

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

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LONG TERM GOALS

The relationship between the chemistry of toxic metals (especially copper and zinc) and their interactions with phytoplankton in harbors is the focus of the project. Our goals are to ascertain how these metals affect marine ecosystems, through effects on phytoplankton species composition and physiology. Long term goals are to develop new tools for assessment of copper and zinc contamination in harbors, both biological and chemical to ask the question "*What levels of Cu discharge into harbors are acceptable in order to preserve a sustainable ecosystem?*".

SCIENTIFIC/TECHNICAL OBJECTIVES

A major objective is to show that *chemical* measurements of Cu chemistry in seawater can be used to predict *biological* effects for a metal such as Cu, where toxicity is influenced by chelators and other metals. Specific objectives to this end include

- To identify and interpret correlations in the water column between measurements of Cu speciation (total Cu, free Cu²⁺ and ligand concentrations) and effects on phytoplankton physiology and species composition.
- To conduct bioassays involving key phytoplankton species isolated by Larry Brand, enabling us to predict how spatial and temporal variability in metal ratios, as well as total concentrations, affect aquatic ecosystems.
- To develop *in situ* sampling probes to assess more accurately the spatial and temporal variability of the chemical parameters which control Cu toxicity (Cu speciation, concentrations of metals like Mn and Fe which ameliorate Cu toxicity).
- To ground-truth these probes by comparing the results with electrochemical analyses.
- To compare directly the data obtained from the *in situ* sampling probes with toxicity testing on waters collected from contaminated and pristine locations.
- To quantify the relationship between water chemistry and the bioaccumulation of metals within natural phytoplankton assemblages. This process will influence the fate and transport of the metals and probably contributes to the toxicity of Cu and Zn to small grazers, an important basis for EPA regulatory criteria for these metals.

A second, important component of this work, is the characterization of strong, selective Cu chelators produced by marine cyanobacteria in relation to Cu stress. Identification of

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this ligand was made during our initial MEQ award, when we were focusing on cyanobacteria as indicator species in contaminated harbors. Our goal is to isolate these materials from culture media in sufficient quantities for structural characterization.

to this end, we are developing a semi-permeable membrane device for the *in situ* collection and subsequent determination of a suite of bioactive metals. These measurements are coupled with

APPROACH

We have compared the relationship between Cu speciation and phytoplankton effects in harbors of increasing complexity. Initially, we examined four coastal lagoons on the south coast of Cape Cod, which had varying degrees of anthropogenic Cu inputs. Subsequently, we have moved on to more complex urbanized systems - San Diego Harbor. The approach has been to collect discrete water samples and characterize metal chemistry and assess phytoplankton effects by measuring phytochelators (produced by phytoplankton in response to metal stress). Effects on species composition have been assessed by using indicator species (cyanobacteria) and by isolating predominate algae in contaminated regimes to determine metal tolerance in culture for comparison with algae in pristine regimes. Metal bioaccumulation as a function of water chemistry is being determined by coupling flow cytometry/cell sorting with elemental analysis by ICP-MS.

A semi-permeable membrane device is being developed for *in situ* accumulation of small bioavailable complexes of a suite of biologically active metals. This device has been deployed in Cape Cod harbors, San Diego Bay and the Elizabeth River (Norfolk, VA). The approach is to deploy the device at stations around the harbors for several days and compare results with electrochemical measurements performed on discrete samples. The waters surveyed vary in contamination, salinity, organic matter concentration and so forth. Deployment of the probes in Norfolk was coordinated with toxicity testing in the waters using EPA protocols. Probes were also deployed in mesocosms to simulate the actual conditions of the test organisms.

Procedures for the isolation of chelators produced by the marine cyanobacterium *Synechococcus* are evaluated by coupling isolation strategies with titrations by polarography. s currently being carried out using a liquid-liquid extraction methodology.

WORK COMPLETED

Results of a 3 year survey of Falmouth coastal ponds were published in 2 papers in *Limnology and Oceanography*. A chelate scale was developed relating half wave potential derived from pseudopolarograms to thermodynamic stability constants for Cu chelators in seawater, as an alternative electrochemical for the characterization of algal produced chelators(submitted).

