# 16 Trace Metals and Harmful Algal Blooms

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## 16.1 Introduction

Trace metals influence phytoplankton both as limiting nutrients and as toxicants. Until recently, phytoplankton productivity in the ocean was thought to be primarily limited by major nutrients (N, P, and Si). However, enrichment experiments in bottles and in mesoscale patches of surface water have shown that iron limits algal growth in major regions of the ocean (Coale et al. 1996; Tsuda et al. 2003; Boyd et al. 2000; Coale et al. 2004), and some coastal upwelling systems (Hutchins et al. 1998, 2002). In addition, it now appears that iron limits $N_2$-fixation by cyanobacteria in the ocean, and thus may control oceanic nitrogen inventories (Rueter 1983; Falkowski 1997; Kustka et al. 2003; Mills et al. 2004). Several other micronutrient metals (Zn, Co, Mn, and Cu) have also been shown to stimulate phytoplankton growth in ocean waters, but their effect is usually less than that of iron (Coale 1991; Crawford et al. 2003; Franck et al. 2003). However, these metals may play an important role in regulating the composition of phytoplankton communities because of large differences in trace metal requirements among species (Brand et al. 1983; Coale 1991; Sunda and Huntsman 1995a). For example, the addition of zinc to surface waters of the subarctic Pacific stimulated the growth of coccolithophores over that of diatoms, while the addition of iron preferentially favored the growth of large diatoms (Crawford et al. 2003). In general, iron can have a critical influence on the composition and structure of algal communities because of differences in requirements among species, particularly coastal and oceanic ones (Brand et al. 1983; Sunda and Huntsman 1995b) and large-celled and small-celled species (Price et al. 1994; Sunda and Huntsman 1997). Trace metals are believed to be less important in limiting algal growth in most coastal and freshwater systems. An important issue in the influence of trace metals on harmful blooms is the relative sensitivity of HAB species to trace metal limitation or toxicity relative to that of competing non-HAB algae. Although such differences are likely, they are as yet largely unknown.

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## 16.2 Chemistry and Availability of Metals

The availability of trace metals varies widely in natural waters due to large variations in total metal concentrations and chemical speciation. Like major nutrients, surface concentrations of many bioactive trace metals (Fe, Zn, Co, Cu, Cd, and Ni) in the ocean and in lakes are often depleted substantially relative to concentrations in deeper waters because of biological uptake in sunlit surface waters and regeneration at depth, as occurs for major nutrients (Bruland 1980; Martin et al. 1989). This efficient removal by phytoplankton results in extremely low dissolved (i.e., filterable) concentrations of iron (0.02–0.5 nM), Zn (0.06–0.2), and Cu (0.4–1.4 nM) in surface ocean waters. Concentrations of these metals increase substantially in transects from oceanic to coastal and estuarine waters because of inputs from continental sources, including rivers, ground water, aeolian dust, and coastal sediments. Filterable iron concentrations can approach 10–20 µM in rivers, orders-of-magnitude higher than surface ocean values (Boyle et al. 1977). This filterable iron occurs largely as hydrous iron oxide-organic colloids, which are rapidly lost from estuarine and coastal waters via salt-induced coagulation and particulate settling (Boyle et al. 1977). Because of this efficient removal, very little of the iron in rivers reaches the sea, and most of the iron in ocean waters is derived from the deposition of mineral dust blown on the wind from arid regions of the continents (Duce and Tindale 1991). These aeolian inputs change seasonally with variations in prevailing winds and are highest in waters downwind of large arid regions such as North Africa and Central Asia.

Trace metals exist as a variety of chemical species in natural waters, which strongly influences their chemical behavior, cycling, and availability to phytoplankton. Most occur as cations that are complexed to varying degrees by inorganic and organic ligands or are adsorbed onto, or bound within particles. Many biologically active metals (Fe, Cu, Mn, and Co) cycle between different oxidation states, which have quite different kinetic lability, solubility, and reactivity toward complex formation.

