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Accumulation of chromium and zinc from aqueous solutions using water hyacinth (*Eichhornia crassipes*)

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ABSTRACT

Under present investigation *Eichhornia crassipes* (water hyacinth) has been tested for removal of two important heavy metals chromium (Cr) and zinc (Zn) from metal solution. This species was grown at four concentrations of Cr and Zn, i.e. 1.0, 5.0, 10.0 and 20.0 mg l^{-1} in single metal solution. This plant has performed extremely well in removing the Cr and Zn from their solution and was capable of removing up to 95% of zinc and 84% of chromium during 11 days incubation period. Removal of Cr at lower concentrations (1.0 and 5.0 mg l^{-1}) was found harmless, without any symptom of toxicity but at 10.0 and 20.0 mg l^{-1}, plants have shown some morphological symptoms of toxicity. On the other hand *E. crassipes* removed Zn safely at all the four concentrations, i.e. 1.0, 5.0, 10.0 and 20.0 mg l^{-1}. In this case morphological symptoms of toxicity were not evident in the test plant. Biochemical parameters viz. protein, sugar and chlorophyll in experimental plants have shown a decreasing trend due to accumulation of Zn and Cr. Overall this methodology is safe for the removal of Zn and Cr and can be utilized at large scale after few further investigation.

1. Introduction

The presence of toxic heavy metals in aquatic ecosystems, resulting from the discharges of untreated metal containing effluents into water bodies, is one of the most important environmental concerns for scientists. Heavy metals are reported to be toxic and found associated with the occurrence of several health effects. Considering its effects on human beings and other aquatic organisms, appropriate treatment of the heavy metals from the waste water is of utmost importance. Different methodologies are used for the removal of the different heavy metals viz. electrodialysis, reverse-osmosis, and adsorption. All of these methodologies used are quite costly and energy intensive, none of them could claim to treat all the heavy metals in economically feasible manner [1].

Economies of developing countries like India have other investment priority therefore they cannot afford the high price involved in the removal of heavy metals from waste water. Contrary to this phytoremediation, i.e. removal of metals through plants offers an eco friendly and cost effective methodology for the treatment of heavy metals from waste water. Therefore, under present investigation phytoremediation of two heavy metals zinc (Zn) and chromium (Cr) by aquatic macrophyte *Eichhornia crassipes* is tested. Among various plant groups used for phytoremediation, aquatic macrophytes attain the most important position.

Several species of aquatic macrophytes such as water hyacinth (*Eichhornia* sp.), Duck-weeds (*Lemna* sp., *Spirodella* sp.), small water fern (*Azolla* sp.) and water lettuce (*Pistia* sp.) have been used for the removal of heavy metals from waste water [2–6]. The aquatic macrophytes are free-floating aquatic plants, entire root system of these plants is submerged in water. All of the above species take up metals from water producing an internal concentration several folds greater than surroundings [7]. Water hyacinth (*E. crassipes*), a rooted macrophyte, known to grow profusely in polluted water bodies, eutrophic lakes and has great potential for the heavy metal accumulation. In spite of being noxious weed; this species has been an important choice for phytoremediation of heavy metals from waste water due to its several advantages over other species [8].

Under present investigation the removal efficiency of water hyacinth was evaluated for two important heavy metals Cr and Zn. Zinc is a non-essential toxic heavy metal, released into the environment from power stations, mining, metal-working industries, waste incinerators and phosphate fertilizer plants. Zinc has become an increasing problem and its toxic effects on biological systems have been reported by various authors. Zinc ions are readily taken up by roots and translocated into the leaves in many plant species, depressing growth by affecting photosynthesis, chlorophyll fluorescence and nutrient uptake by plants.

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Chromium, a non-essential micronutrient for normal plant metabolism, has been reported to be one of the most toxic heavy metals present in waste water discharges from electroplating, dye and pigment manufacturing, wood preserving and leather tanning industries. In addition to being highly toxic, Cr is mobile, and has a long residence time in surface water and groundwater; it poses severe health risk to human beings, aquatic animals, impairs the development and growth of plants [9–11]. Excessive Cr accumulation in the plant tissue can be toxic to the plants, affecting several physiological and biochemical processes and growth. Chromium treatment brings changes in nitrogen metabolism with a reduction of total nitrogen [12,13].

