*Journal of Applied Biosciences* (2009), Vol. 13: 726 - 736.
ISSN 1997 – 5902: www.biosciences.elewa.org

# Phytoremediation of Cadmium, Lead and Zinc by *Brassica juncea* L. Czern and Coss

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ABSTRACT

*Objectives*: To determine the potential of *Brassica juncea* to take up heavy metals (Cd, Pb and Zn) from aquatic environment.

*Methodology and results*: The uptake of Cadmium (Cd), Lead (Pb) and Zinc (Zn) was studied at various concentrations, i.e. 0, 5, 10, 20 and 50 µg $ml^{-1}$in Steinberg's solution over a period of 21 days. After 21 days, the plants were harvested, dried and the root and shoot biomass weighed. The uptake of each metal was studied in the root and shoot respectively, to determine the bioaccumulation coefficient of metals in *B. juncea.* The translocation factor was calculated so as to study the efficiency of the plants to bioaccumulate each metal in roots and shoot. The result showed that the heavy metals accumulated more in roots than in the shoots. When plants were exposed to the higher concentration (50 µg $ml^{-1}$) of Cd or Pb, the metals were present at an average of 18.42 and 12.27 mg $g^{-1}$ tissue in the root, respectively, and at 3.35 and 2.48 mg $g^{-1}$ tissue in the shoots, respectively. The average concentration of zinc was 26.52 mg $gm^{-1}$ and 2.59 mg $g^{-1}$ in root and shoot respectively, when exposed to 50 µg $ml^{-1}$ of zinc.

*Conclusions and application of findings*: *Brassica juncea* has been found to have high potential to remediate Cd, Pb and Zn from aquatic environment with up to a maximum concentration of 50 µg $ml^{-1}$. This plant can therefore be grown in aquatic environment that are contaminated with heavy metals, after which the plant biomass can be harvested and burned to ash to recover the metals or to be disposed of appropriately and safely.

Key words: *Brassica juncea*, phytoremediation, heavy metals, bioaccumulation, translocation

Citation: Anamika S., Susan Eapen and M.H.Fulekar, 2009. Phytoremediation of Cadmium, Lead and Zinc by *Brassica juncea* L. Czern and Coss. *Journal of Applied Biosciences* 13: 726 - 736.

## INTRODUCTION

Metals are released into the environment from the natural weathering processes of the Earth's crust, soil erosion, mining, industrial discharge, urban runoff, sewage effluents, air pollution, pest or disease control agents applied to plants, among other sources (Fargasova, 1994; Alloway, 1995; Raskin & Ensley, 2000). Most of the heavy metals are extremely toxic because, as ions or in certain compounds, they are soluble in water and may be readily absorbed into plant tissues. Once released, it is difficult to remediate such heavy metals from contaminated soil-water environments as they cannot be degraded (Pilon-Smith, 2005). However, there are various physical and chemical methods which can be used for the removal of heavy metals from contaminated environments.

Some of the current practices for remediating heavy metals rely on "dig-and-dump" or encapsulation, neither of which addresses the issue of decontamination of the environment (Mai *et al*., 2003). Conventional cleanup technologies are generally too costly to be used to restore contaminated sites (Holden, 1989; Smith *et al*., 1995; Bio- Wise, 2003; Aboulroos *et al.,* 2006). In order to overcome the shortcomings of physical and chemical methods, phytoremediation has been proposed (Utsunamyia, 1980; Chaney, 1983; Baker *et al*., 1991; Wei *et al*., 2008). This technology can be defined as the use of green plants to reduce, remove, degrade or immobilize environmental pollutants/toxins from soil, water or sediments, with an aim of restoring sites to a condition useable for private or public applications (Cunningham *et al*., 1995; Flathman & Lanza, 1998; Salt *et al*., 1998; Weber *et al*., 2001; Wendy *et al.* 2006). The effectiveness of phytoremediation technology depends on the selection of appropriate plant or plant species. Plants are unique organisms that are 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 (Fulekar, 2005).

The remediation of heavy metals in aquatic environments depends on the concentration of metals, plant species, pH and the nutrients available for plant growth. The potential use of a plant in remediation can be assayed by exposing it to the toxic metal in the presence of nutrient medium. Suitable nutrient medium i.e. Stenberg medium has been taken for the proper growth of the plants. The metal uptake by plant roots can be enhanced at pH 5.5- 5.6. The growth of the plants in Steinberg medium spiked with different concentrations of each metal was observed and the metal contamination assessed at various intervals. After the completion of phytoremediation, the plants used can be harvested and incinerated followed by recycling of the metals or disposal in a landfill (Bennett *et al*., 2003; Angel & Linacre, 2005). This would result in reduction of metal contamination from the polluted site.

