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Potential of *Lemna minor* in Ni and Cr removal from aqueous solution

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ABSTRACT: Duckweeds are of special interest, as they are naturally growing weeds that have the capacity to tolerate and remove toxic pollutants, including heavy metals from the environment. Studies have revealed that duckweed (*Lemna minor*) can tolerate and remove heavy metals from aqueous solutions. In the present study, the efficiency of *L. minor* in the removal of Ni and Cr individually from aqueous solutions was investigated at concentrations of 3.05, 3.98 and 4.9 mg/L for Ni and 1.91, 2.98, and 4.2 mg/L for Cr. Experiments were run for 22 days, after which the metal content in the plant was estimated by atomic absorption spectrophotometer (AAS). The duckweed showed higher percentage of Ni removal than Cr. Specific Growth Rate (SGR) was found to be reduced at high concentrations of both Ni and Cr. Statistical analysis suggested that the growth of the plant was affected by the toxic effect of both Ni and Cr. Bioaccumulation of Ni was higher than Cr in *L. minor*. The mechanism of removal of both Ni and Cr followed second order kinetics. It is suggested that these duckweeds can remove Ni and Cr from aqueous solution and can also accumulate the same in considerable concentrations, at low initial metal concentrations.

Keywords: Chromium, accumulation, kinetics, Nickel, phytoremediation, removal.

INTRODUCTION

Today, heavy metal water pollution is an important issue, as a significant amount of heavy metals are discharged into local water bodies due to both industrial and anthropogenic activities. Heavy metals are non-biodegradable toxic pollutants that often accumulate in macrophytes and hence, enter into the food chain. There are several plants, both terrestrial and aquatic that can remove and accumulate metals in their biomass. Lemnaceae is a well known family that has the potential to accumulate metals from the external environment (Boonyapookana et al., 2002; Megateli et al., 2009). These have small fronds with thin roots and are easy to cultivate in a laboratory and are also easy to harvest with a high growth rate.

Ni is an essential micronutrient of plants at low concentrations. At higher concentrations, it is phytotoxic with adverse effect on the plant's biomass (Bres et al., 2012). A few sources of Ni includes burning of fossil fuel, mining of Ni, waste incineration, electroplating and other discharges from domestic wastes (Bres et al., 2012). Chromium (Cr) has two stable forms Cr (VI) and Cr (III) amongst which Cr VI is more mobile and toxic than Cr III (Shankera, 2005). Cr contamination in water is also a major concern, as various

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anthropogenic activities in the leather industry, plating industry, dye industry, galvanic industries (Oporto et al., 2006) etc discharge Cr into water bodies (Shankera, 2005). A variety of technologies including physical, chemical and biological processes are used to remove heavy metal contaminants from the environment. Phytoremediation is a new promising technology in which plants are used to remove contaminants from the environment (Axtell et al., 2003). It is a cost-effective and eco-friendly technology for the removal of contaminants. There are different experiments that have reported on the efficient removal of heavy metals such as Pb, Cr, Cd (Boonyapookana et al., 2002), Zn etc. from aqueous environment. Macrophytes like *Eichhornia crassipes, Spirodela polyrhiza, Salvinia sp.,* etc. (Axtell et al., 2003) have good potential to tolerate and remove heavy metals from water. The objective of this research was to study the efficiency of *L. minor* in the removal and accumulation of Ni and Cr from aqueous environment and also to study the kinetics of their removal.

MATERIALS AND METHODS

Plastic tubs of 100 cm depth were used. A total of 20 L of spiked metal solution were taken in each tub and 40 g of *L. minor* was exposed to the same. In the experiment, three concentrations of Ni (3.05, 3.98 and 4.9) mg/L and Cr (1.91, 2.98, and 4.2) mg/L were used, resulting in six treatments. Ni and Cr were applied as $NiCl_2$ and $K_2Cr_2O_7$ and the experimental period included a period of 22 days. Water loss from the experimental tubs was made up by addition of pond water. After completion of the experimental period, plants were harvested, washed thoroughly with water, followed by distilled water, and dried at 100°C for 10 min, then at 70°C until completely dry. The plant and water samples were first digested with HNO_3-$HClO_4$ (3:1) during which temperature was raised to about 95°C. The temperature was maintained until nitrous gas evolution stopped and the digest was clear (Kara et al., 2004). The digests were then made up to a final volume of 10 ml in polythene tubes with dilution. The concentration of heavy metals was determined with an atomic absorption spectrophotometer.

All the experimental treatments were replicated thrice and the means and standard deviations (SD) were calculated using Microsoft Office Excel 2003. Statistical analysis was carried out with Sigma Plot, using one way analysis of variance (ANOVA).

