Limitation of Productivity by Trace Metals in the Sea

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Limitation of productivity by trace metals in the sea

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Abstract

Some trace metals such as Fe, Ni, Cu, and Zn are essential for the growth of phytoplankton. The concentrations of these essential trace elements in seawater are so low as to limit their availability to aquatic microbiota. Trace element uptake is ultimately limited by kinetics of reaction with transport ligands or by diffusion to the cell. From what we know of the characteristics of the uptake systems of phytoplankton and their trace metal requirements we can estimate that Fe and Zn may at some times in some places limit phytoplankton productivity, which is in accord with available field data on trace metal enrichments.

Although the founding fathers of the field discussed many elements, including Fe, Mn, Zn, and Cu, as potentially limiting algal growth in seawater (Harvey 1945), for the past several decades biological oceanographers have focused almost exclusively on N and, to a lesser extent, on P and Si. Over that period we have learned that surface seawater concentrations of biologically interesting trace elements are much lower than previously thought (Bruelard 1983). The pervasive contamination that distorted early measurements of trace elements would have also obfuscated their possible biological role. The application of so-called clean techniques to biological experiments has rekindled oceanographers' interest in the biological and ecological function of trace elements in the sea, particularly the possible role of Fe in limiting primary production in oceanic regions where surface seawater is relatively rich in N and P.

Several years ago we proposed that many bioactive elements may be colimiting phytoplankton growth in oceanic waters, that the stoichiometric concepts of Redfield might be usefully extended to trace elements, and that the concentrations of biologically essential trace elements in surface seawater might reflect the properties of algal uptake systems (Morel and Hudson 1984). In this paper we re-examine these ideas in light of what has been learned regarding seawater trace metal chemistry and phytoplankton physiology. Focusing particularly on Fe, we discuss the possibility of metal limitation of algal growth rates in light of recent findings on the chemical and physical processes that limit the kinetics of trace metal uptake by algae (Hudson and Morel in press). The case for colimitation of trace and major nutrients appears increasingly convincing.

Biological roles of trace metals

As cofactors of metalloenzymes and proteins, trace metals are involved in all general metabolic processes of phytoplankton, including photosynthesis, respiration, and major nutrient assimilation. They play an essential role by maintaining conformation and tertiary structure in some proteins. In some enzymes, metals are present in the catalytic site and facilitate oxidation-reduction reactions, transferring electrons from one redox couple to another. In other enzymes, such as alkaline phosphatase (a Zn metalloenzyme), metals function in the ac-
tive site as Lewis acids (Hay 1984). The Fe-S and heme proteins (cytochromes) present in the photosynthetic and respiratory chains are examples of electron transfer proteins. They are the most abundant metal redox proteins and account for most of the cellular Fe (Hewitt 1983; Raven 1988). Other Fe-containing proteins include NO$_3^-$, NO$_2^-$, and sulfite reductases, catalase, peroxidase, superoxide dismutase, and nitrogenase (Lehniger 1975).

Our intent here is to consider the biological effects of the cationic trace metals whose chemical speciation in seawater is controlled through complexation reactions with organic and inorganic ligands. We recognize that trace elements that occur as oxyanions in seawater, including Se and V, have important biochemical roles in phytoplankton (Hatfield et al. 1991; Meisch et al. 1977; Price et al. 1987; Price and Harrison 1988). But our ignorance of their transport kinetics and cellular requirements precludes us from considering them further.

**Uptake and growth at low metal concentrations**

As far as we know, the uptake of all necessary trace metals by phytoplankton occurs via binding to a surface ligand and subsequent transfer across the cell membrane. The result is the well-known Michaelis-Menten uptake kinetics (Table 1), a hyperbolic rate law in which uptake rate ($\rho$) is proportional to the reactive substrate [M'] at low concentration and reaches a maximum ($\rho_{\text{max}}$) at concentrations that exceed the "half-saturation constant" $K_s$ (see Fig. 1). We use the notation [M'] to represent the total concentration of inorganic metal species whose effective reaction rate with the uptake ligands (L) determines uptake as discussed below. The half-saturation constant, which is determined by the rates of metal-ligand binding and dissociation and the rate of internalization, has been found to be invariant for a given trace metal and a given phytoplankton species (Harrison and Morel 1986; Sunda and Huntsman 1985). In contrast the maximum uptake rate can be adjusted up and down by a factor of 20–30, depending on the degree of limitation or sufficiency of the trace metal (Harrison and Morel 1986; Sunda and Huntsman 1985), which is achieved by increasing or decreasing the concentration of uptake ligands in the membrane as illustrated in Fig. 1 for the uptake of Fe in the marine diatom *Thalassiosira weissflogii*.