The present study aimed to study the potential of *Brassica juncea* (Indian mustard) to take up heavy metals such as cadmium (Cd), lead (Pb) and zinc (Zn) from aquatic environments.

## MATERIALS AND METHODS

**Plant materials:** Healthy seeds of Indian mustard *(Brassica juncea* L. Czern and Coss) var. Tm4 (Trombay Mustard 4) were selected for the study and procured from Gamma Field, Bhabha Atomic Research Centre, Mumbai. Seeds were pre-soaked in soap and disinfectant (Dettol®) solution for 15 minutes and thoroughly washed in running tap water until the soap solution was completely removed. The seeds were sterilized with 70% ethanol for 30 seconds followed by sterilization with 0.1% mercuric chloride for 3-5 min, and thoroughly rinsed 5 times in sterile distilled water. The sterilized seeds were inoculated in test tubes containing MS (Murashige & Skoog, 1962) basal liquid (broth) medium supplemented with 3% sucrose. Seedlings were allowed to grow for one month *in vitro*.

**Experimental setup:** Uniform plants were selected for the study. MS medium was drained off and replaced with hydroponics media i.e. Steinberg solution (Steinberg, 1953) containing nutrients, and plants allowed to acclimatize for 1 week prior to starting the experiment. After a week plants were transferred to fresh Steinberg solution which contained Cadmium supplied as $Cd(NO_3)_2 \cdot 4H_2O$, or lead supplied as $Pb(NO_3)_2$ or zinc supplied as $ZnSO_4$. The different concentrations of metals used in the study were 5, 10, 20 and 50 μg $ml^{-1}$. Plants grown in nutrient solution

*Journal of Applied Biosciences (2009), Vol. 13: 726 - 736.*
*ISSN 1997 – 5902: www.biosciences.elewa.org*

without metals served as control. Each treatment was carried out in triplicates.

The sampling of aqueous solution containing metals was done on day 0, 1, 3, 7, 14 and 21 (Anamika *et al*., 2008). Aliquots of 500 µl were withdrawn from each plant growth medium at each sampling, and analysed for cadmium, lead and zinc content. 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. Dry weights of roots and shoots were determined.

Analytical methods: Each sample (dried root and shoots) was digested with 10 ml of a mixture of perchloric acid: nitric acid ($HClO_4$: $HNO_3$- 1:5 v/v). Acid digestion was carried out on hot plate at 70-100 °C until yellow fumes of $HNO_3$ and white fumes $HClO_4$ were observed. The digestion process was continued until a clear solution remained after volatilization of acids, and was stopped when the residue in the flask was clear and white. The digested sample was dissolved in distilled water, filtered to remove 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.

Data analysis: Data were analyzed for mean and standard deviation (X±S.D.) using standard statistical methods (Mahajan, 1997). The ability of plants to translocate heavy metals from the roots to the harvestable aerial part is defined as translocation factor, TF (Mattina *et al*., 2003). The phytoextraction rate or bioaccumulation coefficient was described as the heavy metal concentration in plant divided by heavy metal concentration in the solution (Nanda-Kumar *et al*., 1995). Translocation factor and bioaccumulation coefficient were computed using the following formulae:

$$\text{Translocation factor (TF)} = \frac{\text{Shoot metal content}}{\text{Total plant metal content}} \times 100$$

$$\text{Bioaccumulation Coefficient (BC)} = \frac{\text{Cadmium content g}^{-1}\text{ Dry plant tissue}}{\text{Cadmium content ml}^{-1}\text{ nutrient solution}}$$

## RESULTS AND DISCUSSION

The study investigated remediation of heavy metals from aquatic environment using green plant *B. juncea.* After 21 days of uptake, the heavy metals were depleted from the growth solution indicating successful absorption of cadmium, lead and zinc by *B. juncea* (Anamika *et al.,* 2008). The reduction in concentration of these metals in the medium was attributed to their uptake by the plants. Figure 1 (a, b, c) demonstrates the depletion of metals from the aquatic solution between the 1$^{st}$ and the 21$^{st}$ day showing cadmium (35.2 - 88.9%), lead (26 - 80.1%), and zinc (30 - 89.8%), respectively. The metals were taken up in amounts directly proportional to their concentration in the solution proportionately over the entire period of 21 days (Figure 1a, 1b & 1c). Zinc uptake by *B. juncea* was found to be highest as compared to cadmium and lead.

