The species-specific effects of sublethal concentrations of cadmium on freshwater phytoplankton communities in a Canadian Shield lake

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The species-specific responses of natural phytoplankton communities to low cadmium concentrations were measured in Lake 239 (Experimental Lakes Area, northwestern Ontario). Both *in situ* and laboratory 5-L continuous-flow cultures, and 5-L and 100-mL cultures were used. *Asterionella formosa*, *Dinobryon sertularia*, and *Dinobryon bavaricum* showed dramatic negative sensitivity to low cadmium concentrations (5–100 μg/L), while *Rhabdoderma gorskii* and *Elakatothrix* sp. consistently increased in numbers at the same cadmium concentrations. In all experiments, some species exhibited no apparent effect to cadmium addition as measured by cell counts. The "bottle effect" of each technique was evaluated by comparing the community similarity valves of the control cultures to the lake samples and showed the *in situ* continuous cultures to be most similar to the lake followed by the laboratory continuous cultures, the *in situ* 5-L batch cultures, the 5-L laboratory cultures, and the 100-mL batch cultures. Replicate cadmium cultures, all techniques, were more similar to each other than the lake samples. The similarity of the cadmium cultures to the lake sample or control cultures decreased with increased cadmium concentration and incubation time.

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La réaction de chaque espèce à de faibles concentrations de cadmium a été mesurée pour des communautés phytoplanctoniques naturelles dans le lac 239 ("Experimental Lakes Area", nord-ouest de l'Ontario). Des cultures en flux continu de 5 L, ainsi que des cultures en vrac de 5 L et de 100 mL furent utilisées, à la fois *in situ* et au laboratoire. *Asterionella formosa*, *Dinobryon sertularia* et *D. bavaricum* ont réagi très fortement et négativement aux faibles concentrations de cadmium (5–100 μg/L), tandis que *Rhabdoderma gorskii* et *Elakatothrix* sp. voyaient toujours leur nombre de cellules augmenter en présence de ces mêmes concentrations de cadmium. Dans toutes les expériences, quelques espèces n'ont manifesté aucune réaction (mesurée par le dénombrement des cellules) à l'addition de cadmium. "L'effet de bouteille" de chaque technique a été évalué en comparant les valeurs de similarité des communautés des cultures témoins à celles des échantillons du lac; cette comparaison montre que les cultures continues *in situ* étaient les plus semblables au lac, suivies par les cultures continues au laboratoire, les cultures en vrac de 5 L *in situ*, les cultures en vrac de 5 L au laboratoire et les cultures en vrac de 100 mL. Pour toutes les techniques, les réplicats des cultures avec cadmium étaient plus semblables les uns aux autres qu'ils ne l'étaient aux échantillons du lac. La similarité des cultures avec cadmium aux échantillons du lac ou aux cultures témoins diminuait à mesure que la concentration de cadmium et le temps d'incubation augmentaient.

[Traduit par le journal]

Introduction

Cadmium pollution in aquatic ecosystems is potentially hazardous. Unlike some toxicants, cadmium is a nonbiodegradable, persistent metal that is toxic at low concentrations. The ambient cadmium concentration in aquatic ecosystems are primarily due to the weathering of rocks rich in cadmium sulfide. Core samples from preindustrialization depths indicate the natural sediment concentrations of cadmium sulfide were between 1 and 2 ppm dry weight (Anonymous 1977). Natural processes add an estimated 500 t/year of cadmium to the environment (Bertine and Goldberg 1971). Cadmium pollution in aquatic systems has several sources which include ore smelters, electroplating plants, pigment works, textile works, chemical industries, urban runoff, lumber processing, battery plant wastes, and municipal wastes (Yost et al. 1973; Hutchinson 1973). Cadmium concentrations at these point sources range from background to grams per litre. In one example, Bower et al. (1978) studied a small cover of the Hudson River which was polluted by a battery plant for 19 years. They found cadmium concentrations in the sediments ranged from 214 to 261 ppm dry weight with an estimated total cadmium abundance in the cover of 20–50 t.