Iron is the most biologically important metal, and its chemical behavior is also the most complex. Its stable oxidation state in oxygen-containing waters, Fe(III), undergoes substantial hydrolysis and polymerization, which results in the formation of sparingly soluble hydrous iron oxides (Lui and Millero 2002). This oxide formation and the tendency of ferric ions to adsorb onto particle surfaces results in the particulate removal of iron from seawater and consequent low iron concentrations in ocean waters (Johnson et al. 1997). Most (>99 %) of the dissolved ferric iron in seawater is bound to organic ligands, which minimizes iron adsorption or precipitation, and thus minimizes the particulate scavenging of iron from seawater (Rue and Bruland 1995; Wu and Luther III 1995). Some of these organic complexes may be siderophores produced by cyanobacteria and heterotrophic bacteria to facilitate intracellular iron uptake. These ferric chelates do not appear to be taken up by eukaryotic algae; however, the iron in these chelates is released for cellular uptake by cell-surface reductase systems (Maldonado and Price 2001) and by photolytic reactions, which reductively dissociate ferric chelates (Barbeau et al. 2001). The released ferrous ions are unstable in seawater, and are re-oxidized to soluble ferric hydrolysis species on time scales of minutes (Sunda 2001).

Other bioactive metals such as copper and zinc are also heavily chelated (generally >99 %) in marine waters by unidentified organic ligands of likely biological origin (Sunda and Hanson 1987; Bruland 1989; Coale and Bruland 1990; Moffett et al. 1997). Available evidence indicates that organic complexes of zinc and copper are not directly available to phytoplankton, and that uptake of these metals is instead controlled by the concentration of free aquated metal ions or that of kinetically labile dissolved inorganic species (Sunda and Guillard 1976; Anderson et al. 1978). By lowering free metal ion concentrations, these organic ligands act to buffer free metal ion concentrations, and to minimize algal removal of these metals with algal growth in natural waters.

## 16.3 Trace Metals as Limiting Nutrients

Trace metal micronutrients (Fe, Mn, Zn, Co, Cu, Ni, and Mo) are essential for the growth and metabolism of all aquatic algae. They play critical roles in photosynthesis and the assimilation of essential macronutrients (N, P, and Si); thus trace metal requirements can be influenced by the availability of light, $CO_2$, and major nutrients. Of the micronutrient metals, iron is needed in the greatest amount and is the metal that most frequently limits algal growth. Iron serves essential metabolic functions in photosynthetic electron transport, respiration, nitrate assimilation, $N_2$-fixation, and detoxification of reactive oxygen species (superoxide radicals and hydrogen peroxide). Because of its heavy involvement in photosynthetic electron transport, cellular iron requirements increase with decreasing light intensity (Raven 1990; Sunda and Huntsman 1997). Such effects could increase iron requirements during dense algal blooms where light is attenuated to low levels. Because of the high metabolic cost for iron in nitrate reduction, iron requirements are higher for cells grown on nitrate than on ammonium; consequently, iron can limit nitrate assimilation during blooms (Raven 1988; Price et al. 1994; Maldonado and Price 1996). Because of the even higher iron requirement for $N_2$-fixation, iron is needed in much greater amounts by cyanobacteria growing diazotrophically (i.e., growing on $N_2$) than those growing on ammonia (Berman-Frank et al. 2001; Kustka et al. 2003). Iron can thus limit the development of blooms of diazotrophic cyanobacteria in both the ocean (Kustka et al. 2003) and some lakes (Wurtsbaugh and Horne 1983; Evans and Prepas 1997).

Manganese, like iron, is essential for photosynthesis and thus is needed in higher amounts for growth at low light intensities (Sunda and Huntsman 1998). Mn also occurs in superoxide dismutase, an enzyme that removes toxic superoxide radicals (Peers and Price 2004). Because it has fewer metabolic functions, its requirement for growth is much less than that of iron (Raven 1990; Sunda 2000).

Zinc has a variety of metabolic functions and has a cellular requirement similar to that for Mn (Sunda 2000). It occurs in carbonic anhydrase (CA), an enzyme critical to $CO_2$ transport and fixation (Badger and Price 1994). Higher amounts of this enzyme are needed under $CO_2$-limiting conditions, and the cellular requirement for zinc increases at low $CO_2$ (Morel et al. 1994; Lane and Morel 2000; Sunda and Hunstman 2005). Thus, cells may become co-limited by $CO_2$ and zinc during blooms when $CO_2$ is consumed to low levels by high levels of C-fixation (Sunda and Hunstman 2005). In Lake Kinneret, Israel spring blooms of the dinoflagellate *Peridinium gatunense* caused a ten-fold decrease in $CO_2$, and an accompanying up to 100-fold increase in CA activity per dinoflagellate cell (Berman-Frank et al. 1994). Whether this large increase in CA resulted in a Zn-$CO_2$ co-limitation, however, is not known.

