Journal of Marine Research, 49, 339–354, 1991

The accumulation of barium by marine phytoplankton grown in culture

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ABSTRACT

Marine phytoplankton have been implicated as potentially important vectors for the vertical transport of barium in the oceans. To better assess the extent to which phytoplankton can influence the geochemical cycling of barium, its bioconcentration was studied in 21 clones of 19 species of marine phytoplankters belonging to 9 algal classes. Barium levels in the ash ranged from less than 2 $\mu g\ g^{-1}$ for the coccolithophore *Emiliania huxleyi* and the red alga *Porphyridium cruentum* to 589 $\mu g\ g^{-1}$ for the flagellate *Tetraselmis levis.* Concentrations $\geq 4000\ \mu g\ g^{-1}$, previously reported for certain samples of diatom ash were not encountered in this study. Concentration factors on a volume basis (VCF) ranged from 0 to 3.2×10^4; the geometric mean VCF for all species was 225. Diatoms and coccolithophores generally had lower VCFs (geometric means of 90 and 12, respectively) than did other species; dinoflagellates had a geometric mean VCF of 490. Experiments with the diatom *Thalassiosira pseudonana* indicated that Ba $cell^{-1}$ increased linearly with ambient Ba concentration. Experiments to localize the site of Ba deposition in diatom cells indicated that most of the Ba was associated with the frustules rather than with the organic fraction.

Dinoflagellates and several other algae not only concentrated Ba to relatively high levels, but also accumulated Si when grown in Si-enriched medium, although they grew at least as well without added Si as with it. Ba and Si accumulation were generally negatively correlated.

1. Introduction

Interest in the biogeochemistry of barium was initially motivated by the possibility that its behavior would resemble that of the similar heavy divalent IIA element, radium. Radium-226 has been suggested as a tracer for oceanic circulation patterns (Chan *et al.,* 1976), but this application requires knowledge of the extent to which the distribution of ^{226}Ra is influenced by biological activity in the sea (Szabo, 1967; Wolgemuth and Broecker, 1970; Shannon and Cherry, 1971; Li *et al.,* 1973). Since there is no stable isotope of radium by which biological transport can be monitored, the possibility was raised that Ba might serve as a stable chemical analogue of ^{226}Ra in the marine environment.

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Vertical profiles of Ba in the Atlantic and Pacific Oceans show Ba concentrations increasing from surface to depth by a factor of two to three, with concentrations ranging from 40 to 200 nM (Wolgemuth and Broecker, 1970; Chow, 1976), consistent with the hypothesis that Ba is incorporated in the remains of organisms and released to the deep waters via sinking of organic debris. High concentrations in some pelagic sediments have been correlated with biological activity in the overlying water column (Goldberg and Arrhenius, 1958; Turekian and Tausch, 1964). The biological agents involved in Ba transfer are not well identified, and various organisms have been proposed—calcareous foraminifera, radiolaria (Goldberg and Arrhenius, 1958) and, most particularly, diatoms (Brongersma-Sanders, 1967). Table 1 summarizes published Ba analyses made on planktonic or benthic algal material from natural collections or cultures. Phytoplankters probably introduce Ba into the marine food web, and they thus begin the process of lateral and downward redistribution of the element in the water column, which must be understood quantitatively before a Ba budget for the sea can be established. Dehairs *et al.* (1980, 1987) noted that most of the particulate Ba in the Atlantic and Pacific Oceans is in the form of 1 μm barite particles. They concluded that marine biota are involved in barite formation and that most of the Ba in the water column is recycled. They also pointed out that experimental data on Ba accumulation by phytoplankton are scarce, although such information would be valuable in interpreting oceanographic observations and constructing Ba budgets for the oceans.

Therefore, we have conducted a study to address the question of Ba accumulation in a wide variety of marine phytoplankton, including algal groups (particularly dinoflagellates and coccolithophores) for which no data are yet available. We have also examined the Si content of the algae. The relationships between Ba and Si are of interest because diatoms have been implicated as the principal scavengers of Ba from surface waters (Brongersma-Sanders, 1967), attributable to diatoms' supposed ability to coprecipitate Ba accompanying Si deposition (Ng, 1975).

