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GROWTH AND HEAVY METAL UPTAKE IN *B. JUNCEA* L. SEEDLINGS AS AFFECTED BY BINARY INTERACTIONS BETWEEN NICKEL AND OTHER HEAVY METALS

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ABSTRACT

Interactive effects of Ni in binary combinations with other heavy metals (Mn, Co, Cu, Cr and Zn) were investigated on the growth of *B. juncea* L. seedlings. There was a decline in germination percentage, root and shoot lengths and dry weight of the seedlings with increase in concentrations of the metals in the growth medium. Multiple regression interaction models revealed that in all the binary combinations of Ni with other heavy metals, both the metals were detrimental to the seedling growth. However, the metals in combinations mutually decreased the toxicity of each other. Zn acted antagonistic to Ni and increased the germination percentage of *B. juncea* seeds. Zn and Mn were accumulated to the extent of 0.531 and 0.445 mg g^{-1} dw respectively at 100mg l^{-1} concentration of these metals, whereas the lowest uptake was observed for Ni (0.135 mg g^{-1} dw) at a concentration of 100 mg l^{-1}. In binary combinations, (Ni+Cr), (Ni+Mn), (Ni+Co), (Ni+Cu) and (Ni+Zn), both the metals mutually inhibited the uptake of each other.

Keywords: Heavy metal interactions, synergism, antagonism, Mn, Co, Cu, Cr and Zn.

INTRODUCTION

Industrial wastes impregnated with heavy metals, besides being detrimental to the health of man and animals, extensively damage the living and natural resources of the environment. Heavy metals like Cd, Cu, Pb, Cr, Ni, Hg *etc*. pose a major occupational and environmental hazard, as they are non-biodegradable and have a long biological half life (Barbier *et al.,* 2005). Global awareness of underlying detriments of heavy metals in the environment has gained considerable public attention and brought monumental changes in societies to curtail environmental pollution through pollution control technologies. Phytoremediation is an innovative green clean technology involving the use of plants for pollution abatement. Researchers have been successfully delineating the molecular mechanism of metal uptake and the physiology of metal tolerance by hyperaccumulator species used in phytoremediation (Kochian, 2000). It is well known that in natural environment, contamination by a single pollutant rarely occurs. Metal smelting, mining, manufacturing processes and industrial wastes more often result in contamination of environment with a mixture of toxic metals. Recently, it is more widely realized that examining the effects of heavy metals in various combinations is more representative than single metal studies (Krupa *et al*., 2002). Earlier studies have revealed that the combined metal toxicity of pollutants present in multiple contaminated sites, directly or indirectly affects the phytoremediation potential of hyperaccumulators. It was reported that during the uptake of heavy metals in the plants, there are various positive or negative interactions occurring among different metal ions (Martin-Prevel, 1987), which influence the rate of uptake, transfer, accumulation and their subsequent translocation in the plant body. Therefore, thorough understanding of metal interactions is necessary to streamline the technique of phytoremediation to be used successfully for the remediation of soils impregnated with a variety of pollutants at varying concentrations.

B. juncea is considered as a model system to investigate the biochemistry and physiology of hyperaccumulation of various metal ions (Ebbs and Kochian, 1998). Dushenkov *et al.* (1997) reported *B. juncea* as being particularly effective in sorbing divalent cations of toxic metals from soil solution. Since interactions between two or more metal ions have consistently been shown to result in their altered behavior and mode of action inside the plant body, the mechanism of metal interactions needs to be fully explored for improving the practical effectiveness of phytoremediation according to the current changes in the contaminated environment. Some heavy metals like Zn, Mn, Ni, Co and Cu are essential in small quantities for the metabolic activities of the organisms, but these prove to be toxic beyond certain limits. Ni is considered as an essential micronutrient for plants, but is strongly phytotoxic at higher concentrations (Boominathan and Doran, 2002). Ni induced deactivation of proteins involved in antioxidative enzymes and membrane function has been reported in numerous plants. The present investigation was therefore undertaken to study

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the interactive effects of Ni in binary combinations with Cr, Mn, Co, Cu and Zn on the growth and metal uptake in *B. juncea* seedlings.

MATERIALS AND METHODS

Certified seeds of *B. juncea* L. *cv* PBR 91 were procured from Punjab Agricultural University, Ludhiana, India. 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 plates containing different concentrations of heavy metals, single or in binary combinations of Ni.

i) Single metal treatments –0, 25, 50 and 100mg l^{-1} of each metal (Cr, Mn, Ni, Co, Cu and Zn).
ii) Binary treatments – Ni treatments in combination with other metals at 0, 25, 50 and 100mg l^{-1}.

