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Accumulation and toxicity of Cd, Zn, Ag, and Hg in four marine phytoplankters

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ABSTRACT: Accumulation and toxicity of Cd, Zn, Ag, and Hg were measured in the diatom *Thalassiosira pseudonana*, the chlorophyte *Dunaliella tertiolecta*, the coccolithophore *Emiliania huxleyi*, and the cyanophyte *Oscillatoria woronichinii*. Bioaccumulation of the metals was measured over a wide (up to 10^5) range of metal concentrations, using gamma-emitting radioisotopes of each metal. Metal content of cells was related to total external metal concentration in all cases, in accordance with Freundlich adsorption isotherms. Dead cells accumulated metals comparably to living cells, indicating that the initial association of metal with the cell is governed by adsorption. Volume/volume concentration factors computed at equilibrium ranged from 3×10^2 for Cd in *T. pseudonana* to 9.5×10^4 for Hg in *E. huxleyi*. Metal toxicity, as measured by depression of cell division rate, could generally be described as an exponential function of the log of the external (or cellular) metal concentration, consistent with the concept of cell thresholds of safe metal accommodation. Regression analyses of the data were used to calculate EC_{50} and EC_0 metal concentrations for each alga/metal combination. The general order of accumulation and metal toxicity *vs.* external metal concentration was Hg > Ag > Zn ≥ Cd. Expressed on a cellular metal basis, Hg was usually the most toxic, but there was no clear trend for the other metals for all species. Upon normalizing cellular metal data on the basis of cellular weight, volume, and surface area, interspecific comparisons revealed that these algal species can have markedly different surface affinities for metals and that cellular EC_{50} values ranged from 7.9×10^{-5} M for Hg in *O. woronichinii* cells to 0.2 M for Zn in *D. tertiolecta* cells. The most resistant species to Zn, Ag and Hg was *D. tertiolecta*. Enhanced metal tolerance in *D. tertiolecta* was partly attributable to metal exclusion, however these cells were able to tolerate cellular metal concentrations of up to 10 (Zn) or 100 (Ag and Hg) times those in the other species. Since the toxicity of any metal was correlated with its concentration factor in a given cell, these data may provide a basis for predicting the toxicity of other metals to algae from metal concentration factors in the cells.

INTRODUCTION

In studies of metal toxicity to marine algae, the varying toxicity of different metals to any one species and the varying sensitivities of different algal species to any one metal are well documented (Davies, 1978). Some studies have examined the accumulation of different metals by different algae (e.g. Sunda and Guillard, 1976: Cu; Davies, 1974, 1976: Hg; Braek et al., 1980: Zn and Cd; Conway, 1978: Cd), but comparatively few have generated both accumulation and toxicity data (usually in the form of photosynthesis or growth rate depression) (Davies, 1974, 1976; Jensen et al., 1974; Sunda and Guillard, 1976; Bentley-Mowat and Reid, 1977; Conway, 1978; Braek et al., 1980; Fisher and Frood, 1980; Kuiper, 1981), even though interpretation of toxicity data would seem to require simultaneous metal accumulation data as well (Davies, 1983). Few studies have compared the behavior of several metals and several algal species simultaneously. Consequently, it has been difficult to draw conclusions on interspecific and intermetal trends, yet such comparisons are necessary for discerning trends in physiological strategies of different algae in accommodating toxic metals and for assessing the relative toxicities of different metals. Comparisons between studies are often tenuous since different studies employ different techniques, different growth media (including varying levels of metal chelators, which can have marked effects: Sunda and Guillard, 1976), and different strains of the same algal species, which may have pronounced differences in metal sensitivity (e.g. Jensen et al., 1974; Fisher, 1981).

Foster (1977) demonstrated that Cu exclusion was adopted as an effective resistance strategy by *Chlorella vulgaris* living in polluted river water; once

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in the cells, Cu had the same effect as it did in sensitive strains. Other studies involving Cu (Hall, 1981; Hawkins and Griffiths, 1982) and Hg and Zn (DeFilippis and Pallaghy, 1976) invoke a metal exclusion argument to explain enhanced metal tolerance in marine and freshwater algae. Davies (1976) suggested that the high tolerance of the marine green alga *Dunaliella tertiolecta* to Hg was in part attributable to exclusion, although once associated with the cell, the Hg had a less pronounced effect than in a sensitive alga, *Isochrysis galbana*. Davies argued that *D. tertiolecta* was able to tolerate the Hg by sequestering it in a harmless form (apparently as HgS precipitate) within the cell. Fisher (1981) showed that the high Zn tolerance of *Skeletonema costatum* growing in Zn-contaminated waters was not due to Zn exclusion by the cells, as sensitive and resistant *S. costatum* clones accumulated equal amounts of Zn. Cellular detoxification mechanisms have also been identified in marine and freshwater algae for Cu (Silverberg et al., 1976; Daniel and Chamberlain, 1981; Hall, 1981; Reed and Moffat, 1983) and for Cd (Li, 1980). Thus algae appear to use at least 2 strategies to tolerate high external metal concentrations – metal exclusion (presumably by having less metal-reactive surfaces) or internal sequestering of the metal to avoid extensive exposure of sensitive sites within the cell.

Recently, Fisher et al. (1983a) found that transuranic elements such as Am and Pu were concentrated passively by centric diatoms more than by green and blue-green algae; it thus appeared that the latter had less reactive surfaces for these metals. From the literature as a whole, it also appears that the green and blue-green algae are generally more tolerant of toxic metals than are centric diatoms, for example.

The present study was conducted to examine the possibility that greater metal tolerance in algae was due to a less metal-reactive cell surface (i.e. different and/or fewer ligands per μm^2 cell surface). Four phytoplankters, including a diatom, a coccolithophore, a green alga, and blue-green alga were exposed to varying levels of each of 4 metals – Cd, Zn, Ag, and Hg. Metal toxicity and accumulation, using gamma-emitting isotopes of these metals and radiotracer techniques, were measured. The use of these radioisotopes enables rapid determination of metal accumulation in algal cells, even with low metal concentrations and small sample sizes. The results allow for direct interspecies and inter-metal comparison for bioaccumulation of metals and for growth as a function of cellular as well as ambient metal concentration.

MATERIALS AND METHODS

Unialgal cultures of *Thalassiosira pseudonana* (Bacillariophyceae), *Dunaliella tertiolecta* (Chlorophyceae), *Emiliania huxleyi* (Haptophyceae), and *Oscillatoria woronichinii* (Cyanophyceae) were used in all experiments. Clonal designations and cellular dry weights, volumes, and surface areas are given in Table 1. Cell dimensions for these algae are taken from Fisher et al. (1983a), with some modification for average-size *Oscillatoria* filaments. Cultures were handled aseptically throughout the experimentation.

Inocula for all experiments came from late log-phase cultures maintained in f/2 medium (Guillard and Ryther, 1962) modified so that f/20 Si was used and no Cu, Zn, or EDTA were added. The medium for stock cultures was prepared with autoclaved Mediterranean surface water which had been glass-fiber filtered. Experimental media, prepared by sterile filtration (0.2 µm Nuclepore filters), contained f/2 N, P, and vitamin additions and f/20 Si; no metal or EDTA was added. Mn addition was omitted since it can have a modifying effect on metal toxicity to diatoms, as shown at least for Cu (Sunda et al., 1981). The Mn concentration in an aliquot of experimental medium was determined by flameless atomic absorption spectrometry (Perkin Elmer 5000) to be 3×10^{-7} M.

