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CADMIUM UPTAKE BY THE WATER HYACINTH: EFFECTS OF ROOT MASS, SOLUTION VOLUME, COMPLEXERS AND OTHER METAL IONS[1]

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ABSTRACT

At a fixed Cd^{2+} concentration water hyacinth (Eichhornia crassipes) plants with greater root mass (dry weight) take up more metal ions as a function of time, and more metal ions are taken up by a plant as the solution volume is increased. Experiments in which several different metal ion complexers were present suggest that (1) the roots possess sites which initially reversibly bind free Cd^{2+}, (2) some added complexers can compete with these root sites for free Cd^{2+}, and (3) with time Cd^{2+} bound to the roots is translocated into the root tissues effectively removing it from the equilibrium processes in solution. Many metal ions are taken up by the plant but only the micronutrient Zn^{2+} competes well with Cd^{2+} for uptake. Thus, there may be binding sites on the roots for specific metal ions.

INTRODUCTION

A number of laboratories have examined the ability of the water hyacinth (Eichhornia crassipes) to remove heavy metals from water.[2-9] We recently initiated a systematic investigation of a number of solution factors which affect cadmium uptake by the plant.[10] In continuing to investigate factors of importance to the water hyacinth's ability to be an effective biological pollution control device, the effects of root mass, solution volume, competing metal ions and complexing agents have now been evaluated. The results have also allowed us to gain some insight into the metal uptake process.

EXPERIMENTAL

The tracer used for most studies to monitor cadmium uptake was $^{109}Cd^{2+}$, obtained as a carrier-free solution in 0.1 M HCl (New England Nuclear Co.).

Cadmium solutions were prepared from reagent grade $Cd(NO_3)_2 \cdot 4H_2O$ (Fisher Scientific). All other chemicals used were reagent grade where possible and deionized water was used throughout. For studies employing $^{109}Cd^{2+}$ as a tracer, a Nucleus Model 2010 single channel analyzer/scaler equipped with a 2 x 2 inch NaI (Tl) well-type scintillation detector was used. For the volume study a Perkin-Elmer Model 4000 atomic absorption spectrometer, equipped with a cadmium electrodeless discharge lamp, was used to determine cadmium. This instrument was also employed in the assay of the metals used in the competing ions study.

Water hyacinths were grown in the laboratory in half-strength Hoagland's solution as previously described.[10] For most experiments mature plants of approximately one gram dry weight and about three weeks in age were used. Plants varying in size and age were used in experiments designed to test cadmium uptake as a function of root mass. Experiments were performed in a laboratory maintained at about 22°C and at constant relative humidity.

With the exception of the volume study, variations in cadmium concentration were followed by determining the solution $^{109}Cd^{2+}$ activity at regular intervals. For each experimental condition investigated, individual plants were placed in bottles containing deionized water, the desired amount of cadmium and sufficient tracer to allow for an initial activity of at least 1500 cpm/5ml aliquot. For most studies 500 ml bottles (6 cm in diameter, 14 cm in height) containing a total of 400 ml solution were used. For the volume study glass containers of from 500 to 2500 ml total volume containing 400 to 2000 ml of solution were used. Each study was done at least in triplicate. At regular intervals a 5 ml aliquot was withdrawn, analyzed and returned to the experimental solution. For those samples assayed by atomic absorption spectroscopy, any remaining portion of the aliquot was returned. As plant transpiration is high (about 50 ml/day), the solution volume was regularly monitored during the exposure period and additional deionized water added as needed to maintain a constant volume. The effects of various factors on cadmium uptake were studied by varying a single factor in solution, then monitoring the solution cadmium level. In all studies a cadmium concentration of 1 ppm was used. Uptake at this concentration is rapid and no plant damage is observed.[10]

RESULTS

Uptake as a Function of Root Mass.

A total of forty plants were exposed to Cd^{2+} in deionized water and monitored for their ability to take up the metal. The plants were selected so as to examine a wide range of root masses. After 4 hours of exposure the plants were removed from solution, their roots removed, dried and weighed. A plot of micrograms Cd^{2+} removed versus root mass (mg dry weight) (Figure 1a) indicates that a relationship, while not linear, exists between root mass and cadmium uptake. At 24 hours (Figure 1b) a leveling effect becomes apparent as cadmium is depleted

from solution (400 mg total). Values at 48 hours (not shown) indicate that this leveling effect becomes even more pronounced.

