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Phytoremediation Potential of *Lemna minor* for Removal of Cr(VI) in Aqueous Solution at the Optimum Nutrient Strength

M. Thayaparan[1], S. S. Iqbal[1]*, and M. C. M Iqbal[2]

[1] Department of Chemistry, The Open University of Sri Lanka
[2] Plant Reproductive Biology, National Institute of Fundamental Studies

Abstract

Toxic heavy metal pollution of water and soil is a major environmental concern for which conventional remediation approaches do not provide an appropriate solution. Phytoremediation, which involves removal of pollutants from water and soil through plants, is of low cost and environmentally friendly. In this study, the phytoremediation potential of *Lemna minor* for the uptake of Cr(VI) at the optimum nutrient strength for Cr(VI) uptake was investigated. Capacity assessment for chromium absorption by *Lemna minor* was carried out for 7 days at different levels of chromium concentrations. The time required for significant absorption of chromium was estimated in a time course experiment by growing *Lemna minor* in 3 mg/L chromium solution in which the plant showed no toxicity. Plant samples were harvested at 24 hour intervals for 5 days and wet weight was obtained to determine relative growth; the dried samples were analyzed for chromium using Atomic absorption spectrophotometer. Plant growth decreased significantly with increasing concentration of chromium in the nutrient solution and chlorophyll content (greenness) was also affected. Maximum uptake of chromium (5.8×10^3 µg/g dw) was at 8 mg/L in ambient solution. However, the bio-concentration factor (BCF) decreased with increasing chromium in the ambient solution. The BCF was 1000 for chromium up to 3 mg/L. In the time course experiment, growth of *Lemna minor* and chromium accumulation increased significantly with time up to the 3rd day (3119 µg/g dw). These results suggest that *Lemna minor* is an extreme accumulator of chromium and could be considered for chromium (VI) removal from waterways.

Keywords: Phytoremediation, *Lemna minor*, chromium (VI).

Correspondence should be addressed to Prof. S. S. Iqbal, Department of Chemistry, Faculty of Natural Sciences, The Open University of Sri Lanka, Nawala, Sri Lanka (Email: ssiqb@ou.ac.lk).

Introduction

Rapid urbanization, industrialization and over use of fertilizers in agricultural practices have resulted in serious environmental pollution in Sri Lanka. Textile, paper, tannery, metal finishing food and beverage industries and agricultural runoff contribute most to water pollution. There are standards for discharge of effluent in Sri Lanka. However, effluents are discharged into shore waters, lagoons and estuaries with little or no treatment (CZMP 2004).

Contamination of aquatic systems by heavy metals is a serious environmental concern. Heavy metals, unlike organic pollutants, are not degraded by chemical or biological processes. Thus, they persist a long time in the environment and pose a serious health hazard on living organisms. Chromium is one such contaminant released into the environment due to its heavy use in the dyes and pigments, textile, electroplating, wood processing and tanning industries. In nature chromium exists in two stable oxidation states, trivalent and hexavalent. Chromium is biologically inactive in the metallic state. Organisms weakly absorb trivalent chromium, but Cr (VI) is more toxic and highly soluble in water (Chandra and Kulshreshtha, 2004) and it is very harmful to human health as it is carcinogenic. Chronic chromium toxicity causes cancer in the respiratory tract and lungs (Zayed and Terry 2003).

Traditional technologies such as chemical reduction, precipitation and ion exchange to remove heavy metals are often ineffective or very expensive. Phytoremediation has been accepted widely for its potential to clean up polluted sites. It can be defined as the use of plants to remove or sequester hazardous contaminants from media such as soil, water and air. Plants species are selected for phytoremediation based on their potential to accumulate metals, their growth and yield and depth of their root zone. This ability can be used to remove heavy metals in the contaminated sites. Advantages of phytoremediation over traditional treatments include lower cost, ease of monitoring plants, and possibility of the recovery and re-use of valuable metals (phytomining); it is also environmentally friendly.

