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A Morphometric Analysis of Algal Response to Low Dose, Short-Term Heavy Metal Exposure

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Summary

Three algae, *Melosira granulata*, *Fragilaria capucina*, and *Anacystis cyanea*, collected as part of a natural phytoplankton assemblage were found to differ in their cytological responses to low dose short-term exposure to copper and lead. In general, all were more sensitive to copper than to lead. *Fragilaria* was more sensitive to both metals than the other species examined. Most immediate changes in relative volume categories can be ascribed to changes in vacuole volume that are most likely the result of changes in membrane permeability. There was some degree of accommodation in all three species at 24 hours. These results are discussed in view of the natural environment of the algae, as well as in relationship to previous studies.

Keywords: Algae; Copper effects; Heavy metals; Lead effects; Morphometry; Phytoplankton.

1. Introduction

Many of the reported ultrastructural changes in cultured algal cells resulting from heavy metal exposure are based on experiments lasting for several days or weeks, at concentrations that are uncommon in aquatic environments. In a previous study we found that the phosphate nutrient status of algal cells may mitigate the deleterious effects of heavy metals (SICKO-GOAD and STOERMER 1979). In experiments with cultures of the pennate diatom, *Diatoma tenue* var. *elongatum*, we found that the primary effects of lead treatment, when coupled with phosphate uptake, were swelling and reduction in numbers of mitochondria, and incorporation of lead into polyphosphate bodies. The primary effect of copper treatment was the reduction in numbers of polyphosphate bodies. There was no copper incorporation in the polyphosphate bodies. These ultrastructural changes were found after 2 hours of exposure to the low concentration (μg range) heavy metal treatments.

In order to understand how heavy metals may affect algae in their natural environment, experiments were conducted in which natural phytoplankton assemblages were exposed to low doses of copper and lead over a 24-hour period. The assemblage chosen for study was collected from Saginaw Bay, one of the more eutrophic areas of the Laurentian Great Lakes. Detailed morphometric analyses characterizing responses of three selected algae, *Melosira granulata*, *Fragilaria capucina* and *Anacystis cyanea* are presented in this paper.

2. Materials and Methods

Nearshore surface whole water samples were collected from the northeast segment of Saginaw Bay of Lake Huron on 7 August 1977. Samples were kept in a cooler and immediately transported to the laboratory for processing. Upon return to the laboratory, a portion of the whole water sample was withdrawn and fixed in 3% glutaraldehyde in 0.05 M sodium cacodylate buffer (pH 7.2) for 1 hour at 4 °C. The osmolality of the glutaraldehyde fixative was 387 mosmol. Samples for heavy metal incubations were transferred to 1-*l* sterile Corning tissue culture flasks and inoculated with solutions of lead or copper nitrate to give final concentrations of 10 ppb Pb and 5 ppb Cu. The flasks were then incubated under light and temperature conditions similar to those measured at the collection site for a total period of 24 hours. Aliquots from treatment flasks were

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Table 1. *Sampling scheme employed for morphometric analysis.* The number of micrographs examined and the standard magnifications were as follows: *M. granulata*, 30 photographs per treatment at 3,700×; *F. capucina*, 50 per treatment at 8,000×; *A. cyanea*, 30 per treatment at 11,300×. Micrographs were enlarged 3× for actual counts

Taxon		Treatments						
		0 hr	2 hrs Cu	3 hrs Cu	24 hrs Cu	2 hrs Pb	3 hrs Pb	24 hrs Pb
Melosira granulata	Total points counted	8799	5626	8526	9537	5456	7561	7537
	Total area examined (μm^2)	7272	4650	7046	7882	4509	6249	6229
	Total cell volume (μm^3) light microscope*	5129						
Fragilaria capucina	Total points counted	4390	4983	4501	4668	3092	4100	4675
	Total area examined (μm^2)	750	851	769	797	528	700	798
	Total cell volume (μm^3) light microscope*	400						
Anacystis cyanea	Total points counted	4688	5372	5286	4513	3947	4802	5489
	Total area examined (μm^2)	406	465	457	390	341	415	475
	Total cell volume (μm^3) light microscope*	78						

* No difference in cell volume within the range of standard error was found for the three species examined. Consequently, only one value is reported as the mean volume and this number was used to calculate the number/cell in subsequent tables.

withdrawn at 2, 3, and 24 hours and fixed with glutaraldehyde as described above. After primary fixation all samples were washed with cacodylate buffer, post-fixed in 1% OsO_4 in 0.05 M cacodylate buffer (pH 7.2) for 1 hour at 4 °C, dehydrated in a graded ethanol (50%, 85%, 95%, 100%) and propylene oxide series during a time period of approximately 1.5 hours and then embedded in Epon (LUFT 1961).

Thin sections were cut with a diamond knife, collected on formvar coated 200 mesh copper grids and stained with aqueous uranyl acetate (WATSON 1958). Sections were then examined at a standard magnification dependent upon taxon cell size (Table 1) in a JEOL JEM 100 B electron microscope operating at 80 kV. Microscope magnification calibrations were made by use of a grating replica to determine the stability of the standard magnification. Variation was between 1 and 2%.

Three taxa were selected from the assemblage for quantitative analysis. These taxa were easily identifiable by specific cytological features such as the frustule structure in the two diatoms and the thylakoid arrangement in the blue-green.

