

- Walsh, J.J. and K.A. Steidinger. 2001. Saharan dust and Florida red tides: The cyanophyte connection. *J. Geophys. Res.* 106(C6): 11,597-11,611.
- Wheeler, P.A., B.B. North and G.C. Stephens. 1974. Amino acid uptake by marine phytoplankters. *Limnol. Oceanogr.* 19: 249-259.
- Wood, A.M. and T. Leatham. 1992. The species concept in phytoplankton ecology. *J. Phycol.* 28: 723-729.
- Yentsch, C.S. 1980. Phytoplankton growth in the sea: A coalescence of disciplines. pp. 17-32. In P.G. Falkowski [ed.] 1980. *Primary Productivity in the Sea*. Plenum Press, NY, USA.

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## The Trace Metal Composition of Marine Microalgae in Cultures and Natural Assemblages

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### Abstract

Marine microalgae deplete some essential trace metals in surface oceans and thus influence their distribution and cycling in the ocean; relatively, due to the scarce concentrations in the surface waters, the trace metals can be important in controlling algal production and regulating their community structure. However, there is limited information available for the average trace metal composition either from culture or field studies when compared to our understanding on macronutrient composition in marine microalgae. This chapter reviews the reliable field and culture studies to assess the trace metal composition (Fe, Mn, Zn, Cu, Ni, Co, and Cd) and its variability in marine microalgae. Remarkable similarities of the average trace metal composition among different culture and field studies are observed. The average composition estimated from the selected field studies can be expressed as  $P_{1000}$ ,  $Fe_{5.1 \pm 1.6}$ ,  $Mn_{0.68 \pm 0.54}$ ,  $Zn_{2.1 \pm 0.88}$ ,  $Cu_{0.41 \pm 0.16}$ ,  $Ni_{0.70 \pm 0.54}$ ,  $Co_{0.15 \pm 0.06}$ ,  $Cd_{0.42 \pm 0.20}$ . The similar metal/P composition ratios between deep ocean water and phytoplankton for Zn and Cd indicate that the two metals are regulated by microalgae like macronutrients. The metal/P ratios for Fe and Mn are about one order of magnitude smaller in deep oceanic water than in microalgae, suggesting the relative importance of scavenging processes in controlling the cycling of Fe and Mn in the ocean. Overall, these evidences support that the constant stoichiometric concept for algal elemental composition may be usefully broadened from the major macronutrients to the trace metals. The stoichiometric ratios then can provide a quantitative basis to understand how marine microalgae regulate or influence the cycling and distribution of the metals in the ocean.

## INTRODUCTION

### Elemental Composition of Marine Microalgae

Marine microalgae (phytoplankton), with their large biomass and high growth turnover rates in the ocean, are responsible for almost half of global primary production (Field et al. 1998). Considering their critical role on regulating elemental cycling in the ocean, knowledge of elemental composition of marine microalgae is essential to quantitatively understand how marine microalgae influence elemental cycling in the ocean. As early as 1930, based on some field measurements on macronutrient composition of the bulk plankton assemblages and the concentrations of nitrate and phosphate in the seawater samples, Redfield et al. (1963) concluded that the macronutrient composition of microalgae assemblages follow the constant ratios -C:N:P=106:16:1- and the N/P ratio are close to the corresponding nutrient ratios in seawater (Redfield 1934; Redfield et al. 1963; Flemming 1940). Over the past 70 years, this constant stoichiometry concept has been widely applied in numerous oceanography and marine biogeochemistry studies as reviewed in the studies (Broecker and Peng 1982; Hecky et al. 1993; Falkowski 2000; Geider and La Roche 2002; Li and Peng 2002). The ratios are especially useful for modeling plankton processes, nutrient cycling, and estimating primary productivity from the supply of a known limiting nutrient. Likewise, the information of micronutrient (essential trace metals) composition of marine phytoplankton may also provide a basis for examining how microalgae influence the relative distribution and the vertical transport of the trace metals in the ocean. It would thus be very useful if the traditional Redfield-type elemental composition ratios can be extended from the major nutrients to the trace metals-Fe, Zn, Mn, Cu, Ni, Co, and Cd.

### Roles of the Trace Metals in Regulating Oceanic Productivity

It is now well known that the availability of some certain trace metals in seawater are tightly linked with the growth and community structure of marine phytoplankton. Trace metals, including Fe, Zn, Mn, Cu, Ni, Co, Mo, are cofactors of many essential metalloenzymes and proteins that carry out various metabolic processes such as photosynthesis, respiration and major nutrient assimilation in phytoplankton. However, the dissolved concentrations of many of the trace metals are extremely low in oceanic surface waters, ranging from low nM to sub-nM levels in general (Bruland 1980; Martin and Gordon 1988). Moreover, most of the metals are chelated by

organic ligands with strong chelating ability (Bruland 1989; Rue and Bruland 1995; Moffett 1995; Saito and Moffett 2001), which are not bioavailable to most of the marine microalgae. The bioavailable concentrations of some of the essential metals are so low in the surface waters that the growth of marine algae may be limited or colimited by the metals under certain circumstance (Martin and Gordon 1988, Morel et al. 1994; Saito et al. 2002). For example, iron, the most well studied trace element in the ocean, is known to be a major limiting nutrient for algal growth in the high nutrient low chlorophyll (HNLC) regions, which at least include the Southern Ocean, the Northeast Pacific Ocean, and the equatorial Pacific Ocean (Martin and Fitzwater 1988; Martin and Gordon 1988; Martin et al. 1994; Coale et al. 1996; Boyd et al. 2000). In spite of the increasing understanding for the importance of trace metals on influencing algal productivity and regulating community structure in the ocean, compared to the abundant studies on major nutrient composition in marine phytoplankton, there is relatively little information on marine cellular metal composition from field studies.

### Previous Culture and Field Studies

While discovering the important roles of the trace metals in the ocean, a variety of culture studies have been carried out to study marine phytoplankton-trace metal interactions during the past two decades (Brand et al. 1983; Harrison and Morel 1986; Price and Morel 1990, Lee and Morel 1995, Sunda and Huntsman 1983, 1995a, b, c; 1996; 1998 a, b, c; 2000). These algal culture experiments were mainly focused on the following three studies: the interaction between medium concentrations and growth rates, interaction between growth conditions and algal metal requirement, and metal-metal interaction. These culture studies were generally limited to a few model species and to one or two essential trace metals. Among the studies, Sunda and Huntsman have published systematic detailed trace metal-algae culture studies using the model algal species, particularly the coccolithophore *Emiliania huxleyi* and the diatoms *Thalassiosira pseudonana* (neritic) and *Thalassiosira oceanica* (oceanic) in addition to *Thalassiosira weissflogii* (Sunda and Huntsman 1995a, b, c; 1996; 1997; 1998a, b; 2000). These studies have greatly enhanced our understanding on microalgae-trace metal interaction and also reported some trace metal composition for the model species under certain growth conditions.

However, it appears to be inadequate to use the trace metal composition obtained from the model species in the culture studies mentioned above to represent the composition of the whole phytoplankton assemblages in the ocean. The culture studies to determine the average metal composition

simultaneously in various marine microalgae were scarce, which is mainly due to the difficulty in preparing an ideal algal culture medium to simulate the metal concentrations in natural oceanic surface waters, the difficulty in precisely determining the various extremely low trace metal composition in the cells, and the difficulty in choosing representative algal species to represent natural algal assemblage.

The ideal approach is to directly determine the metal composition in natural phytoplankton assemblage. Nevertheless, given the sampling and contamination problems on collecting marine microalgae assemblages in oceanic surface water, there have been only a few reliable field studies reported so far. Bruland et al. (1991) compiled the results of three field studies (Martin and Knauer 1973; Martin et al. 1976; Collier and Edmond 1984) from 15 plankton samples with low Al contents and gave a rough approximate for the metal composition, representing as  $P_{1000}Fe_5Zn_2(Cu, Mn, Ni, Cd)_{0.4}$ . Kuss and Kremling (1999), using large volume pumping sampling technique, also proposed a Redfield-type metal composition from 9 biogenic samples collected in the North Atlantic Ocean, expressed as  $P_{1000}Fe_5(Zn, Mn)_2Ni_1Cd_{0.5}Cu_{0.4}Co_{0.2}$ . It should be noted that the trace metal composition obtained from field studies are not only easily biased by the interferences of lithogenic and other biogenic particles but also influenced by different sampling methods. Intercomparison of the trace metal data among the field studies using different sampling methods needs to consider the influence of their sampling methods on the data. Alternatively, comparison of the trace metal data between culture and field studies may be an appropriate approach to establish a representative trace metal composition in marine phytoplankton. A recent algal-trace metal culture study (Ho et al. 2003; 2004) was designed to test the feasibility of this idea. The study examined trace metal composition in a variety of marine microalgae species – 15 marine eukaryotic phytoplankton representing five major marine phyla – and grew the species under an identical culture medium designed to mimic natural seawater condition. The major findings of the study would be briefed in the following section.

In 1985, Morel and Hudson has already initiated the concept of extending the Redfield ratio to the trace metals, expressed as  $P_{1000}(Fe, Zn, Mn)_{10}(Cu, Cd, Ni)_1$ , based on scant culture studies on a couple of algal species. After two decades, limited field data sets and the possibility of the field data biased by the contamination of abiogenic particles still hinder our confidence to conclude whether it is appropriate and useful to establish an average trace metal composition for marine microalgae. By comparing the trace metal composition and its variability obtained from reliable culture

and field studies, and also comparing the average algal composition with deep water metal/P ratios, whether the Redfield ratio may also be usefully extended to the trace metals is evaluated.

