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Heavy metal toxicity: Effect on plant growth, biochemical parameters and metal accumulation by *Brassica juncea* L.

R. John[a], P. Ahmad[b,*], K. Gadgil[a], S. Sharma[b]

[a]*Centre for Energy Studies, Indian Institute of Technology Dehli, Hauz Khas, New Dehli, 110016, India.*
[b]*Biochemistry Lab, Centre for Rural Development and Technology, Indian Institute of Technology Dehli, Hauz Khas, Dehli, 110016, India.*
*corresponding author; Email: pervaiz_iitd2002@yahoo.com

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Abstract

Plant growth, pigment concentration, biochemical parameters and uptake of heavy metals were determined for *Brassica juncea* L. in response to cadmium and lead stress. The plant exhibited a decline in growth, chlorophyll content and carotenoids with Cd and Pb but Cd was found to be more detrimental than Pb treatment in *B. juncea*. The protein content was decreased by Cd (900 µM) to 95% and 44% by Pb (1500 µM) at the flowering stage. Proline showed increase at lower concentrations of Cd and Pb but at higher concentrations it showed decrease. More accumulation of Cd and Pb was observed in roots than shoots in *B. juncea.* Cd was found to be more accumulated than Pb but higher concentrations of Pb hampers the Cd absorption.

Keywords: Cd; Pb; heavy metal toxicity; *Brassica juncea*

Introduction

Cadmium (Cd), being a highly toxic metal pollutant of soils, inhibits root and shoot growth and yield production, affects nutrient uptake and homeostasis, and is frequently accumulated by agriculturally important crops and then enters the food chain with a significant potential to impair animal and human health (di Toppi and Gabrielli, 1999). The application of sewage sludge, city waste, and Cd-containing fertilizers causes the increase of Cd content in soils (Williams and David, 1973). The reduction of biomass by Cd toxicity could be the direct consequence of the inhibition of chlorophyll synthesis and photosynthesis (Padmaja et al., 1990). Excessive amount of Cd may cause decreased uptake of nutrient elements, inhibition of various enzyme activities, induction of oxidative stress including alterations in enzymes of the antioxidant defence system (Sandalio et al., 2001).

Lead (Pb), a potentially toxic heavy metal with no known biological function, has attracted more and more considerable attention for its widespread distribution and potential risk to the environment. Pb contamination in soils not only aroused the changes of soil

microorganism and its activities and resulted in soil fertility deterioration but also directly affected the change of physiological indices and, further more, resulted in yield decline (Majer et al., 2002). Ultimately, lead enriched in the body of humans through the food chain and endangered their health (Liu et al., 2003). Soil microbial activity has a great potential as an early and sensitive indicator of stress in soil and has been employed in national and international monitoring programs (Yao et al., 2000).

The sensitivity of plants to heavy metals depends on an interrelated network of physiological and molecular mechanisms such as (i) uptake and accumulation of metals through binding to extracellular exudates and cell wall constituents; (ii) efflux of heavy metals from cytoplasm to extranuclear compartments including vacuoles; (iii) complexation of heavy metal ions inside the cell by various substances, for example, organic acids, amino acids, phytochelatins, and metallothioneins; (iv) accumulation of osmolytes and osmoprotectants and induction of antioxidative enzymes (v) activation or modification of plant metabolism to allow adequate functioning of metabolic pathways and rapid repair of damaged cell structures (Cho et al., 2003).

Brassica juncea belongs to family brassicaceae and is a very important oil crop. Mustard oil is one of the major edible oils in India. Mustard oil has also got medicinal importance. Residual part of seeds is used as cattle feed and in fertilizer. Indian mustard (*Brassica juncea* L.) is a fast growing plant which produces a high biomass even in heavy metal polluted soils. Thus this plant might be a potential candidate for phytofiltration and /or phytostabilization of heavy metal contaminated waste waters. So far this plant species has been used in studies of the effects of heavy metals like cadmium (Qadir et al., 2004; Anjum et al., 2008) and arsenic (Gupta et al., 2008) stresses on plants.

