Effect of cadmium and lead on growth, biochemical parameters and uptake in *Lemna polyrrhiza* L.

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ABSTRACT

Aquatic plants are known to accumulate heavy metals. In this study, Duckweed plants (*Lemna polyrrhiza* L.) were exposed to different concentrations of Cd and Pb. Various physio-biochemical parameters (fresh weight, chlorophyll content, soluble protein, soluble sugars, proline content and metal absorption) were studied. At lower metal concentrations, an increase in proline, protein and sugar was observed but at higher concentrations (above 30 mg/l) their decrease was noticed. Uptake of the metals was concentration and time dependent. Treatment with 1, 10 and 20 mg/l of Cd and Pb showed synergistic relation while 30 and 40 mg/l treatments showed antagonistic relation during the metal uptake. The results suggest that the *L. polyrrhiza* can be effectively used as a phytoremediator for wastewater polluted with more than one heavy metal at moderate concentrations.

Keywords: absorption; biochemical parameters; heavy metal; *Lemna polyrrhiza* L.; phytoremediation

Pollution of environment by toxic metals arises as a result of various industrial activities and has turned these metal ions into major health issue (Waisberg et al. 2003). Although several adverse effects of the toxic metals have been known for a long time, exposure to heavy metals continues, and is even increasing in some parts of the world, in particular in less developed countries. Heavy metal pollution is also a multi-element problem in many areas (An et al. 2004). Under these circumstances, synergistic and antagonistic interactions may be important, and predicted impact based on individual effects of each metal species is likely to be erroneous (Ting et al. 1991). There is therefore a clear need to understand the interactive effects produced by combinations of metal ions at different concentrations.

Of all toxic heavy metals, cadmium (Cd) ranks the highest in terms of damage to plant growth and human health. Moreover, its uptake and accumulation in plants poses a serious health thread to humans via the food chain (Shah and Dubey 1998). The presence of excessive amounts of Cd in soil commonly elicits many stress symptoms in plants, such as reduction of growth, especially root growth, disturbances in mineral nutrition and carbohydrate metabolism (Moya et al. 1993), and may thus strongly reduce biomass production.

Lead (Pb) is one of the most abundant toxic metal in the earth crust. Exposure to lead in the environmental and occupational settings continues to be a serious public health problem (WHO 1995). Elevated Pb in soils may compromise soil productivity and even a very low concentration can inhibit some vital plant processes, such as photosynthesis, mitosis and water absorption with toxic symptoms of dark leaves, wilting of older leaves, stunted foliage and brown short roots (Mohan and Hosetti 1997, Patra et al. 2004).

More recently, the use of plants in metal extraction (phytoremediation) appeared as a promising alternative in the removal of heavy metal excess from soil and water (Glass 2000). Ting et al. (1991) showed the uptake of cadmium and zinc by alga *Chlorella vulgaris*. *Lemna minor* was used to reclaim the lead contaminated water (Rahmani and Sternberg 1999). Prasad et al. (2001) also showed the bioaccumulation of cadmium and copper by *Lemna trisulca*.

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Duckweed (family *Lemnacea*) is a small, fragile, free-floating aquatic plant that flourishes in quiescent, shallow water bodies (Rahmani and Sternberg 1999). Due to its special features, it is used as a test organism for aquatic studies and for wastewater treatment. Constructed wetlands/phytoremediation claim to be low-cost, low-technology system able to treat a variety of wastewaters. In the present study, the effect of Cd and Pb on *L. polyrrhiza* was examined by exposing the aquatic plant separately to each of the two metal species and then to combination of the two at various concentrations. Effects on biomass growth, biochemical parameters (chlorophyll *a*, total soluble sugar, soluble protein, and proline content) and bioaccumulation of heavy metals were studied.

MATERIAL AND METHODS

Duckweed plants, obtained from oxidation pond at Wazirabad on the outskirts of Delhi, identified as *Lemna polyrrhiza*, were kept in a water tank (containing tap water and compost), and maintained as stock in Micro model, Indian Institute of Technology, New Delhi, India.

The plants were taken out of the stock tank and exposed to different concentrations of Cd ($CdSO_4.7H_2O$) and Pb $(C_2H_3O_2)_2.3H_2O$ i.e., 1.0 mg/l, 10 mg/l, 20 mg/l, 30 mg/l and 40 mg/l and different concentrations of Pb $(C_2H_3O_2)_2.3H_2O$ i.e., 1 mg/l, 10 mg/l, 20 mg/l, 30 mg/l and 40 mg/l separately as well as in combination (1 mg/l Cd + 1 mg/l Pb, 10 mg/l Cd + 10 mg/l Pb, 20 mg/l Cd + 20 mg/l Pb, 30 mg/l Cd + 30 mg/l Pb, 40 mg/l Cd + 40 mg/l Pb). The experiments were conducted in plastic tubs with three replicates for each treatment. Initially in each tub 5.0 mg of biomass was added. Comparisons of metal-exposed plants were made with untreated (control) plants. The data pertaining to plant growth (biomass yield), chlorophyll, soluble protein, total soluble sugar, proline content and metal accumulation were obtained after 6, 12, 18, 24 and 30 days after treatment. All the chemicals used were of analytical grade reagent (Merck, India).

