Bull. Environ. Contam. Toxicol. (1999) 62:591-599

Bulletin of Environmental Contamination and Toxicology

Effect of Selenium on the Growth of the Cyanobacterium *Anacystis nidulans*

L. H. Lee, B. Lustigman, S. Murray, S. Koepp

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

Received: 25 August 1998/Accepted: 19 March 1999

Cyanobacteria, such as Anacystis nidulans, provide an excellent indicator to study toxic metabolic effects of heavy metals since they are simple, sensitive and ubiquitous in water systems. Cyanobacteria can also provide an indication of the toxic effects of heavy metal pollution on metabolic activities of higher organisms in the ecosystem. Anacystis nidulans is a rod-shaped, unicellular cyanobacterium and an obligate photoautotroph with a photosynthetic apparatus similar in function to the eukaryotic chloroplast. Several heavy metals on the EPA major toxic contaminant list have previously been studied using Anacystis nidulans (Lee et al. 1991, 1994, 1996).

Selenium, a trace element essential for many organisms, is toxic in high concentrations. It is part of formic dehydrogenase, which forms acetate from carbon dioxide (Andreesen and Ljungdahl 1973). As a nutrient, it can help to prevent heart disease and cancer by working with glutathione peroxidase to reduce peroxides (Disimplicio and Leonzio 1989). At low carbon dioxide levels, selenium induces glutathione peroxidase in the green alga, Chlamydomonas (Yokota et al. 1988).

Selenium is introduced into the environment through coal-fired power plants, smelting, and the combustion of fossil fuels (Cassarett and Doull 1980). Its toxicity has been linked to bioaccumulation in freshwater ecosystems. Increased toxicity results in interference with sulfur uptake and metabolism damaging the sulfhydryl group of cysteine, which acts to link amino acids by disulfide bridges. In seven species of microalgae tested, all demonstrated strong growth inhibition toward selenate and partial growth inhibition to selenite (Pargasova 1994).

In this study, direct count and turbidity were used to determine the effect of various concentrations of selenium dioxide (SeO_2) on the growth of Anacystis nidulans. Light microscopy and scanning electron microscopy (SEM) were used to study morphology. Cells were grown in medium with or without the chelator ethylene diamine tetraacetic acid (EDTA) to determine its effectiveness in reducing toxicity. Previous studies have shown that EDTA can reduce the toxicity of heavy metal ions in microorganisms (Lee et al, 1991, 1994, 1996).

Offprints to: L. H. Lee

MATERIALS AND METHODS

The culture of A. nidulans was obtained from Dr. R. McGowan, Brooklyn College, N.Y. The cells were grown in 250 ml sterile shake flasks with 100 ml Mauro's Modified Medium (3M) (Kratz and Meyer 1955). The cultures were grown under constant fluorescent light at ambient temperature with continuous agitation at 100 rpm until stationary phase was achieved. The flasks were inoculated with approximately 1 X 10^7 cells/ml of A. nidulans. 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 read on days 1, 4, 8, 12, 15, 18, and 21. Cultures were checked for contamination by plating on nutrient agar.

The stock solution of selenium dioxide was prepared at a final concentration of 10,000 mgL Series dilution with final concentrations of 0, 10, 25, 50, and 100 mg/L SeO_2 were used to study the effect of selenium on the growth of A. nidulans. Another set of experiments was carried out with the same protocol as described above, but without EDTA. Four cultures were prepared for each concentration of SeO_2. For each experiment, a control was prepared of untreated A. nidulans in 100 ml of 3M medium and grown under the same conditions. Culture pH was read at days 1, 11 and 22. One-way ANOVA and Duncan's Multiple Range Test were used to analyze the data and to determine the differences between the various concentrations using the SAS system(McClave and Dietrich 1979).