For determining growth effects of metal addition, 20 ml of experimental medium, contained in an autoclaved, 80 ml capacity bottle sealed with a polyurethane stopper (double Parafilm seal for Hg-treated cultures), received 1×10^4 cells ml^{-1} of clones 3H, Dun, and MCH and 1×10^3 filaments ml^{-1} of clone

Table 1. *Thalassiosira pseudonana, Dunaliella tertiolecta, Emiliania huxleyi, Oscillatoria woronichinii.* Clonal designations, dry weight $cell^{-1}$, volume $cell^{-1}$, and surface area $cell^{-1}$ ($filament^{-1}$ of *O. woronichinii*) of the algal species employed in metal accumulation and toxicity experiments

Species	Clone	Cell dry weight (pg)	Cell volume (μm^3)	Cell surface area (μm^2)
Thalassiosira pseudonana	3H	22	61	125
Dunaliella tertiolecta	Dun	15	91	178
Emiliania huxleyi	MCH	50	144	207
Oscillatoria woronichinii	Osc N–1	1204	2445	2630

Osc. For measuring metal accumulation by cells, 200 ml of experimental medium, contained in an autoclaved 250 ml capacity bottle sealed with a Teflon-coated screw cap, received 1×10^5 cells ml^{-1} of clones 3H, Dun, and MCH and 1×10^4 filaments ml^{-1} of Osc. Use of greater biomass in the metal uptake study facilitated subsequent metal detection when using low metal concentrations. Cells for both metal uptake and growth kinetics studies came from the same stock cultures. To determine whether metal accumulation was metabolically mediated, identical numbers of heat-killed cells of clones 3H, Dun, and Osc (killed by placement in a 45 °C water bath for 5 min, 10 min for Dun) were exposed to metal (one concentration each of Zn, Ag, and Hg) and metal accumulation compared with live cells. Preliminary experiments showed that this heat treatment prevented subsequent cell division (and movement: Dun) by the cells, but the cells appeared to maintain their structural integrity for a period of about 1 d. Heat treatment appeared to disrupt the MCH cells so that intact dead cells could not be maintained.

Immediately following inoculation, stable metal was added to the experimental cultures by Eppendorf micropipet. The metals were taken from freshly prepared stocks, held in sealed Nalgene bottles, which consisted of Merck analytical grade metal salts dissolved in distilled water. Stock concentrations were diluted so that additions generally did not exceed 0.002 of the volume of the experimental media. A range of concentrations was used for each metal for the uptake measurements and growth studies. Solutions of stable Cd, Zn, and Hg were prepared by dissolving their chlorides while the solution of stable Ag was prepared with $AgNO_3$. All metal uptake experiments included uninoculated blanks for each concentration of each metal. Immediately following stable metal addition, radioisotopes of each metal were added, via Eppendorf pipet, to each bottle containing media for measuring metal uptake by the algae. Radioisotopes were provided by Commissariat à l'Energie Atomique, France. ^{109}Cd ($t_{½}$ = 454 d), dissolved as $CdCl_2$ in 1 N HCl, was added to give 3.9 kBq l^{-1}; ^{65}Zn ($t_{½}$ = 244 d), dissolved as $ZnCl_2$ in 0.1 N HCl, was added to give 79 kBq l^{-1}; ^{110m}Ag ($t_{½}$ = 250 d), dissolved as AgCN in 0.24 N NH_4OH, was added to give 41.6 kBq l^{-1}; ^{203}Hg ($t_{½}$ = 46.6 d), dissolved as $Hg(CH_3COO)_2$ in 5 % CH_3COOH, was added to give 646 kBq l^{-1}. Addition of stable and radiolabelled metal never necessitated more than a total of 15 min. Total metal concentration in solution at time zero (i.e. total stable plus labelled metal) in 'uptake cultures' ranged from 6.3×10^{-8} M to 4.4×10^{-3} M Cd, from 1.1×10^{-7} M to 1.5×10^{-3} M Zn, from 7.4×10^{-9} M to 9.5×10^{-6} M Ag, and from 1.5×10^{-9} M to 5.1×10^{-5} M Hg. In unlabelled cultures, Cd ranged from 4.4×10^{-8} M to 4.4×10^{-3} M, Zn from 1.5×10^{-6} M to 1.5×10^{-3} M, Ag from 9.3×10^{-10} M to 9.3×10^{-5} M, and Hg from 5×10^{-10} M to 5×10^{-5} M. Total background levels of these metals in this seawater (Fukai et al., 1980) do not exceed 10 % of the lowest added metal concentration.

All bottles were then sealed, swirled, and sampled at time zero. These samples consisted of 5 ml from the radioactive cultures (and blanks) and 1 ml from the unlabelled cultures (preserved with 0.05 ml filtered Lugol's solution), for eventual radioactive counting and cell enumeration, respectively. The cultures were then inoculated unshaken, at 17 °C ± 1 C° on a 14 : 10 L : D light cycle provided by 'cool-white' fluorescent lamps (~ 200 μEin m^{-2} s^{-1}). Subsequent samples were taken from the swirled radioactive cultures (and blanks) at exposure times ranging from 4 h to 96 h, and from the swirled unlabelled cultures at 24 h to 96 h. From the labelled cultures, 1 ml samples were taken and preserved with Lugol's solution for microscopic cell counting and 5 ml unfiltered samples were removed for radioactive counting at each sample time. At the same time, cells were harvested from the media by filtering 25 ml samples through 1 μm Nuclepore filters (25 mm diameter), after which the filters were immediately washed with 10 ml (5 ml gave similar results) of seawater solution of 10^{-4} M EDTA (prepared by dissolving EDTA in glass-fiber filtered seawater and then sterile filtering through 0.2 μm Nuclepores). The EDTA wash was employed to remove loosely bound metal from the filtered cells (see Davies, 1973, and Bates et al., 1982 for detailed discussion of this phenomenon). The filters were then removed for radioactive counting. All samples for radioactive counting (filters, water) were put in sealed plastic counting tubes for eventual counting. The blanks (i.e. the uninoculated radioactive 'cultures') were sampled identically to the algal cultures to assess metal retention (due to metal precipitation in the media, adsorption to the filter, etc.) by the filters at the different metal concentrations. Immediately following the final sampling of the radioactive cells, the sorption of metal to bottle walls was determined. The remaining liquid in the bottles was discarded and the glass walls rinsed two times with 20 ml 3 N HCl; the acid was then placed in counting tubes for radioactive counting. From the unlabelled cultures, 1 ml samples were taken, fixed with Lugol's solution, and cells counted microscopically with a modified Fuchs Rosenthal hemacytometer.

Radioactive samples were counted by gamma spectroscopy using a multichannel analyzer connected to three 7.6 cm well-type NaI (Tl) crystals. The ^{109}Cd photons were detected at 88 KeV, the ^{65}Zn detected at 1115 KeV, the ^{110m}Ag detected at 764 KeV, and the

^{203}Hg at 279 KeV. Standards of each isotope were counted each day and corrections were made for radioactive decay and the slightly varying efficiencies of the different crystals; all results shown represent corrected values normalized to time zero activity. Counting times were such that propagated 1 σ counting errors were generally < 5 %.

