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Toxic binding of cupric ion by marine phytoplankton

by Jerome Gavis[1]

ABSTRACT

The data presented by Gavis *et al.* (1981, J. Mar. Res., *39,* 315–333) for the specific growth rate of phytoplankton in nutrient replete medium as a function of cupric ion activity can be described quantitatively by equations that relate the equilibrium binding of cupric ions to a cell receptor site. Statistically significant conditional binding constants could be estimated from the data for at least eight clones among three species. One clone could bind only one cupric ion per site. The others could bind a maximum of two cupric ions per site. For one of these clones $K_1 > K_2$. For the remaining six $K_2 > K_1$. For three clones K_2 was so much larger than K_1, that only their product β_2 could be determined. Values of K_1 ranged between 8.6×10^8 and 5.7×10^{10}, values of K_2 ranged between 3.1×10^8 and 1.9×10^{10}, while those of β_2 ranged between 3.5×10^{16} and 3.8×10^{20}.

1. Introduction

The toxicity of heavy metal ions in solution to algae is well known. Even at sub-lethal activities heavy metal ions can influence the number and kinds of algae in an aquatic ecosystem and, therefore, its ecological balance. This has led to extensive investigation of the interaction between algae, especially phytoplankton, and heavy metal ions in solution. The mechanism by which they affect phytoplankton is not yet well understood, however.

One of the most significant advances in knowledge about the effect of heavy metal ions on phytoplankton was the realization that toxicity is a function of free metal ion activity, regardless of the total metal concentration (Spencer, 1957; Johnston, 1964; Steemann-Nielsen and Wium-Anderson, 1970; Sunda and Guillard, 1976; Jackson and Morgan, 1978). Moreover, several investigators (Sunda and Guillard, 1976; Anderson and Morel, 1978; Reuter and McCarthy, 1979) have shown that cupric ions, at least, are sub-lethally toxic to some phytoplankton species at activities as low as 10^{-11}-10^{-10} M. Recently, Gavis *et al.* (1981) showed that the specific growth rates of 13 clones among 11 species of marine phytoplankton in nutrient replete media decreased as the cupric ion activity increased

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beyond a threshold value between 10^{-11} and 10^{-10} M. The specific growth rates of seven clones decreased at thresholds between 10^{-10} and 10^{-9} M, while that of one clone decreased at a threshold smaller than 10^{-11} M. Three clones were almost completely unaffected by cupric ion at activities as high as 3×10^{-9} M.

When specific growth rates of those clones exhibiting toxicity to cupric ions were plotted against pCu (minus the logarithm of cupric ion activity), the data points fell along curves that resembled titration curves or, more correctly, formation curves for the binding of cupric ion to cell receptor sites that can regulate specific growth rate.

The purpose of this paper is to demonstrate that the specific growth rate-pCu data obtained by Gavis *et al.* (1981) for at least eight clones among three species can be described quantitatively by equations that relate the equilibrium binding of either one or two cupric ions to a cell receptor site. Moreover, statistically significant binding equilibrium constants can be estimated from the data. The values of the constants are not sufficient to specify the nature of the binding sites, however.

2. Cupric ion binding and growth rate

When a single cupric ion binds to a cell receptor site, —S, according to the reaction (in which charge is omitted)

$$—S + Cu = —SCu \tag{1}$$

the average number of cupric ions bound per site, $\bar{n}_1$, is a function of the cupric ion activity, {Cu}, given by

$$\bar{n}_1 = \frac{S_T - S_O}{S_T} = \frac{K_1\{Cu\}}{1 + K_1\{Cu\}} \tag{2}$$

where S_T and S_O are the total number of sites and the number of unbound sites, respectively, and K_1 is the equilibrium binding constant for the reaction.

When a second cupric ion is able to bind to a site, according to the reaction

$$—SCu + Cu = —SCu_2 \tag{3}$$

the average number of cupric ions bound per site, $\bar{n}_2$, becomes

$$\bar{n}_2 = \frac{K_1\{Cu\} + 2K_1K_2\{Cu\}^2}{1 + K_1\{Cu\} + K_1K_2\{Cu\}^2} \tag{4}$$

where K_2 is the equilibrium binding constant for reaction (3)[2].
In the event $K_2 >> K_1$ equation (4) reduces to

$$\bar{n}_2 = \frac{2\beta_2\{Cu\}^2}{1 + \beta_2\{Cu\}^2} \tag{5}$$

2. For a derivation of equations (2) and (4) see, for example, Tanford (1961; Ch. 8)

where β_2, the product of K_1 and K_2, is the equilibrium binding constant of the combined reaction

$$—S + 2\mathrm{Cu} = —S\mathrm{Cu}_2 . \tag{6}$$

It is not possible to distinguish K_1 and K_2 individually in this case.

