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Plant Screening and Comparison of *Ceratophyllum demersum* and *Hydrilla verticillata* for Cadmium Accumulation

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Cadmium is a pollutant that has been released into the environment for decades. In recent years, interest has been focused on the study of aquatic macrophytes as promising candidates for pollutant uptake and biological indicators of heavy metal concentrations in aquatic ecosystems (Maine *et al.*, 2001). Thailand has a great diversity of aquatic plant species. Some of them can be exploited for the removal of various pollutants including heavy metals from waste water because of their fast growth rates and simple growth requirements. In order to develop a phytoremediation strategy for cadmium, the first objective of the present study was to screen thirteen aquatic macrophytes, each of which had a high biomass, a rapid growth rate, and an ability to accumulate metals. *Ceratophyllum demersum* and *Hydrilla verticillata*, which are rootless submerged plants, were previously identified as potential accumulators of toxic metals (Garg and Chandra, 1991). *C. demersum* was reported to be a powerful scavenger of cadmium (Ornes and Sajwan, 1993), while *H. verticillata* showed the potential to remove toxic metals from waste water (Chandra *et al.*, 1993; Rai *et al.*, 1995) and could take up contaminants from both the sediment pore water and the overlaying water column (Hinman and Klaine, 1992). Both species are common aquatic angiosperms found throughout the world and are tolerant of a broad range of environmental conditions and grow at a rapid rate. In natural habitats around Thailand, *C. demersum* and *H. verticillata* are found together. Hence, the second objective of the present study was to compare the cadmium accumulation and tolerance in both plant species in separate and combined cultures.

MATERIALS AND METHODS

Thirteen plant species, namely *Pistia stratiotes*, *Colocasia esculenta*, *Lemna minor*, *Azolla pinnata*, *C. demersum*, *Neptunia oleracea*, *Typha angustifolia*, *H. verticillata, Hygrophilla difformis*, *Ipomoea aquatica*, *Cyperus strigosus*, *Trapa bispinosa*, and *Pandanus amaryllifolius* were collected from unpolluted water bodies around Bangkok, Thailand and grown in 10% Hoagland's nutrient solution (EPA, 1975) in the laboratory under controlled conditions (at 25 ± 2ºC, illuminated for 12 h daylight period, light intensity approximately 2200 LUX) for four weeks. The plants were exposed to cadmium added nutrient solution. Cadmium prepared from analytical grade $CdCl_2.H_2O$, was added at 0.1, 1 and 10 mg/L. The water pH was 7.2-7.4. Control groups were also prepared and used

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without addition of cadmium. Each treatment was performed in triplicate. Plants were harvested after 7 days, thoroughly washed with distilled water, blotted dry and whole plant fresh weight determined. Plants were oven dried at 100°C overnight and acid digested (APHA, 1998). Total cadmium content in the entire plant was determined using a flame atomic absorption spectrophotometer (APHA, 1998). In addition, the bioconcentration factor (BCF) of each plant species was determined by dividing the metal concentration in plant tissue at harvest (mg/kg) by initial concentration of metal in the external solution (mg/L). The BCF provides an index of the ability of the plant to accumulate the trace element with respect to the trace element concentration in the substrate.

C. demersum and *H. verticillata* were collected from local water bodies at Pathumthanee, Thailand, and grown in of 30% Hoagland's nutrient solution in the laboratory under controlled conditions for four weeks. Selected healthy plants (5 g wet wt) were then treated with different concentrations (0.01, 0.05, 0.25 and 1.25 mg/L) of cadmium in 700 mL of 10% Hoagland's nutrient solution. Plants cultured in the nutrient without cadmium served as controls. There were three replicates for each treatment. Both plant species were exposed to cadmium separately and together in 1000 mL plastic containers for 30 days. Plants from each container were separately harvested at day 7, 14, 21 and 30 and were processed for biomass and metal content measurement as described previously. The relative growth of plants was calculated by dividing the final biomass(g) with the initial biomass(g) of treated plants and multiplied by one hundred. The mean values of biomass and metal content were calculated and subjected to the analysis of variance (ANOVA) using a randomized block design and Least Significant Difference Method (LSD) on the SPSS for Windows program.

