



Effect of macronutrient enrichment on the size distribution, sorption, and bioconcentration factor of iron by coastal phytoplanktonic diatoms

Shun-Xing Li^{a,b,*}, Feng-Ying Zheng^{a,b}

^a Department of Chemistry and Environmental Science, Zhangzhou Normal University, Zhangzhou 363000, PR China

^b Fujian Province University Key Laboratory of Analytical Science, Zhangzhou Normal University, Zhangzhou 363000, PR China

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ABSTRACT

The influences of macronutrient enrichment on iron absorption, adsorption, and the bioconcentration factor (BCF) of iron by coastal diatoms were examined. In addition, the distribution of different iron size fractions in seawater in relation to two diatom species exposed to nutrient enrichment over an extended period (6 days for *Thalassiosira weissflogii*, and 4 days for *Skeletonema costatum*), was investigated. Iron concentrations were measured of seven size fractions: particles ($>0.4 \mu\text{m}$), colloidal particles ($0.4 \mu\text{m} - 100$, $100 - 50$, $50 - 30$, $30 - 10$, $10 - 3 \text{ kDa}$), and soluble species ($<3 \text{ kDa}$).

The absorption, adsorption, uptake, BCF of iron by the diatoms, and the iron species distribution in seawater were affected by the diatom species, as well as the nitrate (N) and phosphate (P) concentration. The addition of P could affect the iron internalization strategy. The content of soluble iron species was positively correlated with the amount of iron absorption by *T. weissflogii*, but it was not suitable for *S. costatum*.

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1. Introduction

In coastal waters, iron predominantly occurs in the particulate form (e.g. $0.05 - 10 \mu\text{mol L}^{-1}$), with a lower dissolved concentration, between 5 and 400 nmol L^{-1} (Achterberg et al., 2000; Öztürk et al., 2003a). Iron, as a key nutrient in many crucial biological processes, has ecological significance in coastal waters (Annett et al., 2008). Iron-limited growth is reported in coastal waters (Hutchins et al., 1998), and even in estuaries (Zhang, 2000). Barbeau et al. (1996), Behrenfeld et al. (1996), and La-Roche et al. (1996) reviewed the toxic effect of iron enrichment on phytoplankton. The enrichment of iron and its speciation, therefore, should have an impact on coastal ecology. On the other hand, enhanced primary productivity and macronutrient enrichment in coastal waters may have significant effects on iron speciation, bioavailability, transformation, and transport to offshore waters.

Phytoplankton passively adsorbs and biologically assimilates iron from seawater. It also releases extracellular organic substances (Price and Morel, 1998) which are capable of complexing with iron. These organic substances dramatically change the physicochemical forms and behavior of iron by either keeping it in solution or

enhancing its coagulation, and hence removing iron from the sunlit layer or making it either bioavailable or nonavailable (Hutchins et al., 1999; Wells 1999). There is a great deal of evidence suggesting a global increase in nutrient levels of coastal waters through riverine and sewage inputs (Rydberg et al., 1996; Smith et al., 1999). Coastal eutrophication has become worldwide, and consequently, coastal organisms are frequently exposed to macronutrient enrichment (Kucuksezgin et al., 2006).

Nutrient enrichment in coastal ecosystems not only causes an increase in marine phytoplankton biomass but also has the potential to alter the concentration, bioavailability and fate of metals (Skei et al., 2000; Bu-Olayan et al., 2001). In many coastal waters, increasing nutrient enrichment accompanied by a variation in nutrient ratio has been shown to profoundly affect phytoplankton species composition (the presence of rich diatoms than other phytoplankton groups) and production, and thus ecosystem structure and function (Smith et al., 1999; Bu-Olayan et al., 2001; Camargo and Alonso, 2006; Paul et al., 2008). Significant nitrogen, phosphate, and iron enrichment has been found in the coastal waters (Bizsel and Uslu, 2000). Recent studies have shown that nitrate can markedly influence metal uptake (both intracellular and cellular surface uptake) and accumulation in marine phytoplankton cells (Lee and Wang, 2001; Wang and Dei, 2001a, 2001b). These previous studies were focused on metal uptake and accumulation over a short exposure period (8 h), by using metal radiotracers and kinetic measurements. The effects of macronutrient enrichment on the photoproduction of hydroxyl radicals in seawater

