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Toxicity and Accumulation of Cadmium and Zinc in *Hydrocotyle umbellata*

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ABSTRACT: The aquatic plant, *Hydrocotyle umbellata*, was tested for toxicity and accumulation of Cd and Zn under laboratory conditions. *H. umbellata* were cultured in modified 10% Hoagland solution supplemented with 0.2, 0.4, 0.6, 0.8 and 1 mg/L Cd and 2, 4, 6, 8, 10 and 50 mg/L Zn and were separately harvested after 3, 6, 9 and 12 days. The toxicity symptoms of *H. umbellata* exposed to Cd and Zn at different concentrations and exposure times were stunted growth and chlorosis in leaves. Finally, some plants died at higher concentrations of metals. The symptoms were more severe at higher metal concentrations. Cd and Zn caused significant decreases in biomass productivity and chlorophyll content when the exposure times and concentrations of both metals were increased. There were significant increases in metal levels in plant tissues when the exposure times and metal concentrations were increased. Both metals accumulated in roots more than in shoots. The high values of bioconcentration factor (BCF) of Cd (7173, at 0.2 mg/L) and Zn (1717, at 2 mg/L) on day 9 of exposure suggested that *H. umbellata* is a good candidate for removal of Cd and Zn from contaminated water.

KEYWORDS: cadmium, zinc, *Hydrocotyle umbellata,* toxicity, accumulation.

INTRODUCTION

Water pollution is clearly demonstrated by the high concentrations of heavy metals in the water, sediments and aquatic organisms[1]. Heavy metals are nowadays among the most important pollutants in source and treated water, and are becoming a severe public health problem. Many industries discharge heavy metals such as Cd and Zn in their wastewater[2]. Cd and Zn are toxic heavy metals that are being used in a wide variety of industrial processes in Thailand. So, they were selected as toxicants for the present study.

Aquatic and wetland plants are important components of lake, pond, river and stream ecosystems throughout the world. The attention focused on vascular aquatic plants has been directed primarily toward their elimination from water bodies, since their rapid proliferation can present impediments to navigation and a threat to the balance of biota in the aquatic systems. In recent years, several species of aquatic vascular plants have been used as bioindicators for pollutants such as heavy metals. Some have also been used frequently to remove suspended solids, nutrient, heavy metals from contaminated sites, while others have been used in phytotoxicity tests for the development of water quality criteria.

Heavy metal removal from aqueous solutions has been commonly carried out by several processes, including chemical precipitation, solvent extraction, ion-exchange, reverse osmosis, adsorption and other methods, which are expensive and frequently inefficient to reach the minimum desirable metal concentrations[3]. Therefore, alternative methods of heavy metals removal based on biological materials have received increased attention in recent years[3,4,5]. One phytoremediation technique is rhizofiltration, which refers to the use of plant roots to sorb, concentrate, and precipitate metal contaminants from surface or ground water[6,7]. The roots of plants are capable of sorbing large quantities of heavy metal from soil or water[8]. Rhizofiltration is generally applicable to treating large volumes of water with low contaminant concentrations (in the ppb range). It has primarily been applied to metals (Pb, Cd, Cu, Fe, Ni, Mn, Zn and Cr) [9-11].

Hydrocotyle species or pennyworts are aquatic plants commonly found in freshwater swamps and marshes[12]. *Hydrocotyle asiatica* extract has been used to combat excessively dry skin in India. It has also been

used as a diuretic, aperient to combat fever and bowel complaints. Recent studies indicate it is a strong anti-inflammatory agent, effective in the treatment of wounds, ulcers and lymphatic edema[13]. It is also known as "the longevity plant" because of its incredible ability to speed cellular renewal and increase longevity[14]. In Thailand, *H. umbellata* is commonly found throughout the country. It has very rapid growth. In recent years, it has been used as a decorative plant, but its medicinal use has not been well established. Removal of heavy metals and pollutants from aquatic systems by *H. umbellata* has been studied [12,15]. In addition, *Hydrocotyle* spp. is relatively cold tolerant with a very good capacity for nutrient uptake[16]. However, there were only a few reports on the metal uptake potential of *H. umbellata* and metal toxicity to the plant. Therefore, the purposes of this study were to examine the accumulation of Cd and Zn and their toxicities to *H. umbellata*.

