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Acute and Chronic Exposure of *Dunaliella salina* and *Chlamydomonas bullosa* to Copper and Cadmium: Effects on Ultrastructure

I. Visviki, J. W. Rachlin

Department of Biological Sciences, Lehman College of CUNY, 250 Bedford Park Boulevard West, Bronx, New York 10468, USA

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Abstract. The ultrastructural changes of *Dunaliella salina* and *Chlamydomonas bullosa* taking place after short term exposure to established copper and/or cadmium EC(50) or exposure to low levels of these cations for 8 months were examined. Cadmium had a greater impact on the ultrastructure of these species than copper. Metal stress affected a variety of cellular parameters including total cell volume, pyrenoid, nucleus, starch granules, polyphosphate bodies, lipids, vacuoles, cell wall, and periplasmalemmal space. The measurements of these cellular parameters used the technique of electron microscopic stereoscopic morphometry. Differences resulting from acute vs. chronic exposure to the cations are documented.

The examination of pollutant effects on various levels of biological organization is essential for the identification of the most sensitive parameters, and the development of bioassays that will safeguard natural environments. Our research shows that algal growth is more sensitive to heavy metal stress than ultrastructural changes (Visviki and Rachlin 1992). Nevertheless, study of the latter is important because it enhances our understanding of the pathways of metal toxicity, and of algal defenses to cation stress.

The present study investigates the changes in cellular morphology of *Chlamydomonas bullosa* and *Dunaliella salina* following short-term exposure to established EC(50) concentrations of copper, cadmium, and a copper-cadmium mixture (Visviki and Rachlin 1993). In addition, it determines the effects of chronic (8-month) algal exposure to low level concentrations of these metals, which approach the ambient values reported for the waters of the Hudson Estuary and Long Island Sound on the east coast of the United States (Breteler *et al.* 1984).

Materials and Methods

Algal specimens, following exposure to copper and cadmium under conditions described by Visviki and Rachlin (1992) were harvested via centrifugation, fixed in 3.5% gluteraldehyde for 1 h at room temperature, and postfixed in 1% osmium tetroxide for 1 h at room temperature. Subsequently, they were dehydrated in 50%, 70%, 95%, and 100% ethanol series and were embedded in Spurr's low viscosity medium under vacuum following standard procedures. The fixation technique used was a modification of that proposed by Haas and Saghy (reviewed by Hayat 1972). The samples were polymerized overnight in a 65°C oven. Blocks were sectioned using an LKB (type 8801) ultramicrotome with a diamond knife. Ultrathin (90 mμ) sections were picked up on 400-mesh copper grids and post stained with uranyl acetate for 10 min at room temperature. The sections were rinsed in absolute methanol, 100%, 80%, and 50% ethanol, and finally distilled water. In addition, they were post-stained in lead citrate for 30 s and were rinsed with distilled water. The post-staining methods used were modifications of the method proposed by Stampak and Ward (1964) and Reynolds (1963) for uranyl acetate and lead citrate respectively.

The sections were examined by transmission electron microscopy using an Hitachi HS-9. Cells were examined at a magnification of 5000×. A minimum of 30 photographs, each representing a random section through a different cell, were obtained for every treatment. Sections containing only cell wall or cell wall with no identifiable features were excluded. Estimates of volume densities of various cellular components were obtained by grid point-counting (Figure 1). Using the procedures of morphometric analysis (Sicko-Goad and Stoermer 1979; Rachlin *et al.* 1982, 1985) the cells were analyzed for relative volume of nucleus, nucleolus, pyrenoid and chloroplast, the number and relative volume of Golgi, lipids, polyphosphate bodies and vacuoles, and mitochondria. For *Chlamydomonas bullosa* the relative volume of the cell wall, the relative volume of the periplasmalemmal space under the cell wall, the number and relative volume of membranous organelles, and intranuclear vacuoles were also calculated. Additionally, the relative numbers (no./cell volume) of lipids, polyphosphate bodies, vacuoles, membranous organelles, and intranuclear vacuoles were estimated, to ensure that changes in the numbers of these cellular components were genuine, and not due to increases or decreases in cell volume. The mean and standard error were obtained

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Correspondence to: J. W. Rachlin

Fig. 1. Electron micrograph of a *Chlamydomonas bullosa* control cell (magnification 13,865) overlayed with a 1-cm counting grid (magnification 1.08)

Table 1. Summary of the morphometric data (mean ± S.E.) of *Dunaliella salina* control cells and cells exposed to acute sublethal copper or cadmium concentrations (μM)

