# Phytoplankton growth in nutrient rich seawater: importance of copper-manganese cellular interactions

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## ABSTRACT

Matrix experiments were conducted to determine the role of trace metals in limiting phytoplankton growth rate in seawater collected from the deep nitrate maximum (800 m, 25 $\mu$M nitrate) off the North Carolina coast. Additions of $FeCl_3$, $MnCl_2$, and chelators (EDTA and NTA) stimulated the growth of unialgal cultures of *Chaetoceros socialis* or of a natural phytoplankton community inoculated into this seawater. Copper additions reduced growth and caused species shifts in the natural community from diatoms to green flagellates; but these effects could be partially or completely reversed by appropriate iron, manganese or chelator additions. We interpreted these results with the aid of thermodynamic calculations, as a physiological interaction between copper and manganese in which copper competes for manganese nutritional sites, thereby interfering with manganese metabolism. Stimulation of phytoplankton growth by EDTA and NTA appears to result directly from the ability of these chelators to tightly complex copper without appreciably binding manganese, resulting in increased manganous/cupric ion activity ratios. Hydrous iron oxides presumably stimulate growth by a similar mechanism. Our results indicate that a combination of low manganese concentrations and high cupric ion activities may limit growth of phytoplankton in upwelling seawater.

## 1. Introduction

There has been much speculation about the influence of trace metals on primary productivity in the oceans. Johnston (1963, 1964) found that the growth of natural phytoplankton was often stimulated by the addition of synthetic chelators, notably EDTA (ethylenediaminetetraacetic acid). According to Johnston "the supply of chelating substances is frequently the most critical aspect of phytoplankton nutrition in seawater." Barber and Ryther (1969) reported that the addition of EDTA alleviated poor growth of phytoplankton in newly upwelled seawater that was otherwise rich in inorganic nutrients. They suggested that an observed increase in phytoplankton growth rate in upwelled seawater with downstream surface advection resulted from the production of natural chelators by the emerging biological com-

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munity. Subsequent experiments (Barber *et al.*, 1971; Barber, 1973) demonstrated that poor growth of phytoplankton in fresh upwelling seawater was also reversed by additions of Fe or Mn.

A number of hypotheses have been proposed to explain growth stimulation by added chelators. Some investigators have suggested that chelators stimulate phytoplankton growth by enhancing the availability of one or more limiting trace metal nutrients, particularly iron (Johnston, 1963, 1964; Barber *et al.*, 1971). EDTA, they argued, enhanced iron supply by solubilizing hydrous ferric oxides. Others have hypothesized that natural levels of ionic copper were toxic to phytoplankton and that the chelation of copper reduced toxicity by binding copper into a nontoxic form (Steemann Nielsen and Wium-Andersen, 1970). Subsequent experiments in model systems have verified that the toxicity of copper to phytoplankton is determined by cupric ion activity and that chelated copper is not directly toxic (Sunda and Guillard, 1976; Anderson and Morel, 1978). These authors found that cupric ion activities toxic to at least some species of phytoplankton fell within the predicted range of cupric ion activity in seawater in which there was little or no organic chelation. Jackson and Morgan (1978) used chemical equilibrium analysis on a portion of the data presented in this paper to demonstrate that growth stimulation by added synthetic chelators of a phytoplankton clone cultured in deep seawater (800 m) was consistent with an alleviation of cupric ion toxicity, but not with alleviation of zinc or nickel toxicity. These authors calculated that chelation of iron by EDTA could not increase iron availability because the dissociation and exchange kinetics for FeEDTA chelates were too slow. They argued that the stimulation of phytoplankton growth by added $FeCl_3$ was similar mechanistically to that by EDTA in that trivalent iron added to seawater forms hydrous iron oxides, which can adsorb copper, thereby lowering cupric ion activity. Jackson and Morgan, however, did not consider growth stimulation in seawater by added manganese.

In this paper we reassess the role of trace metals in controlling phytoplankton growth in upwelled seawater with emphasis on the role of manganese as an essential micronutrient, and on cellular interactions between manganese and copper. Results are presented for phytoplankton culture experiments conducted in "artificially upwelled" seawater (i.e., nutrient rich seawater collected at depth and brought to the surface). The experiments examine the individual and combined effects of added synthetic chelators, iron, manganese and copper on the growth of unialgal cultures of *Chaetoceros socialis* and natural community inocula. The effect of these additions on algal growth rate is interpreted by considering both the chemical interactions among added chelators, hydrous iron oxides, and cupric and manganous ions in seawater and the physiological interactions of manganese and copper with phytoplankton.

## 2. Materials and Methods

*Chaetoceros socialis* or a natural community inoculum were grown in artificially "upwelled seawater" in the presence of single or multiple additions of trace metals ($MnCl_2$, $FeCl_3$, and $CuSO_4$) and chelators (EDTA and NTA (nitrilotriacetic acid)). Seawater for experiments with *C. socialis* was collected in 30 *l* PVC Niskin bottles with Teflon coated stainless steel closure springs. Water for the natural population experiment was collected with a large volume polyethylene sampler (General Oceanics[3]). Water was sampled from approximately 800 m in water depths of 3000 m and 1500 m off the North Carolina coast. The collection depth was selected to coincide with the deep nitrate maximum (ca. 25 $\mu$M nitrate). After collection the water was immediately transferred to 5 gal polyethylene carboys and stored in the dark at 4°C. Before filling, the carboys were rinsed with 10% HCl, leached overnight with surface seawater and then rinsed three times with the deep seawater sample. Seawater was stored for one day for the natural population experiment and for one to four months for the unialgal culture experiments.

The natural phytoplankton community (dominated by *Nitzschia* and *Chaetoceros*) was obtained immediately before use from a surface water sample collected off the North Carolina coast (34° 13.0′N, 76° 12.6′W). Total water depth at this station was 42 m. Chlorophyll *a* was measured and the inoculum size used (20 ml) gave a chlorophyll *a* concentration of 0.006 $\mu g \cdot l^{-1}$ in the experimental bottles.

Cultures of *C. socialis* (obtained from H. Kayser, Biologischi Anstadt, Helgoland, F.R.G.) were maintained in deep seawater containing $10^{-6}$M EDTA under the light-dark regime described below. Inocula for experiments (0.1 to 1.0 ml) were taken from exponentially growing cultures to give an initial chlorophyll *a* concentration of 0.004 ± 0.001 $\mu g \cdot l^{-1}$. *C. socialis* was selected because it is a common dominant phytoplankter in coastal upwelling regions (Ryther *et al.*, 1971; Blasco, 1971; Hobson, 1971).

