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Cadmium adsorption and accumulation by *Ceratophyllum demersum* L.: A fresh water macrophyte

G.P. Kumar, M.N.V. Prasad*

Department of Plant Sciences, University of Hyderabad, Hyderabad, 500046, India

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ABSTRACT

Ceratophyllum demersum (aquatic macrophyte, popularly known as coontail) plants were treated with cadmium viz., 2.5, 5.0, 7.5 and 10.0μM for a duration of 2, 4, 6 and 8 days to examine the amount of Cd adsorbed and accumulated from the hydroponic system. In all treatments, Cd adsorbed to the plant decreased with increasing treatment duration. Whereas, at a chosen treatment of duration, Cd adsorbed to the plant increased with increasing treatment concentration. On the other hand, Cd accumulation increased with increasing treatment concentration as well as duration. Cd adsorption as well as accumulation was found to be significantly correlated with the treatment duration at all tested concentrations. However, adsorption showed negative correlation with the treatment duration. Significant differences were noticed between the concentrations and treatment periods for adsorption as well as accumulation of the Cd. After 2 days treatment at 2.5μM concentration, Cd present in the hydroponic medium was found to be negligible. In plants treated for second time with the same Cd concentration after washing the plants with 10μM EDTA solution for 5 minutes, Cd adsorption was not observed. EDTA pretreatment prevented the adsorption, and increased Cd accumulation. Based on the present study it can be concluded that *C. demersum* is suitable plant to remediate Cd polluted effluents. EDTA pretreatment would enhance the accumulation of the Cd.

Keywords: Adsorption; Accumulation; Cadmium; *Ceratophyllum demersum*

1. INTRODUCTION

Cadmium is widely used in electroplating, NiCd batteries, stabilizers and cadmium alloys. Contamination of aquatic ecosystem by cadmium containing effluents is a serious environmental concern. Aquatic macrophytes play an important role in heavy metal removal (Abbasi and Ramasami, 1999; Cymerman and Kempers, 2002; Gupta *et al.*, 1995; Kadlec *et al.*, 2000; Lee *et al.*, 1998; Prasad *et al.*, 2001; Rai *et al.*, 1995; Tripathi *et al.*, 1996).

C. demersum, a submerged, free-floating rootless aquatic macrophyte, which grows in stagnated water is cosmopolitan in distribution. Exposure of aquatic macrophytes to short-term and long-term involves metal sorption and internal accumulation (Khummongkol *et al.*, 1982). *C. demersum* was reported to be a scavenger of Cd (Ornes and Sajwan, 1993). However, metal adsorption studies, and levels of Cd contamination where the plant is able to remove Cd completely, and reuse of the Cd exposed plants for Cd removal has not been reported. Therefore, the objective of the present study is to i) investigate adsorption and accumulation of Cd at environmentally realistic levels over a period of 8 days at an interval of 2 days ii) identify the treatment concentration at which plants completely sequesters Cd, and iii) study the feasibility of reusage of treated plants.

2. EXPERIMENTAL

2.1. Materials

C. demersum plants collected from the local ponds were maintained in glass aquaria in the laboratory. Lake water supplemented with $1/10^{th}$ Hoagland's nutrient solution (Bonner and Galston, 1952) was used in aquaria and changed periodically once in a month. Aquaria were fixed with aerators to enrich the oxygen.

* Corresponding author. E-mail: mnvsl@uohyd.ernet.in

They were maintained in normal diffused daylight.

2.2. Procedure

Healthy plant segments (2.5g) were washed with deionized water and transferred to 250ml acid washed glass tumblers containing the treatment solution (2.5, 5.0, 7.5 and 10.0 μM of Cd), which was prepared in 1/10th Hoagland nutrient solution. Atomic Absorption Spectrophotometric standard solution for cadmium obtained from local market (1000 mg l^{-1}) was used as a source of cadmium. Treatment solution pH was adjusted to 6.5. Hoagland's nutrient solution (1/10th strength) without Cd served as control. Treated hydroponic cultures were maintained in normal diffused light and an ambient temperature of 27± 2°C.