Determination of chlorophyll. Chlorophyll content was determined by the method of Hiscox and Israelstam (1979). Fresh leaves (100 mg) were kept in the extraction reagent, dimethylsulphooxide (DMSO). The tubes were kept in the oven at 65°C for 40 min. 1 ml aliquot was mixed with 2 ml DMSO and vortexed. Absorbance was determined photometrically at 480, 510, 645, 663 nm (Beckman 640 D, USA) using DMSO for 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 5000 × g for 10 min. Half ml of freshly prepared trichloroacetic acid (TCA) was added and centrifuged at 8000 × g for 15 min. The debris was dissolved in 1 ml of 0.1N NaOH and 5 ml Bradford reagent was added. Absorbance was recorded photometrically at 595 nm (Beckman 640 D, USA) using bovine serum albumin as a standard.

Estimation of proline. Proline concentration was determined using the method of Bates et al. (1973). Fresh leaves (300 mg) were homogenized in 10 ml of 30% aqueous sulphosalicylic acid. The homogenate was centrifuged at 9000 × g for 15 min. A 2 ml aliquot of the supernatant was mixed with an equal volume of acetic acid and acid ninhydrin (1.25 g ninhydrin in 30 ml acetic acid and 20 ml of 6N H_3PO_4) and incubated for 1 h at 100°C. The reaction was terminated in an 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 soluble sugars. Sugar was estimated by the method of Dey (1990). Leaves (0.5 g) were extracted twice with hot 90% ethanol. The ethanol extracts were then combined. The final volume of the pooled extract was made to 25 ml with double distilled water. A suitable aliquot was taken from the extract and 1 ml 5% phenol and 5 ml concentrated sulphuric acid were added. Final volume of this solution was made to 10 ml by adding double distilled water. Absorbance of this solution was measured at 485 nm using a UV-Vis spectrophotometer.

Estimation of Cd and Pb accumulation. Tissue concentrations of nutrient elements were measured in the solution after wet digestion (HNO_3:$HClO_4$, 10:1, v/v, mixture) of the oven-dried plant material. The Cd and Pb contents in the solution were estimated employing a Perkin-Elmer (Analyst Model 300) atomic absorption spectrometer equipped with an air-acetylene flame atomizer. The heavy metal content was expressed as mg/g dw of the sample.

Statistical data. Data was subjected to analysis of variance (ANOVA) by Agries programme and Microsoft Excel for Standard Error.

RESULTS AND DISCUSSION

Fresh weight

The results pertaining to effect of different concentrations of heavy meals on biomass yield of *Lemna polyrrhiza* are depicted in Tables 1 and 2. It was observed that 1 mg/l of Cd and 1 mg/l of Pb increased growth at the end of 30 days to 13% and 28% of the control, respectively. The concentration of 30 and 40 mg/l of Cd and Pb proved to be toxic, affecting the plant growth severely. Fresh weight after 30 days decreased from 35.30 g to 0.55 g and 1.22 g with 40 mg/l of Cd and 40 mg/l of Pb, respectively.

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). In our study, varied concentrations of Cd and Pb affected fresh weight of *L. polyrrhiza*. The reduction in the growth in *L. polyrrhiza* 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 Okamoto 1993). Parameters such as fresh weight of shoot as well as root length were used as useful indicators of metal toxicity in plants. In our study, Cd stress showed a higher decline in these parameters as compared to Pb.

Chlorophyll content

It was observed that 1 mg/l of each Cd and Pb used individually marginally increased the chlorophyll (chl *a*, chl *b* and total chlorophyll). The prolonged exposure to high concentration of Cd (40 mg/l) reduced chl *a* significantly to about 82% of the control, and Pb showed the decline of 77% of the control after 30 days (Figure 1).

Similarly, chl *b* decreased from 0.36 to 0.01 and 0.04 mg/g fw after 24 days of exposure to 40 mg/l of Cd and 40 mg/l of Pb, respectively (Figure 2).