The surface sterilized seeds were germinated on Whatman filter paper No. 1, lined inside (9cm diameter) sterilized Petri plates containing 5 ml of aqueous solutions of heavy metals. The 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 $ZnSO_4.7H_2O$ procured from Sigma aldrich, Qualigens and Loba chemie. Sterilized seeds grown in double distilled water served as the controls. Petri plates in triplicates, each containing 50 seeds were kept at 25± 0.5 °C temperature and 16 h/8 h dark and light photo period (1700 Lux), for 7 days of the growth period. The rate of germination was recorded daily for 7 days and root and shoot lengths were measured. Thereafter, the harvested seedlings were washed thoroughly with double distilled water and kept in oven for 48 h at 80ºC, 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 Cr, Mn, Ni, Co, Cu and Zn were determined using atomic absorption spectrophotometer (Model 6200, Shimadzu, Japan). All the analyses were carried out in triplicate, and the data was analyzed for descriptive statistics, standard error, ANOVA, Tukey's multiple comparison test, multiple regression and correlation, and β-regression coefficients (Sokal and Rholf, 1981; Bailey, 1995). Self coded software developed in MS-Excel was used. The multiple regression 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. β_1 and β_2 are the unitless β-regression coefficients due to X_1 and X_2. Metal interaction was interpreted as described in table 1.

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

Table 2. 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^{-1})
Cr	0.524
Mn	73.739
Ni	28.881
Co	47.803
Cu	0.563
Zn	77.882

RESULTS AND DISSCUSSION

Individual and combined effects of Ni with Zn, Mn, Co, Cu and Cr on the germination parameters of *B. juncea* are presented in figure 1 and tables 2-5. It was observed that there was a reduction in germination percentage with increase in metal concentration in the medium. Maximum reduction was observed in case of Cr, followed by Ni. With increase in Ni concentration in the medium from 25 to 100 mg g^{-1}, germination decreased to 65%, compared to the control (94.6%). Multiple regression equations for combinations, Ni+Zn, Ni+Mn, Ni+Co, Ni+Cu and Ni+Cr showed that both the metals in binary combinations exerted negative influence on the percentage germination as indicated by their negative β-regression coefficients. However, better correlations were obtained when an interaction model was used (Table 4). Additions of Cr, Mn, Co, Cu and Zn even at low concentration of 25 mg l^{-1} further declined the germination percentage with respect to the control. Maximum decline in germination (41%) was caused at (Ni25+Cr25) mg l^{-1} followed by (Ni25+Cu25) mg l^{-1} The interactive effects of all the binary combinations of Ni were observed to be negative except for Ni+Zn, thereby implying that Mn, Co, Cu and Cr are synergistic to Ni in further retarding the percentage germination of *Brassica* seeds. 2-way ANOVA for germination percentage of *B. juncea* seeds for Ni and

Table 3. Percentage change in root length of *B. juncea* seedlings grown in binary combinations of Ni with other heavy metals, with respect to Ni controls.

Metal conc.	Ni in solution (mg l^{-1})			
(mg l^{-1})	0	25	50	100
	% Change with respect to control			
Control 0	0	0	0	0
Cr	Ni + Cr			
25	-86.9	-62.5	-56.9	-3.9
50	-91.3	-78.2	-70.3	-44.7
100	-98.6	-93.8	-95.0	-80.3
Mn	Ni + Mn			
25	-18.787	42.773	29.707	182.895
50	-26.541	73.156	67.782	305.263
100	-61.133	12.389	16.736	201.316
Co	Ni + Co			
25	-39.463	-4.720	27.197	165.789
50	-58.549	-15.339	0.418	153.947
100	-77.137	-39.823	-28.033	135.526
Cu	Ni + Cu			
25	-83.8	-29.5	-29.5	123.7
50	-93.8	-44.5	-28.3	63.2
100	-97.7	-56.6	-54.9	25.0
Zn	Ni + Zn			
25	-12.1	85.5	143.9	509.2
50	-26.8	84.1	135.9	581.6
100	-58.2	20.1	47.7	255.3

Table 4. Multiple regression with interaction for different parameters of *B. juncea* grown in binary combinations of Ni (X_1, mg l^{-1}) and other metals (X_2 mg l^{-1}).

