Reports

Micronutrients and Kelp Cultures: Evidence for Cobalt and Manganese Deficiency in Southern California Deep Seawater

Abstract. *It has been suggested that naturally occurring copper and zinc concentrations in deep seawater are toxic to marine organisms when the free ion forms are overabundant. The effects of micronutrients on the growth of gametophytes of the ecologically and commercially significant giant kelp (*Macrocystis pyrifera*) were studied in defined media. The results indicate that toxic copper and zinc ion concentrations as well as cobalt and manganese deficiencies may be among the factors controlling the growth of marine organisms in nature.*

Trace elements play significant roles in plant and animal metabolism (*1–3*). Zinc and copper are cofactors in numerous enzyme reactions. Manganese is required for photosynthetic electron transfer. Cobalt is essential for vitamin B_{12} synthesis. Zinc, manganese, copper, and cobalt are among the micronutrients required for production of giant kelp (*Macrocystis pyrifera*) sporophytes from zoospores (*4*).

Excessively high trace element concentrations have inhibitory effects on aquatic organisms. Use of nutrient-rich deep seawater in mariculture studies has resulted in limited success. Spencer (*5*) and Davey *et al.* (*6*) reported that Cu was toxic to phytoplankton in natural seawater and the addition of chelators reduced Cu toxicity. Sunda and Guillard (*7*) ascribed this toxicity reduction to decreased free Cu ion activity. Because of the inhibitory effects of Cu observed in laboratory studies, Cu ions have been referred to as "the inherent toxic factor in natural deep ocean seawater" (*8*).

Micromolar total Zn concentrations have also produced adverse effects on growth and behavior of freshwater and marine organisms (*9–11*). Naturally occurring Zn free ion concentrations may control phytoplankton productivity (*12*).

Trace element studies of *Macrocystis* gametophytes were motivated by an enormous variation in gametophyte development observed in preliminary experiments with unenriched surface water from Monterey Bay, California (MBSW), and with a 1:1 mixture of surface water and 300-m seawater collected 3 miles off Newport Bay, California (NBMW). Gametophytes cultured in MBSW developed rapidly. *Macrocystis* sporophytes were produced from released zoospores within 14 days in culture (Fig. 1A). In contrast, cultures in NBMW showed little or no gametophytic development after 14 days in culture (Fig. 1B). Chemical analyses of these two media indicated that the disparity in developmental rates could not be attributed to differences in NH_4^+, NO_3^- + NO_2^-, or PO_4^{3-} (Table 1). However, differences in trace element concentration (total Fe, Zn, Mn, Cu, and Co) as high as an order of magnitude were observed between the two media (Table 1).

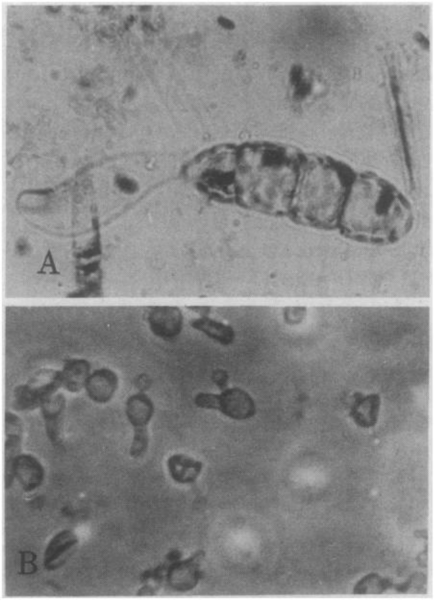

Fig. 1. Light photomicrographs (×400) of *Macrocystis* gametophytes in seawater media. (A) Early sporophytic development observed 2 weeks after spore release in a culture with MBSW. (B) Gametophytes cultured in NBMW 2 weeks after spore release (development scarcely beyond spore germination).

In the experiments described here we examined primary (single variable) and interactive effects of Zn, Mn, Cu, and Co on *Macrocystis* gametophytic growth in culture. The results suggest a deficiency of Co and Mn in southern California deep seawater in addition to growth inhibition by Cu and Zn.

