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Chelating Agent Mediated Enhancement of Phytoremediation Potential of *Spirodela polyrhiza* and *Lemna* minor for Cadmium Removal from the Water

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Abstract- Free floating aquatic macrophytes, *Lemna minor* and *Spirodela polyrhiza*, were exposed to graded concentration of cadmium in a concentration range 1 to10 mg L^{-1} for a period of one month to evaluate cadmium accumulation in the presence of EDTA. The EDTA was added at the rate of 1, 2 and 3 mg L^{-1} separately and the experiment was conducted in triplicate. The water and plant samples were collected at fortnightly interval for analysis of cadmium. At higher concentration of cadmium and EDTA, complete discoloration of plants was observed on the 30th day in the treatment with *L. minor* compared to *S. polyrhiza.* There was a significant difference in the cadmium uptake by the plants in the presence of EDTA when compared to the control ($P<0.05$). Cadmium removal from the water by the macrophytes showed significant difference ($P<0.05$) in the presence of EDTA compared to the control. Based on the result obtained during the study, it is concluded that, the uptake of cadmium by both the species showed a considerable extent of enhancement in the presence of EDTA. Overall observation reveals that a chelating agent such as EDTA can be used as an additive for enhanced phytoremediation potential of *S. polyrhiza* and *L. minor*.

Index Terms- Phytoremediation, EDTA, *Spirodela polyrhiza*, *Lemna minor*, Cadmium

I. Introduction

Heavy metal pollution of the biosphere has increased rapidly since 1900 (Nriagu, 1979), and aquatic ecosystem are directly affected by heavy metal pollution. The major sources of metal pollution are municipal wastes, burning of fossil fuel, fertilizer, sewage, mining and smelting of metalliferous ores and pesticides (Pendias & Pendias, 1989; Rai, 2008). In India, the major sources of heavy metals in the industrial areas are coal mining (Finkelman & Gross, 1999), thermal power plants and chemical industries. High levels of cadmium, copper, lead and Iron act as ecological toxins in both aquatic and terrestrial ecosystem (Balsberg, 1989; Guilizzoni, 1991). Cadmium has become a serious problem because of its high toxicity. The major sources of cadmium pollution are industrial wastes from metallurgical plants, plating works, cadmium pigment manufacturing plants, textiles industries, nickel cadmium batteries etc. The conventional method used for remediation of cadmium contaminated water bodies are costly and time consuming. Phytoremediation is an alternative method to remove the toxic metals from the waste water. It is cost effective and environment friendly, in which hyper accumulating plants are used in order to extract and accumulate contaminants to the harvestable part of plants to clean up the contaminated area (McGrath *et al*., 2002; Salt *et al*., 1998). Uses of hyper accumulating plants are important in the process of phytoremediation. Duckweeds are floating aquatic macrophytes belonging to family *Lemnaceae* and normally found on the surface of nutrient rich fresh and brackishwaters (Zimmo, 2005). Chelating agents such as Ethylene Diamine Tetra Acetic acid (EDTA), citric acid, Ethylene Diamine Di Succinate (EDDS), Nitrilotriacetic acid (NTA) etc. are used in order to enhance the heavy metal uptake from the contaminated area. These chelating agents can make complex with metals thereby increase the bioavailability of metal in the contaminated sites. Therefore the plants can easily uptake the metal from the water. The objective of the study was to evaluate the efficacy of EDTA in the phytoremediation of cadmium contaminated water using *L. minor* and *S. polyrhiza*

II. MATERIAL AND METHODS

In the study, both the plants were collected and acclimatised for about 10 days under natural condition of cultivation site; the plants were kept in an aquarium tank containing 100 L of freshwater. Cadmium was added at the rate of 1, 5 and 10 ppm in triplicate for a period of one month. EDTA was added in each aquarium at a concentration of 1, 2 and 3 ppm separately in triplicate. Water with plants and cadmium but without chelating agents in triplicate served as control. In each aquarium 60 L of tap water was maintained. The plants were added into each tank at the rate of 60 g fresh weight.

