THE RELATIONSHIPS BETWEEN METAL SPECIATION AND METAL-BIOTA INTERACTIONS IN HARBORS

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LONG TERM GOALS

Our long term goal is to understand the interactions between trace metals and phytoplankton in neritic environments. This interaction is envisioned as a two-way process: Trace metals affect the growth, species composition and biomass of phytoplankton. In response, through natural selection, phytoplankton adapt to their chemical environment by genetically altering their responses to trace metals, thus accounting for many of the differences observed among phytoplankton species. These differences lead to trace metals affecting species composition. Another aspect of the interaction between chemistry and biology that we are investigating is how biology in turn affects chemistry--specifically how phytoplankton change the chemical speciation of trace metals in seawater through the production of particular organic compounds. A second long term goal is to use our knowledge of trace metal-phytoplankton interactions to better evaluate the impact anthropogenic trace metal inputs to harbors and neritic waters may have on phytoplankton communities and to evaluate the validity of current water quality regulations.

OBJECTIVES

One objective is to examine the ability of a wide range of phytoplankton species to grow at high concentrations of Cu, Zn, Cd and Pb. The adaptations of different species are compared with respect to their phylogenetic histories and habitat related distributions in order to understand why some species are more resistant to trace metal toxicity than others. Also, clones of the same species from "clean" and "polluted" waters are compared to see if phytoplankton can adapt genetically to high concentrations of trace metals on a

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relatively short time scale. A second objective is to examine phytoplankton species composition along transects in harbors and neritic waters and compare their distributions with the gradients observed in trace metal concentrations and speciation to see if laboratory derived data accurately predicts what is observed in natural waters. A third objective is to determine which organisms and to what extent these organisms alter the chemical speciation of trace metals in natural waters by the production of organic compounds that have high affinities for certain trace metals.

APPROACH

Representative phytoplankton species are being isolated into culture from San Diego Bay, San Francisco Bay, and Los Angeles Harbor and then examined for their ability to tolerate high concentrations of free Cu, Zn, Cd, and Pb. Their steady state growth rates are being measured in incubation chambers in culture media with different buffered trace metal concentrations. These data are being compared with earlier data collected on species isolated from coastal and oceanic waters and Cape Cod embayments. In these harbors and bays, cyanobacteria are being counted and total phytoplankton biomass is being estimated as chlorophyll. These data are being compared to Jim Moffett's data on total and free ionic Cu and Zn concentrations. Various phytoplankton clonal cultures are being challenged with high concentrations of potentially toxin trace metals to determine if they excrete organic compounds that chelate and detoxify them and if they produce phytochelatins.

WORK COMPLETED

Three field trips to San Diego Bay and one to San Francisco Bay have been undertaken and numerous clonal cultures of phytoplankton have been isolated into culture and their growth rates at various free ionic Cu and Zn concentrations have been measured. Copper stressed cultures of phytoplankton have been grown and sent to Jim Moffett for analysis of chelator production and to Beth Ahner for analysis of phytochelatin content. Opportunistic experiments with John Dacey have been conducted in which the effects of Cu, Cd, Zn, Ni, and Cr stress on DMS production were evaluated.

