Indian J. Plant Physiol., Vol. 14, No. 4, (N.S.) pp. 344-352 (Oct.-Dec., 2009)

SCREENING OF *BRASSICA* SPECIES FOR HYPER-ACCUMULATION OF ZINC, COPPER, LEAD, NICKEL AND CADMIUM

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Received on 15 June, 2009, Revised on 28 Dec., 2009

SUMMARY

In a sand culture experiment five different species of *Brassica*, (1) *B. juncea* (Indian mustard) cv. Pusa Bold, (2) *B. campestris* (Yellow mustard) cv. Pusa Gold, (3) *B. carinata* (Ethiopian mustard) cv. DLSC-1, (4) *B. napus* cv. Early napus, (5) *B. nigra* cv. IC-247 were grown in a phytotron for screening possible hyper-accumulator of heavy metals, viz. Zn, Cu, Pb, Ni and Cd. The plants were grown with Hoagland solution (control) and Hoagland solution enriched with medium and high levels of heavy metals up to maximum vegetative stage. Results showed that all the heavy metal contents in stalks of *Brassica* species increased due to enrichment of Hoagland solution with heavy metals. The plant growth was affected at higher level of metal enrichment. Among the species, *B. napus* contained highest amount of Cu, Pb, Ni and Cd in stalks, while *B. carinata* alone and *B. carinata* jointly with *B. juncea* showed highest Zn contents in medium and high metal levels, respectively. This was reflected in highest Zn uptake by *B. carinata* followed by *B. napus*. In case of Cu the trend interchanged among these two species. For Pb, Ni and Cd, *B. napus* accumulated highest amount outperforming all the other species. The most novel aspect of current study remained in the fact that probably for the first time *B. napus* and *B. carinata* reported as promising accumulator of Zn, Cu, Ni, Pb and Cd; which performed better than otherwise most reported *B. juncea*. Succinic acid was characterized and quantified as one of the dominant organic acids in root exudates of promising *Brassica* species. In this respect the greater secretions of this acid by roots of *B. napus* reflected its probable role in metal acquision through complexation.

Key words: **Cadmium, copper, lead, nickel, organic acid, phytoextraction, zinc**

INTRODUCTION

Heavy metal contamination is of special concern due to widespread reports emanating both from India and abroad about various diseases and disorders observed both in human and live stock due to metal toxicity. Agricultural activities in periurban areas and at the periphery of industrial units are under scanner due to potential threat of metal pollution through sewage effluents and sludge coming out as waste from such units; and their subsequent entry to the food chain through various crops (primarily vegetables) growing on fields with these wastes. Other detrimental effects of excessive metal concentrations in contaminated soils can result in decreased soil microbial activity and soil fertility (overall soil quality), yield losses (McGrath *et al*. 1997). In Indian context, long-term applications of sewage effluents and sludge have been reported to increase the concentrations of trace metals significantly in large areas under peri-urban agriculture around Delhi (Rattan *et al.* 2005), Calcutta (Mitra *et al.* 1999), Ludhiana (Arora and Chhibba 1992), Kanpur (Farooq *et al.* 1999), Varanasi (Singh and Singh 1994), Madurai and Coimbatore (Jeyabaskaran and Sree Ramulu 1996). In some of the

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*Corresponding author, E-mail: tpurakayastha@gmail.com above areas, the metal contents in some plant species grown exceeded the critical limits of phytotoxicity (Rattan *et al.* 2002).

Remediation of these heavy metal contaminated soils through specially selected and engineered metal accumulating plants for environmental clean up is an emerging frontline technology called "phytoremediation". It is a cost-effective, non-intrusive, and aesthetically pleasing technology. Phytoremediation (Brown *et al.* 1995, Salt *et al.* 1995) in general and phytoextraction (Kumar *et al.* 1995) in particular, depends on remarkable ability of certain plants to concentrate elements to their harvestable parts of roots and shoots. Such plants are ideally referred as hyper-accumulators. Members of *Brassica* family, with special reference to Indian mustard (*Brassica juncea* L.) have been reported to accumulate several heavy metals in its above ground biomass (Ebbs and Kochian 1998). Recent evidence suggests that Indian mustard (*Brassica juncea*) may accumulate four times more Zn than *Thalspi caerulescens* (Ebbs *et al.* 1997). The *Brassica* species being widely cultivated and adapted in Indo-Gangetic alluvial plains of India, have enormous promise to clean up the contaminated soils around Delhi and similar agro-ecological region. However, very few reports are available on phytoextraction studies from India, despite its importance and that too all reports are confined to studies on water bodies.