The plant and water samples were collected at fortnightly interval. The water samples for metal analysis were collected in polypropylene bottles and in which concentrated nitric acid was added to lower the pH to less than 2.0. After acidification, the water samples were also stored in a refrigerator for further use of digestion. Water samples were also collected in sample bottles for the analysis of water quality parameter such as water temperature, dissolved oxygen, ammonia, nitrite, nitrate, and phosphorus (APHA, 2005) which were carried out on the same day of sample collection. The plants were collected from each tank randomly and debris attached to the plants were removed by washing with distilled water and thereafter were air dried for 2 days or till constant weight was attained. The samples were powdered well and kept at room temperature for wet digestion.

Analysis of cadmium in water and plants: Water samples were subjected to suprapure nitric acid digestion (6 ml) using microwave-assisted Kjeldhal digestion unit (Anton Parr, USA). The digested samples were diluted to 50 ml using distilled water and subjected to heavy metal analysis by atomic absorption spectrophotometer (AAnalyst 800, Perkin Elmer, USA) using flame atomization.

The dried plant samples were digested with a mixture (3:1) of concentrated nitric and hydrofluoric supra pure acids (Merck, Germany) in a microwave-assisted Kjeldhal digestion unit (Anton Parr, USA). Each microwave extraction vessel was added with 8 ml of the acid mixture together with 0.50 g 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 by Atomic Absorption Spectrophotometer (AAnalyst 800, Perkin Elmer, USA) using flame atomization.

Statistical analysis: For all the experiments, the data were analysed by SPSS, 16. Both one-way and two-way analyses were carried out for each experiment to find out the significance between treatments with EDTA and control between 15 days of interval.

III. RESULTS AND DISCUSSION

The result indicated that both *L. minor* and *S. polyrhiza* can effectively remove the cadmium from the contaminated water. Rhizofiltration is a type of phytoremediation in which the heavy metal can be removed from the contaminated water using hydroponically green plants (Raskin et al., 1994). The chelating agent can play a major role in order to enhance the heavy metal uptake from the contaminated area. EDTA is one of the easily available chelating agents which enhance the metal bioavailability in the water, therefore the plant can uptake maximum amount of metal from the surrounding area. Fig.1 indicate that the accumulation of cadmium is higher in the treatment with *S. polyrhiza* than *L. minor* and also greater accumulation was observed in the presence of EDTA at different concentration. EDTA is reported to be the most effective amendment in the phytoremediation process which has been successfully utilized to enhance phytoextraction of Pb and other metals from the contaminated area (Cunningham and Ow, 1996; Chen *et al.*, 2004). *L. minor* can uptake cadmium and copper from the contaminated water (Kara, 2004; Hou *et al*., 2007). Cadmium accumulations by the plant were increased as the concentration of EDTA increased from 1 to 3 mg L^{-1}. The well-developed root system can absorb and accumulate water, nutrients and other non-essential contaminants such as Pb and Cd (Arthur *et al.*, 2005). The graphical representation clearly indicate that the removal of cadmium is higher in all the treatment with *S. polyrhiza* compared to *L. minor.*

The cadmium level (mg L^{-1}) in the water after 15 and 30 days of experiments was given in the Table No. 1. The concentration of cadmium in the water decreased significantly in the presence of EDTA compared to other treatments. There was a significant difference between EDTA 1, 2 and 3 ppm on the 15th and 30th day. In the presence of EDTA (3 ppm), concentration of Cd in the treatment with *L. minor* decreased to 0.202±0.008, 1.181±0.005 and 4.335±0.010 mg L^{-1} with a removal efficiency of 80, 76 and 57% from the treatment containing 1, 5 and 10 mg Cd L^{-1}, respectively. In the presence of EDTA (3 ppm), concentration of Cd in the treatment with *S. polyrhiza* significantly decreased to 0.164±0.006, 1.004±0.004 and 3.147±0.008 mg L^{-1} with a removal efficiency of 84, 80 and 69 % in the treatment containing 1, 5 and 10 mg Cd L^{-1}, respectively. The results show that concentration of cadmium in the water decreases in the presence of chelating agents significantly as compared to the control. The minimum level of cadmium in the water observed in the treatments with 3 mg EDTA L^{-1} at the end of 30 days. The overall cadmium accumulation in the plant shows that there is an increase in uptake of cadmium from 15 to 30 days and the accumulation at different concentration of cadmium (1, 5 and 10 mg L^{-1}) indicated that *S. polyrhiza* can accumulate higher amount of cadmium at different level of concentrations. Similar findings were reported earlier by Hamizah *et al. (2011)* found that, for containers containing *Eichhornia crassipes*, the concentrations of copper decreased from 5.5 to 2.1, 2.5 to 0.11 and 1.5 to 0.04 mg L^{-1} and for containers containing *Centella asiatica*, the concentrations of copper decreased from 5.5 to 0.92, 2.5 to 0.01 and 1.5 to 0.03 mg L^{-1}.