	Control	5.94 Cu^{++}	4.55 Cd^{++}
Cell volume	73.06 ± 0.6	84.74 ± 4.37	124.27 ± 8.00***
Chloroplast r.v.	56.04 ± 4.51	55.00 ± 3.41	69.60 ± 15.00
Golgi no.	2.09 ± 0.17	1.75 ± 0.16	2.05 ± 0.24
Golgi r.v.	1.79 ± 0.23	1.70 ± 0.22	1.35 ± 0.19
Lipid no.	35.34 ± 3.13	32.14 ± 2.80	52.30 ± 4.66
Lipid r.v.	1.45 ± 0.31	1.40 ± 0.22	2.78 ± 0.42*!
Polyphosphate body no.	1.83 ± 0.48	1.25	1.20
Polyphosphate body r.v.	0	0	0
Vacuole no.	6.12 ± 0.60	5.53 ± 0.49	4.18 ± 0.70
Vacuole r.v.	1.94 ± 0.43	1.75 ± 0.22	1.96 ± 0.37*
Mitochondria r.v.[a]	—	—	—
Nucleus r.v.	16.47 ± 1.11	16.95 ± 1.42	12.46 ± 1.32
Nucleolus r.v.	1.66 ± 0.26	1.56	1.00 ± 0.29
Pyrenoid r.v.	6.23 ± 0.72	7.60 ± 0.72	10.60 ± 0.62
Starch r.v.	14.90 ± 1.71	13.70 ± 1.40	10.70 ± 1.22

[a] Mitochondrial relative volumes were omitted because some mitochondrial cross sections under both control and experimental conditions appeared to be swollen

Significance: *P < 0.05, *!P < 0.025, **P < 0.01, **!P < 0.005, ***P < 0.001

for each measurement and the statistical significance of differences was determined by analysis of variance (Visviki and Rachlin 1992).

Results

Comparison of control and metal treated cells of *Dunaliella salina* (Tables 1 and 2) clearly shows that acute exposure to cadmium has greater effects on cellular ultrastructure than copper exposure. Additionally, sublethal cadmium concentration affects a greater number of cellular components than low level concentration or combined sublethal metal concentrations. A concentration of 4.55 μM Cd^{++} caused a 70% increase in total cell volume (Table 1, Figures 2 and 3), and a 24.4% decrease in the relative volume of the nucleus. It did not affect the number of lipids, but caused a 91.7% increase in relative lipid volume.

Table 2. Summary of the morphometric data (mean ± S.E.) of *Dunaliella salina* cells exposed to acute sublethal copper plus cadmium concentrations (μM) and low levels of copper or cadmium for eight months[a]

	5.94 Cu^{++} + 4.55 Cd^{++}	4.9×10^{-4} Cu^{++} 8-month exposure	4.5×10^{-6} Cd^{++} 8-months exposure
Cell volume	69.45 ± 3.99	67.00 ± 3.78	67.87 ± 4.40
Chloroplast r.v.	47.60 ± 3.64	57.34 ± 5.48	53.80 ± 4.99
Golgi no.	1.89 ± 0.17	2.00 ± 0.23	2.00 ± 0.23
Golgi r.v.	1.60 ± 0.35	2.06 ± 0.32	1.36 ± 0.39
Lipid no.	15.19 ± 2.02***	60.44 ± 5.02***	45.50 ± 3.97*!
Lipid r.v.	0.46 ± 0.17*!	4.52 ± 0.55***	3.53 ± 0.43***
Polyphosphate body no.	3.33	—	—
Polyphosphate body r.v.	—	—	—
Vacuole no.	4.76 ± 0.32	—	—
Vacuole r.v.	2.03 ± 0.26	—	—
Mitochondria r.v.[b]	—	—	—
Nucleus r.v.	16.70 ± 1.11	15.19 ± 1.00	16.70 ± 1.11
Nucleolus r.v.	1.19 ± 0.24	—	1.00 ± 0.32
Pyrenoid r.v.	5.60 ± 0.78	5.50 ± 0.56	6.40 ± 0.77
Starch r.v.	16.69 ± 1.87	16.49 ± 1.83	16.95 ± 2.75

[a] Control values are the same as in Table 1

[b] Mitochondrial relative volumes were omitted because some mitochondrial cross sections under both control and experimental conditions appeared to be swollen

