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Effect of Manganese and Zinc on the Growth of *Anacystis nidulans*

L. H. Lee, B. Lustigman, D. Dandorf

Montclair State College, Upper Montclair, New Jersey, USA

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Anacystis nidulans is a unicellular member of the cyanobacteria, one of the largest groups of the Kingdom Monera. It is similar to other bacteria in the structure and chemistry of the cell wall, and its cell division and genetic recombination (Allen 1968). Photoautotrophy is the main mode of nutrition and the photosynthetic apparatus is similar to that of other cyanobacteria (Fogg 1973). Cyanobacteria are excellent organisms to serve as environmental pollution indicators for the investigation of a wide variety of biological problems. There have been several studies on the effects of heavy metals on *A. nidulans* (Lee et al.1991, Lee et al. 1992, Singh and Yadava 1985). Some of these elements, such as manganese, are known to be essential nutrients for cyanobacteria (Casarett and Doull 1980). Others, such as cadmium, are not known to be necessary for normal growth and metabolism (Lee et al. 1992). Large amounts of either essential or non-essential elements can be toxic (Snyder 1982).

Manganese and zinc are essential elements for all living organisms. Manganese is a cofactor for a number of different enzymatic reactions particularly those involved in phosphorylation (Casarett and Doull 1980). Iron deficiency induced by a number of metals, cobalt and manganese in particular, inhibit chlorophyll biosynthesis (Csatorday et al 1984). Zinc deficiency affects early mitotic events and the cells are large and aberrant in appearance. Light is essential for cells to take in zinc. As an industrial contaminant, zinc has been found to block photosynthesis by causing structural damage to the photosynthetic apparatus. In the presence of various pH ranges, high zinc concentrations can be associated with low pH. (Nriagu 1980). It has been indicated that pH value and EDTA (Ethylene Diamine Tetraacetic Acid) have an influence on the effect of some metals (Lee et al. 1991).

The purpose of this study was to determine the effect

Correspondence to: L. H. Lee

of manganese and zinc on the growth of *Anacystis nidulans*, with and without EDTA.

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. In the studies with manganese some of the cultures were monitored for up to 30 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 $MnCl_2$ was diluted to achieve final concentrations of manganese of 0, 50, 75, 100, and 200 ppm with EDTA and 10, 15, 30 and 50 ppm without EDTA. 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. The pH values were measured at the beginning and end of the experiments.

A stock solution of $ZnCl_2$ was diluted to achieve final concentrations of zinc of 0, 10, 25, 50, 75, and 100 ppm. Cultures were prepared and treated as indicated above, both with and without EDTA. PH readings were taken at the beginning and end of the experiments.

RESULTS AND DISCUSSION

The toxicity of manganese to *A. nidulans* was investigated using increasing concentrations of 0 to 200 ppm. Figure 1 shows that *A. nidulans* is able to grow in the medium containing 10 ppm manganese with little effect. At 50 ppm manganese the rate of growth was slower than the control. In medium containing 75 and 100 ppm, there was a long latent period of 8 days for 75 ppm and 16 days for 100 ppm, before the growth of the cells was observed. By 30 days growth was similar to the control. At the concentration of 200 ppm, cell growth was completely inhibited.

The medium containing no EDTA was also studied. Figure 2 shows that *A. nidulans* was able to grow in 10 and 15 ppm manganese without EDTA, but did not reach the stationary phase as quickly as in medium containing EDTA. The growth curve for cultures containing 30 and 50 ppm manganese demonstrated a long latent period prior to the onset of log phase of growth, a latent period of 7 days for 30 ppm and 12 days for 50 ppm without EDTA, respectively. One hundred ppm completely