When no copper is bound by the sites at very low {Cu}, $\bar{n}_1$ or $\bar{n}_2 = 0$, and the cell grows at its maximum specific growth rate, μ_m. As $\bar{n}_1$ or $\bar{n}_2$ increases, the specific growth rate, μ, decreases. The specific growth rate may remain greater than zero at $\bar{n}_1 = 1$ or $\bar{n}_2 = 2$; it may reach zero at $\bar{n}_1 = 1$ or $\bar{n}_2 = 2$; or it may reach zero at smaller $\bar{n}_1$ or $\bar{n}_2$.

If it is assumed that the relationship between μ and $\bar{n}_1$ or $\bar{n}_2$ is linear, then

$$\frac{\mu_m - \mu}{\mu_m - \mu_{\bar{n}}} = \frac{0 - \bar{n}_1}{0 - 1} = \bar{n}_1 \tag{7}$$

and

$$\frac{\mu_m - \mu}{\mu_m - \mu_{\bar{n}}} = \frac{0 - \bar{n}_2}{0 - 2} = \frac{\bar{n}_2}{2} \tag{8}$$

where $\mu_{\bar{n}}$ is a specific growth rate at $\bar{n}_1 = 1$ or $\bar{n}_2 = 2$, positive when $\mu > 0$ at $\bar{n}_1 = 1$ or $\bar{n}_2 = 2$, negative when $\mu = 0$ at $\bar{n}_1 < 1$ or $\bar{n}_2 < 2$, and zero when $\mu = 0$ at $\bar{n}_1 = 1$ or $\bar{n}_2 = 2$.[3]

Equations (7) and (8) may be rearranged to give

$$\mu = \mu_m - (\mu_m - \mu_{\bar{n}})\bar{n}_1 \tag{9}$$

$$\mu = \mu_m - (\mu_m - \mu_{\bar{n}})\bar{n}_2/2 . \tag{10}$$

Combination of equations (9) and (2) or of equations (10) and (4) or (5) provides the relationships that should describe the observed growth rates as a function of {Cu}, or, alternatively, of pCu. If μ_m is known, the methods of nonlinear parameter estimation may be used to test the fit of equations (9) or (10) to the observed data, with $\mu_{\bar{n}}$ and the constants K_1 and K_2, or β_2 determined as parameters. The equations represent the data if the estimates of the parameters are statistically significant.

3. Application to data

When equations (9) and (10) were tested against the data of Gavis *et al.* (1981) by means of the nonlinear parameter estimation routine, LSQ, of Time Series Processor, version 2.7 (Hall, 1975), available at the Johns Hopkins University Computing Center, significant estimates of the parameters and coefficients of de-

3. When $\mu = 0$ at $\bar{n}_1 < 1$ or $\bar{n}_2 < 2$, $\mu_{\bar{n}}$ is an apparent specific growth rate at $\bar{n}_1 = 1$ or $\bar{n}_2 = 2$. In any case $\mu_{\bar{n}}$ can be related to the average number of cupric ions bound per site, $\bar{n}_{\mu=0}$, at $\mu = 0$ by the equation $\bar{n}_{\mu=0} = A\mu_m/(\mu_m - \mu_{\bar{n}})$, where $A = 1$ or 2, depending upon whether 1 or 2 cupric ions can bind per site.

Table 1. Experimentally determined specific growth rates of eight phytoplankton clones as a function of pCu.