RESULTS AND DISCUSSION

Morphological symptoms of *L. minor* exposed to Ni

The morphological adaptive capacity determines the ability of the plant to absorb contaminants from water. When *L. minor* was grown in the presence of different initial concentrations of Ni, symptoms of phytotoxicity appeared after 10 days of exposure with abscission of few fronds and appearance of new chlorotic and smaller fronds. Ni in excess is known to interfere with the iron uptake of plants and their metabolism, causing chlorosis (Rabie et al., 2002). Abscission of daughter fronds has been reported to be beneficial to their survival when Cd stressed mother fronds release them, as it prevents the transportation of the toxic compound from mother to daughter fronds (Li et al., 2004). Abscission or disintegration of fronds and roots in *L. minor,* can be interpreted as a stress response of the toxic compound exposed plant. In the present study, the yellowing of fronds due to chlorosis was another predominant feature visible at the 10^{th} day of incubation. After harvesting on the 22^{nd} day, it was found that fresh biomass was maximum in case of a solution at 3.05 mg/L followed by decreasing biomass with increasing concentrations of Ni. Appenroth et al.

(2010) found similar observations of chlorotic and smaller fronds of *L. minor* and *Spirodela polyrhiza* when they exposed them separately to 40 µM $NiCl_2$ for 4 days. Duman and Ozturk (2010) found increase in biomass of *N. officinale*, when the plants were exposed to 1.0 mg/L Ni. A significant reduction in chlorophyll with increase in Ni accumulation was also reported by Mukhtari et al. (2010), when they exposed sunflowers to Ni in a hydroponic system. It reflects the inhibitory effect of Ni on chlorophyll synthesis.

Morphological symptoms of *L. minor* exposed to Cr

Chromium phytotoxicity on aquatic plants has been studied in less detail. In the present study, duckweed exposed to different concentrations of chromium showed decreased biomass production with increase in initial concentration. The growth of fronds of *L. minor* was affected less in cases of lower initial concentrations of Cr. Chandra and Kulshreshtha (2004) reported duckweeds to have greater tolerance to Cr relative to other aquatic plants.

In the present study, *L. minor* grew normally in the metal solution with no obvious symptoms of metal toxicity until the 10^{th} day of exposure, after which chlorosis of fronds was visible at 2.98 and 4.2 mg/L Cr concentrations. Chlorosis was more severe at higher concentrations with increase in exposure time. The toxic effect of Cr was thus visible in *L. minor* with prominence of chlorosis. Chlorosis of fronds is a characteristic phytotoxicity feature that evidences impairment of photosynthesis in plants (Posada et al., 2013). Cr toxicity was found to affect photosynthesis by causing chloroplast ultrastructure distortion and also inhibition of the synthesis of chlorophyll pigment (Gupta et al., 2011). It is hence clear from the study that the biomass productivity of *L. minor* exposed to different concentrations of Cr decreased with increase in Cr concentration. This decrease may be due to decreased tissue permeability as well as loss of integrity in the membrane of plant tissue as reported by Boonyapookana et al. (2002). They also observed reduction in biomass productivity of *Wolffia globosa* with increase in concentration of Cr in aqueous solution. In the present study, at 4.2 mg/L Cr concentration, frond abscission was also visible. Paiva et al. (2009) found visible symptoms of leaf chlorosis, petiolar chlorosis and necrosis in *Eichhornia crassipes* after 4 days of the experimental period on exposure to 1 mM Cr (VI). Chandra and Kulshreshtha (2004) reported the EC of Cr for *L. minor* to be 5 mg/L after an exposure period of 14 days. EC 50 is defined as the statistically derived toxicant concentration that can be expected to cause a defined nonlethal effect in 50% of a given population of plants under defined conditions (Boonyapookana, 2002). Sinha et al. (2005) reported a decrease in the chlorophyll content in *Pistia stratiotes*, such that at 160 µM Cr exposure for a period of 6 days, there was 42.91% chlorophyll content reduction to that of the control. Maine *et al.* (2004) found no toxicity symptoms when they exposed *P. stratiotes* and *S. herzogii*to Cr (III) at 6 mg/L. Some of the phytotoxic effects of Cr include chlorosis, growth reduction, chromatin condensation, swelling of mitochondria, and finally death (Gupta et al., 2011).