For light microscopy, cell morphology was observed at 1000x magnification using a Reichert Microstar IV microscope. It appeared by light microscopy that the surviving cells in the 50 and 100 mg/L SeO_2 were increasing in length. To further determine the effect of selenium on the length, width and general morphology of the cells, SEM was performed using a small aliquot of A. nidulans cultures grown in concentrations of 0, 50, and 100 mg/L SeO_2 in 3M medium removed during log phase of growth. Aliquots were heat-fixed onto aluminum stubs and coated in a Denten gold sputter-coater for 25 seconds. Samples were observed at 4,500X and 12,500X magnifications at 15kv, using a Hitachii S-246N SEM. The length and width of at least ten cells from each of the three concentrations were measured and compared. The 4,500X micrograph images can be assumed to be representative of the possible effects of similarly treated A. nidulans cells.

RESULTS AND DISCUSSION

Using Duncan Multiple Range test (at alpha =.05) we can conclude in cultures with EDTA, growth at 25 mg/L SeO_2 closely resembled that of the control as measured by turbidity and direct count (Figure 1). Growth at 10 mg/L SeO_2 was significantly greater than the control in terms of cell number, but not in optical density. However, at 50 mg/L SeO_2 and higher, growth was completely inhibited. Growth was similar between the 50 and 100 mg/L SeO_2 cultures, which were