Significance: *P < 0.05, *!P < 0.025, **P < 0.01, **!P < 0.005, ***P < 0.001

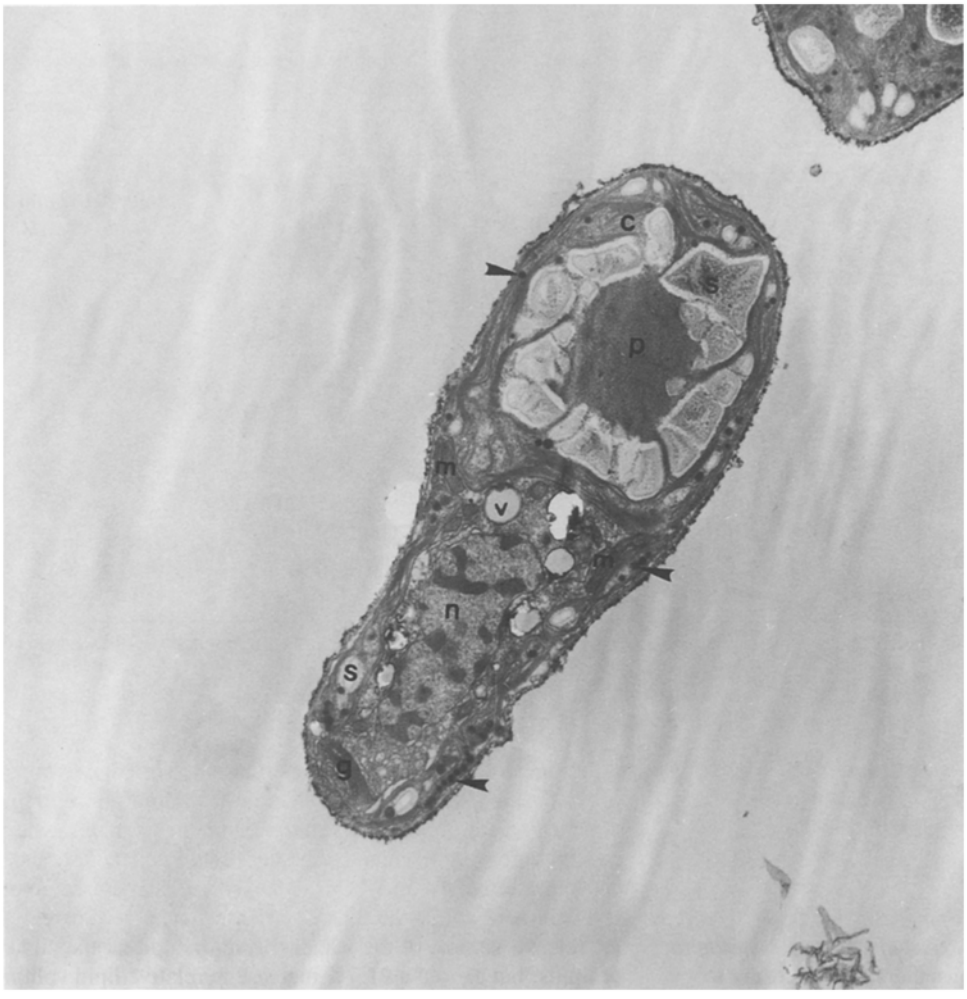

Fig. 2. Electron micrograph of a *Dunaliella salina* control cell (magnification 9,200). c = chloroplast, g = Golgi, m = mitochondrion, n = nucleus, p = pyrenoid, s = starch, v = vacuole. Small arrowheads indicate the position of lipids

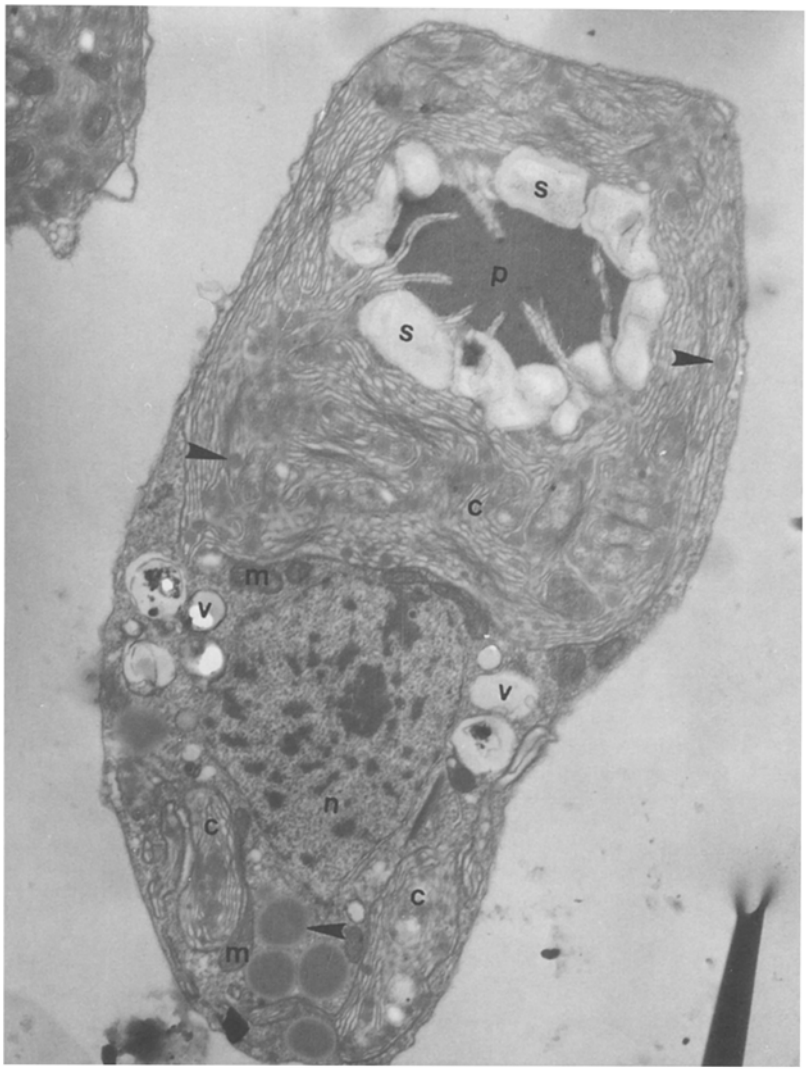

Fig. 3. Electron micrograph of a *Dunaliella salina* cell (magnification 9,200) exposed to 4.55 μM cadmium for 96-h. c = chloroplast, g = Golgi, m = mitochondrion, n = nucleus, p = pyrenoid, s = starch, v = vacuole. Small arrowheads indicate the position of lipids

