CADMIUM ACCUMULATION AND BIOAVAILABILITY IN COONTAIL (*CERATOPHYLLUM DEMERSUM* L.) PLANTS

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Abstract. The aquatic vascular plant (*Ceratophyllum demersum* L.) was investigated as a potential biological filter for removal of Cd from wastewaters. Plants were grown in and harvested weekly from 0.10 *M* Hoagland nutrient solutions containing concentrations of Cd from 0.01 to 1.03 μg Cd mL^{-1}. Tissue Cd was positively correlated to increased concentrations of Cd in solution. Concentration factors (CFs) of Cd in plants after one week were 13.3 for the 0.01 μg Cd mL^{-1} treatment; 451.4 for plants treated with 0.04 μg Cd mL^{-1}, and 506.5 for plants treated with 1.03 μg Cd mL^{-1}. Plants treated with 0.01 μg Cd mL^{-1} sustained tissue Cd concentrations almost 9-fold over those at week 1. However, after 5 weeks tissue Cd concentration in plants exposed to 1.03 μg Cd mL^{-1} had decreased 97% compared to the week 1 concentration. Growth measurements of dry weight, stem lengths, and lateral shoot growth were nagatively correlated to increased Cd treatments. Our results suggest that Coontail exposed to very low Cd concentrations (0.01 μg Cd mL^{-1}) can take up and accumulate Cd. However, plants exposed to Cd at 0.04 μg Cd mL^{-1} or above did not accumulate Cd past one week.

1. Introduction

The release of various heavy metals into aquatic and wetland environments is a worldwide problem of increasing magnitude. Primary sources of these metals are stack emissions and wastewater from zinc smelting, incineration of plated metals and coal fired power plants. Additional sources of these metals are sewage sludge and municipal wastewater that are contaminated through industrial soures. One of the heavy metals that is found in wastewaters and in the aquatic environment is Cd and it has been documented as a potentially toxic metal in the food chain (Lagerwerff, 1972; Page and Bingham, 1973; Logan and Chaney, 1983; Adriano, 1986; Chaney, 1990). The primary producers at the base of many aquatic food chains are vascular plants. A chracteristic of the vascular plants found in aquatic and wetland environments is that they accumulate metals and other elements in excess of their physiological need. Several aquatic plants have been suggested for use in wastewater

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renovation systems (Dymond, 1948; Ornes and Sutton, 1975; Sutton and Ornes, 1977; Wolverton and McDonald, 1978; Sloey *et al.*, 1978; Cooly and Martin 1979; Ornes and Wildman, 1979; Low and Lee, 1981; Jana and Chaudhury, 1984; Hardy and O'Keeffee, 1985). Coontail (*Ceratophyllum demersum* L.) has been suggested (Ornes and Wildman, 1979) and is one of the very common aquatic vascular plants worldwide. The plant's finely dissected or feathery leaves may enhance its potential as a living filter for treated effluents. However, information concerning the degree to which Coontail can accumulate Cd from wastewaters and its role in biomagnification of Cd in aquatic food webs is lacking. An assessment of the accumulation of Cd as a pollutant in aquatic ecosystems is also important for establishing legal limits of this metal in natural waters. The objectives of this study were (1) to investigate the potential for Cd accumilation by Coontail and, (2) to assess the possibility of using Coontail plants to accumulate Cd from waters with Cd and nutrient concentrations similar to those commonly found in wastewaters and treated effluents.

2. Materials and Methods

Coontail plants were grown for 7 d in modified 0.10M Hoagland solution as a pretreatment (Hoagland and Arnon, 1950). Selected plants were transferred into 2800-mL Fernbach flasks containing fresh nutrient solution, prepared in deionized distilled water, with addition of Fe and C. The Fe source was Fe-330 Sequestrene® (Ciba-Geigy Co., Ardsley, New York). Carbon was added as $NaHCO_3$. The 0.10M Hoagland solution contained nutrients in concentrations (mg L^{-1}) of approximately: 21.0 N, 3.1 P, 23.4 K, 20.0 Ca, 4.9 Mg, 6.4 S, 2.5 10^{-2} B, 2.8 10^{-2} Mn, 1.0 10^{-3} Cu, 5.0 10^{-4} Mo, and 2.4 10^{-3} Zn mg L^{-1}. The Fe concentration was 2.0 and C was 67.3 mg L^{-1}. The 0.10*M* Hoagland solution was selected as having nutrients similar to those in a secondary treated sewage effluent (Ornes and Sutton, 1975; Sutton and Ornes, 1977; Gakstatter *et al.*, 1978).