Experiments were conducted in 500 ml of seawater placed in 500 ml borosilicate bottles with Teflon lined screw caps. After addition of seawater, the bottles were allowed to equilibrate to room temperature for 24 h after which reagents, $^{14}C$-bicarbonate, and then phytoplankton were added. The experimental bottles were incubated at 22°C and pH 8.1 ± 0.1 using a 12:12 light:dark cycle. Light was supplied by 16 Sylvania "cool white" fluorescent lights at an intensity of 370 microeinsteins $\cdot m^{-2} \cdot sec^{-1}$. Growth was monitored by long term $^{14}C$ uptake as described by Barber (1973) and Huntsman and Barber (1975). Culture subsamples were filtered daily (0.8 $\mu$m pore size Millipore) to determine carbon fixation and chlorophyll *a* concentration, and growth rate was computed via linear regression of

3. Reference to trade names or products does not imply endorsement by the National Marine Fisheries Service, NOAA.

log base 2 of carbon fixed *vs* time or of chlorophyll *a vs* time during the period of exponential growth. Growth rates determined from chlorophyll *a* measurements were essentially the same as those from fixed carbon data. To avoid redundancy, only carbon doubling rates are presented.

Table 1. Experimental treatments and growth rates for *C. Socialis* cultures in 800 m seawater.

| Experiment | Addition (molar) | | | | | Growth rate (doubling • day$^{-1}$ ± SD) |
|---|---|---|---|---|---|---|
| | $CuCl_2$ | $MnCl_2$ | $FeCl_3$ | EDTA | NTA | |
| 1A | — | — | — | | | |
| 1A | — | — | — | — | — | 0.81 ± 0.07 |
| | | | | $10^{-9}$ | | 0.70 ± 0.08 |
| | | | | $10^{-8}$ | | 1.48 ± 0.10 |
| | | | | $10^{-7}$ | | 2.35 ± 0.15 |
| | | | | $10^{-6}$ | | 3.23 ± 0.16 |
| | | | | $10^{-5}$ | | 2.06 ± 0.19 |
| | | | | $10^{-4}$ | | 0.39 ± 0.19 |
| 1B | — | — | — | — | — | 0.57 ± 0.09 |
| | | | | | $10^{-9}$ | 0.70 ± 0.02 |
| | | | | | $10^{-8}$ | 0.45 ± 0.11 |
| | | | | | $10^{-7}$ | 1.32 ± 0.08 |
| | | | | | $10^{-6}$ | 1.62 ± 0.09 |
| | | | | | $10^{-5}$ | 3.20 ± 0.10 |
| 2A | — | — | — | — | — | 0.61 ± 0.05 |
| | | | | $10^{-6}$ | | 2.86 ± 0.20 |
| | | $10^{-6}$ | | | | 2.37 ± 0.10 |
| | | $10^{-6}$ | | $10^{-6}$ | | 3.14 ± 0.26 |
| 2B | $10^{-7}$ | | | | | 0.04 ± 0.26 |
| | $10^{-7}$ | | | $10^{-6}$ | | 2.34 ± 0.28 |
| | $10^{-7}$ | $10^{-6}$ | | | | 1.83 ± 0.08 |
| | $10^{-7}$ | $10^{-6}$ | | $10^{-6}$ | | 3.00 ± 0.09 |
| 3A | — | — | — | — | — | 0.81 ± 0.19 |
| | | $10^{-9}$ | | | | 1.30 ± 0.08 |
| | | $10^{-6}$ | | | | 2.74 ± 0.07 |
| | | $10^{-7}$ | | | | 2.78 ± 0.07 |
| | | | | $10^{-7}$ | | 2.00 ± 0.03 |
| | | | | $5\times10^{-7}$ | | 2.51 ± 0.10 |
| | | | | $10^{-6}$ | | 2.58 ± 0.03 |
| 3B | $10^{-8}$ | | | | | 0.69 ± 0.10 |
| | $10^{-8}$ | $10^{-8}$ | | | | 2.18 ± 0.11 |
| | $10^{-6}$ | $10^{-7}$ | | | | 2.82 ± 0.03 |
| | $10^{-8}$ | $10^{-6}$ | | | | 2.93 ± 0.13 |

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Table 1. (continued)

| Experiment | Addition (molar) | | | | | Growth rate (doubling • day$^{-1}$ ± SD) |
|---|---|---|---|---|---|---|
| | $CuCl_2$ | $MnCl_2$ | $FeCl_3$ | EDTA | NTA | |
| | $10^{-8}$ | | | $10^{-7}$ | | 1.94 ± 0.10 |
| | $10^{-8}$ | | | $5\times10^{-7}$ | | 2.67 ± 0.02 |
| | $10^{-8}$ | | | $10^{-6}$ | | 2.58 ± 0.12 |
| 3C | $10^{-7}$ | | | | | No growth |
| | $10^{-7}$ | $10^{-7}$ | | | | 1.72 ± 0.15 |
| | $10^{-7}$ | $10^{-6}$ | | | | 1.89 ± 0.16 |
| | $10^{-7}$ | $10^{-5}$ | | | | 1.96 ± 0.01 |
| | $10^{-7}$ | | | $10^{-7}$ | | 0.45 ± 0.39 |
| | $10^{-7}$ | | | $5\times10^{-7}$ | | 2.24 ± 0.08 |
| | $10^{-7}$ | | | $10^{-6}$ | | 2.42 ± 0.11 |
| 4A | — | — | — | — | — | 0.59 ± 0.04 |
| | | | $10^{-6}$ | | | 1.70 ± 0.02 |
| | | | $10^{-6}$ | | | 2.66 ± 0.13 |
| | | | $10^{-6}$ | | | 2.35 ± 0.12 |
| 4B | $10^{-8}$ | | | | | 0.83 ± 0.08 |
| | $10^{-8}$ | | $10^{-6}$ | | | 1.25 ± 0.04 |
| | $10^{-8}$ | | $10^{-5}$ | | | 2.49 ± 0.15 |
| | $10^{-8}$ | | | $10^{-6}$ | | 1.83 ± 0.10 |
| 4C | $10^{-7}$ | | | | | 0.23 ± 0.07 |
| | $10^{-7}$ | | $10^{-6}$ | | | 0.52 ± 0.04 |
| | $10^{-7}$ | | $10^{-5}$ | | | 0.91 ± 0.05 |
| | $10^{-7}$ | | | $10^{-6}$ | | 1.95 ± 0.06 |
| 5 | — | — | — | — | — | 0.78 ± 0.05 |
| | | $10^{-7}$ | | | | 3.08 ± 0.03 |
| | $10^{-8}$ | | | | | 0.78 ± 0.03 |
| | $10^{-8}$ | $10^{-7}$ | | | | 2.80 ± 0.02 |
| | $2\times10^{-8}$ | | | | | 0.54 ± 0.11 |
| | $2\times10^{-8}$ | $10^{-7}$ | | | | 2.54 ± 0.13 |
| | $4\times10^{-8}$ | | | | | 0.49 ± 0.03 |
| | $4\times10^{-8}$ | $10^{-7}$ | | | | 2.06 ± 0.22 |
| | $6\times10^{-8}$ | | | | | 0.19 ± 0.23 |
| | $6\times10^{-8}$ | $10^{-7}$ | | | | 0.88 ± 0.13 |
| | $8\times10^{-8}$ | | | | | 0.49 ± 0.15 |
| | $8\times10^{-8}$ | $10^{-7}$ | | | | 0.59 ± 0.11 |
| | $10^{-7}$ | | | | | 0.08 ± 0.18 |
| | $10^{-7}$ | $10^{-7}$ | | | | 0.70 ± 0.14 |
| 6A | — | — | — | — | — | 0.63 ± 0.06 |
| | | $10^{-6}$ | | | | 2.84 ± 0.06 |

