AN INTERSPECIFIC COMPARISON OF TRACE METAL TOXICITY TO MARINE PHYTOPLANKTON

by
David J. Hughes

Ralph M. Parsons Laboratory
Division of Water Resources & Hydrodynamics
Department of Civil Engineering
Massachusetts Institute of Technology

This work was supported in part by
NSF Grant OCE-7919549
and
NOAA Grant NA79AA-D-00077.

Technical Note No. 25

December 1981

MIT

DEPARTMENT
OF
CIVIL
ENGINEERING

SCHOOL OF ENGINEERING
MASSACHUSETTS INSTITUTE OF TECHNOLOGY
Cambridge, Massachusetts 02139

AN INTERSPECIFIC COMPARISON OF TRACE METAL TOXICITY TO MARINE PHYTOPLANKTON

By

David J. Hughes

Ralph M. Parsons Laboratory
Division of Water Resources & Hydrodynamics
Department of Civil Engineering
Massachusetts Institute of Technology

This work was supported in part by
NSF Grant OCE-7919549
and
NOAA Grant NA79AA-D-00077.

Technical Note No. 25
T81-01

INTRODUCTION

Over the past few years, the emphasis placed on quantifying the metal speciation in culture media has clarified considerably our understanding of trace metal effects on phytoplankton. For example, it is now generally accepted that copper toxicity is controlled by the activity of the cupric ion (Davey et al., 1974; Sunda, 1975; Sunda and Guillard, 1976; Anderson and Morel, 1978; Morel, N.M.L. et al., 1978). It has also been demonstrated that the availability of metals for nutrition is dependent on their free ion activities (Manahan and Smith, 1973; Anderson et al., 1978; Anderson and Morel, in prep.). While there is no practical analytical method for determining free metal activities in seawater, the availability of convenient computer programs and a suitable range of synthetic chelators make it possible to calculate metal activities for controlled laboratory experiments performed in chemically defined (and buffered) culture media and to relate these results to field conditions, where metals and ligands are generally present at lower concentrations (Sunda and Guillard, 1976; Morel et al., 1979).

The limited available data on metal toxicity in phytoplankton cultures demonstrate wide differences in metal sensitivities among species (e.g., compare Anderson and Morel, 1978; and Morel et al., 1978), leading to the hypothesis that trace metals may partially control the composition of species assemblages in natural waters. This hypothesis is supported by the results of _in situ_ experiments. For example, addition of copper in the plastic enclosures of the CEPEX experiments resulted in a shift of dominant algal species from centric diatom and dinoflagellates to pennate diatoms and microflagellates (Goering et al., 1977). In lakes and reservoirs such a shift in algal species is commonly observed upon copper sulfate treatment for control of nuisance algae (McKnight, 1981). In order to begin documenting the possible role of trace metals as natural modulators of phytoplankton species assemblages in marine systems, we need to know a great deal more about what metals, in addition to copper and zinc, may be toxic or

limiting in the range of metal activities that prevail in the ocean; we need also to undersand what differences in metal sensitivities there might be among the principal algal species.

The toxic effects of metals may be observed in several ways, some of which are not consistent with each other. There are numerous reports of depressed photosynthesis and growth rates under metal stress. Morel et al. (1978) reported a systematic increase in the lag phase of Skeletonema costatum as a function of cupric ion activity. However, the subsequent division rate of the organism during exponential growth was not affected by the copper. Stewart (1977) measured the biomass of batch cultures after eight days incubation in lead-treated media, which would fail to discriminate between a prolonged lag phase and a depressed growth rate. Even a careful study of growth rate sometimes yields ambiguous results. Sunda (1975) reported a delay of up to three days in development of acute copper toxicity. Several of his cultures displayed declining growth rates with time. Other methods of assessing toxicity include loss of motility in dinoflagellates (Anderson and Morel, 1978) and chlorosis in higher plants (Foy et al., 1978).

In this report we are reporting data on the toxicity of seven metals (copper, cadmium, zinc, nickel, cobalt, manganese, and lead) to an assortment of clones of marine phytoplankton. The experimental protocol was designed under the assumption--which was subsequently verified--that the free metal ion activities were the critical parameters for all the metals. For example, to study the effect of a given metal we adjusted the total concentrations of all other metals present in the medium so as to maintain constant activities. We have chosen exponential growth rates as our principal measure of the organisms' response to the metals, on the grounds that growth rate is an essential parameter governing diversity in natural populations, and that the requisite time series data might permit us to identify other time-dependent parameters relating to toxicity.

MATERIAL AND METHODS

Most of the phytoplankton species used in this study were obtained from Dr. R.R.L. Guillard of the Woods Hole Oceanographic Institution. The two strains of Biddulphia aurita were supplied by Peter Underhill of the University of Delaware (Underhill, 1978). Table 1 lists the species used.

Stock cultures were maintained in f/2 medium (Guillard and Ryther, 1962) prior to experimentation. The defined medium "Aquil" (Morel et al., 1979) was used for the experiments. Minor changes were made in some nutrient and chelator concentrations, but the rigorous control of trace metal impurities was retained. The nutrient concentrations are shown in Table 2. Some of the experiments were performed with two EDTA concentrations to verify the hypothesis that toxicity was effected by the ionic activity of the metal under consideration, rather than the total metal concentration. All of the copper toxicity experiments were performed with 10^{-3}M Tris* in addition to EDTA*, and the nickel toxicity experiment employed 10^{-5}M NTA* in addition to EDTA. Metal speciation was calculated by the equilibrium program MINEQL (Westall et al., 1976). The use of this program permitted the adjustment of the trace metal suite so that activities of the various nutrient metals were not altered by the different chelator concentrations, or by the addition of large amounts of other metals (with concomittant titration of the organic ligands).

All cultures were maintained in a constant temperature environmental chamber (20°C) continuously illuminated from below with cool-white fluorescent lamps. The light intensity was 175-2000 $\mu E\ m^{-2}\ sec.^{-1}$. Standard sterile techniques were employed, though several of the algal cultures were not axenic. All culture apparatus was soaked in 10% HCl and rinsed three times in deionized, double-distilled water prior to use. Media were sterilized by filtering through acid-washed 0.1 µm or 0.2 µm Nuclepore filters.

---

*abbreviations: Tris: trishydroxy amino methane
EDTA: Ethylenediamine tetraacetate
NTA: Nitrilotriacetate

In the first phase of this study, T. weissflogii was grown in one liter filter polycarbonate flasks. Culture growth was monitored by subsampling and counting the aliquot on a Coulter model TA particle counter. Stock cultures were maintained for one week on a medium lacking the particular metal under investigation before inoculating the test media (e.g., the algae were maintained in zinc-free media for one week before beginning the zinc limitation/toxicity tests).

Carbon incorporation experiment were performed in polycarbonate centrifuge tubes with polypropylene screw closures. Algae were preincubated in the test media for one week prior to the experiment. On the day of the experiment, cultures were inoculated into fresh media (same trace metal suites as the preincubation) and incubated with ca. 10 µCi of $H^{14}CO_3^-$. The labelled bicarbonate was prepared by acidifying Ba $^{14}CO_3$ and trapping the evolved $^{14}CO_2$ in NaOH which had been previously passed through a Chelex-100 column to remove trace metal impurities. The NaOH-$H^{14}CO_3^-$ solution was diluted, dispensed in acid-washed glass ampoules, sealed, and autoclaved.

Cultures were incubated with the label for 4-5 hours, then filtered onto Whattman GF/C filters under mild vacuum (< 12mm Hg). The filters were placed directly into scintillation vials containing ca. 2 ml of acidified methanol (5% acetic acid/ 95% methoanol) to eliminate inorganic ^{14}C. The methanol was dried off and scintillation cocktail added to each vial. A liquid scintillation counter with external standard correction was used to measure the activity of each sample. A "zero-time" sample (a bottle which was inoculated with ^{14}C, mixed, and immediately filtered) was used as a blank. The activity of the ^{14}C stock was measured by couting 1 ml of the "zero-time" sample in a vial containing scintillation cocktail plus phenylethylamine. Chlorophyll was measured fluorometrically by a modification of the method of Yentsch and Menzel (1963).

