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Interactive Effects of Cr(VI) with Other Heavy Metals on the Growth and Metal Uptake Potential of *Brassica juncea* L. Seedlings

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ABSTRACT

The study was undertaken to assess the suitability of *Brassica juncea* L. cv. 'PBR-91' for phytoremediation of multi-heavy element contaminated soils. Growth and heavy metal uptake potential of *B. juncea* seedlings were determined in binary combinations of Cr(VI) with Mn, Ni, Co, Cu and Zn at concentrations varying up to 100 mg/l. Multiple regression interaction models revealed that all the metals, whether applied singly or in combinations, inhibited the growth of seedlings. In a single metal treatment, Cr(VI) (100 mg/l) decreased the germination percentage, root length, shoot length and dry weight to the maximum extent. The interactive effects of binary combinations of Cr(VI) with other metals were generally mutually antagonistic and decreased the toxicity of each other on seedling growth. The maximum uptake was recorded for 100 mg/l each of Zn and Mn, being 0.531 and 0.445 mg/g dw, respectively. The lowest heavy metal uptake was observed for Ni (0.135 mg/g dw) at a concentration of 100 mg/l. Multiple regression interaction models also revealed that the interaction between Cr and the other metals in binary combinations decreased the uptake of Cr by seedlings. This study established that Zn and Mn significantly reduce the deleterious effects of Cr(VI) on seedling growth in *B. juncea*.

Keywords: antagonism, binary interactions, Co, Cu, Mn, Ni, Zn
Abbreviations: ANOVA, analysis of variance

INTRODUCTION

Soil contamination by heavy metals is one of the most serious ecological problems all over the world. Although trace elements are required in microquantities to sustain metabolic activities in organisms, these prove to be lethal beyond certain limits. A high concentration of these metals in the environment results in their incorporation, and subsequent biomagnification at higher trophic levels, which adversely affect the behavioral, structural and functional activities of living organisms (Garbisu and Alkorta 2001; Prasad 2004; Lone *et al.* 2008). Phytoremediation is one of the most promising green technologies, which involves the use of plants to scavenge toxicants (inorganic and organic) from contaminated environments (Raskin *et al*. 1997; Dickinson *et al*. 2009). Phytoremediation research gained momentum after the discovery of several metal hyperaccumulator plants (Reeves and Baker 1999; Cunnigham and Ow 1996). Over the past decade, researchers have sought to perfect the remediation techniques by carefully selecting suitable plants which can sequester more than one metal in appreciable amounts (Lai and Chen 2009). Biotechnology also has been successfully employed to manipulate metal uptake and tolerance properties in various species of hyperaccumulators (Lasat 2000; Reisinger *et al*. 2008; Dowling and Doty 2009). Particular importance has been given to *Brassica* species, because of their relation to wild mustards having high biomass production capability (Dushenkov *et al*. 1995; Kumar *et al.* 1995; Blaylock *et al.* 2000).

Among all the members of Brassicaceae, *B. juncea* emerged out as a suitable candidate for phytoremediation. Dushenkov *et al*. (1997) reported that *B. juncea* is effective, particularly in sorbing divalent cations of toxic metals from soil solutions. It is reported that although *B. juncea* is not a hyperaccumulator, it has demonstrated high tolerance to several heavy metals (Lasat 2000). It has been shown to be effective in phytoextraction of Zn, particularly after EDTA amendments (Ebbs and Kochian 1998). Also that *B. juncea* is being tolerant to heavy metals, came from the experiments by Shahandeh and Hossner (2000) who screened a series of crops for phytoextraction. Thirty-six plant species of different agronomic importance, size, dry matter production, and tolerance to heavy metals were evaluated for Cr(III) and Cr(VI) uptake and accumulation as influenced by rate, form, source, and chelate application to a Cr-contaminated soil and it was found that Indian mustard (*B. juncea* cv. '426308') accumulated more Cr than other agricultural plant species. Simnova *et al.* (2007) reported higher tolerance of *B. juncea* to Cd than *V. radiata* in terms of effects of different concentrations of Cd on Hill reaction, and the contents of chlorophyll and carotenoids. Turan and Esringü (2007) examined the positive effects of EDTA amendments on metal accumulation and uptake in *B. juncea*. At 12 mM/kg EDTA, shoot and root uptake of Cu, Cd, Pb, and Zn uptake was observed to be four fold higher than the control. Shiyab *et al.* (2008) demonstrated *B. juncea* as a potential candidate for phytofilteration of contaminated water and phytostabilization of mercury contaminated soils by inducing an efficient metabolic defence system (especially catalase) to scavenge H_2O_2. Ghodoke *et al.* (2009) examined the potential of *B. juncea* in the treatment of textile effluents. It showed up to 79% discoloration of textile effluent, and also significant induction of intracellular laccase (266%), indicating its crucial role in degradation of textile effluent. Further, it has been widely used as a model system to investigate the physiology and biochemistry of metal uptake and accumulation in plants. Various studies have been performed on *B. juncea* to investigate the modulation of antioxidative defence system, and generation of phytochelatins and metallothioneins under metal stress con-

Received: 16 February, 2008. Accepted: 7 July, 2009.

Original Research Paper

ditions (Nouairi *et al*. 2008; Seth *et al*. 2008; Ansari *et al*. 2009; Khan *et al*. 2009).

Siedlecka *et al.* (1995) revealed that during the uptake of heavy metals by plants, there are various interactions among different metal ions. Since 70% of all metal-contaminated sites involve two or more metals, a thorough study of metal interactions is necessary to streamline the technique of phytoremediation. Metal interactions may be additive, synergistic or antagonistic, and may influence the rate of uptake, transfer and accumulation during phytoremediation (Martin-Prevel *et al.* 1987). Because of the real problem faced by plants, it is more widely recognized that examining the effects of heavy metals in various combinations is more representative than single metal studies (Chaoui *et al.* 1997). Because of their high degree of complexity, higher plants are not very often used in such experiments. Algal models were used by Rasko and Rachlin (1977), Taylor and Stadt (1990) and others to study the interactions of multiple heavy metals in terms of their action. However, with growing interest in the field of phytoremediation, several researchers focused on metal-metal interactions in higher plants. Coughtrey and Martin (1978) explored the tolerance of *Holcus lanatus* to Pb, Zn and Cd in factorial combinations and revealed the greater tolerance of this plant to Cd than to Pb or Zn. Miles and Parker (1979) studied heavy metal interactions in *Andropogon scoparius* and *Rudbeckia hirta* grown on soils from urban and rural sites with heavy metal additions in all combinations of Cd, Zn Pb and Cu. Luo and Rimmer (1994) examined metal interactions affecting the growth of spring barley grown in a soil in which Cd, Cu, Pb and Zn were added singly or in combinations. The most consistent effect on plant growth was found to be that of Zn-Cu interaction. Fargašová and Beinrohr (1997) studied bioaccumulation and interactions of V, Ni, Mo, Mn and Cu in under- and above-ground parts of *Sinapsis alba*. It was reported that all individual metals except Cu, were accumulated more in the above ground parts than in the roots. In metal combinations, V was inhibited by Ni, Mn and Cu; accumulation of Ni by Cu, Mo by V, Mn and Cu and Mn by Cu. Sharma *et al.* (1999) investigated combinational toxicology of Cu, Zn and Cd in binary mixtures on root growth in *Silene vulgaris*, and found non-additive (Cu/Zn, Cu/Cd) or antagonistic (Zn/Cd) responses with respect to root growth inhibition under slightly toxic concentrations, whereas synergistic response was observed at higher concentrations. Chen *et al.* (2003) performed a rapid ecotoxicological assessment of heavy metal contaminated soils (Cu, Pb, Cd and Zn) using canonical analysis. An *et al.* (2004) studied the combined effects of Cu, Cd and Pb on *Cucumis sativum* growth and bioaccumulation, and showed all the three interactions: additive, synergistic and antagonistic by binary combinations of Cu+Cd, Cu+Pb and Cd+Pb. However, ternary combinations of Cu+Cd+Pb produced antagonistic response for the plant growth.

