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Preferential Accumulation of Cadmium and Chromium: Toxicity in *Bacopa monnieri* L. under Mixed Metal Treatments

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Metals are released into the environment from a wide spectrum of anthropogenic activities such as smelting of metallic ores, industrial fabrication and commercial application of metals, which are polluting our aquatic bodies. Amongst these, Cd and Cr are two environmental toxicants that cause poisonous effects on both animals and plants (Sharma et al., 1995) and their concentrations in the environment are escalating. This situation is being aggravated, which needs a technically affordable eco-technological solution. The discovery of metal hyper-accumulating properties in certain plants suggests the potential of using plant-based systems for treatment of wastes containing metals (Ensley, 2000).

In this context, the use of aquatic plants for wastewater treatment has been well documented, which has led to the development of several phytoremediation systems. Phytoremediation is considered to be an effective, low cost, preferred clean-up option for moderately contaminated areas. The ability of wetland plants such as *Typha* sp., *Phragmites* sp., *Scirpus* sp., *Leersia* sp., *Juncus* sp. and *Spartina* sp. to reduce the levels of heavy metals in polluted waters has been demonstrated (Rai et al., 1995; Vajpayee et al., 1995; Lim et al., 2003; Weis and Weis, 2004; Shankers et al., 2005). Therefore, for wetland plants, storing metals below ground is a preferable alternative. While many engineering studies on wetland treatment use a black-box approach, analyzing levels in the influent and effluent (Cheng et al., 2002; Mbuligwe, 2004), more studies are required to determine the pattern and process of metal uptake, distribution and removal by different species of wetland plants. Recently *Leersia hexandra* Swartz has been found to be a new Cr hyper-accumulator (Zhang et al., 2007). Furthermore, in an aquatic ecosystem, metals are usually found in mixed conditions and their uptake is considerably influenced by synergistic or antagonistic interactions of the metals existing in the ambient medium. Such a study focusing on metal interactions with regards to their uptake and toxicity seems to be a prerequisite while assessing suitability of a macrophyte for wastewater treatment programs.

Bacopa monnieri is an aesthetically appealing plant that has the potential for hyper-accumulation of nutrients and metals since it has extensive roots and provides a large surface area that enhances the microbial activities (Sinha et al., 1994; Shukla et al., 2005). The paper reports the effectiveness of *B. monnieri* to remove toxic metals (Cr and Cd) under mixed metal treatment and reports the related toxicity.

Materials and Methods

Young shoots of *B. monnieri* were cultured in 10% Hoagland solution for eight weeks under a fluorescent tube light at an intensity of 2500 lux for 14 hours per day at a temperature of 25 ± 2°C. Stock solutions of Cr and Cd were made using the salt ($K_2Cr_2O_7$ and $CdCl_2$, Himedia Laboratories). Various concentrations (0.01, 0.1, 1.0, 2.5, 5.0 and 10 μg ml^{-1}) for Cr and Cd were made by diluting the stock solution with 10% modified Hoagland medium (Hoagland and Arnon, 1950). The plants cultured in different concentrations of Cr and Cd were supplied with 10% Hoagland solution. Plants were harvested after 24, 48 and 72 h treatment and used for the determination of various