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Table 1. (continued)

| Experiment | Addition (molar) | | | | | Growth rate (doubling • day$^{-1}$ ± SD) |
|---|---|---|---|---|---|---|
| | $CuCl_2$ | $MnCl_2$ | $FeCl_3$ | EDTA | NTA | |
| 6B | $10^{-8}$ | | | | | 0.34 ± 0.24 |
| | $10^{-8}$ | $10^{-9}$ | | | | 0.48 ± 0.09 |
| | $10^{-8}$ | $5\times10^{-9}$ | | | | 0.90 ± 0.11 |
| | $10^{-8}$ | $10^{-8}$ | | | | 1.91 ± 0.06 |
| | $10^{-8}$ | $10^{-7}$ | | | | 2.77 ± 0.01 |
| | $10^{-8}$ | $10^{-6}$ | | | | 2.37 ± 0.02 |
| 6C | $3\times10^{-8}$ | | | | | 0.31 ± 0.05 |
| | $3\times10^{-8}$ | $10^{-9}$ | | | | 0.56 ± 0.08 |
| | $3\times10^{-8}$ | $10^{-8}$ | | | | 0.80 ± 0.05 |
| | $3\times10^{-8}$ | $4\times10^{-8}$ | | | | 1.32 ± 0.05 |
| | $3\times10^{-8}$ | $6\times10^{-8}$ | | | | 1.13 ± 0.06 |
| | $3\times10^{-8}$ | $8\times10^{-8}$ | | | | 1.57 ± 0.07 |
| | $3\times10^{-8}$ | $10^{-7}$ | | | | 1.57 ± 0.06 |
| | $3\times10^{-8}$ | $10^{-6}$ | | | | 2.54 ± 0.05 |
| 6D | $6\times10^{-8}$ | | | | | 0.30 ± 0.16 |
| | $6\times10^{-8}$ | $4\times10^{-8}$ | | | | 0.89 ± 0.04 |
| | $6\times10^{-8}$ | $6\times10^{-8}$ | | | | 0.94 ± 0.02 |
| | $6\times10^{-8}$ | $8\times10^{-8}$ | | | | 1.13 ± 0.03 |
| | $6\times10^{-8}$ | $10^{-7}$ | | | | 1.14 ± 0.03 |
| | $6\times10^{-8}$ | $2\times10^{-7}$ | | | | 1.46 ± 0.03 |
| | $6\times10^{-8}$ | $4\times10^{-7}$ | | | | 1.74 ± 0.05 |
| | $6\times10^{-8}$ | $10^{-6}$ | | | | 1.93 ± 0.04 |

Six separate experiments were conducted with *C. socialis* with a combined total of 82 experimental cultures (Table 1). Although treatments were not replicated within a given experiment, they often were replicated among the different experiments.

Cupric and manganous ion activities in experimental media containing different additions of $MnCl_2$, $CuSO_4$, EDTA and NTA were obtained from estimates of total dissolved metal concentrations and from equilibrium calculations that compute the extent of complex formation with both inorganic and added organic ligands. Calculations of metal complexation are similar to those given by Sunda (1975) and Sunda and Gillespie (1979). In the absence of added chelators, the estimated ratio of free metal ion activity to the total dissolved metal concentration in seawater at pH 8.1 and 35‰ was $10^{-1.8}$ for copper (Sunda and Gillespie, 1979) and $10^{-0.7}$ for manganese (Mantoura *et al.*, 1978). These estimates ignore any possible complexation by natural organic ligands and, therefore, may represent minimum values. Stability constants used to compute metal complexation by EDTA and NTA are listed in Table 2.

Table 2. Stability constants ($K$) for NTA and EDTA (T = 20°C; I = 0.1M)*

| Metal | Log $K$ (EDTA) | Log $K$ (NTA) |
|---|---|---|
| Ca | 10.59 | 6.41 |
| Mg | 8.69 | 5.41 |
| Cu | 18.79 | 12.96 |
| Mn | 14.04 | 7.44 |

* Values selected from Sillen and Martell (1964).

Concentrations of total dissolved copper and manganese were computed from estimated ambient concentrations plus the added concentration of the metals. Ambient concentrations were estimated from current literature values for concentrations in seawater and from minimum additions at which biological effects were recorded. For example, we observed copper toxicity in our experiments at $10^{-8}$M additions indicating that the ambient copper concentrations must have been approximately equal to or less than $10^{-8}$M. Subsurface copper concentrations in North Atlantic sea water have been reported at $\sim 3 \times 10^{-9}$M (Moore and Burton, 1976) and based on this value and the level of addition required to cause toxicity, we estimate ambient copper in the experimental seawater to be in the range $\sim 3 - 10 \times 10^{-9}$M or $7 \pm 4 \times 10^{-9}$M. One of the reasons for the uncertainty in ambient copper is the possibility that the samples were inadvertently contaminated with copper during collection (Boyle and Edmond, 1975). Observed copper toxicity at a $10^{-8}$M addition suggests an upper limit for such contamination of about this value. Estimates of background concentration of manganese ($4 \times 10^{-10}$M) are based on recent analyses for North Atlantic seawater reported by Bender *et al.* (1977). Stimulation of phytoplankton growth in our experiments by $10^{-9}$M manganese additions is consistent with this value.

## 3. Results and Discussion

*Experiments with Chaetoceros socialis in 800 m seawater.* Growth rates of *C. socialis* in 800 m seawater were stimulated to 2.8 ± 0.4 divisions per day by the addition of chelators (EDTA and NTA), $MnCl_2$ or $FeCl_3$ (Table 1, Fig. 1A and B). Cultures maintained in unamended deep seawater had exponential growth rates of only 0.7 ± 0.1 doublings • day$^{-1}$ (n = 6). Maximum growth rates (i.e., $\geq$ 2.4 doublings • day$^{-1}$) were obtained with the following minimum additions: $10^{-8}$M $MnCl_2$; $10^{-5}$M $FeCl_3$; $10^{-6}$M EDTA; or $10^{-5}$M NTA (Table 1, Fig. 1A and B). Thus, growth of *C. socialis* was most sensitive to added manganese and growth was stimulated by this metal at a concentration three orders of magnitude below the iron concentration required to produce a similar effect. The addition of as little as $10^{-9}$M $MnCl_2$ stimulated growth over that of controls (Fig. 1A).

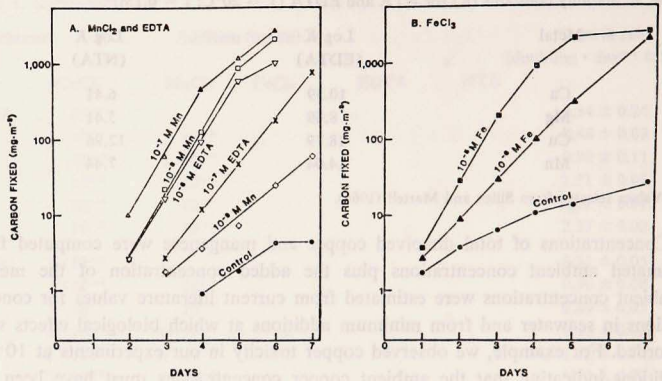


Figure 1. A. Effect of $MnCl_2$ and EDTA on growth of *C. socialis* in 800 m seawater.
B. Effect of $FeCl_3$ on growth of *C. socialis* in 800 m seawater. Results from Exp. 4A.

