Plant Uptake/Bioavailability of Heavy Metals from the Contaminated Soil after Treatment with Humus Soil and Hydroxyapatite

Virendra Misra · Pranav Kumar Chaturvedi

Abstract Uptake /bioavailability study using the Indian mustard plant (*Brassica juncea*) was undertaken at the interval of 7, 14 and 21 days to test the immobilization of heavy metals from contaminated soil that were amended with humus soil and/or hydroxyapatite. For this, four sets consisting of non-humus soil + metals (Cd, Cr, Ni and Pb), humus soil + metals, non-humus and humus soil in the ratio of 1:3 + metals and non-humus soil: humus soil in the ratio of 1:3 + metals + 1% hydroxyapatite were prepared. The bioavailability of Pb, Cd, Cr and Ni in non-humus soil system was 58%, 67%, 65% and 63%, respectively in 7 days, more than 80% in 14 days and more than 90% in 21 days. Use of non-humus, humus soil in the ratio of 1:3 and addition of 1% hydroxyapatite decreased the bioavailability of lead around 21 to 22.5%, Cd 35 to 36%, Cr 25.5 to 26.9%, Ni 34 to 39% in 7, 14 and 21 days. Apart from this increase in the fresh weight of the plant was also noticed during the experiment. The data showed that addition of 1% hydroxyapatite in the non-humus-humus soil system caused the increase in the fresh weight around 90% in 7, 14 and 21 days as compared to plant grown in non-humus and metal soil system.

Keywords Contaminated soil/sites · Humus soil · Immobilization · Bioavailability · Remediation · Heavy metals · Hydroxyapatite

1 Introduction

Release of heavy metals in soil as a result of industrial and anthropogenic activities are known to have potential impact on environmental quality and on human health via ground water and surface water (Rulkens et al. 1998). Non-ferrous metallurgical industries and pyrometallurgical processes are the main source for causing severe contamination of soils (Adriano 2001). Moreover, concentrations of heavy metals in soil may render them nonproductive because of phytotoxicity and may cause bioaccumulation of heavy metals in animals and human exposure (Abdel-Sahab et al. 1994).

A contaminated site may be relatively stable but there is always risk of mobilizing the contaminants by volatilization or flushing (Romantschuk et al. 2000). The technological solutions for treatment of contaminated sites are usually costly, ecologically unsafe and many a times not practically feasible, especially in developing countries. Among the various methods employed earlier, the technique of in situ immobilization is one of the key techniques for the remediation

V. Misra (✉) · P. K. Chaturvedi
Ecotoxicology Section,
Industrial Toxicology Research Centre,
Post Box No. 80, M.G. Marg,
Lucknow, Uttar Pradesh 226 001, India
e-mail: virendra_misra2001@yahoo.co.in

of polluted soils. It reduces the risk of worker's exposure during remediation and is less expensive and much less disruptive to ecosystem than conventional ex-situ methods involving excavation and treatment followed by disposal (Chen et al. 2000).

Immobilization limits the solubility of contaminants contained in the soil and blocks the pollutants within the soil by process of precipitation, adsorption or complexing metals (Mench et al. 1994). Plants are important components of ecosystem as they transfer elements from abiotic into biotic environments. The bioavailability of elements to plants is controlled by many factors associated with soil and climatic conditions, plants genotype and agronomic management, active/passive transfer processes, sequestration and speciation, redox states, type of plant root system and the response of plants to elements in relation to seasonal cycles (Kabata-Pendias and Pendias 1992). Mench et al. (2000); Mench et al. (2006) and Vangronsveld et al. (1995) studied the effect of several soil amendments on the growth of some crops.

