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Transport, Fate and Effects of Silver in the Environment

The University of Wisconsin-Madison
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Editors

Anders W. Andren
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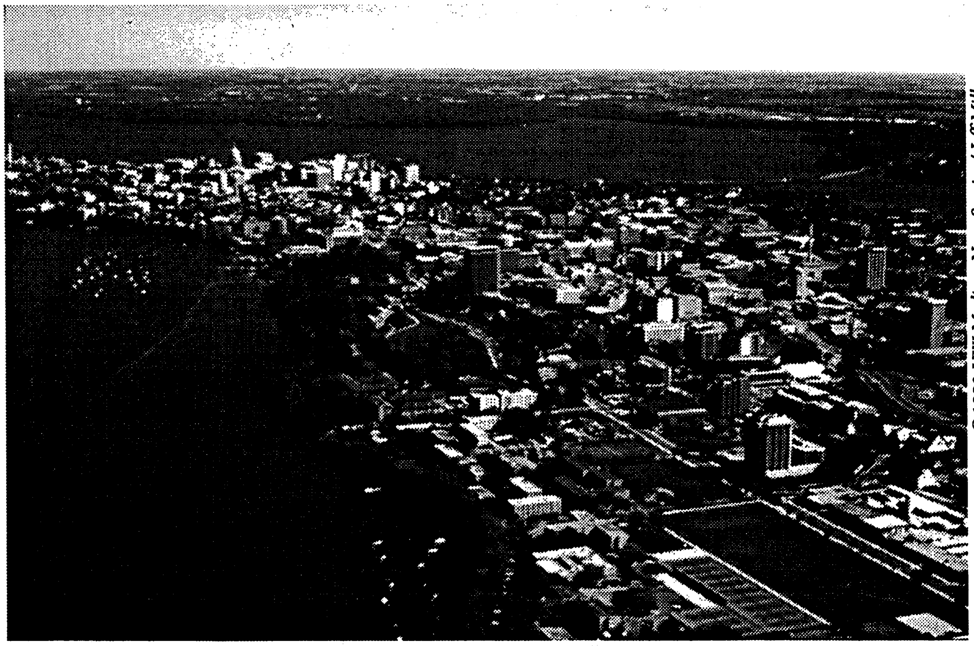
Welcome to Madison



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It is a pleasure to welcome you to this conference at the University of Wisconsin-Madison. The UW-Madison is a world-class university, and those of us associated with it are proud of our accomplishments. We are especially proud of our long history in the area of environmental and multidisciplinary research. The conference brings together experts from the international research community to exchange state-of-the-art information on the transport, fate and effects of silver in the environment.

The behavior of heavy metals in the environment is not a new issue. Indeed, a number of recent conferences have concerned lead, mercury and cadmium. But to our knowledge, no similar comprehensive conference has been devoted to silver. Widespread societal use and recent analytical advances make it an ideal time to assess the environmental cycling and potential impact of anthropogenic silver. The conference, then, provides a forum for up-to-date research results on sources, biogeochemistry, environmental cycling and biological effects of silver in the global environment. By synthesizing current knowledge and identifying informational needs, we hope to more accurately interpret environmental behavior and the potential impacts of silver.

Again, it is indeed a pleasure to welcome you on behalf of the organizing committee and the UW-Madison. We have gathered some of the best scientists involved in the environmental research of silver. I hope you enjoy both the conference and what Madison has to offer.

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EXTENDED ABSTRACTS

Transport, Fate and Effects of Silver in the Environment

University of Wisconsin-Madison

FIRST INTERNATIONAL CONFERENCE

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SESSION A: Environmental Analytical Chemistry of Silver

A.W. Andren and E.A. Crecelius,
Session Chairs

Application of Analytical Voltammetric Methods to the Determination of Silver at Sub-ng/mL Levels

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Introduction

Processing of sensitized photographic materials can result in some release of silver to the environment. Normally the procedures used for silver analysis determine the total amount of silver in the samples. However, in the environment, "free" silver (labile silver complexes) are of great interest. It is, therefore, desirable to establish an upper limit for concentrations of "free" silver (labile silver complexes) that may be present in effluents and surface waters. Potentiometric measurements of silver ions utilizing a silver sulphide ion selective electrode is a generally accepted method of analysis for ionic silver. However, the potentiometric method is not applicable to all types of environmental samples, especially those that are relatively free of complexing agents such as river surface water samples. Therefore, a square-wave voltammetric anodic stripping voltammetry at a glassy carbon electrode was utilized to determine silver ion concentrations at sub-ng/mL levels in aqueous solutions.

Electroanalytical techniques appear to be one of the best approaches available to study bioavailability of metal ions and their complexes with organic and inorganic ligends (1). Electrochemical methods allow the measurement of labile metal ion concentration which consists of the "free" metal ion and the metal that can dissociate in the double layer to be detected at the electrode. Even though electrochemical techniques have their limitations, a good correlation exists between the electrochemical lability and the toxicity of several metal ions found in natural waters (1). Measurement of silver ions at concentrations as low as 0.4 ng/mL (2) have been reported using ion-selective and chemically modified electrodes (3, 4, 5), graphite (2, 6), glassy carbon (7, 8), carbon fiber (9), and gold electrodes (10).

This study was initiated to develop an electroanalytical method to determine silver ions and labile silver complexes present in effluents and surface waters. Calibration curves were established and the response of the electrodes was studied using silver nitrate standard solutions at concentrations ranging between 0.1 ng/mL and 10 µg/mL. Various types of aqueous samples were also analyzed.

Background

Osteryoung square-wave stripping voltammetry is one of the most sensitive electroanalytical techniques which combines multielement measurement capability and speciation of metal ions with speed. Stripping techniques involve two steps. First, "free" metal ions and metal ions from labile metal complexes are deposited onto an inert electrode surface at a constant potential. During the deposition step the analyte of interest is brought to the surface of the electrode by diffusion and/or convection. This preconcentration step is followed by a stripping step which causes dissolution of the deposited metal. In Osteryoung square-wave stripping voltammetry, a symmetrical potential waveform superimposed on a ramp changing at a fixed frequency is applied to the electrode during the stripping step. The peak current that is measured is directly proportional to the amount of metal deposited on the electrode. The preconcentration step can be viewed as an effective electrochemical extraction in which the analyte is preconcentrated on the electrode surface to a considerably higher concentration than it exists in solution.

Experimental

A Bioanalytical Systems (BAS) Model 100A or a Princeton Applied Research Model 384B electrochemical analyzer was used in conjunction with a Pine Instrument Company Model AFCPRB rotator. Glassy carbon (GC) rotating disc electrodes were purchased from Pine Instrument Company. Silver standard solutions were prepared daily by appropriate dilutions of a Spex Industries 1000 µg/mL $AgNO_3$ standard and protected from room light. Chemicals used were all reagent grade or better (>99.99% pure). Electrodes were polished using different grades of alumina or diamond paste before analysis. All glassware was cleaned with acid and stored in dilute nitric acid solution when not being used.

Results

Standard silver nitrate solutions at concentrations between 0.2 ng/ml and 1 ng/mL resulted in one, two or three dissolution peaks depending on the electrode pretreatment procedures and the age of the electrode. The presence of more than one dissolution peak for silver suggests that the deposition step is a complex process, and the coverage is multilayer. The response was very dependent on the electrode pretreatment conditions. It was critical that extreme care had to be taken when preparing solutions, cleaning glassware and preparing the electrodes. The acetate buffer blank showed a broad oxidation peak around 0 V vs SCE. This peak is believed to be due to the oxidation of the carbon functional groups present on the glassy carbon electrode surface. The silver dissolution peak normally appeared at 251 mV vs SCE. However, silver dissolution peaks at around 0 V and +123 mV vs SCE were also observed. In case there was more than one dissolution peak for silver, the peak currents of the different peaks were added to establish the calibration curves. The relative standard deviation (RSD) for 0.2 ng/mL samples varied between 7% to 25%, whereas the RSD for 1 ng/mL samples averaged 4%. Two types of environmental water samples were analyzed to learn more about silver speciation using anodic stripping voltammetry. The environmental samples were prepared by taking 90 mL of the sample and adding 10 mL of acetate buffer to it before analysis. It was found that addition of a complexing agent such as EDTA was necessary when analyzing some of the analytical samples to mask the response due to other metal ions that may be present. The addition of EDTA corresponding to a concentration of 10 µg/mL resulted in the decrease of the silver peak by 20%, but was effective in masking the response due to other electroactive species which interfered with silver speciation. The total silver found in environmental samples by inductively coupled plasma analysis and the "free" silver ions determined by anodic stripping voltammetry were in good correlation. It was necessary to calibrate the electrode every day before analyzing environmental samples. A typical calibration curve may be seen in Figure 2. Calibration curves obtained had slopes which varied between 0.7 to 3.3 µA per nanogram silver per mL solution as a function of electrode used and the activity of the electrode surface. It was normal to not see a response for silver ions if the electrode was prepared improperly for electroanalysis. It was also observed that the stripping process was not 100% complete. This required the application of a positive potential to the electrode surface before each analysis to ensure the removal of residual silver. The measurements at sub-ng/mL levels are still quite challenging because of the difficulty in reproducing electrode surface conditions.

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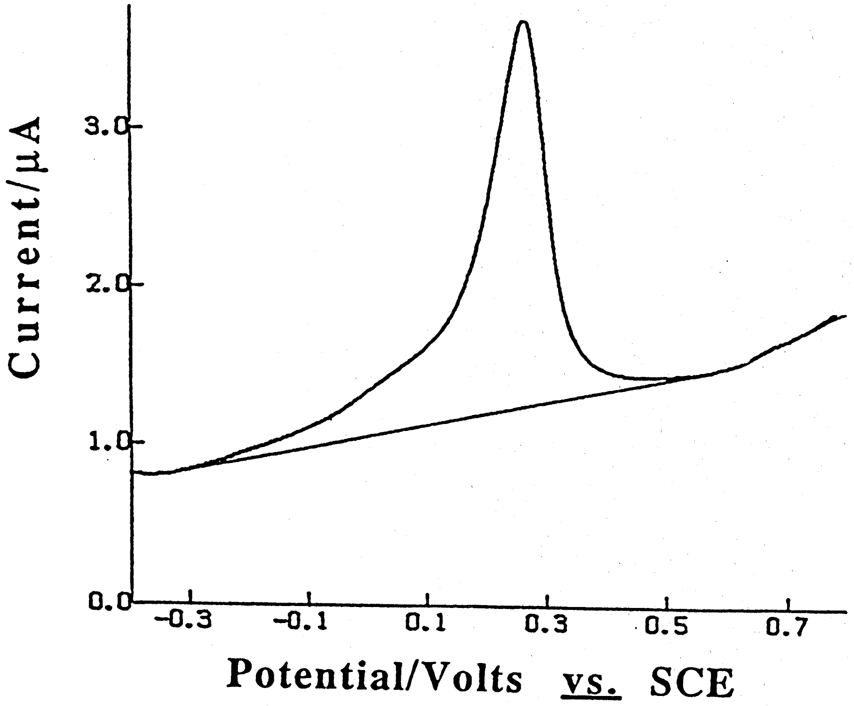

Figure 1. Typical square-wave voltammogram for the stripping of 0.4 ng/mL silver in 0.1 M sodium acetate buffer; pH = 4.5 at a rotating glassy carbon electrode, rotation rate of 3000 rpm, deposition potential of -0.4 V vs SCE for 5 minutes.

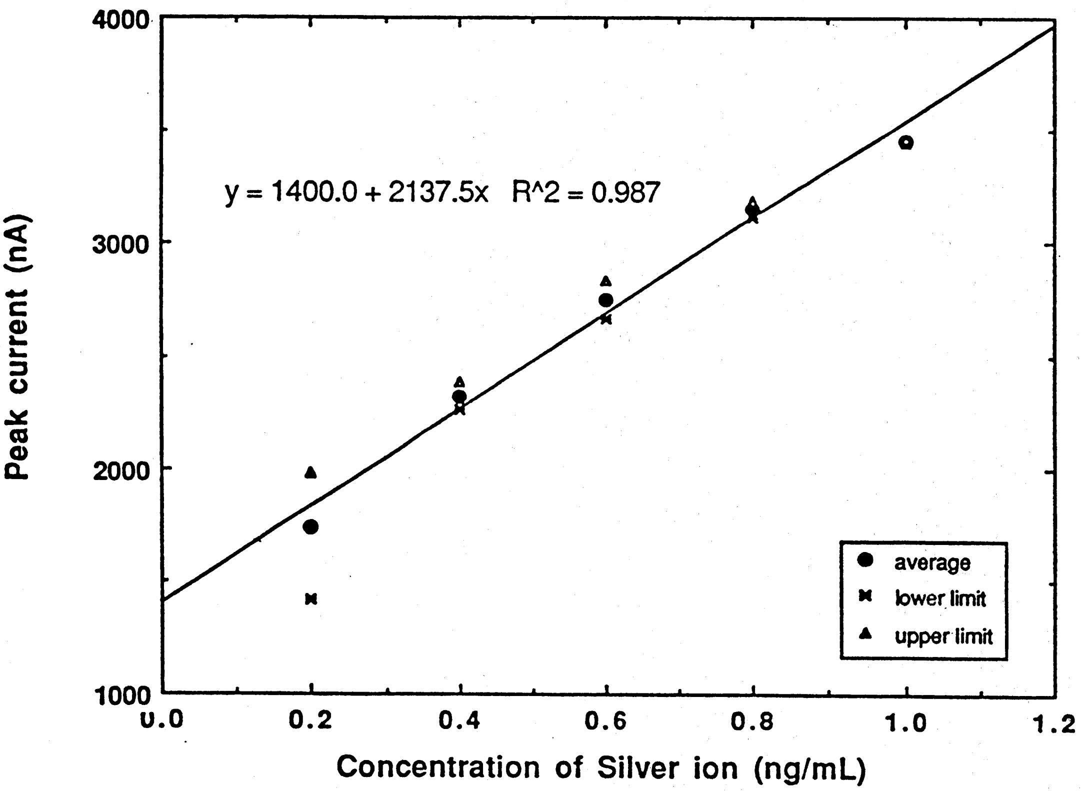

Figure 2. Calibration curve obtained for silver ion concentrations 0.2 ng/mL to 1.0 ng/mL. Experimental conditions are the same as in figure 1

Adsorption Characteristics of Fe Oxide-Coated Granular Activated Carbon: Implications for Silver

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Introduction

In these studies, the ability of GAC to adsorb Cu(II) increased with increasing Fe oxide surface coating. In contrast, removal capacity for a silver-thiosulfate complex decreased when an Fe oxide coating was present. Reasons for these differences are related to the removal mechanisms. Whereas Cu(II) removal primarily involves adsorption, Ag(I) removal probably involves reduction at the GAC surface.

Discussion

The adsorption capacity for Cu(II) increased from 1.3 mg/g for GAC to about 5 mg/g for 37FeGAC (37 mg Fe/g adsorbent). Column tests with 72FeGAC were not conducted, but the results of the batch adsorption tests suggest this composite adsorbent would have a capacity of about 7 mg Cu(II)/g. Although these capacities are considerably less than the approximately 200 mg/g estimated for the ion exchange resin, these tests did not identify an upper limit for the Fe oxide coating. Furthermore, alternative coating processes might result in an Fe oxide surface with increased capacity.

Although some Cu(II) was retained by the composite adsorbent during regeneration, the residual adsorbate did not interfere with subsequent adsorption cycles. In fact, tests through several cycles demonstrated that the fraction of slowly reversible adsorbate actually decreased with increasing cycles. It seems that a fraction of the surface binding sites are high energy, slowly or irreversible sites that accumulate Cu(II) during each adsorption cycle. Even when these sites become full, other lower energy, reversible sites are still able to adsorb and desorb Cu(II) through numerous cycles. None of the adsorption cycling tests, however, ran until the column reached 100% breakthrough, and the site characteristics may change when the adsorbent is fully loaded. This concept seems to be supported by results suggesting that surface precipitation may play a role in adsorbate removal.

Effective regeneration using a small volume adjusted to pH = 2 may be a significant advantage relative to ion exchange processes. A typical ion exchange process would be regenerated with a solution of either 10%H_2SO_4 or 15%HNO_3. As a result, much larger volumes of regenerant must be managed.

The composite adsorbent performed well during tests conducted at low influent Cu(II) concentrations. Removal was consistently down to trace levels, and it seems likely that regeneration could be used to concentrate Cu(II) to levels appropriate for recovery by other techniques.

Finally, removal of complexed Cu(II) depends on the ligand and the characteristics of the complex. Even when a very strong ligand such as EDTA was present removal was possible but the capacity for total Cu(II) removal decreased from about 5 mg/g to about 0.5 mg/g. Adsorption of the Cu(II)-EDTA complex was probably due to exposed GAC.

This Fe oxide-GAC composite adsorbent was not effective at removing a silver-thiosulfate complex from solution. Removal of complexed silver by GAC may involve adsorption followed by a surface reduction reaction. This removal mechanism is consistent with the observed change in pH during column tests and with the minimal silver recovery during column regeneration. When an Fe oxide coating is added to the GAC surface, the surface redox sites may be blocked or inhibited.

Conclusions

Fe oxide-coated GAC performed well as a composite adsorbent for the removal of low levels of Cu(II) from solution. Results from these studies indicate that this solid can be used in column adsorption processes to not only remove but also recover adsorbed Cu(II). This technique looks especially promising for treatment to low levels; a column loaded with 100 µg Cu(II)/L produced an effluent with no more than 3 µg Cu(II)/L through 1000 bed volumes processed. Removal capacities of at least 5 mg Cu(II) per g adsorbent were achieved in column tests. The adsorbent was regenerated using a solution adjusted to pH = 2 and the amount of Cu(II) recovered increased from about 30% in the first cycle to over 90% in subsequent cycles. Retention of adsorbed Cu(II) by solid did not interfere with subsequent adsorption steps through 15 cycles.

The oxide-GAC combination could provide a useful framework for developing specific adsorbents. Future research will examine the effect of the Fe oxide formation pathway, the influence of the GAC substrate characteristics, and adsorbate behavior in the presence of cocontaminants. For example, use of a pre-oxidized GAC substrate may yield a composite adsorbent that does not "trap" Ag(I) through reduction to the elemental state.

SESSION B: Metal Speciation in Aquatic and Terrestrial Systems

J. Kramer, Session Chair

Metal Speciation in Aquatic Systems

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The accumulation of toxic metals in the biosphere will continue to be a serious environmental problem, even if the immediate sources of pollution will be removed in the near future. Thus leachates from waste deposits, transfers from contaminated sediments to the water column and releases due to ground and surface water acidification will cause continuing problems.

To present suitable steps for lowering the concentrations of toxic metals circulating in biota, it has become of urgent importance to understand the conversion, transport and bio-uptake of these metals. These processes are often closely related to the speciation of the elements, *i.e.*, a characterization of the individual physico-chemical forms of the element that together make up its total concentration. It is now well established that a knowledge of the speciation of a metal is a necessity to be able to understand its toxicity as well as its transport in natural waters. It has been shown that biological availability of dissolved trace metals often can be correlated through the activity of the free metal ion. Investigations of biological availability of trace metals therefore require means of determining the activities (or concentrations) of free metal ions, and of other relevant chemical species in case the free metal ion activity concept is not valid.

The speciation of a metal in natural aquatic environments is affected by a large number of different, simultaneously occurring processes. This web of processes comprises various complexation reactions in the aqueous phase, precipitation and dissolution reactions, sorption at surfaces of particulate matter and so on.

Trace metal speciation in natural waters has been based on the use of two different techniques: experimental measurements and computer chemical modelling. The first approach is still limited by the lack of experimental techniques sufficiently sensitive and selective to detect individual species at the concentrations normally found in natural systems.

The computer modelling approach to trace metal speciation in waters involves the use of thermodynamic data, together with known concentrations of reacting components, to compute the equilibrium concentrations (or activities) of the various species. The validity of the assumption that the system being modelled at equilibrium depends on the processes included in the model. Inorganic complexation reactions usually equilibrate quickly, whereas organic complexation reactions are slower, especially when natural organic substances are involved. Precipitation/dissolution processes are also slow, and frequently accompanied with time dependent phase transformations. Many adsorption reactions of trace metals onto particles are accompanied by slow incorporation of the adsorbed metal into the solid matrix so that no true equilibrium is obtained.

The validity of the equilibrium assumption should be considered carefully in speciation modelling exercises. A comparison of results from the model with data for the real system will help to identify gaps in our information and to reveal limitations in applying equilibrium model.

Besides scrutinizing the equilibrium concept, we have to face other limitations when applying an equilibrium model:

i) Important chemical equilibria with pertinent thermodynamic data have not been considered in formulating the model. The naturally occurring solid phases are very seldom pure substances but rather solid solutions. At the present time, reliable thermodynamic data for most of these solid solutions are lacking (especially at low temperatures). Instead idealized end-members of the different solid-solution series have been used as approximations. Furthermore, the chemical nature of naturally occurring organic substances are difficult to characterize. This is also the case thinking of particle surfaces in terms of functional groups and binding site concentrations.

ii) Available thermodynamic data are not always as accurate as one would wish. Temperature, pressure and medium dependences of formation constants may require refinements.

As a concluding remark, it should be stressed that a presented equilibrium model is to be regarded as a working hypothesis of how a real system may function chemically. It is inevitable that some results will be subject to revision as more detailed information of the real system becomes available. Computer modelling has made an important contribution towards an understanding of speciation in natural waters, and will continue to be an important technique in the future.

Silver Speciation in Seawater: The Importance of Sulfide and Organic Complexation

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Introduction

Silver, one of the most toxic of the heavy metals in aquatic ecosystems, has a $^{96}LC_{50}$ level (concentration resulting in 50% mortality in 96 hours) for the American oyster, *Crassostrea virginica*, of about one-hundredth of that for zinc or copper. The toxicity of silver to aquatic organisms has been of little concern because concentrations in the environment were considered to be too low to produce adverse effects. However, the amounts of silver that continue to enter surface waters from industry and municipal sewage may result in increased accumulation of silver by various aquatic organisms in local environs and could result in toxic effects.

Studies with other heavy metals show that their toxicity is often more dependent upon the concentration of complexing ligands than on the total dissolved concentration of the metal. Assuming that this also applies to silver, its distribution among the various aqueous complexes and the thermodynamic activity of its bioavailable complex(es) will determine its bioaccumulation in and toxicity to aquatic organisms. The limited research on silver suggests, based on a plot of toxicity versus the aqueous complex activity, that the neutral complex, $AgCl^0$, may be the primary bioavailable species for yeast. Recently, the data of Andrew et al. on Cu toxicity to *Daphnia magna* has been reexamined using geochemical modeling and multivariant statistical testing. The results suggest that the hydroxide species of Cu are the most toxic species whereas the uncomplexed Cu^{2+}, and other highly correlated species, showed little toxicity in contrast to previous statements based on plotting that the Cu^{2+} ion is the most toxic species. These results indicate that although $AgCl^0$ may be a toxic species, determining whether it is the primary toxic species will require consideration of all the aqueous species and application of a quantitation technique.

The inorganic composition of open ocean water shows minimal spatial and temporal variation in chemical constituents except in upwelling areas. For example, the pH of open ocean water varies over a very narrow range of 8.0 ± 0.7. In contrast, fresh water inflow may cause significant variations of dissolved constituents in coastal waters. For a small group of constituents of important to silver speciation, concentrations may vary notably both spatially and temporally even in the open ocean. For example, sulfide concentrations of 1.7 to >100 $\mu g\ L^{-1}$ have been measured in estuarine water columns and above anoxic coastal sediments. The disequilibrium coexistence of sulfide and oxygen is apparently due to a kinetic constraint on the oxidation of S[-II].

Dissolved organic carbon (DOC) concentrations in seawater varies spatially and temporally. For open ocean water, Duursma reported a DOC range of 1.32 to 3.32 mg L^{-1} of C, Dawson gave 3.40 mg L^{-1} of C as the average concentration and Stumm and Morgan state that the average DOC concentration in seawater is 5.00 mg L^{-1} of C. Organic carbon concentrations of estuarine water compiled by Jenne range from 1.9 to 17 mg L^{-1}, although most values fall between 2 and 6 mg L^{-1}. Some of the dissolved organic compounds in the sea may complex some heavy metals. However, the importance of this complexation for silver is unknown because of the difficulty of measuring complexation capacity at the observed concentrations of silver. Jenne et al. suggested that the calculated oversaturation of San Francisco Bay water with respect to $Ag_2S(s)$ might be due, at least in part, to organic complexation.

Our immediate objective was to investigate the effect of several variables on the equilibrium distribution of silver complexes in coastal waters via sensitivity analysis using a comprehensive geochemical model. The longer range objective was to provide rational for the design of laboratory experiments for the evaluation of silver bioavailability in coastal marine waters including the identification of any additional analytical parameters needed.

Methods

Speciation and saturation indices were calculated with the MINTEQ (version K1) geochemical model. The following complexes were added temporarily to the thermodynamic data base for this study: Ag-amino acid, Ag-2 amino acid, Ag-carb, 2Ag-carb, Ag-2 carb, Ag-phenol. Silver concentrations of 0.5 and 1.0 ng L^{-1}, at pH values of 7.0, 7.5 and 8.2, S[-II] concentrations of 0.0, 0.1, 1.0 and 10.0 µg L^{-1} and DOC concentrations of 1.0 and 5.0 mg L^{-1} for salinities of 27.0, 29.5 and 32.0 ‰ were used. The concentrations of other dissolved constituents at the indicated salinity levels were computed from their concentration at 35 ‰ given in Nordstrom et al. In these sensitivity analyses, the concentration of one constituent was varied while the remaining constituents were held at their base concentrations (e.g., Ag = 0.5 µg L^{-1}, salinity = 30 ‰, pH = 8.2, DOC = 1 mg L^{-1}, S[-II] = 1 µg L^{-1}).

Results

Acanthite, Ag_2S, calculates to be one-half to two orders of magnitude oversaturated at a sulfide and silver concentration of 0.1 to 10 µg L^{-1} and 0.05 ng L^{-1}, respectively. All other silver solids calculate to be undersaturated.

Inorganic Complexes

The sensitivity analyses show that the aqueous species that constitute greater than 1% of the total dissolved Ag are $AgHS^0$, $AgCl_2^-$, $AgCl_3^{2-}$, $AgCl_4^{3-}$. The activities of Cl complexes increase with salinity as the Cl^- activity increases. The most abundant aqueous species is the silver bisulfide complex $AgHS^0$, even at S[-II] concentrations only slightly above 0.01 µg L^{-1} is the activity of this complex of the same order of magnitude as the most abundant Cl complex.

Organic Complexes

No conditional stability constants for natural dissolved organic matter complexes of Ag were found. As a first approximation of the content of the organic matter, we used the available compilations of specific organic compound concentrations in seawater and compilations of stability constants for complexes of silver with organic compounds to obtain a series of representative reactions and stability constants. Stability constants for Ag are available for only a few of the many organic compounds whose concentrations have been determined in seawater.

Using the above data, the organic complexation with silver can be calculated by: 1) assuming three functional group categories for the organics, which form silver complexes and can be represented by the stability constants; 2) using the average molecular weight; and 3) using the percent distribution of the organic compounds by class calculated from Duursma and alternatively by Dawson.

The activities of the silver-organic complexes were very small, comprising much less that 1% of the total dissolved Ag. A five times increase in the total DOC from 1 mg L^{-1} to 5 mg L^{-1} resulted in a significant increase in the activities of silver-organic complexes but did not change the activity of the free metal ion or the chloride complexes. A dissolved organic matter concentration of several hundreds of milligrams per liter would be required to decrease the neutral Cl complex activity by even as much as 5% at S[-II] = 0 and a much higher concentration of DOC would be required at S[-II] concentrations above zero.

