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The Effect of Heavy Metals on the Cytoplasmic Fine Structure of *Skeletonema costatum* (Bacillariophyta)

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Summary

The marine diatom *Skeletonema costatum* (Greville) Cleve was grown in batch culture in the presence of mercury (6.5 μg l^{-1}), cadmium (50 μg l^{-1}) and zinc (265 μg l^{-1}). Alterations in cytoplasmic morphology were observed in cells treated with these sublethal concentrations of the metals. Swollen organelles, dilated membranes and vacuolated cytoplasm indicated plasma membrane damage and subsequent osmotic disorganization. Electron-dense inclusions in vesicles, multivesiculate bodies and cytoplasmic "tubules" apparently derived from the Golgi body are interpreted as mechanisms of sequestering the metals. Mercury and zinc had a marked effect on the fine structure whereas treatment with cadium resulted in little to no cytoplasmic damage.

Keywords: Cytoplasmic fine structure; Diatom; Heavy metals.

1. Introduction

Skeletonema costatum is an important coastal diatom in most parts of the world (GUILLARD and KILHAM 1977). It has been reported in large densities in enclosed, polluted areas such as the Mediterranean Sea (BERNARD 1967) and the Sørfjord (JENSEN, RYSTAD, and MELSOM 1974) where metal tolerant physiological races have been found. It is a relatively important food species for crustacea and mollusca (BARLOW and MONTEIRO 1979) and some zooplankton populations graze selectively on *S. costatum* when provided with a mixed phytoplankton assemblage (PAFFENHÖFER and KNOWLES 1978, WIMPENNY 1973). This centric diatom has been used extensively in physiological studies and the cytoplasmic fine structure has been recently described (SMITH 1981).

Mercury, cadmium and zinc are known to be toxic to marine organisms (MERLINI 1971) and occur in coastal and estuarine regions of the sea at concentrations which may affect biological processes (ABDULLAH and ROYLE 1974, BAKER 1977). The three metals belong to the same group in the periodic table and might therefore be expected to behave chemically in a similar manner. Cadmium has been reported to replace zinc in some biochemical reactions (VALLEE and ULMER 1972), but mercury often reacts irreversibly with membranes and the sulfhydryl groups of proteins and enzymes (PASSOW and ROTHSTEIN 1960, VALLEE and ULMER 1972).

There are very few reports on the morphology of metal-treated algal cells at the ultrastructural level. The formation of morphologically abnormal cells in the presence of heavy metals has been observed at the light microscope level in several species of phytoplankton. Cell deformities, increase in cell volume or osmotic disorganization due to injury to the semi-permeable cell membrane have been noted for *Scripsiella faeroense* (KAYSER 1976), *Asterionella formosa* (TOMPKINS and BLINN 1976), *Isochrysis galbana* (DAVIES 1974), and *Phaeodactylum tricornutum* (NUZZI 1972) grown in the presence of mercury; and for *Ditylum brightwellii* (BENTLEY-MOWAT and REID 1977), *Thalassiosira aestivalis* (THOMAS, HOLLIBAUGH, and SEIBERT 1980), *T.*

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pseudonana (ERICKSON 1972), and *T. weiissflogii* (BENTLEY-MOWAT and REID 1977) grown in the presence of copper and zinc. CANTERFORD (1980) investigated the effects of heavy metals on the morphology of *D. brightwellii* in detail and found that cells exposed to 300 μg 1^{-1} copper were enlarged, the protoplast was withdrawn from the cell wall and the chloroplasts were concentrated in a group rather than evenly distributed throughout the cell; in some cases pseudo-resting spore-like cells were formed. It was reported that these copper-treated cells recovered when transferred to a metal-free culture medium. Similar effects were demonstrated with zinc-, mercury-, silver-, thallium-, cobalt-, and nickel-treated *D. brightwellii* cells (CANTERFORD 1980).

At the ultrastructural level there are published reports on the effects of heavy metals on the fine structure of a small number of diatoms. FUJITA, IWASAKI, and TAKABATAKE (1977) investigated the intracellular distribution of mercury in *Synedra ulna* var. *danica*; although electron micrographs were used to identify the sonicated cell fraction, morphological effects of the mercury treatment were not reported. SICKO-GOAD and STOERMER (1979) found that lead treatment resulted in a decreased number of mitochondria and an increase in membranous organelles in *Diatoma tenue* var. *elongatum*. While lead was incorporated in experimentally-induced polyphosphate bodies, copper-treated cells contained fewer polyphosphate bodies and no other significant morphological changes in cellular organelles were observed. Frustule abnormalities in *Nitzschia liebethrutti* grown with mercury and tin were described by SABOSKI (1977). The abnormalities were thought to arise from enzyme disruption either at the silicon deposition site or at the nuclear level. Changes in frustule fine structure common to both mercury and tin included a reduction in the length to width ratios, fused pores and a reduction in the number of pores per frustule; only tin was detected on the surface of the frustule by X-ray microanalysis.

