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The Toxicological Response of the Alga *Anabaena flos-aquae* (Cyanophyceae) to Cadmium

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Abstract. The toxicological response of the cyanophycean alga *Anabaena flos-aquae* to cadmium was investigated by three integrated approaches: 1) the determination of the incipient lethal concentration of the metal, 2) study of metal incorporation and cellular compartmentalization using X-ray energy dispersive analysis, and 3) the quantification of intracellular structural changes, after metal exposure, using morphometric analysis. After 96 hr of exposure, the incipient lethal concentration was calculated to be 0.118 ± 0.04 μM cadmium. At concentrations three orders of magnitude higher than the incipient lethal concentration, cadmium was incorporated into both the cellular cytoplasm and the cell's polyphosphate bodies. Cadmium also caused the polyphosphate bodies to lose Mg and Ca, resulting in ionic changes in the elemental composition of these cellular inclusions. The utilization of stereological techniques for electron microscopic morphometric analysis established that all concentrations of cadmium tested caused significant reductions in the surface area of the cell's thylakoids. Cadmium induced changes in the numbers and relative volume of the cell occupied by polyhedral bodies, polyphosphate bodies, lipid inclusions, cyanophycin granules, membrane limited crystalline inclusions, and changes in the volume of the cell wall layers were also documented. The physiological significance of these findings are discussed in terms of the toxic action of cadmium and the cellular mechanisms for detoxification of cations once they enter the cell.

An understanding of the toxicological and physiological responses of algae to metals is of particular relevance when attempting to predict the impact of cations on the aquatic environment. This is particularly germain when studying metal effects in waters adjacent to large urban centers where rivers, estuaries and the coastal waters receive large quantities of cation-laden domestic and industrial wastes (Rachlin and Rivlin 1975; Mueller *et al.* 1976; Segar and Cantillo 1976). One of the cations which is probably nonessential for most algal species (Lewin 1962) and has been shown to be prevalent in municipal and industrial effluents (Carmody *et al.* 1973; Preston 1973) is cadmium. That this cation can exert a toxic action on algal populations was demonstrated by Rosko and Rachlin (1977) for *Chlorella vulgaris,* De Filippis *et al.* (1981) for *Euglena gracilis,* and Rachlin *et al.* (1982a, 1982b) for *Plectonema boryanum, Chlorella saccharophila, Navicula incerta* and *Nitzschia closterium.*

The purpose of this study was to continue investigations into the toxicological and physiological effects of cadmium on algal populations. A common freshwater cyanophyte, *Anabaena flos-aquae,* was chosen and three approaches were used to characterize its response to cadmium exposure. These approaches involve: 1) determinations of the incipient lethal concentration of the metal, which under the experimental conditions will reduce population growth by 50% (EC_{50}), 2) determination of incorporation of the metal and cellular compartmentalization of the metal using X-ray energy dispersive analysis techniques, and 3) the utilization of morphometric analysis techniques to quantify intracellular structural changes in response to the metal toxicant.

Materials and Methods

Cultures of the freshwater blue-green alga, *Anabaena flos-aquae* (UTEX #1444; Starr 1978) were obtained from the Starr Culture Collection of Algae, University of Texas at Austin, and were

grown in modified Fitzgerald's medium (Fitzgerald *et al.* 1952). Stock and test cultures were maintained in a Sherer-Gillett RI-24-LTP growth chamber illuminated with Arc-Ray cool white fluorescent lamps supplemented with a 25 watt incandescent lightbulb. The day/night program within the chamber was 16:8 hr, with a mean light intensity around the culture vessels of 5.5 Klux (500 ft. candles, 14 Watts/M^2), and photosynthetically active radiation (400–700 nm), as measured by a Li-Cor Quantum Sensor Model LI-190 SB, of 60 $\mu E/M^2/sec.$, and with an incubation temperature of 19 ± 1°C which was continuously monitored with a Tempscribe Ambient Air Temperature Recorder.

Stock solutions of $CdCl_2$ (A.C.S. grade) were prepared as previously described by Rachlin, *et al.* (1982b). Stock concentrations of Cd were confirmed by flameless atomic absorption spectrophotometry using a Perkin-Elmer Atomic Absorption Spectrophotometer Model 403, fitted with a Perkin Elmer HGA 400 Graphite Furnace.

After preliminary runs to determine the range within which the EC_{50} would be expected to fall, actual 96-hr test runs were prepared using a fixed volume of stock culture, shown to contain 20 μg/ml (dry wt) of *A. flos-aquae,* taken from stock cultures in log growth phase; these were then centrifuged and the medium removed. The cells were overlayed with 5 ml of sterile Cd-containing medium. Tests were performed in 10 ml sterile plastic Corning centrifuge tubes and the cultures were vibrated twice daily, on a Vortex-Genie model K-550g table top mixer to prevent clumping of the cells and consequent differential exposure to the test medium. The pH of each test culture was taken, using a Corning Model 10 pH meter, at the start and end of each test run. All tests were performed in triplicate. The test cultures were then placed in the growth chambers for 96 hr under the light and temperature regime previously described. The final test concentrations used to determine the 96-hr EC_{50} were 0.0120, 0.0435, 0.1201, and 0.4359 μM Cd.