The addition of copper inhibited the growth rate of *C. socialis* with no growth at a concentration *ca.* $10^{-7}$M (Table 1, Fig. 2). The addition of EDTA, $FeCl_3$, or $MnCl_2$ partially or completely reversed copper toxicity. For example, the toxicity of copper at additions of $1 \times 10^{-8}$M and $3 \times 10^{-8}$M was totally reversed by the addition of $10^{-7}$M and $10^{-6}$M $MnCl_2$ (Fig. 2A and B).

*Experiment with a natural community.* The growth of a natural community of phytoplankton inoculated into deep seawater was also stimulated by additions of EDTA, $MnCl_2$, $FeCl_3$ and was inhibited by additions of copper (Fig. 3). The effect of two levels of EDTA and $MnCl_2$ ($10^{-7}$ and $10^{-6}$M) and one of $FeCl_3$ ($10^{-5}$M) was tested at four levels of $CuCl_2$. With no added copper, $10^{-7}$ and $10^{-6}$M $MnCl_2$, $10^{-6}$M EDTA and $10^{-5}$M $FeCl_3$ all gave maximum stimulation of phytoplankton growth. Manganese was more effective than EDTA on a per molar basis, but this relationship was reversed at the two highest additions of copper (0.5 and $1.0 \times 10^{-7}$M). At these high copper additions, $10^{-6}$M EDTA and $10^{-5}$M $FeCl_3$ were equally effective in stimulating growth and both caused greater stimulation than $10^{-6}$M $MnCl_2$. Unlike EDTA, growth stimulation at $10^{-6}$M $MnCl_2$ was similar to or only slightly greater than that at $10^{-7}$M manganese at all copper additions.

Overall, the effect of added Mn, Fe, Cu, and EDTA on the natural community was similar to that observed with cultures of *C. socialis*. An exception, however, was the natural communities response to copper in which pronounced time lags in initial growth were observed with increasing concentrations of this metal (Fig. 3).

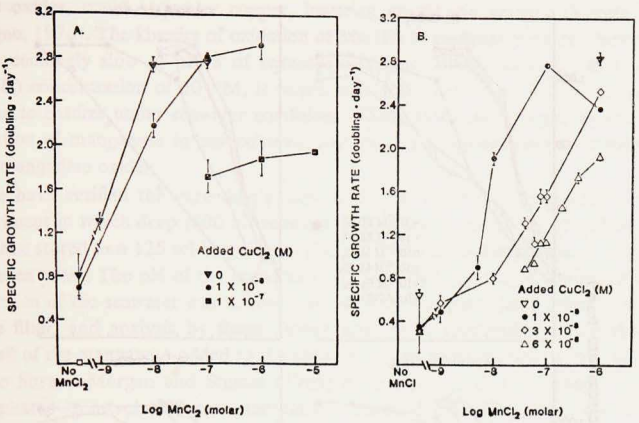


Figure 2. Growth rate of *C. socialis* in 800 m seawater with addition of different combinations of $MnCl_2$ and $CuCl_2$. A. Results from Exp. 3. B. Results from Exp. 6.

The natural community was dominated by species of *Chaetoceros* and *Nitzschia.* Microscopic examination at the end of the experiment indicated that cultures exhibiting pronounced lags due to copper showed a shift in species dominance from diatoms to small green flagellates. Cultures with highest growth rates and no extended lag in initial growth, on the other hand, showed no apparent shift in species dominance. Thus, copper inhibited the growth of the sensitive dominant species of diatoms and allowed less sensitive species to flourish. The observed lags can be explained by the initial lower biomass of copper resistant flagellates. Similar shifts in species composition resulting from copper additions have been observed in large bag seawater enclosure experiments (Thomas and Seibert, 1977) and in a lake treated for nuisance algal blooms (McKnight, 1979).

*Role of manganese and copper in controlling phytoplankton growth.* Stimulation of algal growth in deep seawater by added chelators and iron has been interpreted by Jackson and Morgan (1978) as an alleviation of growth inhibition resulting from ambient toxic levels of copper. They argued that chelators reduced cupric ion activities in seawater to non-toxic levels and that iron did likewise through co-precipitation and adsorption of copper. Arguments by Jackson and Morgan are especially pertinent in that they were based on an analysis of a portion of the data

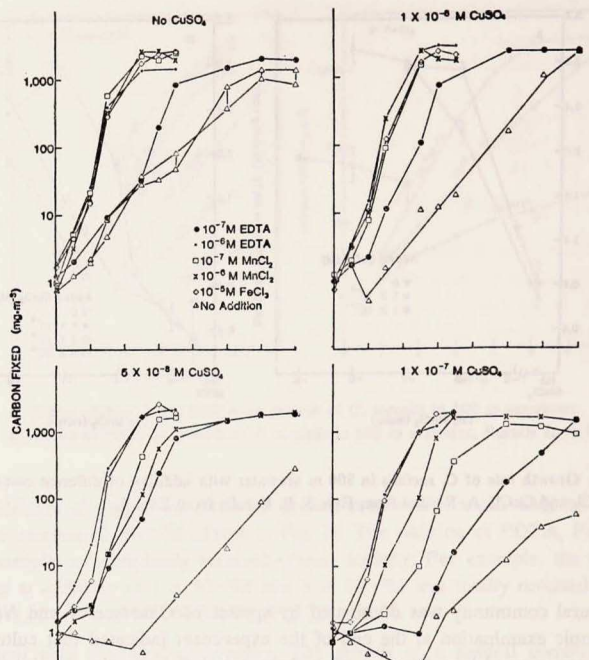


Figure 3. Growth of a natural phytoplankton community inoculated into 800 m seawater: effect of manganese, iron, and EDTA at different concentrations of added copper.

set presented in this paper: that for growth rate of *C. socialis* in deep seawater containing different combinations of added synthetic chelators, iron and copper. The effects of added manganese or combinations of manganese and copper, however, were not dealt with by these authors.

Although reduction in cupric ion activity and therefore copper toxicity provides an adequate explanation for phytoplankton growth stimulation by added chelators or Fe (III), it does not, as we discuss below, readily explain stimulation by Mn (II). Based on chemical equilibria, Mn (II) should oxidize to $MnO_2$ which, like hydrous

ferric oxides, could sequester copper, lowering cupric ion activity (Vuceta and Morgan, 1978). The kinetics of oxidation of Mn (II) to manganese oxides, however, are exceedingly slow at pH 8 of seawater. Morgan (1967) calculated that, at a Mn (II) concentration of $10^{-7}$M, it would take 1000 years for 90% of the manganese to oxidize under seawater conditions. These calculations argue against the oxidation of manganese in our cultures, and therefore, against copper adsorption onto manganese oxides.

