Bull. Environ. Contam. Toxicol. (2000) 64:565-573

DOI: 10.1007/s001280000040

Environmental Contamination and Toxicology

Effect of Thallium on the Growth of *Anacystis nidulans* and *Chlamydomonas reinhardtii*

B. Lustigman, L. H. Lee, J. Morata, F. Khan

Biology Department, Montclair State University Upper Montclair, NJ 07043, USA

Received: 22 September 1999/Accepted: 26 January 2000

Anacystis nidulans is a rod-shaped, unicellular cyanobacterium and an obligate photoautotroph with a photosynthetic apparatus similar in function to the eukaryotic chloroplast. *Chlamydomonas reinhardtii* is a unicellular green algae frequently found in fresh water environments. The cell contains a single nucleus, chloroplast, contractile vacuoles, and two anterior flagella. These organisms can be described as desirable indicators of contamination because they are simple, sensitive, and ubiquitous. Since these organisms are primary producers in the food chain, the metabolic and morphological effects on them by environmental toxicants play an important role in assessing aquatic ecosystems' responses to heavy metal contamination. The EPA has listed major toxic target contaminants, and these contaminants have been used in previous studies with *A. nidulans* and *C. reinhardtii* as models for heavy metal toxicity (Lee et al. 1991, 1994; 1999; Lustigman et al. 1997).

Thallium is a soft, bluish-white metal, which commonly occurs in trace amounts in potash and other mineral compounds. Its use is limited to alloys and certain applications in electrical circuitry. The sulfate, nitrate, and carbonate forms are water-soluble, and the sulfate was formerly used as a pesticide. Thallium isotopes are currently used in cardiac scanning. Thallium enters the environment primarily from coal burning and smelting, in which it is a trace contaminant of the ores. Although thallium is a highly toxic metal, it has been the focus of less research than cadmium, mercury, and lead. Studies of contaminant concentration levels in Lake Erie showed that the level of thallium was higher than cadmium (Cheam et al. 1996). Human exposure to thallium can result in extremely harmful health effects including death (Klaassen 1996). Within the cell, it can substitute for potassium, as in the sodium potassium ATPase pump. It has a strong attraction to sulfhydryl groups, thereby inhibiting and inactivating enzymes (Collins and Stotzky 1992).

In this study, direct count and turbidity were used to determine the effect of various concentrations of thallium nitrate ($TlNO_3$) on the growth of *A. nidulans*, while turbidity and chlorophyll concentration were used to determine the effect on *C. reinhardtii*. Light microscopy was used to study morphology.

Correspondence to: B. Lustigman

MATERIALS AND METHODS

The culture of *A. nidulans* was obtained from Dr. McGowan, Brooklyn College, N.Y. and the *C. reinhardtii* culture was obtained from Carolina Biological Co. Burlington, N.C.,USA. The cells were grown in 250 ml sterile shake flasks containing 100 ml Mauro's Modified Medium (3M) (Kratz and Meyer 1955). For *C. reinhardtii,* a vitamin mix was added which contained thiamine, biotin, and B_{12}. The cultures were grown under constant fluorescent light at ambient temperature with continuous agitation (100 rpm) until stationary phase was achieved. The flasks were inoculated with either approximately 1 X 10^7 cells/ml of *A. nidulans* or 1 X 10^5 cells/ml of *C. reinhardtii.* Growth of the cultures was determined by two methods for *A. nidulans:* 1. direct count using a Spencer hemocytometer, and 2. indirect turbidometric measurement using a Beckmann Spectronic 1001 spectrophotometer at 750 nm. For *C. reinhardtii,* growth was determined by: 1. chlorophyll extraction and measurement by spectrophotometer at 663 and 645 nm, and 2. indirect turbidometric measurement as indicated above. Chlorophyll extraction was performed following a modification of the method of Aron (1949). In the modification for unicellular algae, 3 mls were removed from the flasks and treated with 100% ethanol. Direct counts were not possible because the cells form clumps when exposed to the heavy metal ions, but microscopic evaluation of the cells was performed. Cultures were read every 3-4 days for 21 days. Cultures were checked for contamination on nutrient agar.