Most investigators have used unialgal cultures to measure the effects of cadmium on algae and therefore they have measured species-specific responses. A review of these studies reveals several species are very sensitive to cadmium addition. *Scenedesmus* sp. exhibited toxic symptoms at 100 μg/L (Bringmann and Kuhn 1959). *Scenedesmus quadricauda* (Klass et al. 1974) and *Anabaena inaequalis* (Stratton and Corke 1979) had significant growth rate depression at 6.1 and 20.0 μg/L, respectively. Berland et al. (1976) found significant inhibition of growth at concentrations from 10 to 500 μg/L in 18 species of marine unicells and lethal concentrations ranging from 20 to 1000 μg/L. *Asterionella formosa* had its growth rate reduced an order of magnitude by 2 μg/L of cadmium (Conway 1978).

Few studies have attempted to localize the subcellular effects of cadmium in algae. Cadmium is known to bind to sulfur groups (Vallee and Ulmer 1972) and metallothionein (Kagi and Vallee 1960), a protein with a molecular mass of approximately 6–7 thousand daltons with over 30% cysteine, has been cited as an inducible sequestering and detoxifying agent.

Fractionation of *Anacystis* and separation on a Sephadex G-75 column indicated 65% of the accumulated cadmium was associated with a protein with a molecular mass of 10–12 thousand daltons (MacLean et al. 1972). Silverberg (1976) found ultrastructure damage in the mitochondria of *Ankristrodesmus falcatus, Chlorella pyrenoidosa*, and *Scenedesmus quadricauda* after 8 days of incubation with 20, 50, and 100 μg/L of cadmium. Overnell (1975, 1976) found the Hill reaction of photosynthesis was depressed by short-term incubation with cadmium.

In contrast to the unialgal studies, there have been few natural phytoplankton community studies on the effects of heavy metals. Patin et al. (1974) and Ibragin and Patin (1975) found carbon fixation was depressed at 10 μg/L Cd (and higher concentrations) with 5-day incubation times. They also found a temporary increase in carbon fixation in the 10.0 and 1.0 μg/L Cd cultures on day 3. Gachter (1976) recorded a variable response from the phytoplankton to the same cadmium concentrations to at different times throughout the year and he suggested the variable response was related to the large seasonal differences in species composition and total communty biomass. deNoyelles et al. (1980) measured the response of four species to cadmium incubation as part of an evaluation of the suitability of *in situ* continuous-flow culture operation in Canadian lakes. In this study we investigated the effects of sublethal concentrations of cadmium on natural phytoplankton communities using several bioassay techniques and incubation times with emphasis on an evaluation of the suitability of *in situ* continuous-flow cultures for lake studies.

Materials and methods

A range of cadmium concentrations (5.0–100.0 μg/L) were used to evaluate the effects of cadmium on natural phytoplankton communities in Lake 239 at the Experimental Lakes Area, northwestern Ontario. All glassware and tubing were acid (HCl) washed, rinsed with distilled water, and autoclaved before each incubation, except the small batch culture flasks, which were not autoclaved before each incubation, and the silicone tubing, which was not acid washed. Fresh cadmium stock solutions were made before each experiment and diluted so that no more than 1 mL was added to a culture.

Phytoplankton samples were preserved in acid Lugol's and 40-mL subsamples were concentrated in settling chambers. Random fields (100–250) were counted using either a Wild or Olympus inverted microscope. The raw counts were converted to cells per millilitre using the equation given by the American Public Health Association (Anonymous 1975). Total biomass (cubic micrometres per millilitre) was estimated by multiplying the number of cells per millilitre by the measured volume (cubic micrometres) for each species. Summing the estimates for each species produced the total community biomass estimate. Detection limits varied depending on the number of fields counted, the volume of sample settled, and the magnification used to count the organisms.