Materials and Methods

H. umbellata were obtained from uncontaminated ponds around Bangkok, Thailand. They were thoroughly washed with running tap water to remove all the dirt and dead plant biomass, then kept in a greenhouse. *H. umbellata* were cultured in a modification of 10 % Hoagland's solution, the most suitable nutrient medium for aquatic plants, at pH 6.5 without aeration, with a 12-h photoperiod (under a 100 watt fluorescent lamp) at the temperature of 25 $\pm$ 2°C. The plants were acclimatized for at least 15 days before the experiments.

Healthy *H. umbellata* plants with 8-10 mature leaves (6.92 – 7.42 g) were isolated from the stock culture and rinsed with distilled water. Six plants were placed in each container, which contained 500 mL of a control (metal free) and 0.2, 0.4, 0.6, 0.8 and 1 mg/L of Cd solution and 2, 4, 6, 8, 10 and 50 mg/L of Zn solution prepared from $CdCl_2$ and $ZnSO_4 \cdot 7H_2O$, respectively. The final pH was adjusted to 6.5. Each container was covered with a clear plastic film to prevent contamination. The pH was adjusted every day. All experiments were performed in triplicate.

After 3, 6, 9 and 12 days, plant samples from each container were harvested separately and analyzed for toxicity symptoms, biomass productivity, total chlorophyll content and metal content. In addition, the bioconcentration factors (BCF) for Cd and Zn in *H. umbellata* were determined.

For biomass productivity, treated and control plant samples were dried to a constant weight in an oven (100°C) for 24 hours and weighed after each harvest. The dry weight for each metal concentration and exposure time was expressed as the percentage of biomass productivity relative to controls.

The total chlorophyll content was determined by absorption spectra of plant sample extracts in a spectrophotometer according to the method described by the American Society for Testing and Materials[17] and Mackinney[18]. The absorbance of the extract was measured at both 663 and 645 nm (UV spectrophotometer, CECIL 7200, England).

After harvest, plant samples were thoroughly washed with tap water and rinsed with deionized water. Then, they were separated into shoots and roots, and dried at 85°C in a hot air oven. Total accumulations of Cd and Zn in plant samples were determined by a flame atomic absorption spectrophotometer (FAAS; Perkin Elmer 3100), following digestion with concentrated nitric acid[19].

The BCF is defined as the ratio of metal concentration in the biomass to the initial concentration of metal ion in the feed solution[20]. The BCFs for different concentrations of Cd and Zn at different exposure times of *H. umbellata* were determined by the method of Jain et al.[21].

All measurements were made on samples drawn in triplicate and the data were statistically analyzed (ANOVA) by the Least Significant Difference method (LSD at $P<0.05$) on the SPSS for windows program, after analysis of the homogeneity of variance according to Cochran's test[22].

Results and Discussion

The toxicity symptoms observed in both Cd and Zn treatments were rather similar. *H. umbellata* exhibited chlorosis, stunted growth and no production of new plantlets. In both metals, the symptoms were more severe with the increase in exposure time. On day 12, at 1 mg/L Cd and 50 mg/L Zn, 50% and almost 100% of *H. umbellata* died, respectively.

The effects of Cd and Zn on biomass productivity (% of control) of *H. umbellata* at different concentrations and exposure times are shown in Figure 1. The biomass productivities of plant samples exposed to Cd and Zn at every concentration were significantly lower than those of controls ($P \leq 0.05$). In both metals, the biomass productivity decreased significantly from the first three days for all concentrations. The lowest biomass productivities were found in plant samples treated with Cd at 1 mg/L (60.73 %; Fig. 1A) and Zn at 50 mg/L (44.96%; Fig. 1B).

