# Interactions Among Ligand Production, Chemical Complexation and Speciation, Algal Accumulation, And Sediment-Water Cycling Of Toxic Metals In A Major US Naval Harbor (Elizabeth River, VA)

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## LONG-TERM GOAL

Our long-term goal is to determine the processes controlling the concentrations, chemical complexation and speciation, biological uptake, and cycling of pollutant metals in the water column and sediments of anthropogenically-impacted harbors.

## OBJECTIVES

In conjunction with other investigators funded through the ONR Harbor Processes Program we are performing an integrated study of the biogeochemical cycling of three potentially toxic metals (Cu, Cd and Zn) and two micronutrient metals (Mn and Zn) in the water column and sediments of a major US Naval harbor, the Elizabeth River (VA). We are studying the interrelationships among:
(1) concentrations and chemical complexation and speciation of toxic and micronutrient trace metals (Donat); (2) *in situ* production of Cu chelators by natural microbial populations in response to elevated Cu levels (in collaboration with A. Gordon/J. Donat [ODU]); (3) uptake of metals by phytoplankton and abiotic particles which transport metals to the bottom via particulate settling (in collaboration with W. Sunda/S. Huntsman [NOAA/NMFS]); and (4) fluxes of metals and chelators from the sediments back into the water column (Donat and Burdige). We are focusing on phytoplankton for the metal uptake studies because they are: (a) the principal primary producers, providing the entry point for metals into the food chain, (b) important particulate vectors for vertical metal transport, and (c) may be important *in situ* sources of chelators.

## APPROACH

Our approach consists of both field and laboratory efforts. In conjunction with the Gordon/Donat and Sunda/Huntsman groups, we performed the first of our major field studies of the Elizabeth River in late July <99. We collected surface water samples along a 6-station estuarine transect from Hampton Roads up the Elizabeth River, and bottom water samples and sediment cores from 2 of these stations.

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Water column samples were collected directly into high-density polyethylene (HDPE) and fluorinated linear polyethylene (FLPE) with a peristaltic pumping system, through acid-cleaned Teflon tubing attached to a weighted plastic Afish-vane@, either filtered in line (through 1.0•m and 0.22•m cartridge filters), or not filtered, during collection. Water samples were then double-bagged in plastic ziplock bags and returned to Donat=s clean lab at ODU. Filtered and unfiltered water samples designated for total metal and total dissolved metal determinations were acidified to pH 2 and stored for several months prior to analysis. Filtered water samples designated for metal complexation and speciation determinations were frozen as soon as possible (within 6 hours) and kept dark and frozen until analysis.

Sediment samples were collected in order to determine sediment pore water concentrations of Cu, Cd, Zn, and Mn and complexation and speciation of Cu, Cd, and Zn (Donat), and DOC, DON, alkalinity, and nutrients (Burdige). Sediment cores were collected using a stainless steel or plexiglass box corer, and subsampled using acid-cleaned plexiglass core tubes. Sediment cores were sectioned into 2-cm intervals under $N_2$, and the sediment intervals were centrifuged at in situ temperatures. Porewater samples were filtered (0.45•m); subsamples for total dissolved metals were acidified (pH 2); subsamples for metal complexation and speciation analyses were kept anoxic, cold and dark until analysis. Benthic fluxes were determined using core incubation techniques (Burdige and Homstead, 1994; Skrabal et al., 1997; Burdige and Zheng, 1998).

Water column total and total dissolved metal concentrations were determined by graphite furnace atomic absorption after APDC/DDDC chelation/chloroform extraction (Bruland et al., 1979; Statham, 1985). Complexation and speciation of Cu in the water column samples were determined by competing ligand (salicylaldoxime) equilibration/adsorptive cathodic stripping voltammetry (Campos and van den Berg, 1994; Bruland et al., 1999). Complexation and speciation of Zn and Cd in the water column samples were determined by differential pulse anodic stripping voltammetry at a thin mercury film rotating glassy carbon disk electrode (DPASV/TMFRGCDE, Bruland, 1989, 1992).

Sediment pore water total dissolved Cu concentrations will be determined by chemiluminescence (Sunda and Huntsman, 1991). Sediment pore water total dissolved Zn and Cd concentrations will be determined by DPASV at a hanging mercury drop electrode (HMDE) after UV photooxidation (Donat and Henry, 1997). Cu-, Zn- and Cd-ligand concentrations and conditional stability constants will be determined by DPASV at an HMDE (Donat et al., 1994).

The approach in the Gordon/Donat work involved preliminary laboratory incubations of the Elizabeth River=s natural microbial population to determine the optimum copper concentration (within realistic limits) and the time required to elicit detectable production of copper-complexing ligands by microbial populations at two sites on the Elizabeth River (Norfolk Naval Base and Norfolk Naval Shipyard). In each of the incubation bottles we measured: changes in concentrations and conditional stability constants of the copper ligands produced; the numbers of bacterioplankton, picoplankton, microzooplankton and phytoplankton; and frequency of dividing cells.

