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Barium uptake and adsorption in diatoms

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Abstract—Using trace metal-defined culture conditions, we measured the cellular barium concentration in cultures of the diatom *Thalassiosira weissflogii*. In cultures with low Fe concentration, at a typical surface seawater Ba concentration of 35 to 40 nmol/L, the cellular Ba was 0.5 $\mu\text{mol/mol P}$ ($\sim 0.04 \mu\text{g/g dw}$), much below all previously published values for phytoplankton. When the Fe concentration in the growth medium was increased, the measured “cellular” Ba increased proportionally to the particulate Fe. Under conditions of ferric hydroxide precipitation, similar particulate Ba concentrations were measured in the absence and the presence of cells for a given concentration of particulate Fe. Thus, the bulk of the particulate Ba in such experiments was not truly intracellular but adsorbed on precipitated Fe hydroxides associated with the cell surface. A similar mechanism likely explains the high cellular Ba concentrations reported by previous investigators who utilized relatively high ratios of Fe to EDTA concentrations in the growth medium, resulting in Fe precipitation. On the basis of our results, it seems highly unlikely that intracellular uptake by living phytoplankton explains the vertical flux of Ba in oceanic waters. But the adsorption of Ba on Fe hydroxides that is seen in laboratory experiments must also occur in the oceans and contribute to Ba transport in the upper part of the water column. To be significant, however, such a mechanism would require that the particles that transport Ba out of the surface have a markedly lower Ba/Fe ratio than is measured in the average suspended material. Copyright © 2005 Elsevier Ltd

1. INTRODUCTION

Barite, a primary Ba mineral (BaSO_4), is among the most studied proxies used to reconstruct primary production in the paleo-ocean. High barite concentrations have been measured in sediments underlying highly productive waters (Goldberg and Arrhenius, 1958; Turekian and Tausch, 1964; Paytan et al., 1993; Gingele and Dahmke, 1994) and the flux of particulate biogenic barium correlates well with the flux of organic carbon (Dehairs et al., 1980; Stroobants et al., 1991; Dymond et al., 1992; Francois et al., 1995; Paytan et al., 1996; Jeandel et al., 2000). But interpretations of barite abundance in sediments are hampered by the absence of a fundamental understanding of the mechanisms producing marine barite.

It is generally agreed that barite is undersaturated in the upper water column (except at some locations in the Southern Ocean; Monnin et al., 1999; Rushdi et al., 2000), where it is assumed to form (Legeleux and Reyss, 1996), and that its precipitation is the direct or indirect result of biologic activity. Some benthic and freshwater organisms are known to precipitate barite (Arrhenius and Bonatti, 1965; Tendal, 1972; Bertram and Cowen, 1997). However, since no such pelagic marine organism has ever been identified, the most commonly accepted hypothesis is that barite forms as a result of passive precipitation through enrichment of sulfate and/or barium in decomposing organic material (Chow and Goldberg, 1960; Dehairs et al., 1980; Bishop, 1988; Bernstein et al., 1992; Jeandel et al., 2000). According to Bernstein et al. (1992, 1998), barite formation may involve

acantharians. Their skeletons and cysts, made of celestite (SrSO_4), are enriched in Ba compared to seawater and, when degrading and aggregating with other biogenic particles, release sulfate and barium into the flocs, thus possibly allowing barite precipitation. However, a recent study, in which a significant barium depletion was observed in surface waters off the Pacific coast of Baja California, without corresponding strontium depletion, argues against the role of acantharians as the primary source of biogenic barium (Esser and Volpe, 2002). Another hypothesis, based on Vinogradova and Koval'skiy's (1962) work, is that diatoms accumulate enough Ba to induce barite formation during their decay (Bishop, 1988). The fair correlation between silicate and dissolved barium in the water column (Ku et al., 1970; Bacon and Edmond, 1972; Jeandel et al., 1996; Monnin et al., 1999) strengthens the hypothesis of a possible influence of diatoms on the Ba cycle.

Few laboratory experiments have investigated the uptake of Ba by diatoms. Dehairs et al. (1980), as well as Fisher et al. (1991), observed Ba accumulation in the cells and proposed that most of it, if not all, was associated with the frustules. Ganeshram et al. (2003) found high Ba concentrations in phytoplankton from cultures and natural samples and showed that phytoplankton decomposition could be the source of barite in seawater. But none of the culture studies have used media with defined trace metal chemistry and the published results may have been affected by precipitation of barite and/or adsorption of Ba^{2+} on amorphous iron hydroxide precipitated in the medium. Here we report on the Ba accumulation in the model diatom *Thalassiosira weissflogii* under chemically well-defined culture conditions with particular attention to the formation of Fe hydroxide precipitates in the culture medium.

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Table 1. Composition of the culture medium (based on Price et al., 1988/1989).

