Bull. Environ. Contam. Toxicol. (1992) 49:272–278

Environmental Contamination and Toxicology

Effect of Mercury and Cadmium on the Growth of *Anacystis nidulans*

H. L. Lee, B. Lustigman, V. Schwinge, I-Yu Chiu, and S. Hsu

Montclair State College, Upper Montclair, New Jersey 07043, USA

Anacystis nidulans is a member of the cyanobacteria, one of the largest and most diverse groups in the prokaryotic kingdom. The structure and chemistry of the cell wall, cell division and genetic recombination are all similar to those of other bacteria (Allen 1968). Photoautotrophy is the main mode of nutrition and its photosynthetic apparatus is similar to that of other cyanobacteria (Fogg 1973). Cyanobacteria are excellent organisms to serve as models for the investigation of a wide variety of biological problems such as acting as environmental pollution indicators. There have been several studies on the effects of heavy metals on *A. nidulans*(Lee et al.1991, Singh and Yadava 1985, Whitton and Fahni 1982). Some of these elements, such as copper, are known to be essential nutrients for cyanobacteria (Gupta 1989). Others, like cadmium, are not known to be necessary for normal growth and metabolism (Trevors et al 1986). Large amounts of either essential or non-essential elements can be toxic (Snyder 1982).

Toxic metals, such as mercury and cadmium are a major water pollution problem. Metals come from natural weathering processes of the earth's crust, industrial discharge, pest or disease control agents applied to plants, urban run-off, mining, soil erosion, sewage effluents, air pollution fallout and other sources (Mitchell 1972).

Mercury is one of the more common and potentially more toxic contaminants. It is widely distributed in compound form in rocks and soil. There are three forms of mercury: elemental (Hg^{o}), inorganic and organic compounds. Inorganic mercury is far less dangerous than organic methyl mercury. Due to limited solubility, mercury compounds are deposited in bottom muds of rivers, lakes etc. In these waterways mercury

Send reprint requests to Dr. Lee Lee at above address

compounds are converted into dimethyl and methyl mercury by aerobic and anaerobic bacteria. Dimethyl mercury evaporates into the air. Methyl mercury, remains in the bottom and is slowly released into the water where it enters organisms in the food chain (Snyder 1982) The maximum allowable amount of mercury in drinking water in New Jersey is 0.002 ppm (NJAC 7:10 Sub.Chpt 5).

Cadmium is an important environmental pollutant and potent toxicant to bacteria, algae and fungi (Trevors et al. 1986). A major source of cadmium is vapor emissions that contaminate surrounding soil and water through fallout during smelting. High amounts of cadmium in surface waters are usually due to metallurgical plants, plating operations, cadmium pigments, batteries, sewage effluent, mine drainage and plastics manufacturing (Snyder 1982). Sewage sludge is often contaminated with cadmium which then concentrates in plants grown on contaminated soils. It is one of the most readily absorbed and accumulated metals in plants. Algae and Cyanobacteria are reported to be more sensitive than bacteria and fungi (Trevors et al 1986). Concentrations of cadmium in fresh waters are usually less than 1.0 ppb and in marine about 0.15 ppb (Snyder 1982). The maximum allowable in drinking water is 0.01 ppm in New Jersey (NJAC 7:10 Sub.Chpt 5)

Many factors affect the toxicity of metals, such as pH and water temperature. There also appears to be a protective effect with zinc and calcium against cadmium toxicity as there is with selenium against mercury. (Snyder 1982). It is indicated that EDTA (Ethylene Diamine Tetraacetic Acid) acts to chelate the metal ions and reduce their toxicity (Lee et al.1991). Before we can restore our waters to health, we must study the ecological processes occurring in either healthy or polluted waters. These studies will enable us to determine which materials disturb the ecology of natural waters.