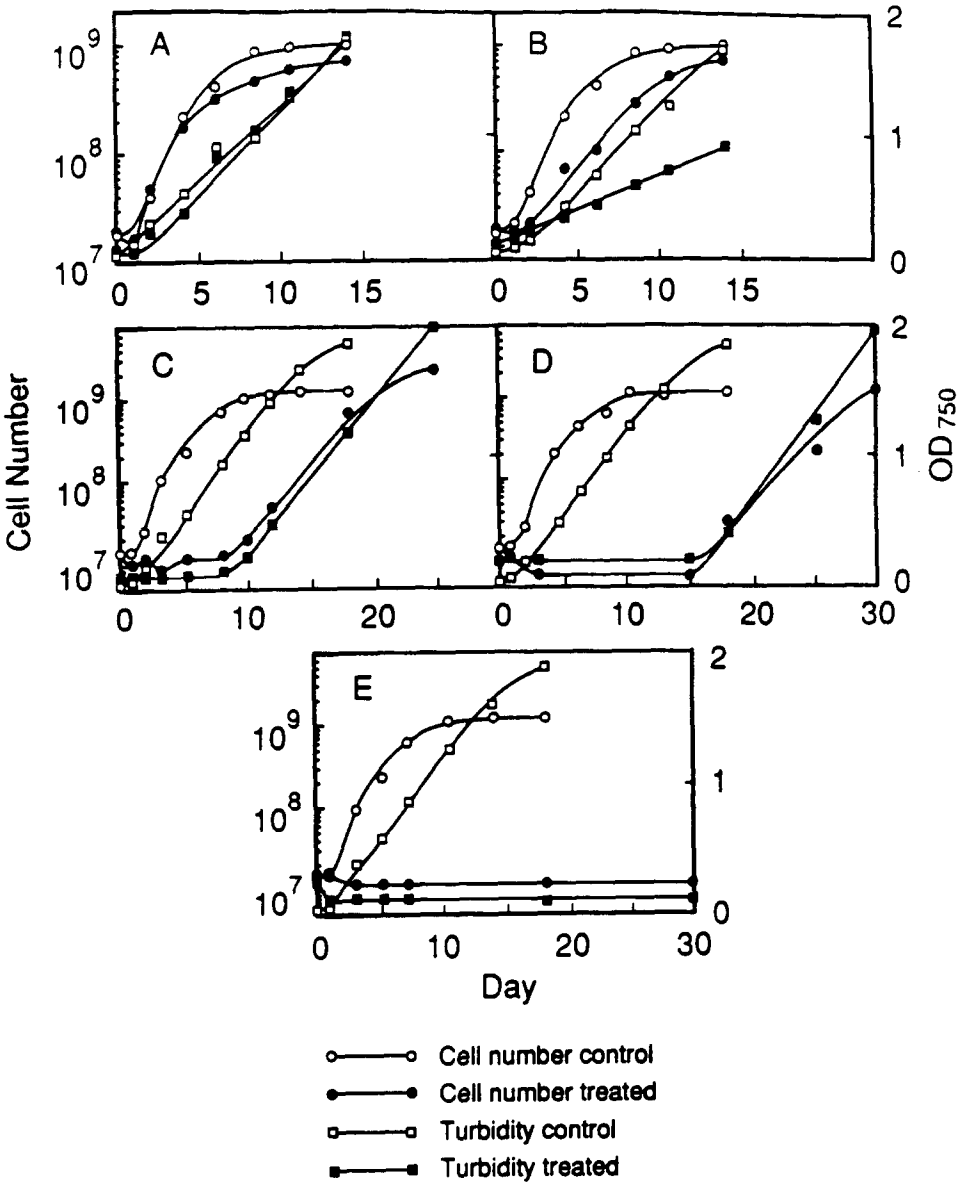

Figure 1. Growth of *Anacystis nidulans* in 3M media in the presence of manganese with EDTA A) 10 ppm B) 50 ppm C) 75 ppm D)100 ppm E) 200 ppm

inhibits the growth of *Anacystis nidulans*. This suggests that EDTA has a chelating effect on manganese ions. Two sets of experiments were performed, one in which the pH was adjusted to 7.9 and one in which the pH was not adjusted at the start of the experiment (pH 6.7). The results of both experiments were very similar. It appears that pH has little effect on growth of *Anacystis nidulans* with manganese. The pH