Zinc also occurs in zinc finger proteins, involved in DNA transcription, and in alkaline phosphatase, needed to acquire phosphorus from organic phosphate esters, which dominate phosphate pools under P-limitation. Consequently, Zn and P may co-limit algal growth in regions where both nutrients occur at low concentrations such as the Sargasso Sea (Wu et al. 2000). Cobalt, and sometimes cadmium, can substitute for zinc in many zinc enzymes such as CA, leading to complex interactions among the three metals in marine algae (Morel et al. 1994; Sunda and Huntsman 1995a; Lane and Morel 2000). Cobalt has a unique requirement in vitamin $B_{12}$, but the need for this cofactor is usually quite low in phytoplankton (Sunda and Huntsman 1995a). A specific requirement for cobalt is seen in marine cyanobacteria and bloom-forming prymnesiophytes (*Chrysochromulina polylepis* and *Emiliania huxleyi*), but the biochemical basis for this is not known (Granéli and Risinger 1994; Sunda and Huntsman 1995a; Saito et al. 2002). Leaching of Co from acidified soils has been suggested to have played a role in the 1988 *C. polylepsis* bloom in Scandinavian coastal waters (Granéli and Haraldsson 1993).

Copper occurs in cytochrome oxidase, a key protein in respiratory electron transport, and in plastocyanin, which can substitute for the iron protein cytochrome $c_6$ in photosynthetic electron transport (Raven et al. 1999). It is also an essential component of the high-affinity iron transport system of many eukaryotic algae (LaFontaine et al. 2002; Peers et al. 2005). Because copper is needed for iron uptake and can metabolically substitute for iron, co-limitations can occur for Cu and Fe, as observed in centric diatoms (Maldonado unpubl. data) and toxic species of *Pseudo-nitzschia* (Wells et al. 2005).

Nickel and molybdenum, like iron, play important roles in nitrogen assimilation. Ni occurs in the enzyme urease, and thus is required by phytoplankton grown on urea (Price and Morel 1991). Mo occurs with iron in nitrate reductase and nitrogenase, and thus is needed for nitrate assimilation and $N_2$ fixation (Raven 1988). It occurs in natural waters primarily as the oxyanion molybdate ($MoO_4^{2-}$). Because of its high concentration in seawater (~100 nM), Mo should not limit algal growth in marine and estuarine environments.

## 16.4 Trace Metal Toxicity

Many reactive nutrient metals, such as Cu, Zn, Ni, and Cd, are toxic at high concentrations (Brand et al. 1986). For these metals, optimal growth occurs at intermediate concentrations, below which growth rate is limited, and above which growth is inhibited.

Toxic metals often are taken up into cells via the transport systems of nutrient metals such as Mn and Zn (Sunda 2000). In addition, a common mode of toxic action is interference with nutrient metal uptake and metabolism (Harrison and Morel 1983; Sunda and Huntsman 1996). As a consequence, antagonistic interactions occur between toxic and nutrient metals, which can influence algal growth. A good example is the interaction between toxic levels of Cu, Cd, or Zn and the nutrient metal Mn (Sunda and Huntsman 1996; Sunda and Huntsman 1998). In these interactions, growth rate inhibition by these metals is related to an inhibition of cellular Mn uptake and the induction of Mn-deficiency. The deficiency is alleviated either by increasing the concentration of Mn ions or by decreasing that of the toxic metal.

Large differences exist in algal sensitivity to toxic metals. In a survey of 38 clones, wide variations were observed in growth inhibition by Cu both within and between algal groups (Brand et al. 1986). Isolates of the cyanobacterium *Synechococcus* were the most sensitive to Cu, and showed a 50 % growth rate inhibition at an average free cupric ion concentration of 50 pM, a level that had little effect on the growth of diatoms, the least-sensitive algal group. Thus Cu pollution in coastal estuaries could influence the species composition of algal communities by selectively inhibiting the growth of more sensitive species. This prediction was confirmed in a study of coastal bays in Cape Cod, Massachusetts. In this study, very low *Synechococcus* cell abundances were found in bays contaminated with copper (Eel Pond and Falmouth Harbor) where free cupric ion concentrations (~100 pM) exceeded toxic levels for this genus (Moffett et al. 1997). In contrast, much higher *Synechococcus* cell concentrations were seen in adjacent bays where there was no measurable Cu contamination and free Cu ion concentrations were <0.1 pM, well below the toxic range.

Although trace metals can inhibit algal growth, algal grazers such as copepods (Sunda et al. 1987) and ciliates (Stoecker et al. 1986) often are more sensitive to toxic metals (e.g., Cu and Zn) than most phytoplankton. Consequently, heavy metal pollution may promote algal blooms by inhibiting algal grazers and thereby decreasing grazing pressure (Sunda et al. 1987). This effect was observed in a mesocosm study in Saanich Inlet, British Columbia, where the addition of 79 nM Cu resulted in a ten-fold increase in chl-*a* relative to a no-addition control owing to a preferential poisoning of algal grazers (Thomas et al. 1977).

