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Toxicity and Accumulation of Lead and Cadmium in the Filamentous Green Alga *Cladophora fracta* (O.F. Müller ex Vahl) Kützing: A Laboratory Study

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ABSTRACT: The toxicity and accumulation of the heavy metals, lead (Pb) and cadmium (Cd) in a common filamentous green alga, *Cladophora fracta*, were studied. *C. fracta* were cultured in a modified Chu No. 10 medium, which was supplemented with 5, 10, 20, 40 or 80 mg/L of Pb or 0.5,1, 2, 4 or 8 mg/L of Cd, and were separately harvested after 2, 4, 6 and 8 days. The toxicity symptoms of Pb and Cd to *C. fracta* showed damage and reduced number of chloroplasts, disintegrated cell wall and death. There were significant decreases in the relative growth and total chlorophyll content when the exposure time and concentration were increased. The accumulation study showed that there were significant increases of metal levels in algal tissue when the exposure time and concentration were increased. The bioconcentration factor (BCF) of Pb was higher than that of Cd at the same duration, suggesting that the accumulation potential of *C. fracta* for Pb was higher than that for Cd.

KEYWORDS: *Cladophora fracta,* lead, cadmium, toxicity, accumulation.

INTRODUCTION

Water pollution by heavy metals in industrial waste effluents is now a global problem[1]. The non-degradability of inorganic pollutants like heavy metals creates a hazard when they are discharged into a water body. The main sources of heavy metal pollution are mining, milling and surface finishing industries, which discharge a variety of toxic metals into the environment[2]. Industrial effluents may be discharged directly into the sea, or into waterways or sewer but whatever the disposal route, these constitute an important source of contamination of the environment[3]. Many industries discharge the heavy metals lead (Pb) and cadmium (Cd) in their wastewaters[4]. Lead and cadmium are toxic heavy metals and are considered non-essential for living organisms. They are being used in a wide variety of industrial processes in Thailand, for example, the use of Pb in battery, paint and ammunition, and the use of Cd as a coloring agent, a stabilizer and in alloy mixtures. Thus, they were selected as toxicants in the present study.

Traditional technologies for the removal of heavy metals, such as chemical reduction and precipitation or ion exchange, are often ineffective and/or very expensive when used for the removal of heavy metal ions to very low concentrations. Moreover, these methods are specific to each metal ion. New technologies are required that can reduce heavy metal concentrations to environmentally acceptable levels at affordable costs. Bioremoval offers a potential alternative to existing methods and is defined as the accumulation and concentration of pollutants from aqueous solutions by the use of biological materials[5]. Bioremoval of heavy metals is one of the most promising technologies involved in the removal of toxic metals from industrial waste streams and natural waters. It is a potential alternative to conventional processes for the removal of metals[6]. The major advantages of the bioremoval technology are its effectiveness in reducing the concentration of heavy metal ions to very low levels, and its use of inexpensive biosorption materials and environmentally friendly technologies[7]. Bioremoval is most effective in treatment of waters containing low concentrations of cationic heavy metals. It has been exploited to observe metal contamination in water bodies where the contaminant level is below detection limits.

Algae accumulate heavy metals from their aquatic environment. Using living algae to remove toxic metals from contaminated water could be advantageous, since they are ubiquitous and have colonized almost all parts of the world. They can be grown easily and have very simple growth requirements. An advantage of using living organisms over dead biomass is that they have fast growth rates and hence produce a regenerating supply of metal – removal material[8]. There is much evidence that algae could accumulate heavy metals in their tissues when grown in polluted waters, including the species *Ulva rigida* (Fe, Mn)[9], *Padina gymnospora* (Zn)[10], *Gracilaria tenuistipitata* (Cd)[11], *Undaria pinnatifida* (Pb)[4], *Cladophora* sp. (Cd)[8], and *Cladophora glomerata* (Zn)[12].

Cladophora sp. is a common filamentous green alga in lake shores, rivers and irrigation channels. It is possibly the most ubiquitous freshwater macroalgae worldwide[13]. Metal binding to non-living cells of *Cladophora* sp. is well documented[14-17]. However, there are only a few reports on the metal uptake potential of living *Cladophora* sp. and the metal toxicity to this genus[8,12]. Hence, the purposes of this study were to examine the Pb and Cd accmulation and their toxicities to *Cladophora fracta*.

