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A trace metal clean reagent to remove surface-bound iron from marine phytoplankton

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Abstract

Many recent studies have investigated Fe biogeochemical cycling, chemical speciation, and limitation of phytoplankton growth in the ocean. In current models of marine iron biogeochemistry, however, two critical parameters remain uncertain. These are the partitioning of particulate iron into scavenged and interior pools, and the iron quotas (Fe/C ratios) of natural plankton communities. These values have not been measured in natural samples, because the only reagent that is available to remove surface adsorbed Fe from cells and other particles (Ti(III) citrate/EDTA [Limnol Oceanogr 34 (1989) 1113]) contains substantial levels of contaminating Fe, and is therefore useful only for Fe radiotracer experiments. We developed a new reagent that differentiates between intra- and surface adsorbed Fe pools in marine phytoplankton as effectively as the Ti wash, but that is also trace metal clean, chemically stable, and harmless to cells. This reagent uses oxalate as a reductant to remove surface adsorbed Fe from phytoplankton cells and other particles. A simple cleaning protocol reduces Fe concentrations in the oxalate solution to levels suitable for trace metal clean field measurements. The oxalate reagent was used to measure scavenged and interior Fe pools in suspended particles collected at four stations in the Southern Ocean. Sixteen percent to eighty-six percent of the total Fe associated with these samples was found to be surface-adsorbed. The oxalate reagent provides a new tool to accurately measure the physical partitioning of Fe in marine particles and could be used along with appropriate corrections for lithogenic Fe to estimate the intracellular Fe quotas of natural plankton communities.

Keywords: Surface-bound and intracellular iron; Phytoplankton; Oxalate solution

1. Introduction

For more than a decade, field and laboratory experiments have demonstrated that Fe plays a key role in marine ecosystems (reviewed by Geider and LaRoche, 1994; Wells et al., 1995; Hutchins, 1995; Price and Morel, 1998). Fe limits phytoplankton growth rates and controls community structure in the open ocean high nitrate low chlorophyll areas (HNLC) (Martin et al., 1991; Coale et al., 1996; de Baar et al., 1990, 1995; Boyd et al., 2000) and in some coastal waters (Hutchins and Bruland, 1998; Hutchins et al., 1998, 2002). Detailed knowledge of

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biological and geochemical pools and fluxes of Fe is thus essential for a complete understanding of carbon and nutrient biogeochemistry in these regimes.

There are two distinct pools of Fe associated with plankton and other suspended marine particles. One is scavenged Fe adsorbed to particulate surfaces and the other is an interior pool. In plankton, this latter pool corresponds to the intracellular ''biological'' fraction that comprises the cellular Fe quota (Hutchins, 1995). Laboratory radiotracer experiments under iron limiting conditions demonstrate the importance of those two physical pools to the turnover and recycling of phytoplankton cellular Fe (Hutchins and Bruland, 1995; Hutchins et al., 1995, 1999), and show that the specific growth rate of phytoplankton is closely correlated with intracellular Fe content (Sunda et al., 1991; Sunda and Huntsman, 1995, 1997).

Despite years of research, however, both the physical partitioning of Fe in marine particles and the Fe quotas (or intracellular Fe/C ratios) of field populations of phytoplankton are still unknown. The lack of methods to determine scavenged and interior pools in natural plankton communities has hindered our understanding of ocean biogeochemical cycling. Current quantitative models of Fe cycling in the world ocean are based on phytoplankton cellular Fe/C ratios obtained solely from laboratory studies (e.g.,Coale et al., 1996; Johnson et al., 1997; Fung et al., 2000; Bruland et al., 2001; Christian et al., 2002; Moore et al., 2002), and the relative importance of scavenged versus particle-incorporated pools to Fe export fluxes is unknown. Recent laboratory and field experiments have shown that phytoplankton ratios of C, N, Si, and P are strongly influenced by external concentrations of Fe (Hutchins and Bruland, 1998; Hutchins et al., 1998, 2001, 2002; Takeda, 1998; Franck et al., 2000; Firme et al., 2003), but we have no information on how intracellular Fe content relates to these changes in major elemental composition.