2. Materials and methods

2.1. Experimental set-up

Water hyacinth (*E. crassipes*) with approximately the same size and weight, 7–8 weeks old were used for the removal of Cr and Zn. The plants were washed thoroughly with tap water followed by de-ionized water prior to the experimentation. All the plants were grown in 15 l experimental tanks filled with 10 l of water. A plant control, i.e. plant grown in tap water and metal control, metal solution without any plants were also established. All the experimental sets were maintained in duplicate. The concentrations of metal ions in solution were determined before the commencement of the experiment. Solution samples were collected periodically from day 0 to day 11 from experimental tank for the determination of metal concentrations with time span elapsed. Loss of water due to evaporation was made up daily by adding tap water to the mark in the experimental tank.

2.2. Heavy metal preparation

Chromium and zinc were added to make their concentrations 1, 5, 10 and 20 mg l^{-1} in water. All of these metals were added as single metal solution, i.e. one experimental set contained a single metal in particular concentration. These heavy metals were added as potassium dichromate ($K_2Cr_2O_7 \cdot 5H_2O$) and zinc nitrate ($(Zn(NO_3)_2 \cdot 6H_2O$). The reagents were dissolved in distilled water to get the desired contamination level. These contaminants were added to all the experimental sets.

2.3. Heavy metal analysis in plants

Experimental plants were harvested seven times during 11 days experiment, i.e. on days 1, 2, 3, 5, 7, 9 and 11 of the exposure. Harvested plants were washed thoroughly twice with tap water followed by de-ionized water. These plants were separated into roots, leaves and stem. All the parts were then oven dried at 80 °C to remove all the moisture. The oven dried samples were grounded to powder and stored for wet digestion for the analyses of metal (Cr and Zn), protein and sugar concentrations. Duplicate portions of ground plant material (2.0 g) were weighed for analysis of metal concentrations while 0.5 g plant materials were used for protein and sugar analyses. After drying the plant samples were grounded by mortar and pestle. These samples were digested with HNO_3–$HClO_4$ in 2:1 ratio (v/v) and diluted to 100 ml with de-ionized water. The digested plant samples were analyzed for heavy metals by means of atomic absorption spectrophotometry (AAS, PerkinElmer). The method of Lowry et al. [14] and the phenol–sulphuric acid method [15] were adopted for protein estimation and sugar assay, respectively.

Table 1
Average physico chemical composition of the water used for the study.

Parameters	Results
Temperature (°C)	28.5 ± 0.7
pH	8.3 ± 0.20
DO (mg l^{-1})	0.87 ± 0.13
BOD (mg l^{-1})	30 ± 2.5
COD (mg l^{-1})	45 ± 3.5
Total suspended solids (mg l^{-1})	1098 ± 10
Total dissolved solids (mg l^{-1})	746 ± 7
Total nitrogen (mg l^{-1})	48.2 ± 4.03
Total phosphorus (mg l^{-1})	6.03 ± 0.33

Table 2
Removal of Zn and Cr by *Eichhornia crassipes*.

Heavy metals	Concentrations (mg l^{-1})	% Removal
Zn	1	94
	5	91
	10	95
	20	88
Cr	1	84
	5	79
	10	72
	20	63

2.4. Statistical analysis

Metal concentrations in water were reported in mg l^{-1} and concentrations in plant parts are reported in mg g^{-1} dry weight and are means of three replicates. Correlation and regression analysis was performed by using SPSS 12 package.

3. Results and discussion

3.1. Removal of Cr and Zn from water

Chemical composition of the water used for the removal experiment is shown in Table 1. All the parameters except heavy metal concentration were approximately same. Four different heavy metal concentrations, i.e. 1, 5, 10 and 20 mg l^{-1} were used in different experimental sets. Removal efficiencies of the plant at different concentrations (1.0, 5.0, 10.0 and 20.0 mg l^{-1}) of metals used for experiment are shown in Table 2. The initial and final concentrations of heavy metals within the plants are presented in Table 3. The variations of the metal concentrations with increasing time span in the different experimental sets are shown in Figs. 1 and 2. Chlorophyll, protein and sugar content in roots and leaves of macrophytes before and after 11 days of exposure to Zn and Cr are shown in Tables 4 and 5, respectively. Mean concentrations of Zn and Cr in different parts of *E. crassipes* are shown in Table 6.

Results revealed increasing trend of removal with the increasing incubation period. Analysis of variance showed significant ($p < 0.001$) differences between the removal and incubation period for all the experimental sets. Analysis of metal concentration with increasing time has suggested that metal concentrations decreased from day 1 to day 11 in different experimental sets. High removal

Table 3
Initial and final concentrations of Zn and Cr in *Eichhornia crassipes*.