On the basis of toxicant concentrations that induce 50% inhibition of root length of *Lepidium sativum* and plant amount of *Spirodela polyrrhiza* (EC50 values), Montvydienė and Marčiulionienė (2004), concluded that the metals most toxic to these plants were Cu, Cr and Cd, whereas the metals least toxic were Zn and Mn. Tea *et al.* (2007) performed toxicity assessment of heavy metal mixture on *Lemna minor* using wastewater discharged from an electroplating unit, in terms of relative growth rate with respect to dry to fresh weight ratio, frond area and guaiacol peroxidase (GPX) activity. Guo *et al.* (2007) documented various physiological changes in two varieties of barley plants under combined toxicity of Al, Cu and Cd, with respect to plant growth, metal accumulation, protein and sugar content, SOD and POD activities. Binary metal combinations of Al+Cd and Al+Cu, produced synergistic responses for the growth of barley seedlings, whereas, ternary combination produced different types of interactions in the two varieties. Cu and Fe homeostasis, and putative interactions between the metals at different levels in *Arabidopsis* were thoroughly investigated by Puig *et al.* (2007). Tappero *et al.* (2007) studied metal cotolerance in *Alyssum murale* using Ni, Co and Zn, and reported hyperaccumulation of Ni and Co (>1000 μg /g dw) and negligible effect of elevated Co and Zn on Ni accumulation. Abou-Shanab *et al.* (2007) studied phytoremediation potential of various crops and wild plants for multimetal contaminated sites, and reported *Conyza discoridis* as the best species for phytoremediation of Zn, Cu and Pb. Kalavrouziotis *et al.* (2008) explored the interrelationships between heavy metals, macro- and micronutrients, and properties of a soil cultivated with *Brassica oleracea*, under the effect of treated municipal waste water. One of the metal-metal interactions most widely studied is Cd-Zn interaction (Aravind and Prasad 2005; Papoyan *et al.* 2007; Bunluesin *et al*. 2007; Ebbs and Uchil 2008). Joint effects of As and Cd on growth and metal accumulation in different plants were also studied by various researchers (Liu *et al.* 2007; Xiao *et al*. 2008; Sun *et al*. 2009).

It is now well established that the presence of two or more metals in contaminated sites significantly affects the uptake and accumulation pattern of heavy metals in plants. The degree of influence varies for different metals, and Cr is considered to be a highly toxic element. Davies *et al.* (2002) reported that Cr is toxic to higher plants at 100 μM kg^{-1} dw. In nature, Cr exits in two different stable oxidation states, Cr(III) and Cr(VI). Both the oxidized forms, however, have the capacity to form complexes with other species (NRC 1999). The hexavalent form of Cr is a biologically toxic state, and to date there is no evidence indicating its potential role in biological systems, as it causes severe damage to plants and animals (O' Brien *et al.* 2003; Panda and Choudhury 2005; Wise *et al.* 2008; Raghunathan *et al.* 2009). Further, it was reported that Cr interferes and influences the behaviour of various essential and non-essential elements such as Ca, Mg, S, N, P, K, Cu, Mn, Mo, etc. (Turner and Rust 1971; Wallace *et al.* 1982; Baddappa and Bopaiah 1989; Morel *et al.* 1996) during the uptake and transport in plants. These metal interactions may have positive or negative effect on the growth and metal accumulation potential, which can be further exploited for enhancing the phytoremediation potential of plants growing on multielemental contaminated sites. *B. juncea* is a confirmed phytoremediator which can grow effectively on multielemental contaminated soils (Abou-Shanab *et al*. 2007; Saraswat and Rai 2009) and is capable of accumulating considerable amounts of Cr (Mei *et al.* 2002; Ghosh and Singh 2005; Hsiao *et al.* 2007; Diwan *et al.* 2008).

The present study was designed to assess the interactive effects of Cr(VI) in binary combinations with other heavy metals, on the growth and Cr uptake of *B. juncea* seedlings with a view to determine the suitability of using this plant for phytoremediation of Cr(VI) in multi-heavy metal contaminated sites.

MATERIALS AND METHODS

Certified seeds of *B. juncea* L. cv. 'PBR-91' were procured from Punjab Agricultural University, Ludhiana, India. This cultivar was chosen from among the several commercial varieties of *B. juncea*, *viz*., PBR-97, PBR-91, Laha-101 and Pusa Agrani, since it shows stability for most of the important yield contributing characters under the prevailing conditions in the area of study. The seeds were surface sterilized with 0.1% $HgCl_2$ solution, washed and rinsed thoroughly with distilled water. These seeds were then cultured in Petri dishes containing different concentrations of heavy metals, singly or in binary combinations.

(i) Single metal treatments – 0, 25, 50 and 100 mg/l of each metal (Cr, Zn, Mn, Ni, Co and Cu).

(ii) Binary treatments – Cr (VI) treatments in combination with other metals at 0, 25, 50 and 100 mg/l.

Fifty surface-sterilized seeds were germinated on Whatman No. 1 filter paper, lined inside 9 cm diameter sterilized Petri dishes containing 5 ml of aqueous solutions of heavy metals either singly or in binary mixtures. Solutions were prepared using AR grade, K_2CrO_4, $MnSO_4·H_2O$, $NiSO_4·6H_2O$, $CoCl_2·6H_2O$, $CuSO_4·5H_2O$ and $ZnSO_4·7H_2O$. All chemicals were procured from Sigma-

Table 1 Interaction in terms of β regression coefficients.

Variables			Interaction
X_1	X_2	$X_1 X_2$	
β regression coefficients			
β_1	β_2	β_3	
+	+	+	Synergistic
-	-	-	Synergistic
+	+	-	Antagonistic
-	-	+	Antagonistic
+	-	+	Mixed: X_1 antagonistic to X_2, but X_2 synergistic to X_1
+	-	-	Mixed: X_1 synergistic to X_2, but X_2 antagonistic to X_1
+/-	+/-	0	Additive

Aldrich. Sterilized seeds grown in double distilled water served as the control. For the initial 7 days of the growth period, Petri plates were kept at 25 ± 0.5°C and a 16-h photoperiod at 1700 Lux. The rate of germination was recorded daily for 7 days, and root and shoot lengths were measured. Thereafter, harvested seedlings were washed thoroughly with double distilled water and kept in oven for 48 h at 80°C, and the dry weights were recorded.

The dried seedlings of different treatments were ground and digested in H_2SO_4:HNO_3:$HClO_4$ (1:5:1) digestion mixture (Allen 1976). The samples were diluted with double-distilled water and filtered. The concentrations of Zn, Mn, Ni, Co, Cu and Cr were determined using an atomic absorption spectrophotometer (Model 6200, Shimadzu, Japan).

All the analyses were carried out in triplicate, and the data was analyzed for descriptive statistics, ANOVA, Tukey's multiple comparison test, multiple regression and correlation, and β-regression coefficients (Sokal and Rholf 1981; Bailey 1995). The interaction model used for binary combinations was

$$Y = a + b_1X_1 + b_2X_2 + b_3X_1X_2$$

where, Y is the studied parameter, X_1 and X_2 are metals in binary combinations, b_1 and b_2 are partial regression coefficients due to the effects of X_1 and X_2 respectively, and b_3 is the partial regression coefficient due to interaction between X_1 and X_2. Unitless β-regression coefficients were computed to determine the relative effects of X_1 (β_1) and X_2 (β_2) and the interaction between X_1 and X_2 (β_3) on the dependent variable (Y). β-coefficients were computed as follows:

$$\beta = b(S_{X1}/S_Y),$$

where S_{X1} and S_Y are the standard deviations of X_1 and Y respectively. Metal interaction was interpreted as described in **Table 1**. Self coded software developed in MS-Excel was used.

RESULTS

There was reduction in germination percentage at higher metal concentrations in the culture medium (**Fig. 1**). Maximum reduction was observed for Cr(VI) at 100 mg/l. However, the presence of Zn and Mn, even at higher concentrations, resulted in seed germination up to 85%. In binary combinations, the addition of Zn and Mn ameliorated the toxicity of Cr(VI) as observed with an increase in the germination percentage at all the combinations of Cr(VI) having Zn and Mn. In the Cr+Mn combination, the germination percentage significantly increased from 45 to 56% at Cr100+Mn25 mg/l and in the Zn+Cr combination, the increase observed was up to 28% compared to the control at Cr100+Zn25 mg/l. Two-way ANOVA for germination percentage of *B. juncea* seeds for Cr(VI) and other metals in binary combinations (**Table 2**) shows statistically significant differences among mean germination percentage values on treatment with both metals. The interaction between Cr(VI) and Mn was also found to be significant.

