EQA – Environmental quality / Qualité de l'Environnement / Qualità ambientale, 17 (2015) 13-23

PHYTOEXTRACTION BASED ON INDIAN MUSTARD (*BRASSICA JUNCEA ARAWALI*) PLANTED ON SPIKED SOIL BY ALIQUOT AMOUNT OF LEAD AND NICKEL

Leela Kaur [(1)]*, Kasturi Gadgil [(2)], Satyawati Sharma [(3)]

[(1)] Department of Environmental Science,
Maharaja Ganga Singh University, Bikaner (Rajasthan) India
[(2)] ABES Engineering College, Ghaziabad, U.P., India
[(3)] Centre for Rural Development & Technology, IIT, New Delhi, India

*Corresponding author E-mail: leela.kaur@gmail.com

ABSTRACT

Phytoextraction is one of the mechanisms of phytoremediation for removal of heavy metals from contaminated soils. The objective of the present study was to investigate the effect of metal chelants, ethelyne diamine tetraacetic acid (EDTA) and salicylic acid (SA), on the accumulation of lead and nickel by Indian mustard (*Brassica juncea arawali*) plants in contaminated soil. Plants were treated with Pb and Ni, each at concentrations of 800 mg/l. EDTA and SA were amended at 0.1 M and 1.0 mM respectively. Plants were harvested and oven-dried. Pb and Ni content were estimated using ICP-OES (Varian Vista-MPX CCD Simultaneous ICP-OES). The samples thus prepared were investigated for metals distribution and morphology in different tissues (root, stem and leaf) using energy dispersive X-ray analysis (EDAX) spectrometer attached to the scanning electron microscope (SEM). Metal distribution study in different tissues (root, stem, leaf) was done by SEM/EDS. The results showed that EDTA increased Pb and Ni uptake as compared to SA.

Key words: *Lead, Nickel, salicylic acid, Indian mustard, ICP-OES, SEM.*

Introduction

Heavy metal pollution in the environment is a great concern in today's world which is a result of release by natural processes and a long history of anthropogenic use of heavy metals. Sources of Pb contaminated soil are mainly mining and smelting, automotive emissions, Pb-based paints and industrial activity (e.g. Haack et al., 2003, Ettler et al., 2004, Monastra et al., 2004, Wong and Li, 2004). While the largest sources of nickel in soil are the waste from metal manufacturing, commercial waste, urban refuse, coal ash and sewage sludge. The negative impacts of heavy metals on the environment and human health demand the need for remediation of contaminated soil but its remediation is quite challenging as metals are non-biodegradable. Some methods, such as immobilisation or extraction by physicochemical techniques, have an adverse effect on soil structure and require engineering costs (Pulford and Watson, 2003). Hence, scientists and environmentalists have been put emphasis on phytoremediation as it is

DOI: 10.6092/issn.2281-4485/5817

environmentally friendly and cost-effective as compared to conventional remediation techniques (Salt et al., 1998; McGrath et al., 2002).

Phytoextraction, the use of metal-accumulating plants to remove toxic metals from soil, is one of the mechanisms of phytoremediation which offers a great promise for commercial development (Chaney et al., 1997).

Heavy metals are bound primarily to organic, oxide and residual fractions in soils resulting thus into low mobility (Adriano, 2001). The low solubility and bioavailability of some toxic metals (e.g., Pb) is the major limiting factor in induced phytoextraction (Lasat, 2000). Synthetic chelating agents have the potential to remobilise metals and to form strong soluble complexes (Nowack et al., 2001; Sun et al., 2001). Increasing Metal–chelant complexes in the soil solution promote the uptake by plants and the translocation of heavy metals from roots to shoots and their accumulation in the harvestable parts of the plants (Blaylock et al., 1997; Huang et al., 1997; Epstein et al., 1999; Grčman et al., 2001; Schmidt, 2003). Ethylenediaminetetraacetic acid (EDTA) has been proved to be an effective chelating agent that has potential for soil remediation applications (Ghestem and Bermond, 1998; Hong et al., 1999; Piechalak et al., 2003; Komárek et al., 2007). Salicylic acid (SA) is an endogenous plant growth regulator which is known to be involved in plant responses to abiotic stresses such as ozone (Koch et al., 2000), UV-B (Surplus et al., 1998), heat stress (Clark et al., 2004; Dat et al., 2000) drought (Singh and Usha, 2003; Nemeth et al., 2002; Senaratna et al., 2000), oxidative stress (Shim et al., 2003), salt and osmotic stress (El-Tayeb, 2005; Khodary, 2004; Borsani et al., 2001). Interestingly, salicylic acid is present at elevated concentrations in metal-hyperaccumulator plants in the genus *Thlaspi*, where it is implicated in the high degree of cellular tolerance towards nickel (Freeman et al., 2005).