Aquatic plants are known to absorb and accumulate heavy metals (Kamel 2013 and Aisien *et.al*. 2010). In this study *Lemna minor* which is a floating aquatic plant covering the surface of a water body as a mat, was investigated, for its ability to remove chromium from aqueous medium. It has an average lifespan of 5-6 weeks and a production rate of 0.45 fronds per day, doubling its mass in 2-3

days (Isaksson *et al.* 2007). Its small size and rapid growth rate make it a potentially useful tool for phytoremediation.

Materials and Methodology

Stock chromium solution (1000 mg/L) was prepared by dissolving Analytical Reagent grade $K_2Cr_2O_7$ in distilled water. Hoagland nutrient solution was prepared according to Hoagland and Arnon (1950). Total chromium content in the effluent was determined by using Atomic Absorption Spectrophotometer (GBC 932AB plus). The pH of the test solution was adjusted to pH 6 which is the optimal pH for growth of *Lemna minor* (Isaksson *et al.* 2007) using conc. HNO_3 and conc. NH_4OH. The pH meter (OHAUS -STARTER 3000 bench pH meter) was used to measure the pH of the solution.

Lemna minor was collected from Boralesgamuwa Lake, Kesbewa Lake, Attidiya marshy land and Diyawanna Oya in the Colombo district. Plants were rinsed with tap water to remove any epiphytes and insect larvae growing on the plants. Plants were acclimatized for 3-7 days in the green house in a large fiber glass tank containing fresh water.

Optimum nutrient strength

In order to find the optimum strength of nutrient solution for the uptake of chromium by *Lemna minor*, the aquatic plants were grown in solutions of varying concentration of chromium at different strengths of Hoagland nutrient medium**.** The experiment was carried out for eight days (Zayed et al. 1998). One experimental set-up with zero metal concentration served as control. Aged water (about 25 ml) was added to compensate for water loss through plant transpiration and evaporation. During the experiment morphological changes were observed. After 8 days of hydroponic culture, the plants were harvested, rinsed twice in distilled water for two minutes, in 20 mM EDTA solution for five minutes and then finally rinsed twice with distilled water for two minutes to remove any Cr on the plant surface (Gothberg *et al.* 2004).

The plant biomass was air dried for six hours and oven-dried at 60 ^{o}C for 24 hours (Radojevic and Baskin, 1999) and ground using a mortar and pestle. The ground samples were placed in clean glass bottles and dried again for 24 hours at 60 ^{o}C. After drying, the bottles were sealed and allowed to stand in a dry and cool place. The powdered plant biomass was dried in a crucible at 105 ^{o}C to

constant weight and prepared for analysis for Cr by acid digestion according to Hoenig *et al.* 1998.

Absorption capacity assessment

Test solutions containing different concentrations of Cr(VI) at the optimum strength of 10% of Hoagland nutrient medium as found in the previous experiment were prepared. Black plastic basins were filled with 2-liter test solutions. The pH of the solution was maintained as 5.8 – 6.0. The aquatic plants were carefully blotted on filter paper and their initial wet weight was recorded and introduced into the test solution. The experiment was run in triplicate for seven days (Leblebici and Aksoy 2011). One experimental set-up with zero metal concentration served as control. Aged water (about 25 ml) was added every day to compensate for water loss through plant transpiration and evaporation. After seven days, the plants were harvested and rinsed as described previously to remove any Cr on the plant surface.

The Relative growth (R.G) of the plant species was calculated as follows (Lu *et al.* 2004).

$$R.G = \frac{\text{Final wet weight}}{\text{Initial wet weight}}$$

Bio Concentration Factor (BCF) which is a useful parameter (Lu *et al.* 2004) to evaluate the potential of plants for accumulating metals was calculated as follows.

$$BCF = \frac{\text{Concentration of metal in plant tissue}}{\text{Initial concentration of metal in external solution}}$$

The plant biomass was prepared for Cr analysis by acid digestion according to Hoenig *et al.* (1998).