There was a progressive decrease in the shoot and root length of the seedlings as metal concentration increased

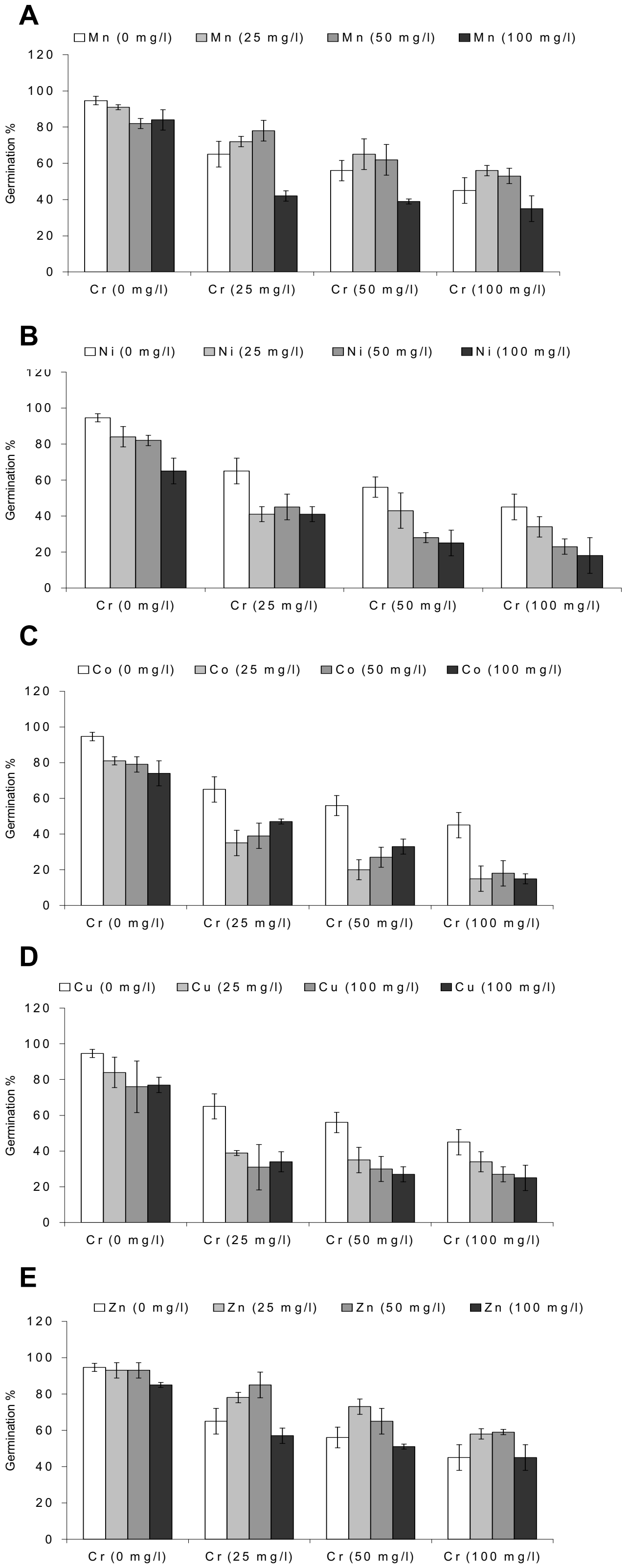

Fig. 1 Germination percentage of *B. juncea* (mean ± SD) grown in binary combinations of Cr(VI) with other heavy metals.

(**Figs. 2, 3**). The IC_{50} values calculated on the basis of root length inhibition are given in **Table 3**. Cr(VI) was found to be the most toxic metal. Root growth was drastically reduced to the minimum value of 0.14 cm as compared to the control. The effects of metals on shoot growth were almost similar to those on root growth. The percentage change in root length of *B. juncea* seedling growth in binary combinations of Cr(VI) with other metals is given in **Table 4**. There was an increase in the root length in Cr(VI) solutions containing Mn and Zn. For solutions containing 25 and 50 mg/l

Table 2 Two-way ANOVA and honestly significant difference (HSD) using Tukey's multiple comparison test for germination percentage and dry weight of *B. juncea* seedlings grown in binary combinations of Cr (VI) and other metals. The first metal in the binary combination is the treatment, and the second the dose.

	Germination percentage					Dry weight (mg/seedling)				
Cr+Mn										
Source of variation	df	SS	MSS	F-ratio	HSD	df	SS	MSS	F-ratio	HSD
Treatment	3	7332.4	2444.1	102.4*	19.6	3	23.2	7.7	24.0*	2.2
Dose	3	2145.4	715.1	30.0*		3	5.3	1.8	5.5*	
Treatment x Dose	9	877.1	97.5	4.1*		9	5.6	0.6	1.9	
Error	16	382.0	23.9			16	5.2	0.3		
Total	31	10736.9				31	39.3			
Cr+Ni										
Treatment	3	12577	4192.3	108.2*	24.9	3	31.3	10.4	43.1*	1.3
Dose	3	3580	1193.3	30.8*		3	16.9	5.6	23.3*	
Treatment x Dose	9	463	51.4	1.3		9	1.1	0.1	0.5	
Error	16	620	38.8			16	3.9	0.2		
Total	31	17240				31	53.2			
Cr+Co										
Treatment	3	15656.4	5218.8	190.6*	20.9	3	24.2	8.1	31.4*	2.2
Dose	3	3759.4	1253.1	45.8*		3	12.0	4.0	15.6*	
Treatment x Dose	9	472.1	52.5	1.9		9	1.2	0.1	0.5	
Error	16	438.0	27.4			16	4.1	0.3		
Total	31	20325.9				31	41.6			
Cr+Cu										
Treatment	3	12738.4	4246.1	80.5*	29.1	3	14.7	4.9	24.7*	1.3
Dose	3	3109.4	1036.5	19.6*		3	7.9	2.6	13.3*	
Treatment x Dose	9	267.1	29.7	0.6		9	2.1	0.2	1.2	
Error	16	844.0	52.8			16	3.2	0.2		
Total	31	16958.9				31	27.8			
Cr+Zn										
Treatment	3	6994.0	2331.3	97.1*	19.6	3	39.9	13.3	28.0*	2.2
Dose	3	1188.0	396.0	16.5*		3	3.8	1.3	2.7*	
Treatment x Dose	9	386.0	42.9	1.8		9	6.7	0.7	1.6	
Error	16	384.0	24.0			16	7.6	0.5		
Total	31	8952.0				31	58.0			

	Root length (cm)					Shoot length (cm)				
Cr+Mn										
Source of variation	df	SS	MSS	F-ratio	HSD	df	SS	MSS	F-ratio	HSD
Treatment	3	739.4	246.5	1205.5*	0.5	3	31.7	10.6	156.5*	0.5
Dose	3	258.7	86.2	421.8*		3	4.3	1.4	21.0*	
Treatment x Dose	9	217.6	24.2	118.2*		9	10.2	1.1	16.8*	
Error	144	29.4	0.2			144	9.7	0.1		
Total	159	1245.1				159	56.0			
Cr+Ni										
Treatment	3	386.4	128.8	1814.1*	0.5	3	44.3	14.8	229.2*	0.5
Dose	3	149.2	49.7	700.4*		3	19.9	6.6	103.2*	
Treatment x Dose	9	355.1	39.5	555.7*		9	7.8	0.9	13.5*	
Error	144	10.2	0.1			144	9.3	0.1		
Total	159	900.9				159	81.3			
Cr+Co										
Treatment	3	767.7	255.9	2666.6*	0.5	3	47.6	15.9	147.1*	0.5
Dose	3	103.7	34.6	360.1*		3	16.7	5.6	51.6*	
Treatment x Dose	9	230.4	25.6	266.8*		9	6.8	0.8	7.0*	
Error	144	13.8	0.1			144	15.5	0.1		
Total	159	1115.5				159	86.7			
Cr+Cu										
Treatment	3	201.9	67.3	821.8*	0.5	3	22.1	7.4	92.7*	0.5
Dose	3	191.9	64.0	781.0*		3	27.4	9.1	114.6*	
Treatment x Dose	9	464.9	51.7	630.7*		9	14.0	1.6	19.5*	
Error	144	11.8	0.1			144	11.5	0.1		
Total	159	870.5				159	74.9			
Cr+Zn										
Treatment	3	791.4	263.8	982.3*	0.8	3	37.2	12.4	138.6*	0.5
Dose	3	130.4	43.5	161.9*		3	3.5	1.2	13.1*	
Treatment x Dose	9	280.7	31.2	116.1*		9	8.8	1.0	10.9*	
Error	144	38.7	0.3			144	12.9	0.1		
Total	159	1241.2				159	62.3			

$^*p \leq 0.05$.