Batch cultures

The techniques used for the small batch cultures (100 mL) were similar to those used by most workers in primary productivity experiments. The sample water (100 mL) was added to the vessels, which already contained the desired concentration of cadmium. The laboratory incubations were performed in a controlled light chamber (Shearer 1976). The culture vessels were held on a clear Plexiglas wheel and rotated in a temperature-controlled water bath. *In situ* incubations were performed using a clear Plexiglas rack which held the culture vessels parallel to the surface of the water at a depth of 90 cm and a midday light intensity of 150 μE m^{-2} s^{-1}.

The 5-L *in situ* batch cultures were contained in 5-L round-bottom flasks that were mounted on the frame of the *in situ* continuous-flow culture apparatus described below. Mixing was achieved by a magnetic stirring bar. The culture vessels were initially filled quickly by allowing air to escape as lake water entered. Cadmium solutions were added by injection through a rubber portal.

Continuous-flow cultures

The continuous-flow cultures (lab and *in situ*) used in this study have been described and illustrated in detail elsewhere (deNoyelles and O'Brien 1974; deNoyelles et al. 1980) and will only be briefly described here. The continuous-flow cultures used in the laboratory and *in situ* consisted of two or four vessels (5-L round-bottom flasks) and were mixed by magnetic stirring bars. In the laboratory, the cultures were illuminated by fluorescent light (150 μE m^{-2} s^{-1}) on a 12 h day : 12 h night cycle. The *in situ* vessels were each connected to a large reservoir jug. Flow and flow rate was achieved by the controlled release of air from each reservoir jug. Laboratory continuous-flow cultures used jacketed flasks (5 L) connected to a water bath for temperature control and raw lake water was replenished by peristaltic pumps. The laboratory vessels were initially filled with raw lake water and connected to a large (18.9-L) central reservoir that was filled every other day with raw lake water. The central reservoir was constantly mixed by a magnetic stirring bar. The *in situ* vessels were initially filled simultaneously with raw lake water. Cadmium was added to the *in situ* vessels by injection and to the laboratory vessels by peristaltic pump.

Experiment 1

The first experiment was conducted (August 1977) in Lake 239 and consisted of two control *in situ* continuous-flow cultures and two experimental *in situ* continuous-flow cultures that maintained a cadmium concentration of 3–4 μg/L. The cadmium cultures were sampled on six different dates between 2 August and 16 August to check the cadmium concentrations. The analytical laboratory at the Freshwater Institute, Winnipeg, Man., Canada, measured the actual cadmium concentration to be between 2.9 and 4.2 μg/L. The cultures were incubated at a depth of 174 cm and a flow rate of 50 mL/h. The cultures were filled and allowed to run 20 h before cadmium injections began. The cultures operated with flow for 188 h and without flow for another 94 h. Stock cadmium solutions were injected every 24 h. The responses of the phytoplankton to containment (control vs. lake) and to cadmium addition (experimental vs. control and lake) were evaluated by cell counts for the 23 most abundant species.

Experiment 2

Experiment 2 was conducted from 27 July to 10 August 1978 in Lake 239. Two continuous cultures were run to separate the effects of enclosure (controls vs. lake), with a flow rate of 50 mL/h at a depth of approximately 2 m. Four *in situ* 5-L batch cultures were attached to the *in situ* continuous cultures frame and run simultaneously with the continuous cultures. Two batch cultures received a perturbation of 5 μg/L cadmium. Small (100-mL) batch cultures were incubated in a controlled-light chamber (Shearer 1976) for 24 h. *In situ* incubations were run for 24, 54, and 244 h. Usually, two or three replicate cultures were run for each treatment. Cadmium concentrations varied from 5.0 to 100.0 μg/L. An independent check on the cadmium concentrations was provided by the analytical laboratory at the Freshwater Institute, Winnipeg, Man., Canada (Table 1). The responses of the phytoplankton to containment (control vs. lake) and to cadmium addition (experimental vs. control and lake) were evaluated by cell counts for the 24 most abundant species.