Discussion and Conclusions

The importance of the variables examined on the speciation of aqueous Ag decreased in the order S[-II], (0.0 to 10.0 µg L^{-1}), pH (7.0 to 8.2), salinity (27 to 32‰) and dissolved organic carbon (1 to 5 mg L^{-1}). The overwhelming effect of even low S[-II] concentrations on speciation indicates the importance of accurate analytical determination of sulfide concentrations in order to reliably compute the Ag speciation. The aqueous speciation of Ag is also dependent on the pH and Cl concentration; predictably, the activity of the silver chloride complexes is dependent on the salinity of the solution. Assuming the $AgCl^0$ is the principal toxic species, then any decrease in pH to below 8.2 would result in a significant increase in toxicity for silver, because of the decrease in the silver hydroxide species and corresponding increase in the silver chloride species with decreasing pH. This suggests that pH must be closely monitored in toxicity studies with an accuracy of 0.05 pH units clearly required but an accuracy of 0.01 pH units desirable. Assuming that the $AgCl^0$ complex is the one that is bioavailable, then the accumulation and toxicity of the Ag will decrease significantly with increases in S[-II] and will decrease significantly with decreases in salinity. We combined the limited available data on organic carbon content in marine waters with the available data on the stability constants of silver with organic compounds to obtain a series of reactions to represent the complexation of Ag and DOC in seawater. Using these reactions and constants, organic complexation of Ag was found to be insignificant.

Acanthite Ag_2S(s) was calculated to be oversaturated by 0.5 to ~ 2 orders of magnitude in waters containing S[-II] in the 0.1 to 10 µ L^{-1} range. Jenne et al. similarly reported acanthite to be oversaturated by many orders of magnitude in Sacramento River water assuming a sulfide concentration of 0.01 µg L^{-1}. Although acanthite is a common silver mineral and known to form in hydrothermal veins, it is not clear if it forms at ambient temperatures. Although it is not known if acanthite or amorphic Ag_2S precipitates from seawater at ambient temperatures, the potential for acanthite formation further emphasizes the importance of determine the sulfide concentrations of waters used in studies of silver speciation and toxicity.

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Cowan, C.E., E.A. Jenne and E.A. Crecelius. 1985. "Silver Speciation in Seawater: The Importance of Sulfide and Organic Complexation." *Marine and Estuarine Geochemistry.* Lewis Publishers, Inc., Chelsea, Michigan. Reprints can be obtained from Eric Crecelius, Battalle/Marine Sciences Laboratory, 1529 West Sequim Bay Road, Sequim, Washington 99368.

Adsorptive Behavior of Silver to Synthetic and Natural Sediments in Aqueous Systems

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In natural waters, silver can exist in oxidation states (0) and (I) and both of these can occur both in dissolved or particulate forms. Reduction to elemental silver (oxidation state 0) can take place in intermediate reducing conditions or via photoreaction. In turn, the reduced silver can remain in dissolved form, or more likely, as a colloidal suspension. It is also possible that, due to induced charge effects in the surrounding hydrate ions, Ag(0) becomes attached to solid matter such as suspended organic and inorganic particles, bottom sediment or soil.

Modeling efforts indicate, however, that monovalent silver compounds will dominate in natural waters [1]. In solution, Ag(I) can be found as the hydrated, so-called "free" cation, or it can participate in reactions leading to a variety of charged and uncharged forms. For instance, the formation of the chloro species $AgCl^0$ and $AgCl_n^{(n-1)-}$ (with n = 2 - 4) seem to be very important, especially in brackish and marine waters. The association with dissolved organic material, such as humic or fulvic acids, may also be very important. Other possible dissolved reaction products may be $Ag(OH)^0$, $AgSO_4^-$, Ag_2CO_3, etc.; anoxic conditions can lead to formation of sulfide and hydrogensulfide compounds. Precipitation of oversaturated solids like $AgCl_{(s)}$ or $Ag_2S_{(s)}$, or adsorption to the various types of solid material present, result in the formation of particulate silver species. Which of these reactions are actually preferred is determined by parameters such as pH and the ionic strength of the solutions, presence of reducing or anoxic regimes, the concentrations of possible reaction partners and of other cations capable of competing with silver for these partners, the amount of solid matter present, etc. [4].

A knowledge of the distribution of the various species is important for judging the pathways Ag can take in a water body. All reaction products mentioned above can be transported in dissolved form or become themselves bound to particles, depending on their individual thermodynamic and kinetic properties. Dissolved forms are more available for uptake by biota. Particle-bound species are probably less available to biota and tend to settle out in association with sediments. Certain sedimentary conditions may cause desorption and redissolution of silver back into the water column. All these reactions are part of a dynamic distribution process and change in response to altered surrounding conditions.

In order to understand the mobility of silver in a given environment, it is necessary to specify the details of this speciation/adsorption process. To date, only a few species can be experimentally identified and their concentrations measured. To obtain some of the needed information, we determined the silver adsorption potential of selected model and natural sediments in equilibrated suspensions under a variety of conditions. We then used these experimental data to calculate the speciation of silver for various types of simulated natural waters. Computer simulations were carried out with MINTEQA2 [2], a geochemical equilibrium speciation program, which calculates the distribution of silver in a user-defined environmental setting. For our experiments, we prepared solutions simulating conditions found in natural waters of fresh and marine type and used MINTEQA2 to predict the dominant silver forms for these solutions. The "free" silver cation, Ag^+, and, to a somewhat lower extent, dissolved silver chloride, $AgCl^0$, should be the most prominent species for oxygenated regions of low salinity. In oxygenated marine waters, polychlorinated species are predicted to be the main forms. Modeling anoxic conditions by including the presence of HS^- showed $Ag(HS)^0$ to become the dominant species in fresh water solutions and even to be a major form in marine type waters. Precipitation of $AgCl_{(s)}$ or $Ag_2S_{(s)}$ was only to be expected for high concentrations of the respective anions. Other silver compounds like $Ag(OH)^0$ or $AgSO_4^-$ were not found to play a significant role under our experimental conditions.

The adsorbing materials we selected to prepare the sediment suspensions were bentonite, a common clay; $MnO_{2(s)}$ and $Fe(OH)_{3(s)}$, both known strong adsorbers for trace metals; goethite, the main iron oxide in soil; and a lake bottom sediment taken from Lake Michigan. In general, the surface charge of an adsorbing particle plays an important role for its adsorption capacity. This charge can be characterized by the material's zero point of charge, ZPC; that is the pH at which the particle suspended in a solution appears to be uncharged. We mainly worked at a pH value of 8, so we could expect bentonite (with a ZPC of 2.5) and MnO_2 (2.8) to be negatively charged and the iron oxides (with ZPCs of about 8) to be either uncharged or slightly positively charged. Due to its heterogeneous composition, the lake sediment has no defined ZPC; it contains probably all of the substances mentioned above and additionally a large amount of organic carbon.

The individual sediment suspension were spiked with a known amount of aqueous silver nitrate solution, equilibrated for a defined period of time, and then analyzed for the Ag contents in the equilibrated solution and on the sediment by graphite furnace atomic absorption spectroscopy. We determined the adsorption density and the equilibrium concentration of dissolved silver over a range of initial silver concentrations to generate adsorption isotherms for each sediment material. Conditional adsorption coefficients were obtained by using an equilibrium approach suggested by Oakley, et al. [3]. Here, parameters with influence on the adsorption equilibrium like pH, ionic strength and concentrations of major ions, are considered to be fixed and figured into the equilibrium constant. The adsorption equilibrium can then be written as:

$$Ag(I) + SO_T \overset{K_{ads}}{\leftrightarrow} Ag\text{-}OS$$

$$\text{with } K_{ads} = \frac{[Ag\text{-}OS]}{[Ag(I)](SO_T)}$$

where [Ag(I)] is the soluble silver concentration of all species, (SO_T) is the amount of sediment in g/l and [Ag-OS] is the concentration of the silver-solid complex. The equation can be rearranged to give:

$$[Ag\text{-}OS]/(SO_T) = \Gamma = K_{ads}\,[Ag(I)]$$

For regions where the silver concentration is much smaller than the total number of available adsorption sites, plotting the adsorption density Γ vs. the dissolved silver equilibrium concentration [Ag(I)] over a range of silver starting concentrations results in straight line with K_{ads} as its slope. Comparison to conditional adsorption coefficients obtained for trace metals in other studies showed silver to lie in the range of strongly bound metals, like mercury, cobalt or zinc [3].

In general, under all experimental conditions, the lake sediment showed the highest adsorption capacity of all the solid phases we examined. This is perhaps expected since there are a large number of adsorption sites present in this material. For a sediment loading of 100 mg/l, the adsorption potential of the substances for freshwater conditions was found to follow the order: lake sediment > bentonite ≈ manganese dioxide > iron hydroxide > goethite, with a hundred-fold difference between the strongest and weakest adsorbers. This order is consistent with the surface charges of the sediments. Since the major silver species present here was predicted to be the silver cation, a higher silver amount is adsorbed by the negatively charged materials. In sea water, the order was found to be lake sediment >> bentonite ≈ goethite > iron hydroxide > manganese dioxide, again with a hundred-fold difference between the strongest and weakest adsorbers. In high salinity regions, negatively charged chloro complexes should be the dominating silver species, so the adsorption potential for the three weakest adsorbers is consistent again with their surface charge. The relatively high adsorption capacity of bentonite is somewhat surprising, as it cannot be explained by charge effects alone. It may be a hint for an additional mechanism (surfaced catalyzed precipitation, perhaps) taking place. Further investigation of this result is planned. The amount adsorbed in the low and high salinity solutions was surprisingly similar. In fresh water, the three strongest adsorbers took up greater than 95% of the initially added Ag in solution, which was only slightly more efficient than that adsorbed in higher salinity waters. For most of the adsorption experiments, we found Langmuir type adsorption isotherms. Prior to modeling this kind of adsorption with MINTEQA2, some modeling parameters had to be determined using the experimental results. For this, the following adsorption equilibrium between a free silver cation Ag^+_f and an unoccupied surface site SO was assumed:

$$Ag^+_f + SO \overset{K_L^{act}}{\leftrightarrow} Ag\text{-}OS$$

$$\text{with } K_L^{act} = \frac{\{Ag\text{-}OS\}}{\{Ag^+_f\}\{SO\}} = \frac{[Ag\text{-}OS] \quad \alpha_{AgOS}}{[Ag^+_f][SO]\ \alpha_{Ag+f}\alpha_{SO}}$$

where {} represents an activity, [] a concentration and α an activity coefficient. Activity coefficients for solid phases are defined to equal unity, so the activity dependent equilibrium constant is connected to the experimentally obtained concentrations in a very simple way:

$$K_L^{act} = K_L/\alpha_{Ag+f}$$

$$\text{with } K_L = \frac{[Ag\text{-}OS]}{[Ag^+_f][SO]}$$

$[Ag^+_f]$ and α_{Ag+f} were calculated with MINTEQA2 from the experimentally obtained equilibrium concentration of dissolved silver. The total molar concentration of adsorption sites is given by the following mass balance equation as a sum of the concentrations of free and of silver occupied adsorption sites:

$$[SOH_T] = [SO] + [Ag\text{-}OS]$$

Using this expression in the equilibrium constant equation and rearranging leads to:

$$\frac{[Ag^+_f]}{[Ag\text{-}OS]} = \frac{[Ag^+_f]}{[SOH_T]} + \frac{1}{K_L\,[SOH_T]}$$

Plotting $[Ag^+_f]/[Ag\text{-}OS]$ vs. $[Ag^+_f]$ over a range of initial silver concentrations allowed the derivation of the needed Langmuir modeling parameters K_L^{act} and $[SOH_T]$. With this, we were able to include an adsorption reaction in the MINTEQA2 modeling of silver speciation. Calculations were carried out for a variety of conditions. As an example, the silver adsorption to lake sediment in fresh water was predicted to change with the sediment amount as follows: At a loading of 10 mg/l sediment, representing the average amount of suspended particles in a lake, 85% of the initial silver concentration will be present as adsorbed, 15% as dissolved species. At a sediment load of 100 mg/l (average value for river waters), more than 98% of the silver is expected to be adsorbed.

Overall, our method of combining experimental work and model calculations allows us to predict silver to be highly reactive towards dissolved ligands and readily adsorbed by solid material present.

In the future, we plan to investigate possible silver desorption from the same sediment materials and use the combined results for further calculations of silver residence times, silver fluxes across water/sediment interfaces, etc. This can help us to re-evaluate reported toxicity data and environmental concentration levels and to generally improve our understanding of the transport and fate of silver in natural waters.

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Geochemical Investigations of the Behavior of Silver In Sedimentary Porewaters

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The U.S. Environmental Protection Agency (USEPA) is assessing various methods and procedures for managing contaminated sediments in the national waterways (USEPA 1992). Because the Environmental Protection Agency (EPA) regulatory mandate is based on the potential of a contaminant to cause significant, unacceptable adverse effects to either human health or the environment, the present study was conducted to provide a geochemical assessment of silver within the context of existing EPA regulatory endpoints.

The equilibrium partitioning approach to the development of sediment quality criteria (USEPA 1992) is predicated on two basic assumptions: a) that sedimentary porewaters provide the dominant biological exposure pathway to solid-associated contaminants, and b) that partitioning relationships are at equilibrium. Given a knowledge of metal partitioning behavior (K_D) and a maximum permissible porewater concentration (e.g., a water quality criterion value; WQC), sediment criteria values can then be estimated:

Given:
$$K_D = [Ag]_{sediment}/[Ag]_{solution} \qquad (1)$$

Therefore:
$$[Ag]_{sediment,max} = K_D{*}[Ag]_{solution,max} \qquad (2)$$

Reported water quality criterion values (protective of aquatic life) for silver include acute and chronic values (USEPA 1980, 1988, 1991). For the purpose of clarification, the values are given in units of both ug/L (ppb) and molality:

	Freshwater	Marine
Chronic	0.12 ug/L (1.11×10^{-9} M)	0.92 ug/L (proposed) (8.53×10^{-9} M)
Acute	$e^{(1.72[\ln(\text{hardness})]-6.52)}$	2.3 ug/L (2.13×10^{-8} M)
	0.92 ug/L (proposed) (8.53×10^{-9} M)	7.2 ug/L (proposed) (6.67×10^{-8} M)

where the hardness-dependent, freshwater acute criterion is calculated by inclusion of hardness in units of mg/L $CaCO_3$ (e.g., at 100 mg/L $CaCO_3$, WQC = 4.06 ug/L; 3.76×10^{-8} M).

Based on previous work linking sedimentary sulfides to metals bioavailability (DiToro et al. 1990; USEPA 1992), Figure 1 was constructed (Loux et al. 1990). The MINTEQA2 geochemical speciation model (Brown and Allison

1987; Allison et al. 1991) was used to assess whether excess porewater sulfides (1 mg/L HS^-) were capable of suppressing porewater dissolved silver concentrations. It should be noted that, although Ag reduction may be significant in some environmental settings, evidence exists that many environmental redox transformations may never reach equilibrium (e.g., see Lindburg and Runnells 1984); hence, Ag reduction was ignored in constructing this (and subsequent) figures. The reader can discern from Figure 1 that between pH 6.5 and 9 (pH water quality criterion values), excess porewater sulfides can suppress porewater Ag concentrations to below hardness-dependent, acute freshwater criterion values (the upper three curves in Figure 1).

Table 1 depicts typical freshwater chemical parameters suitable for continuing the geochemical investigation of sedimentary Ag partitioning. To avoid AgCl precipitation, the initial dissolved Ag concentration was set at 3.67E-7 M (40 ppb). The concentration of reactive hydrous ferrous oxide (HFO) adsorption sites was estimated based on five assumptions: a) sediment porosity of 50%, b) density of 2.5 g/cm^3, c) 6,400 mg/kg sedimentary iron content (Barnwell 1990), d) 12 % HFO content (of the total iron; Chafin and Loux 1991), and e) 0.005 moles of reactive sites/HFO Fe atom (Dzombak 1986). Table 2 displays the more significant chemical reactions with associated formation constants used in MINTEQA2 simulations using the data given in Table 1. Aside from the polysulfide complexation and adsorption reactions, all numerical constants are generally consistent with those reported by Lindsay and Sadiq (1978) and Lindsay (1979). In sulfide-free environments, Ag partitioning may be dominated by adsorptive processes (represented by adsorption on amorphous iron oxide in these simulations). The reader is referred to Dzombak (1986) for further information concerning the application of the MIT Diffuse Layer Model.

Figure 2 illustrates the MINTEQA2-predicted speciation of Ag using the data in Table 1. As in Figure 1, Ag reduction was not included in the simulations used to develop Figure(s) 2 (and 3). The reader can observe that Ag^+ and $AgCl^o$ represent the dominant soluble Ag species; adsorption on HFO (the >FeOAg species in Figure 2) can serve to significantly limit soluble Ag concentrations. Figure 3 illustrates that $AgHS^o$ and $Ag(HS)_2^{1-}$ can dominate Ag solubility in sulfidic environments (in the absence of significant Ag reduction). For the purpose of examining relative binding affinities, adsorption on HFO also was included in the simulations used to generate Figure 3 (Fe^{3+} is likely to be reduced to Fe^{2+} in real-world sulfidic environments). As shown in Figure 3, sulfide complexation can clearly serve to solubilize Ag even in the presence of HFO adsorption.

Figure 4 was constructed from the simulations used to generate Figures 2 and 3 and illustrates an idealized, predicted porewater concentration profile (at pH 7) for Ag as a function of depth in a sediment (where a sulfide-free volume exists atop a sulfidic subregion). The dashed line connecting the predicted silver concentrations in the two regions was added by the author for the purpose of clarification. From both Figures 1 and 4, one sees that excess porewater sulfides can render soluble Ag concentrations below freshwater, hardness-dependent acute WQCs. In addition, as shown in Figure 4, soluble Ag concentration differences between the two regions conceivably could lead to a net diffusive migration of Ag from the sulfide-free to the sulfidic region.

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Table 1. Chemical parameters used in MINTEQA2 simulations of porewater silver concentrations.

Species	Sulfide-free	Sulfidic
Ag^{1+}	3.670×10^{-07} M	3.670×10^{-07} M
SO_4^{-2}	1.150×10^{-04} M	1.150×10^{-04} M
H_4SiO_4	2.500×10^{-04} M	2.500×10^{-04} M
Cl^-	2.000×10^{-04} M	2.000×10^{-04} M
Mg^{2+}	1.650×10^{-04} M	1.650×10^{-04} M
Na^{1+}	2.600×10^{-04} M	2.600×10^{-04} M
Ca^{2+}	3.700×10^{-04} M	3.700×10^{-04} M
>FeOH	1.720×10^{-04} M	1.720×10^{-04} M
HS^{1-}	--------------	3.02×10^{-5} (M const.)
pCO_2	3.5×10^{-4} atm.	3.5×10^{-4} atm.
T	13 °C	13 °C
IS	0.012 M	0.012 M
HFO[1]	3.106 g/L	3.106 g/L

1- Hydrous ferric oxide; discussed in text.

Table 2. Significant reactions and formation constants used in MINTEQA2 simulations of Ag in fresh-water sedimentary porewaters.

Reaction	$\log_{10}(K)$	Reaction	$\log_{10}(K)$
$Ag^{1+} + Cl^{1-} <=> AgCl^{\circ}$	3.27	$Ag^{1+} + 2Cl^{1-} <=> AgCl_2^{1-}$	5.27
$Ag^{1+} + 3Cl^{1-} <=> AgCl_3^{2-}$	5.29	$Ag^{1+} + 4Cl^{1-} <=> AgCl_4^{3-}$	5.51
$Ag^{1+} + H_2O <=> AgOH^{\circ} + H^+$	-12.0	$Ag^{1+} + SO_4^{2-} <=> AgSO_4^{1-}$	1.29
$Ag^{1+} + HS^{1-} <=> AgHS^{\circ}$	14.05	$Ag^{1+} + 2HS^{1-} <=> Ag(HS)_2^{1-}$	18.45
$HS^- + H^+ <=> H_2S$ (aq.)	6.99	$HS^- <=> H^+ + S^{2-}$	-12.92
$Ag^{1+} + 2H_2O <=> Ag(OH)_2^{1-} + 2H^+$	-24.0		
$Ag^{1+} + 2SH^{1-} + 6S^{\circ} <=> Ag(S_4)_2^{3-} + 2H^+$	0.991		
$Ag^{1+} + 2SH^{1-} + 7S^{\circ} <=> AgS_4S_5^{3-} + 2H^+$	0.68		
$Ag^{1+} + 2SH^{1-} + 3S^{\circ} <=> AgS_4HS^{2-} + H^+$	10.43		
Solid Reactions			
$2Ag^{1+} + HS^{1-} <=> Ag_2S + H^+$	36.05		
$>FeOH + Ag^+ + H_2O <=> >FeOAgOH_2^{\circ} + H^+$	-1.72		

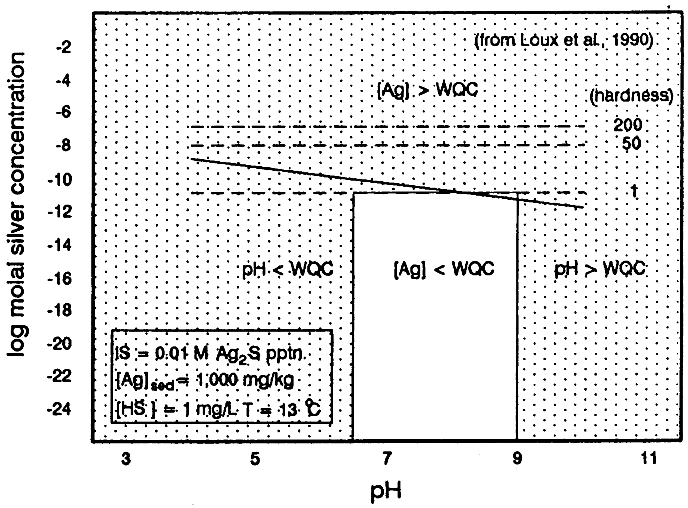

Figure 1. Comparison of MINTEQA2-predicted porewater Ag concentrations with Ag and pH water quality criteria.

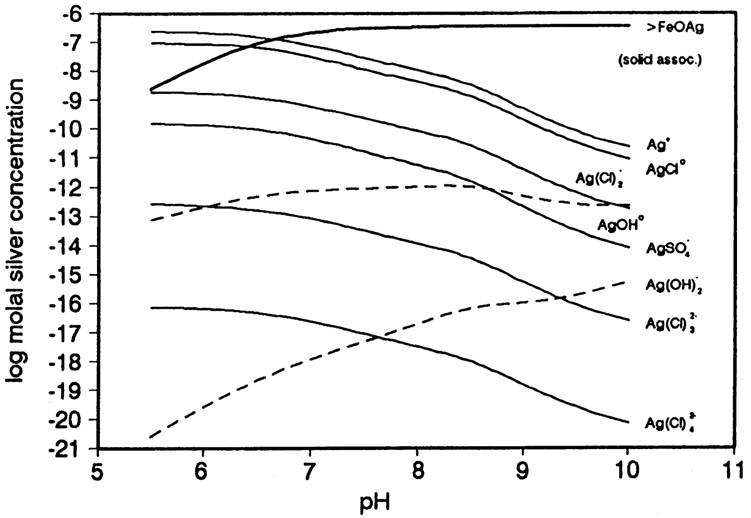

Figure 2. MINTEQA2-predicted speciation of Ag in a sulfide-free environment.

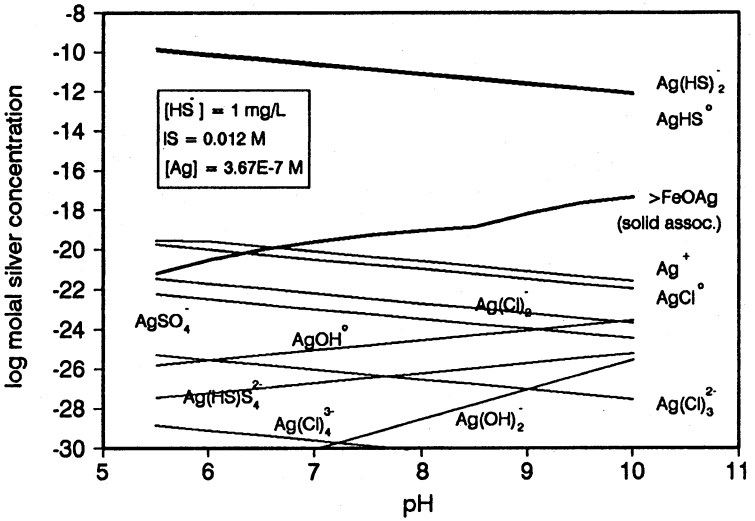

Figure 3. MINTEQA2-predicted speciation of Ag in a sulfidic environment; adsorption included for comparison.

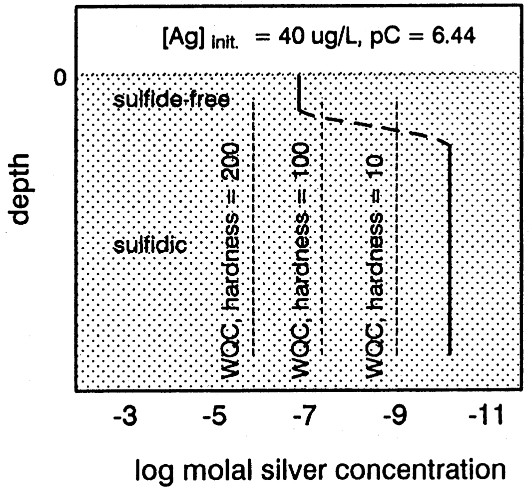

Figure 4. Comparison of predicted sulfidic and sulfide-free Ag concentrations with WQCs at pH 7.

SESSION C: Environmental Cycling of Silver

A. Sodergren, Session Chair

Transport and Fate Model of Silver and Other Metals in New York Harbor

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The purpose of this paper is to present the methodology, calibration results, and projections obtained from a model of metals in New York Harbor. In addition to silver, the metals considered are: cadmium, copper, lead, mercury, nickel, zinc and arsenic. The model extends from the heads of tide of all the major New York and New Jersey tributaries to the ocean boundary in the New York Bight Apex. The model uses transport information derived from a hydrodynamic model of the region. It comprises interactive water column and sediment segments. Suspended solids are modeled in order to properly account for partitioning in the water column and the influence of resuspension and settling. All the known sources of metals to the harbor are included: tributary inflows; municipal and industrial point sources; combined and separate sewer overflows; and atmospheric deposition.

The model is calibrated for two different freshwater flows, a high and a low flow, using a comprehensive set of metals data that was collected using modern clean techniques. During the periods of the sampling, the loss of metals to the sediments appears to be negligible. The system appears to be in equilibrium with the sediment, with the exception of mercury for which there appears to be a net source in addition to resuspension. The relative importance of the various source categories will be examined. In addition, the intertidal behavior of total and dissolved metal will be examined.

Silver Biogeochemistry in Estuaries, with Emphasis on Pathways Controlling Biological Uptake

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The transport and eventual fate of silver is governed by a complex mixture of physical, chemical and biological constraints. Silver is extremely particle reactive and sorbs readily to both living (phytoplankton) and non-living particles (sediment) in freshwater or water of low salinity. As salinity increases, complexation with chloride reduces sorption reactions by both living and dead particles. A salinity increase from 10 to 20 ‰, for example, reduces silver sorption to phytoplankton by 50% and by over 75% to sediment particles.

Biological constraints also govern silver partitioning. Although uptake by phytoplankton appears to be a passive process, a function of surface area and charge, once associated with a cell, silver is not easily removed. The binding of silver by algal cells is quite strong. Silver remained bound to cells exposed to increasing salinity, when held at low Ph, in the presence of strong chelating solutions, and even when the cells were subjected to digestive enzymes. In all instances, less than 5% of the total silver was released by the cells. In contrast, silver sorbed to suspended sediments at low salinities largely desorbed (60-80% loss) as the particle moved down a salinity gradient. Thus, this apparent difference in the degree of silver sorption and the potential for silver desorption in the changing environment of the estuary can greatly affect the transport of silver through the coastal zone and the potential for uptake by higher trophic levels. The desorption of silver from sediments as salinity increases suggests that a greater percentage could remain dissolved and perhaps be available for uptake.

As with phytoplankton, silver uptake by higher trophic levels is dependent upon a suite of chemical and biological variables, the most important being partitioning between dissolved and solid phases. Silver associated with particles does not appear to be available. Both the filter-feeding oyster, Crassostrea virginica, and the omnivorous grass shrimp, Palaemonetes pugio, did not incorporate silver associated with particles, regardless of particle type. Only dissolved silver was taken up in significant quantities.