## METHODS

### Preparation of Algae-trace Metal Culture Medium

Due to the extraordinarily low bioavailable concentrations in natural oceanic surface waters, the trace metal concentrations rarely act as toxicants in the ocean, though many of them can be at elevated levels (Sunda and Guillard 1976; Brand et al. 1986). The studies at the high and toxic metal concentration levels would not be the interest of this chapter. To mimic natural surface seawater conditions, bioavailable trace metal concentrations in trace metal culture medium, compared to traditional algal culture media like *f/2* (Guillard 1975), have to be maintained at relatively low and constant concentration levels. Trace metal ion buffer system has been proved to be an effective and easy approach to sustain low and constant supply for the bioavailable metal ions in the culture medium (Sunda and Guillard 1976). The bioavailable (inorganic) concentrations in culture medium, denoted as  $M'$ , can be buffered by organic chelators like EDTA (ethylenediamine-tetraacetic acid), which have strong chelation ability on the transition metals to keep relatively low bioavailable metal concentrations in batch culture medium. By using organic chelators to regulate  $M'$  in algal culture medium, it was revealed that both algal growth rates and metal uptake rates of the model algal species used are directly related to  $M'$  but are independent of the total metal concentrations (Sunda and Guillard 1976; Anderson et al. 1978). Given the concentrations of the total trace metals and organic chelators, the bioavailable metal concentrations and aquated free metal ions concentrations can be calculated with the complexation constants. The bioavailable and aquated free concentrations for all of the trace metals used may be precisely calculated by using chemical equilibrium computer models like MINEQL (Westall et al. 1986). Aquil, an EDTA-trace metal ion buffered medium designed for algae-trace metal studies, has been widely used in marine microalgae-trace metal studies (Morel et al. 1979; Price et al. 1988/1989; Price and Morel 1990; Yee and Morel 1996; Morel et al. 1994). The procedures for preparing the Aquil medium are briefly described here. Details for the preparation of the medium and trace metal ion buffer system may be found in Price et al. (1989/1990) and Sunda et al. (2004).

The seawater used for trace metal-algae growth studies should contain relatively low trace metal impurities, which can be obtained either directly

from natural oceanic surface waters with low metal concentrations or from trace metal free synthetic ocean water (SOW). To obtain the natural seawater, trace metal clean techniques are required during the collection and storage procedures to avoid contamination. To prepare the trace metal free SOW, the trace metal impurities in raw SOW can be removed by passing SOW through trace metal chelating resins like Chelex-100 (Price et al. 1989/1990). The low trace metal seawater is then first enriched with sterile and metal free major nutrients and vitamins. In Aquil medium, the final concentrations of the major nutrients and vitamins are 150  $\mu\text{M}$   $\text{NaNO}_3$ , 10  $\mu\text{M}$   $\text{Na}_2\text{HPO}_4$ , and 40  $\mu\text{M}$   $\text{Na}_2\text{SiO}_3$ , plus 0.1  $\mu\text{M}$  vitamin  $\text{B}_{12}$ , 0.1  $\mu\text{M}$  biotin, 20  $\mu\text{M}$  thiamin. After adding EDTA stock solution to reach a final 100  $\mu\text{M}$  final concentration, sterile trace metal stock standards are then added into the culture medium. The medium should stay for at least 3h to reach complete complexation equilibrium before inoculating algae (Price et al. 1988/1989). In Aquil medium, total trace metal concentrations are prepared to the following concentrations:  $\text{Fe}_T = 8600$  nM,  $\text{Mn}_T = 120$  nM,  $\text{Zn}_T = 80$  nM,  $\text{Cu}_T = 20$  nM, and  $\text{Co}_T = 50$  nM, calculated in the presence of 100  $\mu\text{M}$  EDTA to yield unchelated constant concentrations of  $\text{Fe}' = 20$  nM,  $\text{Mn}' = 10$  nM,  $\text{Zn}' = 20$  pM,  $\text{Cu}' = 0.2$  pM, and  $\text{Co}' = 20$  pM (Westall et al. 1986, Price et al. 1988/1989). Due to its broad application in marine biogeochemistry, cadmium is usually added with  $\text{Cd}_T = 15$  nM to yield unchelated concentrations of  $\text{Cd}' = 20$  pM (Westall et al. 1986). It should be noted there are uncertainties regarding the exact unchelated concentrations in the medium because the complexation constants for metal-EDTA in seawater are not all precisely known. Comparison among different data sets thus requires careful matching of total metal and ligand concentrations (Ho et al. 2003).

### Choice of $\text{Fe}'$ in the Culture Medium

With the exception of Fe, the choices of  $M'$  in Aquil medium are fairly low and generally near what are thought to be the bioavailable concentrations in natural surface seawater: in the range of 1-20 pM for  $\text{Cd}'$  and  $\text{Zn}'$  (Bruland 1989, 1992), 0.4-5 pM for  $\text{Cu}'$  (Moffett 1995), and 0.1-100 fM for  $\text{Co}'$  (Saito and Moffett 2001). Previous studies have shown that the metal concentration levels in Aquil medium are high enough to allow maximum growth rates for the model microalgae (Brand et al. 1983, 1986, Brand 1991, Sunda and Huntsman 1992; 1997). However, to avoid limiting the growth of coastal species, the  $\text{Mn}'$  is regulated at 10 nM in Aquil medium, a value that is typical of coastal waters but about ten times higher than in the open ocean (Landing and Bruland 1980). Likewise, the Fe concentration maintained in

Aquil culture medium is set to be extremely high to avoid limiting the growth of some coastal algal species (Morel et al. 1979; Price et al. 1989/1990). The bioavailable Fe concentration ( $\text{Fe}'$ ) is set at 20 nM in Aquil medium, which is much higher than the bioavailable concentration in the open ocean – ca 0.05-1 pM (Rue and Bruland 1995). Precipitation of Fe oxides on algal cell surfaces is unavoidable under the high concentration, and the extracellular wash for the precipitate is thus required (Hudson and Morel 1989).

To obtain meaningful trace metal concentration of microalgae to mimic natural algal assemblage, it is critical to choose an appropriate Fe concentration in algal culture medium. On one hand, low unchelated Fe medium concentrations usually lead to low growth rates for coastal algal species (Brand et al. 1983); on the other hand, high Fe medium concentrations unavoidably result in precipitation of hydrous ferric oxide onto cell surfaces (Hudson and Morel 1989). Since ferric oxide may adsorb other trace metals, without removing the surface Fe oxide, the precipitation can lead to a significant overestimation for the adsorbed trace metals in addition to Fe (Morel and Hering 1993). To obtain high growth rates and minimize the problem of extracellular Fe precipitation, Ho et al. (2003) examined the change of intracellular and extracellular Fe concentrations and growth rates for the model diatom *Thalassiosira weissflogii* by varying  $\text{Fe}'$  in culture medium. The results showed that more than 98% of Fe measured under the Aquil recipe (a total Fe concentration of 8200 nM with 100  $\mu\text{M}$  EDTA) was extracellular Fe and the percentage decreased sharply with the decreasing  $\text{Fe}'$ . At the Fe concentration of a total Fe concentration of 82 nM with 100  $\mu\text{M}$  EDTA, corresponding to an unchelated Fe concentration,  $\text{Fe}' = 0.2$  nM at 250  $\mu\text{E}$  light intensity, *Thalassiosira weissflogii* grew at 85% of its maximum growth rate and the extracellular Fe were less than 30% of the total Fe measured. This choice of  $\text{Fe}'$  (0.2 nM) thus allows one to obtain reasonably accurate value for algal cellular Fe concentrations without the tedious procedures to wash the cells for removing Fe oxides precipitate (Ho et al. 2003).

### Culturing and Sampling

To avoid contamination during culturing and pretreatment, all apparatus used for the culture medium preparation, algal culturing and sampling, and elemental analysis are prepared according to rigorous acid cleaning procedures (Cullen and Sherrell 1999; Ho et al. 2003). The algae are usually grown in polycarbonate bottles at 20°C using a 12h:12h light dark cycle with a Cool White light source between 125 and 250  $\mu\text{mol}$  quanta  $\text{m}^{-2}\text{s}^{-1}$  light

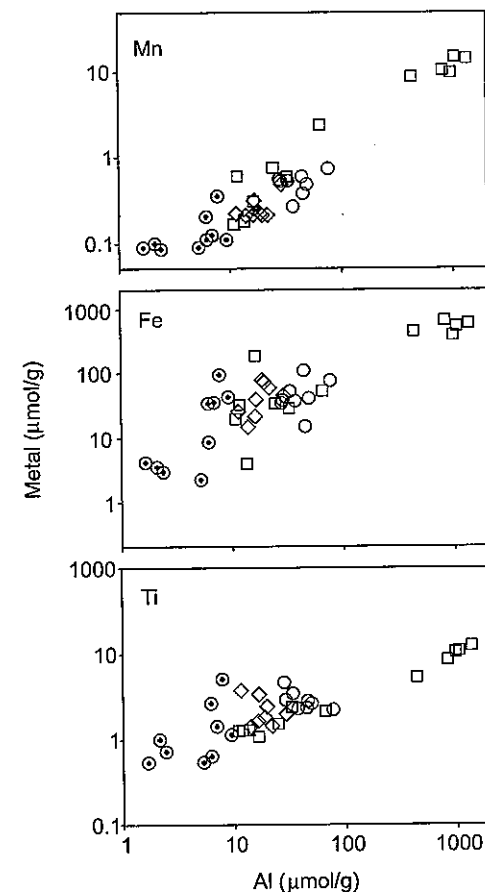
intensity. Replicate culture samples (bottles) are usually maintained in exponential growth through a minimum of 6 generations before harvesting. Cell density and size are determined daily, usually with a Coulter particle counter to calculate growth rates and cell volumes. Cells are usually harvested with a plastic filtration apparatus consisting of polypropylene filter holder and filters, which are also pre-washed with 10% trace metal grade HCl to avoid trace metal contamination. Right after harvesting the cells, the filters with the cells should be thoroughly rinsed with trace metal free seawater or NaCl solution to remove the residues of the culture medium. The filters with the cells are then digested with trace metal grade concentrated  $\text{HNO}_3$  in pre-cleaned Teflon vials for metal analysis (Ho et al. 2003).