Statistical method

The statistical technique used in this study is a previously unpublished modification of the percent similarity (PS) method (Whittaker 1975). There is no commonly used statistical definition of similarity and statistical definitions may vary depending on the investigator's point of view. Consider the following examples. Community A consists of 24 species and community B contains the same 24 species, but at one-half the abundance: are A and B 100%, 50%, or some other percentage similar? Community C has 12 species of equal abundance

TABLE 1. Independent analysis (by the analytical laboratory of the Freshwater Institute, Winnipeg): expected versus observed (mean) cadmium concentrations (micrograms per litre) from experiment 2

Expected	Observed	No. checked	SD
5.0	3.4	4	0.24
10.0	7.7	6	0.76
30.0	30.0	4	1.63
100.0	84.0	8	11.61

TABLE 2. Initial and final community parameters including Shannon and Weaver diveristy (H'), Brillouin diversity (B), evenness (J'), total biomass (TB) ($\times 10^6$), and total counts (TC) ($\times 10^3$) for experiment 1 from Lake 239 for four *in situ* continuous cultures, two controls (C2 and C4), and two with 3–4 μg/L cadmium (Cdl and Cd3) as compared with the lake

	Lake	C2	C4	Cdl	Cd3
3 August 1977					
H'	1.988	1.965	1.921	1.982	1.931
B	1.960	1.938	1.892	1.956	1.905
J'	0.433	0.428	0.418	0.439	0.421
TB($\times 10^6$)[a]	1.726	1.839	1.719	1.755	1.879
TC($\times 10^3$)[a]	2.155	2.289	2.091	2.213	2.332
16 August 1977					
H'	1.907	2.215	2.003	2.319	2.413
B	1.881	2.170	1.968	2.276	2.369
J'	0.416	0.480	0.435	0.510	0.531
TB($\times 10^6$)	1.8375	0.8847	1.1939	0.6537	0.5717
TC($\times 10^3$)	2.350	1.260	1.609	1.214	1.183

[a]See Materials and methods for details of calculating total biomass and total counts.

in common with community A and 12 unique species: is C 50% similar to A just because they share 12 species? Community D has 23 species in common with Community A, but the 1 species which makes up 95% of the total biomass (or total number of individuals) is unique to sample D: is sample D 95%, 5%, or some other percentage similar to A?

Percent similarity as described by Whittaker (1975) would produce a similarity value of 100% for A to B, A to C would vary depending on the proportion of the 12 species in A and C, and A to D would be 5% or less. We believe the major problem with the PS technique lies in its lack of sensitivity to proportional differences. The modification of the PS technique described here consists of an element which compares the abundance of the *i*th species in each sample and uses this comparison as a correction factor (for example, if the *i*th species in sample A and B made up 5% of the total, but was half as abundant in B, the *i*th species would add 5% to the similar value using PS, but only 2.5% using the modification). Unlike the PS technique, this modification requires equal sample size. The similarity values were calculated as follows:

$$\text{Mod } I = \left(\frac{\text{min. abundance (Ai or Bi)}}{\text{max. abundance (Ai or Bi)}}\right) \cdot \text{min \% (PAi or PBi)}$$

where mod I is the index of similarity, Ai is the abundance of the *i*th species in sample A, Bi is the abundance of the *i*th species in sample B, PAi is the percentage of the *i*th species in sample A, and PBi is the percentage of the *i*th species in sample B. The second element of this calculation is the standard form of PS as described by Whittaker (1975).

Diversity methods summarize data on communty structure into one value (Wilhm and Dorris 1968). Pielou (1966) stated that for collections where all individuals can be identified and counted, the most appropriate diversity index is that of Brillouin (1962), while Shannon and Weaver (1963) should be used for subsamples. Both diversity methods and an equitability component (Lloyd et al. 1968) were calculated on all data sets using the DIVER statistical package (University of Kansas library program). Decimal values of the number of cells per millilitre had to be rounded to the nearest whole integer for this package.