DANIEL and CHAMBERLAIN (1981) have observed that copper was localized in polyphosphate or "copper" bodies in the fouling diatom *Amphora veneta*. Spherical polyphosphate bodies were located within cell vacuoles and X-ray microanalysis revealed that these bodies contained high concentrations of phosphorus in association with calcium and in some cases with copper. The "copper" bodies were found to be associated with membranes and contained high concentrations of sulphur, copper and calcium. It was suggested that the polyphosphate and "copper" bodies maintained low concentrations of free copper within the cells thus reducing its toxic effects.

Thus at the fine structural level the effects of heavy metals have been visualized as various types of inclusion, for example electron dense granules, membrane-bound vesicles or deposits in cell walls, whereby the cell immobilizes the metal and removes it from sensitive metabolic sites. Mercury, zinc and lead have been observed to be localized in nuclei either as inclusions (CHOIE and RICHTER 1972, SKAAR, OPHUS, and GULLVÅG 1973) or evenly distributed throughout the nucleoplasm (DE FILIPPIS and PALLAGHY 1975). Inclusions containing lead have also been found in mitochondria (WALTON 1973) and mercury and cadmium have been reported immobilized in membrane-bound vesicles (JANSSEN and SCHOLZ 1979).

In cells which may not have a means of sequestering or resisting metals, injury was observed in the form of dilated membranes or swollen organelles due to direct damage to the membranes or disorganization of osmotic control, through the inhibition of membrane-associated enzymes. The disruption of lamellar structure has been observed in chloroplasts (BASZYŃSKI *et al.* 1980) and hypertrophied Golgi bodies have been reported in fish liver cells (FERRI 1980) after cadmium treatment. Swollen mitochondria and dilated endoplasmic reticulum have been described in the gill epithelial cells of a crustacean (BUBEL 1976) and in fish kidney cells (TRUMP, JONES, and SAHAPHONG 1975, KOYAMA, FUJITA, and ITAZAWA 1979) after mercury treatment.

The work reported here was undertaken in order to identify the fine structural morphological changes which take place in cells treated with sublethal concentrations of some heavy metals. The concentration of metal chosen was the one at which maximum metal uptake occurred without any significant effect on the growth of the culture. The importance of studying the uptake of heavy metals at sublethal concentrations is self-evident when the wide distribution and large population size of *S. costatum* is taken into account along with its contribution within the plankton as a food species. The observation of cytoplasmic fine structural changes attributable to the uptake of heavy metals provides further evidence in support of the effects of the metals on biochemical pathways and may link these effects to reported physiological observations.

2. Materials and Methods

2.1. Culture Conditions

An inoculum of *S. costatum* was obtained from the Marine Biological Association, Plymouth. Cells were cultured under continuous light (3-5 klux, Northlight) at 14.5 ± 0.5 °C, aerated and rotated on an inclined turntable. Natural seawater collected well offshore in the English Channel was filtered, sterilized and nutrients added at sufficient concentrations to allow growth to continue for at least one week. The seawater was routinely checked for metal contamination; seawater samples with levels of zinc greater than 10 μg Zn l^{-1} were rejected for use in experiments. The final concentrations of the added nutrients were: nitrate (as sodium nitrate) 100 μM, phosphate (as disodium hydrogen orthophosphate) 10 μM, vitamin B_{12} 0.74 nM and thiamine HCl 740 nM, iron (as ferric chloride) 1.8 μM, and manganese (as manganese sulphate) 0.18 μM.