Simultaneous exposure to copper and cadmium (Table 2) had a negative effect on both lipid number and relative lipid volume, leading to decreases of 57.1% and 68.3%, respectively. Chronic treatment with 4.9×10^{-4} μM copper, on the other hand, increased lipid numbers by 71% and relative lipid volume by 211.7%. Long term exposure to 4.5×10^{-6} μM Cd increased the lipid numbers by 28.8% and the relative lipid volume by 143%. ANOVA showed that these mean ultrastructural changes were significant.

The growth data of *D. salina* cultures exposed to 5.94 μM copper and 4.55 μM cadmium (Visviki and Rachlin 1993) are not consistent with our morphometric results. Copper and cadmium acted synergistically to limit the growth of this chlorophyte, but at the cellular level their action was either synergistic or antagonistic, depending on the parameter examined. Acute exposure to cadmium led to significant increase in total cell volume, decrease in relative nuclear volume, and increase in relative lipid volume. In joint exposure, copper appeared to counteract these Cd-induced effects. No changes in mean total cell volume or relative nuclear volume were apparent, but relative lipid volume decreased by approximately 160% when compared with Cd-challenged cells, apparently the result of synergistic copper and cadmium action.

Comparison of the morphometric data (Tables 3 and 4) indicate that 96-h exposure to sublethal copper and cadmium concentrations is more toxic to this chlorophyte than acute exposure to either copper or cadmium alone. Acute sublethal exposure to the former led to a 31% decrease in the number of polyphosphate bodies, but did not alter their relative volume. Cells exposed to 0.025 μM Cd^{++} had total volume 49.7% larger than controls. Their relative pyrenoid volume, however, was reduced by 50.2%. Lipid numbers increased under both low level cadmium and combined cadmium and copper treatments with no increase in lipid relative volume, indicating fragmentation rather than increase in lipid content. The cation combination of 0.78 μM Cu^{++} and 0.025 μM Cd^{++} caused a 72.6% increase in total cell volume. (Tables 3 and 4, Figures 4 and 5) and a 64.9% decrease in relative pyrenoid volume. At the same time this cation combination increased the relative starch volume 96.5%, which effectively reduced the active chloroplast volume (total chloroplast volume minus pyrenoid and starch volume) by 27% ($P < 0.005$). Exposure to 4.9×10^{-4} μM Cu^{++} for eight months led to a 24.2% increase in total cell volume and a 28.4% decrease in relative cell wall volume. The periplasmalemmal space (space between the cell wall and the cell membrane) was also reduced by 24.7%. Cells

Table 3. Summary of the morphometric data (mean ± S.E.) of *Chlamydomonas bullosa* control cells exposed to acute sublethal copper or cadmium concentrations (μM)

	Control	0.78 Cu^{++}	0.025 Cd^{++}
Cell volume (A)	109.16 ± 6.92	125.55 ± 7.13	163.45 ± 8.35***
Cell without cell wall (B) r.v.	85.80 ± 5.64	84.90 ± 5.10	82.00 ± 4.43
Periplasmalemmal space r.v.	14.15 ± 1.02	15.00 ± 1.12	16.60 ± 1.16
Cell wall r.v.	8.65 ± 0.42	10.42 ± 0.95	9.30 ± 0.43
Chloroplast r.v.	49.22 ± 4.33	47.90 ± 3.87	48.50 ± 3.16
Golgi no.	1.62 ± 0.14	1.58 ± 0.19	1.67 ± 0.13
Golgi r.v.	0.63 ± 0.16	0.60 ± 0.17	0.46 ± 0.08
Lipid no.	19.42 ± 2.03	12.25 ± 1.08	32.27 ± 3.08*
Lipid r.v.	0.45 ± 0.10	0.20 ± 0.06	0.44 ± 0.13
Polyphosphate body no.	22.45 ± 2.33	15.50 ± 1.74*	28.39 ± 3.02
Polyphosphate body r.v.	1.70 ± 0.23	2.10 ± 0.33	2.20 ± 0.28
Vacuole no.	7.92 ± 0.74	10.12 ± 0.80	13.82 ± 1.13
Vacuole r.v.	9.68 ± 0.01	11.00 ± 1.18	10.70 ± 0.84
Membranous organelle no.	1.75 ± 0.37	2.00 ± 0.25	1.87 ± 0.31
Membranous organelle r.v.	0.80 ± 0.20	0.79 ± 0.20	0.60 ± 0.16
Mitochondria r.v.	2.82 ± 0.40	2.83 ± 0.29	2.18 ± 0.22
Nucleus r.v.	9.96 ± 1.02	9.00 ± 0.95	9.10 ± 1.04
Nucleolus r.v.	1.56 ± 0.30	1.36 ± 0.20	1.40 ± 0.30
Pyrenoid r.v.	7.75 ± 1.06	4.98 ± 0.01	3.86 ± 0.70**!
Starch r.v.	4.60 ± 0.77	4.80 ± 0.91	4.10 ± 0.55