## 16.5 Trace Metal Effects on HABs: Domoic Acid Production in *Pseudo-nitzschia*

Because trace metal concentrations are much higher in rivers and estuaries than in the ocean, they are much less likely to limit algal growth and influence algal species composition in these waters than in the ocean. This should also be true of HAB species whose emergence in blooms is determined by their ability to outcompete non-HAB species under prevailing conditions. Biogeochemical models indicate that carbon fixation in ~40 % of the ocean's surface is limited by an insufficient supply of iron (Moore et al. 2002). Most of these regions are oceanic upwelling systems, in which deep waters rich in major nutrients (N, P, and Si), but with insufficient iron to support maximal algal growth, are advected to the surface. These low-iron upwelling regions occur in the subarctic and equatorial Pacific and most of the Southern Ocean. They also occur in many coastal upwelling systems, as found off the west coasts of North and South America (Hutchins et al. 1998, 2002). In upwelling waters off California, high nitrate concentrations (15–35 μM) can support diatom blooms with chl-*a* values of up to 10–35 μg $L^{-1}$, but for this to occur the upwelling ocean water must be supplemented with iron from external sources, such as rivers or continental margin sediments (Bruland et al. 2001). Off California there is little river input during the upwelling season, and the major external iron source is from shelf sediments. Regions with broad continental shelves generally receive sufficient iron to support substantial diatom blooms, while upwelling waters in areas with narrow shelves, such as the Big Sur region, receive little additional iron, which curtails bloom development. Because of these differences in iron inputs, iron limitation in these waters is spatially and temporally quite variable (Bruland et al. 2001). A similar situation is found in other upwelling regions along the western margins of North and South America and in New Zealand where there are coastal mountain ranges and associated narrow continental shelves.

The diatom blooms in upwelling waters off the west coast of North America frequently contain toxic species of the diatom genus *Pseudo-nitzschia* (see Bates and Trainer, Chap. 7). These algae produce the neurotoxin domoic acid, which has caused poisoning in marine mammals, seabirds, and humans

(Bates et al. 1998; Scholin et al. 2000; Trainer et al. 2002). Not all species of this genus produce domoic acid, and at present 11 are confirmed to produce the toxin (Bates and Trainer, Chap. 7). But even within toxic species, domoic acid production can vary substantially with a number of environmental variables, including limitation by Si, P, and trace metals (Fe and Cu) (Bates 1998; Maldonado et al. 2002; Wells et al. 2005). The genus appears to be widespread in iron-limited oceanic upwelling systems and its growth was stimulated by iron-addition in mesoscale iron fertilization experiments in the eastern equatorial Pacific (Coale et al. 1996), the western subarctic Pacific (Tsuda et al. 2003) and the subantarctic ocean (Coale et al. 2004). Stimulation of *Pseudo-nitzschia* growth by iron addition has also been observed in shipboard bottle-incubation experiments conducted in upwelling systems off Big Sur, California (Hutchins et al. 1998) and the Juan de Fuca Eddy off Washington (Wells et al. 2005). In the latter experiment, the addition of a non-marine siderophore, ferrichrome (which strongly chelates iron and reduces its uptake by eukaryotic algae), reduced cellular iron uptake rates by >80 % and reduced *Pseudo-nitzschia* cell concentrations by 33 % after 2 days of incubation. Interestingly, the ferrichrome addition also increased the average domoic acid content of *Pseudo-nitzschia* cells by 3.4-fold and increased the total cellular domoic acid concentration in the seawater by over two-fold (Wells et al. 2005). Thus, although increased iron concentrations may stimulate blooms of *Pseudo-nitzschia*, the effect of iron on cellular domoic acid concentrations, and thus toxin transfer to higher trophic levels (including humans) is not so easily predicted.