Materials and Methods

Algal Materials

Cladophara fracta (O.F. Müller ex Vahl) Kützing were collected from natural ponds and grown in a modified Chu No.10 medium[18] in the laboratory under controlled conditions (25 ± 2°C, 45 mmol $m^{-2}s^{-1}$ photon flux intensity, 16h/8h light and dark cycle). The final pH of the solution was 5.0. Two-week-old *C. fracta* was used in the experiments.

Toxicity and Accumulation

The modified Chu No. 10 medium was supplemented with five nominal concentrations of Pb prepared from $Pb(NO_3)_2$ (5, 10, 20, 40 and 80 mg/L) and Cd prepared from $CdCl_2$ (0.5, 1, 2, 4 and 8 mg/L). The final pH of the solutions were adjusted to 5.0. One gram fresh weight of algae was inoculated into each flask containing various concentrations of Pb and Cd. Algae cultured in the nutrient medium without heavy metals served as controls. All experiments were performed in triplicate.

Toxicity Symtoms After the exposure of *C. fracta* to Pb and Cd, the algae were harvested at the end of each test duration (2, 4, 6 and 8 days). Toxicity symptoms of treated and control algae were observed under a compound transmission light microscope.

Relative Growth Treated and control algae were gently blotted and weighed after each harvest on day 2, 4, 6 and 8. Relative growth of control and treated algae were calculated as follows:

$$\text{Relative growth} = \frac{\text{Final fresh weight (g)}}{\text{Initial fresh weight (g)}}$$

Total Chlorophyll Content The total chlorophyll content was determined by the absorption spectra of algal extract in a spectrophotometer according to the methods described by Arnon[19] and MacKinney[20]. The absorbance of the extract was measured at both 663 and 645 nm.

Metal Accumulation The procedures of digestion of algal materials were performed according to Anderson[21] and Katz and Jennis[22]. Total accumulations of Pb and Cd in algae were determined using a flame atomic absorption spectrophotometer[23].

Bioconcentration Factor (BCF) The BCF was calculated for quantifying the metal removal potential of the plants. The factor is defined as the ratio of the metal concentration in the dry plant biomass (ppm) to the initial concentration of metal in the feed solution (ppm)[24]. The BCFs for Pb and Cd of *C. fracta* at different concentrations and exposure times were determined[25].

Statistical Analysis

The mean values of relative growth, total chlorophyll content, and metal accumulation were calculated and subjected to analysis of variance (ANOVA) using randomized block design and Least Significant Difference method (LSD) on the SPSS for Windows program after analysis of the homogeneity of variance according to Cochran's test[26].

Results

Toxicity Symptoms

The toxicity symptoms observed in both Pb and Cd treatments were rather similar. The symptoms included the damage of chloroplasts, reduction in number of chloroplasts, disintegrated cell wall and cell death. These symptoms were more severe when the metal concentration and exposure time were increased.

Relative Growth

The effects of Pb and Cd on the relative growth of *C. fracta* at different concentrations and exposure times are shown in Figure 1. The relative growth of algae exposed to Pb and Cd at every concentration were significantly decreased ($P \leq 0.05$) from those of controls. At high metal concentrations, algal growth was reduced (50%) after 8 days. The lowest relative growth was found in algae treated with Pb at 80 mg/L (0.52; Fig. 1A) and Cd at 8 mg/L (0.47; Fig. 1B) on day 8.

