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Effect of Lead and Cobalt on the Growth of *Anacystis nidulans*

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Anacystis nidulans is a typical rod-shaped unicellular cyanobacterium. It is an obligate photoautotroph with a photosynthetic apparatus very similar to the eukaryotic chloroplast in function and molecular aspects (Fogg et al 1973). The adaptability of microorganisms to toxic substances is greater than that of organisms of higher evolutionary level. The study of blue green algae can therefore provide an indication of the toxic effects of a pollutant and its effect on metabolic activities (Rai and Raizada 1989).

The mining and processing of lead dates back several centuries. Its use as an additive to gasoline has decreased in recent years, but batteries and paint pigment remain as current uses of lead. Lead enters aquatic systems from the run-off, or as fallout of insoluble precipitates and it is found in sediments. Typical freshwater concentrations lie between 1-10 ppm. Natural lead concentrations in the soil range from 2-200 ppm and average 10-15 ppm (Snyder 1982).

Lead is toxic to most living things at high exposure and there is no demonstrated biological need. The limit for lead in drinking water as set by US Public Health Service is 0.05ppm (Casarett 1980). Previous reports have indicated that algae are not as sensitive to lead as many other organisms (Pace et al 1977). Chelating agents, such as EDTA (Ethylene Diamine Tetraacetic Acid) are able to reduce the toxicity of lead and other metals. In natural habitats this may be due to the effect of reducing agents and amino acids (Rai and Raizada 1988). PH value has been reported to influence the effect of metals (Lee et al 1991).

Cobalt is a relatively rare metal produced primarily as a by-product of other metals, chiefly copper. It is

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used in high temperature alloys and in permanent magnets. Its salts are used in paint dryers as catalysts, and in the production of pigments. Cobalt is an essential component of vitamin B_{12}. Cobalt has been shown to inhibit chlorophyll biosynthesis and the site inhibition by cobalt has been identified (Csatorday et al 1984).

In this study the effect of lead and cobalt on the growth of *Anacystis nidulans* will be studied.

MATERIALS AND METHODS

The culture of *Anacystis nidulans 625* was obtained from Dr. McGowan, Brooklyn College, N.Y. The cells were grown in sterile shake flasks containing 100 ml Mauro's Modified Medium (3M) (Kratz and Myers 1955). The cultures were grown under constant fluorescent light at ambient temperature with continuous slow agitation until stationary phase was achieved. The flasks were inoculated with approximately $1X10^7$ cells/ml of *A. nidulans*. For each experiment a control was prepared of untreated *A. nidulans* in 100 ml of 3M medium and kept at the same conditions. Growth of the cultures was determined by two methods: 1. direct count using a Spencer hemocytometer or 2. indirect turbidometric reading using a Beckmann Spectronic 1001 spectrophotometer at 750 nm. Cultures were checked for contamination by plating on nutrient agar. PH readings were taken at the beginning and end of each experiment.

The stock solution of $Pb(NO_3)_2$ was prepared in 3M medium or 3M medium without EDTA at a final concentration of 1000 ppm. Series dilutions with final concentrations of 5,50,200 and 1000 ppm were used to study the effect of lead on the growth of *A. nidulans*. Another set of experiments was carried out with the same protocol as described above, but in this set, the pH value of the cultures was adjusted to pH 7.9 before autoclaving using KOH.

The stock solution of cobalt was prepared by adding $Co(NO_3)_2 \cdot H_2O$ to a final concentration of 250 ppm. Series dilutions with final concentrations of 0.1,1,10,15,30 and 50 ppm were used to study the effect of cobalt. The pH of the cultures was checked. An additional set of experiments was prepared as above, but without EDTA added to the medium.

All cultures were grown and monitored as described above. Viability testing of each culture was also carried out, using streak method on 3M agar. The cell morphology was observed at 1000X magnification.