At the end of the treatment period (2, 4, 6 and 8 days), plants were carefully taken out from the medium, washed with double distilled water and then soaked in 10μM EDTA solution for 5 minutes then washed with water. This step was repeated for two times and the washouts were collected and analyzed for Cd adsorbed to the surface of the plant. The plants were allowed for further treatment or subjected to acid digestion to determine the Cd accumulation.

Plants washed with EDTA were allowed to dry in hot-air oven at 80°C for three days. The plants were weighed for its dry weight, crushed into fine powder and then acid digested with Perchloric acid : Sulphuric acid in 1: 3 ratio until the plant become white flakes. The residue was dissolved in 5ml of H_2O_2 and allowed to dissolve, and then it was made up to desired quantity by using double distilled water. This solution was used for AAS analysis to determine the Cd concentration accumulated in the plant. At the end of the treatment, the residual Cd concentration in the medium was determined. Cd was determined by Atomic Absorption Spectrophotometer (GBC 932 plus) equipped with Graphite furnace system (GF 3000).

2.3. Evaluation of maximum adsorption and uptake amount of Cd

In order to determine the maximum Cd adsorption to the plant surface, the equilibrium isotherm of Cd adsorption was calculated using the Langmuir sorption model (Langmuir, 1918). The Cd adsorption (q) for the construction of sorption isotherms was determined as follows:

$$q= q_{max}bC_f/(1+bC_f) \quad (1)$$

where, q is the Cd adsorption (μM Cd/g dry mass), q_{max} is the maximum adsorption (μM Cd/g dry mass), C_f is the final concentration of Cd in the solution (μM/l), b (1/μM) is the Langmuir constant. For the fitting of experimental data, the Langmuir model was linearlized as follows:

$$C_f/q = (1/q_{max}b) + (C_f/q_{max}) \quad (2)$$

2.4. Statistical analysis

Data was collected from five replications of each of the sample. The data on Cd budget (adsorption and / accumulation) were subjected to correlation and two-way analysis of variance (Snedecor and Cochran, 1968).

3. RESULTS AND DISCUSSION

In all treatments, Cd adsorbed to the plant decreased with increasing treatment duration. However, at a chosen treatment of duration, Cd adsorbed to the plant increased with increasing treatment concentration. Cd accumulation increased with increasing treatment concentration as well as treatment duration. Cd adsorbed to the plants treated with 2.5 and 5.0μM for a duration of 2 days was more when compared to accumulation, whereas at 7.5 and 10.0μM adsorption as well as accumulation of Cd were more or less equal or adsorption was more than the accumulation (Figure 1 A-D), which confirmed the transport of metal onto the surface is faster (Holl and Sonthiemer, 1977) than the accumulation since adsorption is based on ionic interactions (Crist *et al.*, 1994). In addition, it also indicated that incase of plants treated for 2 days, Cd uptake involves the adsorption, whereas Cd treatment for 8 days involves the accumulation (Khummonglo *et al.*, 1982). *C. demersum* completely scavenged Cd when grown in hydroponic medium containing 2.5μM Cd for 2 days (Figure 1A).

Adsorption and accumulation of Cd significantly correlated with the tested concentrations at all treatment durations and vice versa. However, adsorption showed negative correlation with the treatment duration (Table 1) at all treatment concentrations. Data on Cd adsorbed and accumulated at different treatment concentrations and durations were analyzed, the ANOVA revealed significant differences between treatment concentrations as well as periods for adsorption and accumulation of Cd (Table 2).

The primary site of metal accumulation is root in aquatic plants (Naqvi and Rizvi, 2000; Srivastav *et al.*, 1994; Wolverton and McDonald, 1975) as well as in terrestrial plants (Bagatto and Shorthouse, 1991; Minnich *et al.*, 1983; Otte, 1991; Pip, 1990). In the present study, *C. demersum*, a rootless aquatic macrophyte, accumulated Cd in its leaves and stem segments. Hence, the entire plant has been analyzed for the metal accumulation.