2. Materials and methods

a. Algal species. The 21 clones of the 19 species studied are listed in Table 2. All inocula came from axenic stocks (except for clones MCH 1 and 451-B) and cultures were handled aseptically throughout the experiments; no rigorous checks were made of bacterial contamination at the end of the experiments, although there were no indications that any of the cultures became appreciably contaminated. Clone MCH 1 of the coccolithophore *Emiliania huxleyi* produces abundant coccoliths (in suitable medium); the other two clones of this species did not produce coccoliths. All clones used may be obtained from the Center for Culture of Marine Phytoplankton, Bigelow Laboratory for Ocean Sciences, West Boothbay Harbor, Maine.

Table 1. Ba concentrations reported in marine algae.

Species	Source*	Ba μg/g ash wt	Ba μg/g dry wt	Analytical Method**	Reference
Bacillariophyceae					
Chaetoceros curvisetum	P	4000		AA	Vinogradova & Koval'skiy (1962)
Melosira granulata	P	25–30		AA	Vinogradova & Koval'skiy (1962)
Nitzschia seriata	P	20–30		AA	Vinogradova & Koval'skiy (1962)
Rhizosolenia calcar-avis	P	20000–30000		AA	Vinogradova & Koval'skiy (1962)
Rhizosolenia alata	CN		58	NA	Dehairs *et al.* (1980)
Rhizosolenia alata	CA		69	NA	Dehairs *et al.* (1980)
Asterionella japonica	CA	311	75	AS	Riley & Roth (1971)
Phaeodactylum tricornutum	CA	1200	95	AS	Riley & Roth (1971)
Skeletonema costatum	CN	7–90	3–36	ID	Ng (1975)
Diatoms, Irish Sea	P	542	248	AS	Riley & Roth (1971)
Diatoms, N. Atlantic	P	44–140		ES	Thompson *et al.* (1967)
Diatoms, S.E. Atlantic	P	70–450	34–226	ID	Ng (1975)
Diatoms, Antarctic	P	140–1000	69–503	ID	Ng (1975)
Prymnesiophyceae					
Monochrysis lutheri	CA	1320	70	AS	Riley & Roth (1971)

Table 1. (Continued)

Species	Source*	Ba μg/g ash wt	Ba μg/g dry wt	Analytical Method**	Reference
Chrysophyceae					
Olisthodiscus luteus	CA	467	35	AS	Riley & Roth (1971)
Pseudopedinella pyriformis	CA	1158	86	AS	Riley & Roth (1971)
Cryptophyceae					
Hemiselmis virescens	CA	945	67	AS	Riley & Roth (1971)
Hemiselmis brunescens	CA	1720	262	AS	Riley & Roth (1971)
Prasinophyceae					
Heteromastix longifilis	CA	392	55	AS	Riley & Roth (1971)
Micromonas squamata	CA	2840	145	AS	Riley & Roth (1971)
Tetraselmis tetrathele	CA	1710	128	AS	Riley & Roth (1971)
Chlorophyceae					
Dunaliella primolecta	CA	2290	80	AS	Riley & Roth (1971)
Dunaliella tertiolecta	CA	1170	38	AS	Riley & Roth (1971)
Chlamydomonas sp.	CA	2620	76	AS	Riley & Roth (1971)
Chlorella salina	CA	743	71	AS	Riley & Roth (1971)
Stichococcus baciliaris	CA	1010	49	AS	Riley & Roth (1971)
Enteromorpha intestinalis	B		0.4	NA	Bowen (1956)
Ulva lactuca	B		2.5	AS	Riley & Roth (1971)

Table 1. (Continued)

Species	Source*	Ba μg/g ash wt	Ba μg/g dry wt	Analytical Method**	Reference
Rhodophyceae					
Gigartina stellata	B		2.1	NA	Bowen (1956)
Chondrus crispus	B		5.6	NA	Bowen (1956)
Rhodymenia palmata	B		0.6	NA	Bowen (1956)
Phaeophyceae					
Fucus serratus	B		14	NA	Bowen (1956)
Fucus vesiculosus	B		10	NA	Bowen (1956)
Laminaria digitata	B		7	NA	Bowen (1956)
Laminaria saccharina	B		11	NA	Bowen (1956)
Ascophyllum nodusum	B		6	NA	Bowen (1956)
Chorda filum	B		19	NA	Bowen (1956)
Sargassum sp.	P	75–170	19–980	NA	Bowen (1956)
Organic fraction of phytoplankton	P		5–154	AA	Martin & Knauer (1973)

*P = plankton
*B = benthos
*CA = culture, artificial seawater
*CN = culture, enriched natural seawater

**AA = atomic absorption
**AS = DC arc spectrographic analysis
**ID = isotope dilution
**ES = DC emission spectrographic analysis
**NA = neutron activation

Table 2. Algal species studied in culture. Nomenclature according to Parke and Dixon (1976).