Despite the clear need to differentiate the partitioning of trace metals into scavenged and interior pools, there are at present no data available for natural marine particulate samples or phytoplankton communities. The limited data published using trace metal clean techniques have only reported total trace metal concentrations of field-collected plankton communities (Collier and Edmond, 1984; Martin and Knauer, 1973; Cullen and Sherrell, 1999; Sañudo-Wilhelmy et al., 2001; Berman-Frank et al., 2001).

The Ti-EDTA-citrate reagent developed by Hudson and Morel (1989) has been nearly universally used to study the biogeochemical cycling of Fe using radiotracers such as ^{55}Fe and ^{59}Fe. The removal of the surface-adsorbed Fe pool by the Ti reagent has allowed the determination of cellular Fe requirements and uptake kinetics under laboratory conditions (reviewed by Hutchins, 1995).

While the Ti reagent is useful for investigating Fe partitioning in radiotracer experiments, it has serious drawbacks. For one thing, its rapid reaction with oxygen makes it hard to manipulate and its shelf life is very short (hours). There is also evidence that the Ti-EDTA-citrate wash could produce cell damage, breakage or lysis in some delicate phytoplankton species (Sunda and Huntsman, 1995). The most serious problem with the Ti reagent, though, is that it cannot be used to measure ''cold'' Fe in marine particles because the reagent is itself contaminated with Fe (Fig. 3 in Section 3).

In this study, we attempted to find alternative reagents that would overcome these limitations by testing the ability of several reductants (e.g. oxalate, stannous, and thiosulfate) to differentiate between surface-scavenged and interior Fe pools in phytoplankton. The highest removal efficiency was obtained using oxalate to reduce and wash off extracellular Fe. In contrast to the Ti wash, our new reagent is chemically stable, making it suitable for extended field sampling campaigns, and is also harmless to cells. Most important though, it is almost completely free of contaminating Fe after the application of a simple cleaning protocol. We used the oxalate reagent to make the first trace metal clean measurements of surface-scavenged and interior iron concentrations of suspended particle collections from the HNLC Southern Ocean.

2. Methods

2.1. Reagent preparation

Oxalic acid has been extensively used to remove noncrystalline inorganic forms of Fe and Al and

organic-complexed Fe and Al from soils (McKeague, 1967). The redox potential of oxalic acid ($E^0_H = -631$ mV; Morel and Hering, 1993) suggests that the application of our new reagent could potentially reduce most of the amorphous iron hydroxides ($E^0_H = -295$ mV; Hudson and Morel, 1989) bound to suspended particles. Except for the substitution of oxalate for Ti(III), the reagent is designed after the formula of Hudson and Morel (1989) and is therefore isotonic with seawater (Table 1). In the Ti reagent, the addition of citrate and EDTA is needed to chelate and thus retard premature oxidation of the titanous ion (Hudson and Morel, 1989). For the stable oxalate reductant, this chelation capacity is unnecessary, but the metal-binding properties of EDTA and citrate likely increase the removal efficiency of the reagent for both Fe and for other metals such as Zn (Hutchins et al., 1999), and so were retained in the new reagent.

Table 1
Composition and preparation of the oxalate reagent

Chemicals[a]	Basic oxalate reagent	Trace metal clean oxalate reagent	Concentration (mol/L)
(a) EDTA ($EDTA\text{-}Na_2\cdot H_2O$)	1.86 g	2.23 g	0.05
(b) Sodium citrate ($C_6H_5Na_3O_7\cdot 2H_2O$)	1.47 g	1.76 g	0.05
(c) KCl	0.074 g	0.089 g	0.01
(d) NaCl	0.5 g	0.6 g	0.25
(e) NaOH (10 M)	drops until pH 6–7		
(f) Oxalic acid ($C_2H_2O_4\cdot 2H_2O$)	1.26 g	1.51 g	0.1
(g) NaOH (10 M)	buffer to pH 8		
(h) Hydroxylamine (1.44 M, $NH_2OH\cdot HCl$)[b]	–	0.5 ml	7.2e – 3
(i) Perchlorate (0.008 M, $NaClO_4\cdot H_2O$)	–	6.5 ml	5.2e – 4
(j) 1,10 phenanthroline (0.055 M, $C_{12}H_8N_2\cdot H_2O$)[c]	–	13 ml	7.2e – 3

[a] To prepare the basic, non-trace metal clean reagent, mix consecutively from (a) to (f) in 60 ml of MQ water and take to 100 ml with MQ water after (g); for the trace metal clean oxalate reagent continue to (j) and follow the procedure outlined in the text to a final volume of 120 ml.

