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Original Research Paper

Efficacy of Chelating Agents in Phytoremediation of Cadmium Using *Lemna minor* (Linnaeus, 1753)

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ABSTRACT

Free floating aquatic macrophyte namely *Lemna minor* (Linnaeus, 1753) was exposed to different concentrations of cadmium (1, 5 and 10 mg/L) for a period of 30 days to evaluate its cadmium (Cd) accumulation capability in the presence of chelating agents such as EDTA and citric acid. The chelating agents were added at the rate of 1, 2 and 3 mg/L separately and the experiment was conducted in triplicate. The water and plant samples were collected at 15 days interval for the analysis of cadmium. There was a significant difference in the Cd uptake ($P<0.05$) by the plant in the presence of chelating agents when compared to the control. Bioconcentration factor (BCF) of cadmium by the plants showed an increasing trend in the presence of chelating agents. The percentage uptake of cadmium by *L. minor* in the presence of EDTA was significantly higher than that of citric acid ($P<0.05$). The overall results suggest that EDTA can be effectively used to enhance phytoremediation efficiency of cadmium by *L. minor* in the contaminated water.

INTRODUCTION

Heavy metals released due to the modern industrial activities have significant impact on the land and water (Niagu 1988, Roy et al. 2005). The metals such as cadmium (Cd), mercury (Hg), chromium (Cr) and lead (Pb) are highly toxic to the aquatic organisms, as they do not vanish easily from the environment. The indiscriminate disposal of agricultural and municipal wastes reaches finally to the lotic and lentic system, leading to water pollution, which may contain large quantities of organic and inorganic materials and also varying amounts of heavy metals (Brar et al. 2000). Cadmium is one of the most potent metal pollutants due to its high toxicity. In order to resolve the environmental problems, environmental regulations are made to maintain the quality of ground and surface water. Removal of heavy metals by conventional methods is costly and time consuming process and may produce large quantities of chemical sludge. Hence, considerable interest has been generated to use biological materials for the removal of the same (Samecka-Cymerman & Kempers 1996).

Phytoremediation is one of the cost effective and environmentally safe method in which the plants are used to remove, destroy or sequester toxic contaminants from the water, soil and air (Prasad 2003). The most important aspect is the selection of hyper accumulating plants for remediation. The free floating, emergent and marginal aquatic plants are effectively used for the remediation of heavy metals from the contaminated sites. Duckweeds are free floating macrophytes which can accumulate N, P, Cu, Cd, Zn, As, Al, Cr, Hg, Ni and Pb from the polluted water (Objegba 2004, Wang et al. 2004, Mkandawire & Dudel 2005, Olguin et al. 2005). Some researchers found that the application of certain chelating agents can enhance the heavy metal uptake from the contaminated soil. As chelating agents can increase the mobility of metal, plant can easily uptake the metal via roots and later translocate into the shoots (Blaylock et al. 1997). A chelate can free a metal from the soil cation exchange site by making a complex with the metal and allowing the chelated metal species to migrate more readily in soil as a chelate metal complex. Hence, it can easily be uptaken via root system from the immediate vicinity. Chelating agents have potential to perturb the natural speciation of metal and to influence metal bioavailability thereby enhance the uptake of metal from water by aquatic plants (Anderson 1985). The chelating agents, namely EDTA, N-(2-hydroxyethyl) ethylene diamine triacetic acid (HEDTA) and citric acid, can enhance the phytoextraction process (Elless & Blaylock 2000, Chen & Cutright 2001, Chen et al. 2003).

Considering the above facts, the present study was conducted with the objective to evaluate and compare the effect of chelating agents in the phytoremediation of cadmium using *L. minor*.

