Chapter 6
Phytoremediation of Heavy Metals by *Brassica juncea* in Aquatic and Terrestrial Environment

Anamika Singh and M.H. Fulekar

Abstract Plant-based environmental remediation has been widely pursued by academic and industrial scientists as a favorable low-cost clean-up technology. Phytoremediation is being developed as an alternative technology for removing or, more accurately, reducing the concentration of toxic pollutants to clean up the environment. In the present research, potential of green plants have been screened for phytoremediation of heavy metals both from aquatic and terrestrial environment. Indian mustard *(Brassica juncea)* has been found as a potential candidate for phytoremediation of heavy metals. *B. juncea* has been used for remediation of Cd, Pb and Zn at varying concentrations, *viz*., 0, 5, 10, 20 and 50 ppm. The depletion of heavy metals was observed at the intervals of 0, 1, 3, 7, 14 and 21 days and metal uptake was studied in the roots/shoots of the plants. The percentage removal of Cd, Pb and Zn was found 88.9%, 80% and 89.8%, respectively at the higher exposure concentration (50 ppm). Similarly *B. juncea* has also been used for phytoremediation of heavy metals (Cd, Pb and Zn) at varying concentrations, *viz*., 0, 5, 10, 20 and 50 mg/kg from mycorrhizal soil in pot culture technique and uptake was studied in the roots/shoots; after harvesting the plants. The uptake of metals in roots was found 25,000 μg g^{-1} – Cd, 32,750 μg g^{-1} -Pb and 30,550 μg g^{-1} –Zn; whereas uptake in shoots was found 4,596 μg g^{-1} Cd, 3,469 μg g^{-1} Pb and 15,878 μg g^{-1} Zn at higher exposure concentration (50 ppm). The research study has proved effective remediation of heavy metals (Cd, Pb and Zn) by *B. juncea* in water-soil environment.

Keywords *Brassica juncea* • Heavy metals • Metal uptake • Phytoremediation

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N.A. Anjum et al. (eds.), *The Plant Family Brassicaceae: Contribution Towards Phytoremediation*, Environmental Pollution 21, DOI 10.1007/978-94-007-3913-0_6,

Abbreviations

Cd	Cadmium
cm	Centimeter
kg	Kilogram
µg	Micro gram
µl	Micro liter
mg	Milli gram
ml	Milli liter
MS	Mycorrhizal soil
Pb	Lead
ppm	Parts per million
VAM	Vesicular arbuscular mycorrhiza
Zn	Zinc

6.1 Introduction

The industrialization and urbanization of the modern world has led to the proliferation of many different metals and compounds in our environment. Metals such as zinc, copper, nickel, manganese, magnesium, iron etc. are essential in very low concentrations for the survival of all forms of life, but are toxic in excess. Metal concentrations have a range of 1–100 mg kg^{-1} soil. If the concentration of a particular metal crosses the normal threshold, then it becomes potentially toxic and may lead to lethal changes. They build up in biological systems through food chain and become a significant health hazard. The most common heavy metal contaminants are: cadmium (Cd), chromium (Cr), copper (Cu), mercury (Hg), lead (Pb), nickel (Ni) and zinc (Zn) (USEPA 1997; Lasat 2002).

Natural contamination originates from either excessive weathering of mineral and metal ions from rocks or from displacement of certain contaminants from the groundwater or subsurface layers of the soil. The most common anthropogenic sources are human activities such as mining, smelting, electroplating, energy and fuel production, power transmission, sludge dumping, and melting operations, disposal of industrial effluents, deposition of air-borne industrial wastes, military operations, land-fill operations, industrial solid-waste disposal and the growing use of agricultural chemicals such as pesticides, herbicides and fertilizers (Okoronkwo et al. 2005; Jing et al. 2007; Igwe and Abia 2006; Lone et al. 2008). The threat of heavy metals to human and animal health is aggravated by their long-term persistence in the environment (Gisbert et al. 2003). There is an urgent need to develop multifaceted approaches for cleaning environment, which consume lesser resources and would be eco-friendly (Fulekar 2005a). Currently, conventional remediation methods of heavy metal contaminated soils include electrokinetical treatment, chemical oxidation or reduction, leaching, solidification, vitrification, excavation, and off-site treatment. These clean up processes of heavy metal pollution are expensive and environmentally destructive (Bio-Wise 2000; Aboulroos et al. 2006). In order to overcome some shortcomings of conventional methods, phytoremediation is introduced into remediation field (Meagher 2000; Wei et al. 2008).