Total Chlorophyll Content

The effects of Pb and Cd on total chlorophyll

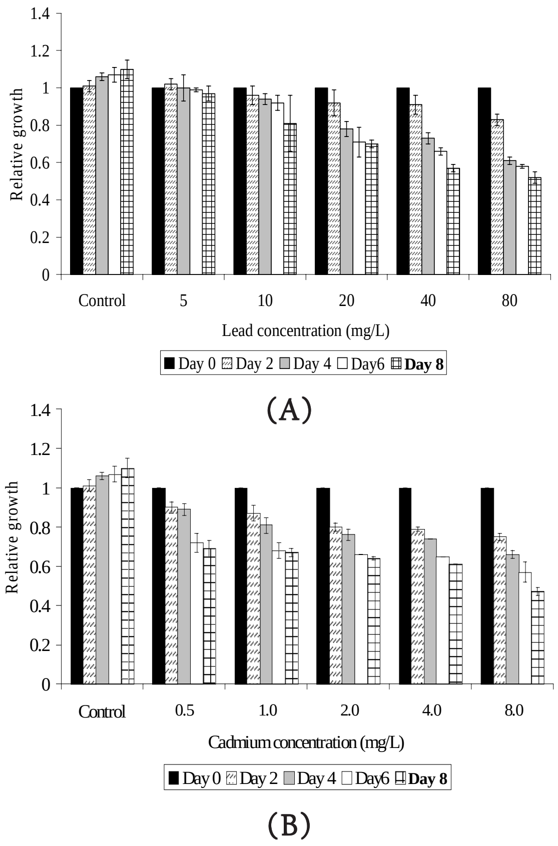


Fig 1. The effects of Pb (A) and Cd (B) on relative growth of *C. fracta* at different concentrations and exposure times.

content of *C. fracta* at different concentrations and exposure times are shown in Figure 2. There were significant decreases ($P \leq 0.05$) of total chlorophyll content when the exposure time and metal concentration were increased. Total chlorophyll contents of *C. fracta* exposed to Pb and Cd at every concentration decreased significantly from those of controls after two days of exposure. The lowest total chlorophyll contents were found in algae exposed to 80 mg/L of Pb (1.0; Fig. 2A) and 8 mg/L of Cd (1.1; Fig. 2B) on day 8.

Metal Accumulation

Pb and Cd accumulations by *C. fracta* at different concentrations and exposure times are shown in Figure 3. There were significant increases ($P \leq 0.05$) of metals in algal tissue when the exposure time and metal concentration were increased. At Pb concentrations of 5, 10, 20, 40 and 80 mg/L, the Pb accumulated in *C. fracta* were 6,170, 11,900, 21,600, 40,000 and 61,400 mg/g dry wt, respectively on day 8 (Fig. 3A). At Cd concentrations of 0.5, 1, 2, 4 and 8 mg/L, the Cd accumulated in *C. fracta* were 603, 1,160, 1,680, 2,630

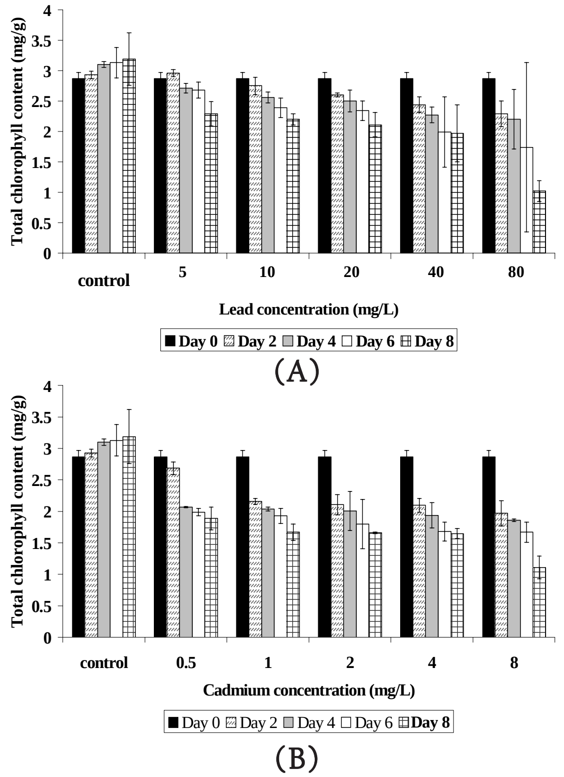


Fig 2. The effects of Pb (A) and Cd (B) on total chlorophyll content of *C. fracta* at different concentrations and exposure times.