The major limitation in phytoremediation of heavy metal contaminated soils is lower solubility of metals in soil. However, plant possess highly specialized mechanisms to stimulate metal bio-availability in the rhizosphere, and to enhance uptake into roots either through secretion of low molecular weight iron chelators called siderophores (mugineic acid and avenic acids) (Fushiya *et al.* 1982, Takagi *et al.* 1984) or organic acids (Chaney *et al.* 1972, Bienfait *et al.* 1982). Besides acidification, organic acids especially di- and tri-carboxylic acids can be potent metal complexers bringing out the difficultly available metal pools (*e.g.* specifically adsorbed, mineral form) to soluble pool (Albuzio and Ferrari 1989, Ritchie 1994, Jones and Kochian 1996). There is a paucity of information available on the characterization and quantification of organic acids, potent metal complexers in the rhizosphere of promising *Brassica* species. The characterization of organic acids have practical significance on their use as soil amendments for faster mobilization of metals to hyper-accumulators.

The hypothesis set for the present investigation was that different *Brassica* species might vary in accumulating heavy metals depending upon their metal acquisition through secretion of organic acids, potent metal chelating agents. Before the onset of any experiment with soil, a preliminary study is required to be conducted in controlled condition. Therefore, a sand culture experiment with five different species of *Brassica* was carried out in phytotron to estimate the metal accumulating efficiency of these species. The characterization and quantification of organic acids secreted by these *Brassica* species was also carried out.

MATERIALS AND METHODS

Selecting hyper-accumulators: Among the reported hyper-accumulators used for scavenging heavy metals from soil, *Brassica* species figure most prominently throughout the world. *Brassica juncea* is most widely reported species out of them having the capacity to accumulate several heavy metals. This along with other species of *Brassica* is widely adapted as oilseed crops in the Indo-Gangetic plains of Northern India. Therefore, immense possibility of using these species for phytoremediation of metal contaminated soils lies in this part of our country. With this hypothesis, five different species of *Brassica,* e.g. *B. juncea* (Indian mustard) cv. Pusa Bold, *B. campestris* (Yellow mustard) cv. Pusa Gold, *B. carinata* (Ethiopian mustard) cv. DLSC-1, *B. napus* cv. Early napus, *B. nigra* IC-247 were chosen for our study. In absence of any cultivar specifically bread for phytoremediation purpose, popular cultivars having profuse vegetative growth were chosen.