RESULTS AND DISCUSSION

All tests were significant at P = 0.05. Table 1 shows the cadmium accumulation by the thirteen plant species. The accumulation increased with the increase in solution concentration except for *P. stratiotes* and *H. difformis* in which 10 mg Cd/L was very toxic. The maximum accumulation of 7942 mg Cd/kg dw was found at 10 mg/L in *H. verticillata*. For most plant species, the increase in cadmium accumulation in plant biomass that occurred when the ambient cadmium concentration in water increased from 1 to 10 mg/L was significant. Many plant species showed toxicity symptoms or reduced biomass when grown in contaminated water at 1 mg/L and 10 mg/L of cadmium. The most visible symptoms were stunted growth and chlorosis. The BCFs of cadmium in the thirteen species of aquatic plants at different concentrations are shown in Figure 1. Low concentrations of cadmium (0.1 and 1 mg/L) resulted in higher BCFs in every plant species when compared with the plants exposed to 10 mg/L cadmium. Among all tested species, *H. verticillata* attained the highest BCF of 3281 at the 1 mg/L concentration level.

The results of cadmium accumulation assays showed that, among the thirteen plant species tested, *H. verticillata, C. demersum, A. pinnata, L. minor* and *N. oleracea* were very good accumulators. They accumulated up to 7941, 7381, 5721, 4578 and 4199 mg/kg dw, respectively, when supplied with 10 mg Cd/L. *L. minor* and *E. crassipes* have been shown to be the top species as cadmium accumulators in the literature (Zayed *et al*., 1998; Zhu *et al*., 1999; Wang *et al*., 2002). Other aquatic plants such as *Spirodela polyrrhiza, Elodea nuttali, Callitriche plataycarpa, Limnanthenum cristatum, Salvinia stolonifer, P. stratiotes* and *Hydromista stolonifera* have also been shown to be very good cadmium accumulators (O'Keefee *et al*., 1984; Chandra and Garg, 1992; Maine *et al*., 2001). In the present study, the bioconcentration factors of cadmium for thirteen species of plants ranged from 0-3300 at various supply levels. Several wetland species have attained higher BCF values. Rai *et al*. (1995) reported BCF values of cadmium ranging from 2125 to 29000 for six wetland plant species (coontail, giant duckweed, bacopa, wild rice, channel grass, and alligator weed) and two algae (*Hydrodictyon reticulatum* and *Chara carallina*). In order to determine what species can be considered a good accumulator, the arbitrary criteria of BCF by Zayed *et al*. (1998) and Zhu *et al*. (1999) were applied, *i.e*., a BCF of over 1000 (100-fold compared on fresh weight) was considered as a hyperaccumulator. Based on this criterion, many tested plant species can be considered as hyperaccumulators, *i.e., L. minor, P. stratiotes, H. difformis, H. verticillata, A. pinnata, C. demersum,* and *N. oleracea.*

The relative growth of *C. demersum* and *H. verticillata* in separate cultures in relation to different exposure times and concentrations of cadmium is shown in Figure 2. There was a significant decrease in relative growth of *H. verticillata* and *C. demersum* when the exposure time and cadmium concentration were increased. At 1.25 mg/L of cadmium, *C. demersum* and *H. verticillata* showed toxicity symptoms, such as chlorosis, after 14 days. The relative growth of *H. verticillata* was significantly different from that of *C. demersum* when they were grown separately. Cadmium accumulation in *C. demersum* and *H. verticillata* is shown in Figure 3. There were significant increases of metal in tissue levels of both species when the concentrations were increased. But the increase of exposure time did not result in the increase of cadmium accumulation in *H. verticillata* and *C. demersum* at all concentrations from day 7. With an increase in the cadmium concentration from 0.25 to 1.25 mg/L, cadmium accumulation in both species was increased significantly.

The relative growth of *C. demersum* and *H. verticillata* which were grown together in different concentrations of cadmium and exposure times are shown in Figure 4. There were significant decreases in relative growth of *H. verticillata* and *C. demersum* when cadmium concentrations were increased. However, in both species, there was not a significant difference in relative growth when the exposure time was increased. The accumulation of cadmium in *C. demersum* and *H. verticillata* at different exposure times and cadmium concentrations is shown in Figure 5. There were significant increases of metal in tissue levels when cadmium concentrations were increased. Whereas, the accumulations were not significantly different when the exposure times were increased. *H. verticillata* showed significantly higher accumulation of cadmium at 0.05, 0.25 and 1.25 mg/L than *C. demersum.* At 0.25 mg/L, both plant species showed no significant increases of metal in tissue levels when the exposure times were increased from

Table 1. The accumulation of Cd by 13 plant species at different concentrations.