We have verified the exceedingly slow oxidation kinetics of manganese in an experiment in which deep (800 m) seawater was amended with $2 \times 10^{-5}$M $MnCl_2$ and then stored in a 125 ml borosilicate glass bottle at room temperature (~ 25°C) for seven years. The pH of the seawater (8.0) remained unchanged during storage. A portion of the seawater was filtered through a 0.02 $\mu$ pore size Nuclepore membrane filter, and analysis by flame atomic absorption spectrophytometry showed that all of the manganese added to the seawater seven years previously was present in the filtrate. Morgan and Stumm (1965) have reported that manganese oxides precipitated from solution are quantitatively retained by 0.22 $\mu$ pore size filters, indicating that the manganese that passed through our smaller pore size filters was present in the soluble manganous form and that no detectible oxidation had occurred.

An additional argument that growth stimulation by added manganese is not due to adsorption of copper by manganese oxides is based on the exceedingly low concentrations of manganese required to increase phytoplankton growth rates. Manganese causes near maximum growth stimulation at additions of $10^{-8}$M whereas 1000 fold higher concentrations of iron are needed for equivalent stimulation (Fig. 1A and B). It is doubtful that such low concentrations of manganese, if converted to manganese oxides, could adsorb sufficient quantities of copper to totally reverse copper toxicity (Vuceta and Morgan, 1978).

In light of the evidence that added manganese remains in solution in the reduced manganous form, the role of manganese as a growth stimulant must be truly physiological; that is, manganese must be acting as a limiting nutrient. Furthermore, the ability of manganese to reverse copper toxicity (Fig. 2) can be explained by a competitive interaction between the two metals at cellular sites involved in manganese nutrition. These could be sites at the cell's surface involved in manganese intracellular uptake or they could be manganese nutritional sites within the cell; for example, enzymes that require manganese as a co-factor.

To further clarify the roles played by copper and manganese, ionic activities of these metals were estimated by thermodynamic calculations for experimental cultures containing different combinations of added copper, manganese, EDTA, and NTA. These calculations are used to assess the effect of added chelators on ionic activities of copper and manganese. Experimental media containing added Fe are

not included in our calculations because of the lack of appropriate thermodynamic data for the adsorption of copper and manganese by hydrous ferric oxides. The role of iron, however, will be discussed later, qualitatively.

Plots of growth rate of *C. socialis* as a function of estimated pCu (− log cupric ion activity) clearly show that growth rate is controlled by this parameter; but the relationship between growth rate and pCu is dependent on the activity of manganous ion (Fig. 4). At intermediate estimated manganous ion activities ($\sim 10^{-10}$ to $10^{-8}$M), the growth rate appears to be dependent on the ratio of maganous ion activity to cupric ion activity; however, at higher cupric ion activities, toxicity curves for copper increase in slope and the dependence of copper toxicity on manganous ion activity is not as pronounced. At low estimated activities of manganese ($< 10^{-10}$M) and copper ($< 10^{-13}$M) associated with high concentrations of EDTA ($\geqslant 10^{-5}$M), growth rate of *C. socialis* decreases with concomitant decreases in the activities of both metals.

*A cellular model for effects of copper and manganese on growth rate.* To explain the relationship between growth rate of *C. socialis* and ionic activities of manganese and copper, a simple two-site cellular binding model was constructed in which copper competes with manganese at one of the sites, but only copper reacts at the second. A two-site model, rather than a single-site model, is required because of the above mentioned differences in the growth rate dependence on copper and manganese at low activities of these metals from that observed at high activities (Fig. 4).

The first site that we have postulated is a cellular manganese activation site. This site may, for example, represent an enzyme that requires binding with manganese for metabolic activity. We further postulate that copper acts at this site as a competitive inhibitor. The reaction of the binding site ($E$) with manganese and copper can be represented as:

$$\mathrm{Mn} + E \rightleftharpoons \mathrm{Mn}E \tag{1}$$

$$\mathrm{Cu} + E \rightleftharpoons \mathrm{Cu}E \tag{2}$$

Since binding with manganese is required for site activation, only $\mathrm{Mn}E$ is metabolically active and $E$ and $\mathrm{Cu}E$ are inactive forms of the site. The metabolic activity of the site will, therefore, be proportional to the fraction of the site in its active manganese complexed form:

$$A = \frac{[\mathrm{Mn}E]}{[E_{\mathrm{tot}}]} = \frac{[\mathrm{Mn}E]}{[\mathrm{Mn}E] + [\mathrm{Cu}E] + [E]} \tag{3}$$

where square brackets denote concentrations of the enclosed chemical species and where $A$ is the fractional site activity on a scale from 0 to 1.

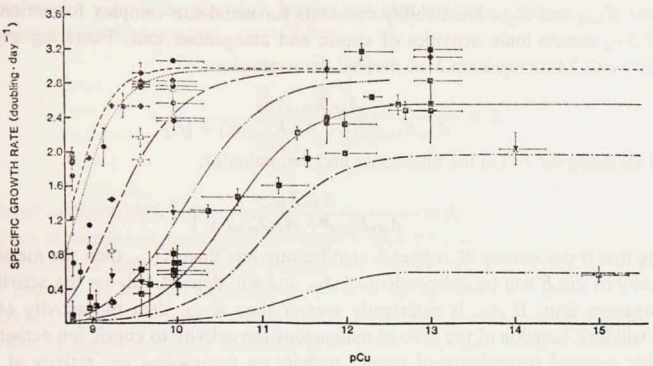


Figure 4. Exponential growth rate of *C. socialis* in 800 m seawater as a function of estimated pCu at different manganese ion activities. Curves are computed from the modeled equation:

$$\mu = \frac{3.0}{1 + A_{Cu^2}\, 10^{17.6}} \cdot \frac{A_{Mn}\, 10^{10.9}}{A_{Mn}\, 10^{10.9} + A_{Cu}\, 10^{11.5} + 1}$$

Symbols represent data points and lines are computed curves for the following experimental conditions.

| | $MnCl_2$ | pMn | EDTA |
|---|---|---|---|
| ---------- XX | $10^{-5}$M | 5.7 | O |
| ---------- ◆ | $10^{-6}$M | 6.7 | 0, $10^{-7}$, $10^{-6}$ |
| .......... ● | $10^{-7}$M | 7.7 | 0 |
| ——— — △ | $10^{-8}$M | 8.7 | 0 |
| ——— . . . ▼ | $10^{-9}$M | 9.5 | 0 |
| ——————— ■ | 0 | 10.1 | O, $10^{-9}$, $10^{-8}$, $10^{-7}$, $5 \times 10^{-7}$, $10^{-6}$ (also NTA = $10^{-9}$, $10^{-8}$, $10^{-7}$, $10^{-6}$, $10^{-5}$) |
| ——— . — X | 0 | 10.6 | $10^{-5}$ |
| ——————— .. ◪ | 0 | 11.5 | $10^{-4}$ |

Vertical error bars represent ± SD for growth rate regressions. Horizontal bars are based on a range in the estimated ambient total copper of 4 to 11 × $10^{-9}$M. Results are from all six *C. socialis* experiments.