Table. 1. Effect of different concentrations of Cd on the fresh weight (g) of *Lemna polyrrhiza*. Values are means of ± SE (n = 3)

Cd (ppm)	Number of days				
	6	12	18	24	30
Control	7.52 ± 0.04	9.52 ± 0.07	12.54 ± 0.09	20.93 ± 0.05	35.30 ± 0.02
1	7.61 ± 0.03	10.89 ± 0.02	15.32 ± 0.10	25.9 ± 0.07	39.98 ± 0.02
10	6.87 ± 0.09	8.35 ± 0.02	9.61 ± 0.07	13.66 ± 0.04	17.01 ± 0.04
20	5.10 ± 0.08	4.71 ± 0.03	4.24 ± 0.07	3.25 ± 0.05	3.21 ± 0.02
30	4.21 ± 0.06	3.95 ± 0.04	2.5 ± 0.07	2.1 ± 0.03	1.24 ± 0.09
40	3.5 ± 0.03	2.21 ± 0.06	1.51 ± 0.06	0.98 ± 0.98	0.55 ± 0.03
CD at 5%	1.43	1.65	2.77	3.21	4.64

Table. 2. Effect of different concentrations of Pb on the fresh weight (g) of *Lemna polyrrhiza*. Values are means of ± SE (n = 3)

Pb (ppm)	Number of days				
	6	12	18	24	30
Control	7.52 ± 0.04	9.52 ± 0.07	12.54 ± 0.09	20.93 ± 0.05	35.30 ± 0.02
1	7.88 ± 0.03	13.64 ± 0.05	20.32 ± 0.06	30.87 ± 0.02	45.32 ± 0.08
10	7.32 ± 0.06	9.43 ± 0.03	11.32 ± 0.11	20.1 ± 0.09	33.56 ± 0.02
20	6.86 ± 0.05	10.22 ± 0.04	14.8 ± 0.09	17.32 ± 0.07	18.44 ± 0.08
30	6.43 ± 0.03	6.23 ± 0.11	5.55 ± 0.09	5.03 ± 0.04	4.65 ± 0.07
40	4.32 ± 0.08	3.23 ± 0.03	2.34 ± 0.10	2.21 ± 0.06	1.22 ± 0.04
CD at 5%	1.21	2.64	3.23	5.73	8.74

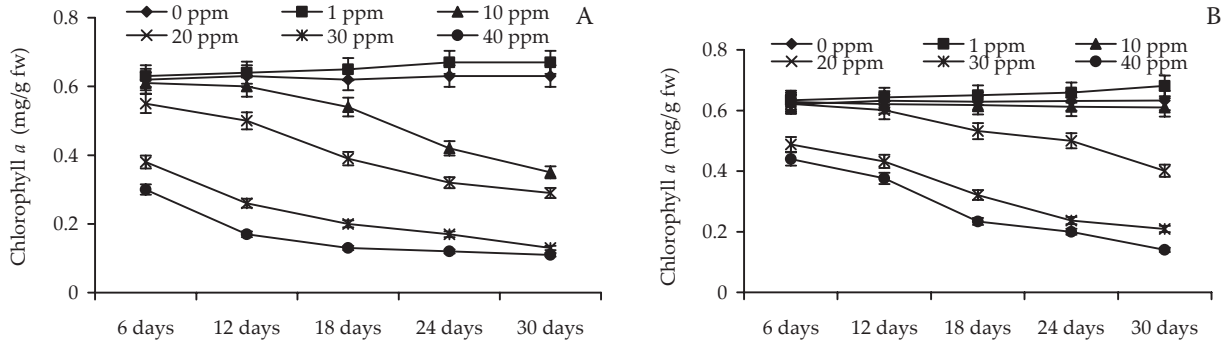

Figure 1. Effect of different concentrations of Cd (A), Pb (B) on chlorophyll *a* of *Lemna polyrrhiza*. Values are means ± SE (n = 3)

10 mg/l of Cd and Pb concentrations decreased the total chlorophyll with time (Figure 3).

Various abiotic stresses decrease the chlorophyll content in plants (Ahmad et al. 2007). Several reports show chlorophyll biosynthesis inhibition by metals in higher plants (Prasad and Prasad 1987). 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) and protochlorophyllide reductase (Van Assche and Clijsters 1990) associated with chlorophyll biosynthesis; (b) impairment in the supply of Mg^{2+} and Fe^{2+} required for the synthesis of chlorophylls; (c) Zn^{2+} deficiency resulting in inhibition of enzymes, such as carbonic anhydrase (Van Assche and Clijsters 1990); (d) the replacement of Mg^{2+} ions associated with the tetrapyrrole ring