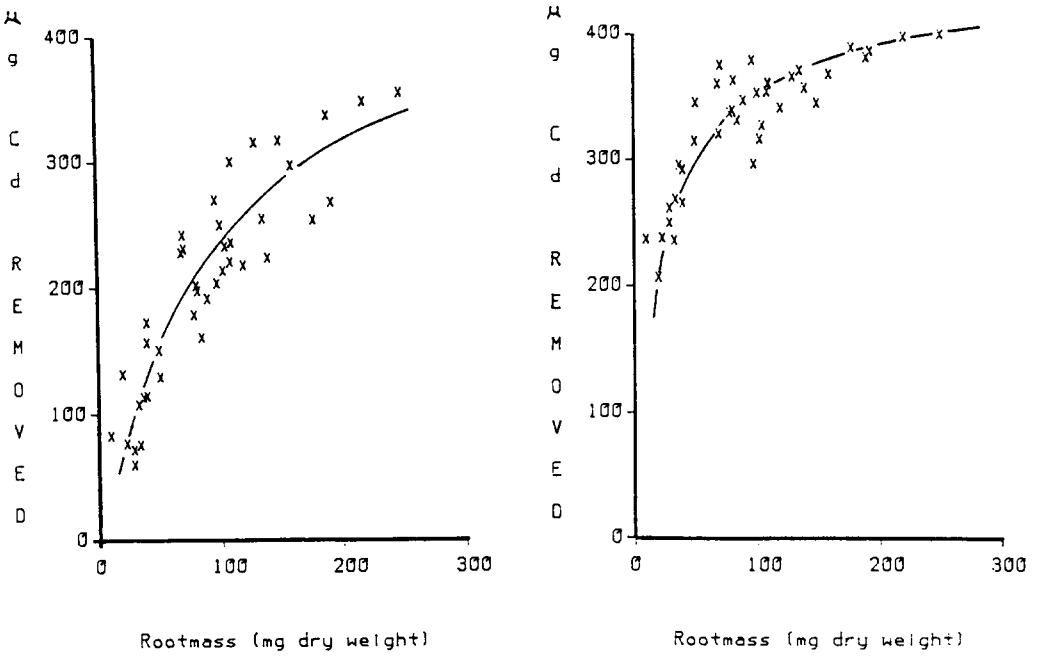

Figure 1. Micrograms cadmium removed at (a) 4 h and (b) 24 h as a function of root mass.

Uptake as a Function of Solution Volume.

Plants were exposed to cadmium solutions of volumes 400, 800, 1200, 1600, and 2000 ml. For this study atomic absorption spectroscopy was used to monitor cadmium uptake as the quantities of tracer required were prohibitive. As observed in Figure 2, apparently linear relationships exist between solution volume and the total amount of cadmium removed at 4, 24 and 48 hours of exposure. As the exposure time increases the slope of the line also increases which is consistent with our earlier results showing increased metal ion uptake at longer exposure times.[10]

Uptake in the Presence of Complexers.

One problem associated with conventional treatment methods for cadmium removal arises from the presence of complexing agents.[11] These species can act to mask the presence of the metal, making it impossible to remove the metal from solution. A number of complexing agents were investigated to determine their effect on cadmium removal by the water hyacinth. In increasing affinity for