MATERIALS AND METHODS

Anacystis nidulans cultures were obtained from Dr. Roy McGowan, Brooklyn, NY. They were grown in 100 ml of Mauro's Modified Medium (3M)(Kratz & Myers 1955) at pH 7.9, at ambient temperature with continuous light and gentle agitation for 14 days. The growth of the cultures was determined by direct count of the cells in a hemocytometer counting chamber and by indirect turbidity reading with a Beckmann spectrophotometer at 750 nm. The cultures were checked periodically for bacterial contamination.
A stock solution of $HgCl_2$ was diluted to achieve final concentrations of mercury of 0, 0.025, 0.05, 0.25, 0.5,

5.0, and 50 ppm. The pH of each flask was adjusted to 7.9 and inoculated with $1X10^7$ cells/ml of exponentially growing *A. nidulans* cells. Direct cell counts and turbidity measurements were used to monitor the growth of the cultures periodically for 14 days. A similar series of flasks were treated the same way, but did not contain EDTA.

A stock solution of $CdCl_2$ was diluted to achieve final concentrations of cadmium of O, 0.025, 0.05, 0.25, 0.5, 5.0, and 50 ppm. Cultures were prepared and treated as indicated above, both with and without EDTA.

RESULTS AND DISCUSSION

The toxicity of mercury to *A. nidulans* was investigated using increasing concentrations of 0 to 50 ppm (Fig 1). Media containing 0, 0.025 and 0.25 ppm of mercury, with or without EDTA, did not have any discernible effect on the growth of *A. nidulans* during the course of the experiment. The results were very similar to the control. In media containing 0.5 ppm mercury and EDTA there is a delay in the onset of log phase of growth and total growth was less than the control. In media without EDTA at 0.5 ppm there was an extreme delay in the onset of log phase and total growth was much less than in the culture with EDTA. The chelating effect of EDTA on mercury was very apparent. In cultures with 5 ppm mercury, with or without EDTA, there was no growth. For 50 ppm the data is the same as 5 ppm. The threshold level thus appears to be 0.5 ppm mercury. It is not known whether recovery is due to overcoming metal shock or if tolerant forms were selected and began to multiply. The appearance of the cultures and cell morphology show that by day 2, cultures at 0.5 ppm were a lighter green, the 5 ppm culture even lighter and the 50 ppm culture was colorless. The cells in the 5 and 50 ppm cultures were small and almost colorless. By day 14 both the 5 and 50 ppm cultures were colorless and the 0.5 ppm was lighter than the control. The pH of the 5 and 50 ppm cultures was 7.9 and 7.7, while that of all the other cultures rose to about 10.0. In the cultures without EDTA, by day 2 the 0.5 ppm and 5 ppm cultures were very pale and the 50 ppm culture was colorless. At day 7, the 0.5, 5 and 50 ppm cultures were colorless. The other cultures were progressively darker, up to the control. The pH of the 0.5, 5 and 50 ppm cultures were 7.5, 7.2,7.2 respectively. The control cultures was about 10.0. By day 14 the 0.5 ppm culture was pale green and the pH was 9.6. All remaining cultures were as previously noted.

The following concentrations of cadmium were added to *A. nidulans* cultures containing 3M with or without EDTA: 0, 0.025, 0.05, 0.5, 5.0 and 50 ppm (Fig 2). The