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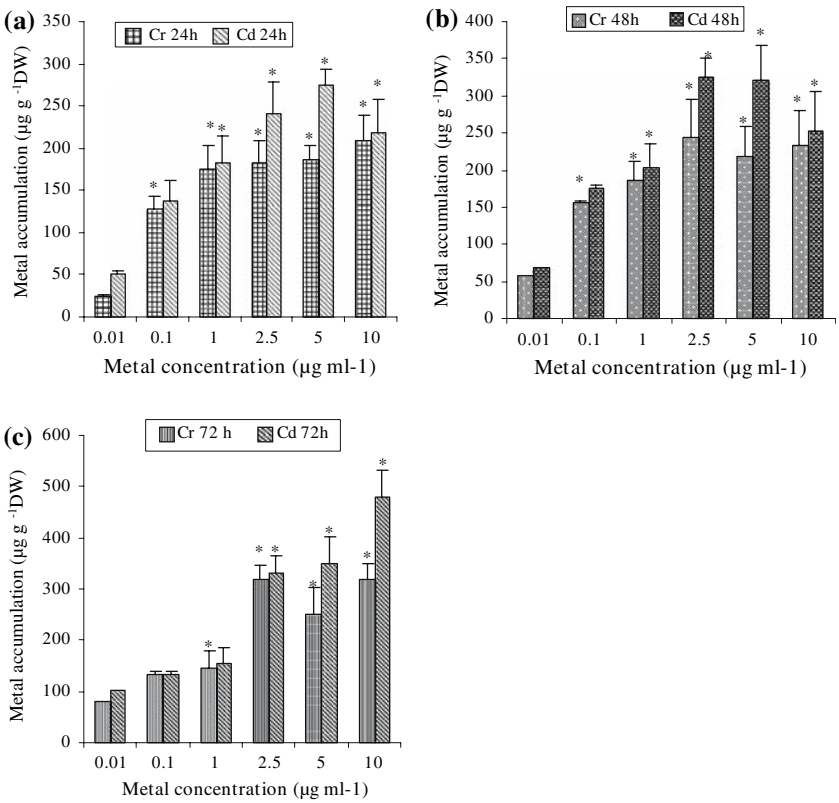

Fig. 1 (a–c) Cr and Cd accumulation potential of whole plant of *B. monnieri* under mixed metal treatment; values are ± SE (n = 5). *LSD $p < 0.05$

parameters. For biomass estimation, plants were oven dried and weighed on a dry weight basis using an electronic balance. Pickford's wet ashing method (Pickford, 1989) was adapted for the analysis of metal contents in plants where samples were digested in an acid mixture. Metal content in treated plants was measured by a Perkin Elmer 2380 atomic absorption spectrophotometer. Harvested plants were washed thoroughly, dried and digested in a HNO_3–$HClO_4$ mixture (3:1, v/v) at 80°C. The metal test concentrations were analytically confirmed by estimating Cr and Cd in the test solutions as described above. Standard solutions of Cr and Cd ($K_2Cr_2O_7$ and $CdCl_2$ Merck, Germany) were used to provide calibration and quality assurance for each analytical batch. Chlorophyll and carotenoid measurements were made from chilled 80% acetone extracts of fresh leaves of control and treated plants following the methods of Arnon (1949) and Duxbury and Yentsch (1956), respectively. Protein was estimated following the methods of Lowry et al. (1951).

Combined Cr and Cd solutions at concentrations of 0.01, 0.1, 1.0, 2.5, 5.0 and 10.0 µg ml^{-1} were used in the study to assess the bioaccumulation potential of *B. monnieri*. All the experiments were conducted in triplicate over a period of three days as preliminary tests to show that plants were able to grow well and stay healthy without nutrient supply in water for ten days. All plants were placed in a 1 L beaker containing 500 ml of mixed metal solution. The pH of the solution was adjusted to 6.8–7.2, which was more suitable for the plants. There was no change in solution pH at the end of the experiment. The experiment was carried out at room temperature (25 ± 2°C) in the laboratory. A plant control free of heavy metals and a metal control free of plants were used for each set of experiments. Solutions and plant samples were collected periodically from 0 to 72 h after exposure of Cr and Cd. The whole plant was washed with tap water followed by deionized water. The materials were then oven dried at 80°C until a constant weight was obtained. The dried samples were then ground and kept in desiccators before being weighed for the analysis of metal and protein etc. In this study, plants at various intervals were used to investigate the bioaccumulation potential and treatment performances under mixed metal treatments.

A two-way analysis of variance (ANOVA) was performed for all the data to confirm their validity. The significant difference between treatment means for different parameters was tested at $P < 0.05$ using a least significant difference (LSD) test (Gomez and Gomez, 1984).