	Substance	Concentration (mol/L)
Gulf Stream Water	(background Ba of 33–40 nmol/L)	
Nutrients	NaH ₂ PO ₄ · H ₂ O	1.00 × 10 ⁻⁵
	NaNO ₃	3.00 × 10 ⁻⁴
	Na ₂ SiO ₃ · 9H ₂ O	1.00 × 10 ⁻⁴
Trace metals	FeCl ₃ · 6H ₂ O	6.10 × 10 ⁻⁸ to 3.36 × 10 ⁻⁶
	MnCl ₂ · 4H ₂ O	1.21 × 10 ⁻⁷
	CuSO ₄ · 5H ₂ O	1.96 × 10 ⁻⁸
	ZnSO ₄ · 7H ₂ O	7.98 × 10 ⁻⁸
	CoCl ₂ · 6H ₂ O	5.04 × 10 ⁻⁸
	Na ₂ MoO ₄ · 2H ₂ O	1.00 × 10 ⁻⁷
	Na ₂ SeO ₃	1.00 × 10 ⁻⁸
	EDTA	1.00 × 10 ⁻⁴
Vitamins	B ₁₂	5.5 × 10 ⁻⁷ g/L
	Biotin	5.0 × 10 ⁻⁷ g/L
	Thiamine HCL	1.0 × 10 ⁻⁴ g/L

2. MATERIALS AND METHODS

2.1. Phytoplankton and Culture Medium

Stock cultures of the marine diatom *Thalassiosira weissflogii*, CCMP 1336 (CCMP, Bigelow, Maine, USA), were maintained in polycarbonate bottles under continuous fluorescent light (150 μmol photons m⁻² s⁻¹) at 20°C. Although we sterilized the medium, cultures were not axenic, but the bacteria accounted for a negligible fraction of the biomass.

T. weissflogii was grown in a medium enriched with filter-sterilized nutrients, vitamins and trace metals (Table 1) based on the Aquil recipe from Price et al. (1988/1989), in which Synthetic Ocean Water (SOW) was replaced by Gulf Stream Water (GSW). Preliminary incubation experiments indicated that SOW was not a good growth medium for our experiments for it contained a high and variable Ba concentration (120–340 nmol/L) despite lengthy cleaning procedure with Chelex 100 ion-exchange resin. GSW, with a background Ba concentration in the range 33 to 40 nmol/L, was filtered through a 0.2-μm filter before use, and microwave sterilized.

Trace metals were added to the growth medium at total concentrations of: Mn_T = 120 nmol/L, Cu_T = 20 nmol/L, Zn_T = 80 nmol/L, Co_T = 50 nmol/L, Mo_T = 100 nmol/L, Se_T = 10 nmol/L. In the presence of 100 μmol/L EDTA, the corresponding unchelated trace metal concentrations are calculated to be: Mn' = 10 nmol/L, Cu' = 0.2 pmol/L, Zn' = 12 pmol/L, Co' = 17 pmol/L, according to calculation based on the conditional stability constant given by Sunda et al. (in preparation). Three major types of experiments were carried out: (1) Ba uptake in *T. weissflogii* cultures at constant Fe (420 nmol/L) and variable Ba (36–260 nmol/L) concentrations; (2) Ba uptake in *T. weissflogii* cultures at variable Fe (61–3360 nmol/L) and constant Ba (40 nmol/L) concentrations; and (3) Ba adsorption on Fe-oxyhydroxide (FeO_x) precipitates in medium containing no cells, (a) at variable Fe concentrations and constant pH in 1-week incubations to allow comparison with (2), and (b) at constant Fe concentration and various pHs in 24 h incubations to maximize adsorption on fresh FeO_x precipitate. In the presence of 100 μmol/L EDTA in the culture medium, Fe precipitates as an oxyhydroxide colloid when its total concentration exceeds a critical value in the range 100 to 1000 nmol/L, depending on the temperature, pH and light regime of the culture (Anderson and Morel, 1980, 1982; Hudson and Morel, 1990; Sunda et al., in preparation). It then becomes difficult to estimate Fe' concentrations as they are governed not only by the total Fe concentration but also by FeO_x solubility, which evolves as the precipitate ages. As will be seen, the critical Fe concentration in our experiments was ~500 nmol/L. Below this concentration, Fe remained dissolved in the medium and its unchelated concentration, Fe', was calculated to range between 0.1 and 1 nmol/L (Fe' ≈ 10^{-2.7} Fe_T under the pH and light conditions of our experiments). Because of the large excess of EDTA over trace metals, the changes in Fe concentration (which never exceeded 3.45% of the EDTA concentration) had no effect on the speciation of the other trace metals in the medium. Ba²⁺, whose affinity for EDTA is less than that of Ca²⁺ (K_{Ba}/K_{Ca} = 10^{-2.8}), is not significantly complexed by EDTA in seawater medium.