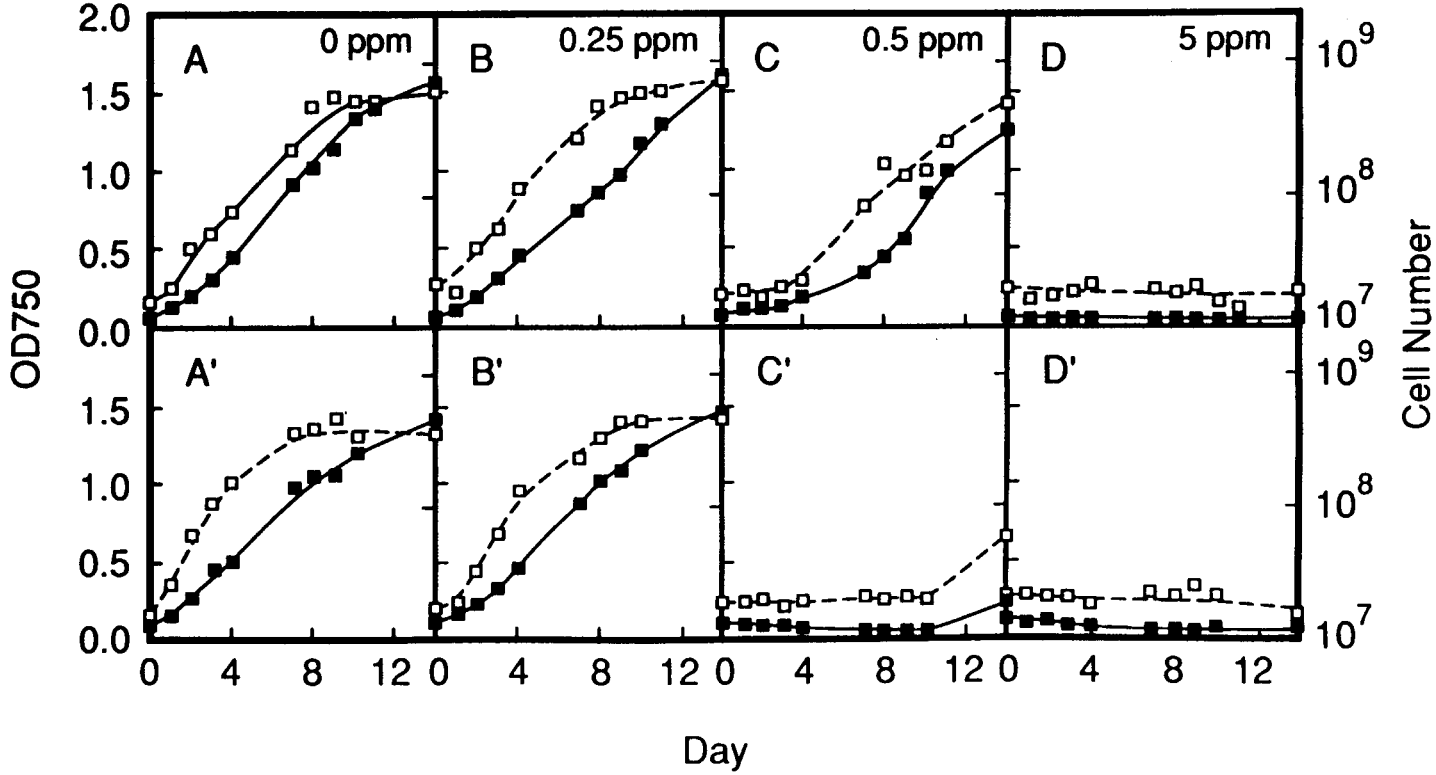

Figure 1. Growth of *A. nidulans* in 100 ml of 3M medium containing mercury at 0(A), 0.25(B), 0.5(C), 5(D) ppm with EDTA; and without EDTA at (A'), (B'),(C'),(D'). Growth was measured by optical density readings at 750 nm (■ --■) and by direct count (□ --□).

