the 3rd International Conference Proceedings

Transport, Fate and Effects of Silver in the Environment

Washington, D.C.
August 6 - 9, 1995

Editors
Anders W. Andren
University of Wisconsin Sea Grant Institute

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TABLE OF CONTENTS

Environmental Cycling, Distribution and Analytical Chemistry

Session 3:

Session 4:

Editors' Note: The extended abstracts included in this document have been printed as submitted by their respective authors and have not been subjected to peer review. The material presented here reflects solely the findings, opinions and conclusions of the individual authors. Questions and answers on the verbal presentations of the papers plus the two panel discussions were recorded at the conference, transcribed and edited for clarity by the editors, including consultation with the authors when necessary.

Argentum III

The apparent general increase of metals in the environment has resulted in several conferences dealing with state-of-the-art assessments of their environmental fates and effects. Recent advances in analytical capabilities have also created a new data base which permits us to better assess the cycling and potential impact of the anthropogenic component of metals in the environment. Silver has a long history of varied uses in society. Yet, very little useful information on environmental concentrations, chemical forms and biological effects are available. Older data on silver behavior, generated from studies made before "clean" sampling techniques and sophisticated analytical methods were available, and before certain complex variables that may influence behavior were recognized, may no longer be considered accurate or reliable.

This conference will provide a forum for the presentation of up-to-date research results on sources, biogeochemistry, environmental cycling and biological effects of silver in the environment. Speakers are chosen from a wide cross-section of scientific disciplines. The conference objectives are to synthesize current knowledge and to identify information needs necessary to more accurately interpret environmental behavior and potential impacts of silver. Keynote and plenary speakers, invited from the international research community, will address recent advances in our understanding of the behavior and effects of metals in the environment, with a particular focus on silver. This year we will be placing special emphasis on chemical and biological behavior of silver in the water column and in sediments.

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Extended Abstracts

Transport, Fate and Effects of Silver in the Environment

Washington D.C.

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Session 1

Behavior of Silver in the Water Column

T.W. Bober
Session Chair

The Occurrence and Behavior of Silver in Natural Waters

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Madison, Wisconsin, USA

In natural waters, silver can exist in oxidation states (0) and (I) and both of these can occur both in dissolved or in particulate forms. From theoretical considerations, reduction to elemental silver (oxidation state 0) can take place under reducing conditions, and laboratory investigations have shown that photoreduction of particle associated Ag (I) may also be important in surface waters.

Modeling efforts, however, indicate that monovalent silver complexes and compounds will dominate in natural waters. 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 $AgCl^0$ $AgCl_n^{(n-1)}$ (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, are likely also very important. Anoxic conditions can lead to the formation of sulfide and sulfhydro complexes, although it is also possible that these complexes may also exist in oxygenated waters. 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 colloidal and particulate silver species. As is the case for other metals, the reactions silver in natural waters is determined by pH, ionic strength of the solution, presence or absence of anoxic regimes, the concentrations of reaction partners and of other cations capable of competing with silver for these partners.

Most measurements of silver concentrations in natural waters prior to the use of "clean techniques" are deemed wrong. Recent measurements of silver in rivers, lakes and estuaries indicate that background values for total silver are less than about 10 ng/L, with most lakes having concentrations below about 2 ng/L. Samples taken downstream of discharges may exceed these levels by one to two orders of magnitude, depending upon volume of flow. Dissolved, or filterable, concentrations indicate very strong particle associations, with log K_D values on the order of 5 - 6. Strong indications of colloidal associations have also been recorded, which have led to suggestions that very low levels of "truly" dissolved levels exist in natural waters. No field data are available which would permit further detailed exploration of chemical speciation.

Until analytical capabilities are developed which go beyond the "dissolved-particulate" classification, we must rely on laboratory and theoretical modeling studies to fully understand chemical speciation of silver in natural waters. Further insight into the kinetics of reaction and adsorption/desorption processes may also be obtained via well-designed laboratory experiments.