The stock solution of $TINO_3$ was prepared at a final concentration of 10,000 mg/L. For *A. nidulans,* series dilution with final concentrations of 0, 5, 10, 15, and 20 mg/L $TINO_3$ were used to study the effect of thallium on growth. Previous studies have shown that ethylene diamine tetraacetic acid (EDTA) can reduce the toxicity of some heavy metal ions for *A. nidulans* (Lee et al. 1991, 1994, 1999), so another set of experiments was carried out with the same protocol as described above, but without EDTA. For C. *reinhardtii,* concentrations of 0, 0.10, 0.25, 0.50, 0.75, and 1.00 mg/L $TINO_3$ were used. *Chlamydomonas* is unable to grow in medium without a chelator, such as EDTA. Four flasks were prepared for each concentration of $TINO_3$. For each experiment, a control was prepared of untreated *A.nidulans* and *C. reinhardtii* in 100 ml of 3M medium, with or without vitamins, grown under the same conditions. Culture pH was read at days 1 and 22.

Comparisons for each pair using Tukey-Kramer HSD were performed to analyze the data and to determine the difference between the various concentrations using JMP computer statistic software (SAS Institute1995).

RESULTS AND DISCUSSION

For *A. nidulans,* growth was similar in all flasks of each concentration, and the standard deviation within each concentration was very small (Fig. 1). At 5 mg/L there was a delay in the onset of log phase until days 11- 15 compared to the rapid onset at day 3 for the control cultures, and cell growth never reached the same