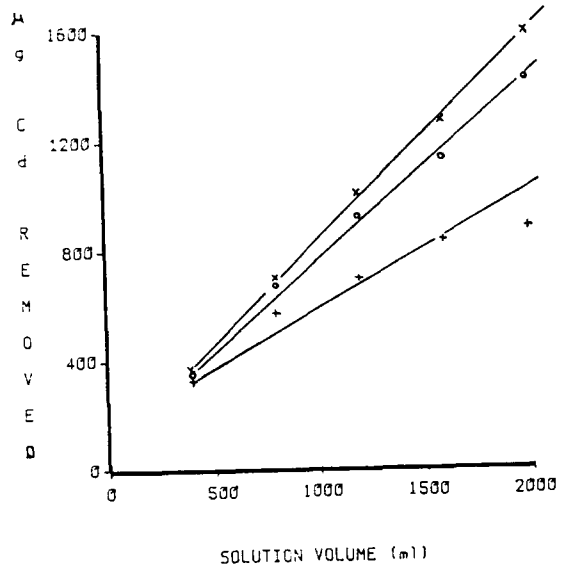

Figure 2. Uptake of cadmium as a function of solution volume (+=4h, o= 24h, x= 48 h)

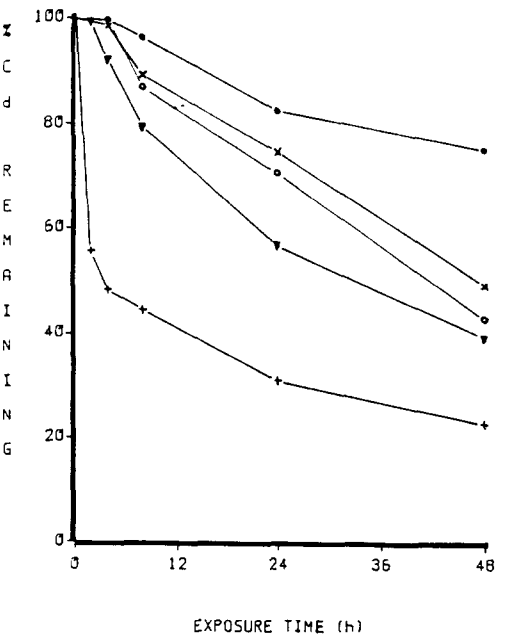

Figure 3. Cadmium uptake in the presence of complexing agents (+= control, ▼=NTA, o= HEDTA, x= EDTA •=CDTA). Average of 6 plants per complexer studied.

cadmium, the complexers used were: acetate, glycine, histidine, salicylic acid, nitrilotriacetic acid (NTA), N-(2-hydroxyethyl)ethylenedinitrilo-N,N',N' -triacetic acid (HEDTA), ethylenedinitrilo-N,N,N',N'-tetraacetic acid(EDTA), and trans-1,2-cyclohexylenedinitrilotetraacetic acid (CDTA) with log K_1 values of 1.70, 4.22, 5.39, 5.55, 9.80, 13.1, 16.28, and 19.84 respectively.[12,13] Phosphate was also included in this set. While this species does not form a soluble cadmium complex, it has the potential for precipitate formation and is an essential nutrient for the plant.

Initial studies were conducted by adding sufficient amount of complexer to represent a 1:1 molar ratio of cadmium to complexer. In each case, the complexer was added to a 1 ppm cadmium solution and the resulting solution adjusted to pH 7 with either 6 M KOH or HNO_3. As compared to a control plant the presence of acetate, glycine, histidine, salicylic acid, or phosphate had no effect on cadmium uptake (data not shown). NTA, HEDTA, EDTA and CDTA each acted to significantly reduce the rate of cadmium uptake as shown in Figure 3. As can be seen, the percent cadmium removed at all time points decreases as the log K_1 for the complexer increases, with the effect most apparent on uptake in the fast uptake phase (0-4 h). As log K_1 increases there is a concomitant reduction in the extent of the fast uptake phase. In all cases though, the rate of cadmium uptake in the slow phase (> 4 h) is about the same.

The effect of complexer concentration on uptake was also briefly examined. NTA/Cd^{2+} molar ratios of 0.1:1, 1:1 and 10:1 yielded average (based upon six plants) percent Cd^{2+} remaining at 48 hrs of 2.9, 28.9 and 64.4 respectively relative to control plants not exposed to NTA. These results help corroborate the idea that the roots only take up free Cd^{2+} in solution.