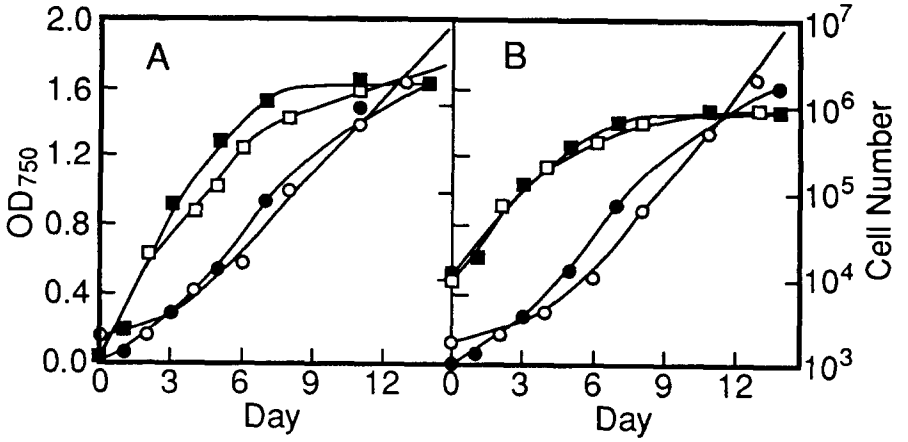

Figure 1. Growth of *A. nidulans* in 100 ml of 3M medium containing lead at 0 (A)and 50 ppm (B). Growth measured by optical density readings at 750nm (●-●) control, (o-o) treated cells,and by direct count(■ -- ■)control, (□ -- □) treated cells

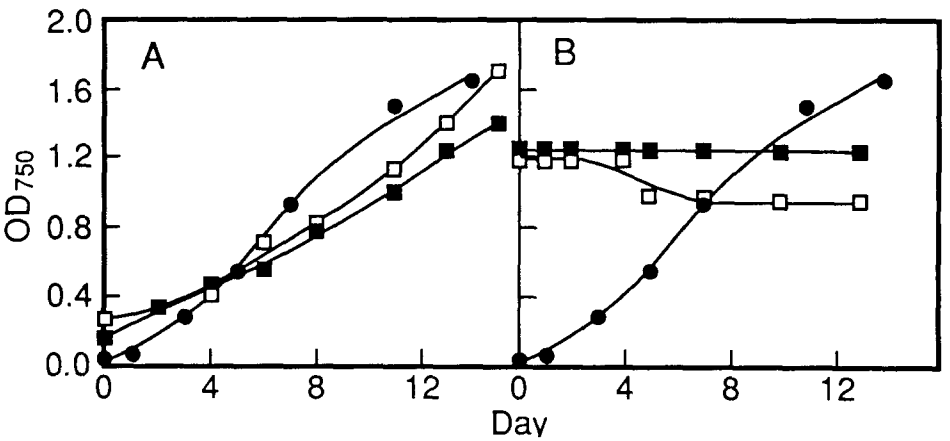

Figure 2. Growth of A.nidulans in 100ml of 3M medium containing lead at 200(A) and 1000 ppm (B).Growth measured by optical density readings at 750nm (●-●)control, (■ -- ■)pH adjusted,(□ -- □)not adjusted

RESULTS AND DISCUSSION

The effect of concentrations of 5, 50, 200 and 1000 ppm of lead on the growth of *A. nidulans* was studied. The growth in concentrations of 50 ppm of lead is very similar to the controls, as measured by both direct count and turbidity (Figure 1). At the concentration of 200 ppm, lead reduced the growth of the cells only slightly. A concentration of 1000 ppm completely inhibited the growth of the cells, regardless of the pH

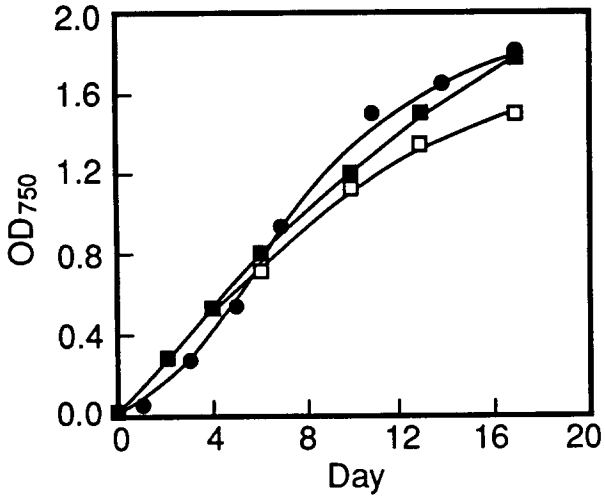

Figure 3. Growth of *A. nidulans* in 100 ml 3M medium containing 200 ppm lead,(● -- ●) control,(■ --■) with EDTA,(□ --□) without EDTA

value (Figure 2). In concentrations of 200 ppm and above, cells either were degraded or combined with cellular debris. Irregular cell morphology was observed, making direct cell count very difficult. PH values of the non-adjusted cultures with 50, 200 and 1000 ppm lead were 6.9, 5.4 and 3.6 respectively. It has been reported that lower pH will affect the growth of *A. nidulans* (Lee et al 1991). In this study, the pH of the cultures with 1000 ppm lead was 3.6. This may add to the effect of lead in repressing growth and decreasing the turbidity of the culture.