RESULTS

Our previous work with coastal and oceanic phytoplankton had shown that most diatoms are killed by free ionic Cu around 10^{-10}M, dinoflagellates at around $10^{-10.5}$M, and cyanobacteria at around 10^{-11}M, and there were no significant differences between coastal and oceanic species. With the species isolated from Cape Cod estuaries, San Diego Bay, and San Francisco Bay, it is being found that estuarine dinoflagellates are not significantly more resistant to Cu than coastal and oceanic species but the estuarine diatoms are considerably more resistant. Most of the coastal and oceanic diatoms are killed at around 10^{-10}M, but the estuarine species survive up to around $10^{-8.5}$ to $10^{-9.5}$M. No significant clonal differences within a species have been found between "clean" and "polluted" estuaries on Cape Cod, or between "clean" and "polluted" areas of San Diego Bay. Clones of diatom species from a bay or estuary are much more resistant to Cu than clones of the same species isolated from out on the continental shelf. These data suggest that estuarine diatoms are "preadapted" to rather high concentrations of Cu and can tolerate substantial amounts of anthropogenic Cu input, but cyanobacteria and dinoflagellates cannot. The laboratory data agree well with our observations in the Cape Cod estuaries and in San Diego Bay. In our previous work on Cape Cod, free Cu concentrations were never high enough to harm eukaryotic phytoplankton, and chlorophyll remained high. Cyanobacteria remained high in Waquoit Bay and Great Pond which have low total Cu concentrations (around 5nM) and free Cu around 10^{-13}M, similar to the adjacent coastal waters. Cyanobacteria declined by a factor of 10 in Eel Pond and Falmouth Harbor, where total Cu concentrations are around 35 to 65 nM and free Cu concentrations are around $10^{-9.5}$ to 10^{-10}M. These results are exactly what would be predicted from the laboratory studies. We have recently obtained similar but more dramatic results in San Diego Bay. Right inside the entrance to San Diego Bay, free Cu is around $10^{-11.5}$M, but further in and throughout most of the bay, free Cu is around $10^{-9.3}$M. Chlorophyll remains high but cyanobacteria abundance drops 100-fold, as predicted by the laboratory studies. In Shelter Island Harbor, free ionic Cu is even higher, around $10^{-8.5}$M. At this point, not only cyanobacteria but also chlorophyll declines, again as predicted. Even estuarine diatoms are dying in Shelter Island Harbor. Microscopic observations indicate that phytoplankton species diversity is also extremely low in Shelter Island Harbor compared to the rest of San Diego Bay. Our laboratory studies show that cyanobacteria excrete chelators that have very strong affinities for Cu. By contrast, most eukaryotic phytoplankton do not have this capability but do produce phytochelatins. Phytoplankton species that produce DMS have been found to produce 10 to 100 times more when under Cu stress, but apparently not under other types of trace metal stress.

Our Zn toxicity studies show that cyanobacteria are about 100 times more sensitive to Zn toxicity than eukaryotic phytoplankton. Cyanobacteria die at a free ionic Zn concentration of around 10^{-9} while most eukaryotic phytoplankton are still alive at a free ionic Zn of 10^{-7}. The exceptions to this are oceanic coccolithophores and some dinoflagellates, which die at a free ionic Zn of around 10^{-8}.

The fact that cyanobacteria are extremely sensitive to both Cu and Zn toxicity indicates that their decline in abundance in San Diego Bay as well as Cape Cod harbors could be the result of high Cu or Zn or both.

A comparison of clones from “clean” and “polluted” areas indicates that on the time scale of decades, phytoplankton species have not been able to adapt genetically to elevated concentrations of Cu and Zn.

IMPACT

The agreement between our laboratory experiments and field observations give us confidence that we will be able to predict what impact various levels of trace metal inputs into harbors and estuaries will have on the phytoplankton community. The knowledge we have gained on the differences among phytoplankton species allow us to choose appropriate species for use in bioassays. The data are also expected to help us evaluate the validity of current EPA guidelines.

TRANSITIONS

These data are not being used by others yet, but we expect new EPA guidelines could be developed based upon these data.

RELATED PROJECTS

This work is being done in close cooperation with Jim Moffett (also funded by ONR). Opportunistic studies are also being conducted with Beth Ahner and John Dacey.

REFERENCES

Moffett, J.W. and L.E. Brand. 1996. Production of strong, extracellular Cu chelators by marine cyanobacteria in response to Cu stress. Limnol. Oceanogr. 41: 388-395.

Moffett, J.W., L.E. Brand, P.L. Croot and K.A. Barbeau. 1997. Cu speciation and cyanobacterial distribution in harbors subject to anthropogenic Cu inputs. Limnol. Oceanogr. (in press).

Boyer, G.L. and L.E. Brand. 1997. Trace elements and harmful algal blooms. In: Physiological Ecology of Harmful Algal Blooms, Ed. by D.M. Anderson, G. Hallegraeff and A. Cembella. (in press).