Phytoremediation is defined as the use of plants to remove pollutants from the contaminated environment (Cunningham et al. 1995; Flathman and Lanza 1998; Salt et al. 1998; Weber et al. 2001). Actually, with some added advantages such as environmental beautification, easy acceptance by the public and potential application to a relatively large pollution area, phytoremediation of heavy metal contaminated soils is widely considered a promising remediation approach in the future (Chaney et al. 1997; Lewandowski et al. 2006; McGrath et al. 2006; Lai and Chen 2009). It utilizes the remarkable ability of the plants to concentrate or remove heavy metals from the environment. Plants are unique organisms equipped with remarkable metabolic and adsorption capabilities, as well as transport systems that can take up nutrients or contaminants selectively from the soil and water. Phytoremediation methods offer significant potential for certain application and permit a much larger site to be restored would generally be possible using more traditional remediation technologies (Fulekar 2005b). There are over 400 different plant species considered suitable to be used as phytoremediator. Indian mustard has been identified as a higher biomass producing plant with the capacity to accumulate zinc and cadmium at higher concentrations in plant cells (Kumar et al. 1995; Salt et al. 1995). After the completion of phytoremediation, the plants used for the study, can be harvested, incinerated and followed by recycling of the metals or as disposal in a landfill (Bennett et al. 2003; Angel and Linacre 2005). It results in reduction of metal contamination from the polluted site.

In the present study, Indian mustard plant has been screened and selected for the phytoremediation of heavy metals such as cadmium, lead and zinc in aquatic and terrestrial environment. Studies are conducted to determine the potential and uptake capacity of cadmium, lead and zinc by *Brassica juncea* from aquatic and terrestrial environment under controlled environmental conditions.

6.2 Screening of Plants

The success of phytoremediation depends on screening and selection of an ideal plant. A plant suitable for phytoremediation possesses the characteristics: ability to tolerate/accumulate metals, fast growth, effective accumulating capacity, high biomass and easily harvestable. Researchers have reported that plant species that are long-term competitors and survivors under adverse conditions normally have an advantage for phytoremediation. They adapt self operative defensive mechanism to survive in adverse environmental conditions. A screening for phytotoxicity and effectiveness of plant's cultivars/varieties is required on a site-specific basis as an initial step in plant selection (Pivetz 2001; Schnoor 2002). The plants which have potential to survive and grow in the contaminated environment are screened and reported by the Scientists for phytoremediation of heavy metals and radionuclides. The green plants which survive in such adverse conditions, but not exploited for phytoremediation of heavy metals have been studied and screened for the present research work. After studying the toxicity of heavy metals at varying concentrations, *Brassica juncea* has been screened as suitable plant for phytoremediation of heavy metals. Indian mustard (*Brassica juncea*), also known as mustard greens, and leaf mustard, is a species of mustard plants and belongs to the family Brassicaceae. The plant was selected for the phytoremediation study based on their fast growth rate/ high biomass along with the tolerance potential to thrive in heavy metals contaminated environment. This plant is used to remove heavy metals from the soil in the hazardous waste sites because it has a higher tolerance towards most of the toxic metals and very high capability of storing them in its parts. *Brassica juncea* is a good candidate for efficient phytoextraction of heavy metals such as Cd from polluted soils (Schneider et al. 1999). The plant is then harvested and disposed off properly. This method is easier and less expensive than traditional methods for the removal of heavy metals. It also prevents erosion of soil from these sites preventing further contamination.

6.3 Methodology Adapted

6.3.1 Plant Materials

Healthy seeds of Indian mustard (*Brassica juncea* L. Czern and Coss) var. TM4 (Trombay Mustard 4) were selected for the research studies. Mustard seeds were obtained from gamma field, Bhabha Atomic Research Centre, Mumbai. Seeds were pre-soaked in soap and dettol solution for 15 min and thoroughly washed in running tap water until the soap solution was completely removed. Then the seeds were sterilized with 70% ethanol for 30 s followed by sterilization with 0.1% mercuric chloride for 3–5 min. The seeds were thoroughly rinsed 5 times with sterile distilled water. These sterilized seeds were inoculated in test tubes containing MS (Murashige and Skoog 1962) basal medium supplemented with 3% sucrose. Seedlings were allowed to grow for 1 month under *in vitro* condition.

6.3.2 Hydroponic Experimental Setup

Uniform plants were selected for the uptake study. MS medium was drained off and replaced with hydroponics media i.e. Hoagland solution (Hoagland and Arnon 1938) containing nutrient solution for the acclimatization for 1 week prior to the experiment. They are then transferred to another Hoagland solution which contained each of the following heavy metal in a separate set up: Cadmium supplied as Cd $(NO_3)_2$. $4H_2O$; lead supplied as Pb $(NO_3)_2$; and zinc supplied as $ZnSO_4$. The different concentrations of metals used in these studies were 5, 10, 20, and 50 µg ml^{-1} with control. Each experiment was carried out in triplicates. Plants grown in nutrient solution without metals served as control. The sampling from aqueous solution containing metals has been carried at an interval of 0, 1st, 3rd 7th, 14th and 21st days *in vitro* condition to ensure that uptake of each metal is being taking place (Anamika et al. 2009). 500 µl of aliquots were withdrawn from each concentration with increasing period. These samples were used for the analysis of cadmium, lead and zinc content. The reduction in concentration of these metals in the medium was attributed to their uptake by the plants. At the end of the experiment the plant samples were collected and washed with de-ionized water twice and rinsed with distilled water. Each sample was divided into root and shoot and oven-dried at 60°C until completely dry. Dry weights of roots and shoots were determined and noted.