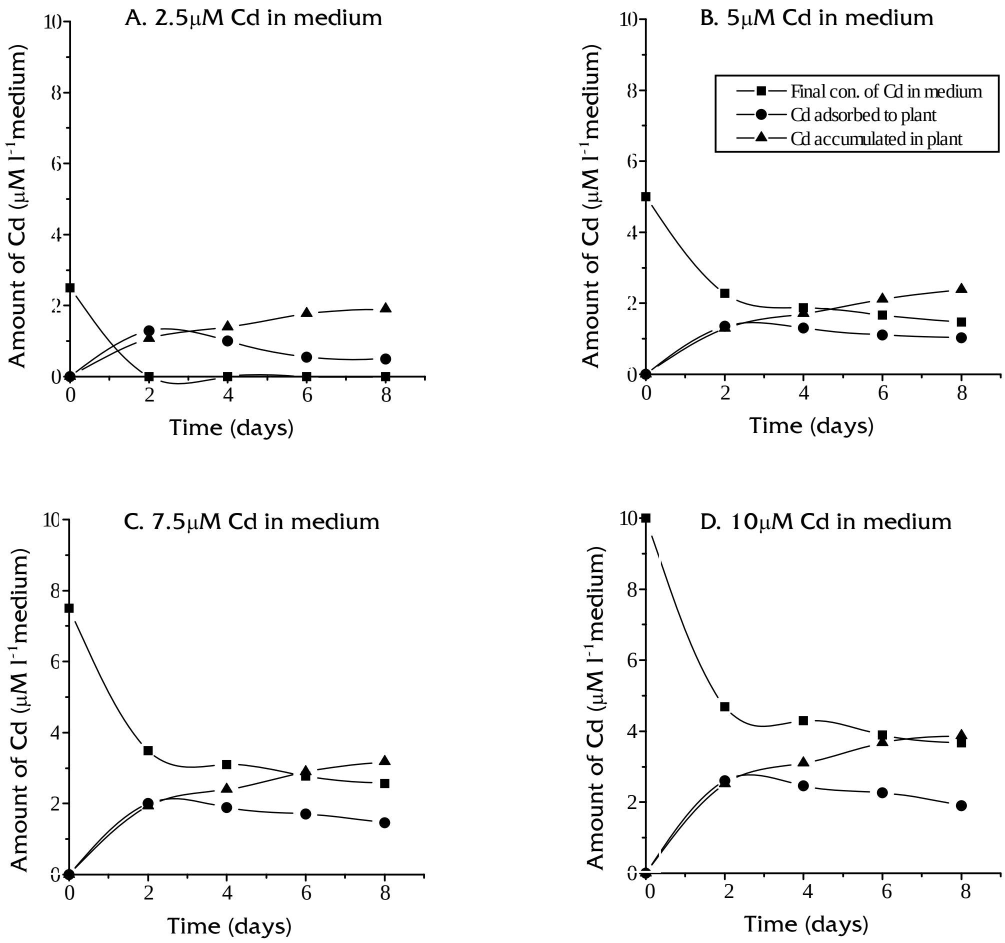

Figure 1. Metal budget (adsorption and accumulation) in *C. demersum* at different treatment concentrations of Cd

Table 1
Correlation of adsorption and accumulation of Cd with treatment period/concentration in *C. demersum*

Treatment Period/ Concentration	Adsorption of Cd		Accumulation of Cd	
	r-value	t-value	r-value	t-value
2 days	0.9600	4.8508*	0.9826	7.4951*
4 days	0.9908	10.4088*	0.9866	8.5801*
6 days	0.9999	110.2320*	0.9867	8.6169*
8 days	0.9987	27.8823*	0.9958	15.5359*
2.5μM	-0.9649	-5.2029*	0.9826	7.4945*
5.0μM	-0.9774	-6.5447*	0.9956	15.1398*
7.5μM	-0.9867	-8.6011*	0.9929	11.8782*
10.0μM	-0.9742	-6.1127*	0.9792	6.8363*

* significant at p=0.05

Table 2
ANOVA for the effect of Cd concentrations and treatment periods on Cd adsorption, accumulation, fresh and dry weight of the plant