At the end of the pretreatment period, plants were rinsed with deionized distilled water, weighed, and measured and cut to 10 cm lengths. Selected and prepared plants were then added to clean flasks (to which deionized distilled water, nutrients, and Cd treatments had been added) and allowed to grow for 1 week under growth chamber conditions of 16.8 hr light @ 400 ± 40 f.c. and temperature of 24 ± 2°C. The Cd was applied as $CdSO_4$. The Cd treatments were 0, 0.01, 0.04, and 1.03 μg Cd mL^{-1}, and were replenished at weekly intervals based on preliminary studies monitoring solution concentrations. These levels were selected based on background (0.01 μg Cd mL^{-1}) and contaminated agricultural and urban wastewater that range from 0.01 to 1.03 μg Cd mL^{-1} (U.S.EPA, 1978). All the flasks were arranged in a randomized complete block design with three replications. At the end of each week, plants were rinsed three times in deionized distilled water. Half of the plant material from each flask was harvested, measured, and kept for dry weight measurements and tissue analysis. Half was placed in fresh treatment solutions to avoid overcrowding and insure potential for vigorous growth. Harvested plant

TABLE I

Dry weights (g m^{-2})[a] of Coontail plants

Concentration of Cd in solution (μg Cd mL^{-1})	Growth period (weeks)					
	0	1	2	3	4	5
0	9.42 A	12.83 A	15.40 B	15.66 B	15.39 B	11.34 B
±0	±2.11	±0.62	±0.84	±1.23	±1.09	±1.29
	a	b	c	c	c	ab
0.01	11.12 A	15.85 B	18.30 C	19.31 C	18.42 B	11.91 B
±0.00	±2.07	±1.07	±1.62	±2.14	±3.39	±1.41
	a	b	c	c	c	a
0.04	12.34 A	16.74 B	17.85 C	17.45 C	15.59 B	14.30 B
±0.01	±2.87	±0.20	±1.31	±0.91	±1.57	±2.81
	c	bc	d	cd	b	ab
1.03	11.52 A	13.15 A	10.41 A	8.06 A	6.53 A	5.06 A
±0.04	±2.18	±1.43	±1.04	±0.71	±0.52	±0.12
	d	e	d	c	b	a
r		−0.4852[c]	−0.9286[c]	−0.9503[b]	−0.9648[b]	−0.9342[c]

[a] Each value is the mean of three replications with standard deviation. Means followed by the same upper case letter within a column or the same lower-case letter within a row are not statistically different at the 0.05 level as determined by Duncan's Multiple Range Test.

[b] $p < 0.05$.

[c] Not significant at the 0.05 level.

material was wrapped in paper towels, placed in brown paper bags, and dried in an oven at 70 ± 2 °C to a constant weight. Dry weights were doubled to estimate total biomass per flask and converted to a m^2 basis with the surface area of the flask being 0.0283 m^2. Dried and ground plant material was wet-digested with concentrated nitric acid followed by perchloric acid (Johnson and Ulrich, 1959). Digests were diluted to volume and analyzed for Cd by flame atomic absorption spectrophotometry with Cd detection limit of $\leq$0.005 μg Cd g^{-1} d.w. Additional aliquots were analyzed for P by a phosphomolybdate method (A.O.A.C., 1975). The concentration factor (CF) of Cd in plants was calculated by dividing the concentration of Cd in the plants by concentration of Cd in the solution. NBS standard tomato leaf tissue (NBS No. 1573) and citrus leaves (NBS No. 1572) were carried through all analyses. One way analysis of variance was applied to compare treatment within weeks and also to compare the effect of time (weeks) within each treatment. The statistics of the experimental design was conducted by ANOVA and Duncan's New Multiple Range Test on the SAS computer program (SAS Institute, Inc., Cary, NC). Regression analysis of parameters among Cd treatments were calculated by substituting tissue parameter (y) and Cd concentration in solution (x) into the prediction equation for a linear, exponential, logarithmic, or power curve fit. These equations allow the investigator to detect whether or not a tissue

