Journal of Environmental Biology
©Triveni Enterprises, Lucknow (India)
Free paper downloaded from: www.jeb.co.in
October 2006, 27(4) 645-652 (2006)
For personal use only
Commercial distribution of this copy is illegal

Toxicity and bioaccumulation of cadmium and lead in *Salvinia cucullata*

Siriwan Phetsombat[1], Maleeya Kruatrachue[2], Prayad Pokethitiyook[1] and Suchart Upatham[3]
[1] Department of Biology, Faculty of Science, Mahidol University, Bangkok 10400, Thailand
[2] Department of Biology, Faculty of Science, and Mahidol University International College, Mahidol University, Bangkok 10400, Thailand, [3]Faculty of Science, Burapha University, Chonburi 20131, Thailand

(Received: 20 June, 2005 ; Accepted: 08 January, 2006)

Abstract: *The toxicity and accumulation of heavy metals, cadmium (Cd) and lead (Pb) in aquatic fern, Salvinia cucullata were studied. Plants were cultured in Hoagland's medium which was supplemented with 0.5,1,2, and 4 mg/l of Cd and 5, 10, and 40 mg/l of Pb and were separately harvested after 2,4,6, and 8 days. The toxicity symptoms of Cd and Pb to S. cucullata showed chlorosis on leaves. There were significant derceases in the relative growth, biomass productivity and total chlorophyll content when the exposure time and concentration were increased. The accumulation study showed the significant increases of both metals when the exposure time and concentration were increased. The roots of S. cucullata had higher Cd and Pb contents than leaves suggesting that the metals were bound to the root cells and were partially transported to the leaves.*

Key words: *Cadmium, Lead, Toxicity, Accumulation, Salvinia cucullata.*

Introduction

In recent years interest has been focused on the study of aquatic plants as promising for pollutant uptake and biological indicators of heavy metals in aquatic ecosystems. Phytotoxicity data for aquatic plants have served a role in regulatory decisions concerning the environmental hazard of most potential contaminant. Aquatic plants presented a variety of algal and macrophyte species in many types of habitat. The use of aquatic plants in water quality assessment has been a common practice for years *in-situ* biomonitors. They have also been used frequently to remove suspended solids, nutrients, heavy metals, toxic organics and bacteria from acid mine drainage, agricultural, landfill, and urban storm-water runoff (Mohan and Hosetti, 1997). Rooted submerged and emergent macrophytes are seldom used in toxicity tests. Their large size and slow growth and lack of established test methods have contributed to this trend. However, the necessity of their use as test species cannot be overlooked due to their ecological significance. In addition, macrophytes are exposed to contaminants through their roots and entire plant surface. Due to their morphology and the recent regulatory focus on contaminated sediments, they may become important test species. Many submerged plants have been used as test species, but there is no widely used single species. The test species may be cultured or obtained from natural or commercial sources. They are exposed in the test chambers containing a nutrient enriched medium, which is often a variation of that used in algal toxicity tested. The tested periods range from several hours to six weeks, and the effects on such parameters as chlorophyll content, peroxidase activity, photosynthesis and growth are monitored.

Studies with macrophytes have been conducted with a variety of metals such as Pb, Cd, Hg, Cr, Cu and several macrophyte species such as *Hydrilla verticillata*, *Potomogenton pectinatus,* and *Vallisneria spiralis* (Jana and Choudhuri, 1982), *Salvinia molesta, Azolla pinnata*, and *Marsilea tninuta* (Gupta and Devi, 1995), *Elodea nuttallia* (Vanderwerff and Pruyt, 1982), and *Lemna* spp. (Wang, 1986 ; Mohan and Hosetti, 1997). There are variations in terms of metal tolerance and ability to accumulate heavy metals among these macrophytes.

In addition, several submerged, emerged and free floating aquatic macrophytes are reported to bioconcentrate heavy metals in natural as well as waste waters. Aquatic macrophytes take up metals from water, producing an internal concentration several folds greater than their surrounding (Greger, 1999). Many aquatic macrophytes are found to be the potential scavenger of heavy metals from aquatic environment and are being used in waste water renovation systems (Kadlec *et al.*, 2000). The benefits of aquatic macrophyte treatment system over conventional method are their natural availability at low operating cost, low energy requirements, offering an alternative to the existing technologies and have potential to effectively remove heavy metals from waste water (Scott, 1992).

Salvinia is an aquatic fern. It is common in tropical and subtropical regions of the world. *Salvinia* grows abundantly in ponds. It is a free floating plant that can be handled easily and economically. It has been used for removal of Hg, Cr, Ni and Pb (Banerjee and Sarker, 1997). The objectives of this study were to study the toxicity and bioaccumulation of Cd and Pb in *Salvinia cucullata* and also to explore the potential of *S. cucullata* in removing Cd and Pb from contaminated water.