A transparent 10 mm square sampling lattice was superimposed over the micrographs for quantitative measurements. Although several sections were collected on one grid, only 1 section/grid was used for the analysis. Individual cells were photographed, including small grazing tip sections. The grazing tip sections of *Fragilaria capucina*, and consequently the frustule relative volume, may be slightly underestimated due to the presence of other pennate diatoms in the assemblage. Such problems were not encountered with either *Melosira* or *Anacystis* because they have a very distinctive cytology (Figs. 1-4). Other stereologic methods used have been described previously (SICKO-GOAD *et al.* 1977, SICKO-GOAD and STOERMER 1979) with the exception that for cells of *Melosira* which exceeded the photographic field of view at the standard magnification, a montage was photographed and used for counting. Volume density was determined using the grid point-counting technique (GLAGOLEFF 1933, CHALKLEY 1943). Surface density was calculated from the relationship

$$S_v = \frac{2I}{L_t} \quad \text{(TOMKEIEFF 1945)}$$

where S_v = surface density, I = number of intersections with a test line system, and L_t = known length of the test line system.

Actual cell volume estimates were obtained from light microscopic examination of cells obtained from the same assemblages as those used for the quantitative analysis. Ten independent measurements for each species and treatment were made from epoxy mounts of the fixed and embedded material. Volume estimates assume a regular geometric shape. Parameter estimates are reported in the paper as the mean ± standard error.

3. Results

General Descriptive Measures

The morphometric results presented in this paper describe both physical and physiological compartments of the cell. In describing the changes that occur during the low dose heavy metal exposure we have made the following operational categories and measured either relative volume (V_v), number per volume (N_v), or surface to volume ratio (S_v).

(1) Wall/Frustule: V_v. This is the area outside the plasma membrane. For *Melosira granulata* we have also measured the relative volume of the "space" or chambers in the siliceous frustule since they contribute significantly to the volume of the frustule.

(2) Mitochondria: V_v and N_v.

(3) Storage: V_v. For the diatoms examined, storage material was almost exclusively lipid in the vacuole.

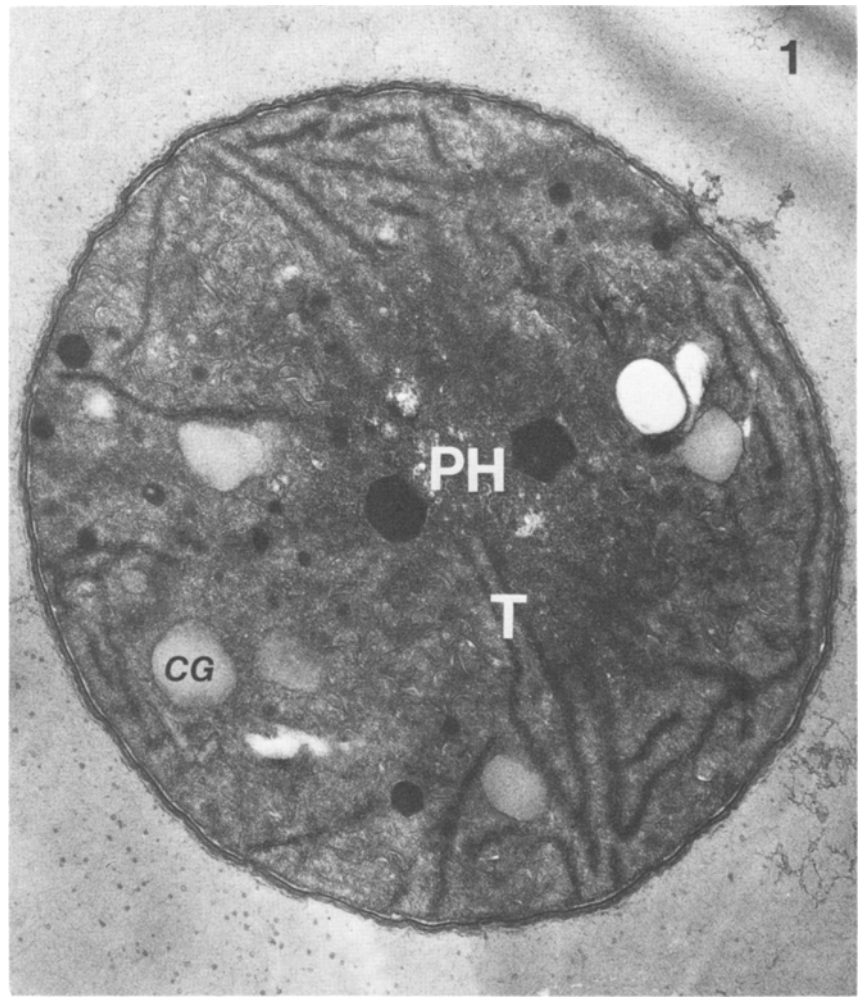

Fig. 1. *Anacystis cyanea*, 24 hours lead incubation. Polyhedral bodies (*PH*), cyanophycin granules (*CG*), Thylakoids (*T*)