One of the most promising, non hyperaccumulating plant species for extracting heavy metals from contaminated soils is *B. juncea* of Brassicaceae family which has been studied well (Kumar et al., 1995; Marchiol et al., 2004; Gisbert et al., 2006).

The aim of the present study were to evaluate the effect of EDTA and SA on the accumulation of Pb and Ni by Indian mustard (*Brassica juncea arawali*) plants in contaminated soil and to determine the distribution of Pb and Ni in the different tissues (leaf, stem, root) of the plant.

Materials and Methods

Experimental site

Under field conditions a pot experiment was conducted to study the comparative effect of EDTA as well as SA on the heavy metal accumulation by mustard and fenugreek plants.

Experiments were conducted at the Micromodel experimental site of the Indian Institute of Technology, Delhi. It is situated at 77.09^{o}E longitude and 20.45^{o}N latitude, and 28 m altitude above sea level. The mean maximum and minimum

temperature during the study period were 18-43°C and 3-15°C, respectively. Some physical and chemical soil characteristics are summarised in Table 1.

Parameter	Unit	Amount
Texture	-	Sandy loam
Clay	%	16.30
Silt	%	14.23
Sandy	%	69.47
Electrical Conductivity	mS/cm	0.28
pH	-	7.5
Cation Exchange Capacity	Cmol/kg	18.4
Organic Carbon	%	0.72
Available N	Kg/ha	272
Available P	Kg/ha	9.0
Available K	Kg/ha	200.7
Total Pb	Mg/kg	0.02
Total Ni	Mg/kg	4.0

Table 1
Some soil characteristics.

Pot experiment

The seeds of *Brassica juncea arawali* were procured from the National seeds Corporation Ltd., Beej Bhawan, Pusa, New Delhi.

About 20 seeds were sown in 11x11 cm pots containing unsterilized field soil, farm yard manure (organic carbon 12.2 %, total N 0.55 %, total P 0.75 %, total K 2.30 % and pH 7.2) and sand in a 2:2:1 ratio in October 2008. In chemical treatment, Pb, Ni, EDTA and SA were added as per the designed treatment. The designed treatment for mustard plants was as follows: (T1) Pb, (T2) Ni, (T3) Pb + Ni, (T4) Pb + Ni + 2.4 mM EDTA, (T5) Pb + Ni + 2.4 mM SA, and (T0) Control or untreated plants. The concentrations of Pb (supplied as $Pb(NO_3)_2$), and Ni (supplied as $Ni(NO_3)_2$) were 800 mg/l in all treatments. Dipotassium salt of EDTA was supplied in the treatment. Plants were watered daily using tap water to maintain optimal growth conditions. Seed germination started after the seventh day of sowing and plants were thinned to 3 plants per pot after 30 days of sowing. Samples of plants were taken one day before and after chelant treatment and monthly after the treatment. At the end of the experiment the plants were harvested and then washed accurately, the aerial part was divided from the roots and the two parts were analysed separately to determine the metal content. Scanning electron microscopic (SEM) observations were also made to determine the localization and translocation path of metals in plant tissues.

Soil and plant analysis

The organic C, N, P and K were estimated by the methods of Walkley and Black, Micro-kjeldahl, Olsen and Flame photometer, respectively, in soil and farm yard manure as described by Rowell (1994). Cation exchange capacity was carried out using the $BaCl_2$ method. Metals concentration in soil was determined using the aqua regia extraction method. Plant samples were washed with tap water and dried

DOI: 10.6092/issn.2281-4485/5817

at 70°C for 48 hours. The dried material was digested with aqua regia. All determinations were performed in triplicate. Metal concentrations in solutions were analysed by ICP-OES (Varian Vista-MPX CCD Simultaneous ICP-OES, Varian Australia Pty. Ltd) with a Ni detection limit of 0.007 mg/l and a Pb detection limit of 0.01 mg/l.