Percentage removal of Nickel by *L. minor*

For phytoremediation of Ni, initial concentrations of 3.05, 3.98 and 4.9 mg/L were selected. In all cases, Ni was significantly reduced from the aqueous solution by *L. minor* with increase in time. During the early days of incubation, Ni removal occurred rapidly, regardless of the initial Ni concentration. A similar removal pattern involving rapid removal towards the earlier stages of the experimental period has been reported by Olguin et al. (2002), who

reported that Cd uptake by *Spirodela polyrhiza* reached equilibrium over a time period of 120 min. Surface adsorption and intracellular uptake are the main procedures reported to be involved for the same.

Figure 1 shows the percentage removal of Ni for different initial Ni concentrations over time. *L. minor* removed 87.33% Ni at 3.05 mg/L, 72.5% Ni at 3.98 mg/L and 65.2% Ni at 4.9 mg/L respectively (Goswami et al., 2014) on 22 days of exposure to the metal. The removal increased but the rate of removal decreased with increase in treatment period in the present study. The removal percentage was dependent on the initial Ni concentration (Fig. 1), such that it decreased with increase in initial Ni concentration. This had similarity with the findings of Axtell et al. (2003), who reported effective removal of Ni from polluted water with low Ni concentration of 3 mg/L. Similar observations have been reported by Bres et al. (2012). They reported 100% Ni removal by *L. minor*, at initial Ni concentrations of 1.0 and 2.76 mg/L to be rapid and occurred within the first 24 h. In an experiment, Axtell et al. (2003) studied the Ni removal potential of *L. minor* and found that it removed 82% of Ni on an average. Hadad et al. (2009) found 62% removal in the first 24 h by *E. crassipes* at 1.0 mg/L Ni. There was 68% removal of Ni after an experimental period of 15 days by *Azolla filiculoides*, at 4.0 mg/L of the initial Ni concentration (Khosravi, 2005).

In the present study, the pH of the aqueous solution was increased to between 7.5 - 8.0. Increase in pH of the aqueous solution might have been responsible for Ni precipitation as hydroxides to some extent. Hussain et al. (2010) obtained similar results and reported the adsorption of Ni hydroxides onto the roots of plants. A positive correlation ($r= 0.95$, $P< 0.001$) was obtained between the initial concentration of Ni in the ambient solution and the concentration of metal removed from the solution. *L. minor* was found to have potential to reduce the level of metal concentration to below discharge standards, as specified by CPCB (2000) for discharge of Ni into inland surface water.

Percentage removal of Chromium by *L. minor*

Lemna gibba and *L. minor* do have high tolerance to chromium and its toxicity and have high potential of Cr uptake and accumulation (Staves and Knaus, 1985). Although it is not at all required by aquatic macrophytes for any of their physiological processes (Mishra and Tripathi, 2008).

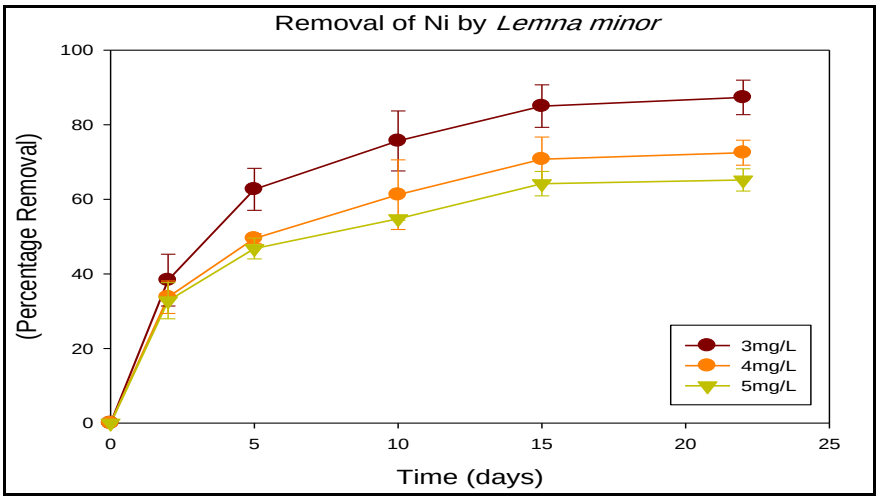

Fig. 1. Percentage removal of Ni by *Lemna minor* at different initial concentrations. Bars denote ± standard deviation from the mean of three replicates