(4) Nucleus: V_v.
(5) Other Cytoplasm: V_v. For the diatoms this category includes endoplasmic reticulum, Golgi, ribosomes, and cytoplasmic matrix. In the blue-green this area also includes the nucleoplasm since it is not easily subdivided into a distinct area. This category also does not include any distinct prokaryote inclusions described below.
(6) Vacuole: V_v. This is the area bounded by the vacuolar membrane and does not include storage that may be present in this area.
(7) Chloroplast: V_v. This includes the pyrenoid.
(8) "Autophagic"-like vacuole: V_v. Category of membranous organelles present in the vacuole, usually near the chloroplast. This is not included in the vacuole category.
(9) Polyphosphate bodies (poly P): V_v.
(10) Polyhedral bodies (carboxysomes): V_v and N_v.
(11) Cyanophycin (structured) granules: V_v and N_v.
(12) PHB granules (poly-β-hydroxybutyrate): V_v.
(13) β-granules (lipid deposits) V_v.
(14) Thylakoids (in the blue-green): S_v. The quantity reported is the surface area/volume ratio of the outer surface of the photosynthetic lamellae per μm^3 cell.
(15) For the diatoms, we have also calculated relative cytoplasmic volume percentages. Cytoplasm was taken to be a composite category (chloroplast, mitochondria, and other cytoplasm) and relative volumes were recalculated disregarding any changes occurring in the vacuole, nucleus, and wall. Consequently, in this case mitochondria V_v is the relative volume of mitochondria in the cytoplasm, not the entire cell.

Melosira granulata

Detailed quantitative results of the copper and lead treatments are presented in Tables 2 and 3. Several trends are apparent. While there were no statistically significant morphometric changes of *Melosira granula-*

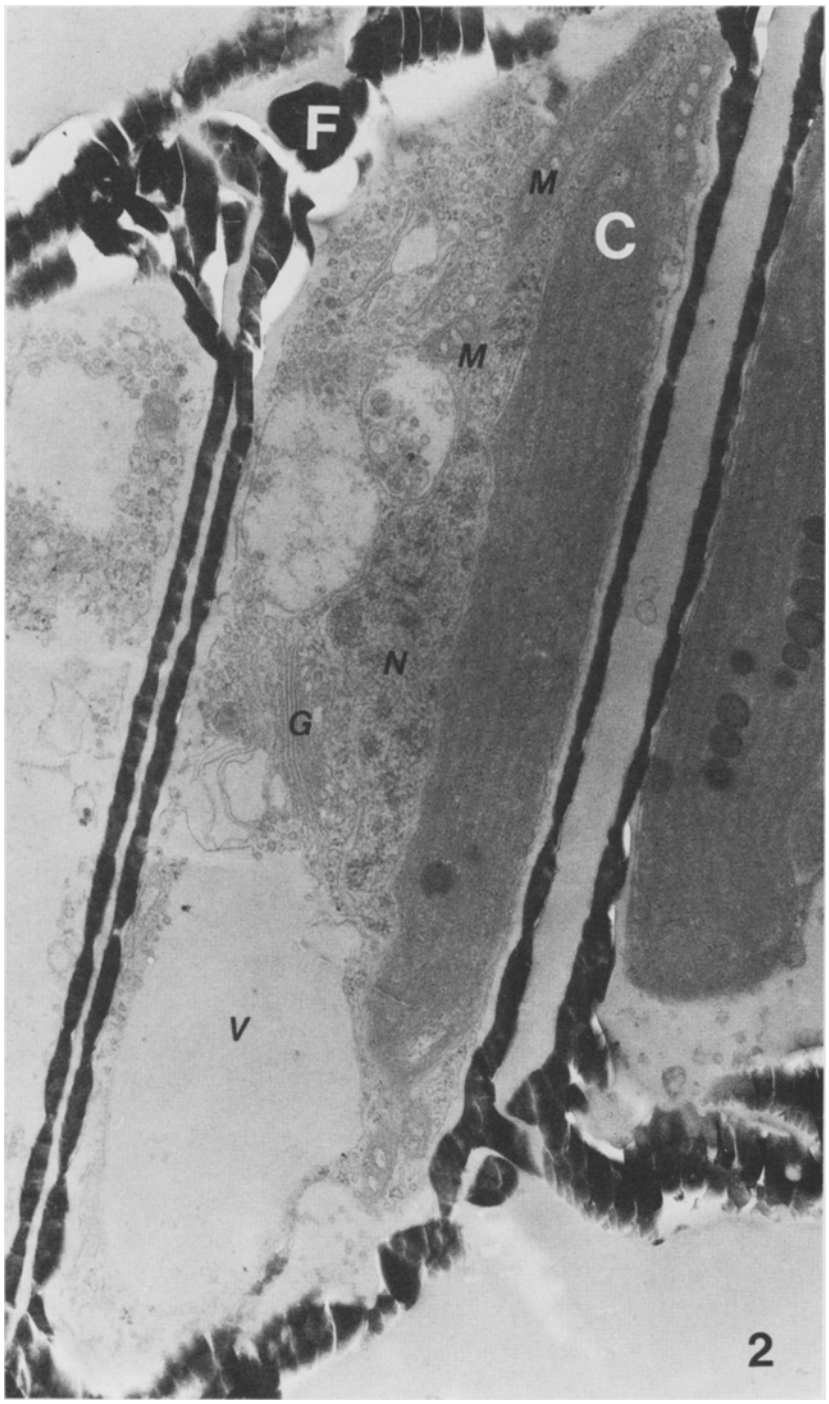

Fig. 2. *Fragilaria capucina*, 2 hours copper incubation. Mitochondria (*M*), chloroplast (*C*), golgi (*G*), nucleus (*N*), vacuole (*V*), siliceous frustule (*F*)