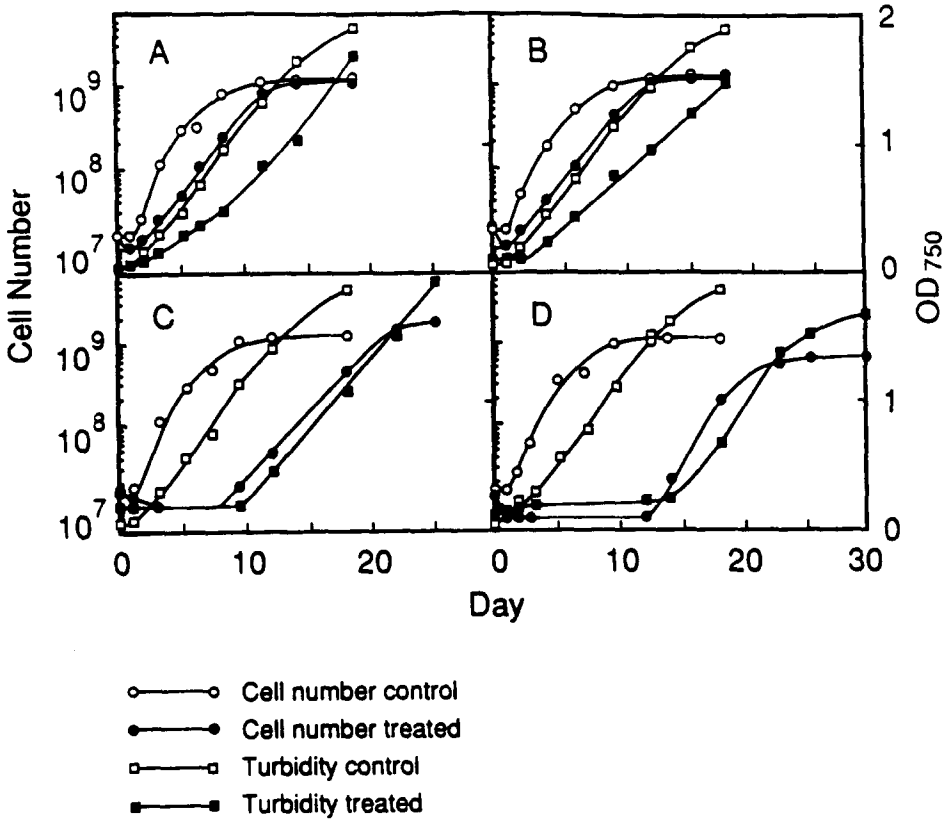

Figure 2. Growth of *Anacystis nidulans* in 3M media in the presence of manganese without EDTA A) 10 ppm B) 15ppm C) 30 ppm D) 50 ppm

measurements taken at the end of experiment showed pH levels of 9-10 in those flasks which contained growing organisms and pH near 7 in those cultures where the organisms were not growing.

The effect of zinc on the growth of *A. nidulans* was determined in media containing EDTA (Figure 3). At 10 ppm concentration, cell growth was slightly inhibited as compared to the control and the cells had lost some of the pigmentation. At concentrations of 25 ppm growth was inhibited as determined by cell number, although the turbidity was effected less severely, probably due to cell debris; pH was 9, which is similar to the control. At 50 ppm zinc, growth was significantly reduced and only recovered in part by the end of the experiment; the cells were small and pale and pH values were near 8. At 75 ppm zinc and higher levels, cell growth was completely inhibited; pH values were 7 at the end of the experiment. The experiment was terminated at 10 days, since stationary phase had been reached by this time. Therefore, the