Sand culture experiment: A sand culture (0.25 – 1.0 mm quartz sand as supporting medium) experiment with five species of *Brassica* as stated earlier, irrigated by graded doses of metal enriched Hoagland solution was carried out in a glasshouse growth chamber in National Phytotron Facility at Indian Agricultural Research Institute, New Delhi. The temperature was maintained at 25°C and 19°C during day and night respectively and the relative humidity was set at 70%. The day period was on an average 12 hours of normal solar radiation. Plastic pots with drainage hole at the bottom were used for this study. *Brassica* species were germinated by placing seeds in pots containing quartz sand. Initially distilled water was used for germination of seeds. Thereafter, two plants were maintained in each pot and irrigated with Hoagland solution throughout growth period. The Hoagland solution comprised of solution A – 1 M $NH_4H_2PO_4$ (1 ml/l), 1 M KNO_3 (6 ml/l), 1 M $Ca(NO_3)_2$ (4 ml/l), 1 M $MgSO_4$ (2 ml/l), solution B - 2.86 g H_3BO_3, 1.81 g $MnCl_2$, 0.22 g $ZnSO_4$, 0.08 g $CuSO_4$ and 0.02 g H_2MoO_4 in 1 liter (1 ml/l), solution C: 0.5% $C_6H_8O_7FeNH_3$ (1 ml/l) was used for irrigating the plants. All the compounds were dissolved in distilled water. Three levels of metal enrichment comprised of (i) 'normal' Hoagland solution (ii) 'medium' level of enrichment of Hoagland solution with Zn (through $ZnSO_4.7H_2O$) = 2 mg/l, Cu (through $CuCl_2.2H_2O$) = 0.2 mg/l, Ni (through $NiCl_2.6H_2O$) = 0.2 mg/l, Pb [through $(CH_3COO)_2Pb$] = 5 mg/l and Cd (through $CdCl_2$. H_2O) = 0.01 mg/l (maximum permissible limits in irrigation water) and (iii) 'high' level of enrichment of Hoagland solution with twice the amount added at 'medium' level. Zinc and Cu being component of normal Hoagland solution had been treated separately (control, medium and high) than Ni, Pb and Cd (medium and high). As Zn and Cu are the essential nutrient elements in normal Hoagland solution, the data relating to their contents and uptake by *Brassica* species were presented in "control", "medium" and "high" metal levels, while for other metals (Cd, Pb & Ni) as the normal Hoagland solution (control) was devoid of these their uptake by *Brassica* sps. was nil. Therefore, the data relating to content and uptake of Cd, Pb and Ni were presented in "medium" and "high" metal levels. The root washings were collected twice in a week after displacing old Hoagland solution with distilled water and each pot was filled with new Hoagland solution during each sampling. This study was carried out in order to produce bench mark data on metal acquisition by selected *Brassica* species. The plants were grown up to maximum vegetative stages (up to 75 days) after which these were harvested. The oven dry weight (70°C for 72 hours) of stalk was recorded. The heavy metal (Zn, Cu, Ni, Pb and Cd) content in acid digested stalk (Jackson 1973) was estimated through atomic absorption spectrophotometer (GBC make, model 301).

Characterization of organic acids: Root exudates of various *Brassica* species were collected periodically with intermittent washings through out the growing season. The organic acids secreted by *Brassica* species were characterized and quantified as per the procedure outlined by Szmigielska *et al.*(1996) in a gas chromatograph (GC Agilent make, model 4890 D). The root washings were concentrated by heating in a hot plate at 50°C temperature (500 ml concentrated to 100 ml). Out of this 50 ml aliquot was taken for methylation. The aliquot was first acidified with 2 ml of 50% H_2SO_4, to protonate the organic anions converting them to acids. It was then methylated with 10 ml methyl alcohol for 30 minutes heated at 60°C water bath. After cooling 10 ml each of distilled water and chloroform was added and the mixture was vigorously shaken in a 100 ml capacity separatory funnel for ten minutes. The methylated organic acids in lower chloroform layer were collected and dehydrated in a desiccator in presence of $CaCO_3$. The common organic acids like malic, maleic, oxalic, tartaric and succinic were methylated following the above procedure and these were used as standards. The 1000 mg l^{-1} organic acid standard was prepared which was later diluted to 100 mg l^{-1} as working standard. The unknown organic acids in root washings was characterized and quantified with reference to known organic acid standards through PC based CERETY QA-QC software. The capillary column (DB-Wax) and Flame Ionization Detector (FID) were used in GC for estimation of organic acids. The oven temperature in GC was maintained between 75°C to 240°C with a run time of 21 minutes for each sample. The inlet and detector temperature were maintained at 250°C. Nitrogen (4 ml min^{-1}) was used as carrier gas, hydrogen (40 ml min^{-1}) and oxygen (300 ml min^{-1}) as auxiliary gas.

Statistical analysis: The statistical analysis of the data was performed using completely randomized design with four replications. The statistical analysis was performed in PC based programme, SPSS 10 version. The separation of means was performed using Duncan's Multiple Range Test at 5% probability level.