In the final phase of this study, growth rates were determined from increases in the in vivo fluorescence of the algae. Initially, cultures were grown in 6.5 ml polycarbonate centrifuge tubes. Five ml of filter

sterilized media was pipetted into sterile tubes and inoculated with the test organism. Growth was monitored by placing the entire tube into the high sensitivity door of a Turner 111 fluorometer, used to measure in vivo fluorescence. Later, the procedure was modified to use a Turner Design model 10 fluorometer with the discrete sample accessory (Brand, et al., in prep.). Cultures were grown in 25 ml Pyrex culture tubes which were previously coated with a silicone film to prevent trace metal adsorption on the glass (Morel et al., 1979). The tubes contained 25 ml of filter-sterilized medium and were covered with standard polypropylene closures.

Initially, these experiments were inoculated with stock cultures grown in media lacking the metal under investigation, as above. However, in several cases, second and third experiments were performed by inoculating fresh test media with cultures grown in the preceding experiment. In those cases where no growth was noted in the previous experiment, the inoculation was taken from the growing culture with the most similar trace metal suite.

Cell volume was determined with a Coulter model Z_f particle counter and a Coulter model P128 size frequency analyzer. The output of the P128 was fed directly to a microprocessor based computer for analysis.

RESULTS

Growth rates are shown in Figure 1 for Thalassiosira weissflogii grown in various free ion concentrations of zinc, copper, cadmium, nickel, and lead. The zinc data appear to form an envelope encompassing the range from zinc limitation to zinc toxicity. Copper and cadmium were deleterious at high metal activities, but lead and nickel had no effect on growth of the algae. During the course of the cadmium experiment, additional EDTA was added to one flask in an attempt to overcome toxicity by reducing the concentration of free Cd^{2+}. In Figure 2, it can be seen that lowering $\{Cd^{2+}\}$, did in fact relieve the growth inhibition in the culture so treated, confirming our contention that the inhibition is mediated by $\{Cd^{2+}\}$, not $(Cd)_T$.

Figure 3 shows the effect of the five metal ions upon carbon uptake in T. weissflogii. The effects of Cd^{2+}, Cu^{2+}, and Pb^{2+} seem qualitatively similar to the previous experiment. Nickel appears to diminish carbon uptake per unit chlorophyll, but this effect is not dramatic. The zinc data are somewhat ambiguous. There appears to be residual Zn^{2+} contamination of approximately 10^{-11}M. The apparent toxicity seen previously at pZn = 8.6 is not seen in this experiment. (The point at pZn = 8.1 is difficult to interpret since this medium is calculated to produce a zinc precipitate.)

Subsequent experiments in which growth was monitored by in invo fluorescence are again qualitatively similar. Figure 4 shows growth curves during the first incubation in the test media. At the end of this experiment, the algae were transferred to fresh test media (Figure 5). This second transfer emphasized the cadmium toxicity, but the algae responded to the other metal ions in essentially the same manner as during the initial exposure. Again, there is evidence for residual zinc contamination but no evidence of zinc toxicity at pZn = 8.6.

Figure 6 compares growth rate as a function of cupric ion activity for 12 strains of marine phytoplankton. If the pCu for 50% inhibition is taken as the onset of copper toxicity, then algae show a range of

about 2.5 orders of magnitude in copper sensitivity, from a high sensitivity at pCu = 11.5 (Biddulphia aurita, clone "B-1," isolated from the Delaware Bay) to a low sensivitity at (Dunaliella terliolecta, clone DUN). Note that the St. Croix isolated of Biddulphia aurita (clone "STX-88") shows much more resistance to copper toxicity than does the Delaware Bay isolate.

Figure 7 presents similar data for cadmium toxicity. Because cadmium toxicity appeared to be enhanced by prior exposure in many species, these data are taken from the second transfer into the respective activities. Again, there is a wide range of sensitivities, and at least one specie (Skeletonema menzelli) seemed to show inhibition over a very wide range of cadmium activity. The species in Figures 6 and 7 are arranged in order of decreasing sensitivity, top to bottom. While some similarity in ranking does exist, the order of sensitivities are not exactly identical, suggesting that high sensitivity to one metal does not necessarily imply high sensitivity to all metals.

The effects of the other metals studied were much less dramatic than copper and cadmium. Figure 8 shows representative plots of growth rate as a function of zinc activity. The salient feature of the zinc studies is the occasional decrease in growth rates at low zinc activities due to zinc limitation. Unfortunately, zinc is a particularly pervasive contaminant, and we were unable to control the zinc residual to a level which permitted interspecific comparison of zinc requirements. The decrease in growth rate at pZn = 8.1 was common to most of the species, but again, the interpretation is difficult due to predicted precipitation.

None of the other metals (nickel, cobalt, manganese, lead) caused reduced growth in the calculated range of solubility in seawater. On one occasion we measured the cell volume of a coccolithophore after two weeks of incubation in the test media (Figure 9). There was a significant increase in volume of the cells exposed to high nickel activities,

but no apparent effect on the growth rate of the organism. By comparison, the cells exposed to copper showed no response in volume, but a strong response in growth rate at high cupric ion activity.

DISCUSSION

For each of the metals in this study, the highest activity shown in the figures is fixed by the formation of one or more solids (as predicted by equilibrium calculations). In the presence of a precipitate, we can no longer guarantee the activities of the various other soluble species, since we have no way of predicting adsorption onto these solids. In light of the recent reports of synergism among metals and other nurients, these data (metal concentration predicted to form precipitates) must be considered separately from those in which no precipitates are expected to form. In addition, the formation of a colloidal precipitate may provide a mechanism of concentrating the metals at the cell surface. Such colloids are believed to be attracted to the cell's surface, where they may be redissolved by metabolic by-products or due to local pH conditions. Thus, it is not surprising that our zinc data show severe toxicity (frequently no growth) at the highest zinc concentrations (pZn = 8.1), while showing little or no toxicity at lower concentrations.

Our results suggest a similar mechanism for lead and nickel toxicity. We found no evidence that lead or nickel impaired the growth of the species studied. However, Steward (1977) reported a depressed growth rate for Dunaliella tertiolecta at lead concentrations above 0.5 to 1.0 mg Pb l^{-1}. We obtained the formula for Stewart's medium (Burkholder's artificial seawater cf. Pacific Northwest Environmental Research Lab, 1974) and analyzed the metal speciation with the aid of the MINEQL program. In this medium solid lead hydroxide [$Pb(OH)_2$] is predicted to form at a lead concentration of about .3 mg/l, which is lower than the concentration at which inhibition occurs.

Our own experiments with nickel showed a similar result. Our early experiments, using 10^{-5}M EDTA as a chelator, showed several species inhibited by nickel calculated in the range of 10^{-10} to 10^{-11}. This turns out to be approximately the point where nickel completely titrated the available chelator, a point where small variations in total nickel cause extremely large changes in free nickel ion--possibly exceeding

the solubility of nickel in the system. In order to gain more control over the activity of free nickel (Ni^{2+}), we increased the metal buffering capacity of the system by adding an additional chelator, NTA (nitrilotriacetate). When the experiments were repeated in the new system, the apparent nickel toxicity disappeared, despite the fact that the new system contained more total nickel at each calculated activity. These inorganic precipitates [$Pb(OH)_2$ and $Ni(OH)_2$] are likely to form in seawater at normal pH at the same metal activities as in our medium, though of course the total metal concentration required to form the precipitates will vary according to available ligands, etc. Thus, it appears that lead and nickel toxicity may be caused not by a soluble fraction (ionic or chelated), but by colloidal precipitates interacting with the phytoplankton.

Of the metals studied by us, only copper and cadmium systematically showed toxicity to phytoplankton within the calculated range of solubility in seawater. The toxicity of both metals for the species studied ranged from a low metal ion activity of 10^{-11}M to a high of $10^{-8.5}$ (50% inhibition), with copper being usually more toxic than cadmium.