Materials and Methods

Culture of plants: *S. cucullata* were collected from natural ponds and cultured in 3% Hoagland's nutrient solution (pH 5.6) in laboratory under controlled conditions (illuminated with a light

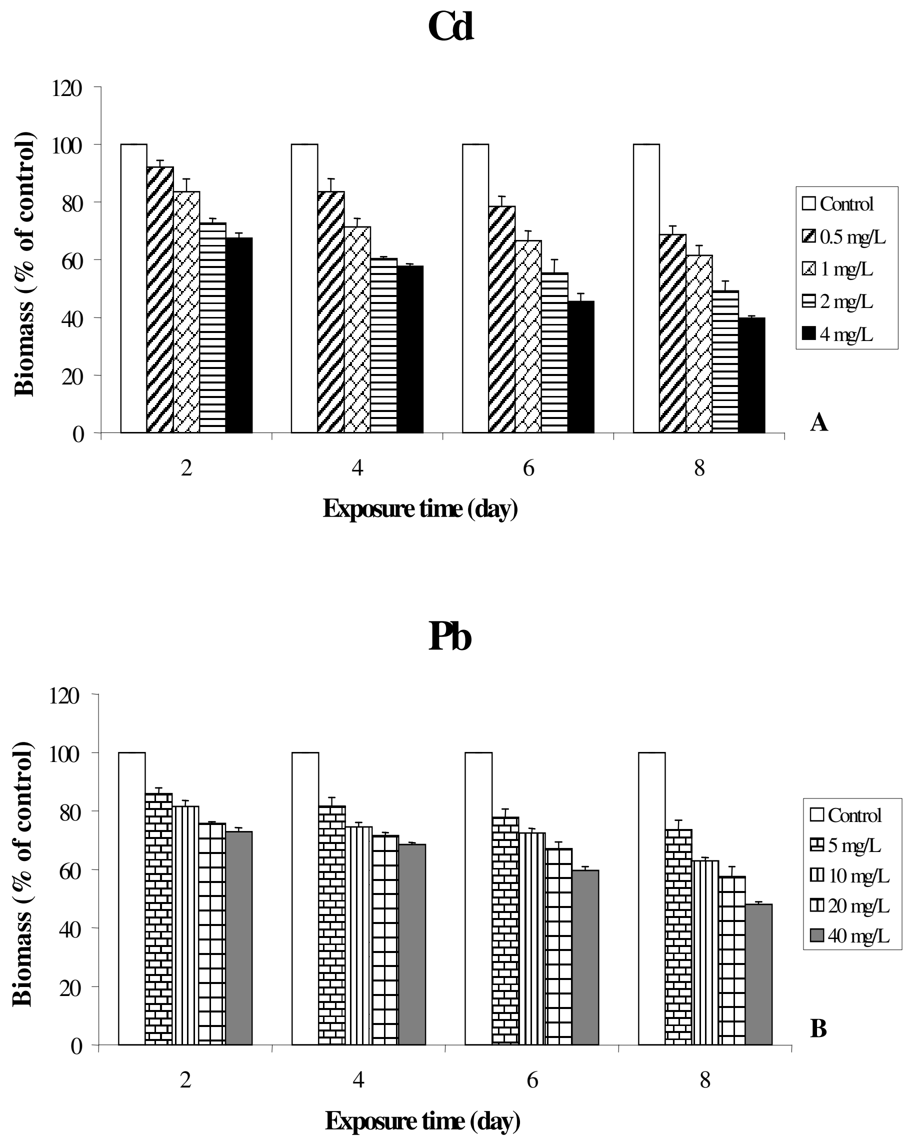

Fig. 1: The effects of Cd (A) and Pb (B) on biomass productivity (% of control) of *S. cucullata* at different concentrations and exposure times.

intensity of 45 µmoles m $^{-2}$s^{-1} , at 12hr/12hr light and dark cycle, under the temperature of 25 ± 2 °C).

Experimental procedures: About 10 g fresh weight of plant was placed in each experimental jar, which contained 300 ml of a control (metal free) and 0.5, 1, 2 and 4 mg/l of Cd solution ($CdCl_2$) and 5, 10, 20 and 40 mg/l of Pb solution [$Pb(No_3)_2$]. The pH of the solutions was adjusted to 5.6. Plant samples from each container were separately harvested after 2, 4, 6 and 8 days to analyze for toxicity symptoms, biomass productivity, total chlorophyll content and metal content. The experiments were set up in triplicate for each concentration and test duration.

Biomass productivity: Plant samples were dried to a constant weight in an oven at 100 °C for 24 hr. The dry weight of plants for each metal concentration and exposure time was expressed as percentage decrease of biomass relative to controls.

Total chlorophyll contents: The chlorophyll content of treated and control plants were measured by the absorption spectra of frond extracts in UV spectrophotometer (CECIL 7200, England) (American Society for Testing and Materials, 1999; MacKinney, 1941). The absorbance of pigment extract was measured at both 663nm (A663) and 645nm (A645).

Heavy metal content: Plants were divided into two parts, leaves and roots. Each part was dried for 24 hr in an oven at 80 °C and weighed. Acid digested plant samples were analyzed for Cd and Pb by a flame atomic absorption spectrophotometer spectrophotometer (Perkin Elmer 3100) (APHA, 1998). The wavelengths used in the determination of Cd and Pb

Table – 1: Cd content in the leaves and roots of *S.cucullata* exposed to various Cd concentrations and exposure times.