Growth rates of cells in the unlabelled cultures were determined from the cell counts, by Eq. 1:

$$\mu = \frac{(\ln Nt - \ln Nt_o)\ (24)}{(t - t_o)\ (\ln 2)} \quad (1)$$

where μ = divisions d^{-1}; Nt and Nt_o = cell densities at times t and t_o, respectively; $t - t_o$ = elapsed time in hours (usually 72 h data were used; growth curves were log-linear for most treatments). Metal accumulation by cells was determined for each sample, by Eq. 2:

$$C_m = \frac{(F_c)\ (F_w)\ (C_t)}{Nt'} \quad (2)$$

where C_m = moles metal $cell^{-1}$ at sample time t'; F_c = fraction of total cell suspension (unfiltered) radioactivity associated with cells at time t' (after subtraction of blank value for corresponding metal concentration); F_w = fraction of t_o radioactivity still in the cell suspension at time t'; C_t = total metal concentration (M) added at t_o; Nt' = cells l^{-1} at time t'. Concentration factors were determined as in Eq. 3, for each metal and each alga, using means from the cultures with the lowest concentrations of each metal (at time of no change):

$$VCF = \frac{\text{moles metal } \mu m^{-3} \text{ cell}}{\text{moles metal } \mu m^{-3} \text{ water}} = \frac{(C_m)\ (10^{15})}{(V_c)\ (C_t)\ (1 - F_c)\ (F_w)} \quad (3)$$

where VCF = volume/volume concentration factor for a given metal and alga; V_c = cell volume (μm^3).

RESULTS

Radioactivity in the cell suspension (unfiltered) of all cultures remained essentially constant over the first 48 h, with the exception of the Hg-treated cultures (Table 2). Cd and Zn generally showed low adsorption (< 2 %) to glass walls, although in Dun cultures treated with Zn there was an inexplicably high loss of Zn to the walls (Table 2). The amount of Ag and Hg retrievable from glass walls ranged from 1.5 to 23 % of the amount added (Table 2). By adding the radioactivity of all the samples removed from each Hg-treated bottle, 93 % of the blank's initial radioactivity could be accounted for, while 92 % of 3H's Hg, 89 % of Dun's Hg, 93 % of MCH's Hg, and 100 % of Osc's Hg were accounted for. For the other metals, essentially all the initial radioactivity of each culture could be accounted for.

As with the transuranic elements (Fisher et al., 1983a), blank cultures showed low uptake of metal on 1 μm Nuclepore filters: generally, < 0.5 % of the water column Cd and Zn at any sample time, < 4 % of the Ag, and < 1 % of the Hg. These blank values were

Table 2. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Percent of time-zero radioactivity detected in suspensions at different times for each metal and each species. Data are means ± 1 SD for all concentrations of each metal. Also shown are percent of time-zero radioactivity recovered from bottle walls by acid treatment immediately after last sample, for each culture. nd: not determined

Metal	Clone	Hours						Walls
		4	12	24	48	72	96	
Cd	3H	99 ± 2	96 ± 7	107 ± 14	108 ± 11			0.6 ± 0.2
	Dun	102 ± 4	96 ± 6	110 ± 13	102 ± 11	nd	nd	1.1 ± 0.9
	MCH	96 ± 4	94 ± 6	106 ± 12	110 ± 8			0.6 ± 0.1
	Osc	99 ± 4	100 ± 5	109 ± 10	105 ± 8			0.9 ± 0.1
Zn	3H	96 ± 2	95 ± 2	106 ± 8	110 ± 5	106 ± 4		1.7 ± 0.2
	Dun	95 ± 4	98 ± 7	99 ± 8	87 ± 18	79 ± 22		22 ± 13
	MCH	98 ± 2	107 ± 7	109 ± 3	108 ± 4	103 ± 3	nd	2.3 ± 0.5
	Osc	96 ± 4	105 ± 12	101 ± 4	109 ± 11	107 ± 9		1.5 ± 0.4
Ag	3H	104 ± 1	101 ± 4	101 ± 6	94 ± 15	90 ± 19	82 ± 28	16 ± 13
	Dun	104 ± 3	99 ± 5	96 ± 8	88 ± 16	81 ± 18	72 ± 20	23 ± 13
	MCH	104 ± 1	108 ± 8	102 ± 6	99 ± 12	96 ± 16	91 ± 19	8 ± 8
	Osc	96 ± 7	97 ± 7	99 ± 13	91 ± 17	101 ± 31	93 ± 17	15 ± 12
Hg	3H	99 ± 1	95 ± 4	75 ± 5	57 ± 11			15 ± 4
	Dun	97 ± 4	98 ± 4	67 ± 8	61 ± 7			13 ± 0
	MCH	94 ± 8	99 ± 3	72 ± 19	45 ± 16	nd	nd	15 ± 4
	Osc	99 ± 9	97 ± 2	83 ± 9	69 ± 17			17 ± 1
	no cells	98 ± 9	102 ± 3	90 ± 16	77 ± 24			7 ± 4

subtracted from the final cellular uptake values, which were usually far greater. Cells accumulated metals rapidly, with final metal cell^{-1} concentrations generally achieved within 1 d. Typical results are given in Fig. 1 for Cd accumulation in MCH. Under most circumstances, the equilibrium metal level per cell did

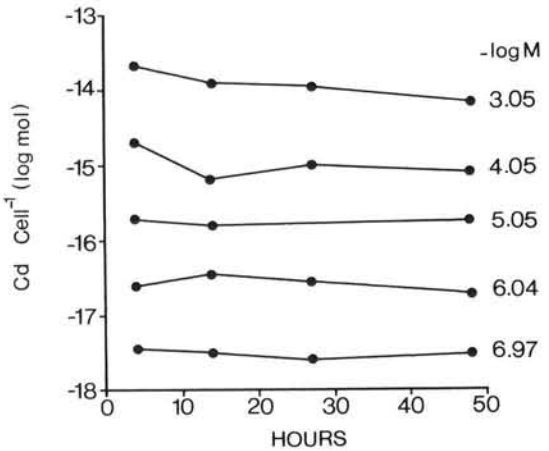

Fig. 1. *Emiliania huxleyi*. Cd content of MCH cells exposed to different Cd concentrations over a 48 h period. Note that the Cd accumulation by these cells was essentially complete at 4 h

not change appreciably after about 12 h uptake, even though cells continued to divide. Dead cells initially accumulated metal in amounts equivalent to those in live cells (Fig. 2), although after about 24 h, dead cells began to disintegrate and metal per cell levels were difficult to determine. At equilibrium, log cellular metal concentrations were linearly related to log total ambient metal concentrations (Fig. 2, Table 3). All regressions were highly significant ($P < .001$). At high external concentrations, there was evidence of precipitation of Zn and Ag and, as well, possible saturation of cells by Ag and Hg (Fig. 2). There was no indication of cells becoming saturated with Cd at high concentrations, even though Cd levels approaching several percent of cells' dry weights were found. Volume/volume concentration factors (VCF) ranged from 3×10^2 for Cd accumulation in 3H to 9.5×10^4 for Hg accumulation in MCH (Table 4). There was never greater than a 5-fold variation among species for accumulation of any one metal, and none of the species consistently accumulated more or less metal than any other. It is noteworthy that the slopes of the regression lines (Table 3) were generally less than 1, possibly due to the EDTA wash which removes loosely bound metal and would lower the slope of total cellular metal (i.e. firmly plus loosely bound metal) *vs.* the log of the external metal concentration to below unity. Thus, the regressions given in Table 3 are only for data on internal cellular metal and firmly-bound surface metal.