Scanning Electron Microscopy (SEM) and Energy dispersive X-ray spectroscopy (EDX)

Plants were separated into roots, stems, and leaves, frozen in liquid nitrogen, and then fractured into small pieces with a blunt knife. The pieces were freeze-dried overnight at −30 °C while under a vacuum using a freeze–dry system. Samples were mounted on aluminium stubs with double-sided carbon tape. Tissue structures and abnormalities of roots and leaves of plants were observed and evaluated using a scanning electron microscope (JSM-840A) coupled with Energy dispersive X-ray spectrometer. The EDX analyses were conducted at accelerating voltages of 20,000 eV and working distance of 1.5 cm. Several randomly selected areas (approximately 0.2 cm × 0.3 cm) on each sample were scanned using the SEM/EDX for 20 frames (approximately 30 min).

Statistical analysis

The experiment was conducted as a factorial randomized block design with each treatment replicated thrice. Statistical analysis of the data was done following analysis of variance (ANOVA); when the ANOVA was significant the means were separated using least significant difference at $P \leq 0.05$ level of significance.

Results and discussion

Plant growth

Effect of treatments on seed germination and plant dry weight is shown in Table 2. Application of SA with heavy metal (Pb^{2+} and Ni^{2+}) treatment helped in reducing the inhibitory effects of these metals on seed germination along with increased dry weight.

Treatment	Seed germination (%)	Dry weight (g)
T0	100±0.8	6.2±0.70
T1	80±1.3	3.42±0.28
T2	85±1.8	2.28±0.30
T3	65±2.2	1.3±0.06
T4	75±1.5	1.5±0.42
T5	90±2.8	2.2±0.60

Values represent mean ± standard deviation (n=3)

Table 2
Effect of treatments on seed germination and dry weight in Brassica juncea arawali.

This finding is supported by Mishra et al. (1997). Seed germination (%) were significantly ($P<0.05$) decreased by EDTA. Seed germination was observed 65% in T3 (Pb + Ni) treatment. However, it was 75% in the presence of EDTA (T4) and 90% in the presence of SA (T5). A potential decrease in total plant dry weight was

observed in plants treated with T4 (Pb + Ni + EDTA). EDTA decreased significantly ($P<0.05$) dry weight, whereas SA stimulated dry weight compared to T3 (Pb + Ni) treatment. It was 1.3 g and 2.2 g in the presence of EDTA and SA respectively. The dry weight significantly ($P\leq0.05$) decreased in T3 and T4-treated plants when compared to the control (T0) plants. No significant ($P\leq0.05$) negative effect on plant growth was observed in the presence of SA.

Plant metal accumulation

Table 3 shows Pb and Ni concentrations in root and shoot part of mustard plants. According to Pugh *et al.* [39] the normal and phytotoxic levels of Pb accumulation in plants were 0.5–10 mg kg^{-1} and 30-300 mg kg^{-1} respectively. According to Kataba-Pendias and Pendias [40] the plant toxicity limits of Ni were 10-100 mg kg^{-1}. Lead bioaccumulation in roots and shoots of plants was significantly different. Pb and Ni concentration in root organs of the plants was higher than that in shoot organs in all treatments. Pb concentration increased in both organs of the plant with EDTA treatment, but it decreased with SA treatment.

Table 3. *Accumulation of Pb and Ni in shoots and roots of* "Brassica juncea arawali"

Treatment	Shoot concentration (mg kg^{-1} DW)		Root concentration (mg kg^{-1} DW)		Translocation Factor (TF)*	
	Pb	Ni	Pb	Ni	Pb	Ni
T0	43±12	79±11	96±16	123±41	0.44	0.64
T1	280±201	65±15	502±182	119±33	0.55	0.54
T2	36±9	91±36	83±15	187±121	0.43	0.48
T3	370±176	217±134	525±246	303±202	0.70	0.71
T4	1010±425	52±27	16395±989	2876±541	0.06	0.02
T5	517±228	69±28	3828±782	610±348	0.13	0.11

(TF)*= Shoot concentration/Root concentration
Values represent mean ± standard deviation (n=3).

Applying EDTA increased Pb concentration in root and shoot organs significantly ($P \leq 0.05$) from 370 mg kg^{-1} (T3) up to 1010 mg kg^{-1} (T4) and 525 mg kg^{-1} (T3) up to 16,395 mg kg^{-1} (T4) respectively. For Ni, EDTA increased the root concentration from 303 mg kg^{-1} to 2876 mg kg^{-1} ($P<0.05$) and decreased the shoot concentration from 217 mg kg^{-1} to 52 mg kg^{-1} ($P\leq0.05$).

Scanning electron microscopic observations

Analysis of leaf, stem and root samples in SEM/EDX indicated some differences in the treatments which is shown in Figure 1-4. This analysis revealed that in the presence of EDTA, the deposition of Pb and Ni particles was predominantly in the vascular tissues of the stem and leaf and in the stele region of the root. However, SEM micrographs of T1, T2 and T3 treatment roots revealed accumulation of Pb and Ni mainly in cell walls while stem and leaf of T1, T2 and T3 treatment showed accumulation of Pb and Ni in the vascular tissues.