Time course experiment

The aquatic species were collected from the fresh water- holding tank and their initial wet weight was recorded. From the capacity assessment experiment it was found that *L. minor* had the capacity to accumulate large quantities of Cr in solutions containing Cr (VI) of initial concentration up to 3.01 mg/L without showing any toxicity symptoms. The plants were cultured in 500 ml of 3.01 mg/L Cr(VI) solution containing10% of Hoagland nutrient solution for five days as the plants started to show chlorosis on the 5th day in the experiment to determine the optimum nutrient strength. The pH of

the test solution was 5.9-6.2. The experiment was run in triplicate. One experimental set up with zero metal concentration served as control. The Aged water was added every day to maintain water level. Plant biomass and test solution in each experimental set up were withdrawn at 24 hours intervals for five days, for analysis.

The data obtained (in three replications) was analyzed by one – way analysis of variance (ANOVA) to determine the significance of differences between the pairs of means. The differences were statistically significant when P-value was less than 0.05. Tukey 95% Simultaneous confidence intervals test was done to determine which mean values were significantly different from the others.

Results and Discussion

Optimum nutrient strength

The morphological change observed during the experiment is given in Table 1. With increasing external Cr concentrations, toxicity effects such as chlorosis on *L. minor* appeared at lower nutrient strength rather than at the higher nutrient strength. Chlorosis appeared in *Lemna minor* on the 2nd day in 12 mg/L Cr concentration at 10% nutrient level, while at 75% nutrient level in the same Cr concentration, there were no toxicity symptoms in *Lemna minor* throughout the experiment. Similarly, *L. minor* treated with 5 mg/L at 10% nutrient strength showed the toxic symptoms on the 5th day of the experiment, while the plants exposed to the same concentration but at 75% nutrient strength were normal and fresh up to 5 days. A similar observation has been reported in water spinach by Gothberg *et al.* (2004).

Table 1. Morphological change in *L.minor* grown in different strengths of Hoagland nutrient with varying concentration of chromium

Cr concentration (mg/L)	Strength of Hoagland nutrient solution			
	10% Nutrient	25% Nutrient	50% Nutrient	75% Nutrient
Control	None	None	None	None
1	None	None	None	None
2	None	None	None	None
3	None	None	None	None
4	None	None	None	None

5	Chlorosis started on 5th	None	None	None
6	Chlorosis started on 4th	Chlorosis started on	None	None
9	Chlorosis started on 3rd	Chlorosis started on	Chlorosis started on 4th	None
12	Chlorosis started on 2nd	Chlorosis started on	Chlorosis started on 4th	None
15	Chlorosis started on	Chlorosis started on	Chlorosis started on 4th	Chlorosis started on 4th

Uptake of chromium by *Lemna minor* in different strengths of Hoagland nutrient solution with varying concentration of chromium is given in Figure 1.

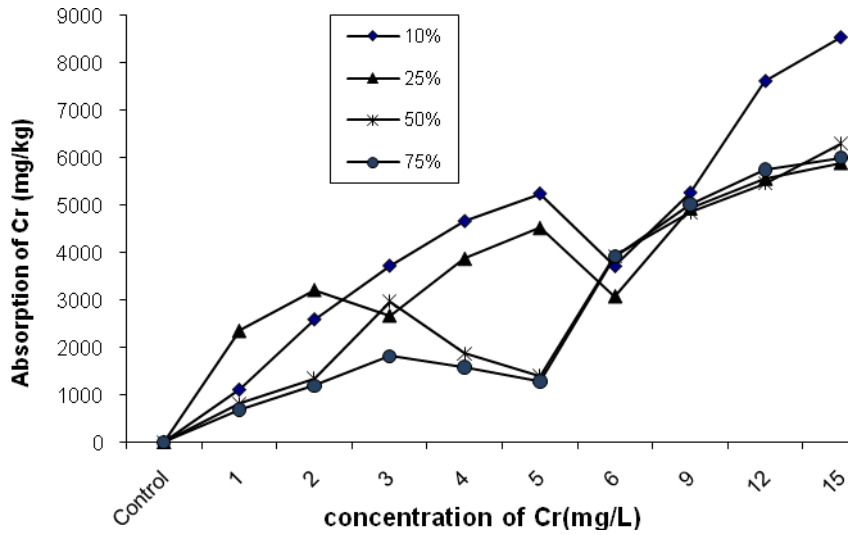

Figure 1. Absorption of chromium by *L. minor* in different strengths of Hoagland nutrient solution with varying concentration of Cr after 8 days of hydroponic culture.