Species	Specific growth rate*† at pCu =														
(Clone)	12.31	12.03	11.79	11.55	11.21	10.89	10.71	10.38	10.13	9.80	9.58	9.25	9.03	8.70	8.50
B. polymorpha (Say 7)	1.48	1.46	1.51	1.40	1.46	1.42	1.39	1.30	1.24	0.91	0.72	0.40	–0–	—	—
T. pseudonana (3H)	1.96	1.90	1.89	1.85	1.72	1.46	1.26	0.90	0.88	0.69	0.65	0.62	0.48	0.29	–0–
T. pseudonana (FCRG 66)	1.57	(1.48)	1.58	1.57	1.58	1.58	1.51	1.41	1.29	0.88	0.58	0.28	0.19	–0–	—
T. pseudonana (W)	2.00	1.99	1.98	1.99	2.07	1.85	1.71	1.18	0.90	–0–	—	—	—	—	—
S. tropicum (A629)	2.07	2.18	2.10	2.06	2.03	2.15	(1.89)	2.16	2.01	1.77	1.47	0.90	0.40	–0–	—
T. pseudonana (C5)	1.53	1.54	1.47	1.46	1.52	1.49	1.51	1.46	1.36	0.78	0.38	0.17	–0–	—	—
T. pseudonana (7-15)	1.42	1.52	1.55	1.49	1.49	1.46	1.39	0.89	0.52	–0–	—	—	—	—	—
T. pseudonana (13-1)	1.65	1.64	1.63	1.61	1.56	1.59	1.35	0.95	0.41	–0–	—	—	—	—	—

* Values in parentheses were omitted in estimation calculations
† Units for specific growth rate-day^{-1}

Table 2. Estimated values of binding constants, $\mu_{\bar{n}}$, and their standard deviations for eight clones of phytoplankton.

Species (Clone)	μ_m*	K_1 (σ_{K_1})	K_2 (σ_{K_2})	β_2 (σ_{β_2})	$\mu_{\bar{n}}$* ($\sigma_{\mu_{\bar{n}}}$)	R^2
B. polymorpha (Say 7)	1.48	1.90×10^9 (0.28×10^9)			−0.75 (0.16)	0.992
T. pseudonana (3H)	2.00	5.70×10^{10} (0.84×10^{10})	3.07×10^8 (0.70×10^8)		−0.74 (0.13)	0.992
T. pseudonana (FCRG 66)	1.58	3.88×10^9 (0.68×10^9)	7.06×10^9 (1.66×10^9)		–0– —	0.998
T. pseudonana (W)	2.00	7.25×10^9 (2.13×10^9)	1.89×10^{10} (1.62×10^{10})		−0.76 (0.45)	0.990
S. tropicum (A629)	2.10	8.62×10^8 (3.55×10^8)	4.26×10^9 (2.31×10^9)		−0.25 (0.12)	0.995
T. pseudonana (C5)	1.53			3.53×10^{19} (0.39×10^{19})	–0– —	0.994
T. pseudonana (7-15)	1.52			2.93×10^{20} (0.37×10^{20})	−0.20 (0.07)	0.993
T. pseudonana (13-1)	1.64			3.84×10^{20} (0.37×10^{20})	−0.18 (0.05)	0.996

* Units for μ_m and $\mu_{\bar{n}}$-day^{-1}

termination, R^2, greater than 0.99 were obtained for eight of the clones they investigated under intermittent illumination. The results for these clones are described and illustrated here. Less significant estimates of the parameters and smaller coefficients of determination were obtained for other clones either because there were too few (μ-pCu) data pairs over the interval in which growth rate changed as a function of pCu (four clones) or there was too much scatter (nine clones) among the data pairs. The data for *M. salina* (Say 2), which was not affected by cupric ions and those for two clones whose growth rates decreased only slightly at low pCu could not be fit by the equations at all. The three clones Gavis *et al.* studied under both intermittent and continuous illumination yielded highly significant fit only under intermittent illumination.

Specific growth rates of the eight clones are listed as a function of pCu in Table 1. Maximum observed growth rates, μ_m, estimates of the constants K_1, K_2, β_2, and of the specific growth rates, $\mu_{\bar{n}}$, are listed in Table 2, along with R^2.

The estimated values of $\mu_{\bar{n}}$ were zero for two clones. These ceased growing only when $\bar{n}_2 = 2$ and the receptor sites were saturated. The remaining clones, for which $\mu_{\bar{n}}$ was negative, ceased to grow before saturation was reached, when $\bar{n}_1 < 1$ or $\bar{n}_2 < 2$.

No clone could grow at a measurable rate after saturation was reached, for which $\mu_{\bar{n}}$ would have been positive.

The (μ-pCu) data pairs for *B. polymorpha* (Say 7), illustrated in Figure 1, were best fit by equation (9) with $\bar{n}_1$ expressed by equation (2) for maximum binding of one cupric ion per site. The curve shown is that of equation (9) with μ_m, and the estimated values of K_1 and $\mu_{\bar{n}}$ given in Table 2.