Although we believe that these properties are likely applicable to all essential trace metals and most if not all phytoplankton species, in fact they have been demonstrated only for Fe and Mn in experiments with merely five marine phytoplankters representing three classes (Sunda and Huntsman 1985, 1986; Hudson and Morel 1990). There is still much work to be done to quantify the kinetics of metal uptake in marine algae.

As a result of the adjustment in the concentration of uptake ligands, the short-term uptake of a trace metal follows a series of hyperbolic Michaelis-Menten kinetics whose maximum is dependent on the prior degree of trace metal stress. At low ambient trace metal concentration—the situation of interest for limiting or near-limiting conditions as may exist in the environment—it is the upper limit of these uptake rate laws that obtains ($L_T = L_T^{\text{max}}$). In addition, because the uptake system is then undersaturated ([M'] $\ll K_s$), the steady state uptake rate is simply proportional to [M'] and given by the expression

$$\rho^{\text{ss}} = \frac{k_{\text{in}} L_T^{\text{max}}}{K_P} [M']$$  \hspace{1cm} (1)

This expression can be simplified further when the rate of internalization is much

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**Table 1. Michaelis-Menten uptake kinetics: M' is the inorganic metal species, L is the cell ligand, ML is the metal-ligand complex, $k_{\text{in}}, k_{\text{out}}$, and $K_s$ are the forward, backward, and internalization rate constants, $\rho$ and $\rho_{\text{max}}$ are the uptake rate and the maximum uptake rate, and $K_P$ is the half-saturation constant.**

$$\begin{align*}
M' + L & \rightleftharpoons ML \rightleftharpoons M \\
\rho &= \frac{\rho_{\text{max}} [M']} {K_s + [M']} \\
\rho_{\text{max}} &= L_T k_{\text{in}} \\
K_s &= \frac{k_{\text{out}} + k_{\text{in}}}{k_{\text{in}}} 
\end{align*}$$
faster than the rate of metal dissociation from the surface ligand \((k_a \gg k_{-a})\). This situation, which corresponds to a quasi-irreversible uptake system, makes good sense under limiting conditions for it would seem uncharacteristically profligate to release back to the medium a recently acquired limiting resource. Indeed the rate of internalization of Fe bound to the surface of \textit{T. weissflogii} is at least four times its rate of dissociation from the surface ligands (Hudson and Morel 1990). (No such kinetic information is yet available for other trace metals.) If the dissociation rate is neglected, the half-saturation constant is then given by \(K_n = k_a/k_{L_a}\), and an upper limit on the uptake rate is obtained simply as the rate of binding of the metal with the surface ligand which is mostly free:

\[
\rho^{as} = k_L L_T^{max}[M'].
\]  

(2)

In addition to increasing their membrane concentration of uptake ligands, phytoplankton under trace metal limitation also reduce their internal concentration (i.e. their quota-\(Q\)) of the metal. Since the growth rate is simply the ratio of the uptake rate to the metal quota

\[
\mu = \frac{\rho^{as}}{Q}
\]  

(3)

by decreasing \(Q\) at low ambient metal concentration, the algae can maintain near-maximum growth rates even though the uptake rate decreases proportionally to \([M']\). At some critical trace metal concentration, however, a minimum possible quota is approached below which the growth rate decreases.

The logarithmic graphs of Fig. 2 illustrate this relationship between metal concentration, uptake rates, cellular quotas, and growth rate (see Morel 1987). The parameters for this figure correspond to the Fe uptake system in \textit{T. weissflogii}. We refer to the conditions where the growth rate is appreciably decreased as “metal limitation” \(([M'] \leq K_n)\), to those for which both the number of uptake ligands and growth rate are near maximum as “metal stress” \((K_n < [M'] < K_n^Q)\), and to conditions where growth rate and quotas are near maximum and the number of uptake ligands below maximum as “metal sufficiency” \((K_n^Q < [M'])\). The condition of most interest to the following discussion is that of the transition between metal limitation and metal stress, where growth rate and the density of uptake ligands are both near maximum. We will thus focus on the cellular quota corresponding to a growth rate of 90% of maximum \(Q_{0.9}\) (Table 2).

When the uptake system is quasi-irre-
versible, the growth rate is given by a simple chemical kinetic expression derived by combining the two previous equations:

\[
\mu = \frac{1}{Q} \times k_L \times [M'] \times L_T^{max}.
\]  

(4)

Thus we can discuss quantitatively the issue of trace metal limitation of phytoplankton growth rate by examining each of the four parameters in this equation: the cellular quota of the trace metal, the rate constant for binding of the metal to the transport ligands, the ambient (reactive) concentration of the metal, and the maximum number of transport ligands in the membrane.