Humic substances are the major organic fraction in humus soils. Among the humic substances, humic acids are natural organic macromolecules with multiple properties and high structural complexity and behave as supramolecules and form supramolecular ensembles with other compounds (Steed and Atwood 2000). Cheng and Hseu (2002) used different soil amendments, including zeolite, bentonite, Penghu soil, manganese oxide and silicate slag to immobilize Cd and Pb in two contaminated soil. The idea of treating the heavy metal contaminated soils with phosphate has recently shown good promise and has been proposed an alternative to soil removal (Berti and Cunningham 1997; Ma et al. 1995). Apatite minerals such as hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$, are known to react with many transition and heavy metals and metalloids to rapidly form secondary phosphate precipitates that are stable over a wide range of geochemical conditions (Zhang and Ryan 1999). Hydroxyapatite, as a metal immobilizing agent has been tested and different indicator plant grown on amended soils (Boisson et al. 1999). Several studies have shown that treatment of synthetic hydroxyapatite phosphate rock or phosphoric acid to contaminated waters and soil effectively reduced lead contamination in aqueous and soil solutions and resulted in formation of pyromorphite, a compound characterized by very

low solubility (Ma et al. 1994; Seaman et al. 2001). In the present study, the plant of Indian mustard (*B. juncea*), which has the capacity to tolerate high levels of heavy metals in soil, was used to investigate the uptake/bioavailability of metals from the contaminated heavy metal soils that were amended with humus soil and hydroxyapatite.

2 Materials and Methods

2.1 Chemicals

Hydroxyapatite $[Ca_{10}(PO_4)_6(OH)_2]$ obtained from Aldrich Chemical Company Inc., Milwaukee, USA was used as a metal immobilizing additive. Solid metal salts $CdCl_2.H_2O$, $(CH_3COO)_2$ $Pb.3H_2O$, $NiSO_4.7H_2O$, $CrCl_3.6H_2O$ and other chemicals used in the study were from E. Merck and AnalaR grade.

2.2 Collection of soil samples

In the present study, humus and non-humus soils collected from orchard and non-orchard areas of Gheru campus of ITRC were used for the preparation of soil matrix. The soil samples were air-dried ground and sieved through 60 mesh sieve pores to remove unwanted material if any.

2.3 Preparation of soil samples and soil analysis

The soil samples were prepared by mixing non-humus soil with humus soil in the ratio of 1:3 to obtain optimal results for immobilization (Misra and Pandey 2004). The background levels of the metals in non-humus/humus soils were Cd (0.09/0.15), Cr (1.9/1.98), Ni (2.1/2.5), Pb (2.0/3.1) μg/g of soil respectively. The cation exchange capacity of non-humus and humus soil determined by the method of Hesse (1971) was 14.72 and 44.32 meq/100 g soil, respectively. The pH of the various soil combinations ranged from 7.04 to 7.40.

2.4 Immobilization studies

For immobilization four sets consisting of (a) non-humus soil + 100 ppm metals (Cd, Cr, Ni and Pb), (b) humus soil+ metals, (c) non humus and humus soil in the ratio of 1:3 + metals and (d) non-humus soil: humus

soil in the ratio of 1:3+ metals+1% hydroxyapatite was prepared in triplicate. For the proper mixing, the soil along with metals and hydroxyapatite was maintained at 80% water holding capacity for two days and dried completely. The soil sample was ground with pestle mortar. For each set 750 g of soil was prepared and divided into three containing 250 g each.

2.5 Uptake/bioavailability studies

Seeds of mustard were surface sterilized with 2% NaClO for 15 min and washed thoroughly with deionized water and then put in petridish having cotton filter paper. The petridish were then placed in a BOD (Culture chamber) at $25\pm1°C$ for germination. After 3 days, germinated seeds (10 No.) were transferred to the beakers (diameter 7.0 cm and 250 ml capacity) containing 250 g of all the above set of soils. 3% Hoagland solution (EPA 1975) was added to these beakers, which served as nutrient. During the germination period, the moisture content (water holding capacity) of the soil was maintained at 80%. Mustard plants were grown in these beakers. After 7, 14 and 21 days the plants were taken, their fresh weights were measured. It is then washed with deionized water and dried at 70°C in oven till constant weight. The dry weights were recorded and

the plant materials were ground with a glass grinder. These tissues were then digested with HNO_3 and $HClO_4$ (5:1 volume) and diluted to 25.0 ml with 0.1N HNO_3 for metal estimation at AAS (Atomic Absorption Spectrophotometer).

2.6 Statistical calculation

The student t-test described by Fisher (1950) was employed to calculate the statistical significance between control and experimental value.