Probes were deployed in waters around Cape Cod spanning a 1000-fold range in free Cu^{2+} concentration. Two sampling trips to San Diego Harbor were carried out in April

and July for analysis of dissolved trace metals, deployment of probes, and for determination of cyanobacterial densities (by Brand). Isolates of ambient phytoplankton were also collected (by Brand). A sampling trip was carried out to Norfolk in October to assess the performance of the probes in an organic rich, low salinity estuary. Toxicity testing was carried out on the same waters by L. Hall (U. Maryland) according to EPA protocols. Results are not available yet.

A protocol for isolation of Cu chelators produced by *Synechococcus* has been established based on a liquid-liquid extraction methodology. An isolate has been obtained and preliminary characterization of its binding and fluorescence properties has been carried out. Detailed measurements on the production rates of strong Cu chelators in *Synechococcus* cultures, including the effects of Cu stress and growth phase have been completed.

RESULTS

The comparative study of harbors on Cape Cod showed that biologically available Cu^{2+} increases by orders of magnitude when strong Cu binding ligands are saturated by anthropogenic copper, which influences phytoplankton species composition in ways that are consistent with predictions from toxicity studies in culture. Phytochelatins, which are produced by phytoplankton in response to metal stress, are elevated in the Cu contaminated harbors relative to the non-contaminated harbors (Ahner, Morel and Moffett, 1997).

San Diego Harbor showed similar features to the contaminated harbors on Cape Cod; saturation of strong ligands leading to very high free Cu^{2+} (up to $10^{-8.5}$ M Cu^{2+}), and (in 1996), a drastic decline in cyanobacteria. In summer 1997 however, a significant residual population of *Synechococcus* was found in San Diego Bay, contrary to predictions, since Cu was high. Possibly a Cu tolerant mutant had evolved. Unfortunately, we were not able to establish the organism in culture.

The intercomparison between the in situ probes and standard electrochemical agreement indicated that for Cu, Zn, Cd, Pb and Mn, there is a direct relationship between metal uptake by the probes and the concentration of inorganic complexes (including the free ion). This suggests that probe-derived data are very useful for estimating the bioavailable concentrations of these metals.

The production of strong ligands by the cyanobacterium *Synechococcus* has been studied using the chelate scale, revealing the presence of at least two strong ligand classes. One forms a reversible complex with a thermodynamic stability constant of 10^{39} (c.f. the conditional stability constant of 10^{13} revealed by cathodic stripping voltammetry). The other ligand forms an even stronger or non-reversible complex which binds 50% or more of the total copper in the culture media.

We have identified an isolation protocol for strong Cu binding ligands produced by *Synechococcus* under Cu stressed conditions. The protocol involves liquid-liquid extraction coupled with HPLC purification. Preliminary characterization of binding and

fluorescent properties has been carried out. We are planning structural characterization work on the new electrospray mass spectrometer acquired by Nelson Frew at WHOI with DURIP funds. That instrument is at WHOI and is being set up.

IMPACTS

Results suggest that Cu has significant effects at the base of marine food chains, consistent with toxicity studies carried out in culture which are based on free ion activity models of metal bioavailability. Validation of models based on biologically available, rather than total metal concentrations, supports the move within EPA towards the adoption of site specific criteria for contaminants such as Cu, which has important implications for the Navy. Our *in situ* passive sampling devices may be an extremely useful tool in ecological assessment, which may augment or replace costly and problematic bioassays which are currently required on a routine basis.

The chelators produced by *Synechococcus* may represent an important class of biological chelators which are highly selective for copper, and may have useful applications in remediation schemes for contaminated wastewater. Our work indicates that they are an extraordinary strong and selective class of Cu chelators.

TRANSITIONS

The *in situ* passive sampling probes may be useful to the navy as an alternative to costly and problematic bioassays which the Navy must perform as part of the NPDES permit process at naval yards around the country. We are currently outlining a work plan to evaluate the probes for this application, in consultation with personnel at the Chief of Naval Operations (Code N45) and at Norfolk and Puget Sound. Evaluation will involve establishing a robust relationship between probe data and toxicity to the most Cu sensitive organisms in the EPA protocols in the waters currently being assayed, with an emphasis on runoff events. A critical part of this process is consultation with scientists in the Health and Criteria division (4304) at the EPA to establish what is required in order for the EPA to be convinced that this is a valid protocol. We have initiated such consultation this year.

Ultimately, the probes could become a useful tool in harbor-scale assessment studies, coupled with physical and biological measurements, to establish realistic site specific criteria for Cu and other contaminants generated by naval activities in harbors.