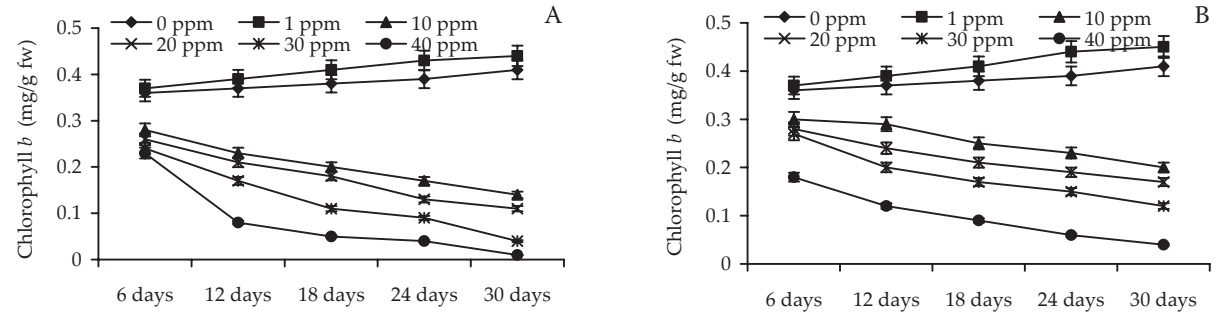

Figure 2. Effect of different concentrations of Cd (A), Pb (B) on chlorophyll *b* of *Lemna polyrrhiza*. Values are means ± SE (n = 3)

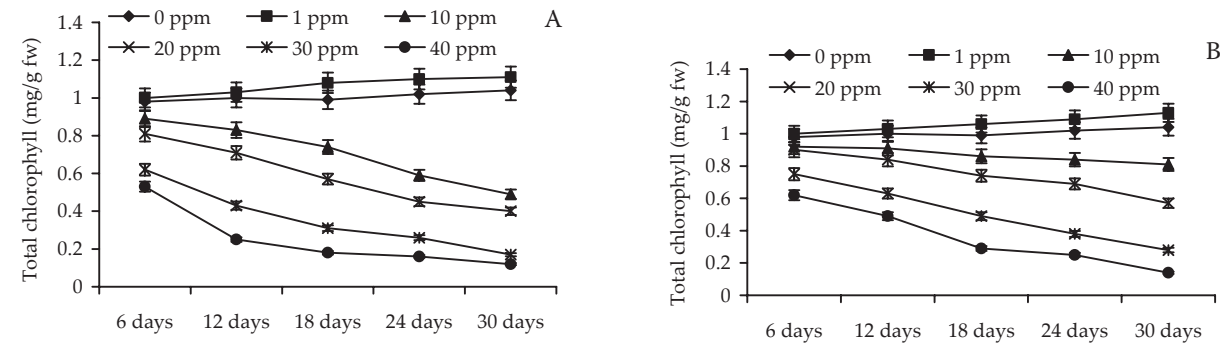

Figure 3. Effect of different concentrations of Cd (A), Pb (B) on total chlorophyll of *Lemna polyrrhiza*. Values are means ± SE (n = 3)

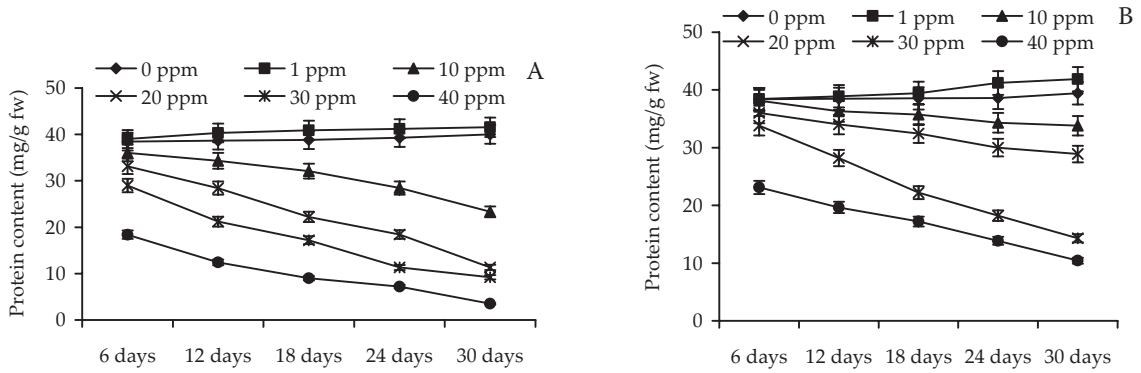

Figure 4. Effect of different concentrations of Cd (A), Pb (B) on soluble protein content (μg/g fw) of *Lemna polyrrhiza*. Values are means ± SE (n = 3)

of chlorophyll molecule. Our results of decrease in chlorophyll content corroborated with the findings of Siedlecka and Krupa (1996) who also found a decrease in chlorophyll content with heavy metal stress in *Zea mays* and *Acer rubrum*. The loss in chlorophyll content can consequently lead to disruption of photosynthetic machinery.

