heavy metal × EDTA × species was considered, maximum shoot fresh weight (70.63 g) was observed in 0 mg kg^{-1} heavy metal treated plants (Table 3). Similar results were also reported by Lombi et al. (2001). They revealed that *B. juncea* suffered from severe phytotoxicity when exposed to heavy metals, that is, Cd and Pb while addition of EDTA increased the phytotoxicity. Qadir et al. (2004) observed reduction in biomass accumulation of *B. juncea* exposed to Cd stress. While these findings are contradictory to Quartacci et al. (2007) who reported that *B. carinata* accumulates high concentrations of heavy metals in shoots without showing biomass reduction in 9 different plant species.

Analysis of the data indicated that EDTA, species and interaction between heavy metal and species had a significant ($p<0.05$) effect on shoot dry weight. While heavy metal and interactions of heavy metal × EDTA, EDTA × species and heavy metal × EDTA × species did not significantly ($p>0.05$) affect shoot dry weight of *Brassica* plant (Table 4). Maximum shoot dry weight (3.19 g) was observed for control plants followed by plants grown in 50 mg kg^{-1} of Cr. Minimum shoot dry weight data (2.39 g) was recorded for plants under 200 mg kg^{-1} Pb stress. When EDTA was applied, maximum shoot dry weight (2.82 g) was noted in plants exposed to 5 mM EDTA. Maximum shoot dry weight was attained by *B. carinata* (10.89 g) in comparison with *B. juncea* plants (9.98 g). These results are in conformity with Ebbs and Kochian (1997) who observed that the shoot dry weight of 3 *Brassica* species decreased significantly in the presence of heavy metals. Similar results are also reported by Quartacci et al. (2006) who revealed that *B. juncea* shoots dry weights was reduced significantly followed by NTA application

EDTA, species and interaction between heavy metal × species significantly ($p<0.05$) affected root length while heavy metal and interactions of heavy metal × EDTA, EDTA × species, heavy metal × EDTA × species showed a non-significant ($p> 0.05$) effect on root length (Table 5). Maximum mean root length (10.84 cm) was observed for the treatments of 5 mM EDTA. Between species, maximum root length was achieved by *B. carinata* plants (10.89 cm) compared with *B. juncea* (9.98 cm). Purakayastha et al. (2008) also observed that root length, among root parameters, appeared as the most powerful parameter to dictate the uptake of metals by *Brassica* species during his research on different *Brassica* species. Statistical analysis of the data obtained also indicated that heavy metal, EDTA, species and interaction between EDTA × species significantly ($p<0.05$) affected root fresh weight of *Brassica* plants while the effect of interactions of heavy metal × EDTA, heavy metal × species and heavy metal × EDTA × species on root fresh weight was not significant ($p>0.05$) (Table 6). Maximum root fresh weight (1.89 g) was produced by control plants whereas minimum root fresh weight (0.82 g) was observed for plants grown under 10 or 20 mg kg^{-1} concentration of Cd. In the case of EDTA addition, maximum mean root fresh weight value (1.28 g) was achieved by plants which were amended with 5 mM EDTA. Similarly, between species, maximum root fresh weight of 1.55 g was noted in *B. juncea* grown in 5 mM EDTA compared with *B. carinata* (0.57 g). These results are confirmed by Wong and Bradshaw (2006) who noted significant toxic effect of

Table 4. Shoot dry weight (g) of *Brassica* species as affected by heavy metals and EDTA application.

Heavy metal (mg kg^{-1})	EDTA (0 m M)		EDTA (5 m M)		Mean
	B. carinata	*B. juncea*	*B. carinata*	*B. juncea*	
Cd 10	1.25	3.71	1.90	3.23	2.52
Cd 20	0.82	4.26	1.62	3.47	2.54
Cd 40	1.06	3.35	2.65	3.10	2.54
Cr 50	1.34	3.97	2.37	3.88	2.89
Cr 100	1.76	3.40	1.34	3.65	2.54
Cr 150	1.82	4.00	1.79	3.42	2.76
Pb 100	1.77	1.44	2.71	3.81	2.43
Pb 150	2.32	2.79	2.59	3.16	2.71
Pb 200	1.92	1.98	2.84	2.81	2.39
Control	2.32	4.40	2.15	3.91	3.19
	2.48 a (1.92 a)		2.82 b (3.39 b)		

Means followed by different letters are statistically different at $p<0.05$. Means in parenthesis refers to species while the other refers to EDTA application.