The present experiment was undertaken to investigate a change in the level of growth, and biochemical aspects, total protein and pigment content in *Brassica juncea* treated with Cd and Pb in order to contribute to an understanding of *B. juncea* adaptation to environmental stress. This crop may further be useful in soil reclamation through the process of phytoremediation. However, selection of *B. juncea* would be of great importance to reclaim the soil with lesser impact on plant metabolism and hence the yield.

Materials and Methods

Biological Material

Seeds of *B. juncea* were sown in the earthen pots, containing homogenously mixed soil with farm yard manure and the pots were watered daily and kept in Micro model (botanical garden) IIT- Delhi, under natural photoperiod of 12 to 13 h and temperature of 28±4 °C. Care was taken to avoid drainage of solution during the treatment by giving water slightly less than field capacity. After germination the plants were treated with heavy metal $CdSO_4.8H_2O$ and Pb $(C_2H_3O_2)2$. *B. juncea* was subjected to six different concentrations of Cd (150, 300, 450, 600, 750 and 900 μM) or Pb (150, 300, 600, 900, 1200 and 1500 μM). The plant leaves were collected at three different growth stages i.e, 25 days (pre-flowering), 45 days (flowering) and 60 days (post flowering) stage for analysis. Pots containing 5 plants and each treatment was replicated 5 times (each replicate is a plant) were taken for

all the treatments arranged in completely randomized block design (CRD). All the chemicals were of analytical grade reagent (Merck, India).

Determination of Pigment content

Chlorophyll content was determined by the method of Hiscox and Israelstam (1979). Fresh leaves (100 mg) were kept in the extraction reagent, dimethyl sulfoxide (DMSO). The tubes were incubated in oven at 65 ^{0}C for 40 min. One ml aliquot was mixed with 2ml DMSO and vortexed. Absorbance was obtained photometrically at 480, 510, 645, 663 nm (Beckman 640 D, USA) with DMSO as a blank.

Protein Estimation

Proteins were estimated by the method of Bradford (1976). Fresh leaves (0.5 g) were homogenized in 1 ml phosphate buffer (pH 7.0). The crude homogenate was centrifuged at 5000x g for 10 min. Half ml of freshly prepared trichloroacetic acid (TCA) was added and centrifuged at 8000x g for 15 min. The debris was dissolved in 1ml of 0.1 N NaOH and 5 ml Bradford reagent was added. Absorbance was recorded photometrically at 595 nm (Beckman 640 D, USA) and bovine serum albumin was used for standard curve drawing.

Estimation of Proline content

Proline concentration was determined using the method of Bates et al. (1973). Fresh leaves (300 mg) were homogenized in 10 ml of aqueous sulphosalicylic acid (3%). The homogenate was centrifuged at 9000 × g for 15 min. A two ml aliquot of the supernatant was mixed with an equal volume of acetic acid and ninhydrin and incubated for 1 h at 100^{0}C. The reaction was terminated on ice bath and extracted with 4 ml of toluene. The extract was vortexed for 20 s and the chromatophore-containing toluene was aspirated from the aqueous phase and absorbance determined photometrically at 520 nm (Beckman 640 D, USA) using toluene for a blank.

Estimation of Cd and Pb Accumulation

The Cd and Pb content were estimated employing a Perkin-Elmer (Analyst Model 300) atomic absorption spectrophotometer equipped with an air-acetylene burner. The heavy metal content was expressed as mg g^{-1} dw of the sample.

Statistical Data

Analysis of variance (ANOVA) for all the measured variables was performed by SPSS Ver. 10, Inc., Chicago, USA. The treatment means were separated using Duncan's multiple range test (DMRT) taking $P<0.05$ as significant.

Results

Growth and Biomass Yield

The effect of Cd and Pb on root length and stem height is presented in table 1-2. Decline in root length ranged from 17% (with 300 µM Cd) to 54% (with 900 µM Cd) at the pre-flowering stage. Similarly, stem height declined from 4% (with 300 µM Cd) to 51% (with 900 µM Cd) at the post-flowering stage. Only the higher concentrations of Pb were toxic and toxicity was found to be highest at the post-flowering stage i.e., 1500 µM Pb declined root length from 28.21cm to 13.97 cm after 60 days time (Table 2).