### Trace Metal Analysis

The trace metal composition in the digested algal culture samples may be determined by any analytical instruments with high sensitivity for determination of trace metals. Sector field high resolution inductively coupled plasma mass spectrometry (HR-ICPMS) and graphite furnace atomic absorption spectrometry (GFAAS) are the most common analytical methods for the purpose. Especially, HR-ICPMS, providing an efficient means for simultaneous multi-elemental analysis including some major elements like P and S, is an ideal analytical tool for the study.

### Sampling Methods for Phytoplankton Assemblages in the Ocean

The trace metal composition in marine microalgae may be obtained directly by determining the composition in phytoplankton assemblages collected in the field. However, due to the extremely low trace metal contents in marine microalgae and extremely high trace metal concentrations in lithogenic particles in natural seawater, it is unavoidable encountering the interference from abiogenic particles in plankton assemblage samples (Fig. 8.1). The problems were clearly noted by Martin and Knauer in 1973 in their classic study for elemental composition of plankton, "the reasons for the scarcity of information concerning the elemental (metal) composition of the primary producers and primary consumers of the sea are not difficult to ascertain...", which include the contamination from terrestrial particles, difficulty to separate phytoplankton from zooplankton, and contamination from vessels and sampling apparatus. After three decades, the sampling problems proposed by Martin and Knauer for the shortage of the field data in



**Fig. 8.1** Comparison of the Al, Mn, Fe, and Ti concentration normalized to dry weight of the suspended particles collected in the surface waters of the South China Sea by size fractionated plankton nets with 10, 60, and 150  $\mu\text{m}$  net apertures (Ho et al. in prep.). The samples were collected from 5 stations across the coastal region, through continental shelf, to deep water regions. The symbols of open circle, diamond, and cross circle represent the data obtained from 10, 60, and 150  $\mu\text{m}$  nets, respectively. Square symbols stand for the data of the samples collected by 0.4  $\mu\text{m}$  polycarbonate filters.

phytoplankton do not lessen much. Although trace metal clean methodology may effectively decrease the contamination from vessels and sampling procedures, it is still challenging to remove the interferences of abiogenic materials and larger particles from the collected suspended particles. While applying the metal data of suspended particles obtained in the field to represent the composition of plankton assemblages, it is essential to exclude the influence of abiogenic composition from the samples.

Moreover, while comparing trace metal composition of suspended particles among different data sets, it should be noticed that different sampling methods represent different size particles collected. Algal assemblages and suspended particles in the oceans are usually collected by planktonic tows (Martin and Knauer 1973; Martin et al. 1976; Collier and Edmond 1984), pumping and centrifuge (Kuss and Kremling 1999), or pumping with membrane filtration (Sherrell and Boyle 1992; Cullen and Sherrell 1999; Cullen et al. 2003). Given the relatively large net aperture (several tenths of  $\mu\text{m}$  in usual), plankton nets are prone to retain larger particles like zooplankton; instead, membrane filtration methods (with 0.45  $\mu\text{m}$  pore size in general) would collect almost all particulate particles, and centrifuge method may collect even smaller particles. Compared to the true phytoplankton elemental composition, the composition in the samples collected by plankton nets are biased with larger particles like zooplankton; samples collected by centrifuge with membrane filtration methods tend to be biased by smaller abiogenic particles as well as larger zooplankton. As witnessed by Al concentration, Figure 8.1 clearly shows that the influence of abiogenic particles on the essential trace metal composition in the suspended particles is severe and the influence is strongly correlated to the particle size, the smaller the particles; the higher percentage of the abiogenic particles in the samples (Fig. 8.1).

The contamination problem from abiogenic particles in natural algal assemblages is commonly observed both in coastal and open oceans studies (Martin and Knauer 1973; Martin et al. 1976; Sherrell and Boyle 1992; Kuss and Kremling 1999). By 76  $\mu\text{m}$  plankton nets, Martin and Knauer (1973), collected the phytoplankton samples in Monterey Bay, California, on 28 different dates. With relative constant concentrations on major cations (e.g., Na, K, Mg), there were more than 80% of the 'phytoplankton' samples in the study containing extremely high amount of Al, Fe, and/or Ti. As Al or Ti are indicators for lithogenic particle concentration, the samples with high Al or Ti are highly likely to be severely biased by the composition of lithogenic particles due to the high concentration of the essential trace metals in lithogenic particles. It was found in the data set of Martin and Knauer (1973) that the essential trace metals of plankton, including Fe, Mn, Cu, Ni, Zn, were all elevated in the samples containing high Al and/or Ti, indicating that the trace metal data set from the planktonic samples with high Al and/or Ti concentration are contaminated by lithogenic particles. By using Al or Ti as an indicator for lithogenic particles, it is clearly shown that most of the particulate materials collected in the field studies, both in neritic and open ocean regions, contained considerable amount of lithogenic particles

(Martin and Knauer 1973; Sherrell and Boyle 1992; Kuss and Kremling 1999).

Since natural planktonic assemblage samples are likely to be hampered by the contamination of lithogenic particles, reliable data for natural algal metal composition must be cautiously assessed from the studies that had sufficient information of the indicative elements like P, Al, and Fe. Given the known and precise metal composition of the crust dust in sampling regions, the influence of lithogenic particles on samples might be corrected. In this chapter, the samples that had a low fraction of lithogenic material and high algal biomass as witnessed by low Al concentrations and high P concentrations are chosen. Following the constraint of Bruland et al. (1991), a cutoff of Al < 100  $\mu\text{g/g}$  dry weight, which corresponds to ca. 1  $\mu\text{mol Fe/g}$  dry weight in crustal rock, equal to the lowest algal cellular Fe concentration (Ho et al. 2003), would be used to exclude the samples that are likely biased by lithogenic particles.

## TRACE METAL COMPOSITION AND ITS VARIABILITY IN MARINE MICROALGAL CULTURE

### Control of the Trace Metal Composition: Internal and External Factors

Before examining the metal composition and its variability in marine microalgal culture, the major factors affecting the composition and the variability are briefly discussed here. Trace metal composition in marine microalgae reflects an interactive balance between external environmental conditions and internal cellular biochemical responses. The variability may come from the change of the external factors such as the nutrient concentrations, light intensity, and temperature; or due to the internal factors like the difference of algal physiological demand on the micronutrients, cellular adaptation to the habitat, or evolutionary heritage.

### Bioavailable metal concentrations ( $M'$ )

Bioavailable metal concentration,  $M'$ , is one of the major external factors controlling the cellular metal quotas. The essential trace metals are transported into cells by membrane proteins, thus algal metal uptake mechanism generally follows the Michaelis-Menten kinetics (Hudson and Morel 1993). Under steady state exponential growth condition, algal metal uptake rates are equal to specific growth rate ( $\mu$ ) multiplied by cellular metal quotas ( $Q$ ). As the bioavailable metal concentration ( $M'$ ) in surface seawater are far

smaller than the Michaelis-Menten half saturation constant, the Michaelis-Menten equation can thus be simplified and metal uptake rate would be proportional to  $M'$ . Laboratory culture studies also found that the metal uptake rates and cellular metal quotas were both proportionally related to external labile inorganic concentrations ( $M'$ ) when the cellular growth rates are limited by the metals (Sunda and Huntsman 1998c). Under metal limitation condition, as specific growth rates are also regulated by  $M'$  and are proportional to  $M'$ , the metal quota increasing extent is lessened due to the simultaneous increase of cellular growth rates.

### Metal-Metal Interaction

Due to the non-specific property of the metal transport sites on algal cellular membrane, trace metals with similar size/charge ratios or chemical nature would compete for the same transport sites as well as intracellular metal binding sites (Hudson and Morel 1993; Bruland et al 1991). The relative concentrations of different metals in culture medium would thus affect cellular uptake for the 'similar' metals. Depending on the relative concentrations of the metals, the interactive relationship among trace metals can be either synergistic or antagonistic (Bruland et al. 1991). For example, the cellular concentrations of Zn, Co and Cd in marine microalgae are highly interdependent because the metals have similar size/charge ratios (Zn and Co) or chemical nature (Zn and Cd) and thus can substitute with each other to carry out same biochemical functions (Price and Morel 1990; Lee et al. 1995; Yee and Morel 1996). In the case of *Thalassiosira weissflogii*, Zn, Co and Cd are known to substitute for each other in carbonic anhydrases which are involved in inorganic carbon acquisition (Lane and Morel 2000; Cullen et al. 1999). In contrast, when Zn' is depleted and Co is available, coccolithophores is able to utilize Co to reach maximum growth rate (Sunda and Huntsman 1995b; Yee and Morel 1996). However, preference exists if three of the metals are all available in culture medium. The Co quota of *Emiliania huxleyi* decreased by a factor of 2 as Zn' increased from 1 to 25 pM (Sunda and Huntsman 1995b) and the Cd quota of *Thalassiosira weissflogii* decreased five fold when Zn' increased from 10 to 100 pM (Sunda and Huntsman 2000), suggesting these two species prefer to take up Zn if available. Due to the metal substitutions, we should expect elevated cellular concentrations of Co and Cd in open ocean phytoplankton growing at very low Zn' concentrations. The variability of Zn, Co, and Cd quotas observed between diatoms and coccolithophores reflects in part the difference of the cellular response to metal-metal substitution (Price and Morel 1990, Morel et al. 1994, Sunda and

Huntsman 1996). Not only for Zn, Co, and Cd, different essential metals groups express various metal-metal interaction patterns, which have been studied in details especially by Sunda and Huntsman (1992; 1995b; 1996; 1998a; 2000) and by Morel and his colleagues. These metal-metal interaction patterns were well discussed and reviewed in the studies (Bruland et al. 1991; Sunda and Huntsman 1998c; Whitfield 2001).