TABLE II

Concentrations of Cd (μg Cd g^{-1} d.w.)[a] in Coontail plants

Concentration of Cd in solution (μg Cd mL^{-1})	Growth period (weeks)					
	0	1	2	3	4	5
0	0[c] A	0 A	0 A	0 A	0 A	0 A
±0	±0	±0	±0	±0	±0	±0
	a	a	a	a	a	a
0.01	0 A	0.13 A	0.68 A	1.28 A	1.21 A	1.23 A
±0.00	±0	±0.13	±0.14	±0.29	±0.08	±0.56
	a	a	b	c	c	c
0.04	0 A	18.06 A	6.89 A	0.99 A	1.22 A	1.40 A
±0.01	±0	±2.31	±1.31	±0.12	±0.16	±0.44
	a	c	b	a	a	a
1.03	0 A	521.72 B	227.73 B	13.60 B	14.41 B	14.55 B
±0.04	±0	±169.37	±52.64	±1.64	±0.62	±2.11
	a	c	b	a	a	a
r		−0.9999[b]	−0.9999[b]	−0.9973[b]	−0.9978[b]	−0.9976[b]

[a] Each value is the mean of three replications with standard deviation. Means followed by the same upper case letter within a column or the same lower-case letter within a row are not statistically different at the 0.05 level as determined by Duncan's Multiple Range Test.

[b] $p < 0.01$.

[c] Tissue concentrations of Cd were below the detection limits of 0.005 μg Cd g^{-1} d.w.

parameter is directly related to Cd concentration in solution.

3. Results and Discussion

Tissue dry weights of control plants over time showed increases of 36 and 20% at weeks 1 and 2, respectively (Table I). From week 2 through week 4 there were no significant changes. From week 4 to week 5, a 26% decrease occurred. This decrease was probably due to crowding within the flasks evidenced by browning of stem tips. Plants exposed to 0.01 μg Cd mL^{-1} and 0.04 μg Cd mL^{-1} showed a similar trend over time, but with dry weights greater than controls. This suggests a stimulatory effect of Cd at these levels. However, dry weights of plants exposed to 1.03 μg Cd mL^{-1} increased from week 0 to week 1 and thereafter decreased 62% through week 5. This suggests that the health of plants exposed to the highest treatment began to decline after only 1 week. In general, dry weights of plants over time within treatments showed increases through week 4 except in plants exposed to the highest Cd treatments that decreased after week 1. Mayes (1975) observed similar increases in dry weights of Coontail except when tissue Cd levels approached 20 μg Cd g^{-1} d.w.

Dry weights across treatments within weeks indicated plants exposed to 0.01

TABLE III

Cadmium Concentration Factors (CFs[a]) of Coontail plants

Concentration of Cd in solution ($\mu g\ Cd\ mL^{-1}$)	Growth period (weeks)				
	1	2	3	4	5
0.01 ±0.00	13.03 A ±12.6 a	68.0 A ±14.0 b	127.7 B ±28.8 c	121.3 B ±7.6 c	122.8 B ±55.9 c
0.04 ±0.01	451.4 B ±57.7 c	172.3 B ±32.8 b	24.7 A ±3.1 a	30.6 A ±4.1 a	35.1 A ±10.9 a
1.03 ±0.04	506.5 B ±164.4 c	221.1 B ±51.1 b	13.2 A ±1.6 a	14.0 A ±0.6 a	14.1 A ±2.0 a
r	0.06065[b]	0.7621[b]	-0.5976[b]	-0.6395[b]	-0.6682[b]

[a] Each value is the mean of three replications with standard deviation. Means followed by the same upper case letter within a column or the same lower-case letter within a row are not statistically different at the 0.05 level as determined by Duncan's Multiple Range Test.

