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Environmental Contamination and Toxicology

Toxicity of Single and Combinations of Lead and Cadmium to the Cyanobacteria *Anabaena flos-aquae*

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Cyanobacteria are prokaryotes that can synthesize chlorophyll a and hence are able to undergo photosynthesis. They are widespread and can be found in many different habitats from aquatic to terrestrial, and known to be tolerant towards conditions of low oxygen, nutrient deficient and free sulphide (Whitton and Potts 2000). The widespread nature of cyanobacteria in different environments makes this class of bacteria useful as indicator of environmental contamination and pollution. Indeed the use of cyanobacteria, such as *Anabaena flos-aquae* to remove heavy metals pollution in contaminated aquatic ecosystems have been studied quite extensively by many researchers. Some of the biological processes that involved biosorption (adsorption) and binding sites have been described by Chong and Volesky (1995), Gadd and White (1993) and Ting et al. (1991).

In this study, *Anabaena flos-aquae* which can be found abundant in lakes are chosen for Cd and Pb toxicity studies. The study is aimed at understanding the effects of Pb and Cd toxicity on this species of cyanobacteria and how the uptake behaviour of these metals affected the toxicity. Such a study is useful for establishing *Anabaena flos-aquae* as a species for environmental monitoring and control.

MATERIALS AND METHODS

Axenic cultures of *Anabaena flos-aquae* strain 15-1710 were obtained from Carolina Biological Supply Co, North Carolina, USA. The cells were then cultured in Bold's Basic Medium (James 1978) to provide a stock for toxicity assays. The stock was cultured under a sterile environment with temperature between 20-25°C and under alternating 12h per day light dark condition, where light source was kept at 4 Wm^{-2}. The growth of the culture was determined by measuring the optical density at a wavelength of 700 nm with a Shimadzu UV-1201 spectrophotometer. A graph was plotted using optical density versus dry weight of *Anabaena flos aquae* cultures to determine the dry weight of cultures use in each exposure. Toxicity evaluations were carried out using culture of *Anabaena flos-aquae* in its active phase, i.e. culture at the 15^{th} day. For toxicity and metal absorption studies, analytical grade of $Pb(NO_3)_2$ and $Cd(NO_3)_2$ were used to prepare test solutions at various concentrations according to Table 1. For combination exposures, the concentrations of Pb and Cd in the test solutions are the same.

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Table 1. The concentrations of Pb and Cd used in single or combination exposure studies.

Exposure/Metal	Concentrations (μg/mL)
Single - Pb	0.20, 0.40, 0.80, 1.00, 1.20
Single - Cd	0.02, 0.05, 0.10, 0.15, 0.20
Combination Pb-Cd (1:1)	0.01, 0.05, 0.10, 0.50, 1.00

The growth inhibition (%) of the cyanobacteria after 96h exposure to each concentration was determined from the initial (before exposure) and final (after exposure) optical density of the culture. The 96h lethal dose (LD_{50}-96h) was then calculated from probit analysis (Finney 1971). The above procedures were repeated for the determination of combined toxicity attributed to Pb-Cd. All metal exposure experiments using *Anabaena flos-aquae* were carried out in Bold Basic Medium in three replicates. Control experiments for each test were performed in a similar manner in the absence of Pb and Cd. The Bold Basic Medium contained nitrate and this did not inhibit the growth of the cyanobacteria. Thus, the counter ion did not affect the toxicity tests.

The accumulation behaviour of all the metals either in single or double-exposure was investigated by exposing the cyanobacteria cells for 96h at the selected metal ion concentrations (Table 1). After the exposure, cells from the culture medium were isolated by centrifugation followed by freeze-drying. A known amount of dried cells was then digested with concentrated nitric-perchloric acids (2:1 v/v). The resulting solution was filtered and analyzed for Cd and Pb using an atomic-absorption spectrophotometer (Perkin-Elmer 1100 B) at wavelength 228.8 nm and 283.3 nm for Cd and Pb respectively. All determinations were carried out in triplicates. The percentage of heavy metal absorption was then calculated.

RESULTS AND DISCUSSION

The growth inhibition of *Anabaena flos-aquae* after exposed to Pb, Cd and combination of Pb-Cd at the stated concentrations are shown in Figure 1. The inhibition values depicted in Figure 1 for Pb, Cd and Pb-Cd exposures are based on 0–1.2, 0–0.15 and 0.01–1.0 μg/mL metal concentrations respectively (Table 1). In general there was increase in inhibition with increase in concentrations of Cd and Pb. For example, in the case of Pb approximately 50% inhibition was achieved after the Pb concentration was increased to 1 μg/mL, and for Cd, similar level of inhibition was achieved at about 0.15 μg/mL Cd. The higher Cd concentrations caused the cyanobacterial cells to die, while the lower concentrations resulted in a slight inhibition of growth. Based on the inhibition data, the LD_{50}-96h values calculated using the probit analysis are shown in Table 2. The LD_{50}-96h values demonstrated that when both Cd and Pb are present together, the toxicity towards *Anabaena flos-aquae* increased by almost 10 and

Table 2. The LD_{50}-96h for *Anabaena flos-aquae* exposed to Pb, Cd and combination of Pb-Cd from probit analysis.