Table 2. *Melosira granulata*. Morphometric results of copper treatments. Results are relative volumes (V_v) and their standard errors unless otherwise designated

	Treatments			
	0 hour	2 hours	3 hours	24 hours
Cellular Volume Percents				
Chloroplast	6.80 (1.15)	5.28 (0.50)	5.91 (0.49)	4.11 (0.93)
Mitochondria	1.44 (0.24)	1.30 (0.18)	0.93 (0.15)	0.90 (0.19)
* Mitochondria (N_v)	0.023 (0.006)	0.030 (0.005)	0.015 (0.002)	0.008 (0.002)
Aver. volume	0.63 μm^3	0.43 μm^3	0.62 μm^3	1.13 μm^3
Number/cell	118	154	77	41
Storage	2.83 (0.57)	5.15 (1.43)	5.63 (1.15)	2.83 (0.69)
Nucleus	0.63 (0.19)	1.24 (0.26)	0.47 (0.21)	0.68 (0.23)
Other cytoplasm	5.80 (1.12)	4.89 (0.58)	3.89 (0.74)	4.16 (0.55)
Vacuole	69.95 (2.70)	69.48 (2.46)	72.06 (2.15)	73.88 (1.93)
Si frustule	10.29 (1.14)	10.04 (1.57)	9.24 (0.94)	11.32 (0.66)
Frustule space	2.26 (0.37)	2.61 (0.35)	1.86 (0.25)	2.12 (0.25)
Auto. vacuole	0.00 (−)	0.00 (−)	0.00 (−)	0.00 (−)
Poly P	0.00 (−)	0.00 (−)	0.00 (−)	0.00 (−)
Cytoplasmic Volume Percents				
Chloroplast	48.42 (3.42)	46.05 (3.43)	55.08 (3.33)	44.85 (3.32)
Mitochondria	10.28 (1.37)	11.32 (1.63)	8.63 (1.03)	9.84 (1.58)
* Mitochondria (N_v)	0.164 (0.030)	0.261 (0.042)	0.136 (0.017)	0.086 (0.015)
Other cytoplasm	41.30 (2.90)	42.64 (3.48)	36.28 (3.56)	45.42 (2.84)

* N_v = number/volume; $N_v = \frac{K\ N_a^{3/2}}{\beta\ V_v^{1/2}}$ (WEIBEL and GOMEZ 1962) where K is assumed to be 1.07 and $\beta = 2.25$. (K = coefficient dependent on relative size distribution of particles. $K > 1$ if size is not uniform. β = shaping constant. N_a = number/area.)

Table 3. *Melosira granulata*. Morphometric results of lead treatments

	Treatments			
	0 hour	2 hours	3 hours	24 hours
Cellular Volume Percents				
Chloroplast	6.80 (1.15)	8.21 (1.33)	4.18 (0.56)	6.32 (1.09)
Mitochondria	1.44 (0.24)	1.37 (0.28)	1.09 (0.18)	1.02 (0.15)
* Mitochondria (N_v)	0.023 (0.006)	0.043 (0.007)	0.022 (0.003)	0.023 (0.004)
Aver. volume	0.63 μm^3	0.32 μm^3	0.50 μm^3	0.44 μm^3
Number/cell	118	221†	112	118
Storage	2.83 (0.57)	2.91 (0.69)	3.73 (0.68)	4.56 (0.99)
Nucleus	0.63 (0.19)	0.82 (0.22)	0.56 (0.21)	0.98 (0.30)
Other cytoplasm	5.80 (1.12)	6.18 (1.01)	3.99 (0.63)	4.98 (0.66)
Vacuole	69.95 (2.70)	67.67 (3.35)	75.21 (1.85)	69.95 (2.81)
Si Frustule	10.29 (1.14)	10.52 (2.01)	9.47 (1.08)	10.10 (1.18)
Frustule space	2.26 (0.37)	2.03 (0.33)	1.65 (0.32)	1.86 (0.33)
Auto. vacuole	0.00 (−)	0.11 (0.16)	0.09 (0.06)	0.23 (0.15)
Poly P	0.00 (−)	0.16 (0.10)	0.01 (0.01)	0.00 (−)
Cytoplasmic Volume Percents				
Chloroplast	48.42 (3.42)	52.09 (3.39)	45.14 (3.37)	51.29 (3.67)
Mitochondria	10.28 (1.37)	8.72 (1.36)	11.71 (1.42)	8.30 (1.15)
* Mitochondria (N_v)	0.164 (0.030)	0.270 (0.041)	0.240 (0.031)	0.183 (0.031)
Other cytoplasm	41.30 (2.90)	39.19 (2.99)	43.14 (3.40)	40.41 (3.49)

* N_v calculated as in Table 2.

† Statistically significant at $\alpha = .05$ calculated by Dunnett's t-test. Compares each time treatment to control values.

ta to copper, there was a steady decrease in both mitochondrial and chloroplast V_v with time. Mitochondrial V_v reduction at 3 and 24 hours is a result of a substantial decrease in number per cell and is not offset by mitochondrial swelling (aver. volume, Tab. 2) that occurs at 24 hours. There also appears to be a short-term increase (at 2 and 3 hours) in storage products with copper treatment (Figs. 3-4).