3 Results

The fresh weight patterns of the plants (B. juncea) grown in different soil system along with metals are shown in Table 1. In case of humus and metals soil system, increase in fresh weight of the plant was 45% in 7 days, 50% in 14 days and 47% in 21 days as compared to the plants grown in non- humus soil and metal soil system. When the soil system non humus and humus in combination of 1:3 along with metal was used the fresh weight enhanced up to 69% in 7 days, 57% in 14 days and 62% in 21 days. Addition of 1% hydroxyapatite in soil system caused further increase in the fresh weight from 69% to 90% in

Table 1 The fresh weight (F.W.) pattern of plant (B. juncea) grown in different soil system

Soil system	7 Days		14 Days		21 Days	
	Weight of parts of plants (g)	Total F. W. (g)	Weight of parts of plants (g)	Total F. W. (g)	Weight of parts of plants (g)	Total F. W. (g)
NHS+Metal (Control)	R=0.055±0.002 S= 0.233±0.015 L= 0.221±0.012	0.510±0.029	R=0.015±0.003 S=0.253±0.016 L=0.280±0.020	0.548±0.039	R=0.009±0.001 S=0.169±0.004 L=0.230±0.011	0.408±0.016
HS+Metal	R=0.060±0.004 S= 0.388±0.021 L= 0.290±0.019	0.738±0.044	R=0.019±0.001 S=0.391±0.025 L=0.411±0.027	0.821±0.053	R=0.013±0.002 S= 0.241±0.012 L= 0.347±0.021	0.602±0.035
NHS: HS +Metal	R=0.064±0.005 S= 0.384±0.020 L= 0.412±0.029	0.860±0.054	R=0.013±0.002 S=0.379±0.023 L=0.467±0.040	0.858±0.065	R=0.014±0.003 S=0.270±0.018 L=0.376±0.023	0.660±0.044
NHS: HS +Metal + 1% HA	R=0.077±0.007 S=0.445±0.032 L=0.446±0.033	0.968±0.072	R=0.024±0.005 S=0.466±0.039 L=0.480±0.038	0.969±0.082	R=0.016±0.003 S=0.296±0.025 L=0.441±0.037	0.753±0.065

NHS=Non-humus soil; HS=Humus soil; HA=Hydroxyapatite

R=Root; S=Shoot; L=Leaf

Values are presented as arithmetic mean of three replicates ± SE

Table 2 The bioavailability of Pb and Cd in the plant grown in different soil system

Soil system	Pb			Cd		
	7 Days	14 Days	21 Days	7 Days	14 Days	21 Days
NHS+Metal (Control)	R=3.05±0.10	R=4.82±0.12	R=5.33±0.17	R=3.45±0.09	R=4.50±0.11	R=5.50±0.15
	S=2.07±0.06	S=2.94±0.09	S=2.98±0.08	S=2.44±0.07	S=2.60±0.08	S=2.60±0.08
	L=0.79±0.01	L=0.94±0.02	L=1.25±0.03	L=0.82±0.01	L=0.85±0.01	L=1.20±0.03
HS+Metal	R=1.78±0.06	R=3.77±0.13	R=3.61±0.09	R=2.00±0.07	R=3.40±0.09	R=3.65±0.10
	S=1.81±0.06	S=2.25±0.06	S=3.55±0.09	S=1.90±0.04	S=2.32±0.08	S=3.56±0.12
	L=0.56±0.01	L=0.72±0.01	L=0.81±0.01	L=0.69±0.01	L=0.69±0.01	L=1.12±0.03
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
NHS: HS +Metal	R=1.28±0.04	R=1.38±0.04	R=1.64±0.06	R=1.80±0.06	R=1.52±0.06	R=1.75±0.06
	S=1.14±0.03	S=1.21±0.04	S=1.09±0.05	S=1.84±0.06	S=1.43±0.05	S=1.69±0.05
	L=0.62±0.01	L=0.74±0.01	L=0.92±0.01	L=0.59±0.01	L=1.32±0.04	L=0.96±0.01
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
NHS: HS +Metal + 1% HA	R=1.02±0.05	R=1.06±0.05	R=1.04±0.04	R=1.20±0.04	R=1.25±0.03	R=1.20±0.04
	S=0.90±0.01	S=1.00±0.04	S=0.95±0.01	S=1.24±0.03	S=1.20±0.03	S=1.18±0.03
	L=0.21±0.00	L=0.19±0.00	L=0.26±0.00	L=1.04±0.03	L=1.12±0.03	L=1.15±0.03
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