of Cr(VI), root length was enhanced most with 50 mg/l Zn, and for solutions containing 100 mg/l Cr(VI), 25 mg/l Mn was effective. Multiple regression analyses (**Table 5**) revealed significant correlations among both metal ions in all binary combinations. Although all the metal ions exerted a negative influence on seedling growth as indicated by their negative β-regression coefficients, their interactive effects were positive in nature, implying thereby that in binary

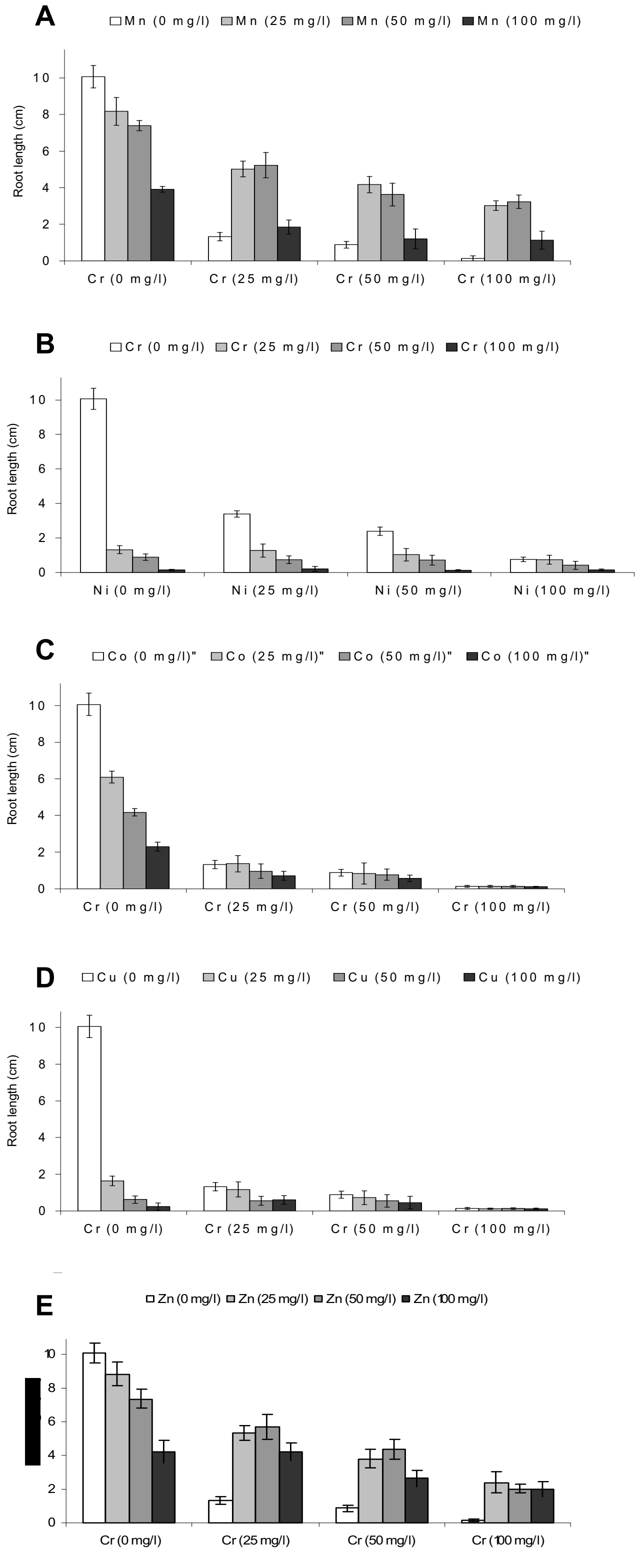

Fig. 2 Root length of *B. juncea* (mean ± SD) seedlings grown in binary combinations of Cr(VI) with other heavy metals.

combinations of Cr(VI) with Mn, Ni, Co, Cu and Zn, these ions showed antagonistic behavior by mutually decreasing each other's toxicity. 2-way ANOVA for root and shoot growth of *B. juncea* seedlings for Cr(VI) and other metals in binary combinations (**Table 2**) shows that there are statistically significant differences among mean root lengths and shoot lengths for the treatments *i.e.* Cr(VI), and doses, *i.e.* other metals. The interactions between Cr(VI) and the other metals in all binary treatments were also found to be significant.

The dry weights of the seedlings decreased considerably except for Zn and Mn, with an increase in the concentration

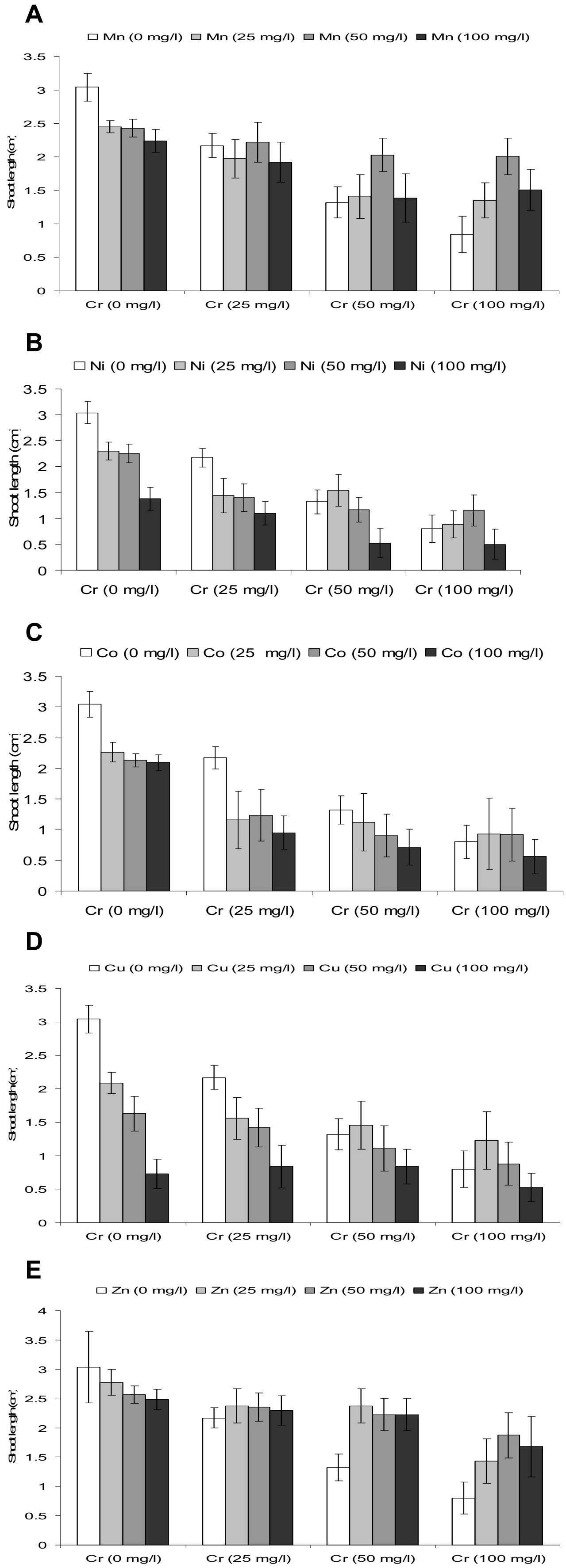

Fig. 3 Shoot lengths of *B. juncea* (mean ± SD) seedlings grown in binary combinations of Cr(VI) with other heavy metals.

of heavy metals (**Fig. 4**). At 25 mg/l, Cr, Co, Ni and Cu reduced the biomass of seedlings by 39, 16, 22 and 15%, respectively. Cr(VI) inflicted the maximum negative effects on seedlings. Further, the results depicted that in binary

Table 3 IC_{50} values of different heavy metals calculated on the basis of inhibition of root length of *B. juncea* seedlings.

Metals	IC_{50} (mg/l)
Cr	0.524
Mn	73.739
Ni	28.881
Co	47.803
Cu	0.563
Zn	77.882

Table 4 Percentage change in root lengths of *B. juncea* seedlings grown in binary combinations of Cr(VI) with other heavy metals, with respect to Cr(VI) controls.