It has often been noted that the relative toxicities of metals to organisms are correlated to the insolubilities of the corresponding metal sulfides (Shaw, 1954). Such correlation ought to be particularly applicable in phytoplankton since reactions with sulfhydryl groups have been implicated as a mechanism of metal toxicity in algae (Shaw and Grushkin, 1957). Indeed, the decreasing order of toxicity of Cu > Cd > Zn fits with the corresponding sulfide insolubilities and Ni, Co and Mn, which have relatively more soluble sulfides are essentially not toxic. However, lead, which forms a very insoluble sulfide ($K_s \cong 10^{-30}$), would be expected to be greatly more toxic than it is observed to be, and the image of sulfhydryl group blockage as a general mode of metal toxicity to phytoplankton, must thus be questioned.

To what extent are laboratory data, such as those presented here, generally applicable to the same species in nature or, more simply, to the same clones under different culture conditions? It is clear from

our widely different results with the two clones of Biddulphia aurita that a particular metal sensitivity cannot be assigned to a particular algal species. It appears, in fact, rather certain that species adapt to environmental metal concentrations (Jensen et al., 1974; Foster, 1977) and that species exhibit large temporal and spatial variations in metal sensitivities. Our data on laboratory clones then simply cannot be applied to the same species in nature. On the other hand, laboratory clones seem to respond consistently to metal ion activities and we have been able to obtain quantitatively similar toxicity data for the same clones over a span of several years. Recognizing that antagonistic and synergistic effects among metals and among metal and nutrients may be important, our results cannot be applied blindly to widely different culture conditions; nonetheless, these data should serve as a useful indication of metal sensitivity for designing experiments with algal clones in culture collections. In addition, the consistency of the data for each individual metal shows that the toxicity of Cu, Cd, and perhaps Zn, may play a role in controlling productivity or phytoplankton assemblages in natural and polluted marine waters while lead, cobalt, nickel, and manganese are practically not toxic.

TABLE 1 SPECIES USED

	Clone
Bacillariophyceae	
Biddulphia aurita	B-1 and STX-88
Skeletonema menzelii	Men-5
Thalassiosira fluviatilis	Actin
Haptophyceae	
Emiliana huxleyi	BT-6
Isochrysis galbana	Iso
Pavlova lutheri	Mono
Hymenomonas	Cocco II
Prasinophycea	
Pyramimonas sp.	Pyr-1
Chlorophyceae	
Dunaliella tertiolecta	Dun
Eustigmatophyceae	
Monallantus sp.	GSB Sticho

TABLE 2 NUTRIENT CONCENTRATIONS IN MEDIUM

PO_4^{3-}	10 μM
NO_3^-	300 μM
$Si(OH)_4$	100 μM
Biotin	5×10^{-7} g/l
Thiamin	10^{-4} g/l
Vitamin B_{12}	5.5×10^{-7} g/l
EDTA (see text)	10^{-5}M or 10^{-6}M
Fe	$10^{-7.8}$M
Mo	$10^{-8.81}$M
Co^{2+} (fixed ionic concentration)	$10^{-10.87}$M
Cu^{2+} (fixed ionic concentration)	$10^{-13.83}$M
Mn^{2+} (fixed ionic concentration)	$10^{-8.26}$M
Zn^{2+} (fixed ionic concentration)	10^{-10}M
Tris (see text)	10^{-3}M
NTA (see text)	10^{-5}M

BIBLIOGRAPHY

Anderson, D.M. and F.M.M. Morel. 1978. Copper sensitivity on Gonyaulax tamarensis. Limnol. Oceangr. 23:283-295.

Anderson, M.A., F.M.M. Morel and R.R.L. Guillard. 1978. Growth limitation of a coastal diatom by low zinc ion activity. Nature 276:70-71.

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

Boyle, E.A., F. Sclater and J.M. Edmond. 1976. On the marine geochemistry of cadmium. Nature 263:42-44.

Boyle, E.A., F. Sclater and J.M. Edmond. 1977. The distribution of dissolved copper in the Pacific. E & PSL 37:38-54.

Brand, L.E., R.R.L. Guillard and L.S. Murphy. A method for the rapid and precise determination of acclimated phytoplankton reproduction rates. Woods Hole Oceanographic Institution, contribution #4396 (in prep.)

Bruland, K.W., G.A. Knauer and J.H. Martin. 1978B. Cadmium in Northeast Pacific waters. Limnol. Oceanogr. 23(4):618-625.

Davey, E.W., M.J. Morgan and S.J. Erickson. 1973. A biological measurement of copper complexation capacity of seawater. Limnol. Oceanogr. 18:993-997.

Falchuk, K.H., D.W. Fawcett and B.L. Valee. 1975. Competitive antagonism of cadmium and zinc in the morphology and cell division of Euglena gracilis. J. Submicrosp. Cytol. 7:139-152.

Foster, P.L. 1977. Copper exclusion as a mechanism of heavy metal tolerance in green algae. Nature 269:322-323.

Foy, C.D., R.L. Chaney and M.C. White. 1978. The physiology of metal toxicity in plants. Annual Review of Plant Physiol. 29:511.

Goering, J.J., D. Boisseau and D. Hattori. 1977. Effects of copper on silicic acid uptake by a marine phytoplankton population: A control ecosystem pollution experiment. Bull. Mar. Sci. 27(1):58-65.

Guillard, R.R.L. and J.H. Ryther. 1962. Studies on marine planktonic diatoms. I. Cyclotella nana and Detonula confervacea (Cleve) Gran. Can. J. Microbiol. 8:229-239.

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

Jensen, A., B. Rystad and S. Melsom. 1974. Heavy metal tolerance of marine phytoplankton. I. The tolerance of three algal species to zinc in coastal seawater. J. Exp. Mar. Biol. Ecol. 15:145-157.

Manahan, S.E. and M.J. Smith. 1973. Env. Sci. & Techn. 7:829.

Morel, N.M.L., J.G. Rueter and F.M.M. Morel. 1978. Copper toxicity to Skeletonema costatum. J. Phycol. 11:43-48.

Morel, F.M.M., J.G. Rueter, D.M. Anderson and R.R.L. Guillard. 1979. Aquil: A chemically defined phytoplankton media for trace metal studies. J. Phycol. 15:135-141.

Rueter, J.G. 1979. The effects of copper and zinc on growth rate and nutrient uptake in the marine diatom Thalassiosira pseudonana. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, MA 02139.

Shaw, W.H.R. 1954. Toxicity of cations toward living systems. Science 120:361.

Shaw, W.H.R. and B. Grushkin 1957. The toxicity of metal ions to aquatic organisms. Arch. Biochem. Biophys. 67:447-452.

Stewart, J. 1977. Relative sensitivity to lead of a naked green flagellate, Dunaliella tertiolecta. Water, Air & Soil Poll. 8:243-247.

Sunda, W.G. 1975. The relationship between cupric ion activity and the toxicity of copper to phytoplankton. Ph.D. thesis, Woods Hole Oceanographic Institution, Woods Hole, MA.

Sunda, W.G. and R.R.L. Guillard. 1976. Relationship between cupric ion activity and the toxicity of copper to phytoplankton. J. Mar. Res. 34:511-529.

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.

Underhill, P.A. 1977. Nitrate uptake kinetics and clonal variablity in the neritic diatom, Biddulphia aurita. J. Phycol. 13:170-176.

Yentsch, C.S. and D.W. Menzel. 1963. Deep-Sea Res. 10:221.

Westall, J.C. 1977. Chemical methods for the study of metal-ligand interactions in aquatic environments. Ph.D. thesis, Massachusetts Institute of Technology, Cambridge, MA 02139.

Westall, J.C., J.L. Zachary and F.M.M. Morel. 1976. MINEQL: A computer program for the calculation of chemical equilibrium composition of aqueous systems. Technical Note #18, Massachusetts Institute of Technology, Cambridge, MA 02139.