Cd conc. (mg/l)	Cd content (µg/g dry wt)									
	Day 0		Day 2		Day 4		Day 6		Day 8	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
Control	0	0	0	0	0	0	0	0	0	0
0.5	0	0	1.4 ± 3.8^{a}	0.5±3.5^{a}	5.1±2.6^{ab}	1.4±3.5^{a}	16.0±4.8^{a}	2.1±4.6^{a}	17.5±4.4^{a}	3.8±4.6^{a}
1	0	0	29.8±2.8^{b}	5.5±6.7^{ab}	60.9±5.0^{a}	6.6±5.4^{ab}	83.1±6.9^{b}	12.5±7.0^{b}	84.6±4.7^{ab}	13.8±6.3^{b}
2	0	0	320.2±3.7^{c}	125.5±4.5^{c}	590.6±3.5^{c}	138.4±4.7^{c}	789.2±6.3^{c}	154.4±3.5^{c}	829.1±6.3^{c}	189.2±4.2^{ab}
4	0	0	767.2±4.3^{d}	223.1±2.8^{d}	830.2±4.9^{d}	290.6±3.7^{d}	1530.3±7.1^{d}	310.1±4.0^{d}	1636.1±9.6^{d}	679.2±5.6^{c}

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

concentration were 228.8 nm and 217 nm, respectively.

Bioconcentration factor: Bioconcentration of chemical by aquatic organisms is generally expressed as the bioconcentration factor (BCF), which is the ratio of substance's concentration sorbed to organism to that dissolved in the surrounding medium (Walker, 1987). It is calculated by the following equation:

$$BCF = \frac{\text{Concentration of metal in dried plant}}{\text{Initial concentration of metal in solution}}$$

Statistical analysis: The mean number of biomass, chlorophyll content and metal content were calculated and subjected to analysis of variance (ANOVA) using randomized block design and least significant different methods (LSD) on the SPSS for windows program after analysis of the homogeneity of variance according to Cochran's test (Winer, 1971).

Results and Discussion

Toxicity symptoms: The toxicity symptoms of *S. cucullata* treated with Cd and Pb at different concentrations and exposure times were quite similar. Chlorosis was observed in the leaves starting from the margin of leaves and extending towards the inner portion of the blades. Finally, they were brown and easily separated from other parts on day 8. The toxicity symptoms increased with increasing concentration and exposure time. However, no morphological changes were observed in the roots of *S. cucullata.* They were still brown in color and healthy looking. The results indicated that roots of *S. cucullata* were more tolerant to heavy metals than leaves.

Biomass productivity: The effects of Cd and Pb on biomass productivity (% of control) of *S. cucullata* are shown in Fig. 1. There were significant decreases of biomass when the exposure time and metal concentration were increased ($p \leq 0.05$). The highest biomass (100%) was found in the control, while the lowest was found at 4 mg/l Cd (39.7%; Fig. 1A) and at 40 mg/l Pb (48.0%; Fig. 1B). Comparatively, the biomass decrease in Cd treated plants was significantly lower than that of Pb treated plants ($p \leq 0.05$), at the highest concentration of metal tested on day 8.

Total chlorophyll content: The effects of Cd and Pb on total chlorophyll content of *S. cucullata* is shown in Fig. 2. The total chlorophyll content of control increased with increasing exposure time. The total chlorophyll contents in treated plants were significantly decreased from that of control ($p \leq 0.05$). The highest total chlorophyll content (2.8 mg/g) was observed in the control, and the lowest was observed at 4 mg/l Cd (0.8 mg/g; Fig 2A) and at 40 mg/l Pb (1.3 mg/g; Fig. 2B), on day 8. Comparatively, at the highest concentration of both metals, the total chlorophyll content of plants treated with Cd was significantly lower than that of Pb treated plants ($p \leq 0.05$).

Heavy metal content: Metals (Cd and Pb) were not observed in the control plants at all exposure times. Cd and Pb contents in the roots and leaves of *S. cucullata* are shown in Tables 1 and 2. The metal contents significantly increased when the exposure time and metal concentration were increased ($p \leq 0.05$). At Cd concentrations of 0.5, 1, 2 and 4 mg/l, the Cd contents in the leaves increased to the maximum levels of 3.8, 13.7, 189.2, and 679.2 µg/g dry wt, respectively, and 17.5, 84.6, 829.0 and 1,636.1 µg/g dry wt in the roots, respectively, on day 8 (Table 1). At Pb concentrations of 5, 10, 20 and 40 mg/l, the Pb contents in the leaves increased to the maximum levels of 273.4, 1040.7, 1340.3 and 3,982.6 µg/g dry wt, respectively and 1,861.8, 7,661.3, 10,064.7 and 14,305.6 µg/g dry wt in the roots, respectively, on day 8 (Table 2). Fig. 3 shows the metal contents in the whole plant at various metal concentrations and exposure times. The highest Cd content (2315.3 µg/g dry wt) was found in plants exposed to 4 mg/l Cd and the highest Pb content (18288.2 µg/g dry wt) was found in plants exposed to 40 mg/l Pb.

The results showed that *S. cucullata* could accumulate Cd and Pb at much higher concentrations in the roots. However, when the biomass productivity was considered together with the accumulation capacity, *S. cucullata* could accumulate high concentrations of Cd (> 100 µg/g) and Pb (>1000 µg/g) and still remained healthy at 2 mg Cd/l and 10 mg Pb/l, respectively.

Bioconcentration factor (BCF): The BCFs of Cd and Pb in *S. cucullata* at different concentrations and exposure times are

Table – 2: Pb content in the leaves and roots of *S.cucullata* exposed to various Pb concentrations and exposure times.