Macrocystis gametophytes were cultured by using ultraclean techniques (*4*) in Aquil, a chemically defined artificial seawater medium (*13*). Chemical equilibrium program REDEQL2 (*14*) was used to compute chemical speciation of media constituents. Three concentrations of each micronutrient (Zn, Mn, Cu, and Co) were used. The variables x_{Zn}, x_{Mn}, x_{Cu}, and x_{Co} were used to represent normalized total Zn, Mn, Cu, and Co concentrations, respectively ($|x_{Me}| \leq 1$, where Me represents one of the four metals) (*15*). Total concentrations of these micronutrients were placed on the same scale by this transformation so that Zn, Mn, Cu, and Co effects were directly comparable from the least squares fit of the culturing data (Eq. 1 below) (*16*). The transformation equation was $x_{Me} = (1/HR_{Me})(Me_T - \overline{Me})$, where Me_T is the total concentration of metal Me, $\overline{Me}$ is the middle concentration of the three used for that metal ($x_{Me} = 0$ when $Me_T = \overline{Me}$), and HR_{Me} is the difference between the high and middle (or low and middle) concentrations (that is, half the concentration range for that metal). The values of HR_{Me} for Zn, Mn, Cu, and Co were 155, 20, 15, and 50 n*M*, respectively; $\overline{Me}$ values were 160, 25, 15, and 55 n*M*, respectively (Table 1).

To reduce the number of experimental treatments, a fractional factorial experimental design was employed (*16, 17*). This enabled first- and second-order primary and interactive effects to be estimated with 27 instead of 81 (3^4) treatments. After 12 days in culture at 14°C, lengths of 40 randomly selected gametophytes from each of the 27 treatments were measured. Aside from the four micronutrient variables, Aquil nutrient concentrations were fixed (*4*) (Table 1). EDTA was added to Aquil to prevent ferric hydroxide precipitation and to control micronutrient chemical speciation (*4*). Table 1 shows that trace metal speciation in Aquil was dominated by EDTA complexation. Because of the abundance of EDTA, free metal ion concentrations were directly proportional to total metal concentrations in Aquil. Thus micronutrient effects could be examined in terms of total or free ion concentrations.

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Table 1. Computed chemical speciation for nine inorganic nutrients and for EDTA in the Aquil formulation (A) and MBSW (assuming no adsorption). Analytical concentrations in MBSW and NBMW were reported by North (*40*). Free ion concentrations in MBSW were computed under the assumption that concentrations of organics in MBSW could be represented by an equivalent EDTA concentration (~ 30 n*M*) required to produce 20 percent Cu-organic ligand complexation (*42*).

Nutrient or compound	Analytical concentration (n*M*)			Computed free ion concentration (n*M*)		Major species (nearest percent)	
	A	MBSW	NBMW	A	MBSW	A	MBSW
Metals							
Fe^{3+}	350	72	18	7×10^{-11}	2×10^{-10}	FeEDTA (100)	$Fe(OH)_3(s)$* (99)
Zn^{2+}	160	61	34	8×10^{-2}	6	ZnEDTA (100)	$ZnCl^+$ (67)
Mn^{2+}	25	22	2	3	7	MnEDTA (65)	$MnCl^+$ (64)
Cu^{2+}	15	16	31	5×10^{-5}	8×10^{-3}	CuEDTA (99)	$Cu(BOH_4)_2$ (68)
Co^{2+}	55	30	2	5×10^{-2}	4	CuEDTA (99)	$CoCl^+$ (67)
Ligands							
MoO_4^{2-}	100	N.R.†	N.R.	100	N.R.	Free ion (100)	N.R.
$EDTA^{4-}$	6.0×10^3	N.R.	N.R.	7×10^{-5}	N.R.	CaEDTA (89)	N.R.
$NO_3^- + NO_2^-$	20×10^3	6×10^3 $(0.3 \times 10^3$ as $NH_4)$	13×10^3 $(1.1 \times 10^3$ as $NH_4)$	2×10^4	13×10^3	Free ion (100) HPO_4^{2-} (51)	Free ion (100) HPO_4^{2-} (51)
PO_4^{3-}	2.0×10^3	1.1×10^3	1.3×10^3	0.3	0.1	$MgHPO_4$ (47)	$MgHPO_4$ (47)
I^-	100	N.R.		100	N.R.	Free ion (100)	N.R.

*(s), solid. †N.R., not reported.