[b] Hydroxylamine solution is buffered to pH 8 with NaOH.

[c] Several drops of sulfuric acid are added to completely dissolve solution.

The concentrations of the other two solutions tested in this study (stannous and thiosulfate) were the same as those reported by Hudson and Morel (1989) for the Ti-EDTA-citrate solution, but substituted $TiCl_3$ with $SnCl_2\cdot 2H_2O$ and $Na_2S_2O_3\cdot 5H_2O$. All of the chemicals used in these experiments were reagent grade.

2.2. Cleaning protocol

For the quantification of scavenged and interior Fe in natural samples, it is necessary to eliminate contaminating Fe in the oxalate reagent using a cleaning protocol that does not modify its chemical properties. The cleaning protocol was developed as a modification of that reported by Knizek and Provaznik (1965). To the oxalate solution, we added hydroxylamine, perchlorate, and 1,10-phenanthroline (Table 1). Next, the pH was readjusted to 8 with 10 M NaOH and heated in a water bath to 50 °C for 15 min (at this point, the solution acquires a reddish orange color due to the complex formed between the phenanthroline and the ferrous ion). Immediately while still hot, the solution was transferred to a 250-ml Teflon separatory funnel and extracted twice with 6 and 4 ml of 1,2-dichloroethane ($C_2H_4Cl_2$). The solution was then transferred to a trace metal clean Teflon separatory funnel and extracted again with 4 ml of 1,2-dichloroethane. In each extraction, the mixture was shaken vigorously for 2 min and allowed to stand for 15 min for phase separation; then the organic phase was discharged. Aliquots of the reagent were collected after each extraction and Fe levels were measured by graphite furnace atomic absorption spectrometry (GFAAS) using the technique of standard additions. The clean oxalate solution was then transferred to an acid-washed polyethylene bottle. The bottle was left open for two days in a class-100 laminar flow unit, where it was periodically shaken to remove the excess volatile solvent.

This method is suitable for cleaning the oxalate reagent for several reasons; first, Fe(II)–1,10-phenanthroline perchlorate is stable over a wide pH range (1.5–9.5; Vydra and Kopanica, 1963) bracketing the pH 8 of the oxalate solution. Second, the excess of 1,10-phenanthroline is extracted in the organic phase during the extraction procedure. And third, the residual 1,2-dichloroethane in the solution is easily

removed at room temperature in a class-100 laminar flow bench. Thorough solvent removal is necessary to avoid potential damaging effects on living cells and could be further facilitated by carrying out the volatization step in a warm-water bath.

2.3. Iron removal efficiency experiments

Laboratory studies for the removal efficiency of the several reagents were conducted with cultures of the diatom *Thalassiosira weissflogii* (CCMP strain 1048) pulse-labeled for 5 min with 0.5 μCi ml^{-1} ^{55}Fe, a procedure that results in >95% of the Fe radiotracer being located in extracellular pools (Hutchins et al., 1999). Triplicate aliquots of the same *T. weissflogii* culture were washed with the oxalate, stannous, thiosulfate, or Ti reagents, or with 0.2 μm filtered seawater only.

For each sample, 15 ml of exponential growth phase, ^{55}Fe-labeled diatom culture ($\sim 5 \times 10^5$ cells ml^{-1}) was filtered onto 3 μm polycarbonate filters under low vacuum (2–3 kPa). When the volume in the filter tower was reduced to $\sim$ 5 ml, the vacuum pump was turned off and the cells were exposed to 5 ml of the appropriate wash solution for 5 min. The vacuum pump was then turned back on and the filters were rinsed three times with 5 ml of filtered seawater. ^{55}Fe activity was determined using liquid scintillation counting. The amount of extracellular iron activity removed by each treatment was calculated as the difference between the mean of the triplicate samples washed only with filtered seawater, and the mean of the three reagent-washed samples.

We tested the long-term stability of the reagent, in order to determine whether the Fe-free oxalate solution is suitable for use on oceanographic cruises that last up to several months. The cell surface-adsorbed Fe removal efficiency of the oxalate solution was measured as described above every 30 days for 3 months. Between experiments, the solution was stored in the dark at 4 °C.