RESULTS AND DISCUSSION

Stalk biomass yield: Brassica carinata and *B. napus* produced highest stalk yield in control and medium metal level but at high metal level, *B. carinata* alone produced highest stalk yield (Table 1). This clearly indicated the difference in biomass distribution pattern among different *Brassica* species. Generally, the stalk yield of *Brassica* species decreased due to enrichment of Hoagland solution with metal. But with the exception, stalk yields of *B. campestris* and *B. nigra* were not affected due to medium level of enrichment, while at high level the yields of these species decreased. Though at moderate level, the degree of metal enrichment was done as per the permissible limits of these metals in irrigation water, the reduction of stalk biomass yield was perhaps be due to additive toxic effects of all the five metals on plant growth. If the metals were added separately, the picture could have been different. Our results could be supported by the findings of Agarwala *et al.* (1997) who reported the order of decrease in dry matter yield of plants due to various heavy metals as: Ni > Co > Zn > Mn > Cu. In a hydroponic experiment, *B. juncea* plants exhibited signs of severe toxicity when grown in the presence of elevated concentrations of Zn (Ebbs and Kochian 1998).

Table 1. Stalk biomass yield (g pot^{-1})of various *Brassica* species grown in metal enriched Hoagland solution in sand culture.

Species	**Levels of metal**		
	Control¶	**Medium#**	**High£**
B. juncea	4.67bA§	3.69bB	3.50bB
B. campestris	4.78bA	4.23abAB	3.49bB
B. carinata	6.00aA	5.00aB	4.48aB
B. napus	6.11aA	4.31abB	3.02bC
B. nigra	4.33bA	3.91bA	3.06bB

¶Control: Hoagland solution, #Medium: Zn-2, Cu-0.2, Ni-0.2, Pb-5 mg l^{-1} and Cd-0.01 mg l^{-1} (maximum permissible limit in irrigation water), £High: Twice the amount in medium solution. §numbers followed by different lower case letters in a column (different species) or numbers followed by different upper case letters in row (different levels of metals) in a particular measurement are statistically different according to Duncan's Multiple Range Test at P=0.05.

Zinc, copper, lead, nickel and cadmium contents: Zinc and Cu being essential elements in Hoagland solution, these two metals have been handled separately form the other three metals of study (*e.g.* Cd, Pb and Ni). Zinc content in stalk was highest in *B. napus* at control treatment, while at medium and high levels of metal enrichment, *B. juncea* along with *B. napus* and *B. carinata* showed greater Zn content (Table 2). One of the criteria for a plant to be hyper-accumulator is production of larger above-ground biomass along with high metal content in it. In this respect *B. carinata* definitely performed better than the rest by producing large stalk biomass as well as exhibiting higher amount of Zn in its stalk. Besides this, our results are in conformity with the findings of Ebbs *et al.* (1997) who also reported Zn content between 500 and 600 mg kg^{-1} dry weight in *B. juncea*, *B. rapa* and *B. napus*. But with regard to Cu, Pb, Ni and Cd contents in stalks, *B. napus* excelled over others. However, *B. napus* was reported to be hyper-accumulators of Se (Banuelos *et al.* 1997), Zn and Cu (Ebbs and Kochian 1997). *Brassica juncea*, *B. campestris* and *B. carinata* ranked second to *B. napus* with respect to Cu contents in stalks both at medium and high levels of metal enrichments. *Brassica campestris* ranked second with respect to Pb content in stalk at medium level of metal enrichment, while at high level, *B. napus* ranked first and other four species ranked second. Likewise, Begonia (1997) reported a greater Pb accumulation (500 µg Pb g^{-1} dry biomass) in *B. juncea*. At both medium and high levels of metal enrichments, *Brassica* species maintained almost similar trend. *Brassica juncea* and *B. carinata* ranked second to *B. napus* with respect to Cd contents in stalk.