### Major Growth Conditions (Light, Temperature, Macronutrient Availability et al.)

Since cellular metal quotas are related to cellular growth rates, the major growth factors like light, macronutrients, temperature which influence growth rates can directly or indirectly influence cellular metal quotas. For example, the Fe and Mn quotas in marine algae are highly dependent on the intensity of the light regime. Light intensity affects the cellular concentration of Fe as it is an integral part of a host of electron carriers involved in photosynthesis. A change in light intensity from 50  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  to 500  $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$  resulted in a decrease by a factor of two in the Fe quota of the dinoflagellate *Prorocentrum minimum* (Sunda and Huntsman 1997). As many of the essential metals are involved in the uptake of the major nutrients (C, N, and P), the availability of the major nutrients is also an important parameter affecting cellular metal concentrations. For example, the Zn, Co and Cd quotas in diatoms also depend on the  $p\text{CO}_2$  in the growth medium due to the demand on synthesizing carbonic anhydrases. Under low  $p\text{CO}_2$  condition in culture medium, Zn, Co and Cd quotas in diatoms tend to increase, and vice versa. The concentration and chemical form of nitrogen in the medium also influence Fe requirements and quotas algal. For example, *Thalassiosira weissflogii* cultures growing nitrate were found to have a 60% higher Fe quota than cultures grown on ammonium (Maldonado and Price 1996). In addition, habitat and/or cell size (Sunda and Huntsman 1997) are the other important factors affecting trace metal quotas in marine algae. The laboratory culture studies showed that oceanic (smaller) isolates are able to survive under metal limitation due to their lower Fe', Mn' and Zn' requirement and higher cell surface area to take up the metals when compared to neritic or larger species (Brand et al. 1983; Sunda and Huntsman 1992, 1995a, 1997).

External conditions can only explain part of the elemental composition variability in marine microalgae. Different physiological and biochemical characteristics among different species certainly are the other major factors causing the quota variability.

### Trace Metal-Algae Studies

The research groups in the laboratories of Drs William G. Sunda and Francois M.M. Morel are the pioneers in applying the trace metal buffer system to study algal trace metal concentrations in marine microalgae culture. The results of these early studies may be found in the review papers (Morel et al. 1991, Sunda and Huntsman 1998c, Whitfield 2001). Morel et al. (1991) reported that the trace metal quotas of Fe, Mn, Zn, Co, Ni, and Cd mainly based on the model coastal diatom *Thalassiosira weissflogii* growing at 90% of the maximum growth rate were 6.7, 6.7, 4.2, 2.5, 1.7, and 1.7  $\mu\text{mol/mol C}$  respectively, corresponding to 0.67, 0.67, 0.42, 0.25, 0.17, 0.17  $\text{mmol/mol P}$  if  $C/P=100$ . Later on, the systematic detailed studies carried out by Sunda and Huntsman for the model algal species *Thalassiosira pseudonana*, *Thalassiosira oceanica*, *Thalassiosira weissflogii*, and *Emiliania huxleyi* (Sunda and Huntsman 1995a, b, c; 1996; 1997; 1998a, b; 2000) quantitatively revealed many important factors affecting the metal composition and metal uptake rate as discussed above.

The metal quotas obtained from the model species are certainly inadequate to reasonably estimate a representative value for the metal composition of the whole phytoplankton assemblages in the ocean. With the difficulties on choosing and culturing enough representative species, there had been no systematic culture studies focused on determining the trace metal elemental stoichiometry in various marine algal species until recently (Ho et al. 2003, 2004), which should be the most extensive study in terms of total species number used. All of the 15 species chosen in the study were grown under an identical and low Fe culture medium designed to mimic the trace metal concentration levels in natural seawater. The individual cellular metal concentrations of each species may be found in the paper. (Ho et al. 2003). It is noted that trace metal composition normalized to a major cellular element provides a cell volume independent concentration (also called quota), which presents a meaningful unit for comparison among different species. Carbon and phosphorus are the most often used denominators to calculate metal quotas in trace metal-algae studies. However, the extracellular cellulose or bio-inorganic calcium carbonate materials found in many dinoflagellates and coccolithophores species hamper the use of carbon-normalized unit among different species; also due to the traditional Redfield ratio (Redfield 1934, 1958), metal quota are usually normalized to P. Thus, phosphorus normalized elemental quotas are most often used by marine biologists and oceanographers (Broecker and Peng 1982; Bruland et al. 1991; Hecky et al. 1993; Geider and La Roche 2002; Li and Peng 2002). It should be

pointed out that cellular P concentrations in marine microalgae may also vary plastically especially under extreme P limited condition. The studies compiled by Geider and La Roche (2002) showed that marine microalgae can exhibit large variations in the C/N/P ratios when grown under nutrient depleted conditions. The N/P ratio of some marine microalgae may vary from less than five to larger than 50 when the cells were grown under extreme N or P limited conditions. The critical N/P composition, representing the composition under N and P co-limitation condition, has been determined for several species, which ranged from 20 to 50 for the species tested (Geider and La Roche 2002). These N/P ratios under extreme nutrient depleted conditions are either far above or less than the Redfield ratio, suggesting that marine microalgae in the ocean in general are not under extreme N or P limited conditions. The relatively low variability for P content (normalized to cell volume) among the 15 species found by Ho et al. (2003) suggest that P normalized metal quotas are appropriate when the cells were grown under macronutrient replete condition.

Ho et al. (2003) found that there are relatively significant variabilities for all the metal quotas among the 15 species examined (Table 8.1). The ranges of the metal quotas from 10<sup>th</sup> to 90<sup>th</sup> percentile (excluding the lowest and highest data) are within a factor of 20 in general. In most of the species tested, the cellular quotas follow the order  $\text{Fe} > \text{Mn} > \text{Zn} > \text{Cu} > \text{Co} \approx \text{Cd}$ , from the highest Fe quota of 7.5  $\text{mmol/mol P}$  on average to the lowest Co and Cd quotas around 0.2  $\text{mmol/mol P}$  on average. Considering the numerous essential roles Fe plays in algae, Fe is expected to have the highest quota among the trace metals in spite of the fact that Fe' in natural seawater is extremely low. In contrast, the lowest Co and Cd quotas probably are due to the limited functions that Co and Cd can play in the eukaryotic species. Systematic metal quota differences were also revealed among different phyla (Fig. 8.2).

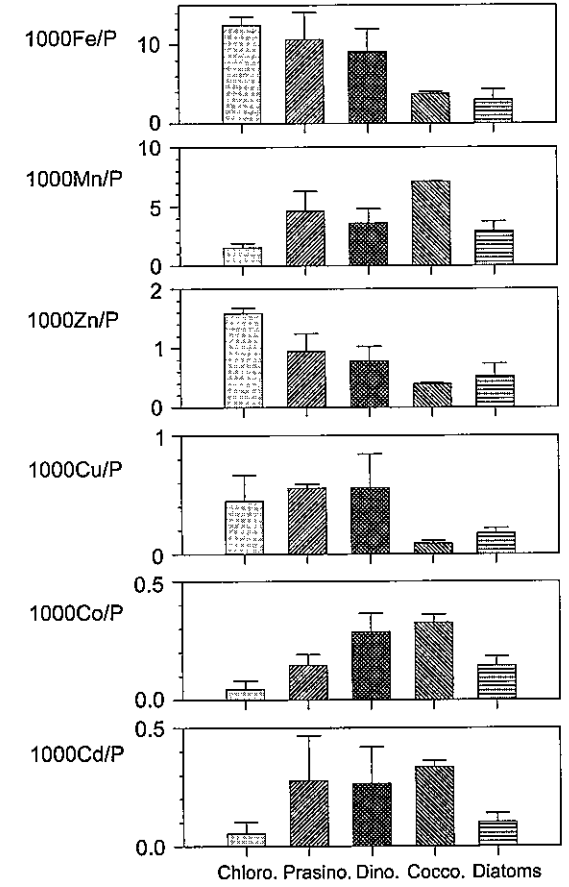
It is not surprising to find significant inter-species and inter-phyla differences for metal quotas in marine microalgae. Large variabilities for macronutrient element composition (C, N, and P) have been reported when various algal species were grown under identical nutrient replete condition (Geider and La Roche 2002), suggesting there is significant variability for the elemental composition among different species and clones. In addition to the interspecific variability, by reviewing numerous culture and field studies on the elemental composition of C, N, and P of marine microalgae and suspended particles, Geider and La Roche (2002) concluded that, depending highly on the availability of the major nutrients, the C/N/P



**Table 8.1** Comparison of trace metal composition in natural plankton assemblages, suspended particulate materials, cultured algae, and deep sea water. All metal quotas are normalized to P. Numbers in the parentheses represent one standard deviation.