MATERIALS AND METHODS

Experimental set-up: *L. minor* was used for phytoremediation enhancement study using chelating agents such as ethylene diamine tetraacetic acid (EDTA) and citric acid. *L. minor* was collected from the Wet-Laboratory of Aquaculture Division, Central Institute of Fisheries Education (CIFE), Mumbai. Plants were acclimatized and grown for 2 weeks in freshwater holding tanks to obtain the required quantity for the experiment. The water and plant samples were analysed 5 times before use for the experiment to estimate the background concentrations of cadmium. The results showed that there was no detectable content of cadmium in the plant and water. Each experiment was carried out in three distinct experimental groups, namely, concentrations of cadmium at 1, 5 and 10 mg/L for each group having three replicates in uniform-size glass aquarium tanks (100 L capacity each) for the accumulation study. Chelating agents, such as EDTA and citric acid (CA), were added in the aquarium tanks separately in triplicate at the same time at a concentration of 1, 2 and 3 mg/L for heavy metal uptake enhancement study (Table 1). Additional tanks in triplicate were set up as control for each treatment to find out the adsorption of metals on the glass aquarium tanks by maintaining the same concentration (1, 5 and 10 mg/L respectively). Aged tap water was used for the preparation of the test medium. Plants were inoculated at the rate of 60 g fresh weight in the tank containing 60 L water. Stock solutions of cadmium, EDTA and citric acid were prepared from analytical grade of cadmium chloride ($CdCl_2H_2O$) ethylenediaminetetra acetic acid disodium salt dehydrate ($C_{10}H_{14}N_2Na_2O_8$ $2H_2O$) and citric acid anhydrous ($C_6H_8O_7$), respectively.

Preparation of sample for heavy metal analysis: The water samples of 50 mL each were collected from each tank in triplicate and preserved by adding concentrated nitric acid (pH < 2.0) until wet digestion. Plant samples (30g) were air-dried at room temperature for 2-3 days. The samples were oven-dried for 3-5 hours to attain a constant weight and ground to a powder using glass mortar and pestle to ensure sample homogeneity with respect to particle distribution. The finely ground material was stored in sealed polyethylene bags and marked properly and kept at room temperature until wet digestion. Also, water samples were collected in other sample bottles for the water quality parameter studies (APHA 2005) which were carried out on the very same day of sample collection. The chlorophyll content in the plant samples was estimated on 15th and 30th day using acetone solvent extraction method (APHA 2005). Three replicates of dried plant samples were digested with a mixture (3:1) of concentrated nitric and hydrofluoric supra pure acids (Merck, Germany) in a microwave-assisted Kjeldahl digestion unit (Anton Parr, USA). Each microwave extraction vessel was added with 8 mL of the acid mixture together with 0.25g of plant sample. The vessels were capped and heated in the microwave unit at 1200 W to a temperature of 190°C for 25 minutes at a pressure of 25 bar. The digested samples were diluted to 50 mL using distilled water and subjected to heavy metal analysis (µg g^{-1}) by atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer, USA) using flame atomization. Water samples were subjected to supra pure nitric acid digestion (6 mL) using microwave-assisted Kjeldahl digestion unit (Anton Parr, USA). The digested samples were diluted to 50 mL using distilled water and subjected to heavy metal analysis (mg/L) by atomic absorption spectrophotometer.

Table 1: Experimental set-up for phytoremediation of Cd using chelating agents.

Chelating agents/Cd	Cd (1 mg/L)	Cd (5 mg/L)	Cd (10 mg/L)
Control (EDTA0/CA0)	C1	C2	C3
EDTA (1 mg/L)	T1	T7	T13
EDTA (2 mg/L)	T2	T8	T14
EDTA (3 mg/L)	T3	T9	T15
CA (1 mg/L)	T4	T10	T16
CA (2 mg/L)	T5	T11	T17
CA (3 mg/L)	T6	T12	T18

The bioconcentration factor provides an index of the ability of plant to accumulate metal with respect to metal concentration in substrate. BCF was calculated for 15th and 30th day. The factor is calculated as the ratio of the metal concentration in the dry plant biomass to the initial concentration of metal in the feed solution (Raskin et al. 1994).

Statistical analysis: For all the experiments, the data were analysed by PROC MEANS and PROC ANOVA procedure (SAS, 9.2). Both one-way and two-way analyses were carried out for each experiment to find out the significance between treatments with and without chelating agents. PROC BOXPLOT procedure was used to draw the boxplots.