At the end of the 96-hr test period, the cultures were removed from the growth chamber and the algal dry wt per ml was determined for each culture. Replicate weights were determined for each culture and the data from the triplicate tests were pooled. To calculate the Cd EC_{50} value for *A. flos-aquae* the pooled data were treated according to the statistical method of probit analysis (Bliss 1952; Finney 1964a, 1964b). The calculated EC_{50} was tested in triplicate on log-phase cultures to confirm its accuracy. In addition to the EC_{50}, the number of days per division T(d) was calculated for each test concentration (Guillard 1973; Honig *et al.* 1980).

After the 96-hr EC_{50} was determined, *A. flos-aquae* cultures were set up as previously described using the calculated EC_{50} value and test concentrations equal to one, two, and three orders of magnitude higher than the EC_{50}. After 96 hr of exposure, the cells were concentrated by centrifugation in a clinical centrifuge, washed three times in distilled water and air dried at room temperature on formvar coated nylon grids (Baxter and Jensen 1980a).

Cells were located using the STEM mode (dark field setting, but with a bright field image) of a JEOL U-3 SEM. Analysis was carried out with a PGT 1000 XCEL energy dispersive X-ray spectrometer as described by Baxter and Jensen (1980b) and Jensen *et al.* (1982a, 1982b).

For studies using the techniques of morphometric analysis, batch cultures were set up for 96 hr exposures to Cd using the same concentrations described above for the X-ray energy dispersive analysis tests. After the exposure period, the cells were harvested, washed, fixed in Epon, sectioned, and examined by transmission electron microscopy, using an HITACHI HU- 11E electron microscope, as previously described by Jensen and Sicko (1974). Photomicrographs were obtained at a total magnification of 31,360 and these were overlayed with a transparent sampling lattice, containing 1.0 cm square spacings, for quantitative measurements. Following the techniques for morphometric analysis (Weibel and Bolender 1973; Sicko-Goad *et al.* 1977; Sicko-Goad and Stoermer 1979; Mori and Christensen 1980; Rachlin *et al.* 1982a), the cells were analyzed for relative size, surface area of the thylakoids, relative volume of the intrathylakoidal spaces, number and volume of the polyhedral bodies, polyphosphate bodies, lipid bodies, cyanophycin granules, membrane limited crystalline inclusions, and the relative volume of the cell wall layers. Thirty photomicrographs, each representing a separate cell, were analyzed for each of the test conditions. The mean and standard error was obtained for each measurement and significance of the differences was determined by both a standard t-distribution and an ANOVA procedure (Zar 1974). A total of 150 individual photomicrographs were morphometrically analyzed.

Table 1. Estimation of the EC(50) value of cadmium from the percent response of *Anabaena flos-aquae* after 96 hr. exposure, with representative regression equation (Data based on triplicate runs)

Conc. (μM)	Log Conc.	Response %	Empirical Probit
Control	—	100	—
0.0120	−1.9208	84.1	5.9986
0.0435	−1.3615	69.8	5.5187
0.1201	−0.9205	49.2	4.9799
0.4359	−0.3606	27.0	4.3872

$Y = -1.047X + 4.027$; $r^2 = 0.9956$; Log EC(50) = −0.9296; s Log EC (50) = −0.1510; S.E. = 0.04; EC(50) = 0.118 ± 0.04 μM; Probability >95%

Results

Table 1 shows the calculated regression equation and EC_{50} concentration representing the response of *Anabaena flos-aquae* to cadmium during a 96-hr exposure under the experimental conditions as described. The EC_{50} was found to be 0.118 ± 0.04 μM cadmium. Confirmation runs using 0.12 μM cadmium demonstrated that *A. flos-aquae* cultures responded with 48.3% of control growth or within 3.4% of the expected response.

Table 2 presents data showing that the pH remained relatively constant throughout the exposure period.

Table 3 presents the initial and final weights of the cultures for each of the test conditions. From this data, the number of days per division, T(d), can be calculated. This calculation is based on the relationship $W_t = W_o e^{K(t)}$; where W_t equals the algal weight at time t, W_o equals the algal weight at time 0, and K is the instantaneous coefficient of change. As expected, since the percentage of control growth

Table 2. Average pH values (based on triplicate readings) of control and metal concentrations of test media at the start and end of the 96 hr. exposure trials of *Anabaena flos-aquae*

Metal	Conc. (μM)	Starting pH	Final pH
Cadmium	Control	7.8	7.8
	0.0120	7.8	7.6
	0.0435	7.8	7.7
	0.1201	7.8	7.8
	0.4359	7.7	7.7

Table 3. Estimation of the number of days per division T(d) of *Anabaena flos-aquae* during cadmium exposure for 96 hours

Conc. (μM)	Average Initial Weight μg/ml	Average Final Weight μg/ml	T(d)
Control	20	83	1.95
0.0120	20	73	2.14
0.0435	20	64	2.38
0.1201	20	51	2.96
0.4359	20	37	4.51

diminishes with increasing cadmium concentrations (Table 1), the respective T(d) values increase in a coinciding pattern. At 0.12 μM cadmium the T(d) is calculated to be 2.96 as compared to a calculated control value of 1.95. A comparison of the toxic response of *A. flos-aquae* and several other algal species to Cd, Cu, Pb, and Zn is shown in Table 4.