Brassica napus and *B. carinata* were dissimilar with respect to Zn contents in stalk at medium and high levels of metal enrichments, while other species showed a reverse trend. The magnitude of increase in the content of Zn in stalk biomass of different *Brassica* species ranged between 5.0 to 16.3 times and 4.2 to 18 times (over control) at medium and high level of metal enrichments, respectively. The highest increase was observed in *B. carinata* at medium level of metal enrichment and it was in *B. campestris* at high level of metal enrichment. The magnitude of increase at high level was greater than at medium level in *B. juncea* and *B. campestris*. In case of Cu, all the species showed significantly higher Cu contents in stalk at high level of metal enrichment over those in either control or medium level. The magnitude of increase in the content of Cu in stalk biomass of different *Brassica* species ranged between 0.8 to 12.3 times and 10.8 to 23.9 times (over control) at medium and high level of metal enrichments, respectively. Irrespective of levels of metal enrichment, the highest increase in Cu content was observed in *B. napus*. *Brassica juncea*, *B. carinata* and *B. napus* showed significantly higher Pb contents at high level of metal enrichment over medium level. The magnitude of increase in the content of Pb in stalk biomass of various *Brassica* species at high level of metal enrichment ranged between 0.7 (*B. campestris*) to 2.0 (*B. carinata*) times over medium level. Interestingly, *B. campestris* showed higher Pb contents at medium than at high level. This indicates the susceptibility of this species at higher metal dose. In case of Ni, all the species except *B. nigra* showed higher accumulation at high level than at medium level. The magnitude of increase in the content of Ni in stalk biomass of various *Brassica* species ranged between 1.1 (*B. campestris*) to 1.7 (*B. carinata*) times over medium level. In case of Cd, all the species except *B. campestris* and *B. nigra* showed greater accumulation of Cd in their stalks at high level of metal enrichment. The magnitude of increase in the content of Cd in stalk biomass of various *Brassica* species ranged between 1.0 (*B. carinata, B. nigra*) to 1.5 (*B. juncea*) times over medium level. In general, the contents of Zn, Cu, Ni, Pb and Cd in different plant parts of *Brassica* species grown in metal stressed Hoagland solution were higher over corresponding plants grown in ordinary Hoagland solution. It was quite obviously related to the magnitude of their accumulation in solution.

These results are in accordance with the findings of other workers who also reported higher accumulation of heavy metals in different plant parts in sewage-sludge amended soil (Valdares *et al.* 1983, Burridge and Berrow 1984) or in hydroponics system where heavy metals were added externally (Ebbs and Kochian 1997). It should be further noticed that the metal contents in the stalks of the various *Brassica* species were much below some earlier reported values for these metals (Ebbs *et al.* 1997, Begonia *et al.* 1998) which might be ascribed both to the potential limitation of accumulation

Table 2. Contents of zinc (Zn) copper (Cu), lead (Pb), nickel (Ni) and cadmium (Cd) in stalks of various *Brassica* species grown in metal enriched Hoagland solution in sand culture.

Species	**Zn content (mg kg^{-1})**			**Cu content (mg kg^{-1})**			**Pb content (mg kg^{-1})**		**Ni content (mg kg^{-1})**		**Cd content (mg kg^{-1})**	
	Levels of metal			**Levels of metal**			**Levels of metal**		**Levels of metal**		**Levels of metal**	
	Control¶	**Medium#**	**High£**	**Control¶**	**Medium#**	**High£**	**Medium#**	**High£**	**Medium#**	**High£**	**Medium#**	**High£**
B. juncea	25.7bC§ (0.0)	189.5bB (7.4)	460.2aA (17.9)	13.0aC (0.0)	100.6bB (7.7)	139.9bA (10.8)	14.6cdB (0.0)	25.0bA (1.7)	66.5bB (0.0)	106.8bA (1.6)	1.97bB (0.0)	2.88bA (1.5)
B. campestris	20.9bC (0.0)*	104.0cB (5.0)	375.7cdA (18.0)	8.2aC (0.0)	88.2bB (108)	141.0bA (17.2)	30.5bA (0.0)	21.3bB (0.7)	43.3cB (0.0)	49.4cA (1.1)	1.69bA (0.0)	2.15bcA (1.3)
B. carinata	25.0bB (0.0)	407.8aA (16.3)	444.4abA (17.8)	13.3aC (0.0)	106.7bB (0.8)	144.7bA (10.9)	12.8dB (0.0)	25.3bA (2.0)	53.8bcB (0.0)	93.8bA (1.7)	2.13bA (0.0)	2.07cA (1.0)
B. napus	98.2aB (0.0)	399.4aA (4.1)	413.4bcA (4.2)	15.2aC (0.0)	193.8aB (12.8)	362.8aA (23.9)	40.0aB (0.0)	61.6aA (1.5)	126.5aB (0.0)	168.0aA (1.3)	3.81aB (0.0)	4.60aA (1.2)
B. nigra	32.3bC (0.0)	309.4bB (9.6)	362.8cdA (11.2)	7.8aC (0.0)	52.0cB (6.7)	91.8cA (11.8)	21.8cA (0.0)	24.9bA (1.1)	30.7dA (0.0)	42.3cA (1.4)	0.86cA (0.0)	0.88dA (1.0)