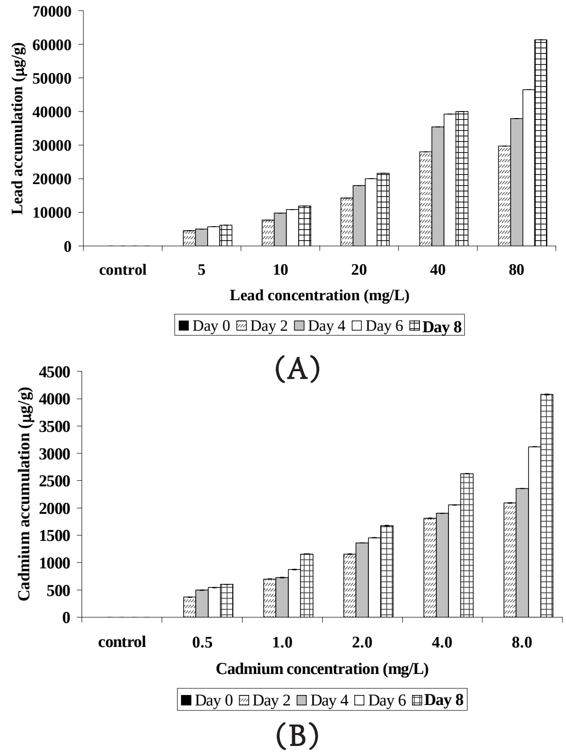
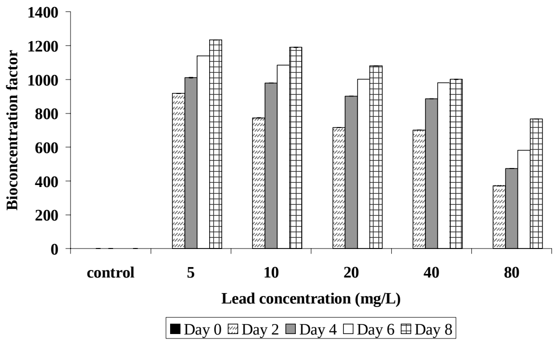


Fig 3. The accumulation of Pb (A) and Cd (B) by *C. fracta* at different concentrations and exposure times.

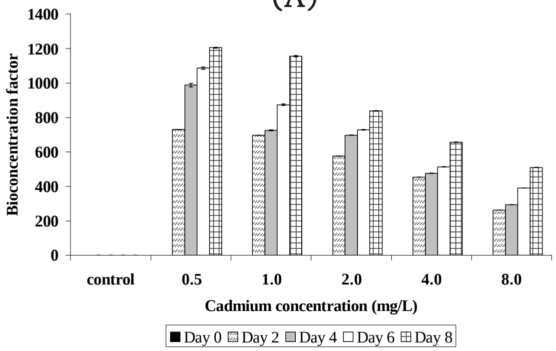
and 4,080 mg/g dry wt, respectively on day 8 (Fig. 3B). The metals were not detected in the controls.

Bioconcentration Factor

The BCFs for Pb and Cd in *C. fracta* at different concentrations and exposure times are shown in Fig. 4. The BCFs of both metals significantly decreased ($P \leq 0.05$) when metal concentrations in feed solutions were increased at each exposure time. On day 8, the BCFs of Pb at 5, 10, 20, 40 and 80 mg/L were 1,230, 1,190, 1,080, 1,000 and 767, respectively (Fig. 4A), while those of Cd at 0.5, 1, 2, 4 and 8 mg/L were 1,205, 1,160, 838, 657 and 510, respectively (Fig. 4B).



(A)



(B)

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

Discussion

In the toxicity symptom study, *C. fracta* exposed to Pb and Cd showed a reduction in number of chloroplasts when compared with those of the control. Cell walls were crumbled and chloroplasts were severely damaged in most cases at high concentrations of metals, but the symptoms were not specific. Similar results were obtained from the toxicity symptom study of metal on *Chlorella*[27]. *Chlorella* cells were useful in the characterization of the toxicity of both metals and organic contaminants. The chloroplast is the organelle most affected by metal contamination. In Ni- treated plants (*Brassica oleracea*), the higher the Ni concentration, the smaller the number of chloroplasts in mesophyll cells[28]. The number and size of chloroplasts decreased, and their internal membranes (especially grana) were reduced and swollen. Uptake and excess of metals by plants and algae can initiate a variety of metabolic reactions, finally leading to global phytotoxic responses, *e.g.*, dwarf growth and chlorosis. They are generally considered to affect membrane permeability and to induce cell decompartmention. An important harmful effect of metals at the cellular level is the alteration of the plasma membrane permeability, leading to leakage of ions like potassium and other solutes[29].