Cells observed in the STEM mode of the SEM show little internal organization except for polyphosphate bodies which were observed as dense inclusions (Figure 1). When the probe is placed over those cell sectors containing the polyphosphate bodies one obtains the spectra shown in Figure 2. The spectra show that the polyphosphate bodies of *A. flos-aquae* contain Mg, P, K and Ca, with the K peak lower than the Ca peak. The Mg peak is only slightly higher than the Ca peak and the largest peak is produced by the presence of P. After 96 hr of exposure to selected cadmium concentrations, no incorporation of Cd was found at 0.12 μM (0.013 ppm), 1.18 μM (0.13 ppm), or 11.83 μM (1.33 ppm). At 118.33 μM (13.3 ppm) Cd, a concentration three orders of magnitude greater than the calculated 96-hr incipient lethal concentration, incorporation of Cd into the polyphosphate bodies could be found in those few cells in which the polyphosphate bodies could still be recognized. The spectra from one of these cells is shown in Figure 3; both the Mg and Ca peaks are absent and the Cd peak exceeds the height of the P peak.

Figure 4 shows a cross section through a typical control cell of *A. flos-aquae* and many of the intracellular organelles (polyhedral bodies, polyphosphate bodies, thylakoids) which were morphometrically analyzed are labelled. Figures 5, 6 and 7 are enlargements of portions of control *A. flos-aquae* cells demonstrating, at higher resolution, intrathylakoidal spaces, membrane limited crystalline inclusions (Figure 5), cyanophycin granules and lipid bodies (Figure 6), plasma membrane and wall layers (Figure 7). An examination of Figures 8 and 9 demonstrate the progressive deterioration of cells respectively exposed for 96 hr to 11.83 μM (1.33 ppm) and 118.33 μM (13.3 ppm) Cd. It is evident that much of the internal integrity of the cellular inclusions is gone and that the plasma membrane has shrunk away from the cell wall increasing the relative volume of cell wall layer number 1.

Table 4. The 96-hr-EC(50) of Cd, Cu, Pb, and Zn (μM) to algae

	Metal			
Algae	Cd	Cu	Pb	Zn
(Cyanophyta)				
Anabaena variabils	0.11			52.00
Anabaena flos-aquae	0.12		5.60	
(Chlorophyta)				
Chlorella saccharophila	0.93	8.60	310.00	11.00
(Bacillariophyta)				
Navicula incerta	27.00	160.00	53.00	150.00
Nitzschia closterium	4.20	0.25	29.40	2.87

Table 5 shows the average number of intercepts of control cells and cells exposed to 0.12 μM, 1.18 μM, 11.83 μM and 118.33 μM Cd and Table 6 shows the results of the morphometric analysis of the data presented in Table 5; only cells of *A. flos-aquae* exposed to 11.83 μM (1.33 ppm) Cd show a significant ($P < 0.01$) reduction in overall size. All other concentrations of Cd produced no significant ($P > 0.05$) alterations in either the cell size or volume of the *A. flos-aquae* cells after 96 hr of exposure, although 118.33 μM (13.3 ppm) Cd produced significant deterioration of the cell's internal integrity (Figure 9).

The results of the morphometric analysis of intracellular components are given in Tables 5 and 6, which present data on the surface area of the thylakoids, relative volume of the intrathylakoidal spaces, number and volume of polyhedral bodies, polyphosphate bodies, lipids, cyanophycin granules, membrane limited crystalline inclusions, and relative volumes of the cell wall layers all after 96 hr exposure to selected concentrations of Cd. All tested concentrations of Cd produced significant ($P < 0.01$) reductions in the surface area of the cell's

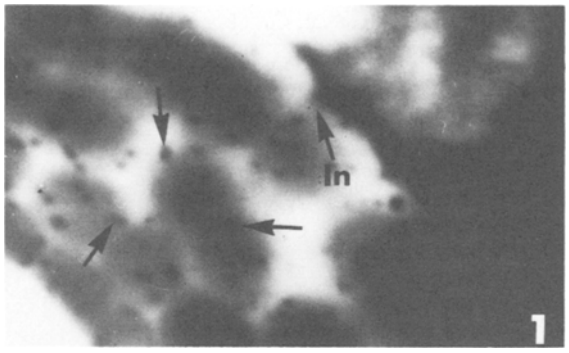

Fig. 1. Scanning transmission electron microscope image of control cells of *Anabaena flos-aquae*. The image is of a series of filaments lying side by side. The individual cells are marked by indentations (In). Numerous polyphosphate bodies can be seen as dark inclusions (arrows). (×5,600)