Factors which affect metabolic processes (temperature, organism size, physiological condition) also affected silver uptake, and the resulting interactions between variables make prediction of silver content problematic. For example, natural populations of Crassostrea within the Chesapeake Bay exhibit quite variable concentrations of silver, with no general seasonal pattern. Further examination determined that variation in silver content in the population is predominantly controlled by salinity and physiological condition of the individuals.

The interactions between biological and geochemical controls of silver availability in estuaries and other aquatic systems underscore the need to fully understand the biogeochemistry of reactive trace elements in order to make predictions of ecosystem impact and to allow for effective system management.

Silver in San Francisco Bay Estuarine Waters

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Spatial gradients of silver concentrations in the surface waters of San Francisco Bay reveal substantial anthropogenic perturbations of the element's biogeochemical cycle throughout the estuarine system. The most pronounced perturbations are in the South Bay, where dissolved (< 0.45 μm) silver concentrations are as high as 250 pM. This is more than one order of magnitude above baseline concentrations in the northern reach of the estuary (6 pM) and approximately two orders of magnitude above natural concentrations in adjacent coastal waters (3 pM). The excess silver is primarily attributed to wastewater discharges of industrial silver to the estuary on the order of 20 kg d^{-1}. The contamination is most evident in the South Bay, where wastewater discharges of silver are on the order of 10 kg d^{-1} and natural fresh water discharges are relatively insignificant.

The limited amount of fresh water flushing in the South Bay was exacerbated by persistent drought conditions during the study period. This extended the hydraulic residence time in the South Bay ($\geq$ 160 days), and revealed the apparent seasonal benthic fluxes of silver from anthropogenically contaminated sediments. These were conservatively estimated to average ~ 16 nmol $m^{-2}d^{-1}$, in the South Bay, which is sufficient to replace all of the dissolved silver in the South Bay within 22 days. Benthic fluxes of silver throughout the estuary were estimated to average ~ 11 nmol $m^{-2}d^{-1}$ with an annual input of approximately 540 kg yr^{-1} of silver to the system. This dwarfs the annual fluvial input of silver during the study period (12 kg yr^{-1}), and is equivalent to approximately 10% of the annual anthropogenic input of silver to the estuary (3,700 to 7,200 kg yr^{-1}). It is further speculated that benthic fluxes of silver may be $\geq$ waste water fluxes of silver during periods of intense diagenic remobilization. However, all inputs of dissolved silver to the estuary are efficiently sorbed by suspended particulates, as evidenced by the relatively constant conditional distribution coefficient for silver throughout the estuary ($K_d \sim 10^5$).

Pore Water Fluxes of Silver in Estuaries

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San Francisco Bay is an urban estuary with anomalously high concentrations of silver in the water, sediments and benthic organisms (Luoma and Phillips 1988; Smith and Flegal 1993; Flegal and Sanudo-Wilhelmy 1993). Total dissolved (< 0.4 µm) silver concentrations in the southern reach of the estuary are as high as 250 pM, which is two orders of magnitude above baseline concentrations of silver in adjacent coastal waters (3 to 8 pM; Sanudo-Wilhelmy and Flegal 1992); silver concentrations of sediments within the southern reach are ≈ 9 µmol kg^{-1} (Luoma and Phillips 1988), which is an order of magnitude greater than the average crustal abundance of silver (0.7 µmol kg^{-1}); and silver concentrations of benthic organisms in the bay are equal to or greater than those of benthic organisms in any other estuary. These include mussels, *Mytilus californianus*, whose concentrations in San Francisco Bay range from 6,500 to 26,900 nmol kg^{-1} (Luoma and Phillips 1988; Smith et al. 1986), which is one to two orders of magnitude greater than the baseline concentration of mussels in adjacent waters (370 nmol kg^{-1}; Goldberg et al. 1983).

Seasonally elevated concentrations of dissolved silver in the southern reach of San Francisco Bay appear to be influenced by the diagenic remobilization of silver from benthic sediments in the estuary (Smith and Flegal 1993). This is consistent with seasonal variations of other trace elements in the estuarine waters, which appear to be influenced by benthic processes (Flegal et al. 1991). It is also consistent with the apparent influence of diagenic processes on the distribution of silver in the surface waters of San Diego Bay (Flegal and Sanudo-Wilhelmy 1993). This was further indicated by the following results of a preliminary study that was designed to quantify the flux of silver from benthic sediments in the San Francisco Bay estuary (Rivera-Duarte and Flegal 1993).

Silver concentrations of sediment cores from San Francisco Bay are up to 100-fold greater than those in cores from adjacent estuaries. Silver concentrations of sediments from four core in the bay ranged from 470 to 7,900 nmole kg^{-1} (dry weight), with the exception of one anomalously high value (18,200 nmole kg^{-1}). In contrast, silver concentrations of sediments from two adjacent estuaries (Drakes Estero and Tomales Bay) ranged from 80 to 440 nmole kg^{-1}, with most concentrations less than 180 nmole kg^{-1}. Therefore, silver concentrations in sediment cores from San Francisco Bay appear to be 3- to 100-fold above baseline concentrations in northeast Pacific estuarine sediments.

Total dissolved silver concentrations in the pore waters of sediment cores from San Francisco Bay also appear to be elevated above baseline concentrations. Dissolved silver concentrations in pore waters from the bay ranged from 0.02 to 4.63 nM. Those pore water concentrations were up to one order of magnitude greater than the highest concentration of dissolved silver in surface waters of the bay and three orders of magnitude greater than the baseline concentration of dissolved silver in adjacent surface waters in the northeast Pacific.

The concentrations of dissolved silver in pore waters of San Francisco Bay are less than previously reported dissolved silver concentrations of pore waters (2 to 9 nM) in Long Island Sound sediments (Lyons and Fitzgerald 1983). However, the limit of detection for the latter measurements (2 nM) was also at least two orders of magnitude greater than the limits of detection for measurements of pore water concentrations of silver in San Francisco Bay (0.02 nM). Since the concentrations of silver in sediments from Long Island Sound and San Francisco Bay are comparable (Di Toro, personal communication), the previously reported concentrations of silver in estuarine sediments are circumspect.

The partition coefficient of silver decreased with depth in the sediment cores from San Francisco Bay. The logarithm of the apparent molar partition coefficient ($D_{Ag^*} = [Ag]_{sediment}$ nmole $Kg^{-1} \div [Ag]_{pore\ water}$ nM) ranged from 3.7 to 5.4 in the top $\leq$ 8 cm of the sediment, and from 2.1 to 3.7 in the bottom $\geq$ 10 cm. The range in those values was comparable with the reported (Smith and Flegal 1993) range in log of the conditional particle-water distribution coefficient (K_d) for surface waters in San Francisco Bay (4.31 to 6.10). The successive decrease in the average partition coefficient from surface waters to deep pore waters could be attributed to the changes in pH, increase in reducing conditions and relatively higher concentrations of dissolved ligands in the sediments. Those factors would tend to increase the relative amount of silver in solution (Stumm 1992).

Diffusive benthic fluxes of silver from the sediments were calculated from the pore water concentration gradients of silver, using a sediment diffusion coefficient for silver based on the relationship suggested by Iversen and Jorgensen (1993). The change in concentration was measured between the highest subsurface concentration in pore waters and an ambient concentration of 25 pM in surface waters in San Francisco Bay (Smith and Flegal 1993). The diffusive benthic fluxes of silver were estimated at 0.04 nmol m^{-2} day^{-1} in the northern reach of the estuary and 0.4 to 4 nmole m^{-2} day^{-1} in the southern reach of the estuary. The resultant diffusive flux of silver to San Francisco Bay was estimated to range from 2 to 164 kg $year^{-1}$.

This conservatively low estimate of the benthic flux of silver to the estuary is $\geq$ the fluvial input of dissolved silver to the estuary (12 kg $year^{-1}$; Smith and Flegal 1993), but it is one to two orders of magnitude lower than anthropogenic inputs of silver (dissolved and particulate) to the estuary (3,700 to 7,200 kg $year^{-1}$; Davis et al. 1991). However, the total benthic flux of dissolved silver to the estuary is comparable to the total anthropogenic flux of silver to the estuary, based on mass balance calculations that indicated that the sediments are a sink for silver in the northern reach of the estuary (-2 kg day^{-1}) and a source for silver in the southern reach of the estuary (9 to 160 kg day^{-1}). Resultant estimates of the net benthic flux of silver to the estuary ranged from 2,500 to 5,800 kg $year^{-1}$, which is equivalent to the net anthropogenic flux of silver to the system. Therefore, the elevated concentrations of silver in surface waters in San Francisco Bay appear to be a consequence of both contemporary discharges of silver from wastewater outfalls and the diagenic remobilization of silver from contaminated sediments within the estuary.

Finally, San Francisco Bay appears to be a trap for silver. The residence time of silver in bay waters (<1 to 13 days) is much shorter than the hydraulic residence time of water in the southern reach of the estuary (60 to $\geq$ 160 days; Walters et al. 1985). This is attributed to the rapid scavenging of silver onto suspended particulates which are deposited within the estuary. Consequently, the high seasonally concentrations of silver in South Bay waters indicates that silver is rapidly recycled between dissolved and particulate phases within the estuary. It also suggests that elevated levels of silver may persist in both phases in spite of efforts to decrease silver contamination in San Francisco Bay through reductions in wastewater discharges of industrial silver.

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Transport and Fate Models of Metals in Aquatic Systems

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Silver Levels in "Background" Streams, Municipal Effluents, and Effluent-Receiving Streams in Wisconsin

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A reevaluation of trace element geochemistries in surface waters throughout the world has been prompted by the recent development of both "clean" sampling and processing techniques, and ultra-sensitive analytical methods. Our research group has been developing and validating these techniques, and is applying these methods to research on levels of trace metals in streams and lakes in Wisconsin and the Upper Midwest, and to investigations of the factors and processes controlling trace metal levels. We have shown that, in comparison with best available data, actual trace element levels in many Wisconsin streams could be up to two orders-of-magnitude lower. Our reported levels of trace elements at some river sites from a recently completed study of 41 rivers in the Upper Midwest are in the range of modern accepted oceanic values.

Reliable data on Ag in freshwaters is particularly limited. We conducted an exploratory study of Ag levels in selected Wisconsin streams during the spring and summer of 1993. Silver measurements were made on 14 indicator streams sampled as part of a research program on background concentrations of trace metals in Wisconsin streams. In unperturbed systems, geochemical processes regulate "background" Ag emissions to low ng L^{-1} levels. However, there is concern that point discharges of Ag from municipal wastewater treatment plants may produce acute and/or chronic toxic responses in aquatic organisms in the receiving streams. In addition, there is concern that the quality of existing Ag data from treatment facilities is poor. To address these concerns additional sampling was conducted at three treatment facilities, to determine if measurable and significant levels of Ag are being discharged to receiving waters from municipal wastewater treatment plants which are collecting waste from major silver end-users.

Samples for Ag and other trace metal analyses were collected using "clean" techniques, which include the use of exhaustively acid-cleaned and double-bagged Teflon sample bottles, all-Teflon sampling apparatus, and Tyvek suits for field personnel. Samples were filtered in the field in an argon-flushed glove box through acid-leached 0.4 µm track-etched polycarbonate filters held in an all-Teflon filter column. Sampling gear is prepared under CLASS 100 laminar-flow benches. Field QC samples including field spikes, bottle and method blanks, and replicates are collected in roughly equivalent numbers as actual samples.

Laboratory analyses were conducted in HEPA filtered clean laboratories using both Zeeman graphite furnace atomic absorption spectrophotometry (z-GFAA) and inductively coupled plasma-mass spectrometry (ICP-MS). Using multiple pipetting z-GFAA, the detection level for Ag in stream waters was 4 to 10 ng L^{-1}. The detection and quantification levels of Ag on the recently acquired ICP-MS are 1.5 and 3 ng L^{-1} respectively, when pneumatic nebulization is used. By employing a CETAC 5000AT ultrasonic nebulizer, we can lower the detection level to 0.1 ng L^{-1}.

The 14 indicator sites were selected from a larger study group of 41 watersheds distributed throughout the state of Wisconsin that reflect homogeneous characteristics of land-use and principal geological variables. The principal variables considered in developing the matrix of sites were: (1) land use/land cover; (2) surficial glacial geology; (3) soil type; and (4) drainage basin size. Six categories of land use/cover were examined. Within each of the land/use classifications we have selected a variety of watersheds with homogeneous soil and surficial geology characteristics. Most streams were sampled at headwater sites where metal concentrations are not influenced by point source discharges. The sub-set of 14 sites chosen for the Ag study represent a wide range of conditional influences on stream water chemistry.

Concentrations of trace metals, including Zn and Cu, varied considerably among sites, for some elements by over an order-of-magnitude. This large natural variance reflects influences of soil type, dissolved organic matter (DOC), and suspended particulate matter on metal levels. Highly significant positive correlations between DOC levels and filtrable trace metal concentrations are observed (Fig. 1), demonstrating the important role that DOC plays in metal speciation and behavior. Ag, however, was not detected above the quantification limit of 8 ng L^{-1} using z-GFAA at any of these sites. We are in the process of re-analyzing these samples by ICP-MS to quantify natural levels of Ag in headwater streams. Preliminary results show Ag concentrations ranging from 4 to 8 ng L^{-1}. Ag partition coefficients (K_p, L Kg^{-1}) to these natural river suspended sediments were in the range of 10^4 to 10^5.

Three municipal treatment plants were investigated: Jackson, Wis., an activated sludge plant using alum/polymer tertiary treatment, sand filtration of the final effluent, and discharging to Cedar Creek; Brookfield, Wis., an activated sludge plant using ferric chloride tertiary treatment, sand filtration of the final effluent, and discharging to the Fox River; and Fall River, Wis., a lagoon system discharging intermittently to the Crawfish River. Samples of plant influent, treated effluent, as well as upstream/downstream samples from receiving waters were collected. Receiving water samples were not collected at the Fall River site, as the treatment system was not discharging during the summer. Concentrations of Ag entering the plants ranged from 4 to 100 µg L^{-1}. In-plant removal, calculated by comparing inflow and outflow concentrations (or water column concentrations in the lagoon), was greater than 98% at all three plants (Table 1). Data on sludge properties and Ag content supplied by the Jackson treatment plant supports our mass balance with near quantitative removal of Ag in the sludge. Upstream and downstream concentrations of Ag were < 8 ng L^{-1} at Jackson and Brookfield.

Table 1. Silver Fate Through Municipal Treatment Plants

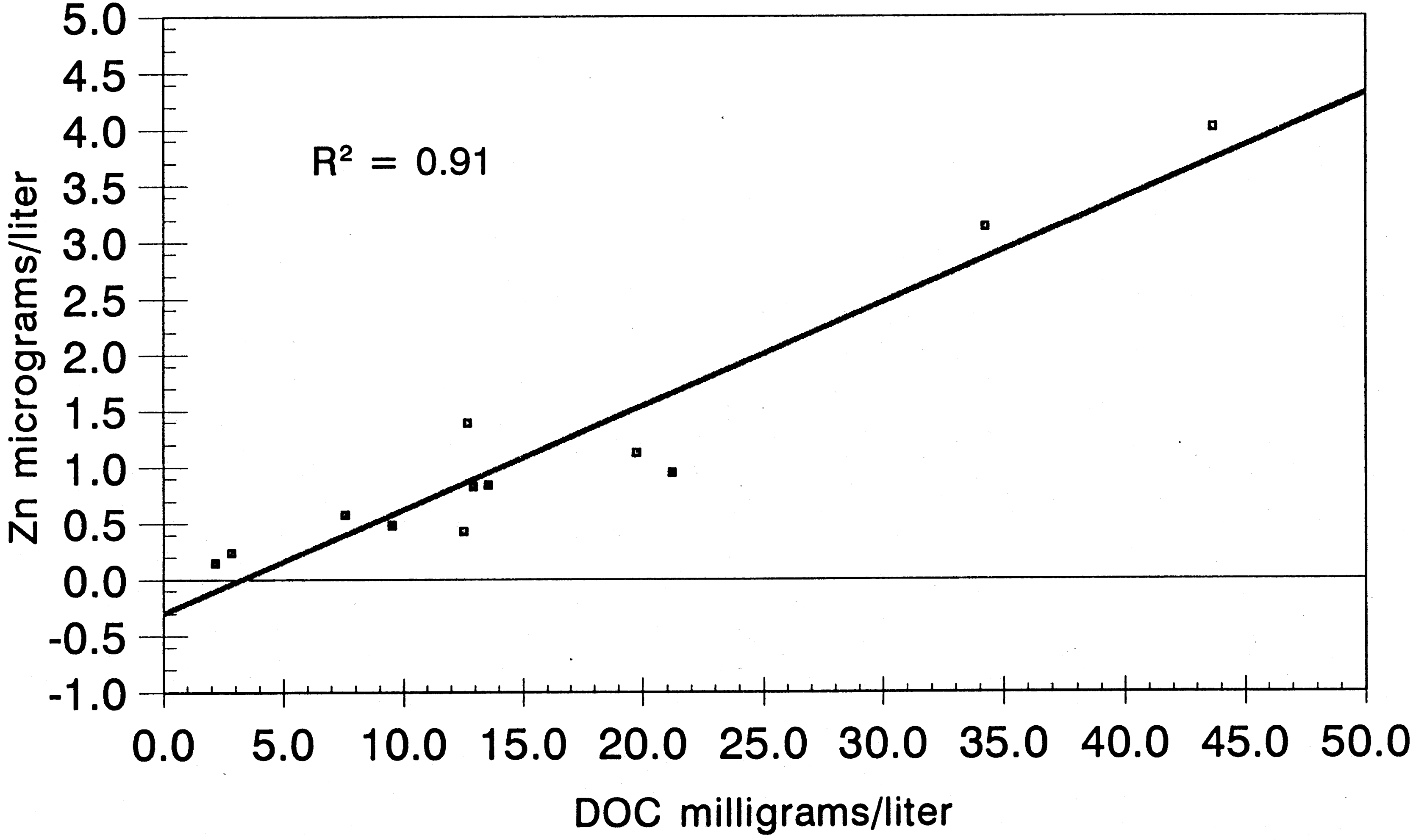
Ag concentrations in µg L^{-1}

Site	[Ag] Inflow	[Ag] Effluent	% Removal
Jackson	103.	0.875	99.2
Brookfield	4.27	0.072	98.3
Fall River	54.4	0.780	98.6

Our results show that Ag entering the treatment facilities was largely removed within the plant, probably through incorporation into sludge solids. Very high levels of partitioning of Ag to suspended particles in the plant effluents was observed (K_p: 8.7 x 10^5 at Jackson, and 4.5 x 10^5 at Brookfield), again suggesting an important role of particles in regulating Ag concentrations. The Ag leaving the plant was rapidly reduced to levels below our z-GFAA quantification limit of 8 ng L^{-1} by dilution and/or incorporation into stream sediments. We will be conducting further investigations of the Ag leaving the plants through modeling of dilution and analysis of stream sediments above and below the treatment facilities.

Regression of Filtrable Zn on DOC

Low Calcium Sites (Fall 1992)



SESSION D: Food Chain Transfer of Metals in the Environment

A. Sodergren, Session Chair

Accumulation of Metals in Aquatic Organisms — Geochemical Implications

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This presentation will review principles relating to the bioavailability and bioaccumulation of metals in marine systems. The focus of the presentation will be on the lower end of food webs, particularly on phytoplankton and phytoplankton-herbivore interactions. There will be discussion of the biochemical and cytological sites of metal deposition in organisms and how these can influence the fate of the metals.

The presentation will consider the trophic transfer of metals in marine food webs and the factors which influence this process for different types of metals.

The presentation will also consider the roles that marine organisms (and their debris) play in mediating the cycling and vertical flux of diverse metals in the oceanic water column.

A Tiered Research Strategy for Evaluation of Silver Bioavailability in Aquatic Systems

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Abstract

A multitiered research program has been initiated to elucidate factors in aquatic environments that influence the bioavailability of silver. The initial focus is on freshwater and estuarine sediments as sinks or sources for silver. The first phase of this research involves sediment amendment procedures using silver compounds. Sediments representing a range of characteristics anticipated to influence silver speciation (e.g., pH, organic carbon, AVS) have been selected for evaluation as well as formulated sediments. Silver concentrations in interstitial water and sediment are measured to determine the partitioning of silver in these compartments.

The second research phase involves measurements of silver bioavailability by using "routine" sediment toxicity tests with *Hyalella azteca* and *Chironomus tentans.* These organisms are exposed to characteristically dissimilar sediments that have been amended with silver. *H. azteca* and *C. tentans* responses to silver in these sediments are used to further evaluate sediment characteristics regulating silver bioavailability.

Finally, the third phase involves a time-course study of silver bioavailability in laboratory microcosms in an attempt to verify information developed in the sediment testing phase. A common thread throughout this laboratory-based research has been to improve our understanding and predictive capabilities regarding the speciation and temporal fate and effects of silver in aquatic ecosystems.

Introduction

Silver in association with other metals is widely distributed throughout the United States as well as most of the continents of the world. Much of the silver produced is mined in North America by Mexico, the U.S. and Canada. The refined silver is used for a variety of industrial, medical and domestic purposes. Throughout the mining, manufacturing and utilization processes, there are opportunities for silver to enter aquatic ecosystems (Sanders and Abbe 1989; Lytle 1984; Freeman 1979). Since ionic silver is relatively toxic (Nebeker 1982; Nebeker et al. 1983), some concerns have arisen regarding the environmental fate and effects of silver in aquatic systems (U.S. EPA 1979, U.S. EPA 1980, Cooley et al. 1988).

We have initiated a multitiered research program to elucidate factors in aquatic environments that influence the bioavailability of silver. The initial focus is on freshwater and estuarine sediments as sinks or sources for silver. It is clear that understanding speciation is crucial for predicting bioavailability (Chudd 1983; Cooley et al. 1988; Kramer and Allen 1988; LeBlanc et al. 1984; Lytle 1984; Sanders and Abbe 1989). In the absence of accurate and precise analytical techniques for important species of silver (Chudd 1983; Wang et al. 1989), perhaps progress can be made using extraction techniques and environmental manipulations to assess the potential for and kinetics of ecologically important silver speciation in aquatic systems coupled with sediments (Brookins 1988; Dyck 1988).

This ongoing research program has three phases: 1) evaluation of sediment amendment procedures using silver compounds; 2) measurement of the bioavailability of silver using typical freshwater and marine sediment toxicity testing organisms; and 3) evaluation of silver speciation kinetics and bioavailability in laboratory microcosms. This paper is a summary of our progress on this research to date.

Evaluation of Sediment Amendment Procedures Using Silver Compounds

Silver compounds for sediment amendments were selected based upon their frequency of use in industry and environmental relevance. We are currently studying silver nitrate. Silver chloride and silver thiosulfate will also be studied.

Sediments were selected for amending experiments based upon the criteria listed below:

1. Representative of range in U.S. (pH 5-9, O.C. < 1%-2.5%, Eh neg.-pos.) [Suedel and Rodgers 1991].
2. Characteristics that may be important in silver speciation and bioavailability (e.g., Redox, pH, O.C.).
3. Accessible: 1-2d drive or colleague lives nearby.
4. Represent both karst topography and granitic (quartz) topography.
5. Need overlying water, also.
6. Need sufficient volume to obtain interstitial water for analysis.
7. Need approximately 10 sediments.
8. Sediments must be unconfounded with toxicity.
9. Matching some current discharge site, geography.

Based upon the sediment selection criteria, seven field-collected and one formulated sediment (Suedel and Rodgers, in review) have been chosen and characterized to date (Table 1). These sediments have widely ranging characteristics and encompass much of the range found in sediments from throughout the continental U.S. (Suedel and Rodgers 1991).

To evaluate the partitioning of silver in sediment-water systems, sediments were amended with silver nitrate (Kodak silver nitrate crystals 173-1082) by adding a 10,000 mg/L solution directly to sediments and allowing a 24 h equilibration period. The overlaying water was then added carefully and samples of sediment, overlaying water and interstitial water were collected through time to evaluate silver partitioning in the diverse sediment-water systems. Concentrations of silver were measured in sediments and water using atomic absorption spectrophotometry (AA). The target silver concentration in each sediment was 320 mg/kg on a dry weight basis. On the initial day of the experiment, concentrations of silver ranged from 28.7 mg/kg in the particulate phase to 153.5 mg/kg for sediment in the formulated sediments and Hemlock Lake sediments, respectively (Table 2). In the overlaying water, silver concentrations generally corresponded with sediment concentrations although there were considerable differences in the response of the diverse sediment and overlaying waters. For example, the field collected sediment 1 and the "similar" formulated sediment responded quite differently to silver amendment. In sediment pore or interstitial waters, silver concentrations ranged from 58 mg/L to 0.07 mg/L on the initial day of this experiment. After 10 days, silver concentration in interstitial water declined in most cases (Figures 1-3).

In overlaying waters, concentrations of silver were also measured using an ion-selective electrode (ISE). The ISE analyses verified that most of the silver in overlaying waters was in ionic form (Ag^+) and corresponded well with the AA analyses (Figure 4).

Analyses of the sediment particulate fractions for silver concentrations illustrated that most of the silver (on a concentration basis) was associated with the fine fraction of the sediment (silt + clay) [Figure 5]. However, a significant amount of silver was also associated with the course or sand fraction of some of the sediments (Figure 5).

Responses of Freshwater Toxicity Testing Species to Aqueous and Sediment-Associated Silver

Silver concentrations were relatively stable for a 10d period in aqueous experiments with pond water (Figure 6). In aqueous range-finding toxicity tests with silver, *C. tentans* was the least sensitive of the five species tested (Table 3). For the two common freshwater sediment testing species, *C. tentans* and *H. azteca*, the amphipod was an order of magnitude more sensitive with a 10d EC50 of 5.8 ug Ag/L compared to 259 ug Ag/L for *C. tentans* (Table 3). In sediment toxicity tests with silver amended sediments. *H. azteca* was again one of the most sensitive species tested although the 10d LC50s for the four test species (*H. azteca, Pimephales promelas, Daphnia magna,* and *C. tentans)* were similar, ranging from 40 to 160 mg Ag/ kg sediment for the field collected sediment (Table 4). Information on toxicity of aqueous silver to these species is not reported in the 1980 USEPA Ambient Water Quality Criteria document. For comparative purposes with those species that are reported in the criteria document, the hardness, pH and alkalinity for these experiments were 6, 7.0-7.5, and 26, respectively, for aqueous tests, and 16-24, 6.5-7.0, and 15-30, respectively, for sediment tests. Ongoing studies will permit evaluation of organism responses to silver speciation in a variety of sediments.

Time-Course Study of Silver Bioavailability in Laboratory Microcosms

Time-course changes in silver bioavailability will be evaluated in laboratory microcosms. Sediments will be amended with silver and the experiments will be conducted for at least three months. During the course of these experiments, sediment samples will be tested for toxicity, silver speciation and bioavailability. Bioavailability of resuspended particulates with associated silver will also be evaluated. These experiments will begin soon and results will be available within the next year.

Summary and Conclusions

1. Based on results from our research to date and published results of other workers, it is clear that bioavailable silver is not related to total silver, the silver concentration that can be measured after a rigorous acid extraction. Clearly, silver speciation in aquatic systems regulates bioavailability and toxicity.

2. Silver speciation can change with time in aquatic systems and sediments can serve as an important "sink" for silver. This change in silver speciation with time should be reflected by altered bioavailability and toxicity to both benthic species and species inhabiting the water column.

3. The sensitivity of aquatic organisms to aqueous and sediment-associated silver varies widely. This will be advantageous as we use these organisms to identify the bioavailable fraction of total silver in these matrices (Luoma, et al. 1991).

4. Both ongoing and planned experiments have been designed to evaluate change in silver speciation and bioavailability in sediment-water systems. These experiments will rely on both theoretical (thermodynamic) predictions of silver speciation as well as chemical/physical measurements of "readily available," loosely bound and tightly bound fractions. Carefully controlled microcosm experiments will play a major role in accomplishing this objective.

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Table 1. Characterization of sediments selected for silver amendment experiments.

