MECHANISMS AND FACTORS REGULATING THE UPTAKE AND TOXICITY OF HEAVY METALS IN PHYTOPLANKTON

William G. Sunda and Susan A. Huntsman
Beaufort Laboratory, National Marine Fisheries Service,
NOAA, Beaufort, NC 28516
Phone: 919/728-8754; Fax: 919/728-8784
E-mail: BSUNDA@HATTERAS.BEA.NMFS.GOV

Award # N00014-96-F0061

LONG TERM GOALS

Our long-term research goal is to determine the influence of pollutant metals on the productivity and species composition of phytoplankton communities in coastal waters and to determine the role of algal metal uptake in controlling the particulate removal and biogeochemical cycling of metals.

OBJECTIVES

To achieve these goals, we need to determine the environmental factors and underlying physiological mechanisms that regulate the uptake and effects of toxic metals (Cu, Cd, and Zn) and interactive nutrient metals (Mn, Zn and Fe) in representative phytoplankton species. We will then combine this information with data on environmental variations in controlling factors (e.g. free ion concentrations of metals) to construct conceptual and mathematical models for algal uptake and biological effects.

APPROACH

Our research effort consisted of (l) long-term growth experiments with algal cultures to quantify relationships among key dependent variables (growth rate, cellular metal concentrations, and steady-state metal uptake rates) and independent controlling variables (free ion concentrations of important metals and light); and (2) short-term kinetic studies to investigate physiological mechanisms of cellular metal uptake and binding characteristics of metal uptake systems. The experiments focused largely on metal/metal interactions, particularly those between toxic and nutrient metals. Effects of light were examined because of its controlling influence on photosynthetic rate, growth rate, and biochemical demand for nutrient metals (Mn and Fe). The toxic metals investigated were Cu, Cd and Zn while the nutrient metals included Mn, Fe, and Zn. Zinc can act as either a toxicant or a nutrient depending on its concentration. Free metal ion concentrations were controlled in these experiments by metal ion buffer systems employing the chelators EDTA or NTA. Cellular metal levels in long-term experiments and metal uptake rates in short-term experiments were measured with radiotracers ^{109}Cd, ^{65}Zn, ^{54}Mn, and ^{59}Fe.

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
30 SEP 1997		**00-00-1997 to 00-00-1997**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Mechanisms and Factors Regulating the Uptake and Toxicity of Heavy Metals in Phytoplankton	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **National Oceanic and Atmospheric Administration (NOAA),National Marine Fisheries Service,325 Broadway,Beaufort,NC,28516**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			
unclassified	**unclassified**	**unclassified**	**Same as Report (SAR)**	**4**	

Results from short-term experiments were modeled using competitive saturation kinetics to determine saturation uptake rates (V_{max}) and affinity constants for cellular metal uptake sites. These results were then combined with data from long term experiments (steady state cellular metal levels, metal uptake rates, and specific growth rate as functions of free metal ion concentrations) to yield conceptual and mathematical models of cellular metal uptake, cellular metal regulation, and metal effects on growth rate. The data from long term experiments were combined with field data for total and free ion concentrations of pertinent metals to predict algal removal of trace metals in coastal waters or metal effects on algal growth.

TASKS COMPLETED AND SCIENTIFIC FINDINGS

Control of Cd concentrations in a coastal diatom by free ionic Cd, Zn, and Mn in seawater - A paper on this topic was written and submitted for publication in the journal Limnology and Oceanography. Cadmium concentrations in seawater are thought to be controlled by algal uptake and regeneration, but the factors controlling Cd levels in phytoplankton are poorly known. Experiments with a coastal diatom *Thalassiosira pseudonana* revealed that cellular Cd:C ratios within the Cd ion concentration ($[Cd^{2+}]$) range in seawater (10^{-13} to 10^{-10} M) were generally proportional to $[Cd^{2+}]$, and inversely related to concentrations of Zn and Mn ions ($[Zn^{2+}]$and $[Mn^{2+}]$) and specific growth rate. Effects of Mn and Zn reflect cellular uptake of Cd by two regulated transport systems: the Mn system whose activity is enhanced at low $[Mn^{2+}]$, and a separate system induced at low cellular zinc. At the low $[Zn^{2+}]$ of surface oceanic waters ($\leq 10^{-11.0}$ M), Cd uptake is controlled by this latter system and, therefore, is inversely related to ionic zinc levels. However, at the higher $[Zn^{2+}]$ in coastal waters, Cd uptake by this system is strongly suppressed and Cd instead is taken up by the Mn system; and as a result, is inversely related to $[Mn^{2+}]$ and largely independent of $[Zn^{2+}]$. Because of the suppression of Cd uptake by high $[Zn^{2+}]$ and $[Mn^{2+}]$ in coastal waters, algal Cd concentrations may actually be lower in these waters than in the ocean despite the presence of higher coastal $[Cd^{2+}]$.

Interactions among Cu^{2+}, Zn^{2+}, and Mn^{2+} in controlling cellular Mn, Zn, and growth rate in a coastal alga - A paper on this topic was written and submitted to Limnology and Oceanography. Culture experiments with the green alga *Chlamydomonas* sp. revealed antagonistic interactions between toxic metals (Cu and Zn) and nutrient metals (Zn and Mn) in regulating cellular Mn and Zn uptake and specific growth rate. High levels of Cu and Zn inhibited cellular Mn uptake while high Cu inhibited Zn uptake rates. These effects were associated with growth rate inhibition at combined conditions of high Zn and low Mn and at high Cu and low Zn. Zinc inhibited Mn uptake by competitively blocking Mn binding to a high-affinity Mn uptake system which was under negative feedback regulation. Kinetic modeling suggested that zinc was taken up by this system, and as a result, cellular Zn concentrations increased as external $[Mn^{2+}]$ was decreased. Zinc uptake behavior was complex, and in addition to the Mn system, uptake appeared to involve a high-affinity, Zn-selective system induced at low cell zinc levels and a separate constitutive low-affinity system. High $[Cu^{2+}]$ inhibited Zn uptake by the high-affinity Zn transport system, but not the low-affinity system. Such complex competitive interactions

among metals provide important controls on cellular metal accumulation, toxicity, and nutrition.

