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Effect of Barium and Nickel on the Growth of *Anacystis nidulans*

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Anacystis nidulans is a simple, unicellular, prokaryotic microorganism. Like other cyanobacteria it is an obligate photoautotroph that is similar to gram-negative bacteria in cell wall structure, replication, and ability to harbor plasmids. Cyanobacteria are excellent organisms to serve as models for the investigation of a wide variety of biological problems, including indicators of environmental pollution. There have been several studies on the effects of heavy metals on A. nidulans (Lee et al. 1991, 1992, 1993, 1994, Singh and Yadava 1985, Whitton and Fahni 1982).

Toxic metals are a major water pollution problem. Metals come from natural weathering processes of the earth's crust, but industrialization and urbanization have led to an increase in contamination of aquatic environments, mainly from industrial discharge, pest or disease control agents applied to plants, urban run-off, mining, soil erosion, sewage effluents, air pollution fallout, and other sources. Among these contaminants are nickel, barium, and their derivatives.

Nickel is found in small quantities in the earth's crust (80ppm) (Cassarett and Doull 1980). Nickel smelting, nickel ore extraction, electronic electroplating, fossil fuels, incineration, coins, steel alloys, batteries and other sources all add to endangerment of the ecosystem by nickel pollution. Nickel carbonyl, formed by nickel in the presence of carbon monoxide, is the most toxic form of nickel. The binding capacity of waste biomass for silver, chromium, lead and copper was reported as greater than that for nickel (Mattuschka and Straube 1993). Some ions may also play a role as essential cofactors in metalloenzymes,as is the case for nickel (Sunda 1989). The widespread distribution of barium in living organisms and the increasing use and consequent dissemination of the compounds in the ecosystems have enhanced the importance of the study of their toxic

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effects (Das 1988). Barium compounds are used for a variety of industrial purposes including white pigment in paints, filler in paper production, as opaque compounds for x-ray studies of internal organs, drilling mud, and as additives for protection against rust in lubricants (Cassarett and Doull 1980). Barium extraction, industrial usage and disposal, as well as crustal sources, produce contamination (Jickells 1992). Soluble barium salts exhibit acute toxicity and the use of barium is regulated because it may affect drinking water (Wang 1986). Barium has been shown to cause myocardial toxicity in humans and animal models due to modifications in membrane permeability and possible alterations in cell function (Delfino 1988, Parry 1989).

The effects of selected concentrations of nickel chloride and barium chloride on the growth of A. nidulans were studied, with and without addition of EDTA (ethylene diamine tetraacetic acid). EDTA has been known to act to chelate metal ions and reduce their toxicity (Lee et al. 1991). Since chelators, both natural and synthetic, effect the available concentration of metal ions in waters, we wished to determine the influence of chelation with EDTA on heavy metal ions as determined by growth of A. nidulans. 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 and Myers 1955) at pH 7.9, ambient temperature, constant fluorescent light, and continuous agitation at 100 rpm for 14 days. The chelato EDTA is a constituent of 3M at a concentration of 0.1% The flasks were inoculated with approximately 1 X 10^7 cells/ml of A. nidulans. For each experiment, a control was prepared of untreated A. nidulans in 100 ml of 3M medium and maintained at the same conditions. 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. Cultures were checked periodically for bacterial contamination by plating on nutrient agar. The pH readings were taken at the beginning and end of each experiment. Cell morphology was observed at 1000X magnification. Duplicate flasks were prepared for each concentration of heavy metal and each experiment was performed at least twice, so that a minimum of four cultures were studied for each concentration of metal ion. The results represent the pooled average of these cultures. Statistical analysis was not performed because the variance of the samples at each concentration was consistently extremely small.

A stock solution of nickel chloride was prepared at a final concentration of 10,000 ppm. Series dilution with final concentrations of 0, 10, 25, 50, and 100 ppm within the flasks were used to study the effect of nickel chloride on the growth of A. nidulans. The pH of each flask was adjusted to 7.9. Each flask was inoculated with approximately 1 X 10^7 cells at the start of the experiment. Direct cell counts and turbidity measurements were used to monitor the growth of the cultures periodically for 14 days after the addition of nickel chloride. A similar protocol was followed for cultures in 3M media without EDTA, but with the same concentrations of metal ions.

A stock solution of barium chloride was prepared at a final concentration of 10,000 ppm. Series dilutions with final concentrations 0, 50, 100, 250, 500, 750 and 1000 ppm in each flask were used to study the effect of barium on the growth of A. nidulans. Cultures were prepared, treated, and monitored as indicated above, both with and without 0.1% EDTA in the 3M media.