Pb conc. (mg/l)	Pb content (µg/g dry wt)									
	Day 0		Day 2		Day 4		Day 6		Day 8	
	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf	Root	Leaf
Control	0	0	0	0	0	0	0	0	0	0
5	0	0	877.1 ± 8.03[a]	66.1±6.0[a]	1237.4±9.5[a]	176.2±6.5[a]	1522.6±6.4[a]	194.4±2.2[a]	1861.8±5.9[a]	273.4±4.2[a]
10	0	0	4034.4±5.3[b]	580.9±5.1[b]	5652.5±5.9[b]	831.7±5.3[b]	6939.7±6.6[b]	882.5±3.1[b]	7661.3±5.4[b]	1040.7±4.9[b]
20	0	0	5302.1±6.5[c]	632.3±2.6[b]	7241.4±9.5[c]	837.1±4.1[b]	9429.3±7.8[b]	945.4±2.6[b]	10064.4±6.9[c]	1340.3±6.2[bc]
40	0	0	9388.9±7.1[d]	2005.6±3.3[c]	12432.5±8.2[d]	2308.99±2.7[c]	13009.0±9.5[c]	3453.5±6.0[c]	14305.6±7.8[d]	3982.6±2.8[d]

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

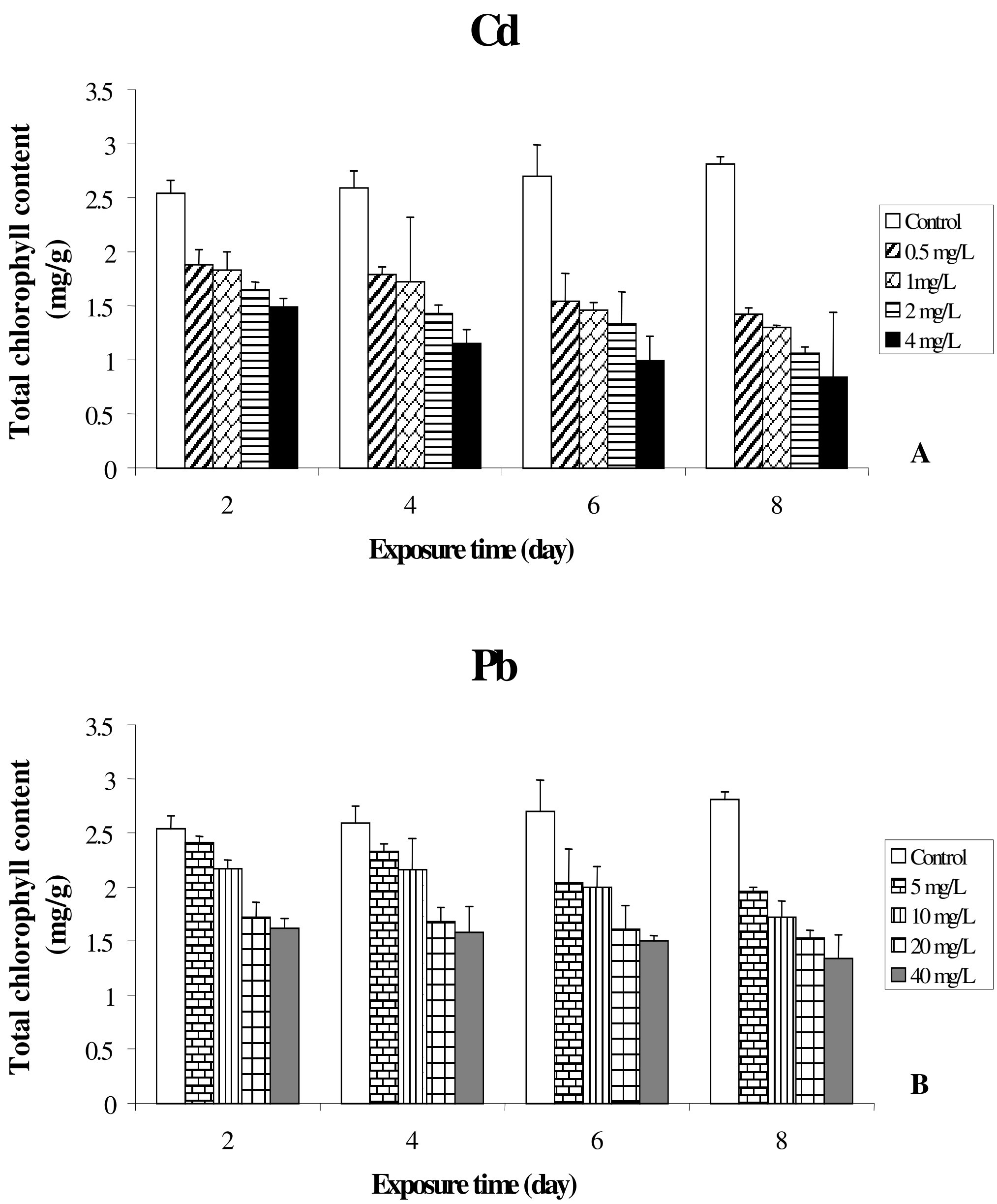

Fig. 2: The effects of Cd (A) and Pb (B) on total chlorophyll content of *S. cucullata* at different concentrations and exposure times.