In chromium treated *L. minor*, the concentration of Cr in the biomass increased with decreasing ambient nutrient strength (Figure 1). *Lemna minor* showed high absorption of chromium at

10% nutrient level. The plants grown in a controlled set up did not show any chromium in its tissue.

The metal uptake in plants varies considerably. In a nutrient-enriched environment, due to competition between the metal ion and the cations in the nutrients for the uptake sites, the uptake of metal ion under investigation may decrease (Greger M., 1999). On the other hand, a generous availability of nutrients promotes plant growth, which in turn creates an increasing number of uptake sites for metal in the plants, thus encouraging uptake; however, the extent of metal absorption by plants depends on the relative responses of metal uptake and growth rate.

The metal uptake by *L. minor* at a particular metal concentration decreased with increasing nutrient strength. Previous studies (Greger *et al.* 1991) also showed the influence of nutrient enrichment on the uptake of toxic metals by plants. The Cd uptake rate in *E. crassipes* was much higher in deionized water than in 50% Hoagland nutrient solution (O'Keeffe *et al.*1984). The Cd net uptake in the roots of sugar beet (*Beta vulgaris L.)* was greater when the nutrient concentration was minimal, rather than optimal (Greger *et al.* 1991). The strength of the external nutrient solution is of importance for the accumulation and toxicity of heavy metals in water spinach (Gothberg *et al.* 2004).

The nutrient strength of 10% was considered the optimum strength for Cr uptake and had been used for the rest of the experiments.

Absorption capacity

The most obvious initial effect of heavy metal stress is manifested as change in plant growth. The initial wet weight of *L. minor* was about 3.6 g and metal concentrations in the test solution were 1 to 8 mg/L. *L. minor* plants in control grew at a higher rate so that after 7 days, its RG was 3.99 while the relative growth of plants in the Cr test solutions were in the range of 0.79- 2.48. The relative growth significantly decreased ($P \leq 0.05$) with increasing Cr in the test solution. Goswami and Majumdar (2015) reported a significant reduction in specific growth rate of *Lemna minor* with increase in Cr(VI) concentration in ambient solution.

At the end of the experiment period (after 7 days) Cr(VI) caused a distinct limitation of *Lemna*'s growth compared to the control (Table 2). Similar effects had been observed in *Azolla caroliniana* by Bennicelli *et al.* (2004). In this study it was observed that Cr(VI)

caused inhibition of *Azolla caroliniana* biomass growth by 20-27 %. Arrora *et al*. (2004) also observed inhibited growth of three *Azolla* spp. (*A. microphylla*, *A. filiculoides* and *A. pinnata*) by Ni and Cd. Khosravi *et al*. (2005) had further reported that the presence of Pb, Cd, Ni and Zn caused an inhibition of biomass growth by 25%, 42%, 31% and 17% respectively in comparison to the weight of *Azolla* in control experiment, in which there was no heavy metal present.

Table 2. Percentage relative growth of *L.minor* in different concentrations of chromium

Initial concentration of Cr solution (mg/L)	Average relative growth	% R.G compared to control
0.0	3.99	--
0.79	2.48	62
1.85	1.42	36
3.01	1.09	27
4.31	0.91	22
6.72	0.80	20
8.32	0.79	20

The highest relative growth of 2.48 was observed in 0.79 mg/L solution. It appeared that low concentration of Cr did not affect plant growth. Higher doses of Cr (*i.e*. above 4 mg/L) limited the relative growth by more than 70%.

Cr Uptake by *L. minor*

In general there was an increase in total Cr in the plant biomass as the Cr concentrations in the feed solution increased (Control plant showed absorption of 0.16 mg Cr/kg DW which was presumably present in the plants at the time of sampling). Uptake of chromium by *L. minor* in different concentrations of chromium is shown in Figure 2.