Initial concentration (mg l^{-1})	Zn concentration in plants (mg g^{-1} dry weight)	Cr concentration in plants (mg g^{-1} dry weight)
1	0.88	0.10
5	1.22	1.13
10	1.83	1.41
20	2.32	1.71

Table 4
Chlorophyll and protein changes in *Eichhornia crassipes* 7 days after exposure to Cr.

Plant part	Cr concentration ($mg\,l^{-1}$)	Chlorophyll content ($mg\,g^{-1}$ dry weight)	Protein content ($mg\,g^{-1}$ dry weight)	Sugar ($mg\,g^{-1}$ dry weight)
Leaves	0.0 (control)	11.53 ± 0.22	93.34 ± 3.16	51.93 ± 1.32
	1	9.67 ± 0.17	89.55 ± 3.1	37.25 ± 1.41
	5	9.13 ± 0.14	78.17 ± 1.27	31.79 ± 0.65
	10	7.83 ± 0.13	72.54 ± 0.69	25.79 ± 0.57
	20	5.84 ± 0.08	55.65 ± 1.10	16.51 ± 0.40
Stem	0.0 (control)	–	39.25 ± 0.31	11.87 ± 0.25
	1	–	34.35 ± 0.30	8.12 ± 0.10
	5	–	31.29 ± 0.30	7.51 ± 0.10
	10	–	20.61 ± 0.25	5.21 ± 0.10
	20	–	23.55 ± 2.89	1.35 ± 0.10
Roots	0	–	50.03 ± 1.1	09.52 ± 0.12
	1	–	41.03 ± 0.90	8.65 ± 0.24
	5	–	37.49 ± 1.26	8.57 ± 0.17
	10	–	33.72 ± 1.80	7.23 ± 0.15
	20	–	33.29 ± 1.15	2.57 ± 0.04

and accumulation of Zn and Cr by *E. crassipes* were observed at 1.0, 5.0, 10.0 and 20.0 $mg\,l^{-1}$. With increasing metal concentration water hyacinth was able to remove and accumulate high amount of heavy metals. Removal of heavy metals by *E. crassipes* in present study was higher and this is in agreement with Denga et al. [5], Miretzky et al. [7] and Mishra et al. [10]. The results of the regression analysis confirm that the metal removal by *E. crassipes* was proportional to the metal concentrations ($p < 0.01$). The results obtained from the present study indicated that metal removal percentage for Cr was highest at 1.0 $mg\,l^{-1}$ (84%) while for Zn it was highest at 10.0 $mg\,l^{-1}$ (95%). This species was able to remove the 63–84% Cr and 88–94% Zn in 11 days incubation period. The removal efficiencies for Cr and Zn varied with varying concentration of these heavy metals.

The metal removal efficiency for Zn was increased initially with increasing time and concentrations as shown by greater removal efficiency at 10.0 $mg\,l^{-1}$ where as for Cr it decreased with increasing concentrations. Decrease in metal concentration with time frame showed up to 60% removal within the first 3 days of the experiment. Control experimental sets showed loss of 2.1–5.7% heavy metals from the water. This loss might be due to precipitation, adsorption to clay particles and organic matter, co-precipitation with secondary minerals. Study reveals significantly higher ($p < 0.001$) accumulation of heavy metals in *E. crassipes*. Higher removal of heavy metals may be attributed to the several special characters of *E. crassipes* like fibrous and dense root system, broad leaves, and fast growth [16].

The decrease in metal removal efficiency at 20 $mg\,l^{-1}$ may be due to the saturation of Zn selective sites and also the tolerance of the plants towards Zn when the concentration was further increased. Many plant species illustrate low mobility of heavy metals due to the fact that there are barriers or lack of transport mechanism suitable for transport from roots to shoots [17]. A similar trend was reported by Hassan et al. [18]. Low concentration of metals in the shoot of *E. crassipes* may be due to the slow mobility of metal from root to shoot and also the formation of complex compounds with COOH groups that may inhibit the translocation of metal to shoot [19]. Although translocation of metals has been reported in the process of phytoextraction [20], it may not be the main mechanism of metal transport in aquatic vascular plants. The containment, immobilization and accumulation of metals in the root structures may be due to the process of rhizofiltration, which is commonly observed in aquatic plants. Roots exudates in the rhizosphere may also cause the metals to precipitate onto the root surfaces [20]. Metal ions can be actively absorbed into the root cells via plasmalemma, and adsorbed on the cell walls via passive diffusion or moved acropetally in the roots of aquatic macrophytes. Acropetal transport played a major role in metal ion transport in