2.2. Culture Methods

All labware was soaked in 5% detergent overnight, then in 10% HCl overnight and finally rinsed with Milli-Q water. Polycarbonate culture bottles with seawater were sterilized by microwaving before addition of nutrients, vitamins and trace metals. Cultures were grown under constant light (150 μmol photons m⁻² s⁻¹) at 20°C and monitored daily with a Coulter Multisizer. In the first set of experiments, the polycarbonate culture bottle with 2 L of growth medium amended with BaCl₂ as needed was inoculated with 10 mL of a stock culture. Cells were harvested in triplicates (600–700 mL) in late exponential phase (~70,000 cells/mL, ~5 d) by filtration onto acid-washed 5-μm polycarbonate (PC) filters (which have a low Ba blank) and the filters were then rinsed with ~40 mL of NaCl previously cleaned by Chelex 100 ion-exchange resin. In the second set of experiments, pH was measured just before harvest and only 120 mL of diatom culture was filtered through 5-μm PC filters, while in the third set of experiments, 40 mL of growth medium was filtered through 0.2-μm PC filters to collect the Fe precipitates. The filters were rinsed four times with 2 mL of buffered NaCl solution (0.7 mol/L with 2.38 mmol/L of HCO₃⁻, pH = 8.2). Filters covered with diatoms were placed into 10-mL Teflon tubes and the cells digested as described below. Filters with only FeO_x precipitates (no cells) were placed into cleaned polypropylene centrifuge tubes; the FeO_x was dissolved by addition of 2 mL of 5% HNO₃ and the samples were measured directly.

2.3. Analysis

The elemental concentrations of Ba, P and Fe in samples were determined using Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) on a Finnigan MAT Element2 (Thermo Finnigan, Bremen, Germany). For each filter with cells in Teflon tube, 800 μL of 50% HNO₃ (Optima Grade) was added, and the tube was tightly capped before being heated below the boiling point for 4 h (Analysis of barite crystals showed that there are ~50% dissolved by this technique, so that the low cellular concentrations reported below cannot result from undigested cellular barite). After digestion, the tubes were filled with Milli-Q water up to 8 mL, and centrifuged at 3000 rpm for 10 min, at 20°C, to separate the silica from the acid-soluble fraction. Ba concentrations in filtrates were measured (after a 20× dilution) in the growth medium before inoculation and after harvest. In one preliminary experiment, we also digested the silica frustules by overnight digestion with HF (0.4 mL/5 mL sample), after HNO₃ digestion. Blanks were made following all the procedural steps, but instead of cell samples, only medium was filtered. At low Ba and low Fe, blanks accounted for up to 50% of the signal. However, the limit detection (defined as 3 times the standard deviation of the blanks) was at least 8 times lower than the signal. The instrument was calibrated using certified standards (High Purity Standards) diluted in Milli-Q water acidified with 5% Optima Grade HNO₃. Direct measurement of standard river water (SLRS-4, National Research Council Canada) ensured the accuracy of

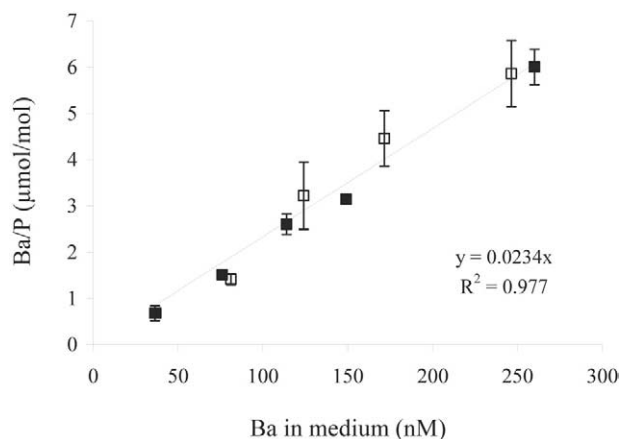


Fig. 1. Ba/P ratios in *Thalassiosira weissflogii* cells vs. Ba concentration in culture medium (filled squares = experiment 1; open squares = experiment 2).

the detection, giving average Ba and Fe concentrations of 12.7 ± 0.6 and 102.6 ± 4.5 ppb respectively ($n = 41$), compared with the certified values of 12.2 ± 0.6 and 103 ± 5 ppb. Sc was used as the internal standard.

3. RESULTS

In a first set of experiments, we measured the cellular accumulation of Ba as a function of external Ba concentration in the presence of a total Fe concentration in the medium of 420 nmol/L (Fig. 1). The cellular quotas are reported as Ba/P (mol/mol) ratios based on simultaneous Ba and P measurements.

The measured cellular barium quotas are proportional to the Ba concentration in the medium (Table 2, Fig. 1). These quotas are also much lower than all previous data on Ba concentrations in phytoplankton (Table 3). For example, the Ba/P ratio of $1.5 \mu\text{mol/mol}$ observed at 76 nmol/L external Ba concentration (which is close to the concentrations most often used in previous studies) corresponds approximately to a dry weight concentration of $0.10 \mu\text{g/g}$ (given a C:P ratio of 86 (Ho et al., 2003) and a dry mass twice that of carbon). In comparison, the culture data of Fisher et al. (1991), Dehairs et al. (1980) and Riley and Roth (1971) show cellular Ba concentrations that are in the range 3 to 95 $\mu\text{g/g}$ (Table 3). In an early experiment, we found little difference between the particulate Ba concentrations measured with or without HF digestion. Thus, the Ba content of the frustule (which would be included in the concentrations reported by Fisher et al., 1991, and Dehairs et al., 1980, but not in ours) cannot account for this discrepancy. The difference between our data and others must thus result from inherent differences among organisms or methodologic differences among investigators. Published data on the composition of various phytoplankton species (Ho et al., 2003) do not show such large differences among diatoms for other trace elements and it seems unlikely that *T. weissflogii* would fortuitously have an extraordinarily low Ba content. The use of natural seawater (which could contain some unknown Ba chelator) rather than a purely artificial medium cannot account for the difference either, since neither we nor Dehairs et al. (1980) found any difference between the two. Indeed, Ba/P ratios measured at high Ba concentration in our natural seawater medium were