The data pairs for *T. pseudonana* (3H), (FCRG66), (W), and *S. tropicum* (A629) were best fit by equation (10), with $\bar{n}_2$ expressed by equation (4) for binding of up to two cupric ions per site. These are illustrated in Figures 2-4. The curves shown are those corresponding to equation (10), calculated with the values of μ_m, K_1, K_2, and $\mu_{\bar{n}}$ listed in Table 2. Because $K_1 >> K_2$, the curve for *T. pseudonana* (3H), Figure 2, has three inflection points. For *T. pseudonana* (FCRG66), Figure 3, *T. pseudonana* (W), Figure 4, curve A, and *S. tropicum* (A629), Figure 4, curve B, K_2 is somewhat larger than K_1. As a result the inflection points coalesce into one.

The data pairs for *T. pseudonana* (C5), (7-15), and (13-1) could only be fit by equation (10) with $\bar{n}_2$ expressed by equation (5). For these clones $K_2 >> K_1$ so that only their product, β_2, could be estimated. Figure 5 a, b, c illustrates the data pairs and the curves corresponding to equation (10), calculated with the values of μ_m, β_2, and $\mu_{\bar{n}}$ listed in Table 2.

4. Discussion

It is immediately evident that a two-binding constant equation, like (10), is needed to represent the three-inflection point plot of the (μ-pCu) data pairs of *T. pseudonana* (3H), Figure 2. On the other hand, both equations (9) and (10) are able to represent the one-inflection point plots of the (μ-pCu) data pairs of the other seven clones. The estimated parameters listed in Table 2 and the curves drawn in Figure 1 and Figures 3-5 are those corresponding to whichever equation best fit the data. For example, application of equation (9), alternative to equation (10), to the data for *T. pseudonana* (FCRG66) is illustrated by the dashed curve in Figure 3, with $K_1 = 2.72 \times 10^{10}$ and $\bar{n} = -0.37$ day^{-1}. Even visually, the conclusion is that equation (10) provides a better fit to the data than does equation (9).

The smallest estimated value of the individual binding constants K_1 and K_2 listed in Table 2 is 3×10^8. This is considerably larger than the known binding constants for copper complexes of a large number of simple ligands, as can be ascertained by comparison with available tabulations of binding constants (Martell and Smith, 1974, 1977; Smith and Martell, 1975, 1976). Evidently the cell receptor sites are multiligand, or chelating, sites. There is sufficient difference among the binding constants that sites common to two or more clones even within the same species cannot be identified. It is possible, however, that otherwise identical receptor sites in different clones are influenced by the proximity of different substituent groups

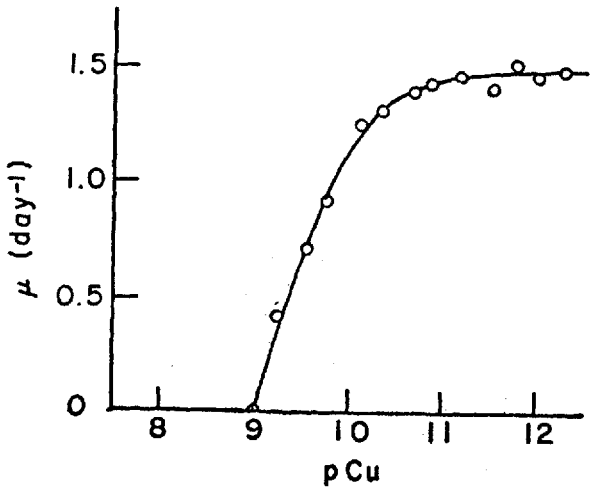

Figure 1. Specific growth rate of *B. polymorpha* (Say 7) as a function of pCu. Curve drawn corresponds to equation (9), with $\mu_m = 1.48$ day^{-1}, $\mu_n = -0.75$ day^{-1}, $K_1 = 1.90 \times 10^9$.