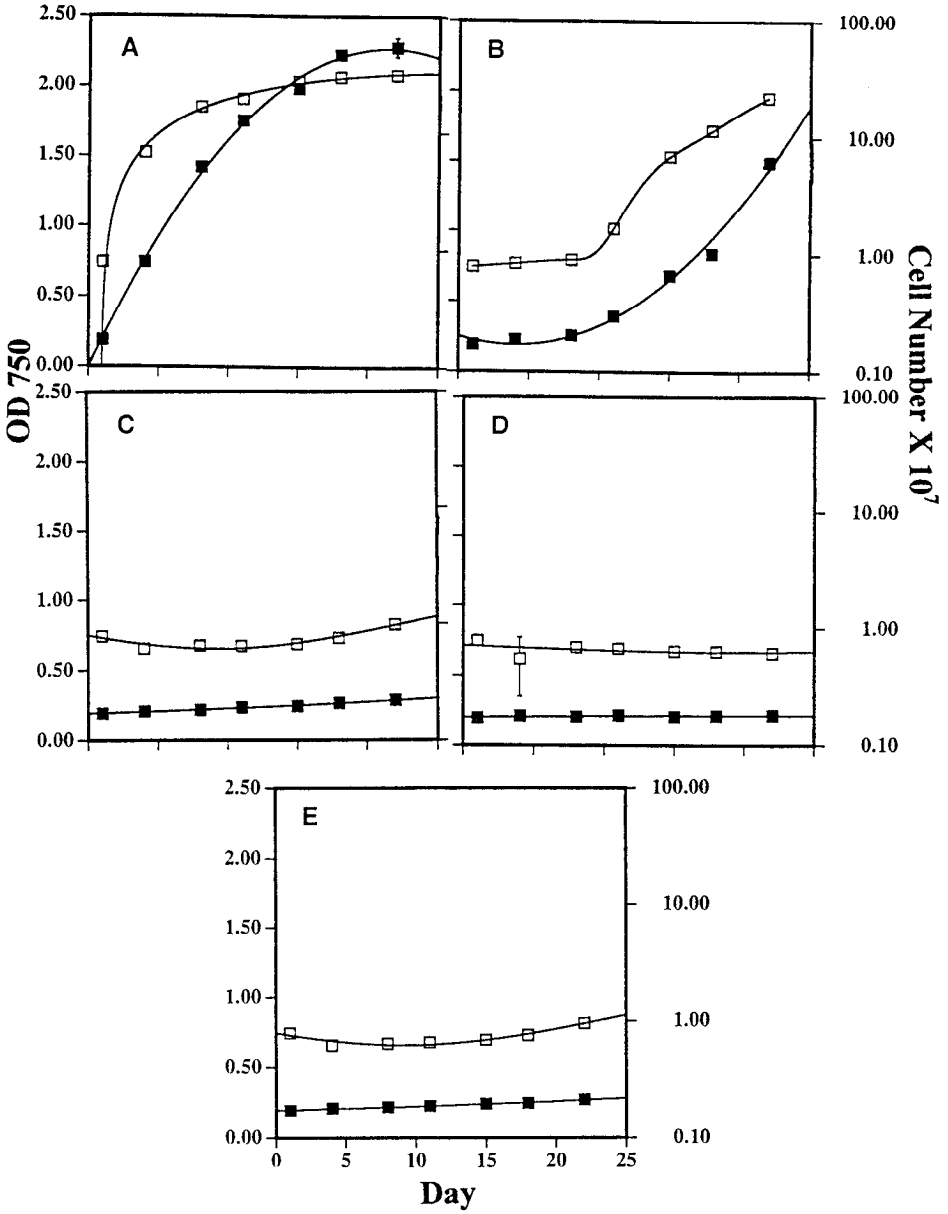

Figure 1. Growth of Anacystis nidulans in 100 ml of 3M medium containing $TINO_3$ at different concentrations (O-20 mg/L). Error bars represent standard deviations. Curves were fit by regression analysis.

A) 0 mg/L B) 5 mg/L Ç) 10 mg/L D) 15 mg/L E) 20 mg/L

❑ Represents mean cell number■ Represents mean optical density (OD).