RESULTS

All the treatments with chelating agents showed significantly higher cadmium uptake than the control irrespective of the concentration of cadmium or chelating agents (Table 2). Cadmium uptake in the presence of EDTA was significantly higher than citric acid in their corresponding doses (Figs. 1-6). The cadmium uptake in the treatment with EDTA1 was at par or even significantly higher than that of higher doses of citric acid (CA2 or CA3). At cadmium 1

Table 2: Cd concentration (µg g^{-1}) in *L. minor* on dry weight basis (Mean±SE).

Chelating agent (mg/L)	Cd- 1 mg/L 15 days	Cd- 1 mg/L 30 days	Cd- 5 mg/L 15 days	Cd- 5 mg/L 30 days	Cd- 10 mg/L 15 days	Cd- 10 mg/L 30 days
Control	133.24^{d}±7.44	222.10^{d}±11.36	747.40^{e}±19.04	1508.90^{f}±18.64	1290.50^{f}±18.28	2242.95^{f}±9.21
EDTA- 1	229.10^{b}±9.57	351.70^{bc}±11.31	1069.00^{c}±2.64	2303.60^{d}±10.53	2131.30^{c}±18.09	2712.33^{b}±11.92
EDTA- 2	309.77^{a}±5.54	401.50^{ab}±7.50	1180.00^{b}±4.85	2452.30^{b}±6.06	2417.70^{b}±10.90	2954.67^{a}±5.70
EDTA- 3	329.67^{a}±10.10	447.20^{a}±11.12	1315.00^{a}±8.89	2522.30^{a}±12.35	2664.30^{a}±4.70	2991.67^{a}±7.13
CA- 1	185.47^{c}±8.56	295.10^{c}±22.01	928.98^{d}±8.50	2186.10^{e}±4.36	1751.30^{e}±7.88	2451.33^{e}±6.69
CA- 2	233.33^{bc}±6.86	348.73^{bc}±10.17	1050.00^{c}±8.57	2297.70^{d}±9.52	1898.30^{d}±2.33	2557.00^{d}±7.21
CA- 3	245.67^{b}±6.26	405.20^{ab}±6.82	1085.00^{c}±12.25	2359.30^{c}±6.49	1916.30^{d}±6.74	2610.33^{c}±7.53

Mean values in a column under each category bearing different superscripts vary significantly (P<0.05).

Table 3: Percentage of cadmium removed from water after phytoremediation with *L. minor* (Mean±SE).

Chelating agent (mg/L)	Cd- 1 mg/L 15 days	Cd- 1 mg/L 30 days	Cd- 5 mg/L 15 days	Cd- 5 mg/L 30 days	Cd- 10 mg/L 15 days	Cd- 10 mg/L 30 days
Control	15.47^{e}±0.70	45.03^{f}±0.90	15.82^{f}±0.11	46.54^{f}±0.29	13.76^{h}±0.14	37.71^{g}±0.05
EDTA- 1	25.97^{c}±0.85	64.67^{cd}±0.64	24.05^{d}±0.14	69.79^{d}±0.14	23.46^{c}±0.09	50.61^{c}±0.16
EDTA- 2	32.07^{ab}±1.00	73.47^{b}±1.64	27.69^{b}±0.12	75.97^{a}±0.11	26.02^{b}±0.03	54.47^{b}±0.08
EDTA- 3	35.57^{a}±1.07	79.83^{a}±0.79	29.45^{a}±0.06	76.37^{a}±0.11	27.46^{a}±0.12	56.65^{a}±0.11
CA- 1	20.33^{d}±0.62	56.60^{e}±1.50	22.31^{e}±0.19	65.98^{e}±0.08	18.88^{g}±0.08	43.86^{f}±0.08
CA- 2	25.53^{c}±0.97	62.40^{de}±1.11	25.82^{c}±0.13	72.17^{c}±0.13	20.11^{f}±0.09	46.08^{e}±0.06
CA- 3	30.77^{bc}±0.83	69.43^{bc}±1.75	24.19^{d}±0.22	73.90^{b}±0.16	20.95^{d}±0.06	47.00^{d}±0.06