The plant biomass was determined with respect to uptake of cadmium, lead and zinc by *B. juncea.* There were no significant differences in the biomass of *B. juncea* when exposed to cadmium, lead and zinc at various concentrations ranging from 0 to 50 µg ml$^{-1}$ (table 1). This shows that *B. juncea* is tolerant and has potential to grow in contaminated environments and to efficiently take up heavy metals.

In the harvested plant biomass, the metals showed an increasing trend as their concentration in the nutrient solution increased (figure 2). Heavy metals were 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). Our study showed higher accumulation of all metals (cadmium, zinc and lead) in the roots than in the shoots. The maximum accumulation of cadmium (at 50 µg ml$^{-1}$) in roots and shoots of *B. juncea* were 18.42 mg gm$^{-1}$ and 3.35 mg gm$^{-1}$ , respectively, while the highest accumulations of Pb (at 50 µg ml$^{-1}$) averaged 12.26 mg gm$^{-1}$ and 2.47 mg gm$^{-1}$ respectively, in roots and shoots. Zinc concentration in root and shoot tissues reached its highest values of 26.52 mg gm$^{-1}$ and 2.58 mg gm$^{-1}$, respectively, at 50 µg ml$^{-1}$ concentration. 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 heavy metals accumulation by *B. juncea* in the order: Zn> Cd>Pb

Table 1: Biomass of *B. juncea* after 21 days of exposure to heavy metals Cadmium, Lead and Zinc in contaminated Steinberg nutrient solution.

| Metal | Concentration (µg $ml^{-1}$) | Dry Weight (g) | |
|---|---|---|---|
| | | Roots | Shoots |
| 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 |
| | Control | 0.020±0.008 | 0.063±0.012 |
| Zn | 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 |

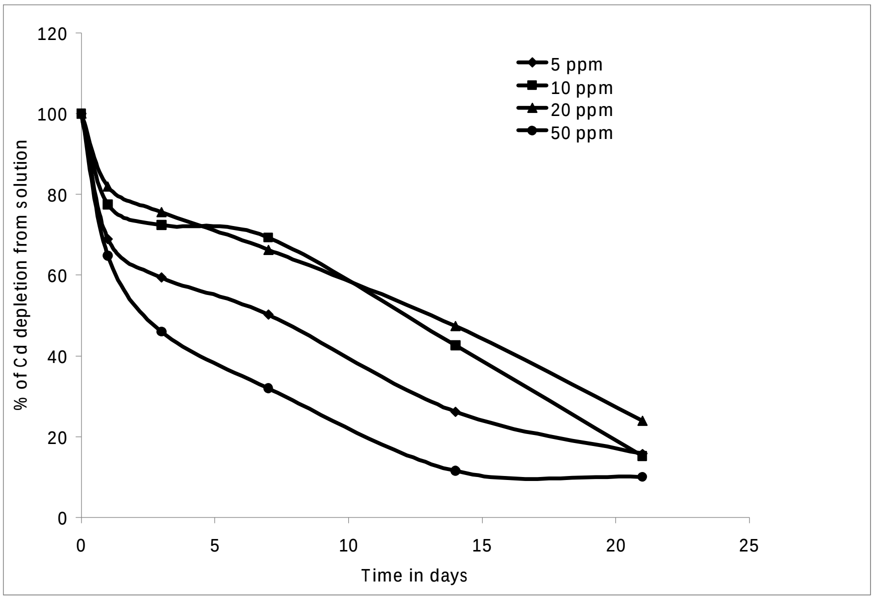
*Values are averages of three replicates ± S.D.

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 and within a plant, cadmium and zinc are more mobile than lead and copper (Greger, 2004). Cadmium is one the most dangerous heavy metals due to its high mobility and the small concentration at which its effects on the plants begin to show (Vazquez *et al*.., 1992).