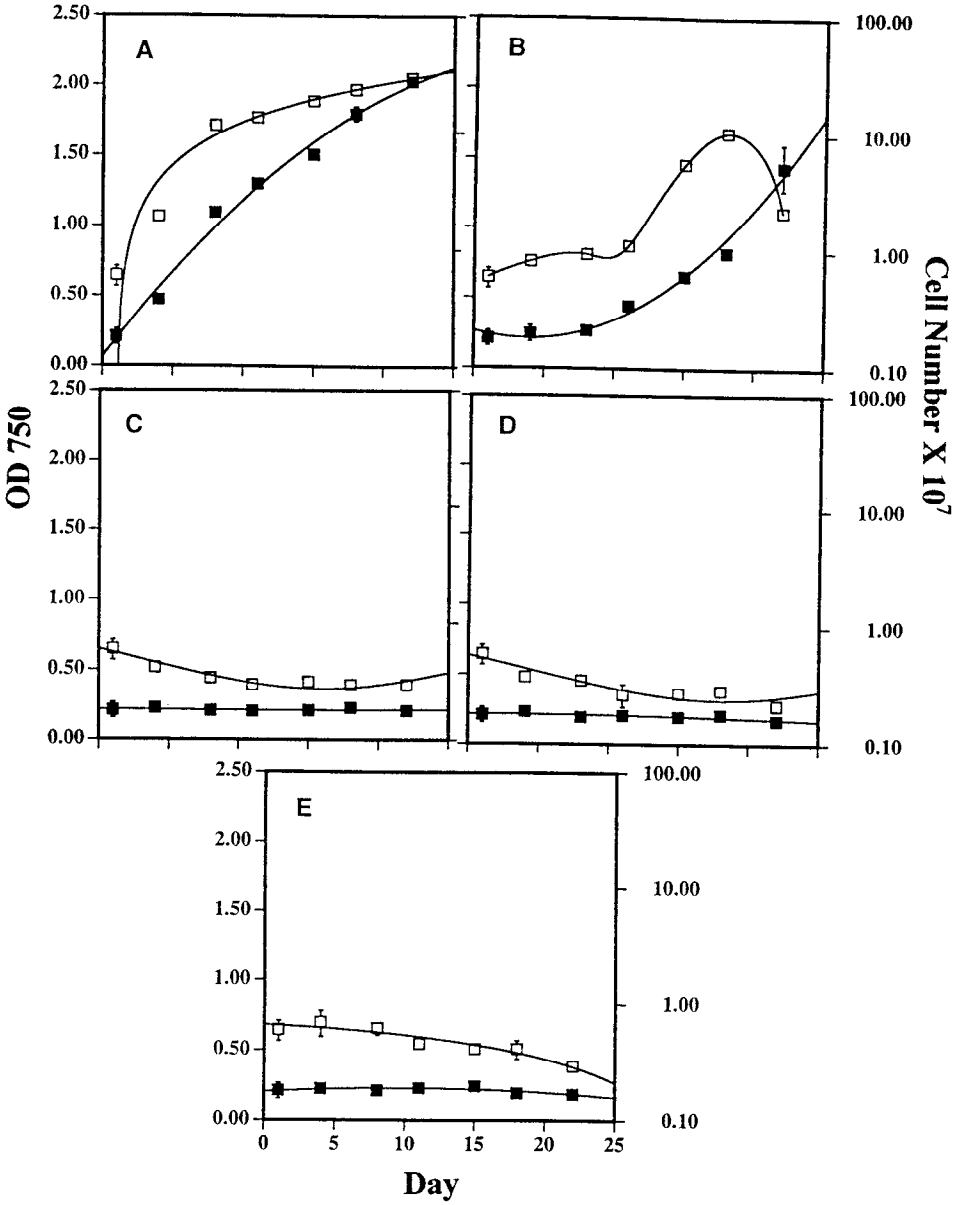

Figure 2. Growth of Anacystis nidulans in 100 ml of 3M medium containing $TINO_3$ at different concentrations (0-20 mg/L) without EDTA. Error bars represent standard deviations. Curves were fit by regression analysis.
A) 0 mg/L B) 5 mg/L C) 10 mg/L D) 15 mg/L E)20 mg/L
❑ Represents mean cell number. ■ Represents mean optical density (OD).