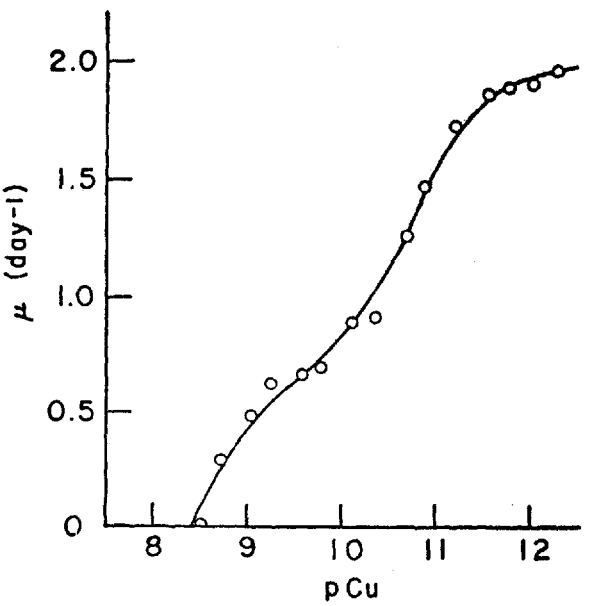

Figure 2. Specific growth rate of *T. pseudonana* (3H) as a function of *pCu*. Curve drawn corresponds to equation (10), with $\mu_m = 2.00$ day^{-1}, $\mu_n = -0.74$ day^{-1}, $K_1 = 5.70 \times 10^{10}$, $K_2 = 3.07 \times 10^8$.

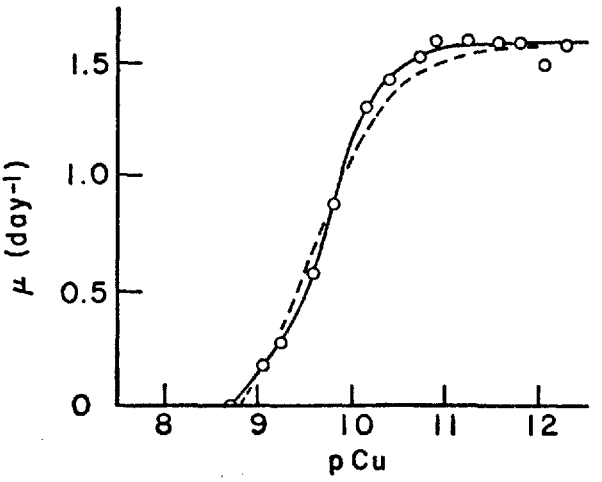

Figure 3. Specific growth rate of *T. pseudonana* (FCRG66) as a function of pCu. Solid curve corresponds to equation (10), with $\mu_m = 1.58$ day^{-1}, $\mu_{\tilde{n}} = 0$, $K_1 = 3.88 \times 10^9$, $K_2 = 7.06 \times 10^9$. Dashed curve corresponds to equation (9), with $\mu_m = 1.58$ day^{-1}, $\mu_{\tilde{n}} = -0.37$ day^{-1}, $K_1 = 2.72 \times 10^{10}$.

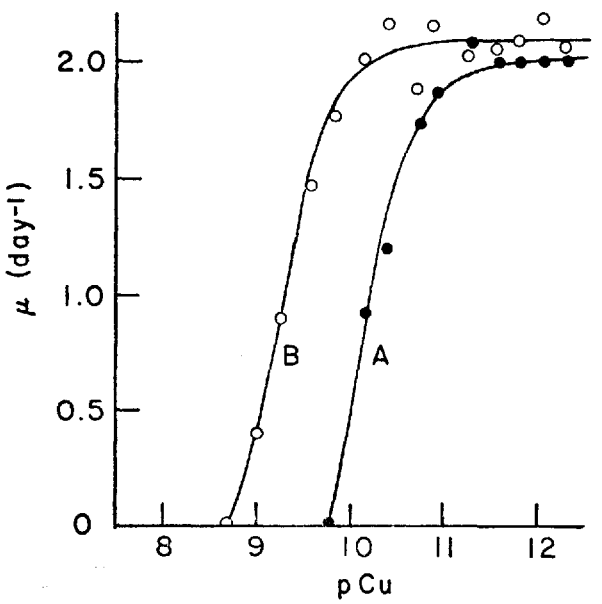

Figure 4. Specific growth rates of *T. pseudonana* (W), curve A, and *S. tropicum* (A629), curve B, against pCu. Curve A corresponds to equation (10), with $\mu_m = 2.00$ day^{-1}, $\mu_{\tilde{n}} = -0.76$ day^{-1}, $K_1 = 7.25 \times 10^9$, $K_2 = 1.89 \times 10^{10}$. Curve B corresponds to equation (10), with $\mu_m = 2.10$ day^{-1}, $\mu_{\tilde{n}} = -0.25$ day^{-1}, $K_1 = 8.62 \times 10^9$, $K_2 = 4.26 \times 10^9$.

