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Cadmium Uptake and Toxicity to Water Hyacinth: Effect of Repeated Exposures Under Controlled Conditions

Raphael Nir, Avital Gasith, and Albert S. Perry

Institute for Nature Conservation Research, George S. Wise Faculty of Life Sciences, Tel-Aviv University, Ramat-Aviv 69978, Israel

Water hyacinth, Eichornia crassipes (Mart) Solms, has drawn attention as a plant of rapid growth and high biomass production, (Knipling et al. 1970; Del Fosse 1977;) and capable of removing pollutants from domestic and industrial waste effluents (Wolverton 1975; Tokunaga et al. 1976; Tatsuyama et al. 1977; Wolverton and McDonald 1978; Cooly and Martin 1979; Tatsuyama et al. 1979; McDonald and Wolverton 1980; Chigbo et al. 1982; Muramoto and Oki 1983; Kay et al. 1984; O'Keeffe et al. 1984; Rosas et al. 1984; Hardy and O'Keeffe 1985). Most of the above studies were static assays of short duration (1 to 3 days) and with a single exposure of the plants to cadmium. We presumed that repeated exposures to Cd might change the rate of uptake of the metal as well as the growth and physiological state of the plant. This prompted us to undertake the present study in order to evaluate the potential capacity of water hyacinth to remove Cd from solution under conditions of repeated exposures but otherwise favorable growth conditions and without interference from other toxic compounds.

Removal of metals from effluents by plants is expected to be compounded by the influence of specific conditions of the medium such as temperature, pH, ionic strength, presence of other metals or complexing ligands (Nir et al. 1989). The results of the present study will serve as a comparative reference for evaluating the effect of effluent conditions on Cd toxicity to water hyacinth and the plant's capacity for metal removal.

MATERIALS AND METHODS

Water hyacinth plants were obtained from the botanical garden of Tel-Aviv University. The experiments were carried out outdoors during summer and fall (June to November : temperature range- 23 to 30°C; light intensity - 1100 to 1600 $\mu E/m^2/sec$) and in a greenhouse during winter and spring (January to May: temperature range - 15 to 25°C; light intensity - 300 to 1400 $\mu E/m^2/sec$). Orienting tests showed that under conditions of weekly renewal, maximum biomass production was obtained in 25% Hoagland nutrient solution (Hoagland and Arnon 1950).

Send reprint requests to Dr. A. Gasith at the above address.

The plants were held in nine liter, opaque, low-density polyethylene buckets (23 cm diameter by 24 cm deep). Each bucket contained eight liters of 25% Hoagland solution (initial pH= 4.75 ± 0.25), either free of or with Cd. A 1000 ppm $Cd(NO_3)_2$ stock solution was used for the preparation of 0.05, 0.1, 0.4, 1.0 and 2.5 ppm concentrations of Cd in 25% Hoagland solution. The plants were repeatedly exposed by replacing the medium once a week following flushing of the buckets with tap water. Usually one bucket was used for each tested concentration and for control. Each bucket contained 3 plants of approximately equal initial wet biomass. Prior to testing, the plants were acclimatized in buckets containing 25% Hoagland solution for one week. The experiments lasted from one to three weeks.

Tissue concentration of Cd was analyzed in all the plants following rinsing with tap water for one minute. No loss of Cd was detected after rinsing the plants and immersion in tap water for up to 90 minutes. Leaves and roots were oven dried at 95°C for 48 hrs, ground separately and strained through 40-mesh wire gauze to obtain a fine powder. Subsamples of 0.2 g of roots and 0.4 g of leaves were digested in 20 ml of boiling 7.2N HNO_3 for 30 minutes. After cooling, the solution was filtered through Whatmann No. 1 filter paper and was diluted to 100 ml with double distilled water. Final pH was <2.0. Cd concentration was determined by atomic absorption spectrophotometry (Perkin - Elmer 460, 228.8 nm, Air-Acetylene flame, Varian Techtron Hollow cathode). Redigestion of the particulate material collected on the filter paper revealed no trace of Cd. Using this particular method the minimum detectable level was 3 µg Cd/g dry weight of plant material. The efficiency of the method was tested by measuring the total recovery in the plants and the aqueous medium. The total recovery was greater than 95%.

The effect of Cd on plant growth was determined by analysis of biomass production. Once a week, upon renewal of the growth medium, the plants were removed from the buckets, washed in tap water and blotted gently with paper towels until no more moisture came off the plants. The plants were then weighed (wet biomass) to the nearest 0.1 g and were returned to their respective buckets. At the end of each experiment the final wet biomass was determined, the plants sectioned into roots and leaves (including the petioles), oven dried at 95°C for 48 hrs and weighed to obtain dry biomass. The dry to wet weight ratio of Cd-free plants was used for estimating the initial dry weight of all the plants used in the experiment.