Once accumulated, all metals exerted toxic effects on the growth of the cells. Depression of growth rates (μ) was exponentially related to the external metal concentration and the cellular metal level (Fig. 3 to 6; Table 5). The equation form which best describes all the toxicity data is

$$y = 100 - ae^{bx} \quad (4)$$

where $y = (\mu$ treated cells $\div \mu$ control cells$) \times 100$; $x =$

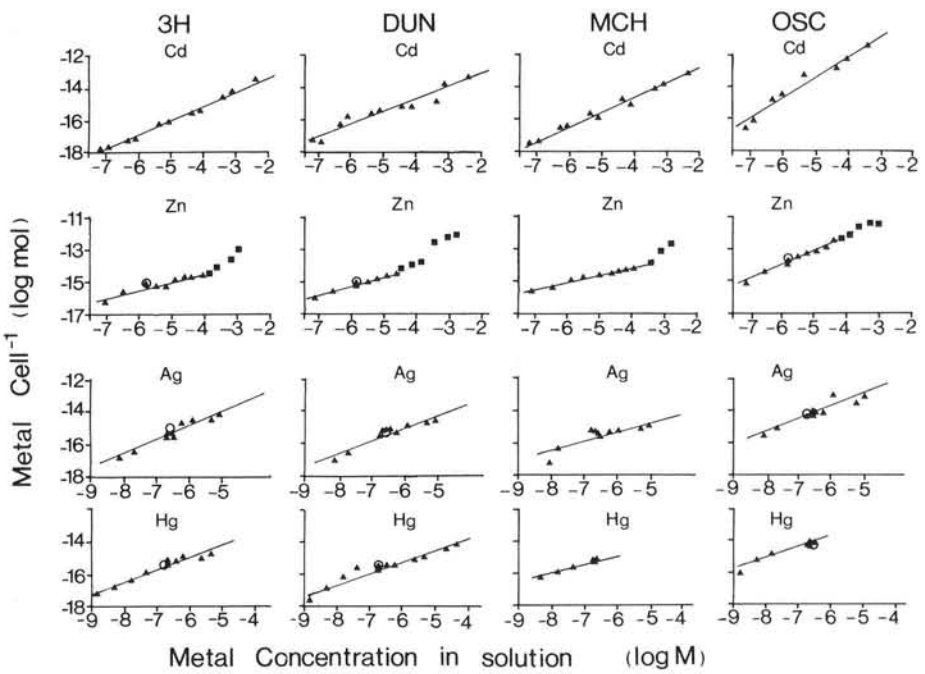

Fig. 2. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Cd, Zn, Ag, and Hg contents of 3H, Dun, MCH and Osc cells as a function of external metal concentrations. Each data point is the mean plateau concentration at time of no change. Triangles: live cells; circles: heat-killed cells (for 3H, Dun, and Osc); squares: heavy precipitation of Zn (not included in statistical analysis). Least squares fits are presented for live cells, and are described in Table 3

Table 3. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Regression analysis of metal accumulation data. Equation for lines is $y = ax + b$; where y = metal cell^{-1} (log mol); x = metal concentration in solution (log M). All values of a, b, and r significant at $P < .001$. All data weighted equally

	Cd				Zn			
	a	b	r	SE est.	a	b	r	SE est.
3H	.906 ±.031	−11.566 ±.158	.995	.143	.503 ±.064	−12.469 ±.346	.948	.157
Dun	.714 ±.066	−11.970 ±.339	.964	.306	.580 ±.021	−11.842 ±.123	.996	.040
MCH	.880 ±.042	−11.178 ±.216	.990	.195	.436 ±.027	−12.525 ±.147	.985	.079
Osc (filament)	1.222 ±.087	−7.345 ±.487	.985	.282	.897 ±.063	−8.716 ±.352	.983	.137
	Ag				Hg			
3H	.859 ±.091	−9.777 ±.592	.958	.231	.734 ±.063	−10.634 ±.441	.968	.199
Dun	.750 ±.087	−10.664 ±.566	.950	.221	.660 ±.051	−11.362 ±.341	.966	.224
MCH	.627 ±.096	−11.537 ±.626	.918	.244	.577 ±.064	−11.445 ±.465	.976	.080
Osc (filament)	.719 ±.128	−9.528 ±.838	.893	.326	.760 ±.080	−9.102 ±.603	.979	.140

Table 4. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Volume/volume concentration factors of Cd, Zn, Ag, and Hg in four algal species

Clone	Cd ($\times 10^2$)	Zn ($\times 10^3$)	Ag ($\times 10^4$)	Hg ($\times 10^4$)
3H	3.0	12	3.4	9.3
Dun	10	10	1.3	3.2
MCH	3.7	4.6	2.4	9.5
Osc	10	5.2	6.6	7.6

metal concentration (log M for dissolved, log mol cell^{-1} for cellular). Equation 4 can be converted as follows:

$$y = 100 - ae^{(b \log M)} \quad (5)$$

$$y = 100 - ae^{(b \ln M/2.303)} \quad (6)$$

$$y = 100 - aM^{(b/2.303)} \quad (7)$$

It follows that when $b = 2.303$, y is linearly related to M; however, b generally differed from 2.303 for all species and metals, particularly for Cd and Zn, for which there are the most growth rate data. Thus, it appears that the data are consistent with the concept that a cellular threshold exists for metal accomodation/toxicity. The data depicted in Fig. 3 to 6 suggest that these cellular thresholds for safe metal accomodation vary widely among metals and among algal species.

Growth of Osc in the Cd-treated cultures was erratic and reliable data on Cd toxicity to this species were not obtained. Regression analysis showed that comparable r values for μ depression *vs.* external or *vs.* cellular metal levels were computed (Table 5). To facilitate comparison of the four algal species, which varied in size and weight, the metal toxicity data are also expressed on cell colume, surface area, and dry weight bases (Fig. 3 to 6). Regression analysis (Table 5) of the data generated EC_{50} and EC_0 values for all species and metals, where EC_{50} is the metal concentration (either external or cellular) at which $\mu = 50\ \%\ \mu$ of control cells and EC_0 is the minimum metal level giving $\mu = 0$. The EC_{50} and EC_0 values are presented in Table 6. The general order of metal toxicity (as measured by EC_{50} calculations) *vs.* external metal concentrations was Hg > Ag > Zn ≥ Cd. *Dunaliella* appeared far less sensitive to external Zn, Ag, and Hg than were the other species. When EC_{50} values are based on cellular metal concentrations (normalized, e.g. to cell volumes), all clones have comparable sensitivity to Cd, while Dun is more resistant than are the other species to Zn, Ag, and Hg (Fig. 7). The greater resistance of Dun to Zn, Ag, and Hg appears not to be due solely to metal exclusion, as it takes significantly more of these metals per unit volume of Dun cell to depress μ than per unit volume of the other cells; in the case of Hg, the Dun cells are about 2 orders of magnitude more resistant per unit volume of cell material.