With lead treatment, *Melosira* mitochondria N_v increased significantly. There was also variation in chloroplast V_v at 2 and 3 hours. Neither mitochondrial numbers nor their average volume change at 24 hours. Storage products appear to increase steadily, although the increase is not significant ($\alpha = .05$, Dunnett's t-test, STEEL and TORRIE 1960). Unlike copper treatments, there is a small increase in the category "autophagic"-like vacuoles, in lead treatments.

Fragilaria capucina

Detailed quantitative results of lead and copper treatments on *F. capucina* are presented in Tables 4 and 5. With copper treatment (Table 4) there were statistically significant reductions in chloroplast and cytoplasm volumes as well as statistically significant increases in vacuole volume, at 2 and 3 hours exposure. Other cellular compartments did not change significantly. "Autophagic"-like vacuoles also increase in all copper samples. There were no significant differences in any cellular component volumes nor any readily observable trends toward increase or decrease with lead treatment (Table 5) of *Melosira.*

Anacystis cyanea

Detailed morphometric results for *Anacystis cyanea*, a cyanobacterium, are presented in Tables 6 and 7. Copper treatment (Table 6) results in statistically significant differences ($\alpha = .05$) in the number of cyanophycin granules at 3 hours and poly-β-hydroxybutyric acid granules at 24 hours. β-granules and membranous inclusions also increase. Thylakoid surface area to volume ratio decreases steadily with time. The most noticeable difference resulting from lead treatment (Table 7) is the constant decrease in thylakoid surface area, with a decrease that is significantly larger than copper treatments at 24 hours.

4. Discussion

Previous reports in the literature have demonstrated that heavy or trace metals are often found as deposits in various cellular organelles such as mitochondria (SILVERBERG 1976, STUVE and GALLE 1970), chloroplasts (FUJITA *et al.* 1977), nuclei (SKAAR *et al.* 1973, CHOIE and RICHTER 1972, MOORE and GOYER 1974), and cell walls. Evidence of movement from the wall or plasma membrane area to the vacuole or cytoplasm is often reported (MURRAY and KIDBY 1975, GERRARD *et al.* 1974, BROWN and SMITH 1976, 1979, BEVERIDGE and MURRAY 1980). We found no evidence of such deposits in any organelles or cellular compartments in the three algae we examined in this study. However, photosynthetic membranes and mitochondria appeared to be most sensitive to metals, especially copper. While there appeared to be some chloroplast recovery in *Fragilaria*, mitochondrial numbers were still greatly reduced after 24 hours. In addition, there were changes in the vacuole relative volume in both diatoms that ranged from 6 to 64%. When chloroplast relative volumes were recalculated as a cytoplasmic percentage rather than a cellular percentage, there was an indication that the main effects observed in the diatoms were a result of membrane leakage, perhaps at the tonoplast, resulting in a greater water content and subsequent size increase of the vacuole. Since the cells we examined and measured showed no indication of active division or size change and since the frustule is rigid, additional water in the vacuole could result in apparent changes in the volume of the other organelles.

Class B ions (nitrogen and sulfur binding, as defined by NIEBOER and RICHARDSON 1980) have been shown to produce stress at the molecular level which results in an increase in membrane permeability that in some cases leads to loss of intracellular contents (DAVIES 1978, OVERNELL 1975, SHIEH and BARBER 1973, RICHARDSON *et al.* 1979, NIEBOER *et al.* 1979, KAMP-NIELSEN 1971). BAZZAZ and GOVINDJEE (1974) reported that lead induces conformational changes in chloroplast thylakoid membranes which result in an increase in absorbance at 540 nm. The absorbance change can indicate either chloroplast shrinkage or tighter thylakoid packing.

The ultrastructural responses of *Anacystis* to heavy metal incubations are consistent with physiological responses reported in the literature. The decrease in surface area of thylakoid membranes observed with both lead and copper treatments can be correlated with the reduction in photosynthesis reported as a result of exposure to these metals (STEEMAN NIELSEN and WIUM-ANDERSON 1971, GUPTA and ARORA 1978, THOMAS *et al.* 1977, BAZZAZ and GOVINDJEE 1974). Copper treatment also resulted in significant differences in the number of cyanophycin granules and PHB granules.