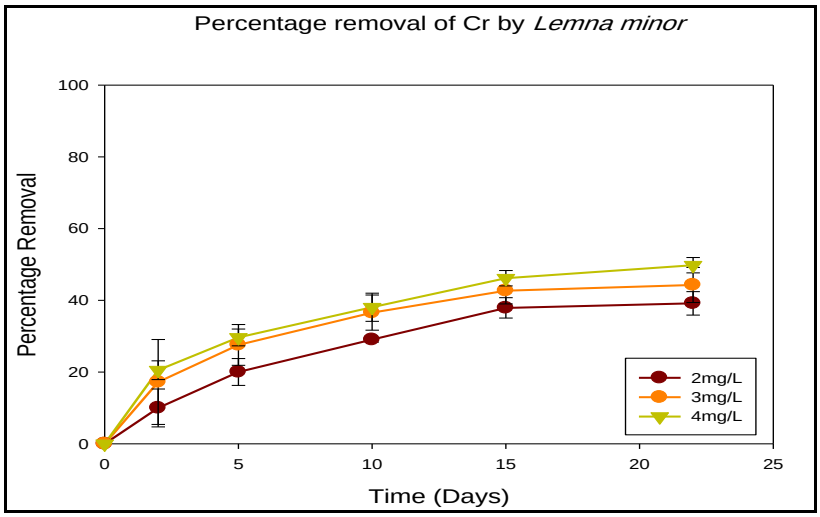

Fig. 2. Percentage removal of chromium by *Lemna minor*. Bars denote ± standard deviation from the mean of three replicates

The three initial Cr concentrations selected for removal by *L. minor* are 1.91, 2.98, and 4.2 mg/l. The experimental period was of 22 days. The percentage removal of Cr was found to increase with increase in initial concentration of Cr (Fig. 2). The maximum removal, 49.79%, was achieved by *L. minor* at initial Cr concentration of 4.2 mg/l on the 22nd day. The results also showed increase in removal of Cr with increase in exposure time. The rate of removal was found to decrease with increase in the number of treatment days. Since Cr has low mobility and it is also a fact that macrophytes do not require Cr for any physiological purposes, Cr removal by macrophytes is of low percentage (Mishra and Tripathi, 2008). Obek et al. (2009) reported that during the first 24 h of exposure of *L. gibba* to the external solution, a high ability to concentrate heavy metals was observed. Quinones et al. (2008) found that *E. crassipes, P. stratiotes* and *S. auriculata* are able to remove 50, 70 and 90% of hexavalent Cr, respectively from aqueous solution over an experimental period of 35 days. They explained that biosorption and bioaccumulation processes were simultaneously involved for accumulation of Cr in the biomass of the plants. Reduction of concentration of Cr after an exposure period of 22 days by *L. minor* was found to be positively correlated ($r = 0.98$, $P < 0.001$) with the initial Cr concentration in the aqueous solution.

Growth Parameter

Specific Growth Rate of *L. minor* exposed to Ni

There are a few standards that recommend the biomass of plants to be a good reliable parameter for bioassay (EPA, 1996; ISO, 2001; OECD, 2002; Mkandawire et al., 2006). At a low Ni concentration of 3.05 mg/L, the effect of Ni on *L. minor* was visibly less, such that the specific growth rate was found to be 0.0304 ± 0.0005 (Fig. 3). Increase in concentration brought about a statistically significant effect on the growth of *L. minor*. Plants when subjected to 3.05 mg/L Ni, showed no significant differences from control treatments, whereas when *L. minor* were exposed to 3.98 and 4.9 mg/L Ni, the specific growth rates were 0.0265 ± 0.001 and 0.0201 ± 0.002 respectively, with significant differences with that of control. Hence, the toxicity threshold lies in the range of 3.05 and 3.98 mg/L Ni. Hadad *et al.* (2006) reported the findings of Ingole and Bhole (2003) that at 5 mg/L Ni, *E. crassipes* had normal growth with good removal efficiency.