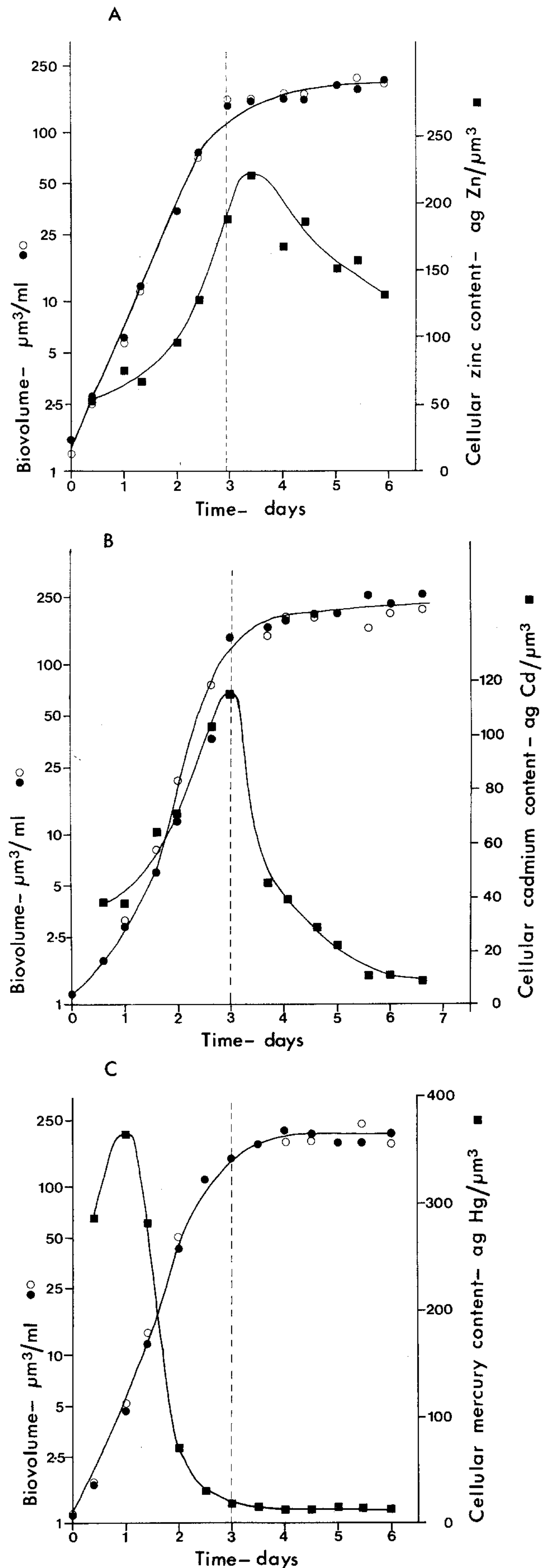
2.2. Growth Measurements

In order to determine the sublethal concentration of the three metals for *S. costatum*, cultures were grown at a range of metal concentrations (up to 45 μg Hg l^{-1}, 415 μg Cd l^{-1}, and 2,300 μg Zn l^{-1}). Cultures were sampled twice daily during the course of an experiment and the cells counted using a model T Coulter counter (100 μm diameter aperture) which measured the size spectrum of the particles. Using the mean channel volume and the number of particles counted per channel, the volume of cell material per ml of culture was calculated (SHELDON and PARSONS 1967). Because of the wide range of size differences possible during the growth of diatom cultures, growth was measured as increments in volume of cell material rather than as increase in cell numbers. The "biovolume" was calculated from the total of the products of Coulter counter channel volume and the number of particles per channel (minus a background particle value, corrected for sample size and dilution of the sample). Parameters such as specific growth rate and cellular metal concentration were compared at the same biovolume values, rather than at the same time intervals, so that populations could be compared at the same stage of physiological development. If the parameters are compared at the same time intervals the cultures are likely to be at different stages of growth in different treatments, and therefore nutrient consumption and hence the protein content (available binding sites) are likely to be different.

2.3. Metal-uptake Measurements

Metal uptake was measured using radioactive isotopes of the metals and a Nuclear Enterprises Sr 3 model ratemeter (well-type gamma scintillation counter). The final value for cellular metal content was expressed as attograms (10^{-18} g) of metal per cubic micrometer of cellular material. Metal stocks were added to the cultures aseptically at least twelve hours after subculturing. This allowed equilibration of the nutrient additions and reduced the possibility that the added metals might precipitate out of solution as co-precipitates with the nutrient additions, for example with iron.

The level of activity in the cells was monitored twice daily for the duration of an experiment. A 5 ml aliquot of culture was measured directly to give a value for total radioactivity in the sample. A second 5 ml aliquot of culture was filtered through two 0.45 μm pore diameter membrane filters, placed one on top of the other. The difference in activity between the two filters gave a value for the amount of radioactivity associated with the cellular material. The



radioactivity data after being corrected for decay and counting geometry was finally expressed as a fraction of the initial reading from time 0. This fraction was then multiplied by the initial metal concentration of that flask, to obtain a value for the concentration of metal associated with the cellular material. The concentration of metal per unit cell material was obtained by dividing the above value by the biovolume corresponding to that sample.

For the morphological studies reported here the cultures were grown in the presence of 265 μg Zn l^{-1} (as zinc chloride), 50 μg Cd l^{-1} (as cadmium chloride), and 6.5 μg Hg l^{-1} (as mercuric chloride). These concentrations represent sublethal values of the metals to *S. costatum*. Maximum uptake of the metals at these concentrations occurred after three days while the cultures were still growing exponentially, except for mercury where losses of the metal occurred from the culture due to volatilization. Growth curves for control and metal-treated cultures and metal-uptake curves are given in Figures *A-C* (see also Tables 1-3). The times at which the cultures were sampled for electron microscopical fixation are shown by the broken lines on the figures.

Table 1. *Biovolume and cellular metal content values for the zinc treatment; cells were fixed for electron microscopy at time 2.95 days. The biovolume figure in brackets is for the control culture*

Sampling time (days)	Biovolume (μm^3 ml^{-1}) $\times 10^{-6}$		Metal remaining in solution (μg Zn l^{-1})	Cellular metal content ag μm^{-3})
0.00	1.67	(1.41)	265	
0.41	2.80	(2.48)	261	54.0
0.99	6.37	(5.97)	260	74.8
1.41	13.7	(12.8)	258	66.3
1.99	36.7	(36.5)	255	96.0
2.39	81.8	(74.9)	249	128
2.95	164	(181)	233	178
3.37	173	(180)	227	221
3.97	183	(199)	203	168
4.39	178	(194)	232	187
4.97	210	(211)	231	151
5.41	208	(230)	230	158
5.85	225	(215)	234	132

