1994

# Relative Sensitivities of Diatoms to Selected Heavy Metals

Jennifer E. Carlson

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# RELATIVE SENSITIVITIES OF DIATOMS TO SELECTED METALS

(TITLE)

BY

JENNIFER E. CARLSON

**THESIS**

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1994
YEAR

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**ABSTRACT**

Carlson, Jennifer E. M.S. Eastern Illinois University. August, 1993. Relative sensitivities of diatoms to selected heavy metals.

Baseline data, including diatom community structure, metal concentrations of water, sediments and periphyton of the Embarras River were determined previously (Vaultonburg, 1991). Data on diatom community structure in conjunction with efforts to identify relative sensitivity of diatoms may be useful in establishing an effective method for biomonitoring metal pollution in the Embarras River and other aquatic systems. The main objectives of this project were: i) develop a bioassay method; ii) evaluate relative toxicities of several metals towards diatoms; and, iii) evaluate relative sensitivities of diatoms toward dissolved metals.

Unialgal cultures of *Cyclotella meneghiniana, Navicula vaucheriae,* and *Nitzschia palea* were isolated from Embarras River water and associated substrata (e.g. mud, stones, twigs). Standard 14-day, non-renewal bioassay procedures were used to investigate the effects of various concentrations of Al, Cu, Ni, and Zn on diatom population growth and survival. Copper was found to be the most toxic metal to all three diatoms with $EC_{50}$ values of 5-10 $\mu$M. Nickel $EC_{50}$ values were 18 $\mu$M for *N. palea*, and 10-12.5 $\mu$M for *C. meneghiniana* and *N. vaucheriae*. Zinc was found to be

less toxic than Ni with an $EC_{50}$ of 17 μM for *C. meneghiniana* and 50-100 μM for *N. vaucheriae* and *N. palea*. Aluminum was the least toxic to all diatoms with $EC_{50}$ values of 150 μM for *C. meneghiniana*, 360 μM for *N. vaucheriae* and 500-1000 μM for *N. palea*. All three species appear to be equally sensitive to Cu. *Cyclotella meneghiniana* was found to be the most sensitive to Al and Zn. Both *C. meneghiniana* and *N. vaucheriae* were more sensitive to Ni than was *N. palea*.

**ACKNOWLEDGEMENTS**

I would like to thank Dr. Charles Pederson for all of his help with the research and writing of this thesis. Also, thanks for listening to all of my problems throughout the years and for making me learn to love country music. I would like to express my appreciation to Dr. Andrew Methven and Mr. Hank Nilson as well for serving as members of my committee.

To Jim Johnson, Mike Thon, Eric Grunder, Bill Petersen, Mary Hruska, Marla Faver, Scott Phipps, John Ensign, Dave Vaultonburg, Dr. Pederson and Dr. Methven: thanks for helping me stay sane by making it fun to be a Botany graduate student! I will never forget all of the good times we had in the classroom, on field trips, at stress reduction seminars, at ISAS meetings, etc.

I would like to dedicate this thesis to my parents, Les and Jane Carlson. Without your love and your financial and emotional support, I might have never made it this far. Thank you very much.

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# INTRODUCTION

Increased concern has been expressed over the degradation of fresh water resources due to various pollutants, including dissolved heavy metal ions. Toxic metals are released into the environment by natural sources, such as leaching and volcanic activity, and by anthropogenic sources including smelting operations, fossil fuel combustion and effluents from industry, mines and municipalities. In excess of six million metric tons of Hg, Cd, As, Pb, Ni, Cu and Zn are estimated to be released into the biosphere each year (Nriagu and Pacyna 1988).

Although limited amounts of heavy metals are required for metabolic activities in organisms, increased amounts of dissolved heavy metal ions are potentially toxic. Excessive concentrations of heavy metals can reduce species diversity, alter community structure, and decrease productivity (Takamura et al. 1989). Whereas organic toxins may be dissociated into harmless elemental components, heavy metals precipitate into and remain in sediments. Gradually, they may recirculate into the water, posing a potential problem to aquatic food webs as metal ions are accumulated by algae and aquatic macrophytes (Rai et al. 1981).

Heavy metals enter streams as solutes and particulates which may contaminate sediments a great distance downstream from the pollution source. The Clark Fork Complex in Rhode Island receives drainage from a water-filled open pit mine formed following 125 years of Cu and Ag mining and smelting

activities. Metal concentrations 380 km downstream from the source are ten times higher than levels in non-polluted tributaries. Numbers derived through extrapolation suggest that detectable levels of metals would extend more than 500 km downstream. High levels of Cu, Zn, and other metals in rivers as a result of metal extraction as of 1990 have adversely affected fisheries in more than 21,000 km of rivers worldwide (Moore and Luoma 1990). Rivers receiving extremely high levels of heavy metals may become less able to self-purify causing loss of fish, invertebrate, and plant life (Hargreaves 1981). In as much as the subjects of concern from heavy metal poisoning are living organisms, the most logical way to gather data is through the use of biomonitors (Dixit et al. 1992).

Algae, including diatoms are particularly good biomonitors in that: i) algae incorporate metals in proportion to metal concentration in the water; ii) biomonitoring offers increased sensitivity since metal concentrations of algal materials are higher than the surrounding medium; iii) algae provide an integrated picture of intermittent and low level pollution; iv) algal materials indicate bioavailability; and v) algal materials can be stored for extended periods of time (Whitton 1984). Diatoms have been found to be excellent bioindicators of heavy metal ion pollution in lotic ecosystems due to their abundance and relative ease of sampling (Round 1991).

Phytoplankton communities exposed to high levels of heavy metal ions often undergo succession in which the more resistant species dominate, thus creating a more unstable community in which primary production may be impaired (Sorentino 1979). Generally, there is a decrease in phytoplankton diversity as the concentration of heavy metals increase. Species able to tolerate high levels of pollution proliferate due to lack of competition for resources from less tolerant species. Normally, a small number of abundant species are found with a few rare species limited in number (van Dam 1973). Increased concentrations of heavy metal ions in lotic ecosystems can potentially bring about changes in periphyton species composition, even if it involves a metal ion normally present in lesser quantities. In oligotrophic Myra Creek, it was found that species diversity upstream of a Pb-Zn-Cu mining site was 1.08, whereas the downstream site had a species diversity of only 0.14 during summer. (Deniseger et al. 1986).