	Sed 1	Sed 2	Sed 3	Sed 4	Sed 5	Sed 6	Sed 7	Sed 8
pH	6.0	6.95	6.8	6.87	7.9	7.3	7.48	7.38
Redox	+57	+62	-232	-168	-248.5	-297	-291	-300
% O.C.	0.26	0.03	0.18	2.54	0.08	0.45	1.02	--
% O.M.	1.68	1.75	0.29	6.05	0.47	3.94	2.31	4.56
CEC	1.5	1.58	0.11	14.29	0.67	7.77	2.40	6.91
% Water	27.1	19.9	23.3	56.3	21.9	44.6	30.5	48.3
% Sand	75.9	77.05	94.47	28.5	95.59	34.38	95.19	--
% Silt	21.4	22.95	5.53	71.5	4.23	65.62	4.25	--
% Clay	2.7	0	0	0	0.18	0	0.56	--

Table 2. Initial silver concentrations in sediment, overlaying water and pore water. Target concentration in sediment samples was 320 mg/kg Ag.

Sample	Sediment (mg/kg)	Overlaying Water (mg/L)	Pore Water (mg/L)
Sed 1	39.58	0.55	0.64
Sed 2	28.66	5.07	14.56
Sed 3	34.46	3.91	12.98
Sed 4	137.33	0.08	0.07
Sed 5	121.49	2.76	58.25
Sed 6	153.54	0.14	0.18
Sed 7	109.30	0.06	0.16
Sed 8	102.43	0.03	0.21

Sed 1: UMBFS 1 (Lafayette Co., MS)
Sed 2: Formulated Sediment (Suedel and Rodgers, in review)
Sed 3: UMBFS Sandy (Lafayette Co., MS)
Sed 4: Snake Pit (Lafayette Co., MS)
Sed 5: Hamlin Beach (Monroe Co., NY)
Sed 6: Hemlock Lake (Ontario Co., NY)
Sed 7: Powder Mills Park (Monroe Co., NY)
Sed 8: Oatka Creek (Monroe Co., NY)

Table 3. Results from aqueous exposures of toxicity testing organisms to silver nitrate in range-finding and definitive tests.

		Endpoint (ug Ag/L)		
Organism	Test Type	96h EC50	10d EC50	10d NOEC
C. dubia	Range-finding	1-5	1-5	<5.0
D. magna	Range-finding	1-5	1-5	<5.0
P. promelas	Range-finding	5-10	<5.0	<5.0
H. azteca	Range-finding	1-10	1-10	<10
C. tentans	Range-finding	100-500	100-500	<500
H. azteca	Definitive	6.8 (6.22-7.53)	5.8 (5.21-6.51)	4.0 (survival)
C. tentans	Definitive	676 (444-2033)	259 (192-371)	125 (growth and survival)

Table 4. Results from sediment exposures of toxicity testing organisms to $AgNO_3$ amended to sediments in range-finding tests.

		Endpoint (mg Ag/kg)	
Organism	Sediment	96h LC50	10d LC50
D. magna	UMBFS 1	40-80	40-80
P. promelas	UMBFS 1	80-160	80-160
H. azteca	UMBFS 1	80-160	80-160
C.tentans	UMBFS 1	40-160	40-160
H. azteca	Sed. 3	<20	<20
C. tentans	Sed. 3	160	<20
H. azteca	Sed. 4	>320	---
C. tentans	Sed. 4	>320	---

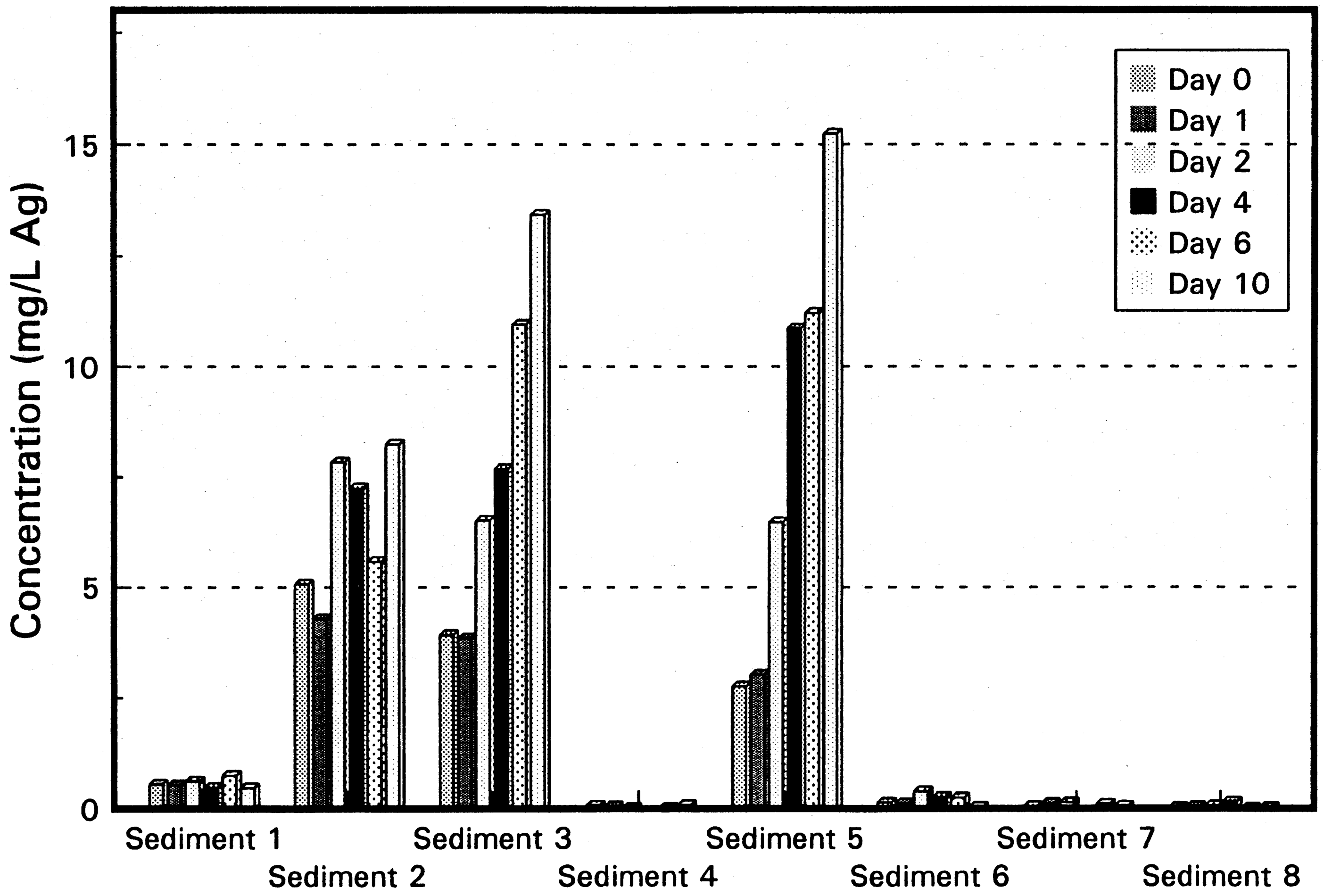

FIGURE 1. Ag CONCENTRATION IN OVERLAYING WATER (SEDIMENTS AMENDED WITH 320 mg Ag/kg as $AgNO_3$).

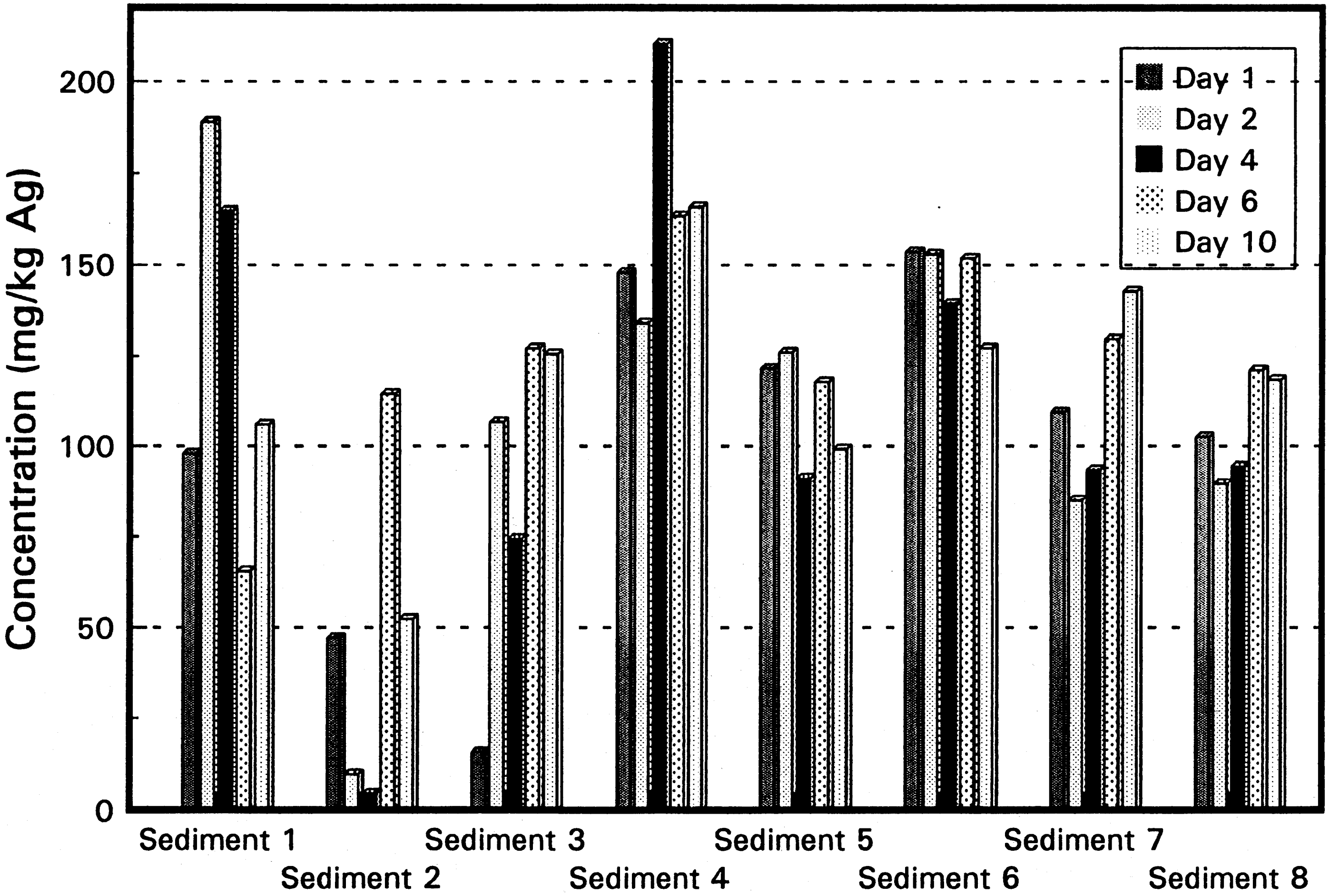

FIGURE 2. Ag CONCENTRATION IN TOTAL SEDIMENT
(SEDIMENTS AMENDED WITH 320 mg Ag/kg as $AgNO_3$).

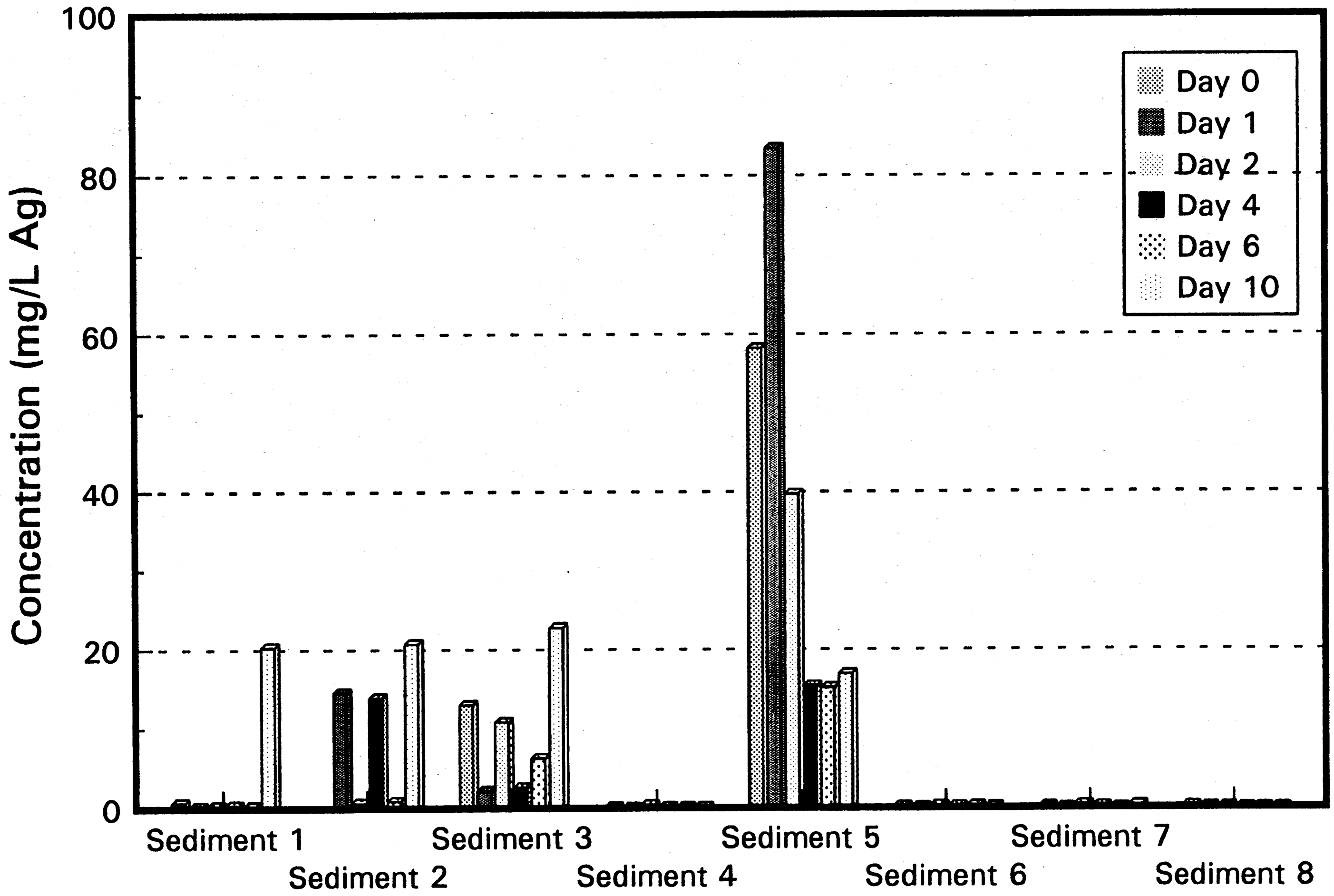

FIGURE 3. Ag CONCENTRATION IN INTERSTITIAL WATER
(SEDIMENTS AMENDED WITH 320 mg Ag/kg as $AgNO_3$).

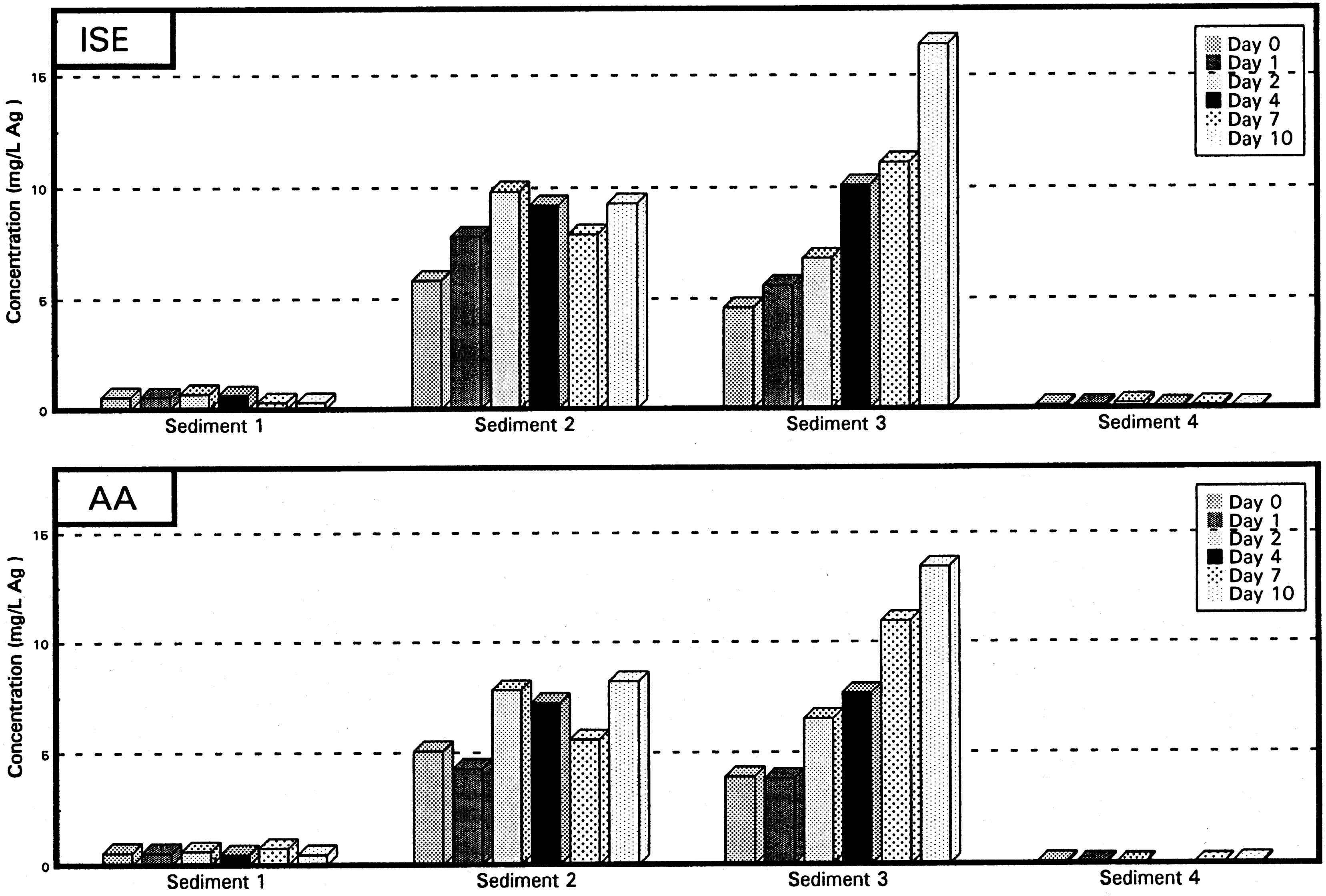

FIGURE 4. ISE AND AA Ag CONCENTRATION IN OVERLAYING WATER.
(SEDIMENTS AMENDED WITH 320 mg Ag/kg as $AgNO_3$).

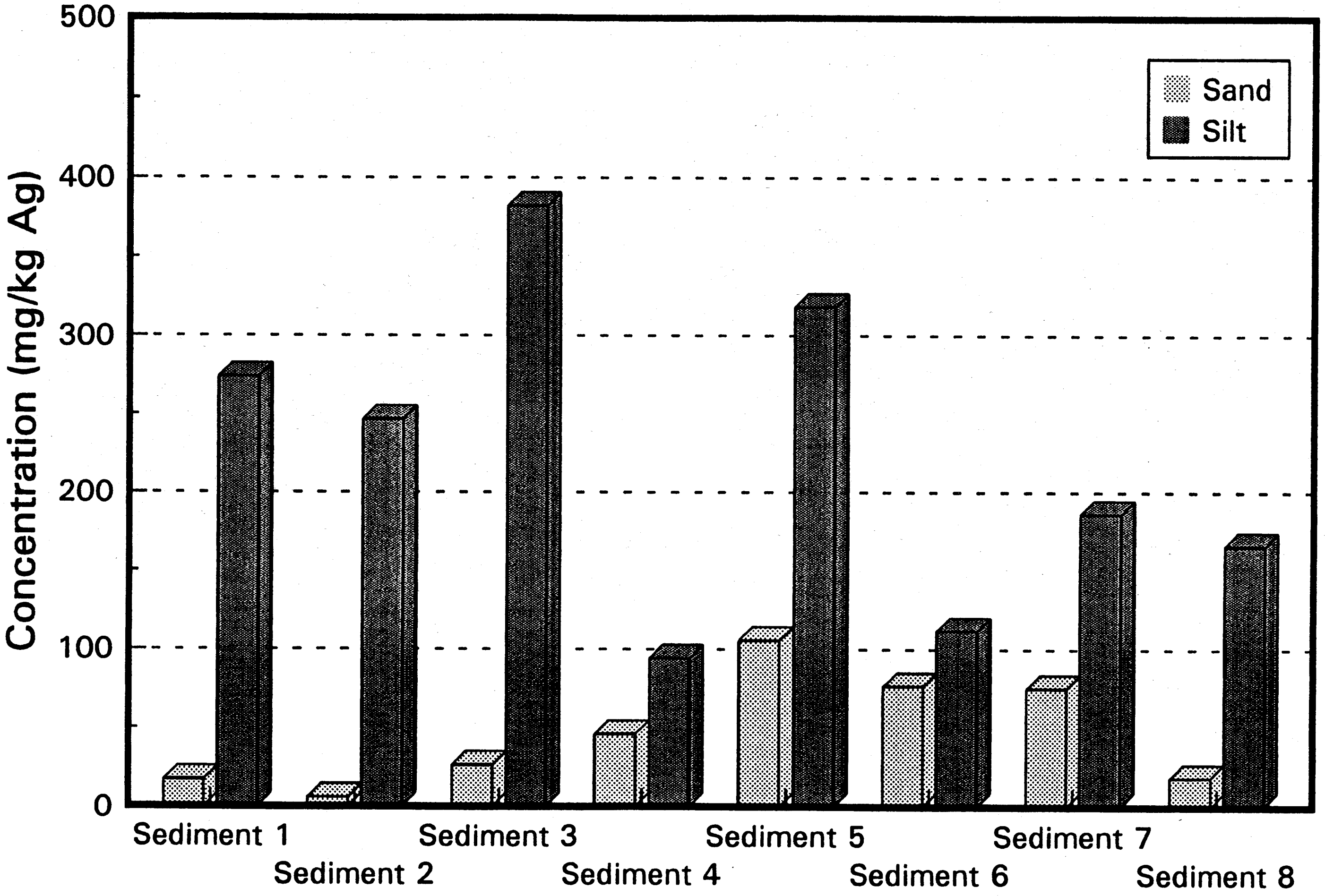

FIGURE 5. Ag CONCENTRATION IN SAND AND SILT SEDIMENT FRACTIONS (SEDIMENTS AMENDED WITH 320 mg Ag/kg as $AgNO_3$).

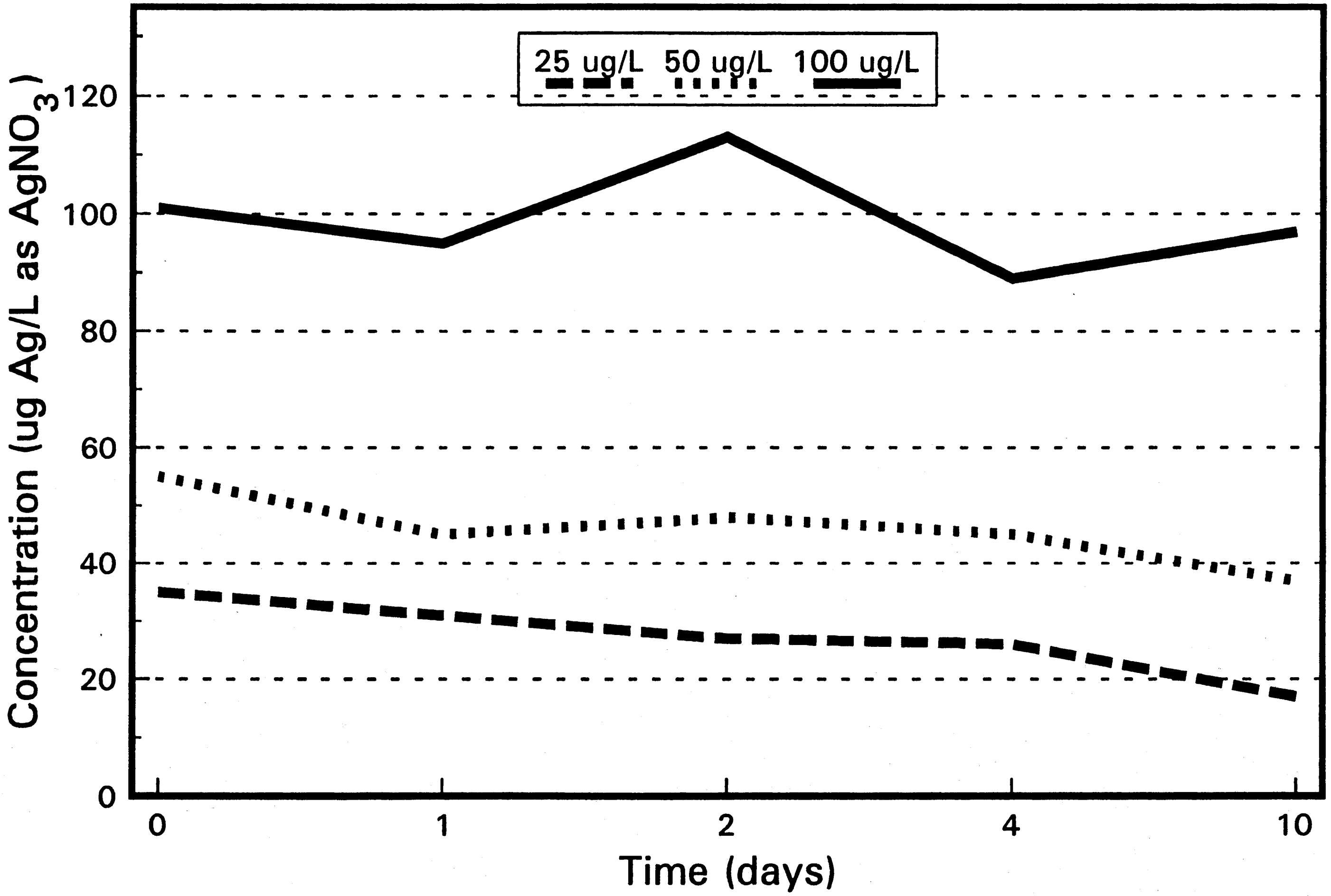

FIGURE 6. Ag FATE IN UMBFS POND WATER.

The Concentration of Silver in Mussels and Oysters from NOAA National Status and Trends Mussel Watch Sites

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Introduction

The objective of the National Oceanic and Atmospheric Administration (NOAA) National Status and Trends (NS&T) Mussel Watch Project is to quantify the current status of and identify trends in environmental quality of the nation's coastal and estuarine regions through the analysis of bivalve molluscs and surficial sediments for specific chemical contaminants. The levels of selected organic and trace-metal contaminants are compared on a site and regional basis, and temporal changes in bivalve contaminant burdens at each site are determined.

Bivalve molluscs are pervasive, sedentary biointegrators of both particulate-bound and dissolved trace-metal and organic compounds. The types and concentrations of contaminants found in the bivalves reflect man's activities near the shoreline of major waterways that feed into affected coastal areas and, to a lesser extent, the degree of atmospheric input of certain contaminants to the aquatic environment. Because bivalves can acquire contaminants directly from seawater and ingested food, changes in concentrations in bivalve tissue can provide a time-integrated measure of the abundance of bioavailable contaminants in the water column. Also, because bivalves concentrate contaminants by factors of 10^2 and 10^5, contaminants that are likely to be below the detection limits of even the best available analytical methods in water samples can be measured in their tissues.

NOAA Mussel Watch sampling began in 1986. Throughout the first four years of the Mussel Watch Project (Period 1), bivalves and sediments were collected by Battalle at least once at 80 East Coast and 60 West Coast sites, including sites in Alaska and Hawaii. Bivalve collections were made once annually at most sites, and sediments were collected at each site at least once.