Significance: *P < 0.05, *!P < 0.025, **P < 0.01, **!P < 0.005, ***P < 0.001

Table 4. Summary of the morphometric data (mean ± S.E.) of *Chlamydomonas bullosa* control cells exposed to acute sublethal copper plus cadmium concentration (μM), and cells exposed to low levels of copper or cadmium for eight months[a]

	0.78 Cu^{++} + 0.025 Cd^{++}	4.9×10^{-4} Cu^{++} 8-month exposure	4.5×10^{-6} Cd^{++} 8-month exposure
Cell volume (A)	188.37 ± 13.49***	135.62 ± 5.65**!	163.80 ± 9.40***
Cell without cell wall (B) r.v.	84.40 ± 5.47	89.30 ± 3.88	88.00 ± 5.17
Periplasmalemmal space r.v.	15.60 ± 2.17	10.65 ± 0.83**	11.45 ± 1.26
Cell wall r.v.	9.65 ± 0.90	6.19 ± 0.43***	7.02 ± 0.57*!
Chloroplast r.v.	43.28 ± 3.23	46.90 ± 2.86	45.00 ± 3.00
Golgi no.	2.33 ± 0.23	1.50 ± 0.14	1.60 ± 0.13
Golgi r.v.	0.50 ± 0.10	0.56 ± 0.08	0.60 ± 0.09
Lipid no.	31.13 ± 3.50*	28.26 ± 4.41	22.07 ± 3.44
Lipid r.v.	0.46 ± 0.10	0.17 ± 0.07	0.12 ± 0.06
Polyphosphate body no.	20.14 ± 3.41	23.59 ± 2.13	24.79 ± 3.27
Polyphosphate body r.v.	1.40 ± 0.19	2.40 ± 0.42	3.30 ± 0.39
Vacuole no.	17.17 ± 1.57*	10.50 ± 0.49	10.60 ± 0.84
Vacuole r.v.	13.35 ± 1.33	12.28 ± 0.70	17.69 ± 1.50***
Membranous organelle no.	2.42 ± 0.22	1.79 ± 0.17	1.69 ± 0.26
Membranous organelle r.v.	0.47 ± 0.12	0.50 ± 0.17	0.50 ± 0.17
Mitochondria r.v.	2.50 ± 0.30	2.20 ± 0.21	2.20 ± 0.22
Nucleus r.v.	7.56 ± 0.79	9.30 ± 0.95	9.90 ± 0.77
Nucleolus r.v.	1.10 ± 0.23	1.40 ± 0.14	1.70 ± 0.10
Pyrenoid r.v.	2.72 ± 0.40***	5.40 ± 0.54	5.00 ± 0.71
Starch r.v.	13.64 ± 1.44***	4.40 ± 0.74	4.60 ± 0.97

[a]Control values are the same as in Table 3
Significance: *P < 0.05, *!P < 0.025, **P < 0.01, **!P < 0.005, ***P < 0.001

chronically exposed to low levels of cadmium had total volumes 51.9% larger than controls. It is obvious from Tables 3 and 4 that their mean total cell volume is very similar to that of cells challenged with 0.025 μM Cd^{++} for 96 h, thus indicating that acute sublethal exposure and chronic low level exposure had the same effect on the cell volume of this chlorophyte. Combined cation exposure increased the number of cellular vacuoles, and chronic exposure to cadmium alone led to an increase in the relative volume of these vacuoles. Analysis of variance showed that all mean ultrastructural changes discussed above were significant.

An additional difference between control and experimental cells was the presence of membranous organelles (Figure 6). Even though the relative number and relative volume of these structures (Tables 3 and 4) was not significantly different in challenged and control cells, the frequency of cells carrying these organelles increased with metal exposure; 21.1% of control cells had membranous organelles compared to 45.5% and