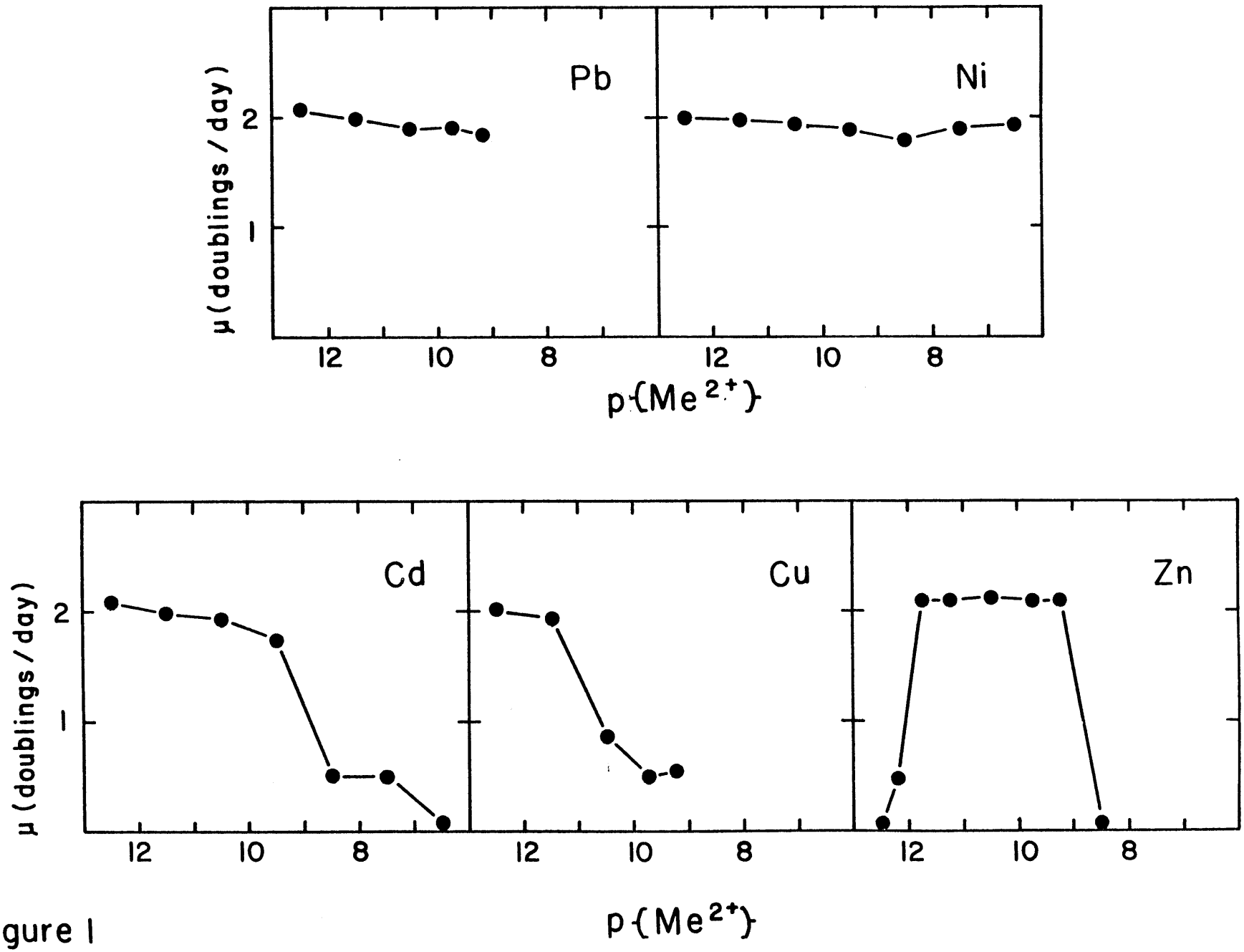

Figure 1

Figure 1: Growth rates for Thalassiosira weissflogii grown in Aquil plus various free ion activities of Zn, Cu, Cd, Ni, and Pb.

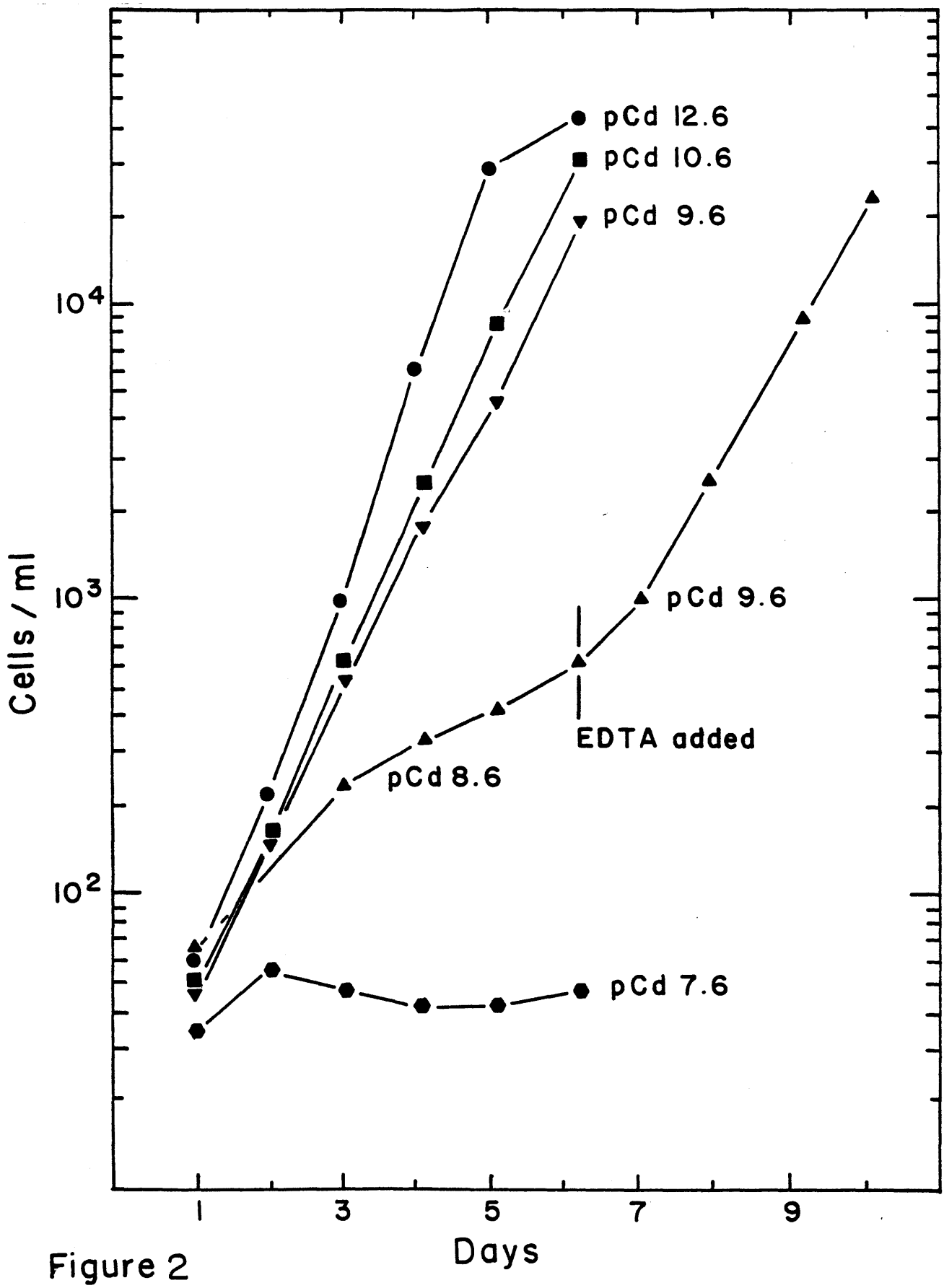

Figure 2

Figure 2: Growth of Thalassiosira weissflogii in Aquil with pCd of 12.6, 10.6, 9.6, 8.6 and 7.6. After six days of growth the pCd 8.6 culture was amended with EDTA to lower the pCd to 9.6.