Table 5. Root length (cm) of *Brassica* species as affected by heavy metals and EDTA application.

Heavy metals (mg kg^{-1})	EDTA (0 m M)		EDTA (5 m M)		Mean
	B. carinata	*B. juncea*	*B. carinata*	*B. juncea*	
Cd 10	9.92	8.70	13.75	10.50	10.72
Cd 20	10.67	8.43	11.33	8.50	9.73
Cd 40	11.50	8.05	13.92	9.80	10.82
Cr 50	9.00	10.75	10.17	9.75	9.92
Cr 100	10.08	8.30	11.25	10.98	10.15
Cr 150	9.83	11.33	10.67	10.42	10.56
Pb 100	10.50	8.43	10.67	11.30	10.23
Pb 150	11.50	11.42	10.33	11.83	11.27
Pb 200	12.00	8.40	11.17	10.67	10.56
Control	10.67	11.00	8.83	11.00	10.38
	10.02 a (10.89 a)		10.84 b (9.98 b)		

Means followed by different letters are statistically different at $p<0.05$. Means in parenthesis refers to species while the other refers to EDTA application.

heavy metals on the growth of rye grass roots. Analysis of the data also suggested that root dry weight was significantly ($p<0.05$) affected by EDTA and species while heavy metals and interactions of heavy metal × EDTA, heavy metal × species, EDTA × species and heavy metal × species × EDTA had a non-significant ($p>0.05$) effect on root dry weight (Table 7). Maximum root dry weight (0.25 g) was achieved at 5 mM EDTA concentration. Between species, maximum root dry weight (0.26 g) was noted in *B. juncea* compared with *B. carinata* (0.15 g). Similar results were also reported by Ebbs and Kochian (1997) who reported significant decrease in root dry weight in 3 *Brassica* species.

Table 8 indicates heavy metal accumulation levels in the shoots of *Brassica* species as affected by heavy metals and EDTA application. Statistical analysis of the data revealed that heavy metal, EDTA, species, interaction between heavy metal × species, EDTA × species and heavy metal × EDTA × species significantly ($p<0.05$) affected the accumulation of heavy metals in shoots of *Brassica* plants while the effect of interaction between heavy metal × EDTA was non-significant ($p>0.05$). It is evident from the data that maximum accumulation of heavy metals (95.42 mg kg^{-1}) was achieved by plants exposed to 150 mg kg^{-1} of Pb, followed by 88.34 mg kg^{-1}, by plants grown on 200 mg kg^{-1} Pb concentration. Minimum accumulation (0.82 mg kg^{-1}) was noticed for Cd in control plants. When subjected to EDTA, maximum

Table 6. Root fresh weight (g) of *Brassica* species as affected by heavy metals and EDTA application.

Heavy metal (mg kg^{-1})	EDTA (0 m M)		EDTA (5 m M)		Mean
	B. carinata	*B. juncea*	*B. carinata*	*B. juncea*	
Cd 10	0.34	0.72	0.58	1.63	0.82b
Cd 20	0.27	0.75	0.38	1.89	0.82b
Cd 40	0.27	0.76	0.69	1.76	0.87b
Cr 50	0.30	1.31	0.49	1.43	0.88b
Cr 100	0.61	1.07	0.27	2.13	1.02b
Cr 150	0.53	1.39	0.35	1.57	0.96b
Pb 100	0.52	0.43	0.79	2.70	1.11b
Pb 150	0.72	1.01	0.64	2.47	1.21b
Pb 200	0.55	0.79	0.78	1.83	0.99b
Control	1.52	2.84	0.77	2.42	1.89a
	0.84 a (0.57 a)		1.28 b (1.55 b)		

Means followed by different letters are statistically different at $p<0.05$. Means in parenthesis refers to species while the other refers to EDTA application.

Table 7. Root dry weight (g) of *Brassica* species as affected by heavy metals and EDTA application.

Heavy Metals (mg kg^{-1})	EDTA (0 m M)		EDTA (5 m M)		Mean
	B. carinata	*B. juncea*	*B. carinata*	*B. juncea*	
Cd 10	0.08	0.14	0.13	0.28	0.16
Cd 20	0.05	0.14	0.08	0.44	0.18
Cd 40	0.06	0.13	0.27	0.38	0.21
Cr 50	0.07	0.30	0.15	0.21	0.18
Cr 100	0.15	0.21	0.11	0.31	0.20
Cr 150	0.11	0.29	0.15	0.22	0.19
Pb 100	0.13	0.06	0.23	0.50	0.23
Pb 150	0.26	0.14	0.20	0.40	0.25
Pb 200	0.21	0.09	0.20	0.23	0.18
Control	0.23	0.43	0.17	0.34	0.29
	0.16 a (0.15 a)		0.25 b (0.26 b)		

Means followed by different letters are statistically different at $p<0.05$. Means in parenthesis refers to species while the other refers to EDTA application.