Both Cd and Zn, at high concentration and long exposure time, are toxic to *H. umbellata*. The plants exposed to Cd and Zn showed decreases in biomass productivity. A decline in biomass productivity may be due to increased tissue permeability and loss of the membrane integrity of the plant tissue[23]. Pahlsson[24] observed cytological abnormalities in Cd-treated plants and found lowered cell division and cellular growth.

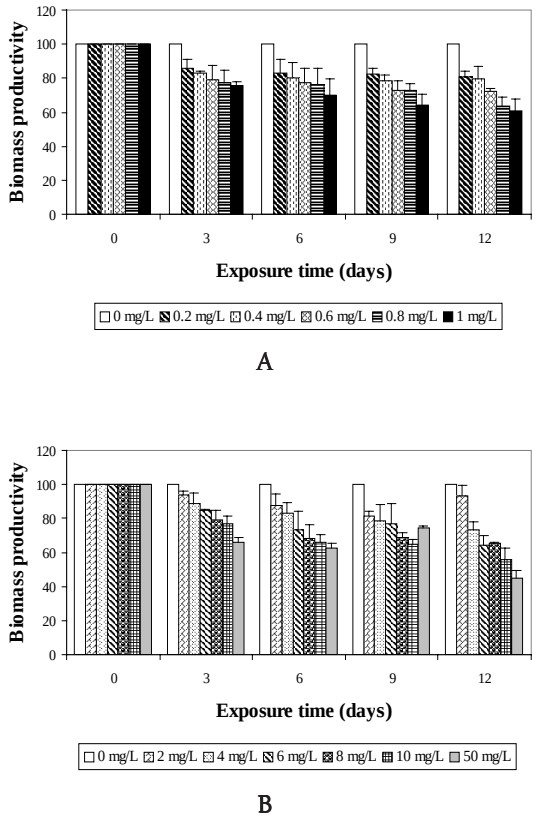

Fig 1. The effects of Cd (A) and Zn (B) on biomass productivity (% of control) of *H. umbellata* at different metal concentrations and exposure times. Error bars represent ± SD (n = 3). Different letters in the same group indicate a significant difference at P≤ 0.05 according to the Least Significant Difference method.

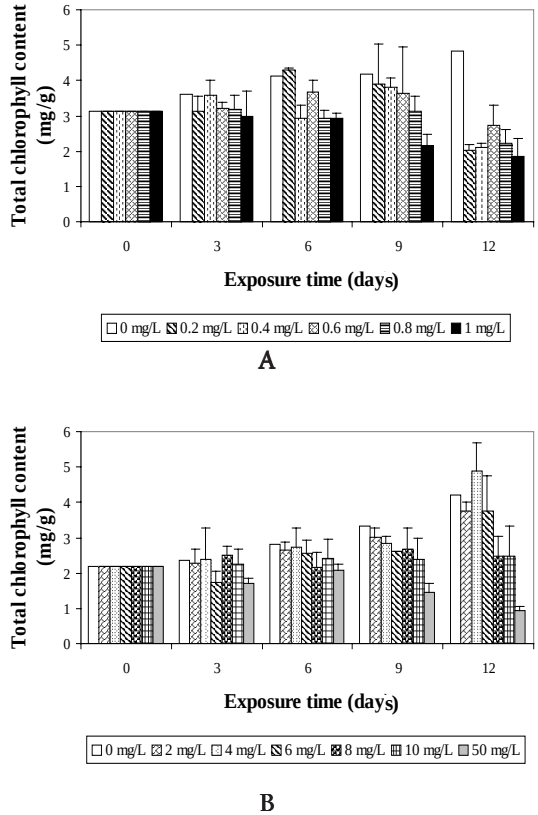

Fig 2. The effects of Cd (A) and Zn (B) on total chlorophyll content of *H. umbellata* at different metal concentrations and exposure times. Error bars represent ± SD (n = 3). Different letters in the same group indicate a significant difference at P≤ 0.05 according to the Least Significant Difference method.