Before embarking on a systematic discussion of these four parameters, we note that the uptake of a trace metal cannot be considered in isolation of the other trace metals in the medium. This is clear from the coordinative nature of the uptake system described above. No ligand is ever perfectly specific so that some degree of competition for ligand binding must occur among all trace metals. This issue is discussed by Sunda (1991) and Bruland et al. (1991). An amusing and extreme example of competitive uptake inhibition is provided by the “Zn squeeze” phenomenon (Sunda 1991). At low [Zn], Zn may be a limiting nutrient and at high [Zn] it is a toxicant due, for example, to interference in the metabolism of other essential metals. Because Cu competitively inhibits Zn uptake, Zn requirements are elevated at high [Cu]. Conversely, Zn competitively inhibits Mn uptake so that at low ambient [Mn], even relatively low [Zn] interfere with the Mn nutrition of the cells. At high [Cu] and low [Mn], the result can be a paradoxical situation in which Zn is simultaneously limiting and toxic.

**Minimum metal quotas**

Adaptation to different ambient concentrations of trace metals is clearly one of the characteristics that distinguishes phytoplankton species from different environments. In particular, when the concentrations of metals such as Fe, Zn, or Mn are sufficiently low, oceanic species that are adapted to low trace metal conditions can outgrow neritic species that thrive in metal-rich coastal waters (Brandt et al. 1983). This adaptation is not necessarily the result of a better uptake system, but may represent a lower requirement for the metals. For example, as discussed later, the uptake rate of Fe is constrained by fundamental chemical and physical limits. However, there is evidence that oceanic species reduce their Fe require-

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Table 2. Concentration (amol cell\(^{-1}\)) of trace metals in *Thalassiosira weissflogii*. Cells were of 750 fl size, contained \(\sim\)12 pmol C cell\(^{-1}\) and were growing at \(\sim\)90% of \(\mu_{max}\).

<table>
<thead>
<tr>
<th>Metal</th>
<th>Conc quota (amol cell(^{-1}))</th>
<th>Metal:C ((\mu)mol mol(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zn(*)</td>
<td>50</td>
<td>4.2</td>
</tr>
<tr>
<td>Cd(†)</td>
<td>20</td>
<td>1.7</td>
</tr>
<tr>
<td>Co(‡)</td>
<td>30</td>
<td>2.5</td>
</tr>
<tr>
<td>Fe(§)</td>
<td>80</td>
<td>6.7</td>
</tr>
<tr>
<td>Ni(§)</td>
<td>20</td>
<td>1.7</td>
</tr>
<tr>
<td>Mn(§)</td>
<td>80</td>
<td>6.7</td>
</tr>
</tbody>
</table>

\(*\) Sunda and Huntsman 1992.
† Price and Morel 1990.
‡ Harrison and Morel 1986.
ments (Sunda et al. 1991; Brand 1991). The recently measured Fe quotas of oceanic phytoplankton are even lower than values expected based on the cellular concentrations of Fe catalysts necessary to achieve rapid growth (Raven 1988). How such a biochemical feat is accomplished is unknown, but it is a dramatic illustration that minimum metal quotas are species-specific and a reflection of the selective pressures of the species’ habitat.

Minimum metal cellular quotas of a given species are also affected by environmental conditions such as major nutrient sources, other trace elements, and light. A simple and important case is that of the replacement of Zn by Cd (Price and Morel 1990). When the ambient [Zn] is too low and limits the growth of *T. weissflogii*, addition of Cd to the medium restores near maximum growth rate. Although the exact biochemical nature of this substitution remains unknown, it could be the cause of the Cd depletion observed in surface seawater. A more precise understanding of the biochemical function of Cd and an elucidation of its uptake kinetics are necessary before we can demonstrate and quantify the role of Cd in oceanic productivity and the effect of the biota on the cycling of Cd in surface seawater.

Ni provides the clearest example of the dependence of a minimum metal quota on a major nutrient (Price and Morel 1991). Ni is probably not required by eucaryotic phytoplankton growing on Ni sources other than urea (Q_{min}^{Ni} = 0). However, in the sea, phytoplankton obtain from 20 to 50% of their N from urea (McCarthy et al. 1977; Harrison et al. 1985), and they thus require Ni, a constitutive element in the urease enzyme which is necessary to hydrolyze urea. The minimum Ni quota is then relatively high, ~20 amol cell^{-1} for *T. weissflogii*.