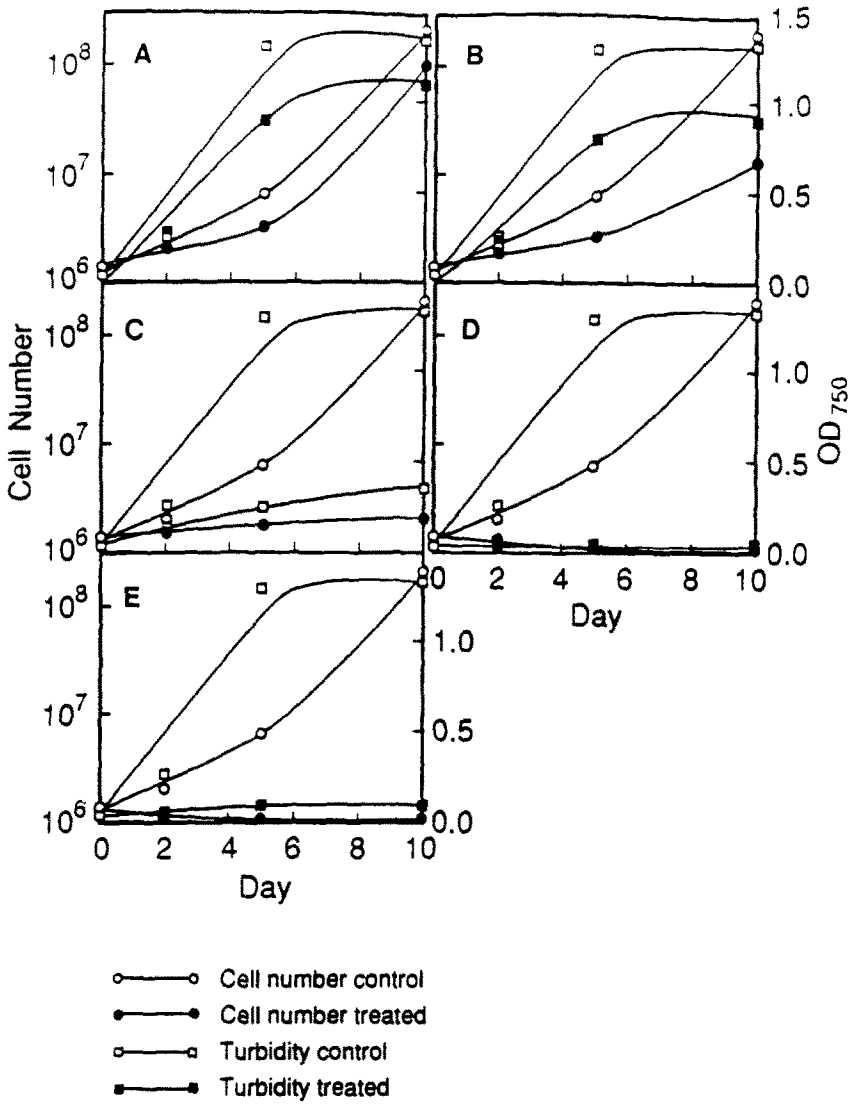

Figure 3. Growth of *Anacystis nidulans* in 3M media in the presence of zinc with EDTA A) 10 ppm B) 25 ppm C) 50 ppm D) 75 ppm E) 100 ppm

number of data points for this experiment are fewer.

A similar series of experiments were conducted in media which did not contain EDTA, using values of 0, 10, 25, 50, 75 and 100 ppm (Figure 4). At 10 ppm $ZnCl_2$ without EDTA, both cell growth and turbidity were significantly inhibited as compared to the control. PH values were lower for both the control and the zinc treated flasks, less than 8. Concentrations of 25 ppm and higher showed a complete inhibition of cell growth, with no recovery. This suggests that EDTA is important for chelating zinc ions.