TABLE 3. Final community similarity values from experiment 1 based on species specific counts of the 23 most common species

	C2	C4	Cdl	Cd3
C4	70.37 / 71.78			
Cdl	59.01 / 55.22	44.37 / 49.46		
Cd3	51.32 / 50.48	39.63 / 46.82	83.54 / 83.24	
Lake	48.89 / 46.22	59.88 / 62.16	27.71 / 35.84	25.03 / 33.97

NOTE: For each comparison, the top value is community similarity based on total biomass and the bottom value is community similarity based on total number of individuals or colonies. See Materials and methods for a detailed explanation of the statistical methods and Table 2 for an explanation of the symbols.

Results

Experiment 1

The initial community structure and community similarity values of the 5-L continuous-flow cultures were all similar (Table 2). The final total biomass values of the *in situ* cultures were lower in both control and cadmium cultures than the lake samples. The control cultures contained twice the total biomass of the cadmium cultures and two-thirds the total biomass of the lake samples. There was a 40% reduction of total numbers in the control *in situ* cultures as compared with the lake samples. Both control cultures contained more total cells than either cadmium culture. Both diversity measures showed the lake samples to have the lowest diversity, followed by the control cultures. The cadmium cultures had the highest diversity. The same trend was present in the evenness values. The unusually low diversity and evenness values of the lake samples were caused by two diatom species (*Tabellaria fenestrata* and *Asterionella formosa*) that were at the peak of their seasonal occurrence and accounted for approximately 94% of the total biomass.

The final community similarity values (Table 3) showed the control cultures to be more similar to the lake than the cadmium cultures and the replicate cultures more similar to each other than any other sample. Several species showed consistent patterns in the cultures. *Tabellaria fenestrata* dropped to one-half the lake abundance in the control cultures and to one-quarter of the lake abundance in the cadmium cultures. *Dinobryon sertularia* and *Dinobryon bavaricum* (10.8 and 8.5 cells/mL) maintained the same population concentrations in the lake and control cultures, but dropped below the detection limit in the cadmium cultures. *Rhodomonas pusilla* dropped to less than 10 cells/mL in both the control and cadmium cultures, while the lake maintained 115.6 cells/mL. *Cryptomonas* sp. also dropped in the *in situ* cultures, control and cadmium (to less than 1 cell/mL), with the lake maintaining a population of 16.4 cells/mL. *Elakatothrix* sp. maintained equal population sizes in the lake and the control cultures, but increased

TABLE 4. Initial and final communty parameters for experiment 2 from Lake 239 for two *in situ* continuous cultures run without perturbation (C1 and C2) and compared with the lake

	Lake	C1	C2
31 July 1978			
H'	2.940	2.983	2.986
B	2.856	2.893	2.892
J'	0.672	0.659	0.639
TB($\times 10^5$)	2.133	2.035	2.106
TB($\times 10^2$)	5.938	5.747	5.892
10 August 1978			
H'	3.058	2.798	2.741
B	2.953	2.678	2.629
J'	0.683	0.610	0.598
TB($\times 10^5$)	2.861	2.106	2.260
TC($\times 10^2$)	4.948	4.004	4.099

NOTE: See Table 2 for an explanation of the symbols.

almost 50% above the lake population in the cadmium cultures. *Rhabdoderma gorskii* decreased in the control cultures, but increased above the lake concentration in the cadmium cultures (lake, 313.7; controls, 248.5; cadmium cultures, 343.6 cells/mL).

Experiment 2

Initial samples from the 5-L continuous-flow cultures had similar community structure parameters. Final samples showed that the lake samples had not only the highest diversity, but higher total cell counts and higher total biomass than the two control cultures (Table 4). The final similarity values showed the two control cultures to be equally similar to the lake (62.3 and 66.8), but more similar to each other (81.9%).