6.3.3 Soil Sampling and Characterization

The alluvial soil used in the pot experiment, was collected from a depth of about 0–15 cm along the banks of Surya River, Palghar (located 100 km away from Mumbai). The soil was air-dried and then passed through 2 mm sieve, and large stones and plant root debris were removed. This prepared soil was stored in a plastic bag at room temperature (27–30°C) until the use. The physico-chemical characteristics of the soil were measured by standard methods (Table 6.1). The content of heavy metals (Cd, Pb and Zn) in soil was estimated by atomic absorption spectrophotometer (APHA 1998).

6.3.4 Mycorrhizal Inoculum

Soil based mycorrhizal inoculum was developed by pot-culture technique at laboratory scale with the help of starter inoculums and using sorghum as a host plant. A starter culture of mycorrhizal fungi (VAM) was procured from Division of Microbiology, IARI, New Delhi. The physico-chemical parameters (Table 6.1) and microbial characterization (Table 6.2) of soil were done after the development of mycorrhizal soil. The developed mycorrhizosphere provide a direct link between soil and roots, and are renowned for their ability to increase plant mineral nutrients, notably P and enhance phytoremediation (Leyval et al. 1997; Gaur and Adholeya 2004; Bush 2008; Anamika and Fulekar 2010).

Table 6.1 Physico-chemical characterization of Alluvial soil and mycorrhizal soil[a]

Parameters	Methods used	Alluvial soil	Developed mycorrhizal soil
pH	APHA (1998)	7.2	7.3
Electrical conductivity (mMohs)	APHA (1998)	0.2	0.34
Moisture (%)	APHA (1998)	35	42.2
Water holding capacity (%)	APHA (1998)	65	67
Organic carbon (g/kg)	Walkley-Black method (Jackson 1973)	72	259
Nitrogen (g/kg)	APHA (1998)	5.8	8.4
Phosphorus (g/kg)	APHA (1998)	0.72	0.81
Sodium (mg/kg)	APHA (1998)	23	32
Potassium (mg/kg)	APHA (1998)	21	22
Heavy metal (ppm)	APHA (1998)		NA
Zn	APHA (1998)	10.5	
Cd	APHA (1998)	BDL	
Pb	APHA (1998)	BDL	

APHA American Public Health Association, *BDL* Below Detection Limit
[a]All the values are mean of three replicates

Table 6.2 Microbial status of developed mycorrhizal soil

Microorganisms	Species
Bacteria	*Alcaligens* spp.
	Bacillus spp.
	Pseudomonas spp.
	Sarcina spp.
	Serratia spp.
	Streptococcus spp.
Fungi	*Asergillus flavus*
	A. fumigatus
	A. niger
	Penicillium spp.
	Rhizopus spp.
	Mucor spp.
Actinomycetes	*Micromonospora* spp.
	Nocardia spp.

6.3.5 Pot Culture Experiment

Pot culture experiments were conducted in the green house. The growth medium in the pots consisted of alluvial soil and mycorrhizal inoculum (5:1) and treated as Mycorrhizal soil (MS). The alluvial soil without the mycorrhizal inoculum was treated as control. Mycorrhizal soil filled in each 1 kg capacity of pot (having perforated base for proper aeration and drain connecting system) and amended with heavy metals: Cd as Cd $(NO_3)_2$. $4H_2O$; Pb as Pb $(NO_3)_2$ and Zn as $ZnSO_4$, separately. The various concentrations applied for each heavy metal was; 0, 5, 10, 20, 50 mg kg^{-1}.

The healthy seeds of Indian mustard (*Brassica juncea* L. Czern and Coss) var. TM4 (Trombay Mustard 4) were surface-sterilized with 0.1% mercuric chloride for 5 min and subsequently washed several times with distilled water to avoid fungal contamination. Six seeds were sown into each pot and the pots were randomly placed in a green house at an average diurnal temperature of 25–27°C, and a relative humidity 40–60%. Plants were watered to maintain soil moisture at 60–70% of water holding capacity by adding water during the experiment. One hundred milli liter of Hoagland solution (Hoagland and Arnon 1938) was given to growing plants once in a week. The drainage collected at the bottom of the pot was also added in the pots to avoid the loss of metals through leachate. The plants were grown for a period of two and half months.