Plant species	Cd accumulation (mg/kg dw)			
	0 mg Cd/L	0.1 mg Cd/L	1 mg Cd/L	10 mg Cd/L
Lemna	ND*	279.47[a]**	1725.55[c]	4578.9[bc]
Pistia	ND	182.09[bc]	2554.52[b]	ND
Hygrophilla	ND	131.49[c]	1451.45[cd]	ND
Hydrilla	ND	263.79[a]	3281.0[a]	7941.86[a]
Cyperus	ND	78.0[d]	545.64[e]	1859.6[d]
Azolla	ND	254.8[a]	1049.19[d]	5721.03[bc]
Ceratophyllum	ND	226[ab]	1532.34[d]	7381[b]
Trapa	ND	ND	553.02[e]	2545.97[d]
Neptunia	ND	158.52[c]	1394.37[d]	4199.51[c]
Pandanus	ND	0.76[e]	263.3[e]	1744.88[de]
Ipomoea	ND	ND	539.15[e]	1356.65[de]
Colocasia	ND	22.8[d]	136.33[e]	947.73[de]
Typha	ND	ND	243.64[e]	1244.47[de]

*ND = not detectable, detection limit 0.1 mg/L

** Means within a column followed by different letters indicate significant differences at P<0.05.

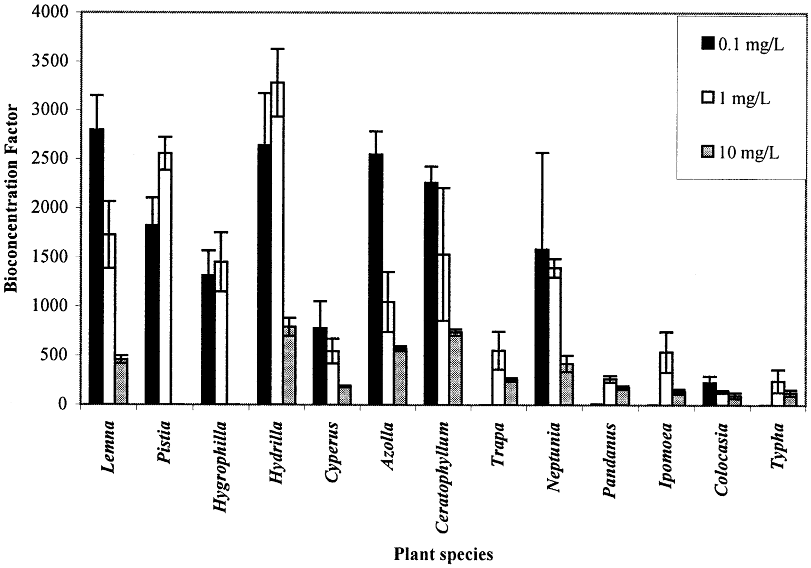

Figure 1. The bioconcentration factors (BCFs) for cadmium in 13 plant species at different cadmium concentrations.

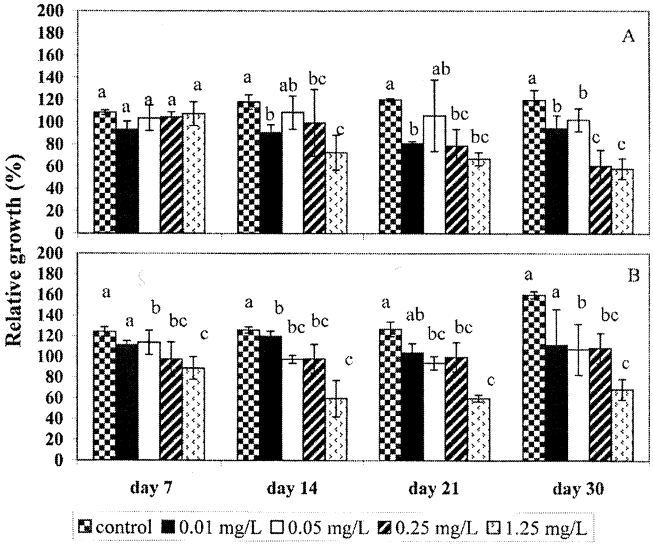

Figure 2. The effects of Cd on relative growth (%) of *C. demersum* (A) *and H. verticillata* (B) at different concentrations and exposure times (separate culture).

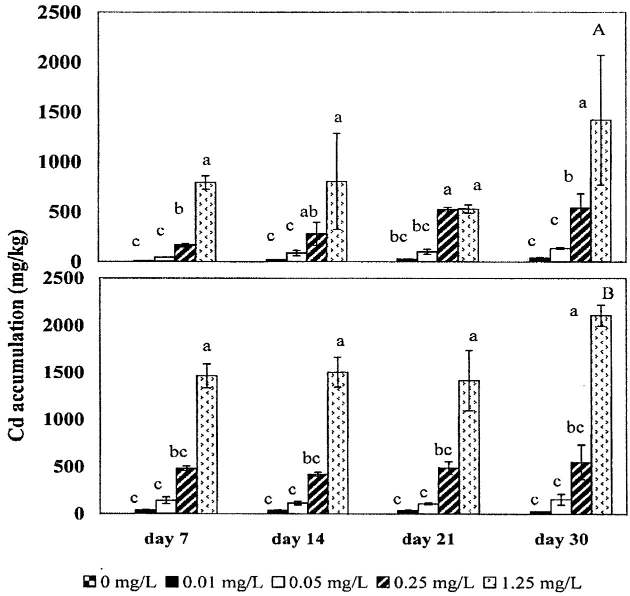

Figure 3. The accumulation of Cd by *C. demersum* (A) and *H. verticillata* (B) at different concentrations and exposure times (separate culture).