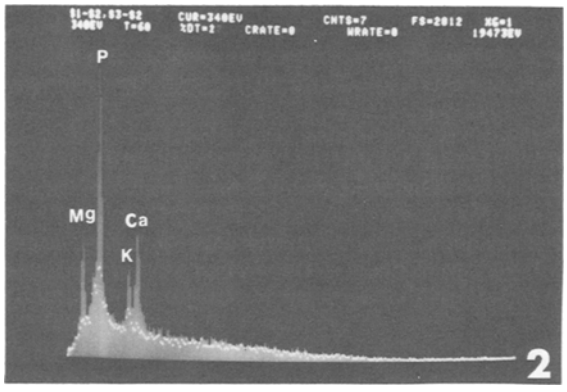

Fig. 2. Spectra from control *Anabaena flos-aquae* cell. Bars show elements in a cell sector containing polyphosphate bodies, while the dots show elements in a cell sector not containing polyphosphate bodies

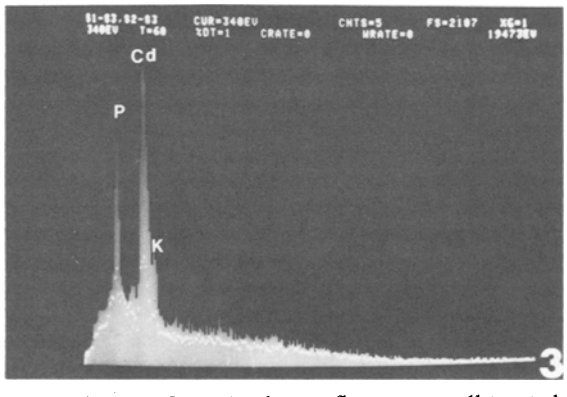

Fig. 3. Spectra from *Anabaena flos-aquae* cell treated with 118.33 μM Cd (13.3 ppm) for 96 hr. Bars show elements in a cell sector containing polyphosphate bodies and dots show elements in cell sector containing no polyphosphate bodies. Note that P, Cd and K show significant peaks in this cell sector (dots). The Cd-K peak is generated from both elements. The Cd lines were identified in the spectrometer

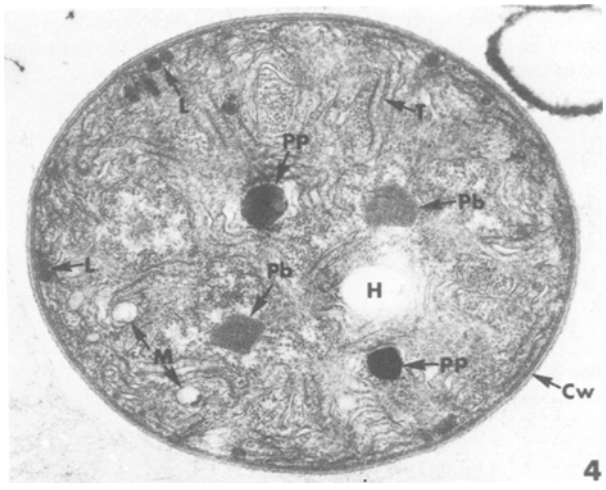

Fig. 4. Cross section of a control cell of *Anagaena flos-aquae* showing most of the cellular inclusions. Shown in the micrograph are polyhedral bodies (Pb), polyphosphate bodies (Pp), a hole left where a polyphosphate body was lost from the section (H), lipid bodies (L), membrane limited crystalline inclusions (M), thylakoids (T), and the multilayered cell wall (Cw). (×21,800)

thylakoids, but only 1.18 μM Cd produced a significant ($P < 0.05$) reduction in the relative volume of the intrathylakoidal spaces. Though all other concentrations produced a general trend towards reduction of the relative volumes of the intrathylakoidal spaces, these reductions were not significant ($P > 0.05$).

None of the concentrations of Cd tested produced a significant change in the average number of polyhedral bodies of the *A. flos-aquae* cells, but all concentrations of Cd higher than the calculated EC_{50} (0.12 μM) resulted in significant ($P < 0.05$) reductions in the relative volume of the cell occupied by these bodies. Only 11.83 μM (1.33 ppm) Cd caused a significant ($P < 0.01$) reduction in the average number of polyphosphate bodies found in the *A. flos-aquae* cells. In the photomicrographs of cells used in this phase of the study, those cells exposed to 118.33 μM Cd had no structures clearly identifiable as polyphosphate bodies. When these bodies are observed, they are found to have incorporated Cd to a rather high degree (Figure 3).

The data shows that all concentrations of Cd tested caused a significant ($P < 0.05$) reduction in the relative volume of the cell occupied by the polyphosphate bodies, and that only 1.18 μM (0.13 ppm) Cd caused a significant ($P < 0.01$ and $P < 0.05$ respectively) increase in both the number and volume of the cell's lipid bodies. No lipid inclusions were identified in cells exposed to the highest concentration (118.33 μM) of Cd tested. Cells exposed to either 0.12 μM or 11.83 μM Cd showed no significant ($P > 0.05$) change in either their lipid num-