6.3.6 Analytical Methods

Each sample (dried root and shoots) was digested with 10 ml of perchloric acid: nitric acid ($HClO_4$: HNO_3- 1:5 v/v) mixture separately. Acid digestion was carried out on hot plate at 70–100°C until yellow fumes of HNO_3 and white fumes $HClO_4$ were evolved. The digestion process was continued until a clear solution remains after volatilization of acids. The digestion was stopped when the residue in the flask was clear and white. The digested sample was dissolved in distilled water, filtered for the removal of impurities (APHA 1998) and made up to the desired volume. The samples were analyzed by GBC 932 B+ Atomic Absorption Spectrophotometer (Australia) using air-acetylene flame to estimate cadmium, lead and zinc contents in the plant samples.

6.3.7 Data Analysis

The heavy metals are taken up by the plants through their roots from the solution. Experimental data were analyzed for uptake of cadmium, lead and zinc by Indian mustard plants. The experiment was carried out in triplicates and average values are reported. Data were analyzed for mean and standard deviation ($X \pm S.D.$) using standard statistical methods (Mahajan 1997).

6.4 Results

6.4.1 Phytoremediation of Heavy Metals in Aquatic Environment

The remediation of heavy metals (Cd, Pb and Zn) were carried out using *Brassica juncea* (Indian Mustard) from aquatic environment at the concentrations ranging from 0, 5, 10, 20 and 50 ppm for a period of 21 days. The healthy plants of *B. juncea* were grown in Hoagland solution spiked with various concentrations of Cd, Pb and Zn, separately. After the growth, the plants were harvested and analyzed for biomass and metal uptake of heavy metals in the roots/ shoots.

6.4.1.1 Depletion of Metals from the Solution

Metal depletion was studied to understand the potential of plant- *B. juncea* at various exposure levels. Figure 6.1a–c demonstrates the depletion of Cd, Pb and Zn from aquatic environment after 21 days of exposure period. *B. juncea* has remediated 35.2–88.9% Cd, 26–80.1% Pb and 30–89.8% Zn from lower to higher concentration of heavy metals. Pb uptake was found lower than Cd and Zn by *B. juncea*.

6.4.2 Dry Biomass Analysis

Brassica juncea was harvested after 21 days of phytoremediation of heavy metals from aquatic environment. Biomass of plant was found to be 0.009 g in roots and 0.063 g in shoots for Cd, 0.028 g in roots and 0.107 g in shoots for Pb and 0.015 g in roots and 0.061 g in shoots for Zn at 50 ppm exposure; after 21 days period. The significant difference in biomass was found for each metal exposure with increasing period as demonstrated in Table 6.3. The statistical analysis has been given for each heavy metal (Cd, Pb and Zn) for minimum 0–50 ppm concentrations. Result shows that *B. juncea* has tolerance potential to grow in contaminated environment and effectively phytoremediated metals.

6.4.3 Bioaccumulation of Metals in the Roots and Shoots of Plants

Bioaccumulation of metals has been studied in root/shoots of plant- *B. juncea*. Bioaccumulation of Pb, Cd and Zn in roots was found to be 12,264, 18,419 and 26,517 μg g^{-1}, respectively at higher exposure concentration (50 ppm), after 21 days exposure period. Whereas bioaccumulation of Pb, Cd and Zn in shoots of *B. juncea* was found 2,477, 3,349 and 2,585 μg g^{-1}, respectively at 50 ppm after phytoremediation of heavy metals in aquatic environment. Result shows that all the metals studied have bioaccumulated more in roots as compared to shoots (Fig. 6.2a–c) of *B. juncea*.

Phytoremediation studies show that Pb- 1,258 μg g^{-1}, Cd- 2,230 μg g^{-1} and Zn- 2,250 μg g^{-1} in roots of the plants at lower exposure level (5 ppm); whereas bioaccumulation of metals in shoots was recorded 254 μg g^{-1} Pb, 344 μg g^{-1}