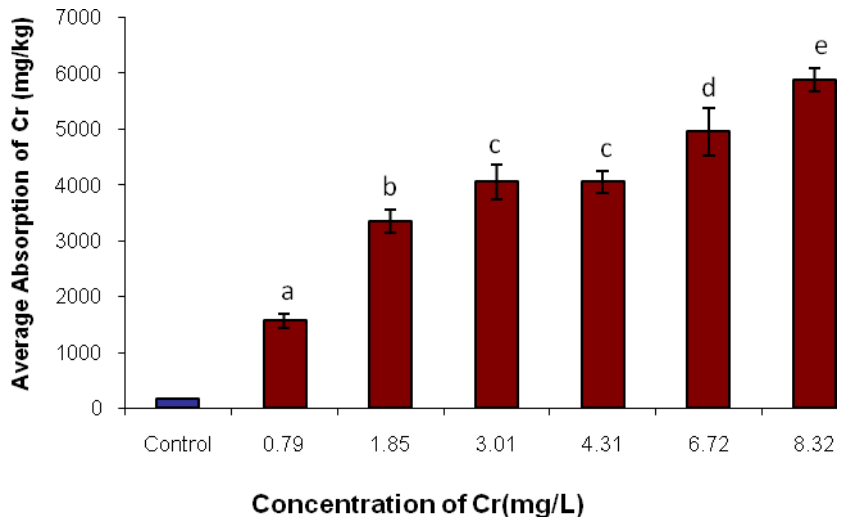

Figure 2. Uptake of Cr by *L. minor* with different concentrations (Error bars are standard deviations)

Mean values of absorption denoted by the different letters significantly differ at P≤ 0.05 by Tukey's 95% simultaneous confidence intervals. The absorption of chromium by *L. minor* increased significantly (P ≤ 0.05) when concentration of chromium was increased. After the experiment, control plants showed a very low Cr concentration (160 mg/kg). Plants treated with 8 mg/L, accumulated the highest level of metal and these plants did not survive. It appeared that this concentration was very toxic to *L. minor*. However, plants showed toxicity symptoms such as complete chlorosis or whiteness and transparency of fronds after 4.31 mg/L Cr concentration.

Several studies have been reported on the effects of chromium on aquatic plants. Roots of water hyacinth showed an accumulation of 18.92 µmol (g dry tissue wt^{-1}) Cr (Chandra and Kulshreshtha, 2004).Under experimental conditions, duckweed *(Lemna minor)* accumulated 2.87g Cr/kg (Zayed *et al.*1998). In *Bacopa monnieri* and *Scirpus lacustris,* accumulation of 1600 and 739 µg Cr/g DW respectively has been reported when exposed to 5 mg/L Cr for 168 hours in solution culture (Chandra and Kulshreshtha, 2004).

Bio Concentration Factor (BCF) is a useful parameter to evaluate the potential of the plants in accumulating metals and this value is

calculated on a dry weight basis. Ambient metal concentration in water is a major factor influencing the metal uptake efficiency. The variation of BCF of *L. minor* for chromium with varying concentration of chromium is shown in Figure 3. Mean values of BCF denoted by the different letters significantly differ at $P \leq 0.05$ by Tukey's 95% simultaneous confidence intervals. The BCF values decreased significantly ($P \leq 0.05$) with increasing concentration of the feed solution. The maximum BCF value for Cr was 1978. However BCF value was more than 1000 for Cr concentration up to 3.01 mg/L and BCF decreased to 701 in 8.32 mg/L Cr solutions (Figure 3). In general, when the metal concentration in the feed solution increases, the amount of metal accumulating in plant increases, whereas, the BCF value decreases (Lu *et al.* 2004). Similar results were reported (Jain et al 1990) wherein BCF values for water velvet (*Azolla pinnata*) and *L. minor* treated with Pb and Zn gradually decreased with increasing metal concentration in feed solution. Zhu *et al.* (1999) also reported that BCF of water hyacinth were very high for Cd, Cu, Cr and Se at low external concentration. Zayed *et al.* (1998) reported that *L. minor* bio concentrated the six elements Cu, Se, Pb, Cd, Ni and Cr to different levels at low supply concentrations compared with those at high supply concentrations.