¶Control: Hoagland solution, #Medium: Zn-2, Cu-0.2, Ni-0.2, Pb-5 mg l^{-1} and Cd-0.01 mg l^{-1} (maximum permissible limit in irrigation water), £High: Twice the amount in medium solution. §Numbers followed by different lower case letters in a column (different species) or numbers followed by different upper case letters in row (different levels of metals) in a particular measurement are statistically different according to Duncan's Multiple Range Test at P=0.05. *The figures in parentheses indicate the times increase over control/medium treatment.

capacity of species as well as the additive toxic effects of all the metals added through Hoagland solution.

Uptake of Zinc, copper, lead, nickel and cadmium: The highest Zn uptake by stalk was observed in *B. carinata* at both medium and high levels of metal enrichments (Table 3). But it was earlier reported that *B. juncea* was a better hyper-accumulator over *B. carinata*, *B. nigra* and *B. campestris* for Zn *(*Ebbs *et al.* 1997). In control treatment, though *B. napus* showed greater Zn uptake, at medium level *B. napus* ranked second next to *B. carinata*. At high level, *B. juncea* ranked second next to *B. carinata* in this respect. In case of Cu the trend interchanged among these two species (*B. carinata* and *B. napus*). On the other hand, the higher phytoextraction capacity has been reported in *B. juncea* for Cu (Ebbs and Kochian 1997), Pb (Salt *et al.* 1997), Pb, Zn and Cd (Rio *et al.* 2000). The differences in the trend observed in the present investigation might possibly be due to differences in the uptake capacity of these cultivars used. In the current study, *Brassica napus* was far superior accumulator of Cu, Ni, Pb and Cd and *B. carinata* for Zn than *B. juncea*. It might be due to the fact that *B. napus* possessed more fibrous root system with greater amount of root hairs as compared to other species as observed in our laboratory (unpublished data) which might help in extracting metals more efficiently than others.

Brassica juncea, *B. campestris* and *B napus* showed significantly higher Zn uptake at high levels of metal enrichment as compared to control as well as medium level, while *B. carinata* and *B. nigra* showed similar Zn uptake at medium and high metal level. The magnitude of Zn uptake by stalk biomass of different *Brassica* species ranged between 2.9 to 13.6 times and 2.1 to 13.4 times (over control) at medium and high level of metal enrichments, respectively. The highest increase was observed by *B. carinata* at medium level of metal enrichment and it was by *B. juncea* at high level of metal enrichment. All the *Brassica* species except *B. nigra*, showed significantly higher Cu uptake at both medium and higher metal levels over control. The magnitude of Cu uptake by stalk biomass of different *Brassica* species ranged between 6.2 to 9.3 times and 8.1 to 12.3 times (over control) at medium and high level of metal enrichments, respectively. The highest increase was observed by *B. napus* at medium level of metal enrichment and it was by *B. campestris* at high level of metal enrichment. Among the *Brassica* species, *B.*

Table 3. Uptake of zinc (Zn) copper (Cu), lead (Pb), nickel (Ni) and cadmium (Cd) by stalks of various *Brassica* species grown in metal enriched Hoagland solution in sand culture.