The established Mussel Watch sampling sites represent a wide range of geographic locations; these include estuaries, embayments and harbors known to be contaminated, as well as estuaries and offshore locations remote from known sources of pollution. Site locations were selected by NOAA to represent areas that integrate contaminant accumulations but that are outside the zone of initial mixing or dilution of any point source. The sites were selected so as to be in depositional sedimentary environments. Further, the criteria include the availability of appropriate-sized bivalves (5- to 8-cm mussels; 7- to 10-cm oysters) in numbers sufficient to sustain repeated samplings. Where feasible, and assuming the above criteria were met, sites were established to coincide with historical monitoring sites of the United States Environmental Protection Agency Mussel Watch program.

Methods

Mytilus edulis were collected at sites on the East Coast from Maine south into Delaware Bay, and sites on the West Coast from Alaska to southern California. The American Oyster *Crassostrea virginica* was collected at sites from Delaware Bay south along the east coast of Florida. The California, or surf, mussel *Mytilus californianus* was collected at 30 sites on the West Coast over generally the same range as *Mytilus edulis*. The oyster *Ostrea sandvicensis* was collected at two sites located in Hawaii.

The bivalves from each site were sized and shucked in preparation for sample processing. After shucking, the tissues of all animals collected from each station were homogenized, and an aliquot was freeze-dried to a constant weight and ground to a powder in a plastic mixer mill. For analysis of silver, a 0.3-g aliquot of dry tissue homogenate was weighed in an acid-cleaned, preweighed Teflon digestion bomb. The sample was digested with 5 mL nitric acid in a microwave oven. After cooling, the sample was diluted with deionized distilled water. Silver was quantified in tissue digested by either inductively coupled plasma mass spectrometry or graphite furnace atomic absorption with zeeman background correction.

Results and Discussion

The concentrations of silver in mussels and oyster collected from rural sites (remote from known sources of silver) were approximately 0.3 µg/g dry weight for mussels from the northeastern United States (U.S.), 0.1 µg/g for mussels from the west coast of the U.S., and 1.0 µg/g for oysters from the southeastern U.S.

Elevated concentrations of silver occurred in mussels and oysters collected from urban areas with the highest silver concentrations observed in bivalves near major wastewater treatment plant outfalls. The East Coast sites with elevated concentrations of silver in bivalves include sites in Boston Harbor, Hudson-Raritan Estuary, Delaware Bay and Chesapeake Bay. Maximum silver concentrations for East Coast mussels were 2 µg/g and for oysters were 5 µg/g.

The West Coast sites with significant silver contamination were from California. The highest silver concentrations in West Coast mussels were 4 to 5 µg/g for sites near the Los Angeles and San Diego wastewater treatment plant outfalls. Mussels from several San Francisco Bay sites also contained elevated silver concentrations (about 1 µg/g) compared to rural sites.

Although the concentrations of silver and other metals (Cu, Hg, Pb and Sn) were occasionally elevated at the same urban sites, there was not a high degree of correlation between silver and other metals.

Silver in mussels and sediment is a relatively sensitive indicator of urban contamination because of naturally low concentrations of silver in water and sediment and the high bioaccumulation factor for silver in bivalves.

Additional information may be obtained from Dr. Tom O'Conner, NOAA National Status and Trends Office, Coastal Monitoring Branch, NOAA N/ORCA21, 6001 Executive Blvd., Rockville, MD 20852.

Bioavailability of Silver to Bivalves in Estuaries

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Chloro-complexes dominate the speciation of Ag in brackish waters, in contrast to freshwater. In laboratory experiments, the strong chloro-complexes affect the adsorption of Ag on iron oxide surfaces. Sorption to natural sediments is stronger than indicated by experiments with iron oxides alone, and is affected by the nature of the sediment. As indicated in the latter experiments, sediments contaminated by anthropogenic Ag in San Francisco Bay are enriched in the metal, with a distribution coefficient of approximately 10^4 - $5X10^4$.

Deposit feeding clams rapidly concentrate Ag spiked into sediments at low concentrations (0.5 - 4.0 ug/g). The goechemical characteristics of the sediment affect bioaccumulation. Enrichment of Ag in the tissues of deposit feeders from estuarine ecosystems also can occur where sediments are contaminated. In uncontaminated environments (Ag in sediments 0. 1ug/g), Ag concentrations in deposit feeding clams are < 1.0 ug/g dry wt. These clams accumulate 100 ug/g Ag or higher in environments where Ag is enriched to only 1 - 2 ug/g in sediments. Thus small changes in Ag to estuaries appear to result in a strong bioaccumulation response in these bivalves. Bioaccumulation of Ag does not correlate well with total Ag in sediments. Strong correlations are observed with weak-acid extractable (1N HCl) Ag, however. Macoma balthica from 17 English estuaries and from San Francisco Bay all followed the same correlation between HCl-extractable and tissue Ag. The decline in Ag concentrations in M. balthica near a local sewage treatment plant also followed declining concentrations in sediments over a 17-year study. The form of HCl-extractable Ag is difficult to demonstrate, but approximately forty percent appears to be associated with organic materials in English estuaries. In the laboratory, some types of organic materials can enhance Ag bioavailability. Nevertheless, when bulk Ag concentrations and assimilation efficiencies are employed models of Ag uptake from food alone do not appear to explain tissue concentrations observed in nature. Quantitative understanding of Ag uptake from solution, better geochemical understanding of Ag partitioning between sediments and pore water/surface waters, and better understanding of Ag bioavailability from different food types, including sulfide-rich sediments, are necessary to understand Ag bioavailability in estuaries.

The Effect of Silver on Plants Grown in Sludge-Amended Soils

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Introduction

Land application of sewage sludge is one disposal option following wastewater treatment. Sewage sludges arising from the treatment of photo processing solutions may contain silver in the form of silver sulfide, Ag_2S. Two studies were performed to evaluate the effect of silver sulfide on plants grown in sludge-amended soils.

Methods

Study 1: The first study was performed to evaluate the effect of silver sulfide on the emergence, growth and yield of corn, lettuce, oat, turnip and soybean seedlings grown in soil amended with 1% anaerobically digested sewage sludge. The anaerobically digested sewage sludge was obtained from the drying beds of the Avon Wastewater Treatment Plant (Avon, New York). The treatment plant is a publicly-owned treatment works (POTW) serving the Village of Avon, New York (population of approximately 3,200) and treating approximately 2.75 million gallons of wastewater per day. There is no major source of industrial effluent impacting this treatment plant.

Crystalline silver sulfide was first mixed with the anaerobically digested sewage sludge to generate two different sludges containing silver at the concentrations of 1,000 and 10,000 mg Ag/kg sludge. The silver-amended sludges were then blended with the soil at a rate of 1% by weight (equivalent to approximately 10 tons of sludge per acre). The blending of the silver-amended sludge with the soil mixture produced final concentrations of 10 and 100 mg Ag/kg soil mixture. A sludge-amended soil control was prepared by blending the sludge (without silver) with the soil at a rate of 1% by weight. The sludge-amended soils were then transferred to growing containers.

Forty seeds of each plant species were sown in each of the prepared soils. The growing pots were placed in a greenhouse equipped with supplemental lighting (sodium vapor lamps). All pots received a 16-hour photoperiod. The growing pots were observed daily for evidence of germination/emergence. Approximately 14 days after 50 percent of the seedlings of each plant species emerged, the heights of one half of the randomly selected seedlings of each species in each soil treatment were measured and these seedlings were then harvested. Wet and dry weights of the shoots and roots of each of the harvested seedlings were measured. The silver concentrations of the dried shoots and roots of the harvested seedlings were determined by atomic absorption spectroscopy.

Ten remaining seedlings of each species in each soil treatment were transplanted to larger pots and grown to maturity. The heights of the mature plants were measured and the plants were then harvested. After harvest, the stalk wet and dry weights, root wet and dry weights and plant yields (e.g., number of seeds, size of turnip bulb, etc.) were measured. The silver concentrations of the roots, stalks and edible portions of each plant species were determined by atomic absorption spectroscopy.

Study 2: The second study was performed to evaluate the effect of crystalline silver sulfide on the germination/emergence, growth and yield of lettuce, spinach and Chinese cabbage seedlings grown in soil amended with 1% anaerobically digested sewage sludge. The anaerobically digested sewage sludge was again obtained from the drying beds of the Avon Wastewater Treatment Plant (Avon, New York).

The silver sulfide was first mixed with the anaerobically digested sewage sludge to generate three different sludges containing silver at concentrations of 1,000, 5,000 and 10,000 mg Ag/kg sludge. The silver-amended sludges were then blended with the soil at a rate of 1% by weight (equivalent to approximately 10 tons of sludge per acre). The blending of the silver-amended sludge with the soil mixture produced final nominal concentrations of 10, 50 and 100 mg Ag/kg soil mixture. A sludge-amended soil control was prepared by blending the sludge (without silver sulfide) with the soil at a rate of 1% by weight. The sludge-amended soils were then transferred to growing containers.

Twenty-one seeds of each plant species were sown in each of the prepared soils. The growing pots were placed in a greenhouse equipped with supplemental lighting (sodium vapor lamps). All pots received a 16-hour photoperiod. The growing pots were observed daily for evidence of germination/emergence. All plants were grown to maturity, then harvested. Wet and dry weights of the above soil portions and roots of each of the harvested plants were measured. The silver concentrations of the dried edible portions and roots of the plants were determined by atomic absorption spectroscopy.

Results and Discussion

Study 1: The presence of silver, as silver sulfide, in sludge-amended soils at levels of up to 100 mg Ag/kg did not adversely affect the germination and emergence of corn, lettuce, oat, turnip or soybean seeds sown in the sludge-amended soils. Turnip and lettuce seedlings were unaffected by the presence of levels up to 100 mg Ag/kg soil. The oat, corn and soybean seedlings exposed to silver had heavier and/or taller ($\alpha \leq 0.05$) shoots than the corresponding control plants. (Refer to Figure 1.) When the plants were transplanted and allowed to grow to maturity, oat, corn, turnip and lettuce plants did not appear to be physically affected by levels of silver sulfide up to 100 mg Ag/kg soil. Mature soybeans exposed to silver sulfide at a level of 10 mg Ag/kg soil had significantly ($\alpha \leq 0.05$) greater stalk dry weights than those of control plants grown in 1% sludge-amended soil. The mean dry weights of stalks of soybean plants grown in soil containing 100 mg Ag/kg were not significantly different from the mean dry weights of stalks from soybeans grown in soil which had been amended with anaerobically-digested sewage sludge. (Refer to Figure 2.)

The silver concentrations of the edible portions of mature corn, turnips, oats and soybeans were below the 0.08 µg/g limit of quantitation for the analytical method. The silver content of the leaves of lettuce plants grown in sludge-amended soil containing silver sulfide at 10 mg Ag/kg soil was not significantly different than that of lettuce plants grown in the 1% sludge control soil. The leaves of lettuce plants grown in sludge-amended soil containing silver sulfide at 100 mg Ag/kg soil had significantly (approximately 8 times) more silver than the leaves of the corresponding control lettuce plants grown in 1% sludge-amended soil. (Refer to Figure 3.)

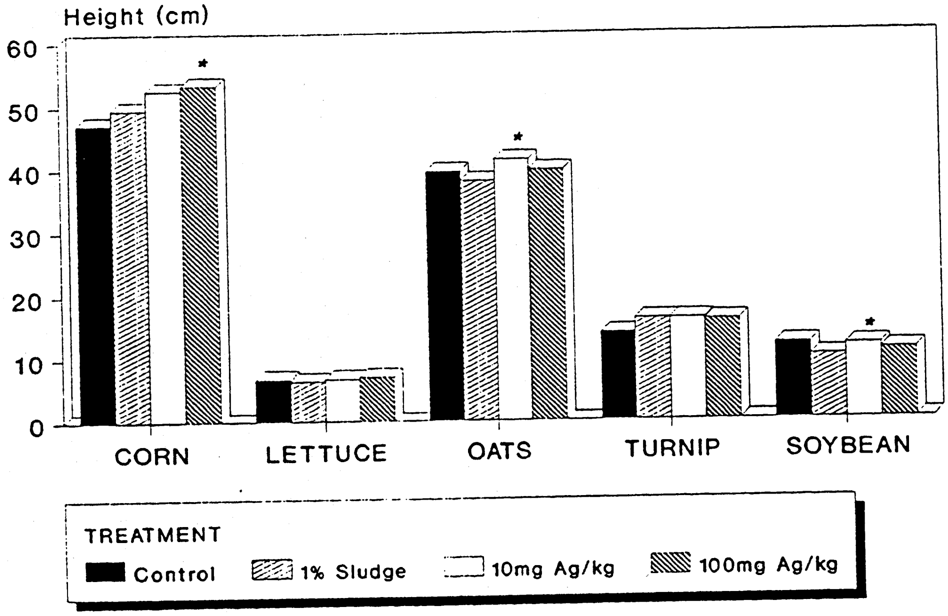
Study 2: There was no effect of silver sulfide on the mean emergence time of Chinese cabbage, lettuce or spinach seeds sown in the soil mixtures used in this study. Chinese cabbage plants grown in the sludge-amended soils containing silver sulfide were significantly lighter in dry weight ($\alpha \leq 0.05$) than the plants grown in the control soil containing 1% anaerobically digested sewage sludge, although they were heavier than the plants grown in soil that was not amended with 1% sludge. Similar results were obtained from the lettuce plants grown in the silver-amended soil mixtures. Silver sulfide in sludge-amended soil had no effect on the mean dry weight of spinach plants. There was no apparent concentration-response relationship observed between the amount of silver sulfide in the test soil and the mean dry weight of the mature plants. (Refer to Figure 4.)

The silver concentrations of Chinese cabbage and spinach plants grown in the sludge-amended soils containing silver sulfide were not greater than those of the plants grown in the control soils containing 1% anaerobically digested sludge. The concentrations of silver in lettuce plants grown in soils containing 50 mg Ag/kg and 100 mg Ag/kg were significantly higher (approximately 2 and 4 times, respectively) ($\alpha \leq 0.05$) than those of plants grown in the 1% sludge control soils. There appears to be evidence of a concentration-response relationship between the amount of silver in the test soils and the concentration of silver in the leaves of mature lettuce plants. (Refer to Figure 5.)

Conclusions

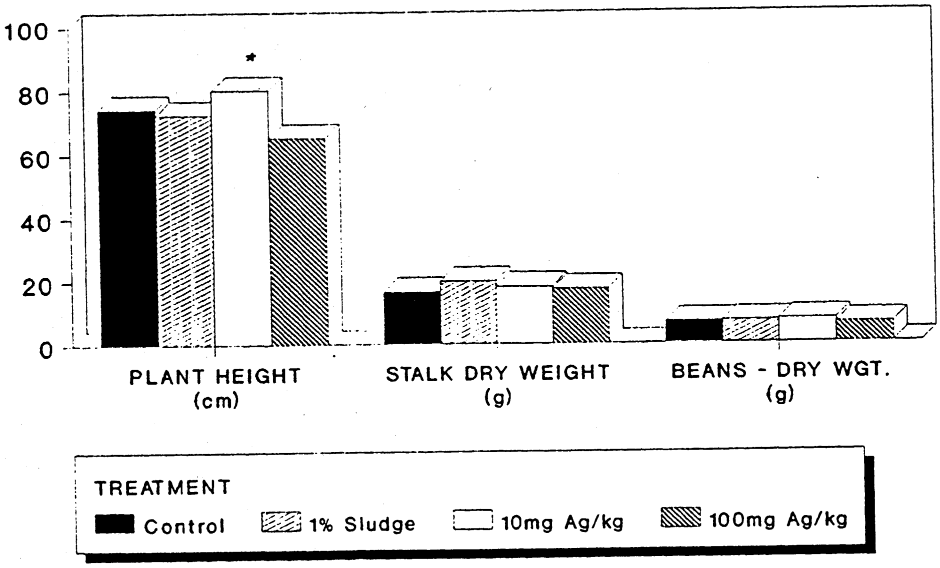
The presence of silver, as silver sulfide, at levels of up to 100 mg Ag/kg soil mixture did not adversely affect the germination or emergence of corn, lettuce, oat, turnip, soybean, spinach or Chinese cabbage seeds. Silver sulfide, at levels up to 100 mg Ag/kg appears to have no effect on the size of corn, oat or turnip plants grown in sludge-amended soils. The presence of up to 100 mg Ag/kg soil did not adversely affect the yield (number of seeds produced, etc.) of soybean, corn, turnip or oat plants. The presence of silver sulfide at a level of 10 mg Ag/kg soil, appears to result in slightly smaller lettuce, soybean and Chinese cabbage plants grown in soils amended with 1% anaerobically digested sewage sludge, however, the apparent effect of silver does not appear to remove the beneficial effects of applying sewage sludge to land as a fertilizer.

FIGURE 1. Heights of Corn, Lettuce, Oat, Turnip, and Soybean Seedlings Harvested 14 Days After 50% Emergence



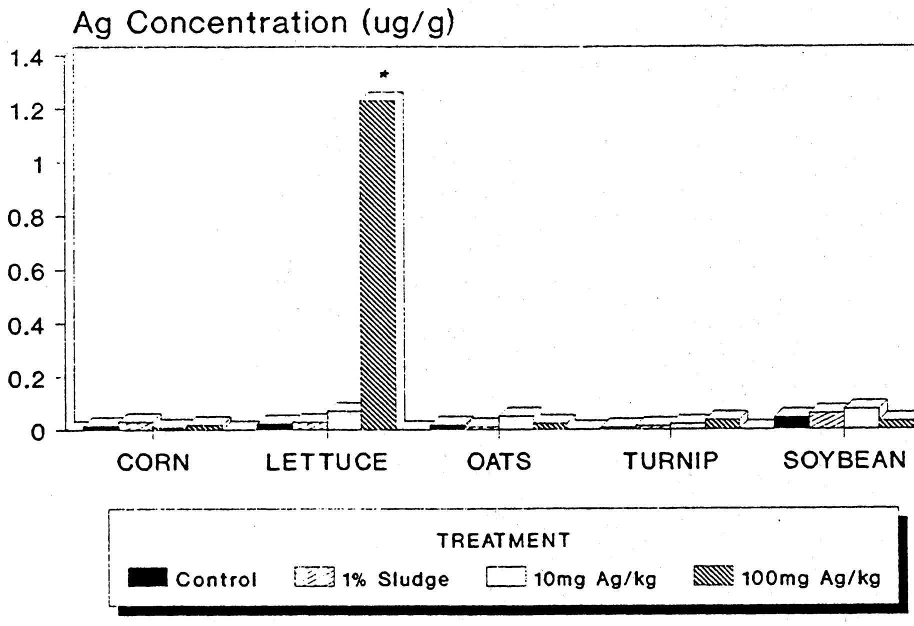
(Symbol (*) indicates samples which are significantly different ($\alpha \leq 0.05$) from the control group)

FIGURE 2. Mean Height and Dry Weights of Soybean Plants Harvested at Maturity



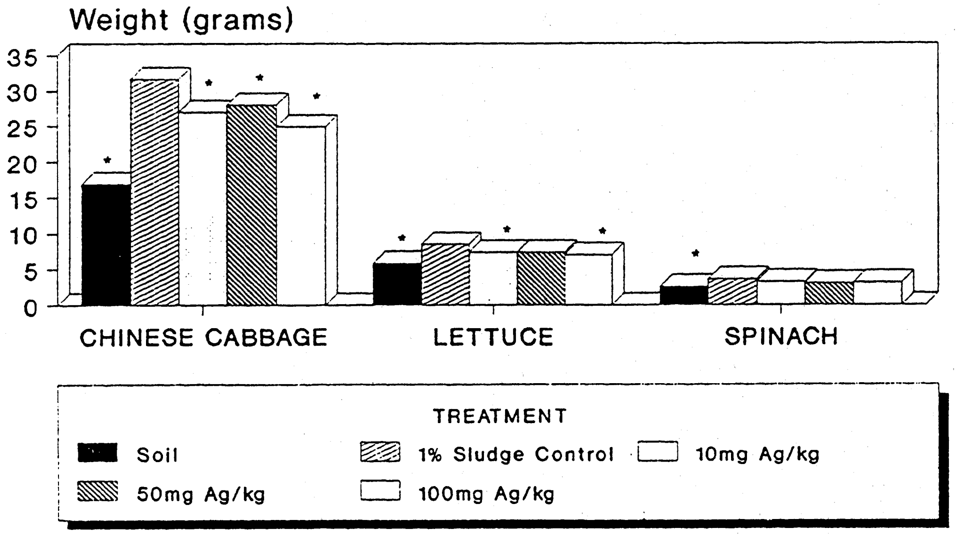
(Symbol (*) indicates samples which are significantly different ($\alpha \leq 0.05$) from the control group)

FIGURE 3. Silver Concentration of Edible Portions of Corn, Lettuce, Oats, Turnips, and Soybeans Grown in Sludge-Amended Soils Containing Silver Sulfide



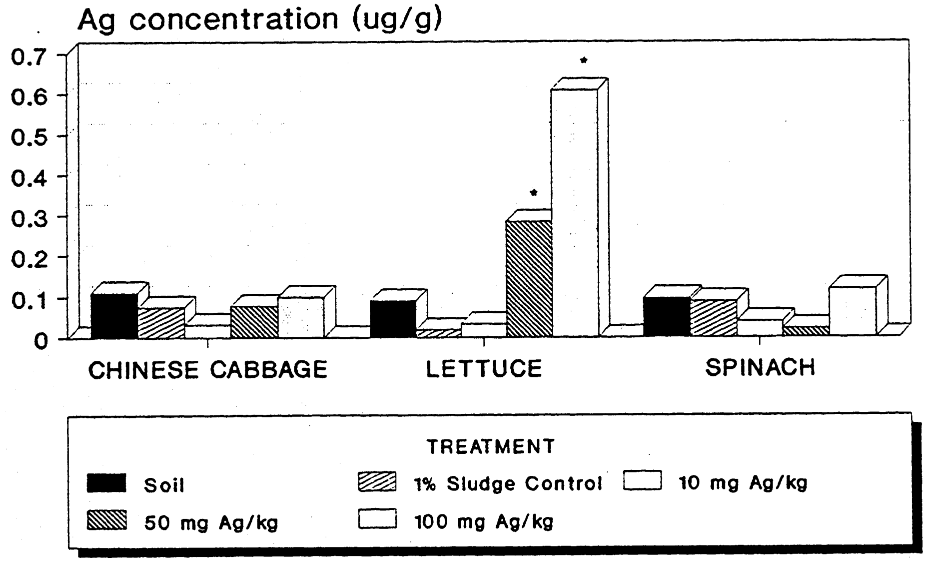
(Symbol (*) indicates samples which are significantly different ($\alpha \leq 0.05$) from the control group)

FIGURE 4. Mean Dry Weights of Chinese Cabbage, Spinach, and Lettuce Plants Grown in Sludge-Amended Soils Containing Silver Sulfide



(Symbol (*) indicates samples which are significantly different ($\alpha \leq 0.05$) from the control group)

FIGURE 5. Silver Concentration of Edible Portions of Chinese Cabbage, Spinach, and Lettuce Plants Grown in Sludge-Amended Soils Containing Silver Sulfide



(Symbol (*) indicates samples which are significantly different ($\alpha \leq 0.05$) from the control group)

Hazard Assessment of Metal Pollution

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Bioindicators

Only a fraction of the metals in an aquatic environment will be taken up by biota (Luoma 1983; Hodson 1988). It follows that the levels of heavy metals in the environment need not to correlate to the impact of metals on organisms. Better information on the bioavailability of potential toxic metals is obtained by measuring the accumulation of the metals in biota. However, the levels of heavy metals in organisms provide little information about the toxic effects of the metals. Hence, large efforts have been directed towards the development of methods that can detect the effects of pollutants on aquatic organisms (Bayne *et al*,, 1988; Landner 1989; Addison & Clarke 1990). Such methods range from effects on the ecosystem level to the detection of biochemical alterations. An advantage of the biochemical approach is the potential early warning capacity (Haux & Forlin 1988; Thomas 1990). The basic concept for biochemical indicators of environmental pollution is that all effects of chemicals originate in the perturbation of a biochemical reaction in an individual organism (Hodson 1986). Thus, it may be possible to take action before effects are apparent at a higher level of biological organization. Furthermore, in some cases, biochemical alterations are relatively specific to a single chemical or a group of chemicals (Haux & Forlin 1988, 1989; Thomas 1990).

Metallothionein

Metallothionein (MT) is an ubiquitous, low-molecular weight, cysteine-rich protein that avidly binds various transition elements, especially group IB and IIB metals (Waalkes *et al.*, 1984; Hamer 1986). A central feature of MT is that the synthesis of the protein can be induced by metals (Klassen & Lehman-McKeeman 1989; Waalkes & Goering 1990; Hogstrand *et al.*, 1991). In mammals, a variety of hormones and other factors are able to induce MT, but such factors have little, if any, influence on the synthesis of MT in teleost fish (George & Young 1986; Overnell *et al.*, 1987; Hyliner *et al.*, 1989; George *et al.*, 1992; Burgess *et al.*, 1993). The exact function of MT is yet to be demonstrated, but the protein is generally regarded to be involved in processes including the intracellular homeostasis of Cu and Zn, enzyme activation, the acute-phase response to infections, free-radical scavengering, and detoxification of metals (Bremner & Beattie 1990).

Metallothionein as Bioindicator of Exposure to Cu, Zn and Cd

There is increasing evidence that an elevated level of MT in fish indicates that the fish has been exposed to Cu, Zn or Cd (Roch *et al.*, 1982; Roch & McCarter 1984a; Olsson & Haux 1986; Hogstrand & Haux 1990; Hogstrand *et al.*, 1991; Sulaiman *et al.*, 1991; Hylland *et al.*, 1993). Specific, sensitive and rapid analyses of MT can be performed by immunoassays developed for MT from fish (Hogstrand & Haux 1990a; Norey *et al.*, 1990) or differential pulse polarography (Olafson & Sim 1979; Thompson & Cosson 1984). Thus, it is possible to carry out the quantification of MT within the framework of biomonitoring programs.

A biochemical bioindicator must have an early warning capacity (Haux & Forlin 1989). That is, the effect has to be detected before the population is affected. Roch and McCarter (1984b) plotted the level of hepatic MT of rainbow trout, caged in a water system polluted by a mixture of Cu, Zn and Cd, against data on acute toxicity to the metal mixture. The data showed that the LC_{10} concentration of the metal mixture corresponded to a four-fold increase in MT (Roch & McCarter 1984a). Further, during a field study on Bermuda, elevated MT contents were found in fish collected from moderately metal contaminated areas (Hogstrand & Haux 1990b). The relationship between toxicity data and the MT level, together with the presence of increased hepatic MT levels in feral fish from moderately polluted areas, suggest that MT does have an early warning capacity.

Another criterium for bioindicators is that a dose-response relationship should be established. In several studies, it has been shown that the level of MT in fish is dose-dependently increased by treatment of Cu, Zn or Cd. This includes induction following injection of Cd (Sabourin *et al.*, 1985; George 1989; Hogstrand & Haux 1990b), exposure to waterborne Cu (McCarter & Roch 1983; Hogstrand & Haux 1993), or a mixture of Cu, Zn and Cd (Roch & McCarter 1984b), and environmental exposure to heavy metal mixtures, mainly containing Cu and Zn (Roch *et al.*, 1982; Roch & McCarter 1984a; Hogstrand & Haux 1990b; Hogstrand *et al.*, 1991).

A response of a bioindicator should preferably enable interpretations in terms of decreased fitness of the individual and effects on population and ecosystem levels. Such connections have not been demonstrated for any specific biochemical indicator (Thomas 1990). A thorough understanding of the physiological function of the system will provide information that can be used for this health assessment. For example, the involvement of MT in the normal trace metal metabolism implies that an increased MT level and an altered metal composition of the protein could severely disturb cellular processes where Cu and Zn are involved. The ability of MT to protect the cell from metal toxicity suggests that cellular toxicity is to be expected from heavy metal that is not bound to MT and other detoxifying systems (Din & Frazer 1985). Thus, an increased amount of non-MT bound metal in the target organ of exposed fish can be indicative of an exceeded capacity of the animal to deal with heavy metals. At present, the only shown correlation between MT and the health status of the fish is the relationship between the level of hepatic MT and the data on acute toxicity (Roch & McCarter 1984a; Roch *et al.*, 1986).