At equilibrium the following mass action equations would apply:

$$\frac{[MnE]}{A_{Mn}[E]} = K_{MnE} \tag{4}$$

and

$$\frac{[CuE]}{A_{Cu}[E]} = K_{CuE} \tag{5}$$

$$[MnE] = A_{Mn}[E]\, K_{MnE} \tag{6}$$

and

$$[CuE] = A_{Cu}[E]\, K_{CuE} \tag{7}$$

where $K_{MnE}$ and $K_{CuE}$ are stability constants for metal-site complex formation $A_{Cu}$ and $A_{Mn}$ denote ionic activities of cupric and manganous ions. Factoring expressions 6 and 7 into equation 3 we derived the expression:

$$A = \frac{A_{Mn}K_{MnE}[E]}{A_{Mn}K_{MnE}[E] + A_{Cu}K_{CuE}[E] + [E]} \tag{8}$$

and factoring out $[E]$ in the numerator and denominator,

$$A = \frac{A_{Mn}K_{MnE}}{A_{Mn}K_{MnE} + A_{Cu}K_{CuE} + 1} . \tag{9}$$

Note that if the activity of copper is significantly less than $K_{CuE}$, then the metabolic activity of site $E$ will be independent of $A_{Cu}$ and will depend only on the activity of manganous ions. If $A_{Cu}$ is sufficiently greater than $K_{CuE}$, then the activity of the site will be a function of the ratio of manganous ion activity to cupric ion activity.

The reduced dependence of copper toxicity on manganous ion activity at high cupric ion activities suggests binding of copper at a second copper inhibition site that is not involved in manganese nutrition. The steepness of the toxicity curve also suggests the reaction of more than one cupric ion at this site:

$$nCu + S \rightleftharpoons Cu_nS \tag{10}$$

where $S$ is the free matabolically active site and $Cu_nS$ is the inactive site after reaction with n cupric ions. The proportion of this site in its active form would be:

$$\frac{[S]}{[S_{tot}]} = \frac{[S]}{[S] + [Cu_nS]} . \tag{11}$$

If we again assume equilibrium and apply an appropriate mass action expression, then

$$A^* = \frac{1}{1 + A_{Cu^n}K_{Cu_nS}} \tag{12}$$

where $A^*$ is the fractional activity of the site and $K_{Cu_nS}$ is the stability constant for the reaction of copper with the inhibition site.

Finally, in constructing our molecular model, we will assume that if all other factors are nonlimiting, then the growth rate of the cells will be proportional to the fraction of the two sites in their active forms. We further assume that the activity of the two sites regulate growth rate in a simple multiplicative fashion. The final mathematical form of the model is therefore

$$\mu = \mu_{max} \cdot \frac{A_{Mn}K_{MnE}}{A_{Mn}K_{MnE} + A_{Cu}K_{CuE} + 1} \cdot \frac{1}{A_{Cu^n}K_{Cu_nS} + 1} \tag{13}$$

where $\mu$ is the growth rate and $\mu_{max}$ is the optimal growth rate in the absence of copper toxicity or manganese limitation.

The model was fit to the data by an iterative procedure and the following parameters were determined:

$$\mu_{max} = 3.0$$
$$n = 2$$
$$K_{Cu_2S} = 10^{17.6}$$
$$K_{MnE} = 10^{10.9}$$
$$K_{CuE} = 10^{11.5}.$$

Modeled relationships between growth rate and cupric ion activity at varying activities of manganese are plotted in Figure 4, and there is a reasonable fit between the experimental data and the curves computed from the modeled equation.

A two-site model was necessary to explain all the observed growth data for *C. socialis,* including that with added copper in the absence of chelators. Cupric ion activities in seawater should normally be $\leqslant 10^{-9.8}$M (based on total copper concentrations of $\leqslant 10^{-8}$M) and according to our model, the second site is appreciably affected by copper only at activities above $10^{-9.5}$M. Thus, only the first cellular site, involving competitive binding between cupric and manganous ions, should affect phytoplankton growth at natural levels of copper in seawater.

Although the site of copper-manganese competition has not been identified, evidence suggests that it may be associated with the chloroplast. Manganese is required in small amounts to activate certain nonphotosynthetic enzymes, but there is a much greater requirement in photosynthesis, specifically photosystem II, where it is essential for oxygen evolution (Cheniae and Martin, 1970; O'Kelley, 1974; Diner and Joliot, 1976). Eyster *et al.* (1956, 1958) found that while an external manganese concentration of $10^{-11}$M would satisfy the requirement for nonphotosynthetic processes in *Chlorella,* photosynthetic reactions required concentrations two to three orders of magnitude higher. Habermann (1969) observed that both the Hill and Mehler reactions in isolated chloroplasts exhibit manganese reversible copper inhibition and suggested that both copper and manganese compete for the same enzymatic sites. Copper inhibition of flagellar motility in *Gonyaulax,* on the other hand, was unaffected by manganese concentration (Anderson and Morel, 1978), indicating that manganese reversal of copper toxicity is not a general phenomena; rather it probably occurs only at specific loci, such as manganese activated enzymes.

Our model postulates the presence of a second site of copper inhibition at which manganese does not compete. Copper can be a rather nonspecific metabolic inhibitor due to its tendency to bind tightly with the sulfhydryl groups of proteins (Passow *et al.*, 1961). A number of cellular functions can be affected including membrane permeability (McBrien and Hassall, 1965), late stage cell division (Kanazawa and Kanazawa, 1969) and respiration (McBrien and Hassall, 1967).

*Role of added chelators and $FeCl_3$.* We are now in a position to discuss the

stimulatory roles played by added chelators and ferric iron. To do this we need to consider the chemical nature of cupric and manganous ions and their tendencies toward complex formation with soluble ligands, such as EDTA and NTA, and at surfaces, such as that of hydrous ferric oxides. Copper has a strong tendency toward complex formation and is highest in the Irving-Williams order for stability in complex formation. Manganese, on the other hand, associates much more weakly with ligands. Thus, ligands (soluble and surface) added to seawater will tend to have a much larger effect on activities of cupric ions than manganous ions. Furthermore, competition by calcium ions will often prevent added ligands from having any effect at all on manganese ion activities.

Stability constants for both EDTA and NTA are five orders of magnitude higher for copper than for manganese (Table 2). Consequently, the addition of EDTA at concentrations $\leqslant 10^{-6}$M reduces the activity of cupric ion without having an appreciable effect on the activity of manganous ions. EDTA, therefore, at low concentrations stimulates algal growth by increasing the manganous/cupric ion ratio while having no effect on manganous ion activity. At higher concentrations of EDTA ($10^{-5}$ and $10^{-4}$M), manganese as well as copper is appreciably complexed and there is a concomitant decrease in growth rate of *C. socialis*. Increases in EDTA concentrations in this range have little effect on ionic ratios, but have a rather marked effect on manganous ion activities. We must caution, however, that since ionic activities of a number of essential transition metals (e.g., Zn and Co) will be affected as well as those of Mn and Cu, it is impossible to unequivocably ascribe growth limitation at high chelator concentrations solely to manganese deficiency.