[b] Not significant at the 0.05 level.

or 0.04 $\mu g\ Cd\ mL^{-1}$ had significantly greater dry weights than controls, except during weeks 4 and 5. However, plants exposed to the highest level of Cd resulted in significant reduction of dry weights after week 2 through 5.

Control plants were not exposed to Cd and tissue levels were below the detection limits of $\leqslant 0.005\ \mu g\ g^{-1}$ d.w. (Table II). In plants exposed to low levels of Cd (0.01 $\mu g\ Cd\ mL^{-1}$), tissue Cd concentration increased through week 3 and did not change thereafter. However, in plants exposed to the higher levels of Cd (0.04 or 1.03 $\mu g\ Cd\ mL^{-1}$) tissue Cd concentrations were greatest at week 1 and thereafter decreased about 95% through week 5. This suggests that Coontail can tolerate tissue levels up to 18.06 $\mu g\ Cd\ g^{-1}$ d.w. Mayes (1975) reported that Coontail plants exposed to natural levels of Cd (0.01 $\mu g\ Cd\ mL^{-1}$) accumulated Cd up to 6 weeks and thereafter tissue levels declined.

Tissue Cd concentrations across treatments within weeks showed no significant differences in uptake except in plants exposed to the highest Cd level (1.03 $\mu g\ Cd\ mL^{-1}$), which showed many-fold increases in tissue Cd. Regression analyses of tissue Cd concentrations with increasing Cd treatments showed a linear model provided the best fit with positive correlation coefficients, significant at the 0.01 level during each of the 5 weekly harvests.

The amount of Cd concentration in the tissue from each treatment was calculated and presented in the form of concentration factors (CFs). Plants exposed to only 0.01 $\mu g\ Cd\ mL^{-1}$ continued to concentrate Cd through week five, contrasted to CF of plants exposed to higher Cd treatments which decreased about 95% during this period (Table III). This indicates that Coontail exposed to this level of Cd will

TABLE IV

Concentrations of P (μg P g^{-1} d.w.)[a] in Coontail plants

Concentration of Cd in solution (μg Cd mL^{-1})	Growth period (weeks)					
	0	1	2	3	4	5
0	9082 A	9706 B	17872 B	25233 B	20667 B	16471 A
±0	±235	±626	±3228	±1360	±529	±1040
	a	a	b	d	c	b
0.01	9167 A	15455 C	23131 C	32501 C	29139 C	26203 B
±0.00	±251	±727	±2267	±1821	±1294	±805
	a	b	c	f	e	d
0.04	9153 A	15775 C	18563 B	21290 A	18379 AB	16915 A
±0.01	±120	±568	±453	±559	±961	±880
	a	b	c	d	c	b
1.03	9037 A	7418 A	13157 A	18954 A	18108 A	17989 A
±0.04	±136	±438	±1078	±2238	±2015	±1125
	a	a	b	c	c	c
r		−0.7283[b]	−0.8231[b]	−0.6377[b]	−0.4603[b]	−0.2121[b]

[a] Each value is the mean of three replications with standard deviation. Means followed by the same upper case letter within a column or the same lower-case letter within a row are not statistically different at the 0.05 level as determined by Duncan's Multiple Range Test.

[b] Not significant at the 0.05 level.

tolerate and accumulate Cd over time. Plants exposed to higher Cd treatments did not accumulate past week 1 presumeably because of metabolic processes being affected by Cd. Similar patterns of Cd uptake by Coontail grown in ponds and barrow pits in Cd contaminated areas in Indiana have been documented by Mayes (1975).

The concentration factors (CFs) across treatments within weeks showed CFs in plants exposed to 0.04 or 1.03 μg Cd mL^{-1} during week 1 and 2 were significantly greater than plants exposed to 0.01 μg Cd mL^{-1}. From week 3 through week 5, however, CFs of plants exposed to the 0.01 μg Cd mL^{-1} were significantly greater than those of exposed to 0.04 or 1.03 μg Cd mL^{-1}. This indicates that plants exposed to the 0.01 μg Cd mL^{-1} treatment were not saturated and/or inhibited as were plants exposed to higher treatments. Regression analyses showed linear correlations provided the best fit between CFs and concentrations of Cd in solution but were slightly below the 0.05 level of significance.