Leborans and Novillo[25] also found that Cd caused a decrease of the cellular volume, of the growth rate and of the level of photosynthetic pigments. However, at low concentrations, the present study showed a stimulation in the relative growth of *H. umbellata* at 2 and 4 mg/L Zn on day 3. It may be noted that the beneficial effect on the growth of plants of Zn at low concentrations may also partially be due to the fact that zinc is an essential micronutrient for plant growth.

Jain *et al*.[21] carried out experiments using *Azolla pinnata* and *Lemna minor* exposed to Cd and Pb and observed similar results. Several studies also showed similar results, such as a reduction in growth of bean plants treated at 96 hours with 5 µM of Cd and 100 µM of Zn[26], a decrease in biomass of pea plants treated with 4 and 40 µM of Cd in hydroponic culture for 7 days[27], and a decrease in biomass of duckweed treated with Cd at 1, 2, 4 and 8 mg/L with increasing concentration and exposure time [28].

The effects of Cd and Zn on the total chlorophyll content of *H. umbellata* at different concentrations and exposure times are shown in Figure 2. There were significant decreases of the total chlorophyll content when the exposure time and metal concentration were increased (P≤0.05). Total chlorophyll contents of *H. umbellata* exposed to Cd at every concentration decreased significantly from those of control after three days of exposure. However, the total chlorophyll content increased with increasing duration of zinc at 2, 4 and 6 mg/L. In Zn treatment, the chlorophyll content at 50 mg/L decreased significantly from the first six days (P≤0.05). The lowest total chlorophyll contents were found in *H. umbellata* exposed to 1 mg/L Cd (0.69 mg/g; Fig. 2A) and 50 mgZn/L (0.94 mg/g; Fig. 2B) on day 12.

The decline in chlorophyll concentrations might be caused by a reduction in the synthesis of chlorophyll, by increasing chlorophyllase activity, by increased

disorderness of chloroplast membrane and interaction of electron transport of photosystem II [23]. In these reports, it was speculated that the metals inhibit the final reductive steps of chlorophyll formation. This was confirmed by the data obtained with leaf discs of barley (*Hordeum vulgare*) floating on Cd containing solutions[29]. This metal was found to inhibit protochlorophyllide reductase activity. Decreases of chlorophyll content imply a reduction in photosynthetic activity, and therefore reduced carbon fixation and possible effects at the whole plant level. In addition, Cd and Zn may inhibit electron transport and photophosphorylation in photosynthesis[30]. Besides this, Cd and Zn may induce stomatal closure that can be indirectly responsible for a decrease in CO_2 fixation in photosynthesis[30]. Pahlsson[24] reported that Cd and Zn are also known to inhibit chlorophyll biosynthesis, leading to lower chlorophyll content. It is well known that Cd can cause disorganization of chloroplasts, modify the mitochondrial structure and affect the photosystems[25].

Cd and Zn accumulation by *H. umbellata* at different concentrations and exposure times is shown in Figs 3 and 4. There were significant increases of metals in plant organs (shoots and roots) when the exposure time and concentration were increased ($P \leq 0.05$). Both metals were found to accumulate more in roots than in shoots. The highest metal contents were 2014 (for Cd) and 7036 μg/g (for Zn) after the plants were exposed to 1 mg/L Cd (Fig. 3B) and 50 mg/L Zn for 12 days (Fig. 4B). Cd was not detected in the control plants at all exposure times. Zn was detected in the controls, because it is a micronutrient, but at very low concentration when compared to treated groups.