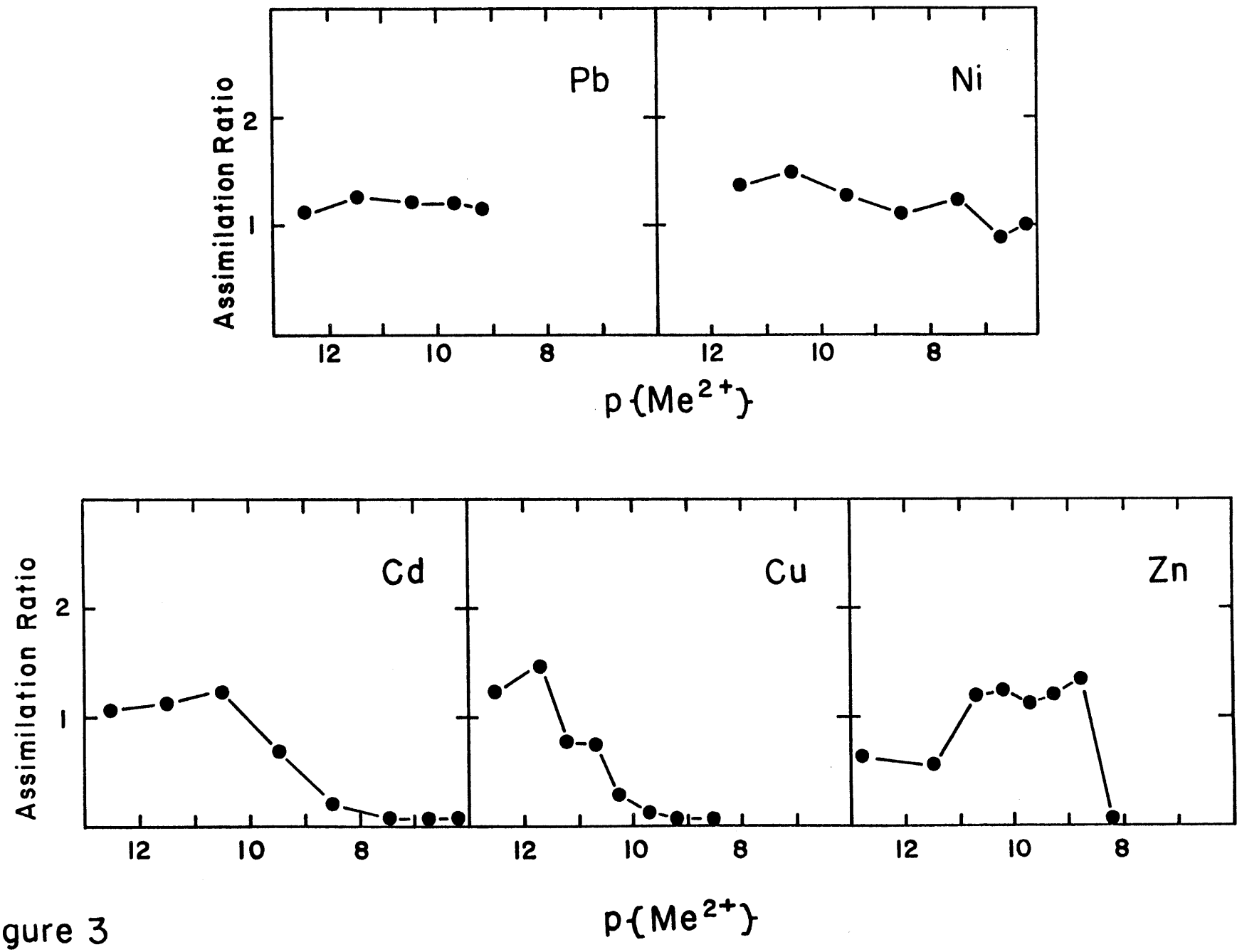

Figure 3

Figure 3: The effect of the metal ions Pb, Ni, Cd, Cu and Zn on carbon uptake in Thalassiosira weissflogii.

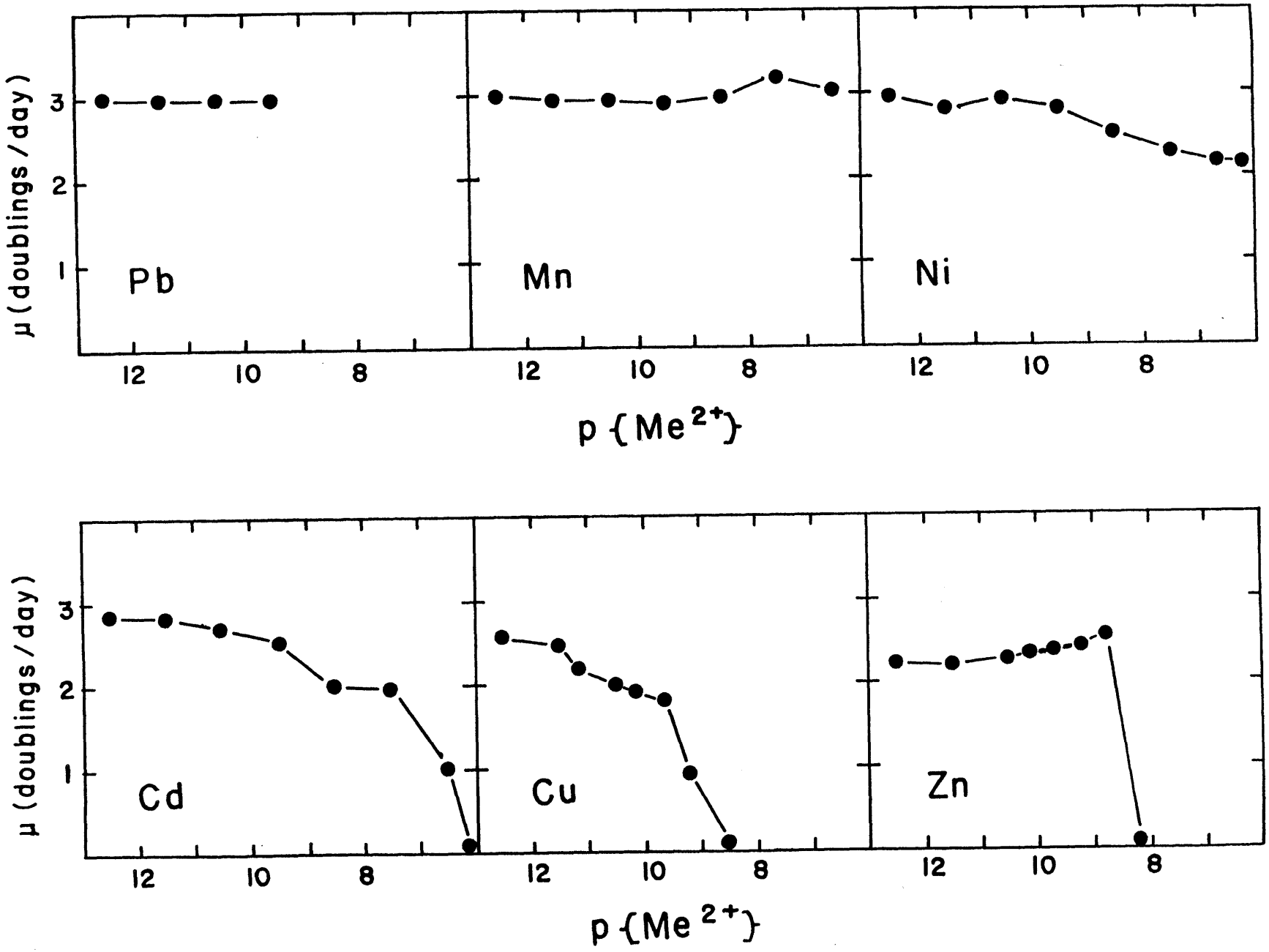

Figure 4

Figure 4: Growth rates for Thalassiosira weissflogii grown in Aquil plus various free ion activities of Zn, Cu, Cd, Ni, and Pb. Growth was measured in vivo fluorescence.