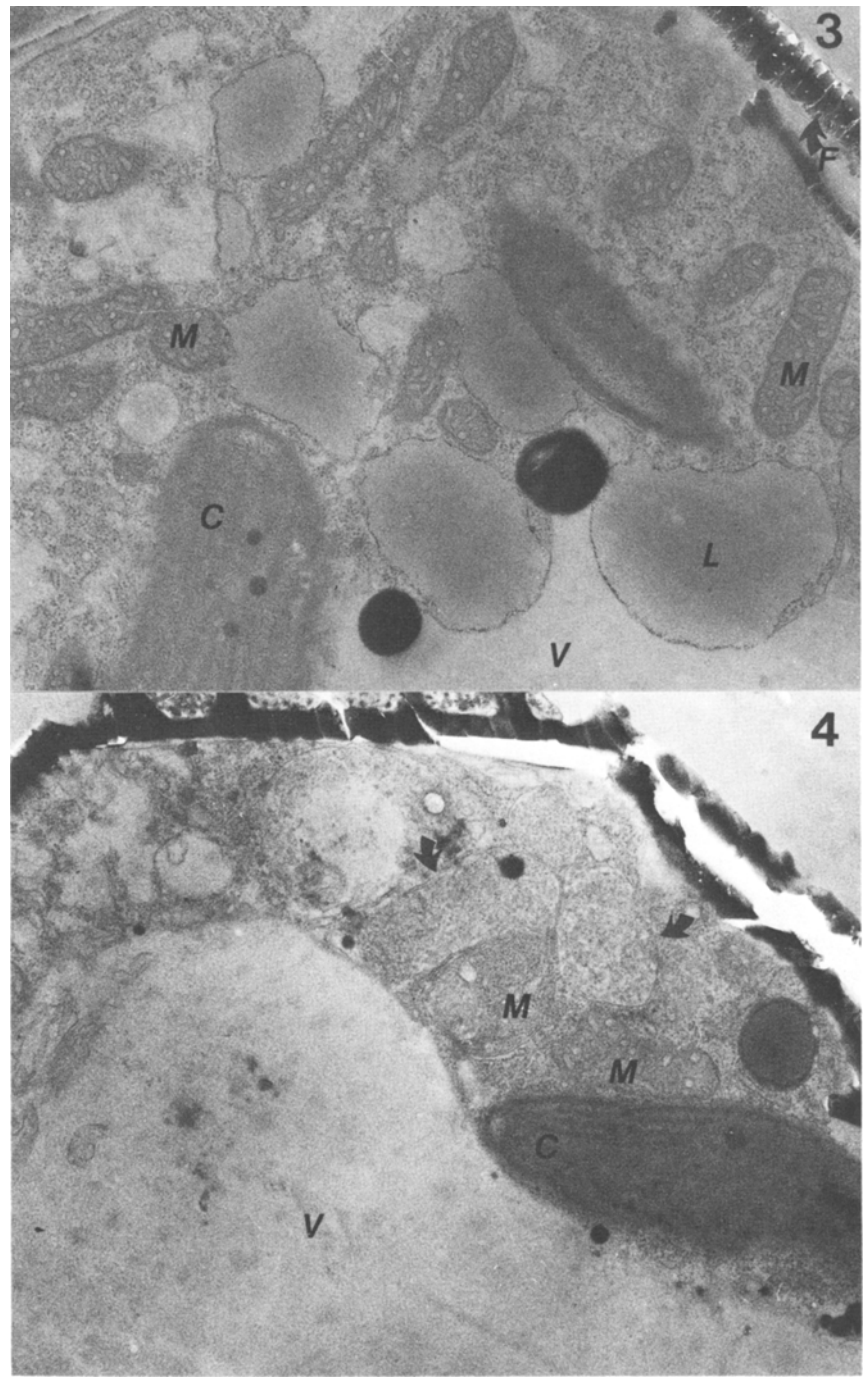

Fig. 3. *Melosira granulata*, time 0 sample. Note numerous mitochondria (*M*), chloroplast (*C*), vacuole (*V*), siliceous frustule (*F*) and lipid-like inclusions (*L*)

Fig. 4. *M. granulata*, 3 hours lead incubation. Note what appear to be "abnormal" mitochondria (arrows)

Table 4. *Fragilaria capucina*. Morphometric results of copper treatments. Results are relative volumes (V_v) and their standard errors unless otherwise designated

	Treatments			
	0 hour	2 hours	3 hours	24 hours
Cellular Volume Percents				
Chloroplast	20.73 (1.54)	12.92 (1.53)†	14.42 (1.51)†	20.01 (1.94)
Mitochondria	1.89 (0.22)	1.61 (0.17)	1.16 (0.13)	1.22 (0.22)
* Mitochondria (N_v)	0.319 (0.056)	0.306 (0.059)	0.276 (0.060)	0.129 (0.027)
Aver. volume	0.06 μm^3	0.05 μm^3	0.04 μm^3	0.09 μm^3
Number/cell	128	122	110	52
Storage	12.35 (2.34)	16.28 (2.49)	15.11 (2.63)	14.07 (2.64)
Nucleus	4.67 (0.87)	3.93 (0.91)	3.15 (0.67)	3.34 (0.68)
Other cytoplasm	14.19 (0.92)	8.93 (0.91)†	9.66 (0.83)†	9.04 (0.68)†
Vacuole	22.00 (1.62)	35.84 (2.41)†	35.95 (2.75)†	29.65 (2.15)
Frustule	24.20 (1.76)	20.11 (0.57)	20.26 (0.68)	21.74 (0.80)
Auto. vacuole	0.00 (−)	0.36 (0.47)	0.27 (0.35)	0.64 (0.52)
Poly P	0.02 (0.04)	0.00 (−)	0.00 (−)	0.00 (−)
Cytoplasmic Volume Percents				
Chloroplast	56.35 (2.23)	55.09 (3.59)	57.13 (3.98)	66.10 (4.14)
Mitochondria	5.14 (0.66)	6.84 (1.17)	4.58 (0.72)	4.03 (1.72)
* Mitochondria (N_v)	0.867 (0.184)	1.305 (0.297)	1.095 (0.233)	0.427 (0.104)
Other cytoplasm	38.58 (1.98)	38.07 (3.15)	38.29 (3.66)	29.87 (3.93)

* N_v calculated as in Table 2.