NHS=Non-humus soil; HS=Humus soil; HA=Hydroxyapatite

R=Root; S=Shoot; L=Leaf

Values are presented as arithmetic mean of three replicates ± SE

7 days, 57 to 90% in 14 days and 62 to 85% in 21 days. The increase in the fresh weight showed the improvement in the growth of *B. juncea* after addition of humus soil and 1% hydroxyapatite as compared to the soil with out humus and hydroxyapatite. The data on bioavailability of different metals in different parts (root, shoot, leaf) of the plants grown in different soil systems in 7, 14 and 21 days are shown in Tables 2

Table 3 The bioavailability of Cr and Ni in the plant grown in different soil system

Soil system	Cr			Ni		
	7 Days	14 Days	21 Days	7 Days	14 Days	21 Days
NHS+Metal (Control)	R=3.60±0.09	R=4.45±0.14	R=5.5±0.17	R=3.50±0.09	R=4.40±0.12	R=5.47±0.14
	S=2.10±0.06	S=2.71±0.07	S=2.4±0.06	S=2.04±0.06	S=2.66±0.06	S=2.23±0.06
	L=0.77±0.01	L=0.94±0.01	L=1.2±0.02	L=0.75±0.01	L=0.96±0.01	L=1.17±0.03
HS+Metal	R=1.99±0.07	R=3.43±0.07	R=3.5±0.08	R=1.96±0.07	R=3.57±0.08	R=3.60±0.08
	S=1.78±0.06	S=2.26±0.05	S=3.4±0.08	S=1.77±0.05	S=2.32±0.06	S=3.45±0.08
	L=0.63±0.01	L=0.87±0.02	L=1.0±0.02	L=1.24±0.02	L=0.90±0.02	L=1.02±0.02
	P<0.001	P<0.001	P<0.01	P<0.001	P<0.001	P<0.02
NHS: HS +Metal	R=1.70±0.05	R=1.46±0.05	R=1.62±0.05	R=1.43±0.05	R=1.50±0.05	R=1.57±0.05
	S=1.80±0.07	S=1.40±0.05	S=1.60±0.04	S=1.44±0.05	S=1.38±0.05	S=1.47±0.04
	L=0.57±0.01	L=1.30±0.03	L=1.10±0.02	L=1.24±0.02	L=1.28±0.03	L=1.26±0.02
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001
NHS: HS +Metal + 1% HA	R=1.14±0.02	R=1.20±0.03	R=1.24±0.03	R=1.18±0.04	R=1.44±0.04	R=1.68±0.05
	S=1.12±0.02	S=1.26±0.02	S=1.16±0.02	S=1.14±0.02	S=1.24±0.02	S=1.26±0.03
	L=0.29±0.00	L=0.32±0.00	L=0.29±0.00	L=1.04±0.02	L=1.00±0.02	L=1.02±0.02
	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001	P<0.001

NHS=Non-humus soil; HS=Humus soil; HA=Hydroxyapatite

R=Root; S=Shoot; L=Leaf

Values are presented as arithmetic mean of three replicates ± SE

and 3. The total bioavailability percent of metals by plants are shown in Fig. 1a, b, c, and d. The bioavailability of Pb, Cd, Cr and Ni in non-humus metal soil system was 59%, 67%, 65% and 63% respectively in 7 days, 87%, 80%, 81% and 80% in 14 days and more than 90% in 21 days. In case of