Percentage of cadmium removed from water showed that maximum removal was observed in the treatment with EDTA 3 on 30th day. Earlier studies by Devaleena *et al*. (2013) found that *S. polyrhiza* and *L. minor* could remove 52-75 % and 42-78 % of cadmium from the contaminated water. As the concentration of EDTA increased from 1 to 3 mg L^{-1}, the percentage of cadmium removed from water increased (Fig. 2). The Cd removal in terms of percentage was maximum in the presence of *S. polyrhiza* than *L. minor* using EDTA. The application of 0.1M Na_2EDTA solution at pH 4.5 showed a removal of 85.26% of lead from the contaminated soil (Ayejuyo., 2012). The total Bio concentration Factor (BCF) value of cadmium indicated that higher BCF was observed in the treatment with *S. polyrhiza* and maximum concentration was observed in the presence of EDTA compared to the control (Fig. 3). According to Dipu *et al*. (2012), BCF of cadmium was more in the presence of EDTA compared to the control by using aquatic macrophytes. BCF of cadmium indicated that the *S. polyrhiza* can accumulate high amount of cadmium in the presence of EDTA compared to *L. minor*.

The water quality parameters indicated that dissolved oxygen, pH, and available phosphorous and ammonia-N level varied among the treatments with EDTA and control (Table No. 2). The dissolved oxygen levels in the water decreased after the treatment with EDTA (Satyakala and Jamil, 1992). The chlorophyll-a decreased in the treatment with increase in concentration of cadmium and chelating agent. Growth rate of *L. minor* and *S. polyrhiza* decreased with increased Pb concentrations and chlorophyll content was negatively correlated with Pb exposure (Lablebici and Ahmet, 2011). In this study, the chlorophyll-a decreased significantly as the concentration of Cd increased from 1 to 10 mg L^{-1}. Leblebici and Ahmet (2011) found that *S. polyrhiza* when exposed to Pb concentration of 5 mg L^{-1} or higher, a decrease in chlorophyll pigments of the plants were observed with minimum chlorophyll-a value being 0.414 mg g^{-1} fresh weight on day 7 at 50 mg L^{-1} compared to 1.601 mg g^{-1} in the control. The morphological parameters indiacated that discoloration of the plant appeared at high concentration of cadmium (10 mgL^{-1}) and EDTA (3 mgL^{-1}). Nitrate and phosphorus content decreased with increase on concentration of cadmium. Nitrate, phosphate and sulfate concentration in water decreased with time in the nutrient enriched treatments in *L. minor* and *S. polyrhiza* (Leblebici and Ahmet, 2011).