The Chlorophyll-a (chl-a) content in the blade of the last fully developed leaf was used for determination of chlorosis as a measure of the physiological state of the plants. Three discs, 9 mm in diameter, were removed from each leaf using a cork borer. A template was used to standardize the position of the borer on the leaf blade. Each disc was extracted with 3 ml of DMF (N, N-Dimethylformamide) for 24 hours in the dark at 4°C (Moran 1981). Under these conditions complete chlorophyll extraction was obtained within 24 hours. Chl-a was measured spctrophotometrically

in a Varian Techtron UV - VIS Spectrophotometer model 635 and the content per disc was calculated as follows:
Chl-a in micrograms/disc = $12.64 \times A_{664} - 2.99 \times A_{647}$

where, 12.64 and 2.99 are chl-a constants and A_{664} and A_{647} are the absorbance readings at the respective wavelengths (Moran, 1981).
The Student - Newman - Keuls (SNK) test (Sokal and Rohlf, 1969) was used for statistical evaluation of the results.

RESULTS AND DISCUSSION

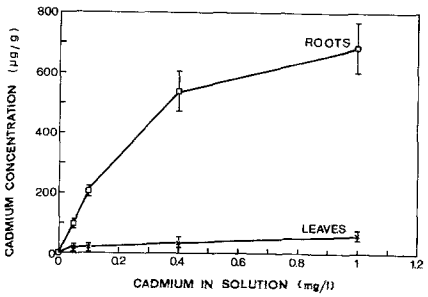
Exposure of water hyacinth to Cd in concentrations >0.1 ppm resulted in accumulation of Cd in roots and leaves, in chlorosis of the leaves and suppression of plant growth. These plants had darker, brittle roots with fewer rootlets than those of Cd-free plants.

No trace of Cd was found in unexposed plants. The Cd concentration in roots and leaves of exposed plants was positively correlated with the Cd concentration in solution (Fig. 1, $r>0.9$) and to the time of exposure (Fig. 2, $r>0.9$).

The total amount of Cd in the plants was linearly correlated with the time of exposure ($r=0.94$, $n=12$, Y mg/plant= $0.11t + 0.12$, t= days). A positive correlation was found also between the total amount of Cd in plant tissue and the net gain of wet and dry biomass of plants exposed to 0.4 ppm Cd for two weeks ($r=0.87$, $n=12$). Similar results were reported by others (Tokunaga et al. 1976; Wolverton and McDonald 1978; Tatsuyama et al. 1979; Kay et al. 1984; O'keeffe et al. 1984; Rosas et al. 1984). Accordingly, water hyacinth may be expected to remove Cd to the limit of the toxic effects which inhibit growth.

Maximum Cd concentration in plants exposed to 0.4 ppm for 3 weeks was found in the roots (703 and 67 ppm in the roots and leaves, respectively). Accordingly, the roots constitute the main sink for the Cd removed from solution (>80%). This is confirmed by other studies (Rosas et al. 1984; Wolverton 1975). Fujita (1985) has shown that roots of water hyacinth exposed to Cd contain two Cd-binding substances (resembling mammalian Cd-thionines). These substances are inductively formed and may play an important role in the accumulation of, as well as tolerance to, Cd.

The total plant biomass per bucket at the end of each week, and the respective amount of Cd removed by the plants, are shown in Table 1. Although the plants continued to grow, the net weekly uptake of Cd per plant remained relatively unchanged (0.7-0.9 mg Cd/week). This meant a reduction in the Cd removed per unit biomass. The data reveal about 30% reduction in the Cd removed per week, after one week of exposure. Other studies have shown that metal sorption was rapid at the outset but decreased to a slower rate thereafter. (Wolverton, 1975; Wolverton and McDonald, 1975; Tatsuyama et al., 1977; O'Keeffe et al., 1984).



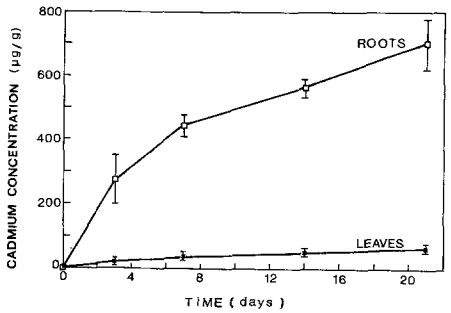


Figure 1. (left) Cd concentration in water hyacinth (µg/g dry weight) after one week of exposure, as a function of initial Cd concentration in solution (n= 3, mean ± SD).