RESULTS AND DISCUSSION

The toxicity of nickel, both with and without EDTA, to A. nidulans was investigated using increasing concentrations of 0 to 100 ppm (Fig. 1). In media containing EDTA there was a slight decrease in the growth at 10 ppm NiCl as measured by optical density, but not direct count. This may be due to the presence of smaller and some bleached cells, compared to the control. At 25 ppm NiCl, growth was very severely inhibited, and at 50 and 100 ppm, it was completely inhibited. Results of cultures grown without EDTA in the medium demonstrated that EDTA was an effective chelator of nickel (Fig. 1). Growth of cells at concentrations of 10 ppm nickel and higher were completely inhibited. This indicates that nickel chloride is toxic to A. nidulans at concentrations >10 ppm NiCl with EDTA and <10 ppm NiCl without EDTA. Results of the measurement of pH at the end of the experiment indicated that all cultures where growth occurred achieved pH values of approximately 9. This is similar to results previously seen with cultures of A. nidulans (Lee et al. 1991).

The data of experiments using barium demonstrates a completely different set of results (Fig. 2). Growth was comparable to the control at 50 and 100 ppm $BaCl_2$ with EDTA in the medium. At 250 ppm there was a slight decrease in growth and a greater decrease at 500 ppm. At 750 ppm there was severe inhibition and at 1000 ppm, almost complete inhibition of cell growth. These results show that it requires extremely high levels of barium to inhibit the growth of A. nidulans. Results in medium lacking EDTA show that at 50 ppm $BaCl_2$ growth was similar to the control (Figure 3). At 100

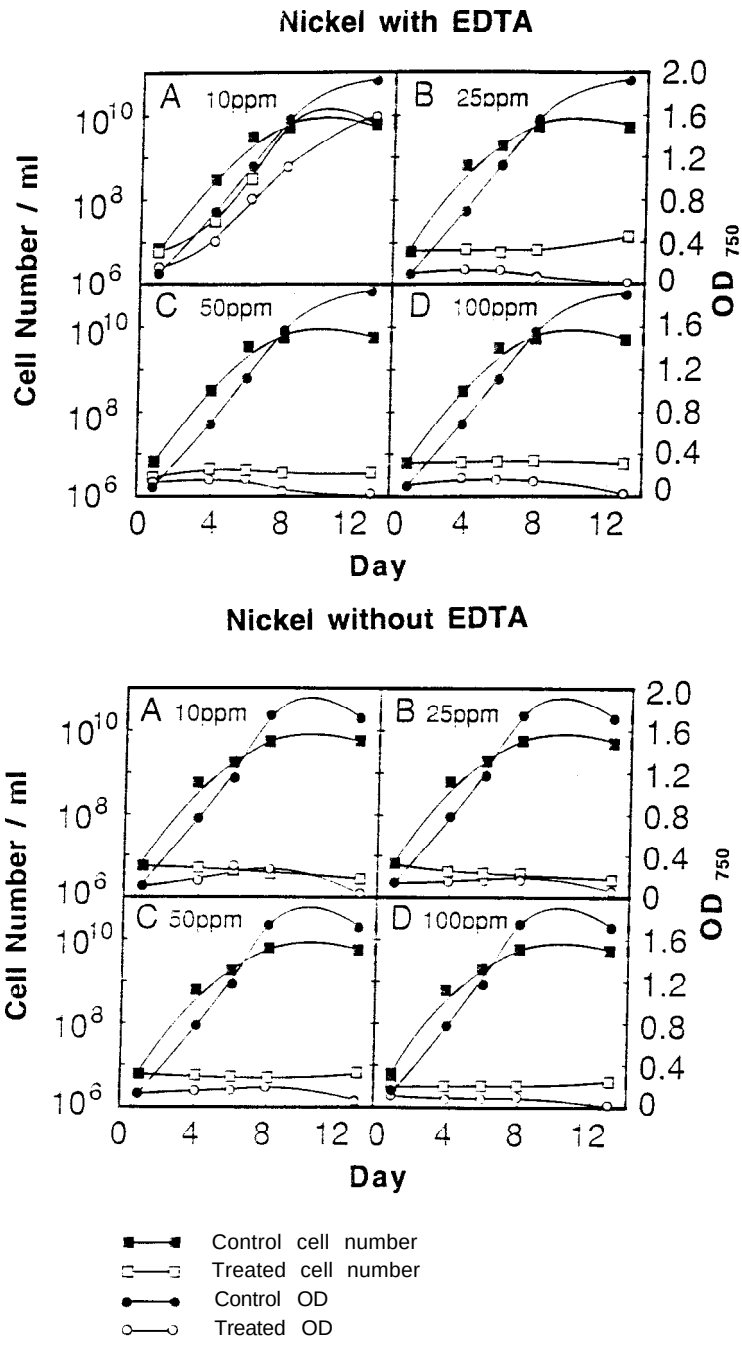

Figure 1. Growth of A. nidulans in 100 ml of 3M medium containing nickel chloride at 10(A), 25(B), 50(C), or 100(D) ppm with EDTA and without EDTA. Cell growth was measured by optical density (OD) readings at 750 nm and direct counts.