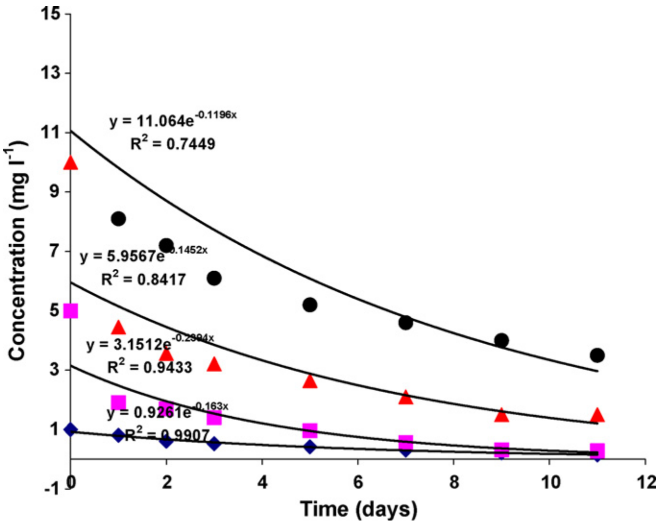

Fig. 1. Removal of chromium by *Eichhornia crassipes* during 11 days exposure.

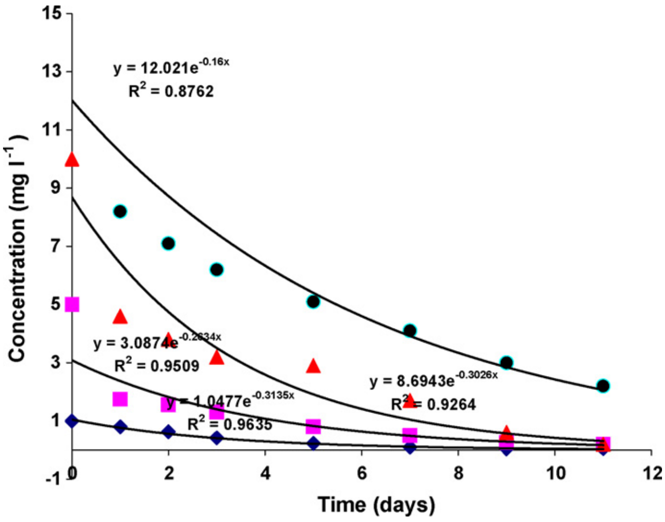

Fig. 2. Removal of zinc by *Eichhornia crassipes* during 11 days exposure.

Table 5
Chlorophyll and protein changes in *Eichhornia crassipes* 7 days after exposure to Zn(II).

Plant part	Zn concentration (mg l^{-1})	Chlorophyll content (mg g^{-1} dry weight)	Protein content (mg g^{-1} dry weight)	Sugar (mg g^{-1} dry weight)
Leaves	0.0 (control)	11.53 ± 0.25	113.3 ± 3.10	40.12 ± 1.20
	1	10.31 ± 0.18	98.4 ± 2.70	28.5 ± 0.80
	5	8.67 ± 0.15	81 ± 1.50	20.4 ± 0.40
	10	6.2 ± 0.06	65.25 ± 2.10	16.3 ± 0.40
	20	4.17 ± 0.01	41.10 ± 1.10	13.36 ± 0.20
Stem	0.0 (control)	–	77.26 ± 1.0	10.16 ± 0.10
	1	–	67.34 ± 0.9	9.26 ± 0.10
	5	–	61.24 ± 0.6	7.16 ± .08
	10	–	54.23 ± 1.10	5.52 ± 0.05
	20	–	23.55 ± 0.86	2.35 ± 0.10
Roots	0.0 (control)	–	51.42 ± 1.10	26.1 ± 0.2
	1	–	43.71 ± 0.45	17.2 ± 0.2
	5	–	27.11 ± 0.30	10.5 ± 0.2
	10	–	24.33 ± 0.22	6.8 ± 0.08
	20	–	16.8 ± 0.20	9.8 ± 0.08

Table 6
Mean concentration of heavy metals in different parts of *Eichhornia crassipes*.