Although an exact quantitative assessment is impossible, the addition of ferric iron and subsequent formation of hydrous ferric oxides should have a similar effect on cupric and manganese ion activities as the addition of chelators. Measurements by Swallow *et al.* (1980) have shown that copper strongly associates with ferric precipitates. Experimental data, however, is not available for manganese association, although on the basis of its hydrolysis constants, we would not expect manganese to adsorb to hydrous ferric oxides as readily as copper (Schindler, 1975).

Can iron be acting in our experiments in a truly physiological role? Jackson and Morgan (1978) argued against this on the basis of slow dissociation kinetics of ferric-EDTA chelates. Their calculations, however, did not consider photoreduction to Fe (II) (Anderson and Morel, 1980) and, therefore, did not consider all possibilities. But we can argue against iron limitation along several other lines. The first is the extremely high concentration of iron, $10^{-5}$M, needed to bring about maximum growth rate stimulation (Table 1, Exp. 4A), a concentration that is two to three orders of magnitude above ambient levels (Brewer, 1975). A second and stronger argument is that if cells were limited by iron, then how can we explain the complete stimulation of growth to maximum rates by addition of low levels of

manganous ions? In such a case we would have to invoke action at a metabolic site whereby the site could be activated by either manganese or iron.

*Effect of manganese-copper-cellular interactions on phytoplankton growth in seawater.* We found that addition of as little as $10^{-9}$M manganese stimulated growth of *C. socialis* in deep seawater and $10^{-8}$M brought about near maximum stimulation. In a much earlier study, Harvey (1947) showed that addition of manganese at 5 to 10 $\times$ $10^{-9}$M brought about a sizable stimulation of growth of *Dunaliella* or *Chlorella* in heat sterilized Plymouth Sound seawater.

If one examines recent values for concentrations of manganese in seawater, it becomes readily apparent why manganese might be deficient in at least some marine waters. Bender *et al.* (1977) reported dissolved manganese concentrations in surface Sargasso Sea water of about 2 $\times$ $10^{-9}$M, which decrease rapidly with depth to a concentration of about 4 $\times$ $10^{-10}$M below 300 m. Similar profiles have been found in the Pacific Ocean where manganese concentrations at the surface ranged from 0.3 to 3.0 $\times$ $10^{-9}$M and decreased with depth below the mixed layer at 10 of 13 stations (Klinkhamer and Bender, 1980). Thus, manganese concentrations in oceanic seawater are exceedingly low; about three orders of magnitude less than in standard algal culture media such as F/2 (Guillard and Ryther, 1962). Filterable manganese concentrations in some estuaries, on the other hand, can approach micromolar levels (Holliday and Liss, 1976; Evans *et al.*, 1977). It would appear, therefore, from our data and that of Harvey (1947) that natural variations in manganese abundance could influence phytoplankton growth in seawater.

It is also apparent from our data that manganese growth limitation is affected in a competitive fashion by copper and that under certain conditions growth rate is controlled by the ratio of manganous ion activity to cupric ion activity. Thus, the growth of manganese deficient cells can be as readily affected by changes in cupric ion activity as by changes in concentrations of ionic manganese.

It is possible that at least some of the reduced growth of *C. socialis* in our experimental sea water results from contamination by copper during seawater collection (Boyle and Edmond, 1975) rather than from ambient natural concentrations of copper. But as discussed previously, observed growth inhibition at $10^{-8}$M additions suggests ambient background copper concentrations in our experiments (including that arising from contamination) at less than or equal to about $10^{-8}$M. Relationships between growth rate of *C. socialis* and computed cupric ion activity in the absence of added manganese show that copper inhibits growth rate at activities $> 10^{-12}$M with 50% inhibition at ca. $10^{-10.7}$M (Fig. 4). By considering only growth data in media that contain added copper ($10^{-8}$ to $10^{-7}$M) or added copper plus added EDTA, we eliminate much of the uncertainty in estimates of cupric ion activity resulting from uncertainties in ambient concentrations of copper. Reducing this source of uncertainty, however, does not change the overall patterns

of toxicity; we still observe copper toxicity at pCu values < 12.

We now readdress the question of whether natural levels of copper could affect the growth of *C. socialis* in upwelling seawater. Boyle and Edmond (1975) recently reported copper concentrations of $3 \times 10^{-9}$M in upwelling seawater south of New Zealand. In the absence of chelation we compute that this concentration would correspond to a cupric ion activity of about $10^{-10.3}$M, well within the activity range for copper inhibition of growth rate of *C. socialis*.

An intriguing factor is that unlike other trace metals such as Cd, Cu, Ni, and Zn whose concentrations increase with depth in the ocean, (Bruland, 1980), manganese concentrations usually decrease (Bender *et al.*, 1977; Klinkhammer and Bender, 1980). Higher concentrations of manganese in surface waters probably result from terrestrial inputs from runoff and aolian transport (Bender *et al.*, 1977). As a result of vertical distributional patterns newly upwelled seawater may often have relatively higher concentrations of copper (as well as other toxic trace metals) and lower concentrations of Mn than surface water which it displaces. As the water "ages" at the surface, the activity of cupric ion may tend to decrease due to biological scavenging. Boyle and Edmond (1975), for example, found that copper was enriched in upwelling seawater collected south of New Zealand, but recorded a three-fold decrease in copper concentrations along with a decrease in nitrate with increasing residence time at the surface. Concomitantly, the concentration of dissolved manganese in upwelled seawater may increase with time due to inputs from riverine and aolian sources, inputs from bottom sediments (in the case of coastal upwelling) or dilution with surface water containing higher manganese concentrations. As a result of such changes, phytoplankton growth could be stimulated due to an alleviation of manganese deficiency. We note that we need not invoke production of extracellular chelators to explain increases in the growth rate of algal cells in upwelled seawater as it "ages" at the surface (Barber and Ryther, 1969). However, if chelators are produced, they should also have a stimulatory effect similar to that of EDTA or NTA since natural organic ligands isolated from seawater bind copper by four to five orders of magnitude more strongly than they do Mn (II) (Mantoura *et al.*, 1978). Finally, in coastal upwelling areas such as occur along the eastern margins of the Pacific and South Atlantic, the proximity of land or reducing bottom sediments may be especially beneficial in supplying manganese to upwelling seawater and thereby alleviating manganese growth limitation.

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## REFERENCES

Anderson, D. M. and F. M. M. Morel. 1978. Copper sensitivity of *Gonyaulax tamarensis*. Limnol. Oceanogr., *23*, 283–295.

Anderson, M. A. and F. M. M. Morel. 1980. Uptake of Fe(II) by a diatom in oxic culture medium. Mar. Biol. Letters, *1*, 263–268.

Barber, R. T. 1973. Organic ligands and phytoplankton growth in nutrient-rich seawater, *in* Trace Metals and Metal Organic Interactions in Natural Waters, P. C. Singer ed. Ann Arbor Science Publishers, Ann Arbor, Mich., 321–338.

Barber, R. T., R. C. Dugdale, J. J. MacIsaac, and R. L. Smith. 1971. Variations in phytoplankton growth associated with the source and conditioning of upwelling water. Invest. Pesq., *25*, 171–193.

Barber, R. T. and J. H. Ryther. 1969. Organic chelators: Factors affecting primary production in the Cromwell Current upwelling. J. exp. Mar. Biol. Ecol., *3*, 191–199.