2.4. Cell lysis and growth effects experiments

We examined whether the application of the oxalate reagent could cause cell breakage or lysis using several eukaryotic and prokaryotic phytoplankton cultures. Triplicate samples of the cultures were exposed to 50% (v/v) oxalate reagent or 50% 0.2 μm filtered seawater for 5 min. Abundances of visibly intact cells (defined as chlorophyll *a*-autofluorescing, whole cells) in the two treatments were then determined using epifluorescence microscopy counts. Eukaryotic algal species used included three diatoms, the coastal centric *T. weissflogii* (CCMP 1048), the oceanic centric *Thalassiosira oceanica* (CCMP 1335), and the pennate *Fragillaria pinnata* (CCMP 395). Other eukaryotes tested were a prasinophyte (*Tetraaselmis* sp.) and a dinoflagellate (*Prorocentrum* sp.), both local Delaware estuarine isolates, and the harmful algal bloom pelagophyte *Aureococcus anophagefferens* (CCMP 1708). Prokaryotic phytoplankton used were two species of *Synechococcus*, one that is an oceanic phycoerythrin-dominated strain (CCMP 1334) and one that is a coastal phycocyanin-rich isolate (PCC 7002).

We also tested how washing with the oxalate reagent affects the growth of live diatom cells. *T. weissflogii* cultures were washed with either the oxalate reagent or with 0.2 μm filtered seawater alone, as described above. After washing, the cells were resuspended from the filters into fresh medium under normal growth conditions. The lag period until growth resumed after washing and the specific growth rates over the next 3 days were recorded in triplicate cultures using in-vivo fluorescence measurements.

2.5. Field study

We used the oxalate solution to measure levels of scavenged and interior Fe in suspended particle samples from the Southern Ocean. Near-surface water ($\sim$ 15 m depth) was collected in November–December 2001 south of Australia using a trace metal clean Teflon pump (Hutchins et al., 1998). The 1–5-l samples were filtered at sea immediately after collection onto acid-washed 0.2 μm filtered 47 mm polycarbonate filters using trace-metal clean filtration equipment and handling methods under a class-100 laminar flow hood. Four stations were sampled along a latitudinal transect representative of the different oceanographic regimes of the Southern Ocean (Arrigo et al., 1998). These included a station in the Subantarctic water mass (46°45S, 141°20E), one at the Subantarctic Front (51°20S, 142°59E), one at the Polar Front (53°40S, 141°42E), and one within surface waters of

the Antarctic Circumpolar Current at the SOIREE site (60°46S, 139°25E; Boyd et al., 2000).

Fe concentrations in the total particulate (unwashed) and interior (oxalate-washed) pools were measured by GFAAS after an acid digestion (Eggimann and Betzer, 1976), and the scavenged pool was estimated by difference. All reported values were corrected for field filter blanks.

3. Results and discussion

The initial objective of this research was to test several potential Fe reductants (oxalate, stannous, and thiosulfate) and establish how efficiently they removed extracellular Fe from the surface of marine phytoplankton cells. The removal efficiencies of extracellular ^{55}Fe were relatively low for the stannous and thiosulfate solutions, 50% and 72%, respectively. However, the extracellular ^{55}Fe removal efficiency of the oxalate solution was high (97%) and indistinguishable from the Ti-EDTA-citrate wash (Fig. 1). This high removal efficiency was consistent for several independently prepared batches of the oxalate solution (data not shown). Results obtained using the oxalate reagent are thus closely comparable with published results using the Ti solution (Hudson and Morel, 1989).

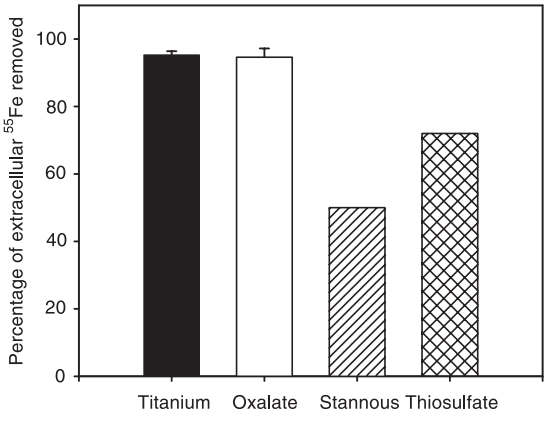

Fig. 1. Comparison of removal efficiencies of extracellular ^{55}Fe by the Ti-EDTA-citrate wash, oxalate solution (Table 1), stannous ($SnCl_2{\cdot}2H_2O$)and thiosulfate ($Na_2S_2O_3{\cdot}5H_2O$) in cultures of the diatom *Thalassiosira weissflogii*. The laboratory studies were conducted according to Hutchins et al. (1999). The removal efficiencies of the Ti-wash and the oxalate solution are high and indistinguishable, while the stannous and thiosulfate reagents are less effective.