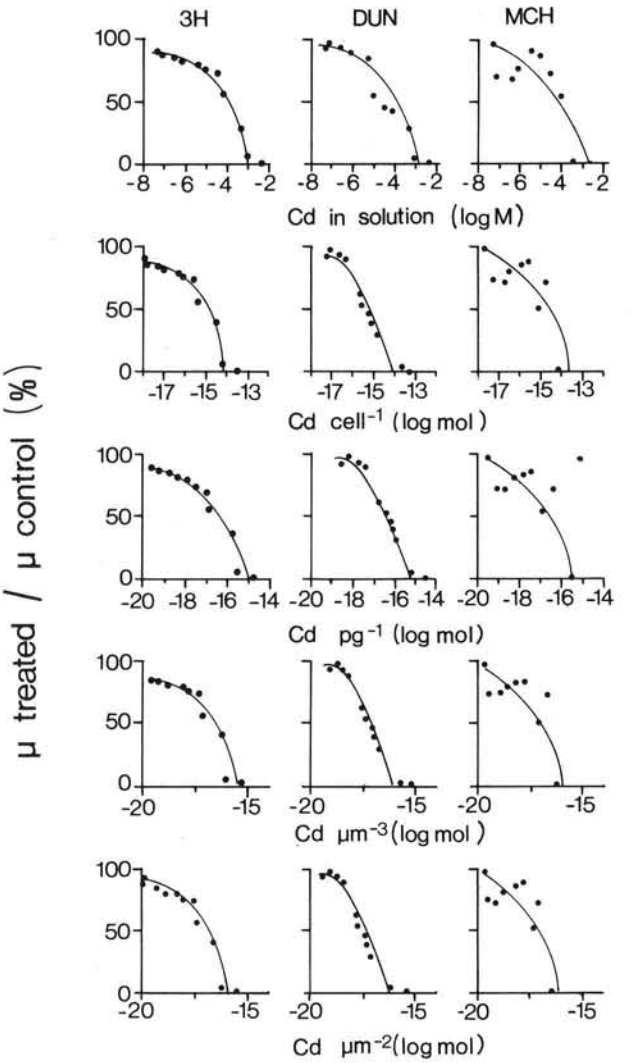

Fig. 3. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH). Effects of external and cellular Cd on growth rates (μ) of 3H, Dun, and MCH cells. To facilitate interspecific comparisons, data are also presented on cellular dry weight (pg), volume (μm^3), and surface area (μm^2) bases. See Table 5 for statistical treatment of data. Control μ = 1.61 (3H), 1.70 (Dun), 0.78 (MCH)

Upon pooling all the data, EC_{50} and EC_0 values (based on external metal concentrations) were exponentially related to the log of the VCF's in the algae (Fig. 8), suggesting that metals which are more reactive for algal surfaces, as reflected by the bioaccumulation (and VCF) data, are also more toxic.

DISCUSSION

Accumulation

The degree of metal association with the cells was in direct proportion to the external metal concentration, although saturation of some algae by Ag and Hg may have been approached at very high concentrations. Linearity of the results is best described by the Freundlich isotherm:

$$\log C_m = a \log C_t + b \quad (8)$$

where values of a and b are given in Table 3. Equilibrium with respect to metal partitioning between dissolved and solid (i.e. glass wall and suspended particulate) phases was rapidly achieved (comparable to many other reports: Davies, 1978) and maintained despite growth of the cells. That is, as cells divided to produce new cells, and hence new reactive surface material, the total particulate metal content also increased but the metal cell^{-1} remained essentially constant. Davies (1976) similarly observed that the reactivity of *Dunaliella tertiolecta* cell surfaces for Hg did not change with the age of the culture, although the surface reactivity of the haptophyte *Isochrysis galbana* for Hg did change significantly over an eight-day period (i.e. a and b in Eq. 8 changed with time) (Davies, 1974). Moreover, heat-killed cells in our study

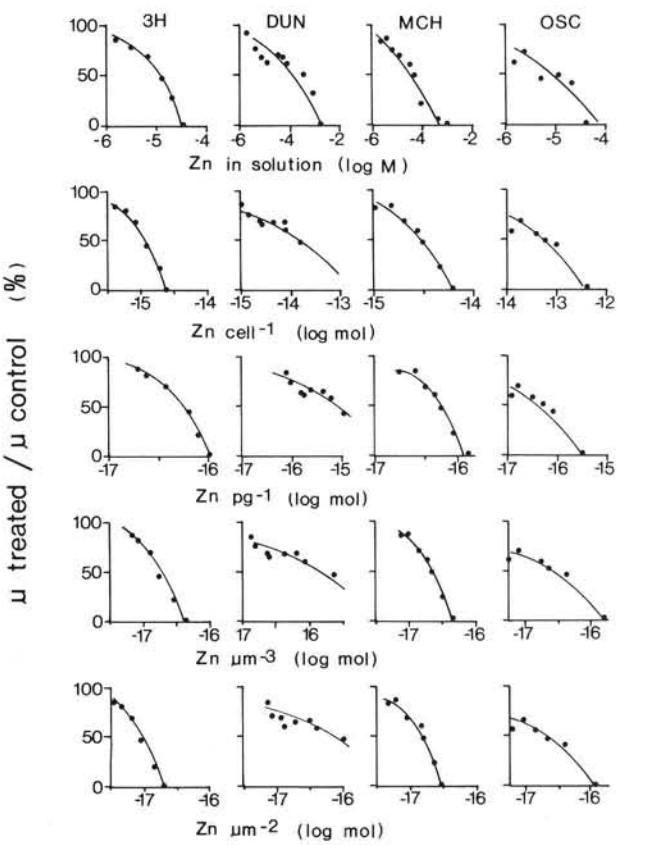

Fig. 4. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Effects of external and cellular Zn on growth rates (μ) of 3H, Dun, MCH, and Osc cells. See Table 5 for statistical treatment of data. Control μ = 2.07 (3H), 1.59 (Dun), 1.26 (MCH), 0.72 (Osc)

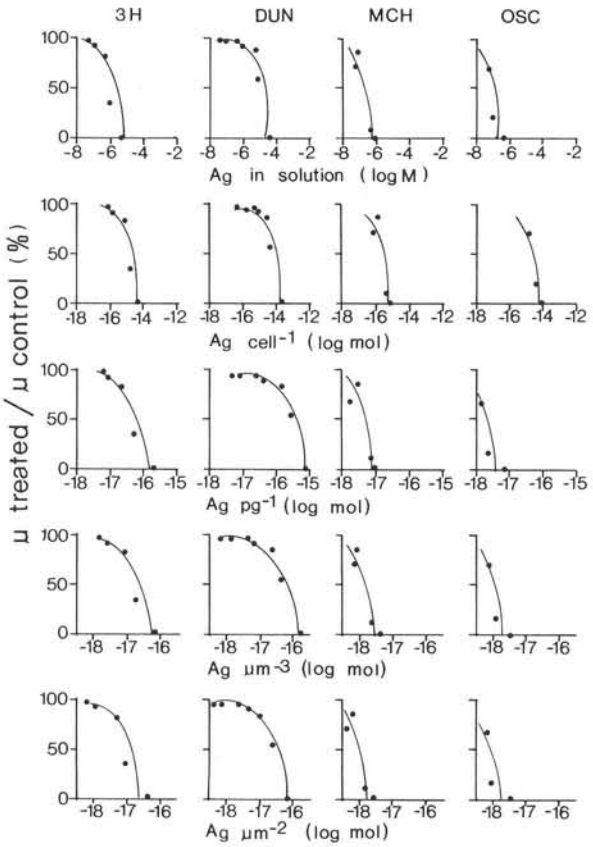

Fig. 5. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Effects of external and cellular Ag on growth rates (μ) of 3H, Dun, MCH, and Osc cells. See Table 5 for statistical treatment of data. Control μ = 1.62 (3H), 1.48 (Dun), 0.70 (MCH), 0.57 (Osc)