Table 1. Biomass yield of *B. juncea* with different concentrations of Cd at different developmental stages.

	Root Length (cm)			Stem Height (cm)		
Cd concentration(µM)	25 days	40 days	60 days	25 days	40 days	60 days
0	17.68±3.2^{a}	24.54±3.1^{a}	27.86±1.8^{a}	32.34±2.4^{a}	46.86±4.2^{a}	57.89±4.4^{a}
150	18.01±4.1^{a}	23.95±2.4^{b}	27.21±1.4^{a}	33.79±1.4^{b}	45.95±3.4^{b}	58.12±4.3^{a}
300	14.75±2.2^{b}	21.86±2.1^{b}	25.65±1.3^{b}	32.65±1.3^{ab}	43.56±2.9^{c}	55.33±3.8^{a}
450	12.6±1.1^{ab}	21.01±1.4^{c}	23.86±2.1^{b}	30.34±1.9^{c}	40.23±2.5^{d}	49.18±4.2^{b}
600	10.75±1.4^{c}	18.75±2.0^{c}	21.75±2.3^{c}	26.56±2.4^{d}	36.54±2.7^{d}	40.6±2.8^{c}
750	8.75±3.1^{c}	12.75±2.8^{cd}	15.12±1.5^{d}	21.57±2.2^{d}	30.56±2.5^{e}	33.32±3.3^{d}
900	8.12±1.9^{d}	10.32±1.4^{d}	11.87±1.2^{d}	18.73±1.2^{e}	24.11±1.7^{e}	28.32±2.2^{d}

Different letters indicate significant difference between means at *P<0.05* (DMRT).Values are means±S.E (n=5).

Table 2. Biomass yield of *B. juncea* with different concentrations of Pb at different developmental stages.

	Root Length (cm)			Stem Height (cm)		
Pb concentration(µM)	25 days	40 days	60 days	25 days	40 days	60 days
0	18.01±2.2^{a}	25.12±2.7^{a}	28.21±1.7^{a}	33.56±3.3^{a}	45.79±3.2^{a}	58.79±3.8^{a}
150	18.12±2.2^{a}	24.21±2.4^{b}	27.98±2.8^{a}	32.57±2.9^{b}	47.12±3.5^{b}	57.32±4.1^{b}
300	18.02±2.5^{a}	24.1±2.7^{b}	27.31±3.1^{a}	32.47±3.5^{b}	46.74±4.3^{b}	56.83±4.2^{b}
600	16.86±1.2^{b}	22.32±2.3^{c}	26.23±3.2^{ab}	30.46±3.2^{c}	43.85±4.9^{bc}	53.75±3.9^{bc}
900	15.53±2.2^{b}	20.42±2.0^{c}	21.86±2.9^{c}	28.42±2.4^{c}	37.53±4.0^{d}	45.31±4.2^{d}
1200	12.53±2.8^{c}	18.64±2.0^{d}	19.52±1.2^{d}	25.31±1.2^{d}	34.13±2.2^{e}	35.42±2.2^{e}
1500	11.63±1.3^{c}	12.61±2.3^{e}	13.97±2.2^{e}	24.53±1.2^{d}	30.32±1.3^{e}	33.42±2.3^{e}

Different letters indicate significant difference between means at *P<0.05* (DMRT). Values are means ±S.E (n=5).