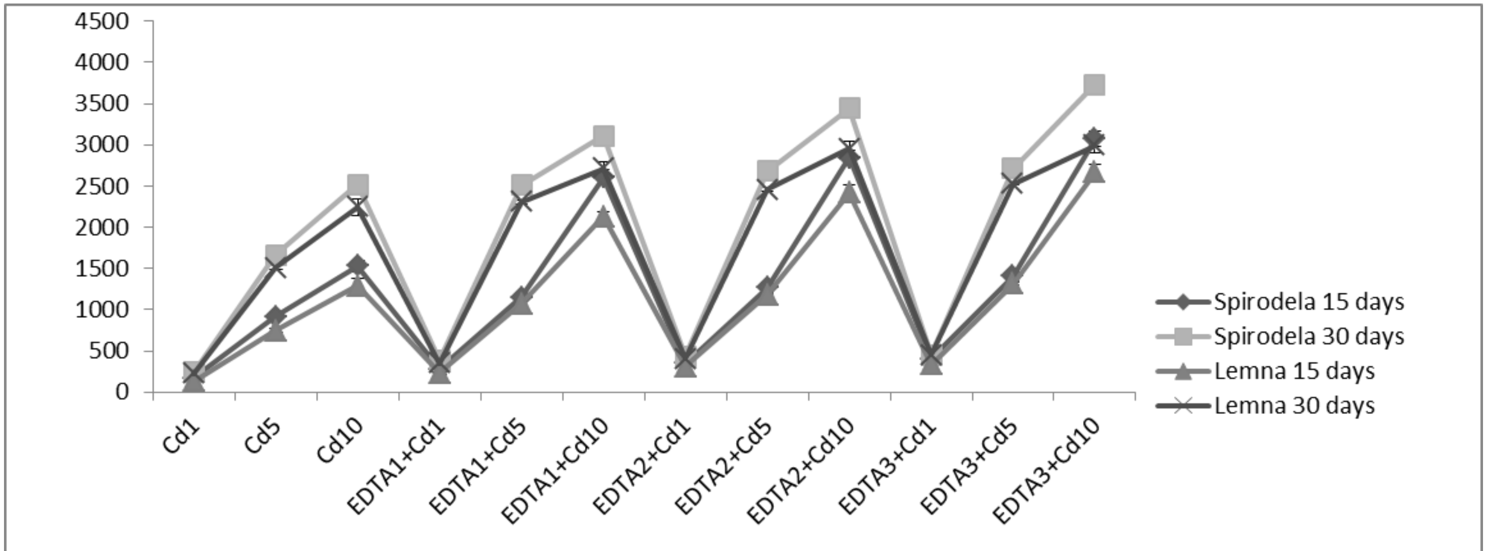

Fig. 1: Cd concentration (µg g^{-1}) in *S. polyrhiza* and *L. minor* on 15 and 30th day (Mean±SE)