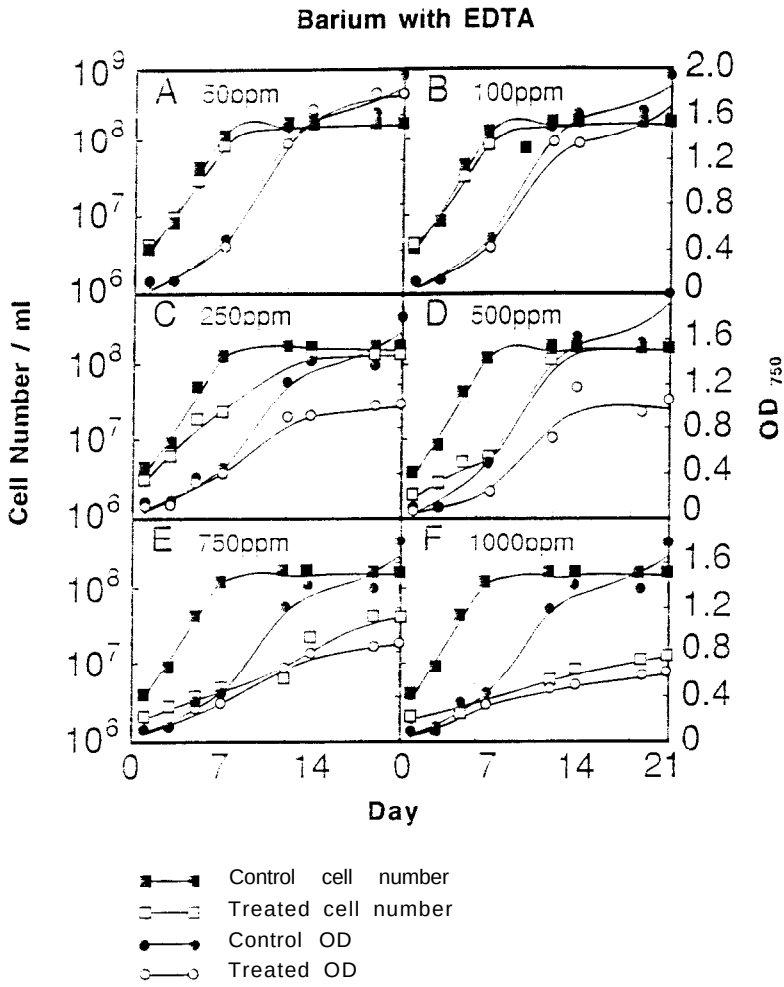

Figure 2. Growth of A. nidulans in 100 ml of 3M medium containing barium chloride at 50(A), 100(B), 250(C), 500(D), 750(E), or 1000(F) ppm with EDTA. Cell growth was measured by optical density (OD) readings at 750 nm and direct count.

ppm there was a slight decrease in growth, which was greater at 250 ppm. A severe decrease was seen at 500 ppm and almost complete inhibition at ≥750 ppm $BaCl_2$. These results indicated that EDTA had a slight effect on chelating barium: the levels of barium needed to produce an effect were quite high. A. nidulans appeared to show high tolerance to extremely high concentrations of barium, both with or without EDTA in the medium.

The extent of environmental pollution and human exposure to hazardous toxic chemicals in the environment is often difficult to assess. It has been