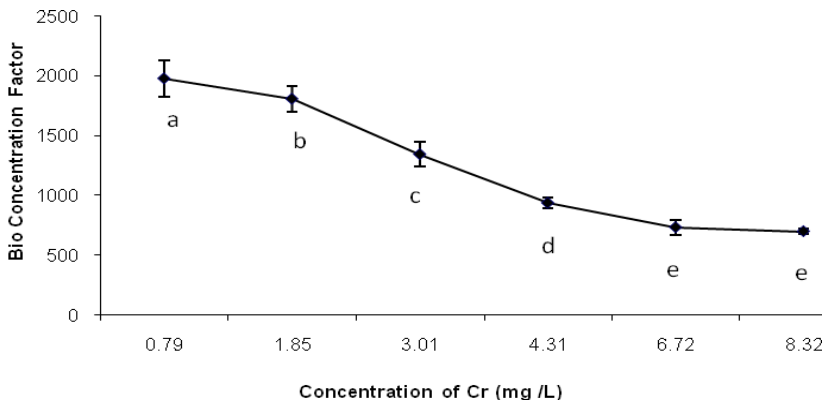

Figure 3. Bio Concentration Factor of *L. minor* for chromium (Error bars are standard deviations)

In terms of phytoremediation, a good accumulator should have the ability to concentrate the elements in its tissue, for example, a BCF of more than 1000 (Zayed et al 1998). According to this arbitrary criterion, *Lemna minor* can be considered as an extreme

accumulator of Cr at low external concentration (up to 3.01 mg/L). Phytotoxicity of chromium in an aquatic environment has not been studied in detail. In aquatic species, namely, *Myriophyllum spicatum*- the maximum increase in shoot length was found at 50 µg/L Cr. *Lemna minor* showed relatively greater tolerance to chromium. However, an inhibition of growth in *Spirodella* and *Lemna* species was found at 0.02 mmol and 0.00002 mmol Cr concentrations, respectively. The effective concentrations (EC-50) for some aquatic species have been reported: *Lemna minor*, 5.0 mg/L, 14 days. *L. paucicostata*, 1.0 mg/L, 20 days; *M. spicatum*, 1.9 mg/L , 20 days; *Spirodela polyrrhiza*, 50 mg/L , 14 days. Chromium toxicity on biochemical parameters was shown in terms of reduction in photosynthetic rate at 50 µg/L Cr (VI) solutionin *Myriophyllum spicatum*. Decrease in chlorophyll and protein contents were also recorded in *N. indica*, *V. spiralis* and *Alternanthera sesilis* with an increase in chromium concentration (Chandra and Kulshreshtha, 2004). Chromium–induced morphological and ultrastructural changes have been reported in several aquatic vascular plants: In *L. minor* and *Ceratophyllum demersum*, chromium–induced changes in chloroplast fine structure disorganized thylakoids with loss of grain and caused formation of many vesicles in the chloroplast (Chandra and Kulshreshtha, 2004).

Time course Experiment - *Lemna minor* for chromium

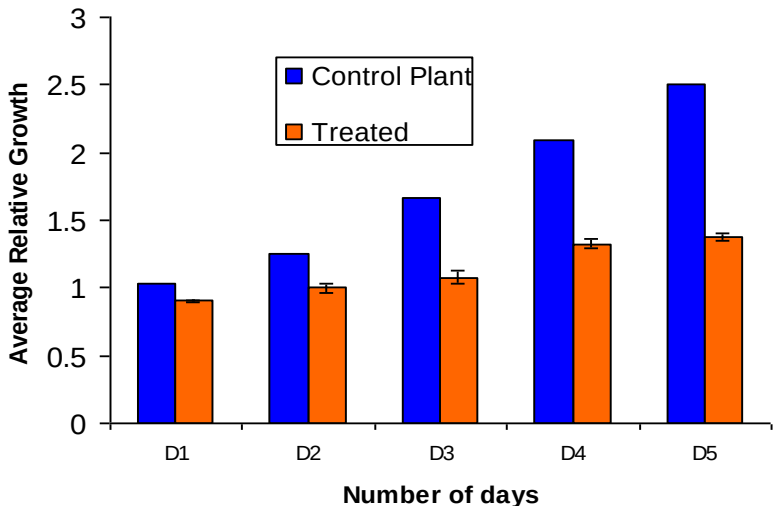

Figure 4. Average relative growth of *L. minor* in chromium solution with time (Error bars are standard deviations)