	Source of Variation	DF	SS	MS	F-value
Cd	Bet. treatment periods	3	0.8326	0.2775	21.0404*
adsorption	Bet. treatment concs.	3	5.0288	1.6763	127.0793*
	Error	9	0.1187	0.0132	
Cd	Bet. treatment periods	3	3.0324	1.0108	90.7645*
accumulation	Bet. treatment concs.	3	7.3278	2.4425	219.3352*
	Error	9	0.1002	0.0111	
Fresh weight	Bet. treatment periods	3	2.1174	0.7058	1.3646
of the plant	Bet. treatment concs.	4	12.5179	3.1294	6.0506*
	Error	12	6.2065	0.5172	
Dry weight	Bet. treatment periods	3	0.0015	0.0005	1.0804
of the plant	Bet. treatment concs.	4	0.0059	0.0014	3.0865
	Error	12	0.0057	0.0004	

* F-value significant at p=0.05

Fresh and dry weights of the treated plants at different treatment concentrations and periods were presented in Table 3. In general, fresh weight of the plant decreased with increasing treatment concentration as well as treatment duration, however, at the end of 2 days treatment, plants showed increased growth (fresh weight) over their corresponding control at all treatment concentrations except 10 μM. Plant growth gradually increased up to 7.5μM (4.85% over the control) and declined there after (at 10 μM) (Table 3). When the fresh and dry weights of the plants treated with different concentrations for different durations were analyzed by two-way ANOVA; significant difference was found only between treatment concentrations for fresh weight of the plant. On the other hand, no significant difference was observed either between treatment concentrations or periods for dry weight of the plant (Table 2).

Analysis of variance revealed that the amounts of Cd adsorbed and accumulated were regulated by the treatment concentrations as well as duration. Likewise, it also revealed the lack of significant effect of treatment duration either for fresh weight or dry weight of the plant. On the other hand, treatment concentrations were found to have significant effect on fresh weight of the plant, which was not reflected in dry weights.

Table 3
Changes in fresh weight (gm) and dry weight (mg) of *C. demersum* plants treated with different concentrations of Cd during different treatment periods

Treatment Period	Treatment Concentration				
	Control	2.5μM	5.0μM	7.5μM	10.0μM
Initial	10.0	10.0	10.0	10.0	10.0
2 Days	10.72 (820)	10.80 (820)	11.12 (820)	11.24 (832)	10.08 (808)
4 Days	11.76 (832)	10.60 (820)	10.60 (820)	10.04 (808)	9.80 (808)
6 Days	12.04 (840)	10.32 (820)	9.76 (808)	9.68 (808)	9.12 (800)
8 Days	12.80 (860)	9.72 (808)	9.32 (800)	9.12 (800)	8.80 (720)

values in parentheses indicate dry weight of the plant in mg.

Plants at 2.5μM treatment concentration completely removed the Cd from the medium. Hence, plants were treated for second time with the same Cd concentration after washing the plants with 10μM EDTA solution for 5 minutes. Interestingly, in the second treatment, Cd adsorption to the plant was not observed. More over, plants could not remove the Cd completely from the medium. Therefore, after the first two days treatment, plants were allowed to grow in Cd free nutrient solution for six days and then subjected to treatment (2.5μM Cd), where the plant able to remove Cd completely from the medium. In those plants Cd accumulation was found to be 2.5175μM/g dry wt, which is 92.38 per cent more than its corresponding sample where plants were subjected to Cd stress single time. This indicated that pause in the treatment would allow reusage of plants again to remediate the Cd contaminated solutions. In the other set of experiments, prior to the Cd treatment, fresh plants were subjected to EDTA pre-treatment and then allowed to 2.5μM Cd concentration. Cd budget was done after 2 days. No adsorption was observed and significant increase (p=0.05) in accumulation was noticed than the plants untreated with EDTA (1.9743μM/g dry wt).

The adsorption profiles of Cd on *C. demersum* under these different concentrations were studied in order to determine the maximum adsorption of Cd on the plant surface. The adsorption was found to be saturated in 2 days and intracellular accumulation in plant was observed to increase significantly with the treatment duration (Figure 1A-D). The data obtained for adsorption (extra cellular) and accumulation (intra cellular) were analyzed using the Langmuir sorption model. The equilibrium data was well fitted by Langmuir isotherms and was represented in Figure 2. The linear regression line of adsorption rate using equation (2) was also presented in set of the same figure (Figure 2). The maximum Cd adsorption (q_{max}) and the Langmuir constant (b) were estimated from this linear regression line to be 2.01E-2mg Cd/g and 1.5003, respectively.