Results and Discussion

The data presented in Fig. 1(a–c) show the accumulation of Cr and Cd in *B. monnieri* at different concentrations and treatment durations. The results showed a concentration

and duration dependent accumulation of Cr and Cd inside plant tissue. However, the accumulation of Cd was more than Cr at all the concentrations. During metal treatment it was 480.5 μg g^{-1} DW for Cd and 319.5 μg g^{-1} DW for Cr at 10.0 μg ml^{-1} of both metals. Uptake of metal may vary with the metal combinations existing in the ambient medium. In an earlier study, where essential (Cu) and non-essential (Cd) metal coexisted in the medium, uptake of Cu was stimulated by Cd (Sinha et al., 1994). While in the present study, where two non-essential metals coexist in the medium, Cr enhances the uptake of Cd. Cr is also reported to enhance Mn uptake when both exist together in the medium which in turn ameliorates Cr toxicity in *Hydrilla verticillata* (Sinha et al., 1993). Similarly Cr was found to enhance Cd accumulation in *H. verticillata* and *Chara corallina* (Rai and Chandra, 1992; Rai et al., 1995). The concentration factor shown in Table 1 also confirms our observation on preferential uptake of Cd over Cr. Cd and Cr toxicity in *B. monnieri* was evaluated in terms of decrease in biomass. By increasing the concentrations of Cr and Cd in nutrient media, a gradual reduction in biomass of a plant was observed (Fig. 2). A maximum decrease in biomass was found at 10.0 μg ml^{-1} of Cr and Cd, which was 0.057 g FW in comparison to 0.193 g FW in the control after 72 h of treatment. Since the metals were not tested under single metal conditions, the possibility of such an interaction is ruled out. Besides, Cr and Cd concentration proved to be inhibitory with respect to biomass production (Tokalioglu and Kartal, 2006).

Table 1 Concentration factor of Cr and Cd after 72 h of treatment

Metal concentration (μg ml^{-1})	Concentration factor (Cf)	
	Cr	Cd
0.01	2535	5190
0.1	1284	1369
1.0	174	182
2.5	73	97
5.0	37	55
10.0	21	22

The data presented in Fig. 3(A) show the effects of different concentrations of Cr and Cd together on chlorophyll, a component of *B. monnieri*, at different treatment durations. Like other parameters, chlorophyll, a component of the plant, decreased significantly with increased Cr and Cd concentrations in the medium. Chlorophyll a content at 10.0 μg ml^{-1} Cr and Cd concentration was found to be 0.679 mg g^{-1} FW against 2.530 mg g^{-1} FW in the control after 72 h of treatment. Data presented in Fig. 3(B) show the effect of various Cr and Cd concentrations on chlorophyll b content at different treatment periods. There was a gradual decrease in the chlorophyll b content of the plant by increasing metal concentrations. Minimum chlorophyll b content was recorded in a plant treated with 10.0 μg ml^{-1} Cr and Cd concentration, i.e., 0.049 mg g^{-1} FW as compared to 0.761 mg g^{-1} FW in the control after 72 h of treatment. The total chlorophyll was 0.720 mg g^{-1} FW in 10.0 μg ml^{-1} as compared to 3.290 mg g^{-1} FW in the control after 72 h of treatment. Total chlorophyll content indicates the sum of chlorophyll a and b, therefore the effect of different concentrations of Cr and Cd have affected total chlorophyll content in a similar way.

Metals have been reported to inhibit the final reduction stage in chlorophyll formation by interacting with the functional –SH group of the enzyme synthesizing chlorophyll (Prasad and Prasad; 1987). Degradation of the photosynthetic pigment chlorophyll is routinely observed in response to exposure of plants to elevated concentrations of various heavy metals. The resulting decrease in pigments causes deficiency in light harvesting capacity (Ouzounidou, 1996; Mazhoudi et al., 1997) and consequently decreases photosynthetic activity of the cell. Results obtained during the present study confirm the earlier study on a submerged plant *i.e. Potamogeton pectinatus*, *H. verticillata* and *Vallisneria spiralis* (Guilizzoni 1991; Jana and Chaudhary 1982; Rai et al., 2003). The effects of various combinations of Cr and Cd on the carotenoid content of *B. monnieri* at different exposure concentrations are shown in Fig. 4. Carotenoid content was 0.36 mg g^{-1} FW in 10.0 mg l^{-1} against 3.08 mg l^{-1} FW in the control after 72 h of treatment. The data showed a differential response on the carotenoid content of