In the case of *Lemna minor,* the best concentration of chromium was found to be 3.01 mg/L. In this concentration, growth of *Lemna minor* was normal with greater accumulation. Initial fresh weight of *Lemna minor* was 2.6 g. *Lemna minor* was normal and very fresh during the test period. The effect of chromium on relative growth of *Lemna minor* with exposure time is shown in Figure 4.

The relative growth of control plant increased with the passage of time. The average relative growth of chromium-treated plants significantly ($P \leq 0.05$) increased with exposure time. The lowest value of relative growth was observed to be 0.90 on Day 1. Relative growth reached its maximum (1.37) on Day 5.

The variation of uptake as given by mean values of absorption of chromium by *Lemna minor* with exposure time is shown in Figure 5 (Control plants showed absorption of 0.02×10^3 mg/kg of Cr). The uptake denoted by the different letters significantly differs at $P \leq 0.05$ by Tukey's 95% simultaneous confidence intervals.

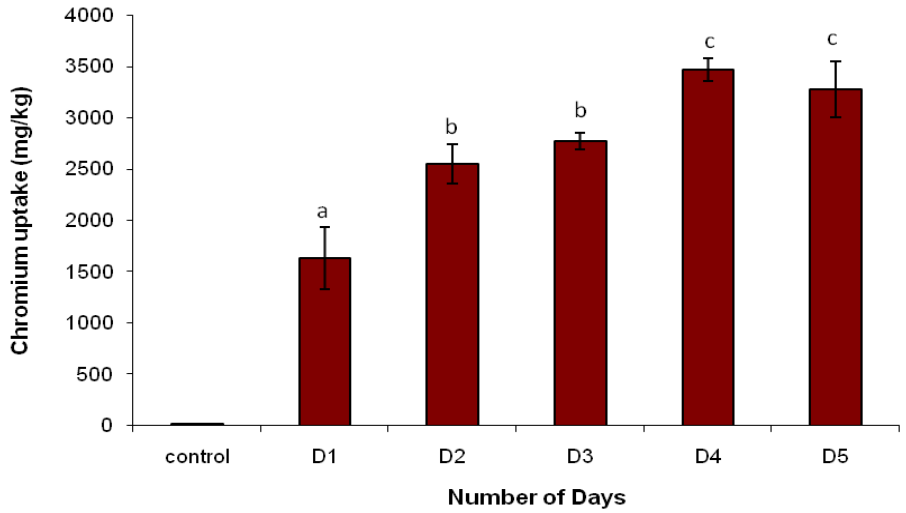

Figure 5. Uptake of chromium by *L. minor* with time. (Error bars are standard deviations)

The results revealed that under the experimental condition, the accumulation of chromium increased significantly ($P \leq 0.05$) when the exposure time was increased. In this study *Lemna minor* accumulated highest chromium concentration (3.46×10^3 mg/kg DW) when exposed to 3.01 mg/L chromium on Day 4. According to the Tukey's test, at $P \leq 0.05$, the difference between the absorption

means on Day 4 and Day 5 was not significant, *i.e.* the removal efficiency on Day 4 and 5 are almost the same. This shows that maximum absorption can be achieved by Day 4.

Conclusion

The strength of external nutrient solution is important for accumulation and toxicity of metals in *Lemna minor.* 10% nutrient strength was found to provide the optimum condition for Cr uptake. *L.minor* has the capacity to accumulate large quantities of Cr(VI) and also the ability to grow in solutions containing Cr(VI) with the initial concentration up to 3 mg/L within 7 days, although its growth was inhibited by 64% relative to *Lemna* plants that were not exposed to Cr(VI) (control). *Lemna minor* has the ability to bio-concentrate 3460 mg Cr(VI) /kg DW from a solution with an initial concentration of about 3 mg/L within 4 days.

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