Cadmium Release

In our previous study we reported that plants initially exposed to cadmium for 48 hours and then transferred to a Cd^{2+} free solution exhibited no significant release of the metal.[10] To determine if the presence of complexing agents could result in the release of previously taken up cadmium, plants were initially exposed to 1 ppm cadmium for 24 hours and then transferred to solution containing 9×10^{-5} M CDTA, EDTA or HEDTA. This concentration represented a 10:1 molar ratio of complexer to Cd^{2+}. A plot of the solution cadmium/cadmium originally removed ratio versus time (Figure 4) shows that the larger is the log K_1 of the complexer used the greater is the amount of Cd^{2+} released in the 8-24 hour time frame up to a maximum of 60% of the cadmium originally taken up by the plant when CDTA was the complexer present in the solution. This initial release appears to be a mirror image of the fast uptake phase for the metal.[10] This rapid release phase is then followed by a slow re-uptake of the metal at a rate comparable to the initial slow uptake phase.

Since CDTA, with a log K_1 of 19.84 for formation of Cd(CDTA), represents

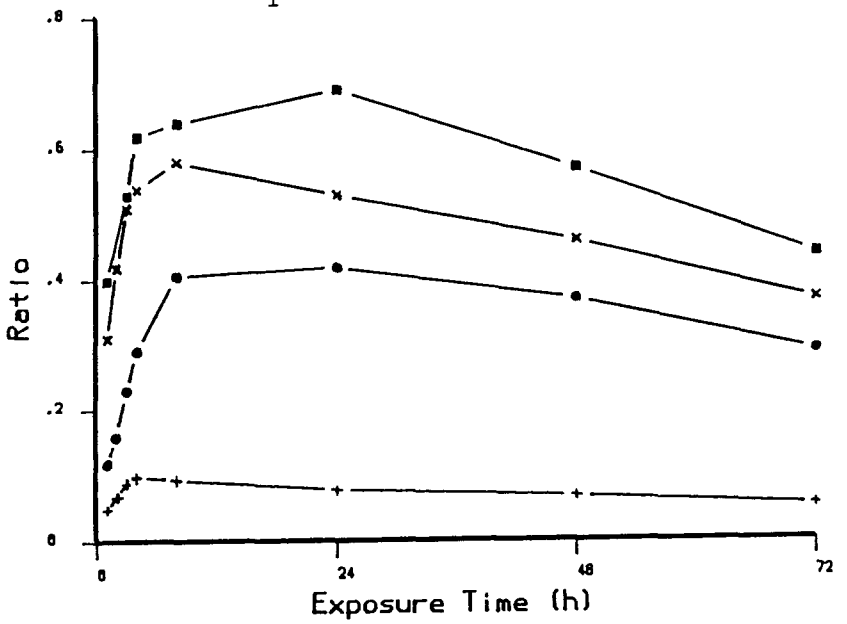

Figure 4. Cadmium release in the presence of complexing agents (+=control, ●=HEDTA, x=EDTA, ■=CDTA). Ratio = micrograms Cd^{2+} in solution per micrograms Cd^{2+} originally removed. Average of 6 plants per complexer studied.