	Clone	Source
Class Bacillariophyceae		
Phaeodactylum tricornutum	Phaeo	Great South Bay, LI, NY
Skeletonema costatum	Skel	Milford, CN
Fragilaria pinnata	13-3	33°11′N, 65°15′W
Thalassiosira rotula	289-1	Peru upwelling region
Thalassiosira pseudonana	3H	Forge River, LI, NY
Thalassiosira oceanica	13-1	33°11′N, 65°15′W
Class Prymnesiophyceae		
Emiliania huxleyi	BT-6	32°10′N, 64°30′W
Emiliania huxleyi	451-B	Oslofjord
Emiliania huxleyi	MCH 1	off Bermuda
Prymnesium parvum	Prym	? (from M. Droop)
Class Dinophyceae		
Gonyaulax polyedra	GP60	? (from W. Hastings)
Gymnodinium nelsoni	GSBL	Great South Bay, LI, NY
Gymnodinium sp.	Gymno	Galveston, TX
Prorocentrum minimum	Exuv	Great South Bay, LI, NY
Scrippsiella trochoidea	Peri	? (from M. Parke)
Class Cryptophyceae		
Chroomonas salina	3C	Milford, CN
Class Chlorophyceae		
Dunaliella tertiolecta	Dun	? (from H. Davis)
Class Prasinophyceae		
Tetraslemis levis	Platy-1	Falmouth Great Pond, MA
Class Eustigmatophyceae		
Nannochloropsis salina	GSB Sticho	Great South Bay, LI, NY
Class Rhodophyceae		
Porphyridium cruentum	Porph	Woods Hole, MA
Class Cyanophyceae		
Oscillatoria sp. (*woronichinii?*)	Sm-24	Falmouth Great Pond, MA

b. Culture methods. General techniques and media are described in detail elsewhere (Guillard, 1975), but some of the main aspects are presented below. Glassware was cleaned with dilute HCl or HNO_3, then detergent, and rinsed finally with deionized distilled water having no detectable Ba. Flask stoppers were silicone rubber or cotton; the latter contained Ba but too little to contribute measurable contamination. Small quantities of Ba were found to leach out of glass walls into seawater stored in glass vessels for periods of 18 days, so that on average the dissolved Ba concentration increased by 31%; no such leaching was detected in water stored in polypropylene vessels. Ba showed no evidence of sorption to any container surface.

Medium used for stock cultures was Vineyard Sound seawater (approx. 32 ppt) filtered through a 10 μm nylon cartridge filter and enriched as half-strength medium *f* (Guillard and Ryther, 1962). The experimental media were *f*/2 (Si addition of

107 μM) or modifications thereof: clone MCH 1 was grown in *f*/50 to ensure coccolith formation; for culturing certain species (not diatoms), silicon was omitted from the medium, autoclaved in polycarbonate vessels, to see if Si abundance in the medium influenced Ba accumulation by the cells.

Cultures were incubated at 20 C under cool-white fluorescent lamps producing about 60 to 80 μEin $m^{-2}sec^{-1}$ at the culture surface (L:D cycle: 14:10 h). Most experimental cultures were grown in 2 l borosilicate Erlenmeyer flasks equipped with aerators, while some species were cultured in larger quantities in carboys equipped with aerators. The complete medium was autoclaved in these vessels after the pH had been reduced to about 3.5 using HCl to minimize precipitation. The pH was then returned to 8.0 with NaOH after the medium had been cooled and aerated; no apparent precipitation resulted. In several cases (clones Platy-1, Exuv, Peri, GSBL, MCH 1, BT-6, Sm-24, 3C, GSB Sticho, Prym), nutrients were added aseptically to the seawater after autoclaving. Each batch of seawater used was analyzed for Ba content before and after enrichment and sterilization. The Ba content of the medium, measured immediately prior to inoculation, typically lay in the range 69 to 73 nM. [Ng (1975) reported similar values in her modified *f*/2 enrichment of Vineyard Sound seawater sterilized by filtration and Bernat *et al.* (1972) measured 73 nM Ba in surface Mediterranean waters.] Exceptions are noted in the text. The percent removal of Ba by the algal cells was calculated by dividing the Ba in algal ash harvested l^{-1} by the dissolved Ba l^{-1} present initially in the culture medium (assumed for calculations to be 73 nM).