Metal conc. (mg/l^{-1})	Cr(VI) in solution (mg/l)			
	0	25	50	100
	% change with respect to control			
Control (0)	0	0	0	0
Mn	**Cr+Mn**			
25	-18.7	280.3	373.8	2057.1
50	-26.5	296.2	312.5	2207.1
100	-61.1	40.1	36.3	707.1
Ni	**Cr+Ni**			
25	-66.3	-3.7	-15.9	50.0
50	-76.2	-21.9	-19.3	-14.2
100	-92.4	-44.6	-52.2	7.1
Co	**Cr+Co**			
25	-39.4	3.7	-5.6	-7.1
50	-58.5	-27.2	-12.5	-7.1
100	-77.1	-46.2	-35.2	-21.4
Cu	**Cr+Cu**			
25	-83.7	-11.3	-18.1	-14.2
50	-93.	-58.3	-37.5	-7.1
100	-97.7	-54.5	-48.8	-21.4
Zn	**Cr+Zn**			
25	-12.1	303.0	332.9	1607.1
50	-26.8	331.8	395.4	1350.0
100	-58.1	217.4	198.8	1335.7

combinations with Zn and Mn, the seedlings' biomass was higher than that of seedlings cultured in Cr(VI) alone. Results of the multiple regression interaction model (**Table 4**) showed that except for Zn, all other metal ions exhibited a deleterious effect on the dry weight of seedlings. However, these metal ions in binary combinations showed a significant antagonistic interaction among themselves by mutually decreasing each other's toxicity, thereby showing a positive effect on the dry weight, except for (Cr+Ni), in which the interactive effect was negative on the dry weight. This suggests a synergism between the two metal ions. 2-way ANOVA for the dry weight of *B. juncea* seedlings for Cr(VI) and other metals in binary combinations (**Table 5**) shows that there are statistically significant differences among mean dry weight values for treatments of Cr(VI) and doses (other metals) in all binary combinations.

The results of uptake analysis (**Fig. 5, Table 6**) indicate that the uptake of each metal was directly proportional to its concentration in the medium. *B. juncea* seedlings showed maximum uptake of Zn ions, followed by Mn. At 100 mg/l of Zn and Mn, uptake was found to be 0.531 and 0.445 mg/g dw, respectively. On the other hand, the metal that accumulated the least was Ni (0.135 mg/g dw) followed by Cr (0.180 mg/g dw). Co and Cu showed moderate accumulation of 0.224 and 0.235 mg/g dw, respectively at a metal concentration of 100 mg/l.

Uptake analysis of seedlings grown in the binary treatments revealed that the presence of Zn and Mn strongly inhibited the uptake of Cr in seedlings, and *vice versa*. At the highest Cr(VI) treatment of 100 mg/l, addition of even low doses of Mn (25 mg/l) and Zn (25 mg/l), greatly inhibited the uptake of Cr by 66 and 60%, respectively. Similar patterns were also observed in other binary combinations of (Cr+Ni) and (Cr+Cu), where Ni and Cu ions inhibited the

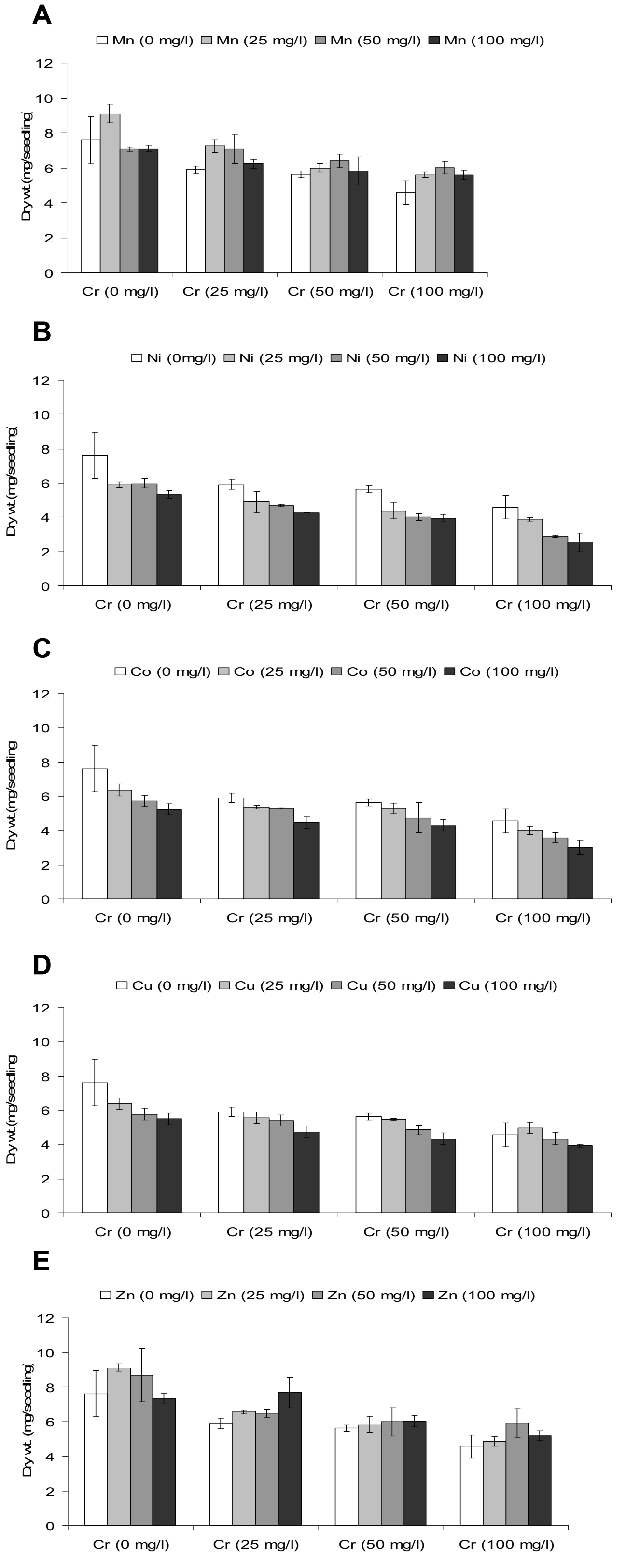

Fig. 4 Dry weight (mg/seedling) of *B. juncea* (mean ± SD) seedlings grown in binary combinations of Cr(VI) with other heavy metals.

uptake of Cr (**Table 7**). However, Co facilitated the uptake of Cr, but the overall interactive effect of the (Cr+Co) combination on Cr uptake was also negative. Multiple regression interaction model (**Table 8**) showed a significant correlation among both metal ions for the uptake of Cr in these binary combinations. β-regression coefficients for Zn, Mn, Ni and Cu were negative, and the interactive effects of these combinations on the uptake of Cr(VI) were also negative.

Table 5 Multiple regression with interaction models for different parameters (Y) of *B. juncea* in binary combinations of Cr (X_1, mg/l) and other metals (X_2, mg/l).

Metal	**Multiple regression equation**	**r**	**β regression coefficients**		
			β_1	**β_2**	**Interaction (β_3)**
Germination percentage					
Mn	$Y = 87.90 - 0.38\ X_1 - 0.18\ X_2 + 1.5{\times}10^{-4}\ X_1X_2$	0.8432*	-0.78	-0.37	0.02
Ni	$Y = 80.34 - 0.47\ X_1 - 0.26\ X_2 - 3.6{\times}10^{-5}\ X_1X_2$	0.8702*	-0.76	-0.42	-4.2×10^{-3}
Co	$Y = 75.54 - 0.51\ X_1 - 0.14\ X_2 - 7.5{\times}10^{-4}\ X_1X_2$	0.8342*	-0.75	-0.20	-0.08
Cu	$Y = 77.74 - 0.44\ X_1 - 0.21\ X_2 - 3.4{\times}10^{-6}\ X_1X_2$	0.8109*	-0.73	-0.36	4.1×10^{-4}
Zn	$Y = 89.10 - 0.40\ X_1 - 0.11\ X_2 + 7.9{\times}10^{-4}\ X_1X_2$	0.8393*	-0.90	-0.24	0.13
Root length (cm)					
Mn	$Y = 7.58 - 0.07\ X_1 - 0.04\ X_2 + 5.0{\times}10^{-4}\ X_1X_2$	0.7308*	-0.95	-0.51	0.54
Ni	$Y = 5.32 - 0.06\ X_1 - 0.05\ X_2 + 6.7{\times}10^{-4}\ X_1X_2$	0.7573*	-1.00	-0.82	0.75
Co	$Y = 5.98 - 0.07\ X_1 - 0.05\ X_2 + 6.0{\times}10^{-4}\ X_1X_2$	0.7789*	-1.01	-0.66	0.78
Cu	$Y = 4.69 - 0.05\ X_1 - 0.05\ X_2 + 7.0{\times}10^{-4}\ X_1X_2$	0.6898*	-0.88	-0.85	0.69
Zn	$Y = 7.68 - 0.08\ X_1 - 0.03\ X_2 + 6.0{\times}10^{-4}\ X_1X_2$	0.7911*	-1.07	-0.37	0.54
Shoot length (cm)					
Mn	$Y = 2.64 - 0.02\ X_1 - 0.01\ X_2 + 1.3{\times}10^{-4}\ X_1X_2$	0.8262*	-1.15	-0.41	0.65
Ni	$Y = 2.65 - 0.02\ X_1 - 0.01\ X_2 + 1.1{\times}10^{-4}\ X_1X_2$	0.9235*	-1.01	-0.78	0.45
Co	$Y = 2.51 - 0.02\ X_1 - 0.01\ X_2 + 1.1{\times}10^{-4}\ X_1X_2$	0.8909*	-1.01	-0.73	0.45
Cu	$Y = 2.60 - 0.02\ X_1 - 0.02\ X_2 + 1.7{\times}10^{-4}\ X_1X_2$	0.9470*	-1.02	-1.08	0.71
Zn	$Y = 2.82 - 0.02\ X_1 - 3.4\text{x}10^{-3}\ X_2 + 1.3{\times}10^{-4}\ X_1X_2$	0.8900*	-1.19	-0.26	0.60
Dry weight (mg/seedling)					
Mn	$Y = 7.73 - 0.03\ X_1 - 0.01\ X_2 + 1.8{\times}10^{-4}\ X_1X_2$	0.8051*	-1.05	-0.29	0.46
Ni	$Y = 6.62 - 0.02\ X_1 - 0.02\ X_2 - 2{\times}10^{-5}\ X_1X_2$	0.9342*	-0.72	-0.49	-0.04
Co	$Y = 6.90 - 0.03\ X_1 - 0.02\ X_2 + 5.5{\times}10^{-5}\ X_1X_2$	0.9700*	-0.88	-0.64	0.63
Cu	$Y = 6.88 - 0.02\ X_1 - 0.02\ X_2 + 1{\times}10^{-4}\ X_1X_2$	0.9203*	-0.90	-0.73	0.30
Zn	$Y = 2.77 - 0.02\ X_1 - 3.4\text{x}10^{-3}\ X_2 + 1.3{\times}10^{-4}\ X_1X_2$	0.9070*	-1.21	-0.22	0.61