A similar interaction has been hypothesized between Fe and NO_{3}^{-}. With NO_{3}^{-} rather than NH_{4}^{+} as a nitrogen substrate, the functioning of NO_{3}^{-} and NO_{2}^{-} reductases (NR and NiR) should increase the Fe requirement of marine algae (Raven 1988). We can calculate this increase by considering the quantity of Fe in these enzymes and in the additional redox catalysts needed to facilitate NO_{3}^{-} reduction. The NR and NiR are only present in actively growing phytoplankton using NO_{3}^{-} as a source of N. Their Fe content is calculated from their Fe stoichiometry [mol Fe (mol enz.)^{-1}], their turnover number [mol substrate transformed time^{-1} (mol enz.)^{-1}], and their maximum cellular activity (mol substrate transformed time^{-1} cell^{-1}). The Fe stoichiometries of the enzymes are well known in higher plants (Hewitt and Notton 1980) and green algae (Galvan et al. 1987) (NR: 4Fe/enzyme; NiR: 5Fe/enzyme) and we assume that they are similar in marine phytoplankton. In *T. weissflogii*, grown in Fe-replete medium, the maximum in vitro rate of NR is 5.7 fmol NO_{3}^{-} reduced cell^{-1} min^{-1} and that of NiR is 7.5 fmol NO_{3}^{-} reduced cell^{-1} min^{-1} (Price unpubl.). Their corresponding turnover numbers are 3.8 × 10^{3} min^{-1} (calculated for spinach NR from data of Notton 1983) and 5.3 × 10^{3} min^{-1} (Hewitt 1975). Thus, by analogy to other organisms these NO_{3}^{-} assimilating enzymes should contain 13 amol Fe cell^{-1}, representing >15% of the total cellular Fe in *T. weissflogii*.

An even more significant Fe requirement exists for the production of cellular reductant to reduce NO_{3}^{-}. The reductive assimilation of CO_{2} and NO_{3}^{-} to produce carbohydrates and amino acids requires 36 mol e^{-} (mol N)^{-1}, assuming a C:N of 6.6 (see Thompson et al. 1989), which represents ~20% more reductant than needed for assimilation of NH_{4}^{+} because 8 of the 36 mol e^{-} are used to reduce NO_{3}^{-} to NH_{4}^{+}. If the production of reductant is proportional to the quantity of Fe-containing redox proteins and is as efficient as it can be during growth on NH_{4}^{+}, roughly 20% more cellular Fe is needed for cells to grow on NO_{3}^{-}. Thus, theoretically, growth on NO_{3}^{-} should require altogether ~40% more cellular Fe than does growth on NH_{4}^{+}.

In laboratory experiments, however, we have been unable to confirm this expected interaction between Fe and NO_{3}^{-}. Our results show that under Fe deficiency *T. weissflogii* grows at the same rate with NO_{3}^{-} or NH_{4}^{+} as a source of N, suggesting that no additional Fe is required for growth on NO_{3}^{-}. Such a result is surprising in view of the theoretical arguments presented above and the greater light dependence (reductant requirement) of NO_{3}^{-} than NH_{4}^{+} assim-
lution by phytoplankton under Fe sufficiency (Larsson et al. 1985) and deficiency (Rue-ter and Ades 1987). However, these latter short-term measurements may not account for the potential adaptive strategies of phytoplankton to obtain and allocate reductant for NO$_3^-$ and C assimilation under Fe deficiency. In contrast to the laboratory results, the field data discussed later support the predicted increase in Fe requirement for NO$_3^-$ assimilation. We note that the interdependence of Fe and N nutrition is also likely to be affected by light in a complex way in view of the high energetic requirement for NO$_3^-$ and NO$_2^-$ reduction, of the sensitivity of NR and NiR to UV light, and of the role of Fe in some UV detoxification processes (Palenik et al. 1990).

Rate of metal binding to uptake ligands

The kinetics of metal-ligand coordination reactions in the laboratory have been well studied and we know a great deal about the molecular processes that limit the overall reaction rates. In the most common and simplest case (to which we limit our discussion), a hydrated or inorganically complexed metal ion such as Ni(H$_2$O)$_6^{2+}$ initially forms an outer-sphere complex with a complexing ligand (L) on the cell’s surface:

$$k_{os} \approx \frac{[Ni(H_2O)_6^{2+} \ldots L^{n-}]}{Ni(H_2O)_6^{2+} \ldots L^{n-}}.$$  