The bioaccumulation coefficient (BC) of cadmium varied from 514.8 to 435.36 as exposure increased from the minimum (5 µg $ml^{-1}$) to the higher rate (50 µg $ml^{-1}$); lead BC varied from 300.6 to 294.82 at exposure from 5 to 50 µg $ml^{-1}$, while BC for zinc ranged from 544.2 to 582.04 between 5 µg $ml^{-1}$ to 50 µg $ml^{-1}$. The BC of different metals by *B. juncea* was found to be in the order of Pb<Zn<Cd at almost all concentrations tested.

The translocation factor which can describe the movement and distribution of heavy metals in plants was also determined in order to assess the uptake of heavy metals by roots of *B. juncea* from the hydroponics solution and their translocation from roots to the shoots. After 21 days of exposure, only 15.38% of cadmium (at 50 µg $ml^{-1}$), 16.80% of lead (at 50 µg $ml^{-1}$) and 17.30 % of zinc (at 5µg $ml^{-1}$) was translocated from roots to shoots (fig. 3). The translocation factor was found in the order of Pb< Zn<Cd. It was observed that lead accumulated in roots of *B. juncea* in higher concentrations as compared to cadmium and zinc. Zinc being a micronutrient was translocated proportionately in root and shoot; whereas cadmium uptake from root to shoot is similar to that of zinc. Transport across root cellular membrane is an important process which initiates metal absorption in plant tissues. Tanhan *et al.* (2007) have studied the uptake and accumulation patterns of Pb, Cd and Zn in *Chromolina odorata* and reported that accumulation increased in order of Cd< Zn < Pb, while the BC increased in order of Cd< Pb< Zn. Similar translocation pattern was reported in

*Polygonum thunbergii* but with a different accumulation pattern i.e. Cd< Pb<Zn<Cu (Kim *et al*., 2003).



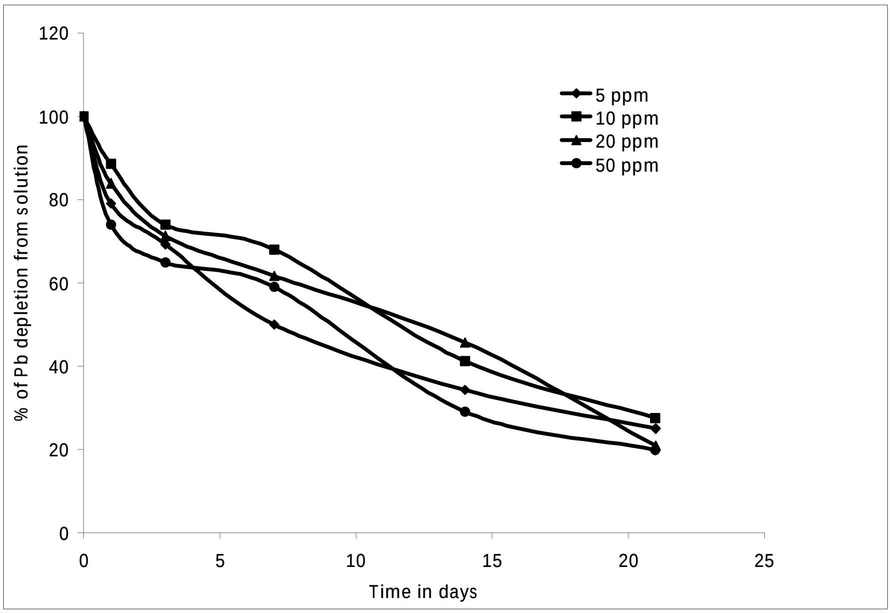




Figure 1 a & b: Depletion of Cadmium and Lead from solution during 21 days of uptake by *Brassica juncea*.

*Journal of Applied Biosciences (2009), Vol. 13: 726 - 736.*
*ISSN 1997 – 5902: www.biosciences.elewa.org*