DOI: 10.6092/issn.2281-4485/5817

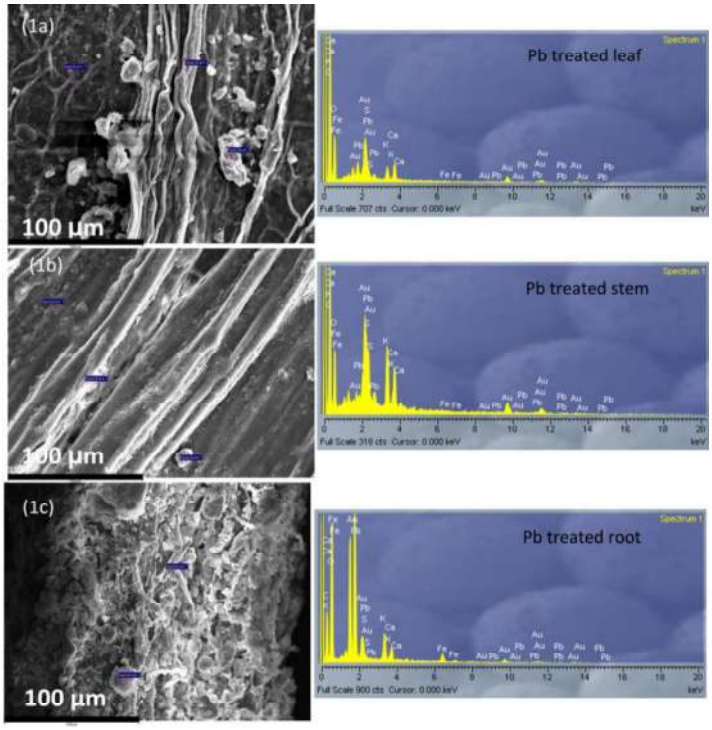

Figure 1
Scanning electron micrographs and EDX spectra of Pb and Ni distribution in leaf, stem and root of "Brassica juncea arawali" grown in the presence of 800 mg l^{-1} Pb treatment. (1a) Pb treated leaf, (1b) Pb treated stem, (1c) Pb treated root. The EDX spectrums are taken from the area indicated by the square.

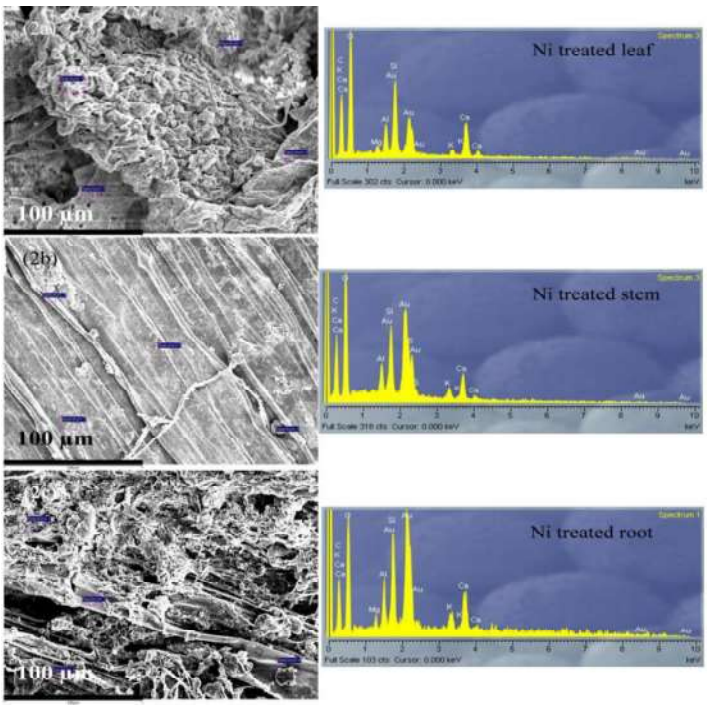

Figure 2
Scanning electron micrographs and EDX spectra of Pb and Ni distribution in leaf, stem and root of "Brassica juncea arawali" grown in the presence of 800 mg l^{-1} Ni treatment. (2a) Ni treated leaf, (2b) Ni treated stem, (2c) Ni treated root. The EDS spectrum is taken from the area indicated by the square.