H. umbellata possesses the potential to accumulate metals in its tissues. Under the experimental conditions, the accumulations of Cd and Zn by *H. umbellata* were increased when the exposure time and metal concentration were increased. In the present study, it was found that Cd and Zn accumulated more in roots than in shoots. Similar results have been reported by several investigators in different species of plants[1,27,31-36]. The differences in the ability of plants to accumulate heavy metals have been related to differences in their root morphology[37]. It was suggested that a plant with

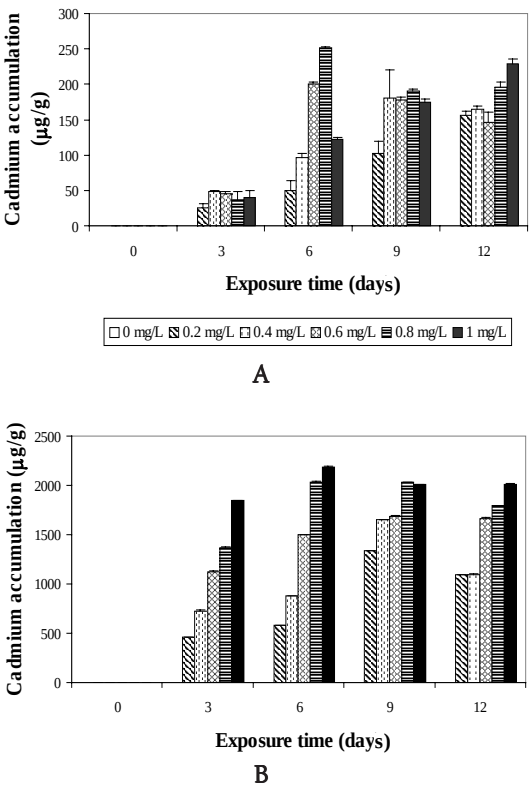

Fig 3. The accumulation of Cd in shoots (A) and roots (B) of *H. umbellata* at different metal concentrations and exposure times. Error bars represent ± SD (n = 3). Different letters in the same group indicate a significant difference at $P \leq 0.05$ according to the Least Significant Difference method.

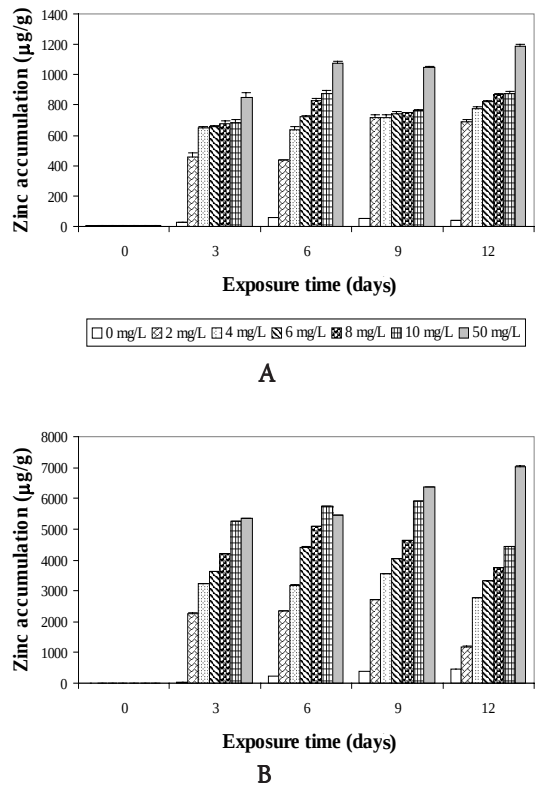

Fig 4. The accumulation of Zn in shoots (A) and roots (B) of *H. umbellata* at different metal concentrations and exposure times. Error bars represent ± SD (n = 3). Different letters in the same group indicate a significant difference at $P \leq 0.05$ according to the Least Significant Difference method.

numerous thin roots would accumulate more metals than one with a few thick roots.

Several studies have found high levels of metal accumulation in aquatic plants such as water milfoil (*Myriophyllum spicatum*) which accumulated 2800 µg/g of Cd [8]. Tokunaga et al. [38] found that in water hyacinth, exposure to 5 mg/L Cd resulted in an ultimate leaf concentration of 1010 µg/g and a root concentration of 2230 µg/g. In comparison, other aquatic plant species were proven to be poor accumulators of metal. Zayed *et al.*[39] reported that the highest concentration of any trace element accumulated in duckweed tissue was 13.3 µg/g of Cd. Bulrush (*Scirpus robutus*) and saltmeadow cordgrass (*Spartina pateus*) accumulated 200 and 250 µg/g when exposed to 0.5 and 1 mg/L of Cd, respectively[39]. In comparison, *H. umbellata* can be considered a good accumulator for Cd and Zn.