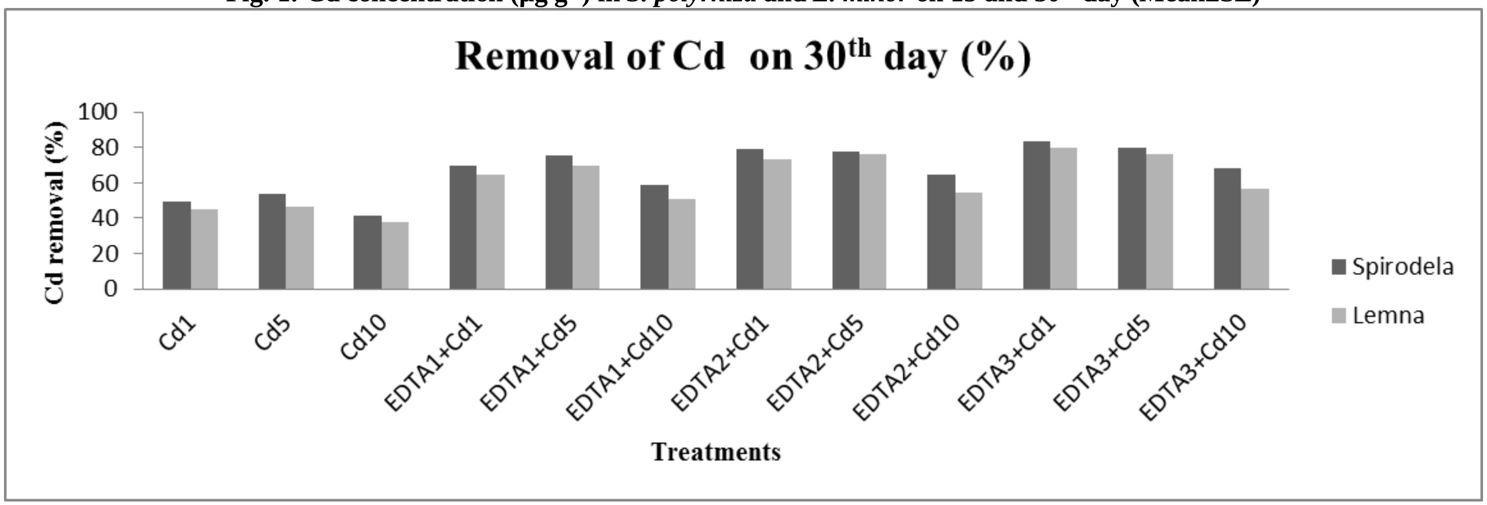

Fig. 2: Percentage of cadmium removed from water on 30th day with *S. polyrhiza* and *L. minor* (Mean±SE)

Table No. 1: Concentration of cadmium in water (mg L^{-1}) after phytoremediation by *S. polyrhiza* and *L. minor* (Mean±SE)

Treatment	*Spirodela polyrhiza*		*Lemna minor*	
	15th Day	30th Days	15th Day	30th Day
Cd1	0.805^{d}±0.006	0.504^{f}±0.035	0.845^{e}±0.007	0.550^{e}±0.009
Cd5	3.976^{c}±0.017	2.301^{d}±0.005	4.209^{c}±0.005	2.673^{c}±0.014
Cd10	8.312^{a}±0.014	5.828^{a}±0.017	8.624^{a}±0.014	6.229^{a}±0.005
EDTA1+Cd1	0.701^{d}±0.008	0.301^{g}±0.004	0.740^{f}±0.009	0.353^{f}±0.006
EDTA1+Cd5	3.699^{c}±0.006	1.208^{e}±0.007	3.798^{d}±0.007	1.510^{d}±0.007
EDTA1+Cd10	7.314^{a}±0.009	4.113^{b}±0.012	7.654^{b}±0.009	4.938^{b}±0.016
EDTA2+Cd1	0.648^{e}±0.006	0.212^{h}±0.007	0.679^{f}±0.010	0.265^{g}±0.016
EDTA2+Cd5	3.597^{c}±0.006	1.109^{e}±0.003	3.615^{d}±0.006	1.201^{d}±0.006
EDTA2+Cd10	7.009^{b}±0.009	3.525^{c}±0.007	7.398^{b}±0.003	4.553^{b}±0.008
EDTA3+Cd1	0.595^{e}±0.009	0.164^{h}±0.006	0.644^{g}±0.010	0.202^{h}±0.008
EDTA3+Cd5	3.514^{c}±0.006	1.004^{e}±0.004	3.528^{d}±0.003	1.181^{d}±0.005
EDTA3+Cd10	6.830^{b}±0.010	3.147^{c}±0.008	7.254^{b}±0.011	4.335^{b}±0.010