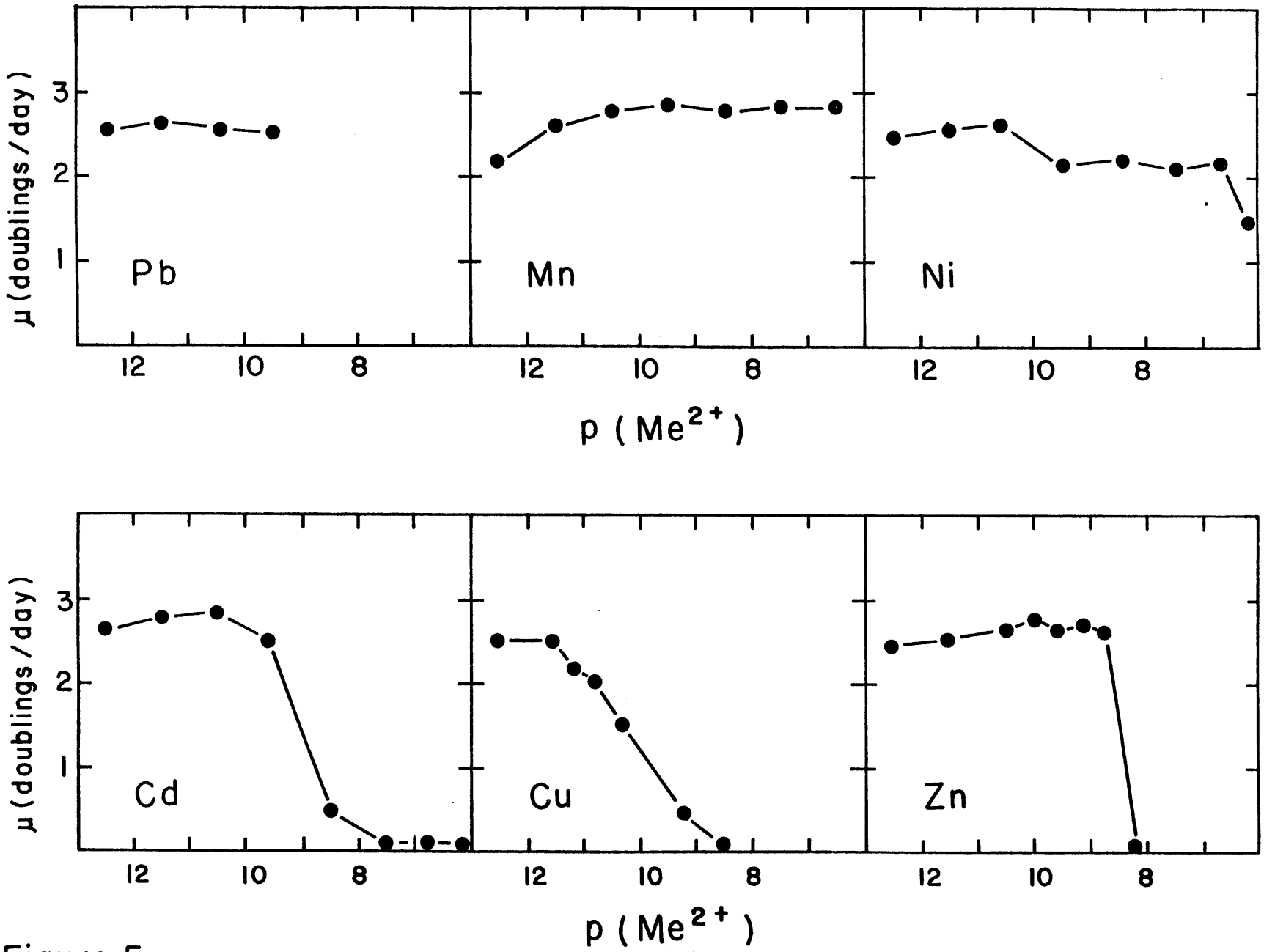

Figure 5

Figure 5: Growth rates for Thalassiosira weissflogii grown in Aquil plus various free ion activities of Zn, Cu, Cd, Ni, and Pb. Algal inoculum was obtained from the experiments described in Figure 4.

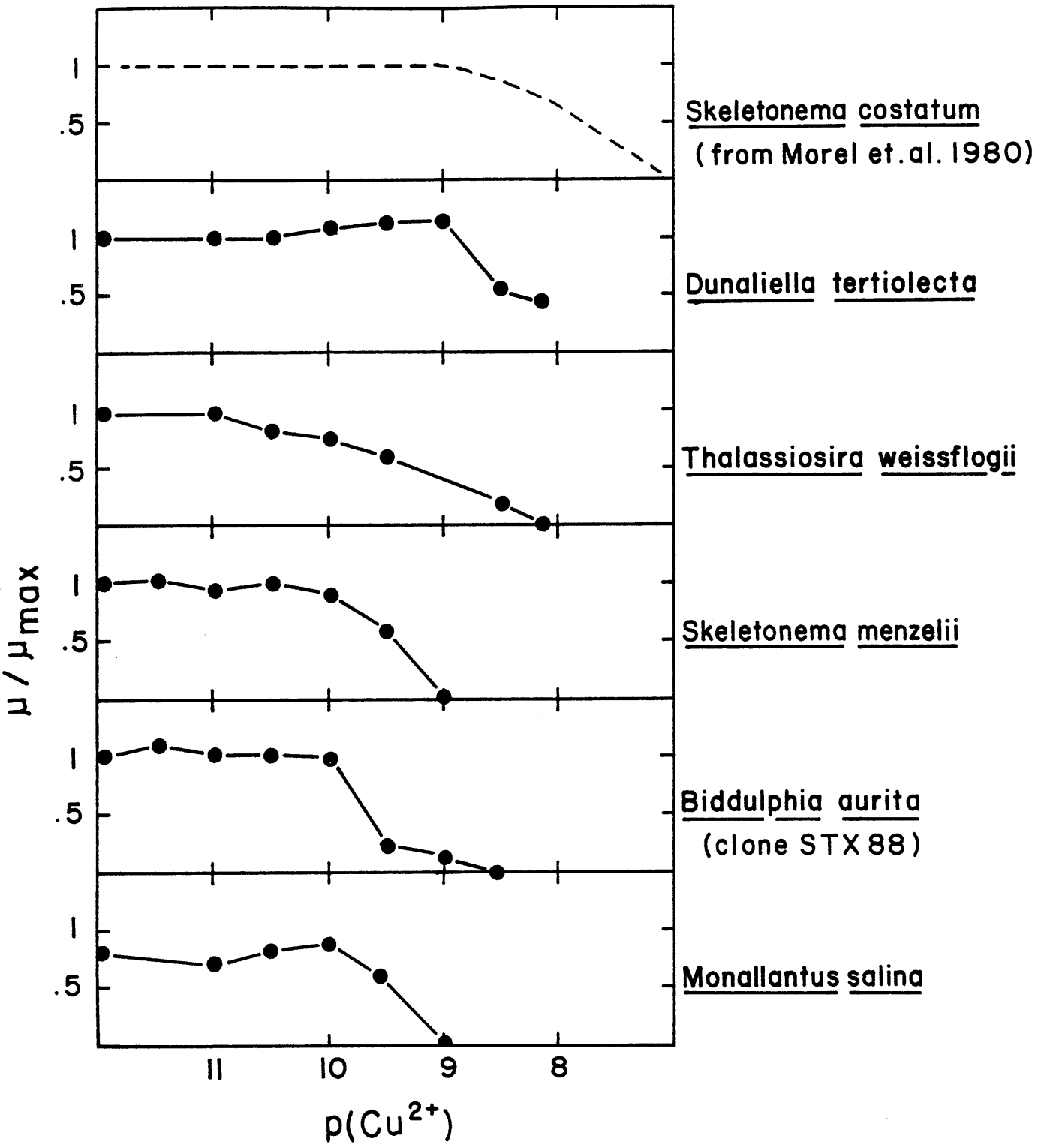

Figure 6

Figure 6: Comparison of growth rates as a function of cupric ion activity for 13 strains of marine phytoplankton.