Reference	Martin and Krauer 1973 (n=4) <sup>a</sup>	Martin et al. 1976 (n=6) <sup>b</sup>	Collier and Edmond 1984 (n=2) <sup>c</sup>	Kuss and Kremling 1999 (n=9) <sup>d</sup>	Cullen et al. 2003 w./w.o. 1 nM Fe <sup>e</sup>	Ho et al. 2003 (n=15)	Bruland and others <sup>f</sup>
Sampling method	Net	Net	Net	Centrifuge	Filtration	Culture	Deep water
Sampling site	Coastal	Pacific ocean	Pacific ocean	Atlantic ocean	Southern ocean	Laboratory	Pacific ocean
Fe	7.4 (5.5)	3.6 (1.3)	4.6 (0.7)	4.6 (1.3)	N.A.	7.5 (5.3)	0.33
Zn	0.86 (0.63)	1.9 (1.2)	3.0 (1.3)	1.9 (0.7)	2.9/11	0.80 (0.52)	2.7
Mn	0.39 (0.21)	0.36 (0.11)	0.34 (0.04)	1.6 (0.2)	0.70/1.6	3.8 (2.4)	0.33
Cu	0.18 (0.10)	0.38 (0.06)	0.52 (0.05)	0.37 (0.06)	0.60/1.4	0.38 (0.35)	1.7
Ni	0.21 (0.16)	0.34 (0.14)	0.86 (0.17)	1.4 (0.4)	N.A.	N.A.	3.0
Co	N.A.	N.A.	N.A.	0.19 (0.02)	0.10/0.15	0.19 (0.13)	0.01
Cd	0.07 (0.02)	0.53 (0.08)	0.54 (0.10)	0.51 (0.09)	0.44/1.2	0.21 (0.22)	0.35

<sup>a</sup>Data are obtained from the group 1 raw data in Table 1 of Martin and Krauer (1973). Samples were collected in Monterey Bay, California, with 76 μm aperture phytoplankton net during blooming condition. Only data with Al content less than 100 μg/g (dry weight) are included. High Al concentration in the samples indicates that the data may be biased by the presence of aluminosilicate minerals (Bruland et al. 1991). The phosphorus concentration was obtained from Bruland et al. (1991).  
<sup>b</sup>Data are obtained from Table 7-3 in Martin et al. (1976). Samples were collected in the oligotrophic water of North Pacific open ocean with 64 μm aperture plankton net. Only data with Al less than 100 μg/g are included, i.e. data from Station 69, 73, 75, 77, 78, and 85. The data of Station 54, 81, and 88 are not included as P concentrations were too low (81, 88) or some elements (esp. Fe) were abnormally high (54).  
<sup>c</sup>Data are obtained from Table 3 in Collier and Edmond (1984). Samples were collected in the open ocean of North Pacific with 44 μm aperture net. Only data of MANOP C (Tow 1 and Tow 2) in the table are included. Other samples had either high Al contents (>100 μg/g) or no Al data reported.  
<sup>d</sup>Data are obtained from Table 3 and 4 in Kuss and Kremling (1999). Only data of 'biogenic samples' are included in the table are included. Suspended particulate matters were collected in the surface water of the Atlantic open ocean with pumping and centrifuge method.  
<sup>e</sup>Data are obtained from Table 3 and Table 2 in Cullen et al. (2003). Data with (left data) and without Fe added (right data) are both presented. The quotas obtained with 1 nM Fe added approximate the metal quotas without Fe limitation (Table 2). Suspended particulate matters were collected in the surface water of the Antarctic ocean by pumping and membrane filtration (0.45 μm). The data plotted in Figure 3 are the data with Fe added to exclude the influence of low growth rates caused by severe Fe limitation.  
<sup>f</sup>Reference sources: Fe (Martin and Gordon 1988); Mn (Landing and Bruland 1980); Zn, Cu, Ni, and Cd (Bruland 1980); Co (Knauer et al. 1982).



**Fig. 8.2** Comparison of the average trace metal quotas among different phyla. For n > 2, the error bars represent 1 s.e.; for n = 2, the error bars represent 1 average deviation. Chlorophyceae (n = 3); Prasinophyceae (n = 3); Dinophyceae (n = 4); Prymesiohyceae (n = 2); Bacillariophyceae (n = 4). Figure is modified from the Fig. 4 of Ho et al. (2003).

ratios may range and vary dramatically in marine microalgae, though in general the elemental composition in most natural suspended materials collected in the ocean still follows the Redfield ratio. In spite of the significant variability found for the trace metal composition among different species or phyla, the possibility should not be excluded that the marine microalgae assemblage may still have a relatively constant trace metal composition as found in macronutrient composition in marine algae by Redfield. In fact, Redfield (1958) was aware that the elemental composition of plankton was uniform only in a 'statistical' sense, thus the significant

variabilities of the composition among different species or phyla should exist.

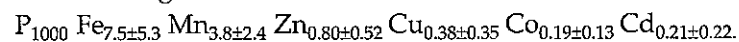
In culture studies, to choose a truly representative species to stand for the entire marine microalgae population in the ocean is operationally impractical. The 15 eukaryotic species chosen in the study by Ho et al. (2003) certainly should not be considered as exact representatives of the marine microalgal assemblage in the oceans. However, since the 15 species have included the major eukaryotic phyla in the ocean, if the metal quotas in culture studies can approximately reflect the cellular quotas in nature, the distribution ranges of the metal quotas represented by the species may stand for the quota range of algal species in natural planktonic assemblages; the metal quota average obtained from the 15 species might then approximate the average found in nature.

Even though the  $M'$  chosen in the study of Ho et al. (2003) were designed to mimic the conditions in natural seawater, whether the theoretical  $M'$  value can reflect the conditions in nature are remained unanswered. The growth conditions like temperature or light intensity for the culture studies in the laboratory are also not exactly the same as the natural conditions in the field. Examining the metal quota variability affected by varying  $M'$  and other major growth conditions would thus help us understand the variability extent of the quotas affected by the external and internal factors.

The question can be answered by the laboratory studies in which the quotas of a given trace metal in a given phytoplankton species are measured under a variety of growth conditions, and over a range of  $M'$ . Such detailed studies have already been performed by Sunda and Huntsman (1995b, 1998b, 2000). The studies of Sunda and Huntsman showed that the cellular trace metal quotas in marine phytoplankton are relatively well regulated over a certain range of bioavailable metal concentrations, a range that actually embraces the natural metal concentrations in the oceans but not severely limiting the algal growth. Using Fe as an example, when the bioavailable Fe concentration ( $Fe'$ ) range roughly from 10 to 750 pM (Sunda and Huntsman 1995a), the cellular Fe quotas varied with  $Fe'$  are listed as the following: Fe quotas of *E. huxleyi* varied from 11 to 24  $\mu\text{mol/mol C}$  when  $Fe'$  ranged from 10 to 750; Fe quotas of *Pelagomonas calceolata* varied from 8 to 10  $\mu\text{mol/mol C}$  when  $Fe'$  ranged from 11 to 580; Fe quotas of *Thalassiosira oceanica* varied from 5 to 34  $\mu\text{mol/mol C}$  when  $Fe'$  ranged from 10 to 760; Fe quotas of *Thalassiosira pseudonana* varied from 13 to 70  $\mu\text{mol/mol C}$  when  $Fe'$  ranged from 24 to 760; Fe quotas of *Thalassiosira weissflogii* varied from 11 to 31  $\mu\text{mol/mol C}$  when  $Fe'$  ranged from 28 to 750; Fe quotas of *Prorocentrum minimum* varied from 11 to 37  $\mu\text{mol/mol C}$  when  $Fe'$  ranged from 23 to 750.

To summarize, an increase by factors of 20 to 75 in the unchelated Fe concentration ( $Fe'$ ) only results cellular quota change by factors of 1.3 to 7 for all the algae examined, which included diatoms, coccolithophore, and dinoflagellate. Likewise, when  $Zn'$  increased from 15 to 2454 pM, the Zn quotas only increased by factors of 3 to 10 for the algae examined (Sunda and Huntsman 1996). Similar results were also found for other trace metals. The detailed comparison for all the metals are compiled in the study of Ho et al. (2003).

In conclusion, bioavailable trace metal concentrations only modestly affect algal metal quota at the low concentration levels which are close to the natural concentrations. Trace metal quotas are generally regulated within a factor of 2 to 4 when the  $M'$  varies over 1 order of magnitude. Similarly, the influence of other external factors, like metal-metal interaction, the light intensity, Fe limitation and so on, can rarely change the cellular quota of a given trace metal by more than a factor of 2 to 5 in the natural growth conditions (Sunda and Huntsman 1997; Cullen et al. 2003). Thus, it can be said that the quota variabilities affected by the major external factors are much smaller than the intrinsic variabilities that are seen among the 15 species, which span one or two orders of magnitude depending on the metal. Thus, the metal quotas reported from the culture study (Ho et al. 2003) mainly reflect the intrinsic biochemical requirements for the essential trace elements and the quotas determined in the culture study should be close to the metal quotas for the species in the ocean. The range of the metal quotas observed in the laboratory study can thus be taken as a first approximation of the range expected for the trace element composition of phytoplankton in the sea. The quota average of the culture study can be taken as a first approximation of the trace metal quotas of various species of phytoplankton. The average with one standard deviation of the metal quotas in the culture study of Ho et al. (2003) yields the following trace metal stoichiometry for marine microalgae:



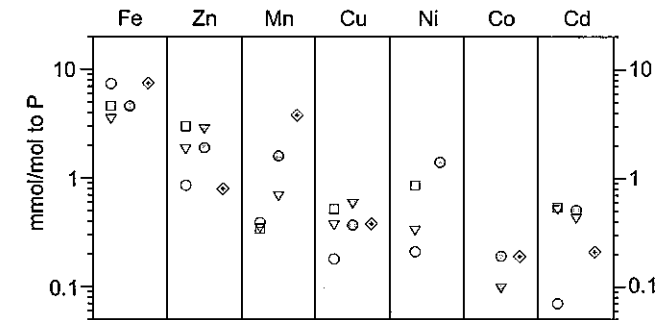
In other algal studies focusing on metal composition, the three model species of *Thalassiosira pseudonana*, *Thalassiosira weissflogii*, and *Emiliania huxleyi* have the most complete metal quota data. Two of the species *Thalassiosira weissflogii* and *Emiliania huxleyi* were already included in the study of Ho et al. (2003). The trace metal quotas of the two species obtained from Ho et al. (2003, 2004) are generally close to those reported by Sunda and Huntsman (Ho et al. 2003, 2004). The data in Ho et al. can thus well represent the value of the same species from other studies.