Treatments	Mutiple regression with interaction								r	β regression coefficients		
	Germination percentage (Y)									(β_1)	(β_2)	Interaction (β_3)
Ni+Cr	Y=	80.7	-0.25	X_1-	0.46	X_2-	$1.7x10^{-4}$	X_1X_2	0.8780*	-0.42	-0.75	-0.02
Ni+Mn	Y=	91.26	-0.21	X_1-	0.12	X_2-	$6.8x10^{-4}$	X_1X_2	0.8956*	-0.67	-0.37	-0.15
Ni+Co	Y=	87.78	-0.32	X_1-	0.29	X_2-	$1.5x10^{-4}$	X_1X_2	0.8740*	-0.64	-0.57	-0.02
Ni+Cu	Y=	86.50	-0.31	X_1-	0.14	X_2-	$1.6x10^{-3}$	X_1X_2	0.8951*	-0.64	-0.28	-0.23
Ni+Zn	Y=	93.50	-0.31	X_1-	0.12	X_2+	$2.5x10^{-4}$	X_1X_2	0.9073*	-0.89	-0.33	0.05
	Root length (cm)(Y)											
Ni+Cr	Y=	5.32	-0.05	X_1-	0.06	X_2+	$6.7x10^{-4}$	X_1X_2	0.7573*	-0.82	-1.00	0.75
Ni+Mn	Y=	8.04	-0.08	X_1-	0.04	X_2+	$6.2x10^{-4}$	X_1X_2	0.8570*	-1.18	-0.57	0.69
Ni+Co	Y=	7	-0.07	X_1-	0.05	X_2+	$7x10^{-4}$	X_1X_2	0.8529*	-1.15	-0.81	0.87
Ni+Cu	Y=	5.49	-0.05	X_1-	0.06	X_2+	$7x10^{-4}$	X_1X_2	0.7245*	-0.84	-1.00	0.85
Ni+Zn	Y=	8.35	-0.06	X_1-	0.04	X_2+	$5.9x10^{-4}$	X_1X_2	0.7309*	-1.03	-0.58	0.68
	Shoot length (cm)(Y)											
Ni+Cr	Y=	2.64	$-1.4x10^{-2}$	X_1	$-1.8x10^{-2}$	X_2+	$1.1x10^{-4}$	X_1X_2	0.9240*	-0.78	-1.01	0.45
Ni+Mn	Y=	2.7	-8.8	X_1	$-6x10^{-3}$	X_2+	$1.9x10^{-4}$	X_1X_2	0.8010*	-0.75	-0.49	0.12
Ni+Co	Y=	2.7	-0.01	X_1	$-1.4x10^{-2}$	X_2+	$1.2x10^{-4}$	X_1X_2	0.8757*	-0.83	-1.06	0.63
Ni+Cu	Y=	2.7	-0.01	X_1	$-1.7x10^{-2}$	X_2+	$1.3x10^{-4}$	X_1X_2	0.8789*	-0.75	-1.06	0.56
Ni+Zn	Y=	2.85	-0.01	X_1	$-5.2x10^{-3}$	X_2+	$9.9x10^{-5}$	X_1X_2	0.7891*	-1.14	-0.56	0.77
	Dry weight (mg/seedling)(Y)											
Ni+Cr	Y=	6.48	-0.02	X_1	$-2.3x10^{-2}$	X_2-	$1.2x10^{-5}$	X_1X_2	0.9180*	-0.54	-0.72	-0.03
Ni+Mn	Y=	7.52	-0.02	X_1	$-1.1x10^{-2}$	X_2+	$8.4x10^{-5}$	X_1X_2	0.7178*	-0.8	-0.4	0.22
Ni+Co	Y=	6.86	-0.02	X_1	$-1.7x10^{-2}$	X_2+	$1.2x10^{-4}$	X_1X_2	0.8963*	-0.89	-0.85	0.42
Ni+Cu	Y=	6.6	-0.02	X_1	$-1.3x10^{-2}$	X_2+	$9.9x10^{-5}$	X_1X_2	0.8139*	-0.85	-0.75	0.41
Ni+Zn	Y=	7.88	-0.02	X_1	$-9x10^{-3}$	X_2+	$4.4x10^{-5}$	X_1X_2	0.7463*	-0.78	-0.27	0.1

*$p \leq 0.05$

other metals in binary combinations (Table 5) shows that there are statistically significant differences among mean germination percentage values on treatment with both the metals. The interaction between Ni and Mn was also found to be significant.

Table 5. ANOVA for different parameters of *B. juncea* seedlings grown in binary combinations of Ni and other metals.

		Germination percentage		Root lengths		Shoot lengths		Dry weights	
	Cr+Ni								
Source of variation	df	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD
Treatment	3	108.2*		1814.1*		229.2*		39.8*	
Dose	3	30.8*	24.93	700.4*	0.48	103.2*	0.37	14.5*	1.48
Treatment x Dose	9	1.3		555.7*		13.5*		7.01*	
	Ni+Mn								
Source of variation	df	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD
Treatment	3	31.7*		1258.9*		87.2*		16.6*	
Dose	3	11.5*	21.47	150.5*	0.68	32.5*	0.4	16.4*	1.51
Treatment x Dose	9	2.4*		131.8*		11.5*		10.9*	
	Co+Ni								
Source of variation	df	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD
Treatment	3	49.8*		368.0*		97.01*		8.55*	
Dose	3	57.0*	22.92	1248.5*	0.48	51.22*	0.4	12.27*	1.12
Treatment x Dose	9	1.7		295.1*		13.93*		7.15*	
	Cu+Ni								
Source of variation	df	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD
Treatment	3	18.5*		786.6*	0.48	131.98*		16.21*	
Dose	3	36.7*	30.5	273.1*		69.86*	0.4	12.97*	2.21
Treatment x Dose	9	1.1		465.7*		16.82*		6.64*	
	Ni+Zn								
Source of variation	df	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD	F-ratio	HSD
Treatment	3	56.2*		759.6*		90.15*		20.91*	
Dose	3	13.7*	19.9	416.0*	0.68	22.44*	0.31	20.65*	2.06
Treatment x Dose	9	1.1		146.0*		19.43*		6.59*	

*$p \leq 0.05$

Table 6. Multiple regression equations for uptake of metals in *B. juncea* grown in binary combinations of Ni (X_1, mg l^{-1}) and other metals (X_2 mg l^{-1}).