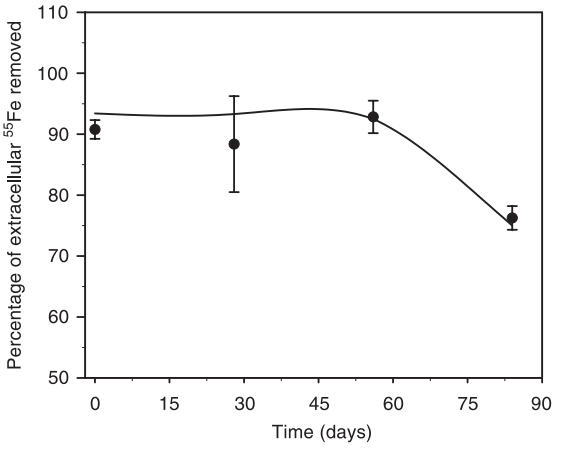

Fig. 2. Extracellular Fe removal efficiency of the oxalate solution after 30, 60 and 80 days. The oxalate solution is stable for up 2 months stored in the dark in a refrigerator.

The oxalate reagent is stable under oxic conditions, making it easy to handle and manipulate in the laboratory. Our long-term stability study showed that the removal efficiency of the oxalate solution was unchanged for at least 60 days following preparation of the reagent (Fig. 2). After nearly 3 months of refrigerated storage, the reagent did begin to lose effectiveness, as removal of extracellular Fe was 20% lower than at the start of the experiment. In contrast, the relatively unstable Ti wash must be prepared fresh daily (Hudson and Morel, 1989). The greater shelf life of the oxalate reagent should make it the method of choice for lengthy shipboard studies. The chemical stability of the new reagent may also make it preferable for use in laboratory radiotracer experiments, although for this application the rigorous trace metal cleaning protocol is not essential and could be omitted (see Table 1).

Table 2 shows the results of our cell lysis and growth experiments. For all species examined, live cell counts were indistinguishable between oxalate- and seawater-washed treatments. The application of the oxalate solution did not appear to cause cell damage or lysis in any phytoplankton species we examined, including the notoriously delicate *A. anophagefferens*. No significant differences in the length of time until resumption of growth (lag period, days)

Table 2
Average cell density (±1 standard deviation) and growth rate comparison between phytoplankton cultures washed with the oxalate reagent and with filtered seawater

Phytoplankton culture[a]	Filtered seawater wash (10^5 cells ml^{-1})	Oxalate wash (10^5 cells ml^{-1})
Thalassiosira weissflogii (diatom)	10.3 ± 5.99	88.8 ± 2.85
Thalassiosira oceanica (diatom)	32.6 ± 4.34	31.0 ± 6.61
Fragilaria pinnata (diatom)	12.5 ± 2.51	14.4 ± 3.74
Prorocentrum sp. (dinoflagellate)	5.38 ± 1.69	6.00 ± 2.33
Tetraselmis sp. (prasinophyte)	26.4 ± 8.62	25.6 ± 4.77
Aureococcus anophagefferens (pelagophyte)	24.7 ± 4.52	26.9 ± 6.23
Synechococcus WH7803 (cyanobacterium)	28.1 ± 20.9	31.3 ± 25.9
Synechococcus PCC 7002 (cyanobacterium)	62.5 ± 17.9	50.6 ± 14.5
Growth parameters[b] (*T. weissflogii*)	Filtered seawater wash	Oxalate wash
Lag period after washing before resumption of growth (days)	1	1
Specific growth rate following lag period (μ, day^{-1})	0.36 ± 0.06	0.43 ± 0.08

[a] Cell abundances of intact, autofluorescing cells in eukaryotic and prokaryotic phytoplankton cultures after 5 min exposure to 50% (v/v) mixtures of the oxalate wash, or to 0.2 μm filtered seawater.
[b] Growth parameters (lag period and specific growth rate) after washing an exponential phase *T. weissflogii* diatom culture with the oxalate wash or with filtered seawater.

or in subsequent specific growth rates (μ, day^{-1}) were found after washing diatom cultures with filtered seawater or with the oxalate reagent (Table 2). The oxalate reagent thus appears to be almost completely nontoxic to phytoplankton, and is suitable for experiments involving live cells.