In order to determine if the EDTA in the medium was acting as a chelating agent for the lead and affecting growth, cultures were prepared without EDTA. The curve of cell growth was very similar to the controls at concentrations of 50 ppm Pb (Data not shown). At concentrations of 200 ppm, there was some reduction in growth when EDTA was absent from the medium (Figure 3). Previous studies have indicated that algae are resistant to concentrations of lead that are toxic to other organisms (Pace et al 1977). Our results show that toxicity first occurred at 1000 ppm, far above the typical natural values.

Concentrations of 0.1,1,10,15,30 and 50 ppm cobalt were used. Figure 4 shows that there was a delay in the onset of log phase of growth at concentrations of 15 ppm cobalt and the cell numbers remained lower than the controls. Concentrations of 30 ppm and higher were able to prevent the growth of the cells completely. PH values were not altered by cobalt. At concentrations

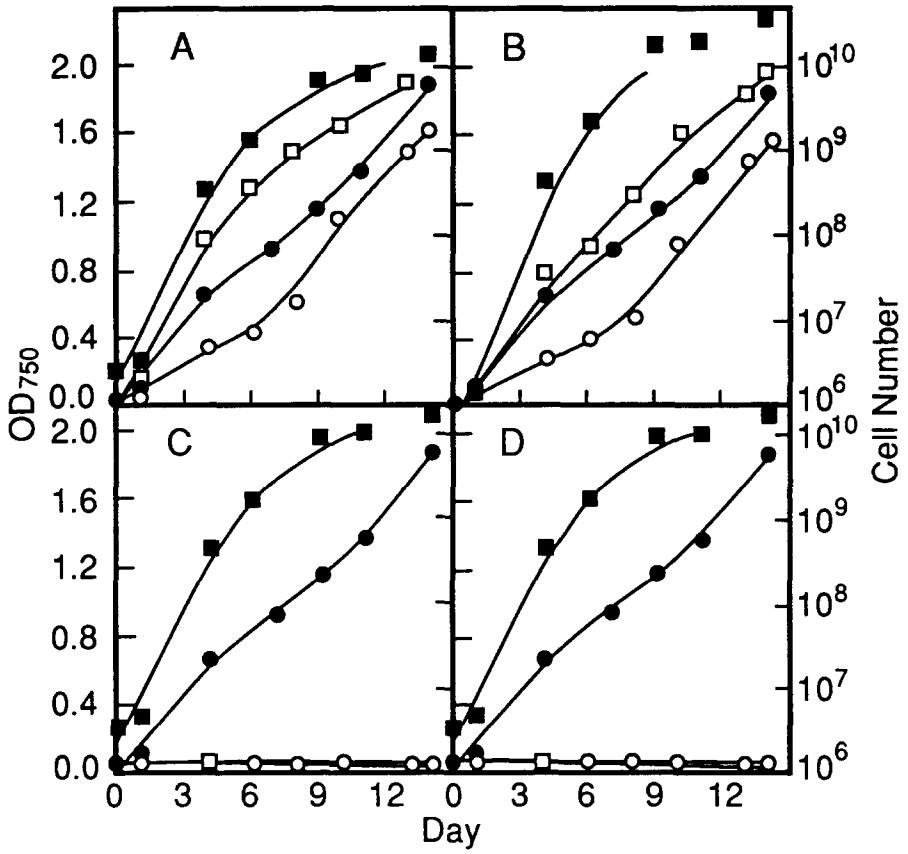

Figure 4. Growth of *A. nidulans* in 100 ml of 3M medium containing cobalt at 10 (A), 15(B), 30(C), 50(D)ppm. Growth measured by optical density readings at 750 nm (●-●)control, (o-o)treated cells, and by direct cell count (■ --■)control, (□ --□)treated cells

of 10 and 15 ppm, the color of the cultures was more blue than green, and the cells that were observed were very small. The absorption spectrum of the pigments (Figure 5) indicated that the medium containing cobalt did not have the peak at 680nm which represents the absorption of chlorophyll a. These results suggest that cobalt affects the biosynthesis of chlorophyll a in *A. nidulans cells.*

Cultures were also grown in cobalt media without EDTA (Figure 6). Cell growth was inhibited at 10 ppm and the total number of cells remained reduced. At 15 ppm, cobalt completely prevented the growth of the cells. This is in contrast to the controls where growth was only reduced. EDTA therefore was effective in reducing the toxic effect of cobalt on the growth of *A. nidulans*.