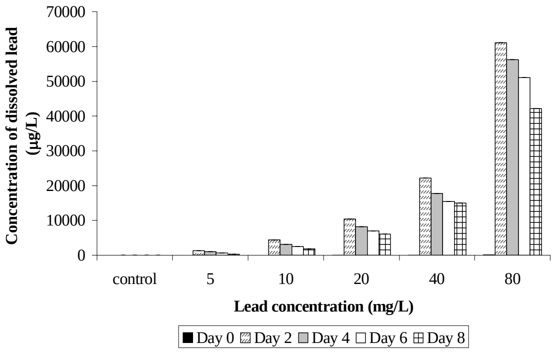
In the present study, the relative growth of *C. fracta* exposed to Pb and Cd decreased significantly when the exposure time and metal concentration were increased. Miranda and Hangovan[30] studied the Pb influence on specific growth rate of *Lemna gibba*. They found that high Pb concentrations (200-500 mg/L) in the media significantly inhibited the specific growth rate of *L. gibba* under continuous and discontinuous illumination. This might be due to the fact that Pb induces the activity of the enzyme peroxidase that is involved in the degradation of indoleacetic acid (IAA), the hormone which stimulates plant growth and multiplication.

Several studies have reported on the effects of Cd and algal growth. Fargasova[31] studied the effects of Cd, Cu, Zn, Pb and Fe on the green alga *Scenedesmus quadricauda* and found that the toxicity for all the observed parameters increased with the concentration of these metals in the cultivation medium. Lasheen et al[32] reported that Cd had slight inhibitory effects on algal growth at low concentration (0.05 mg/L), while it severely inhibited algal growth at higher concentrations (>1.0 mg/L). Leborans and Novillo[33] found that Cd caused a decrease of the cellular volume, the growth rate and of the level of photosynthetic pigments.

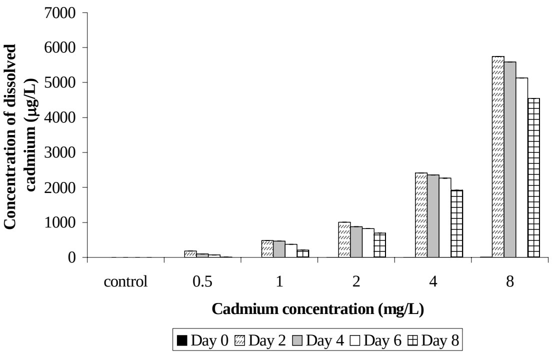
The total chlorophyll content of *C. fracta* significantly decreased when the exposure time and Pb or Cd concentration were increased. Pb and Cd at high concentrations destroyed chloroplasts of *C. fracta,* as shown in the toxicity symptom study. It is well known that Cd can cause disorganization of chloroplasts leading to a reduction of the photosynthetic pigments[33]. Both Cd and Pb were reported to inhibit chlorophyll biosynthesis, leading to the lowered chlorophyll contents[34]. Sen and Mondal[35] reported that the decline in chlorophyll content might be caused by a reduction in the synthesis of chlorophyll, possibly by increasing chlorophyllase activity, by disorderness of chloroplast membrane and by inactivation of electron transport in photosystem I.

C. fracta possess the potential to accumulate metals in their tissues. The results revealed that, under the experimental condition, the accumulations of Pb and Cd by *C. fracta* were increased when the exposure time and metal concentration were increased. Similarly, Costa and Leite[36] found that the amount of Cd and Zn accumulated by *Chlorella* was dependent on the external metal concentration, with increasing metal accumulation at increased external metal concentrations. The total Pb sorption by *Stichococcus bacillaris* cells increased with the increasing external Pb concentration and time of exposure[37]. Maine et al[38] also concluded that both initial Cd concentration and time had a statistically significant effect on the sorption of Cd by *Pistia stratiotes*.