*$p \leq 0.05$.

Table 6 Two-way ANOVA and honestly significant difference (HSD) using Tukey's multiple comparison test for metal uptake in *B. juncea* seedlings grown in binary combinations of Cr (VI) and other metals.

	Mn+Cr					**Cr+Mn**			
	Cr uptake					**Mn uptake**			
Source of variation	df	SS	MSS	F-ratio	HSD	SS	MSS	F-ratio	HSD
Treatment	3	0.020	0.007	11.128*	0.097	0.130	0.043	26.101*	0.159
Dose	2	0.001	0.000	0.553		0.019	0.010	5.759*	
Treatment x Dose	6	0.007	0.001	1.985		0.031	0.005	3.092*	
Error	12	0.007	0.001			0.020	0.002		
Total	23	0.035				0.201			
	Ni+Cr					**Cr+Ni**			
	Cr uptake					**Ni uptake**			
Treatment	3	0.021	0.007	3.516*	0.173	0.007	0.002	3.813*	0.097
Dose	2	0.013	0.007	3.338*		0.011	0.005	9.296*	
Treatment x Dose	6	0.006	0.001	0.537		0.000	0.000	0.092	
Error	12	0.024	0.002			0.007	0.001		
Total	23	0.064				0.024			
	Co+Cr					**Cr+Co**			
	Cr uptake					**Co uptake**			
Treatment	3	0.011	0.004	7.595*	0.089	0.037	0.012	19.500*	0.097
Dose	2	0.004	0.002	3.570*		0.002	0.001	1.848	
Treatment x Dose	6	0.006	0.001	1.915		0.012	0.002	3.069*	
Error	12	0.006	0.000			0.008	0.001		
Total	23	0.026				0.058			
	Cu+Cr					**Cr+Cu**			
	Cr uptake					**Cu uptake**			
Treatment	3	0.028	0.009	28.159*	0.069	0.030	0.010	24.290*	0.079
Dose	2	0.002	0.001	2.596		0.029	0.014	35.343*	
Treatment x Dose	6	0.006	0.001	2.877		0.007	0.001	2.781	
Error	12	0.004	0.000			0.005	0.000		
Total	23	0.039				0.070			
	Zn+Cr					**Cr+Zn**			
	Cr uptake					**Zn uptake**			
*Treatment	3	0.021	0.007	28.666*	0.056	0.167	0.056	30.502	0.168
Dose	2	0.001	0.001	2.927		0.068	0.034	18.614*	
Treatment x Dose	6	0.007	0.001	4.646*		0.048	0.008	4.359*	
Error	12	0.003	2.4E-04			0.022	0.002		
Total	23	0.032				0.304			

The first metal in the binary combination is the treatment, and the second the dose

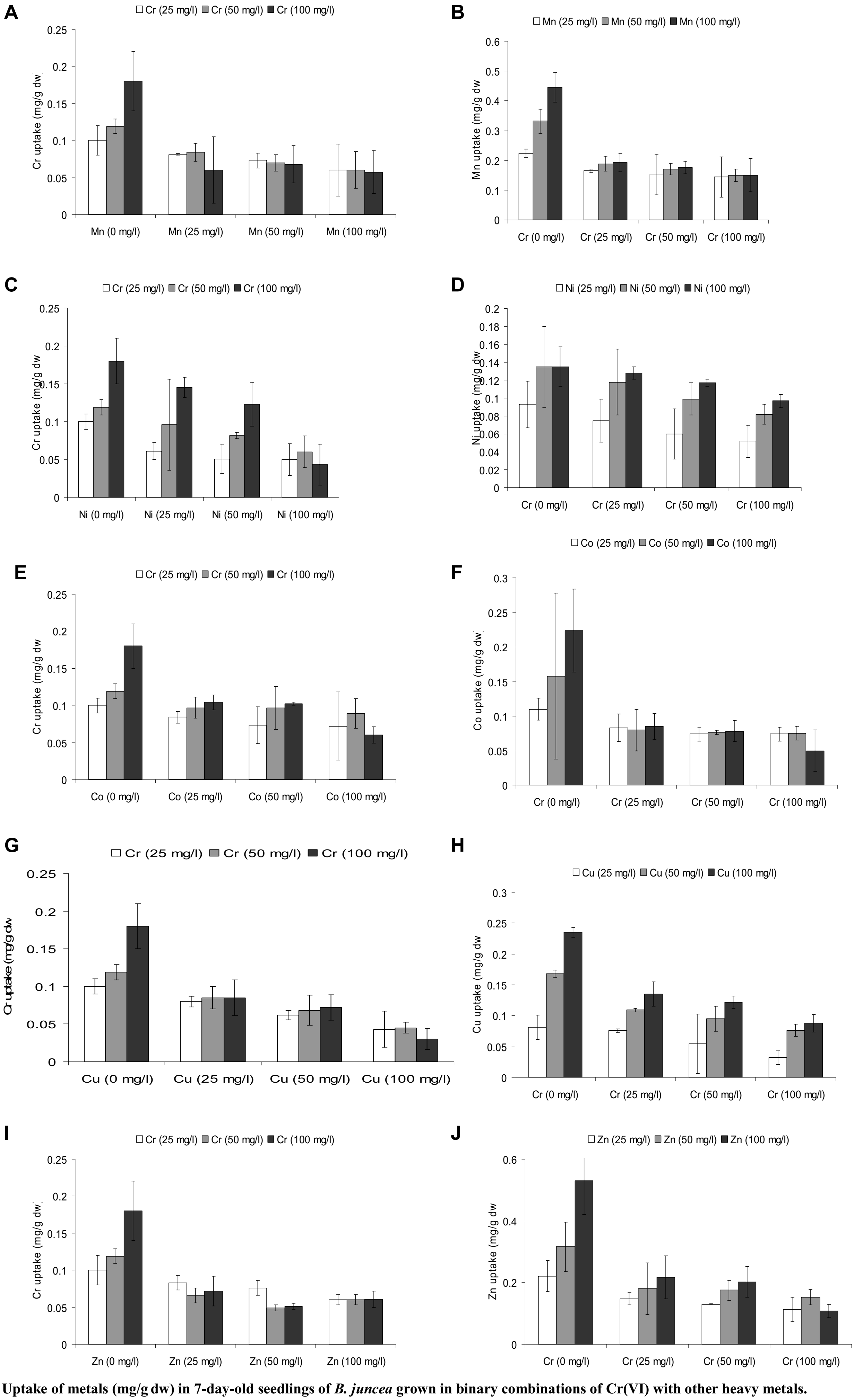

Fig. 5 Uptake of metals (mg/g dw) in 7-day-old seedlings of *B. juncea* grown in binary combinations of Cr(VI) with other heavy metals.