Mean values in a column under each category bearing different superscripts vary significantly (P<0.05).

mg/L, the treatment with EDTA2 and EDTA3 did not differ significantly whereas in higher cadmium concentration, EDTA3 showed significantly higher cadmium uptake than treatment with EDTA2 except for cadmium at 10 mg/L on 30th day. On 15th day, the treatment with CA2 and CA3 did not differ significantly at cadmium 1, 5 and 10 mg/L, therefore CA3 can be replaced by CA2. But treatment with CA3 showed significantly high cadmium uptake among the treatment with citric acid on 30th day. In general, the overall results showed significant differences in cadmium uptake by the *L. minor* in the presence of chelating agents compared to control. As the concentration of EDTA and citric acid increased from 1 to 3 mg/L, there was an increase in the cadmium accumulation by *L. minor* in the different treatments.

In the treatment T3 and T6, a significant percentage of cadmium was removed from the water (35.37 and 76.37% respectively) compared to other treatments (Table 3). In all the treatments, significantly higher removal in terms of percentage was observed in the presence of EDTA3 except treatment T6 on 30th day. In the treatment with citric acid, there was a significant difference in removal of cadmium in terms of percentage among different concentrations. In comparison to the control, the rate of Cd removal in the presence of citric acid (3 mg/L) increased from 45.03 to 69.43%, 46.54 to 73.90% and 37.71 to 47% on the 30th day. The overall result showed that there was a significant difference in percentage of Cd removal between the control and treatments with chelating agents. Considering the removal of Cd in terms of percentage, maximum removal took place in the presence of EDTA.

The BCF of Cd by *L. minor* showed that significantly high BCF was observed in the treatment T6 (i.e. 362.48). BCF in the presence of EDTA2 and EDTA3 showed that EDTA3 can be replaced by EDTA2 in the treatments containing 1 mg Cd L^{-1}. Similarly, CA3 can be replaced by CA2 in all the treatments (Table 4). The overall results suggest that BCF of Cd by *L. minor* was significantly high on the 15th day in the presence of EDTA compared to citric acid.

The water quality parameters showed that the dissolved oxygen level in the water decreased as the concentration of cadmium and chelating agents increased (Table 5). The water temperature was in the range of 25.1 to 28.4°C. The pH of the water decreased at greater concentration of cadmium and chelating agents. Available phosphorus showed decreasing trend. The chlorophyll content in the plant decreased significantly at higher concentration of cadmium which led to the discoloration at the end of the experiment.

DISCUSSION

The result of cadmium accumulation by *L. minor* in the presence of chelating agents such as EDTA and citric acid shows an increasing trend in all the treatments compared to the control from 15 to 30 days. Significantly high accumulation of cadmium was observed in the presence of EDTA3 (2991.67