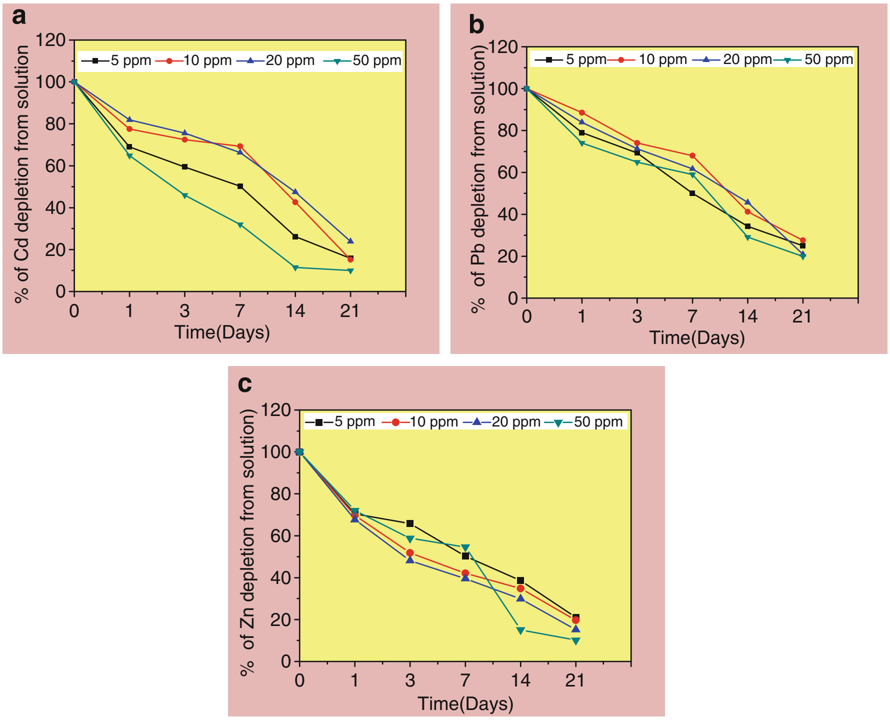

Fig. 6.1 Depletion of (**a**) Cd, (**b**) Pb and (**c**) Zn from the solution during phytoremediation experiments. The results show the depletion at various concentrations of Cd, Pb and Zn. All the values are mean of three replicates

Table 6.3 Dry biomass of *B. juncea* after 21 days of exposure to the heavy metals contaminated Hoagland solution[a]

Metal	Concentration (ppm)	Dry weight (g)	
		Roots	Shoots
Zn	Control	0.020 ± 0.008	0.063 ± 0.012
	5	0.016 ± 0.003	0.057 ± 0.007
	10	0.014 ± 0.002	0.056 ± 0.003
	20	0.013 ± 0.004	0.057 ± 0.008
	50	0.015 ± 0.004	0.061 ± 0.009
Cd	Control	0.011 ± 0.002	0.080 ± 0.017
	5	0.008 ± 0.004	0.076 ± 0.019
	10	0.005 ± 0.002	0.055 ± 0.006
	20	0.007 ± 0.003	0.063 ± 0.014
	50	0.009 ± 0.004	0.079 ± 0.009
Pb	Control	0.037 ± 0.007	0.124 ± 0.007
	5	0.022 ± 0.003	0.064 ± 0.009
	10	0.014 ± 0.004	0.072 ± 0.013
	20	0.017 ± 0.002	0.095 ± 0.017
	50	0.028 ± 0.005	0.107 ± 0.014

[a]Values are averages of three replicates ± S.D.

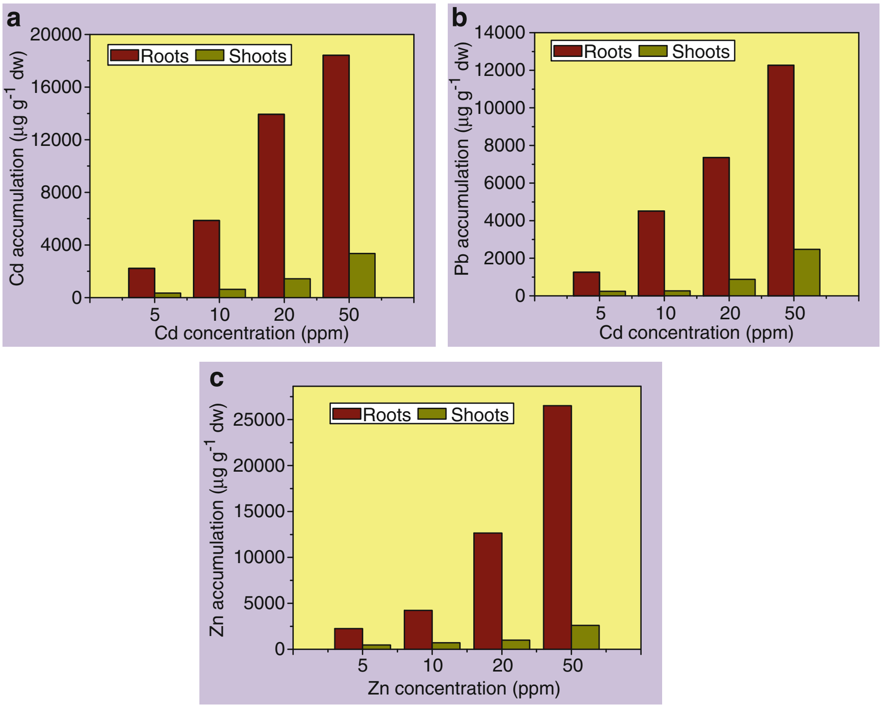

Fig. 6.2 Accumulation of (**a**) Cd, (**b**) Pb and (**c**) Zn in the roots and shoots of *B. juncea*. All the values are mean of three replicates