Species	**Zn content (mg kg^{-1})**			**Cu content (mg kg^{-1})**			**Pb content (mg kg^{-1})**		**Ni content (mg kg^{-1})**		**Cd content (mg kg^{-1})**	
	Levels of metal			**Levels of metal**			**Levels of metal**		**Levels of metal**		**Levels of metal**	
	Control¶	**Medium#**	**High£**	**Control¶**	**Medium#**	**High£**	**Medium#**	**High**	**Medium#**	**High**	**Medium#**	**High£**
B. juncea	0.12bC§	0.70dB	1.61bA	0.06aB	0.37bA	0.49bcA	53.9cA	87.5bcA	245.6bB	373.6bA	7.28cB	10.1bA
	(0.0)*	(5.8)	(17.9)	(0.0)	(6.2)	(8.2)	(0.0)	(1.6)	(0.0)	(1.5)	(0.0)	(1.4)
B. campestris	0.10bC	0.44dB	1.31cA	0.04aB	0.37bA	0.49bcA	129.0bA	74.3cA	183.2bcA	172.2cA	7.15cA	7.50dA
	(0.0)	(4.4)	(18.0)	(0.0)	(9.3)	(12.3)	(0.0)	(0.6)	(0.0)	(0.9)	(0.0)	(1.0)
B. carinata	0.15bB	2.04aA	1.99aA	0.08aB	0.53bA	0.65bA	64.0cB	113.3bA	269.1bB	423.0abA	10.7bA	9.27cB
	(0.0)	(13.6)	(17.8)	(0.0)	(6.6)	(8.1)	(0.0)	(1.8)	(0.0)	(1.6)	(0.0)	(0.9)
B. napus	0.60aC	1.72bB	1.25cA	0.09aB	0.83aA	1.10aA	172.3aA	186.3aA	544.8aA	508.3aA	16.4aA	13.9aB
	(0.0)	(2.9)	(4.2)	(0.0)	(9.2)	(12.2)	(0.0)	(1.1)	(0.0)	(0.9)	(0.0)	(0.8)
B. nigra	0.14bB	1.21cA	1.11cA	0.03aA	0.20cA	0.28cA	85.3cA	76.2cA	120.1cA	129.4cA	3.36dA	2.69cB
	(0.0)	(8.6)	(11.2)	(0.0)	(6.7)	(9.3)	(0.0)	(0.9)	(0.0)	(1.1)	(0.0)	(0.8)

¶Control: Hoagland solution, #Medium: Zn-2, Cu-0.2, Ni-0.2, Pb-5 mg l^{-1} and Cd-0.01 mg l^{-1}, (maximum permissible limit in irrigation water), £High: Twice the amount in medium solution. §Numbers followed by different lower case letters in a column (different species) or numbers followed by different upper case letters in row (different levels of metals) in a particular measurement are statistically different according to Duncan's Multiple Range Test at P=0.05. *The figures in parentheses indicate the times increase over control/medium treatment.

carinata is the only species which showed significantly higher Pb uptake at high metal level over medium level. The increase in Pb uptake by stalk biomass of various *Brassica* species ranged between 1.0 (*B. carinata*) to 1.5 (*B. juncea*). *Brassica juncea* and *B. carinata* are the two species which exhibited significantly higher Ni uptake at higher metal level. The Ni uptake by *B. juncea* and *B. carinata* in high level of metal enrichment was 1.5 and 1.6 times higher (over medium level), respectively. Except *B. campestris*, all other species showed significantly higher Cd uptake at high metal level over medium level. The Cd uptake by *B. juncea* was 1.4 times higher in high level than in medium level. The Cd uptake by *B. carinata*, *B. napus* and *B. nigra* at high metal level was lower than that at medium level. This was mainly due to proportionately higher decrease in biomass yield over concomitant increase in metal content due to metal enrichment of Hoagland solution. In general though the biomass yield decreased due to metal enrichment of Hoagland solution, the uptake of several metals by different *Brassica* species greatly enhanced. This was probably be due to enhanced metal concentration in stalks of certain promising *Brassica* species which increased metal uptake in spite of yield loss due to metal enrichment of Hoagland solution. Our findings could be supported by Brown *et al.* (1995) who reported greater translocation of Zn and Cd in shoots of *Thlaspi caerulescens* due to spiking of Hoagland solution with Zn (3160 µM) and Cd (63 µM).