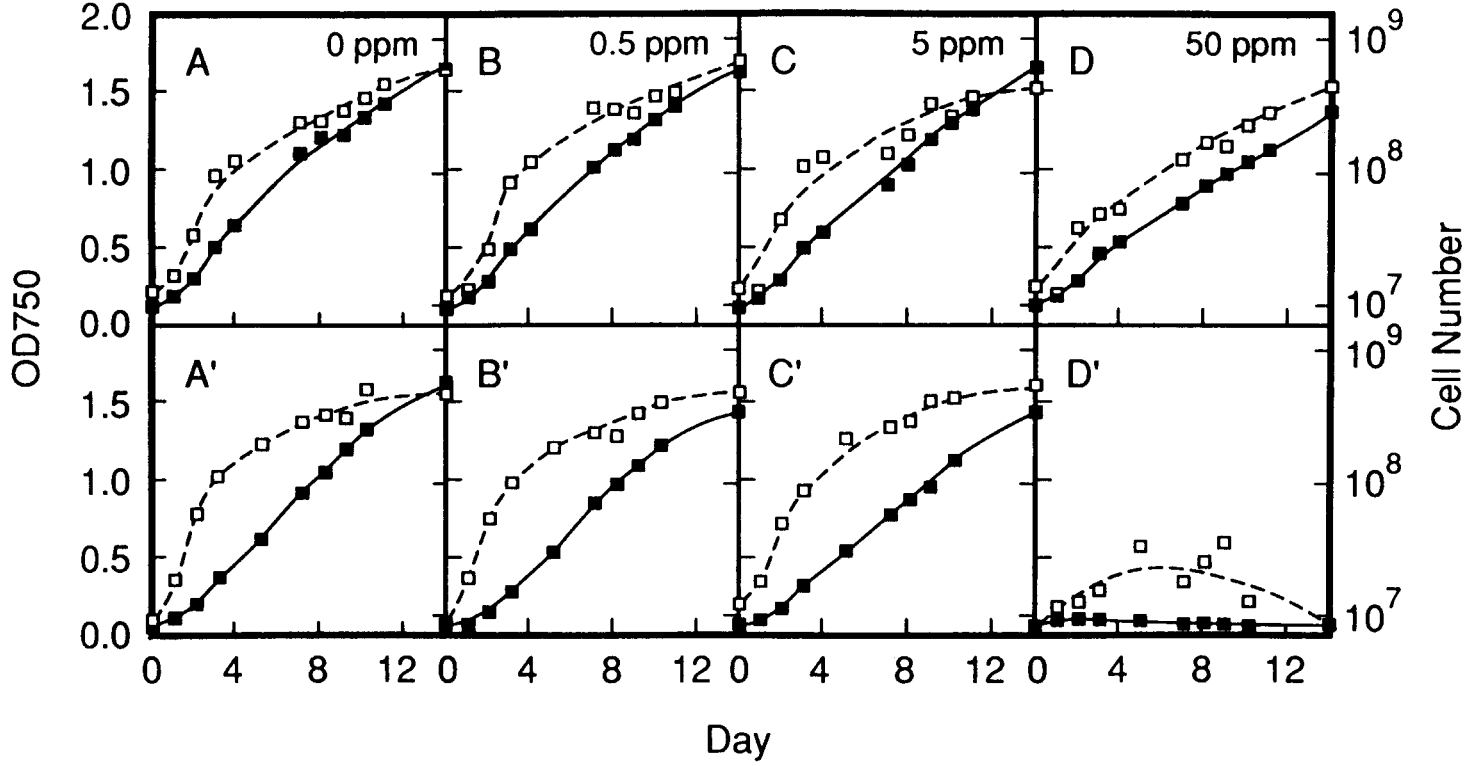

Figure 2. Growth of *A. nidulans* in 100 ml of 3M medium containing cadmium at 0(A), 0.5(B), 5 (C), 50(D) ppm with EDTA; and without EDTA at (A'), (B'),(C'),(D'). Growth was measured by optical density readings at 750 nm (■ --■) and by direct count (□ --□).

data at concentrations of 0.025, 0.05, 0.5, and 5.0 ppm showed the same results as the control, with or without EDTA. The culture of 50 ppm cadmium without EDTA showed no growth, indicating that EDTA was effective in chelating cadmium. After day 3 the 50 ppm culture was almost clear. In contrast to the other cultures, for the first eight days, the 5 ppm culture without EDTA displayed some aggregation and the cells were small and faint. By day 14, recovery had occurred. The pH of the cultures with EDTA rose to about 10 in all concentrations. The same effect occurred in the cultures without EDTA, except the 50 ppm culture which rose to about 14.

There are other reports concerning the role of pH in regulating metal toxicity. Increased metal uptake seems to be favored by low pH in some species while alkalinity seems to have the same effect in others (Singh and Yadava 1985). EDTA appears to be an effective chelator for both mercury and cadmium ions. It has been reported that the toxicity of metals, including mercury, to *A. nidulans*, was decreased by light (Whitton 1968). This may have been a factor in these results as well.