2.4. *Transmission Electron Microscopy*

Several conventional fixation techniques, including Karnovsky (1965) and Oakley and Dodge (1974), for examination of the fine structure of *S. costatum* were attempted. In all cases poor results were obtained; the plasma membrane was broken and the cell contents all or partially lost. After measuring the osmotic pressure of solutions used in primary fixation, seawater was indicated as the most appropriate vehicle for the primary fixative chosen, glutaraldehyde. Cells were fixed by slowly dripping glutaraldehyde directly into the culture. After fixation for 1-2 hours the cells were centrifuged gently to a pellet and rinsed with sodium cacodylate buffer made up in seawater. The cells were post-fixed with osmium tetroxide and after an alcohol dehydration series, they were embedded in Spurr's medium (Spurr 1969). Pale gold sections were cut using a diamond

Figs. *A-C*. Metal uptake and growth curves plotted against time; the control cultures are represented by the open circles. At the concentrations of the three metals used, the growth of the treated cultures was unaffected when compared with the control cultures. The times at which the treated cells were fixed for electron microscopy are indicated by the broken lines

Table 2. *Biovolume and cellular metal content values for the cadmium treatment; cells were fixed for electron microscopy at time 3.00 days. The biovolume figure in brackets is for the control culture*

Sampling time (days)	Biovolume (μm^3 ml^{-1}) $\times 10^{-6}$		Metal remaining in solution (μg Cd l^{-1})	Cellular metal content (ag μm^{-3})
0.00	1.22	(1.19)	50.0	
0.60	1.94	(1.99)	49.6	37.9
1.04	3.07	(3.37)	49.3	38.1
1.60	6.68	(8.55)	49.3	63.6
2.04	13.5	(22.3)	48.7	70.8
2.65	38.6	(80.1)	45.0	103
3.00	70.3	(160)	39.1	115
3.67	188	(177)	37.1	44.8
4.06	202	(209)	38.5	38.6
4.60	209	(213)	41.9	29.4
5.02	212	(216)	44.3	22.3
5.63	259	(181)	46.5	12.3
6.04	239	(218)	48.3	11.7
6.63	270	(211)	48.5	9.13

Table 3. *Biovolume and cellular metal content values for the mercury treatment; cells were fixed for electron microscopy at time 2.98 days. The biovolume figure in brackets is for the control culture*

Sampling time (days)	Biovolume (μm^3 ml^{-1}) $\times 10^{-6}$		Metal remaining in solution (μg Hg l^{-1})	Cellular metal content (ag μm^{-3})
0.00	1.13	(1.23)	6.50	
0.44	1.81	(1.98)	5.13	286
0.96	4.83	(5.34)	2.71	364
1.44	12.4	(14.8)	0.195	281
2.02	45.5	(53.8)	0.182	73.4
2.48	118	(118)	0.104	28.6
2.98	168	(160)	0.156	19.5
3.50	199	(193)	0.195	16.2
3.98	229	(200)	0.228	13.6
4.50	223	(199)	0.214	13.6
5.02	212	(211)	0.169	14.3
5.44	210	(237)	0.195	14.5
6.02	225	(196)	0.221	13.6

knife and after post-staining with uranyl acetate and lead citrate, the sections were examined in an AEI-801 transmission electron microscope.

The cultures were fixed for electron microscopy after three days' growth; this ensured that the cells were examined during the exponential period of growth. The fixation time was also chosen so that it coincided with the maximum uptake of the metals. The cellular metal contents of the cells illustrated in the electron micrographs are shown in Table 4.

Table 4. *Metal remaining in solution and cellular metal contents at the time of fixation for electron microscopy*

Metal	Initial metal concentration ($\mu g\, l^{-1}$)	Metal remaining in solution ($\mu g\, l^{-1}$)	Cellular metal content ($ag\, \mu m^{-3}$)	Concentration factor
Zn	265	233	178	7.65×10^{2}
Cd	50.0	39.1	115	2.94×10^{3}
Hg	6.50	0.156	19.5	1.25×10^{5}

3. Results

The fine structure of control cells has been described in detail by SMITH (1981). Figures 1–4 show electron micrographs of control cells from a culture in early exponential growth. The cytoplasmic fine structural organization in *S. costatum* is essentially similar to that described for other diatoms, although there is a reduced number of organelles. A section through a control cell contains a nucleus, one Golgi body, two chloroplasts, several mitochondria and the cytoplasm densely packed with ribosomes.