A least squares fit of gametophytic growth data in the defined medium (*17*) generated the expression

$$Y = 136 + 8x_{Mn} - 5x_{Cu} + 7x_{Co} - 7x_{Zn}x_{Cu} - 15x_{Zn}^2 - 27x_{Mn}^2 - 12x_{Cu}^2 - 18x_{Co}^2 - 6x_{Cu}x_{Zn}^2 - 6x_{Cu}x_{Mn}^2 \qquad (1)$$

where Y is mean gametophytic length in micrometers. Analysis of variance indicated that the ten effects represented in Eq. 1 were significant at the 99.9 percent confidence level by the F test ($f_1 = 1$, $f_2 = 1069$). Equation 1 indicated optimal total concentrations of 169, 28, 12, and 65 n*M* for Zn, Mn, Cu, and Co, respectively. Predicted maximum length under these conditions (138 μm) compares favorably with the previously determined optimal length (139 μm) (*4, 15*). The fit of the experimental data to Eq. 1 was considered excellent. Lengths determined by Eq. 1 were within 10 percent of observed mean lengths for all 27 treatments. Only at the micronutrient concentrations nearest optimal among the 27 treatments (that is, $x_{Me} = 0$ for all four metals; Table 1) were embryonic sporophytes produced from released zoospores within 14 days.

Equation 1 was used to compare the results of a later culturing experiment to gametophytic length data predicted for that experiment (*17*). Variations between predicted and observed mean gametophytic lengths ranged from 2 to 13 percent, with six of nine length predictions within 10 percent of the observed mean lengths.

Certain plants can use complexes between Fe and organic or inorganic ligands (*18*). If *Macrocystis* can readily use complexed forms of Fe, Zn, Mn, Cu, and Co, it may be inferred that MBSW was deficient in Fe, Zn, and Co for optimal gametophytic growth in the defined medium (Table 1). The rapid growth of *Macrocystis* in MBSW (Fig. 1A) contradicts this. However, trace metals in free ionic form (Cd^{2+}, Cu^{2+}, Pb^{2+}, and Zn^{2+}) are apparently more readily assimilated by many organisms than are complexed forms (*7, 19–25*). If this is the case for *Macrocystis*, then only Mn and possibly Fe in MBSW were close to concentrations suited for gametophytic culturing (Table 1). The high computed free ion concentrations of Zn, Mn, Cu, and Co in MBSW relative to the optimal Aquil formulation indicate that plants cultured in this medium should have been strongly inhibited. The Zn, Mn, Cu, and Co free ion concentrations were apparently 70, 2, 300, and 60 times too great, respectively. To achieve the optimal values, the free ion concentrations of Zn, Mn, Cu, and Co in MBSW should be decreased by 98.6, 50.0, 99.7, and 98.3 percent, respectively [for example, $98.6 = 100(1 - 1/70)$].

Amorphous ferric hydroxide as well as other inorganic particulates and organic detritus remove trace elements from solution by sorption. Grimme (*26*) and Balistrieri and Murray (*27*) observed relative trace metal adsorption efficiencies on goethite (α-FeOOH) that correspond to relative overabundances in MBSW (in descending order: Cu, Zn, Co, and Mn), suggesting adsorption on iron hydroxide as a mechanism for the required free metal ion concentration decrease in MBSW.

Total concentrations of Cu, Zn, Co, Mn, and Fe in NBMW during the culturing period were 31, 34, 2, 2, and 18 n*M*, respectively. Assuming that the amount of adsorbed micronutrients increases linearly with total Fe in natural seawater—that is, with increased adsorbing surface—NBMW (18 n*M* total Fe) would have roughly 25 percent of the capacity of MBSW (72 n*M* total Fe) to remove micronutrients from solution; that is, removal from NBMW would amount to 4 n*M* Cu (0.25 × 0.997 × total Cu in MBSW), 15 n*M* Zn, 7 n*M* Co, and 3 n*M* Mn. This would leave NBMW with 27 n*M* total dissolved Cu (0.01 n*M* as free ion) and 19 n*M* total dissolved Zn (1 n*M* as free ion), but virtually depleted in dissolved Co and Mn. A comparison of the Cu and Zn free ion concentrations in NBMW with those in the optimal Aquil formulations indicated that Cu and Zn were overabundant by factors of 250 and 11, respectively.

Free ion concentrations may be decreased in MBSW by other mechanisms. Even if all the Fe (72 n*M*) as amorphous ferric hydroxide were site-filled with these trace nutrients, calculations based on data of Davis and Leckie (*28*) indicate that only 54 percent of the required 116 n*M* decrease in total dissolved Zn, Mn, Cu, and Co can be accounted for by this mechanism (*17*).