Treatments (mg l^{-1})	Uptake of metals (mg g^{-1} dw)(Y)								r	β regression coefficients	
	Multiple regression equations									β_1	β_2
Cr+Ni	Y (Cr) =	0.083	+	7.5×10^{-4}	X_1	-	7.9×10^{-4}	X_2	0.9088*	0.569	-0.708
	Y (Ni) =	0.083	+	6.1×10^{-4}	X_1	-	4.4×10^{-4}	X_2	0.9139*	0.698	-0.590
Mn+Ni	Y (Mn) =	0.157	+	3.2×10^{-3}	X_1	-	1.4×10^{-3}	X_2	0.9298*	0.824	-0.431
	Y (Ni) =	0.100	+	4.4×10^{-5}	X_1	-	2.9×10^{-4}	X_2	0.5113	0.065	-0.507
Ni+Co	Y (Ni) =	0.099	+	2.8×10^{-4}	X_1	-	3.1×10^{-4}	X_2	0.6467*	0.391	-0.516
	Y (Co =	0.135	+	8.5×10^{-4}	X_1	-	9.8×10^{-4}	X_2	0.7524*	0.443	-0.608
Ni+Cu	Y (Ni) =	0.083	+	3.9×10^{-4}	X_1	-	3.9×10^{-4}	X_2	0.6933*	0.446	-0.531
	Y (Cu) =	0.102	+	5.3×10^{-4}	X_1	-	6.8×10^{-4}	X_2	0.5470	0.302	-0.456
Ni+Zn	Y (Ni) =	0.102	+	-1.6×10^{-5}	X_1	-	2.6×10^{-4}	X_2	0.4021	-0.021	-0.402
	Y (Zn) =	0.218	+	1.9×10^{-3}	X_1	-	1.8×10^{-3}	X_2	0.8133*	0.535	-0.612

*$p \leq 0.05$

The data corresponding to root and shoot growth of the seedling versus the treatment of heavy metals is presented in figures 2-3. It was found that the inhibitory effects of metals on the growth of the seedlings were more pronounced at the higher concentration, thereby demonstrating a dose dependent inhibition of the shoot and root growth. The IC_{50} values calculated on the basis of root length inhibition were given in table 2. Cr (VI) was found to be most toxic metal as the lowest observed value of root length is 0.14 cm at the concentration of Cr

Table 7. ANOVA for metal uptake (mg g^{-1} dw) by the seedlings of *B. juncea* grown in water cultures containing different binary combinations of Ni with other metals.

		Cr+Ni		Ni+Cr	
		Ni uptake (mg g^{-1} dw)		Cr uptake (mg g^{-1} dw)	
Source of variation	df	F-ratio	HSD	F-ratio	HSD
Treatment	3	3.9*	0.097	3.5*	0.177
Dose	2	9.3*		3.3	
Treatment x Dose	6	0.09		0.5	
		Mn+Ni		Ni+Mn	
		Ni uptake (mg g^{-1} dw)		Mn uptake (mg g^{-1} dw)	
Source of variation	df	F-ratio	HSD	F-ratio	HSD
Treatment	3	6.2*	0.079	6.1*	0.25
Dose	2	0.2		34.5*	
Treatment x Dose	6	1.1		1.4	
		Co+Ni		Ni+Co	
		Ni uptake (mg g^{-1} dw)		Co uptake (mg g^{-1} dw)	
Source of variation	df	F-ratio	HSD	F-ratio	HSD
Treatment	3	2.7	0.008	21.5*	0.105
Dose	2	3		11.8*	
Treatment x Dose	6	1.8		5.2*	
		Cu+Ni		Ni+Cu	
		Ni uptake (mg g^{-1} dw)		Cu uptake (mg g^{-1} dw)	
Source of variation	df	F-ratio	HSD	F-ratio	HSD
Treatment	3	6.1*	0.079	66.1*	0.056
Dose	2	2.7		15.6*	
Treatment x Dose	6	1.6		14.5*	
		Zn+Ni		Ni+Zn	
		Ni uptake (mg g^{-1} dw)		Zn uptake (mg g^{-1} dw)	
Source of variation	df	F-ratio	HSD	F-ratio	HSD
Treatment	3	5.6*	0.097	13.7*	0.217
Dose	2	0.03		26.4*	
Treatment x Dose	6	1.4		1.9	