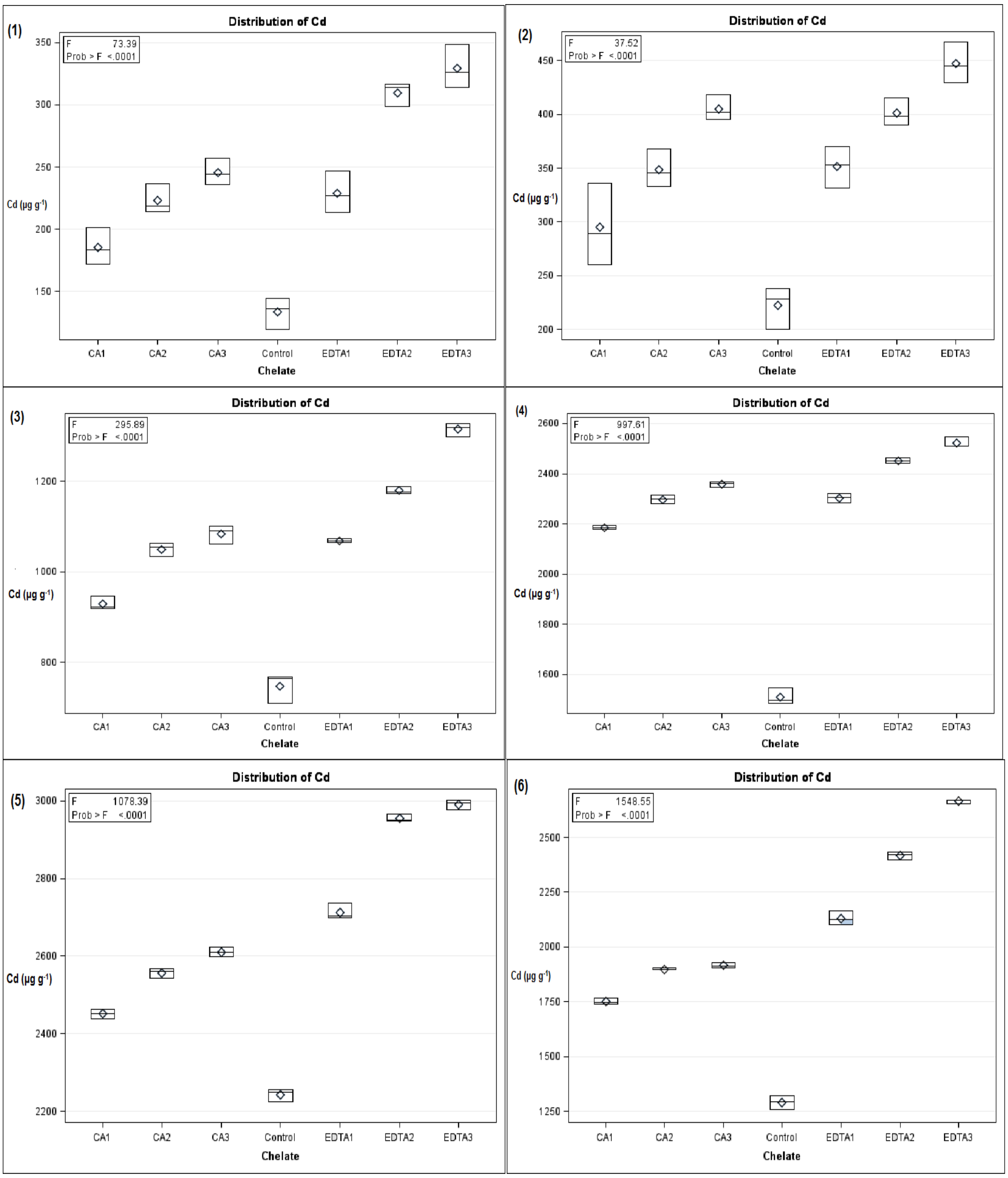

Fig. 1, 2: Cadmium accumulation (µg g^{-1}) by *L. minor* on 15 and 30th day from 1 mg Cd L^{-1} respectively; Fig. 3, 4: Cadmium accumulation (µg g^{-1}) by *L. minor* on 15 and 30th day from 5 mg Cd L^{-1} respectively; Fig. 5, 6: Cadmium accumulation (µg g^{-1}) by *L. minor* on 15 and 30th day from 10 mg Cd L^{-1} respectively.

µg g^{-1}) than EDTA1 and EDTA2 at the end of experiment. Dipu et al. (2011) also observed a significant increase ($p<0.05$) in absorption of cadmium (1 mg/L) by *Pistia* sp. (0.322 mg g^{-1}) when EDTA (1 mg/L) was added along with cadmium. This indicates that EDTA can increase the bioavailability of cadmium in the water. Significantly high concentration of Cd (2610.33 µg g^{-1}) accumulation was observed in the treatment with CA3. Sinhal et al. (2010) found that marigold efficiently accumulated the Zn, Cu, Pb and Cd from treated soil and addition of EDTA and citric acid, increased the accumulation of these metals many fold. The chelating agents enhanced the bioavailability of metal from the soil particles, hence the plant could easily uptake the metal from the soil contaminated site (Taiz & Zeiger 2002). The

Table 4: Bioconcentration factor (BCF) of cadmium in *L. minor* (Mean±SE).