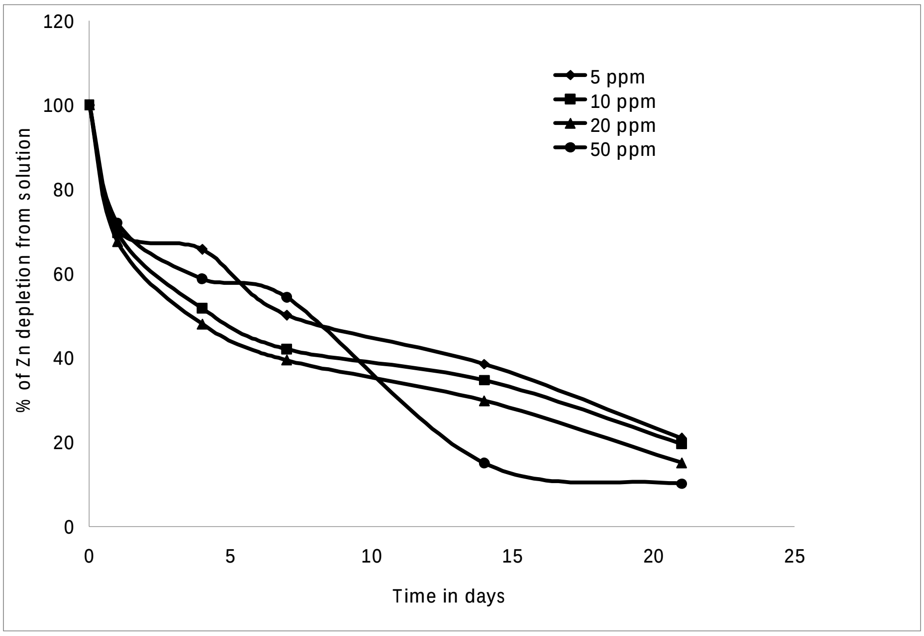



Figure 1 c: Depletion of Zinc from solution during 21 days of uptake by *Brassica juncea*.

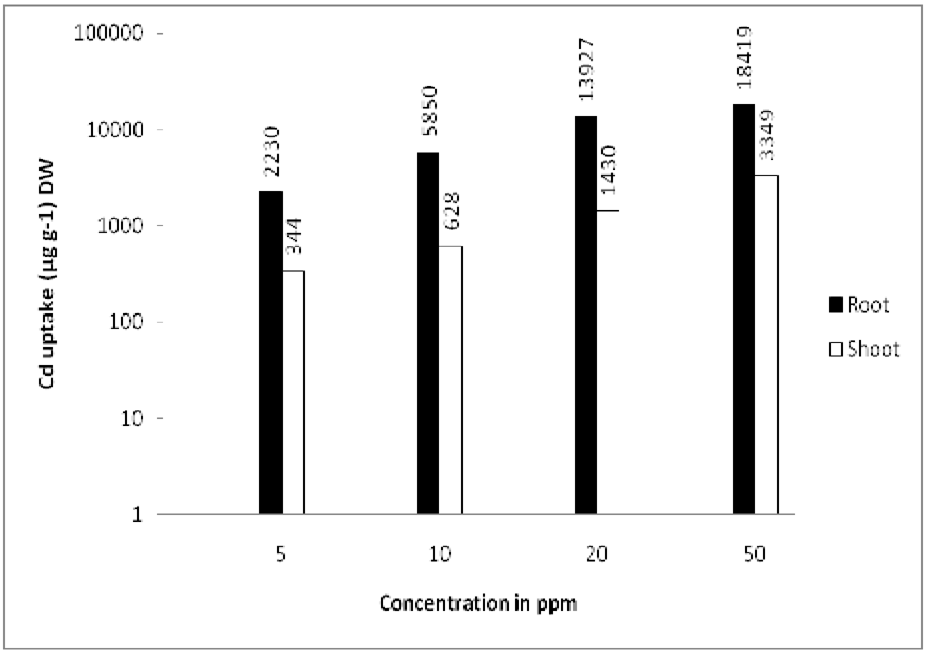



Figure 2 a: Accumulation of cadmium in the dry biomass of roots and shoots of *Brassica juncea* cultivated in hydroponics spiked with varying concentrations of the heavy metal.