Chlorophyll and Carotenoid Content

The levels of chlorophyll 'a', chlorophyll 'b'; and total chlorophyll in control plants were found maximum in *B. juncea* at the flowering stage. Pb proved to be less toxic as compared to Cd and 900 µM of Pb decreases chl 'a' to about 35% of the control at the flowering stage of *B. juncea* (Figure 1, B). At higher levels of Cd treatment (900 µM), chl 'b' declined from 0.74 mg/g fw to 0.15 mg/g fw at the flowering stage (Figure 2, A) while, 900 µM of Pb decreased it from 0.74 to 0.56 mg/g fresh weight of leaves (Figure 2, B). Total chlorophyll also showed the decline at higher concentration of Cd and Pb (Figure 3)

With 900 µM of Cd treatment, carotenoid content decreased from 0.413 to 0.083 mg/g fresh weight of the phytobiomass after 60 days (Figure 4, A).

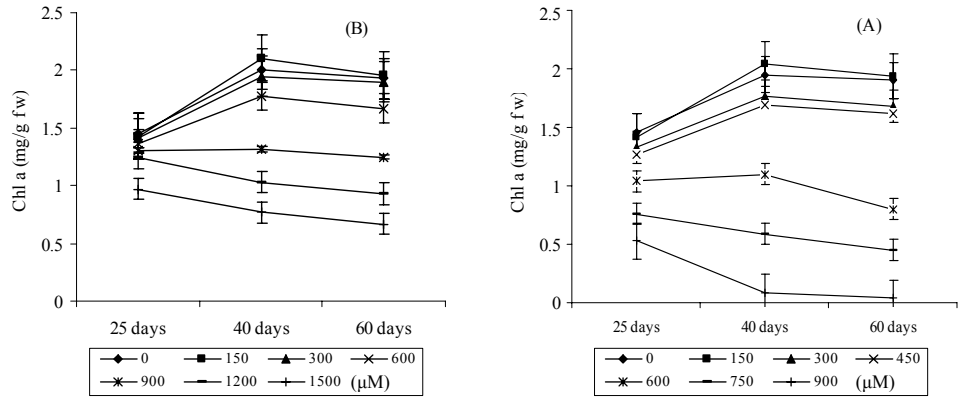

Figure 1. Effect of different conc. of Cd (A) and Pb (B) (µM) on Chl 'a' content of *B. juncea* at different developmental stages. Values are significantly different $P<0.05$ from control (DMRT). Values are means ± S.E (n=5).

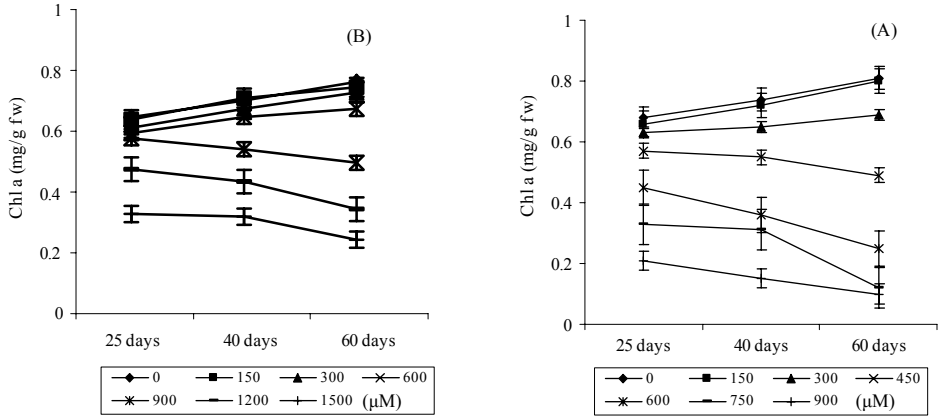

Figure 2. Effect of different conc. of Cd (A) and Pb (B) (µM) on Chl 'b' content of *B. juncea* at different developmental stages. Values are significantly different $P< 0.05$ from control (DMRT). Values are means±S.E (n=5).

Soluble Protein Content

The results related to the effect of Cd and Pb on protein content is depicted in figure5. 450 µM Cd declined protein content to 24.9% and 900 µM decreased it to 87.3% of the control at the flowering stage (Figure 5, A). Pb did not show much impact on protein content except 1200 and 1500 µM which decreased protein content to 58.7% and 77.4% respectively of the control (Figure 5, B).