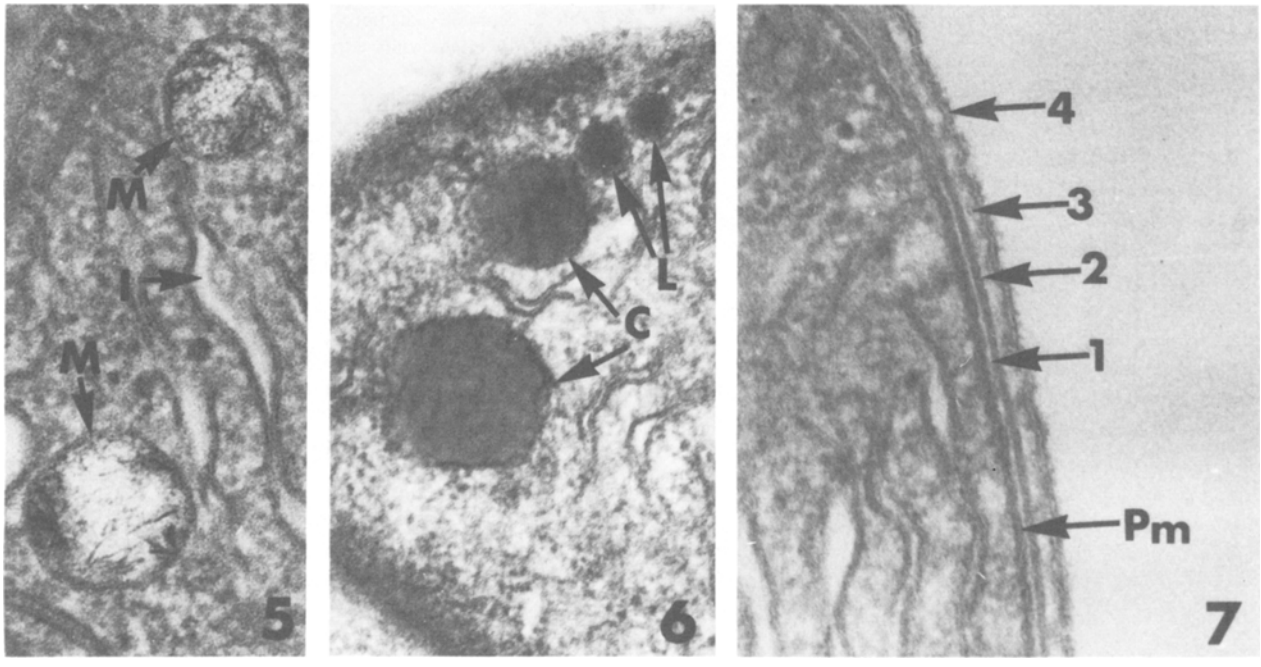

Fig. 5. Portion of a control cell of *Anabaena flos-aquae* showing an enlargement of the membrane limited crystalline inclusions (M) and an intrathylakoidal space (I). (×121,000) **FIG. 6.** Portion of a control cell of *Anabaena flos-aquae* showing cyanophycin granules (C) and lipid bodies (L). (×94, 560) **FIG. 7.** Portion of a control cell of *Anabaena flos-aquae* showing the cell wall layers which are numbered 1 to 4 starting adjacent to the plasma membrane (Pm). (×121,000)

bers or the relative volume of these inclusions. No significant change was observed in the numbers or volume of the cell's cyanophycin granules except for those cells exposed to 11.83 μM Cd which produced a significant ($P < 0.01$) reduction in both the numbers and volume of these structures.

Membrane limited crystalline inclusions were observed to significantly ($P < 0.05$) increase in numbers in cells exposed to 1.18 μM Cd and to significantly ($P < 0.05$) decrease in numbers in cells exposed to 118.33 μM Cd for 96 hr. None of the concentrations of Cd tested produced any significant ($P > 0.05$) changes in the relative volume of the cell occupied by the membrane limited crystalline inclusions. A significant ($P < 0.01$) increase in the relative volume of wall layer 1 in cells exposed to both 11.83 μM (1.33 ppm) and 118.33 μM (13.3 ppm) Cd, with the higher concentration producing the greater increase in relative volume was observed, while a significant ($P < 0.01$) change, *i.e.*, reduction in the combined relative volume of cell wall layers 2, 3 and 4 was found only in *A. flos-aquae* cells exposed to 11.83 μM (1.33 ppm) Cd for 96 hr.

Discussion

We concerned ourselves with characterizing the toxicological and physiological responses of the freshwater cyanophyte *Anabaena flos-aquae* to selected cadmium concentrations. The algal responses were characterized by: 1) determining the concentration of cadmium which reduced population growth by 50% of control values (EC_{50}), 2) use of x-ray energy dispersive analytical techniques to evaluate metal incorporation and cellular compartmentalization, and 3) the use of morphometric analysis to quantify intracellular structural changes.

The data in Table 1 indicates that the 96 hr EC_{50} (incipient lethal concentration) of cadmium for *Anabaena flos-aquae* was 0.118 ± 0.04 μM. Preliminary studies (Rachlin and Jensen, unpublished) indicate the incipient lethal concentration of Cd for a related cyanophyte, *Anabaena variabilis,* to be 0.11 μM, showing the consistency of response of this algal genus to Cd. Table 4 shows that these cyanophytes are more sensitive to Cd intoxication than are the eukaryotic algae, *Chlorella saccharophila* (0.93 μM Cd), *Nitzschia closterium* (4.23μM Cd) and *Navicula incerta* (26.69 μM Cd) (Rachlin *et al.* 1982b).