Plants were analyzed for P as an indicator of plant vigor (Table IV), because P is a major nutrient required for numerous metabolic compounds and pathways. Tissue P in control plants increased from week zero through week 3, and thereafter slightly decreased (similar to dry weights). This decrease in tissue P suggests a natural loss in plant vigor associated with crowding within the flasks. Plants exposed to 0.01 and 0.04 μg Cd mL^{-1} showed a similar trend to that of control plants. Tissue P in plants exposed to 1.03 μg Cd mL^{-1}, followed a similar

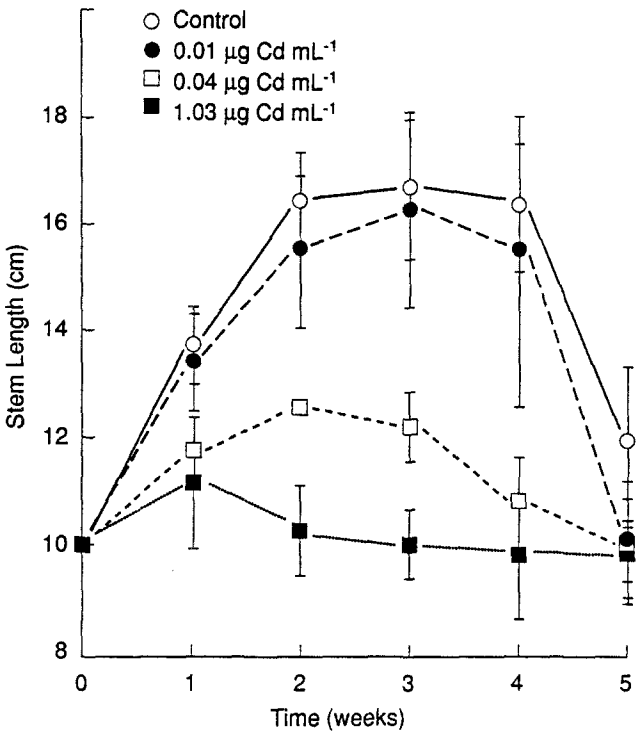

Fig. 1. Stem lengths of Coontail plants.

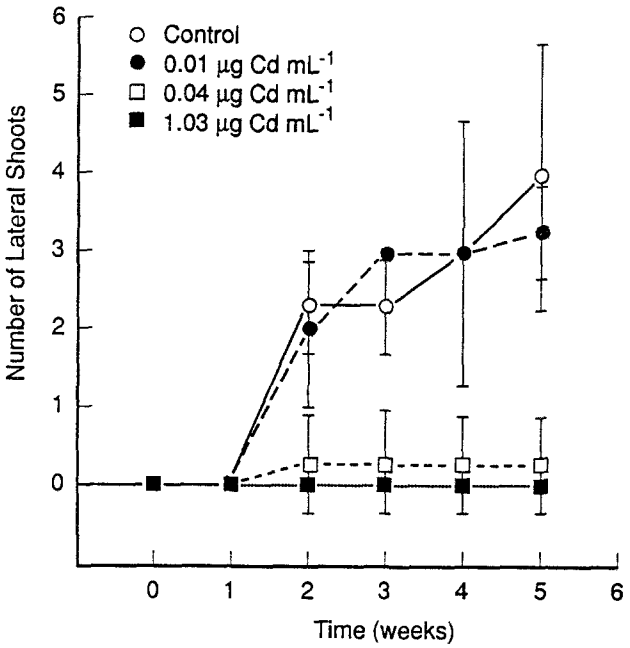

Fig. 2. Number of lateral shoots of Coontail plants.