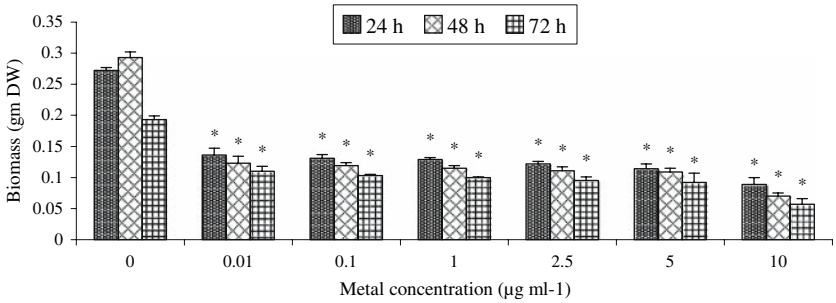

Fig. 2 Effect of mixed metal treatment of Cr and Cd on biomass production (g DW) by *B. monnieri*; values are mean ± SE (n = 5). *LSD $p < 0.05$

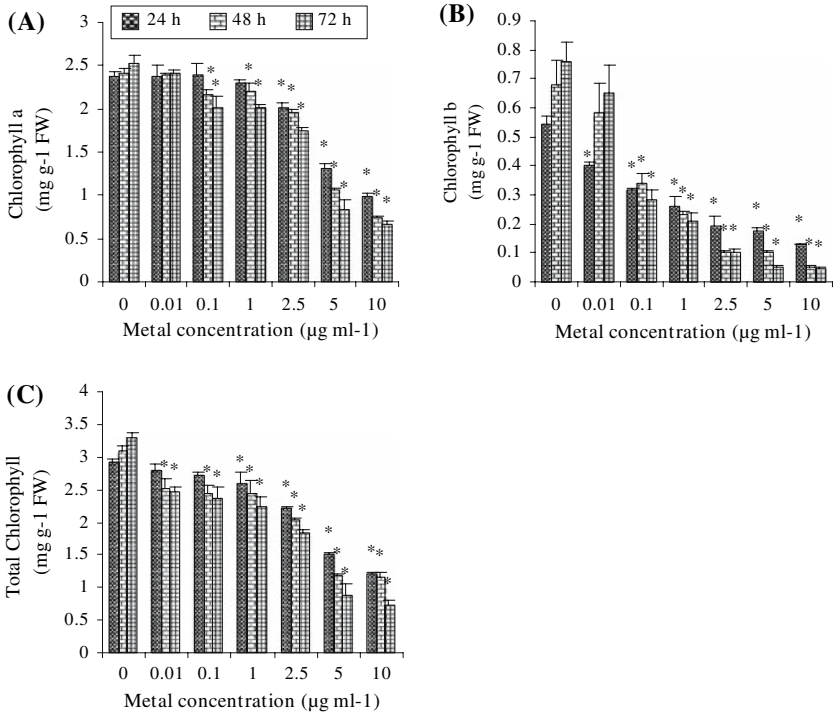

Fig. 3 (a–c) Effects of Cr and Cd accumulation on photosynthetic pigment [A—chlorophyll a; B—chlorophyll b; and C—total chlorophyll (mg g^{-1} FW)] of *B. monnieri*; values are mean ± SE ($n = 5$). *LSD $p < 0.05$

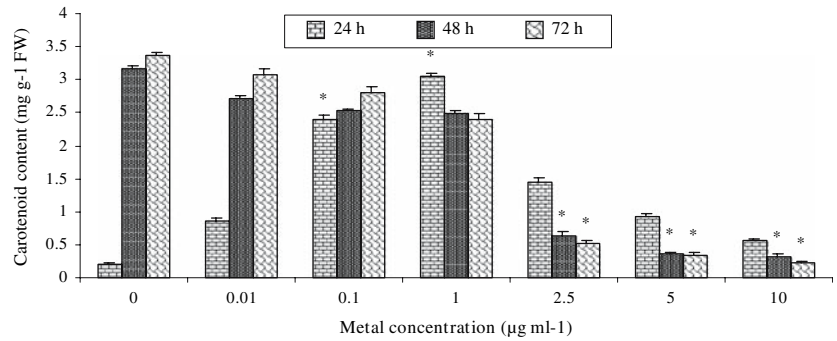

Fig. 4 Effects of Cr and Cd accumulation on carotenoid content (mg g^{-1} FW) of *B. monnieri*; values are mean ± SE ($n = 5$). * = LSD $p < 0.05$