## TRACE METAL COMPOSITION IN NATURAL PLANKTONIC ASSEMBLAGES

### The Average and Variability of the Metal Composition

As discussed in the method section, while applying the metal data of suspended particles obtained in the field to represent the composition of plankton assemblages, it is essential to exclude the influence of abiogenic composition from the samples. Using Al and P concentrations as thresholds to evaluate the data obtained from field studies, the data sets compiled in Table 8.1 can be considered to be reliable in representing the average metal composition for plankton assemblages in the sampling regions. The three earlier data sets all came from plankton tows samples, including the coastal sample (Martin and Knauer 1973) and two open ocean samples (Martin et al. 1976; Collier and Edmond 1984), collected with 76, 64, and 44  $\mu\text{m}$  net apertures respectively. The data set of Kuss and Kremling (1999) came from pumping and centrifuge; compared to the data set of Cullen et al. (2003) which came from pumping plus membrane filtration. In spite of no available information of Al and Fe in the data set of Cullen et al. (2003), the data are included, as it is unlikely that the samples collected in the Antarctic region were severely influenced by lithogenic particles, where it is known to have low terrestrial dust input. In the study of Cullen et al. (2003), the metal quotas with and without Fe treated (adding 1 nM) are both included. To exclude the influence of the low growth rates due to the severe Fe limitation (Cullen et al. 2003), the metal quotas with Fe added are used for calculating the average. To visualize the difference among different data sets from different sampling methods, the data in Table 8.1 are presented in Fig. 8.3.

It should be noted that some of the average metal composition value in the data sets have large standard deviations (Table 8.1). This is partially due to the limited numbers of reliable data in each data set and partially due to the influence of abiogenic particles (Table 8.1). It can be justified by larger standard deviation for Fe and Mn, and smaller standard deviation for Co and Cd. As seen in Fig. 8.3, in spite of the fact that the data were obtained from different sampling methods and sampling regions, the variability of each metal composition from all the data sets are fairly constrictive. The metal composition follow the order  $\text{Fe} > \text{Zn} \approx \text{Mn} > \text{Cu} > \text{Co} \approx \text{Cd}$ , from the highest Fe quota of 7.4 mmol/mol P to the lowest Co and Cd quotas around 0.1 mmol/mol P, which are similar to the metal quota distribution trends observed in the culture studies (Ho et al. 2003). The Fe quotas are remarkably consistent with each other in the field datasets, ranging from 3.6 to 7.4 mmol/mol P. The highest Fe value, 7.4 mmol/mol P, is from the solo coastal



**Fig. 8.3** Comparison of the average trace metal quotas among field and culture studies. All of the metals are normalized to P with the units of mmol/mol P. Open symbols represent the data obtained from plankton tow sampling method (Circle: Martin and Knauer 1973; Reverse triangle: Martin et al. 1976; Square: Collier and Edmond 1984); solid symbols represent the data obtained from centrifuge or filtration methods (Circle: Kuss and Kremling 1999; Reverse triangle: Cullen et al. 2003); the culture data is labeled with the crossed diamond symbol (Ho et al. 2003; 2004). Details for how the data were obtained are described in Table 8.1.

data (Martin and Knauer 1973). Neritic species are expected to have higher Fe quotas than oceanic species as culture studies have shown that neritic species have higher Fe demands to sustain maximum growth than oceanic species (Brand et al. 1983; Sunda and Huntsman 1995a). Overall, the Fe value among the field data sets only range by factors of 2. Similarly, the quotas of Zn and Cu in the data sets only vary approximately by factors of 3, ranging from 0.9 to 3.0 and from 0.2 to 0.6 mmol/mol P respectively. In both cases (Zn and Cu), the Zn and Cu value from the study of Martin and Knauer (1973) are the lowest among the data sets, using planktonic tow with a larger net aperture (76  $\mu\text{m}$ ) to collect plankton samples during a diatom bloom in coastal region.

Compared to Fe, Zn, and Cu, the variabilities of the Mn and Ni quotas are much larger, ranging from 0.36 to 1.6; and from 0.21 to 1.4 mmol/mol P respectively. Interestingly, the Mn quotas of the three data set that came from plankton nets are almost identical to one another, and both the Mn and Ni quotas from plankton nets are all significantly lower than the other 2 data sets obtained from pumping and filtration methods, suggesting the larger particles collected by plankton tows may contain lower Mn and Ni quotas than the particles collected by the pumping and centrifuge methods. The result obtained by analyzing the 15 Mn data from Ho et al. (2003) shows that the averaged Mn quota (with one standard error) of the four largest species, with cell volume more than 1000 ( $\mu\text{m}$ )<sup>3</sup>, *Gymnodinium chlorophorum*, *Thalassiosira heimii*, *Ditylum brightwellii*, and *Thalassiosira eccentrica*, is

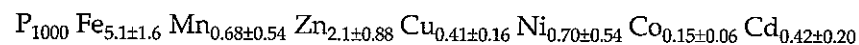
1.8±0.2 mmol/mol P, which was much smaller than the average of the other 11 smaller species, 4.5±0.6 mmol/mol P, indicating smaller phytoplankton may have higher Mn quotas or there are significant Mn adsorption on the cell surface. If the first situation is the case, samples collected by plankton nets would underestimate Mn quotas in phytoplankton assemblage; and the Mn quotas obtained from the samples collected by pumping and filtration should be more representative for total plankton. The Ni quotas determined in the samples collected by the plankton tows were also lower than the ones obtained from the centrifuge method. It is believed that prokaryotic algae may have kept using Ni and Co in the modern ocean and contain higher Ni and Co quotas due to the reducing conditions of early Proterozoic oceans that prokaryotic algae appeared from and the higher Ni and Co availability under anoxic conditions than other trace metals (Saito et al. 2002). Thus it is expected to see higher Ni quotas in the samples collected by the centrifuge method, which probably also included most of the prokaryotic algae (Fig. 8.3). Unfortunately, there were no Co data reported for the studies with plankton tow sampling methods. The two field Co data from pumping method were a close match for the data of the culture study, ranging from 0.1 to 0.19 mmol/mol P and remarkably close to the culture value (0.19), which is also the lowest average quota among the metals. All of the field studies listed in the Table 8.1, except Kuss and Kremling (1999), probably have undersampled the picoplankton and thus cyanobacteria. Due to their high total biomass in the open ocean, more future culture and field studies should focus on examining the trace metal composition of the bacteria.

The most noteworthy features in Fig. 8.3 is the fairly high and remarkably consistent Cd quotas in the open ocean samples collected from different sampling methods, ranging from 0.44 to 0.54 mmol/mol P, which is almost one order of magnitude higher than the coastal Cd value from Martin and Knauer (1973). These high Cd values from the oceanic samples, possibly reflect extensive substitution of Cd for Zn in the algae live in the Zn-poor open ocean and intensive uptake of Cd by calcareous plankton organisms.

Compared to the culture data (Table 8.1), only with the notable exception of Mn, the field quotas for trace elements fall within the range of laboratory values (Fig. 8.3) and the individual field value of Fe, Zn, Cu, Co, and Cd are relatively close to one another among the different field studies and also close to the average of the culture values (Table 8.1 and Fig. 8.3).

The consistency of the average metal composition of the reliable field and culture studies raises a fundamental question about why the metal composition can be relatively constant in marine phytoplankton assemblage.