Chelating agent (mg/L)	Cd- 1 mg/L		Cd- 5 mg/L		Cd- 10 mg/L	
	15 days	30 days	15 days	30 days	15 days	30 days
Control	$131.92^{d}\pm7.37$	$105.20^{b}\pm5.53$	$149.18^{e}\pm3.80$	$140.90^{c}\pm8.70$	$128.02^{f}\pm1.81$	$110.44^{a}\pm3.23$
EDTA- 1	$226.83^{b}\pm9.47$	$166.00^{ab}\pm24.54$	$213.35^{c}\pm0.53$	$155.11^{b}\pm3.58$	$211.44^{c}\pm1.79$	$75.91^{cd}\pm1.31$
EDTA- 2	$306.70^{a}\pm5.48$	$134.84^{b}\pm12.41$	$335.49^{b}\pm0.96$	$151.97^{a}\pm1.44$	$239.85^{b}\pm1.08$	$72.58^{d}\pm1.41$
EDTA- 3	$326.40^{a}\pm10.00$	$181.72^{ab}\pm26.88$	$362.48^{a}\pm1.77$	$342.24^{ab}\pm2.52$	$264.32^{a}\pm0.46$	$45.12^{e}\pm1.40$
CA- 1	$183.63^{c}\pm8.47$	$137.85^{b}\pm17.80$	$225.43^{d}\pm1.69$	$203.60^{b}\pm2.83$	$173.74^{e}\pm0.78$	$86.28^{b}\pm0.94$
CA- 2	221.12^{b}c±6.79	$167.96^{ab}\pm16.42$	$209.65^{c}\pm1.71$	$106.29^{ab}\pm4.60$	$188.33^{d}\pm0.23$	$82.44^{bc}\pm1.10$
CA- 3	$243.23^{b}\pm6.19$	$230.63^{a}\pm9.13$	$286.50^{c}\pm2.44$	$276.27^{ab}\pm2.52$	$190.11^{d}\pm0.66$	$87.79^{b}\pm0.17$

Mean values in a column under each category bearing different superscripts vary significantly ($P<0.05$)

Table 5: Water quality parameters in the different treatments with *L. minor*.

Parameters							
				Cd-1 mg/L			
	Control	EDTA1	EDTA2	EDTA3	CA1	CA2	CA3
DO	5.53- 6.07	5.08-5.40	4.76-5.02	4.37-4.77	5.05-5.35	4.59-4.85	4.28-4.93
pH	7.9-8.2	7.4-7.8	7.2-7.4	7.1-7.3	7.4-7.7	7.3-7.4	6.9-7.3
AP	0.33-0.41	0.21-0.30	0.20-0.29	0.16-0.30	0.22-0.39	0.14-0.32	0.13-0.24
NH_3-N	0.06-0.11	0.08-0.12	0.09-0.16	0.08-0.19	0.06-0.18	0.11-0.21	0.09-0.24
NO_2-N	0.25-0.28	0.26-0.33	0.25-0.26	0.20-0.26	0.25-0.28	0.17-0.28	0.20-0.28
				Cd-5 mg/L			
	Control	EDTA1	EDTA2	EDTA3	CA1	CA2	CA3
DO	5.54-6.04	5.06-5.36	4.73-5.07	4.34-4.74	4.99-5.38	4.52-4.86	4.26-4.92
pH	7.7-8.1	7.4-7.6	7.4-7.7	7.0-7.2	7.1-8.0	7.0-7.1	7.1-7.3
AP	0.24-0.38	0.22-0.36	0.17-0.36	0.14-0.23	0.14-0.28	0.10-0.22	0.13-0.21
NH_3-N	0.03-0.14	0.10-0.19	0.10-0.18	0.12-0.19	0.08-0.17	0.12-0.19	0.05-0.19
NO_2-N	0.24-0.27	0.29-0.31	0.23-0.35	0.31-0.32	0.23-0.32	0.29-0.30	0.27-0.32
				Cd-10 mg/L			
	Control	EDTA1	EDTA2	EDTA3	CA1	CA2	CA3
DO	5.50-6.01	4.99-5.36	4.69-5.01	4.33-4.66	5.00-5.29	4.47-4.79	4.20-4.91
pH	7.8-8.0	7.3-7.6	7.1-7.2	6.7-7.0	7.3-7.9	7.0-7.4	6.7-7.0
AP	0.16-0.32	0.16-0.27	0.15-0.24	0.06-0.23	0.11-0.18	0.09-0.13	0.04-0.17
NH_3-N	0.08-0.17	0.10-0.13	0.03-0.15	0.13-0.21	0.09-0.14	0.15-0.22	0.10-0.31
NO_2-N	0.26-0.28	0.22-0.34	0.22-0.33	0.31-0.36	0.28-0.33	0.19-0.33	0.19-0.29