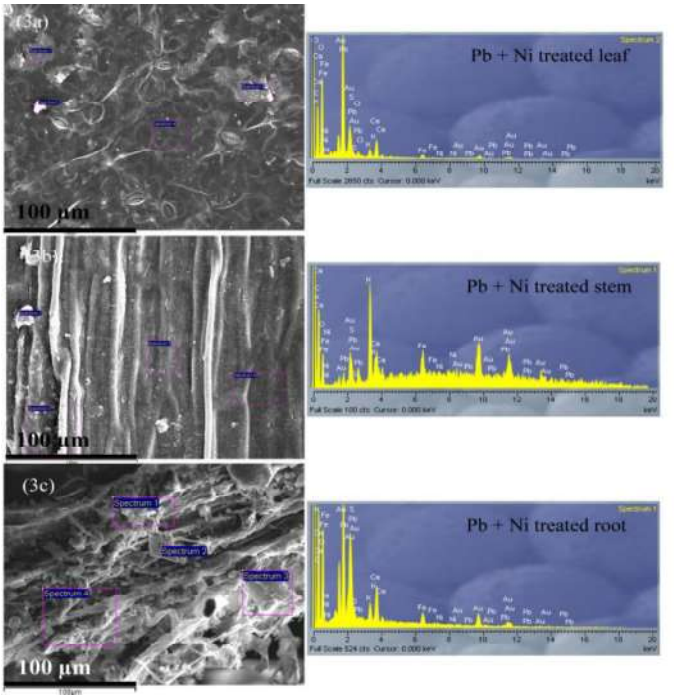

Figure 3
Scanning electron micrographs and EDX spectra of Pb and Ni distribution in leaf, stem and root of "Brassica juncea arawali" grown in the presence of 800 mg l^{-1} Pb + 800 mg l^{-1} Ni treatment.
(3a) Pb + Ni treated leaf,
(3b) Pb + Ni treated stem,
(3c) Pb + Ni treated root.
The EDS spectrum is taken from the area indicated by the square.

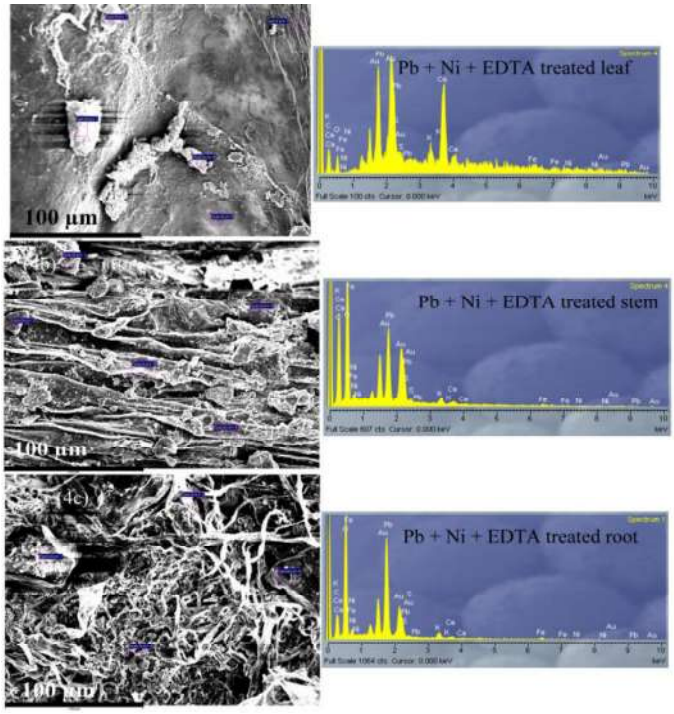

Figure 4
Scanning electron micrographs and EDX spectra of Pb and Ni distribution in leaf, stem and root of "Brassica juncea arawali" grown in the presence of 800 mg l^{-1} Pb + 800 mg l^{-1} Ni + EDTA treatment.
(4a) Pb + Ni + EDTA treated leaf,
(4b) Pb + Ni + EDTA treated stem,
(4c) Pb + Ni + EDTA treated root.
The EDS spectrum is taken from the area indicated by the square.

DOI: 10.6092/issn.2281-4485/5817

Percentage element accumulation in leaf, stem and root part of mustard with different treatment is shown in Table 4.

Order of metal accumulation in plant tissues was found as follows: leaf > stem > root. This comparative study shows that percentage Ni accumulation was more in T3 treatment while percentage Pb accumulation was higher in T4 treatment.