Characterization of organic acids: Four organic acids, namely succinic, oxalic, tartaric and maleic were characterized and quantified in the root exudates of various *Brassica* species at varying levels of metals (Table 4). The maleic acid was not detected in the root exudates in any of the *Brassica* species. Only the succinic acid was found to be present in root exudates of all the *Brassica* species both in control and medium level of metal enrichments. At the medium level of metal enrichments, the concentration of succinic acid in root exudates of all the *Brassica* species except *B. carinata* decreased drastically. The order of per cent decrease in the concentration was 62, 79, 70, 25% in *B. juncea*, *B. campestris, B. napus* and *B. nigra*, respectively. The concentration of succinic acid in the root exudates of *B. carinata* due to metal enrichment at medium level increased by 191% which could indirectly suggests its role in acidification and metal complexation followed by faster mobilization to metallophytes in the rhizosphere. Earlier it was reported that plants are capable of secreting organic acids (Chaney *et al.* 1972, Bienfait *et al.* 1982) especially di- and tri- carboxylic acids can be potent metal complexers bringing about the difficultly available metal pools (*e.g.* specifically adsorbed, mineral form) to soluble pool (Albuzio and Ferrari 1989, Ritchie 1994, Jones and Kochian 1996). Further it was reported that *B. napus* could secrete citrate and malate in its rhizosphere to mobilize phosphorus from rock phosphate (Hoffland 1992). Oxalic acid was non traceable under normal Hoagland solution, while it was present under medium toxicity level in the root exudates of *B. juncea*, *B. campestris* and *B. napus*. In *B. carinata*, only high toxicity level showed its presence. The tartaric acid was detected only in the root exudates of *B. carinata* in the

Table 4. Quantification of various organic acids in root exudates collected from sand culture experiment.

Species	Succinic acid (µg)			Oxalic acid (µg)			Tartaric acid (µg)		
	Control¶	Medium#	High£	Control¶	Medium#	High£	Control¶	Medium#	High£
B. juncea	17.5	6.62 (62%)*	nd	nd	4.50	nd	nd	nd	nd
B. campestris	6.92	1.44 (79%)	nd	nd	0.84	nd	nd	nd	nd
B. carinata	1.12	3.26 (191%)	nd	nd	nd	9.25	0.24	nd	nd
B. napus	22.0	6.50 (70%)	nd	nd	28.0	nd	0.48	nd	nd
B. nigra	1.35	1.01 (25%)	nd	nd	nd	nd	nd	nd	nd

¶Control: Hoagland solution, #Medium: Zn-2, Cu-0.2, Ni-0.2, Pb-5 mg l^{-1} and Cd-0.01 mg l^{-1}, (maximum permissible limit in irrigation water), £High: Twice the amount in medium solution, nd – not detected, *The figures in parentheses indicate the per cent increase/decrease over control treatment.

control treatment. The study confirms variability of nature of organic acid secretions among various *Brassica* species and indicates a possible role of succinic and oxalic acid in metal acquisition.

Among the various *Brassica* species investigated, *B. napus* cv. Early napus emerged as the most promising phytoextractor of Cu, Ni, Pb and Cd, while *B. carinata* cv. DLSC-1 was the most efficient phytoextractor of Zn. Succinic acid being major organic acid as secreted by the *Brassica* species could be used as a potent soil amendment for further enhancing the efficiency of phytoextraction of heavy metals.

ACKNOWLEDGEMENTS

The authors are indebted to Indian Council of Agricultural Research, New Delhi for providing the financial support through National Agricultural Technology Project.

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