Use of periphyton to monitor heavy metal pollution helps demonstrate which metals are available for uptake by aquatic plants which form the base of the food chain. Diatom community structure as well as metal concentrations of water, sediments and periphyton of the Embarras River drainage have been described by Vaultonburg (1991). These baseline data were obtained for use in monitoring heavy metal pollution which may occur in conjunction with

industrialization in the watershed. However, diatom community structure can only be useful as a biomonitor of heavy metals once relative toxicities of metals have been determined.

The Embarras River drainage system acquires pollutants chiefly from agricultural runoff, municipal and industrial effluents, and urban and highway runoff. Of the heavy metals previously detected in periphyton in the Embarras River, Al, Ni, and Zn were utilized in toxicity tests performed in this study. While Cu was not detected, it was included in the study because of its value as a reference toxicant. Of three diatom species assayed, *Cyclotella meneghiniana* and *Nitzschia palea* were previously detected in the Embarras River (Vaultonburg 1991). Exposure of these three species to varying concentrations of heavy metals was used to develop an effective bioassay method which evaluates relative toxicity and sensitivity.

# METHODS AND MATERIALS

**Sample collection**

Samples were collected from a site on the Embarras River, near Oakland, Douglas County, Illinois, SW1/4, Sec. 35, T5N, R10E. This site was chosen because of its close proximity, ease of access, and because it was one of the sites used in Vaultonburg's (1991) study. Samples were collected by filling Whirlpak bags with water sediment, rocks, leaves or substrata appearing to have attached diatom colonies. Samples were returned to the laboratory and processed at once.

**Laboratory cultivation**

Sterile agar plates were prepared by solidifying 25% Bristol's medium (Lylis and Trainor 1973) with 2% agar. The spray plating technique (Wiedeman et al. 1964) was employed in order to obtain unialgal colonies. Following completion of spray plating, plates were inverted and placed in a Biotronette Mark III Environmental Chamber in a 12-hr light/dark period, at 24-31 °C. Within two days, golden brown colonies of diatoms could be detected. Using a Nikon Phase Contrast microscope, individual cells were picked off of the agar with micropipets and transferred to 125 mm screw cap culture tubes containing 10 mL of 100% filter sterilized (Gelman CA membrane filters, d = 50mm, pore size = 0.22 $\mu$m) Bristol's medium. These cultures were checked periodically and transferred to fresh medium until unialgal cultures had

been produced. Unialgal stock cultures were produced by inoculating 250 mL Erlenmeyer flasks containing 100 mL of 100% Bristol's medium.

**Toxicity tests**

Range-finding tests were run in order to determine a range of toxicity of each organism for each metal. Metal spikes of 10,000, 1,000, 100, 10 and 1 $\mu$M Al, Cu, Ni and Zn (added as chloride salts) were prepared for use in range-finding toxicity tests. Glass-deionized water was used to make metal solutions which were subsequently filter sterilized. Eight mL of sterile 25% Bristol's medium was added to 125 mm screw cap experimental tubes. One mL of each metal solution was added to each designated tube. Control tubes containing 9 mL of medium were prepared for each diatom species. Four replicates of each treatment were used for each species. Dilutions of unialgal cultures in sterile glass deionized water were used to ensure consistent inoculum size of 100,000 cells per mL. Cultures of *Navicula vaucheriae* and *Nitzschia palea* were homogenized using a tissue grinder prior to dilution. Cell density of stock cultures was determined using a Petroff-Hauser counting chamber. One mL of an appropriately diluted culture was put into each control and experimental tube bringing total volume per tube up to 10 mL, resulting in an initial cell density of 10,000 $mL^{-1}$.

Following inoculation, tubes were positioned diagonally in racks and placed in the environmental chamber for a period of 14 days. At the end of this time, 3 mL of concentrated sulfuric acid were added to each tube in order to terminate growth and to clear the diatom frustules. After 48 hr, each tube was centrifuged and the supernatant aspirated. Each tube then was filled with water, agitated, centrifuged, and the supernatant was aspirated. The washing procedure was repeated a second time before frustules were resuspended in 10 mL of deionized, distilled water.

Once ranges were determined, definitive tests were run between the lowest observed effect concentration (LOEC) and the no observed effect concentration (NOEC). Definitive tests were executed using concentrations of 125, 250, and 500 $\mu$M Al; 12.5, 25, and 50 $\mu$M Ni and Zn; and 125, 250, and 500 $\mu$M Cu respectively. Definitive tests were used to determine the $EC_{50}$ of each metal to each diatom species. The $EC_{50}$ is the concentration at which 50% of the test organisms exhibit a particular response in a particular time relative to the control. The response in these tests was a reduction in cell yield relative to the control. $EC_{50}$ values were calculated via straight-line interpolation (Walsh 1988).

**Slide preparation and cell enumeration**

One mL of the contents of each tube plus 5-10 mL of deionized water were transferred to Gelman cellulose acetate membrane filters (d = 25 mm, pore size = 0.45 $\mu$m) using a vacuum filtration apparatus. This was done in a manner which ensures even distribution of the diatoms on the filter to facilitate accurate counting of cells. After filtration, each millipore filter was placed on a glass slide and several drops of immersion oil added to clear the filter. A cover slip was placed on top of the filter taking care to avoid production of air bubbles. Clear nail polish was applied to the edges of the cover slip to obtain a semi-permanent mount.

Using 400x magnification on a Nikon phase contrast microscope with attached video monitor, 30 fields (16,500 $\mu m^2$) of each filter were observed. Mean number of cells per field and mean number of cells per mL of the original suspension were determined.

**Statistical analyses**

Results of definitive tests (mean cells per mL) were analyzed using single factor analysis of variance. Utilizing the Scheffé F-test, significant ($p < 0.05$) differences in cell yield among treatment groups were determined (Neter and Wasserman 1974).