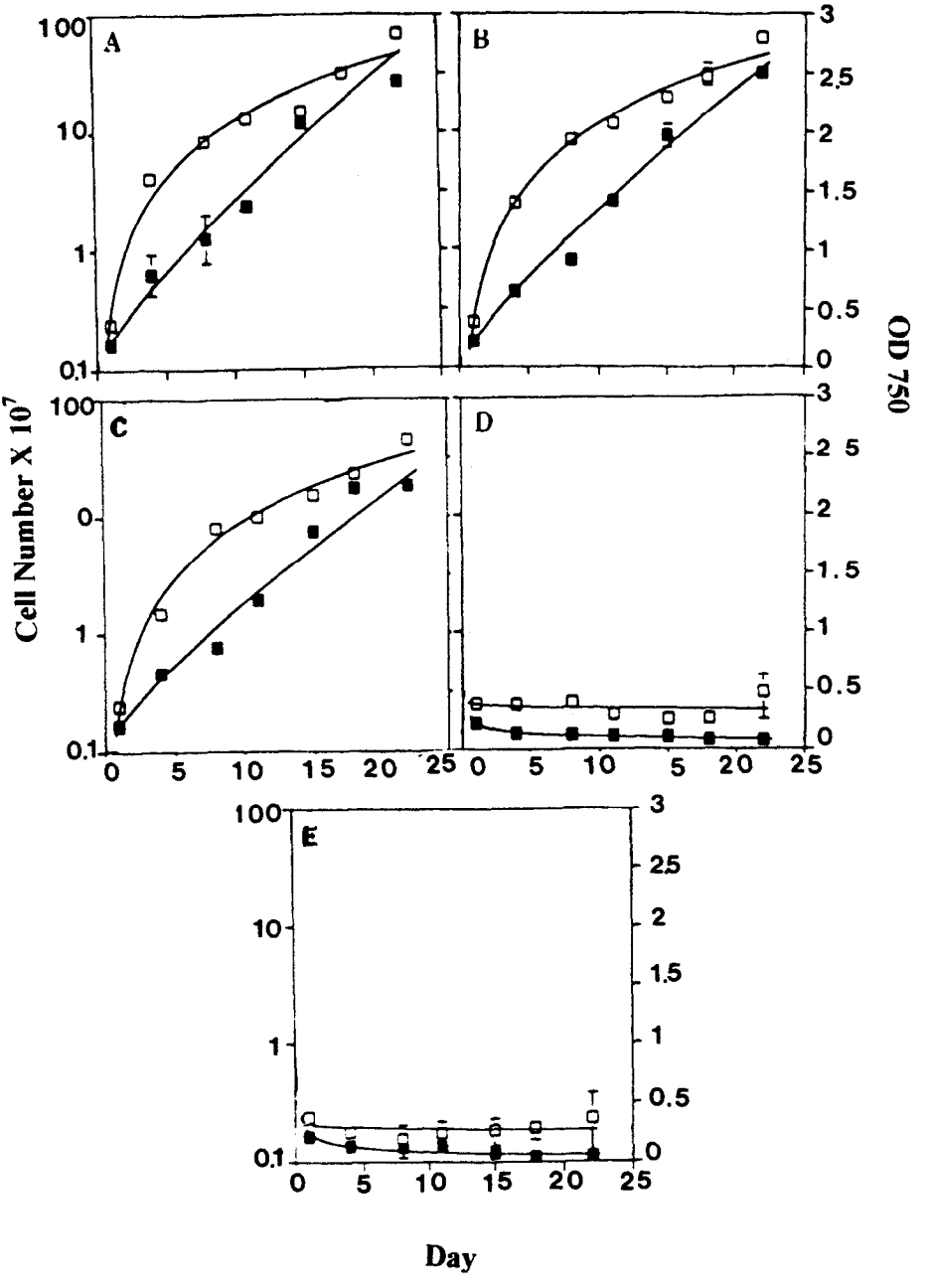

Figure 1. Growth of Anacystis nidulans in 100 ml of 3M medium containing SeO_2 at different concentrations (0-100 mg/L) with EDTA. Error bars represent standard deviations. Curves were fit by regression analysis.
A) 0 mg/L SeO_2 B) 10 mg/L SeO_2 C) 25mg/L SeO_2 D) 50 mg/L SeO_2
E) 100 mg/L SeO_2 □ Represents cell number mean. ■ Represents optical density mean (OD).