*$p \leq 0.05$

100 mg l^{-1}. The IC_{50} value for Ni was found to be 28.88 mg l^{-1}. The effects of heavy metals on root growth were more pronounced as compared to shoot growth. All the binary combinations of Ni had inhibitory effects on the seedling growth at all the tested concentrations. Percentage change in root lengths of *B. juncea* seedlings in binary combinations of Ni with other metals is given in table 3. Maximum inhibitory effect was caused by Cr followed by Cu. Even at low concentration of these metals in the medium, there was a decline in root length by 62% in case of (Ni25+Cr25) and by 29% in case of (Ni25+Cu25) mg l^{-1}as compared to the control. Similarly shoot length was also adversely affected by all the binary combinations with respect to the controls. The shoot lengths of the seedlings were most affected by (Ni25+Cr25) and (Ni25+Co25) which decreased the shoot length by 52% and 31% respectively. The multiple regression interaction models (Table 4) for both root and shoot growth of the seedlings showed that, although all the metals exerted negative influence on the seedling growth, the interactive effects of Ni in combination with Cr, Mn, Co, Cu, and Zn are antagonistic. 2-way ANOVA for root and shoot growth of *B. juncea* seedlings for Ni and other metals in binary combinations (Table 5) showed that there are statistically significant differences among mean root and shoot lengths on treatment with both the metals. The interactions between Ni and the other metal in all binary treatments were also found to be significant.

The effects of heavy metals on the seedling biomass varied with different concentrations, applied individually or in combination with Ni (Fig. 4). Since all the metals induced negative effects on the seedling growth, corresponding dry weight was also greatly reduced. Both Ni and Cr at a concentration of 100 mg l^{-1} caused maximum reduction by 66% as compared to the control. However, in the presence of Zn and Mn, there was a slight increase in the biomass, by 17% at (Ni25+Zn50) and by 6% at (Ni25+Mn50) concentrations as compared to the control (Ni 25 mg l^{-1}). However, at (Ni25+Cu25)

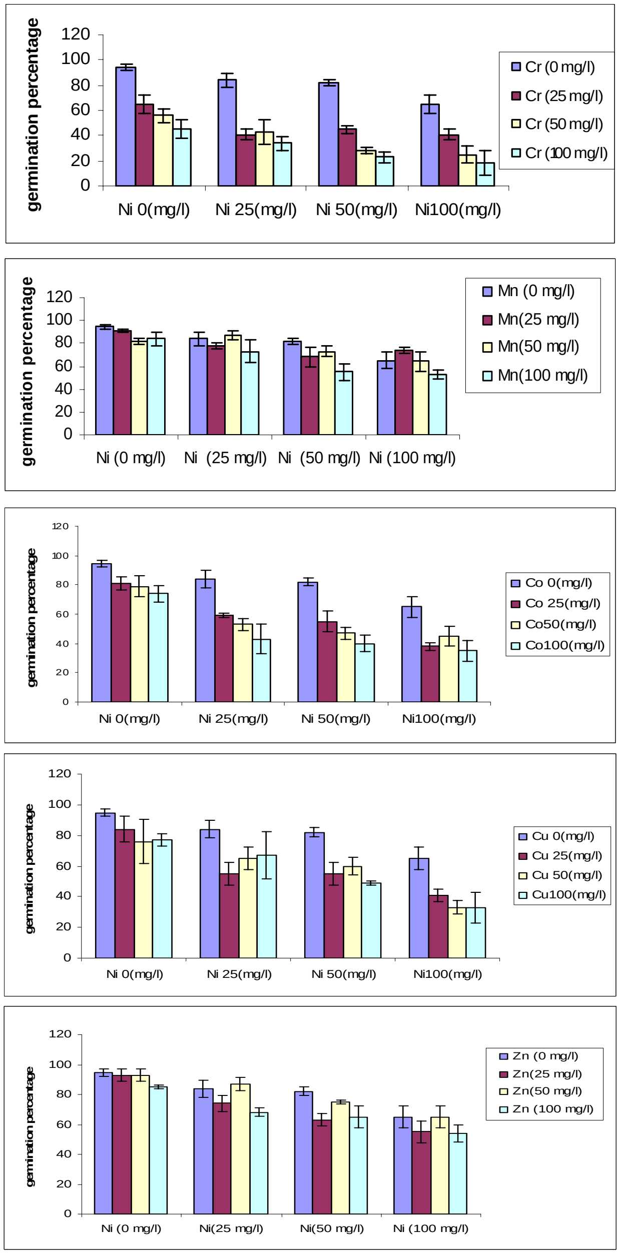

Fig.1. Germination percentage (mean±SD) of *B. juncea* grown in binary combinations of Ni with other heavy metals.