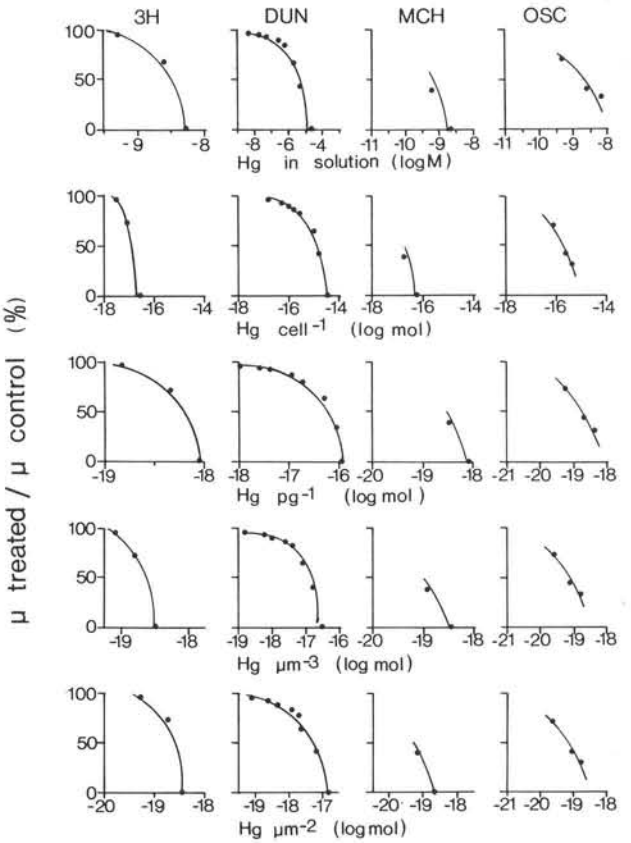

Fig. 6. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Effects of external and cellular Hg on growth rates (μ) of 3H, Dun, MCH, and Osc cells. See Table 5 for statistical treatment of data. Control μ = 1.50 (3H), 1.41 (Dun), 0.70 (MCH), 0.62 (Osc)

accumulated metal comparably to living cells, further indicating that bioaccumulation of these metals in these algae proceeds by non-metabolic adsorption. Rabsch and Elbrächter (1980) noted that heat killed (50 °C) diatoms *(Coscinodiscus granii)* accumulated 3 times more Cd and 4 times more Zn than did living cells, and Glooschenko (1969) found that formalin-killed diatoms *(Chaetoceros costatum)* accumulated more Hg than did living cells; the differences presumably resulted from significant changes in the surface chemistry caused by the heat or formalin treatment. Conway and Williams (1979) found equivalent Cd accumulation by live and cold-killed cells of one freshwater diatom *(Fragilaria crotonensis)* but not another *(Asterionella formosa)*. The linear relation of log metal cell^{-1} *vs.* log external metal concentration is similar to findings for Cd and Zn uptake in diatoms (Braek et al., 1980), Cd accumulation in a haptophyte (Li, 1980), a green alga (Rebhun and Ben-Amotz, 1984), and a freshwater diatom (Conway, 1978), Zn accumulation in two green algae (Bates et al., 1982), Hg in a haptophyte and a green alga (Davies, 1974, 1976), and Cu in a diatom and a coccolithophore (Bentley-Mowat and Reid, 1977). As such, the concentration dependence of algal adsorption of these metals is similar to that of the reactive transuranic elements (e.g. Pu, Am, Cm, Cf) (Fisher et al., 1983a).

Sunda and Guillard (1976) and subsequent investigators concluded that bioaccumulation and consequent toxicity of Cu in a diatom *(Thalassiosira pseudonana)* was a function of the ionic Cu level, and was not linearly related to total ambient Cu concentration in water where there was appreciable organic complexation of Cu. In our study we found that the cellular accumulation of Cd, Zn, Ag, and Hg was directly related to the total metal concentration in solution. Since we employed natural rather than artificial seawater and used no artificial chelators to control the speciation of the metals, we do not know the degree to which the metals were in ionic form. At the higher concentrations, all the metals would certainly have exceeded the organic complexing capacity of the

Table 5. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Regression analysis of toxicity data, for metal concentrations dissolved in water and metals associated with cells. The equation for lines is $y = 100 - ae^{bx}$; where y = (μ treated ÷ μ control cells) (100); x = metal concentration (log M for dissolved, log mol $cell^{-1}$ for cellular). All data were weighted equally. nd = not determined

Dissolved metal		3H	Dun	MCH	Osc
Cd	a	.327	1136	526	
	b	.504	.778	.610	nd
	r	.978**	.889**	.697*	
	SE est.	.168	.635	.812	
Zn	a	3.0×10^4	382	906	1839
	b	1.299	.542	.672	.721
	r	.998**	.909**	.957**	.856*
	SE est.	.039	.234	.191	.210
Ag	a	2.3×10^7	1.2×10^5	9.5×10^5	1.1×10^5
	b	2.227	1.678	1.509	1.091
	r	.953*	.954**	.863 *n.s.*	.842 *n.s.*
	SE est.	.508	.529	.461	.292
Hg	a	2.1×10^{14}	3.7×10^4	4.8×10^9	6.6×10^7
	b	3.432	1.258	2.011	1.620
	r	.995**	.997**	.982*	.984**
	SE est.	.136	.115	.389	.302
Cellular metal					
Cd	a	1.6×10^5	2.1×10^8	8.1×10^5	
	b	.548	1.028	.667	nd
	r	.997**	.861**	.679*	
	SE est.	.173	.704	.831	
Zn	a	1.2×10^{15}	3.6×10^5	1.7×10^{17}	1.7×10^6
	b	2.069	.649	2.467	.790
	r	.811*	.752*	.985**	.933**
	SE est.	.369	.770	.103	.147
Ag	a	2.4×10^{18}	2.4×10^{23}	1.5×10^{18}	1.7×10^{11}
	b	2.592	3.512	2.427	1.503
	r	.953*	.941**	.867 *n.s.*	.837 *n.s.*
	SE est.	.506	.950	.455	.297
Hg	a	1.3×10^{36}	2.5×10^{14}	5.8×10^{39}	1.9×10^{24}
	b	4.703	1.967	5.257	3.312
	r	.966*	.997**	.974 *n.s.*	.971*
	SE est.	.133	.101	.708	.646

**P < .001; *P < .05; *n.s.* not significant

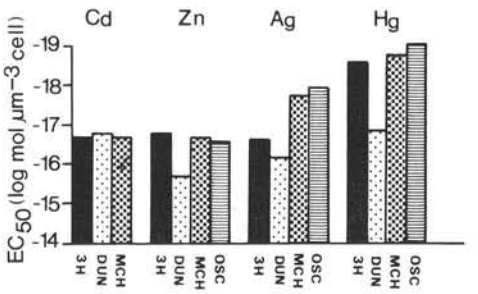

Fig. 7. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Comparison of EC_{50} values for all metals and all species, based on μ *vs.* cellular metal concentrations

seawater and were probably ionic or in inorganc complexes. Even the lowest Cd and Zn concentrations in our study were probably sufficient to exceed the seawater's complexing capacity (Fisher and Fabris, 1982); the organic complexing capacity of seawater for Ag and Hg has been little studied and it is unknown whether the lowest concentrations of these metals exceeded this capacity in the experimental seawater.