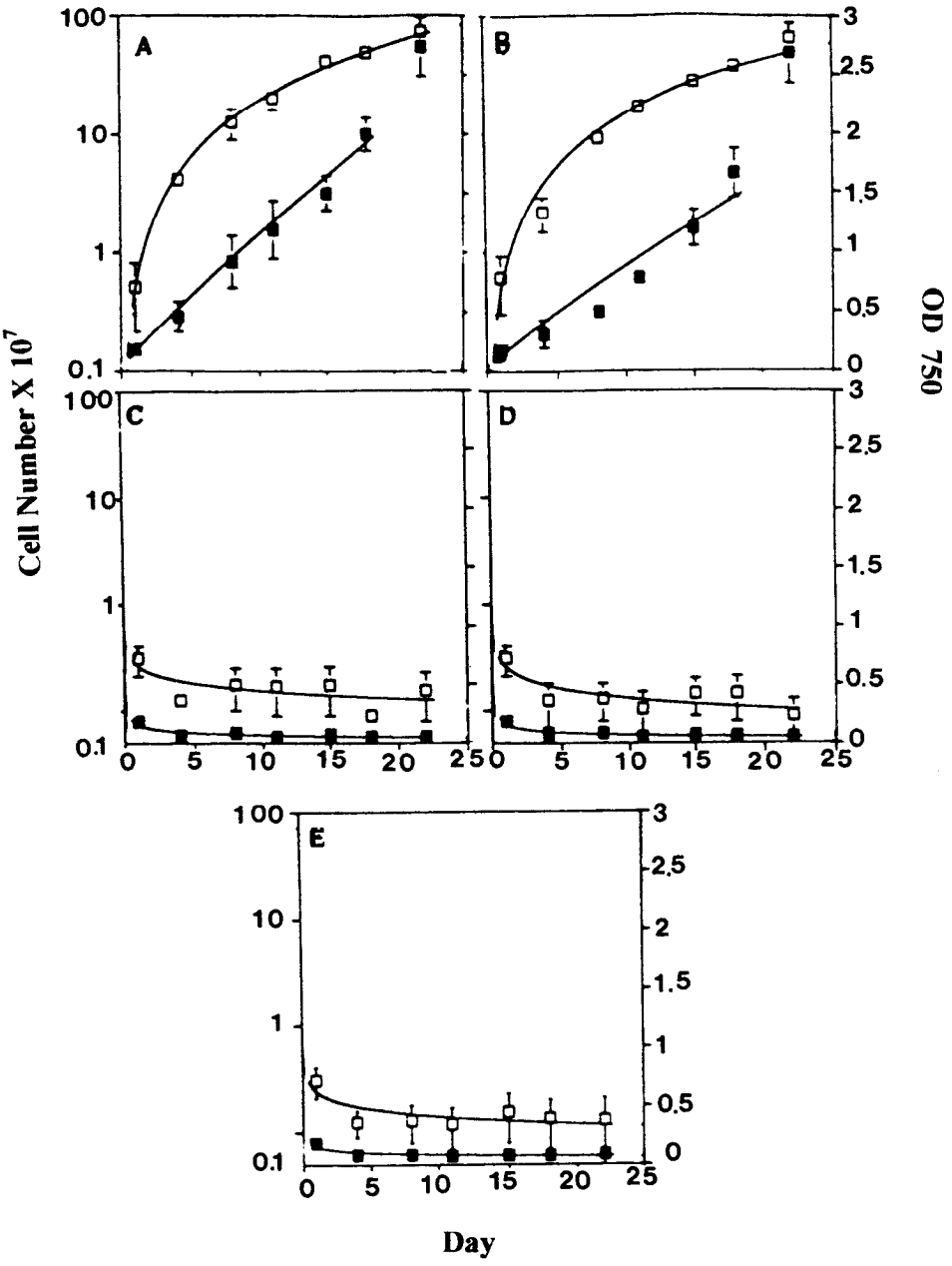

Figure 2. Growth of Anacystis nidulans in 100 ml of 3M medium containing SeO_2 at different concentrations (0-100 mg/L) without EDTA. Error bars represent standard deviations. Curves were fit by regression analysis. A) 0 mg/L SeO_2 B) 10 mg/L SeO_2 C) 25mg/L SeO_2 D) 50 mg/L SeO_2 E) 100 mg/L SeO_2 □ Represents mean cell number. ■ Represents mean optical density (OD).

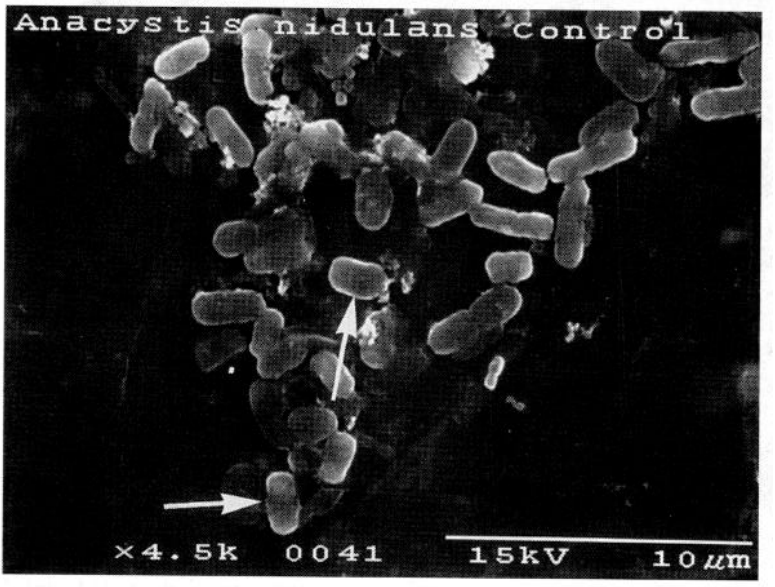

Figure 3. Scanning electron micrograph of Anacystis nidulans in control 3M with EDTA at 4,500X.