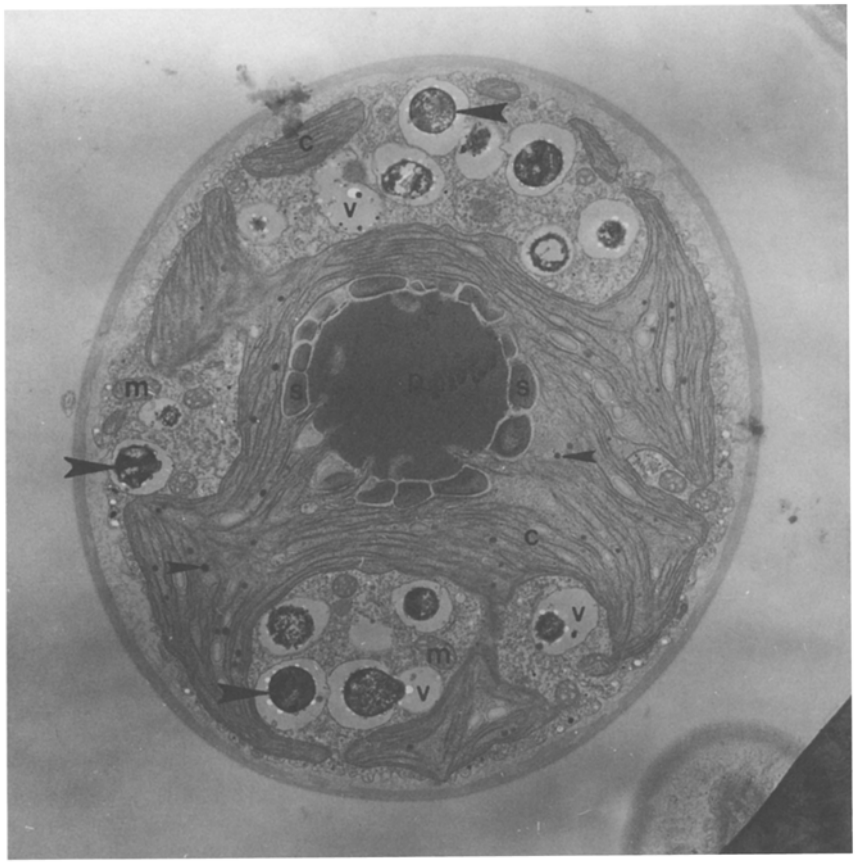

Fig. 4. Electron micrograph of a *Chlamydomonas bullosa* control cell (magnification 9,200). c = chloroplast, m = mitochondrion, p = pyrenoid, s = starch, v = vacuole. Small arrowheads indicate the position of lipids. Large arrowheads indicate the position of polyphosphate bodies

43.3% of cells exposed to 0.025 μM Cd^{++} and 4.5×10^{-6} μM Cd^{++}, respectively. Sixty-three percent of cells challenged with both cations, 55.9% of cells chronically exposed to 4.9×10^{-4} μM Cu^{++}, and 60.6% of cells exposed to 0.78 μM Cu^{++} had these organelles. Also, 39.4% of *C. bullosa* cells exposed to the latter treatment had intranuclear vacuoles compared to 5.3% of controls. There was no significant difference in the relative number or relative volume of intranuclear vacuoles of control or Cu-treated cells.

Overall *C. bullosa* was the most sensitive of the two species examined in this study. All experimental treatments, but one (0.78 μM Cu^{++}), caused a significant increase in total cell volume. We suggest that this is due to metal inhibition of normal cell division rather than to changes in either cell wall or cell membrane permeability. We are led to this conclusion because the periplasmalemmal space was not significantly different in treated cells (except after long term Cu treatment) when compared to controls.

Comparison of morphometric data with toxicological results indicate no simple relation between the effects of copper and cadmium on growth (Visviki and Rachlin 1993), and their effects on algal ultrastructure. These cations acted synergistically to limit the growth of *C. bullosa,* but their relationship at the cellular level varied depending on the cellular component under consideration. Exposure to 0.78 μM Cu^{++} caused significant reduction in the relative number of polyphosphate bodies, whereas dimetal exposure had no effect. This shows that the action of the two cations was antagonistic towards this parameter. Exposure to 0.025 μM Cd^{++} gave rise to cells with larger volumes than controls. Exposure to both metals enhanced that trend, indicating that copper and cadmium acted either additively or synergistically to inhibit normal cell division.

Copper- or Cd-treated cultures subsequently challenged with Cu EC(50) or Cd EC(50), respectively, demonstrated greater sensitivity to these cations (Visviki and Rachlin 1993). Morphometric analysis revealed that chronically exposed cells showed significantly smaller relative cell wall volume than controls. Their increased sensitivity, thus, could be, at least in part, attributed to this change, since a thinner cell wall might be a less effective barrier to heavy metal uptake.

Discussion

Comparison of the morphometric data obtained after short-term, sublethal, monometal exposures shows that cadmium has far greater impact on the ultrastructure of the two species examined than does copper. This agrees with the growth data of the chlorophytes (Visviki and Rachlin 1993). The cellular targets of these cations include: chloroplast, pyrenoid, lipids, nucleus, polyphosphate bodies, vacuoles, and cell wall.

Chlamydomonas bullosa cells exposed to sublethal concentrations of copper and cadmium showed no changes in overall chloroplast volume, but there was a decrease in their active chloroplast volume. This reduction is an indirect effect, brought about by an inhibition of normal cell division, and the subsequent accumulation of starch granules; thus, the cell's ability to photosynthesize is compromised.