# RESULTS

**Range-finding tests**

All three diatom species tolerated high concentrations of Al. One hundred percent reduction in cell yield relative to the control was observed at 1000 μM and decreasing concentrations of Al did not affect cell yield. At concentrations at or above 10.0 μM Cu, 100% reduction in cell yield was observed while concentrations of 1.0 and 0.1 μM Cu appeared to have no effect on any of the three species. Nickel and Zn shared identical results in that 100% mortality relative to the control was observed at concentrations of 100 and 1000 μM. Lower concentrations of Ni and Zn did not effect cell yield.

**Definitive tests**

At all three concentrations of Al (Table 1), no significant differences were noted in growth of *N. palea* relative to the control. However, cell yield of *N. vaucheriae* was only 10.5% of the control at a concentration of 500 μM Al. Growth at 250 and 125 μM Al did not differ significantly from each other or the control. *Cyclotella meneghiniana* exhibited higher mortality with exposure to increasing concentrations of aluminum. Fifty-seven percent cell yield relative to the control was observed at 125 μM Al, 27.1% at 250 μM Al, and 3.1% at 500 μM Al. Differences in *C. meneghiniana* cells per mL of each treatment group were significant. Therefore,

Table 1. Mean cells $mL^{-1}$ of each of three diatom species after 14 day exposure to concentrations of Al. Treatment groups which differ significantly ($p<0.05$) from other treatment groups and/or control are indicated in parentheses (C = control, 1 = treatment 1, 2 = treatment 2, 3 = treatment 3).

| Aluminum (μM) | *C. meneghiniana* | *N. vaucheriae* | *N. palea* |
|---|---|---|---|
| 0 (Control) | 264,122 (1,2,3) | 257,587 (3) | 234,889 |
| 125 (Treatment 1) | 151,205 (C,2,3) | 302,869 | 180,552 |
| 250 (Treatment 2) | 71,647 (C,1,3) | 193,620 | 210,816 |
| 500 (Treatment 3) | 8,253 (C,1,2) | 27,168 (C) | 214,225 |

*C. meneghiniana* is most sensitive to aluminum with an $EC_{50}$ of 150 μM (Figure 1), *N. vaucheriae* exhibits intermediate sensitivity to aluminum with an $EC_{50}$ of 360 μM (Figure 2), and *N. palea* is the least sensitive with an $EC_{50}$ of greater than 500.

Definitive tests using Cu did not yield significant differences relative to different treatments or to the control (Table 2). All diatoms tested tolerated all concentrations of copper to which they were exposed. Thus, the $EC_{50}$ may lie between 5 and 10 μM Cu for all three species.

Nickel (Table 3) was found to be more toxic to *C. meneghiniana* and *N. vaucheriae* than to *N. palea*. *Cyclotella meneghiniana* exhibited a 16.1% cell yield relative to the control at 12.5 μM Ni, 1.2% at 25 μM and 1.3% at 50 μM. Although results of all three treatments are not significantly different from each other, they are significantly different from the control. The $EC_{50}$ of Ni to *C. meneghiniana* is greater than 10 but less than 12.5 μM. *Navicula vaucheriae* is sensitive to nickel in that only 0.50, 0.29, and 0.048% cell yield were determined relative to the control at concentrations of 12.5, 25, and 50 μM Ni respectively. The $EC_{50}$ of Ni to *N. vaucheriae* should be between 10 and 12.5 μM. When *N. palea* was exposed to 12.5 μM Ni, there was no significant difference relative to the control; however, cell yield was reduced to 11% at 25 μM,

Figure 1. Mean response (percent cell yield relative to control) of *Cyclotella meneghiniana* to concentrations of 125, 250, and 500 μM Al.