Since metals would initially associate with the cells by binding to some ligand on the cell surface, the extent of metal adsorption to cells in suspension would depend on the number of cells, the number and kind of available ligands per unit cell surface, and the reactiv-

Table 6. *Thalassiosira pseudonana* (3H), *Dunaliella tertiolecta* (Dun), *Emiliania huxleyi* (MCH), *Oscillatoria woronichinii* (Osc). Calculated EC_{50} and EC_0 values for Cd, Zn, Ag, and Hg on ambient metal concentration and cellular metal concentration bases. Also shown are cellular EC_{50} and EC_0 values nomalized to cellular volume (μm^3), surface area (μm^2), and dry weight (pg). nd: not determined

		Cadmium		Zinc		Silver		Mercury	
		EC_{50}	EC_0	EC_{50}	EC_0	EC_{50}	EC_0	EC_{50}	EC_0
Metal in solution	3H	3.7	2.4	4.9	4.4	5.9	5.5	8.5	8.3
−log M	Dun	4.0	3.1	3.7	2.4	4.6	4.2	5.3	4.7
	MCH	3.9	2.7	4.3	3.3	6.5	6.1	9.1	8.8
	Osc	nd	nd	5.0	4.0	7.1	6.4	8.7	8.3
Metal $cell^{-1}$	3H	14.9	13.7	15.0	14.6	14.8	14.5	16.85	16.7
−log mol	Dun	14.8	14.2	13.7	12.6	14.2	14.0	14.9	14.5
	MCH	14.5	13.5	14.5	14.2	15.6	15.4	16.67	16.54
	Osc	nd	nd	13.2	12.3	14.6	14.2	15.7	15.5
Metal μm^{-3}	3H	16.7	15.5	16.8	16.4	16.6	16.3	18.64	18.5
−log mol	Dun	16.8	16.2	15.7	14.6	16.2	16.0	16.9	16.5
	MCH	16.7	15.7	16.7	16.4	17.8	17.6	18.83	18.70
	Osc	nd	nd	16.6	15.7	18.0	17.6	19.1	18.9
Metal μm^{-2}	3H	17.0	15.8	17.1	16.7	16.9	16.6	18.95	18.8
−log mol	Dun	17.1	16.5	16.0	14.9	16.5	16.3	17.2	16.8
	MCH	16.8	15.8	16.8	16.5	17.9	17.7	19.0	18.86
	Osc	nd	nd	16.6	15.7	18.0	17.6	19.1	18.9
Metal pg^{-1}	3H	16.3	15.1	16.4	16.0	16.2	15.9	18.2	18.1
−log mol	Dun	16.0	15.4	14.9	13.8	15.4	15.2	16.1	15.7
	MCH	16.2	15.2	16.2	15.9	17.3	17.1	18.37	18.24
	Osc	nd	nd	16.3	15.4	17.7	17.3	18.8	18.6

ity of the metal in question. If we assume that metals initially associate with an algal cell by attaching to some surface ligand, then by normalizing the metal uptake data shown in Fig. 2 and Table 3 on a surface area basis, an interspecific comparison of cell surface reactivity becomes possible for each metal (Fig. 9). The regression lines in Fig. 9 suggest that the surfaces of all 4 species have roughly comparable affinities for Ag over the indicated range of Ag concentrations, while Osc cell surfaces appear to be most reactive for Cd and Zn and Dun cell surfaces are less reactive for Hg than are those of the other species (which are nearly identical to each other). Davies (1976) also found that the Hg reactivity of the Dun cell surface was significantly lower than that of *Isochrysis galbana*, a more Hg-sensitive form. The nature of the surface ligands is, at present, a matter of speculation, although they may well include sulfhydryl, amino, phosphatidic, and hydroxyl groups. Once associated with the plasmalemma, the metals may be translocated into the cell via carrier protein molecules (Williams, 1981), or they may remain bound to the cell surface (Fisher et al., 1983b). Because the EDTA wash to which the cells were subjected is sufficient to remove loosely bound metal (Davies, 1973; Bates et al., 1982; Fisher, unpubl.), it is assumed that the cellular metal values reported here are for metals firmly bound either on the cell surface (i.e. wall or membrane) or in the interior of the cells. The nature of the binding of metals to algal surfaces is still largely uncharacterized and probably varies among metals and algal species, including covalent bonding to proteins for the highly reactive metals and ionic charge bonding for the less reactive metals (Crist et al., 1981).

The volume/volume concentration factors of Zn and Ag reported here are of the same order as Lowman et al. (1971) report for natural phytoplankton populations (on wet weight bases); Lowman et al. give no values for Cd and Hg concentration factors in marine phytoplankton. The concentration factor of Hg in Dun cells was nearly identical to the average value (2.5×10^4) calculated from Davies' (1976) results for this alga. The Cd VCF in *Thalassiosira pseudonana* was comparable to that ($\sim 3 \times 10^2$) estimated for another diatom, *Skeletonema costatum*, using the data of Braek et al. (1980), but lower by up to 2 orders of magnitude than Cd concentration factors in freshwater diatoms (Conway, 1978). The difference between Cd accumulation in freshwater and marine diatoms is possibly due to differences in chloride complexing and in the magnesium concentration of the two water types (Braek et al., 1980). Kayser and Sperling (1980) report Cd concentration factors of $\sim 10^3$ in the marine dinoflagellate *Prorocentrum micans*. It was apparent that the general order of VCFs for all species was Cd < Zn < Ag < Hg. The VCFs of these cells for the IB and IIB transition

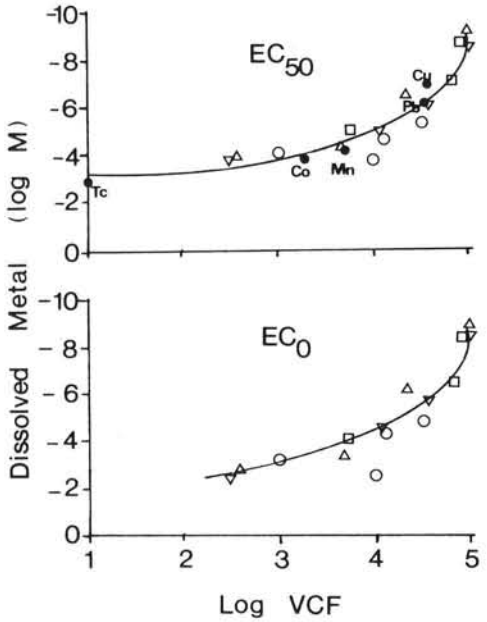

Fig. 8. *Thalassiosira pseudonana, Dunaliella tertiolecta, Emiliania huxleyi, Oscillatoria woronichinii, Nitzschia closterium, Asterionella japonica.* EC_{50} and EC_0 values *vs.* volume/volume concentrations factors (VCF) for all metals and all species. (▽) 3H; (○) Dun; (△) MCH; (□) Osc. Also shown (●) are literature data for other metals: Tc VCF for *T. pseudonana* (Fisher, 1982); estimated Tc EC_{50} for *T. pseudonana* (Gearing et al., 1975); Co, Mn, and Cu VCFs for natural phytoplankton (Lowman et al., 1971); Co toxicity for the diatom *Nitzschia closterium* (Rosko and Rachlin, 1975); Mn, Pb, and Cu toxicity for the diatom *Asterionella japonica* (Fisher and Jones, 1981); Pb VCF data for *T. pseudonana* (Fisher et al., 1983b). All experimental data derived from media containing no chelators