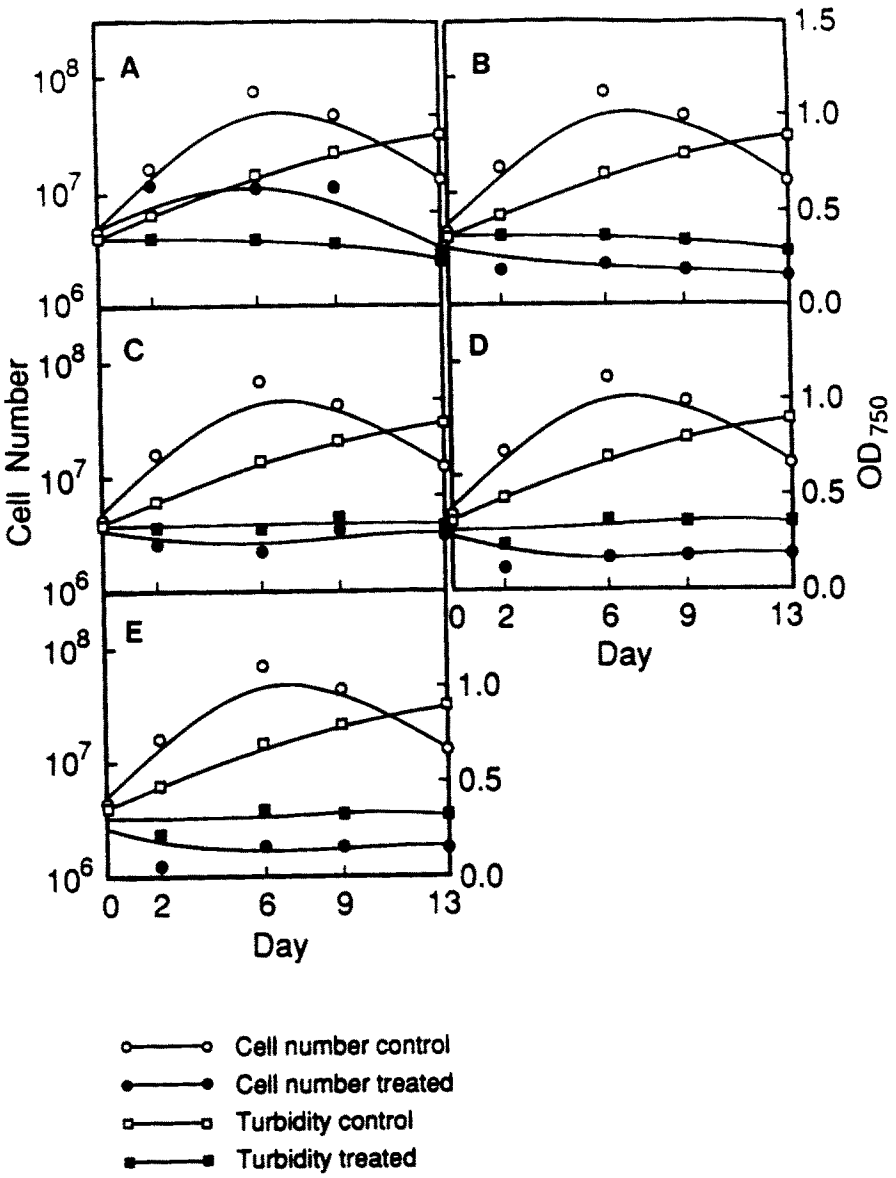

Figure 4. Growth of *Anacystis nidulans* in 3M media in the presence of zinc without EDTA A) 10 ppm B) 25 ppm C) 50 ppm D) 75 ppm E) 100 ppm

While manganese and zinc are essential metal ions needed for growth of *A. nidulans*, when concentrations become very high, they become toxic. This is more evident with zinc, where 75 ppm zinc with EDTA and 25 ppm without completely inhibited growth. With manganese this concentration was in the range of 200 ppm with EDTA and 100 ppm without. The higher magnitude of toxicity to zinc, as seen in Figure 4 as

compared to Figure 3, is due to the chelating effect of EDTA on zinc ions. Thus, while EDTA can be effective chelator for both zinc and manganese, its effect is greater for zinc. Previous reports of the effect of EDTA and other substances as chelators in algal growth studies have shown similar results of varying effectiveness. The variables which appear to effect the capacity of chelation are dependent upon the metallic ion involved (Lee et al. 1992a, 1992b), the chelating agent and the algal species involved (Lee et al. 1992a, 1992b; Rai and Raizada, 1988).

The effect of pH on metal toxicity has been reported. Increased metal uptake seems to be favored by low pH in some species while alkalinity seems to affect others (Singh and Yadava 1985). Previous studies on the effect of pH and aluminum with *A. nidulans* shows greater toxicity at low pH (Lee et al. 1991). In this study low pH accompanied higher metal toxicity for zinc, but not manganese. It has also been 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). Whitton and Fahni (1982) reported resistance to cadmium with repeated subculturing at inhibitory levels. Similarly our studies found that long term exposure to manganese was necessary for the cells to overcome the inhibitory effect of the heavy metal. This effect may be due to the release of extracellular chelating factors by the cells which alleviate heavy metal uptake and toxicity (Rai and Raizada 1988), or the passage of plasmid encoded genes for heavy metal resistance (Trevors et al., 1986). *Anacystis nidulans*, like other bacteria, has been shown to contain plasmids (Law and Doolite 1979). The synergistic action of the other metal ions in 3M medium, such as calcium, which compete for the same active uptake sites in the cell membrane (Singh and Yadava 1985) may also have an effect.

In summary, both manganese and zinc produce toxic effects on the growth of *Anacystis nidulans*. Zinc is the more toxic ion, producing reduced growth with 25 ppm and lethality at 50 ppm in standard 3M medium. In media without EDTA this effect is greater and lethality is present at 25 ppm. On the other hand, lethality to manganese was shown at 200 ppm. Lower concentrations of manganese produced a delay in the onset of exponential phase. EDTA was an effective chelator with manganese as well. Resistance to heavy metals may be due to the production of extracellular chelating factors or to the presence of heavy metal resistance genes present in a plasmid (Rai and Raizada, 1988; Trevors, 1986).

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