comparable to those observed in our artificial medium, which had a high Ba background.

The major methodologic difference between our experiments and previous ones is the buffering of the free trace metal concentrations achieved by adding a large excess of EDTA in the medium. As explained in the Methods section, EDTA has no effect on the speciation of Ba^{2+} in seawater. We thus wondered if the presence of an amorphous iron hydroxide (FeO_x) precipitate forming on the surface of cells in previous culture studies might be the reason for the high particulate Ba concentrations that were observed. The formation of such Fe precipitates in culture media is a difficult problem to control as full growth of the coastal species like *Thalassiosira weissflogii* phytoplankton typically necessitates saturating or near-saturating concentrations of Fe. The solubility of hydrous ferric oxide is a contentious subject (Liu and Millero, 2002; Nakabayashi et al., 2002) and the chemistry of iron in culture medium is complicated by an active redox cycle driven by light and by the organisms (Anderson and Morel, 1980, 1982; Maldonado and Price, 2001; Sunda and Huntsman, 2003). Furthermore, the colloidal nature of the precipitate and its association with the cell surface make its quantification difficult.

To test the possible importance of Ba adsorption on FeO_x , we performed a second set of experiments, maintaining a background Ba concentration (~ 40 nmol/L) in the GSW medium but varying the total Fe concentration from 61 to 3360 nmol/L. Over this range, the hydrous ferric oxide associated with the cells accounts for a variable percentage of the measured "cellular" Fe, from negligible to practically all of it (Ho et al., 2003; Sunda and Huntsman, 1995; Hudson and Morel, 1990). As seen in Figure 2 and Table 4, the measured cellular Ba did indeed increase dramatically with the Fe concentration in the growth medium. At low Fe concentrations, in the range where we expect no FeO_x precipitation, the measured particulate Ba was $0.53 \pm 0.08 \mu\text{mol/mol}$ P, independent of Fe and in good accord with the

Table 2. Ba and P concentrations, and Ba/P ratios in *T. weissflogii* for various Ba concentrations of the culture medium.

[Ba]-in medium (nmol/L)	μ (d^{-1})	Cellular Ba/P ^a ($\mu\text{mol/mol}$)	Cellular [Ba] ^b (nmol/L)
Experiment 1			
37	1.20	0.69 (0.07)	97
76	1.16	1.51 (0.08)	211
114	1.18	2.61 (0.22)	366
149	1.24	3.15 (0.09)	440
260	1.22	6.00 (0.39)	840
Experiment 2			
36	1.18	0.68 (0.16)	95
81	1.17	1.41 (0.12)	198
124	1.22	3.22 (0.73)	451
172	1.18	4.46 (0.60)	625
246	1.23	5.86 (0.71)	821

^a Errors on Ba and P concentrations measured by ICPMS, from which Ba/P errors are derived (in parentheses), were calculated as the standard deviation of the mean divided by the root square of the number of replicates.

^b Cellular Ba concentrations were calculated by multiplying the cellular Ba/P ratio by the cellular P concentration of *T. weissflogii* given by Ho et al. (2003).

Table 3. Ba concentrations reported in diatoms.

Diatom species	Origin ^a	Ambient Ba (nmol/L)	Ba in cells ($\mu\text{g/g}$ dry wt)	Reference ^b
<i>Asterionella japonica</i>	A	No data	75	Riley and Roth (1971)
<i>Phaeodactylum tricornutum</i>	A	No data	95	Riley and Roth (1971)
<i>Rhizosolenia alata</i>	N	74	58	Dehairs et al. (1980)
	A	No data	69	Dehairs et al. (1980)
<i>Phaeodactylum tricornutum</i>	A	69–73	7.2	Fisher et al. (1991)
<i>Thalassiosira rotula</i>	A	69–73	7.0	Fisher et al. (1991)
<i>Thalassiosira oceanica</i>	A	69–73	5.5	Fisher et al. (1991)
<i>Thalassiosira pseudonana</i>	A	69–73	3.0	Fisher et al. (1991)
Diatoms, Irish Sea	S	No data	248	Riley and Roth (1971)
Diatoms, Vineyard Sound (MA)	S	60	5600 ^c	Ganeshram et al. (2003)
<i>Thalassiosira weissflogii</i>	N	76	0.1 ^c	This study (i)
	N	40	0.4 ^c	This study (ii)

^a A = culture in artificial medium; N = culture in natural seawater medium; S = phytoplankton collected from sea.