Can Metallothionein be Used as a Bioindicator of Ag Exposure?

There has previously been no reports on the ability of Ag to induce the synthesis of MT. However, given that all other group IB and IIB transition elements induce MT production, it seems likely that Ag would have this ability too. With the objectives to evaluate MT as a bioindicator of Ag exposure, we studied the effect of three different silver salts ($AgNO_3$, $Ag[S_2O_3]_m$, and $AgCl_n$) on the levels of MT in liver and gills.

Ten juvenile rainbow trout (*Oncorhynchus mykiss*), 1.0±0.3 g (mean±SD, N=36) were exposed to either $AgNO_3$ at 4.3, 7.2, or 9.3 µg l^{-1}, $Ag[S_2O_3]_n$ at 98 or 164 mg l^{-1}, AgCl at 1.0 mg l^{-1}, and $AgCl_n$ in 50 mM NaCl, 0.93 mM NO_3^- at 120 µg l^{-1}. The exposure to 98 mg $Ag[S_2O_3]_n$ l^{-1} was performed in duplicate. An untreated group of fish for each of the salts tested served as control. In addition, one group was treated with 9.55 mM $Na_2[S_2O_3]$ to eliminate the possibility that $Na_2[S_2O_3]_n$ itself might cause changes in tissue MT levels. Silver chloride in 50 mM NaCl, 0.93 mM NO_3^- was prepared by mixing 0.93 mM of $AgNO_3$ ([Ag] = 100 mg l^{-1}) with 50 mM NaCl. However, according to the aquatic chemistry program MINEQL+ (Schecher 1991), 99.9% of the added Ag formed cerargyrite and the actual [Ag] left in solution was 120 µg l^{-1}. Using the same program, 93% of the dissolved Ag was calculated to be present as $AgCl_n$. A stock solution of silver thiosulphate was made by mixing AgCl and $Na_2[S_2O_3]$ in a molar ratio of 1 to 4 in deionized water (Nanopure). The fish were exposed for seven days at 15-17oC in covered 50-L polypropylene tanks supplied by air lines. Exposure of the fish was performed statically in dechlorinated Hamilton tap water ($[Na^+]$=0.6 mM; $[Cl^-]$=0.7 mM; $[Ca^{2+}]$=1.0 mM; $[HCO_3^-]$=1.9 mM; pH=7.9-8.2). Both the $AgNO_3$ and the $AgCl_n$ exposure media were exchanged daily. For economical reasons the same $Ag[S_2O_3]_n$ media were used throughout. Due to the high concentrations of Ag given (LC_{50}: $AgNO_3$=12 µg Ag l^{-1}; $Ag[S_2O_3]_n$=137 mg Ag l^{-1}, $AgCl_n$ > 10 mg Ag l^{-1} (Wood *et al.*, 1994)) there were mortalities in some of the treatments. The number of analyzes that the average MT values are based on are given in the figures. After seven days of exposure, the fish were killed by a blow to the head. The whole gill basket and the liver were dissected out, weighed and frozen in liquid nitrogen. Samples were stored at -70oC until used. The samples were subsequently thawed, processed and analyzed for MT by RIA as outlined by Hogstrand & Haux (1990a).

Exposure to $AgNO_3$ for seven days slightly increased the level of MT in liver but not in gills (Fig. 1). Silver thiosulphate dramatically increased the hepatic MT and also the induction of branchial MT was highly significant (Fig. 2). Compared to the response found for other metals, the increase in MT caused by $Ag[S_2O_3]_n$ was remarkably large. For example, exposure of juvenile rainbow trout for 12 weeks to waterborne $CuSO_4$ at 28% of the LC_{50}, increased the level of MT in the liver by 230% (Hogstrand & Haux 1993). Sixteen weeks of exposure to a mixture of Zn, Cu and Cd, at a level corresponding to 32% of the 96-h LC_{50}, induced hepatic MT in rainbow trout by approximately 320% (Roch & McCarter 1986). The present study shows that hepatic MT was increased by 480% in fish that were exposed to 71% of the 168-h LC_{50} for one week. Silver chloride was extremely difficult to get into solution and 1.0 mg l^{-1} of added AgCl had no effect on the hepatic MT content (Fig. 3). An easier way to get $AgCl_n$ into solution was to add $AgNO_3$ to water containing 50 mM NaCl. Exposing rainbow trout in this way to 120 µg Ag l^{-1} markedly induced MT in both liver and gills (200% and 140%, respectively (Fig. 4).

The reason for the much higher MT content found in fish exposed to $Ag[S_2O_3]_n$ or $AgCl_n$, than in $AgNO_3$ exposed fish, is probably directly related to the extremely high toxicity of $AgNO_3$. During short-term exposure, $AgNO_3$ is a surface-acting toxicant that severely, and seemingly specifically, interferes with the Na^+ and Cl^- transport over the gills (Wood *et al.*, 1994). Osmoregulatory disturbance followed by hypovolemic collapse of the circulatory system and death probably occurred before appreciable amounts of Ag accumulated in the animal. Silver thiosulphate and $AgCl_n$ are several orders of magnitude less toxic to rainbow trout during short-term exposure (Wood *et al.*, 1994). Nevertheless both these salts are clearly bioactive since they were capable to markedly elevate both hepatic and branchial MT levels.

The concentrations of Ag used in the present study were unrealistically high compared with levels normally found in aquatic systems. However, the time-course of the induction was relatively rapid and it is unlikely that a steady state in MT was established during the experiment. The time-course of the induction will depend on the retention rate of the inducing metal. Usually, it takes more than four weeks to develop a full MT response in fish during exposure to waterborne metals. Silver is accumulated very slowly by fish (Garnier & Baudin 1990; Garnier *et al.*, 1990), which would indicate that the time period required to obtain maximal MT content is comparably long. The most important information from the present study is that Ag is a very potent inducer of MT. To further evaluate MT as a biomarker for Ag exposure, it is necessary to conduct long-term experiment where low levels of Ag are administrated via the water and/or the diet. There is also a need to measure MT in feral fish from a water that contains Ag as single major pollutant to find out whether or not MT is elevated in fish environmentally exposed to Ag.

Acknowledgements

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Wood, C.M., S. Munger and C. Hogstrand. 1994. The physiological mechanisms of toxicity of silver and other metals to freshwater fish. (*c.f.* this volume)

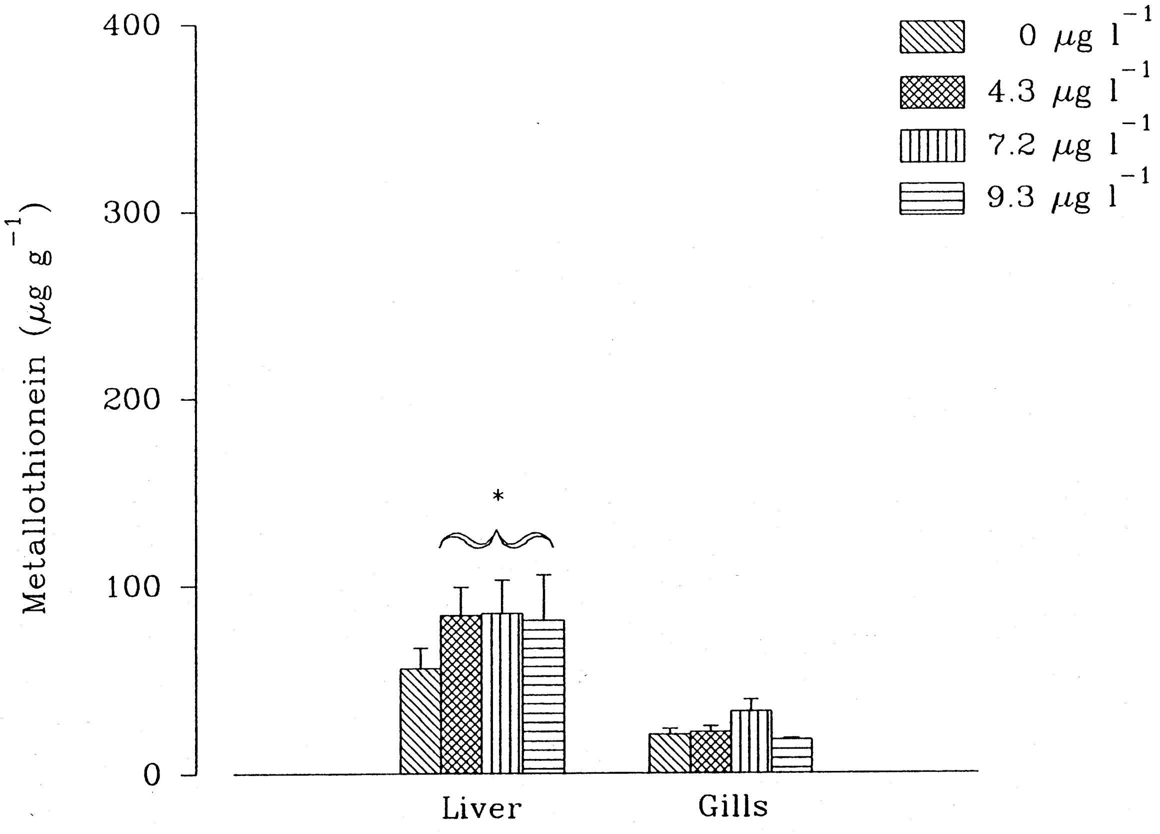
Figure Legends

Fig. 1. — Metallothionein content in liver and gills of juvenile rainbow trout, exposed for seven days to $AgNO_3$ at 4.3 (N=9), 7.2 (N=5), and 9.3 (N=2) µg Ag l^{-1}. A control group held under identical conditions served as control (N=10). Vertical error bars denote one way SE. The hepatic MT level was significantly increased by $AgNO_3$ treatment at $p<0.05$ in the Equality Against Ordered Alternatives test (Lehman 1975).

Fig. 2. — Metallothionein content in liver and gills of juvenile rainbow trout, exposed for seven days to $Ag[S_2O_3]_n$ at 98 (N=18), or 164 (N=3) mg Ag l^{-1}. A control group held under identical conditions served as control (N=10). In addition, one group of fish were exposed 9.55 mM of $Na_2S_2O_3$ (N=6). Vertical error bars denote one way SE. Groups with common letter subscripts were not statistically different in a Tukey HSD-test following a one-way ANOVA.

Fig. 3. — Metallothionein content in liver of juvenile rainbow trout, exposed for seven days to AgCl at 1.0 mg Ag l^{-1} added to the water (N=10). Gill MT was not determined (N.D.). A control group held under identical conditions served as control (N=10). Vertical error bars denote one way SE. The groups were not statistically different at $p<0.05$ in the Mann-Whitney U-test.

Fig. 4. — Metallothionein content in liver and gills of juvenile rainbow trout, exposed for seven days to $AgCl_n$ at 100 mg total Ag l^{-1} (N=10). The concentration of waterborne Ag was calculated to be 120 µg l^{-1}, using the MINEQL+ aquatic chemistry program (Schecher 1991). A control group held under identical conditions served as control (N=10). Vertical error bars denote one way SE. An * denotes a statistically significant difference from the control at $p<0.05$ in the Mann-Whitney U-test.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Metallothionein ($\mu g\ g^{-1}$)
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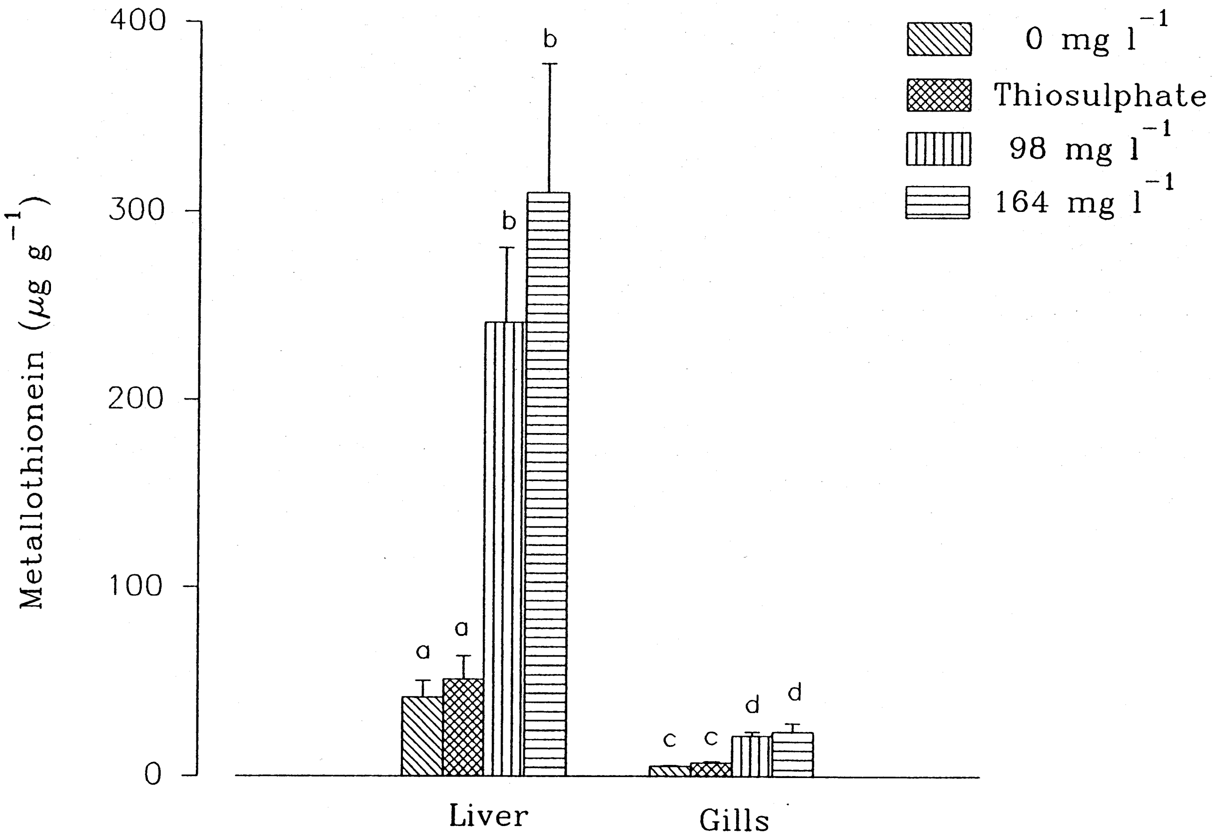
9.3 $\mu g\ l^{-1}$
*
Liver
Gills


Metallothionein ($\mu g \ g^{-1}$)
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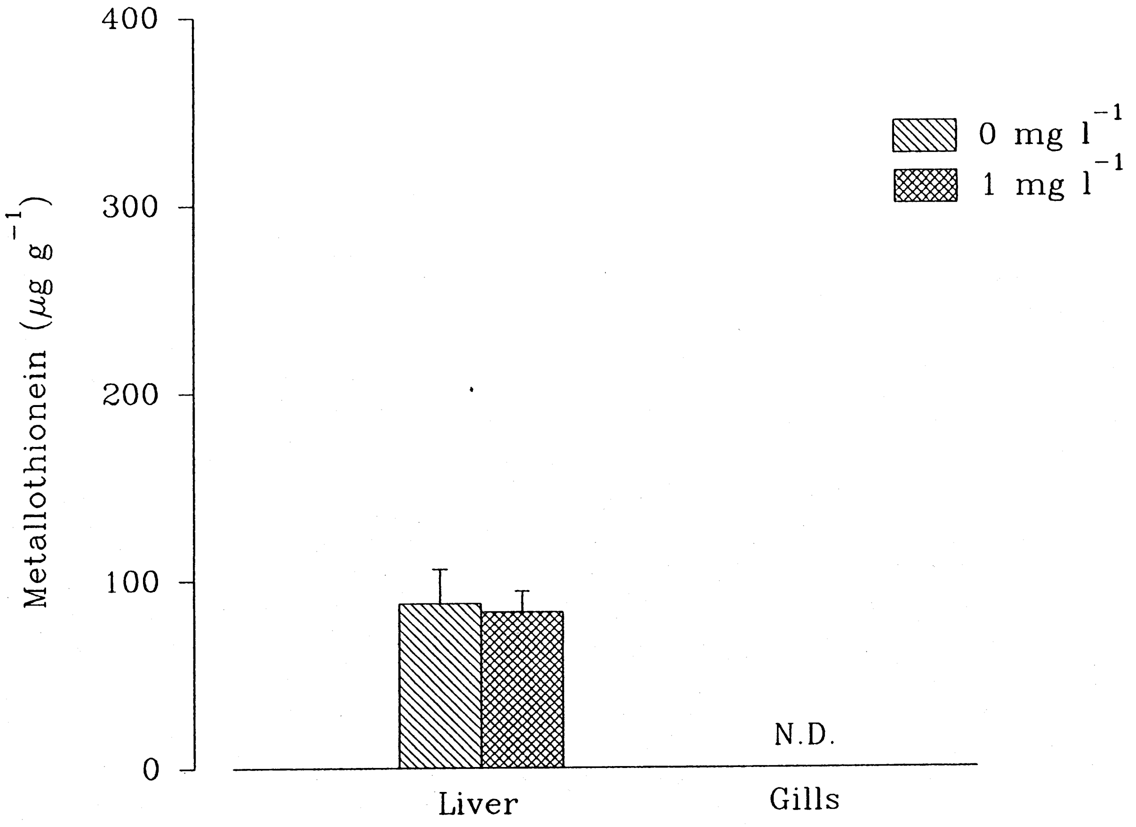
0 mg l^{-1}
Thiosulphate
98 mg l^{-1}
164 mg l^{-1}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1 mg l⁻¹
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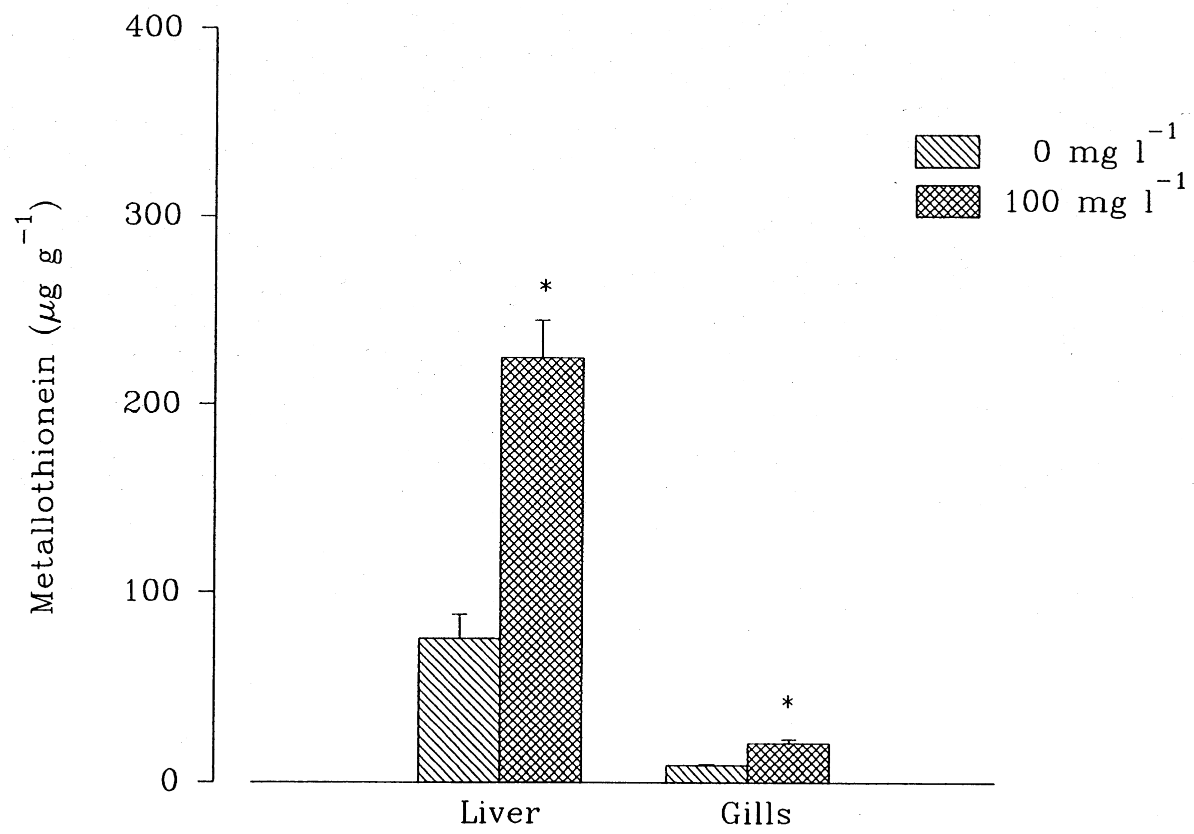
Liver
Gills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SESSION E: Toxicity of Metals to Aquatic Organisms

J.H. Rodgers and T.W. Bober,
Session Chairs

Factors and Mechanisms Regulating the Uptake and Effects of Metals in Aquatic Organisms

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Trace metals can exist in natural waters in numerous chemical species, profoundly affecting their uptake by organisms. Many occur as inorganic complexes with chloride, hydroxide, and carbonate ions while others [e.g., copper (II)] are heavily complexed by natural organic ligands. Metals may also exist in numerous particulate forms, including ions adsorbed onto particle surfaces, metals present within specific mineral phases (metal oxides or sulfides) or bound within organic particles. A large body of experimental evidence has shown that the uptake and biological effects of metals are regulated by the concentration of either free metal ions, or in some cases, the concentration of kinetically labile dissolved inorganic species (free metal ions plus inorganic complexes). Consequently, the formation of organic complexes and many inorganic complexes decreases biological uptake of metals by decreasing their free ion concentrations.

This "ionic dependence" is a consequence of the predominant mechanism of cellular metal uptake: facilitated or active transport of metals across the cell's external bilayer membrane by binding with specific transport ligands. The rate of cellular metal uptake will be related to the thermodynamic and kinetic factors regulating the reaction of metal species with these membrane sites. Under steady state conditions the rate of metal uptake by the transport ligand will be described by the Michaelis Menten or saturation kinetics equation:

$$V = \frac{V_{max}\,[M]}{[M] + K_s} \qquad (1)$$

where V_{max} is the maximum rate achieved when all of the transport ligands are bound to the metal, [M] is the concentration of either free metal ions or kinetically labile inorganic species, and K_s is the half saturation constant equal to the available metal concentration at which half of the carrier ligands are complexed.

The half saturation constant, K_s, is defined by the equation:

$$K_s = (k_d + k_{in})/K_f \qquad (2)$$

where k_f, k_d and k_{in} are rate constants for, respectively, formation of the membrane transport ligand complex, dissociation of metal back into the external medium and transfer of the metal from the membrane complex into the cytoplasm. If $k_d >> k_{in}$, a pseudoequilibrium will be established between metal species in the medium and metal bound to the transport ligand, and at equilibrium, the concentration of the carrier complex, and therefore, the uptake rate will be related to the external free metal ion concentration. However, in cases where $k_{in} >> k_d$, the system would be under kinetic control and would be controlled by the concentration of labile inorganic species, which possess sufficiently rapid ligand exchange kinetics to permit efficient donation of metal ions to the transport ligand. In cases of kinetic control, organic chelates and most bound particulate forms of metals (e.g., metal oxides) would not be directly available for uptake since their dissociation and ligand exchange kinetics (or their diffusion rates in the case of colloids and particulates) are too slow to permit efficient metal donation to the transport ligand.

The membrane transport systems involved are used by the cell for the uptake of necessary nutrient metals (e.g., Mg, K, Fe, Zn, Mn, Co). To regulate uptake, and thus, internal concentrations of essential nutrient metals, cells usually increase V_{max} as the available concentration of metal outside the cell is decreased. Toxic metals often gain entry into the cell by utilizing these nutritive metal transport sites, resulting in competitive (i.e., antagonistic) interactions between toxic and nutrient metals in terms of both cellular uptake and resulting biological effects. For example, the toxic metal cadmium can be taken up by algal cells through the cells' transport systems for zinc, manganese, or iron. As a result, cadmium uptake will be related not only to the free cadmium ion concentration, but also to the available concentrations of these three nutrient metals.

Specific transport ligands are necessary for cellular metal uptake because external bilayer lipid membranes are impermeable to charged species or highly polar neutral metal complexes. However, some neutral, less polar metal species, such as $HgCl_2$ or CH_3HgCl, are sufficiently lipohylic to diffuse directly through bilayer membranes. Other neutral complexes (e.g., $CdCl_2$ and $CuCO_3$) are too polar to permit appreciable uptake by this mechanism, while AgCl complexes may also be sufficiently nonpolar to permit some cellular uptake by direct bilayer diffusion. Thus, cellular uptake of inorganic mercury and silver is often controlled by the concentration of neutral $HgCl_2$ and AgCl complexes while that of Cd is related to the concentration of free Cd^{2+}. Effects of chloride ion complexation are particularly important in estuaries where chloride ion concentrations can vary by 1,000-fold.

It is clear that many complex chemical and biological factors control the cellular uptake of both nutritive and toxic metals. Although we have obtained a general understanding of many of these factors, along with their underlying causative mechanisms, there is still much to be learned about this extremely complex subject.

The Physiological Mechanisms of Toxicity of Silver and Other Metals to Freshwater Fish

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The gills of fish are delicate, anatomically complex structures which comprise over 50% of the total surface area of the animal. In the gills, blood is brought into intimate contact with the external water for exchanges of respiratory gases, ions, and other substances by transport processes comparable to those of lungs, kidney and digestive tract in humans. It is therefore not surprising that most metals of regulatory concern exert their primary effects as "surface-active" toxicants on the gill epithelium. At very high, acutely lethal levels, most metals cause gross epithelial damage, inflammation, edema and mucification of the gills resulting in rapid death due to blockage of respiratory gas exchange ("suffocation"). However, at *environmentally realistic* metal levels, the bulk of available evidence suggests that the more slowly developing toxic effects are due mainly to *free ionic metal* binding to specific sites on the gill. Therefore, water chemistry can have a profound effect on metal toxicity. In general, water hardness (Ca^{2+} and Mg^{2+}) is protective, though the extent of the ameliorative effects of hardness varies greatly amongst metals. The effects of water pH and alkalinity cannot be generalized, but rather are metal specific through their influence on both metal speciation and the availability and avidity of binding sites on the gill. However, the presence of alternate ligands dissolved or suspended in the water column is often the principal protective feature, especially in natural exposures, in contrast to laboratory exposures with reconstituted or defined media. For example, organic acids, fluoride, chloride, sulfate, thiosulfate, carbonates, *etc.*, occurring in natural waters may all serve to reduce or prevent toxicity, either by removing ionic metal from solution, and/or by out-competing gill ligands for the metal in question.

Knowledge of the *toxic mechanism(s) of action* for a pollutant is central to environmental toxicology. The toxic mechanism is a key piece of evidence required by regulatory agencies and advisory bodies in order to make reasonable and informed decisions. In the past decade, fish physiological research has made considerable progress in this area for several metals of concern, as well as for H^+, often considered as an "honorary metal." In each case, toxicity appears due to interaction with specific sites on the gills resulting in an interference with one or more gill functions (Wood 1992). In turn, this "surface-active" toxicity is reflected *internally* by changes in blood chemistry. Indeed the first clues as to the nature of the toxic effect have always been provided by blood chemistry data.