## WORK COMPLETED

As described above, we performed the first of our major field studies of the Elizabeth River in late July <99, in conjunction with the Gordon/Donat and Sunda/Huntsman projects. We collected surface water samples along a 6-station estuarine transect from Hampton Roads up the Elizabeth River, and bottom

water and sediment core samples from 2 of these stations, in the Elizabeth River. We have completed the total metal, total dissolved metal, and metal complexation and speciation determinations for Cu, Cd, and Zn in the water column samples. Metal-complexing ligand concentrations, conditional stability constants, and the dissolved speciation of these metals have been calculated. We have also completed the metal benthic flux experiments for the two sites. The total dissolved metal, and metal complexation and speciation analyses for Cu, Cd, and Zn in the sediment pore waters, and in the water-over-core samples for the benthic flux studies, are currently in progress.

To better understand the role of sediment processes in the production of metal-complexing ligands, we have also continued to study dissolved organic matter cycling in marine sediments. Specifically, we have used pore water fluorescence (Burdige et al., 1999a) and carbohydrate data (Burdige et al., 1999b) to begin to quantify a recently-proposed conceptual model for DOM cycling in marine sediments (Burdige, 1999; Burdige and Gardner, 1998).

In the Gordon/Donat work, Gordon and Donat selected incubator-mooring sites, and the necessary clearances were obtained for the experiments at Navy-controlled stations. Water samples were collected from the sites and preliminary copper ligand concentrations and conditional stability constants were measured. Preliminary studies were performed to determine the required Cu concentration and incubation time to elicit detectable changes in ligand concentration in water samples. Evaluation of a metabolic inhibitor for use in these studies was completed. The *in situ* incubation apparatus was designed, constructed and field-tested. Initial experiments were successfully completed at both sites to determine the effect of an elevated copper concentration and addition of a metabolic inhibitor on ligand production by the intact microbial community.

**RESULTS**

All total (unfiltered) and total dissolved (0.22•m-filtered) metal concentrations increased along the transect from Hampton Roads Harbor (Stn 3) up the Elizabeth River (to Stns 7 and 8). Total Cu ranged from 7nM (Stn 3) to 64nM (Stn 8); total dissolved Cu ranged from 7nM (Stn 3) to 51nM (Stn 8) (Fig. 1). Total Cd ranged from 96pM (Stn 3) to 400pM (Stn 8); total dissolved Cd ranged from 98pM (Stn 3) to 338pM (Stn 8). Total Zn ranged from 7nM (Stn 3) to 131 nM (Stn 8); total dissolved Zn ranged from 8nM (Stn 3) to 89nM (Stn 8).

In all surface water samples, dissolved Cu was observed to be nearly completely (>99.86%) complexed by two strong organic ligands. The concentration of the stronger (log K== 12.5) ligand, $L_1$, averaged 42nM (range: 31-60nM), and the concentration of the weaker (log K== 11) ligand, $L_2$, averaged 71nM

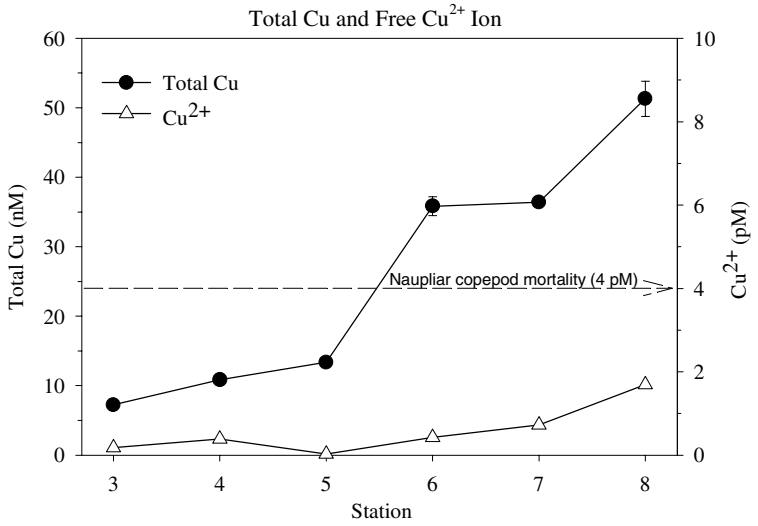


***Fig. 1. Concentrations of Total Dissolved Cu (nM) and the free $Cu^{2+}$ ion (pM) in surface waters of Hampton Roads Harbor (Stns 3,4) and the Elizabeth River (Stns 5-8). Dashed line indicates the lowest free $Cu^{2+}$ ion concentration reported to be toxic to the naupliar stage of the common estuarine copepod Acartia tonsa (Sunda et al., 1990).***

(range: 32-128nM). The concentrations of both ligands generally exceeded that of dissolved Cu. In general, the resulting free $Cu^{2+}$ ion concentrations (the potentially toxic form; Fig. 1) were buffered at levels from 0.025-0.72pM (pCu range: 13.6 to 12.74); however, at Stn 8 the free $Cu^{2+}$ ion concentration increased to 1.69pM (pCu: 11.8). All free $Cu^{2+}$ ion concentrations calculated are below the lower limit concentration of 4pM reported to be toxic to the naupliar stage of the common estuarine copepod *Acartia tonsa* (Sunda et al., 1990).