^b Concentrations of Fe and EDTA in culture medium are 23 $\mu\text{mol/L}$ Fe and 180 $\mu\text{mol/L}$ EDTA for Riley and Roth (1971), 6.5 $\mu\text{mol/L}$ Fe and 15 $\mu\text{mol/L}$ EDTA for Dehairs et al. (1980), 12 $\mu\text{mol/L}$ Fe and 12 $\mu\text{mol/L}$ EDTA for Fisher et al. (1991), and in this study, (i) 420 nmol/L Fe or (ii) 3.4 $\mu\text{mol/L}$ Fe and 100 $\mu\text{mol/L}$ EDTA.

^c Estimated from the Ba/P ratio assuming a dry mass twice that of carbon.

results of Figure 1. At higher Fe concentrations, where FeO_x precipitates, the measured particulate Ba concentration increased proportionally to the particulate Fe, reaching values many times higher than at low Fe concentrations. This is consistent with adsorption of Ba on FeO_x .

If Ba could be removed along with the colloidal Fe attached to the surface of the cells with appropriate washing techniques, it would demonstrate that the bulk of the particulate Ba measured in cultures with high Fe concentration is indeed adsorbed on FeO_x . Our attempts with the Ti-citrate-EDTA (Hudson and Morel, 1989) and the oxalate wash techniques (Tovar-Sanchez et al., 2003) were unsuccessful, however, due to Ba contami-

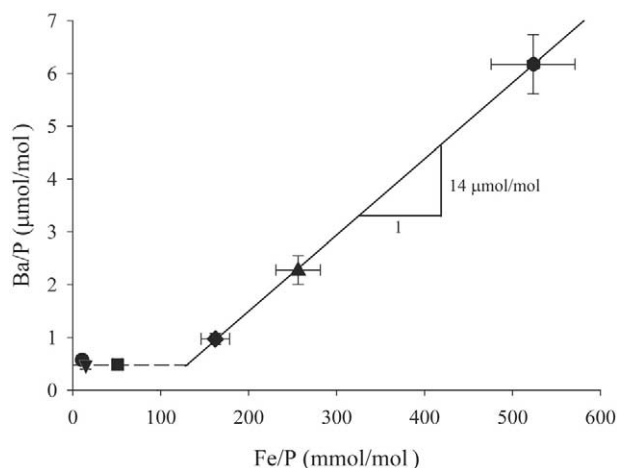


Fig. 2. Ba and Fe quotas in *T. weissflogii*. The circle, inverted triangle, square, diamond, triangle and hexagon symbols correspond to total Fe additions, in the presence of 100 $\mu\text{mol/L}$ EDTA, of 61, 84, 304, 840, 1680, and 3360 nmol/L, respectively. At $\text{Fe/P} < 100$ mmol/mol, Ba/P mole ratios are constant in *T. weissflogii* (0.53 $\mu\text{mol/mol}$, horizontal dashed line) and little accumulation of Ba is observed (see also Table 3). At $\text{Fe/P} > 100$ mmol/mol however, the Ba concentration in the cells increases with increasing Fe concentrations, most likely due to extracellular Fe precipitation on the diatom frustules and Ba adsorption on the Fe-oxhydroxides.

nation of the wash solutions. We thus performed an experiment in which growth medium containing background Ba (40 nmol/L) and various Fe concentrations but not inoculated with cells was left to age for 1 week. Figure 3a shows the concentrations (normalized to the original volume of medium) of Fe and Ba collected on a 0.2- μm filter at the end of that experiment, along with the results of the culture experiments of Figure 2 (also using concentration normalized to the volume of the medium). At high Fe concentrations, the measured particulate Ba follows the same trend as a function of particulate Fe in both sets of experiments, confirming that the bulk of particulate Ba is not cellular but adsorbed on FeO_x . Note that the particulate Fe collected on the filter from un-inoculated media only accounts for a small fraction of the total Fe added, much of the colloidal FeO_x passing through the 0.2- μm filter and being collected in the filtrate in the absence of cells (Fig. 3b). The filtration of the colloidal FeO_x by the 0.2- μm filter increases with the concentration of Fe and becomes almost quantitative in the presence of cells. This phenomenon underlies both the difficulty in collecting FeO_x colloids quantitatively and the efficiency of the association between these colloids and the diatoms in cultures. At low Fe concentrations, below the point where we expect FeO_x precipitation, we collected from the un-inoculated media a few percent of the Fe and a very small fraction of the Ba ($\sim 2.5 \times 10^{-5}$ of the total Ba) on the filter. These small concentrations most likely reflect an imperfect washing of the filters. But since Ba concentrations are similar in both the presence and absence of cells, it is possible that the very low cellular concentrations we obtained in Figures 1 and 2 are, in fact, overestimates of the true cellular values.