It now appears that at least part of the variation in domoic acid production with changes in iron availability may be related to a role of this molecule in cellular iron uptake. Rue and Bruland (2001) noted that domoic acid has a chemical structure similar to that of the siderophore mugineic acid, which is produced by some terrestrial plants to complex iron and facilitate its uptake from soils. They demonstrated that domoic acid chelates both iron and copper and suggested that it may be released by *Pseudo-nitzschia* cells to either facilitate iron uptake or detoxify copper. This hypothesis has since been confirmed in both laboratory and field experiments. In culture experiments with *P. multiseries* and *P. australis*, isolated from Monterey Bay, California, iron limitation of growth increased the rate of extracellular release of domoic acid by five- to ten-fold and the addition of domoic acid increased iron uptake rates in *P. multiseries* by three-fold (Maldonado et al. 2002). Moreover, in the deck-incubation experiments described above in the Juan de Fuca Eddy, the addition of domoic acid increased iron uptake rates by the algal community and had a similar stimulatory effect on the growth of the *Pseudo-nitzschia*-dominated algal community as the addition of iron (Wells et al. 2005). Thus, by promoting iron uptake, domoic acid production could convey a competitive advantage to toxigenic *Pseudo-nitzschia* species in iron-limited oceanic and coastal upwelling systems.

Copper toxicity also increased the rate of extracellular production of domoic acid by over ten-fold in *P. multiseries* cultures, and the addition of an excess of domoic acid alleviated copper toxicity (Maldonado et al. 2002). Thus another physiological function of domoic acid may be copper detoxification. Copper toxicity could be particularly problematic in freshly upwelled seawater due to very high ratios of free Cu to Mn ions in some upwelling source waters, and to a physiological antagonism between Cu toxicity and Mn limitation in diatoms (Sunda 1988/89).

Paradoxically, while Cu toxicity promoted domoic acid production, Cu limitation of *P. australis* caused an equally large increase in intracellular and extracellular domoic acid concentrations (Wells et al. 2005). As noted earlier, copper is needed for intracellular transport of iron by a high-affinity transport system; thus copper limitation may give rise to iron limitation, and thereby trigger domoic acid production. Alternatively, as suggested by Wells et al. (2005), domoic acid may be involved in facilitating copper uptake by *Pseudo-nitzschia* by a yet-unidentified mechanism. Such an effect could be important given the high level of copper complexation by natural chelators, and the resultant low free cupric ion concentrations in surface seawater (Coale and Bruland 1990).

It is clear that iron and copper can have important direct effects on domoic acid production. However, iron may also indirectly influence cellular domoic acid concentrations through its effect on silicification in diatoms. In both laboratory and field experiments, iron limitation was shown to increase Si:N ratios in diatoms by up to three-fold (Hutchins and Bruland 1998; Takeda 1998). As a result, there is a higher depletion of silica relative to nitrate in iron-limited ocean waters, which drives these systems toward Si-limitation (Hutchins and Bruland 1998). Because domoic acid content per cell increases substantially under silica limitation in *Pseudo-nitzschia* (Bates 1998), iron limitation could indirectly increase cellular domoic acid levels by promoting Si-limitation. This effect will act in concert with the direct effects of iron limitation on domoic acid production discussed above.

## 16.6 Trace Metal Effects on Other HAB Species

Although iron and other trace metals are likely to influence the growth and toxin production in other HAB species, there is little firm evidence for such effects. One of the more recently proposed linkages between HABs and trace metals is that among atmospheric iron inputs to the ocean, blooms of the $N_2$-fixing cyanobacterium *Trichodesmium*, and toxic blooms of the dinoflagellate *Karenia brevis* along the west coast of Florida (Lenes et al. 2001; Walsh and Steidinger 2001). These authors noted that *Karenia brevis* blooms were often preceded by or co-occurred with blooms of *Trichodesmium* in stratified outer-shelf waters during summer and fall. They argued that fixed nitrogen produced by *Trichodesmium* was subsequently used to fuel *Karenia brevis* blooms. As noted earlier, $N_2$-fixation requires high levels of cellular iron, which is largely supplied from aeolian deposition. During the summer, the west Florida shelf receives large inputs of Saharan dust transported by the Trade Winds from North Africa, which have been argued to fuel *Trichodesmium* blooms (Lenes et al. 2001). An average of 71 % of the atmospheric dust loadings along the south Florida coast occur during June through August (Prospero 1999), the same period when 15 of the 16 of the most intense *K. brevis* blooms were initiated in the last 50 years (Walsh and Steidinger 2001). Although these patterns are intriguing, further research is needed to verify cause-and-effect linkages among atmospheric iron deposition and blooms of *Trichodesmium* and toxic dinoflagellates in the southeastern United States, and possibly in other tropical and subtropical coastal environments.

Much of the limited and inconclusive earlier evidence for trace metal effects in HABs has been previously reviewed (Boyer and Brand 1998). Because of limited space here, we refer the reader to that review for a discussion of that material.