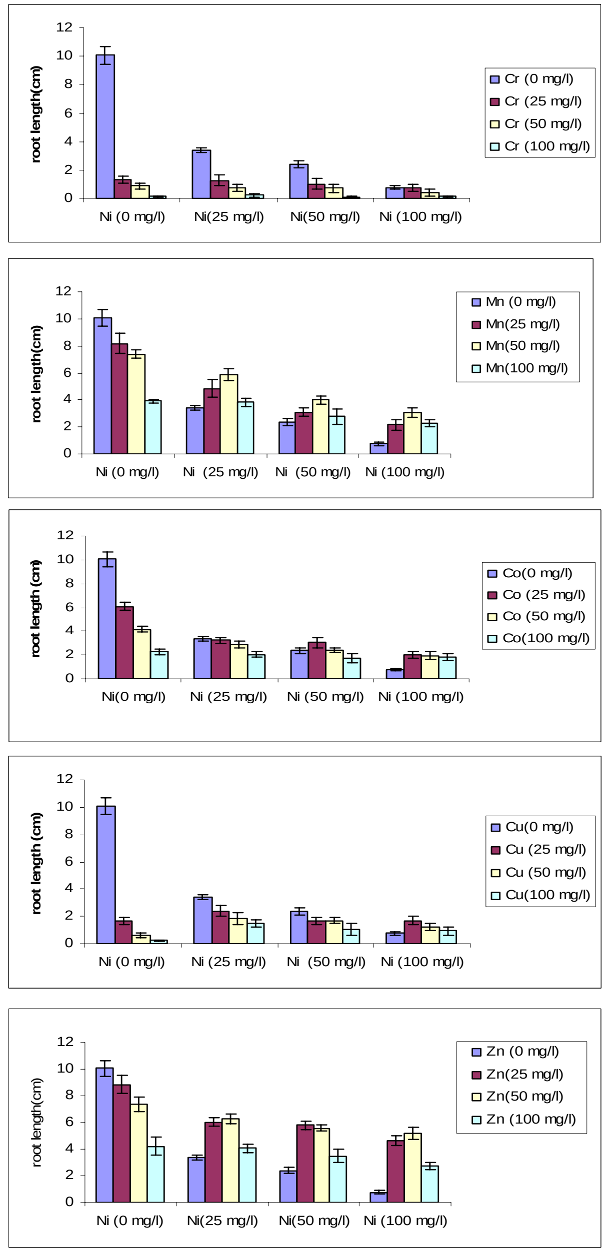

Fig. 2. Root length (cm) (mean±SD) of *B. juncea* seedlings grown in binary combinations of Ni with other heavy metals.

and (Ni25+Co25), the dry weight of the seedlings was further reduced by 6% and 5% respectively. The multiple regression interaction model (Table 4) for the dry weight of the seedlings depicted that in binary combinations, Ni along with the other metal ions exerted negative influence on the dry weight as represented by their negative β-regression coefficients. However, the interactive effect of Ni with Mn, Co, Cu and Zn, was observed to be positive, showing mutual decrease in the toxicities caused by the antagonistic interaction of the metal ions in binary combinations. 2-way ANOVA for dry weight of *B. juncea* seedlings for Ni and other metals in binary combinations (Table 5) showed that there are statistically significant differences among mean dry weight values on treatment with both the metals in all binary combinations. The interaction of Ni with Cu, Cr, Co and Zn was also found to be significant.

Figure 5 gives the uptake potential of all the six metals in the *B. juncea* seedlings, applied singly or in binary combinations of Ni with other heavy metals at various concentrations. The results fairly indicated that the uptake

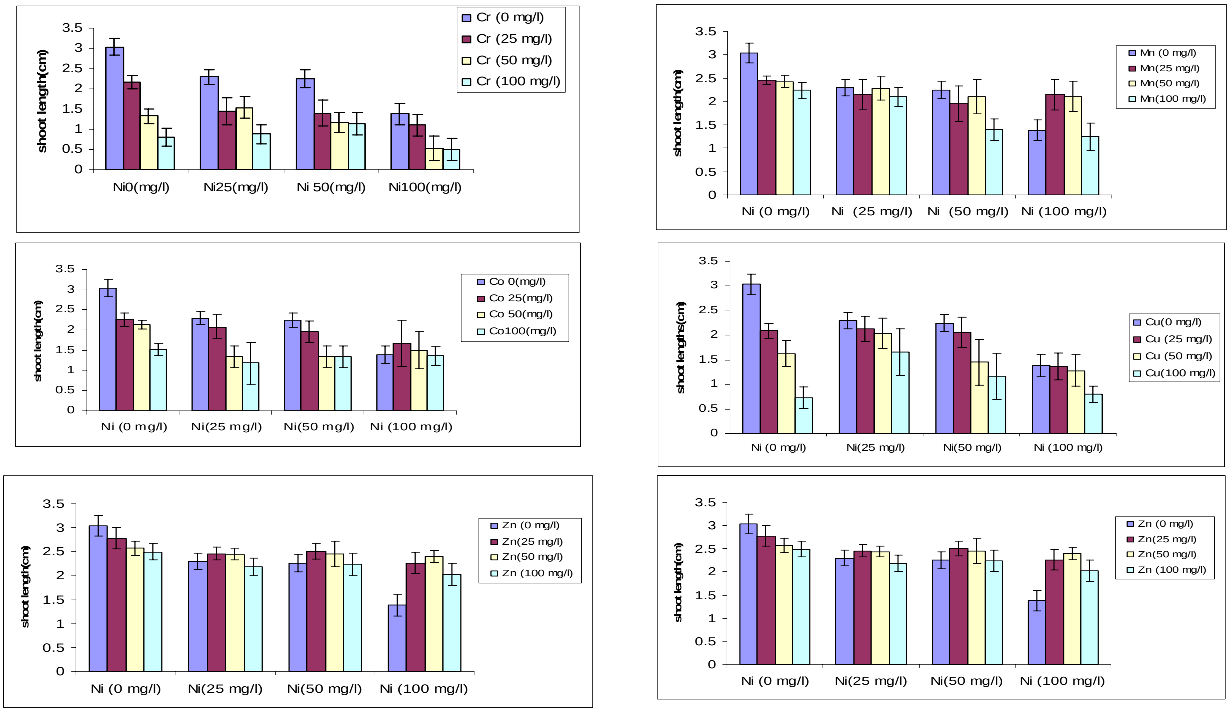

Fig. 3. Shoot length (cm) (mean±SD) of *B. juncea* seedlings grown in binary combinations of Ni with other heavy metals.