A water molecule is then expelled from the inner hydration sphere of the metal,

$$k_{H_2O} \rightarrow Ni(H_2O)_5^{2+} \ldots L^{n-}$$

$$+ H_2O,$$  

and finally the ligand binds to the free coordination site on the metal,

$$Ni(H_2O)_3^{2+} \ldots L^{n-} \rightarrow Ni(H_2O)_3 L^{(n-2)-}.$$  

The slow step in this process is the loss of a water molecule from the hydration sphere of the metal in the outer-sphere complex (expression 6). As a result the outer-sphere complex formation step can be considered to be at equilibrium with the metal ion and the ligand. The rates of the subsequent ligand-binding steps, since they are faster than the loss of the initial water molecule, are irrelevant. The overall rate constant for the complexation reaction is thus simply given by

$$k_L = k_{os} \times k_{H_2O}. \quad (8)$$

The outer-sphere complex stability constant, $K_{os}$, depends only on the electrical charges and ionic radii of the metal and the ligand. In seawater, $K_{os}$ is typically on the order of 0.3–8 M$^{-1}$ for complexes of metal ions of charge +2 through −1 with ligands of charge −1, separated by 0.5 nm. The water loss constant is little affected by the nature of the ligand. Thus, to a first approximation, the rate of binding to a variety of ligands is constant for any given metal (at given conditions of pH and major ion concentrations) and simply determined by its water loss rate.

Inorganic complexation of metal ions can, however, increase water loss rates by decreasing the net charge of the metal center (Crumbliss and Garrison 1988). For example, in the ferric iron hydrolysis series the water loss rate increases from $2 \times 10^2$ s$^{-1}$ for Fe$^{3+}$ to $>10^7$ s$^{-1}$ for Fe(OH)$_2^+$ and Fe(OH)$_3^-$. Chloride complexes of Ni also exhibit enhanced solvent exchange rates—7 $\times$ 10$^5$ s$^{-1}$ for NiCl$^+$ relative to 3 $\times$ 10$^4$ s$^{-1}$ for the aquo complex (Marguer and et al. 1978). The effects of inorganic complexation have not been quantified for all metals or complexes, however. As long as the inorganic ligand binds the metal ion more weakly than the incoming ligand L, the net effect of major anion complexation should be to increase complexation rates.

The hydrated and inorganically complexed metal species react much faster with ligands than do the organically complexed forms. Such complexes have been observed in seawater for Cu and Zn (Bruland 1989; Coale and Bruland 1988, 1990). Thus, the concentration of labile inorganic species (including the hydrated and hydrolyzed ions) defines an upper limit for the availability of the metal for biological uptake. Practically, in seawater where the concentration of major inorganic ligands is constant, we can define for each metal an effective rate constant, $k_L$, that takes into account both the inor-
ganic speciation of the metal and the lability of each species. For the metals of interest this effective reaction rate in seawater (Table 3) varies from $10^2$ s$^{-1}$ for Ni which is very inert to $10^9$ s$^{-1}$ for Cu which is very labile. With ligands at 10 pM concentrations, as would be typical for uptake sites on ambient phytoplankton populations ($\sim 10^6$ cells liter$^{-1}$), the corresponding characteristic metal reaction time goes from 10 d to 2 min.

The speed with which a metal can react with a transport ligand provides a measure of its bioavailability. This bioavailability differs from the concept of “free metal ion activity” controlling metal availability (Morel 1983; Sunda 1991), although in practice the proportionality of free metal ion and total inorganic complex concentrations (e.g. [M$^{2+}$] $\propto$ [M$^+$]) means both interpretations of most experimental data are equivalent.

Reactive metal concentrations in seawater

The question of what forms of trace metals are available to phytoplankton in seawater has long been discussed by biological oceanographers (Harvey 1945; Goldberg 1952). The main focus has been, and continues to be, on the biological availability of Fe colloids. The dissolved concentration of Fe is so low in seawater that one is naturally inclined to wonder if the more abundant particulate and colloidal forms may not be the principal source for the nutrition of phytoplankton. However, we have just shown that in the context of a ligand-mediated uptake system biological availability means chemical reactivity and the bioavailable fraction is effectively the dissolved inorganic fraction. Except for Fe, which is 50–90% in the particulate phase (Martin et al. 1989), the essential metals are chiefly present in the dissolved (or at least 0.4-$\mu$M filterable) phase. Thus, the central issue in terms of their bioavailability is that of the extent of complexation in kinetically inert (or slow) organic complexes. The work of Bruuland and coworkers has established that Cu and Zn are almost completely bound to organic ligands in open-ocean surface seawater. For these metals, we consider Bruuland’s (1989) and Coale and Bruuland’s (1988, 1990) estimation of the inorganic metal concentrations (see Table 3) to represent the reactive (i.e. bioavailable) concentrations. For the other metals, in the absence of evidence for organic complexation, we assume the measured total dissolved metal concentrations to be those of interest.