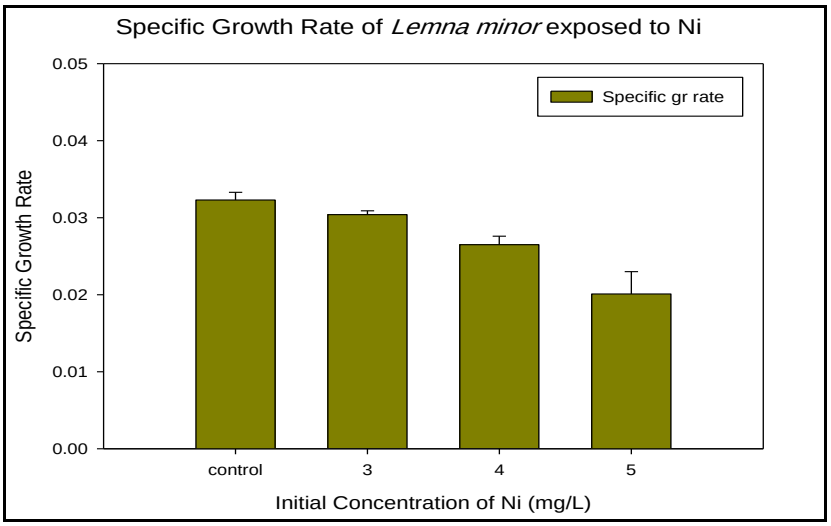

Fig. 3. Bars denote ± standard deviation from the mean of three replicates

Hadad et al. (2009) found reduced relative growth rate in *E. crassipes* at 1 mg/L Ni exposure over a period of 30 days and was significantly lower than control. Chlorosis was another toxicity symptom of the plant observed in 75% of the leaves. A highly significant ($P < 0.005$) and negative correlation ($r = -0.91$) was found between the initial Ni concentration exposure to *L. minor* and their specific growth rate.

Specific Growth Rate of *L. minor* exposed to Cr

While studying the removal of Cr by *Lemna minor*, at the initial Cr concentrations of 1.92, 2.98 and 4.2 mg/L, specific growth rate (SGR) was found to be 0.0163, 0.0148 and 0.0102, respectively (Fig. 4). Thus, significant reduction in growth rate was obtained with increase in Cr concentration in ambient solution. Vajpayee *et al.* (2001) found *Vallisneri aspiralis* to have Cr tolerance at lower concentration, such that 64% of plant biomass reduction was obtained on exposure to 10 mg/L Cr for 3 days. Correlation between initial Cr concentration in solution and the specific growth rate of *L. minor* was negative ($r = -0.61$).

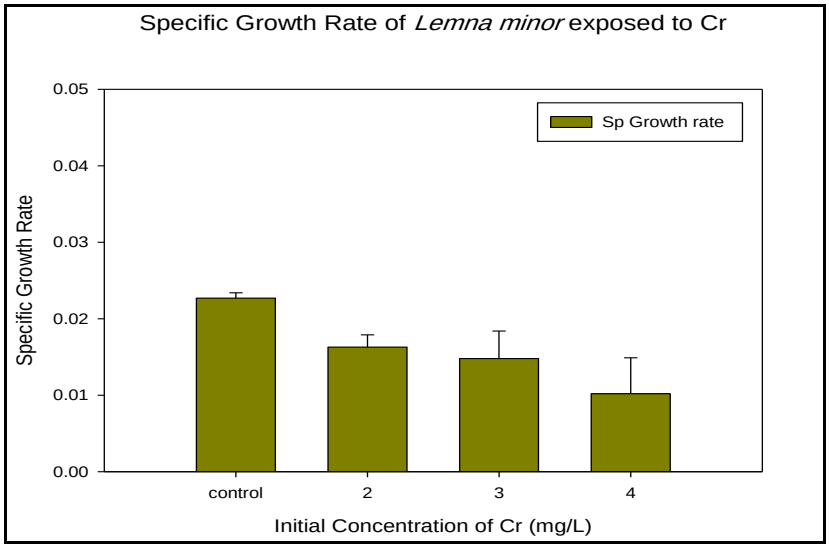

Fig. 4. Specific Growth Rate of *Lemna minor* at different initial Cr concentrations. Bars denote ± standard deviation from the mean of three replicates

Nickel accumulation by *L. minor*

Metal uptake characteristics are of three types: exclusion, indication and accumulation (Baker et al., 1981). A small concentration of the metal was taken up by excluders and no further metal uptake occurred even when the metal concentration varies in the external environment. Indicators were found to have a linear relationship with metal concentration in the surrounding external environment. Accumulators accumulate metals in concentrations greater than that found in the surrounding external environment (Baker et al., 1981). Uptake of metals by plants also depends on the bioavailability of the metal in the aqueous phase which depends upon metal retention time (Yasar et al., 2013) and dissolved metal concentrations (Gusek and Figueroa, 2009).

In the control set-up of plants without Ni, no Ni was found in the dry biomass of *L. minor*, when analyzed. Ni accumulated in the dry biomass of *L. minor* (test samples) at the end of the experimental period of 22 days was determined using an AAS (APHA, 1998) and shown in Table 1. The accumulation of Ni by *L minor* showed an increasing trend, such that at the highest initial Ni concentration of 4.9 mg/l, 12881.1 mg/kg of Ni accumulation was found. At lower concentrations of 3.05 and 3.98 mg/L, Ni accumulation was found to be 7111.99 and 9653.88 mg/kg. Ni has been known to reach the highest concentration in a plant. The statistical analysis (ANOVA) showed that there were significant differences between Ni accumulations in the tissue of *L. minor* at the end of the experimental period at different initial concentrations ($P< 0.001$). Zayed (1998) classified plants with accumulation of more than 1000 mg/kg Ni and a BCF of more than 1000 as hyper-accumulators. *L. minor* was thus able to accumulate a good amount of Ni from the external solution and could be considered as a hyperaccumulator of Ni. Pajevic et al. (2002) reported that macrophytes can accumulate ten to several thousand times higher concentration of heavy metals, than the concentration of heavy metals present in the aquatic environment in the surroundings. The ability to bioaccumulate metals depends not only on the plant species, but also on abiotic factors including the concentration of the elements present in the ambient solution, exposure time etc. A high positive correlation ($r = 0.97$) existed between initial Ni concentrations and accumulated metal in the dry biomass.