humus soil the bioavailability of Pb decreased from 59% to 42%, Cd from 67 to 46%, Cr from 65% to 44% and Ni 63% to 44% in 7 days. In 14 days, the bioavailability of Pb decreased from 87% to 68%, Cd from 80% to 64%, Cr from 81% to 66% and Ni from 80 to 68%. Similar trend was found in 21 days, the
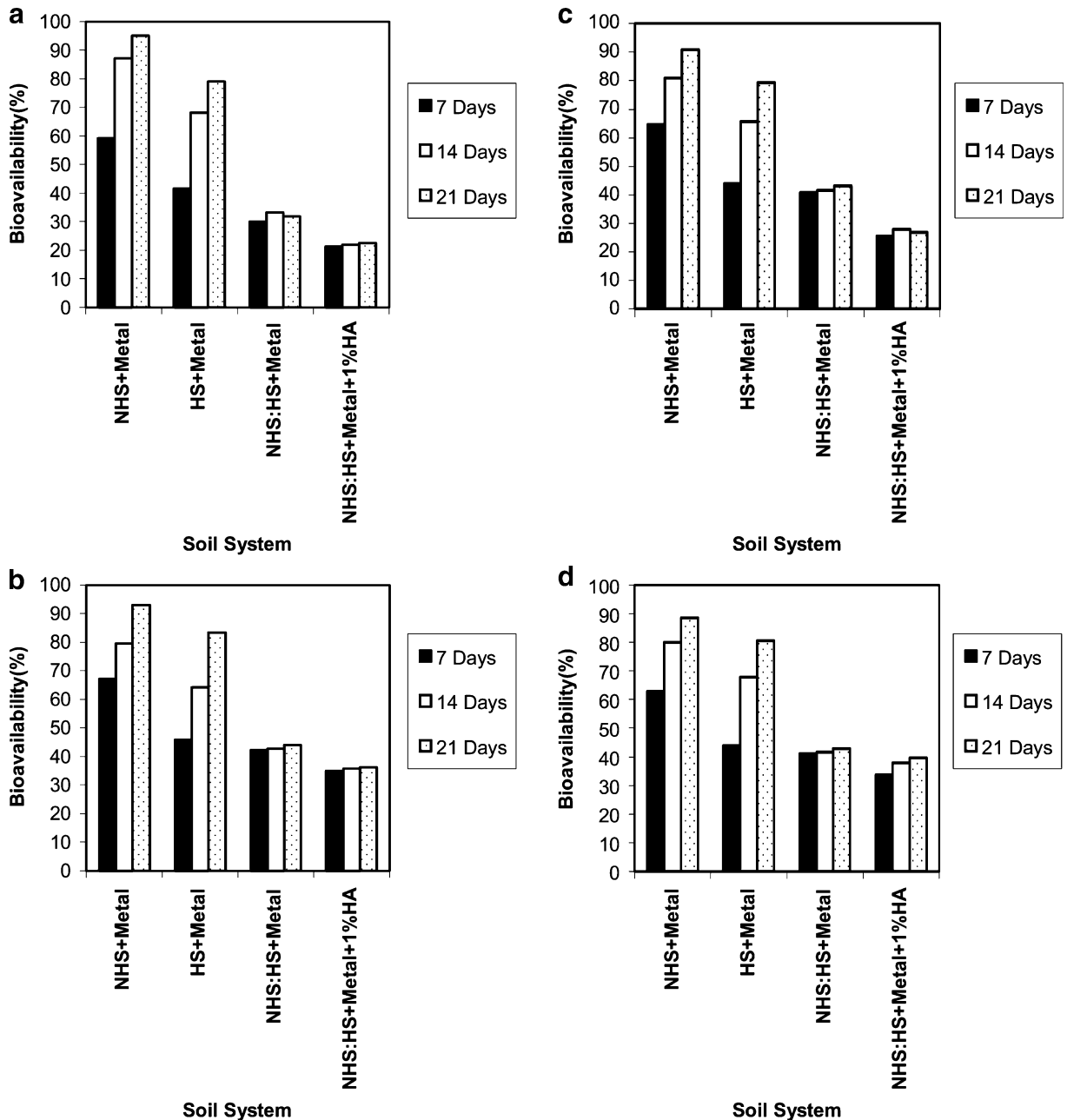

Fig. 1 **a** Percent bioavailability of Pb in different parts of the plant in different soil system. **b** Percent bioavailability of Cd in different parts of the plant in different soil system. **c** Percent bioavailability of Cr in different parts of the plant in different soil system. **d** Percent bioavailability of Ni in different parts of the plant in different soil system

bioavailability of Pb decreased from 95 to 75%, Cd from 93 to 83%, Cr 91 to 79% and Ni 89% to 81%. Use of non-humus and humus soil in the ratio of 1:3 and addition of 1% hydroxyapatite decreased the bioavailability of lead around 21 to 22.5%, Cd 35 to 36%, Cr 25.5 to 26.9%, Ni 34% to 39% in 7, 14 and 21 days.