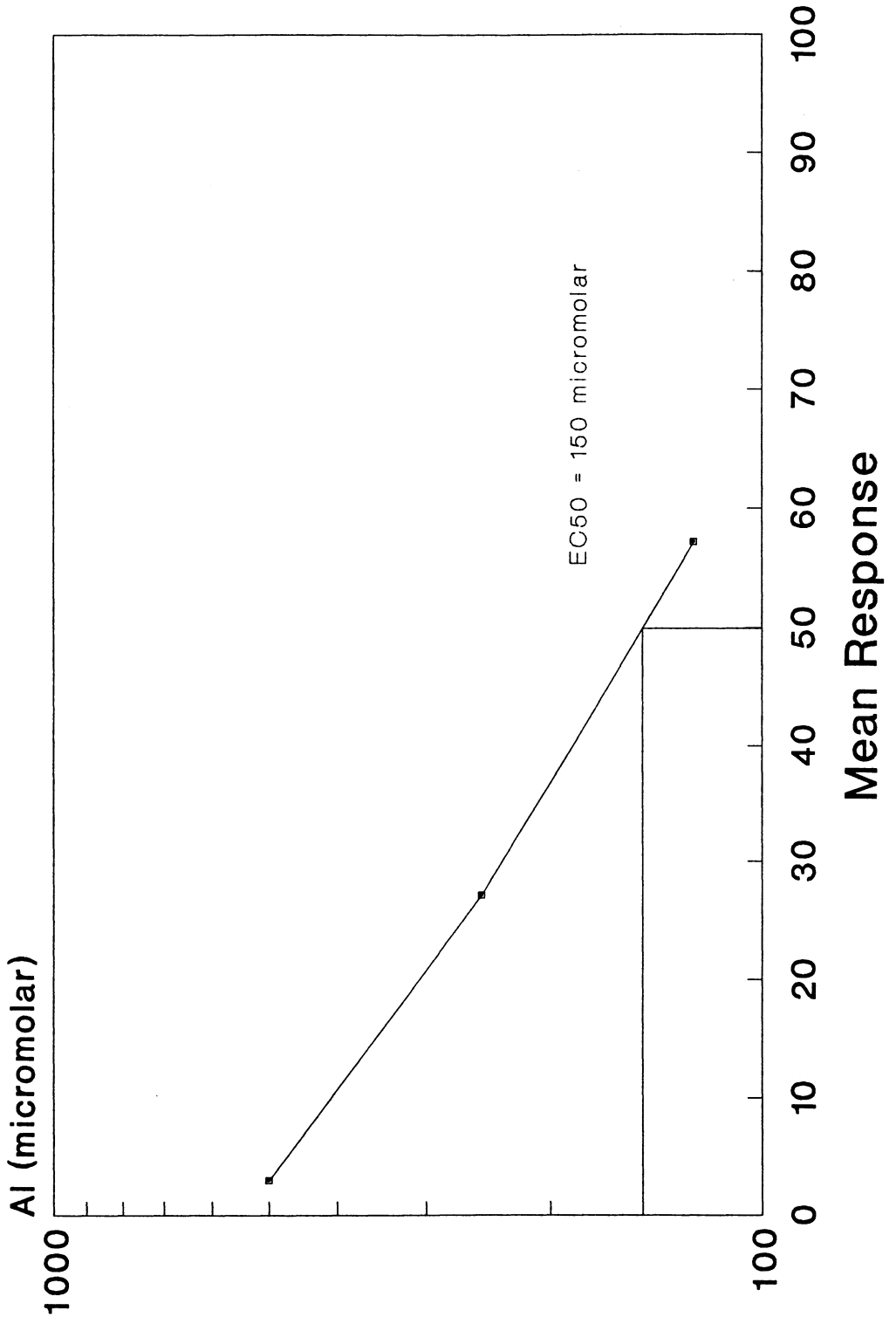

AI (micromolar)
1000
100
0
10
20
30
40
50
60
70
80
90
100
Mean Response
EC50 = 150 micromolar

Figure 2. Mean response (percent cell yield relative to control) of *Navicula vaucheriae* to concentrations of 125, 250, and 500 $\mu$M Al.

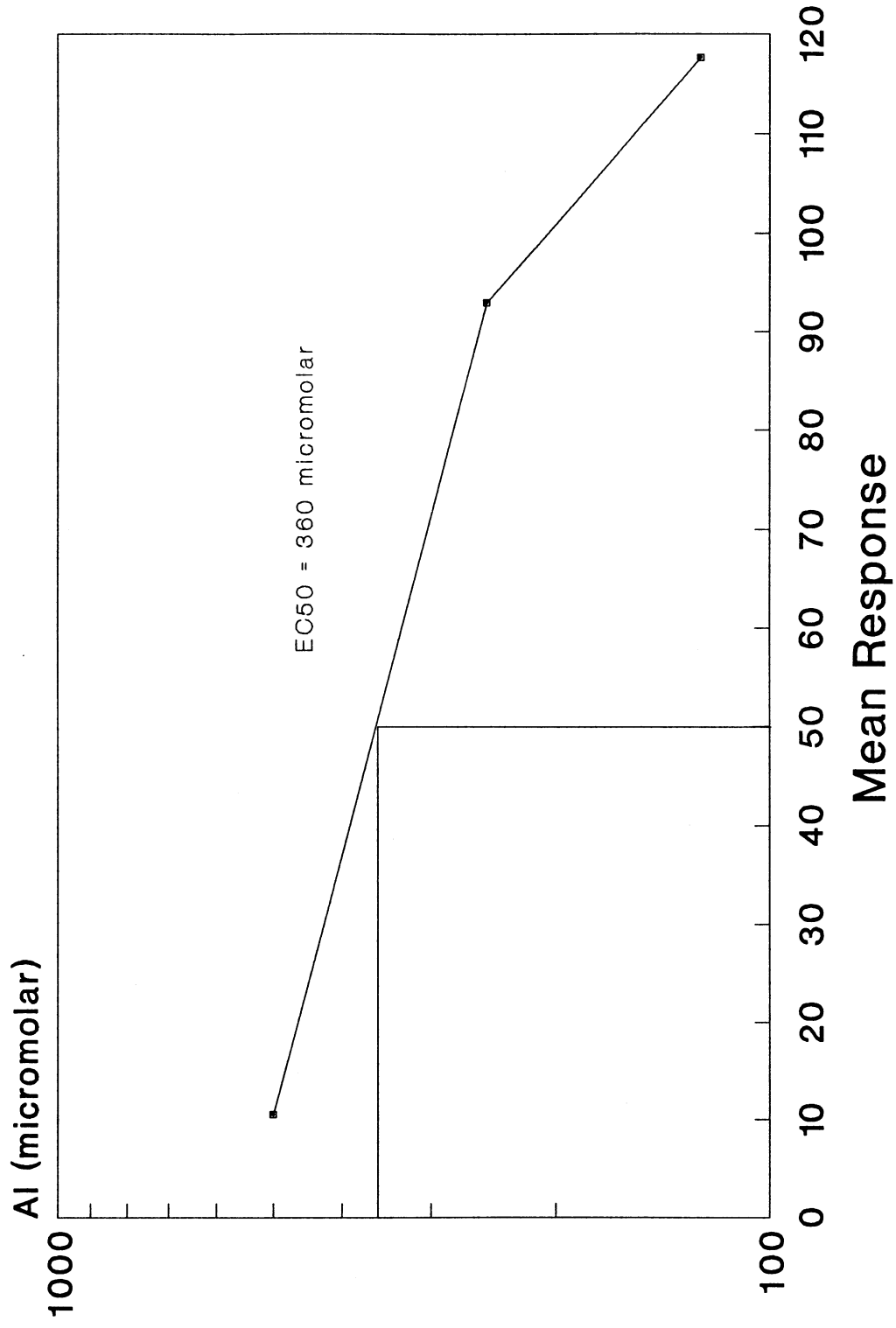

Al (micromolar)
1000
100
EC50 = 360 micromolar
0
10
20
30
40
50
60
70
80
90
100
110
120
Mean Response

Table 2. Mean cells $mL^{-1}$ of each of three diatom species after 14 day exposure to concentrations of Cu. Treatment groups which differ significantly ($p<0.05$) from other treatment groups and/or control are indicated in parentheses (C = control, 1 = treatment 1, 2 = treatment 2, 3 = treatment 3).