Table 8. Heavy metals (mg kg^{-1}) accumulation by *Brassica* species as affected by heavy metals and EDTA application.

Heavy metal (mg kg^{-1})	EDTA (0 m M)		EDTA (5 m M)		Mean
	B. carinata	*B. juncea*	*B. carinata*	*B. juncea*	
Cd 10	5.97	6.47	10.55	14.57	9.39b
Cd 20	5.67	6.19	10.69	16.62	9.79b
Cd 40	6.05	6.29	10.52	15.59	9.61b
Control	0.90	0.75	0.00	0.00	0.82
Cr 50	8.67	11.59	10.75	11.33	10.59b
Cr 100	8.58	13.96	11.01	10.75	11.08b
Cr 150	9.57	6.83	11.52	12.92	10.21b
Control	3.75	8.00	0.00	0.00	5.88
Pb 100	28.32	108.25	84.13	122.58	85.82a

Table 8. Contd.

Pb 150	29.70	142.88	108.50	100.58	95.42a
Pb 200	29.54	115.12	98.13	110.58	88.34a
Control	35.00	71.75	0.00	0.00	53.38
	27.48 a (24.39 a)		38.57 b (41.66 b)		

Means followed by different letters are statistically different at $p<0.05$. Means in parenthesis refers to species while the other refers to EDTA application.

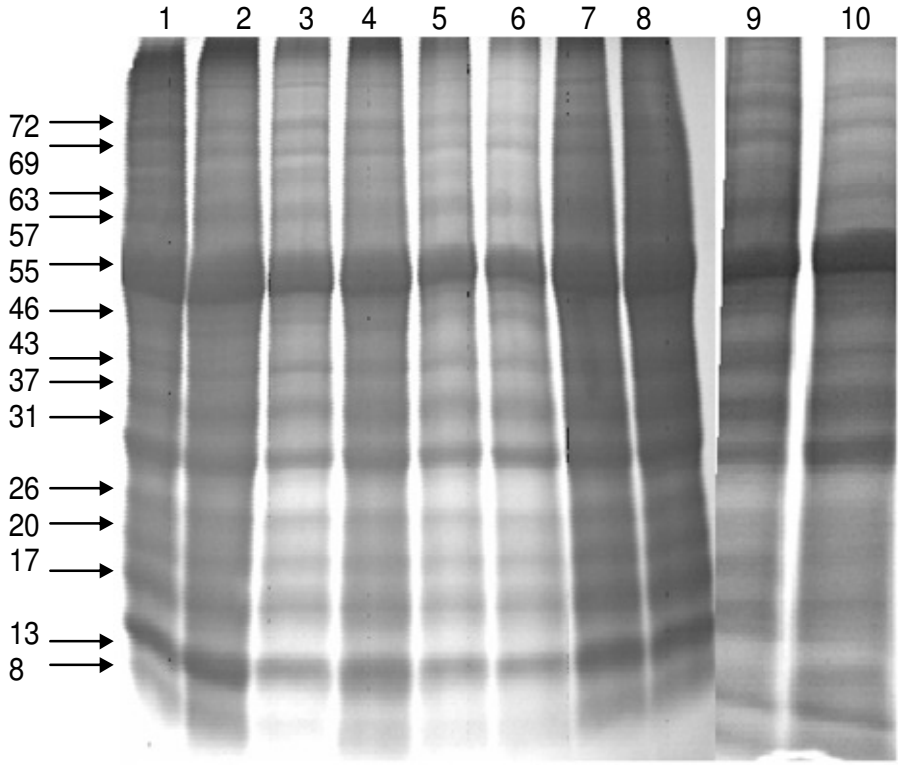

Figure 1. SDS-PAGE protein profile of *Brassica carinata* grown under different heavy metals (mg kg^{-1}) and EDTA (0 mM). Lane 1, Control; lane 2, Cd10; lane 3, Cd20; lane 4, Cd40; lane 5, Cr50; lane 6, Cr100; lane 7, Cr150; lane 8, Pb100; lane 9, Pb150; lane 10, Pb200.