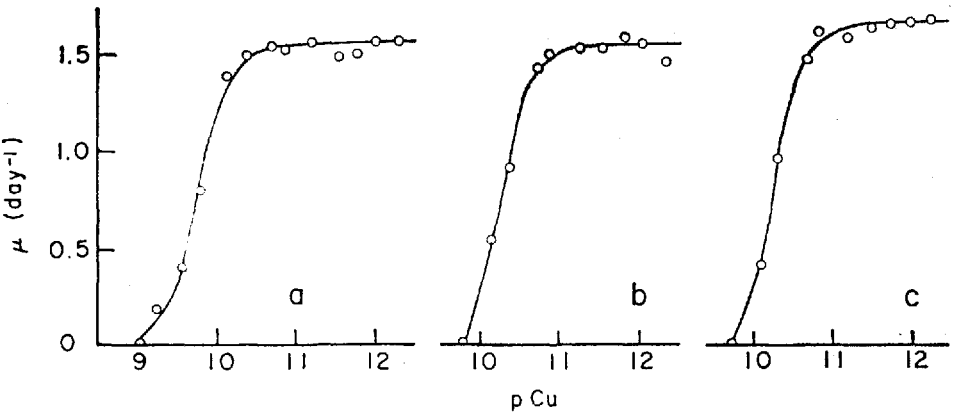

Figure 5. Specific growth rates of *T. pseudonana* (C5), (7-15), and (13-1), respectively, against pCu. (a) Curve drawn corresponds to equation (10), with $\mu_m = 1.53$ day^{-1}, $\mu_{\hat{n}} = 0$, $\beta_2 = 3.53 \times 10^{19}$. (b) Curve drawn corresponds to equation (10), with $\mu_m = 1.52$ day^{-1}, $\mu_{\hat{n}} = -0.20$ day^{-1}, $\beta_2 = 2.93 \times 10^{20}$. (c) Curve drawn corresponds to equation (10), with $\mu_m = 1.64$ day^{-1}, $\mu_{\hat{n}} = -0.18$ day^{-1}, $\beta_2 = 3.84 \times 10^{20}$.

that do not themselves bind cupric ions, and only appear to be different. Fisher and Jones (1981), in fact, suggested that the receptor sites are sulfhydryl groups, which exist in different arrangements and, therefore, have different binding constants for metal ions, in different proteins.

As a cation cupric ion must compete with protons for cell receptor sites. The proton binding ability of the sites is not known. Therefore, the binding constants must be considered conditional constants, valid at the pH of the medium, 8.1, in the experiments of Gavis *et al.* (1981).

The constant for the second cupric ion bound, K_2, is larger than that for the first, K_1, for six clones. In fact, K_2 is so much larger than K_1 for three clones that only their product, β_2, could be estimated. This implies that the first cupric ion increases the affinity of the site for the second. While unusual, this phenomenon has been observed elsewhere in biological systems. For example, Breslow and Girotti (1970) showed that the binding of the first cupric ion increased the affinity of the 3′-cytidilic acid-ribonuclease complex for a second cupric ion by a factor of at least 45 relative to the first. Cupric ion, perhaps because it carries two positive charges, may cause conformational change in cell macromolecules that bind copper and can control specific growth rate. As the charge contributes to, or is the cause of, the decrease in specific growth rate, it may also enhance the binding of the second cupric ion in those clones for which $K_2 > K_1$.

Sunda *et al.* (1981) also observed two-cupric ion receptor sites that affected growth rate of *Chaetoceros socialis*. They determined a value of 4×10^{17} for the constant equivalent to β_2 from their data. Their experiments led them to attribute

the toxic effect of cupric ion at least partly to competition with manganese, an essential trace element. Because the manganese concentration in the experiments reported by Gavis *et al.* (1981) was 10^{-6} M (f/2 medium), the curves shown in Figures 1-5 here may be compared with those at high manganese ion activities illustrated by Sunda *et al.* (1981; Fig. 4).

These results do not determine the nature of the receptor sites, nor how the molecules of which they are part contribute to the cellular processes that control the specific growth rate. The results leave other questions unanswered as well. Do most phytoplankton respond to cupric ion in the manner described here? How does the response to cupric ion depend upon pH in the medium? Do other metal ions like cadmium, nickel, zinc that are toxic to phytoplankton act in the same manner as cupric ion? The answers must be left for the future.

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