Fig. 3 shows removal of contaminating Fe from the reagent during the three organic extractions. Our cleaning protocol reduced the level of Fe in the oxalate solution by 30-fold, from 550 to <17 nmol/l. The low levels of contaminating Fe obtained after the cleaning procedure make the oxalate reagent the ideal tool to measure scavenged and interior particulate Fe pools in field collected samples.

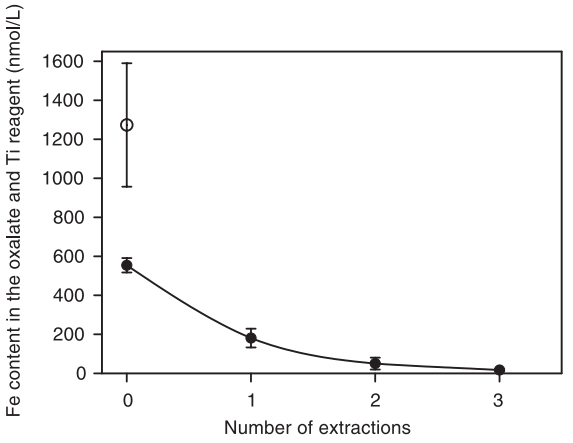

Fig. 3. Reduction in Fe content of the oxalate solution during the organic extraction cleaning procedure. The levels of contaminating Fe were reduced from 500 to <17 nmol/l after the three organic extractions. Open circle represents the concentration (average ± standard deviation) of iron in the Ti(III) citrate/EDTA reagent.

We carried out measurements of total and interior Fe in natural particle samples collected in the surface Southern Ocean (Fig. 4). Our total particulate Fe measurements at the SOIREE site (~0.30 nmol/l) were consistent with the total particulate Fe concentrations (0.28 nmol/l) reported for that location by Bowie et al. (2001) for samples collected in 1999. Our

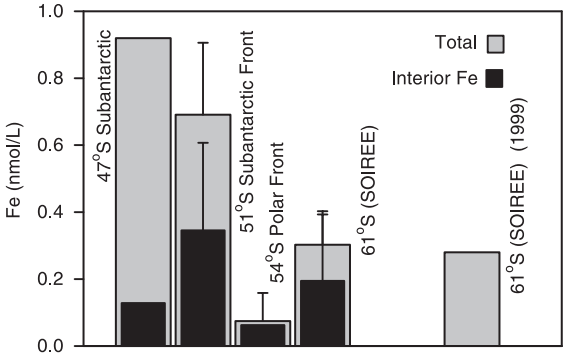

Fig. 4. Levels of total (>0.2 μm, unwashed) and interior (>0.2 μm, not removed by the oxalate wash) particulate Fe measured at four different locations in the surface Southern Ocean. The total Fe level measured at the SOIREE site in 2001 was consistent with total particulate Fe concentrations reported by Bowie et al. (2001) for samples collected in 1999 at that location.

results indicated that surface-adsorbed Fe accounted for 86% (47°S), 50% (51°S), 16% (54°S), and 36% (61°S) of the total particulate Fe measured at each location. The remainder of the particulate Fe (14–84%) was in interior pools not removed by the oxalate wash, including phytoplankton intracellular Fe.

Partitioning between surface adsorbed and interior Fe pools in our Southern Ocean samples (Fig. 4) is consistent with previous results obtained using radioactive Fe and the Ti wash. In Fe recycling experiments, 39–71% of the ^{55}Fe transferred from added radiolabeled cells to natural phytoplankton communities was surface-associated (Ti labile). Samples from the equatorial Pacific HNLC area had the highest proportions of interior (or intracellular) Fe, while coastal Monterey Bay communities had the lowest fraction of total particulate Fe in these "biological" pools (Hutchins et al., 1993; Hutchins and Bruland, 1995). In the experiments presented here, samples from the two Subantarctic stations had substantially lower proportions of the total Fe in interior pools (<50%), while at the Polar Front and Antarctic Circumpolar Current (SOIREE site) stations most of the particulate Fe (>64%) was located in the interior of the phytoplankton cells.