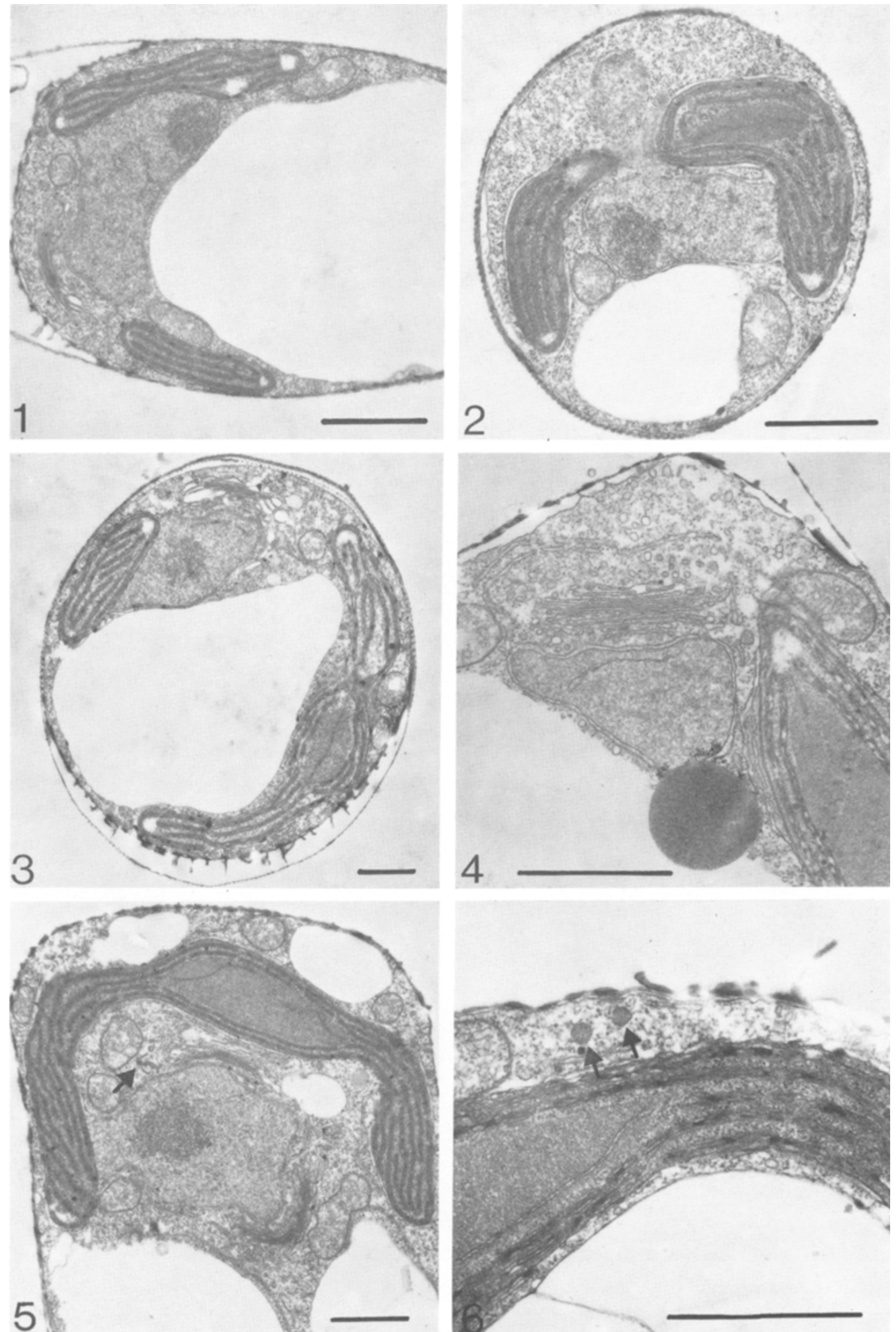
The nucleus of cells from exponentially growing cultures consisted of a double nuclear membrane with nuclear pores, granular nucleoplasm and a more densely staining nucleolus made up of ribosomes. Inclusions or infoldings of the nuclear membrane were often seen in the nucleoplasm. The mitochondria were similar to those observed in other diatoms and varied in shape from spherical to oval with tubular cristae. The golgi body consisted of six to eight cisternae and was always found on the epivalve side of the nucleus, closely associated with the nuclear membrane. The chloroplasts contained a few lamellae which ran parallel to the long axis of the chloroplast; the lamellae consisted of three thylakoids. The chloroplast lamellae were bounded by an envelope composed of a double membrane which in turn was bounded by a vesicle of endoplasmic reticulum. Each chloroplast contained a single lens-shaped pyrenoid traversed by a single thylakoid.

Growth of the zinc-treated culture was unaffected when compared with the control culture (see Fig. *A* and Table 1). The cellular zinc content reached its maximum value of 221 $ag\, \mu m^{-3}$ at 3.37 days (see Table 1). Cultures grown in the presence of 265 $\mu g\, l^{-}1$ zinc were fixed during exponential growth and examined for morphological changes. Cells exposed to sublethal concentrations of zinc consistently formed cytoplasmic "tubules". The "tubules" (see Figs. 5, 7, and 8) appear to be made up of two parallel unit membranes with open ends; they occurred at the periphery of the oldest cisternae of the golgi body and it is suggested that they derive from the breakdown of the cisternae. Other morphological effects, such as the breakdown of chloroplast and mitochondrial membranes, were observed in only some of the zinc treated cells. In Figure 6 two multivesiculate bodies are indicated. These bodies were occasionally found after zinc treatment.

Growth of the cadmium-treated culture was unaffected when compared with the control culture (see Fig. *B* and Table 2). The cellular cadmium content reached its maximum value of 115 $ag\, \mu m^{-3}$ at 3.00 days (see Table 2). *S. costatum* cells grown in the presence of 50 $\mu g\, l^{-1}$ cadmium contained organelles which appeared normal when compared with sections of control cells (Fig. 9). There was, however, evidence of swollen membranes particularly of the chloroplast, endoplasmic reticulum and the cytoplasm was often highly vacuolate (Fig. 10). In some cells multivesiculate bodies were present in the cytoplasm (Fig. 11).