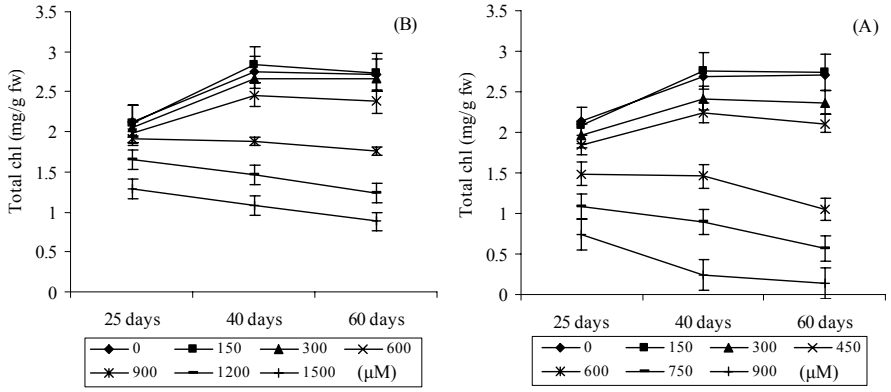

Figure 3. Effect of different conc. of Cd (A) and Pb (B) (μM) on total Chl. Content of *B. juncea*. at different developmental stages. Values are significantly different $P<0.05$ from control (DMRT). Values are means± S.E (n=5).

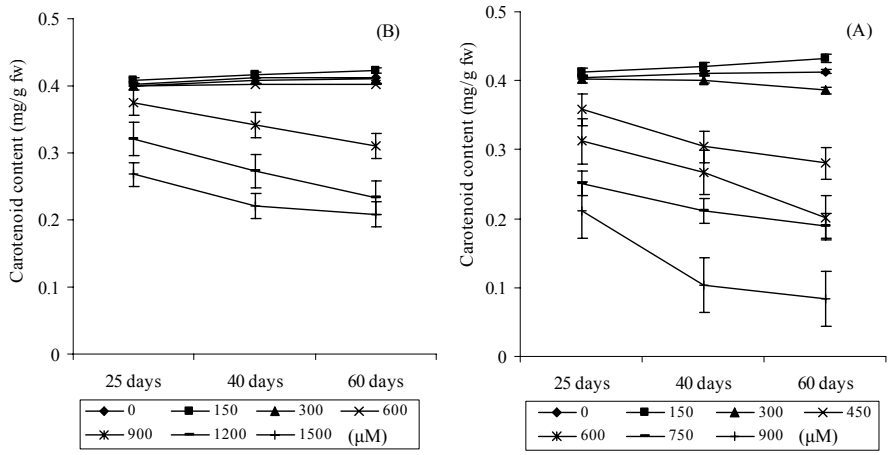

Figure 4. Effect of different conc. of Cd (A) and Pb (B) (μM) on carotenoid content of *B. juncea*. at different developmental stages. Values are significantly different $P<0.05$ from control (DMRT). Values are means±S.E (n=5).

Proline Content

The results pertaining to the effect of Cd and Pb on proline content is presented in figure 6. The higher concentrations of Cd (750 and 900 μM) decreased proline content to 9% and 28% of the control respectively at post-flowering stage (Figure 6, A). Treatment with different Pb concentrations showed a very little effect on the proline storage (Figure 6, B).