In spite of the fact that the interspecific variability is fairly large, ranging around one order of magnitude for most of the metals, the inter-phyla variability narrows the variability down to factors of 5 or even less after averaging the metal quotas among the different species from the same phyla (Fig. 8.2). The moderate inter-phyla metal variability can be smoothed further when averaging the metal contents from the whole algal community structure. Although  $M'$  and other growth conditions are different in different regions, as discussed previously, the metal quotas are relatively well regulated by varying  $M'$  and other growth factors under natural conditions. In addition, several lines of evidence suggest that marine microalgae that survive under environments with extremely low metal concentrations, develop various mechanisms to take up the essential trace metals, which would narrow down the metal quota differences for the algae grown under high and low  $M'$  regions. For example, the cell surface/volume ratios are much smaller in the open oceans where the metal concentrations are extremely low and some open ocean species are able to take up organically bound Fe in natural seawater (Maldonado and Price 1999, Hutchins et al. 1999). However, it is possible that the generalities of the average metal composition may be lost under certain growth environments that are dominant by few algal species such as in the regions with *Emiliania huxleyi* blooming or red tide. To summarize, the average metal data with one standard deviation from the field studies yields the following trace metal stoichiometry:

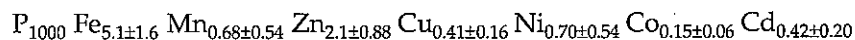


## CONCLUSIONS

### Extend the Redfield ratio?

The concept of the Redfield ratio may refer to the following two aspects: the first one is the consistency of the elemental composition of the plankton assemblage collected from different oceanic regions; the second one is the relationship between plankton composition and seawater composition. Can the Redfield ratio concept be usefully extended to the trace metals in terms of the two aspects? In this review, the intercomparison of the metal quotas between the culture and field datasets shows that the average metal quota from the field datasets obtained from various sampling methods and regions are relatively consistent to each other; and the average metal quotas measured in the field assemblages are also consistent with the average composition determined in culture studies. These striking similarities not

only indicate that the metal composition obtained from laboratory culture studies can precisely reflect algal trace metal composition in the field but also support the data reported from the field studies listed in Table 8.1 may well represent the average metal composition for plankton assemblages. It can be said that it is very likely that there is constant trace metal composition for phytoplankton assemblages in the ocean. In this aspect, the traditional Redfield ratio may be extended to the trace metals. The overall trace metal stoichiometry obtained from the five field studies listed in Table 8.1 is:



It appears more field studies are needed to confirm the consistency of the average metal quotas in the ocean. Comparing the metal composition in plankton with water metal/p ratios in the deep Pacific ocean water (Table 8.1), expressed as  $P_{1000} Fe_{0.33} Mn_{0.33} Zn_{2.7} Cu_{1.7} Ni_{3.0} Co_{0.01} Cd_{0.35}$ , the result shows that the composition for Cd and Zn are similar between the phytoplankton composition and the water composition, indicating that the two metals are mainly regulated by biological processes, taken up in the surface ocean by microalgae and regenerated in the deep oceans. Relatively, it is not surprising to obtain the low metal/P value in the deep water for Fe and Mn due to their property to be scavenged in the water column of the oceans. The higher Cu/P and Ni/P ratios in the deep water than the algal composition suggest that the sources of dissolved Cu and Ni in oceanic deep water are mainly from abiogenic materials instead of the decomposition of biogenic particles. Overall, the metal stoichiometry in marine microalgae provides a basis for modeling and examining how marine microalgae influence the relative distribution and the vertical transport of the trace metals in the ocean, especially for Cd and Zn.

### Final Remark

Laboratory algal culture experiments observed that the essential metals at low concentrations can limit marine microalgal growth (Anderson and Morel 1982; Brand et al. 1983). Driven partially by the findings, John Martin and his colleagues eventually revealed and proved the importance of Fe on controlling algal productivity in the ocean and proposed the prominent 'The Iron Hypothesis' (Martin and Gordon 1988). Trace metal-algae study is a splendid example showing that marine microalgal culture study is a powerful analogue tool to understand the roles of marine microalgae play in nature.

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### REFERENCES

- Anderson, M.A., F.M.M. Morel, and R.R.L. Guillard. 1978. Growth limitation of a coastal diatom by low zinc ion activity. *Nature*. 276: 70-71.
- Anderson, M.A. and F.M.M. Morel. 1982. The influence of aqueous iron chemistry on the uptake of iron by the coastal diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 27: 789-813.
- Boyd, P.W., A.J. Watson, C.S.B. Law, E.R. Abraham, T. Trull, R. Murdoch, D.C.E. Bakker, A.R. Bowie, K.O. Buesseler, H. Chang, M. Charette, P. Croot, K. Downing, R. Frew, M. Gall, M. Hadfield, J. Hall, M. Harvey, G. Jameson, J. LaRoche, M. Liddicoat, R. Ling, M.T. Maldonado, R.M. McKay, S. Nodder, S. Pickmere, R. Pridmore, S. Rintoul, K.K. Safi, P. Sutton, R. Strzepek, K. Tanneberger, S. Turner, A. Waite, and J. Zeldis. 2000. A mesoscale phytoplankton bloom in the polar southern ocean stimulated by Fe fertilization. *Nature*. 407: 695-702.
- Brand, L.E. 1991. Minimum iron requirements of marine phytoplankton and the implications for the biogeochemical control of new production. *Limnol. Oceanogr.* 36: 1756-1771.
- Brand, L.E., W.G. Sunda, and R.R.L. Guillard. 1983. Limitation of marine phytoplankton reproductive rates by zinc, manganese, and iron. *Limnol. Oceanogr.* 28: 1182-1198.
- Brand, L.E., W.G. Sunda, and R.R.L. Guillard. 1986. Reduction of marine phytoplankton reproduction rates by copper and cadmium. *J. Exp. Mar. Biol. Ecol.* 96: 225-250.
- Broecker, W.S. and T.-H. Peng. 1982. *Tracers in the Sea*. Lamont-Doherty Geological Observatory Columbia University, Palisades, New York, USA.
- Bruland, K.W. 1980. Oceanographic distributions of cadmium, zinc, nickel, and copper in the north Pacific. *Earth Planet. Sci. Lett.* 47: 176-198.
- Bruland, K.W. 1989. Complexation of zinc by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 34: 269-285.
- Bruland, K.W. 1992. Complexation of cadmium by natural organic ligands in the central North Pacific. *Limnol. Oceanogr.* 37: 1008-1017.
- Bruland, K.W., J.R. Donat, and D.A. Hutchins. 1991. Interactive influences of bioactive trace metals on biological production in oceanic waters. *Limnol. Oceanogr.* 36: 1555-1577.
- Coale, K.H., S.E. Fitzwater, R.M. Gordon, K.S. Johnson, and R.T. Barber. 1996. Control of community growth and export production by upwelled iron in the equatorial Pacific Ocean. *Nature*. 379: 621-624.
- Collier, R. and J. Edmond. 1984. The trace element geochemistry of marine biogenic particulate matter. *Prog. Oceanogr.* 13: 113-199.

- Cullen, J.T. and R.M. Sherrell. 1999. Techniques for determination of trace metals in small samples of size-fractionated particulate matter: phytoplankton metals off central California. *Mar. Chem.* 67: 233-247.
- Cullen, J.T., T.W. Lane, F.M.M. Morel, and R.M. Sherrell. 1999. Modulation of cadmium uptake in phytoplankton by seawater  $p\text{CO}_2$ . *Nature*. 402: 165-167.
- Cullen, J.T., Z. Chase, K.H. Coale, S.E. Fitzwater, and R.M. Sherrell. 2003. Effect of iron limitation on the cadmium to phosphorus ratio of natural phytoplankton assemblages from the Southern Ocean. *Limnol. Oceanogr.* 48: 1079-1087.
- Falkowski, P.G. 2000. Rationalizing elemental ratios in unicellular algae. *J. Phycol.* 36: 3-6.
- Field, C.B., M.J. Behrenfeld, J.T. Randerson, and P. Falkowski. 1998. Primary production of the biosphere: Integrating terrestrial and oceanic components. *Science*. 281: 237-240.
- Flemming, R.H. 1940. Composition of plankton and units for reporting populations and production. pp. 535-539 *In* Proceedings of the Sixth Pacific Science Congress of the Pacific Science Association. 1939. Uni. California Press, California, USA.
- Geider, R.J. and J. La Roche. 2002. Redfield revisited: variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* 37: 1-17.
- Guillard, R.R.L. 1975. Culture of phytoplankton for feeding marine invertebrates. pp 26-60. *In* Culture of Marine Invertebrate Animals., W.L. Smith, and M.H. Chanley [eds.], Plenum Press, New York, USA.
- Harrison, G.I. and F.M.M. Morel, 1986. Response of the marine diatom *Thalassiosira weissflogii* to iron stress. *Limnol. Oceanogr.* 31: 989-997.
- Hecky, R.E., P. Campbell, and L.L. Hendzel. 1993. The stoichiometry of carbon, nitrogen, and phosphorus in particulate matter of lakes and oceans. *Limnol. Oceanogr.* 38: 709-724.
- Ho, T.-Y., A. Quigg, Z.V. Finkel, A.J. Milligan, K. Wyman, P.G. Falkowski, and F.M.M. Morel. 2003. The elemental composition of some marine phytoplankton. *J. Phycol.* 39: 1145-1159.
- Ho, T.-Y., A. Quigg, Z.V. Finkel, A.J. Milligan, K. Wyman, P.G. Falkowski, and F.M.M. Morel. 2004. The elemental composition of some marine phytoplankton (Corrigendum). *J. Phycol.* 40: 227-227.
- Ho, T.-Y., L. S. Wen, D.C. Lee, and C.F. You, (in prep.) Sources and abundance of trace metals in the suspended particles collected by size-fractionated plankton nets in the South China Sea.
- Hudson, R.J.M. and F.M.M. Morel. 1989. Distinguishing between extracellular and intracellular iron in marine phytoplankton. *Limnol. Oceanogr.* 34: 1113-1120.
- Hudson, R.J.M. and F.M.M. Morel. 1993. Trace metal transport by marine microorganisms - implications of metal coordination kinetics. *Deep-Sea Res. I.* 40: 129-150.
- Hutchins, D.A., A.E. Witter, A. Butler, and G.W. Luther. 1999. Competition among marine phytoplankton for different chelated iron species. *Nature*. 400: 858-861.
- Knauer, G.A., J.H. Martin, and R.M. Gordon. 1982. Cobalt in northeast Pacific waters. *Nature*. 297: 49-51.
- Kuss, J. and K. Kremling. 1999. Spatial variability of particle associated trace elements in near-surface waters of the North Atlantic (30 degrees N/60 degrees W to 60 degrees N/2 degrees W), derived by large-volume sampling. *Mar. Chem.* 68: 71-86.
- Landing, W.M. and K.W. Bruland. 1980. Manganese in the north Pacific. *Earth Planet. Sci. Lett.* 49: 45-56.