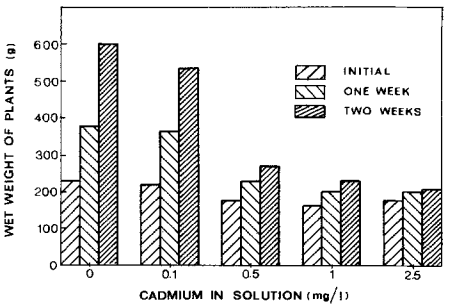
Figure 2. (right) Cadmium concentration in water hyacinth (µg/g dry weight) exposed to 0.4 ppm Cd in a weekly renewal test, as a function of time. (n=3, mean ± SD).

Tissue concentration of Cd in daughter plants which developed during the exposure period was twice as high as that found in the parent plants (Table 2). This may be attributed to the fact that the daughter plants were exposed to the metal from the outset. Translocation of the metal from parent to daughter plants may not be excluded. The rapid chlorosis and growth inhibition in the daughter plants correlated well with the high tissue-Cd concentration.

Exposure of water hyacinth to Cd caused considerable reduction in the net production of wet biomass (Fig. 3). For example, at the end of one and two weeks the net gain of wet biomass of plants exposed to 0.5 ppm Cd dropped by 60 and 75%, respectively, with reference to the control. Plants exposed to 1.0 and 2.5 ppm Cd gained little or no weight (Fig. 3), and ultimately died within two weeks.

Table 1. Plant biomass, total amounts and weekly net uptake of Cd in plants exposed to 0.4 ppm Cd for three weeks. (Each bucket contained 3 plants).

Week	Total Wet Biomass g/Bucket	Total Cd In Plants mg/Bucket	Net Cd Uptake Per Plant mg/Week	Net Cd Uptake Per g Plant mg/Week
1	205	2.52	0.84	0.0123
2	250	4.68	0.72	0.0086
3	297	7.35	0.89	0.0090



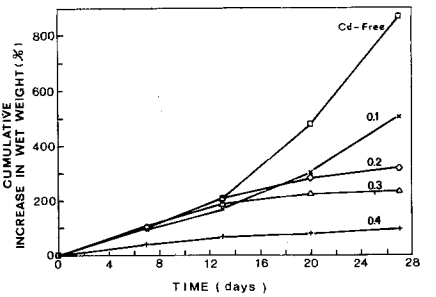


Figure 3. (left) Total wet biomass production by water hyacinth as a function of the cadmium concentration in solution (mg/l, 6 plants for each concentration).

Figure 4. (right) The cumulative increase in wet biomass of water hyacinths as a function of cadmium concentration in solution (mg/l) and the time of exposure (3 plants for each concentration).

Table 2. Tissue concentration of Cd (mean ±SD) in roots and leaves of parent and daughter plants of water hyacinth exposed to 0.4 ppm Cd for 3 weeks. (n= 3 parent plants, 6 daughter plants).

	Cadmium Concentration (µg/g)	
	Parent Plants	Daughter Plants
Root:	617 ±17	1030 ±239
Leaf:	56 ±4	107 ±35

The net gain of wet biomass was negatively correlated with the Cd concentration in the medium and the period of exposure (Fig. 4). A 50% reduction in plant growth was recorded in plants exposed to 0.4 ppm Cd within the first week, while at the lower concentration of 0.1 ppm an effect was noticeable after three weeks.

There was no significant difference in dry-weight gain between Cd-free plants and plants exposed to 0.1 up to 1.0 ppm Cd (Table 3). Correspondingly, the percentage of dry weight of plants exposed to Cd was higher than that of Cd free plants. For example, the percentage of dry weight of plants exposed to 0.4 ppm Cd for two weeks was approximately 18% higher than that of Cd-free plants (7.82 ±0.42, n=12 and 6.45 ±0.44, n=24, respectively). This may indicate suppression of water absorption and/or increase in water loss via evapotranspiration. Reduction in the number of side roots as well as the change in the roots texture supports the former, however, an effect on water loss cannot be excluded.

Table 3. Effect of cadmium concentration on the gain of wet and dry biomass and on the chlorophyll-a content per leaf disc in water hyacinth exposed to different Cd concentrations for one week. (n= 3 plants, values followed by the same letter are not significantly different at the 5% level as determined by the SNK test).