Cadmium, with or without EDTA, did not appear to have as great a toxic effect as mercury. PH is reported to be a factor in cadmium uptake; with pH 8.5 favoring maximum uptake (Singh and Yadava 1985). As the experiment progressed, the pH rose to around 10 in all cultures except 50 ppm, thereby decreasing Cd uptake. It is also reported that uptake levels of heavy metals vary with concentration of the ion in the medium as well as the length of time of exposure (Singh and Yadava 1985). Resistance to cadmium is also increased by repeated subculturing at inhibitory levels, due to selection of spontaneous mutants (Whitton and Fahni 1982). Plasmid encoded genes may play a role in Cadmium resistance as well (Trevors et al.1986). Another factor that may have lessened the effect of cadmium was the presence of Ca^{++} and Zn^{++} which reduce cadmium uptake. This effect has been attributable to competition for the same active sites on the cyanobacteria. The ions may also stabilize and maintain cell wall integrity (Singh and Yadava 1982). Lastly, resistance may also be due to the production of extracellular metabolites, which play a role in alleviating heavy metal uptake/toxicity (Rai and Raizada 1988).

In summary, low concentrations of mercury have no significant effect on the growth of *A. nidulans*. At concentrations of 0.5ppm the effect becomes apparent and at concentrations of 5 and 50 ppm, the growth is completely inhibited. Low concentrations of cadmium

had no effect on the growth of *A. nidulans*. At 5 ppm, growth was observed to be inhibited without EDTA in the medium. At high concentrations(50 ppm) with or without EDTA, the effect becomes pronounced, especially in the cultures without EDTA. While it does not appear that *Anacystis nidulans* concentrates mercury or cadmium, uptake studies would need to be performed in order to determine if concentration or exclusion of the metals occurs. If they do concentrate these metals, perhaps the organism could be used to remove toxic metals from contaminated soil or water.

REFERENCES

Allen MM (1968) Ultrastructure of the cell wall and cell division of unicellular blue-green algae. J Bact 96:842-851.

Fogg GE, Stewart WDP, Fay P, Walsby AE (1973) The blue-green algae. Academic Press, London, p1-373.

Gupta SL (1989) Interactive effects of nitrogen and copper on growth of cyanobacterium Microcystis. Bull Environ Contam Toxicol 42:270-275.

Kratz WA, Myers J (1955) Nutrition and growth of several blue-green algae. Amer J Bot 42:282-287.

Lee LH, Lustigman B,Chu I-Yu, Jou HL (1991) Effect of aluminum and pH on the growth of *Anacystis nidulans*. Bull Environ Toxicol Contamin 46:720-726

Mitchell R (1972) Water Pollution Microbiology. John Wiley & Sons, New York, pp 1-65,205-288.

New Jersey Administrative Code 7:10-Sub Chpt.5 New Jersey Safe Drinking Water Act. Department of Environmental Protection Agency;, Safe Drinking Water Division, State of New Jersey.

Rai LC, Raizada M (1988) Impact of chromium and lead on *Nostoc muscorum*:regulation of toxicity by ascorbic acid, glutathione, and sulfur-containing amino acids. Ecotoxicol Environ Safety 15:195-205.

Singh SP, Yadava V (1985) Cadmium uptake in *Anacystis nidulans*: effect of modifying factors. J Gen Appl Microbiol 33:39-48.

Snyder A (1982) Environmental Toxicology. Macmillan Publishing, New Jersey, pp.582-628, 842-845.

Trevors JT, Stratton GW, Gadd GM (1986) Cadmium transport, resistance and toxicity in bacteria, algae and fungi.Can J Microbiol 32:447-464.

Whitton BA (1968) Effect of light on toxicity of various substances to *Anacystis nidulans*. Plant Cell Physiol 9:23-26.

Whitton BA, Fahni AAS (1982) Influence of cobalt, nickel, copper and cadmium on the blue-green alga *Anacystis nidulans*. Environ Poll 27:275-281.

Received September 20, 1991; accepted January 15, 1992.