Sorption experiments at very low concentrations are inherently difficult since insufficient rinsing of the filter will lead to an overestimation of the particulate concentration but too much rinsing will result in desorption of the Ba from the FeO_x . To better quantify Ba adsorption on FeO_x , we thus performed another experiment with a higher Ba concentration (80 nmol/L), a much higher Fe concentration (2.8 $\mu\text{mol/L}$, in the absence of EDTA) and incubating for 24 h at various pHs from 7.7 to 8.6. As seen in

Table 4. Ba, Fe, and P concentrations, Ba/P, Fe/P and Ba/Fe ratios in *T. weissflogii* for various Fe concentrations of the culture medium, at background Ba concentration (40 nmol/L).

[Fe] in medium (nmol/L)	μ (day ⁻¹)	pH ^a	Cellular concentrations and ratios in biovolume ^b					
			[Ba] (nmol/L)	[Fe] (mmol/L)	[P] (mmol/L)	Ba/P (μ mol/mol)	Fe/P (mmol/mol)	Ba/Fe (μ mol/mol)
61	1.16	8.76	40.4 (5.3)	0.731 (0.053)	69.8 (5.3)	0.58 (0.09)	10.5 (1.10)	55.3 (8.28)
84	1.20	9.02	42.2 (6.8)	1.28 (0.079)	87.7 (5.9)	0.48 (0.08)	14.6 (1.33)	33.0 (5.69)
304	1.28	9.01	42.2 (4.8)	4.26 (0.28)	83.8 (6.1)	0.51 (0.07)	50.9 (5.03)	9.91 (1.30)
840	1.30	9.00	86.0 (6.8)	14.4 (1.01)	88.6 (6.3)	0.97 (0.10)	162 (16.2)	5.97 (0.63)
1680	1.29	9.04	192 (18.6)	21.6 (1.46)	84.5 (6.1)	2.27 (0.27)	256 (25.3)	8.89 (1.05)
3400	1.32	9.08	522 (31.6)	44.3 (2.71)	84.6 (5.7)	6.17 (0.56)	523 (47.7)	11.8 (1.01)

^a pH was measured just before harvest.

^b Errors (in parentheses) were calculated from the standard deviation of the measurement. Cellular concentrations were calculated by dividing concentrations measured by ICPMS by the biovolume (obtained from Coulter Multisizer II).

Figure 4, the measured particulate Ba increased sharply with pH, as expected for Ba adsorption on FeO_x (Dzombak and Morel, 1990).

4. DISCUSSION

Our experimental data indicate that the diatom *T. weissflogii* takes up very little Ba intracellularly, but that a relatively large amount of Ba becomes adsorbed on the hydrous ferric oxide (FeO_x) that is associated with the cell surface. Three questions are

raised by this result: (1) Is the result consistent with previously published data? (2) How significant is Ba uptake by the diatom and does it matter physiologically? (3) What are the roles of Ba uptake and adsorption on oxides in Ba geochemistry?

4.1. Comparison With Previous Studies

There have been a few studies of Ba adsorption on FeO_x (reviewed by Dzombak and Morel, 1990). Under the conditions of interest, adsorption is a function of pH and proportional to the concentrations of dissolved Ba and particulate Fe:

$$[\text{Ba}_{\text{ads}}] = K_{\text{ads}}(\text{pH}) \times [\text{Ba}^{2+}] \times [\text{FeO}_x] \quad (1)$$

Thus, at a given pH, the ratio $[\text{Ba}_{\text{ads}}]/[\text{FeO}_x]$ should be simply proportional to the Ba in solution. Our data obtained in the presence and absence of cells and in 1-week and 24-h incubations are thus reasonably consistent with each other: in Figures 2 and 3, the correlation between particulate Ba and Fe concentrations (at high Fe concentrations) corresponds to a ratio $[\text{Ba}_{\text{ads}}]/[\text{FeO}_x] = 1.4 \times 10^{-5}$ mol/mol; in Figure 4, at pH ~ 8.5 to 9.0 typically observed for cultures in late exponential growth, we extrapolate a ratio $[\text{Ba}_{\text{ads}}]/[\text{FeO}_x]$ in the range 4 to 5 $\times 10^{-5}$ mol/mol, somewhat