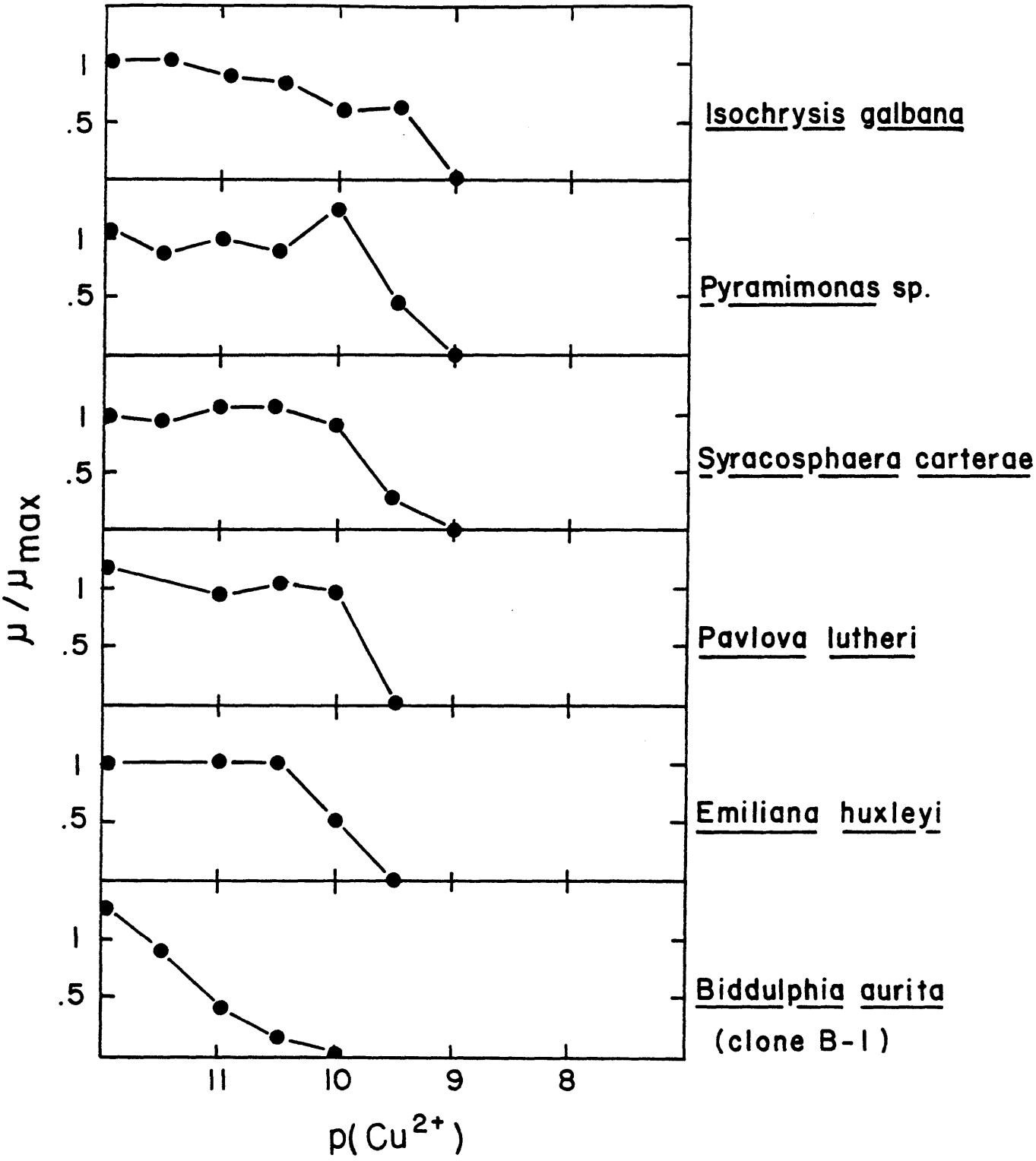

Figure 6 (cont.)

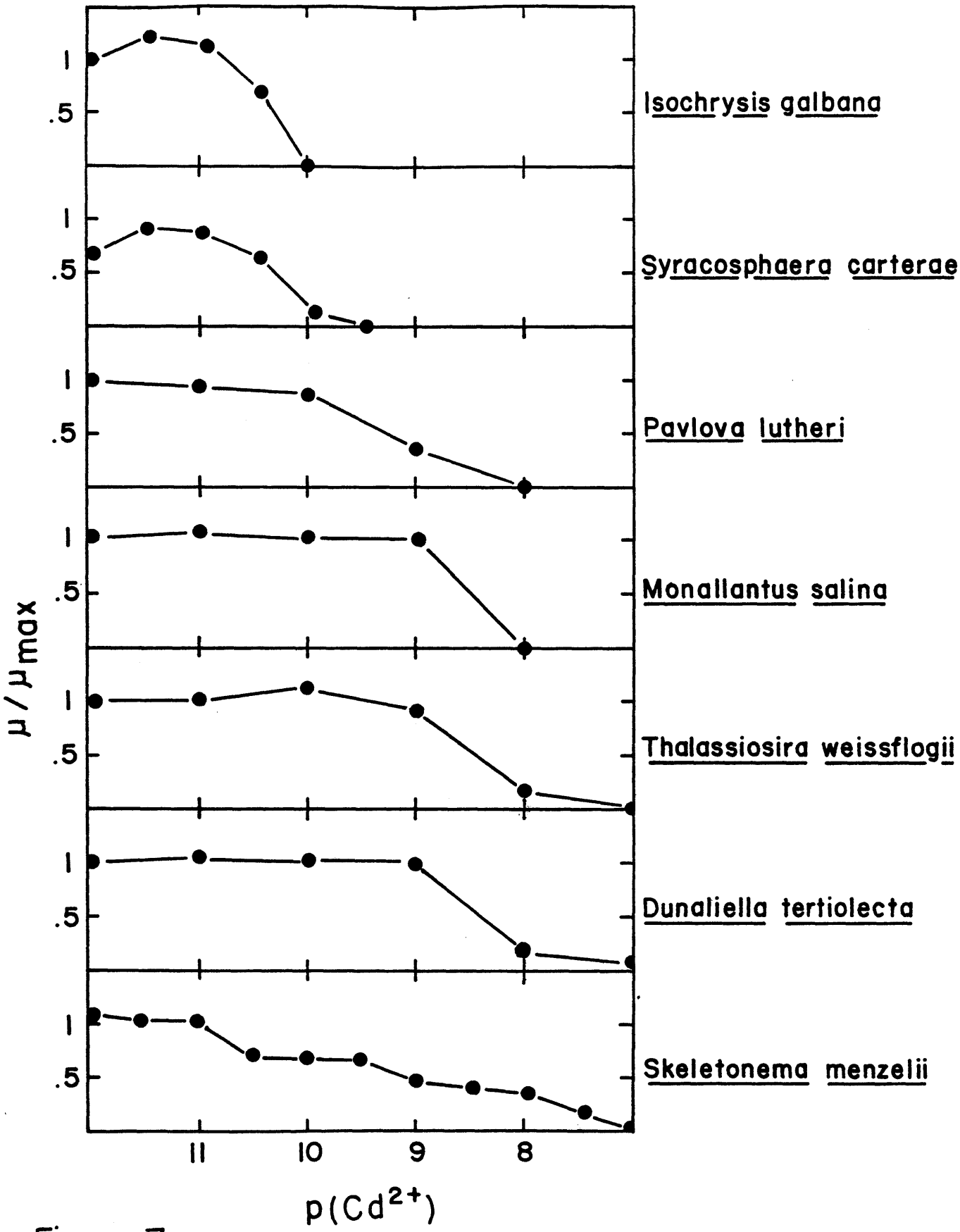

Figure 7

Figure 7: Comparison of growth rates as a function of cadmium ion activity for 7 strains of marine phytoplankton.