Interactive effects of light, and ionic Mn and Cu in controlling cellular Mn and growth of a coastal diatom - A paper on this topic was written and is now in press in Limnology and Oceanography. Manganese is an essential component of the photosynthetic electron transport chain and copper is known to inhibit cellular Mn uptake. Experiments were conducted to examine the interactive effects of free Mn and Cu ion concentrations, and light on cellular Mn, chlorophyll *a*, and specific growth rate in the estuarine diatom *Thalassiosira pseudonana*. Copper inhibition of growth rate occurred only at low [Mn^{2+}], an effect that was independent of light intensity and was totally accounted for by Cu's inhibition of Mn uptake rate. Under growth limiting light, cells contained high Chl *a* and Mn concentrations. These cells required a higher cell Mn:C to achieve maximum growth rate than cells grown under saturating light, due at least in part to an increased need for Mn for synthesis of additional photosynthetic units during low-light acclimation. The higher cell Mn required under low light was not provided by higher uptake rates, since these rates were the same in light-saturated and light-limited cells. However, since cell Mn equals the uptake rate divided by the specific growth rate, the cells were able to accumulate the additional Mn needed for low light acclimation by maintaining their uptake rates constant during light limitation of growth rate. Our results indicate that [Mn^{2+}], [Cu^{2+}], and light are likely to interact in controlling algal growth rate since all three vary within the euphotic zone. Algal removal of Mn from seawater should be enhanced under low illumination due to increased cellular Mn concentrations.

Influence of dissolved inorganic iron concentration, light, and cell size on cellular iron and cellular iron growth requirements in coastal algae - A paper on this topic was written and is now in press in Nature. Iron is an important limiting micronutrient, and like Mn, one of its primary requirements is photosynthetic electron transport. Iron limitation of growth rate and cellular iron uptake were examined at high and low light in coastal diatoms (*Thalassiosira pseudonana* and *T. weissflogii*) and coastal dinoflagellates (*Prorocentrum minimum* and *P. micans*) representing a range of cell diameters (~3.5, 11, 12, and 30 µm, respectively). At saturating light (500 µEinst m^{-2} s^{-1}), larger cells required higher external iron concentrations than smaller cells to achieve the same iron-limited growth rate. All species showed a similar linear relationship between iron-limited specific growth rate and cellular Fe:C ratio, and a similar hyperbolic relationship between iron uptake rate normalized to cell surface area and external iron concentration. The larger cells' increased growth requirement for external iron was accounted for by their low surface to biovolume ratios, and resulting low iron uptake rates per unit of biomass. At growth limiting light (50 µEinst m^{-2} s^{-1}), the relationships between specific growth rate and cell Fe:C was also linear, but cells required 4-5 times higher Fe:C to achieve the same growth rate due to the light under-saturation of photosystems and resultant decrease in electron flow per photosynthetic unit. Cells compensated for this reduced efficiency by increasing the number of photosynthetic units, and as a result, cellular iron concentrations increased due to the increased demand for iron-containing electron transport proteins. As with Mn, the higher cell Fe:C needed for low light acclimation was not provided by an increased iron uptake rate as uptake rates were the same in both high- and low-light

acclimated cells. Rather, again as with Mn, the additional Fe was provided by the light-limited decrease in specific growth rate, the effective biodilution rate. Our results indicate that Fe availability, light, cell size, cellular Fe:C and growth rate are all integrally linked. They indicate the cells are more likely to be Fe-limited under low irradiance and that Fe-limited cells will have greater difficulty adapting to low light. Adaptation to low light at the bottom of the euphotic zone in stratified regions will lead to high cell Fe:C ratios and, therefore, high removal of Fe relative to other nutrients (C, N and P). This higher removal should drive these systems toward greater Fe limitation. In the open ocean, such effects may largely explain the observed depletion of Fe within deep chlorophyll maxima. The combined Fe/light limitation favors the growth of small cells, consistent with the observed dominance of small procaryotes and the paucity of larger eucaryotes typically observed in light limited regimes such as deep Chl maxima.

IMPACT

Our results indicate that the uptake and toxicity of Cu, Cd, and Zn are not only controlled by the free ion concentrations of these metals, but also by the availability and cellular concentrations of nutrient metals (Mn, Zn and Fe) with which these metals competitively interact. Thus, factors that influence the cellular concentrations of Mn, Zn, and Fe can greatly influence whether or not a toxic metal will exert an inhibitory effect on algal growth rate or whether a metal (e.g., Cd) is effectively scavenged by phytoplankton. Low light increases cellular concentrations of Mn and Fe and, therefore, should not only influence algal removal of these metals, but also will influence uptake and effects of toxic metals with which these nutrient metals interact.

RELATED PROJECTS

By combining our culture results with measurements of $[Cu^{2+}]$, $[Zn^{2+}]$, $[Cd^{2+}]$, and $[Mn^{2+}]$ in estuarine and coastal waters provided by other contractors (Ken Bruland at UC, Santa Cruz; John Donat at Old Dominion and Jim Moffett at WHOI), we are beginning to formulate the theoretical framework for predicting or assessing the toxicity and biological scavenging of metals. Such modeling for Zn, for example, correctly predicts the extent of Zn removal from waters in Narragansett Bay. By contrast, there is a lack of algal removal of copper in that estuary and our models also correctly predict this observation.