Growth of the mercury-treated culture was unaffected when compared with the control culture (see Fig. *C* and Table 3). The cellular mercury content reached its maximum value of 364 $ag\, \mu m^{-3}$ at 0.96 days (see Table 3). Cells of *S. costatum* grown in the presence of 6.5 $\mu g\, l^{-1}$ mercury exhibited varying degrees of membrane damage, atrophied Golgi bodies, and contained cytoplasmic vesicles with electron-dense inclusions (Fig. 12). In the control cells it was observed that the golgi body was usually located at the epivalve face of the cell, between the nucleus and the plasmamembrane. However, in mercury-treated cells the Golgi body was often found towards the centre of the cell or between the chloroplast and nucleus.

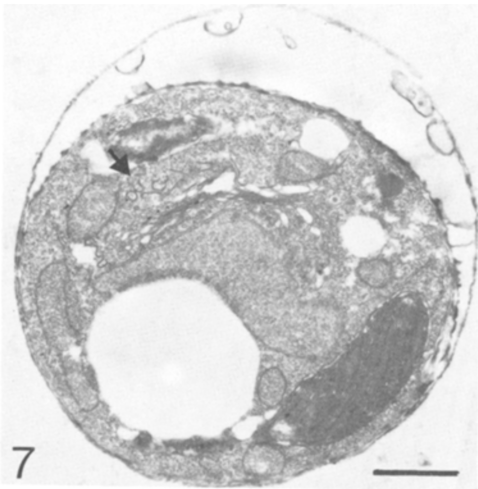
Treatment with zinc, cadmium and mercury caused no observable change in frustule fine structure or filament formation. Table 4 gives the cellular metal contents for the three metal treatments. During mercury-uptake experiments, mercury was lost from the culture medium as well as from cellular material (see Table 3 and

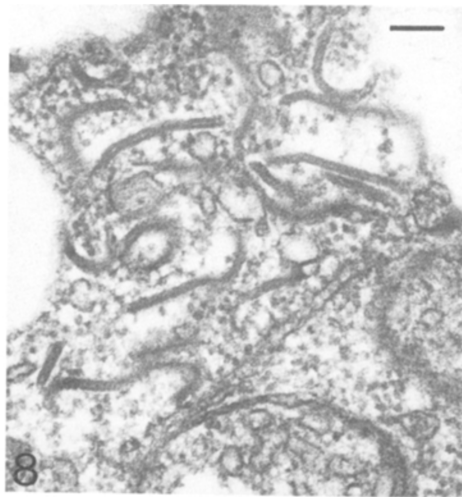


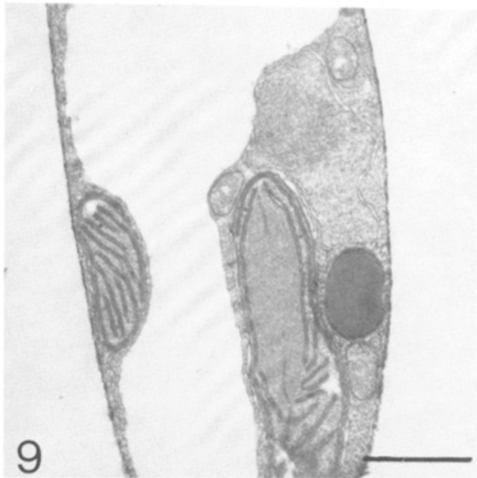
Figs. 1-4. Sections through control cells from a culture in early exponential growth. Note the position of the Golgi body, between the nucleus and epivalve face, in Figs. 1, 3, and 4. (Scale bar 1 μm)

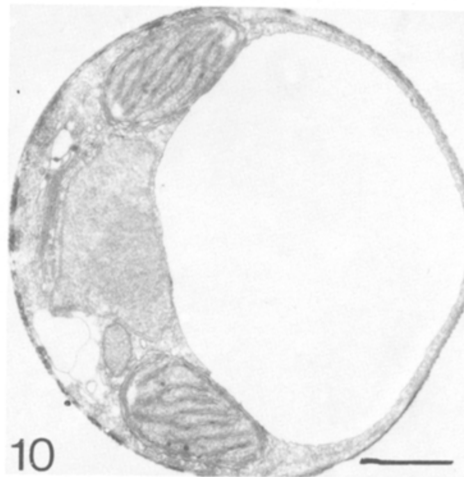
Fig. 5. Longitudinal section through a zinc-treated cell. An area close to the golgi body (arrowed) contains electron-dense "tubules". (Scale bar 1 μm)