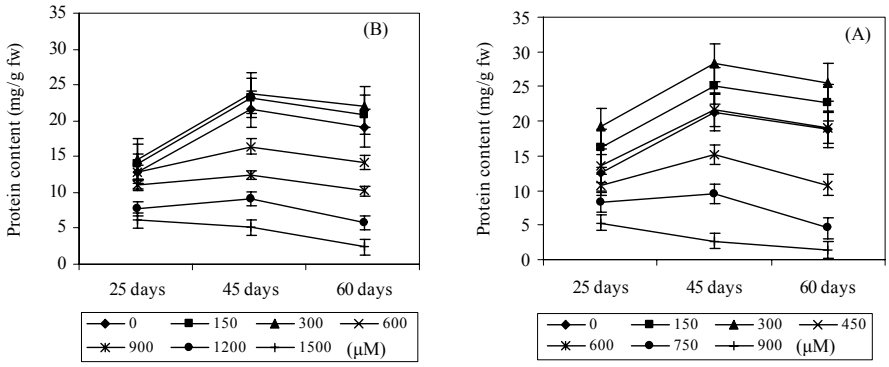

Figure 5. Effect of different conc. of Cd (A) and Pb (B) (µM) on soluble protein content (mg/g fw) of *B. juncea*. at different developmental stages. Values are significantly different $P<0.05$ from control (DMRT). Values are means ± S.E (n=5).

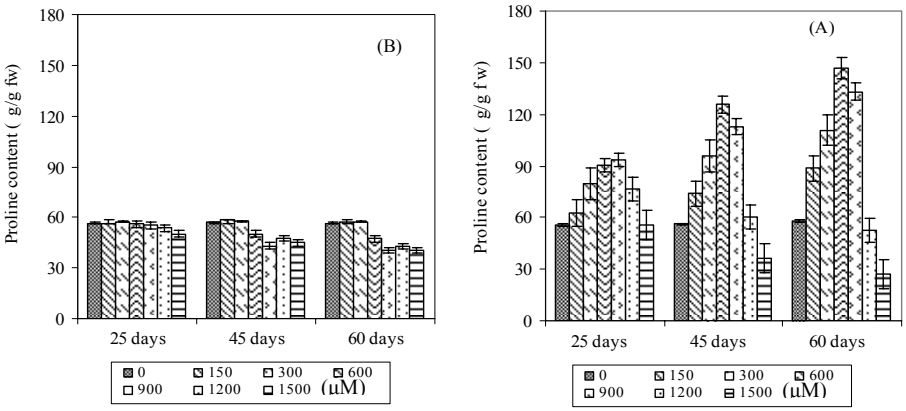

Figure 6. Effect of different conc. of Cd (A) and Pb (B) (µM) on proline content (µg/g fw) of *B. juncea*. at different developmental stages. Values are significantly different $P<0.05$ from control (DMRT). Values are means±S.E (n=5).

Table 3. Accumulation of Cd in roots and shoots of *B. juncea* at different developmental stages.

Cd concentration (µM)	Accumulation of Cd in Shoots(mg/g dw)			Accumulation of Cd in roots(mg/g dw)		
	25 days	40 days	60 days	25 days	40 days	60 days
0	0.12± 0.01^{a}	0.23±0.03^{a}	0.28±0.05^{a}	0.32±0.02^{a}	0.44±0.02^{a}	0.51±0.02^{a}
150	4.21±0.03^{b}	6.75±0.07^{b}	7.85±0.08^{b}	16.01±1.32^{b}	35.81±2.54^{b}	51.11±2.5^{b}
300	8.64±0.06^{c}	10.74±0.22^{c}	12.74±0.15^{c}	20.01±1.94^{bc}	41.11±3.12^{b}	60.23± 3.7^{c}
450	10.11±0.05^{c}	11.76±0.85^{c}	13.86±0.10^{c}	42.12±2.77^{c}	61.11±4.32^{c}	85.91±4.45^{d}
600	12.65±0.11^{cd}	14.75±0.96^{d}	16.43±1.03^{d}	63.53±4.32^{d}	83.11±5.37^{d}	95.21±7.31^{d}
750	11.32±0.08^{e}	14.87±1.21^{d}	13.21±1.21^{cd}	60.43±3.54^{d}	75.53±4.98^{d}	116.32±8.32^{d}
900	8.56±0.08^{e}	9.83±0.92^{e}	11.64±0.95^{e}	38.32±3.31^{e}	50.91±4.21^{e}	55.21±3.76^{e}

Different letters indicate significant difference between means at $P<0.05$ (DMRT). Values are means ± S.E(n = 5).

Table 4. Accumulation of Pb into roots and shoots of *B. juncea* at different developmental stages.