Maximum Cd adsorption was found during 2 days treatment period at 10μM (28.9935μgm/g fresh wt) and maximum Cd accumulation was observed at the end of 8 days (603.29μgm/g dry wt) (Table 4).

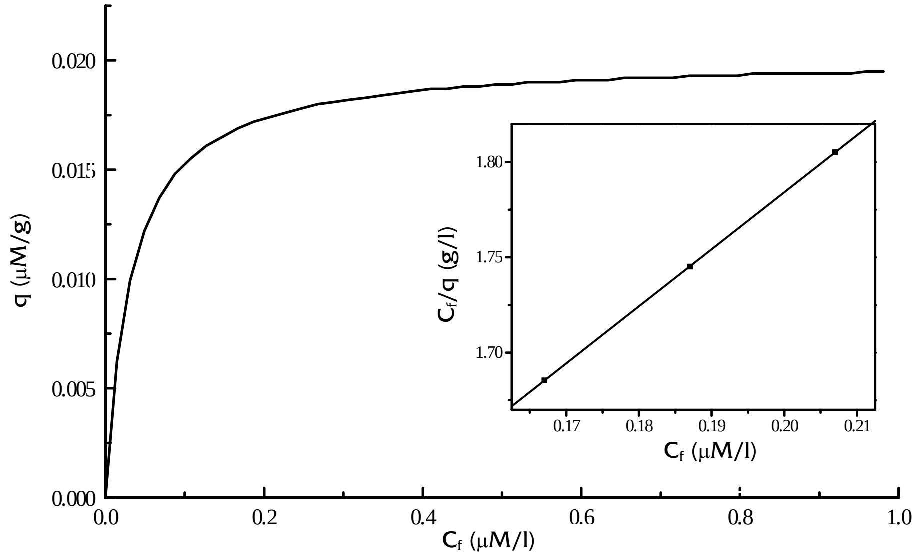

Figure 2. Adsorption of Cd by *C. demersum* plants at various Cd concentrations. The inset shows the linear regression line of the adsorption rate using Langmuir model

Table 4.
Biosorption and bioaccumulation of Cd in *C. demersum* as a function of treatment concentrations and durations of exposure to Cd

Treatment Period	Initial Cd. Con. (mg/l)	Cd adsorbed μg/g	Cd adsorbed μmol/g	Cd accumulated μg/g	Cd accumulated μmol/g
2 Days	0.0 (Control)	0.0	0.0	0.0	0.0
	0.2810	13.3809	0.1190	147.1063	1.3086
	0.5620	13.7025	0.1218	177.5941	1.5798
	0.8430	19.9987	0.1779	259.7320	2.3105
	1.1241	28.9935	0.2579	349.5283	3.1094
4 Days	0.0 (Control)	0.0	0.0	0.0	0.0
	0.2810	10.5750	0.0941	190.8365	1.6976
	0.5620	13.7808	0.1225	233.3878	2.0762
	0.8430	21.0847	0.1875	333.7381	2.9689
	1.1241	28.1919	0.2507	431.9994	3.8430
6 Days	0.0 (Control)	0.0	0.0	0.0	0.0
	0.2810	5.9298	0.0527	242.9290	2.1610
	0.5620	12.7382	0.1133	294.1859	2.6170
	0.8430	19.8052	0.1761	403.0065	3.5851
	1.1241	27.9669	0.2487	515.7230	4.5878
8 Days	0.0 (Control)	0.0	0.0	0.0	0.0
	0.2810	5.6794	0.0505	263.8574	2.3472
	0.5620	12.3614	0.1099	334.8694	2.9790
	0.8430	17.9473	0.1596	446.0288	3.9678
	1.1241	24.2345	0.2155	603.2982	5.3669

4. CONCLUSIONS

C. demersum, an aquatic macrophyte, is thus proved to be an effective scavenger of Cd by adsorbing as well as accumulating Cd from hydroponic cultures. Aqueous solutions having Cd up to 2.5μM can be effectively remediated by using this plant in 2 days time. Pause in the treatment would allow reusage of plants again for Cd remediation. EDTA pre-treatment would enhance accumulation of the Cd. These results indicate, that this plant can be used in phytoremediation technology to treat the Cd containing effluents.

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