Note that the order of MBSW overabundance (Cu > Zn > Co > Mn) corresponds to the Irving-Williams order of stabilities of chelates formed by metal ions with ligands (Hg > Cu > Ni ≈ Zn > Co > Mn ≈ Cd > Ca > Mg). Although trace metal complexation with dissolved humic substances may be significant in freshwaters with a dissolved organic carbon (DOC) content ≥10 mg/liter (*29–32*), Mantoura *et al.* (*29*) and Kremling *et al.* (*33*) found that metal chelation with dissolved humic materials in seawater, with a typical DOC content of ~ 1 mg/liter (*34*), was appreciable only for Cu (3 to 10 percent total Cu chelated). Because MBSW speciation was computed on the basis of an estimated 20 percent complexation for EDTA (Table 1), a much stronger metal chelator than natural organic ligands (*29, 35*), it is unlikely that complexation to dissolved organics is an important mechanism in reducing free ion concentrations in MBSW. Work by Balistrieri *et al.* (*36*) and Hunter (*37*) indicated that metals may be scavenged by particulates coated by a film of surface-active organic matter. If the surface chemistry of such coated particles controls the adsorption properties of marine particulates (*36*), one might expect the stability of metal complexation with these particle films to follow the Irving-Williams order. Thus metal complexation by organic films on marine particulates may be another mechanism for trace metal removal from MBSW.

Crist *et al.* (*38*) found that bonding between metallic ions (Ca^{2+}, Sr^{2+}, Zn^{2+}, Mg^{2+}, and Na^{+}) and algal cell walls of *Vaucheria* followed the Irving-Williams order. This trend would enhance effects due to Cu and Zn overabundance and Co and Mn deficiency. In trace element studies with *Chaetoceros socialis* cultures and natural phytoplankton communities, Sunda *et al.* (*39*) observed that additions of FeCl$_3$, MnCl$_2$, and the chelators EDTA and NTA (nitrilotriacetic acid) to 800-m seawater collected off the North Carolina coast stimulated phytoplankton growth. A physiological interaction was suggested in which Cu interferes with Mn uptake by competing for nutritional sites. North (*40*) observed growth stimulation by *Macrocystis* juveniles (macroscopic plants of ~ 1 g wet weight) with Mn additions to 300-m seawater, collected 3 miles off Newport Bay, California, in batch cultures. Microscopic *Macrocystis* sporophytes were also produced in 12 to 14 days in flowing 300-m or surface seawater from the same site (*41*). These observations support trace element deficiency over Cu-Zn growth inhibition.

The results presented here are consistent with the idea that inhibition of the growth of marine organisms observed in cultures prepared with deep seawater is due to inherent toxic Cu and Zn free ion activities; they also suggest that there are Co and Mn deficiencies in southern California deep seawater.

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Localization of Calcium in Amyloplasts of Root-Cap Cells Using Ion Microscopy

Abstract. *An ion microscope has been used to demonstrate that the calcium ion is present in the amyloplasts of root-cap cells of corn, pea, and lettuce. The localization of calcium in the gravity-sensing organelle suggests a possible role of calcium in the gravity-sensing mechanism of plant roots.*

Calcium has been implicated in plant geotropism and associated processes in several ways. (i) As growth curvature commences, calcium has been found to move to the upper or concave side of the stem or coleoptile (*1*). (ii) The transport of auxin, which must be involved in the lateral redistribution of growth, is dependent upon calcium (*2*). (iii) The application of inhibitors of the calcium-binding protein calmodulin results in an erasure of geotropic sensitivity (*3*).

The initial process involved in gravity-sensing in higher plants is the lateral displacement of amyloplasts upon reorientation of a plant organ with respect to gravity. In roots this sensing function is carried out in the root cap, which contains abundant amyloplasts; when the root cap is removed, geotropic response is lost until new amyloplasts have been formed (*4*). The mechanism by which gravity-induced amyloplast movement might be transduced into a physiological gradient that would lead to curving growth is notably lacking. A relevant first question would be whether the calcium requirement for geotropism might be related to the amyloplast sensing mechanism. The intent of this study has been to examine the possibility that the redistribution of amyloplasts by gravity might bring about some redistribution of calcium in the gravity-sensing cells of the root cap.

With the ion microscope, a direct-imaging, secondary-ion mass spectrometer, it is possible to carry out the mass