* Corresponding author. Department of Chemistry and Environmental Science, Zhangzhou Normal University, Zhangzhou 363000, PR China. Tel.: +86 596 2523251; fax: +86 596 2520035.

E-mail address: lishunxing@fjzs.edu.cn (S.-X. Li).

by the alga have been observed (Li et al., 2008). Photochemical processes can significantly affect the speciation of iron and its bio-sorption and bioconcentration factor (Zuo and Hoigné, 1992, 1993; Zuo and Jones, 1997).

Both the Joint Global Ocean Flux Study and the Land–Ocean Interactions in the Coastal Zone Project, core projects of the International Geosphere–Biosphere Program, are focused on (a) the biogeochemical processes, cycles and fluxes of macronutrients, and (b) the distribution, transport, transformation, cycling, end-result, production and consumption of trace metals.

In spite of growing interest in iron research, especially in the open oceans (Gerringa et al., 2000; Nishioka et al., 2001, 2005; Boye et al., 2006), our understanding of the chemical, biological and physical mechanisms that regulate the iron biogeochemical cycle and its bioavailability is still limited, especially for coastal waters. We have a very restricted knowledge of the role of phytoplankton in controlling the iron biogeochemical cycle in coastal water, because only phytoplankton blooms, i.e., special phenomena, have been studied (Gobler et al., 2002; Öztürk et al., 2003a, 2003c; Öztürk and Bizsel, 2003b; Rose and Waite, 2003; Wells and Trick, 2004). There is surprisingly little information available on the quantitative and qualitative impact of major nutrient additions on iron species distribution. Some of the central concepts related to iron removal in coastal seawater, including iron absorption and adsorption by marine phytoplankton, are still vague and not fully understood. Furthermore, the relationship between iron species distribution and iron sorption by marine phytoplankton has not been mentioned to date.

It is important to distinguish metal absorption (intracellular uptake) and adsorption (cellular surface uptake) (Wang et al., 1997) for two reasons. Firstly, metal biogeochemical fates, ecological effects, and bioavailability or toxicity are quite different when trace metals were absorbed or adsorbed by marine phytoplankton. After absorption (i.e. assimilation) by algal cells, trace metals combine with organic compounds such as proteins and enzymes and accumulate through the aquatic food chain; whereas trace metals adsorbed by cell surfaces can be partly desorbed into the seawater, and then exist as inorganic compounds transferred by seawater. Secondly, a correct bioconcentration factor (BCF) can be obtained; BCF is a measurement which characterizes the accumulation of a chemical in an organism, and so BCF is defined as the concentration of a chemical in an organism (intracellular uptake, absorption) divided by the concentration in a reference compartment (e.g. the surrounding water).

We carried out experiments to address the following questions for the first time: (a) does macronutrient enrichment affect iron sorption (absorption and adsorption) by coastal diatoms, and iron species distribution in the water over long exposure period?; (b) are the influences of macronutrient concentrations on iron absorption and adsorption different or the same?; (c) what relationships are there between iron absorption and adsorption?; (d) does the coexistence of coastal diatoms and macronutrient enrichment affect the distribution of different iron size fractions in seawater?; (e) which iron size fraction is available to coastal diatoms?; and (f) are there species-specific differences between the effects of macronutrient enrichment on iron sorption, BCF, and iron species distribution? Answers to none of these questions have been reported previously. All of these questions, we think, are pertinent to the nutrient loading effects on the biogeochemical cycling of iron in coastal seawater, and iron availability.