† Statistically significant at $\alpha = .05$ calculated by Dunnett's t-test. Compares each time treatment to control values.

Table 5. *Fragilaria capucina*. Morphometric results of lead treatments

	Treatments			
	0 hour	2 hours	3 hours	24 hours
Cellular Volume Percents				
Chloroplast	20.73 (1.54)	17.98 (1.71)	22.78 (1.38)	16.73 (1.56)
Mitochondria	1.89 (0.22)	2.07 (0.22)	1.63 (0.20)	2.16 (0.27)
* Mitochondria (N_v)	0.319 (0.056)	0.541 (0.079)	0.414 (0.050)	0.269 (0.039)
Aver. volume	0.06 μm^3	0.04 μm^3	0.04 μm^3	0.08 μm^3
Number/cell	128	216†	166	108
Storage	12.35 (2.34)	15.14 (2.69)	10.15 (2.19)	14.97 (2.27)
Nucleus	4.67 (0.87)	4.88 (0.97)	6.12 (0.99)	3.14 (0.81)
Other cytoplasm	14.19 (0.92)	11.03 (0.93)	11.49 (0.73)	12.00 (0.88)
Vacuole	22.00 (1.62)	26.91 (2.21)	26.00 (1.91)	28.90 (2.32)
Frustule	24.20 (1.76)	21.96 (0.63)	21.85 (0.55)	21.93 (0.95)
Auto. vacuole	0.00 (−)	0.06 (0.05)	0.00 (−)	0.15 (0.18)
Poly P	0.02 (0.04)	0.00 (−)	0.00 (−)	0.00 (−)
Cytoplasmic Volume Percents				
Chloroplast	56.35 (2.23)	57.92 (3.51)	63.45 (2.42)	54.12 (2.75)
Mitochondria	5.14 (0.66)	6.67 (1.10)	4.55 (0.68)	6.99 (1.17)
* Mitochondria (N_v)	0.867 (0.184)	1.742 (0.307)	1.154 (0.334)	0.870 (0.187)
Other cytoplasm	38.58 (1.98)	35.52 (3.12)	32.00 (2.09)	38.82 (2.44)

* N_v calculated as in Table 2.

† Significant at $\alpha = .05$ (see Table 4).

Table 6. *Anacystis cyanea.* Morphometric results of copper treatments. Results are relative volumes (V_v) unless otherwise designated

	Treatments			
	0 hour	2 hours	3 hours	24 hours
Cell wall	10.03 (0.87)	7.82 (0.34)	8.19 (0.40)	10.26 (0.68)
Other cytoplasm	82.27 (1.15)	83.71 (1.17)	86.00 (0.62) †	79.79 (0.84)
Polyhedral bodies	1.15 (0.23)	1.17 (0.23)	1.27 (0.20)	0.75 (0.16)
* Polyhedral (N_v)	0.332 (0.076)	0.550 (0.093)	0.580 (0.090)	0.410 (0.075)
Aver. volume	0.03 μm^3	0.02 μm^3	0.02 μm^3	0.02 μm^3
Number/cell	26	43	45	32
Cyanophycin granules	4.35 (0.60)	4.19 (0.80)	2.04 (0.29) †	4.72 (0.44)
* Cyanophycin (N_v)	1.125 (0.131)	0.743 (0.104)	0.675 (0.114)	1.181 (0.106)
Aver. volume	0.04 μm^3	0.06 μm^3	0.03 μm^3	0.04 μm^3
Number/cell	88	58	53	92
PHB granules	1.32 (0.32)	1.23 (0.29)	1.25 (0.25)	2.81 (0.52) †
β-granules	0.87 (0.19)	1.62 (0.20) †	1.14 (0.17)	1.31 (0.17)
Membranous organelles	0.00 (−)	0.24 (0.10)	0.11 (0.07)	0.35 (0.11)
Polyphosphate	0.00 (−)	0.00 (−)	0.00 (−)	0.00 (−)
Surface area/volume thylakoids ($\mu m^2/\mu m^3$)	5.90 (0.287)	5.48 (0.167)	5.40 (0.199)	5.16 (0.281)

* $K = 1.07$; $\beta = 1.38$ (sphere).
† Significant at $\alpha = .05$ (see Table 4).

Table 7. *Anacystis cyanea.* Morphometric results of lead treatments. Results are relative volumes (V_v) unless otherwise designated

	Treatments			
	0 hour	2 hours	3 hours	24 hours
Cell wall	10.03 (0.87)	8.99 (1.05)	8.37 (0.63)	8.76 (0.74)
Other cytoplasm	82.27 (1.15)	82.90 (1.17)	83.94 (0.89)	82.97 (0.68)
Polyhedral bodies	1.15 (0.23)	0.89 (0.16)	1.02 (0.25)	1.51 (0.21)
* Polyhedral (N_v)	0.332 (0.076)	0.446 (0.097)	0.410 (0.067)	0.364 (0.061)
Aver. volume	0.03 μm^3	0.02 μm^3	0.02 μm^3	0.04 μm^3
Number/cell	26	35	32	28
Cyanophycin granules	4.35 (0.60)	4.64 (0.69)	4.27 (0.55)	4.66 (0.50)
* Cyanophycin (N_v)	1.125 (0.131)	1.330 (0.179)	1.168 (0.138)	0.988 (0.097)
Aver. volume	0.04 μm^3	0.04 μm^3	0.04 μm^3	0.05 μm^3
Number/cell	88	104	91	77
PHB granules	1.32 (0.32)	1.22 (0.32)	0.90 (0.19)	0.98 (0.21)
β-granules	0.87 (0.19)	1.24 (0.18)	1.50 (0.18)	1.06 (0.17)
Membranous organelles	0.00 (−)	0.15 (0.06)	0.00 (−)	0.00 (−)
Polyphosphate	0.00 (−)	0.00 (−)	0.00 (−)	0.05 (0.04)
Surface area/volume thylakoids ($\mu m^2/\mu m^3$)	5.90 (0.287)	5.59 (0.235)	5.47 (0.187)	4.44 (0.232) †