To be sure, some exceptions are likely. In some cases metal-ligand complexes may be transported across the membrane. This may be the case if sufficiently lipophilic complexes form or if specific transport ligands such as siderophores are released into the medium for subsequent transmembrane transfer of the metal-ligand complex via specialized porter proteins. In other cases, the close association of a phytoplankton colony with an Fe particle may create a microenvironment in which the Fe particle is made effectively available to the organisms. This has been hypothesized for Trichodesmium which apparently forms its characteristic “puffs and tufts” in the Sargasso Sea around Fe particles (J. G. Rueter pers. comm.). Finally, pinocytosis of Fe colloids by some species cannot be ruled out, al-
though the relatively slow diffusion rate of colloids should not make this an advantageous strategy despite its intuitive appeal. For example, if 80% of the Fe were present in particles of 0.1-μm average diameter, the diffusion-limited encounter rate of these particles with phytoplankton cells would be 50 times smaller than that of the 20% of the Fe that is dissolved (molecular diam ~5 nm). Because Fe limitation of phytoplankton growth occurs when [Fe'] is near the diffusion limit (see below), particles, even small particles, are an inefficient Fe source.

The dissolution/precipitation kinetics of Fe must thus play the key role in its bioavailability. By extension to what has been observed at low pH in model laboratory systems and in the field (Waite and Morel 1984; McKnight et al. 1988), it has been postulated that Fe may undergo a rapid photocycle in seawater. Through photoreduction, Fe(III) oxide minerals may produce dissolved Fe(II) which would then reoxidize in the presence of oxygen. In inorganic model systems this process is ineffective in maintaining significant dissolved Fe(II) concentrations (<1% of the total Fe), both because the photoreduction rate is slow (10^{-4}–10^{-2} h^{-1} depending on the size and nature of oxide phase at typical illumination intensities) and because the reoxidation rate of Fe(II) is fast (~20 h^{-1}). Organic compounds which may both enhance the photoreduction by providing an electron source from material sorbed on the oxide and slow the reoxidation rate by complexing Fe(II) in solution may make the Fe photocycle in seawater more effective.

However, because of the much more rapid oxidation rate of Fe(II) at the surface of Fe(III) oxides than in solution, it seems likely that the major consequence of the photoreduction of Fe oxides in seawater is the formation of amorphous Fe(III)-oxide coatings on the mineral surface. Such coatings which are both more soluble and more labile than the original oxides should result in an increase in dissolved Fe(III) species concentrations and hence in increased Fe bioavailability, as has been observed by Wells and coworkers (Wells et al. 1983; Wells 1989) who have shown increased bioavailability of Fe suspensions upon illumination and decreased bioavailability upon subsequent aging in the dark.

Although these experiments do not provide the direct chemical information one would ultimately like to obtain, they are in fact easier to interpret than present chemical attempts at measuring Fe(II) in seawater. These measurements invariably depend on the complexation of Fe(II) by strong ligands. In the presence of some readily available electron sources from organic groups, such as semiquinones or perhaps thiols, these ligands will pull the Fe(III)/Fe(II) equilibrium toward Fe(II) and measure not the steady state, in situ reduced Fe concentration but the reducible Fe concentration. This measured concentration may correspond to the reactive Fe(III) or to the available electrons and bear no relationship with the Fe(II) actually in solution. One should note that the analytical Fe(II) reagent itself, or impurities in it, may often be the electron source.

**Uptake ligands**

We do not know the chemical nature of metal uptake ligands in phytoplankton outer membranes. By analogy with cyanobacteria (Brown et al. in press) we suspect that the Fe transport molecules are membrane-bound siderophores. In one study with dinoflagellates, such siderophores were released to the medium (Trick et al. 1983). Nonetheless we have some measures of the concentrations of these ligands (Table 4). These concentrations are at first blush extraordinarily high. For example, the concentration of cell-surface ligands involved in Fe transport in Fe-limited *T. weissflogii* is only 5–10 times lower than the required Fe concentration. In urea-grown *T. weissflogii*, the Ni transport ligand may even be as much as a fourth of the required Ni. For Fe, these ligands are in fact so numerous that they must occupy a sizable fraction of the cell membrane, ~50% if they are typical transport proteins (mol wt = 100 kD) (Hudson and Morel 1990), ~2% in the more likely condition that they are low molecular weight ligands such as siderophores (mol wt = 1 kD). It may seem paradoxical for cells to devote so much of their resources to the acquisition of elements required in such minute quantities.
Table 4. Cell surface metal transport sites \((L_T)\) in *Thalassiosira weissflogii*.

<table>
<thead>
<tr>
<th>Metal</th>
<th>((L_T)) (mol cell(^{-1}))</th>
<th>((L_T)) (mol cm(^{-2}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe*</td>
<td>(17 \times 10^{-18})</td>
<td>(4 \times 10^{-12})</td>
</tr>
<tr>
<td>Ni†</td>
<td>(5 \times 10^{-18})</td>
<td>(1 \times 10^{-12})</td>
</tr>
</tbody>
</table>

* Hudson and Morel 1990.
† Price and Morel 1991.