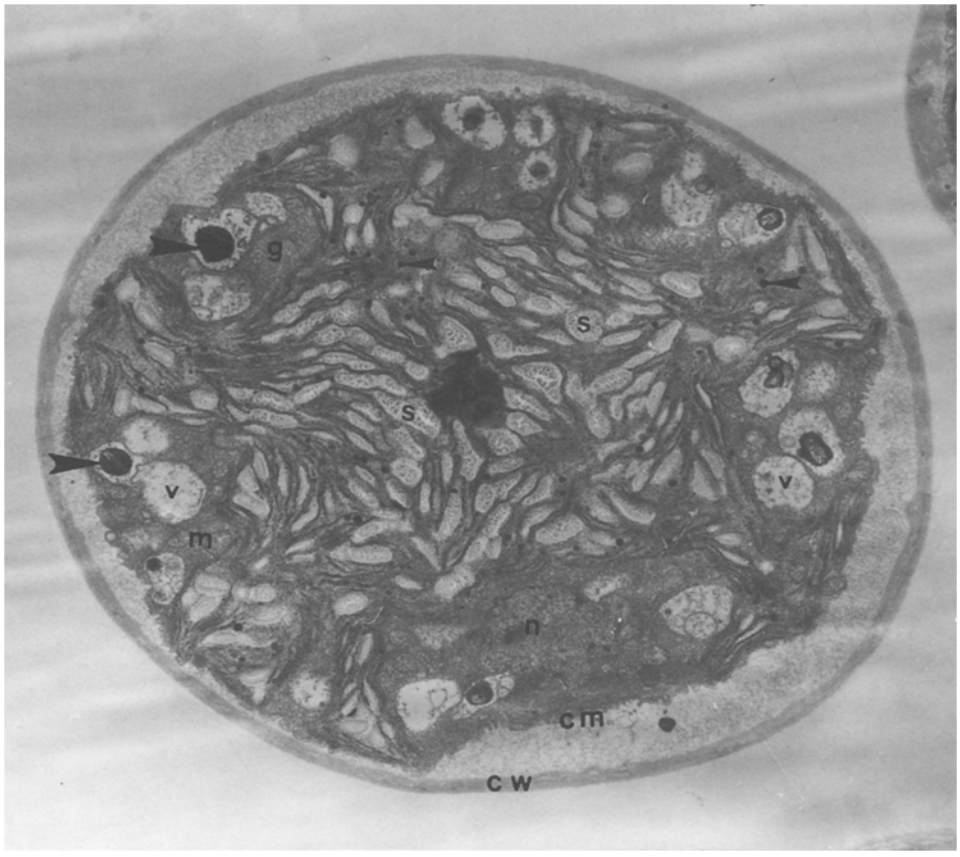

Fig. 5. Electron micrograph of a *Chlamydomonas bullosa* cell (magnification 9,200) exposed to 0.78 μM copper and 0.025 μM cadmium for 96-h. cm = cell membrane, cw = cell wall, g = Golgi, m = mitochondrion, n = nucleus, p = pyrenoid, s = starch, v = vacuole. Small arrowheads indicate the position of lipids. Large arrowheads indicate the position of polyphosphate bodies

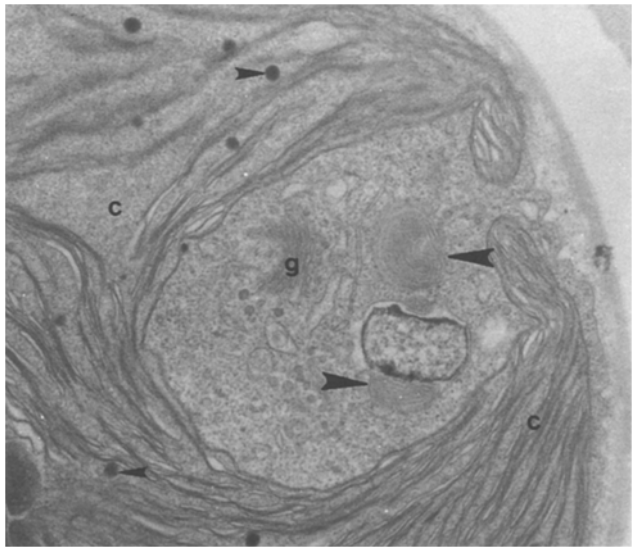

Fig. 6. Electron micrograph of a *Chlamydomonas bullosa* control cell (magnification 18,113) with membranous organelles. c = chloroplast, g = Golgi. Small arrowheads indicate position of lipids. Large arrowheads indicate position of membranous organelles

The pyrenoid was affected by two cation treatments. Its volume was decreased in *C. bullosa* cells exposed to sublethal concentration of cadmium and to sublethal copper-cadmium combination. Reduction in pyrenoid volume was observed in *Dunaliella minuta* cells chronically treated with low copper concentration (Visviki and Rachlin 1993). Reduction in the number of pyrenoids was reported by Chan and Wong (1987) for *Chaetomorpha brachygona* subjected to high multimetal concentrations. Since the pyrenoid is the site of the production of starch synthetase, which polymerizes glucose molecules into starch (Bold and Wynne 1978), reduction in size of this organelle might signal the inability of the cell to produce energy storage molecules.