For example, we now know that H^+ specifically causes a severe disturbance of plasma salt regulation (Na^+ and Cl^-; "ionoregulatory toxicity") by reducing the uptake and stimulating the passive loss of Na^+ and Cl^- across the gills. This occurs because H^+ competitively inhibits the Na^+ carrier (and apparently also the Cl^- carrier by secondary effect) on the apical membrane of the gill transport cells, and opens the pathways between cells for diffusive Na^+ and Cl^- leakage by a Ca^{2+}-dependent mechanism (Wood 1989). Cu has virtually identical effects on Na^+ and Cl^- regulation, but mainly by a different mechanism - inhibition of the basolateral Na^+/K^+ ATPase enzyme which provides the energy for transport and opening of the pathways between cells by a Ca^{2+}-independent effect (Lauren and McDonald 1986, 1987). Cd, on the other hand, has no effect on Na^+ and Cl^- regulation, but rather causes potentially lethal plasma hypocalcemia by specifically inhibiting the basolateral Ca^{2+}ATPase, thereby blocking Ca^{2+} uptake from the water (Verbost *et al.*, 1987, 1988). Zn is superficially similar to Cd in its toxic action, causing hypocalcemia by an inhibition of Ca^{2+} uptake. However, in this case, the detail of the toxic mechanism differs, because Zn appears to competitively block the apical Ca^{2+} channels (Spry and Wood 1988, 1989; Hogstrand *et al.,* 1993). Al, at environmentally realistic concentrations, is unique in causing two toxic mechanisms, a "respiratory toxicity" due to an inflammatory thickening of the blood-to-water diffusion distance, and an "ionoregulatory toxicity" due to inhibition of basolateral Na^+/K^+ATPase and opening of leakage pathways by a Ca^{2+}-dependent effect (Playle *et al.,* 1989; Playle and Wood 1989, 1991). In all cases, these actions appear largely or entirely attributable to the free ion form(s) of the metals.

In the presently ongoing study on freshwater rainbow trout, *oncorhynchus mykiss*, we have measured LC50 values for $AgNO_3$ and $Ag(S_2O_3)_n$ and followed changes in blood chemistry during six days of sublethal exposure to $AgNO_3$ as a first step in characterizing the toxic mechanism(s) of various forms of Ag. All experiments were performed in dechlorinated Hamilton tapwater (Lake Ontario: $(Na^+) = 0.6$, $(Cl^-) = 0.7$, $(K^+) = 0.05$, $(Ca^{2+}) = 1.0$, $(Mg^{2+}) = 0.2$, (titractable alkalinity) = 2.0 $mmol.l^{-1}$, hardness = 140 ppm as $CaCO_3$,pH = 8.0, 15 ± 1°C). LC50 values were determined by standard methods on juvenile fish (1 - 4 g), and physiological studies were performed on adult trout (200 - 400 g) fitted with chronic indwelling dorsal aortic catheters for repetitive blood sampling without disturbance. Flow-through exposures were employed in these physiological studies to avoid changes in speciation or complexation, and comparably sampled control fish were run to check for non-specific effects due to blood sampling.

The four and seven day LC50 values (ug $Ag.l^{-1}$, means with 95% C.L.) for $AgNO_3$ were 11.8 (10.9 - 13.8) and 9.1 (7.3 - 11.3), comparable to earlier reports on rainbow trout (Davies *et al.,* 1978; Nebeker *et al.,* 1983). The comparable values for $Ag(S_2O_3)_n$ were 161,000 (146,000 - 177,000) and 137,000 (118,000 - 159,000) $ug.l^{-1}$. This indicates that $Ag(S_2O_3)_n$, the major form of Ag discharged by the photographic industry (Terhaar *et al.,* 1972; Bard *et al.,* 1976), is over four orders of magnitude *less toxic* than $AgNO_3$, the form of Ag traditionally used in toxicity testing. Whereas $AgNO_3$ is fully dissociated in solution, $Ag(S_2O_3)_n$ is not; the high affinity of the thiosulfate anion for Ag^+ (dissociation constant = 3.5×10^{-14}) is presumably responsible for the dramatically lower toxicity of the latter.

Adult trout exposed to $AgNO_3$ (10 $ug.l^{-1}$) for six days exhibited a severe stress response manifested as a progressive increase in plasma glucose, which reached five-fold control levels by the end of the experiment. However, exposed fish showed no evidence of respiratory toxicity. Indeed, blood lactic acid concentrations remained extremely low, while arterial P_{O2} actually increased, and P_{CO2} decreased, effects likely associated with hyperventilation. Arterial pH exhibited a modest decline (0.15 units), but measurements of P_{CO2} and plasma HCO_3^- indicated that the fall in P_{CO2} served as a "respiratory compensation" to prevent a much larger "metabolic" acidosis associated with a significant loss of plasma HCO_3^-. Plasma Ca^{2+} levels remained constant throughout the exposure, and there was only minor increase in plasma K^+ levels, indicating that Ag^+ did not interfere with the branchial processes regulating these ions. These results suggest that the mechanism of action of Ag is different from that of Cd or Zn (Ca^{2+} transport antagonists) and at least partially different from that of Al (respiratory toxicity).

The most pronounced effect of $AgNO_3$ exposure was a progressive decline in plasma Na^+ and Cl^- concentrations to 70% of control values after six days (Fig. 1). The form of this response was very similar to that seen during exposure to sublethal H^+ (Wood 1989) or Cu^{2+} (Lauren and McDonald 1986). The plasma Na^+ and Cl^- levels reached by day six of Ag at 10 $ug.l^{-1}$ were comparable to those associated with death from much higher concentrations of these other two toxicants. At least for H^+, it is known that the loss of Na^+ and Cl^- entrains an osmotic shift of fluid out of the blood plasma and extracellular space into the intracellular compartment. This hemoconcentration induces an eventual circulatory collapse which appears to be the proximate cause of death (Wood 1989). Measurements of hematocrit, hemoglobin, and plasma protein during $AgNO_3$ exposure indicated a similar intense hemoconcentration such that plasma volume had decreased to about 60% of control levels by day six.

We conclude that ionic Ag^+, presented in the form of $AgNO_3$, is extremely toxic to fish, exerting effects on Na^+ and Cl^- regulation similar to those of low pH and Cu, likely by a "surface-active" mechanism on the gills. $Ag(S_2O_3)_n$ is very non-toxic. Future studies should address the physiological effects of $Ag(S_2O_3)_n$ and other naturally occurring forms (*e.g.,* AgCl); the details of the Ag^+ action and binding on the gills; and the possibility of synergistic effects between Ag^+, H^+, and Cu^{2+}.

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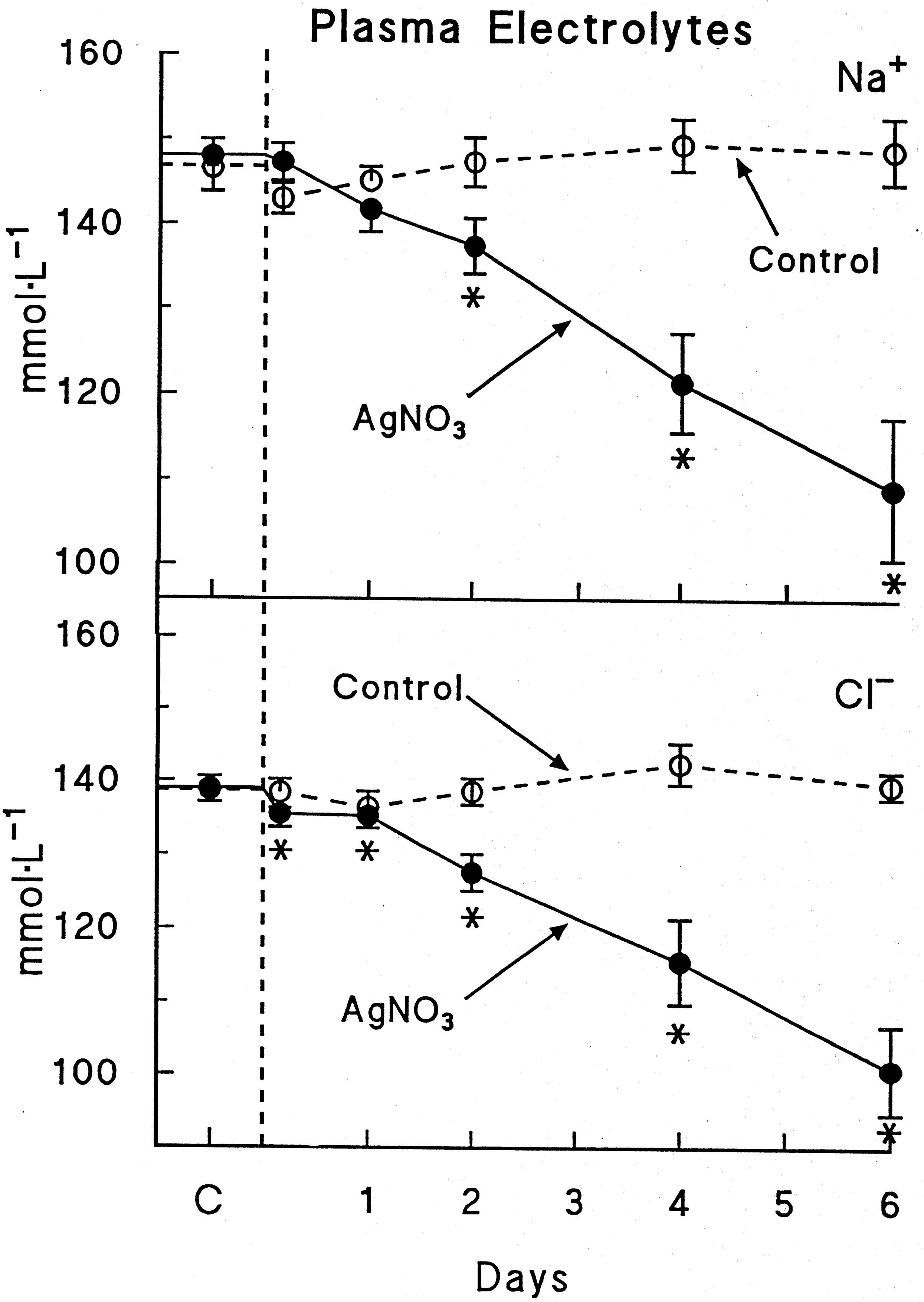

Figure 1. The influence of 6 days exposure to 10 ug.l⁻¹ Ag (as $AgNO_3$; solid circles; N = 10 - 12) *or* control conditions (open circles; N = 8)) on plasma Na^+ and Cl^- concentrations in freshwater rainbow trout. Means ± 1 SEM. * indicates mean significantly different ($P < 0.05$) from pre-exposure value (C).

Metal-Gill Conditional Stability Constants: What About Silver?

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Powerful aquatic chemistry computer programs such as MINEQL$^+$ (Schecher 1991) and MINTEQA2 (US EPA 1991) calculate metal speciation and complexation using conditional equilibrium stability constants. However, calculations of water chemistry may not reflect how a metal interacts with biological surfaces such as fish gills. My approach is to calculate stability constants for metal-gill interactions, and to insert these *biological* values into what have been, until now, strictly *chemical* programs. This approach will prove useful in predicting toxicological and physiological effects of silver on fish living in waters of differing chemistry.

The method to accurately determine the Ag-gill binding constant (K) is to expose fish to low, environmentally realistic concentrations of Ag, in the presence or absence of chelating agents such as EDTA (ethylenediaminetetraacetic acid). Fish gills are then assayed for bound Ag using graphite furnace atomic absorption spectroscopy (AAS). Free Ag^+ in solution is estimated using MINEQL$^+$ or MINTEQA2. Free Ag^+ and gill-bound Ag are used to construct a Langmuir isotherm, from which are determined $K_{Ag\text{-}gill}$ and the number of gill binding sites. This approach has successfully been applied to Cu and Cd (Playle et al. 1993a, b), and is explained more fully in those two papers.

Preliminary data for Ag have been obtained, using 1-2 g rainbow trout (Oncorhynchus mykiss) exposed 2-3 h to 0.1 µM Ag (= 10.8 µg·L^{-1} Ag). Fish were exposed to Ag in aerated, synthetic soft water (3 fish per 2 L), containing ~80 µM Na, ~20 µM Ca, and ~120 µM Cl (at pH 6.4 and 17.5°C). Extracted gills were heat-digested in 5x their weight of HNO_3 and diluted 10x in ultrapure water. Injections on the graphite furnace were 10 µL, using standard furnace operating parameters for Ag. The 0.1 µM concentration of Ag yields silver accumulation on the gills about 10x higher than background, but is still a low, realistic Ag concentration.

Rainbow trout exposed to 0.1 µM Ag showed decreases in the amount of Ag bound to the gills as the concentration of EDTA in the water was increased from 0.025 µM to 0.5 µM. Gill Ag (in nmole Ag·g^{-1} wet tissue) correlated directly with the amount of free Ag^+ in solution, as calculated by MINEQL$^+$ ($r=0.882$, $P \leq 0.001$, $n=20$). A Langmuir isotherm constructed using the EDTA data yielded log $K_{Ag\text{-}gill}$~8.9, with the number of gill binding sites per fish ~3×10^{-10} moles. MINTEQA2 yielded essentially identical values.

Preliminary data were also obtained for trout exposed to 0.1 µM Ag in soft water modified by the addition of Ca, Cl, H^+, dissolved organic carbon (DOC), and thiosulphate ($S_2O_3^{2-}$). The addition of 1,030 µM Ca and 2,240 µM Cl did not affect the amount of Ag binding to trout gills, whereas acidic conditions (pH 4.7) and 5 to 10 mg·L^{-1} DOC reduced gill Ag to about half. Addition of 0.05 to 0.5 µM $S_2O_3^{2-}$ also reduced Ag binding to the gills.

From all these data, I was able to insert five new components into the MINEQL$^+$ program, to allow simulation of Ag interactions at fish gills. The log K values used were: Ag-gill = 8.9, H-gill = 6.5, Ca-gill = 4.0, Ag-DOC = 8.3, and H-DOC = 4.0. The number of gill binding sites used was 3×10^{-10} moles, and the number of Ag binding sites on DOC was entered as 0.05 µmoles/mg DOC.

Using these values, and entering measured water chemistry for the initial, soft water conditions, about 80% of the 0.1 μM Ag in solution was in the form Ag^+, and ~20% as aqueous AgCl ($MINEQL^+$ simulation). At the gills, ~96% of the gill sites were occupied by Ag, ~2% by H^+, with ~2% remaining as free gill sites. Simulating the addition of 2.2 mM Cl to the soft water yielded ~15% free Ag^+, ~69% AgCl, and ~16% $AgCl_2$, a substantial reduction of free Ag^+ in solution. At the gills, however, ~83% of the gill sites were still occupied by Ag, ~9% by H^+, ~1% by Ca, with about 7% remaining as free gill sites. The reason for such high Ag binding to the gills, in spite of reduced amounts of free Ag^+ in solution, was the high Ag binding constant for the gill (log $K_{Ag\text{-}gill}$~8.9) in comparison to the AgCl binding constant (log K_{AgCl}=3.3). That is, the gills were able to out-compete Cl for Ag^+.

Other computer simulations using $S_2O_3^{2-}$ and DOC reinforced the idea that decreases in free Ag^+ in solution do not reduce the amount of Ag binding to trout gills as much as might be expected, due to the relatively strong binding of Ag to the gills. Log $K_{Ag\text{-}gill}$~8.9 is similar to that determined for Cd (log $K_{Cd\text{-}gill}$=8.6); both are higher than the stability constant of Cu (log $K_{Cu\text{-}gill}$=7.4; Playle et al. 1993b). Inserting metal-gill stability constants into aquatic chemistry programs such as $MINEQL^+$ and MINTEQA2 will be a powerful tool for predicting toxicological and physiological effects of metals on aquatic biota.

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ARGENTUM

AN INTERNATIONAL CONFERENCE

Session F: Panel Discussion: Setting Effluent Standards for Metals: Information Requirements for Regulatory Agencies

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Panel Chair: J.Sullivan
Panel Members: D.M. Di Toro, M. Heber, J. Kramer,
C. Ingersoll, J. Rodgers, A. Sodergren

Panel Discussion: Setting Effluent Standards for Metals: Information Requirements for Regulatory Agencies

J. Sullivan, D.M. Di Toro, M. Heber, J. Kramer, C. Ingersoll, J. Rodgers, and A. Sodergren

ANDREN: Most of you probably have not met him, but our panel chairman is Jack Sullivan from the Wisconsin Department of Natural Resources. Jack is head of the Standards Program for the Wisconsin Department of Natural Resources, and for those of you who, perhaps, like to shoot some arrows, Jack is also the principal author of the wildlife criteria for the Great Lakes Initiative. You can ask him some questions on this issue if you are so inclined. Most of the other panel members you have already met: John Rodgers from the University of Mississippi; Margaret Heber from the Office of Water in the Office of Science and Technology, from the Aquatic Life Criteria section; Anders Sodergren from Lund University, Lund, Sweden, an ecotoxicologist, and he tells me that they will have — what is it? — a 16,000 or 20,000 sq. ft. Ecotoxicology Center in Lund, which will be inaugurated next year, one of the first of its kind that I'm aware of. Also on the panel is Dominic Di Toro, whom many of you know already. Dominic is a consultant at HydroQual as well as a professor at Manhattan College. And, finally, Jim Kramer who is a geochemist — a low temperature geochemist — from McMaster University in Hamilton — or is it Burlington? — Hamilton, Ontario, Canada. So, are we ready to record? Watch what you're saying, folks, Big Brother is watching — and listening.

SULLIVAN: Thanks, Anders. I am with the Wisconsin Department of Natural Resources and in my current role I am a regulator. My involvement with metals is that it is one of the many classes of substances that we are required to set standards for in the state of Wisconsin. We do have a delegated program from the EPA and we do use toxicology information and come up with criteria to use to regulate both industries and municipalities where necessary. So we derive the standards and it's also my staff and I who derive the limitations that then go into point source discharge permits and other types of regulatory functions within our agency. So I am familiar with metals problems with respect to regulation. Well, there's not a problem. If the level's too high, you're regulated. That should be the regulator's viewpoint, right? No, that's not true. I am pleased to participate in this, because I think if there's one thing that you can say about metals chemistry and metals toxicity and the discharge of metals and metals in the environment in general, it is that they're the most commonly regulated substance in our state, from a discharge standpoint. Certainly, they're the least understood, and we have a very long history of regulating metals in the state of Wisconsin. I haven't been here for the last day and a half to hear what people are saying, but I would guess that we've heard a lot about the difficulties in analyzing for metals at low levels, the clean techniques that are necessary, the uncertainty about dissolved or particulate forms and how toxicity is expressed. Indeed, the understanding of all this with respect to metals is not simple, and yet we, and people like me across the country, are charged with trying to make some sense of all that information and try to regulate metals where necessary.

To get this panel discussion going, I wanted to talk a little bit about the occurrence of metals in discharges, and I'd like to use Wisconsin as an example. We've talked about toxicity, the analysis, bioavailability, all the many issues that surround metals. But what about their occurrence? I pulled data off our computer and the results are current as of July 20, 1993. I will present data on the number of times the metals are detected in discharges and the number of times that Wisconsin DNR has put a permit requirement on these discharges. I will also briefly talk about the basis for these actions. I said that metals are most often regulated — and let me just throw out a few numbers at you. With respect to metals, and for the first category, its industries in the state, if you're not familiar with Wisconsin, we're largely pulp and paper; we have one or two active mines in the state; one petroleum refinery; many Superfund sites that the data would be included here; and several metal platers as well across the state. How about occurrence of the different metals? First, with respect to industries, let me just pick out a couple. **Cadmium:** we've looked at a hundred different industries with respect to the concentration of metals and their effluent. For cadmium, we've found it in 38 of them, or about a third, and we've put limits and permits for protection of aquatic life in about 20 percent of those, that is about 20 of the 38. **Chromium:** chromium shows up fairly frequently. Chromium (III) is found about 50 percent of the time in the effluents, where about 10 percent of the time we'll put a limit in the permit for the control of this element. **Lead:** lead is found about 50 percent of the time in effluents. It is, however, not that often that we have to put a limit in the permit with respect to this compound. And again, almost all the time for the protection of aquatic life. **Nickel:** nickel is an element that shows up about 33 percent of the time in industry effluents. I guess not surprising then is the fact that we see the occurrence of these metals from 30 percent to 50 percent of the time in effluents. **Silver:** now let's look at silver once before I finish up here. Silver has shown up in 32 of 100 effluents for industries in the state of Wisconsin and has been regulated about 20 times. That's the substance that's been of interest for this conference. We ask that your acute criteria be read in the pipe in Wisconsin. Of the 20 times that silver has been regulated, 15 times were for acute toxicity. **Zinc:** zinc is the most commonly occurring metal we see in point source discharges, at about 70 percent of the time. It is regulated for aquatic life protection approximately 40 percent of the time. It is not surprising that metals show up, since they are naturally occurring, but the frequency of zinc is on the high side at 70 percent of the time.

Let's contrast the metals with some of the organic substances that show up in effluents so you can get a better perspective. Let me pick a couple of organic substances that people would be familiar with. Dioxin has shown up in five of 100 effluents in the state and has been limited all five times. DDT, detected once out of 100 times. PCBs, seven times, regulated one out of seven. Generally speaking, if you look at all the 100 or so substances (organics that we generally look at), the frequency of occurrence is much less than the metals. That's true for a variety of reasons that we don't need to get into. But the fact of the matter is for the substances we look for, metals show up most often and people get limits and permits most often for metals.

Municipalities in the state of Wisconsin are not that much different. We've reviewed 138 facilities across the state and cadmium has shown up in 58 of those. Of the 58, 27 were regulated for aquatic life protection. Copper shows up in almost every municipal effluent. We see it 106 times out of 140. In 75 of those facilities we've regulated the discharge of copper. Mercury has been detected 71 times and regulated or controlled 36 times, and limited in permits 30 times. I'll point out, since Anders opened up the door on that, it's been regulated 30 times for the protection of wildlife in the state of Wisconsin. Our criteria is higher than that which is proposed in the Great Lakes Initiative, but we commonly still detect mercury in effluents. My guess, however, is that 80-90 percent of the mercury data that are submitted to our agency are flawed data, and that when we go back in and get good analytical data, eight or nine out of 10 times the problem goes away with respect to mercury. Silver in municipalities shows up 54 out of 138 times and may be regulated 20-25 percent of the time.

The story is the same for organics in municipalities. However, these substances do not show up with the same frequency that metals do. Dioxin has been detected twice in municipalities and regulated twice. DDT again has one occurrence. These data have been collected from facilities over approximately an eight-year period. Some of the data are good, some bad, of course. PCBs have not been regulated but have been detected six times out of 138 point source discharges. So again, the frequency of detection is comparable to that of metals.

We have a heck of a time with regulatory requirements surrounding metals. Oftentimes, we may see high metals concentrations, but the permittee is passing all their whole effluent toxicity requirements. The metals don't seem to be showing toxicity. We have some guidance regarding water effects ratios usage which is coming out at the national level. These, however, are a band-aid on the problem and not the ultimate solution. We do have several people in the state moving forward with trying to modify their metal limits using water effects ratios. Perhaps the most common method used in our state to meet limits, however, for metals toxicity, is high rate diffusion of the effluent. We do allow what we call zones of initial dilution, where, if you diffuse your effluent at high velocities, we give you better dilution credits. Oftentimes, people will meet their limits using high rate diffusion or zones of initial dilution to meet these limits.

The best way to deal with your metals problem then, is to get good data, but you can't always do a lot with respect to pollution prevention. Sometimes chemical substitution solves the problem, but for the most part it does not. In summary, we do have a difficult time dealing with metals and permits when you sift through all the information that we most commonly deal with in terms of metal discharge permits, whether it be a small metal-plating facility, a municipality, a paper mill, a mining facility, or a Superfund site. So with that, let's open up our discussion for questions.

Q: Do you issue your permits or restrictions on total recoverable metal or on dissolved metal in the effluent?

SULLIVAN: We allow discharges, if your limit is based on aquatic life, to use a total recoverable data in the state of Wisconsin. We do entertain the submittal of information leading to, as I said earlier, some form of a water effects ratio. However, we're not totally sold on that as the solution. As a rule, we do use total recoverable in our state.

HEBER: Maybe I should add a response to that. The federal regulation for NPDS permitting require that metals be determined as total recoverable, not as dissolved. And most states do their permitting based on total recoverable. We don't anticipate changing that.

Q: That was an impressive list of regulations and licensing procedures. Now I would like to know a little more about the strategy behind this. As I see it, you can look upon every pollutant and question whether there are local effects or whether there is a large-scale influence. Depending on answers to that question, I think you have to look at the pollutant differently. Namely, if you have local effects, they can be corrected. If you have large-scale, widespread effects, it's an unacceptable way, and you have to treat those pollutants differently.

SULLIVAN: I guess I need to put on my EPA hat here. I run a state-delegated program, but nationally the thrust of the programs is that we have a list of substances, largely created by attorneys, that we regulate in the United States and it is a dual approach. One that is chemical-specific and one that is of whole-effluent toxicity. You carry out this dual application and that is the approach that has been taken across this nation and has been used for some years. I won't say that there might not be other ways to do it or better ways to do it, but it's the way we currently are doing it here in Wisconsin and elsewhere.

Q: What happens when you do not pass your metals limits on a chemical-specific basis, but pass the whole effluent toxicity test?

SULLIVAN: Good question. The national program is based on independent application. You have to meet your metals limits from a chemical-specific standpoint and you must also pass your whole effluent toxicity. So you can pass your whole effluent test, have all your fathead minnows and all your *Daphnia* swimming around real happy, but if you exceed your metals limit, you still will be considered to have a permit violation. You must do something to deal with the level of that metal in your effluent. There are a lot of people that think they should be able to show biologically that you don't have to meet your metals limit, but right now we work it in our state and my understanding is that the way the program is to be run is independent application of both whole effluent and the chemical-specific limit.

HEBER: The way he answered the question is the way we're going to continue to deal with different kinds of limitations in permits. They're each dealt with independently. I think as time goes on we'll gain more experience about the different tests and what they're actually showing so that we might understand why you'd get that kind of a result. I have a feeling that when sewage treatment plants start applying clean sampling and clean chemical analyses techniques to their metal sampling that a lot of those exceeding their permit limits will probably go away. And most of the municipalities that we've spoken to are very excited that we're starting to push clean sampling, clean analyses, and the water effect ratio guidance that was referred to. There should be some general language about how to do the analytical works. A lot of it is just common sense. But you'd be amazed at how some of these samples are taken. For example, you can't take metal samples in steel buckets, it just doesn't work that way; galvanized buckets are also nonusable.

Q: I guess there are two avenues of approach. One is that it is a policy decision on the independent application of whole effluent versus limits. I'm not sure, however, whether you want to try to change national policy. The other approach is to generate information — toxicity information — which shows that the data that have gone into making up the current criteria that you're interested in may be flawed in that it didn't account for all the different types or ways that we may want to express metal toxicity. Thus, I would try to influence the science aspect of silver toxicity. One other thing, and I think it's really a band-aid, is the water effects ratio work. This approach is obviously only a short-term solution. It looks, however, as if good toxicological data are being developed right now and this information may, hopefully, lead us to standards that are based on good science.

HEBER: We only have an acute number that's really a final number right now for silver. We proposed additional silver criteria, but we haven't gone final with it. Some of the work that you heard presented today that was Russ Erickson's work, is part of that effort to respond to the public comments we got in on the proposed criteria. There have been some things that have happened since then, too. We are trying as an agency to move forward in a consolidated way in the Office of Water in dealing with metals across the board. When I say that, I mean all the way from criteria to permits and enforcement, including modeling. We had a meeting back in January, which a lot of you are probably very aware of, to pull together all of the experts, as many of the experts as we could, in the area of metals. We included chemists, geochemists, analytical chemists, physiologists, modelers, aquatic toxicologists, and made them sit all together in one room and listen to each other, which was horrible for some of them, I'm sure. But I think it worked, because in dealing with metals, in regulating metals, we've been dealing, I think, with just the chemistry or just the aquatic toxicity, and not really looking at the problem as a whole. We can't do that anymore. One of the things I heard from all the participants at the meeting was, "I'm glad somebody finally did this and made us sit down and think about what we're doing and what we know in each of the different fields of metals science," if you want to call it that.