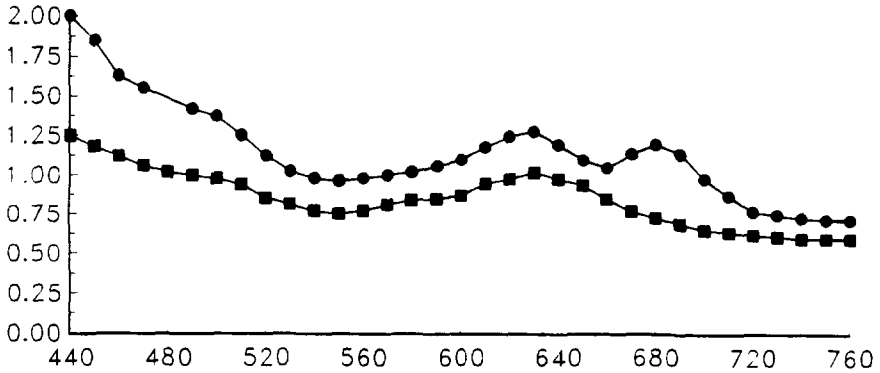

Figure 5. Absorbtion spectrum of *A. nidulans* treated with 15ppm cobalt.(● --●)Control (■ --■)Treated

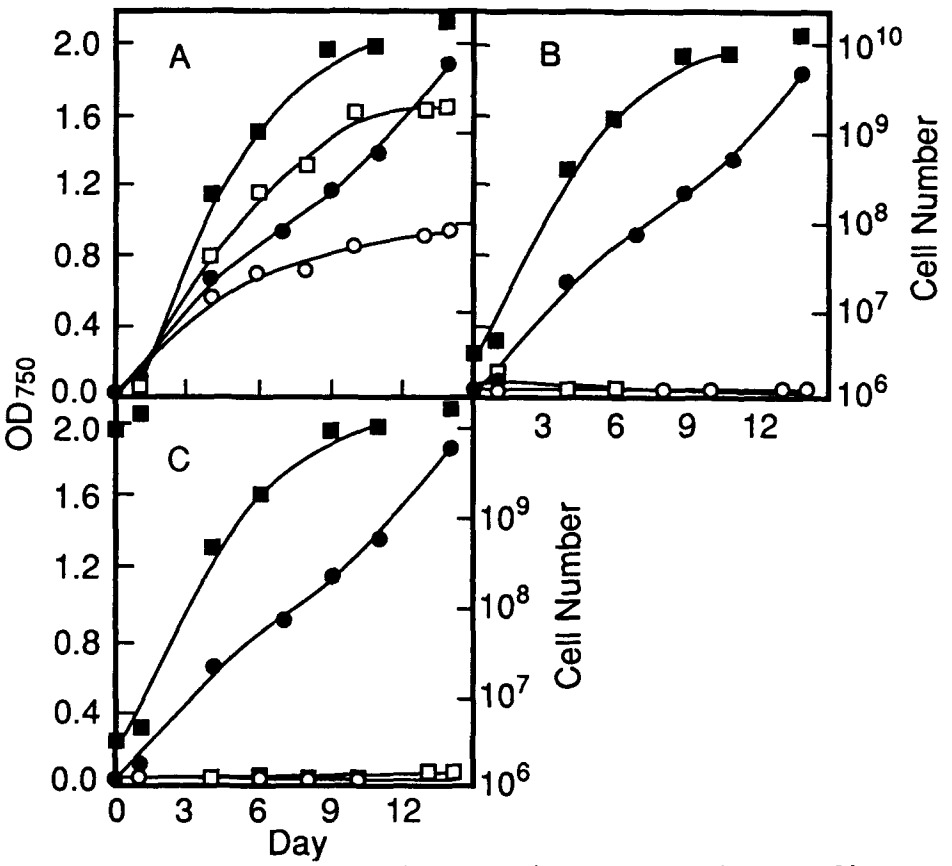

Figure 6. Growth of *A. nidulans* in 100 ml of 3M medium without EDTA containing cobalt at 10(A),15(B),30(C)ppm Growth measured by optical density readings at 750 nm (●-●) control, (o-o)treated cells, and by direct cell count (■ --■)control, (□ --□)treated cells.

Cobalt has previously been found to inhibit the biosynthesis of chlorophyll in *A. nidulans* . The mechanism is considered to be competition with iron for the active site on the chlorophyll molecule. Cells accumulate protoporphyrin IX and at the same time both chlorophyll and phycobiliprotein synthesis are blocked (Csatorday et al,1984). Our results indicate that with cobalt, there is a sharp reduction in biosynthesis of chlorophyll a, but little or no effect on production of phycocyanin. EDTA was more effective as a chelating agent with cobalt than with lead.

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