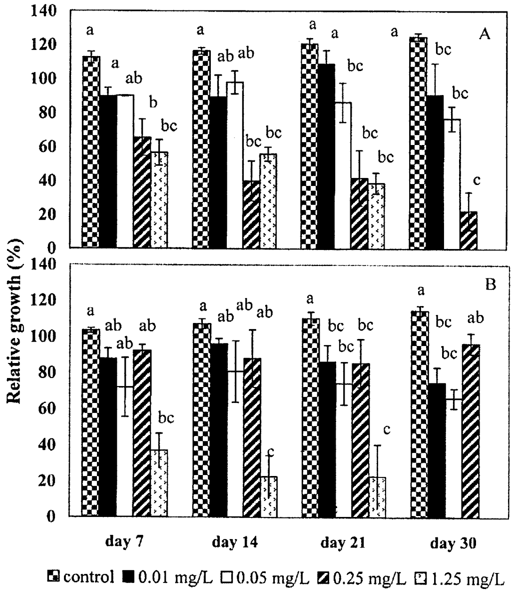

Figure 4. The effects of Cd on relative growth (%) of *C. demersum* (A) *and H. verticillata* (B) at different concentrations and exposure times (combined culture).

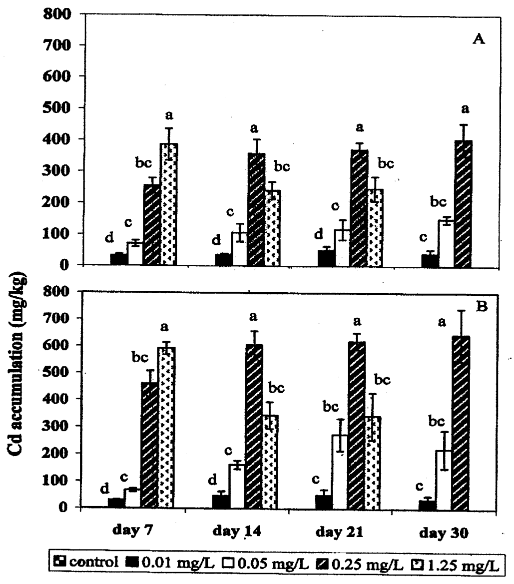

Figure 5. The accumulation of Cd by *C. demersum* (A) and *H. verticillata* (B) at different concentrations and exposure times (combined culture).

day 14 to 30, suggesting that these two species approached their maximum accumulation in 14 days. Similar patterns were found in *C. demersum* and *H. verticillata* at 1.25 mg Cd/L, but at this cadmium level plants reached their maximum accumulation in 7 days.

Several studies have reported on the high metal accumulation by *C. demersum* and *H. verticillata* (Sinha *et al.*, 1993; Rai *et al.*, 1995; Gupta and Chandra, 1996a; 1996b). However, in these studies, they never cultured nor exposed these two plant species together. Our results showed that when *C. demersum* and *H. verticillata* were separately cultured under experimental conditions, the accumulation of cadmium by *H. verticillata* was much higher than that of *C.demersum*. This might be due to the higher relative growth of *H. verticillata* which implies that cadmium is less toxic to *H. verticillata* than *C. demersum*. When these two species were grown in competition at various concentrations of cadmium and durations, we found that the accumulation capacity of *H. verticillata* was still higher than that of *C. demersum* especially at high cadmium concentrations (0.25 and 1.25 mg/L). At low cadmium concentration, the cadmium accumulation in plant tissues of both species was not much different from each other. From a phytoremediation standpoint the actual plant removal efficiency of any given trace metal is the product of plant density and the rate of metal accumulation in harvestible plant parts (Qian *et al.*, 1999). Our data showed that *H. verticillata* was the best plant species for the accumulation of cadmium under hydroponic conditions based on the rate of metal accumulation in whole plant tissues. These plants may be good candidate for phytoremediation strategies because they are fast growing submerged plants that are commonly found all over the world and the entire plant can be harvested.

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