To assess the relationship of Ba $cell^{-1}$ with ambient Ba in the medium, an experiment with replicate cultures of *Thalassiosira pseudonana* (3H) was conducted in which the medium was enriched with Ba (added as $BaCl_2$) up to 970 nM. The Ba content of the diatom ash was examined for each medium.

Initial algal cell densities in the cultures were set at less than 1% of harvest density. Most cultures were harvested at 4–6 d. Since the incubation periods lasted for at least 7 generations and only a small fraction of the available Ba was typically removed by the cells (see below), it is presumed that at the time of cell harvest, an equilibrium for Ba partitioning between dissolved and cellular phases had been reached.

Population densities were measured microscopically by counting cells in chambers appropriate to the cell sizes and culture densities (Guillard, 1973). Cell volumes were measured with a Coulter Counter (Model TA-II) and checked microscopically. For each species, the cell volume used was that of the modal size class. For chain forming species, microscopic estimates of cell volume were used.

Cells were harvested by filtration onto 1.2 μm Millipore cellulose acetate filters which are virtually ashless and noncontaminating (Bankston and Fisher, 1977). Volumes filtered were up to 4.8 l. In a few cases, cells were first concentrated by centrifugation for 15 min (3000 g, 10 C) in noncontaminating polycarbonate centrifuge bottles; no medium contact with metal occurred. Each filter was then rinsed

Table 3. Ba and Si in cultured marine phytoplankton. Also shown: final cell concentrations (per ml); pH of cultures; ash and dry weights; volumes of cells; Ba removed from the medium by cells (%); volume/volume concentration factors (VCFs) and ash weight concentration factors (ACFs) for Ba in the cells.

species	clone	final cell concentration $\times 10^4$	final pH	ash wt. analyzed (mg)	dry wt. per cell (pg)	ash wt. per cell (pg)
Phaeodactylum tricornutum	Phaeo	142	8.4	8.2	22.6	3.3
Skeletonema costatum	Skel	96	9.0	8.1	nd	10.5
Fragilaria pinnata	13-3	147	9.3	9.4	nd	8.0
Thalassiosira rotula	289-1	1.9	8.8	7.60	1258	422
Thalassiosira oceanica	13-1	136	8.6	10.50	31.6	10.2
Thalassiosira pseudonana	3H	380	8.9	21.4	17.5	4.7
Emiliania huxleyi	BT-6	798	8.9	81.5	7.5	0.4
Emiliania huxleyi	451-B	88	8.0	4.1	17.4	1.0
Emiliania huxleyi	MCH 1	55	8.1	111.8	49.9	47.7
Prorocentrum minimum	Exuv	3.8	8.8	6.6	997	87.9
**Prorocentrum minimum*	Exuv	13	8.9	9.6	371	29.7
Gymnodinium nelsoni	GSBL	nd	8.2	7.6	nd	nd
Heterocapsa pygmaea	Gymno	260	8.5	9.7	13.3	2.5
Gonyaulax polyedra	GP60	1.2	8.5	5.2	5996	249
Scrippsiella trochoidea	Peri	0.6	8.2	13.8	3850	758
Dunaliella tertiolecta	Dun	112	8.9	3.6	24.1	1.6
Nannochloropsis salina	GSB Sticho	430	8.0	17.8	5.9	0.9
Tetraselmis levis	Platy-1	53	9.1	22.8	162.6	29.0
†*Oscillatoria* sp.	Sm-24	15	9.7	120.4	500.3	200
*†*Oscillatoria* sp.	Sm-24	5	9.6	2.2	688	44.2
Prymnesium parvum	Prym	34	8.2	5.8	40.4	9.7
**Prymnesium parvum*	Prym	51	8.2	5.3	18.1	3.6
Chroomonas salina	3C	47	9.2	10.2	58.4	6.2
Porphyridium cruentum	Porph	339	9.3	323.4	nd	40.4

*grown without added Si †per filament nd: not determined

with two 5 ml washes of a prefiltered 3.2 ppt NH_4COOH solution (to remove contaminating salts) and dried at 70 C. Dry weight measurements of formate-washed cells were simultaneously made on separate smaller portions of the cultured material using GFF glass fiber filters (Fisher and Schwarzenbach, 1978). Preliminary experiments using replicate samples of *Thalassiosira pseudonana* (3H), washed and unwashed, showed that formate rinsing contributes no Ba to the analytical sample and does not leach measurable Ba from cells. Cultures from some carboys were collected by centrifugation and were not washed with ammonium formate. The ash of precipitate from uninoculated carboy "cultures" caught on filters had 6–8 μg Ba g^{-1}, corresponding to 52–54 ng particulate Ba l^{-1} in autoclaved media with heavy precipitation (Thompson and Bankston, unpubl.).