The 5-L batch cultures (two controls and two cultures with 5 μg/L Cd) had similar community structure parameters initially (Table 5). The final samples showed overlapping total biomass values, total cell count values, and similarity between the two control cultures and the two cadmium cultures (Tables 5 and 6). *Cryptomonas pusilla, Cryptomonas erosa*, and *Botryococcus braunii* dropped below the detection limit in both the control and cadmium cultures. *Cyclotella glomerata* dropped to half the lake density in both the control and cadmium cultures. *Dinobryon sertuliara* showed a dramatic increase above the lake value in the control cultures (75.3 cells/mL in the controls and 17.0 cells/mL in the lake samples); however, in the cadmium cultures the values dropped below 10 cells/mL. *Dinobryon bavaricum* dropped below 1 cell/mL in the cadmium cultures, while the control culture and lake maintained populations of 8.2 and 1.2 cells/mL, respectively. *Rhabdoderma gorskii* was the only species to increase in the cadmium cultures (206.1 cells/mL) above the lake (31.1 cells/mL) and control cultures (156.4 cells/mL).

Several small batch cultures (100 mL) were run as part of experiment 2. The cell count data from the different incubations are not, however, directly comparable. The cultures were started at different times over 2-week period and the ambient lake concentrations of the various species were constantly changing. The cell-count data are especially useful, however, for determining species-specific trends. In general, community diversity, evenness, total biomass, and total cell counts showed an inverse relationship to incubation time. Total biomass and total cell counts were consistently higher and closer to the lake values in the control cultures than in those receiving cadmium. The similarity values showed decreased similarity between control cultures and cadmium cultures with increased incubation time (Tables 7 and 8).

TABLE 5. Initial and final community parameters for experiment 2 from Lake 239 for four *in situ* 5-L batch cultures, two controls (B2 and B4), and two with 5 μg/L cadmium (CdB1 and CdB3) as compared with a lake sample

	CdB1	CdB3	B2	B4	Lake
31 July 1978					
H'	2.897	2.752	2.796	2.801	2.940
B	2.811	2.680	2.715	2.727	2.856
J'	0.650	0.643	0.609	0.631	0.672
TB(10^5)	2.023	2.019	1.909	2.147	2.133
TC(10^2)	5.795	6.390	6.485	6.601	5.938
10 August 1978					
H'	1.853	2.019	2.190	2.230	3.058
B	1.761	1.925	2.101	2.131	2.953
J'	0.476	0.471	0.525	0.521	0.683
TB($\times 10^5$)	1.377	1.936	1.709	2.145	2.861
TC($\times 10^2$)	3.154	3.766	3.868	3.483	4.948

NOTE: See Table 2 for an explanation of symbols.

TABLE 6. Final community similarity values from experiment 2 based on species specific counts of the 24 most common species

	CdB1	CdB2	B2	B4
CdB2	65.01 / 74.05			
B2	70.70 / 61.16	67.36 / 56.95		
B4	50.87 / 45.18	65.96 / 51.64	59.17 / 40.20	
Lake	43.48 / 36.07	61.51 / 43.23	47.96 / 37.02	66.72 / 51.73

NOTE: For each comparison the top value is community similarity based on total biomass and the bottom value is community similarity based on total number of individuals or colonies.

At the end of the 244-h incubation, *Asterionella formosa* had dropped in both the cadmium and control cultures to less than 30 cells/mL while the lake population had increased to 120.2 cells/mL. *Cryptomonas pusilla* dropped below the detection limit in both cultures while the lake population held at a constant 100 cells/mL. *Rhabdoderma gorskii* was reduced in both the control and cadmium cultures as compared with the lake samples, but the cadmium culture had a 30% larger population than the control. The most dramatic change in the 244-h cultures was by *Dinobryon bavaricum*, which increased to 297.8 cells/mL in the control cultures while the cadmium cultures had no detectable individuals and the lake population was less than 5 cells/mL. The same trend evident for *Dinobryon bavaricum* in the 244-h cultures was present in the shorter incubations, but to a lessr extent. One additional species that had a very low populations size when the 244-h cultures were started and therefore was more apparent in the short incubations that were started later during the week was *Dinobryon sertularia*. *Dinobryon sertularia* consistently increased in abundance in the control cultures (all incubation times) and decreased in the cadmium cultures.