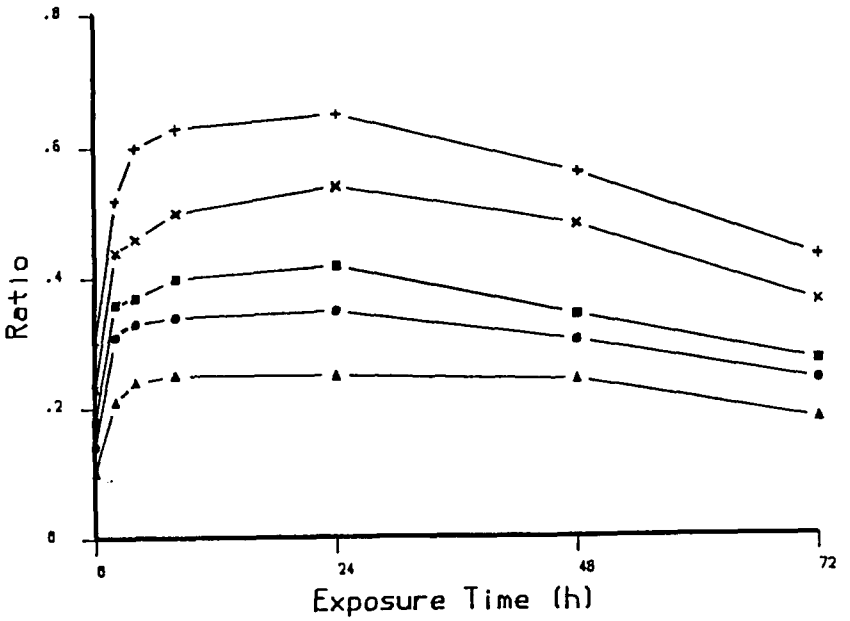

Figure 5. Cadmium release in the presence of CDTA after various Cd^{2+} exposure periods. Ratio=micrograms Cd^{2+} in solution per microgram Cd^{2+} originally removed. (▲=1 day, ●=2 days, ■=3 days, x=4 days, +=5 days). Average of 6 plants per initial exposure period.

the strongest complexer investigated and demonstrated the greatest cadmium release potential, its ability to remove the metal from plants was monitored as a function of cadmium exposure time. Plants were initially exposed to 1 ppm Cd^{2+} for from 1-5 days, subsequently exposed to $9x10^{-5}$ M CDTA and then monitored as a function of time for cadmium release. As seen in Figure 5, there is a decrease in the maximum amount of cadmium released in the 8-24 hour time frame (as compared to the amount initially removed) with increasing initial cadmium exposure time.

Effect of Metal Ions on Cadmium Uptake.

It has been reported that the presence of other heavy metals results in a decrease in cadmium uptake.[4,5] We have already shown that the presence of an increasing concentration of zinc ion results in decreased cadmium uptake.[10] In order to further investigate this effect plants were exposed to cadmium solutions containing various metal ions representing a range of ionic radii and charge, including metals occurring naturally in water. Solutions were prepared which contained 1 ppm cadmium and sufficient metal to result in a 10:1 molar ratio of metal ion to cadmium. This ratio would be expected to make any effect in cadmium uptake readily apparent. Each metal was used as its nitrate salt and the solutions were adjusted to pH 6-7. The lower pH value was used if precipitate formation was evident. Earlier work has shown that this decrease in pH does not

significantly alter cadmium uptake.[10] The percent decrease in cadmium uptake at 24 hours in the presence of these metals as well as the percent of these metals removed from solution by the water hyacinth at 24 hours are reported in Table I. As can be seen, the plant was capable of removing significant amounts of each of the competing metals present, but only in the case of zinc ion was a dramatic decrease in cadmium uptake observed.

TABLE I

Effect of Competing Ions on Cadmium Uptake

Competing Ion	Concentration (ppm)	% Decrease In Cadmium Uptake[a]	% Competing Ion Removed[a]
Ca^{2+}	3.6	2.1 - 5.5	38.0 - 40.7
Fe^{3+}	5.0	3.7 - 5.9	29.3 - 54.1
Hg^{2+}	17.9	6.0 - 7.0	36.5 - 59.8
Co^{2+}	5.3	6.9 - 7.1	32.0 - 58.0
Cu^{2+}	5.7	7.0 -13.2	24.0 - 24.1
Ni^{2+}	5.2	7.8 -12.8	13.5 - 39.1
Mg^{2+}	2.2	21.4 -22.3	19.5 - 25.1
Na^{+}	2.0	21.6 -25.4	29.4 - 39.6
Zn^{2+}	5.8	81.1 -82.5	30.7 - 31.8
Cd^{2+}[b]	1.0		71.2 - 73.8

[a]Range for 3 plants

[b]Control Solution

DISCUSSION

The results shown in Figure 1 clearly demonstrate that plants with greater root mass remove more cadmium from solution per unit time. To determine the nature of the apparent leveling effect of cadmium uptake, additional experiments will need to be performed in which the initial concentration of cadmium is varied and time points beyond 24-48 hours examined. Monitoring uptake as a function of root surface area may also yield more informative results. However, these current results certainly indicate that plants having different sizes, and therefore ages, are all capable of taking up and concentrating cadmium in their tissues.