Table 3. (Continued)

ash/dry (%)	Vol. per cell (μm^3)	Si in ash (%)	Si/cell (pg)	Ba in ash ($\mu g/g$)	Ba/cell (fg)	Ba removed (%)	Ba VCF × 100	Ba ACF × 100
15	150	9	0.30	49	0.16	2.3	1.1	1.57
nd	268	30	3.15	21	0.22	2.1	0.8	0.67
nd	136	26	2.08	21	0.17	2.4	1.2	0.67
34	17000	34	144	21	9.0	1.7	0.5	0.67
32	134	36	3.67	17	0.18	2.4	1.3	0.54
27	67	35	1.66	11	0.05	1.9	0.8	0.35
5	67	0	0	40	0.02	1.6	0.3	1.28
6	76	17	0.16	46	0.04	0.4	0.6	1.47
96	144	0	0	0	0	0.0	0.0	0.00
9	1072	9	8.21	50	4.38	1.7	4.2	1.60
8	1072	0	0	313	9.28	12.1	9.9	10.02
17	34000	10	nd	328	nd	8.3	nd	10.50
19	268	0	0	250	0.62	16.1	2.8	8.00
4	17000	0	0	96	23.9	2.9	1.5	3.07
20	8580	8	71	323	245	14.4	32.7	10.34
7	134	0	0	209	0.34	3.8	2.6	6.69
15	17	7	0.06	155	0.14	6.0	8.7	4.96
18	536	5	1.48	589	17.1	90.1	320.4	18.85
40	nd	15	29.50	9	1.76	2.6	nd	0.29
6	nd	0	0	344	15.21	7.6	nd	11.01
24	134	11	1.04	64	0.62	2.1	4.7	2.05
20	134	1	0.03	192	0.70	3.6	5.5	6.14
11	134	6	0.39	223	1.39	6.5	11.0	7.14
nd	nd	0	0	0	0	0.0	nd	0.00

c. Analysis of samples. Dried, filtered cell samples were ashed at 550 C for 15 h and the ash was weighed. The samples were then treated by a modified lithium metaborate fusion technique (Ingamells, 1970) and analyzed for Ba and Si using an atomic emission spectrometer/spectrograph equipped with a dc argon plasma jet excitation source and an echelle diffraction grating (AESS) as described by Bankston and Fisher (1977). The analytical method can detect Ba levels as low as 2 $\mu g\ g^{-1}$ in algal ash and 14 nM in solution with a coefficient of variation of 7%.

d. Digestion of organic matter. In a first step toward determining the cellular site of Ba deposition, attempts were made to measure Ba in frustules and in the organic content of *Thalassiosira rotula* (289-1) and *Thalassiosira pseudonana* (3H). Three different techniques were used, employing either strong acid (50% HNO_3), ultravio-

let light irradiation (about 9 cm from a 1200 watt tube) or enzymatic digestion (crude protease and DNAase, Sigma Chemical Co.). For each method, cells from one liter of dense culture were concentrated by centrifugation or by filtration on Nuclepore polycarbonate filters. For acid cleaning, the cells were resuspended in their own medium, in glass vessels. For UV treatment, they were resuspended in their own medium slightly acidified, in quartz tubes. For enzymatic treatment, the cells were burst osmotically in deionized distilled water, washed in acetone to remove lipids, again in water, and suspended in water for enzymatic treatment. All digestions took several days. When digestion was essentially complete, the frustules were removed by filtration and both the frustules and liquid digests analyzed for Ba by dc argon plasma-echelle optical emission spectrometry.

3. Results

a. Weights. Ash weights (Table 3) varied from 4% to 96% of the cellular dry weights depending on species and culture conditions. The *Emiliania huxleyi* clone MCH 1 which produces coccoliths in great abundance had the highest relative ash content—96%—although this ash derives largely from coccoliths shed into the medium during growth. The two clones of this species that produced no coccoliths (451-B and BT-6) had ash weights of 5-6% of the dry weight. Values for diatoms were about 30% for most species, but only 15% for *Phaeodactylum tricornutum.* In the triradiate form (as used here), *Phaeodactylum tricornutum* has no frustule at all, even when it occurs in colonies as the triradiate form (Nelson *et al.,* 1984). Ash weights of species other than diatoms seldom exceeded 20% of the dry weights; however, the cyanophyte *Oscillatoria* cf. *woronichinii* (clone Sm-24) attained 40% when cells were grown in medium with silicate added. The ash weight:dry weight ratios of the dinoflagellate *Prorocentrum minimum* (clone Exuv) and the prymnesiophyte flagellate *Prymnesium parvum* were not appreciably affected by silicon additions to the medium.

b. Barium contents. Most species removed only a small fraction of the Ba in the medium—<3%—but the dinoflagellates and *Oscillatoria* sp. removed as much as 10% under some circumstances, and a dense culture of *Tetraselmis levis* removed 90% in one experiment (Table 3).