Bender, M. L., G. P. Klinkhammer, and D. W. Spencer. 1977. Manganese in seawater and the marine manganese balance. Deep-Sea Res., *24*, 799–812.

Blasco, D. 1971. Composition distribution of phytoplankton in the region of upwelling off the coast of Peru. Invest. Pesq., *36*, 61–112.

Boyle, E. and J. M. Edmond. 1975. Copper in surface waters south of New Zealand. Nature, *253*, 107–109.

Brewer, P. 1975. Minor elements in sea water, *in* Chemical Oceanography, 2nd ed., vol. 1, J. P. Riley and G. Skirrow, eds. Academic Press, London, 414–496.

Bruland, K. W. 1980. Oceanographic distributions of cadmium, zinc, nickel, and copper in the north Pacific. Earth Planetary Sci. Letters, *47*, 176–198.

Cheniae, G. M. and I. F. Martin. 1970. Studies on the function of manganese in photosynthesis, *in* Papers in Plant Physiology, W. S. Hillman, ed. Holt, Rinehart and Winston, New York, 245–256.

Diner, B. A. and P. Joliot. 1976. Oxygen evolution and manganese, *in* Photosynthesis, Pt. 1, A. Trebst and M. Avron, eds. Springer-Verlag, New York, 187–205.

Evans, D. W., N. H. Cutshall, F. A. Cross, and D. A. Wolfe. 1977. Manganese cycling in the Newport River estuary, North Carolina. Estuarine Coastal Mar. Sci., *5*, 71–80.

Eyster, C., T. E. Brown, and H. A. Tanner. 1956. Manganese requirement with respect to respiration and the Hill reaction in *Chlorella pyrenoidosa*. Arch. Biochem, Biophys., *64*, 240–241.

Eyster, C., T. E. Brown, H. A. Tanner, and S. L. Hood. 1958. Manganese requirement with respect to growth, Hill reaction and photosynthesis. Plant Physiol., *33*, 235–241.

Guillard, R. R. L. and J. H. Ryther. 1962. Studies of marine planktonic diatoms. I. *Cyclotella nana* Hustedt and *Detonula confervacea* (Cleve) Gran. Can. J. Microbiol., *8*, 229–238.

Habermann, H. M. 1969. Reversal of copper inhibition in chloroplast reactons by manganese. Plant Physiol., *44*, 331–336.

Harvey, H. W. 1947. Manganese and the growth of phytoplankton. J. Mar. Biol. Assoc. U.K., *26*, 562–579.

Hobson, L. A. 1971. Relationships between particulate organic carbon and micro-organisms in upwelling areas off southwest Africa. Invest. Pesq., *36*, 195–208.

Holliday, L. M. and P. S. Liss. 1976. The behaviour of dissolved iron, manganese and zinc in the Beaulieu Estuary, S. England. Estuarine Coastal Mar. Sci., *4*, 349–353.

Huntsman, S. A. and R. T. Barber. 1975. Modification of phytoplankton growth by excreted compounds in low density populations. J. Phycol., *11*, 10–13.

Jackson, G. A. and J. J. Morgan. 1978. Trace metal-chelator interactions and phytoplankton growth in seawater media: Theoretical analysis and comparison with reported observation. Limnol. Oceanogr., *23*, 268–282.

Johnston, R. 1963. Seawater, the natural medium for phytoplankton. I. General features. J.

Mar. Biol. Assoc. U.K., *43*, 427–456.

—— 1964. Seawater, the natural medium for phytoplankton. II. Trace metals and chelation, and general discussion. J. Mar. Biol. Assoc. U.K., *44*, 87–109.

Kanazawa, T. and K. Kanazawa. 1969. Specific inhibitory effect of copper on cellular division in *Chlorella*. Plant Cell Physiol., *10*, 495–502.

Klinkhammer, G. P. and M. L. Bender. 1980. The distribution of manganese in the Pacific Ocean. Earth Planetary Sci. Letters, *46*, 361-384.

Mantoura, R. F. C., A. Dickson, and J. P. Riley. 1978. The complexation of metals with humic material in natural waters. Estuarine Coastal Mar. Sci., *6*, 387–408.

McBrien, D. C. H. and K. A. Hassall. 1965. Loss of cell potassium by *Chlorella vulgaris* after contact with toxic amounts of copper sulphate. Physiol. Plant, *18*, 1059–1065.

—— 1967. The effect of toxic doses of copper upon respiration, photosynthesis and growth in *Chlorella vulgaris*. Physiol. Plant., *20*, 113–117.

McKnight, D. M. 1979. Interactions between fresh water plankton and copper speciation. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge. 283 pp.

Moore, R. M. and J. D. Burton. 1976. Concentrations of dissolved copper in the eastern Atlantic Ocean 23°N to 47°N. Nature, *264*, 241–243.

Morgan, J. J. 1967. Chemical equilibria and kinetic properties of manganese in natural waters, *in* Principles and Application of Water Chemistry, S. D. Faust and J. V. Hunter, eds. Wiley, New York, 561–620.

Morgan, J. J. and W. Stumm. 1965. Analytical chemistry of aqueous manganese. J. Am. Waterworks Assoc., *57*, 107–119.

O'Kelley, J. C. 1974. Inorganic nutrients, *in* Algal Physiology and Biochemistry, W. D. P. Stewart, ed. University of California Press, Berkeley, 610–635.

Passow, H., A. Rothstein, and T. W. Clarkson. 1961. The general pharmacology of the heavy metals. Pharmacol. Rev., *13*, 185–224.

Ryther, J. H., D. W. Menzel, E. M. Hulburt, C. J. Lorenzen, and N. Corwin. 1971. The production and utilization of organic matter in the Peru coastal current. Invest. Pesq., *36*, 43–59.

Schindler, P. W. 1975. Removal of trace metals from the oceans: A zero-order model. Thalassia Jugosl., *11*, 101–111.

Sillen, L. G. and A. E. Martell. 1964. Stability constants of metal-ion complexes. Chem. Soc. Spec. Publ., *17*, 754 pp.

Steemann Nielsen, E. and Wium-Andersen, S. 1970. Copper ions as poison in the sea and in freshwater. Mar. Biol., *6*, 93–97.

Sunda, W. G. 1975. Relationship between cupric ion activity and the toxicity of copper to phytoplankton. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge. 168 pp.

Sunda, W. G. and P. A. Gillespie. 1979. The response of a marine bacterium to cupric ion and its use to estimate cupric ion activity in seawater. J. Mar. Res., *37*, 761–777.

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.

Swallow, K. C., D. N. Hume, and F. M. M. Morel. 1980. Sorption of copper and lead by hydrous ferric oxide. Environ. Sci. Technol., *14*, 1326–1331.

Thomas, W. H. and D. L. R. Seibert. 1977. Effects of copper on the dominance and the diversity of algae: Controlled ecosystem pollution experiment. Bull. Mar. Sci., *27*, 23–33.

Vuceta, J. and J. J. Morgan. 1978. Chemical modeling of trace metals in fresh waters: Role of complexation and adsorption. Environ. Sci. Technol., *12*, 1303–1309.

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