In the surface waters of Stns 3, 4, and 5, dissolved Zn was observed to be nearly completely (>99.1%) complexed by two strong organic ligands. At these stations, the concentration of the stronger (log K== 10.8) ligand, $L_1$, averaged ~10nM (range: 8.7-14.8nM), and the concentration of the weaker (log K== 9) ligand, $L_2$, averaged ~8nM. At these stations, the concentrations of both ligands exceeded that of dissolved Zn. Organic complexation of dissolved Zn decreased steadily upriver from 95% at Stn 6 to 58% at Stn 8, and only one Zn-complexing ligand was detected at Stns 6, 7, 8 (concentration range: 33-62 nM; log K=~ 9). The resulting free $Zn^{2+}$ ion concentrations (the bioavailable or potentially toxic form) ranged almost 3 orders of magnitude from 9- 25pM (pZn: 10.8) in Hampton Roads Harbor (Stns 3,4,5) to 0.9-24nM (pZn: 7.6-9.1) in the Elizabeth River (Stns 6,7,8). The lowest free $Zn^{2+}$ ion concentrations calculated (9-25pM) are equal to, or above, levels reported to be limiting to neritic phytoplankton growth (Brand et al., 1983), while the highest free $Zn^{2+}$ ion concentrations calculated (~24nM) approach levels reported to be toxic to naupliar stages of the common estuarine copepod *Acartia tonsa* (Sunda et al., 1990).

In the Gordon/Donat work, a time series study conducted in May >99 utilizing copper additions of 50 and 100 nM, and sampling times of 0 and 168 hours. Copper ligand concentrations were observed to increase in a dose-dependent fashion when copper was added to water samples containing intact, natural microbial communities. Based on the results of this experiment, an elevated, but environmentally-realistic, copper concentration (100 nM) and incubation time (one week) for

stimulation of ligand production were selected for the July >99 major field study. Under these conditions, the concentrations of copper-complexing ligands increased over the course of the week after and addition of 100nM Cu, causing the free $Cu^{2+}$ ion concentration to decrease dramatically relative to the free $Cu^{2+}$ ion concentration initially resulting from the 100nM Cu addition.

## IMPACT

The waters and sediments of the Elizabeth River/Hampton Roads Harbor (home of the US Atlantic Fleet) have been heavily contaminated by pollutant metals (Cu, Cd, and Zn) and organics from a variety of civilian and military sources. Harbor waters have been shown to be toxic to copepods, apparently due to extremely high concentrations of free $Cu^{2+}$ and $Zn^{2+}$ ions (Sunda et al. 1990). Pollutant metals entering this harbor/estuarine system have a variety of fates including complexation, uptake by phytoplankton and abiotic particles, particulate transport to the bottom, and ultimately, removal by lateral flow and/or burial in sediments. Metals in the sediments are released into sediment pore waters with the remineralization of biotic particles, and can be returned to the water column by diffusion and resuspension. The dissolved ligands that bind and detoxify metals are derived from a variety of sources including production by water column phytoplankton and release from sediments (Skrabal, Donat, and Burdige 1997, 1999). All of these processes strongly influence the concentrations and physical and chemical species of metals in harbor waters. Moreover, because speciation influences the biological and geochemical behavior of metals, it also controls their fate and effects. Total metal concentrations provide little insight into their environmental fate and effects.

Metal accumulation by phytoplankton is important both for metal entry into the food chain and metal transport to the sediments. However, the uptake and effects of toxic metals are controlled not only by free ion concentrations of these metals, but also by those of interactive nutrient metals. Thus, to predict algal uptake, physiological effects, and biogeochemical cycling of toxic metals it is essential to quantify the free ion concentrations of these important interacting nutrient metals.

## RELATED PROJECTS

Our work is part of an integrated, collaborative study of the complex processes controlling the concentrations, chemical complexation and speciation, biological uptake, and cycling of three potentially toxic metals (Cu, Cd and Zn) in a major US Naval harbor, the Elizabeth River, and its adjacent waters. As discussed above, out work is carried out in coordination with studies by W. Sunda and S. Huntsman (NOAA/NMFS) on the mechanisms and factors controlling algal metal accumulation, and by A. Gordon and J. Donat (ODU) on the *in situ* production of Cu chelators by natural microbial populations in response to elevated Cu levels. Both of these projects are also funded through the ONR Harbor Processes Program.

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