Barium concentrations in algal ash of cells grown with added silicon (Table 3) ranged from non-detectable (<2 $\mu g\ g^{-1}$) in the red alga *Porphyridium cruentum* and in the coccolithophore *Emiliania huxleyi* clone MCH 1 (grown in *f*/50) to 589 $\mu g\ g^{-1}$ for *Tetraselmis levis* (Platy-1). Three dinoflagellates, *Gymnodinium nelsoni, Scrippsiella trochoidea* and *Prorocentrum minimum,* exceeded 300 $\mu g\ g^{-1}$ in their ash. As a rule, the diatoms and prymnesiophytes had lower Ba ash contents than representatives of the other groups studied. The one member of the Rhodophyceae we studied (*Porphyridium*) had no detectable Ba or Si (but this is not universal in the class, as shown in Table 1).

Concentration factors for Ba in algal cells were calculated on a volume-volume

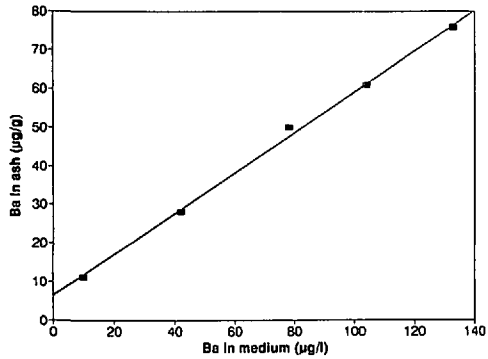

Figure 1. Ba concentration in the ash of *Thalassiosira pseudonana* (3H) as a function of the Ba concentration in the growth medium. $y = 0.531x + 6.248$ ($r = .999$, $P < .001$). The lowest Ba concentration in the medium (10 µg l^{-1}) and the Ba concentration in the diatom cells growing in this medium are mean values from three replicate cultures from separate experiments.

basis, determined as Ba µm^{-3} cell divided by Ba µm^{-3} medium at the time of cell harvest. Concentration factors (VCFs) ranged from 0 to 3.2×10^4 (Table 3), with a geometric mean value of 225 for all species tested. The geometric mean for diatoms was 90, for dinoflagellates 490, for coccolithophores 12, and for other species 2064. *Tetraselmis levis* had the highest VCF value. Concentration factors for Ba were also determined on an ash weight basis (Table 3, denoted ACF), determined as Ba g^{-1} cell ash divided by Ba g^{-1} ash in the ambient seawater used (313 ng g^{-1}). The geometric mean ACF for Ba in all species was 164. There were no significant relationships between Ba concentration factors and the size of the cells or the pH of the cultures at time of harvest.

Ba concentrations in *Thalassiosira pseudonana* samples derived from cultures containing varying concentrations of Ba are presented in Figure 1, which shows a significant linear relationship between the Ba content of the ash and of the medium.

No Ba was detected in the organic fractions (digests) of *Thalassiosira* clones 289-1 or 3H, or in the water from which ultraviolet light-irradiated frustules had been removed, indicating that most (if not all) of the Ba was associated with the frustules. Dehairs *et al.* (1980) similarly concluded that Ba associated with the diatom *Rhizosolenia alata* was entirely associated with the silica of the cells. The localization of Ba in species other than diatoms was not investigated.

c. Barium accumulation in low-silicon medium. Omission of silicon from the enrichment resulted in higher Ba accumulation by the cells harvested. Thus, the Ba concentration in ash of *Prorocentrum minimum* increased from 50 to 313 µg g^{-1}, of *Oscillatoria* sp. from 9 to 344 µg g^{-1}, and of *Prymnesium parvum* from 64 to 192 µg g^{-1} (Table 3). This greater Ba concentration in the ash of low-silicon grown cells was accompanied by increases in absolute amounts of Ba per cell (increases of 112% for