## References

Anderson MA, Morel FMM, Guillard RRL (1978) Growth limitation of a coastal diatom by low zinc ion activity. Nature 276:70–71

Badger MR, Price GD (1994) The role of carbonic anhydrase in photosynthesis. Annu Rev Plant Physiol and Plant Mol Biol 45:369–392

Barbeau K, Rue EL, Bruland KW, Butler A (2001) Photochemical cycling of iron in the surface ocean mediated by microbial iron(III)-binding ligands. Nature 413:409–413

Bates SS (1998) Ecophysiology and metabolism of ASP toxin production. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. NATO ASI Series 41. Springer, Berlin Heidelberg New York, pp 405–426

Bates SS, Garrison DL, Horner RA (1998) Bloom dynamics and physiology of domoic-acid-producing *Pseudo-nitzschia* species. In: Anderson DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. NATO ASI Series 41. Springer, Berlin Heidelberg New York, pp 267–292

Berman-Frank I, Cullen JT, Shaked Y, Sherrell RM, Falkowski PG (2001) Iron availability, cellular iron quotas, and nitrogen fixation in *Trichodesmium*. Limnol Oceanogr 46:1249–1260

Berman-Frank I, Zohary T, Erez J, Dubinsky Z (1994) $CO_2$ availability, carbonic anhydrase, and the annual dinoflagellate bloom in Lake Kinneret. Limnol Oceanogr 39:1822–1834

Boyd PW et al (2000) A mesoscale phytoplankton bloom in the polar Southern Ocean stimulated by iron fertilization. Nature 407:695–702

Boyer GL, Brand LE (1998) Trace elements and harmful algal blooms. In: Andersen DM, Cembella AD, Hallegraeff GM (eds) Physiological ecology of harmful algal blooms. NATO ASI Series 41. Springer, Berlin Heidelberg New York, pp 489–508

Boyle E, Edmond JM, Sholkovitz ER (1977) The mechanism of iron removal in estuaries. Geochim Cosmochim Acta 41:1313–1324

Brand LE, Sunda WG, Guillard RRL (1983) Limitation of marine phytoplankton reproductive rates by zinc, manganese and iron. Limnol Oceanogr 28:1182–1198

Brand LE, Sunda WG, Guillard RRL (1986) Reduction of marine phytoplankton reproduction rates by copper and cadmium. J Exp Mar Biol Ecol 96:225–250

Bruland KW (1980) Oceanographic distributions of cadmium, zinc, nickel and copper in the North Pacific. Earth Planet Sci Lett 47:176–198

Bruland KW (1989) Complexation of zinc by natural organic ligands in the central North Pacific. Limnol Oceanogr 34:267–285

Bruland KW, Rue EL, Smith GJ (2001) Iron and macronutrients in California coastal upwelling regimes: implications for diatom blooms. Limnol Oceanogr 46:1661–1674

Coale KH (1991) Effects of iron, manganese, copper, and zinc enrichments on productivity and biomass in the subarctic Pacific. Limnol Oceanogr 36:1851–1864

Coale KH, Bruland KW (1990) Spatial and temporal variation of copper complexation in the North Pacific. Deep Sea Res 47:317–336

Coale KH et al (1996) A massive phytoplankton bloom induced by an ecosystem-scale iron fertilization experiment in the equatorial Pacific Ocean. Nature 383:495–501

Coale KH et al (2004) Southern Ocean iron enrichment experiment: carbon cycling in high- and low-Si waters. Science 304:408–414

Crawford DW et al (2003) Influence of zinc and iron enrichments on phytoplankton growth in the northeastern subarctic Pacific. Limnol Oceanogr 48:1583–1600

Duce RA, Tindale NW (1991) Atmospheric transport of iron and its deposition in the ocean. Limnol Oceanogr 36:1715–1726

Evans JC, Prepas EE (1997) Relative importance of iron and molybdenum in restricting phytoplankton growth in high phosphorus saline lakes. Limnol Oceanogr 42:461–472

Falkowski PG (1997) Evolution of the nitrogen cycle and its influence on the biological sequestration of $CO_2$ in the ocean. Nature 387:272–275

Frank VM, Bruland KW, Hutchins DA, Brzezinski MA (2003) Iron and zinc effects on silicic acid and nitrate uptake kinetics in three high nutrient, low chlorophyll regions. Mar Ecol Prog Ser 252:15–33

Granéli E, Haraldsson C (1993) Can increased leaching of trace metals from acidified areas influence phytoplankton growth in coastal waters? Ambio 22:308–311

Granéli E, Risinger L (1994) Effects of cobalt and vitamin B12 on the growth of *Chrysochromulina polylepis* (Prymnesiophyceae). Mar Ecol Prog Ser 1994:177–183