metals thus appear to be lower than those for the transuranic elements (Np excepted), where values of $> 10^5$ are regularly observed (Fisher et al., 1983a). Reasons for the different reactivities of these metals may relate to atomic properties such as polarizing power (cation charge^2/ionic radius) which can influence affinity for available ligands (Turner et al., 1981).

Toxicity

The influence of increasing metal concentration on the cell division rate, i.e. the exponential relation between μ depression and log external metal concentration, is similar to results obtained for other species with Hg (Davies, 1974, 1976), Cu (Sunda and Guillard, 1976; Bentley-Mowat and Reid, 1977; Gavis et al., 1981), Zn (Jensen et al., 1974), Cd (Bentley-Mowat and Reid, 1977; Conway, 1978; Li, 1980), and other metals (Davies, 1978). Kuiper (1981) also observed that μ of a marine *Chlamydomonas* was exponentially related to cellular Hg, similar to our findings for all 4 metals. The sudden decline in μ (Fig. 3 to 6) of some metal-treated cells when cellular metal content exceeds an apparent threshold value suggests that each cell is capable of accommodating a certain amount of metal before sensitive sites within the cell are poisoned. These observed threshold phenomena are the reason that an exponential model best describes the relation between μ depression and log cellular metal concentration. Clearly, different cells have different metal sensitivities and different capacities to harmlessly store metal, with the Dun cells best able to accommodate cellular Zn, Ag, and Hg, very possibly as a result of precipitation of the metal as sulfide (Davies, 1976). Other de-toxification mechanisms identified in other algae have included Cd binding to metallothionein-like proteins (Olafson et al., 1979; Li, 1980) and Cu sequestration in intranuclear inclusions (Silverberg et al., 1976) or sulfur-rich, membrane-bound bodies (Daniel and Chamberlain, 1981).

Care should be taken in extrapolating from laboratory derived EC_{50} values to field situations, since the experimental conditions provided the cells with nearly

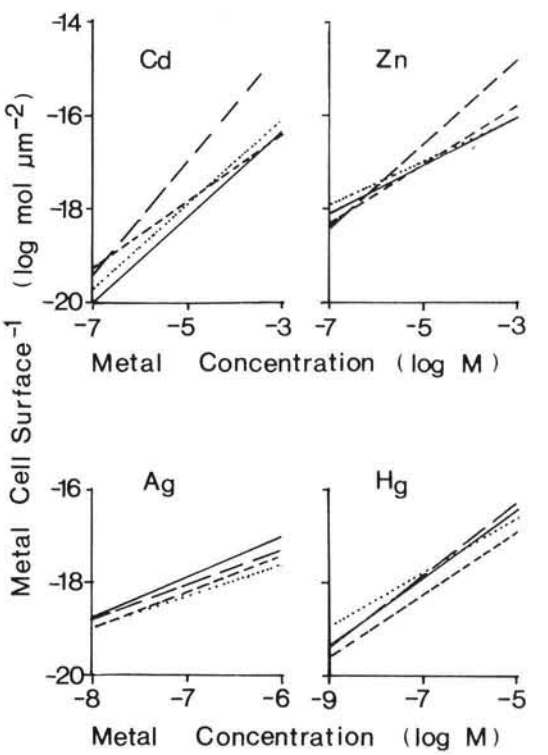

Fig. 9. *Thalassiosira pseudonana, Dunaliella tertiolecta, Emiliania huxleyi, Oscillatoria woronichinii.* Metal content of algal cells, normalized to cell surface area (log mol μm^{-2} cell surface), at different external metal concentrations. Regression lines described in Table 3 were modified to express data on a surface area basis in order to facilitate comparison of the metal reactivities of the various algal cell surfaces. (——) 3H, (– – –) Dun, (· · ·) MCH, (— —) Osc

optimal growing conditions and since the Mn content of the experimental medium might be sufficient to ameliorate the toxicity of the metals as it does for Cu (Sunda et al., 1981). Thus, the EC_{50} values reported here probably underestimate the metal sensitivity of phytoplankton cells in natural waters. Nevertheless, the EC_{50} values calculated in our study suggest that ambient concentrations of Cd ($\leq$ 1 nM), Zn ($<$ 5 nM), Ag ($<$ 10 pM) and Hg ($\sim$ 10 pM) in surface waters of oceanic or unpolluted coastal systems (Bruland, 1980; Martin et al., 1983; Olafsson, 1983) are unlikely to approach levels toxic to marine phytoplankton. Moreover, in comparing EC_{50} values based on cellular metal content of the most sensitive species with metal concentrations found in natural phytoplankton populations in the Pacific and the coastal Mediterranean (Martin and Knauer, 1973; Hardstedt-Romeo, 1982), we see that natural cellular levels (mol metal pg^{-1} dry wt) of Cd are 7×10^{-4} the lowest EC_{50} observed, Ag is around 5×10^{-3} the lowest EC_{50}, Hg is around 10^{-2} the lowest EC_{50}, and Zn is around 5×10^{-2} to 10^{-1} the lowest EC_{50}. In heavily contaminated estuaries and bays, however, cellular concentrations of these metals may conceivably reach toxic concentrations, potentially resulting in alterations in a community's species composition.

An overall picture which emerges when taking into account all the bioaccumulation and toxicity data is that those metals which are concentrated most are the most toxic. The exponential relation of the VCF data with the EC_{50} values suggests that predictions are possible for metal toxicity to phytoplankters based on concentration factor data. Fig. 8 shows that the line correlating log EC_{50} and log VCF data from this study also adequately describes other experimental data taken from diverse sources for other metals and other algal species.

CONCLUSIONS

(1) Accumulation of Cd, Zn, Ag, and Hg by the algae was generally rapid, and equilibria were reached with respect to metal partitioning between dissolved and particulate phases. Uptake, which was passive, was describable by Freundlich adsorption isotherms.

(2) Metal toxicity was an exponential function of cellular metal, but since cellular metal content was linearly related to ambient metal concentration, the toxicity of metals was adequately described as an exponential function of ambient metal as well.

(3) The general order of bioconcentration and toxicity of the metals was Hg > Ag > Zn > Cd. Regression-derived measures of sublethal (EC_{50}) and lethal (EC_0) metal concentrations correlated exponentially with volume/volume concentration factors for the metals.

(4) Variations in metal sensitivity of the algal species examined were sometimes attributable to differential metal reactivity of cell surfaces and sometimes to different abilities of the cells to accommodate higher cellular metal concentrations.

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