accumulation occurred in plants (38.57 mg kg^{-1}) when 5 mM EDTA was applied. Between species, maximum accumulation was found to be 41.66 mg kg^{-1} in *B. carinata* when compared with *B. juncea* (24.39 mg kg^{-1}). For interaction between heavy metal × EDTA × species, maximum accumulation (142.88 mg kg^{-1}) was observed for those plants that were grown under 150 mg kg^{-1} Pb stress (*B. juncea*; 0 mM EDTA) while minimum accumulation (0.75 mg kg^{-1}) was noted for Cd in control plants (*B. juncea*; 0 mM EDTA). The results are in agreement with Blaylock et al. (1997) who reported that accumulation of Pb in the plant tissue corresponds to the Pb and EDTA concentrations in soil after working with *B. juncea.* Ahmed et al. (2001) found that EDTA increases the solubility of Cd helping its enhanced accumulation in *B. juncea* roots, shoots and stem. These results agree with Kos et al. (2003) and Lesage et al. (2005).

Protein analysis by SDS-PAGE

Protein profile by SDS-PAGE of *Brassica* species exposed to different levels of heavy metals and EDTA application showed that *B. carinata* plants treated with Cd (20 mg kg^{-1}) and Pb (100 and 150 mg kg^{-1}) and EDTA expressed one polypeptide each of molecular weight 57 and 60 kDa when compared with other treatments (Figure 2). Similarly, *B. carinata* when exposed to 100 mg

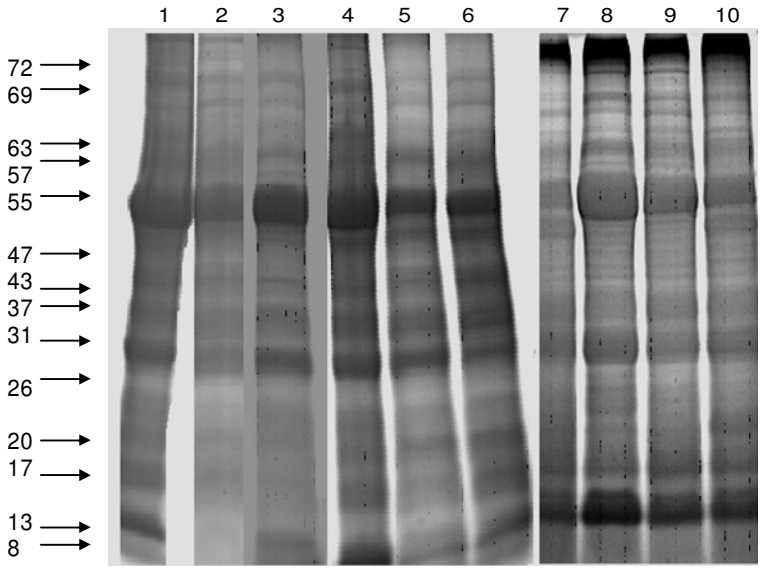

Figure 2. SDS-PAGE protein profile of *Brassica carinata* grown under different heavy metals (mg kg^{-1}) and 5 mM EDTA. Lane 1, Control; lane 2, Cd10; lane 3, Cd20; lane 4, Cd40; lane 5, Cr50; lane 6, Cr100; lane 7, Cr150; lane 8, Pb100; lane 9, Pb150; lane 10, Pb200.