Zayed et al. (1998) found *Lemna* fronds to accumulate 1790 mg/kg of Ni at initial Ni concentration of 10.0 mg/L. An earlier study reported that Ni accumulation by plants is concentration dependent (Duman and Ozturk, 2010) where they found a maximum accumulation of 5757.9 mg/kg dw by *N. officinale* when the plants were exposed to 25 mg/L Ni for 7 days.

In a case study, Kara et al. (2003) found that over a period of 4 days, Ni proved to be toxic to *L. minor*. They also found that maximum accumulation of Ni occurred over the first few days after which a decrease in accumulation was observed, may be due to saturation. Appenroth et al. (2010) studied the toxicity of Ni on the chloroplast of *S. polyrhiza* and *L. minor*. They found Ni accumulation of 3.3 g/kg DW and 2.7 g/kg DW by *S. polyrhiza* and *L. minor* respectively, on exposure to 100 µM for a period of 7 days. On exposure to 4 µM, the accumulation of Ni was 0.19 g/kg DW and 0.10 g/kg DW for *Spirodela* sp. and *L. minor*, respectively. After 6 days of the experimental period, notable accumulation of Ni was found to occur in *L. gibba* (Obek et al., 2009). Kara et al. (2005) reported higher Ni accumulation by water fern *Azolla filiculoide* (9000 mg/kg) followed by *Salvinia natans L* (6295 mg/kg).

Table 1. Accumulation of Ni by *Lemna minor* at different initial Ni concentrations

Desired Initial Ni concentration (mg/L)	Actual Initial Ni concentration (mg/L)	Ni in dry biomass (mg/kg)
3	3.05 ± 0.06	7111.99 ± 616.33
4	3.98 ± 0.03	9653.88 ± 397.95
5	4.9 ± 0.14	12881 ±1434.91

Chromium accumulation in *L. minor*

In the present study, metal accumulation was found to increase with increase in initial Cr concentration and was found to be highest at 4.2 mg/L. Accumulation of Cr by *L. minor* was found to be 1993.84 mg/kg at initial concentration of 1.91 mg/L that increased to 3634.34 mg/kg and 5884.87 mg/kg at 2.98 mg/L and 4.2 mg/L. respectively (Table 2). Statistical differences between Cr accumulation at different initial Cr concentrations were obtained to be significant (P= 0.004). A positive correlation (r = 0.95) existed between the initial Cr concentrations in the external solution and accumulation of Cr in *L. minor*. Accumulation of Cr by plants causes reduction in growth of plants as well as chlorosis (Panda and Choudhury, 2005) which are some of the toxicity symptoms found also in the present study. The concentration remaining as residual concentration was also positively correlated (r = 0.92, P< 0.005) with the Cr accumulated in the dry biomass of *L. minor*.

Table 2. Accumulation of Cr by *Lemna minor* at different initial Ni concentrations

Desired Initial Cr concentration (mg/L)	Actual Initial Cr concentration (mg/L)	Cr in dry biomass (mg/kg)
2	1.91 ± 0.04	1993.84 ± 118.64
3	2.98 ± 0.02	3634.34 ± 709.65
4	4.2 ± 0.08	5884.87 ± 884.48

The potential of duckweeds in accumulating a substantial amount of Cr from ambient solution has been reported (Chandra and Kulshreshtha, 2004). Similar observations were made by Sinha *et al.* (2005) who reported an increase in the accumulation of Cr in roots and leaves of *Pistia stratiotes* with increase in initial Cr concentration. Vajpayee et al. (2001) obtained similar results when they exposed *Vallisneria spiralis* to different concentrations of Cr and found a maximum accumulation of 1050.8 mg/kg Cr accumulation at 10.0 mg/L Cr exposure over a period of 3 days. Again, there are plants like *Leersia hexandra* that have strong tolerance to chromium (III) and Cr (VI) (Zhang et al., 2007). At an initial Cr concentration of 5.0 mg/l, *L. hexandra* accumulated 1359 and 5130 mg/kg (DW) of Cr in the leaf and root, respectively (Zhang et al., 2007). Oporto (2006) studied the removal of Cr by *L. minor* at initial concentrations of 0.5 and 2.0 mg/l in the batch experiment, over a period of 16 days and found that with decrease in the initial concentration of Cr, the uptake of Cr by *L. minor* increased. Although Cr is known to play no role in the metabolic processes of plants, it is accumulated in significant amount (Chandra and Kulshreshtha, 2004). Cr is a non-essential element for plants and hence, have no specific uptake mechanisms and therefore its uptake is mediated by