Reduction in growth rate is further demonstrated by the data presented in Table 3 showing that the days per division, T(d), increase from the control value of 1.95 to 4.51 as the Cd concentration is increased. These reductions in growth rates during metal exposure are in good agreement with the findings of Fisher and Frood (1980) for *Skeletonema*

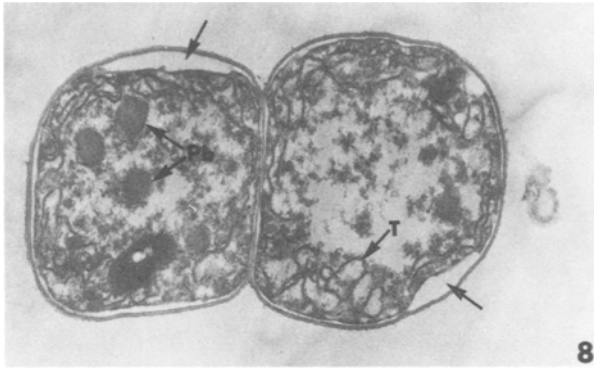

Fig. 8. Thin section of *Anabaena flos-aquae* cells treated with 11.83 μM Cd (1.33 ppm) for 96 hr. Polyhedral bodies (Pb) and thylakoids (T) are clearly visible in the cell. Note the shrinkage of the cell away from the cell wall, greatly enlarging the area counted as cell wall layer 1 (arrows). (×10,800)

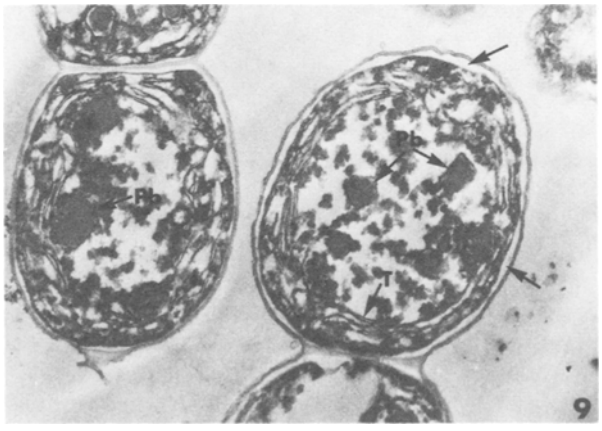

Fig. 9. Thin section of *Anabaena flos-aquae* cells treated with 118.33 μM Cd (13.3 ppm) for 96 hr. The cells are greatly damaged and polyhedral bodies (Pb) and thylakoids (T) can just be recognized. Note the shrinkage of the cell away from the cell wall which results in a greatly enlarged area counted as cell wall layer 1 (arrows). (×10,800)

Table 5. Summary of morphometric data (mean values) of *Anabaena flos-aquae* cells exposed to selected concentrations of cadmium

	Cd Concentration μM				
	0.0	0.12	1.18	11.83	118.33
Relative Cell Size	62.70	63.80	56.90	51.40	61.70
Thylakoid Surface Area μM²	6.56	4.89	5.23	4.06	3.46
Intrathylakoidal Space Relative Volume	1.86	1.67	0.00	1.30	0.59
Polyhedral Body Number	2.33	2.73	1.77	1.73	2.43
Polyhedral Body Relative volume	4.04	3.81	2.69	3.05	3.51
Polyphosphate Body Number	0.46	0.36	0.33	0.06	n.v.
Polyphosphate Body Relative Volume	2.13	0.57	0.76	n.v.	n.v.
Lipid Body Number	3.20	3.40	5.77	3.43	n.v.
Lipid Body Relative Vol.	0.59	0.52	1.46	1.10	n.v.
Cyanophycin Granule Number	0.53	0.27	0.43	0.07	0.40
Cyanophycin Granule Relative Volume	0.64	0.31	0.59	0.06	0.49
Mem. Lim. Cryst. Incl. Number	0.93	0.67	1.73	0.80	0.43
Mem. Lim. Cryst. Incl. Relative Volume	0.69	0.73	0.87	0.52	0.54
Wall Layer Number 1 Relative Volume	n.v.	n.v.	n.v.	2.01	3.84
Wall Layer 2, 3, 4 Combined Relative Volume	5.64	5.48	5.74	4.86	5.24

Key: n.v. = no value obtained

costatum, Nitzschia closterium, Chaetoceros compressum, Asterionella japonica and the reported data of Rachlin *et al.* (1982b) for *Chlorella saccharophila, Navicula incerta* and *Nitzschia closterium*. That these reductions in growth are due to Cd and not responses to changes in pH over time is shown in Table 2. These slight changes in pH have no significant effect on algal growth (Rosko and Rachlin 1975, 1977). Further, Stary and Kratzer (1982) demonstrated that the cumulation factor for Cd logarithmically increases over the pH range 6.0–8.5. Therefore, under the pH conditions of this study (Table 2) Cd cumulation would be optimal.