The BCFs for Cd and Zn in *H. umbellata* at different concentrations and exposure times are shown in Figure 5. The BCFs of both metals significantly decreased when metal concentrations in the feed solutions were increased at each exposure time ($P \leq 0.05$). The highest BCFs were found in plants treated with 0.2 mg/L Cd (7173) and 2 mg/L Zn (1717) on day 9.

BCF is a useful parameter to evaluate the potential for accumulating metals and this value was calculated on a dry weight basis. In this study, the BCF values of *H. umbellata* in each category of Cd were higher than those of Zn, indicating that the uptake of Cd was better than that of Zn. From the view of phytoremediation, a good accumulator should have the ability to concentrate the trace elements in its tissue, such as a BCF of more than 1000 (100-fold accumulation over control compared on a fresh weight)[39]. Based on these criteria, our results showed that *H. umbellata* is a good accumulator of Cd and Zn with the BCF values of 7173 and 1717, respectively.

Conclusion

Requirements for developing a practical process for bioremoval of heavy metals include low-cost production of plant biomass, ease of removing the biomass from suspension, and the capability to reduce metal concentrations to very low residual levels. The aquatic plants studied, *H. umbellata,* are very easy to harvest and are potentially produced in mass culture. The experimental data presented here indicate that these plants may have promising metal absorbing characteristics. The fact that *H. umbellata* had high BCFs for Cd and Zn at low external concentration is also important for phytoremediation, because, to its advantage, the process is more cost-efficient than other conventional techniques in treating large volumes of wastewater with low concentrations of pollutants. However, since the results obtained were from batch experiments, not continuous flow experiments, the accumulations of Cd and Zn presented here may not be valid under natural field conditions with flow of water, and the presence of dissolved oxygen and rhizosphere microorganism activity. Further studies under simulated field conditions should be done. In addition, since both Cd and Zn are closely associated in the natural environment and their chemical similarity can lead to interaction between these two ions, further work is needed to study the interactive effects (antagonistic and synergistic) of Cd and Zn on heavy metal accumulation in *H. umbellata.*

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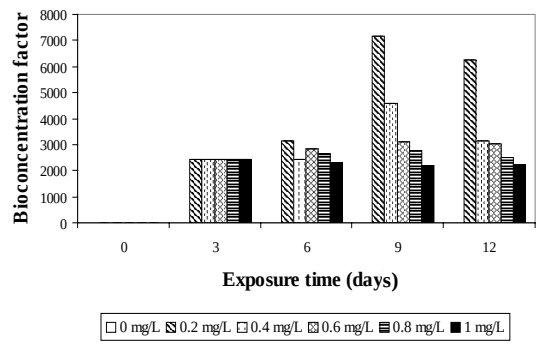

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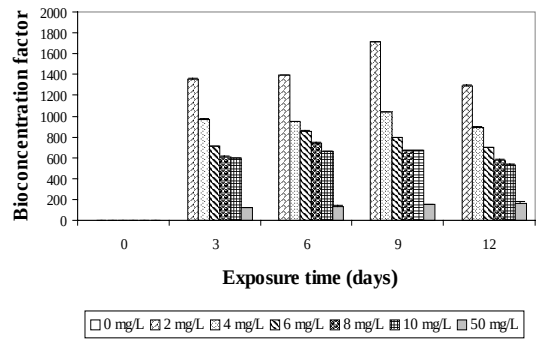

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Fig 5. The bioconcentration factor values of Cd (A) and Zn (B) in *H. umbellata* at different metal concentrations and exposure times. Error bars represent ± SD (n = 3). Different letters in the same group indicate a significant difference at $P \leq 0.05$ according to the Least Significant Difference method.

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