4 Discussion

Treatment of heavy metal contaminated soil with dolomite, phosphates, calcium carbonate or organic matter help to immobilize heavy metals in soil, so that plants cannot take them. Such materials can reduce the concentration of heavy metals by precipitation, adsorption or complexation (Mench et al. 1994). The toxicity and bioavailability of heavy metals, inturn depend in part on reactivity and solubility, which are determined by the nature, concentration and physico-chemical state of metals, their speciation, solubility, specific characteristics of soil sites and nature of additives (Everhart et al. 2006; Rulkens et al. 1998).

The increase in the fresh weight of the plant *B. juncea* grown on the non-humus: humus soil (1:3) matrix and in the presence of hydroxyapatite and decrease in the uptake/bioavailability of Pb, Cr, Ni, Cd from 7 to 21 days in our study could be due to the non-availability of free/soluble metals to the plants. The reason for this may be attributed to the (a) presence of carboxylic, phenolic, amino and quinone with aromatic nucleuses of low degree of condensation, which are incorporated by parts of non-aromatic character on the surface of humus soil. The presence of aromatic nucleuses with mobile p-type electrons and various functional groups cause the ability of humus soil to ionic exchange, complexation, and oxidation-reduction reaction with metals. (b) ion exchange processes at the surface of hydroxyapatite or substitution of calcium in hydroxyapatite by other metals. Apart from this, since the ionic radius of Cd^{2+} (0.97 Å) is closer to Ca^{2+} (0.99 Å) than the radius of Ni^{2+} (0.72 Å), the isomorphic substitution of Ca^{2+} by Cd^{2+} is more favorable than the isomorphic substitution by other metals.

Ma et al. (1994) reported the removal of Pb through dissolution of hydroxyapatite followed by precipitation as hydroxypyromorphite. According to Shashkova et al. (1999) lead would be adsorbed first on the surface of the apatite particle, then, by diffusion, cation exchange would take place between apatite Ca^{2+} ions and Pb^{2+} ions in solution. Several workers have observed that addition of hydroxyapatite in the soil reduces the solubility of zinc and cadmium resulting the formation of insoluble phosphate complexes and thereby reducing the plant uptake, although not to the same extent as lead (Alloway 1995; Lambert et al. 1997). The variation in bioavailability of metal to plants depends upon the complexation sites, affinity of soil matrix and ionic radii of different metals (Singh et al. 2001). A recent study containing biosolids, alkaline cement kiln dust and rock phosphate (carbonated apatite) found that many of these amendments reduced phytoavailability and extractability of heavy metals (Basta and Gradwohl 2000). McGowen et al. (2001) reported that diammonium phosphate treatment increased retardation 2-fold for Cd, 6-fold for Zn, and 3.5-fold for lead. Apart from this, pH is another crucial factor in the extractability of heavy metals in contaminated soil (Laperche et al. 1996). Increasing the soil pH by liming resulted in immobilization of several metals (Cd, Cu, Ni, and Zn) and reduced their bioavailability in wheat, carrot and spinach by many folds (Hooda and Alloway 1996). The sorption behaviour of Zn, Cu and Cd in soils varies from soil to soil and is influenced by soil properties, such as pH, organic matter, charge on the clay and amorphous hydroxide contents, and cation exchange capacity (McBride 1989).

5 Conclusions

The potential utility of humus rich soil along with hydroxyapatite in minimizing the environmental risks of heavy metals from contaminated site through immobilization is highlighted in the present study. Out of four metals (Pb, Cr, Cd, and Ni) applied in the soil, *B. juncea.* grown on the soil system of non-humus: humus soil in the ratio of 1:3 + metal (100 ppm) and 1% hydroxyapatite showed the bioavailability of Pb (21%), Cr (26%), Cd (36%) and Ni (39%) as compared to the bioavailability of these metals in non-humus soil. The bioavailability was minimum in case of Pb and maximum in case of Ni. The data showed that the growth of *B. juncea* improved after addition of humus soil and hydroxyapatite. Further, amending the non-humus soil with

humus soil and hydroxyapatite can immobilize metals and thereby reducing the bioavailability of metals to plants.

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