carriers used for the uptake of essential elements for plant metabolism (Shanker et al., 2005). Emergent plants *Baco pamonnieri* and *Scirpus lacustri* saccumulated 1600 and 739 µg/g Cr, respectively (Chandra and Kulshreshtha, 2004). *Ipomoea aquatica* has been reported to accumulate a considerable amount of Cr from external ambient solution (Rai and Sinha, 2001). Soltan and Rashed (2003) reported that Cr accumulation by plants could also be due to co-precipitation in the plaques of Fe and Mn on roots. A significant amount of Cr accumulation in the tissues of the aquatic plant *Vallisneri aspiralis* and a decrease in biomass was reported by Sen et al. (1987).

Kinetics of removal of Ni by *L. minor*

The kinetic study of Ni removal by *L. minor* was carried out with different initial concentrations of Ni. The results obtained were fitted to second order kinetic models and the linearity plots of 1/ Ct versus time at different initial concentrations of Ni is presented in Figure 5, respectively. The respective kinetic equations have been included in the respective plots (Fig. 5). The rate constants are mentioned in Table 3 and were found to be higher at the lower initial concentrations of metals.

To determine which removal kinetics was adopted by *L. minor* for the removal of Ni from aqueous solution, coefficient of determination (R^2) and rate constant values were estimated in case of each of the concentration and reported in Table 3. The second order kinetic model showed high values of coefficient of determination (R^2) and was selected as appropriate for the different initial concentrations of Ni. Based on the R^2 criteria, results of kinetic study fitted better with the second order kinetics model at all concentrations. Other researchers also studied the removal kinetics of metals and found different kinetic models to fit the removal of different metals.

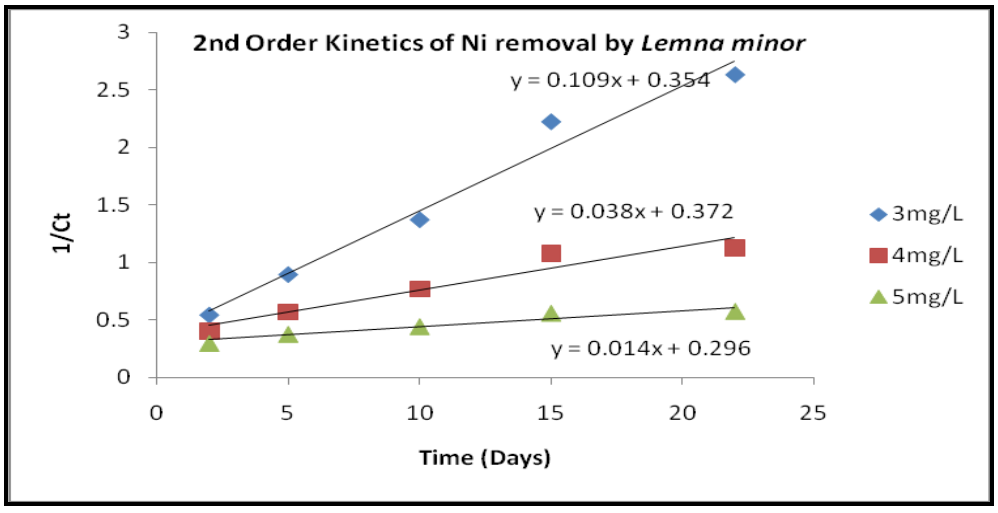

Fig. 5. Second Order Removal kinetics of Ni removal by *Lemna minor*

Table 3. Coefficient of determination and Rate Constant Values obtained at different initial Ni concentrations by Second Order Kinetic Model

Kinetic Model	Desired C_0 (mg/L)	Actual C_0 (mg/L)	R^2	Rate Constant
Second Order	3	3.05	0.975	0.109
	4	3.98	0.930	0.038
	5	4.9	0.921	0.014

The coefficient of determination (R^2) from linear regression and error analysis, determines the optimum kinetic model for the removal of Ni by *L. minor*. However, both might be erroneous based on the various axis settings employed in the linearized form of the kinetic models. The non linear chi-square test compares both first and second order kinetic models on the same abscissa and ordinate and is therefore free from such errors.