Table 7 Percentage change in uptake of different metals in *B. juncea* seedlings grown in binary combinations of Cr(VI) with other heavy metals with respect to the controls.

Metal conc (mg/l)	Cr+Mn			
	Cr uptake (mg/g dw)			
	Control	Mn (25 mg/l)	Mn (50 mg/l)	Mn (100 mg/l)
Cr 25	0	-19.0	-27.0	-40.0
Cr 50	0	-29.4	-41.2	-49.6
Cr 100	0	-66.7	-62.2	-68.3
	Mn uptake (mg/g dw)			
	Control	Cr (25 mg/l)	Cr (50 mg/l)	Cr (100 mg/l)
Mn 25	0	-26.3	-32.1	-35.7
Mn 50	0	-43.2	-48.6	-54.7
Mn 100	0	-56.9	-60.7	-66.3
	Cr+Ni			
	Cr uptake (mg/g dw)			
	Control	Ni (25 mg/l)	Ni (50 mg/l)	Ni (100 mg/l)
Cr 25	0	-39.0	-49.0	-50.0
Cr 50	0	-19.3	-31.1	-49.6
Cr 100	0	-19.4	-31.7	-76.1
	Ni uptake (mg/g dw)			
	Control	Cr (25 mg/l)	Cr (50 mg/l)	Cr (100 mg/l)
Ni 25	0	-19.4	-35.5	-44.1
Ni 50	0	-12.6	-26.7	-39.3
Ni 100	0	-5.2	-13.3	-28.1
	Cr+Co			
	Cr uptake (mg/g dw)			
	Control	Co (25 mg/l)	Co (50 mg/l)	Co (100 mg/l)
Cr 25	0	-16.0	-27.0	-28.0
Cr 50	0	-18.5	-18.5	-25.2
Cr 100	0	-42.2	-43.3	-66.7
	Co uptake (mg/g dw)			
	Control	Cr (25 mg/l)	Cr (50 mg/l)	Cr (100 mg/l)
Co 25	0	-24.5	-32.7	-32.7
Co 50	0	-49.4	-51.9	-52.5
Co 100	0	-62.1	-65.2	-77.7
	Cr+Cu			
	Cr uptake (mg/g dw)			
	Control	Cu (25 mg/l)	Cu (50 mg/l)	Cu (100 mg/l)
Cr 25	0	-20.0	-38.0	-57.0
Cr 50	0	-28.6	-42.9	-62.2
Cr 100	0	-52.8	-60.0	-83.3
	Cu uptake (mg/g dw)			
	Control	Cr (25 mg/l)	Cr (50 mg/l)	Cr (100 mg/l)
Cu 25	0	-6.2	-32.1	-60.5
Cu 50	0	-35.1	-43.5	-54.8
Cu 100	0	-42.6	-48.1	-62.6
	Cr+Zn			
	Cr uptake (mg/g dw)			
	Control	Zn (25 mg/l)	Zn (50 mg/l)	Zn (100 mg/l)
Cr 25	0	-17.0	-24.0	-40.0
Cr 50	0	-44.5	-58.8	-49.6
Cr 100	0	-60.0	-71.7	-66.1
	Zn uptake (mg/g dw)			
	Control	Cr (25 mg/l)	Cr (50 mg/l)	Cr (100 mg/l)
Zn 25	0	-33.5	-41.2	-48.9
Zn 50	0	-43.0	-44.6	-51.6
Zn 100	0	-59.1	-62.0	-79.7

DISCUSSION

The main aim of the present investigation was to find out the binary combinations of Cr(VI) with five other metals, viz. Mn, Ni, Co, Cu and Zn, which could be best suited for the growth of *B. juncea* in multielemental contaminated sites and enhance the metal accumulation potential of this plant. Since seed germination is the first physiological process affected by Cr and other metals, the ability of a seed to germinate in a medium containing Cr is indicative of its level of tolerance to this metal (Peralta *et al*. 2001). Since the transition from an inert quiescent seed to a vital metabolizing system is a very vulnerable phase which can seriously impair the functionality of any phytoremediator, it is important to study the influence of heavy metals on seeds.

A low concentration of Cr(VI) (25 mg/l) significantly reduced the germination percentage by 52% as compared to the control. Parr and Taylor (1982) reported that high levels of hexavalent Cr in the soil reduced the germination in bush beans up to 48%. Peralta *et al.* (2001) found that 40 mg/l of Cr(VI) reduced the ability of *Medicago sativa* seeds to germinate and grow in the contaminated medium by 23%. Zeid (2001) attributed the reduced germination of seeds under Cr stress either to the depressive effect of Cr on the activity of amylases, or its enhancing the activity of protease, and on the subsequent transport of sugars to the embryo axis. In the present study Zn and Mn counteracted the effects of Cr(VI), showing an improvement of germination by 17% in the case of (Cr25+Zn25) mg/l. All other metal ions, Cu, Co and Ni, in combination with Cr(VI), exerted a more negative influence on seed germination (**Fig. 1**). A decrease in root growth is also a well documented effect by toxic heavy metals. Root length is more affected by Cr(VI) than by any other heavy metal (Breckle 1991; Prasad *et al*. 2001; Tang *et al*. 2001). Chen *et al.* (2001) reported that total root length and weight were affected by 20 mg/l Cr(VI) kg^{-1} soil as $K_2Cr_2O_7$. The general response of decreased root growth due to Cr toxicity could be due to the inhibition of root cell division or the cell cycle. At a very high concentration of Cr(VI), reduction in root growth could be due to the direct contact of seedlings with Cr(VI) ions causing a collapse and subsequent inability of the roots to absorb water from the medium due to plasmolysis in root cells (Bassi *et al*. 1990; McGrath 1995). Liu *et al.* (2009) reported the storage of Cr in the cell wall of the roots of the *Leersia hexandra*. Also, the adverse effects of Cr on plant height and shoot growth have been reported by Rout *et al.* (1997). The reduction in shoot growth may be mainly due to reduced root growth and consequently less nutrients and water transport to the aerial parts of the plant, having a direct impact on cellular metabolism of shoots contributing to a reduction in seedling length. Our finding that the root and shoot growth of *B. juncea* seedlings decrease drastically with increasing concentrations of Cr(VI) are in accordance with Peralta *et al.* (2001) who reported a concentration dependant inhibition of root growth at 20 and 40 ppm of Cr(VI) in *Medicago sativa* plants grown on solid medium. Ghosh and Singh (2005) also demonstrated that the growth of *B. juncea* is highly affected with increase in chromium concentration in the soil. Shanker *et al.* (2005) reported that Cr stress can induce 3 possible types of metabolic modifications in plants (a) alteration in the production of pigments, such as chlorophyll and anthocyanin; (b) increased production of metabolities (glutathione, ascorbic acid); and (c) changes in metabolic production of new biochemically related metabolites that may induce resistance to Cr stress such as phytochelatins, metallothioenins and histidine. The investigators studied the mechanism of action of Cr in biological systems, and established that the Halliwell-Asada pathway is the key pathway, whereby Cr toxicity and tolerance is mediated. Cr-DNA interaction is one of the well established mechanisms of action of Cr in causing apotopsis and carcinogenesis. Elbekai and El-Kadi (2007) while studying as to how heavy metals alter the carcinogenicity of AhR ligands demonstrated that As(ii), Cd(II) and Cr(VI) increase Cyp1a1 m RNA levels in Hepa 1c1c7 cells at the transcriptional and post transcriptional levels. Cr(VI) mediates Fenton-like reactions and produces ROS, which are responsible for all the toxicity and genotoxicity caused by the metal. Goupil *et al.* (2009) studied the expression of stress related genes in tomato plants exposed to Cr and As and reported greater tolerance of tomato plants to As due to the induced production of stress proteins as compared to Cr. However Cr tolerance was also observed in various hyperaccumulator species. Sinha *et al.* (2008) reported increased level of antioxidants in *B. juncea* under Cr stress that leads to Cr tolerance. Shanker *et al.* (2004) suggested differential response to AA and H_2O_2 signaling by Cr(III) and Cr(VI) in

Table 8 Multiple regression interaction models for metal uptake in *B. juncea* seedlings grown in binary combinations of Cr (X_1, mg/l) and other metals (X_2, mg/l).