Metal	LD_{50}-96 h (μg/mL)	Probit equation	r^2
Pb	0.99±0.12	*P = 6.11 LogC + 5.02	0.866
Cd	0.14±0.01	P = 2.67 LogC + 7.27	0.997
Pb-Cd combined	0.08±0.01	P = 0.80 LogC + 6.69	0.947

*P – probit value, C – concentration of metal

two folds respectively when compared to single exposures of Pb and Cd alone (Table 2).

At approximately 0.1 μg/mL of the mixed metal concentration, up to 78% inhibition was observed but when the concentration was increased to 1 μg/mL, near 100% inhibition occurred (Figure 1). Clearly, Cd is more toxic to *Anabaena flos-aquae* than Pb but a combination of both metals proved to be even more toxic to this cyanobacteria than exposure to each metal individually. According to Rangsayaton et al. (2002), the external surface sorption appears to be the first defense mechanisms against toxic heavy metals.

The toxicity of Pb on cyanobacteria and algae may be caused by factors involving binding of the metal onto the thylakoid or mitochondria membranes, which eventually lead to membrane damage and impedes photosynthetic activities. Another possible effect of Pb toxicity may be attributed to the interaction of Pb ion with polyphosphates in the cell that resulted in loss of phosphate nutrient through the precipitation of insoluble lead phosphate (Vymazal 1995).

Toxicity of Cd towards cyanobacteria and algae is higher than Pb because of the many more destructive effects of the metal. In the case of *Anabaena doliolum* exposure to Cd and other metals, a non-competitive inhibition of nitrate uptake and nitrogen reductase occurred. Under stress from Cd toxicity, cyanobacterium shifts from N_2/NO_3^- metabolism pathway to the NH_4^+ utilization pathway (Mallick et al. 1994). It has also been suggested that Cd induces toxicity on *Anabaena flos-aquae* probably through interaction with the thio components found in the cell (Mushrifah and Peterson 1990). Therefore, it is not surprising that the toxicity of Cd on *Anabaena flos-aquae* determined in this work is higher than that of Pb (Table 2).

The increase in the toxicity level of these metals may be attributed to the sequestering behaviour of the cyanobacterial biomass. The sequestering of both metals by the cyanobacterial biomass are depicted in Figure 2, which shows the amount of Pb or Cd sequestered under single or double exposure conditions.

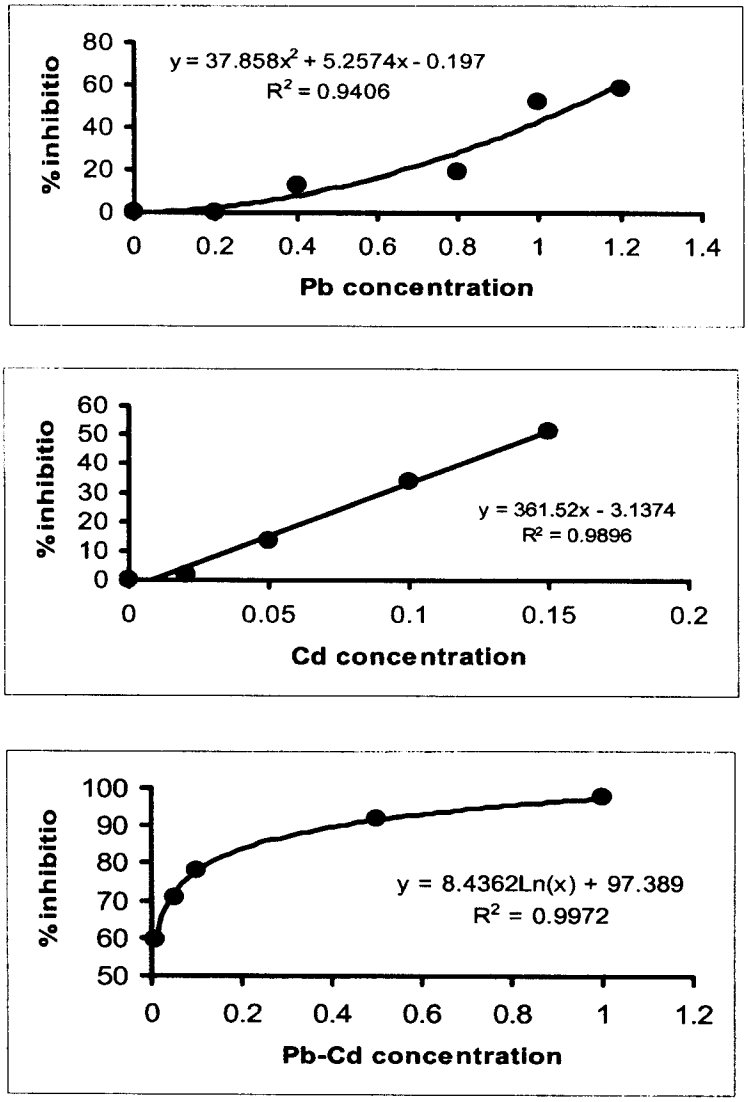

Figure 1: The inhibition of *Anabaena flos-aquae* by various concentrations (μg/mL) of Pb, Cd and combination of Pb and Cd (at the same concentration).