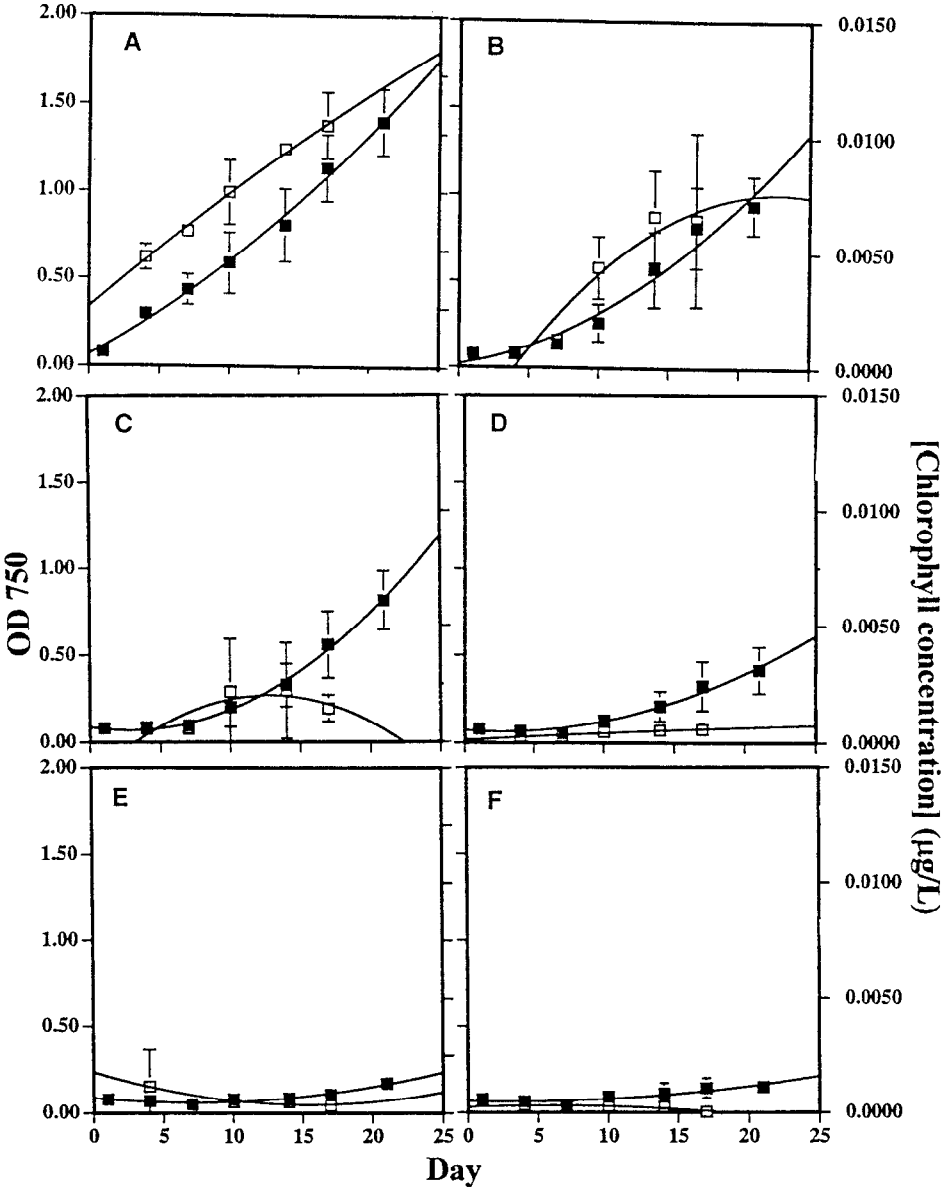

Figure 3. Growth of Chlamydomonas reinhardtii in 100 ml of 3M medium containing $TINO_3$ at different concentrations (0-1.0 mg/L) with EDTA. Error bars represent standard deviations. Curves were fit by regression analysis.
A) 0 mg/L B) 0.1 mg/L C) 0.25 mg/L D) 0.50 mg/L E) 0.75 mg/L F) 1.00 mg/L □ Represents mean total chlorophyll. ■ Represents mean optical density (OD).

values as the control. Growth was completely inhibited at 10, 15, and 20 mg/L $TlNO_3$. The results of statistical analysis using Tukey-Kramer HSD ($p = 0.05$) indicate that control growth of *A. nidulans* cultures with EDTA was significantly greater than any of the other concentrations as measured by turbidity and direct count. Growth at 5 mg/L $TlNO_3$ was significantly less than the control and greater than all other concentrations. There was no significant difference in growth levels between the 10, 15, and 20 mg/L $TlNO_3$, but they were significantly different from 0 and 5 mg/L $TlNO_3$ levels, Results for 0 and 5 mg/L $TlNO_3$ were significantly different from each other. Observations of the cells revealed that control cells displayed normal morphology and blue-green color. At 5 mg/L $TlNO_3$. the ceils were smaller, more slender, and pale, while at 10 mg/L and higher, they were colorless.

In order to determine if EDTA was acting as a chelating agent for thallium, cultures were prepared without EDTA. Results were similar to the cultures with EDTA (Fig. 2). At 5 mg/L $TlNO_3$. the onset of log phase was delayed until days 11-15 with a sharp decrease by day 21. Values were never as great as in the control. Growth was completely inhibited at concentrations of 10, 15, and 20 mg/L $TlNO_3$. Results using Tukey-Kramer HSD ($p = 0.05$) indicate that the control was significantly different from all other cultures. Results for 0 and 5 mg/L $TlNO_3$ were significantly different from each other. Cultures with 5 mg/L $TlNO_3$ displayed significantly greater growth than the other values. At 10, 15, and 20 mg/L $TlNO_3$ results were not significantly different from each other, but were different from the control and 5 mg/L $TlNO_3$. Cells in the cultures displayed morphology similar to those with EDTA, and at 10 mg/L and higher they were colorless. These results indicate that at 5 mg/L $TlNO_3$, EDTA has some effect in chelating thallium, since a sharp decrease in growth was observed at day 21 in the absence of EDTA.

Determination of thallium effects on growth of the chlorophyte *Chlamydomonas reinhardtii* shows that it displays much greater sensitivity to thallium than was seen with the cyanobacterium, *Anacystis nidulans.* Growth was reduced at 0.1 mg/L $TlNO_3$, (Fig. 3). At 0.25 mg/L $TlNO_3$, the optical density began to increase at days 11-15, but chlorophyll concentration remained low. This was similar to the results seen with the 5mg/L $TlNO_3$ cultures of *A. nidulans.* At 0.5, 0.75 and 1.0 mg/L $TlNO_3$, growth was severely inhibited, but at 0.5 mg/L $TlNO_3$ cultures, optical density showed a slight increase at days 15-21. Using Tukey-Kramer HSD ($p = 0.05$), as determined by optical density, results show that the control had significantly greater growth than all of the treatment cultures. At 0.10 and 0.25 mg/L $TlNO_3$ growth was significantly less than the control, but significantly greater than 0.5, 0.75 and 1.0 mg/L $T1NO_3$. Results at 0.5, 0.75, and 1.0 mg/L $TlNO_3$ were not significantly different from each other, but were different than 0, 0.1 and 0.25 mg/L $TlNO_3$. Results at 0.1 and 0.25 mg/L were not significantly different from each other. Statistical analysis of chlorophyll concentration indicates that the control was significantly different from all other values. Results at 0.1 mg/L $TlNO_3$ were not significantly different than 0.25, but were different from the