Soluble protein content

Cd treatment (20 mg/l) declined the soluble protein in *L. polyrrhiza* to 52% and 70% after 24 days and 30 days, respectively, while 30 mg/l of Cd proved to be significantly more toxic decreasing the value to about 76% of the control after 24 days (Figure 4A). Cd showed much more toxic effects as compared to Pb. The exposure to 30 mg/l Pb led to the decline of 62% and 72.8% after 24 days and 30 days, respectively (Figure 4B).

Abiotic stress may inhibit a synthesis of some proteins and promote others (Ericson and Alfinito 1984) with a general trend of decline in the overall content. Our studies coincide with Costa and Spitz (1997) who also reported a decrease in soluble protein content under heavy metal stress in *Lupinus albus.* Mohan and Hosetti (1997) found more pronounced decrease in the protein content with Cd as compared to Pb treatment in *L. minor.* The decrease in protein content in *L. polyrrhiza* may be caused by enhanced protein degradation process as a result of increased protease activity (Palma et al. 2002) that is found to increase under stress conditions. It is also likely that these heavy metals may have induced lipid peroxidation in *L. polyrrhiza* and fragmentation of proteins due to toxic effects of reactive oxygen species led to reduced protein content (Davies et al. 1987).

Proline content

Maximum proline accumulation of 5.6 fold was observed with 20 mg/l Cd 24 days (Figure 5A) but 30 mg/l of Cd declined the proline to about 25% of the control, while in the case of Pb (30 mg/l) the maximum accumulation of proline was found 5.1 fold after 30 days (Figure 5B).

Proline, an amino acid, is well known to get accumulated in wide variety of organisms ranging

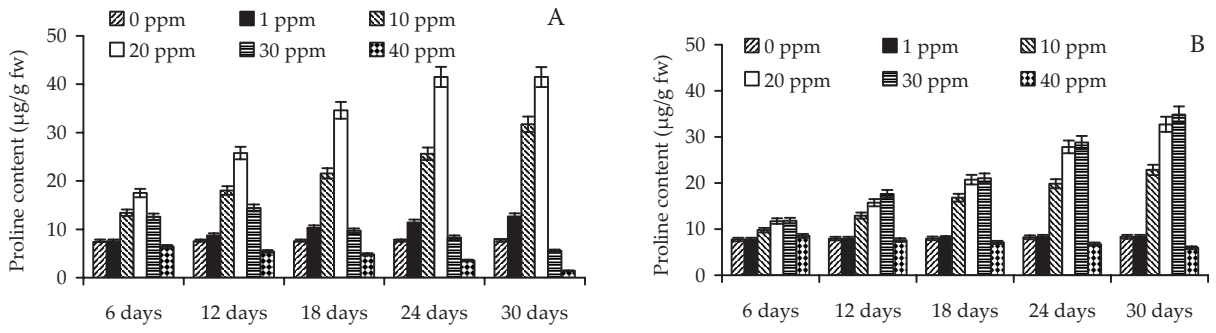

Figure 5. Effect of different concentrations of Cd (A), Pb (B) on proline content (μg/g fw) of *Lemna polyrrhiza*. Values are means ± SE (n = 3)

Table 3. Effect of different concentrations of Cd on soluble sugars (mg/g fw). Values are means ± SE (n = 3)

Cd (ppm)	Number of days				
	6	12	18	24	30
Control	32.44 ± 4.2	33.32 ± 5.1	33.54 ± 5.5	33.88 ± 5.4	34.01 ± 5.7
1	33.56 ± 4.8	34.76 ± 4.9	35.29 ± 5.1	37.25 ± 5.7	39.44 ± 6.2
10	33.11 ± 3.9	32.17 ± 4.7	33.12 ± 5.1	33.55 ± 5.3	34.36 ± 4.8
20	29.32 ± 3.7	27.43 ± 4.6	25.21 ± 3.8	22.11 ± 3.3	20.12 ± 2.9
30	22.43 ± 3.7	20.11 ± 3.5	18.67 ± 3.5	15.66 ± 2.6	11.01 ± 1.9
40	18.13 ± 2.8	16.47 ± 2.5	13.36 ± 2.2	11.59 ± 1.4	8.12 ± 1.1
CD at 5%	7.32	7.11	6.84	5.88	4.32

Table 4. Effect of different concentrations of Pb on soluble sugars (μg/g fw). Values are means ± SE (n = 3)