Table 4. *Element accumulation (%) in plant tissues of "Brassica juncea arawali" treated with different Pb and Ni treatments.*

Treatment	Plant tissues	C	O	S	K	Ca	Fe	Ni	Pb
T1	Leaf	52.6 ±27	42.93 ±24	0.19 ±0.0	1.69 ±1.72	0.81 ±0.36	0.99 ±1.66	ND	0.02 ±0.01
	Stem	56.3 ±11.1	35.1 ±7.5	0.87 ±0.68	1.28 ±1.17	5.60 ±6.22	0.07 ±0.13	ND	0.04 ±0.05
	Root	31.0 ±2.9	60.7 ±2.1	0.14 ±0.08	2.09 ±0.2	3.40 ±3.27	1.86 ±1.06	ND	0.02 ±0.01
T2	Leaf	42.2 ±25	31.5 ±18	0.37 ±0.6	1.6 ±0.9	19.3 ±3.1	0.59 ±0.9	0.12 ±0.3	ND
	Stem	63.4 ±20	26.0 ±13	1.38 ±3	1.27 ±0.80	2.2 ±3	0.41 ±0.2	0.02 ±0.2	ND
	Root	52.0 ±14	33.7 ±9.0	0.21 ±0.4	2.19 ±1.00	4.22 ±4.00	0.84 ±0.30	0.08 ±0.20	ND
T3	Leaf	59.1 ±9.0	37.0 ±9.0	0.29 ±0.20	0.60 ±0.10	1.84 ±0.30	0.17 ±0.30	0.02 ±0.0	0.05 ±0.0
	Stem	34.3 ±18.0	40.2 ±14.0	0.08 ±0.20	7.43 ±3.00	14.6 ±15.0	2.82 ±2.00	1.98 ±5.00	0.02 ±0.10
	Root	45.4 ±7.0	46.5 ±5.0	0.2 ±0.2	3.24 ±3,00	1.78 ±0.20	1.68 ±0.60	0.04 ±0.0	0.02 ±0.0
T4	Leaf	41.8 ±11,0	32.9 ±16,0	0.5 ±0.6	2.84 ±3.00	15.0 ±17.0	1.25 ±2.00	0.31 ±0.30	0.06 ±0.30
	Stem	29.5 ±14.0	59.5 ±12.0	0.03 ±0.20	2.36 ±2.00	0.83 ±0.20	3.23 ±2.00	0.18 ±0.20	0.01 ±0.0
	Root	33.0 ±14.0	56.0 ±11.0	-0.03 ±0.10	3.85 ±4.00	0.75 ±0.40	2.45 ±2.00	0.14 ±0.20	0.04 0.0

Values represent mean ± standard deviation (n=3).

Accumulation of Pb and Ni affected the presence of Ca and Fe as Ca was highest in T2 treatment (Ni) and Fe was highest in T4 treatment. The presence of EDTA caused decrease in Ni accumulation in stem from 1.98% to 0.18%. EDTA increased Fe uptake from 4.67% (T3) to 6.93% (T4). Mari *et al.* [41] identified a chelant (Ni-nicotianamine complex) in *Thalspi caerulescens* which helps in translocation of Ni. A study by Wycisk *et al.* [42] showed free histidine as the most effective translocator of Ni as compared to malic acid and citric acid.

Histidine and nicotianamine mediated transport help in enhanced Ni translocation in the absence of EDTA. Whereas, translocation of Ni is limited in the presence of EDTA as EDTA-bounded Ni was expected not to be able to complex with histidine.

Conclusion

The results indicate that the remediation of Pb by *Brassica juncea arawali* can be enhanced by the addition of EDTA. In the presence of EDTA, plant growth was significantly inhibited, while in the presence of SA, plant growth was good. Scanning electron microscopy analysis revealed that in the presence of EDTA, the deposition of Pb and Ni particles was predominantly in vascular tissues of the stem and leaf. However, in roots, deposition of Pb was in the stele region. The results showed that EDTA increased Pb and Ni uptake as compared to SA. Further experiments are needed to determine the actual mechanisms involved in Pb and Ni uptake.

Acknowledgement
The authors thank University Grants Commission (U.G.C.), New Delhi for providing financial support.

References

ADRIANO D.C. (2001) Trace Elements in the Terrestrial Environments: Biogeochemistry, Bioavailability, and Risks of Metals. Springer, New York.

BLAYLOCK M.J., SALT D.E., DUSHENKOV S, ZAKHROVA O, GUSSMAN C, KAPULNIK Y, ENSLEY B.D., RASKIN I. (1997) Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environmental Science and Technology, 31:860–865.