Cadmium Conc. ppm	Gain Of Wet Biomass g ±SD	Gain Of Dry Biomass g ±SD	Chlorophyll-A µg/leaf disc ±SD
0.0	25.8±2.2 (a)	1.8±0.5 (a)	9.9±0.3 (a)
0.05	26.1±5.0 (a)	1.8±0.6 (a)	9.5±0.6 (a)
0.1	26.3±3.9 (a)	1.9±0.5 (a)	9.6±0.9 (a)
0.4	12.2±2.0 (b)	1.5±0.2 (a)	7.5±0.4 (b)
1.0	11.2±4.6 (b)	1.6±0.3 (a)	7.6±1.2 (b)

Chlorosis of the leaves was apparent in water hyacinth exposed to 0.4 ppm Cd for one week. The chl-a content of the leaves decreased by approximately 24% relative to that in Cd-free plants (Table 3). The extent of chlorosis increased with the time of exposure (Table 4).

Table 4. Chlorophyll-a content in leaves of water hyacinth exposed to 0.4 ppm Cd, as a function of time. (Values followed by the same letter are not significantly different at the 5% level as determined by the SNK test).

Time	Chlorophyll-A (µg/leaf disc ±SD)	
Days	Cd-Free Plants	Plants Exposed To Cd
3	11.4 ±1.9	10.2 ±1.0 (a)
7	10.0 ±2.3	6.9 ±1.9 (ab)
14	12.4 ±1.6	4.6 ±1.9 (b)
21	11.3 ±3.2	4.5 ±3.3 (b)

The above effects were considerably more pronounced in daughter plants. For example, the chl-a content in leaves of daughter plants which developed within 3 weeks of exposure to 0.4 ppm Cd was 75% lower than that found in respective leaves of Cd-free daughter plants (2.3 vis 9.2 ug/leaf disc, respectively).
Symptoms of Cd toxicity were apparent at all concentrations of Cd tested between 0.1 and 2.5 ppm. The extent of the toxic effects was a function of Cd concentration in solution and of the period of exposure. Similar adverse effects were reported by others but

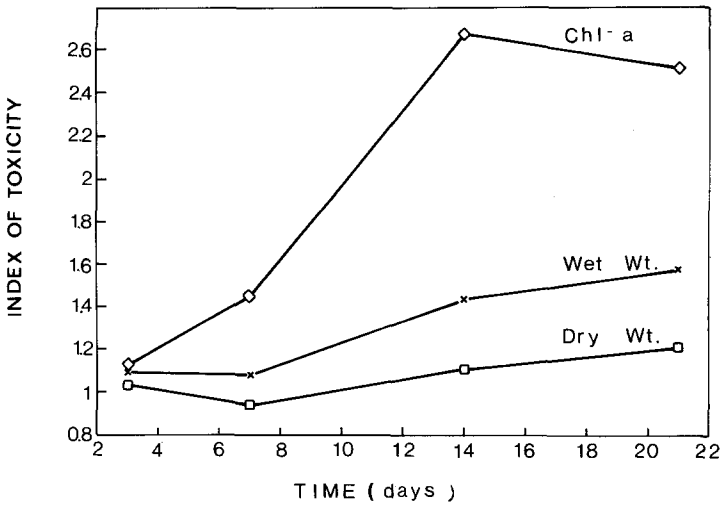


Figure 5. Index of toxicity in plants exposed to 0.4 ppm Cd for 3 weeks (3 plants for each time of exposure).

at higher metal level (e.g., Wolverton 1975; Kay et al. 1984; O'Keeffe et al. 1984;). This may be attributed to a difference in clones of water hyacinth, to difference in experimental conditions (such as different growth media, pH, single or multiple exposures and different duration of the test) and possibly to the difference in the toxic symptoms considered as end points.

We suggest that the ratio obtained by dividing the value of a selected parameter determined in Cd-free plants by the respective value in plants exposed to Cd may be used as a comparative index for the degree of toxicity. Accordingly, a toxicity index (T.I.) of 1.0 indicates no effect. The higher the value of the T.I. the greater is the adverse effect. This could be used for selecting the most sensitive parameter exhibiting acute or chronic effects.

In the present study the highest T.I. for the effect of Cd with time was obtained for chl-a content (Fig. 5). Therefore, chlorosis is suggested as a sensitive parameter for evaluating sublethal and acute toxic effects of Cd, and possibly of other toxic metals, in water hyacinth.

Under favorable nutrient conditions and interference from no other toxic metals, water hyacinth has been shown to have a remarkable capacity for removing Cd from solution (>70%), under conditions of repeated exposure to relatively low concentrations of the metal (<0.5 ppm). Considering the fact that similar efficiencies of Cd removal were reported by others in non-renewal tests (e.g., Kay et al. 1984; Rosas et al. 1984) we may conclude that at least for a period of a few weeks, and at a relatively low Cd concentration,

repeated exposures do not change the plant's performance significantly. In longer exposures and/or higher Cd levels, the efficiency of metal removal is expected to drop in correlation with the progressive suppression of growth. A decrease in the metal uptake capacity is expected under effluent conditions. This aspect is currently being investigated.

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