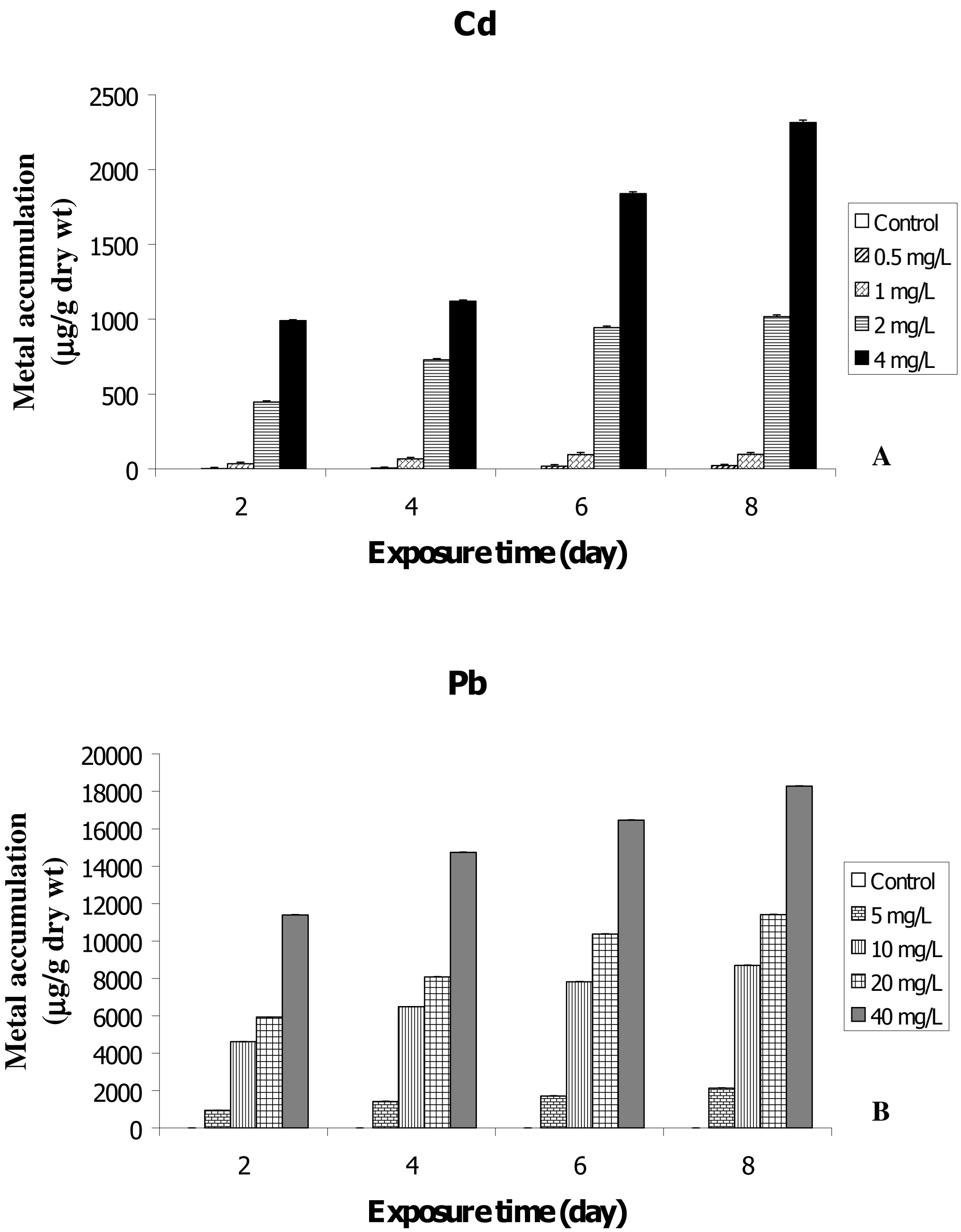

Fig. 3: The accumulation of Cd (A) and Pb (B) by *S. cucullata* at different concentrations and exposure times.

shown in Fig. 4A and 4B, respectively. The BCFs of metals increased when the exposure times were increased. The BCFs of Cd of *S. cucullata* at 0.5, 1, 2, and 4 mg/l were 42.6, 98.3, 492.1 and 578.8 mg/l, respectively on day 8 (Fig. 4A). The BCFs of Pb of *S. cucullata* at 5, 10, 20 and 40 mg/l were 427.1, 870.2, 570.2 and 457.2 mg/l, respectively, on day 8(Fig 4B). The BCFs of Pb in *S. cucullata* were highest at 10 mg/l at all exposure times. The higher value of BCF indicates the ability of plants to concentrate metals in their tissues. Hence, *S. cucullata* could concentrate Pb in their tissues better than Cd. The present study shows that Cd and Pb are toxic to *S. cucullata* as shown by the toxicity symptoms such as chlorosis, decreases in the biomass and total chlorophyll content. The severity of symptoms increased with the increases in metal concentration and exposure time. In comparison, Cd is much more toxic than Pb as shown by the values of biomass and total chlorophyll content. Other studies have also shown that Cd was more toxic than Pb at the same concentration in *L.. minor* (Mohan and Hosetti, 1997), *E. crassipes* (Zhu *et al*., 1999).