The meeting that you had over the last two days does the same thing for silver, and I'm really encouraged to see someone pushing ahead with one particular metal, trying to just push what we know about silver forward so that we can make some decisions as an agency and give some guidance to our states. We're all in kind of a resource bind at this point, none of us have a whole lot of money, and the states are in worse shape than we are, and the recommendations that came out of that Annapolis meeting we published in the federal register. We sent copies of a draft memo out to our regions and we will be going final with that guidance and policy memo for how the Office of Water is going to handle metals. That should be coming out probably towards the end of this month. At least, that's the schedule right now. And what we're trying to do is push clean analysis and sampling techniques, because most historical data, ambient data, for metals are bad, and you've heard people talk about that. As I said before, we will not be changing our regulations for permitting. We feel it's very important to maintain total recoverable metals for permits and for mass balance calculations. One of the other recommendations that's been very controversial that came out of that meeting was that most of the scientists in that room — not all of them, but most of them — felt that when given, or asked to make a choice, whether they thought total recoverable or dissolved metal was a better estimator of the bioavailable fraction of metals in general, not any one particular metal (and I exclude selenium and mercury when I say this), the majority felt that dissolved was a better indicator of the bioavailable fraction. So we will probably be going towards changing our criteria to be expressed in terms of dissolved metal instead of total recoverable. And in order to do that we need to come up with, or we need to provide the states with, percent dissolved numbers so that they can make the transition from total recoverable to dissolved. In addition to that, there is one more piece, what's been referred to as the water effect ratio, and that's a way of changing or modifying our national criteria numbers by using site water and seeing whether or not the effects of the site water change the toxicity of a particular metal and the criteria themselves.

So there are a number of things that we're trying to do as an agency to move forward. Hopefully, in the guidance we're putting together, we will be giving some guidance on monitoring and what to do with old data; how to make some determinations as to which data might be good; and how you would know that ... I'm just trying to think what else. There's quite a laundry list of things that are going on right now. We've been working with USGS to come up with some very concrete methodologies to do better clean sampling and analysis techniques for metals. They are on a much longer time track than we are so we may be coming out with something sooner, but that's about where we are right now.

ANDREN: I have two questions, one directed at Jim Kramer and the other at Dominic Di Toro. First to Dominic. Yesterday, if I remember right, you mentioned something about dissolved versus total metal and your idea about that. I wondered if you could expand upon that a little bit. The question for Jim is, with reference to these new numbers that we get with clean methods, how do you think that they will affect our way of regulating discharge?

DI TORO: The issue of what form of metal should be regulated is, unfortunately, really tied to the issue of bioavailability. From the point of view of what quantity in the effluents should be regulated, I don't think there's any issue there. It's clear that you have to regulate total recoverable metal for no other reason than that nobody knows how to do a calculation which predicts exposure in the receiving water unless you consider all of it. So that is completely independent from the question of bioavailability, but I think those are completely separable issues. So in the first place, in order to get mass balance calculations done correctly you have to know the total concentration of metals coming at you from everywhere. Now, there may be a fraction of your effluent that is absolutely, biologically unavailable (e.g., crystalline, persistent mineral phases). You would still include those in your calculation, but presumably they would not be involved in the question of what is the concentration of bioavailable metal in the receiving water. It's really not a complicated problem if you ask yourself what are you trying to protect. What you're trying to protect is the aquatic organisms in the receiving water, and presumably pretty soon the aquatic organisms in the sediments.

Let's talk about aquatic effects first. The first question that occurs to you is, why are you interested in having acute toxicity absent in effluent pipes? The Clean Water Act doesn't say anything about fishable, swimmable pipes. It talks about fishable, swimmable receiving waters. But that's basically a cheap shot. The reason is, at least in my mind, that you're saying you don't want to allow any acute toxicity even in a small zone. Now, depending on how large the zone of initial dilution is, that can be modified as well. The real question, and it's simply put is, what fraction of the total metal concentration that occurs in the receiving water is bioavailable? That's the fraction that you regulate. Okay? So it's simply put. The question is, how do you do that little trick, how do you calculate what is the bioavailable fraction of the total diluted metal that occurs outside of your doorstep? Well, there are various ways to go at it, and the most conservative way is to just say, all of it is bioavailable, and you apply the criteria to total recovery. That is the simple way out. For people that have to regulate in an infinitely complicated world that isn't a bad way to go because they're covered, they're being conservative — that's one way to do the problem.

On the other hand, if it becomes a burdensome regulation, if you're regulating unnecessarily, the New York City case is the best example. The difference between what you'd have to do if you regulated in total recoverable and if you regulated in dissolved copper in the receiving water is a lot of money — in the billions of dollars. At that point then, it becomes important to ask yourself more directly, is there a better approximation? Well, what came out of the meeting in January that Margaret was talking about was the opinion of the majority of people that if you have to make that choice, your best estimate of what is the bioavailable fraction of total diluted metal out there is the dissolved fraction. After that comes most of the research stuff that we've been hearing about today, which is the proposition that the ionic metal is "the bioavailable fraction." But I think you can see from the stuff we saw relative to the gill modeling, complexation on gills, that that's not right either. It is related to the ionic metal concentration, but it's not related in a straightforward way. It is unrealistic to expect that water quality criteria will be based on, let's say, ionic, "free" copper, or zinc. This is, of course, due to all kinds of other factors that influence toxicity — pH, calcium, magnesium, organic matter, etc. So at this stage of the game, the practical solution, as I think that most of us who think about this see it, is that dissolved metal is the bioavailable fraction. There are some data, however, that suggest that particulates are involved in toxicity, and there are some reasons to think that it might be. For example, when the particulate metal is in the gill chamber, the pH of the gill chamber is slightly lower, there may be a desorption of metal and so on. So there are some mechanisms that one could dream up that may be active. But if you have to guess, and if you're regulating, it looks to me like choosing dissolved metal is the way to go. You are then left with one practical problem: how do you predict the fraction of total metal that is dissolved? And the answer is, there is no good theoretical foundation to do it. You measure it. And the measurements are straightforward. You measure dissolved and particulate, don't go subtracting metals measurements, you make a mess. And then you calculate a partition coefficient. That's the way we see it on the practical side.

KRAMER: The second question was: What is the impact of the new, low numbers on this whole game? Let me make a deja vu comment. I was on the first Science Advisory Board of the International Joint Commission when it was started in '70-something.

I don't know how many times we went around and around with the scientists saying that looking at total metals does not relate well to availability and we asked them to do something else. Back in those days there was research money around and we recommended that EPA labs and state labs at least look at the dissolved and particulate fractions and then maybe we can calculate K_D partitioning coefficients. Let me focus specifically on silver, which I don't know much about, but I know a thousand times more than when I came here two days ago. I'm amazed actually at how much information there is, and I'm sure if we dig a few more people out of the woodwork we'll find that there's even more information out there. But one of the things with regard to this topic of dissolved and particulate fractions, if we look at it the way Dominic does, i.e., in terms of modeling, there's a lot of agreement with the K_D values from San Francisco when compared to many other places. In other words, there's a number there. So the first thing we could do, and I'm thinking as a scientist, not as a regulator, if we had a K_D value by looking at the totals or the particulate and the dissolved fractions, we could ask, is this particular watercourse consistent with this ongoing hypothesis of the distribution of silver in the environment? My feeling would be, when the numbers are not that way, and we've seen some exceptions with silver, we'd better go and make a lot of more careful K_D measurements. So that's one aspect of the silver question.

One of the other aspects, when you look at total recoverable metal which immediately implies the particulate phase particularly with regard to silver, it is clear that humans, over the last century or so, have mobilized silver within the surface of the earth. And one of the things I think of as a geochemist is, you're regulating water, but this material is concentrating, in sediments, and specifically in municipal sludges. You've got to do something with this mass of material. In terms of long-term cycling, the aspect I would like to know is: what is the ultimate fate of these higher levels of silver in the sediment, in the sludge, when you have to put them somewhere? This is my question right now, and I think it's the one question I haven't had answered here, at least to my satisfaction. That means really looking more at the solid phase. I mentioned the black box a couple of days ago, I think it's still a black box. Because we must dispose of these materials, and if we can dispose of them with some confidence, they'll end up in the geological record or something like that, then I don't feel so bad. But if under certain conditions these metals get mobilized, we're going to have regulatory problems again. So I guess this is the way I come at it. Thus, with respect to the question of regulation, I don't think it would be too difficult to ask for two kinds of measurements — a particulate and a dissolved measurement. You may for the time being regulate on, let's say, total recoverable. This also will generate the data down the line for future computations. Scientists like Di Toro and other people will then be able to take all these numbers and be able to say, "well, that's ok except for ... ." So I think that if we look at regulations as changing slowly over some period of time, at least over my kids' lifetime anyhow, I hope that we will, in a logical and in a scientific way, almost demand, if you will, in the regulation the kind of information that can lead to a much better type of system.

Q: You talked about regulations concerning the protection of aquatic plant life, but there's been no discussion yet about public health concerns. In particular, I'm thinking about contaminated waters where certain organisms may concentrate an element like silver in oysters. People like to eat oysters, and oysters can be very rich in silver. If you eat a certain number of oysters is that going to have any immediate implications for human health? Zinc is another element. For example, in parts of Long Island Sound oysters were contaminated to an astounding level of two and a half percent by weight. So the point I have is, these animals may not be exactly passive collectors of metals. But people like to collect and eat oysters and mussels and do that on a regular basis. Thus, a pathway analysis of metals may be good to do from a human health standpoint rather than making calculations based just on protecting aquatic organisms.

SULLIVAN: That's a good comment. And, since we don't have oysters in Wisconsin, I won't punt on this one. In Wisconsin we do consider the uptake of metals into aquatic organisms and we do monitor for the occurrence of metals. And to the extent that we have comparative numbers for where it may become a public health problem, we can put out advisories to advise the public on consumption. We look at wildlife in terms of mostly waterfowl in Wisconsin, as well as most common fish species that are caught. And quite frankly, I can't specifically speak in regard to silver. Mercury clearly is a problem for people that eat a lot of fish. If you happen to be good enough to catch them out of lakes that have high mercury, there is potential concern there. For other metals, we commonly don't see them in organisms because of their low bioaccumulation factors. But I would think that the state of New York and others that are on the coast do monitor oysters and other bivalves that are consumed, for substances like metals including, perhaps, silver. I don't know.

Q: You've got people doing chemical analyses that are not capable of getting accurate data. And it's good for EPA to move away from their cookbook methods that anyone can do towards rigorous analytical methods that will really get accurate numbers. But the problem with that is <u>time</u> and <u>cost</u>. And we're talking about, I think, years and years before all the agencies and municipalities, etc., can come up with fees and spend the money to be able to put up reasonable techniques on the board. Now, you're right that simple common sense can take away a lot of the problem. But there's much more involved with the analytical considerations than that. Do you see agencies playing an active role in helping people change?

HEBER: I don't think we have a choice. But I guess I have to answer your question in two ways. The concentration ranges that we talk about for ambient monitoring are much lower, obviously, than what we see when we monitor for effluents. And for the dischargers in particular, I think that they'll be able to find laboratories, or even within their own facilities, that can give them the clean techniques (the analytical and sampling techniques) they will need to meet their permit limits. That's the first consideration because it costs money if they're out of compliance and there's a good reason for them to want to get laboratories to do these analyses for them. The detection limits and how good those laboratories are probably doesn't have to be quite as good as somebody who's going out and actually doing ambient monitoring because the concentration range is lower. Mercury is going to be another problem because, from what I'm told — I'm not a chemist — even if you have broken a mercury thermometer in your laboratory you probably can never use that laboratory again to analyze for mercury. But that is something we're working on. We've been working on it with USGS, somebody in the Office of Water specifically has been assigned to do that. They're responsible for our EPA part 136 methods which are regulations that our permittees are held to. So we know we've got to do that, we don't have a choice.

Q: This question is for Professor Sodergren. How is the regulation in Europe, how do you compare it to the way we regulate metals in the United States?

SODERGREN: Well, I haven't got that knowledge. But I think that in most major countries we have adopted many of the strategies and thinking which have been developed in the States. With respect to regulatory strategy, we are looking at certain pollutants and their regulation with respect to their ability to escape from the point source. Pollutants which are able to escape and go far away from their point source are regarded more seriously than those which are on the local level, not escaping the local place. This is because you can correct a pollutant situation if it stays nearby, but in a widespread case, for example, those carried across the Baltic Sea, in our case, it is impossible to do anything. So it does mean that we perhaps are looking at pollutants differently due to their behavior.

Q: We have talked about the analytical aspects of metal detection in natural waters and waste streams. We certainly have to keep on improving. But we also do bioassays. There will be a cost in that, too, and are we on the right road, and do we have a long way to go for that as well?

RODGERS: Perhaps we're in better condition for other metals in terms of data and the solidity of those data than we are, perhaps, with silver, although there are certainly some exceptions. The question I got asked today that we wrestle with a lot is the relationship between the laboratory test you do and how you take those numbers to the field. This is a very critical issue. It's something that deserves an awful lot of thought because that's why you do get these adjustments in water effects ratios and so on. When you ask about the testing, these days there's got to be an analytical effort to back up bioassay work. So there's an incremental addition of analytical effort that goes with every test. And one thing that was said here that I don't think escapes you folks is that you can measure the dissolved phase of metals, you can measure the particulate phase of metals, and that may have an unusual and interesting and perhaps not readily predictable relationship to total recoverable metal. So you ought to think that one through a little bit as you charge forth.

Q: I have a question which gets back the dissolved/particulate issue. As I reflect back on the talks, I think that, without exception, when a chemist was speaking about reactions he assumed they are at equilibrium. When a biologist was speaking, there was always a rate involved in the process. Here we have a chemical that's clearly very particle reactive. So I wonder about the rate of dissolution, or release from the solid phase. One of the things we heard about would, perhaps, constitute an interesting natural laboratory test regarding dissolution. That is, study San Diego Bay, which, if I understand correctly, has been cut off from any anthropogenic sources for thirty years or so and has high levels of silver in the sediment. So maybe people could adjust their sampling regimes to address this issue.

SULLIVAN: I won't disagree with that.

Q: I think that they answered my question for themselves a little bit, in that the suggestion is there that the diffusive and bioturbation fluxes of silver out of sediment could be maximized in summer during low flow conditions and Nick's and my work show you're not only dealing with particle reactions and sediment reactions, you're also dealing with phytoplankton uptake, where you have very rapid uptake for that metal. Because the flux rate for that time of year is still on the low side, the metal will not get out. So it's going to settle right back down, and you've got settling rates for all of these particles (probably less than a day in the channel system), and so you've got a nice dynamic system where silver can come in and out of the sediment. And we could see things in a system like Green Bay where silver could come in and out of the system all summer long and never go anywhere. Then comes cold weather, when water is flushing through the system at a lot faster rate, there's not as much silver coming out of the sediment, so it's pretty much trapped there. So it looks like for a particle-reactive element like silver you've got a pretty good trap in an estuary.

Q: On that same comment, I would like to point out that not all the estuaries in the country have the same seasonal patterns of flushing rates. I mean, in Florida, we're wet in the summer much more than in the winter. So maybe we don't have the same conditions and transport mechanisms.

SP: You still have to have an incredibly rapid flushing rate. Particles probably settle and come back up several times a week; I mean, there are more resuspension particles within an estuary than there are productive particles. I didn't mean to generalize for all estuaries, but I think that, in general, an estuary acts as a trap for particle-reactive elements and that's what we see with silver and with lots of other things.

DI TORO: Just to add a little confusion to it all, I would like to remind you that our analysis in New York Harbor tends to indicate that essentially dilution is what happens to silver. When we look at other metals, their behavior is not as complicated as you might think. I think the reason is, that although you have all these interactions going on with the sediments, on balance the only thing that can ultimately happen is burial. That's the ultimate sink. That plus dilution. Certainly it's a transient phenomenon. Also, you should think about the fact that you can do waste load allocations in streams in a really straightforward way.

SULLIVAN: Thank you. I think we're going to wrap it up here and turn it back over to Anders.

ANDREN: Well thank you very much. I'd just like to make a couple of concluding remarks before we go. I certainly have learned a few things. We know that, as we leave here, we should think about silver in terms of icograms and nanograms per liter in natural waters, rather than in terms of micrograms and milligrams per liter. I think we've learned that speciation is still a problem. We have a reductionist approach and we have a simplistic approach, and I think we should work from both directions. I don't think they're mutually exclusive. I know people like Dr. Di Toro will try to simplify as much as he can, as he should. I know that Dr. Sjoberg will try to understand everything there is to know about speciation, as he should. And sometimes perhaps they will meet in the future. We've learned that silver is extremely surface-reactive, it's reactive to reduced sulphur groups, organic matter, and that it can be sequestered. We've talked about different approaches to study silver transport and discharge. Thus, when silver is released, several things may happen: part of it will be diluted, part of it will be partitioned differently, part of it will sediment out, some of it will be resuspended and so forth. There are a lot of interesting research activities around the country that I was not aware of, and I think that in the next year we can make better sense out of all these data, making silver a much better understood element than heretofore. We have learned a lot about how metals are adsorbed and taken up by living organisms. We've learned where these metals attach themselves on biological membranes, how they traverse interfaces, and I think that this aspect is really encouraging. There are a lot of studies that are in progress, and I think it would be fun to have another meeting like this next year. I encourage any one of you who has any purse strings to go and push some buttons. I would also like to thank three people who are not here. They helped design our cover, the layout, all of the correspondence, etc. They are Gloria Gardner, Chris Kohler and Delphine Skinner. Even though they're not here, I'd like to applaud their efforts.

And finally, again, I'd like to thank the sponsors, the organizing committee, and particularly the speakers. So, until next year, thank you very much.

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POSTER SESSION

Transport, Fate and Effects of Silver in the Environment

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Determination of Silver (I) Ion by Anodic Stripping in Flow Injection System

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Photographic processes can cause some release of silver to the environment. Toxicity studies have revealed that free silver ion is the most toxic form of silver. It is therefore of great importance to determine free silver ion at trace levels. A quantitative measurement of total silver by atomic absorption spectroscopy or neutron activation analysis will not provide an accurate assessment of environmental impact. One of the solutions to this problem would be using electrochemical methods. Anodic stripping voltammetry is a well-established electroanalytical technique capable of measuring concentrations of metals at trace levels [1]. This method involves two steps: deposition of the metal of interest at an electrode and then the oxidation of metal from the electrode.

Flow injection analysis is a type of continuous flow analysis that involves injection of reproducible sample volume into a continuously flowing unsegmented carrier stream, followed by quantitation of the species of interest at the down stream detection area.

The combination of anodic stripping voltammetry with flow injection analysis results in an easily automated system capable of precise and sensitive determination of heavy metals in a variety of sample matrices. As the sample is injected into the system, the electrode is held at the deposition potential. While the sample plug passes the electrode surface, some of the analyte is reduced. After the entire sample plug has passed through the cell, the stripping current is recorded. Electrochemical stripping techniques associated with flow injection analysis offer advantages over its counterparts performed in a static cell in terms of sample handling under automated control, protection of reagent and sample from atmosphere gases and other contamination and medium exchange. Although determination of silver has been conducted by several groups in a conventional cell by either stirring solutions or rotation of electrodes [2-4], it is of interest to carry out anodic stripping measurements of silver in flow injection system.

In this work, our goal is to use an automated flow system to carry out the anodic stripping of free silver ion at trace levels. An automated system has been constructed to provide controlled flow for anodic stripping experiments. The diagram of the flow system is shown in Figure 1. The flow system consists of a sample pump, a carrier solution pump, a shunt valve, a sample injection valve and a thin layer channel flow cell detector, all controlled by a laboratory minicomputer (DEC PDP 8/e). The sample pump is a Spectraphysics model SP8700 Ternary Solvent Delivery system. This system has three gas-tight bottles whose contents may be mixed by a ternary proportioning valve. The carrier phase pump is a LKB/Bromma 2150 isocratic pump. This pump provides the carrier solution from its reservoir, through the shunt and sample valves, to the detector and out to waste. All solution reservoirs are sparged with helium and all tubing for the transport of solution is stainless steel. The shunt valve and the sample valve are Valco EC10W, 10-port, electrically actuated valves.

The shunt valve stops the flow of the carrier solution to the system. Stopped flow allows for detection in a quiescent solution. The output of the ternary proportioning valves is introduced to the carrier stream by the sample injector. The carrier solution differs from the sample solution only in that it does not contain the analyte. The carrier solution preceding the sample plug is analyzed in the same manner as the sample to provide a blank signal to be subtracted from the sample signal. The carrier solution that follows the sample plug sweeps out the sample plug from the cell.

The electrochemical detector is an EG&G PARC thin layer channel cell. Preliminary experiments were performed at a BAS glassy carbon electrode with an area of 0.071 cm^2. A Teflon spacer of 0.127 mm was used to form a thin layer between working and counter electrodes, the latter of which is part of the stainless steel block of the cell body. The Reference electrode was a Ag/AgCl. All of the components of the flow system are under computer control. Square wave voltammetry was used for the anodic stripping. The program runs identical experiments on blank (carrier) solutions and analyte solutions, which results in automatic subtraction of blank.

A systematic study of dependence of stripping peak on square wave parameters including step height (ΔE), amplitude (Esw) and frequency (f), mobile phase flow rate, conditioning potential, conditioning time and concentrations has been done for standard silver solutions. The appropriate square wave parameters are ΔE=10 mV, Esw=50 mV and f=100 Hz. A flow rate of 0.5 ml/min was used, which is a compromise between higher deposition efficiency and rapidity of analysis. A deposition potential of -0.4V was suitable for deposition of free silver ion. The analytical signal depends on the amount of material injected into the system. The amount can be altered by changing either sample concentration, sample loop size or both. Experiments show that under conditions of controlled flow with deposition of no more than 10% of a monolayer of silver, reproducible results were obtained at a glassy carbon electrode down to 0.1 ppm. A linear calibration curve shown in Figure 2 was obtained for a concentration range of 0.1-1.0 ppm with a sample loop of 50 µl under the above conditions. At lower concentrations (<0.1 ppm), multiple peaks appear. In order to obtain a single sensitive signal, an attempt has been made to use some other solid electrodes, such as platinum, nickel and carbon paste. Because of the relatively large background current of platinum, it was not a suitable electrode for anodic stripping of silver. Preliminary experiments performed in a stationary cell showed that both nickel and carbon paste electrodes resulted in small background current and improved stripping signal. Easy renewal of the carbon paste electrode surface is an advantage of this electrode over other solid electrodes. The use of carbon paste and nickel electrodes in the flow system continues to be under investigation.

Acknowledgments

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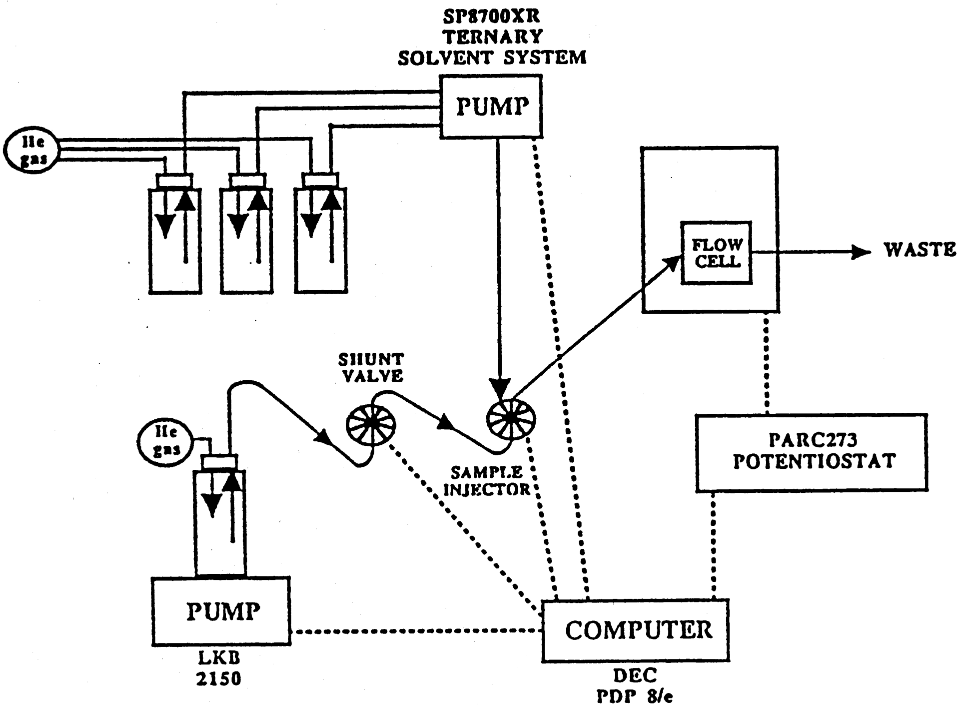

Figure 1. Diagram of flow injection system

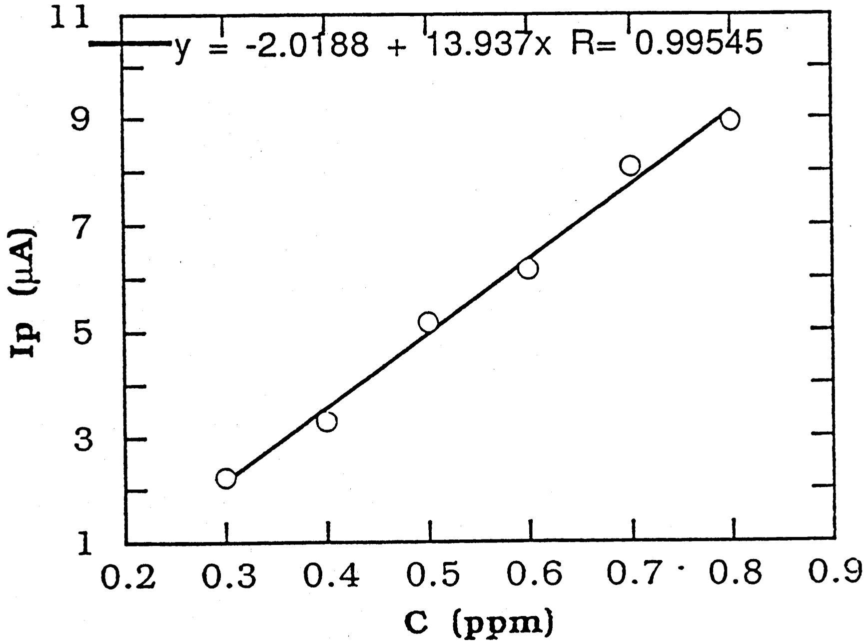

Figure 2. Calibration curve for standard silver solutions

Ecotoxicological Effects of Silver Compounds

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Silver ion may cause ecotoxicological effects in aquatic systems at low levels. However, empirical observations over the years had suggested that certain silver compounds may be less toxic than free silver ion. Because of the potential for such effects and the importance of silver in photographic products, our laboratory conducted ecological effects studies on various forms of silver in aquatic and terrestrial environments over the past two decades. The experiments comprised both acute and chronic studies utilizing plants, waste water treatment microorganisms, and aquatic and terrestrial animals. Plant studies included both economically important species, such as corn and lettuce, and aesthetically important ones, like marigolds. Test species were utilized from all levels of the food chain and included invertebrates, aquatic vertebrates, and terrestrial and aquatic plants. Exposure scenarios varied from 5-hour microbial respiration-inhibition studies to 96-hour flow-through or static aquatic effects studies to 1-12 week toxicity studies. The 96-hour aquatic effects protocols were the most frequently used. Test endpoints included 50% effect responses (inhibition or lethality), no-observed-effect concentrations, occurrence of germination, bioaccumulation/biomagnification factors, and maximum-acceptable-toxicant concentrations.

The range of effects of silver for different compounds spanned eight orders of magnitude (0.0005-10,000 mg/L). The highly soluble compounds had effects primarily in the lower end of the range (more toxic), and the less soluble compounds had effects largely at the upper end of the range (less toxic).

From these accumulated data, observations were made concerning the modes of action of the silver compounds tested. It appeared that the more bioavailable the silver ion was in the testing environment, the less of it was needed to affect the organisms exposed. Aquatic exposure was generally more toxic to organisms than soil exposure. The ionic soluble silver salts had high toxicity in most instances. Silver thiosulfate, which is highly soluble, had unexpectedly low toxicity, and this was attributed to the complexation of the silver by the thiosulfate solution. In all species, silver sulfide was the least toxic compound tested and also the least soluble compound tested. Silver uptake, as expressed by the concentration factors determined during microcosm and pond studies, suggested that silver did not magnify through tropic levels.

In conclusion, the bioavailability of the silver ion in the testing environment determined, to a great extent, the ecotoxicological effects that were observed. The removal of silver ion, by complexation of silver ion or the formation of a highly insoluble compound, reduced the ecotoxicological effects of silver ion in our experiments. The data suggest that the magnitudes of ecotoxicological effects are related to silver speciation.

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