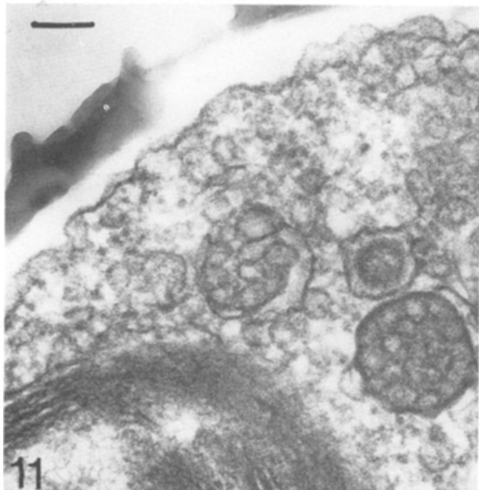
Fig. 6. Longitudinal section through the chloroplast of a zinc-treated cell. Electron-dense multivesiculate bodies in the cytoplasm are indicated. (Scale bar 1 μm)











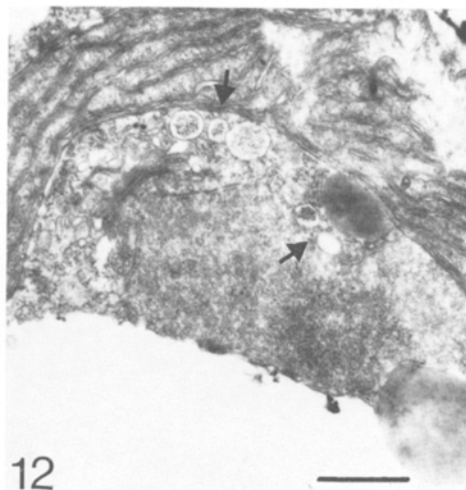


Fig. 7. Transverse section through a zinc-treated cell. An area containing many electron-dense "tubules" is arrowed. Note that the cytoplasm has become vacuolated. (Scale bar 1 μm)

Fig. 8. Detail from Fig. 7. On close examination, the electron-dense "tubules" can be seen to be made up of two membranes with a fine fibrous structure between the membranes. (Scale bar 0.1 μm)

Fig. 9. Oblique section through a cadmium-treated cell. The organelles appear normal when compared to control cells. (Scale bar 1 μm)

Fig. 10. Transverse section through a cadmium-treated cell. The organelles appear undamaged except for some vacuolation of the cytoplasm. (Scale bar 1 μm)

Fig. 11. Transverse section through a cadmium-treated cell containing electron-dense multivesiculate bodies in the cytoplasm. (Scale bar 0.1 μm)

Fig. 12. Oblique section through a mercury-treated cell. Vesicles containing electron-dense material are indicated. The cytoplasm is vacuolate in places and the tonoplast and some chloroplast lamellae are disrupted. The Golgi body is atrophied and located between the nucleus and chloroplast, a position not seen in control cells. (Scale bar 1 μm)

Fig. *C*). The loss of mercury could be due to volatilization of the inorganic mercury by the alga (ZINGMARK and MILLER 1975) or reduction of the inorganic mercury ions by organic matter released during growth (TORIBARA, SHIELDS, and KOVAL 1970).

Table 5. *Cytoplasmic effects of metal-treatments on the fine structure of S. costatum (N = 20 for control, zinc and mercury treatments, N = 14 for cadmium treatment)*

	Control	Zn	Cd	Hg
	(% frequency of occurrence)			
1. Vesicles with electron dense contents	0	0	0	55
2. Multivesiculate bodies	0	20	29	0
3. Cytoplasmic "tubules"	0	75	0	0
4. Concentric membrane whorls	5	15	21	15
5. Dilated double membranes	20	65	71	60
6. Vesiculate cytoplasm	25	85	100	85
7. Golgi body displaced	0	0	0	45
8. Organelle membranes disrupted	0	15	14	25
9. Broken tonoplast	35	55	43	65
10. Broken plasmamembrane	0	0	7	0

Table 5 compares the cytoplasmic effects observed in the four treatments; electron micrographs of sections through *S. costatum* cells which included a nucleus were scored for ten types of cytoplasmic damage. Twenty cells each were scored for the control, zinc and mercury treatments and fourteen cells were scored for the cadmium treatment. The displacement of the Golgi body and the formation of vesicles containing electron dense, granular contents occurred only with mercury treatment. The disruption of organelle membranes including damage to the tonoplast was more likely to occur with mercury treatment than in the other treatments. The formation of cytoplasmic "tubules" was unique to zinc treatment. No particular cytoplasmic damage was associated with cadmium treatment, the effects observed being general to all treatments.

4. Discussion

There is no known biological requirement for mercury or cadmium and their toxicity to cells is due mainly to their strong affinity for ligands of proteins, enabling the metals to inhibit a large number of enzymes and to bind to and affect the conformation of nucleic acids (VALLEE and ULMER 1972). The interaction of mercury with membranes is well documented (PASSOW and ROTHSTEIN 1960, VALLEE and ULMER 1972), mercury treatment resulting in irreversible membrane damage and loss of cell potassium due to the inhibition of the ion transport enzyme Na-K-activated ATPase (SCHMIDT-NIELSEN 1974, SOUTHARD, BLONDIN, and GREEN 1974).