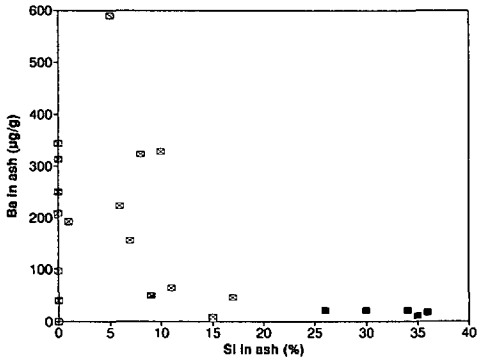

Figure 2. Ba and Si concentrations in the ash of all clones examined. Solid rectangles denote data for diatoms.

Prorocentrum minimum, 764% for *Oscillatoria* sp., and 71% for *Prymnesium parvum*) (Table 3), showing that higher Ba concentrations in the ash are not attributable merely to lower ash content per cell.

d. Silicon contents. The Si levels of all clones are also given in Table 3. [Si contents in diatom ash detected with AESS agree with measurements on several of our samples by D.M. Nelson (unpubl.), made colorimetrically following a Na_2CO_3 fusion according to Nelson and Goering (1977).] Diatom ash values ranged from 9% Si for *Phaeodactylum tricornutum* to 36% for *Thalassiosira oceanica* (13-1), with most nearly 30%. In the coccolithophores, two clones of *Emiliania huxleyi* (MCH 1, with coccoliths, and BT-6, without coccoliths) had no detectable Si. The third clone, 451-B, also without coccoliths, had 17% Si in the ash. The absolute amounts of both ash and Si in the naked clones were small, and this observation requires further consideration. The ash of the prymnesiophyte *Prymensium parvum* had 11–12% Si when the alga was cultured in complete medium but only 1% Si when the alga was cultured (from the same inoculum, in the same batch of water) with Si omitted from the medium. The ash contents of other clones cultured in *f*/2 without Si generally contained no detectable Si. In complete medium (Si added) the range was 5–15% Si with the notable exceptions that in *Porphyridium cruentum* and *Dunaliella tertiolecta,* none could be detected.

For all algae, on an ash concentration basis, Ba was negatively correlated with Si (Fig. 2), although this was largely due to the low Ba content of the diatoms. Excluding diatoms, there was no apparent relationship between Ba and Si content in algal ash. For diatoms alone, on an ash concentration basis, Ba was negatively correlated with Si ($r = -0.953$), although this relationship becomes insignificant ($r = -0.56, P > .05$) by omitting the lightly silicified diatom *Phaeodactylum tricornutum.*

4. Discussion

The barium concentrations in phytoplankton ash samples in this study are well within the range generally reported for natural and cultured populations (Table 1). No exceptionally high barium concentrations were found, in contrast to some findings (Table 1), suggesting that intact phytoplankton cells would not generally act as important vectors for barium transport in the sea. Dehairs *et al.* (1980), working with the diatom *Rhizosolenia calcar-avis,* the highest barium accumulator previously reported (Table 1), came to a similar conclusion. Thus, our data are consistent with the conclusions of Dehairs *et al.* (1980) that most barium associated with total suspended particulates is not associated with siliceous frustules or calcareous tests, although the small fraction that is associated with the diatoms does appear to be silica-bound.

Bishop (1988), analyzing marine particulate matter collected from the upper 1000 m of the N.W. Atlantic, showed that barites are formed in decaying organic matter and the remains of diatoms (including clumps or strings within broken *Rhizosolenia* cells) but are not actively formed by marine plankton and are not found in any intact living cells (including *Rhizosolenia*). Thus, living cells have relatively low Ba concentrations and no barite but decaying cells are rich in Ba, possibly explaining the discrepancy between Ba measurements in *Rhizosolenia*-rich plankton samples (Vinogradova and Koval'skiy, 1962) and laboratory culture studies with these diatoms (Dehairs *et al.,* 1980). While it is now clear that axenic (or nearly so) cultures of phytoplankton do not concentrate Ba to very high levels, future experiments should examine the barium content of senescent, decomposing phytoplankton cells in the presence of bacteria.