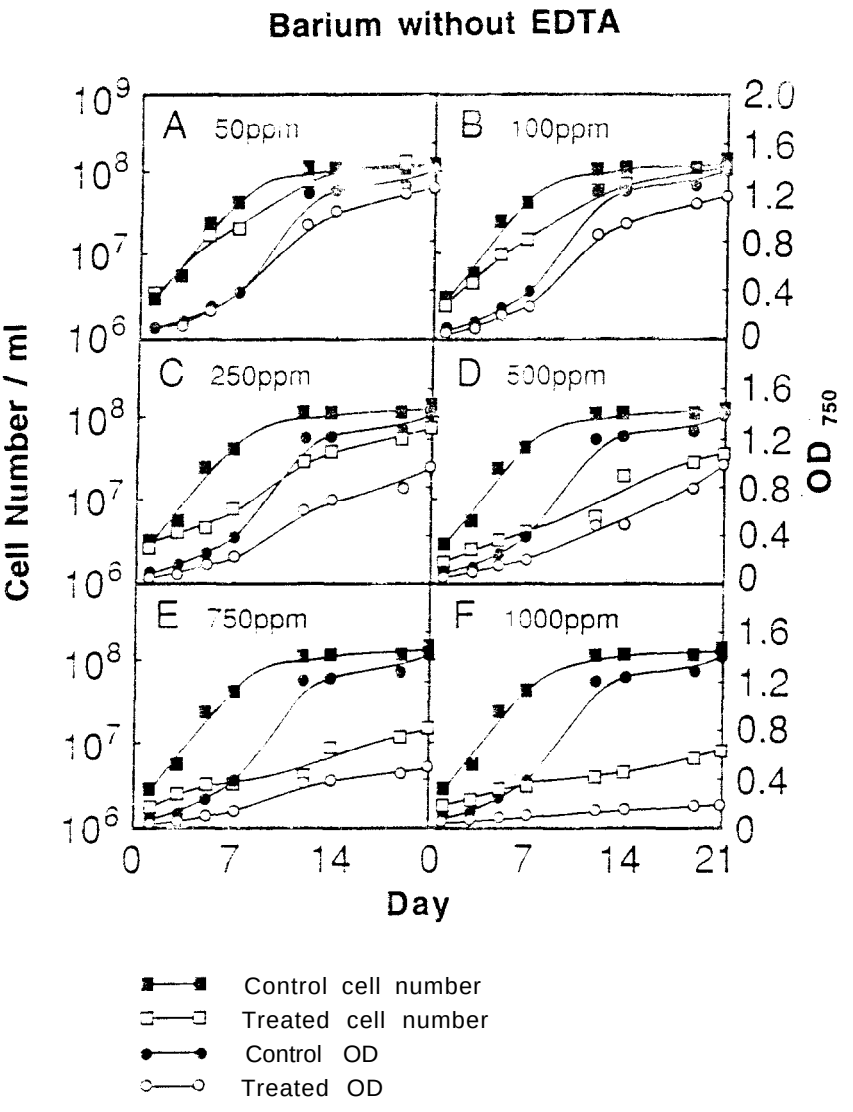

Figure 3. Growth of A. nidulans in 100 ml of 3M medium containing barium chloride at 50(A), 100(B), 250(C), 500(D), 750(E), 1000(F) ppm without EDTA. Cell growth was measured by optical density readings at 750 nm and direct count.

proposed to use biological indicators to demonstrate environmental pollution. This approach is valuable for demonstrating exposure to potentially toxic trace elements. Past reports have indicated varying levels of toxicity dependent upon the metallic ion species and on the biological indicator species. Wide differences exist among algal species in their requirements for nutrient metals and in their sensitivity to toxic metals (Sunda 1989). Resistance by microorganisms to

high levels of toxic metals has been ascribed to a variety of mechanisms. This may be due to either entrapment by cellular components or biosorption and complexation (Harris and Ranelow 1990).
Rai et al. (1981) reported toxicity of heavy metals as the following! from greatest to least: silver, cadmium, nickel, selinium, copper, barium and lead for Chlorella vulgaris. Chlorella isolated from activated sludge was inhibited from growth and uptake of inorganic nitrogen and phosphorous in the presence of nickel, but the inhibition was lower than in the presence of copper or hexavalent chromium (Wong and Chang 1991).

Our results have shown that, in the presence of EDTA, A. nidulans demonstrated some sensitivity to 10 ppm nickel ions, with concentrations >25 ppm being extremely lethal; while without EDTA, severe inhibition of growth was seen at 10 ppm. The role of EDTA in 3M is as a chelating agent. A chelator will aid the cell in surviving in an undesirable environment by binding with excess concentrations of metal ions. It preferentially binds with Fe in 3M medium, but also effects other metal ions present. Chelators play an important role in the bioavailability of metallic ions in the environment. Natural organic ligands in the environment may act as chelators and may serve to influence the effect of the metallic ions on microorganisms (Mattuschka and Straube 1993). In our study, nickel was chelated by the presence of EDTA in the medium, thereby limiting the lethality of nickel.

Mechanisms of toxicity for heavy metals are binding to the cell membrane (Rachlin and Grosso 1993) and binding to metabolic sites normally occupied by other essential nutrient metalloenzymes (Sunda 1989). Nickel is thought to affect the functioning of polymerases involved in the biosynthesis of DNA (Casarett and Doull 1980).

Because of the effect of barium on the human cardiovascular system, the use of barium and its environmental levels are regulated (Wang 1986). We found that extremely high levels of barium had no effect on growth of A. nidulans, suggesting that it would not serve as a useful bioindicator of barium toxicity.

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