* $K = 1.07$; $\beta = 1.38$ (sphere).
† Statistically significant at $\alpha = .05$ (see Table 4).

Table 8. *Comparison of Fragilaria volume categories and collection site information from 1977 and 1980 data.* The 1980 data have been regrouped into categories which are comparable to the 1977 data (see SICKO-GOAD *et al.* 1977 for a complete category description)

	1977 set	1980 set
Date of collection	April 1975	August 1977
Time of day	Afternoon	Morning
Site	Northeast Saginaw Bay	Southeast Saginaw Bay
Total chloroplast (%)	18.9 ± 1.53	20.72 ± 1.54
Total vacuole (%)	35.9 ± 1.91	34.36 ± 2.49
Frustule (%)	20.3 ± 0.69	24.18 ± 1.69
Total cytoplasm (%)	24.9 ± 1.27	20.74 ± 1.43
Carbon-containing cytoplasm (%)	56.8 ± 1.86	53.82 ± 2.04
"Metabolizing biovolume"	43.5 ± 1.98	41.46 ± 2.64

Cyanophycin (structured) granules are copolymers of aspartic acid and arginine with the amino acids present in a 1:1 molar ratio and are believed to be cellular N reserves (SIMON 1971). STEWART *et al.* (1978) believe that cellular inclusions such as cyanophycin granules, polyphosphate bodies, polyhedral bodies (carboxysomes), and lipid droplets may be of critical importance in sustaining blue-green algae in a variety of habitats. These cellular reserve materials are usually stored during unfavorable growth conditions such as nutrient depletion and may be responsible for sustained growth or survival when the depletion becomes critical in the environment.

The results presented in this study demonstrate that some naturally occurring algal species are sensitive to low dose, short-term exposure to heavy metals. In general, all species were more sensitive to copper than to lead. Of the three species examined, *Melosira* was least affected by either metal.

The three algae examined were part of a phytoplankton assemblage collected from Saginaw Bay of Lake Huron, one of the more eutrophic areas of the Laurentian Great Lakes. All three species are widely distributed in eutrophic waters. However, *Melosira granulata* and *Anacystis cyanea* are found with greater frequency in more heavily impacted areas (STOERMER 1978). These species had the least number of significant changes in organelle volumes during metal exposure. This may explain why these species survive in impacted areas and substantiate the hypothesis that certain algae predominate over others when pollutants are introduced into the sea (THOMAS and SEIBERT 1977). Lead and copper concentrations utilized in this experiment were approximately equal to the maximum spring concentration of these metals in Saginaw Bay (IJC 1977). Although the algae are periodically exposed to these metals in the same concentration range in their natural environment, they responded cytologically within hours to laboratory additions.

There is some evidence in our data set, alluded to earlier, which suggests that all three species may be accommodating although to different degrees, to the additional metal exposure. Most statistically significant differences in the volume categories were observed after 2 or 3 hours of incubation. One of the most obvious trends in our data is the short-term reduction of chloroplast volume of *F. capucina* with copper treatment (Table 4). This reduction is also accompanied by an increase in vacuole volume with subsequent return to near time 0 values at 24 hours. SANDMANN and BÖGER (1980) reported physiological evidence of copper adaptation in *Scenedesmus acutus* within 24 hours of exposure to the metal. They found that copper toxicity was transient and that chlorophyll was broken down and membrane lipids were decomposed by peroxidation during the transient period. After 24 hours, lipid peroxidation decreased and normal growth resumed. They found no evidence of copper excretion and suggested that the cells produced copper binding proteins during the metal sensitive period.

Our data also show two other interesting features. First, copper treatment of the algae in this study produced more short-term cellular effects than in our previous study (SICKO-GOAD and STOERMER 1979) where metals were introduced during phosphate uptake. This provides additional evidence that phosphate nutrient status is important during studies of metal toxicity. Second, the *Fragilaria* time 0 data presented in this paper are remarkably consistent with our 1977 data set (SICKO-GOAD *et al.* 1977) for this organism (Table 8). There was more variation in organelle volumes as a result of experimental treatments than

resulted from sampling two completely different populations. The large variation in frustule volume in the present data can be explained by the unusually large number of grazing sections counted. This large variation was not found in the experimental treatments where the standard errors were considerably lower (*cf.* Tables 4–5). This strengthens our belief that electron microscope morphometric techniques can be used to quantitatively assess the impact of perturbations on cytological characteristics of individual species as they occur in their natural environment. Such perturbation studies on phytoplankton assemblages previously have been limited to the assessment of changes in species composition and number.

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