Pb concentration (μM)	Accumulation of Pb in Shoots(mg/g dw)			Accumulation of Pb in roots(mg/g dw)		
	25 days	40 days	60 days	25 days	40 days	60 days
0	0.14±0.01a	0.24±0.02^{a}	0.31±0.02^{a}	0.34±0.02^{a}	0.74±0.02^{a}	1.12±0.91^{a}
150	6.32±0.03b	8.43±0.03^{b}	10.42±1.03^{b}	10.32±0.93^{b}	27.11±2.10^{b}	36.22±2.12^{b}
300	15.74±1.04c	21.23±1.32^{c}	28.32±2.91^{c}	17.42±0.87^{c}	35.32±2.43^{c}	52.43±2.54^{c}
600	19.74±1.87c	24.11±1.99^{c}	26.21±2.4^{c}	32.32±1.76^{d}	53.21±2.82^{d}	62.54±3.03^{d}
900	21.21±1.82c	26.21±2.32^{cd}	28.21±2.65^{cd}	41.54±2.11^{d}	60.21±3.32^{e}	78.21±3.21^{e}
1200	11.43±1.22d	13.32±2.01^{d}	13.87±1.43^{d}	42.86±1.91^{d}	64.64±3.41^{e}	85.97±4.01^{e}
1500	7.21±0.89e	7.44±0.98^{e}	7.97±1.01^{e}	31.21±2.03^{e}	21.44±1.21^{ab}	28.64±1.43^{b}

Different letters indicate significant difference between means at $P<0.05$ (DMRT). Values are means ± S.E (n=5).

Uptake of Cd and Pb

Plant showed maximum accumulation of Cd (116.32 mg/g dry weight of the plant material) in the roots at 750 μM Cd treatment (Table 3). On prolonged exposure (post flowering stage) to higher concentration of Cd i.e. 900 μM, there was significant decline in accumulation rate of Cd. Similarly, Pb accumulation was higher in roots as compared to shoots. Accumulation of Pb was comparatively lower and reached upto 85.97 mg/g dw of *B. juncea* at the post-flowering stage (Table 4).

Discussion

The most common effect of Cd toxicity in plants is stunted growth, leaf chlorosis and alteration in the activity of many key enzymes of various metabolic pathways (Arduini et al., 1996). The reduction in plant growth during stress is due to low water potential, hampered nutrient uptake and secondary stress such as oxidative stress. Further Pb can also disturb microtubule organization in meristematic cells (Eun et al., 2000). In our study, with varied concentrations of Cd and Pb, fresh weight of *B. juncea* got affected. The greater impact of heavy metal was observed on the root growth as compared to shoot leading to a greater reduction in its length and fresh weight (Elloumi et al., 2007). The reduction in the growth of *B. juncea* could be also due to the suppression of the elongation growth rate of cells, because of an irreversible inhibition exerted by Cd on the proton pump responsible for the process (Aidid and Okamote, 1993).

Retarded shoot growth due to the presence of the root environment with excess of Pb has also been found by Seyyedi (1999). A few cases of increase in the plant biomass due to metal pollutants have been reported in the literature, but these were from experiments using low concentrations of metals (Breckle, 1991).

The decline in chlorophyll content in plants exposed to Cd^{2+} and Pb^{2+} stress is believed to be due to (a) inhibition of important enzymes, such as δ-aminolevulinic acid dehydratase (ALA-dehydratase) (Padmaja et al., 1990) and protochlorophyllide reductase (Van Assche and Clijsters, 1990) associated with chlorophyll biosynthesis; (b) impairment in the supply

of Mg^{2+}, Fe^{2+}, Zn^{2+} and Mg^{2+} (Van Assche and Clijsters, 1990; Küpper et al., 1996). Similar decrease in chlorophyll content under heavy metal stress was reported earlier in cyanobacteria, unicellular chlorophytes (*Chlorella*), gymnosperms, such as *Picea abies* and angiosperms, such as *Zea mays*, *Quercus palustrus* and *Acer rubrum* (Siedlecka and Krupa, 1996). The decrease in chlorophyll content was also reported in sunflower (Zengin and Munzuroglu, 2006) and in almond (Elloumi et al., 2007).