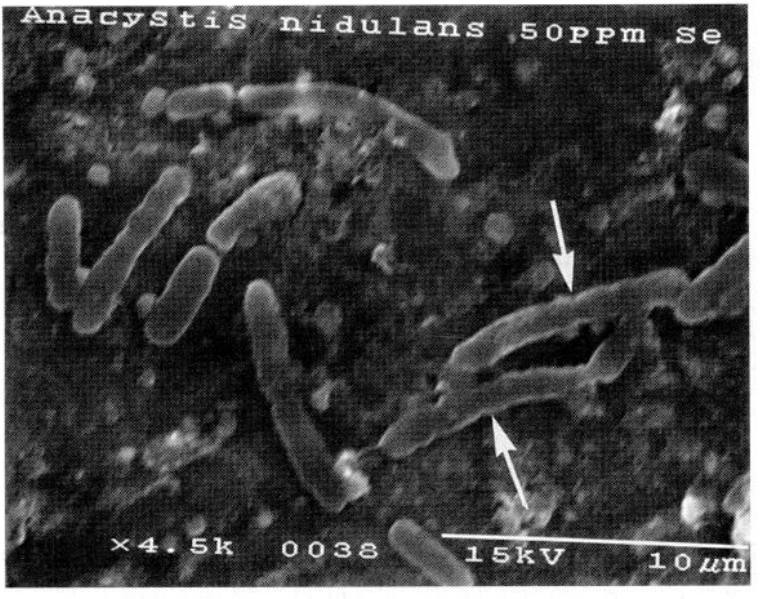

Figure 4. Scanning electron micrograph of Anacystis nidulans in 3M with EDTA containing 50 mg/L SeO_2 at 4,500X. Arrows indicate length of individual cell.

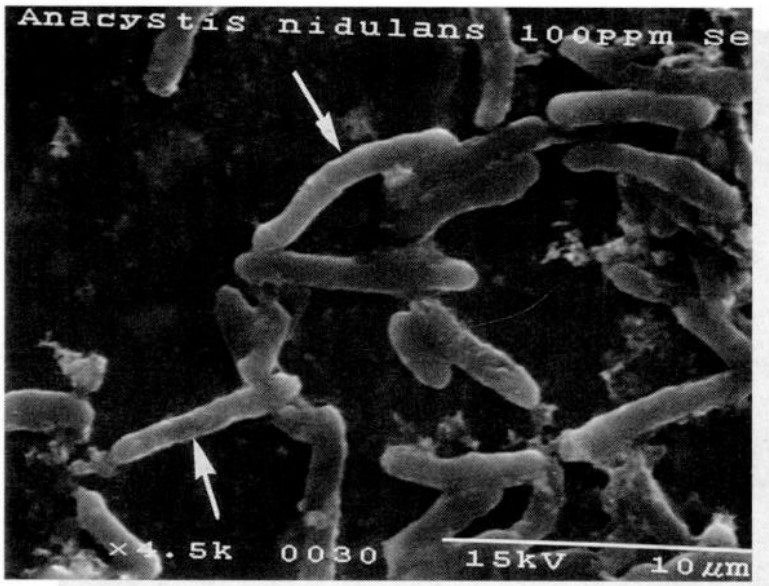

Figure 5. Scanning electron micrograph of Anacystis nidulans in 3M with EDTA containing 100 mg/L SeO_2 at 4,500x. Arrows indicate length of cell.

Table 1. Effect of SeO_2 on the mean size (± SD) of Anacystis nidulans as determined by SEM.

SeO_2 (mg/L)	Length (um)	Width (um)
0	2.04±0.37	1.06±0.06
50	4.35±0.84	1.08±0.02
100	7.39±0.88	1.04±0.08

Table 2. Time dependent changes in mean pH (± SD) in cultures with different SeO_2 concentrations (0-100mg/L) in 3M media containing EDTA.

SeO_2(mg/L)	Day 1	Day 11	Day 22
0	8.53±0.04	10.59±0.13	10.80±0.06
10	8.33±0.04	10.68±0.07	10.69±0.05
25	8.12±0.03	10.62±0.07	10.54±0.05
50	7.79±0.02	8.35±0.05	8.52±0.03
100	7.36±0.04	7.79±0.04	8.12±0.09

significantly different from the control and the 10 and 25 mg/L SeO_2 values. The 10 mg/L SeO_2 cultures showed significantly greater growth in terms of cell number, but not for optical density compared to the other values.