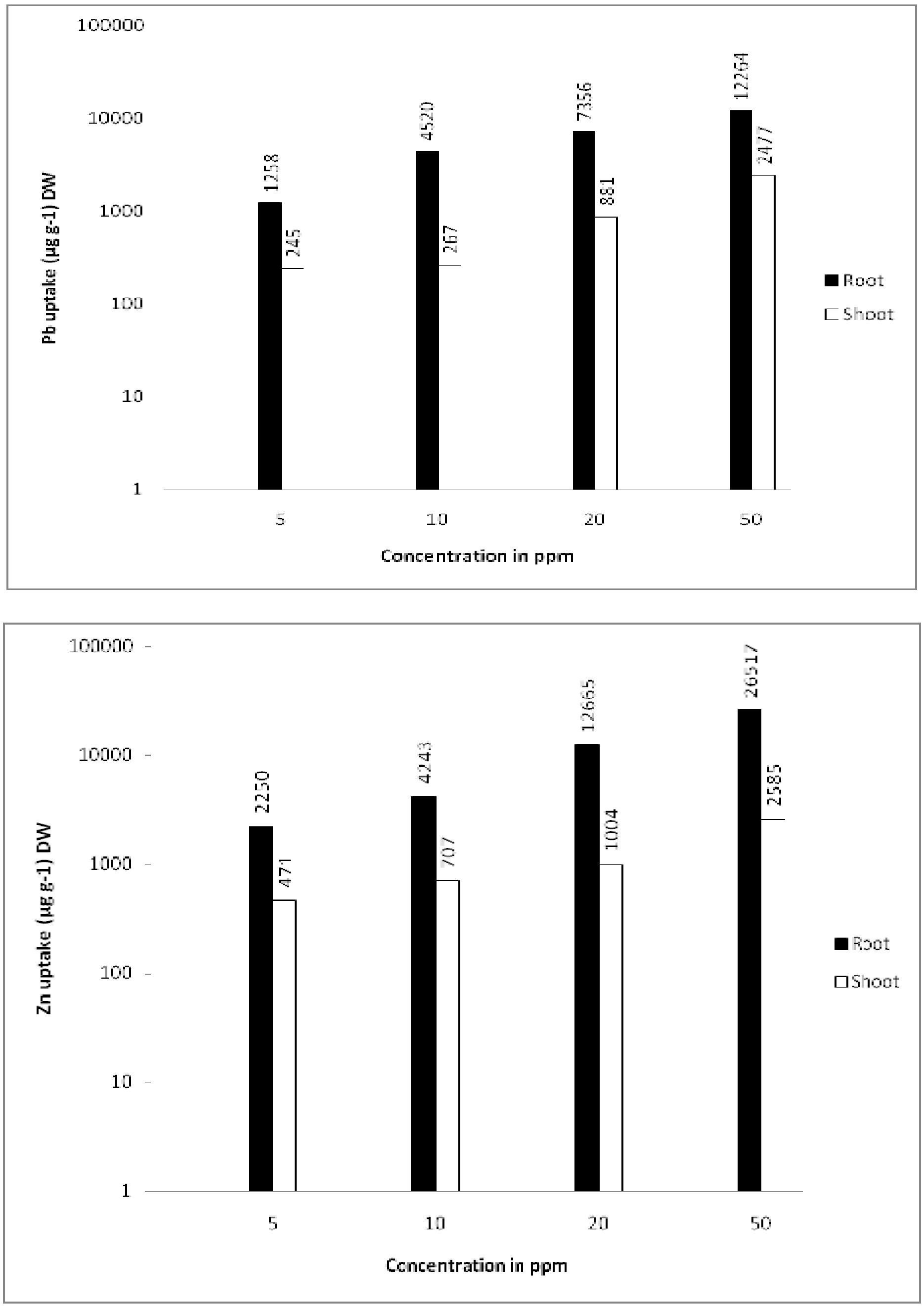



Figure 2 b & c: Accumulation of lead and zinc in the dry biomass of roots and shoots of *Brassica juncea* cultivated in hydroponics spiked with varying concentrations of the heavy metals.

Table 2: Metal accumulation in roots and shoots of *Brassica juncea* and their Bioaccumulation Coefficient (compared to control treatment).

| Metal | Concentration (µg $ml^{-1}$) | Metal uptake (mg $g^{-1}$) | | Bioaccumulation Coefficient |
|---|---|---|---|---|
| | | Roots | Shoots | |
| Cd | Control | ND | ND | ND |
| | 5 | *2.23±0.11 | 0.34±0.02 | 514.8±24.35 |
| | 10 | 5.85±0.40 | 0.63±0.08 | 647.8±18.35 |
| | 20 | 13.93±0.34 | 1.45±0.31 | 767.86±38.89 |
| | 50 | 18.15±0.46 | 3.35±0.39 | 435.36±28.68 |
| Pb | Control | ND | ND | ND |
| | 5 | 1.26±0.17 | 0.24±0.04 | 300.6±30.83 |
| | 10 | 4.52±0.34 | 0.27±0.07 | 478.7±41.47 |
| | 20 | 7.36±0.17 | 0.88±0.04 | 411.85±20.03 |
| | 50 | 12.26±0.99 | 2.48±0.03 | 294.82±22.87 |
| Zn | Control | ND | ND | ND |
| | 5 | 2.25±0.04 | 0.47±0.03 | 544.2±30.83 |
| | 10 | 4.24±0.68 | 0.71±0.08 | 495±35.92 |
| | 20 | 12.67±0.33 | 1.00±0.05 | 683.45±38.77 |
| | 50 | 26.52±0.71 | 2.58±0.14 | 582.04±43.98 |