Harrison GI, Morel FMM (1983) Antagonism between cadmium and iron in the marine diatom *Thalassiosira weissflogii*. J Phycol 19:495–507

Hutchins DA, Bruland KW (1998) Iron-limited diatom growth and Si:N uptake ratios in a coastal upwelling regime. Nature 393:561–564

Hutchins DA, DiTullio GR, Zhang Y, Bruland KW (1998) An iron limitation mosaic in the California upwelling regime. Limnol Oceanogr 43:1037–1054

Hutchins DA et al (2002) Phytoplankton iron limitation in the Humboldt Current and Peru Upwelling. Limnol Oceanogr 47:997–1011

Johnson KS, Gordon RM, Coale KH (1997) What controls dissolved iron concentrations in the world ocean? Mar Chem 57:137–161

Kustka AB, Sañudo-Wilhelmy S, Carpenter EJ, Capone D, Burns J, Sunda WG (2003) Iron requirements for dinitrogen and ammonium supported growth in cultures of *Trichodesmium* (IMS 101): comparison with nitrogen fixation rates and iron:carbon ratios of field populations. Limnol Oceanogr 48:1869–1884

LaFontaine S, Quinn JM, Nakamoto SS, Page MD, Göhre V, Moseley JL, Kropat J, Merchant S (2002) Copper dependent iron assimilation pathway in the model photosynthetic eukaryote *Chlamydomonas reinhardtii*. Eukaryot Cell 1:736–757

Lane TW, Morel FMM (2000) Regulation of carbonic anhydrase expression by zinc, cobalt, and carbon dioxide in the marine diatom *Thalassiosira weissflogii*. Plant Physiol 123:345–352

Lenes JM et al (2001) Iron fertilization and the *Trichodesmium* response on the West Florida shelf. Limnol Oceanogr 46:1261–1277

Lui X, Millero FJ (2002) The solubility of iron in seawater. Mar Chem 77:43–54

Maldonado MT, Hughes MP, Rue EL, Wells ML (2002) The effect of Fe and Cu on growth and domoic acid production by *Pseudo-nitzschia multiseries* and *Pseudo-nitzschia australis*. Limnol Oceanogr 47:515–526

Maldonado MT, Price NM (1996) Influence of N substrate on Fe requirements of marine centric diatoms. Mar Ecol Prog Ser 141:161–172

Maldonado MT, Price NM (2001) Reduction and transport of organically bound iron by *Thalassiosira oceanica*. J Phycol 37:298–310

Martin JH, Gordon RM, Fitzwater S, Broenkow WW (1989) VERTEX: phytoplankton/iron studies in the Gulf of Alaska. Deep-Sea Res 36:649–680

Mills MM, Ridame C, Davey M, La Roche J, Geider RJ (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429:292–294

Moffett JW, Brand LE, Croot PL, Barbeau KA (1997) Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. Limnol Oceanogr 42:789–799

Moore JK, Doney SC, Glover DM, Fung IY (2002) Iron cycling and nutrient-limitation patterns in surface waters of the World Ocean. Deep-Sea Res (II Top Stud Oceanogr) 49:463–507

Morel FMM, Reinfelder JR, Roberts SB, Chamberlain CP, Lee JG, Yee D (1994) Zinc and carbon co-limitation of marine phytoplankton. Nature 369:740–742

Peers G, Price NM (2004) A role for manganese in superoxide dismutases and growth of iron-deficient diatoms. Limnol Oceanogr 49:1774–1783

Peers G, Quesnet S, Price NM (2005) Copper requirements for iron acquisition and growth of coastal and oceanic diatoms. Limnol Oceanogr 50:1149–1158

Price NM, Ahner BA, Morel FMM (1994) The equatorial Pacific Ocean: grazer-controlled phytoplankton population in an iron-limited ecosystem. Limnol Oceanogr 39:520–534

Price NM, Morel FMM (1991) Colimitation of phytoplankton growth by nickel and nitrogen. Limnol Oceanogr 36:1071–1077

Prospero JM (1999) Long-term measurement of the transport of African mineral dust to the southeastern United States: implications for regional air quality. J Geophys Res 104:15917–15927

Raven JA (1988) The iron and molybdenum use efficiencies of plant growth with different energy, carbon and nitrogen sources. New Phytol 109:279–287

Raven JA (1990) Predictions of Mn and Fe use efficiencies of plant growth with different energy, carbon and nitrogen sources. New Phytol. 109:279–287

Raven JA, Evans MCW, Korb RE (1999) The role of trace metals in photosynthetic electron transport in $O_2$-evolving organisms. Photosyn Res 60:111–149