2. Materials and methods

2.1. Reagents

All reagents were diluted with Mill-Q™ water, except for the quartz-distilled acids, which were diluted with quartz-distilled

water. Trace metal grade HCl and HNO₃ (Fisher Scientific) were used. Iron standards, prepared from 1.0 mg mL⁻¹ stock solutions (SPEX, Edison, NJ; Fisher Scientific), diluted with 1 mol L⁻¹ HNO₃ were used to prepare the working standards for particulate and dissolved iron analyses.

2.2. Seawater and marine phytoplankton cultures

The seawater used in all experiments was collected from Zhangzhou Bay, Fujian Province, China. It was filtered with 0.2 μm membrane filters, and then stored at 4 °C for about 6 months before the experiments began. The seawater N and P concentrations were measured three times using a flow injection analyzer, and the background concentrations were 9.3 μmol L⁻¹ for N (as nitrate) and 1.5 μmol L⁻¹ for P (as reactive P). The background concentrations of iron in the seawater were measured using inductively coupled plasma mass spectrometry (ICP-MS), and were 3.6 μmol L⁻¹, similar to the reports from Achterberg et al. (2000) and Öztürk et al. (2003a). The amounts of iron in seawater were determined three times and the relative standard deviation was 1.1%. Consequently, this coastal seawater, with both iron and macronutrient enrichment, could be used for experiments related to iron absorption, adsorption and bioconcentration by coastal diatom, and to determine iron species distribution in seawater.

Two species of coastal diatoms *Thalassiosira weissflogii* and *Skeletonema costatum* were chosen for this study since they are common species found in many coastal areas (McKinney et al., 1997; Ahner et al., 1998; Zudaire and Roy, 2001; Huang et al., 2004; Zhang and Wang, 2004; Radić et al., 2005). They were also species which were suitable for culturing within a laboratory environment. *T. weissflogii* and *S. costatum* were obtained from the State Key Laboratory for Marine Environmental Science, Xiamen University. They were maintained in seawater (with *f/10* levels of Si and vitamins added, but without trace metals) at different N (added as NaNO₃, its concentration was shown in Section 2.3) and P (added as Na₂HPO₄, its concentration was shown in Section 2.3) concentrations at 19 °C, under a light illumination of 140 μmol photons m⁻² s⁻¹ with a 14/10 h light/dark cycle. Exponentially growing cells were used for the experiments.

2.3. Iron absorption and adsorption by the diatoms under different nutrient regimes

T. weissflogii or *S. costatum* cells were filtered and transferred to new medium every 1–2 days, to ensure that the cells were acclimated to these conditions. After 4 transfers, the cells were again filtered and added into 1000 mL, 0.2 μm filtered seawater (with *f/10* levels of Si and vitamins added) held in acid-cleaned polycarbonate bottles, at a cell concentration of 1 × 10⁴ cells mL⁻¹. For N and P, a two-factor experiment was performed to examine the effect of N and P on iron absorption, adsorption and bioconcentration by the cells. The experimental macronutrient treatments included: (a) total N concentrations of 16, 32, 64, and 128 μmol L⁻¹, at a total P concentration of 2 μmol L⁻¹; and (b) total P concentrations of 2, 6, 8, and 10 μmol L⁻¹, at total N concentration of 32 μmol L⁻¹. These experiments were replicated (*n* = 3). The N, P and iron concentrations were maintained in the cultures through compensating addition daily after determination of N, P and iron in the medium, i.e., semi-continuous culture was adopted.

Iron was washed using a trace metal clean reagent (Tovar-Sanchez et al., 2003). In the trace metal clean reagent, oxalate was used as the reductant to remove surface adsorbed trace metals from phytoplankton cells and other particles. To the oxalate solution, hydroxylamine, perchlorate, and 1, 10-phenanthroline were added. Next, the pH was adjusted to 8 with 10 mol L⁻¹ NaOH and

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