A mechanism to account for the observed reduction in algal growth after Cd exposure has been proposed by De Filippis *et al.* (1981). They found that Cd inhibited NADP-oxidoreductase, significantly lowering the cell's supply of NADPH. This action results from this enzyme requiring sulfhydryl groups for activity. Cadmium is a strong sulfhydryl antagonist (Vallee and Ulmer 1972). The level of response would then depend both on the concentration of Cd ions traversing the cell's membrane and the amount of this ion not bound by the cells' inherent detoxifying mechanisms (Simkiss 1979; Jensen *et al.* 1982a, 1982b; Rachlin *et al.* 1982a).

In previous studies, Kuypers and Roomans (1979), Baxter and Jensen (1980a) and Jensen *et al.* (1982a, 1982b) have shown that air drying of cells provides good retention of ions *in situ* and is therefore a method of choice in X-ray energy dispersive analysis studies. The spectra generated when the probe is placed over cell sectors containing polyphosphate bodies (bars) and over cell sectors without polyphosphate bodies (dots) is shown for control *A. flos-aquae* cells (Figure 2), and those cells exposed to 118.33 μM (13.3 ppm) Cd (Figure

Table 6. Results of morphometric analysis of *Anabaena flos-aquae* cells exposed to selected concentrations of cadmium

	Cd Concentration μM			
	0.12	1.18	11.83	118.33
Relative Cell Size	—	—	+ <	—
Thylakoid Surface Area	+ <	+ <	+ <	+ <
Intrathylakoidal Vol.	—	+ <	—	—
Polyhedral Body #	—	—	—	—
Polyhedral Body Vol.	—	+ <	+ <	+ <
Polyphosphate Body #	—	—	+ <	(n.v.)
Polyphosphate Body Vol.	+ <	+ <	+ <	(n.v.)
Lipid Body #	—	+ >	—	(n.v.)
Lipid Body Vol.	—	+ >	—	(n.v.)
Cyanophycin Granule #	—	—	+ <	—
Cyanophycin Granule Vol.	—	—	+ <	—
Mem. Lim. Cryst. Incl. #	—	+ >	—	+ <
Mem. Lim. Cryst. Incl. V.	—	—	—	—
Wall Layer #1 Volume	—	—	+ >	+ >
Wall Layer 2, 3, 4 Volume	—	—	+ <	—

Key: — = No Change; + = Significant Change; > = Increase; < = Decrease; n.v. = no value obtained

3). The polyphosphate bodies of control cells show peaks for Mg, P, K and Ca, with P generating the highest peaks followed by Mg and Ca, and the K peak being the smallest. These findings are consistent with previous reports for the elemental components of polyphosphate bodies (Baxter and Jensen 1980a; Jensen *et al.* 1982a, 1982b). The cell sectors, of control cells, without polyphosphate bodies contain P and K, but no other elements are present in a high enough concentration to be detectable (Figure 2). No Cd was detected in either the polyphosphate body containing cell sectors or cell sectors not containing polyphosphate bodies in cells exposed to 0.12 μM (0.013 ppm), 1.18 μM (0.13 ppm), or 11.83 μM (1.33 ppm) Cd for 96 hr. This does not necessarily negate Cd incorporation at these concentrations, but may reflect Cd incorporation at concentrations below the detectable limits of the analysis technique. At 118.33 μM (13.3 ppm) Cd, incorporation is found in both the polyphosphate bodies, when these are present, and in the cytoplasm itself (Figure 3). It is also evident that the elemental composition of the polyphosphate bodies are changed in that Mg and Ca are no longer present in detectable amounts.

Other studies have also shown that certain heavy metals can cause loss or reduction of other elements from cells and cell inclusion bodies (Passow and Rothstein 1960; Doonan *et al.* 1979; Kuypers and Roomans 1979; Nakajima *et al.* 1979; Baxter and Jensen 1980b; and Jensen *et al.* 1982a) demonstrated the uptake of Mg, Ba, Mn, Cd, Co, Cu, Hg, Ni, Pb and Zn, but not Sr, by the polyphosphate bodies of the cyanophyte *Plectonema boryanum*, while Cd, Cu, Hg, Pb and Zn were also found in the organism's cell sectors without polyphosphate bodies. It has also been reported that Pb is incorporated into the cell wall of *P. boryanum* (Rachlin *et al.* 1982a). Sicko-Goad and Stoermer (1979) have reported that Pb—but not Cu—is incorporated into the polyphosphate bodies of the diatom, *Diatoma tenue*, and Jensen *et al.* (1982b) found that Pb could be detected, after cell exposure, in the cytoplasm, polyphosphate bodies and cell walls of *Chlorella saccharophila* and *Nitzschia closterium*. Jensen *et al.* (1982b) also found that while the polyphosphate bodies and cytoplasm of *Navicula incerta* could incorporate Pb, none was detected in the cell wall. It was further reported that Zn was sequestered in the polyphosphate bodies and cytoplasm of *C. saccharophila* and *N. incerta* but no Zn was detected in cells of *N. closterium* over the concentration range studied. From the current investigation and the reports cited above it is clear that the polyphosphate bodies of algal cells play a prominent role in the compartmentalization and mobilization of heavy metals in the aquatic ecosystem, and that one of the effects of heavy metals on cells is the alteration of the normal ion balance.