Cd and 471 µg g^{-1} Zn. The bioaccumulation of metals increased with increasing concentrations and found 12,517 µg g^{-1} Pb, 18, 419 µg g^{-1} Cd and 26,517 µg g^{-1} Zn in the roots of *B. juncea;* whereas 2,477 µg g^{-1} Pb, 3,349 µg g^{-1} Cd and 2,585 µg g^{-1} Zn in shoots at higher exposure level (50 ppm). The comparison of data analysis revealed that Cd was more accumulated in shoots than roots as compared to Zn and Pb. Metal accumulation in *B. juncea* was found to be 5.4, 4.9 and 5.96 times higher in roots as compared to the shoots in case of cadmium, lead and zinc respectively. The data showed that the heavy metals accumulation by *B. juncea* were found in order of Zn $>$ Cd $>$ Pb. The comparison has been made to demonstrate concentration of Pb, Cd and Zn in roots and shoots of *B. juncea* at varying concentrations. The results of the present study have shown that *B. juncea* has the potential for the uptake and accumulation of cadmium and lead. This plant may be of practical use for the decontamination of polluted water and soil containing these metals.

Brassica juncea – an identified potential green plant has been used for phytoremediation of heavy metals from aquatic environment. The depletion of heavy metals in aquatic solution and uptake by plants with increasing exposure period have been observed. After the proper growth, the plant biomass, uptake of each selected heavy metals in roots/shoots of the plants including their translocation factor to assess bioaccumulation potential of plants have been studied. The biomass of *B. juncea* was found higher upto the concentration of 10 ppm in case of Cd and Pb, and thereafter decreased as the exposure levels of Cd and Pb increased i.e. 20 and 50 ppm. Zn has shown positive effect on plant biomass. Heavy metals were found efficiently taken up mainly by the roots of *B. juncea* plants at all the evaluated concentrations. Similar findings were reported by Jadia and Fulekar (2008) for uptake of heavy metals (Cd, Cu, Ni, Pb and Zn) by fibrous root grass. Once metal ions are absorbed, they can be accumulated in the roots or be exported to the shoots via the transpiration stream (Ximenez-Embun et al. 2001).

Research has shown that metal concentration in plant tissues is a function of the heavy metals content in the growing environment (Cui et al. 2004), and that the uptake and accumulation of different metals may vary from plant to plant species. Kim et al. (2003) suggested that such discrepancies arise due to variation in type of heavy metals, its concentration, form of metal present and plant species. Different metals are differently mobile within a plant; cadmium and zinc are more mobile than lead and copper (Greger 2004). Cd is one the most toxic heavy metals due to its high mobility and the small concentration at which its effects on the plants begin to show (Vázquez et al. 1992).

Our results further showed that Pb is accumulated more in roots as compared to the other two metals (Zn and Cd). Pb uptake studies in plants have demonstrated that roots have an ability to take up significant quantities of lead whilst simultaneously greatly restricting its translocation to above ground parts (Lane and Martin 1977). Liu et al. (2000) have reported that *B. juncea* has considerable ability to remove lead from solutions and accumulate it in roots. Kumar et al. (1995) have also reported the higher accumulation of lead in roots of sorghum species, with indications that lead can be found on the outer surface of plant roots, as crystalline or amorphous deposits, and could be deposited in the cell walls or in vesicles.

The Cd and Zn uptake were found to be higher in shoot as compared to Pb. Zn and Cd have many physical and chemical similarities as they both belong to Group II of the periodic table. They are usually found together in the ores and compete with each other for various ligands. Thus the interaction between Zn and Cd in the biological system is likely to be similar. The fact that cadmium is a toxic metal and Zn is an essential element makes this association interesting as it raises the possibility that the toxic effects of cadmium may be preventable or treatable by Zn (Chowdhury and Chandra 1987).

6.4.4 Phytoremediation of Heavy Metals in Mycorrhizosphere

The research work carried out for phytoremediation of heavy metals by alfalfa in mycorrhizal soil as discussed above has been followed for *Brassica juncea*. Phytoremediation of cadmium, lead and zinc at varying concentrations viz. 0, 5, 10, 20 and 50 ppm using *B. juncea* in the mycorrhizospheric soil for a period of two and half months has been studied.