| Copper ($\mu$M) | *C. meneghiniana* | *N. vaucheriae* | *N. palea* |
|---|---|---|---|
| 0 (Control) | 65,686 | 232,826 | 248,302 |
| 1.25 (Treatment 1) | 129,309 | 232,138 | 264,122 |
| 2.50 (Treatment 2) | 116,929 | 237,641 | 267,561 |
| 5.00 (Treatment 3) | 101,797 | 243,137 | 230,648 |

Table 3. Mean cells $mL^{-1}$ of each of three diatom species after 14 day exposure to concentrations of Ni. Treatment groups which differ significantly ($p<0.05$) from other treatment groups and/or control are indicated in parentheses (C = control, 1 = treatment 1, 2 = treatment 2, 3 = treatment 3).

| Nickel (μM) | *C. meneghiniana* | *N. vaucheriae* | *N. palea* |
|---|---|---|---|
| 0 (Control) | 206,039 (1,2,3) | 500,846 (1,2,3) | 234,889 (2,3) |
| 12.5 (Treatment 1) | 33,244 (C) | 2,510 (C) | 292,552 (2,3) |
| 25.0 (Treatment 2) | 2,407 (C) | 1,478 (C) | 34,035 (C,1) |
| 50.0 (Treatment 3) | 2,751 (C) | 240 (C) | 103 (C,1) |

and to 0.033% at 50 μM. The $EC_{50}$ of Ni was determined to be 18 μM for *N. palea* (Figure 3).

Exhibiting results similar to those of Al and Cu, Zn (Table 4) had no significant effect upon *N. palea* at any of the concentrations tested, resulting in an $EC_{50}$ of 50-100 μM. The $EC_{50}$ of *N. vaucheriae* is 50-100 μM Zn as well. Cell yield of *N. vaucheriae* was not significantly different from the control at concentrations of 12.5 or 25 μM, although it exhibited only 57% growth when exposed to 50 μM Zn. *Cyclotella meneghiniana* is most sensitive to Zn with an $EC_{50}$ of 17 μM (Figure 4). The control is significantly different from treatments of 25 and 50 μM Zn, where cell yield was calculated as 25.6% and 2.6%, respectively. Cell yield of 68.5% was observed with treatments of 12.5 μM Zn which did not differ significantly from the control. Cell yield after exposure to 12.5 μM was significantly different from treatments with 50 μM Zn.

Figure 3. Mean response (percent cell yield relative to control) of *Nitzschia palea* to concentrations of 12.5, 25.0, and 50.0 $\mu$M Ni.