In order to determine if EDTA was acting as a chelating agent for the selenium and affecting growth, culture media were prepared without EDTA. Results show the control had significantly greater growth than all the experimental cultures (Figure 2). At 10 mg/L SeO_2, growth was significantly less than the control, but

Table 3. Time dependent changes of pH in cultures with different SeO_2 concentrations (0-100mg/L) in 3M without EDTA

SeO_2 (mg/L)	Day 1	Day 11	Day 22
0	7.03±0.11	9.15±0.17	9.49±0.04
10	6.76±0.04	8.74±0.09	9.36±0.07
25	6.47±0.06	7.16±0.07	7.47±0.07
50	6.16±0.02	6.83±0.06	7.34±0.08
100	5.30±0.03	6.34±0.06	6.84±0.09

greater than 25, 50 and 100 mg/L SeO_2 groups. Growth was severely limited at concentrations of 25 mg/L and higher. These results indicate that EDTA is an effective chelating agent for selenium since a concentration of 25 mg/L SeO_2 was toxic for A. nidulans when EDTA was not used, while 50 mg/L SeO_2 was toxic with EDTA. Differences in growth between the 25, 50 and 100 mg/L SeO_2 cultures were not significant.

Cells in 0 and 10 mg/L SeO_2 groups, with or without EDTA, displayed normal cellular morphology and pigment color. At concentrations of 50 mg/L SeO_2 and higher, where the number of cells was sharply reduced, cell morphology was irregular with many degraded cells and cellular debris. Cells that were present were longer than the control cells. Therefore, additional studies using cultures at 0, 50 and 100 mg/L SeO_2 with EDTA were undertaken using SEM to determine the effect on cell structure. Results show that cells retained their rod-shape and the same diameter (width), but length increased dramatically (Figures 3, 4, 5, Table 1). At 50 mg/L SeO_2 cell length was 213% greater than the control, and at 100mg/L SeO_2, 313% longer than the control.

The pH increased from the initial pH of 7.9 in flasks in which growth was sustained (Tables 2 and 3). These results are similar to those in previous experiments in which pH values increase to over 8 in cultures demonstrating growth, but not in those without growth. All pH values were within the range described as capable of supporting growth for A. nidulans (Lee et al. 1991).

After 21 days the cultures were centrifuged, washed and resuspended in 100 ml 3M medium without SeO_2. These results indicate that 50 mg/L SeO_2 is cyanostatic and 100 mg/L SeO_2 is cyanocidal with EDTA, while 25 mg/L SeO_2 is cyanostatic and 50 mg/L SeO_2 is cyanocidal without EDTA.

The adaptability of microorganisms to toxic substances is greater than that of organisms of greater size and complexity. The impact of metal ions on microbial growth depends on the concentrations of the metal, temperature, pH, redox potential, other chemicals present and the anatomical and physiological characteristics of the microorganism (Wood 1974). There are two phases for the uptake of metals by microorganisms. They include 1) rapid, in which the metal binds passively to the cell surface and does not enter the cell (Garnham et al.

1992), and 2) extended, in which there is slower, active metabolic uptake of the ions (Ting et al. 1989).

Defensive mechanisms of microorganisms vary considerably from one to another and also between different metal toxicants with several toxic mechanisms existing (Fargasova 1994). Resistance to metal toxicity may be due to the ability of the cell to exclude heavy metals from vital regions of the cell (Ting et al. 1989, Gowrinathan and Rao 1990). Release of chelating agents, both organic and inorganic, is another frequently used defense (Codina et al. 1993). Extracellular amino acids, such as cysteine, bind harmful metallic ions (Kosakowska et al. 1988). Competition between metallic ions for binding sites on the plasma membrane may also effect resistance (Sunda 1989), and genetic mechanisms have been frequently demonstrated for resistance which include chromosomal resistance (Nagel and Voight 1989) and resistance plasmids (Kondo et al. 1974).

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