Table 1. Time dependent changes (± SD) in pH in *A. nidulans* cultures with different $TlNO_3$ concentrations (0-20 mg/L)

$TlNO_3$	EDTA		No EDTA	
(mg/L)	Day 1	Day 22	Day 1	Day 22
0	7.9	9.29±0.15	7.9	9.29±0.11
5	7.9	8.37±0.22	7.9	8.14±0.03
10	7.9	7.87±0.06	7.9	7.81±0.01
15	7.9	7.87±0.04	7.9	7.83±0.02
20	7.9	7.88±0.02	7.9	7.85±0.04

Table 2. Time dependent changes (± SD) in pH in *C. reinhardtii* cultures with different $TlNO_3$ concentrations (0-1.0 mg/L)

mg/L	Day 1	Day 22
0	7.94±0.14	10.07±0.17
0.10	8.03±0.12	10.15±0.15
0.25	8.01±0.13	9.78±0.55
0.50	7.99±0.10	8.50±0.51
0.75	7.80±0.11	7.95±0.11
1.00	7.80±0.05	7.93±0.09

control and 0.5, 0.75, and 1.0 mg/L $TlNO_3$. Results at 0.25, 0.5, 0.75, and 1.0 mg/L $TlNO_3$ were not significantly different from each other.

Control cultures were deep green in color and cells did not form clumps. At 0.10 mg/L $TlNO_3$, cultures were also deep green but had clumps of cells. As the concentration of thallium increased, the cells became increasingly paler and displayed greater clumping (0.25 and 0.50mg/L $TlNO_3$). At 0.75 and 1.0 mg/L $TlNO_3$, cells were colorless. The difference between the results of optical density and chlorophyll measurements were confirmed by observations using light microscopy in which cells at 0.25mg/L $TlNO_3$ were intact, but had lost their green color. The results at 0.50, 0.75, and 1.0 mg/L $TlNO_3$ show loss of chlorophyll in these cells, reduced number of cells, and increased cellular debris. Growth at 0.25 mg/L $TlNO_3$ also displayed a delay in the onset of log phase, until days 11-15, similar to the results seen with *A. nidulans* at 5mg/L $TlNO_3$.

After 21 days the cultures were centrifuged, washed, and resuspended in 100 ml 3M medium without $TlNO_3$. Regrowth of the cultures in new media indicated that, 545454546for *A. nidulans,* 10 mg/L $TlNO_3$ is cyanostatic and 15 mg/L is cyanocidal, both with and without EDTA. The same procedure was followed for

C. reinhardti except for the addition of vitamins to the medium. Results indicate that 0.50 mg/L was algastatic and 0.75 mg/L was algacidal.

Measurement of pH indicates an increase from the initial pH of 7.9 in flasks in which growth was sustained (Tables 1 and 2) for both *A. nidulans* and *C. reinhardtii.* These results are consistent with previous experiments in which pH values increase when growth occurs, but not in cultures without growth.

There are considerable differences in the lethal concentrations of thallium towards the two organisms. These differences may be due to variations in the morphology and physiology of prokaryotic and eukaryotic microorganisms, as well as the ability of the microorganisms to either exclude or detoxify thallium. Microorganisms also differ in their ability to adapt to conditions of toxicity. Microbial growth can vary due to the concentration of the metal ions (Wood 1974). Alterations in the species of microalgae present in water bodies occur because of the impact of toxicants on their nutrient sources. This, in turn, will adversely affect zooplankton and herbivore grazing populations (Havens and Heath 1991).