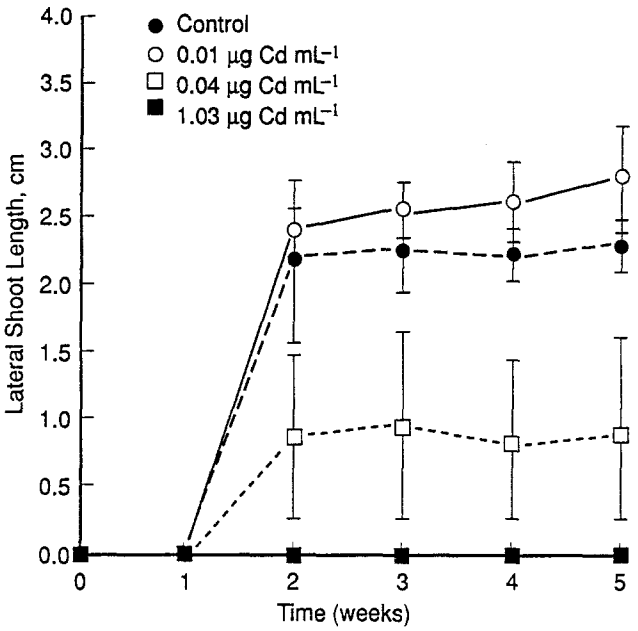

Fig. 3. Lateral shoot lengths of Coontail plants.

trend over time but with less accumulation of P.

Tissue P, in general, decreased with increased Cd treatment. An exception to this trend was plants exposed to 0.01 μg Cd mL^{-1} whose tissue P remained above controls throughout the growth period. This suggests a stimulatory effect on tissue P by Cd at low levels and a supressing effect on tissue P by Cd at high levels. This latter effect has been reported by Sajwan *et al.*, 1990.

Stem lengths of control plants increased through week 2 and did not change from week 2 through week 4 (Figure 1). Stem lengths of plants exposed to 0.01 μg Cd mL^{-1} were similar to those of controls. Plants exposed to 0.04, also showed a significant increase over time. Plants exposed to 1.03 μg Cd mL^{-1} showed no significant change in length over time. This suggests that stem lengths could be a more sensitive parameter than dry weights.

The number of lateral shoots on control plants increased from week 1 to week 2 and thereafter did not change significantly. The number of shoots on plants exposed to 0.01 μg Cd mL^{-1} did not change significantly after week 3 (Figure 2). Plants exposed to 0.04 μg Cd mL^{-1} produced very few lateral shoots. Plants exposed to 1.03 μg Cd mL^{-1} produced no lateral shoots. Thus, lateral shoot production also was a relatively sensitive bioassay for Cd.

The lateral shoot lengths of control plants and plants exposed to 0.01 μg Cd mL^{-1} increased through week 2 and remained at those lengths through week 5 (Figure 3). Plants exposed to 0.04 μg Cd mL^{-1} did not increase lateral shoot lengths over time. There were no lateral shoots produced in plants exposed to 1.03 μg

Cd mL^{-1}. Regression analyses of lateral shoot lengths with increasing Cd treatments showed a negative correlation slightly below the 0.05 level of confidence. The growth parameters in Figures 1, 2, and 3 further support our findings that Coontail is sensitive to Cd when exposed to solution concentrations above 0.04 μg Cd mL^{-1} and when tissue concentrations accumulate above 18 μg Cd g^{-1} d.w.

This study revealed that the aquatic vascular plant Coontail (*Ceratophyllum demersum* L.) accumulated Cd from 0.10*M* Hoagland solution containing concentrations of Cd from 0.01 to 1.03 μg Cd mL^{-1}. Tissue Cd was positively correlated to increasing concentrations of Cd in solution. This indicates a potential for Coontail plants to play an important role in biomagnification of Cd in aquatic food webs and Cd cycling in water. Future studies are needed to identify the Cd concentrations in natural waters below which significant accumulation in aquatic vascular plants would not occur. The ecological importance of Cd cycling by this aquatic vascular plant is clearly demonstrated by its capacity for Cd uptake and release. The ecological importance may be better understood when one considers the role of this plant in natural aquatic habitats, not the least of which is the utilization of these habitats by wildfowl and fish (Martin *et al.*, 1961 and Fassett, 1975).

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