Increasing solution volume (fixed Cd^{2+} concentration) does correlate well with increased Cd^{2+} uptake as shown in Figure 2. However the fact that the slopes are less than one at all three time points also reveals that the uptake

efficiency decreases as the solution volume increases. In terms of the overall amount of Cd^{2+} taken up it is noted that increasing the solution volume, which increases the amount of Cd^{2+} to be taken up, results in increasing uptake per unit time. This demonstrates that the water hyacinth is able to effectively remove Cd^{2+} from solution when the amount of metal ion present is either quite small or quite large.

The influence of metal ion complexers on the uptake process (Figures 3 and 4) is consistent with an equilibrium existing between free Cd^{2+} in solution and Cd^{2+} bound to some type of site on the roots. This equilibrium is being influenced by a competing equilibrium involving free Cd^{2+} and that complexed in solution. The results suggest that only free Cd^{2+} is taken out of solution by the plant. That is, as the amount of Cd^{2+} in solution is steadily decreased by being bound to complexers having increasing log K_1 values, the amount of Cd^{2+} removed from solution by the roots decreases. Notice however (Figure 3) that the effect seems limited to what we have referred to as the fast uptake phase.[10] The slow uptake phase does not seem to be altered by the presence of the complexers. This seems logical as we interpret the slow uptake phase to be the process whereby the plant removes Cd^{2+} from the equilibrium system into the interior of the root cells.

Complexer effects appear to fall into three categories: (1) those which have no apparent influence; (2) those which partially eliminate; and (3) those which almost completely eliminate the fast uptake phase. We believe the fast phase to be due to the saturation of the metal ion binding sites on the roots. This idea is supported by our earlier finding that stirring the solution dramatically increases rate of uptake.[10] Complexers having small log K_1 values: acetate (1.70), glycine (4.22), histidine (5.39), salicylic acid (5.55), as well as phosphate, do not effectively compete with the root binding sites for free Cd^{2+} (Category 1). NTA, which has a log K_1= 9.80, does compete somewhat with the root binding sites for free Cd^{2+} in solution (Category 2). Finally, complexers having large log K_1 values: HEDTA (13.1), EDTA (16.28), and CDTA (19.84), appear to compete much more effectively for free Cd^{2+} than do the root binding sites (Category 3). These data also suggest that the log K_1 for the root binding sites is somewhat larger than the value for NTA (9.80) and smaller than the value for HEDTA (13.1).

Additional evidence in support of the equilibrium involving free Cd^{2+} and that bound to the roots is based upon the results obtained in the Cd^{2+} release experiments. As shown in Figure 4 the amount of Cd^{2+} released from the roots is proportional to the binding strength of complexer present in solution. This release also mirrors the original fast uptake phase. With time, however, the Cd^{2+} is removed from the equilibrium system by the plant as shown by the slow re-uptake even in the presence of the complexers. This conclusion is further strengthened by noting the results presented in Figure 5. The longer any given

plant is exposed to Cd^{2+} prior to its being subsequently treated with a strong complexer (CDTA), the smaller is the amount of Cd^{2+} that can be released from the roots. These observations are also consistent with the idea that while the roots possess binding sites for free Cd^{2+} in solution, eventually the metal ions occupying those sites are translocated into the plant cells and thus removed from the equilibrium processes in solution.

The fact that of all the metal ions tested (Table I) only Zn^{2+}) ions compete well with Cd^{2+} ions for uptake by the roots of the water hyacinth is consistent with binding sites specific for these two metal ions as previously suggested.[10] It is of course quite interesting to note that the water hyacinth took up substantial amounts of every metal ion tested, including the heavy metal ion Hg^{2+}. Presumably the roots have binding sites for metal ions required for normal growth and maintenance of the plant, and some of those sites are prehaps utilized in the uptake of other metal ions (e.g. Cd^{2+}). The actual number of distinctly different metal ion binding sites on the roots remains unknown. Certain plants have been found to be metal tolerant.[14] Consequently, the ability of any given plant to function well as a biological pollution control device must also depend upon the ultimate fate of the metal ion(s) once they are tekan up by those plants.

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