Overall, the barium content of diatom ash was lower than that of the dinoflagellates, green-pigmented algae or cryptomonads. Coccolithophores, whether naked or with coccolith production, had low levels of barium, consistent with the observation that Miocene pelagic coccolith oozes are low (Goldberg and Arrhenius, 1958; Turekian and Tausch, 1964; Thompson and Bowen, 1969). It appears that even the dinoflagellates (when intact) do not concentrate barium to sufficiently high levels to serve as important vectors for the vertical transport of this element in pelagic systems. Fowler (unpubl. data) found that euphausiid fecal pellets collected in the Mediterranean contained 370 μg Ba g^{-1} ash, indicating the potential for Ba transport to ocean depths in Ba-rich fecal pellets, probably in the form of barite (Bishop, 1988).

The barium levels in diatom ash were linearly related to levels in seawater, consistent with observations for many other metals in phytoplankton (Fisher, 1986), including barium (Dehairs *et al.,* 1980). These results are also consistent with the hypothesis that barium uptake is a passive process in which metal on cell surfaces is in equilibrium with the metal concentration in ambient seawater (Davies, 1978).

The barium concentration factors found here are well below the concentration

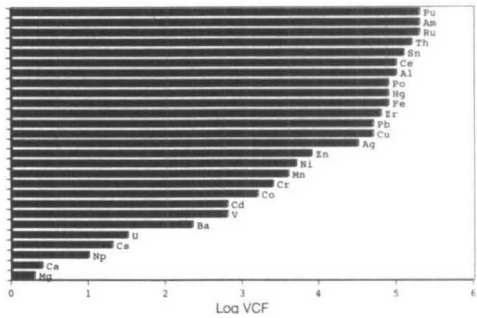

Figure 3. Volume-volume concentration factors (VCFs) for 27 metals, including Ba, in marine phytoplankton. Values shown are logs of the geometric mean values for all species examined. (Values for other metals taken from the compilation in Fisher, 1986; value for Ba from this study.)

factors of many other metals, including both essential and nonessential metals (Fig. 3). By comparison with other metals, barium could be considered as only moderately particle-reactive in seawater. It would be of interest to compare barium accumulation by marine phytoplankters with their strontium and radium accumulation. Owing to similar chemical properties, it is expected that accumulation patterns would be similar for all three metals. Some evidence from natural plankton samples supports this idea. Thus, Li *et al.* (1973) noted that Ra/Ba ratios were constant in particulate matter of the Indian-Antarctic Ocean, and Martin and Knauer (1973) observed that plankton samples off the California coast that were rich in Sr were also rich in Ba. In evaluating field samples, care must be taken to separate acantharians, whose skeletal material is rich in Sr, from phytoplankton. The VCF values measured for Ra in cultured phytoplankton were <300 (Fisher *et al.*, 1987).

The dinoflagellates and green-pigmented algae not only concentrated barium to comparatively high levels, but, curiously, also accumulated silicon when grown in complete medium. These species have no known silicon requirement, and grow at least as well without added silicon. It is unlikely that the finding of silicon in these species is an artifact due to precipitation of silica from the medium, because three species—*Dunaliella tertiolecta, Emiliania huxleyi,* and *Porphyridium cruentum*—were grown and harvested under the same conditions as the other algae, yet had no detectable silicon and because in several instances (see below) the pH of the medium did not get high enough to cause inorganic precipitation of Si. Fuhrman *et al.* (1978) have shown that the prasinophyte *Tetraselmis levis* (clone Platy-1) takes up silicon at a rate proportional to silicic acid concentration in the medium (at high levels) and proportional to the growth rate in the culture. This species, which does not have a macro-requirement for silicon, concentrated silicon and contained large intracellular pools of both water-soluble and water-insoluble silicon. Nelson *et al.* (1984) and

Smith-Palmer *et al.* (1985) concluded that Si uptake by this organism principally occurs by precipitation at pH's >9.5. However, we have found that Si levels were relatively high (7–17%) in the ash of some non-diatoms (e.g., *Emiliania huxleyi* 451-B, *Gymnodinium nelsoni, Scrippsiella trochoidea,* and *Nannochloropsis salina*) even when the pH was ≤ 8.2 (Table 3). The uptake kinetics of Si by nondiatoms warrants further study.

Acknowledgments. We thank V. McAlister and B. Olson for technical assistance, M. H. Bothner for use of the Spectrascan IIB emission spectrometer, and S. W. Fowler for making unpublished data available. S. W. Fowler and R. D. Cherry kindly reviewed an early draft of the manuscript and M. Brzezinski and two anonymous reviewers provided helpful comments on the manuscript. This study was supported by NSF grants OCE 7710876 and OCE 8810657. Contribution No. 783 from the Marine Sciences Research Center.

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Received: *27 September, 1990;* revised: *5 February, 1991.*