TABLE 7. Final community parameter for experiment 2 from Lake 239 small batch cultures (24 h: 100 μg/L Cd and control, lab run and *in situ* run; 54 h: 30 μg/L Cd and control, *in situ* run; 244 h: 30 μg/L Cd and control, *in situ* run). The batch cultures were started at different times over a 2-week period

	24-h run				244-h run		54-h run	
	Lab, 100 μg/L Cd	Lab, control	*In situ*, 100 μg/L Cd	*In situ*, control	Control	30 μg/L Cd	Control	30 μg/L Cd
H'	2.074	2.765	2.458	3.095	1.691	2.023	2.711	2.535
B	1.970	2.643	2.329	2.948	1.623	1.912	2.610	2.413
J'	0.517	0.634	0.548	0.671	0.453	0.603	0.626	0.618
TB($\times 10^5$)	1.039	1.386	1.940	2.152	1.082	0.851	1.576	1.119
TC($\times 10^2$)	2.906	3.235	3.072	3.341	4.301	1.709	4.287	2.696

TABLE 8. Similarity values of control cultures compared with cadmium cultures of the small batch (100 mL) technique run *in situ* in Lake 239

24 h	54 h	244 h
75.96	53.82	37.47
54.02	40.53	16.51

NOTE: The top value is similarity based on biomass and the bottom value is similarity based on total cell counts.

Discussion

The methods used to analyze and operate bioassays greatly influence the type and value of data produced. The community response may (and does) change markedly depending on the sensitivity of the dominant organisms at the time of perturbation. Techniques that measure community parameters (chlorophyll *a*, total biomass, total individuals, optical density, carbon fixation, oxygen evolution, nitrogen fixation, etc.) are measuring the composite response of a multispecies algal assemblage. Unialgal tests that usually measure only total biomass attempt to estimate this composite response, but cannot simulate the constantly changing sensitivity of communities. Studies that analyze unialgal tests with community parameters are the most common in the literature on the effects of cadmium on algae. One alternative to the community parameter technique is species-specific counts and this is the approach we have used.

Based on our data, one of the few generalizations that may be made about the effects of cadmium on algae is that both the positive and negative effects are species specific. Several species were very sensitive to cadmium addition. *Dinobryon sertularia* and *D. bavaricum* populations were reduced to or just below the detection limit by 5 μg/L cadmium addition in both experiments and in all the techniques used. Other species showed a consistent trend to cadmium addition in both experiments and in the various techniques, but not to the same magnitude (e.g., *T. fenestrata*). *Rhabdoderma gorskii* (both experiments) and *Elakatothrix* sp. (first experiment, this taxon was not present during the second experiment) were the only species substantially more abundant in the cadmium cultures (of all techniques) than in the control cultures or the lake.

It is sometimes desirable or necessary to produce a single value that represents the response of a community to some perturbation. Any community response is, however, a composite response of the species present. For example, a community response based on community total biomass may have no net change, but this certainly does not mean that no species changes have occurred. Techniques (e.g., Environmental Protection Agency's algal assay bottle test) that use a single species to estimate community parameters are insensitive to species-specific changes and atypical species shifts with little change of community parameters was the chronic response documented for heavy metal and acid pollution by Yan (1979). We believe that community comparison techniques based on species-specific data are more sensitive than techniques based on community parameters (e.g., total biomass, chlorophyll *a*, etc.). Consequently, we chose to evaluate our samples with a similarity technique. This technique compares the *i*th species in both communities in such a manner that each species makes a contribution to the community similarity value. We believe this procedure for community comparisons gives more sensitive and less ambiguous values.