*Values are averages of three replicates ± S.D; ND= Not detected

The cadmium and zinc uptake were found to be higher in shoot as compared to lead. Zinc and cadmium 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 zinc and cadmium in the biological system is likely to be similar. The fact that cadmium is a toxic metal and zinc 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 zinc (Chaudhury & Chandra, 1987).

Our results further showed that lead is accumulated more in roots as compared to the other two metals (zinc and cadmium). Lead 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 & Martin, 1977). Roots of plants act as a medium for heavy metal translocation and there may be a potential tolerance mechanism operating in the roots (Ernst *et al*., 1992). The comparison data of metal uptake revealed that only small amount of lead is translocated from root to shoot, as compared to zinc and cadmium. Liu *et al*. (2000) have reported that *B. juncea* has considerable ability to remove lead from solutions and accumulate it in roots. Nandkumar *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 findings of the present phytoremediation study have demonstrated the potential use of *B. juncea* for remediation of heavy metals from contaminated aquatic environment. This plant can be grown in fields that are contaminated with heavy metals and thereafter be removed, ashed and buried for safe disposal.

ACKNOWLEDGEMENTS: Authors are grateful to the Board of Research in Nuclear Sciences (BRNS), Department of Atomic Energy (DAE), The Government of India for the financial support. We are thankful to Dr.

S.J. Jambhulkar, who provided the Indian mustard seeds from the gamma field, BARC, Mumbai and Dr. V. Ramachandran, BARC for rendering assistance with AAS for analysis of metals.

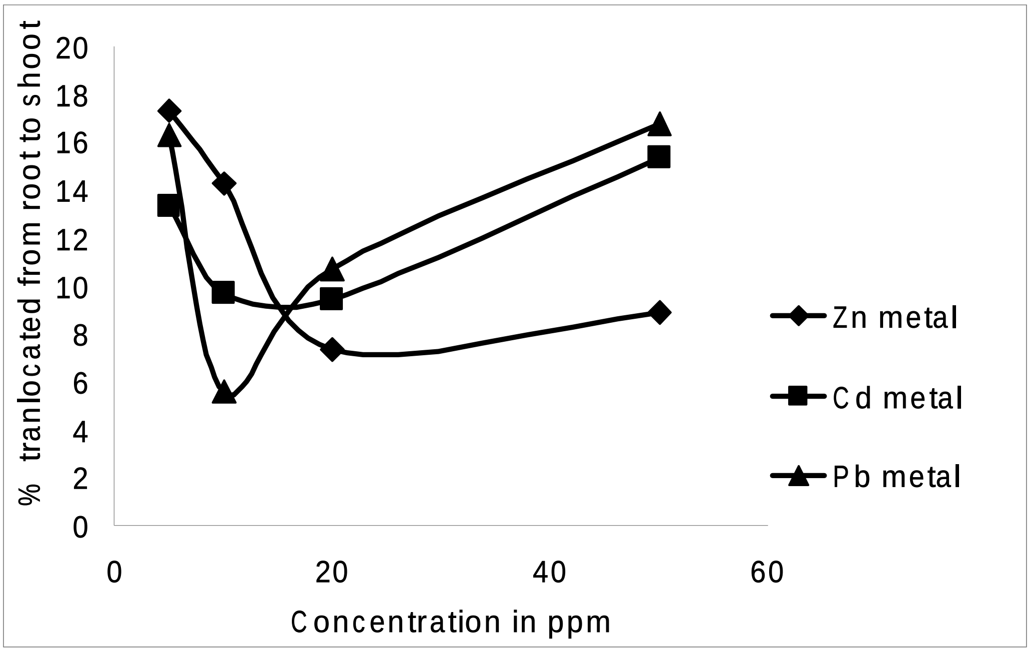



Figure 3: Translocation factor of heavy metals to shoot part in *Brassica juncea.*

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