Increases in the relative lipid volumes of *Dunaliella salina* exposed to acute sublethal concentration of cadmium were accompanied by increase in cell volume. This argues for cation induced inhibition of normal cell division, which effectively uncouples growth rates from photosynthetic rates. *D. salina* cultures chronically exposed to low levels of either copper or cadmium also exhibited increases in lipid volume, but not cell volume. Carotenogenesis in *D. salina* is known to increase under conditions of stress such as: high salinity, high light intensity, high temperature, and nutrient limitation (Loeblich 1982; Ben-Amotz and Avron 1983). Relative to the above tolerances of this organism, the salinity, temperature, and light intensity employed in this study were by no means extreme. It is therefore suggested that the observed lipid accumulation might be due to nutrient limitation induced by copper and cadmium. Evidence supporting this suggestion is provided by Overnell (1975), who showed that short-term low-level Cu exposure limited the potassium uptake of *D. tertiolecta* cultures. In contrast to these results, dimetalic exposure to copper and cadmium reduced the lipid volume of *D. salina* cells by 68%. Similar results have been reported for *Anabaena flos-aquae* exposed to high cadmium doses (Rachlin *et al.* 1984; Rai

et al. 1990). The toxicity mechanism for lipid reduction is not known, but Rai *et al.* (1990) have suggested that extensive Cd binding on lipid bodies could lead to their dissolution.

Polyphosphate bodies serve as cellular phosphate storage sites that can be degraded when phosphate availability is limited. Decreases in their numbers following exposure to either copper or cadmium have been shown for *Diatoma tenue* (Sicko-Goad and Stoermer 1979), and *Anabaena flos-aquae* (Rachlin *et al.* 1984; Rai *et al.* 1990). The reduction in the number of polyphosphate bodies observed in *C. bullosa* cells, following sublethal 96-h exposure to copper without any reduction in polyphosphate volume, indicates that these structures coalesced, and, thus, the copper probably did not interfere with the phosphate uptake mechanism.

The cell nucleus is not immune to the toxic action of cations. McLean and Williamson (1977) demonstrated that cadmium could be incorporated into the nucleus, and could thus alter the DNA content of the affected cell. Cadmium resistant cells were shown by Bonaly *et al.* (1980) to have the same DNA content as control cells. In our study, cadmium reduced the relative nuclear volume of *D. salina* cells by 24% of controls. These results are consistent with those reported above, and for lymphocytes exposed to zinc (Berger and Skinner 1974), and can be attributed to cadmium induced inhibition of DNA replication.

Cytoplasmic vacuolization reported by Smith (1983) for *Skeletonema costatum* following sublethal cation exposure was attributed to osmotic disorganization due to membrane damage. Our *C. bullosa* cells when chronically exposed to low levels of cadmium exhibited a 72% increase in the relative vacuolar volume. The osmotic disorganization mechanism of Smith (1983) does not seem to apply to our *C. bullosa* cells, for although total cell volume of Cd-treated cells increased, and their cell wall volume decreased, the periplasmalemmal space did not change. This indicates the absence of increased osmotic pressure within the cell.

Chronic exposure of *C. bullosa* cells to very low levels of copper or cadmium resulted in decrease in the relative cell wall volume by 24% and 19% respectively. The cell wall of *C. bullosa* is thick and is normally composed of three not easily distinguished layers (Cann and Pennick 1982), which contain glycoproteins with hydroxyproline (Pickett-Heaps 1975). Gradual degradation of the peptidoglycan layer has been observed in Anabaena flos-aquae exposed to high cadmium levels (Rachlin *et al.* 1984; Rai *et al.* 1990). Reduction in the relative volume of the cell wall by cation exposure results in cells that could be more susceptible to mechanical stress, and alter its resistance to cation exposure.

The development of membranous organelles in *C. bullosa* cells following exposure to cation stress is interpreted as a detoxification mechanism by binding cations to protein. Membranous organelles are usually found within vacuoles, and can be described as concentric masses of membranes. They have been observed in several algal species (Marano-LeBaron and Izard 1972; Sicko-Goad and Stoermer 1979; Hoshaw and Maluf 1981). Their role in normal cell function is not known. Sicko-Goad and Stoermer (1979) reported that their numbers increased in *Diatoma tenue* cells exposed to lead. Rachlin *et al.* (1982) detected the presence of membrane whorls, structures comparable to membranous organelles, in *Plectonema boryanum* exposed to Co, Zn, Hg, Cd, Ni, and Cu, and suggested that the lipids and proteins of these membranes may tie up excess quantities of intracellular cations. Indeed, membranous organelles possess a great surface area where metals can bind to sulfhydryl groups and thus reduce the number of cations free to interact with vulnerable internal sites. This mechanism of detoxification is applicable to the present study where the percentage of cells with membranous organelles increased under all cation exposure conditions. Additionally, an increase in the number of cells with intranuclear vacuoles was observed after short-term copper exposure. The membranes of these vacuoles appeared to be several layers thick, and it is suggested that they represent an early stage in membranous organelle formation.

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