The toxicity symptoms observed as the result of Cd and Pb exposure was chlorosis in the leaves of *S. cucullata.* This correlates well with the decrease in total chlorophyll

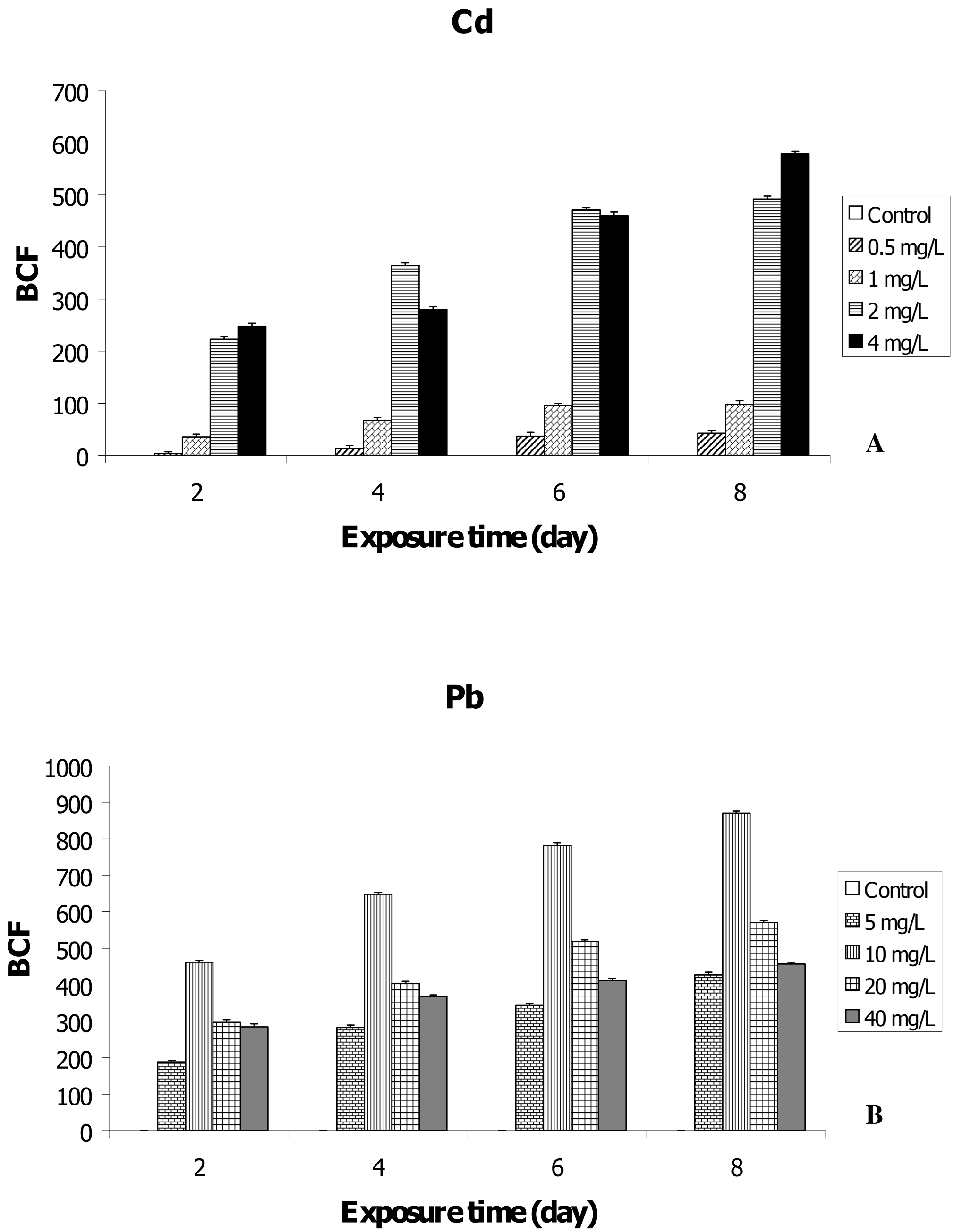

Fig. 4: The bioconcentration factor values of Cd (A) and Pb (B) in *S. cucullata* at different concentrations and exposure times.

content. Similar symptoms were reported in other plants exposed to low concentration of Cd and high concentration of Pb. Heavy metals are reported to inhibit chlorophyll biosynthesis, particularly by inhibiting 2 – aminolevulinic acid hydrogenase and protochlorophyllide reductase (Ouzounidou, 1995). Cd inhibited the synthesis of 5 - aminolevulenic acid and formation of photoreactive and protochlorophyllide complex with its substrate (Gadallah, 1995). Overnell (1975) reported that 0.01- 0.05 mg /l Cd reduced the concentration of ATP and chlorophyll in many species, and decreased oxygen production. While Cd does not appear to inhibit photochemical reductions, it may interfere with different steps of Calvin cycle, resulting in the inhibition of photosynthetic substances and in poisoning of the cell cytoplasm (Pahlasson, 1989). Pb inhibits PSI of photosynthesis and causes iron deficiency, indicating that the light phase of photosynthesis is affected in the treated plants due to the induced iron deficiency in PSII by Pb. Many heavy metals have effectively blocked photosynthetic electron transport at the level of PSII both at oxidizing (donor) and reducing (acceptor) site (Clijster and Van Assche, 1985; Krupa and Baszynski, 1995).

Another toxicity symptom observed as the result of Cd and Pb exposure was decrease in biomass of *S. cucullata* when the exposure time and metal concentration were increased. Both Cd and Pb were found to inhibit growth in many species of aquatic plants such as *Lemna* species (Huebert and Shay, 1993; Miranda and Hangovan, 1978; Mohan and Hosetti, 1997), *Eichhornia* species (Brown and Rattigan, 1979; Zhu *et al*., 1999), *Spirodela* species (Oron *et al*., 1984; Banerjee and Sarker, 1997). Cd was shown to interfere with the increased tissue permeability, hence the increase in toxicity (Sen and Mondal, 1987), the increased cross linking of pectins in the middle lamella of cell wall which might inhibit cell expansion (Poschenrieder *et al.,* 1989), and the direct and indirect effect on the growth hormone, auxin metabolisms or auxin carriers. Similarly, Pb also causes severe impairment of plant metabolic processes including the photosynthetic activity and the induction of enzyme peroxidase that is involved in the degradation of indoleacetic acid which stimulates plant growth and multiplication (Hoffman *et al*., 1985).