- Lane, T.W. and F.M.M. Morel. 2000. A biological function for cadmium in marine diatoms. *Proc. Natl. Acad. Sci.* 97: 4627-4631.
- Lee, J.G. and F.M.M. Morel. 1995. Replacement of zinc by cadmium in marine phytoplankton. *Mar. Ecol. Prog. Ser.* 127: 305-309.
- Lee, J.G., S.B. Roberts, and F.M.M. Morel. 1995. Cadmium: A nutrient for the marine diatom *Thalassiosira weissflogii*. *Limnol. Oceanogr.* 40: 1056-1063.
- Li, Y.H. and T.H. Peng. 2002. Latitudinal change of remineralization ratios in the oceans and its implication for nutrient cycles. *Global Biogeochem. Cycles* 16: 77-1-77-16.
- Maldonado, M.T. and N.M. Price. 1996. Influence of N substrate on Fe requirements of marine centric diatoms. *Mar. Ecol. Prog. Ser.* 141: 161-172.
- Maldonado, M.T. and N.M. Price. 1999. Utilization of iron bound to strong organic ligands by plankton communities in the subarctic Pacific Ocean. *Deep-Sea. Res. II.* 46: 2447-2473.
- Martin, J.H. and R.M. Gordon. 1988. Northeast Pacific iron distributions in relation to phytoplankton productivity. *Deep-sea Res. I.* 35: 177-196.
- Martin, J.H. and G.A. Knauer. 1973. The elemental composition of plankton. *Geochim. Cosmochim. Acta.* 37: 1639-1653.
- Martin, J.H., K.W. Bruland, and W.W. Broenkow. 1976. Cadmium transport in the California current., pp. 159-184. *In* H.L. Windom and R.A. Duce [eds.], Marine Pollutant Transfer. Lexington Books (D.C. Health and Co.). Toronto, Canada.
- Martin, J.H. and S.E. Fitzwater. 1988. Iron deficiency limits phytoplankton growth in the north-east Pacific subarctic. *Nature*. 331: 341-343.
- Martin, J.H., K.H. Coale, K.S. Johnson, S.E. Fitzwater, R.M. Gordon, S.J. Tanner, C.N. Hunter, V.A. Elrod, J.L. Nowicki, T.L. Coley, R.T. Barber, S. Lindley, A.J. Watson, K. Van Scoy, C.S. Law, M.I. Liddicoat, R. Ling, T. Stanton, J. Stockel, C. Collins, A. Anderson, R. Bidigare, M. Ondrusek, M. Latasa, F.J. Millero, K. Lee, W. Yao, J.-Z. Zhang, G. Friederich, C. Sakamoto, F. Chavez, K. Buck, Z. Kolber, R. Greene, P.G. Falkowski, S.W. Chisholm, F. Hoge, R. Swift, J. Yungel, S. Turner, P. Nightingale, A. Hatton, P. Liss, and N. W. Tindale. 1994. Testing the iron hypothesis in ecosystems of the equatorial Pacific Ocean. *Nature*. 371: 123-129.
- Moffett, J.W. 1995. The spatial and temporal variability of copper complexation by strong organic ligands in the Sargasso Sea. *Deep-Sea Res. I.* 42: 1273-1295.
- Morel, F.M.M. and J.G. Hering. 1993. Principles and applications of aquatic chemistry. Wiley, New York, USA.
- Morel, F.M.M. and R.J.M. Hudson. 1985. The geobiological cycle of trace elements in aquatic systems: Redfield revisited. pp 251-281. *In* W. Stumm, [ed.], Chemical Processes in Lakes. John Wiley, New York, USA.
- Morel, F.M.M., R.J.M. Hudson, and N.M. Price. 1991. Limitation of productivity by trace metals in the sea. *Limnol. Oceanogr.* 36: 1742-1755.
- Morel, F.M.M., J.R. Reinfelder, S.B. Roberts, C.P. Chamberlain, J.G. Lee, and D. Yee. 1994. Zinc and carbon co-limitation of marine-phytoplankton. *Nature*. 369: 740-742.
- Morel, F.M.M., J.G. Rueter, D.M. Anderson, and R.R.L. Guillard. 1979. Aquil: a chemically defined phytoplankton culture medium for trace metal studies. *J. Phycol.* 15: 135-141.
- Price, N.M., G.I. Harrison, J.G. Hering, R.J. Hudson, P.M.V. Nirel, B. Palenik, and F.M.M. Morel. 1988/89. Preparation and chemistry of the artificial algal culture medium Aquil. *Biol. Oceanogr.* 6: 443-461.
- Price, N.M. and F.M.M. Morel. 1990. Cadmium and cobalt substitution for zinc in a marine diatom. *Nature*. 344: 658-660.

- Redfield, A.C. 1934. On the proportions of organic derivatives in sea water and their relation to the composition of plankton. pp. 176-92. In R.J. Daniel [ed.], James Johnstone Memorial Volume. Liverpool University Press, UK.
- Redfield, A.C. 1958. The biological control of the chemical factors in the environment. *Am. Scientist*. 46: 205-221.
- Redfield, A.C., B.H. Ketchum, and F.A. Richards. 1963. The influence of organisms on the composition of sea-water. pp. 26-77. In M.N. Hill [ed.], *The Sea*, Interscience Publication New York USA.
- Rue, E.L. and K.W. Bruland. 1995. Complexation of iron III by natural organic ligands in the Central north Pacific as determined by a new competitive ligand equilibration/adsorptive cathodic stripping voltammetric method. *Mar. Chem.* 50: 117-138.
- Saito, M.A. and J.W. Moffett. 2001. Complexation of cobalt by natural organic ligands in the Sargasso Sea as determined by a new high-sensitivity electrochemical cobalt speciation method suitable for open ocean work. *Mar. Chem.* 75: 49-68.
- Saito, M.A., J.W., Moffett, S.W. Chisholm, and J.B. Waterbury, 2002. Cobalt limitation and uptake in *Prochlorococcus*. *Limnol. Oceanogr.* 47: 1629-1636.
- Sherrell, R.M. and E.A. Boyle. 1992. The trace metal composition of suspended particles in the oceanic water column near Bermuda. *Earth Planet. Sci. Lett.* 111: 155-174.
- Sunda, W.G. and R.R.L. Guillard 1976. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. *J. Mar. Res.* 34: 511-529.
- Sunda, W.G. and S.A. Huntsman. 1983. Effect of competitive interactions between manganese and copper on cellular manganese and growth in estuarine and oceanic species of the diatom *Thalassiosira*. *Limnol. Oceanogr.* 28: 924-934.
- Sunda, W.G. and S.A. Huntsman. 1992. Feedback interactions between zinc and phytoplankton in seawater. *Limnol. Oceanogr.* 37: 25-40.
- Sunda, W.G. and S.A. Huntsman. 1995a. Iron uptake and growth limitation in oceanic and coastal phytoplankton. *Mar. Chem.* 50: 189-206.
- Sunda, W.G. and S.A. Huntsman, 1995b. Cobalt and zinc interreplacement in marine phytoplankton: Biological and geochemical implications. *Limnol. Oceanogr.* 40: 1404-1417.
- Sunda, W.G. and S.A. Huntsman. 1995c. Regulation of copper concentration in the oceanic nutricline by phytoplankton uptake and regeneration cycles. *Limnol. Oceanogr.* 40: 132-137.
- Sunda, W.G. and S.A. Huntsman. 1996. Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. *Limnol. Oceanogr.* 41: 373-387.
- Sunda, W.G. and S.A. Huntsman, 1997. Interrelated influence of iron, light and cell size on marine phytoplankton growth. *Nature*. 390: 389-392.
- Sunda, W.G. and S.A. Huntsman, 1998a. Interactions among  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Mn}^{2+}$  in controlling cellular Mn, Zn, and growth rate in the coastal alga *Chlamydomonas*. *Limnol. Oceanogr.* 43: 1055-1064.
- Sunda, W.G. and S.A. Huntsman. 1998b. Control of Cd concentrations in a coastal diatom by interactions among free ionic Cd, Zn, and Mn in seawater. *Envir. Sci. Technol.* 32: 2961-2968.
- Sunda, W.G. and S.A. Huntsman. 1998c. Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems. *Sci. of the Total Envir.* 219: 165-181.
- Sunda, W.G. and S.A. Huntsman. 2000. Effect of Zn, Mn, and Fe on Cd accumulation in phytoplankton: Implications for oceanic Cd cycling. *Limnol. Oceanogr.* 45: 1501-1516.

- Sunda, W.G., N.M. Price, and F.M.M. Morel 2004. Trace metal ion buffers and their use in culture studies. In R. Anderson [ed.], *Algal Culturing Techniques*, Academic Press. Burlington USA.
- Westall, J.C., J.L. Zachary, and F.M.M. Morel 1986. MINEQL: a computer program for the calculation of the chemical equilibrium composition of aqueous systems. Report 86-01, Department of Chemistry, Oregon State University, Corvallis, OR, USA.
- Whitfield, M. 2001. Interactions between phytoplankton and trace metals in the ocean. *Adv. Mar. Biol.* 41: 3-128.
- Yee, D. and F.M.M. Morel. 1996. In vivo substitution of zinc by cobalt in carbonic anhydrase of a marine diatom. *Limnol. Oceanogr.* 41: 573-577.