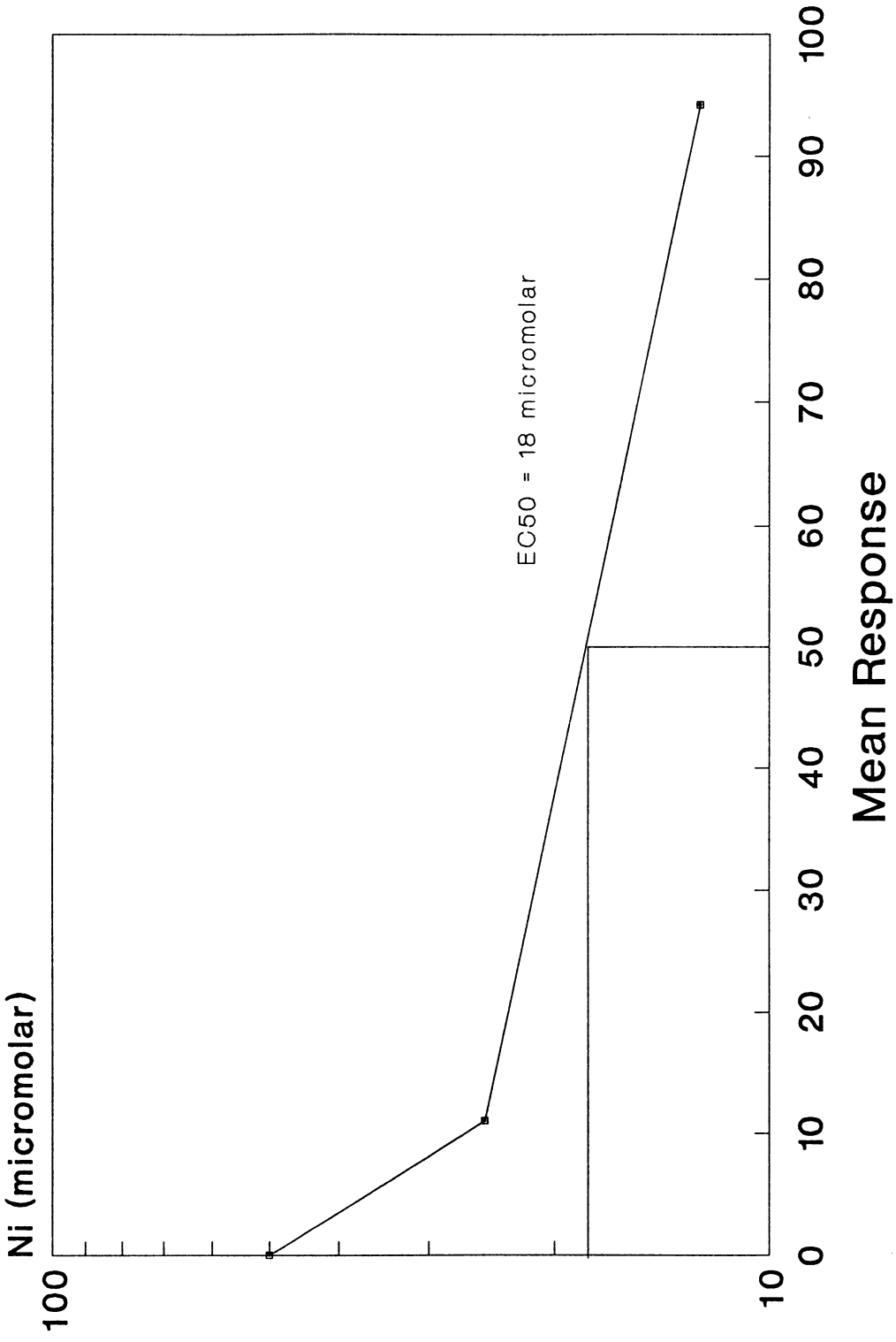
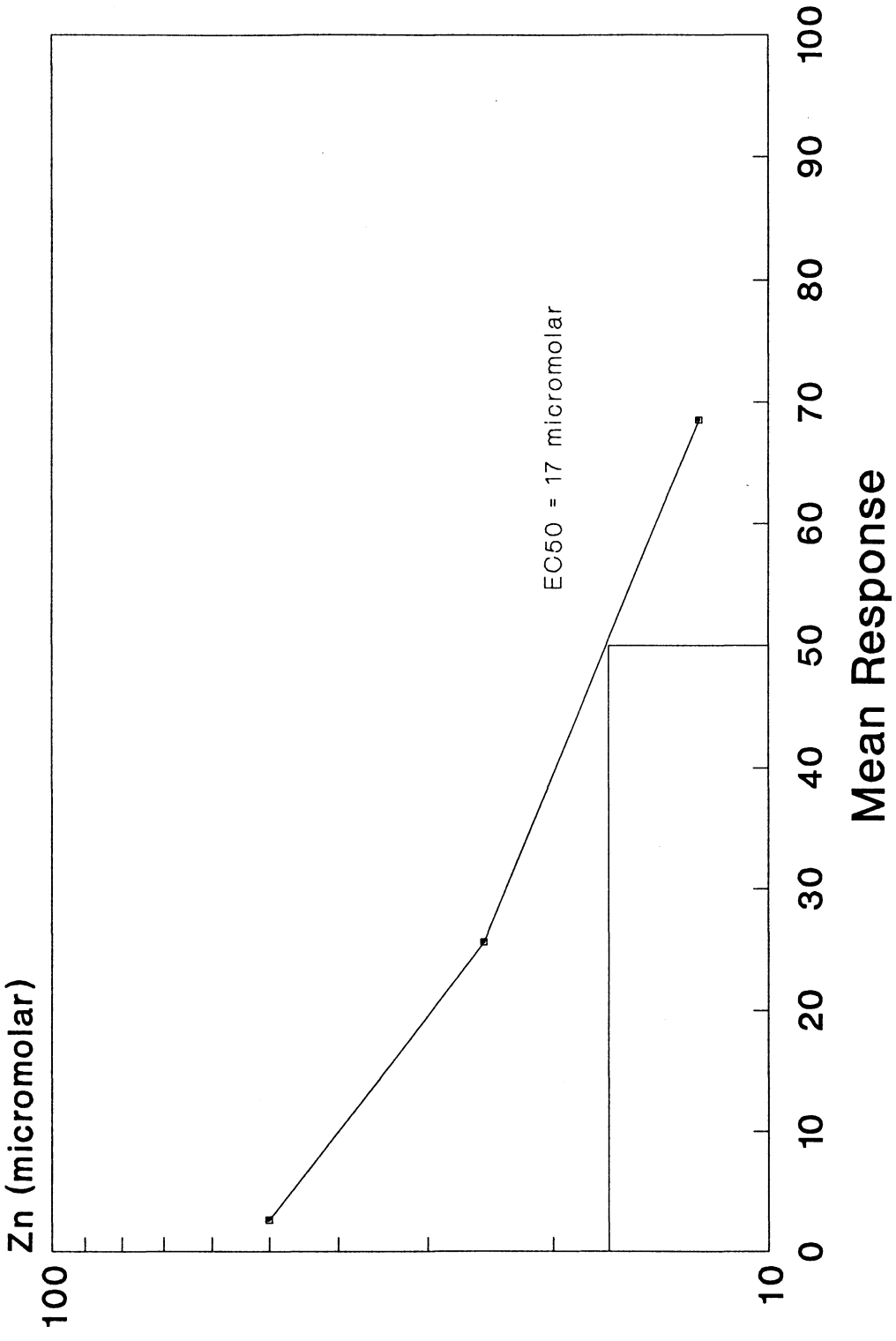

Ni (micromolar)
100
10
0
10
20
30
40
50
60
70
80
90
100
Mean Response
EC50 = 18 micromolar

Table 4. Mean cells $mL^{-1}$ of each of three diatom species after 14 day exposure to concentrations of Zn. Treatment groups which differ significantly ($p<0.05$) from other treatment groups and/or control are indicated in parentheses (C = control, 1 = treatment 1, 2 = treatment 2, 3 = treatment 3).

| Zinc ($\mu$M) | *C. meneghiniana* | *N. vaucheriae* | *N. palea* |
|---|---|---|---|
| 0 (Control) | 146,161 (2,3) | 284,381 (3) | 211,160 |
| 12.5 (Treatment 1) | 100,077 (3) | 259,307 | 162,325 |
| 25.0 (Treatment 2) | 37,486 (C) | 193,629 | 149,027 |
| 50.0 (Treatment 3) | 3,783 (C,1) | 161,673 (C) | 139,283 |

Figure 4. Mean response (percent cell yield relative to control) of *Cyclotella meneghiniana* to concentrations of 12.5, 25.0, and 50.0 μM Zn.


Zn (micromolar)
100
10
0
10
20
30
40
50
60
70
80
90
100
Mean Response
EC50 = 17 micromolar

# DISCUSSION

**Aluminum**

Aluminum is one of the most abundant elements on earth. Acidification of the environment causes solubilization of large amounts of Al, especially when pH falls below 5.5. Ground water with pH lower than 4.0 was found to have concentrations of 300 to 1600 mM Al. Abnormally high levels of Al in acidified bodies of water cause flocculation of phosphate and humic complexes, thereby reducing nutrient content in the water and decreasing growth of phytoplankton. Increased concentrations of Al in lakes causes a net decrease in photosynthesis and phosphorus uptake (Pettersson et al. 1988).