Rue E, Bruland K (2001) Domoic acid binds iron and copper: a possible role for the toxin produced by the marine diatom *Pseudo-nitzschia*. Mar Chem 76:127–134

Rue EL, Bruland KW (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

Rueter JG (1983) Theoretical iron limitation of microbial $N_2$ fixation in the oceans. EOS 63:445

Saito MA, Moffet JW, Chisholm SW, Waterbury JB (2002) Cobalt limitation and uptake in *Prochlorococcus*. Limnol Oceanogr 47:1629–1636

Scholin CA et al (2000) Mortality of sea lions along the central California coast linked to a toxic diatom bloom. Nature 403:80–84

Stoecker DK, Sunda WG, Davis LH (1986) Effect of copper and zinc on two planktonic ciliates. Mar Biol 92:21–29

Sunda WG (1988/89) Trace metal interactions with marine phytoplankton. Biol Oceanogr 6:411–442

Sunda WG (2000) Trace metal-phytoplankton interactions in aquatic systems. In: Lovely DR (ed) Environmental microbe-metal interactions. ASM Press, Washington, DC, pp 79–107

Sunda WG (2001) Bioavailability and bioaccumulation of iron in the sea. In: Turner DR, Hunter KA (eds) The biogeochemistry of iron in seawater. Wiley, New York, pp 41–84

Sunda WG, Guillard RLL (1976) The relationship between cupric ion activity and the toxicity of copper to phytoplankton. J Mar Res 34:511–529

Sunda WG, Hanson AK (1987) Measurement of free cupric ion concentration in seawater by a ligand competition technique involving copper sorption onto $C_{18}$ SEP-PAK cartridges. Limnol Oceanogr 32:537–551

Sunda WG, Huntsman SA (1995a) Cobalt and zinc interreplacement in marine phytoplankton: biological and geochemical implications. Limnol Oceanogr 40:1404–1417

Sunda WG, Huntsman SA (1995b) Iron uptake and growth limitation in oceanic and coastal phytoplankton. Mar Chem 50:189–206

Sunda WG, Huntsman SA (1996) Antagonisms between cadmium and zinc toxicity and manganese limitation in a coastal diatom. Limnol Oceanogr 41:373–387

Sunda WG, Huntsman SA (1997) Interrelated influence of iron, light and cell size on marine phytoplankton growth. Nature 390:389–392

Sunda WG, Huntsman SA (1998) Interactive effects of external manganese, the toxic metals copper and zinc, and light in controlling cellular manganese and growth in a coastal diatom. Limnol Oceanogr 43:1467–1475

Sunda WG, Huntsman SA (2005) The effect of $CO_2$ supply and demand on zinc uptake and growth limitation in a coastal diatom. Limnol Oceanogr 50:1181–1192

Sunda WG, Tester PA, Huntsman SA (1987) Effects of cupric and zinc ion activities on the survival and reproduction of marine copepods. Mar Biol 94:203–210

Takeda S (1998) Influence of iron availability on nutrient consumption ratio of diatoms in oceanic waters. Nature 393:774–777

Thomas WH, Holm-Hansen O, Siebert DLR, Azam F, Hodson R, Takahashi M (1977) Effects of copper on phytoplankton standing crop and productivity: controlled ecosystem pollution experiment. Bull Mar Sci 27:34–43

Trainer VL, Hickey BM, Homer RA (2002) Biological and physical dynamics of domoic acid production off the Washington coast. Limnol Oceanogr 47:1438–1446

Tsuda A et al (2003) A mesoscale iron enrichment in the western subarctic Pacific induces a large centric diatom bloom. Science 300:958–961

Walsh JJ, Steidinger KA (2001) Saharan dust and Florida red tides: the cyanophyte connection. J Geophys Res 106:11597–11612

Wells ML, Trick CG, Cochlan WP, Hughes MP, Trainer VL (2005) Domoic acid: the synergy of iron, copper, and the toxicity of diatoms. Limnol Oceanogr 50:1908–1917

Wu J, Sunda W, Boyle E, Karl, DM (2000) Phosphate depletion in the western North Atlantic Ocean. Science 289:759–762

Wu J, Luther III GW (1995) Complexation of Fe(III) by natural organic ligands in the Northwest Atlantic Ocean by a competitive ligand equilibration method and a kinetic approach. Mar Chem 50:159–178

Wurtsbaugh WA, Horne AJ (1983) Iron in eutrophic Clear Lake, California: its importance for algal nitrogen fixation and growth. Can J Fish Aquat Sci 40:1419–1429