Pb (ppm)	Number of days				
	6	12	18	24	30
Control	32.44 ± 4.2	33.32 ± 5.1	33.54 ± 5.5	33.88 ± 5.4	34.01 ± 5.7
1	33.12 ± 3.8	34.06 ± 4.5	35.35 ± 4.7	36.22 ± 5.3	37.09 ± 5.3
10	33.05 ± 3.1	33.89 ± 3.4	34.12 ± 2.9	36.01 ± 2.9	36.55 ± 2.5
20	32.5 ± 4.7	31.12 ± 3.3	31.55 ± 3.0	32.61 ± 2.8	31.32 ± 2.1
30	29.3 ± 1.4	28.4 ± 2.7	27.37 ± 2.5	26.52 ± 1.5	25.61 ± 1.2
40	25.3 ± 3.1	20.6 ± 2.4	17.53 ± 1.9	15.41 ± 1.2	10.05 ± 1.0
CD at 5%	7.71	6.52	5.11	4.31	4.12

from bacteria to higher plants on exposure to abiotic stress (Saradhi et al. 1993, Ahmad et al. 2006). Proline accumulation in shoots of *Brassica juncea*, *Triticum aestivum* and *Vigna radiata* in response to cadmium toxicity was demonstrated by Dhir et al. (2004) but they found that proline accumulation decreased with the exposure to cadmium in hydrophytes (*Ceratophyllum*, *Wolffia*, and *Hydrilla)*. It has been often suggested that proline accumulation may contribute to osmotic adjustment at the cellular level (Perez-Alfocea et al. 1993) and stabilizes the structure of macromolecules and organelles. Proline also acts as a major reservoir of energy and nitrogen, which can be used in resuming the growth (Chandrashekhar and Sandhyarani 1996) after the stress removal.

Soluble sugar content

The results related to the soluble sugar content are depicted in Tables 3 and 4, which revealed that lower concentrations of Cd and Pb increased the soluble sugar content; however, higher concentrations of 40 mg/l of Cd and Pd showed a decrease of 74.7 and 73.8% in soluble sugar content after 30 days, respectively.

The total carbohydrates got inhibited if Cd concentration is more than 5 mg/kg soil (Saleh and Al-Garni 2006). Our results corroborate with the findings of Ahmad et al. (2006) who found that an increase in soluble sugars at low concentrations of salt stress and decrease at higher concentrations in *Pisum sativum*. The decrease in total sugar content of stressed leaves probably corresponded with the photosynthetic inhibition or stimulation of respiration rate. Higher starch accumulation in damaged leaves of *Tilia argentea* and *Quercus cerris* may result both in the higher resistance of their photosynthetic apparatus (Prokopiev 1978) and low starch export from the mesophyll. The negative effect of heavy metals on carbon metabolism is a result of their possible interaction with the reactive centre of ribulosebisphosphate carboxylase (Stiborova et. al. 1987).

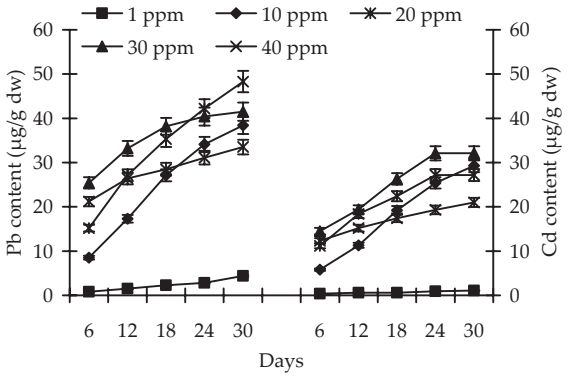

Figure 6. Absorption of Cd and Pb by *Lemna polyrrhiza* from individual metal solutions. Values are means ± SE (n = 3)

Absorption of Cd and Pb

Lemna polyrrhiza absorbed less Cd as compared to Pb. The concentrations of Cd and Pb were 0.42 and 0.81 µg/g dw, respectively (Figure 6). The rate of accumulation of Cd and Pb was higher at lower concentrations. Among different heavy metal treatments, almost linear uptake was observed for 10 mg/l Cd while in the case of Pb linear uptake took place for 10 and 20 mg/l Pb (Figure 6). For lower concentrations, i.e. 10 and 20 mg/l, the uptake was concentration and time dependent. However, at higher concentrations (30 and 40 mg/l) the uptake of both metals was lower and at the end of 30 days the absorption was almost stagnant at 40 mg/l due to the toxicity caused to the plant (Figure 6).