While literature pertaining to the effects of Al upon diatoms is sparse, Pettersson et al. (1985) have shown that Al induces pronounced physiological and structural changes in the cyanobacterium *Anabaena cylindrica*. *Cyclotella meneghiniana* appears to be as sensitive to Al as *A. cylindrica* (Table 5). Pettersson et al. (1985) found that 180 μM Al caused a 50% decrease in culture density of *A. cylindrica*. This is comparable to the $EC_{50}$ of 150 μM found for *C. meneghiniana* in this study. Aluminum concentrations of 370 μM caused cessation of growth of *A. cylindrica* (Petterson et al. 1985) whereas levels of 360 μM caused 50% cell yield relative to control of

Table 5. Effects of Al upon algae.

| Concentration | Alga | Toxic effect | Reference |
|---|---|---|---|
| 180 μM | *Anabaena cylindrica* | 50% decrease of culture density | Pettersson et al. 1985 |
| 370 μM | *A. cylindrica* | cessation of growth | Pettersson et al. 1985 |
| 150 μM | *Cyclotella meneghiniana* | 50% cell yield relative to control | this study |
| 360 μM | *Navicula vaucheriae* | 50% cell yield relative to control | this study |
| 500-1000 μM | *Nitzschia palea* | 50% cell yield relative to control | this study |

*N. vaucheriae* in this study. This study showed that 500-1000 μM of Al were required to cause 50% cell yield relative to control in *N. palea*.

**Copper**

Copper is a trace element important in metabolic processes of algae. When exposed to high concentrations of Cu, algal growth and photosynthesis are inhibited and permeability of plasma membranes is altered causing loss of potassium from the cells (Rai et al. 1981). Diatoms are extremely sensitive to Cu in that Cu ions inhibit uptake of silicic acid, the major component of the frustule (Sorentino 1979). Inhibitory levels of Cu in this study were higher than what had been observed in previous studies (Table 6). An $EC_{50}$ of 5-10 μM was established for *N. palea* in this study, although, Rai et al. (1981) reported that 0.031 μM Cu was toxic toward *N. palea*. Takamura et al. (1989) reported that 3.33 μM Cu causes 50% inhibition of photosynthesis in *N. palea*. Copper at 0.079 μM proved to inhibit *Cyclotella nana* (Bartlett et al. 1974) while I found an $EC_{50}$ value between 5 and 10 μM of Cu for *C. meneghiniana*. Cell yield of *N. vaucheriae* was reduced by 50% when in the presence of 5 to 10 μM Cu in this study, whereas Takamura et al. (1989) reported that only 0.78 μM Cu caused 50% inhibition of photosynthesis in *Navicula secreta*. *Navicula* spp. exhibited adverse effects when exposed to 1.10 μM Cu (Patrick 1977).

Table 6. Effects of Cu upon algae.

| Concentration | Alga | Toxic effect | Reference |
|---|---|---|---|
| 5-10 μM | *Cyclotella meneghiniana* | 50% cell yield relative to control | this study |
| 0.079 μM | *Cyclotella nana* | inhibitory concentration | Erickson 1972 |
| 5-10 μM | *Navicula vaucheriae* | 50% cell yield relative to control | this study |
| 0.78 μM | *Navicula secreta* | 50% inhibition of photosynthesis | Takamura et al. 1989 |
| 1.10 μM | *Navicula spp.* | adverse effects | Patrick 1977 |
| 5-10 μM | *Nitzschia palea* | 50% cell yield relative to control | this study |
| 0.031 μM | *N. palea* | toxic concentration | Steemann Nielson and Wium-Andersen 1970 |
| 1.49 μM | *N. palea* | 50% inhibition of photosynthesis | Takamura et al. 1989 |
| 0.79 μM | *Selenastrum capricornutum* | incipient inhibition | Bartlett et al. 1974 |
| 1.43 μM | *S. capricornutum* | complete inhibition | Bartlett et al. 1974 |
| 4.72 μM | *S. capricornutum* | algicidal effect | Bartlett et al. 1974 |

*Selenastrum capricornutum* showed incipient and complete inhibition at levels of 0.79 and 1.43 μM Cu respectively. A complete algicidal effect toward *S. capricornutum* was observed at a level of 4.72 μM Cu (Bartlett et al. 1974). Growth of *Lyngbya nigra* was inhibited at 0.45 μM Cu and photosynthesis and respiration were severely inhibited at 0.8 μM Cu (Rai et al. 1981).

**Nickel**

Increasing concentrations of Ni, a potentially toxic micronutrient, caused an algal community to shift from mainly diatoms to mostly green and blue-green algae (Patrick 1977). Varied levels of Ni are reported to cause damaging effects upon algae (Table 7). I found $EC_{50}$ values between 10 and 12.5 μM for *C. meneghiniana* and *N. vaucheriae* and 18 μM Ni for *N. palea*. Patrick (1977) reported that only 0.034 μM Ni caused disappearance of sensitive species in a freshwater habitat. Rai et al. (1981) reported severe inhibition of nitrogen fixation in *Nostoc* when exposed to 0.43 μM Ni. *Scenedesmus* maintained 20% growth when exposed to 25.5 μM Ni (Stokes et al. 1983).

**Zinc**

Although an essential micronutrient for algal growth, Zn may be toxic at increased concentrations. Williams and Mount (1965) found that *Cymbella tumida* and *Synedra ulna*,

Table 7. Effects of Ni upon algae.

| Concentration | Alga | Toxic effect | Reference |
|---|---|---|---|
| 10-12.5 μM | *Cyclotella meneghiniana* | 50% cell yield relative to control | this study |
| 10-12.5 μM | *Navicula vaucheriae* | 50% cell yield relative to control | this study |
| 18 μM | *Nitzschia palea* | 50% cell yield relative to control | this study |
| 0.43 μM | *Nostoc* sp. | nitrogen fixation severely inhibited | Henricksson and DaSilva 1978 |
| 25.5 μM | *Scenedesmus* (lake strain) | 20% growth | Stokes et al. 1983 |
| 0.034 μM | diatom community | poor diatom diversity in freshwater habitat | Patrick 1977 |