BORSANI O, VALPUESTA V, BOTELLA M. A. (2001) Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings, Plant Physiology, 126:1024–1030.

CHANEY R.L., MALIK M, LI Y.M., BROWN S.L., BREWER E.P., ANGLE J.S., BAKER A.J.M. (1997) Phytoremediation of soil metals. Current Opinions in Biotechnology, 8:279–284.

CLARK S.M., MUR L.A.J., WOOD J.E., SCOTT I.M. (2004) Salicylic acid dependent signaling promotes basal thermotolerance but is not essential for acquired thermotolerance in *Arabidopsis thaliana*. Plant J., 38:432–437.

DAT J.F., DELGADO-LOPEZ H, FOYER C.H., SCOTT I.M. (2000) Effects of salicylic acid on oxidative stress and thermotolerance in tobacco. Plant Physiology, 156, 659–665.

EL-TAYEB M.A. (2005) Response of barley grains to the interactive effect of salinity and salicylic acid. Plant Growth Regulation, 45:215–224.

Epstein, A.L., Gussman, C.D., Blaylock, M.J., Yermiyahu, U, Huang, J.W., Kapulnik, Y, ORSER C.S. (1999) EDTA and Pb–EDTA accumulation in Brassica juncea grown in Pb-amended soil. Plant and Soil, 208:87–94.

ETTLER V, MIHALJEVIC M, KOMAREK M. (2004) ICP-MS measurements of lead isotopic ratios in soils heavily contaminated by lead smelting: tracing the sources of pollution. Analytical and Bioanalytical Chemistry, 378, 311–317.

FREEMAN J.L., GARCIA D, KIM D, HOPF,A, SALT D.E. (2005) Constitutively elevated salicylic acid signals glutathione-mediated nickel tolerance in Thlaspi nickel hyperaccumulators. Plant Physiology, 137:1082–1091.

GHESTEM J.P., BERMOND A. (1998) EDTA extractability of trace metals in polluted soils: a chemical-physical study. Environmental Technology, 19:409–416.

DOI: 10.6092/issn.2281-4485/5817

GISBERT C, CLEMENTE R, NAVARRO-AVIÑÓ J, BAIXAULI C, GINER A, SERRANO R, WALKER D.J, BERNAL M.P. (2006) Tolerance and accumulation of heavy metals by Brassicaceae species grown in contaminated soils from Mediterranean regions of Spain. Environmental and Experimental Botany, 56:19–27.

GRČMAN, H, VELIKONJA-BOLTA, Š, VODNIK, D, KOS, B, LEŠTAN, D. (2001) EDTA enhanced heavy metal phytoextraction: metal accumulation, leaching and toxicity. Plant and Soil, 235:105–114.

HAACK U.K., HEINRICHS H, GUTSCHE F.H., PLESSOW K. (2003) The isotopic composition of anthropogenic Pb in soil profiles of northern Germany: evidence for pollutant Pb from a continent-wide mixing system. Water Air Soil Pollution, 150:113–134.

HONG P.K.A., LI C, BANERJI S. K., REGMI T. (1999) Extraction, recovery, and biostability of EDTA for remediation of heavy metal-contaminated soil. Journal of Soil Contamination, 8:81–103.

HUANG J.W., CHEN J, BERTI W.R., CUNNINGHAM S.D. (1997) Phytoremediadon of lead-contaminated soils: role of synthetic chelates in lead phytoextraction. Environmental Science and Technology, 31:800–805.

KATABA-PENDIAS A., PENDIAS H. (2001) Trace Elements in Soils and Plants. Boca Raton, Florida, CRC Press.

KHODARY S.E.A. (2004) Effect of salicylic acid on growth, photosynthesis and carbohydrate metabolism in salt stressed maize plants. International Journal of Agricultural Biology, 6, 5–8.

KOCH J.R., CREELMAN R.A., ESHITA S.M., SESKAR M, MULLET J.E., DAVIS K.R. (2000) Ozone sensitivity in hybrid poplar correlates with insensitivity to both salicylic acid and jasmonic acid. The role of programmed cell death in lesion formation. Plant Physiology, 123:487–496.

KOMÁREK M, TLUSTOŠ P, SZÁKOVÁ J, CHRASTNÝ V, ETTLER V. (2007) The use of maize and poplar in chelant-enhanced phytoextraction of lead from contaminated agricultural soils. Chemosphere, 67:640–651.

KUMAR N.P.B.A., DUSHENKOV V, MOTTO H, RASKIN I. (1995) Phytoextraction: the use of plants to remove heavy metals from soils. Environmental Science and Technology, 29:1232–1238.