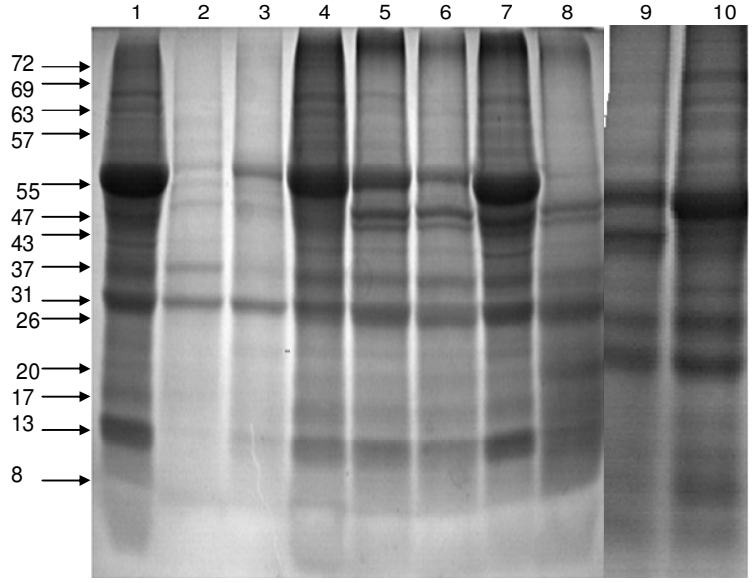

Figure 3. SDS-PAGE Protein profile of *Brassica juncea* grown under different heavy metals (mg kg^{-1}) and EDTA (0 mM). Lane 1, Control; lane 2, Cd10; lane 3, Cd20; lane 4, Cd40; lane 5, Cr50; lane 6, Cr100; lane 7, Cr150; lane 8, Pb100; lane 9, Pb150; lane 10, Pb200.

kg^{-1} and 5 mM EDTA revealed that a band of 55 kDa disappeared when compared with other treatments (Figure 3). The same brassica specie when treated with Cr (100 mg kg^{-1}) indicated that 63 kDa protein was not expressed when compared with other treatments (Figure 1). The data further suggested that *B. carinata* when exposed to Pb (100 mg kg^{-1}) and 5 mM EDTA abundantly expressed two polypeptides of molecular weight 69 and 72 kDa (Figure 2). Banding profile of the treated plants revealed that the same *Brassica* (*B. carinata*) two polypeptides (20 and 43 kDa) were highly expressed when treated with 20 mg kg^{-1} Cd and Cr (100 mg kg^{-1}),

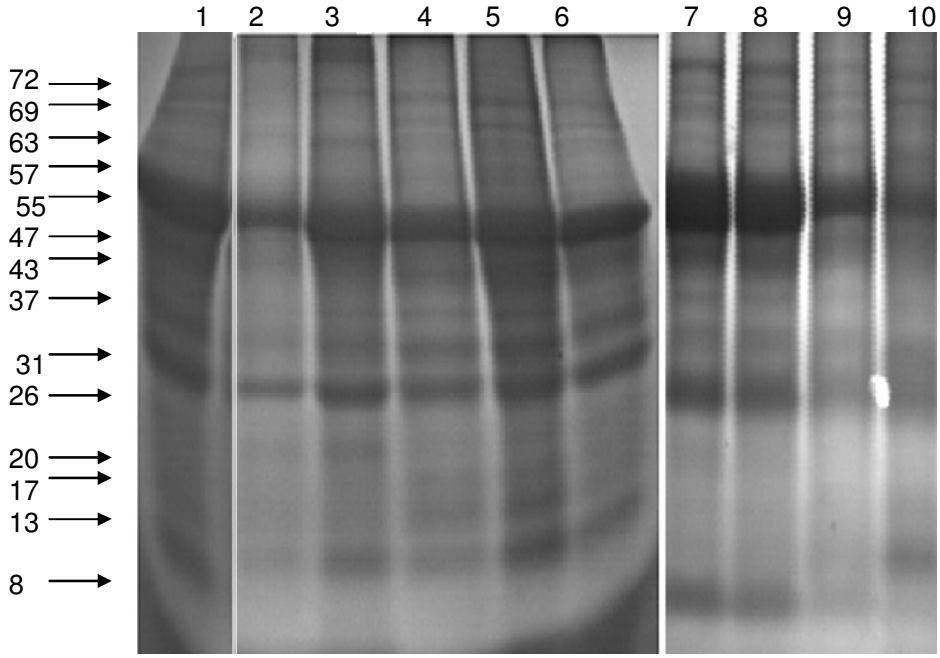

Figure 4. SDS-PAGE Protein profile of *Brassica juncea* grown under different heavy metals (mg kg^{-1}) and 5 mM EDTA. Lane 1, Control; lane 2, Cd10; lane 3, Cd20; lane 4, Cd40; lane 5, Cr50; lane 6, Cr100; lane 7, Cr150; lane 8, Pb100; lane 9, Pb150; lane 10, Pb200.

respectively (Figure 2). The data also suggested that plants treated with Cr (150 mg kg-1) abundantly expressed 13, 43 and 72 kDa protein when compared with other treatments (Figures 3 and 4). Similarly, 69 kDa protein was highly expressed in the case of Cr (50 mg kg^{-1}) treatment (Figure 4). Wu et al. (2011) reported that *CAXcd*-expressing petunia plants showed significantly greater Cd tolerance and accumulation than the controls.

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