Duckweed (*L. minor*) was found to be an efficient hyperaccumulator of heavy metals and to exhibit mortality at higher concentrations of metals. Rahmani and Sternberg (1999) observed the complete die-off in *L. minor* at high doses of Pb. Wojcik et al. (2005) found higher metal accumulation in roots than in shoots of hydroponically grown *Thlaspi caerulescens*. Some literature data show a higher Cd accumulation in shoots than in roots (Roosens et al. 2003) as well, although other authors reported a higher Cd content in roots than in shoots. Our studies corroborate with Brennan and Shelley (1999) who found higher accumulation of Pb in the roots than shoots of maize.

The bioaccumulation of single metal is known to be influenced by the presence of other metals, resulting in inhibited or enhanced bioaccumulation of one metal in the mixture (An et al. 2004). Several studies reported that the presence of one metal influenced the uptake of another metal (Peralta-Videa et al. 2002). Our studies show a higher accumulation of Cd than Pb, which confirms the results of An et al. (2004) who observed lesser uptake of Cd in the shoots of *Cucumis sativus* in presence of Pb.

In conclusion, our results indicate that the exposition of *Lemna polyrrhiza* to different concentrations of Cd and Pb results in an increase in growth, pigment content, proline, protein and sugar content at lower concentration; at higher concentrations their decrease was observed. Cd effect was more significant than that of Pb in hampering plant growth and development. Cd was accumulated more than Pb by *L. polyrrhiza*. Phytoremediation may contribute in the treatment of various sites contaminated with heavy metals/toxic metals. *L. polyrrhiza* can be used to reclaim the water bodies polluted with heavy metals.

REFERENCES

Ahmad P., Sharma S., Srivastava P.S. (2006): Differential physio-biochemical responses of high yielding varieties of Mulberry (*Morus alba*) under alkalinity (Na_2CO_3) stress *in vitro*. Physiol. Mol. Biol. Plants, *12*: 59–66.

Ahmad P., Sharma S., Srivastava P.S. (2007): *In vitro* selection of $NaHCO_3$ tolerant cultivars of *Morus alba* (Local and Sujanpuri) in response to morphological and biochemical parameters. Hort. Sci. (Prague), *34*: 114–122.

Aidid S.B., Okamoto H. (1993): Responses of elongation growth rate, turgor pressure and cell wall extensibility of stem cells of *Impatiens balsamina* to lead, cadmium and zinc. Biometals, *6*: 245–249.

An Y.J., Kim Y.M., Kwon T.I., Jeong S.W. (2004): Combined effects of copper, cadmium, and lead upon *Cucumis sativus* growth and bioaccumulation. Sci. Total Environ., *326*: 85–93.

Arduini I., Godbold D.L., Onnis A. (1996): Cadmium and copper uptake and distribution in Mediterranean tree seedlings. Physiol. Plant., *97*: 111–117.

Bates L.S., Waldren R.P., Teare I.D. (1973): Rapid determination of free proline for water-stress studies. Plant Soil, *39*: 205–207.

Bradford M.M. (1976): A rapid and sensitive method for the quantitation of microgram quantities of protein utilizating the principle of protein dyes binding. Anal. Biochem., *72*: 248–254.

Brennan M.A., Shelley M.L. (1999): A model of the uptake, translocation and accumulation of lead (Pb) by maize for the purpose of phytoextraction. Ecol. Eng., *129*: 271–297.

Chandrashekhar K.R., Sandhyarani S. (1996): Salinity induced chemical changes in *Crotalaria striata* DC. Indian J. Plant Physiol., *1*: 44–48.

Costa G., Spitz E. (1997): Influence of cadmium on soluble carbohydrates, free amino acids, protein content of *in vitro* cultured *Lupinus albus*. Plant Sci., *128*: 131–140.

Davies C.S., Nielsen S.S., Nielsen N.C. (1987): Flavor improvement of soybean preparations by genetic removal of lipoxygenase-2. J. Am. Oil Chem. Soc., *64*: 1428–1433.

Dey P.M. (1990): Oligosaccharides. In: Dey P.M., Harborne J.B. (eds.): Methods in Plant Biochemistry, Vol. 2, Carbohydrates. Academic Press, London: 189–218.

Dhir B., Sharmila P., Saradhi P.P. (2004): Hydrophytes lack potential to exhibit cadmium stress induced enhancement in lipid peroxidation and accumulation of proline. Aquat. Toxicol., *66*: 141–147.

Ericson M.C., Alfinito A.E. (1984): Proteins produced during salt stress in tobacco cell cultures. Plant Physiol., *74*: 506–509.

Glass D.J. (2000): Economic potential of phytoremediation. In: Raskin I., Ensley B.D. (eds.): Phytoremediation of Toxic Metals: Using Plants to Clean up the Environment. John Wiley and Sons, New York: 15–31.