Our studies of soluble protein content coincides with the findings of Singh and Sinha (2005) who found decrease in soluble protein content in *B. juncea* when grown on various amendments of tannery waste containing heavy metals. Decrease in the protein content has also been found in aquatic plants when treated with metalliferous wastewater. However, increased concentration of salt stress increases protein content in *Pisum sativum* (Ahmad and Jhon, 2005). Ahmad et al., (2006) reported the increase in protein content with lower concentrations of salt (Na_2CO_3) and decrease with higher concentration of salt (Na_2CO_3) in mulberry. Heavy metal stress has been shown to induce a variety of proteins resulting in an overall increase in protein content (Shah and Dubey, 1997). The decrease in protein content as observed at higher concentrations of Cd and Pb in *B. juncea* may be because of enhanced protein degradation process as a result of increased protease activity (Palma et al., 2002) which is found to increase under stress conditions. It is also likely that these heavy metals may have induced lipid peroxidation and fragmentation of proteins due to toxic effects of reactive oxygen species which led to reduced protein content.

Proline, an imino acid is well known to get accumulated in wide variety of organisms ranging from bacteria to higher plants on exposure to abiotic stress (Saradhi et al., 1993). Plants have been shown proline accumulation under environmental stress (Ahmad and Jhon, 2005; Ahmad et al., 2006; Ahmad et al., 2008). It has been often suggested that proline accumulation may contribute to osmotic adjustment at the cellular level and enzyme protection stabilizing the structure of macromolecules and organelles. Increase in proline content may be either due to *de novo* synthesis or decreased degredation or both (Kasai et al., 1998). Proline accumulation in shoots of *B. juncea*, *Triticum aestivum* and *Vigna radiata* in response to Cd^{2+} toxicity has been demonstrated by Dhir et al., (2004) but they found that proline accumulation decreased with the exposure to Cd^{2+} in hydrophytes (*Ceratophyllum*, *Wolffia,* and *Hydrilla)*. Similar results of increasing proline content by Cd^{2+} was also reported by Zengin and Munzuroglu, (2006) in sunflower.

The results related to the uptake of metals in this study suggest that roots of *B. juncea* are efficient barriers to Cd and Pb translocation to the above ground plant parts. The uptake of metals by plant is coupled to a chemiosmotic process across the membrane of intact root cells (Mitchell, 1979). It has been shown that Pb is unevenly distributed in roots, where different root tissues act as barriers to apoplastic and symplastic Pb transport and hence Pb transport to shoot gets restricted. Although tolerance and root to shoot metal transport are often negatively correlated, and tolerance is often associated with enhanced metal retention in roots, it does not necessarily mean that increased root retention itself could be the cause of tolerance (Harmens et al., 1993). Further, the translocation of Cd from root to shoot is an important factor affecting accumulation of this metal in aerial tissues of *Brassica juncea*. Our results are in confirmation with that of Zhu et al., (1999) who also observed higher accumulation of Cd into the roots of *B. juncea* as compared to above ground parts by over expressing gamma-glutamylecyteine synthetase. Cd accumulation in stem and inactivation

in root cells are probably related to its binding in cell walls, compartmentalization in vacuoles and complexation with metal binding proteins and peptides, especially phytochelatin and metallothioneins (Gupta and Goldsbrough, 1991). These processes are strategies employed by plants, at least in part, to face unavoidable stress conditions.

Conclusions

In conclusion, our results indicated that the exposure of *B. juncea* to Cd and Pb results is an decrease in growth, pigment content and at lower concentration of heavy metals increase in protein and proline was observed but at higher concentrations it was decreased. Cd was accumulated more than Pb by the roots of *B. juncea*. Cd was more effective than Pb in hampering plant growth and development. *B. juncea* can be used as a heavy metal accumulator in heavy metal affected soils.

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