When exposed to 0.15 μg/mL Cd alone, the cyanobacterial biomass sequestered up to 0.026 μg/g Cd ions per dry weight, which resulted in 78% growth inhibition. For single exposure to Pb, increase in Pb concentration also resulted in increase

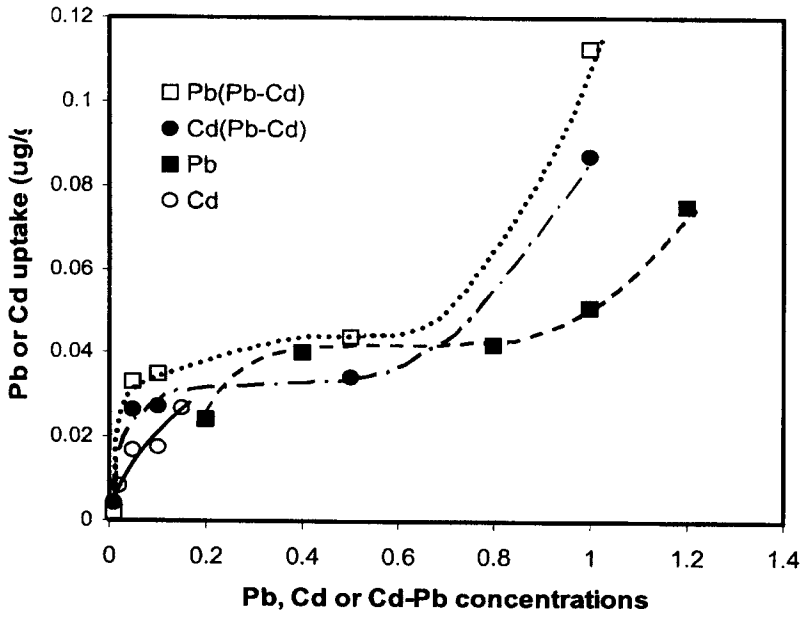

Figure 2: The sequestering of Pb or Cd by *Anabaena flos-aquae* (dry weight basis) at various concentrations (μg/mL) of Pb, Cd, Pb and Cd together (same concentration).

sequestering of Pb and it reached 0.07 μg/g dry weight at an exposure of 1 μg/mL Pb.

It is seen that during simultaneous exposure of the cells to the same Pb and Cd concentrations, i.e. 1 μg/mL, the amount of Pb sequestered is more than twice that of during single exposure to 1 μg/mL of Pb. In the case of Cd, higher sequestering of Cd was also observed during double exposure when the Cd concentrations at 0.05 and 0.1 μg/mL Cd were considered (Figure 2).

A comparison in the amount of metal ions sequestered using ANOVA confirmed that both Pb or Cd sequestered by *Anabaena flos-aqua* under double exposure condition is significantly higher (at 99% level, $p = 0.0006$) than that of during single exposure. Therefore, the presence of both Cd and Pb together appears to cause enhanced absorption of each other, which leads to a synergistic effect on toxicity. It is known that heavy metals exert their toxic effects through competing for protein binding sites, active enzymes and biologically reactive groups. These reactions can interrupt normal metabolic processes (Visviki and Rashlin 1991).

The sequestering behavior of Pb and Cd in cyanobacteria species has been reported before. The kinetic of uptake of Pb and Cd by several cyanobacteria has been known to be rapid and completed within 3 h (Jennett et al. 1979). In the first day exposure

of *S. quadricauda* to Cd, a 500-fold absorption took place (Goryunova and Kuzmina 1979). The process of Cd and Pb uptake has been reported for Chlorophyceae and Cyanophyceae which was attributed to adsorption, where the metals penetrated into the cells (Revis et al. 1989). Other experiments on the absorption of Cd by Spirulina species demonstrated that ion exchange is the dominating mechanism (Chojnacka and Noworyta 2001). For cyanobacteria in general, the mechanism of Pb and Cd uptake involved binding onto the cell wall or cell surface (Mahan et al. 1989; Jennet et al., 1979).

In conclusion the exposure of *Anabaena flos-aquae* to Pb or Cd indicated that Cd was more toxic than Pb. When both metals were present together, the toxicity towards *Anabaena flos-aquae* increased and this seemed to be attributed to the higher sequestration of both metals during double exposure conditions. However, based on these data, the mechanism of how both Cd and Pb is sequestered by the cyanobacteria could not be ascertain. To understand this behavior, further work is necessary.

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