To examine this problem quantitatively, we rewrite Eq. 2 and 3 using the concentration of uptake sites per area of membrane (since it must ultimately be limited by crowding) and the cellular content of the trace metal per cell volume (since it must ultimately limit growth):

\[
\rho_{ss}/V = \mu \times Q/V = k_L \times L_T/A \times \frac{3}{r} \times [M']_{seawater}.
\]

(9)

Thus for a given metal (fixed \(k_L\) and \([M']_{seawater}\)), the volumetric uptake rate is proportional to uptake ligand density and inversely proportional to cell radius. As the uptake ligand density increases, the uptake rate becomes eventually limited by diffusion:

\[
\rho_{ss}/V = \frac{3}{r^2} \times D \times [M]_{seawater}.
\]

(10)

As shown on Fig. 3, for any essential trace metal at a given free concentration in seawater, the volumetric uptake rate and hence the specific growth rate are a function of both cell radius and uptake ligand density.

First consider the case of Fe whose dissolved concentration in the North Pacific, for example, is \(~5 \times 10^{-11}\) M. As seen in Fig. 3A, even with a very low Fe requirement, algal species with a radius of 10 µm require a very high concentration of uptake sites and become almost diffusion limited if they divide once a day. The minimum ligand density of 4 pmol cm\(^{-2}\) required for uptake is near the maximum measured in the laboratory for *T. weissflogii* (10 pmol cm\(^{-2}\)). It is also so large that only small molecules can possibly fit in the membrane thus reinforcing the notion that the Fe uptake ligands may be membrane-bound siderophores. Clearly, the situation is not as critical for smaller species which are at a competitive advantage at such low Fe concentrations.

According to what we know of the chemistry of Ni and Mn in the oceans, neither of these metals should be near diffusion limitation. For Ni, however, if transport were effected by porter proteins in the membrane, the reaction rate with these proteins may become limiting for large phytoplankton (Fig. 3B,C). The situation would be much more critical if a significant fraction of either Mn or Ni in solution were organically bound—a possibility that still needs to be ascertained. As a case in point, consider Zn. The very low inorganic concentration of Zn in the oceans should make it impossible for all but the smallest of phytoplankton to divide once a day and acquire sufficient Zn by diffusion (Fig. 3D). It is thus probably not coincidental that Cd has been found to substitute efficiently for Zn in *T. weissflogii* (Price and Morel 1990) and presumably does so in other species. The question of possible limitation by Zn of phytoplankton growth in the open ocean is of course dependent on the Zn requirement of oceanic species which again may be lower than that of coastal species (Sunda and Huntsman 1992).

Geobiology of trace metals

The observed correlations between the oceanic concentrations of several trace metals—Cd, Zn, Ni, and Fe—and the major nutrients—N, P, and Si—have invited comparison to the Redfield ratio for major nutrients (Boyle et al. 1976; Bruland et al. 1978; Morel and Hudson 1984). Of course in coastal waters where the metals may not be limiting, the cellular ratio of trace elements is unlikely to vary with their availability as is the case with P in freshwaters. In the open ocean, however, where according to our theory many trace elements may be colimiting, a roughly constant cellular ratio of essential elements should obtain. Extension of the Redfield paradigm to trace metals suggests a similarity should be observed between oceanic concentrations, oceanic particles, and the trace metal requirements of phytoplankton in culture. Analysis of the carrier phase responsible for removal of trace met-
Fig. 3. Volume-normalized uptake rates of trace metals as a function of ligand density on cell surfaces. Theoretical curves, calculated according to Eq. 9 and 10, assume the metal complexation kinetics and oceanic concentrations shown in Table 3. Uptake rates are proportional to ligand numbers at low densities and plateau at the diffusion-limited uptake rate for each cell size. Realistic ranges of ligand densities can be gauged by the vertical dotted lines, which represent the number of small proteins (26 kD) that would occupy 20% of the membrane area, and the right edge of each graph, which corresponds to the total number of phospholipids in the plasmamembrane. Note that diffusion-limited uptake rates should not be attainable for Ni due to the large number of sites that would be needed. The horizontal lines correspond to steady state uptake rates required for coastal (-----) and oceanic (· · · · · ·) phytoplankton growing at 1 d⁻¹. These rates were computed for coastal phytoplankton with the quotas given in Table 2 and a cellular C concentration of 16 M. Rates for oceanic phytoplankton were computed from data of Sunda (1991) and Sunda et al. (1991). To avoid further complicating the graphs we have not taken into account the size dependence of cellular [C]−[C] × V⁻⁰·³³ (Strathmann 1967).

als from surface seawater has shown that it is principally the organic matrix of the plankton, although the ratios in particles do not exactly match observed major nutrient-trace metal correlations (Collier and Edmond 1983).