The removal of Ni by *L. minor* showing lower value of chi-square, has been selected as the optimum kinetic model (Table 4). Based on the above said criteria, the second order kinetic model was found to be a better fit with the experimental data obtained for the removal of Ni by *L. minor*.

Kinetics of removal of Cr by *L. minor*

Second Order kinetic model was applied to the data of Cr removal by *L. minor*. The R^2 values of each of the initial concentrations of Cr has already been mentioned in Table 5 which also includes the rate constant values. In general, the rate constants decreased with increase in initial Cr concentration in the aqueous spiked solutions.

Table 4. Values of different Error functions and Chi square

Kinetic Model	Desired C_0 (mg/L)	Actual C_0 (mg/L)	SAE	SSE	ARE	Chi square
Second Order	3	3.05	0.216	0.014	5.483	0.014
	4	3.98	1.181	0.334	19.95	0.226
	5	4.9	0.65	0.123	5.519	0.048

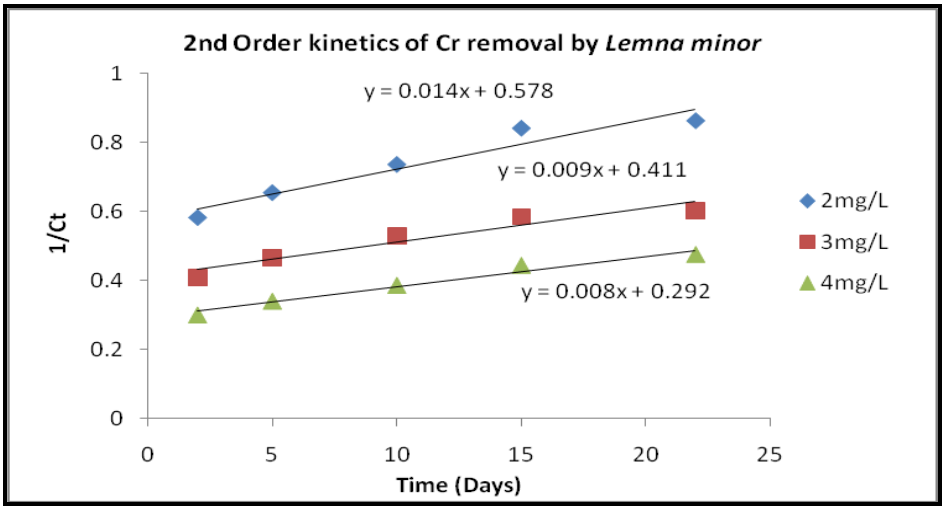

Fig. 6. Second Order removal kinetics of Cr by *Lemna minor*

Table 5. Coefficient of determination and Rate Constant Values obtained at different initial Cr concentrations by Second Order Kinetic Model

Kinetic Model	Desired C_0 (mg/L)	Actual C_0 (mg/L)	R^2	K_1
Second Order	2	1.91	0.929	0.014
	3	2.98	0.914	0.009
	4	4.2	0.97	0.008

From the results, it was found that the kinetic study data fitted better with the second order kinetic model based on R^2 criteria. Error analysis has also been studied further to evaluate the goodness fit of the removal kinetic model and calculated values provided in Table 6. From error analysis by SAE, SSE, ARE, chi square test (all discussed previously), *L. minor* was found to follow second order removal kinetics more suitably at all concentrations for removal of Cr.

Table 6. Values of different Error functions and Chi square

Kinetic Model	Desired C_0 (mg/L)	Actual C_0 (mg/L)	SAE	SSE	ARE	Chi square
Second Order	2	1.91	0.226	0.013	3.334	0.009
	3	2.98	0.417	0.044	4.207	0.021
	4	4.2	0.437	0.048	3.39	0.018

CONCLUSION

As indicated in the results, the hydroponic experiment conducted with spiked metal contaminated water indicated that *L. minor* has a good potential to tolerate Ni and Cr at lower concentrations. When treated with Ni at different concentrations, frond abscission and chlorosis were visible after 10 days of exposure. *L. minor* met the basic characteristics of metal hyperaccumulation and was found to be a hyperaccumulator of both Ni and Cr at all experimental concentrations. Thus, it suggested that duckweed can tolerate both Ni and Cr but at lower concentrations.

L. minor, if cultured in the vicinity of Ni and Cr contaminated effluents, could possibly treat the water and could therefore remove the toxic metals from the water, rendering it less toxic or non-toxic. Therefore, *L. minor* might be useful in the treatment of water contaminated with Ni and Cr, individually.

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