S. cucullata possesses the potential to accumulate metals in its tissue. The results revealed that, under the experimental conditions, the accumulations of Cd and Pb by *S. cucullata* were increased when the exposure time and concentration of metal were increased. In the present study, *S. cucullata* accumulated metals to the highest concentration of 679.2 μg Cd/g in the leaves and 1631.1 μg Cd/g in the roots and of 3982.9 μg Pb/g in the leaves and 14305.6 μg Pb/g in the roots, respectively. The accumulation of Cd and Pb in various parts of aquatic macrophytes under laboratory conditions had been reported in several species of aquatic plants, for example water hyacinth (Tokunaga *et al*., 1976), water milfoil (*Myriophyllum aquatictum)* (Mutsunaga *et al*., 1999), water lettuce (*Pistia stratiotes*; Maine *et al*., 2001), *A. pinnnata*, *L. minor* (Jain *et al*., 1990). Metal concentrations were reported to be higher in the roots in most studies (Cataldo *et al*., 1981; Rauser, 1987). In addition, the difference in the ability of plants to accumulate heavy metals has been related to differences in their root morphology (Hemphill, 1972; Schierup and Larsen, 1981). The latter suggested that a plant with numerous thin roots would accumulate more metal than one few thick roots. *S. cucullata* possess numerous thin roots and they were shown to accumulate both Cd and Pb at the highest concentration in the roots.

The bioconcentration factor is defined as the ratio of metal concentration in the plant to the initial concentration of metal in the feed solutions. In this study, the BCF values of *S. cucullata* in each group of Pb was significantly higher than those in each group of Cd, indicating that the uptake of Pb was better than that of Cd. The ambient metal concentration in water was the major factor influencing the metal uptake efficiency (Rai and Chandra, 1992). Some aquatic plant species have been shown to exhibit higher accumulation of Pb and Cd with very high BCFs of Pb ranging from 2133 to 8064 (8064 for coontail, 2521 for giant duckweed, 2133 for musk-grass and 7174 for *Hygrorhiza*), which would also qualify them as good phytoremediator (Rai and Chandra, 1992). In the present study, the highest BCF of Cd in *S.cucullata* was 578.8 mg/l (at 4 mg Cd/L) while that of Pb was 870.2 (at 10 mg Pb/L). From the view of phytoremediation, a good accumulator should have the ability to concentrate the element in the tissue, for example a BCF more than 1000(100- fold compared on a fresh weight; Zayed *et al*., 1998). Based on these criteria, our results indicated that *S.cucullata* was a moderate accumulator of Cd and a good accumulator of Pb.

The results of the present study indicated that *S. cucullata* is a promising plant for metal accumulation and it may be used as a biological agent in phytoremediation. *S. cucullata* could be employed to remediate either waste water effluent streams or water bodies contaminated with Cd and Pb. The uptake of metal ion from water is influenced by various parameters such as temperature, pH and exposure time. However a suitable harvesting system will be required for obtaining meaningful results. The harvested *S. cucullata* tissue, rich in Cd and Pb can be processed easily.

Acknowledgments

This study was supported by the grants from the Post-Graduate Education, Training and Research Program in Environmental Science, Technology and Management under Higher Education Development Project of the Commission on Higher Education, Ministry of Education, and Mahidol University, Bangkok, Thailand.

References

American Society for Testing and Materials: Standards on biological effects and environmental fate. 2nd ed. Baltimore, Maryland (1999).

APHA.: Standard methods for the examination of water and wastewater. 20nd ed. APHA, AWWA, WEF, Washington D.C.(1998).

Banerjee, G. and S. Sarker: The role of *Salvinia rotundifolia* in scavenging aquatic Pb (II) pollution: a case study. *Bioproc. Engr*., **17**, 295-260 (1997).

Brown, B.T. and B.M. Rattigan: Toxicity of soluble copper and other metal ions to *Elodea canadensis. Environ. Pollut*., **20,** 303-314 (1979).

Cataldo, C.A., T.R. Garland and R.E. Wildung: Cadmium distribution and chemical fate in soy bean plants. *Plant Physiol*., **68**, 835-839 (1981).

Clijster, H. and F. Van Assche: Inhibition of photosynthesis by heavy metals. *Photosynth. Res*., **7**, 40-41 (1985).

Gadallah, M.A.A.: Interactive effect of heavy metals and temperature on the growth and chlorophyll, saccharides and soluble nitrogen in *Phaseolus* plants. *Biol. Plant.,* **36,** 373-382 (1995).

Greger, M.: Metal availability and bioconcentration in plants. *In*: Heavy metal stress in plants-from molecules to ecosystems (*Eds*: M.N.V. Prasad and J. Hagemeyer). Springer, Berlin, pp.1-27 (1999).

Gupta, M. and S. Devi: Uptake and toxicity of cadmium in aquatic ferns. *J. Environ. Biol*., **16(2)**, 131-136. (1995).

Hemphill, D.D.: Availability of trace elements to plants with respect to soil plant interaction. *Ann. New. York. Acad. Sci.,* **199,** 46-61 (1972).