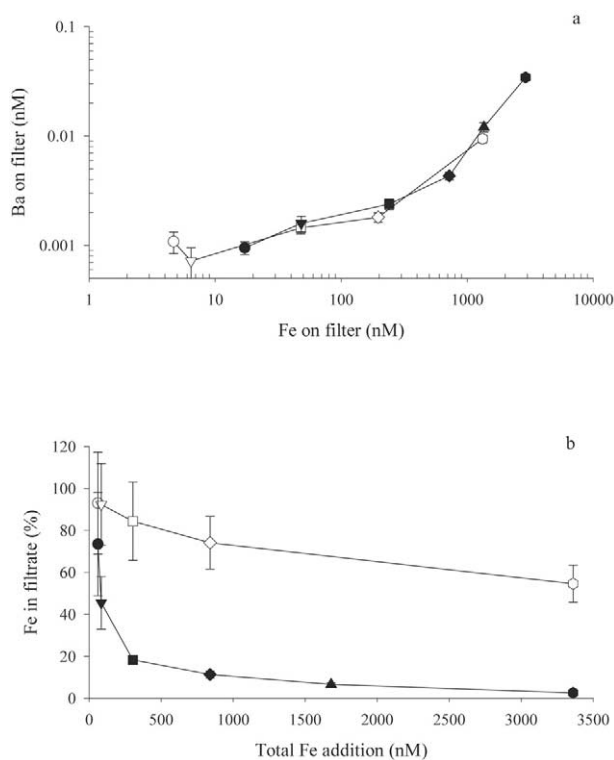


Fig. 3. (a) Particulate Ba and Fe concentrations, and (b) fraction of total Fe added passing through a 0.2- μ m filter, in the absence (open symbols) and the presence (filled symbols) of *T. weissflogii*, after 1-week incubations. Symbols are the same as in Figure 2. No extra Ba was added into the Gulf Stream Water medium (background value of 40 nmol/L).

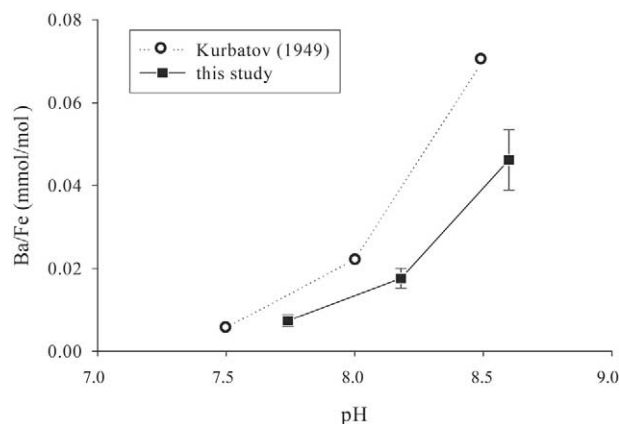


Fig. 4. Ba/Fe molar ratios as a function of pH in a medium containing 80 nmol/L Ba and 2.8 μ mol/L Fe (24-h incubation experiments). Kurbatov's (1949) data were recalculated to match the total Ba concentration of our experiment.

higher than the value of 2.8×10^{-5} mol/mol expected for twice the Ba concentration. This higher apparent affinity of the Ba for FeO_x in the short term experiment may reflect a larger effective surface area for the fresher precipitate.

Of the previous data on Ba adsorption on FeO_x , those of Kurbatov (1949) obtained at high ionic strength (0.3 mol/L NH_4Cl ; Fig. 1EBA3 in Dzombak and Morel, 1990) are most relevant to our seawater conditions. We calculated the ratio $[\text{Ba}_{\text{ads}}]/[\text{FeO}_x]$ from these data and normalized them to the Ba concentration in the medium. As seen in Figure 4, these calculated ratios are reasonably consistent with our data, a remarkable result in view of the fact that they come from 55-yr-old experiments carried out with a very high Fe concentration (590 $\mu\text{mol/L}$).

All previously published laboratory data on Ba uptake by phytoplankton have been obtained with cultures containing a relatively high ratio of Fe to EDTA concentrations. In such experiments, a large fraction of the Fe would have precipitated and much of the FeO_x become associated with the cell surface (Ho et al., 2003). We can estimate how much of the “cellular” Ba concentrations reported in these studies can be attributed to Ba adsorption on FeO_x . For example, in the presence of 12 $\mu\text{mol/L}$ EDTA (in *f/2* medium), most of the 12 $\mu\text{mol/L}$ Fe added in the experiments of Fisher et al. (1991) must have precipitated and adsorbed ~ 0.62 nmol/L of the 70 nmol/L Ba in solution (calculated using the extrapolated ratio $[\text{Ba}_{\text{ads}}]/[\text{FeO}_x] = 5.2 \times 10^{-5}$ mol/mol corresponding to a pH of 8.7 according to Fig. 4, as pH varied between 8.4 and 8.9 in this study). For the range of biomass of 25 to 65 mg/L dry weight in their diatom cultures, this would correspond to an apparent cellular Ba concentration of ~ 1 to 4 $\mu\text{g/g}$ (dry weight), similar to that actually reported by these authors. Not enough information is given in the papers of Riley and Roth (1971) and Dehairs et al. (1980) to allow similar calculations. But the long duration of the batch cultures (20–30 and 5–10 d, respectively) used to obtain sufficient biomass suggests that the pH was high at the time of harvest in all these experiments. As seen in Figure 4, the adsorption of Ba on FeO_x increases sharply around $\text{pH} = 9$, which is typical of batch cultures of phytoplankton in late or post exponential growth phase. (The relatively low Fe concentration used by Dehairs et al., 1980, [6.5 $\mu\text{mol/L}$] may explain why these authors could not measure cellular Ba in cultures of *Chaetoceros lauderi* above their detection limit of 1 $\mu\text{g/g}$, a value 10 times larger than our measurement of cellular Ba in *T. weissflogii*).