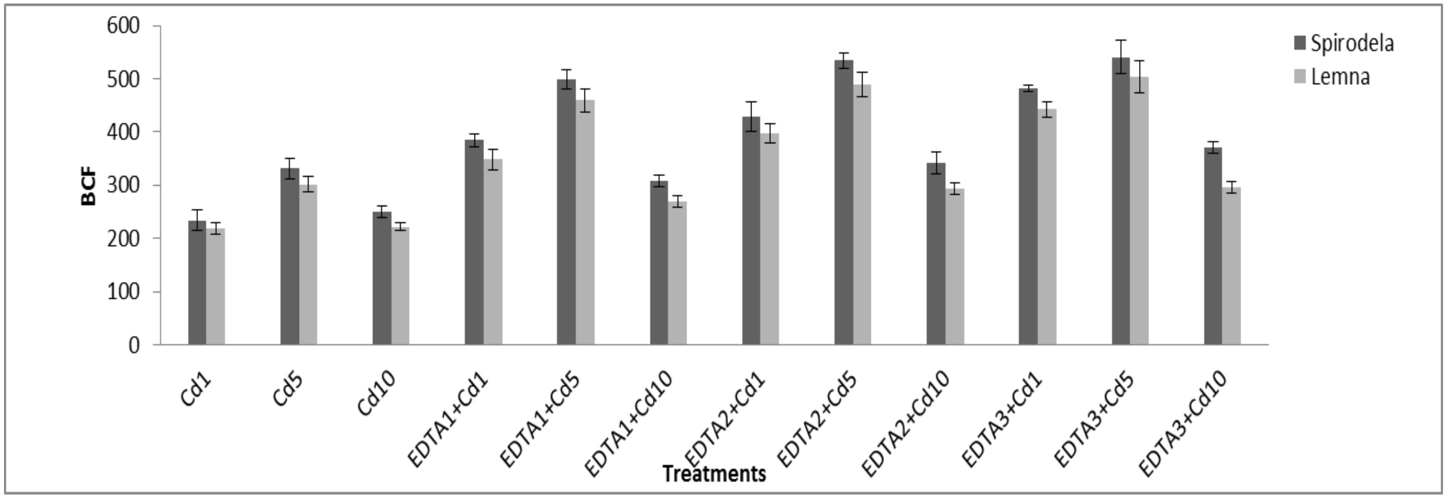

Fig. 3: Bioconcentration factor (BCF) of cadmium in *S. polyrhiza* and *L. minor* (Mean±SE)

Table No. 2: Water quality parameters (Range) in the different treatment with *L. minor* and *S. polyrhiza*

Parameters	Cd (mg L^{-1})							
	L. minor				***S. polyrhiza***			
	control	**EDTA1**	**EDTA2**	**EDTA3**	**control**	**EDTA1**	**EDTA2**	**EDTA3**
DO	5.53- 6.07	5.08-5.40	4.76-5.02	4.37-4.77	5.23-5.60	5.22-5.86	4.87-5.24	4.75-5.06
pH	7.9-8.2	7.4-7.8	7.2-7.4	7.1-7.3	7.4-8.1	7.6-7.8	7.1-7.4	7.0-7.3
AP	0.33-0.41	0.21-0.30	0.20-0.29	0.16-0.30	0.21-0.36	0.17-0.38	0.18-0.35	0.15-0.30
NO_2-N	0.25-0.28	0.26-0.33	0.25-0.26	0.20-0.26	0.37-0.46	0.47-0.48	0.39-0.40	0.48-0.51
NO_3-N	0.08-0.16	0.17-0.23	0.40-0.45	0.42-0.53	0.15-033	0.13-0.26	0.30-0.33	0.25-0.26
NH_3-N	0.06-0.11	0.08-0.12	0.09-0.16	0.08-0.19	0.02-0.09	0.09-0.11	0.08-0.16	0.08-0.24
Chlorophyll-a	0.42-0.54	0.27-0.28	0.17-0.25	0.14-0.21	0.44-0.54	0.34-0.36	0.22-0.26	0.19-0.30
Chlorophyll-b	0.04-0.06	0.05-0.06	0.05-0.07	0.03-0.05	0.07-0.08	0.09-0.11	0.09-0.10	0.09-0.11

DO: Dissolved Oxygen (mg L^{-1}); pH (no unit); AP: Available phosphorus (mg L^{-1}); NO_2-N: Nitrite-N (mg L^{-1}); NO_3-N: Nitrate- N (mg L^{-1}); NH_3-N: Ammonia-N (mg L^{-1}); Chlorophyll-a,b (mg g^{-1})

IV. CONCLUSION

Based on the result obtained during the study, it can be concluded that,

- The free floating plants such as *L. minor* and *S. polyrhiza* are very effective in accumulation of cadmium from contaminated water.
- The cadmium accumulation of *S. polyrhiza* is higher compared to *L. minor.*
- The efficiency of plant can be increased by adding EDTA as chelating agent.
- Maximum accumulation was observed in the presence of EDTA at 3 mgL^{-1}.

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