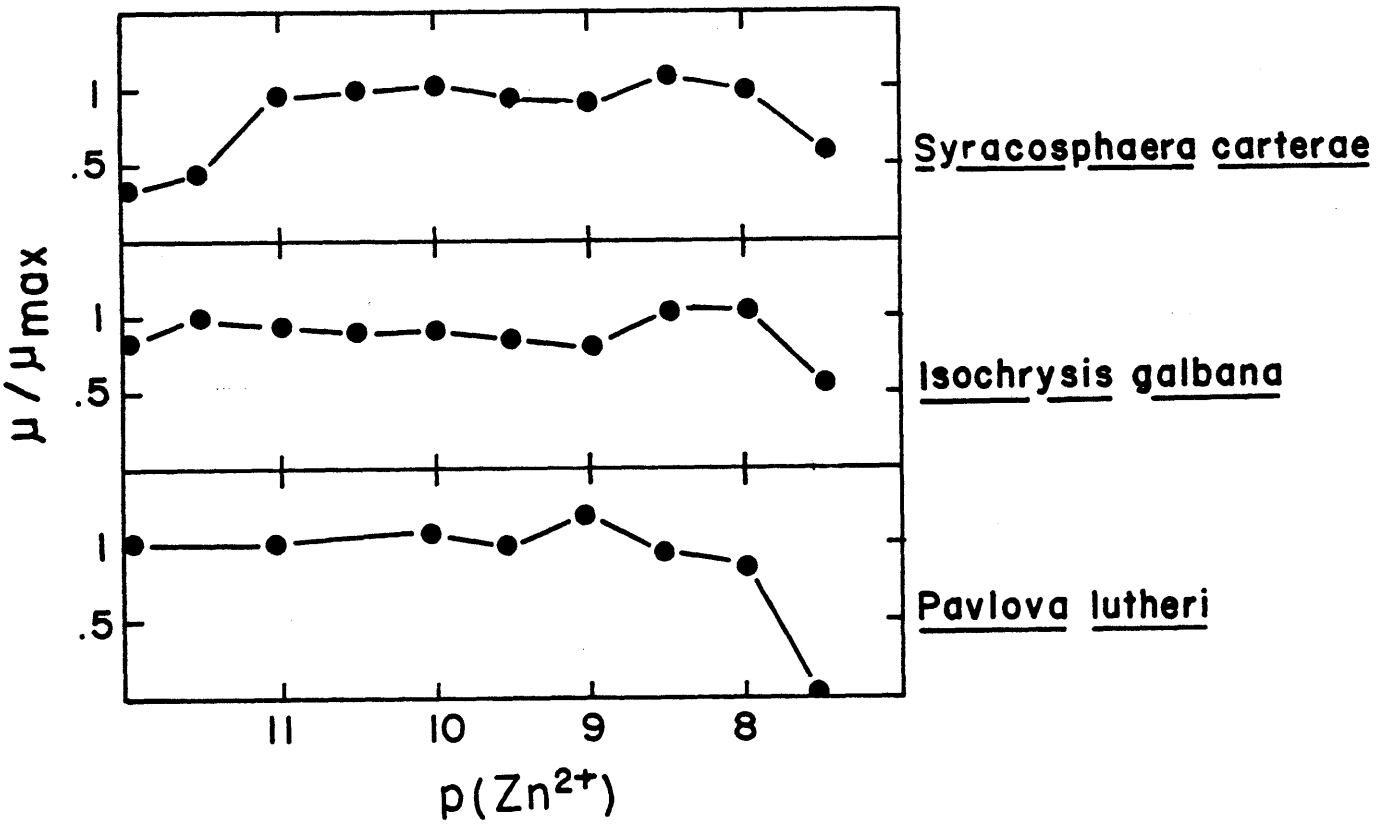

Figure 8

Figure 8: Comparison of growth rates as a function of zinc ion activity for 3 strains of marine phytoplankton.

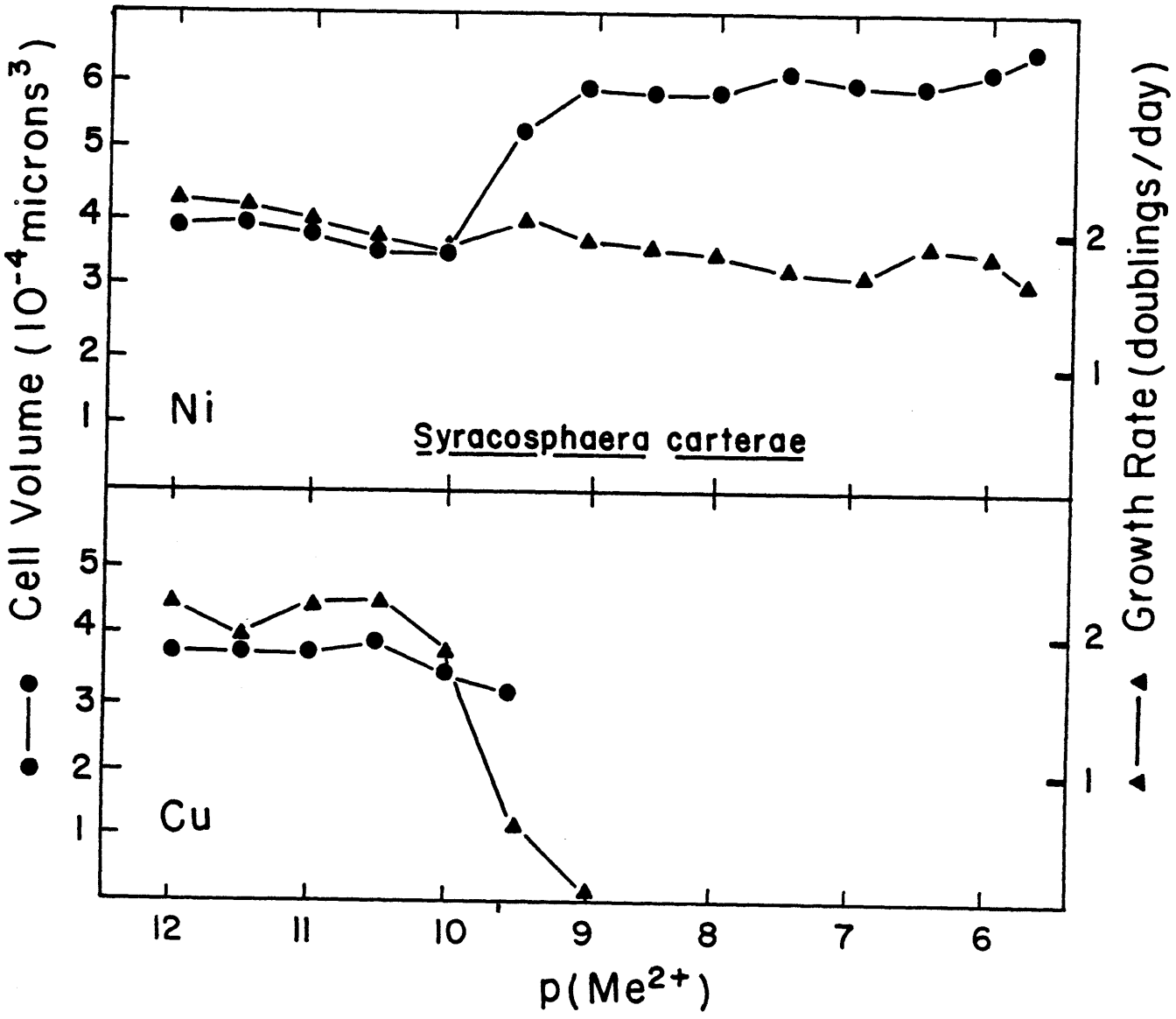

Figure 9

Figure 9: Comparison of growth rates and cell volume as a function of copper and nickel ion activity for Syracosphaera carterae after two weeks of incubation in test media.