Table 6.4 Dry biomass of *B. juncea* after two and half months exposure to the mycorrhizal soil enriched with heavy metals[a]

Metal	Concentration (mg/kg)	Dry weight (g)	
		Roots	Shoots
Zn	Control	0.031 ± 0.003	0.472 ± 0.030
	5	0.050 ± 0.005	0.506 ± 0.043
	10	0.059 ± 0.004	0.622 ± 0.047
	20	0.045 ± 0.003	0.533 ± 0.024
	50	0.037 ± 0.004	0.486 ± 0.030
Cd	Control	0.031 ± 0.003	0.472 ± 0.030
	5	0.049 ± 0.004	0.522 ± 0.029
	10	0.045 ± 0.008	0.472 ± 0.041
	20	0.034 ± 0.005	0.326 ± 0.019
	50	0.021 ± 0.004	0.297 ± 0.046
Pb	Control	0.031 ± 0.003	0.472 ± 0.030
	5	0.045 ± 0.004	0.500 ± 0.018
	10	0.049 ± 0.002	0.466 ± 0.035
	20	0.036 ± 0.002	0.396 ± 0.013
	50	0.027 ± 0.005	0.288 ± 0.038

[a]Values are averages of three replicates ± S.D.

6.4.4.1 Dry Biomass Analysis

The dry biomass of *B. juncea* was recorded and presented in Table 6.4 for Cd, Pb and Zn metals at varying concentrations ranging from 5 to 50 ppm. Biomass of *B. juncea* was found to be 0.021 g in roots and 0.297 g in shoots for Cd, 0.027 g in roots and 0.288 g in shoots for Pb and 0.037 g in roots and 0.486 g in shoots for Zn at 50 ppm of exposure level over a period of two and half months in mycorrhizosphere pot culture experiment. *B. juncea* has produced high biomass in case of Zn as compared to Cd and Pb. Biomass decreased gradually as the concentration of Cd and Pb increased (i.e. 50 ppm) in mycorrhizal soil. The plant's biomass yield affected at the higher ppm levels exposure of Cd. Lead showed low effect on plant biomass. The positive effect has been seen in Zn concentrations exposure from lower to higher, resulting into high biomass yield.

6.4.4.2 Bioaccumulation of Metals in the Roots and Shoots of Plants

The study shows that heavy metals were efficiently taken at all concentrations using high biomass producing plant *B. juncea* grown in mycorrhizal soil. The mean uptake of metals Cd, Pb and Zn by *B. juncea* increased as the concentrations of these metals in mycorrhizal soil increased. In plant, shoots and roots were observed to have a characteristic uptake capacity for different metals. The research findings demonstrated that uptake of Cd from mycorrhizosphere by *B. juncea* was 2,783 μg g^{-1} at 5 ppm and 25,000 μg g^{-1} at 50 ppm in roots and 838 and 4,596 μg g^{-1} at minimum to maximum concentrations (0–50 ppm) in shoot, respectively. The uptake of Pb in roots was recorded 2,938 and 32,750 μg g^{-1} at

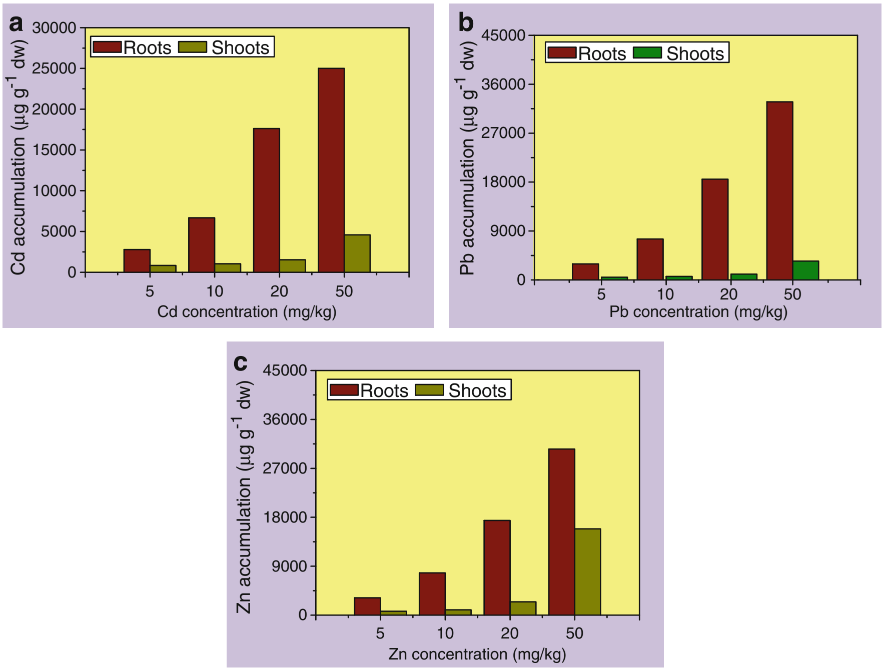

Fig. 6.3 Accumulation of (**a**) Cd, (**b**) Pb and (**c**) Zn in the roots and shoots of *B. juncea* during metal remediation from mycorrhizosphere soil. All the values are mean of three replicates