Microorganisms are capable of coding for enzymes that can immobilize and detoxify toxic heavy metals from their cells. Specific enzymes have been identified that are capable of reducing toxic heavy metals to forms that are non-toxic (Abbas and Edwards 1989,Tsai and Olson 1990). Metallothionein-like compounds have been identified in several types of microorganisms which function in reducing the toxicity of heavy metals, These compounds act by binding cysteine and lysine to heavy metals (Hamer et al 1985), thereby providing a mechanism by which the ions are incapable of adversely interacting with essential metabolic enzymes. Another detoxification mechanism employed by some microorganisms is conformational changes in the cell wall that will trap the heavy metal ions and prevent them from entering the cell (Zhag and Majidi 1994). Various functional groups have been identified that are responsible for the binding of heavy metals to the cell surfaces of microorganisms (Gardea-Torresday et al 1990). Several genes have also been isolated that provide for resistance to heavy metals, in particular mercury in bacteria (Tsai and Olson 1990) and cadmium in *Chlamydomonas* (Nagel and Voight 1989). In procaryotic organisms, plasmids which code for mercury and cadmium resistance have also been isolated (Kondo et al 1974).

REFERENCES

Abbas A, Edward C (1989) Effects of metals on a range of *Streptomyces* species, Appl Environ Microbiol 55:2030-2035

Aron DI (1949) Copper enzymes in isolated chloroplasts. Plant Physio 24: 1-15

Cheam V, Lechner J, Desrosiers R, Azcue J, Rosa F, Mudroch A (1996) LEAFS determination and concentration of metals in Great Lakes ecosystem. Fresenius J Anal Chem 355: 336-339

Colins YE, Stotzsky G (1992) Heavy metals alter the electrokinetic properties

of bacteria, yeast, and clay minerals. Appl Environ Microbiol 58: 1592-1600
Gardea-Torresday JL, Becker-Hapak MK, Hosea JM, Darnall DW (1990) Effect of chemical modification of algal carboxyl groups on metal ion binding. Environ Sci Technol 24: 1372-1378
Hamer DH, Theile DJ, Lemontt JE (1985) Function and autoregulation of yeast copperthionein. Science 228:685-690
Havens KE, Heath RT (199 1) Increased transparency due to changes in the algal size spectrum during experimental acidification. J Plank Res 13:673-679
Klaassen C (1996) In: Casarett and Doull's Toxicology. Fifth edition. McGraw-Hill, NY
Kondo I, Ishikawa T, Kakahara H (1974) Mercury and cadmium resistances mediated by the penicillinase plasmid in *Staphylococcus aureus* J Bacterial 117:1-7
Kratz WA, Meyer J (1955) Nutrition and growth of several blue-green algae. Am J Bot 42:282-287
Lee LH, Lustigman B, Chu I, Jou HL (1991) Effect of aluminum and pH on the growth of *Anacystis nidulans.* Bull Environ Contam Toxicol 46:720-726
Lee LH, Lustigman B, Maccari J (1994) Effect of manganese and zinc on the growth of *Anacystis nidulans.* Bull Environ Contam Toxicol 53: 158-165
Lee LH, Lustigman B (1999) Effect of selenium on the growth of the cyanobacter *Anacystis nidulans.* Bull Environ Contam Toxicol 59: 591-599
Lustigman B, Lee LH, Weiss-Magasic C (1997) Effects of mercury on the growth of *Chlamydomonas reinhardtii.* Bull Environ Contam Toxicol 59: 187-193
Nagel K, Voight J (1989) In vitro evolution and preliminary characterization of a cadmium-resistant population of *Chlamydomonas reinhardtii.* Appl Environ Microbiol 55:526-528
SAS Institute (1995) IN: JMP Statistics and Graphics Guide Version 3, Cary, NC.
Tsai Y, Olson BH (1990) Effects of Hg^{2+}, CH_3Hg, and temperature on the expression of mercury resistance genes in environmental bacteria. Appl Environ Microbiol 56: 3266-3272
Wood JM (1974) Biological cycles for toxic elements in the environment. Science 183: 1049-1052
Zhang W, Majidi V (1994) Monitoring the cellular response of *Stichococcus bacillaris* to exposure of several different metals using *in vivo* PNMR and other spectroscopic techniques. Environ Sci Technol 28: 1577-1581