Hoffman, D.J., B.A. Ratther, G.A. Burton and J. Carins : Handbook of ecotoxicology of laboratory organisms for assessing hazardous waste sites. Lewis Publisher, Florida, pp.163-184 (1985).

Huebert, D.B. and M. Shay: The response of *Lemna trisulca* L. to cadmium. *Environ. Pollut*., **80,** 247-253 (1993).

Jain, S.K., P. Vasudevan and N.K. Jha: *Azolla pinnata* and *Lemna minor* for removal of lead and zinc polluted water. *Water Res*., **24,** 177-184 (1990).

Jana, S. and M.A. Choudhuri: Senescence in submerged aquatic angiosperms: Effects of heavy metals. *New. Phytol*., **90,** 477-484 (1982).

Kadlec, R.H., R.L. Knight, J. Vymazal, H. Brix, P. Cooper and R. Haberl: Constructed wetlands for pollution control - Processes, performance, design and operation. IWA Publisher, London, pp. 1- 156 (2000).

Krupa, Z. and T. Baszynski: Some aspect of heavy metal toxicity towards photosynthetic apparatus-Direct and indirect effects on light and dark reactions. *Acta Physiol. Plant*., **17,** 177-190 (1995).

MacKinney, G.: Absorption of light by chlorophyll solution. *J. Biol. Chem*., **144,** 315-323 (1941).

Maine, A.M., M.V. Duarte and N. Sune: Cadmium uptake by floating macrophyte. *Water Res.,* **35,** 2629-2634 (2001).

Miranda, M.G. and K. Hangovan: Uptake of boron by *Lemna minor*. *Aquat. Bot.,* **4,** 53-64 (1978).

Mohan, B.S. and B.B. Hosetti: Potential phytotoxicity of lead and cadmium to *Lemna minor* grown in the sewage stabilization ponds. *Environ. Pollut*., **2,** 133-238 (1997).

Mutsunaga, T., H. Takeyama, T. Nakao and A. Yamazawa: Screening of marine microalgae for bioremediation of cadmium polluted seawater. *Biotechnol.,* **70,** 33-38 (1999)

Oron, G., L.R Wildschut and D. Porath: Waste water recycling by duckweed for protein production and effluent renovation. *Water Sci. Technol*., **17,** 803-817(1984).

Ouzounidou, G.: Cu-ions mediated changes in growth, chlorophyll and other ion contents in a Cu tolerant *Koeleria splendens*. *Biol. Plant*., **37**, 71-79 (1995).

Overnell, J.: The effect of some heavy metal ions on photosynthesis in a fresh water alga. *Pesticides Biochem. Physiol*., **5**, 19-26 (1975).

Pahlasson, A.M.B.: Toxicity of heavy metals (Zn, Cd, Cu, Pb) to vascular plants. *Water Air Soil Pollut*., **47,** 278-319 (1989).

Poschenrieder, C.H., B. Gunse and J. Barcelo: Influence of cadmium on water relations, stomatal resistance and abscisic acid content in expanding leaves. *Plant Physiol.,* **90**, 1365-1371 (1989).

Rai, U.N. and P. Chandra: Accumulation of copper, lead, manganese and iron by field population of *Hydrodictyon reticulatum* Lagerheim. *Sci. Total Environ*., **116**, 203-211 (1992).

Rauser, W.E.: Compartmental efflux analysis and removal of extracellular cadmium from roots. *Plant Physiol.,* **85**, 62-65 (1987).

Schierup, H.H. and V.J. Larsen: Macrophyte cycling of zinc, copper, lead and cadmium in the littoral zone of a polluted and a non-polluted lake. 1. Availability, uptake, and translocation of heavy metals in *Phragmites australis. Aquat. Bot*., **11**, 197-210 (1981).

Scott, C.D.: Removal of dissolved metals by plant tissue. *Biotech. Bioeng*., **39**,1062-1068 (1992).

Sen, A.K. and N.G. Mondal : *Salvinia natan*-as the scavenger of Hg. *Water Air Soil Pollut.,* **34**, 439-466 (1987).

Tokunaka, T., N. Furuta and M. Morimoto : Accumulation of cadmium in *Eichhornia crassipes*. *J. Hyg. Chem*., **22,** 234-239 (1976).

Vanderwerff, M. and M.J. Pruyt: Long-term effects of heavy metals on aquatic plants. *Chemosphere*, **11,** 727-739 (1982).

Walker, C.H.: Kinetic models for predicting bioconcentration factors. *Environ. Pollut*., **44,** 227-239 (1987).

Wang, W.: Toxicity tests of aquatic pollutant by using common duckweed. *Environ. Pollut.*, **11,** 1-14 (1986).

Winer, B.J.: Statistical principles in the experimental design. McGraw-Hill, New York (1971).

Zayed, A., S. Gowthman and N. Terry: Phytoaccumulation of trace elements by wetland plants: I. Duckweed. *J. Environ. Qual*., **27,** 715-721 (1998).

Zhu, Y.L, A.M. Zayed, J.H. Qian, M. Souza and N. Terry: Phytoaccumulation of trace elements by wetland plants: Water hyacinth. *J. Environ. Qual.,* **28,** 339-344 (1999).

Correspondence to :
Dr. M. Kruatrachue
Department of Biology, Faculty of Science
Mahidol University, Rama 6 Road
Bangkok 10400, Thailand
E-mail: scmkt@mahidol.ac.th
Tel.: +66-2-201-5483
Fax: +66-2-354-7166