DO: Dissolved Oxygen (mg/L); pH (no unit); AP: Available phosphorus (mg/L); NO_2-N: Nitrite-N (mg/L); NH_3-N: Ammonia-N (mg/L)

result showed that there was a significant increase ($p<0.05$) in the cadmium uptake by *L. minor* in the presence of EDTA or citric acid in the order of EDTA>citric acid>control. Chelating agents (also known as sequestering agents) can inhibit undesirable metal-catalysed reactions by forming complexes with the metal ions. The resulting structure, called a chelate, deactivates the metal ion and prevents it from reacting with other components of the system. The chelating agents, namely EDTA and citric acid, significantly enhanced the exchangeable fraction of heavy metal as the residual form of metals was reduced markedly by the addition of chelants (Yeh & Pan 2012).

There was a significant difference in the percentage of cadmium removal from water between the treatments in the presence of EDTA or citric acid. Duckweed removed 100% copper and lead after 8 days of the treatment (Wafaa et al. 2007). In the control tank, percentage of cadmium removal was 37-46% after 30 days. *Pistia stratiotes* was effectively used to remove Pb, Cr, Mn and Zn almost completely after 24 h of exposure (Miretzky et al. 2004). There was a significant difference in cadmium removal between the 15th and 30th day. The result shows that among the chelating agents, the percentage of cadmium removal is more in the presence of EDTA (50-80%) than citric acid (44-69%). Maja & Domen (2009) reported that addition of 10 millimoles EDTA per kg removed 26% of Cu from the contaminated soil. In the presence of cadmium, *L. minor* showed significantly higher BCF in the first 15 days compared to the next 15 days of uptake. Jain et al. (1989) observed that BCF for *Azolla pinnata* and *L. minor* treated with Pb and Zn gradually decreased with increasing the metal concentration in the feed solution. According to Dipu et al. (2012), the BCF of cadmium was more in the presence of EDTA, i.e. 322 compared to the treatment without EDTA, i.e. 206 by using *Pistia stratiotes.* The significantly higher BCF (362) was observed in the treatment with EDTA than citric acid and control. This indicated that the chelating agents can play a major role in increasing the cadmium accumulation in plants. There was a significant increase in BCF between the treatments with EDTA and citric acid. The level of DO in the water decreased during the experiment due to the decomposition of plants in the series from higher to lower concentration of cadmium in the presence of EDTA and citric acid. The available phosphorus significantly decreased in all the treatments during the experimental period. It might be due to the uptake of phosphorus by the plants for their growth.

In conclusion, the overall result shows that chelating agents have significantly higher cadmium uptake than the control irrespective of the concentration of cadmium or chelating agents. *L. minor* can uptake higher concentration of cadmium in the presence of chelating agents in the order of EDTA>citric acid>control. The maximum accumulation was observed in the treatment with 3 mg EDTA L^{-1} on 30^{th} day. It can be summarized that addition of chelating agents like EDTA or citric acid at a rate of 1 to 3 mg/L to wastewater can enhance the heavy metal uptake by increasing their bioavailability in cadmium contaminated system.

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