Morphometric analysis of *A. flos-aquae* cells exposed to Cd revealed that only 11.83 μM had any effect on overall cell dimensions. This Cd concentration caused a significant reduction in cell size. Loss of overall cell size results from loss of fluid from the cell and increased condensation of the wall mucopolymers (Rachlin *et al.* 1982a). All concentrations of Cd tested resulted in a significant reduction in the surface area of the cell's thylakoids, and while there was a general trend towards reduction of the intrathylakoidal spaces, only 1.18 μM Cd induced a significant reduction. Thylakoids are the photosynthetic lamellae of the cell where almost all of the cellular chlorophyll and carotenoid pigments are localized (Wolk 1973). Reduction in thylakoidal surface area may indicate loss of photosynthetic potential in Cd treated cells, both by reduction of the proteinaceous lamellae (Schmitz 1967) themselves and by reduction in the cell's chlorophyll content. Rosko and Rachlin (1977) have demonstrated changes in the chlorophyll-a content of the chlorophycean alga *Chlorella vulgaris* after heavy metal exposure. Reduction of the intrathylakoidal spaces will have the effect of bringing the thylakoids into closer approximation. This may have some value in facilitating photosynthetic activity in metal-stressed cells. Loss of the protein portion of the thylakoid lamellae may represent a detoxifying mechanism in the cell. Rothstein (1959) has demonstrated that metals interact with sulfhydryl groups in cellular

membranes to produce -S-metal-S-bridges. Simkiss (1979) has discussed the importance of proteins and lipoproteins in binding with metals as a means of incorporating them within the cell.

Polyhedral bodies (Carboxysomes) contain the enzyme ribulose 1,5-bisphosphate carboxylase (Codd and Stewart 1976, 1977; Codd *et al.* 1980; Lanaras and Codd 1982) a key enzyme in the Calvin Cycle. None of the concentrations of Cd tested altered the number of polyhedral bodies in the exposed cells. However, 1.18 μM, 11.83 μM and 118.33 μM Cd caused significant reductions in the volume of the cell occupied by these bodies, indicating a reduction in size of these bodies with a probable mobilization of the enzyme.

All of the concentrations of Cd tested caused a significant reduction in the volume of the cell's polyphosphate bodies along with a trend towards reduction in their numbers. The importance of these bodies in sequestering metals has already been discussed. Their reduction in size and numbers represents a significant loss of stored phosphate and potential metal sequestration sites due to ion imbalance in response to Cd toxicity. Polyphosphate bodies were rarely found in cells exposed to 118.33 μM Cd, but when found had high Cd levels.

Only 1.18 μM Cd, a concentration one order of magnitude higher than the calculated incipient lethal concentration, caused a significant increase in the number and volume of lipid inclusions. Wolk (1973) reported that sulfoquinovosyl diglyceride is one of the predominant lipids of cyanophycean algae. Increases in bodies containing this compound may indicate a cellular mechanism of detoxification because the affinity of metals for potential sulfur-binding sites is an established fact.

None of the Cd concentrations tested had a significant effect on the numbers or volume of cyanophycin granules except 11.83 μM (1.33 ppm). At this concentration, both the numbers and relative volumes of these granules were significantly reduced. The cyanophycin granules are composed of a protein reserve made up of a multi-L-arginyl-poly(L-aspartic acid) (Simon *et al.* 1980). The significance of these findings is not clearly understood, although these bodies with their protein reserves may be part of the cell's internal detoxification system (Simkiss, 1979).

The nature and function of membrane limited crystalline inclusions is not well understood at the present time. None of the concentrations of Cd tested caused a change in the relative volume of the cell occupied by these bodies. Cadmium at a concentration of 1.18 μM caused a significant increase in their numbers. Since the volume of these inclusions did not change there is a presumption that these inclusions fragmented into many smaller ones in response to this concentration of Cd. Evidence for coalescence of these inclusions at a Cd concentration of 118.33 μM comes from a significant reduction in their numbers, but again no change in the volume of the cell occupied by these inclusions.

At concentrations of Cd two and three orders of magnitude higher than the calculated incipient lethal concentration, there is a marked shrinking away of the plasma membrane from the cell wall. This is clearly observed in Figures 8 and 9, and results in a significant increase in the cellular volume of the area normally designated as cell wall layer number 1. Only at 11.83 μM Cd was there a measured decrease in the combined volumes of wall layers 2, 3 and 4. All other concentrations tested produced no significant changes in the combined volume of layers 2, 3 and 4. Reduction in the volume of these layers may indicate a depolymerization of wall mucopolymers and a mobilization of this material into the area occupied by wall layer number 1.

This study demonstrates the importance of applying multiple methodologies towards a fuller understanding of the cellular response to heavy metal exposure. We believe that only through similar multifaceted studies can one gain the insight needed to make predictive statements regarding the effects of metal toxicants on the algal base of the aquatic ecosystem. It is suggested that this approach be applied in future toxicological studies.

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