Hiscox J.D., Israelstam G.F. (1979): A method for the extraction of chlorophyll from leaf tissue without maceration. Can. J. Bot., *59*: 1332–1334.

Mohan B.S., Hosetti B.B. (1997): Potential phytotoxicity of lead and cadmium to *Lemna minor* L. growth in sewage stabilization ponds. Environ. Pollut., *98*: 233–236.

Moya J.L., Ros R., Picazo I. (1993): Influence of cadmium and nickel on growth, net photosynthesis and carbohydrate distribution in rice plants. Photosynth. Res., *36*: 75–80.

Palma J.M., Sandalio L.M., Javier Corpas F., Romero-Puertas M.C., McCarthy I., del Rio L.A. (2002): Plant proteases protein degradation and oxidative stress: role of peroxisomes. Plant Physiol. Biochem., *40*: 521–530.

Patra M., Bhowmik N., Bandopadhyay B., Sharma A. (2004): Comparison of mercury, lead and arsenic with respect to genotoxic effects on plant systems and the development of genetic tolerance. Environ. Exp. Bot., *52*: 199–223.

Peralta-Videa J.R., Gardea-Torresdey J.L., Gomez E., Tiermann K.J., Parson J.G., Carrillo G. (2002): Effect of mixed cadmium, copper, nickel and zinc at different pH upon alfafa growth and heavy metal uptake. Environ. Pollut., *119*: 291–301.

Perez-Alfocea F., Estan M.T., Santa Cruz A., Bolarin M.C. (1993): Effects of salinity on nitrate, total nitrogen, soluble protein and free amino acid levels in tomato plants. J. Hort. Sci., *68*: 1021–1027.

Prasad D.P.H., Prasad A.R.K. (1987): Effects of lead and mercury on chlorophyll synthesis in mungbean seedlings. Phytochemistry, *26*: 881–884.

Prasad M.N.V., Malec A., Waloszek A., Bojko M., Strzalka K. (2001): Physiological responses of *Lemna trisulca* L. to cadmium and copper bioaccumulation. Plant Sci., *161*: 881–889.

Prokopiev E. (1978): Afforestation of Industrial Areas. Zemizdat, Sofia. (In Bulgarian)

Rahmani G.N.H., Sternberg S.P.K. (1999): Bioremoval of lead from water using *Lemna minor*. Biores. Technol., *70*: 225–230.

Roosens N., Verbruggen N., Meerts P., Ximenez-Embun P., Smith J.A. (2003): Natural variation in cadmium tolerance and its relationship to metal hyperaccumulation for seven populations of *Thlaspi caerulescens* from western Europe. Plant Cell Environ., *26*: 1657–1673.

Saleh M., Al-Garni S. (2006): Increased heavy metal tolerance of cowpea plants by dual inoculation of an arbuscular mycorrhizal fungi and nitrogen-fixer *Rhizobium* bacterium. Afr. J. Biotechnol., *5*: 133–142.

Saradhi P., Alia, Vani B. (1993): Inhibition of mitochondrial electron transport is the prime cause behind proline accumulation during mineral deficiency in *Oryza sativa*. Plant Soil, *155/156*: 465–468.

Shah K., Dubey R.S. (1998): A 18 kDa cadmium inducible protein complex from rice: its purification and characterization from rice (*Oryza sativa* L.) roots tissues. J. Plant Physiol., *152*: 448–454.

Siedlecka A., Krupa Z. (1996): Interaction between cadmium and iron and its effects on photosynthetic capacity of primary leaves of *Phaseolus vulgaris*. Plant Physiol. Biochem., *34*: 833–841.

Stiborová M., Ditrichová M., Březinová A. (1987): Effect of heavy metal ions on growth and biochemical characteristics of photosynthesis of barley and maize seedlings. Biol. Plant., *29*: 453–467.

Ting Y.P., Lawson F., Prince I.G. (1991): Uptake of cadmium and zinc by alga *Chlorella vulgaris*: Multi-ion situation. Biotechnol. Bioeng., *37*: 445–455.

Van Assche F., Clijsters H. (1990): Effects of metals on enzyme activity in plants. Plant Cell Environ., *13*: 195–206.

Waisberg M., Joseph P., Hale B., Beyersmann D. (2003): Molecular and cellular mechanisms of cadmium carcinogenesis. Toxicology, *192*: 95–117.

WHO (1995): Inorganic Lead. Environmental Health Criteria 165. World Health Organization, Geneva.

Wojcik M., Vangronsveld J., Tukiendorf A. (2005): Cadmium tolerance in *Thlaspi caerulescens*. I. Growth parameters, metal accumulation and phytochelatin synthesis in response to cadmium. Environ. Exp. Bot., *53*: 151–161.

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