In order to correct for the amount of Fe associated with lithogenic particles in our field-collected samples in the Southern Ocean, we have also determined their Al content. This is consistent with other studies, which have successfully used Al concentrations to identify contamination with lithogenic material in phytoplankton (Martin and Knauer, 1973; Cullen and Sherrell, 1999). As a major and relatively invariant component of continental materials (8.0%; Taylor, 1964; Wedepohl, 1995), Al is the ideal normalizer for terrigenous materials. After subtracting the lithogenic fraction (Fig. 5, assuming an average Fe/Al crustal ratio of 0.04; Wedepohl, 1995), the internal biogenic Fe pool in our field-collected samples was essentially the same as that reported by Sunda and Huntsman (1995) for pure open ocean phytoplankton cultures. These field results show that the oxalate reagent can be used effectively to measure scavenged and interior particulate Fe in trace metal clean field collections.

The availability of the oxalate reagent will allow oceanographers to undertake experimental and observational studies to provide quantitative estimates of particulate Fe physical partitioning and biological Fe/C ratios in the ocean. These are values that are critically needed to build realistic models of the marine cycle of Fe. The oxalate reagent promises to be a useful new research tool to further our understanding of how Fe biogeochemistry affects marine primary productivity, fluxes of nutrient elements, and the exchange of carbon between the oceanic and atmospheric reservoirs.

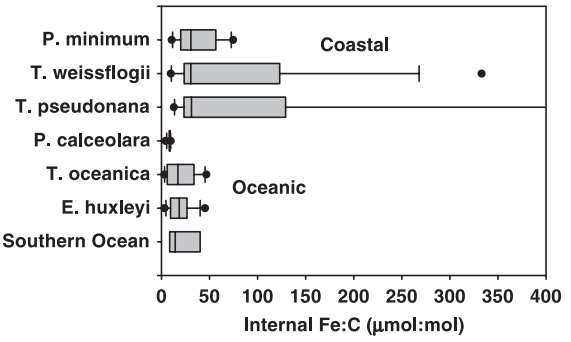

Fig. 5. Box plots comparing internal biogenic Fe concentrations (after correction for the lithogenic contribution) measured in field samples collected for this study in the Southern Ocean, with those reported for pure phytoplankton cultures grown under laboratory conditions by Sunda and Huntsman (1995). The consistency of our field measurements with the lab results suggests that our sampling and analysis protocols may provide some of the first data on Fe content of natural assemblages of phytoplankton.

4. Conclusions

The new oxalate reagent accurately differentiates between intra-and extracellular Fe pools in phytoplankton. It is easy to prepare, chemically stable for at least 2 months, and harmless to cells. The simple cleaning protocol removes contaminant Fe from the oxalate reagent, without modification of its chemical properties. Fe concentrations in the cleaned reagent are suitable for trace metal-clean field measurements (on the order of nmol/l or pmol/ml). The oxalate technique is relatively simple, and readily accessible to any laboratory with an atomic absorption spectrophotometer or ICP-MS.

The availability of the oxalate reagent will allow a key variable in the marine Fe cycle to be measured, the partitioning of particulate Fe between scavenged and interior pools. Our measurements of these fractions on field samples collected in the Southern Ocean

suggest that even in this Fe-limited, HNLC regime a significant fraction of the total particulate pool in surface waters is present as scavenged, non-biological Fe, particularly north of the Polar Front.

The application of the oxalate method to a wide variety of samples from different regimes and depth profiles should help to provide geochemical modelers with quantitative estimates of Fe physical partitioning and scavenging rates to constrain export flux estimates (e.g. Moore et al., 2002). The oxalate reagent will be a useful tool for biological experiments as well. Its relative chemical stability makes the reagent ideal for laboratory radiotracer studies, and this application does not require preparation using the trace metal cleanup procedures. More importantly, though, the trace metal clean version of the reagent could be used to estimate intracellular Fe quotas of natural plankton communities. Fe/C ratios of biological communities from different oceanographic regimes are another set of key parameters in Fe biogeochemical models, but at present these must be estimated and extrapolated from a few laboratory culture studies using model algal species.

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