The final community similarity values from experiment 1 (Table 3) clearly show the control cultures (C2 and C4) more similar to each other than any other sample, as are the cadmium cultures (Cd1 and Cd3). Additionally, the control cultures were twice as similar to the lake samples as the cadmium cultures. The cadmium addition of 3–4 μg/L caused species-specific changes that were well replicated in the cadmium cultures, as is evident in the high similarity values between the cadmium cultures. In this experiment, the total biomass values were only slightly lower in the cadmium cultures, a point not reflected in the total cell-count values (Table 2). The relationship of total biomass and total cell-count parameters indicates a species shift to larger taxa, but the biomass and cell-count values alone provide only limited information about the species shifts when compared with species-specific data.

In experiment 2, the 5 μg/L addition to the 5-L batch cultures produced overlapping similarity values, total cell counts, and total biomass between and among the control and cadmium cultures (Tables 5 and 6). Initially, these results were interpreted as no detectable response to cadmium addition at this concentration using batch methods. However, examination of the species-specific counts showed that several trends present in the continuous-flow cultures were also present in the batch cultures. These trends were not apparent in the community response, because they were masked by the "bottle effect." The "bottle effect" was measured in the control cultures as compared with the lake. The control cultures had several minor taxa disappear, reduced total biomass and total cell-count values and a dramatic atypical increase of two species (*D. sertularia* and *D. bavaricum*). We believe this is an example of a "bottle effect" masking the effects of cadmium addition in the community response. Without species-specific counts, incorrect conclusions could have been made. The community response of

the small batch (100-mL) cultures run with higher cadmium concentrations (30–100 μg/L) were pronounced and while similar "bottle effects" were observed in the small batch cultures, the higher cadmium concentrations produced a distinct negative response.

In this study, cadmium additions (5.0–100.0 μg/L) caused atypical species composition shifts with little or no reduction of communty total biomass, especially at the 5.0 μg/L concentration. The subtle effects of toxic compounds may be masked by the "bottle effect" of the techniques unless great care is taken in sample analysis. Bottle effects, like those of cadmium addition, were species specific. *Botryococcus* and both species of *Cryptomonas* decreased in all control cultures, while *D. sertularia* and *D. bavaricum* increased. The possible causes of "bottle effect" have been previously discussed (deNoyelles et al. 1980; Venrick et al. 1977).

The use of bioassays has become a common practice in ecological studies. When the objective of these bioassays is the realistic prediction of what would happen if a natural ecosystem was perturbed we believe strongly that a basic characteristic of any assay technique using microorganisms (e.g., phyto- or zoo-plankton) should be the ability to replicate the natural system in the absence of the perturbation. Any technique with a large amount of "bottle effect" should be used only with great caution. An assay technique that maintains similar total biomass values, but also has dramatic species composition shifts produces, in our opinion, data of limited value which are not easily interpreted. Considering the specific nature of a community response to a perturbation, investigators claiming to evaluate the effects of some factor on a natural ecosystem must use a subsample of that particular ecosystem. It is also our opinion that exposures of less than 24 h using natural communities may produce misleading results because they must be analyzed using composite community methods. Lastly, unialgal bioassays that are analyzed with a composite community parameter are the least likely to produce realistic results.

Using the criteria mentioned above and considering cost and logistics, we believe that continuous-flow cultures, especially *in situ*, are among the best techniques currently in use. In an evaluation of experimental *in situ* systems, Adams (1982) listed only two techniques that have greater similarity to whole ecosystems: (*i*) large enclosures and (*ii*) whole pond or lake systems. Both have the advantage of containing more of the system being studied, but each also has certain disadvantages. The whole-ecosystem technique has no replication and both are difficult to control, more costly to operate, and are practical in only limited areas. Additionally, quantitative data to support the thesis that a higher higher degree of species-specific replication exists for the large control enclosures versus the natural system as compared with continuous-flow control cultures are not readily available. The techniques involved with each bioassay system limit not only the type of data produced, but also determine the suitability of each assay system to evaluate a specific problem.

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