In addition to explaining the high apparent cellular Ba measured in previous laboratory studies, the adsorption of Ba on FeO_x is also consistent with qualitative features of the published data. For example, Ganeshram et al. (2003) observed that, in natural samples, $\sim 20\%$ of the particulate Ba was removed by rinsing with distilled water and 85% of the rest by rinsing with dilute (10%) nitric acid. The authors suggested biologic uptake of dissolved Ba by plankton and/or Ba adsorption on organic matter. But if this labile particulate Ba was actually adsorbed on FeO_x , in the course of such sequential rinsing, it would have been first partially released as it equilibrated with the aqueous solution and then completely released into the acid solution.

4.2. Significance of Ba Uptake

On the basis of the measured P quotas and cellular volumes, the cellular Ba concentrations in *T. weissflogii* at low Fe concentrations can be calculated to be in the range 40 to 100 nmol/L at an ambient Ba concentration of 35 to 40 nmol/L (Table 2). The true cellular concentration of Ba in exponentially growing cells is thus equal to or a few times greater than the concentration in the external medium. Since the cytoplasm of the cell is at a negative potential compared to the outside (~ -70 mV), passive leakage of Ba^{2+} into the cell through the transporters of other ions (including Ca^{2+} and trace metals) could easily account for a modest cellular accumulation, and no specific Ba transport need be invoked to explain the data.

Barium is not known to have any physiologic function. The measured cellular quota of Ba (Ba/P of 0.5–0.7 $\mu\text{mol/mol}$ in exponentially growing cells for a typical surface seawater Ba concentration of 35–40 nmol/L) is indeed quite low, much smaller than that of other essential trace elements, which range from 20 $\mu\text{mol/mol}$ P for Mo to 20,000 $\mu\text{mol/mol}$ P for Fe in *T. weissflogii* (Ho et al., 2003). Further, an attempt to make the background Ba in the medium unavailable to the cells by adding 10 $\mu\text{mol/L}$ of the cryptand 222 (4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosan, Merck, a strong Ba complexing agent; $K_{\text{Ba}} = 10^{9.6}$) had no negative effect on the growth of *T. weissflogii*. It thus seems highly unlikely that Ba plays a physiologic role in this organism.

4.3. Ba Geochemistry

It is now well established that barite crystals are the main contributors to the vertical flux of Ba into the sediments, and that microorganisms somehow play a role in the precipitation of the mineral (Dehairs et al., 1980, 1990; Bishop, 1988; Dymond et al., 1992). Given that barite is largely undersaturated in the oceanic water column (Monnin et al., 1999; Rushdi et al., 2000), the difficulty is to understand what causes its precipitation and what is the exact role of microorganisms, particularly of diatoms (Vinoogradova and Koval'skiy, 1962; Bacon and Edmond, 1972; Bishop, 1988), in that precipitation. If they can be extrapolated to other species, our laboratory results with *T. weissflogii* rule out direct cellular uptake by living phytoplankton as the concentrating mechanism leading to Ba precipitation. But our data suggest a mechanism to accumulate Ba at intermediate depths, particularly at the oxygen minimum: in surface waters, the high pH caused by phytoplankton growth (8.2–9 and above during blooms; Hansen, 2002; Hinga, 2002; Pedersen and Hansen, 2003) favors both FeO_x precipitation and Ba adsorption onto it (top right of Fig. 4); in deeper water, after sinking in association with biomass, the low pH (~ 7.6) resulting from respiration leads to Ba desorption (bottom left of Fig. 4) and to some FeO_x dissolution. Such “Ba pumping” mechanism, possibly coupled with an increased SO_4^{2-} concentration in decaying phytoplankton aggregates (Chow and Goldberg, 1960; Bishop, 1988; Stroobants et al., 1991), could lead to supersaturation and precipitation of barite, which would then dominate the deep vertical flux of Ba (Dehairs et al., 1980). The presence of some barite crystals in surface waters militates against such an explanation, however, as does the much higher ratio of Ba to Fe concentrations measured in suspended particles collected at

the ocean surface: from 3.3×10^{-4} to 1.2×10^{-1} mol/mol (Risler, 1984; Dehairs et al., 1987, 1990; Martin et al., 1988; Jickells et al., 1990; Sherrill and Boyle, 1992; Sarthou et al., 1997; Kuss and Kremling, 1999; Cardinal et al., 2001) compared to the highest value of 4.7×10^{-5} mol/mol measured in this study of cultured phytoplankton (Fig. 4). But rapidly settling particles that carry Ba downward in the surface ocean probably constitute only a small fraction of the suspended material that is analyzed and they might have a higher Ba/Fe ratio than the average suspended matter. The majority of barite crystals is clearly formed in subsurface and intermediate waters (Dehairs et al., 1990; Legeleux and Reyss, 1996) and it seems that a carrier phase other than BaSO₄ must cause the depletion of dissolved Ba²⁺ at the surface. It may thus be worthwhile examining further the possibility that iron oxyhydroxides and other oxides may be responsible for the downward flux of Ba from the surface ocean and the supersaturation of barite in microenvironments in the subsurface.

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