Treat-ments	Uptake of metals (mg/g dw) (Y)	r	β-regression coefficients		
	Multiple regression equation with interaction		β_1	β_2	Interaction (β_3)
Cr+Mn	$Y(Cr) = 0.081 + 5.3x10^{-4} X_1 - 1.6x10^{-4} X_2 - 8.2x10^{-6} X_1X_2$	0.7626*	0.485	-0.172	-0.676
	$Y(Mn) = 0.166 + 1.9x10^{-3} X_1 - 2.4x10^{-4} X_2 - 2.3x10^{-5} X_1X_2$	0.8018*	0.675	-0.103	-0.738
Cr+Ni	$Y(Cr) = 0.050 + 1.3x10^{-3} X_1 - 4.5x10^{-5} X_2 - 1.3x10^{-5} X_1X_2$	0.9765*	0.992	-0.040	-0.868
	$Y(Ni) = 0.085 + 5.7x10^{-4} X_1 - 4.7x10^{-4} X_2 - 5.2x10^{-7} X_1X_2$	0.9106*	0.659	-0.641	0.054
Cr+Co	$Y(Cr) = 0.071 + 8.7x10^{-4} X_1 + 1.4x10^{-4} X_2 - 1.1x10^{-5} X_1X_2$	0.8959*	0.924	0.175	-1.095
	$Y(Co) = 0.076 + 9.9x10^{-4} X_1 + 8.1x10^{-5} X_2 - 1.6x10^{-5} X_1X_2$	0.8011*	0.673	0.065	-0.951
Cr+Cu	$Y(Cr) = 0.076 + 7.4x10^{-4} X_1 - 2.4x10^{-4} X_2 - 1.1x10^{-5} X_1X_2$	0.9251*	0.602	-0.234	-0.785
	$Y(Cu) = 0.057 + 1.5x10^{-3} X_1 - 2.8x10^{-4} X_2 - 1.0x10^{-5} X_1X_2$	0.9173*	0.901	-0.200	-0.553
Cr+Zn	$Y(Cr) = 0.077 + 5.4x10^{-4} X_1 - 1.3x10^{-4} X_2 - 7.8x10^{-6} X_1X_2$	0.6888	0.479	-0.135	-0.623
	$Y(Zn) = 0.123 + 2.9x10^{-3} X_1 - 1.9x10^{-4} X_2 - 2.7x10^{-5} X_1X_2$	0.8224*	0.825	-0.066	-0.689

*$p \leq 0.05$.

Vigna radiata. Karuppanapandian *et al.* (2008) also reported Cr induced accumulation of peroxide content, stimulation of antioxidative enzymes and lipid peroxidation in *V. radiata*.

Since the main prerequisite for higher yield in phytoremediator plants is an increase in biomass production in terms of dry matter, toxicity to *B. juncea* by Cr is a major hurdle in its phytoremediation potential. Our observation confirms the reports of Han *et al.* (2004) that *B. juncea* is not a good candidate for phytoremediation of soils when Cr is the major pollutant. However, the results of the present investigation showed that supplementation of Zn or Mn to the growth medium helps in overcoming the toxic effects of Cr(VI) and enhance seedling biomass. Aravind and Prasad (2005) reported that antioxidative properties of Zn play an important role in counteracting Cd toxicity in *Ceratophyllum demersum*. Mn is also widely recognized as an antidote to elevated uptake of some heavy metals. Baszynski *et al*. (1980) reported the protective role of elevated Mn content in plants against Cd toxicity towards photosynthetic apparatus. Roy and Bera (2002) reported amelioration of mercury toxicity by Mn in case of mung bean seedlings as in combined solutions; mercury uptake was mostly prevented in the presence of 10 ppm of Mn. Moreover Mn is an important component of stress combating antioxidative enzyme, SOD which can ameliorate the toxic effects of heavy metals (Narang *et al*. 2008). Data regarding the uptake of different ions in the seedlings (applied singly or in combination) indicated that the uptake of one ion directly or indirectly influences the uptake of other ion from a binary mixture. All the metal ions demonstrated a concentration-dependent increase in the uptake potential. It is generally held that the uptake of nutrients by plants is a metabolically regulated process (Salisbury and Ross 1995). Much of the information required to understand the behavior of metal pollutants in plants can be extrapolated from the extensive database available for nutrient species. Although uptake mechanism can be shown to be quite specific for individual ions, competition with respect to absorption can be shown for a group of closely related anions or cations. It was reported that sulphite, thiosulphate, and chromate competitively retard sulphate uptake and behave as analogs of sulphate. Ghosh and Singh (2005) also showed that the dominant forms of Cr in contaminated soils are $Cr_2O_7^{-2}$ and CrO_4^- oxyanions that are actively transported to the cells by the sulphate transport system. It was also reported that Cr, due to its structural similarity with some essential elements, possibly affects mineral nutrition of plants in a complex way.

The interactions of Cr with uptake and accumulation of other nutrients have received maximum attention by researchers, as both the Cr species, Cr(III) and Cr(VI), interfere with the uptake of several other ionically similar elements like Fe and S. As Cr is highly toxic and nonessential element to the plants, plants lack a specific mechanism for its uptake. Therefore, the uptake of this metal occurs through carriers used for the uptake of essential metals for plant metabolism (Shanker *et al.* 2005). The pathway of Cr(VI) transport is active transport involving carriers of essential anions such as sulphate (Cerventes *et al.* 2001). Cr due to its structural similarity with some essential elements can affect mineral nutrition of plants in a complex way (Shanker *et al.* 2005). It is found that Cr(III) and Cr(VI) are taken up by different mechanisms (Zaccheo *et al.* 1985), and both the species can interfere with the uptake of various other ionically similar elements (Skeffington *et al.* 1976). Cr(VI) is reported to be actively taken up, in contrast to Cr(III) which is passively taken up and retained by cation exchangers (Shanker *et al.* 2004). Barceló *et al.* (1985) described the inhibition of P, Zn, Cu and Fe translocation within bean plants. It was further found that in soluble Mn fractions, critical effects on the uptake of Mn, Cu, Zn, Fe and Al were influenced by Cr (Ottabbong 1989). The results of the present investigation where Cr(VI) was applied in combination with Co, Ni, Cu, Zn and Mn also showed that the uptake of all these ions was inhibited by Cr(VI). However, Turner and Rust (1971) reported that in soybean plants, Cr decreased the concentrations of Ca, Mg, P, B and Cu but Fe, Mn and Zn remain unaffected. Our results where Zn and Mn uptake was diminished in the presence of Cr(VI) indicate that the ion interaction mechanism may be species-specific. Moreover, Cr(VI) uptake was also decreased in the presence of Zn, Mn, Ni, Co and Cu. The competitive interaction between Cr and Cu, where these inhibit the uptake of each other in *B. juncea* seedlings has been reported by Morel *et al*. (1995) in tomato seedlings. The present study finds support from Sharma and Pant (1994) in which Mn and Cu concentrations in maize plants decreased with increasing Cr levels in the medium.

The interaction of dissolved metals with cell membranes can affect the transport, chemistry, bioaccumulation, and relative toxicity of metals. The reactions of metal ions with various surface functional groups such as sulphydryl, amino, carboxyl hydroxide, oxide, *etc*. are numerous and difficult to quantify individually, thereby complicating the development of a general relationship between the aqueous chemistry of metals, their interaction among themselves and subsequently their toxicological properties. It is therefore concluded from the study that *B. juncea* is very sensitive to variations in metal concentrations in binary mixtures, but is capable of high metal enrichment. At 100 mg/l of Zn and Mn, uptake was found to be 0.531 and 0.445 mg/g dw, respectively in the seedlings The proposed models can be used to predict the interactive effects of metal ions in the metal accumulation process. The present study specifically highlights the role of Zn and Mn in ameliorating the toxicity of Cr(VI) in *B. juncea* seedlings. By the addition of even low concentration of Mn (25 mg/l) and Zn (25 mg/l) to Cr(VI) (100 mg/l), uptake of Cr was reduced by 66 and 60%, respectively. Multiple regression interaction models showed that the interactive effects of binary combinations of Cr+Zn and Cr+Mn are positive on all the growth parameters studied, by mutually decreasing the toxicity of each other, thereby implying antagonistic interaction of these metal ions with Cr(VI). Zn, Mn, Ni and Cu inhibit the uptake of Cr.

ACKNOWLEDGEMENTS

Thanks are due to the Head of Department of Botanical & Environmental Sciences, Guru Nanak Dev University, Amritsar for providing research facilities.

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