As shown in Table 5, the trace metal : PO₄ ratios for the coastal diatom T. weissflogii
are within factors of 2–10 of the ratios expected from oceanic correlations. In the ocean, the combined effect of various phytoplankton species with different trace element compositions likely results in the observed metal-nutrient correlations. Although culture work suggests the trace metal composition of phytoplankton is not as narrowly constrained as the major nutrients, the coherence of culture and fieldwork suggests that either the compositions of the dominant phytoplankton species are relatively constant or the compositions are constant when averaged over sufficiently large temporal and spatial scales. Redfield’s concept also suggests that trace metal concentrations in seawater may be as much related to phytoplankton nutritional requirements as they are to geochemistry. Further, all the correlated major nutrients and essential trace elements should in some way be colimiting the oceanic biota. According to our discussion, at least Zn and Fe—trace metals for which we have both physiological information and some analytical data on speciation—should be near limiting in some parts of the oceans. As seen in Fig. 3, diffusion or uptake constraints for these two metals should limit the growth rate of all but the smallest phytoplankton. Substitution of these essential elements in particular biochemical functions may make other metals such as Cd or Cu simultaneously limiting. Thus it is not totally beyond the imagination of even sober oceanographers to anticipate that with additional information, particularly as concerns organic binding of essential metals, many trace elements will indeed be found to colimit in some way the growth of oceanic plants.

Another aspect of the biological control of seawater composition is how much phytoplankton can deplete surface concentrations. To the extent that the transport of trace elements out of the photic zone is effected by phytoplankton uptake, the efficacy of the uptake systems should somehow be reflected in the concentrations of the elements. If the reaction rates of metals with uptake sites control their availability to phytoplankton, the surface concentrations of the essential metals should be inversely related to their kinetic lability. Indeed, as can be seen in Fig. 4, there is apparently a direct inverse relationship between [M'] and \(1/k_L\) for the bioactive metals—Mn being a notable exception. Despite uncertainties in the numbers, this relationship is probably not fortuitous and can in fact be rationalized on the basis of phytoplankton physiology (Hudson and Morel in press). At the least, it would seem to indicate that a single kinetic mechanism with a time constant of \(\sim 3\) h \(=k_L\) is responsible for maintaining the low inorganic concentrations of Fe, Zn, Cu, Ni, Cd, and Co in surface seawater. We believe this mechanism to be algal uptake.
Fig. 5. Concentration of NH$_4^+$ calculated to limit by diffusion phytoplankton of different sizes growing at 0.5, 1.0, and 2.0 d$^{-1}$.

Despite all previous arguments, we have no direct evidence demonstrating that the specific growth rates of phytoplankton in oceanic waters are limited by trace metals or for that matter by any major nutrients. In the high-nutrient, low-Fe waters of the North and equatorial Pacific, experimental data simply show that Fe additions increase the biomass in incubation bottles after a few days. In fact, independent measurements of phytoplankton growth rate in these waters indicate that the indigenous community is growing quickly and it is generally agreed that this growth is supported chiefly by NH$_4^+$ as the N source, which is a very interesting observation.

According to the results of diffusion calculations shown on Fig. 5, to double once a day at an ambient NH$_4^+$ concentration of 0.3 $\mu$M, cells >7-$\mu$m radius must utilize NO$_3^-$ to supplement the available NH$_4^+$. Because growth on NO$_3^-$ presumably requires additional cellular Fe supplies, large cells (r > 10 $\mu$m), whose protoplasm is likely the primary component of organic matter departing the euphotic zone (Michaels and Silver 1988), should be Fe limited by virtue of both their size and their need for NO$_3^-$. Thus, NO$_3^-$-replete parts of the ocean could sustain a population of small cells (r < 10 $\mu$m) growing at maximal rates in the microbial loop, while larger cells must remain dormant until dust or upwelling provides sufficient Fe for them to grow rapidly and remove NO$_3^-$ (Hudson and Morel 1990). A direct demonstration of the role of Fe in the utilization of NO$_3^-$ in the equatorial Pacific has been obtained (Price et al. 1991). As seen in Fig. 6, Fe addition increased the N-specific NO$_3^-$ uptake rate of phytoplankton in incubation bottles but did not affect NH$_4^+$ uptake. This result can only be interpreted as a "physiological" stimulation of the phytoplankton community. Thus at least in the equatorial Pacific, it appears that Fe limits NO$_3^-$ utilization and hence new production.

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Trace metal limitation


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