In the present study, *C. fracta* accumulated Pb and Cd to the highest concentrations of 61,400 mgPb/g when exposed to 80 mg Pb/L, and 4,090 mgCd/g when exposed to 8 mg Cd/L (Figure 5). Several studies have found high levels of metal accumulation. Water milfoil (*Myriophyllum spicatum*) exposed to 16 mg Pb/L and 16 mg Cd/L could accumulate 36,500 mgPb/g and 2,800 mgCd/g, respectively[39]. *Chlorella vulgaris* accumulated 5,000 mgCd/g when exposed to 50 mM Cd[40]. Tokunaga et al[41] found that a water hyacinth exposed to 5 mg/L of Cd attained in an ultimate leaf concentration of 1,010 mgCd/g and a root concentration of 2,230 mgCd/g. In comparison, other algae and aquatic plant species were proven to be poor accumulators of metals. Zayed et al[42] reported that the highest concentrations of each trace element accumulated in duckweed tissue were 13.3 mgCd/g and 0.63 mgPb/g when treated with 10 mg Cd/L and 10 mg Pb/L, respectively. Bulrush (*Scirpus robutus*) and saltmeadow cordgrass (*Spartina patens*) accumulated 200 and 250 mgCd/g when exposed to 0.5 and 1.0 mg/L Cd, respectively[43]. In comparison, *C. fracta* can be considered a good accumulator for Pb and Cd.



(A)



(B)

Fig 5. Concentrations of dissolved Pb (A) and Cd (B) remaining in the culture media of *C. fracta* at different concentrations and exposure times.

BCF is a useful parameter to evaluate the potential of plants for accumulating metals and this value was calculated on a dry weight basis[24]. In this study, the BCF values of *C. fracta* in each group of Pb were significantly higher than those in each group of Cd, indicating that the uptake of Pb was better than that of Cd. There was a gradual decrease in the Pb and Cd uptake potential with an increase in Pb and Cd concentration in feed solutions. The ambient metal concentration in water is the major factor influencing the metal uptake efficiency[44-45] and concomitantly the BCF values, as found in the present study. Similar experiments and similar results have also been reported by Zhu et al[46] who found that BCFs of water hyacinth were very high for Cd, Cu, Cr and Se at low external concentration, and they were decreasing as the external concentration increased. Zayed et al[42] reported that duckweed bioconcentrated the six elements (Cu, Se, Pb, Cd, Ni, Cr) under study to different levels at low supply concentrations compared with those at high supply concentrations. Studies have also found high levels of Cd accumulation in floated duckweed, *L. gibba* (5,953)[47] and ivy duckweed, *L. trisulca* (3,594)[48]. Rai et al[49] reported Cd BCF values ranging from 2,125 to 29,000 for six wetland plant species (coontail, giant duckweed, bacopa, wild rice, channel grass) and green algae (*Hydrodictyon reticulatum* and *Chara corallina*). Zhu et al[46] found the Cd BCF value of 2,150 for water hyacinth. From the view of phytoremediation, a good accumulator should have the ability to concentrate the elements in its tissue, for example, a BCF of more than 1,000 (100-fold compared on a fresh weight basis)[42]. Based on this criterion, our results showed that *C. fracta* is a good accumulator of Pb and Cd with BCF values of 1,234 and 1,205, respectively.

Requirements for developing a practical bioremoval process for heavy metals include low-cost production of plant biomass, ease of removing the biomass from suspension, high maximum specific adsorption, and the capability to reduce metal concentration to very low residual levels. The green macroalgae *C. fracta* are very easy to harvest and are potentially produced in mass culture. The experimental data presented here indicated that these algae may have promising metal adsorbing characteristics. The fact that *C. fracta* had high BCFs for Pb and Cd 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, more work is needed to optimize the design and management of an aquatic plant based system so as to get maximum efficiency in metal removal. The system should have a confined environment such as a constructed wetland or lagoon system. In addition, knowledge of water chemistry, presence of humic acid in the system, harvesting techniques, metal recovery technology, and safe disposal of used plants will have to be worked out before large scale application is adopted.

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