5 and 50 ppm, while in shoots it was found 486 µg g^{-1} at 5 ppm and 3,469 µg g^{-1} at 50 ppm. However, the uptake of Zn was found more as compared to Cd and Pb, reached highest accumulation, i.e. 30,550 µg g^{-1} in roots and 15,878 µg g^{-1} in shoots at highest concentration (50 ppm). The heavy metals were taken by the *B. juncea* in the following order: Zn > Cd > Pb. These results could be seen in Fig. 6.3a–c.

The accumulation of Cd, Pb and Zn was compared in roots and shoots of *B. juncea*. The metal uptake in roots was found 2,783 µg g^{-1} (Cd), 2,938 µg g^{-1} (Pb) and 3,175 µg g^{-1} (Zn) at lower doses (5 ppm). The uptake of metals in roots was increased upto 25,000 µg g^{-1} – Cd, 32,750 µg g^{-1} -Pb and 30,550 µg g^{-1} -Zn at higher concentration- 50 ppm. Similarly, the uptake of metal in shoots was found to be 838 µg g^{-1} Cd, 486 µg g^{-1} Pb and 705 µg g^{-1} Zn at lower doses (5 ppm); whereas uptake increased to 4,596 µg g^{-1} Cd, 3,469 µg g^{-1} Pb and 15,878 µg g^{-1} Zn at higher doses (50 ppm). The comparison of data analysis shows that *B. juncea* was found to accumulate 5.4 times more Cd, 9.4 times more Pb and 1.9 times more Zn in roots as compared to the shoots. The research findings show that Pb was bioaccumulated more in roots than shoots of plants as compared to Cd and Zn.

Phytoremediation of heavy metals using *B. juncea* has been studied in mycorrhizal soil using pot culture technique. The results showed an increasing trend for biomass production (Cd and Pb) as the concentrations increased from 5 to 10 mg/kg.

The positive effects have been seen in case of Zn concentrations. However, the biomass yield was found only affected at the higher concentrations, i.e. 20 and 50 mg/kg of Cd and Pb that shows inhibitory effect on plant growth. Higher doses of heavy metal can affect physiology, reduced plant growth and dry biomass. It is reported that Mycorrhizal fungi helps/ protects the plants against metal toxicity, however restricts the translocation of metals from root to shoot. Therefore, in the present research there were no toxicity symptoms like chlorosis, retarded growth etc. observed in case of *B. juncea* plants. In the present research, the significant effect on plant growth in mycorrhizosphere was observed. *M. sativa* plants have produced better biomass which results in higher uptake of heavy metals in mycorrhizosphere. The results showed the enhanced nutrients availability as well microbial communities in mycorrhizal soil, which favour the uptake of heavy metals by green plants. Higher levels of organic matter and nutrients content in the mycorrhizosphere had beneficial influence on soil chemical and biochemical properties and plant growth, thus increasing biomass yields. Various researchers have reported the similar findings where the mycorrhizosphere was shown to change soil structure by stabilizing aggregates (Miller and Jastrow 1990; Bearden and Petersen 2000; Augé et al. 2001), thereby enhancing soil-HM retention capacity in the plants.

The results showed that *B. juncea* efficiently bioaccumulated heavy metals at the varying concentrations range of 5, 10, 20 and 50 mg/kg. The accumulation of Cd, Pb and Zn was found increased with increasing concentrations of metals in the mycorrhizosphere. Root of the plants is the primary source of metals accumulation followed by shoot. The root of plants is being in the direct contact with mycorrhizal soil able to take up the heavy metals, along with their nutrients uptake ability. Once the metal is taken by the root of plants, it translocated to shoot via vascular tissues. In the soil-mycorrhizosphere, the mycorrhizal hyphae also help to take up the heavy metals from contaminated environment.

6.5 Conclusions

The green plant *Brassica juncea* has been identified as potential candidate for remediation of heavy metals – Cd, Pb and Zn from aquatic/ terrestrial environment. The research study shows that the plant has grown in contaminated environment and produced significant biomass (roots/shoots) which has found directly proportionate to uptake of heavy metals. The bioaccumulation of heavy metals was found higher in roots than the shoots of *B. juncea* for each of the heavy metal studied. The present study has proved the effective remediation of heavy metals by the green plant which could be buried or disposed off at safe environment. The higher concentrations of heavy metals bioaccumulated in plants can also be recovered and reused. Therefore, phytoremediation study will be beneficial for decontamination of heavy metals contaminated environment and/or extraction of metals for beneficial purpose.

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