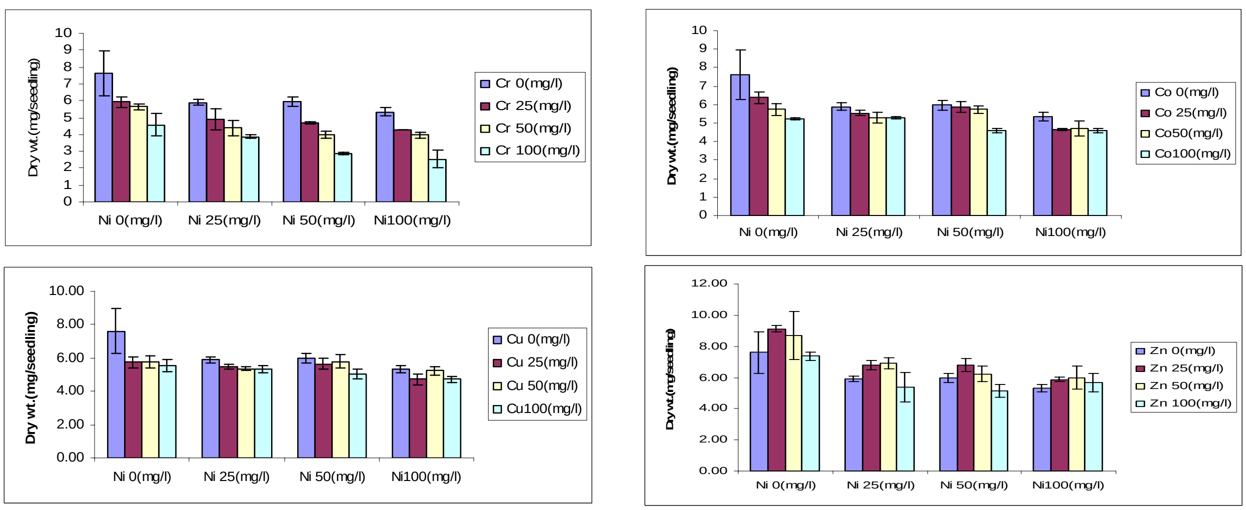

Fig. 4. Dry weight (mg/seedling) (mean±) of *B. juncea* seedlings grown in binary combinations of Ni with other heavy metals.

potential of each metal was directly proportional to its concentration in the medium. As the concentration of the metal ion increases from 25 mg l^{-1} to 100 mg l^{-1}, there is a corresponding increase in the uptake of metal ions, thereby showing dose dependent linear relation of metal uptake in the seedlings. The data obtained revealed that the *B. juncea* seedlings showed maximum uptake of Zn (0.531 mg g^{-1}dw) followed by Mn (0.446 mg g^{-1}dw), and the lowest uptake by Ni (0.135 mg g^{-1}dw) at the highest applied treatment of 100 mg l^{-1} to the seedlings. As given in table 6, uptake of each ion was not only affected by the concentration of the element in the medium but also by the presence of other elements. In binary combinations of Ni with Cr, Mn, Co, Cu and Zn both the metal ion mutually inhibited the uptake of each other. 2-way ANOVA for the uptake of Ni and other metals in binary combinations (Table 7) showed that there are statistically significant differences among mean uptake values on treatment with both the metals in all binary combinations.

Therefore, the results of the present study elucidate various positive and negative interactions among metal ions in binary combination of Ni. Inhibitory effects of Ni in plant growth and development has been reported by