In contrast to cadmium and mercury, zinc is a micronutrient essential to many enzymes and has a role in DNA, RNA and protein synthesis (PRASK and PLOCKE 1971, FALCHUK, FAWCETT, and VALLEE 1975). It has been shown that different clones of *S. costatum* may vary in zinc sensitivity (JENSEN, RYSTAD, and MELSOM 1974). The formation of structures such as the cytoplasmic "tubules" and multivesiculate bodies could provide a large number of binding sites for the zinc and may provide an explanation for the very low sensitivity of the Plymouth clone of *S. costatum* to zinc.

Cytoplasmic and nuclear inclusions capable of binding metals have been reported by SILVERBERG, STOKES, and FERSTENBERG (1976) for *Scenedesmus* spp. with copper and by SICKO-GOAD and STOERMER (1979) for *Diatoma tenue* var. *elongatum* for lead. SICKO-GOAD and STOERMER (1979) found that sublethal levels of lead (0.05 μg at l^{-1}) resulted in a reduction in the number of mitochondria and an increase in membranous organelles in the cytoplasm of the diatom.

The cytoplasmic "tubules" observed after treatment with sublethal concentrations of zinc probably result from the breakdown of Golgi cisternae and at the same time provide membrane surfaces capable of binding the metal and removing it from sensitive metabolic sites. An interesting observation was made by MALONE, KOEPPE, and MILLER (1974) who found that lead taken up by *Zea mays* root cells was concentrated in Golgi vesicles which migrated towards the cell wall where the membrane surrounding the lead deposit fused with the plasma membrane and subsequently with the cell wall. Sublethal cadmium concentrations had no effect on the growth of *S. costatum*; however, fine structural effects were observed: swollen membranes, vacuolate cytoplasm and the appearance of multivesiculate bodies. These effects are consistent with those reported for algal, higher plant and animal cells. Cadmium was found to induce the formation of dense intramitochondrial granules in the freshwater green algae *Ankistrodesmus falcatus*, *Chlorella pyrenoidosa* and *Scenedesmus quadricauda* (SILVERBERG 1976). Early effects of cadmium treatment included swelling and vacuolization in the mitochondria. The disruption of

chloroplast lamellar structure has been observed in cadmium-treated tomato plants (BASZYŃSKI *et al.* 1980). In animal cells, cadmium appears primarily to disrupt the plasma membrane resulting in the inhibition of membrane ATPase activity and the loss of osmoregulation which in turn results in cellular swelling (BUBEL 1976). BUBEL (1976) described dilated endoplasmic reticulum profiles, diffuse (swollen) cytoplasm, dissociated ribosomes, swollen mitochondria and the basal membrane withdrawn from the basal lamina and giving rise to concentric membrane whorls in the gills of a crustacean exposed to cadmium ions.

In cultures grown with sublethal mercury concentrations the nucleus was found in a position not recorded in control cells, and fine structural damage to the cytoplasm was evident. The migration of the nucleus is believed to precede vegetative cell division in *Melosira varians* (CRAWFORD 1973); if a similar mechanism is found to operate in *S. costatum*, the inhibition of the movement of the organelles by mercury would result in the subsequent prevention of cell division. DAVIES (1976) observed the production of abnormally large cells of *Dunaliella tertiolecta* after mercury treatment and proposed that cell division was uncoupled from growth due to the effect of mercury on the intracellular production of methionine.

The effects of mercury on the cytoplasmic fine structure of *S. costatum* cells are similar to the morphological changes reported for mercury-treated animal cells. The most important effect of mercury appears to be the binding with sulphydryl groups on surface membrane proteins, resulting in the inhibition of membrane ATPase and leading to volume changes, including swollen mitochondria and the dilation of endoplasmic reticulum due to the increase in membrane permeability (BUBEL 1976, SCHMIDT-NIELSEN 1974). TINGLE, PAVLAT, and CAMERON (1973) reported swelling of and membrane damage in mitochondria of a ciliate exposed to sublethal concentrations of mercury. The formation of cytoplasmic inclusions after mercury treatment has been observed in kidney cells of flounder (SAHAPHONG and TRUMP 1971) and in gill cells of a crustacean (BUBEL 1976). In both cases, the vesicles containing the inclusions resulted from the breakdown of mitochondria. NAKADA *et al.* (1978) measured significant increases in the permeability of lipid bilayers at mercury concentrations of $5 \times 10^{-4}\,\mu g\,l^{-1}$ and suggest that disruption of the plasma membrane is the primary effect of mercury treatment. SHIEH and BARBER (1973) and OVERNELL (1975) have shown that mercury and copper cause a breakdown in the permeability of the cell membrane of algal unicells, resulting in the loss of internal potassium ions. Increased intracellular levels of mercury could result in increased binding of mercury to the sulphydryl groups of cytoplasmic proteins as well as membrane damage to the organelles.

The ultrastructural studies of the metal-treated *S. costatum* cells indicate that two processes of interaction with the cells were occurring at the cytoplasmic level. There was evidence of membrane damage visualized as swollen organelles, dilated membranes and vacuolated cytoplasm. Possible mechanisms of sequestering the metals were observed in the form of electron-dense inclusions in vesicles, the formation of multivesiculate bodies and the appearance of abnormal cytoplasmic "tubules" apparently derived from the Golgi body.

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