LASAT M.M. (2000) Phytoextraction of metal from contaminated soil: a review of plant/soil/metal interaction and assessment of pertinent agronomic issues. Journal of Hazardous Substances Research, 2:1–25.

MARCHIOL L, SACCO P, ASSOLARI S, ZERBI G. (2004) Reclamation of polluted soil: phytoremediation potential of crop-related Brassica species. Water Air Soil Pollution, 158:345–356.

MARI S., GENDRE D., PIANELLI K., OUERDANE L., LOBINSKI R., BRIAT J.F., LEBRUN M., CZERNIC P. (2006) Root-to-shoot long-distance circulation of nicotinamine and nicotinamine-nickel chelates in the metal hyperaccumulator Thlaspi caerulescens. J. Exp. Bot., 57:4111–4122.

MCGRATH S. P., ZHAO F. J., LOMBI E. (2002) Phytoremediation of metals, metalloids, and radionuclides. Advanced Agronomy, 75:1–56.

MISHRA A, CHOUDHURI M.A. (1997) Ameliorating effects of salicylic acid on lead and mercury: induced inhibition of germination and early seedling growth of two rice cultivars. Seed science and technology, 25(2):263-270.

MONASTRA V, DERRY L.A., CHADWICK O.A. (2004) Multiple sources of lead in soils from a Hawaiian chronosequence. Chemical Geology, 209:215–231.

NEMETH M, JANDA T, HORVATH E, PALDI E, SZALAI G. (2002) Exogenous salicylic acid increases polyamine content but may decrease drought tolerance in maize. Plant Science, 162:569–574.
NOWACK B, KARI F.G., KRÜGER H.G. (2001) The remobilizations of metals form iron oxides and sediments by metal–EDTA complexes. Water, Air, and Soil Pollution, 125:243–257.
PIECHALAK A, TOMASZEWSKA B, BARALKIEWICZ D. (2003) Enhancing phytoremediative ability of *Pisum sativum* by EDTA application. Phytochemistry, 64:1239–1251.
PUGH R.E., DICK D.G., FREDEE A.L. (2002) Heavy metal (Pb, Zn, Cd, Fe and Cu) contents of plant foliage near the Anvil Range lead/zinc mine, Faro, Yukon Territory. Ecotoxicological Safety, 55:273–279.
PULFORD I.D., WATSON C. (2003) Phytoremediation of heavy metal contaminated land by trees–A review. Environment International, 29:529–540.
ROWELL D.L. (1994) Soil Science: Methods and Applications. Longman Scientific and Technical, Harlow, UK, pp. 200-217.
SALT D.E., SMITH R.D., RASKIN I. (1998) Phytoremediation. Annual Review of Plant Physiology, 49:643–668.
SCHMIDT U. (2003) Enhancing phytoextraction: the effect of chemical soil manipulation on mobility, plant accumulation, and leaching of heavy metals. Journal of Environmental Quality, 32:1939–1954.
SENARATNA T, TOUCHELL D, BUMM E, DIXON K. (2000) Acetylsalicylic (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. Plant Growth Regulation, 30:157–161.
SHIM I.S., MOMOSEY, YAMAMOTO A, KIM D.W., USUI K. (2003) Inhibition of catalase activity by oxidative stress and its relationship to salicylic acid accumulation in plants. Plant Growth Regulation, 39:285–292.
SINGH B, USHA K. (2003) Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. Plant Growth Regulation, 39:137–141.
SUN B, ZHAO F.J., LOMBI E, McGRATH S.P. (2001) Leaching of heavy metals from contaminated soils using EDTA. Environmental Pollution 113:111–120.
SURPLU S.L., JORDAN B.R., MURPHY A.M., CARR J.P., THOMAS B, MACKERNESS S.A.H. (1998) Ultraviolet-B-induced responses in Arabidopsis thaliana: role of salicylic acid and reactive oxygen species in the regulation of transcripts encoding photosynthetic and acidic pathogenesis-related proteins. Plant Cell Environment, 21:685–694.
WONG C.S.C., LI W.X.D. (2004) Pb contamination and isotopic composition of urban soils in Hong Kong. Science of Total Environment, 319:185–195.
WYCISK E., KIM E., SCHROEDER J., KRAMER U. (2004) Enhancing the first enzymatic step in the histidine biosynthesis pathway increases free histidine pool and nickel tolerance in Arabidopsis thaliana. FEBS Lett., 578:128–134.

DOI: 10.6092/issn.2281-4485/5817