both of which were documented for the Embarras River (Vaultonburg 1991) were present in artificial canals after exposure to high levels of Zn. All other diatom species are intolerant to even moderate levels of Zn. At higher than ambient concentrations Zn will cause inhibited enzyme synthesis, and result in the cessation of growth of some phytoplankton (Parry and Hayward 1973). Gachter and Mares (1979) demonstrated that increased amounts of Zn caused less efficient photosynthesis, shifts in community structure, and reduction of species number in limno-corrals. Furthermore, Patrick (1977) found that with increased concentrations of Zn, cell divisions in *Navicula seminulum* var. *hustedtii* were reduced by 50% within 5 days of exposure. When exposed to high concentrations of Zn, chlorophyll content of *N. seminulum* var. *hustedtii* tends to become reduced and photosynthesis is affected (Rai et al. 1981). $EC_{50}$ values for Zn in this study were greater than 50 but less than 100 μM for *N. vaucheriae* and *N. palea*, and 17 μM for *C. meneghiniana* (Table 8). According to Takamura et al. (1989), *N. palea* showed 50% inhibition of photosynthesis at a level of 200.4 μM Zn while *Navicula secreta* showed 50% inhibition of photosynthesis when exposed to 86.6 μM Zn. Patrick (1977) found that levels of zinc ranging from 19.9-68.8 μM caused a 50% reduction in division rate of *Navicula seminulum*. *Chlorella vulgaris* underwent a 50% growth rate reduction after exposure to 36.7 μM Zn (Rana and Kumar

Table 8. Effects of Zn upon algae.

| Concentration | Alga | Toxic effect | Reference |
|---|---|---|---|
| 36.7 μM | *Chlorella vulgaris* | 50% reduction in growth rate | Rana and Kumar 1974 |
| 153.0 μM | *Chlorella* sp. | growth severely inhibited | Song 1977 |
| 305.9 μM | *Chlorella* sp. | no growth | Song 1977 |
| 16.7 μM | *Cyclotella meneghiniana* | 50% cell yield relative to control | this study |
| 86.6 μM | *Navicula secreta* | 50% inhibition of photosynthesis | Takamura et al. 1989 |
| 19.9-68.8 μM | *Navicula seminulum* | 50% reduction in division rate | Patrick 1977 |
| 50-100 μM | *Navicula vaucheriae* | 50% cell yield relative to control | this study |
| 50-100 μM | *Nitzschia palea* | 50% cell yield relative to control | this study |
| 200.4 μM | *N. palea* | 50% inhibition of photosynthesis | Takamura et al. 1989 |
| 305.9 μM | *Scenedesmus spinosus* | slight inhibition of growth | Song 1977 |

1974). When subjected to 153 μM Zn, growth of *Chlorella* sp. was severely inhibited (Song 1977), and growth ceased upon exposure to 305.9 μM Zn. Slight inhibition of growth of *Scenedesmus spinosus* was noted at concentrations of 305.9 μM Zn (Rai et al. 1981)

**Bioassay method comparison**

My work involved single species, single perturbation toxicity tests. Comparison is difficult because some of the previous studies were conducted in laboratories while others were conducted *in situ*. Furthermore, variables such as day length, temperature, hardness, flow rate, and pH may cause variation in results from different types of bioassays. For example, Peterson et al. (1984) found that metal toxicity toward algae is highly pH dependent and Sudhakar et al. (1991) reported that hardness and organic matter levels reduce toxicities of trace metals.

Future tests should be conducted using multiple species and/or metals. In lotic ecosystems, competition plays a role in which diatoms become dominant or perish under heavy metal stress. Such tests could reveal additive, antagonistic or synergistic interactions between metals.

**Pollution indicators**

Of the three species I investigated, *Nitzschia palea* appears to be most resistant to exposure to heavy metals. This corresponds to other studies which report this species to be extremely tolerant of many types of pollution. *Nitzschia palea* has long been regarded as an indicator of organic pollution (Patrick and Roberts 1979) and is often found growing profusely near the outfall of sewage treatment plants. However, *N. palea* only becomes common when there is a lack of competition from other diatoms.

*Nitzschia palea* may also serve as an indicator of metals pollution. Metal pollution due to liquid waste discharges from a paper mill on the river Godavari in India caused a large drop in pH and a change in algal community structure. Downstream from the mill diatom species diversity was much reduced and cyanobacteria were more prevalent even though upstream diatoms were more diversified and dominant. Copper, Ni, Zn, and other heavy metals were all detected downstream of the mill, but were not detected upstream. *Nitzschia palea* and *C. meneghiniana* were both found to be pollution tolerant. Neither species was observed upstream of the mill, however, at the site of discharge they replaced many of the original upstream species. One Km downstream of the mill many of the metal sensitive species found upstream reappeared and *N. palea* and *C. meneghiniana* were reduced in number (Sudhakar et al.

1991). Rushforth et al. (1981) collected data on the response of periphytic diatoms to varying concentrations of heavy metals in natural systems. *Nitzschia palea* and *C. meneghiniana* were detected at high ends of heavy metal gradients in the Uintah Basin of Utah. High concentrations of Cu and Ni were found in the spring and of Al and Zn in the summer. Thus, my study confirms *N. palea* as a valid indicator of high levels of heavy metals in river ecosystems.

# APPENDIX A

Table 9. Sources and grades of reagents used in this study.

EM Science, Cherry Hill, NJ (Gibbstown, NJ = *):

$FeCl_3 * 6H_2O$, certified

$CoCl_2 * 6H_2O$, certified *

$ZnCl_2$, certified *

Fisher Scientific Company, Fair Lawn, NJ:

agar, laboratory grade

$AlCl_3 * 6H_2O$, reagent

$Na_2EDTA$, certified

$CuSO_4$, certified

$Na_2MoO_4 * 2H_2O$, certified

CuCl, certified

$H_2SO_4$, reagent

J.T. Baker Chemical Company, Phillipsburg, NJ:

$Na_2SiO_3 * 9H_2O$, chemical reagent

$NiCl_2 * 6H_2O$, reagent

Mallinckrodt Chemical Works, St. Louis, MO:

$CaCl_2 * 2H_2O$, analytical reagent

$MnCl_2 * 4H_2O$, analytical reagent

$ZnSO_4 * 7H_2O$, analytical reagent

$NaNO_3$, analytical reagent

$MgSO_4 * 7H_2O$, analytical reagent

$K_2HPO_4 * 3H_2O$, analytical reagent

$KH_2PO_4$, analytical reagent

NaCl, analytical reagent

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