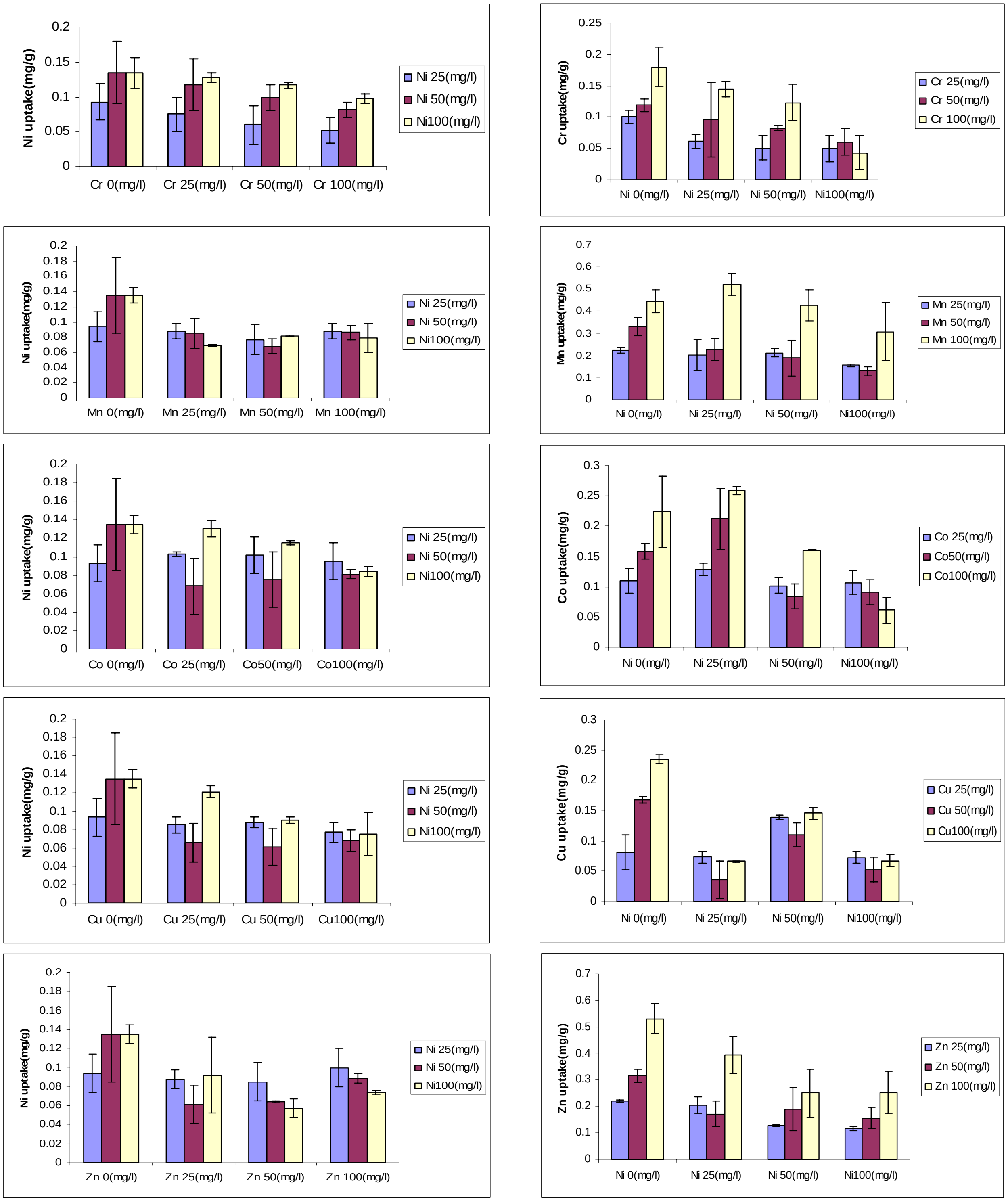

Fig. 4. Metal uptake (mean±) by *B. juncea* seedlings grown in binary combinations of Ni with other heavy metals.

many researchers (Gajweska *et al.*, 2008). Ni is not considered to be an essential element in plant nutrition, yet its uptake behavior is characteristic of nutrients, suggesting thereby that Ni^{2+} may be acting as an analog of an essential species for which effective transport mechanism are operating. Korner *et al*. (2008) studied free space uptake and influx of Ni^{2+} in excised barley roots. Ni inhibits the uptake of Mn, Cr, and Cu probably due to the complexes or allosteric interactions with the carrier complex or proteins which could affect their uptake. It was further observed that Zn and Cu increased Ni uptake which is not consistent with the results of Catalado *et al.* (1978), who showed Cu and Zn are competitive inhibitors with respect to Ni^{2+} uptake. Generally the interaction of metal ions with biological surfaces such as cell membranes, affects the transport, chemistry, bioaccumulation and toxicity of metals. Different surface functional groups such as carbonyl, sulphydryl, hydroxide, oxides and amines act as sites of interactions among metals ions (Dirilgen, 2001). Also the various reactions occurring between surface groups and metal ions are numerous, complicating the aqueous chemistry of metals, their interactions and toxicological properties. The proposed model can be used to investigate critically the phytotoxicity and interactive aspects of metal mixtures to the plant. It was observed that Cr, Mn, Ni, Co Cu and Zn, when applied individually, are toxic to growth of *B. juncea* seedlings beyond the threshold values but in binary combinations, Ni and other studied heavy metals are antagonistic to each other for their effects on the growth of seedlings. The uptake of Ni was inhibited in combinations, (Ni+Mn), (Ni+Co), (Ni+Cu) and (Ni+Zn) due to competitive inhibition of metals. The present study therefore, highlights the importance of understanding the basic mechanism involved in uptake and accumulation of metal ions in a phytoremediator undergoing multiple metal stress which is essential for genetic engineering approaches aimed at improving the cellular defence mechanism, and hence the efficiency of a phytoremediator.

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