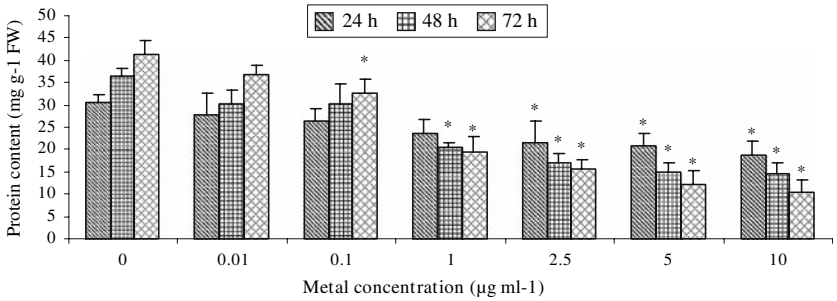

Fig. 5 Effects of Cr and Cd accumulation on protein content (mg g^{-1} FW) of *B. monnieri*; values are mean ± SE ($n = 5$). * = LSD $p < 0.05$

the plant. An increase in carotenoid content was found at low metal concentrations up to 1 µg ml^{-1} Cr and Cd. It declined at higher concentrations (>1.0 µg ml^{-1}), which were inhibitors with respect to carotenoid content. Such an increase in carotenoid content in the plant following metal administration is considered to be an antioxidant response of the plant to reduce metal toxicity.

The effects of different metal combinations on the protein content of *B. monnieri* at different treatment durations are shown in Fig. 5. The soluble protein content of the plant decreased with increasing combined concentrations of both the metals Cr and Cd, which proved to be lethal with regards to protein synthesis as 41.41, 36.87, 32.69, 27.53, 21.12 and 15.0 mg FW protein was found in

Table 2 A summary table of the first effect levels of combined concentrations of Cr and Cd to various parameters of *B. monnieri* exposed to different concentrations of Cr and Cd

Parameters	Combined concentrations of Cr and Cd exhibiting first effect (μg ml^{-1})	
	Stimulatory	Inhibitory
Biomass	-	0.01
Chlorophyll a	-	0.01
Chlorophyll b	-	0.01
Total chlorophyll	-	0.01
Carotenoid	1.0	2.5
Protein	-	0.01

0.0, 0.01, 0.1, 1.0, 2.5, 5.0 and 10.0 μg ml^{-1}, respectively after 72 h. It reduced drastically at 2.5 μg ml^{-1} and 10.0 μg ml^{-1} metal combination. A higher concentration of Cr and Cd (10.0 μg ml^{-1}) has a toxic effect on the protein content of *B. monnieri*. A decrease in protein content in the presence of metal may be due to breakdown of soluble protein or due to the increased activity of protease or other catabolic enzymes, which were activated and destroyed the protein.

The first effect levels of Cr + Cd in this plant ranged between 0.01–2.5 μg ml^{-1} (stimulatory/inhibitory) which was much higher than the concentration of these metals in natural waters (Table 2) and that the adverse impact was not more at lower level of metals.

Further it seems possible that metal accumulation plays an important role in determining toxicity in aquatic species under combined metal treatment. In the case of two non-essential metals, one metal may boost the uptake of the other thereby increasing the toxicity of the plant (Rai et al., 1995). Whereas in the case of essential and non-essential metals, the uptake of essential metal was increased and toxicity was reduced (Sinha et al., 1993). However, for reaching at this type of conclusion, the study of metal uptake in the plant under mixed metal conditions is required, which is beyond the scope of the present study. In the single metal treatment, the accumulation of Cr was found to be greater in comparison to Cd (Shukla et al., 2005; Singh et al., 2006). Thus the results showed that the plant prefers Cd during the mixed metal treatment over Cr during the single metal treatment; Cr accumulation was more than Cd. However, to arrive at this conclusion, studies are required on more plants by taking different groups of metals under single and mixed metal treatments.

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