Initial metal concentration (mg l^{-1})	Cr concentration in plants (mg g^{-1} dry weight)			Zn concentration in plants (mg g^{-1} dry weight)		
	Roots	Leaves	Stems	Roots	Stems	Leaves
1	0.45	0.30	0.25	0.41	0.29	1.8
5	0.67	0.39	0.14	0.75	0.21	0.26
10	1.35	0.59	0.47	2.11	0.16	0.37
20	1.76	0.38	0.13	2.42	0.52	0.66

root tissue than passive diffusion in their study on submerged macrophytes [21]. Metal accumulation in leaves and stems may be largely attributed to the process of ion exchange within the tissues and surrounding solution also via passive penetration of ions into the tangential region. [22].

Figs. 1 and 2 show the Zn and Cr removal pattern shown by *E. crassipes*. Uptake increased in all the treatments for all the 11 days. The rate of removal was higher for the first 3 days and thereafter it decreased. The bioaccumulation pattern of Zn and Cr in leaves, stems and roots of *E. crassipes* showed that the roots have the highest concentration followed by leaves and petioles, respectively. Around 50–60% of total Zn accumulation in the plants was present in the roots. Previous studies on the accumulation of various metal ions by aquatic plants have also shown that the deposition of most metals was higher in roots than the other parts of plants [23,24,7,9].

3.2. *Toxic effects of Zn and Cr on some biochemical parameters of Eichhornia crassipes*

Accumulation of heavy metals in aquatic macrophytes is known to produce significant physiological and biochemical responses towards the growth of roots, stems and leaves [7,8,9,10]. The high solubility and strong reducing ability of Zn may cause phytotoxicity to the experimental plants. The changes in selected biochemical parameters of *E. crassipes*, i.e. sugar, protein and chlorophyll after being exposed to Zn and Cr are shown in Tables 4 and 5, respectively. Some morphological symptoms of metal toxicity such as yellowing, chlorosis of leaves and root shedding were apparent in the macrophytes exposed to 10.0 and 20.0 mg l^{-1} Cr while for Zn such toxicity was not apparent at any of the applied concentrations. Three biochemical parameters sugar, protein and chlorophyll contents of the macrophytes showed a similar trend of decline corresponding to the increase in the concentration of both the heavy metals. Reduction of protein, sugar and chlorophyll contents in the test plants were observed with the increasing duration of exposure [25]. Similar observations on decrease in photosynthetic pigments, protein and sugar contents in various aquatic vascular plants exposed to Cr and Zn were reported by Hassan et al., Mishra et al. and Cheng et al. [18,10,25]. Statistical analysis based on least significant difference showed that exposure to Zn and Cr results in significant effects on selected biochemical parameters.

The macrophytes treated with Cr showed higher reduction of protein, sugar and chlorophyll compared to plants treated by Zn. This suggests greater toxic strength of Cr as compared to Zn on similar concentrations. Excessive Cr accumulation in plant tissue can be toxic to the plants, affecting several physiological and biochemical processes and growth [12]. Exposure to Zn normally leads to oxidative damage and may also change the metalloenzymes of the plant by displacement or replacement of metal ions. The reduction of chlorophyll content in *E. crassipes* may be attributed to inhibition of chlorophyll synthesis which results in the loss of photosynthetic activity due to the disruption of chloroplast. The decline of sugar formation may be associated with reduced rates of photochemical activities and chlorophyll formation. Loss of sugar formation may also be due to the conversion of sugar into energy when the plants were stressed. When the test plants were exposed to Zn, the protein content declined; this may be due to the formation of complexes of protein and Zn, hence changing the conformation and solubility of the protein which eventually resulted in the decrease in enzymatic activity.

4. Conclusion

The present study proved *E. crassipes* as a good accumulator of Cr and Zn. This macrophyte has accumulated Zn and Cr up to 3.542 and 2.412 mg g^{-1} at metal concentration of 10 mg l^{-1} after 11 days of exposure. This plant has successfully removed up to 84% of Cr and 94% of Zn. Upon 11 days exposure to Zn and Cr biochemical parameters such as protein, sugar and chlorophyll was reduced. Regardless of the concentrations of the treatment, the roots were the most efficient of all the plant tissues in accumulating Zn accumulation in water hyacinth followed the order: roots > leaves. Chromium accumulation at the concentration 10.0 and 20.0 mg l^{-1} was found to have associate production of some morphological symptoms of toxicity such as yellowing of the leaves, growth retardation and chlorosis. However Zn was not associated with production of any toxic symptoms at all the concentrations studied. On the basis of results *E. crassipes* can be recommended for the removal of Cr and Zn from waste water, however further study may assure their full utilization in this context.

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