To obtain some of this information, we determined the silver adsorption potential of selected model and natural sediments in equilibrated suspensions under a variety of environmental conditions. We then used these experimental data to calculate the speciation of silver for various types of simulated natural waters. Computer simulations were then carried out with MINTEQA2, a geochemical

speciation program, which calculates the distribution of silver in a user-defined environmental setting. A number of adsorption/desorption experiments were also carried out using similarly well-defined systems. These data indicate that silver is rapidly adsorbed to a variety of solids, whereas desorption kinetics is exceedingly slow. Laboratory determined log K_Ds are higher than those observed in the environment. Our interpretation is that part of the reason for high K_Ds in the laboratory is that Ag^+ is dominant in these well-defined systems, whereas these species are extremely low in rivers, lakes, estuaries and oceans. Ag-organic matter complexes or colloidal associations seem to reduce values of field-measured K_Ds.

Data from experiments on photo-enhanced sorption of silver to bentonite will also be presented. Although further experiments will be necessary to determine rates of photo-reduction of Ag (I) on other representative natural particles, our preliminary results indicate that photochemical reactions do enhance adsorption of silver to solids. If these photochemical reactions result in the formation of Ag^0, it may be necessary to reconsider some of our present concepts of silver cycling, especially as they relate to surface waters.

Questions & Answers: The Occurrence and Behavior of Silver in Natural Waters

Tape malfunctioned during the questions & answers session.

Effect of Water Quality Parameters on Silver Availability

G.P. Cobb, S.J. Klaine, T.W. La Point, R. Jeffers,
M. Wenholz, B. Forsythe, T. Bills and V.C. Waldrop
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Pendleton, South Carolina, USA

Aqueous concentrations of chloride, calcium carbonate, and humic acid were altered to determine if the inorganic and organic composition of water affects silver solubility. As nucleation of solids from solution begins, small particles, that can be suspended in solution, are formed. Silver incorporated in these suspended particles may be measured as aqueous silver during routine chemical analyses. Silver in suspended particles may actually be less bioavailable than are soluble forms of silver. Our data indicate silver solubility is correlated to dissolved organic carbon and the ratio dissolved silver:total silver decreases with time.

Introduction

Inorganic and organic components of water influence silver speciation in waste water effluent and may affect silver toxicity. Cationic and anionic constituents of aqueous systems control ionic strength which affect metal solubility. Organic materials such as humic and fulvic acids are capable of complexing silver and other metals in solution. It is also entirely possible that silver combines with ions and/or organic material to form suspended solids which are measured as aqueous silver. This suspended silver may be less available for bioaccumulation than are soluble forms of silver. Lower bioavailability of silver reduces the toxicity observed for silver solutions. To address silver solubility and availability questions, silver concentrations discussed in this paper were evaluated along with toxic responses of aquatic organisms to test solutions (Klaine et al., this publication).

Methods

Dissolved organic carbon (DOC) in the form of humic acid, chloride, and calcium carbonate concentrations were varied in solutions containing 2 to 40 µg/L of silver to determine resultant effects on silver solubility and toxicity. Soluble silver was determined in aliquots filtered through a 0.45 µm membrane and total aqueous silver was determined in unfiltered aliquots. Water samples were acidified and directly introduced into a graphite furnace atomic absorbtion (GFAA) spectrophotometer. An autosampler was used to limit variance during introduction of the 35 µl sample and 15 µl sodium phosphate modifier solutions into the graphite furnace. Five point calibrations were performed prior to sample analysis each day and after every 50 sample

analyses. Blanks were analyzed after every tenth sample. Samples were analyzed at Day 0 and Day 4 after solution preparation.

Results

Measured total silver concentrations were similar to nominal silver concentrations on day 0 and decreased by day 4 (respectively designated pre and post in Figures 1-6). Suspended particles were generally observed in day zero samples, indicating rapid nucleation of silver containing particles. Silver solubility was not correlated to hardness (Figures 1 and 2) or chloride content (Figures 1&4, 2&5, and 3&6) in the solution at day 0 or day 4. Silver concentrations were altered by DOC (Figures 2&3 and 5&6). Increased amounts of DOC in solution increased soluble silver (that not retained by a 0.45 μm filter) at day 0. DOC increases also raised the amount of total silver suspended in solution as filterable particles at day 4. Differences between heights of dark and light bars within Figure 3 and Figure 6 were larger than height differences within Figure 2 and Figure 5. Increased DOC caused a temporal change in dissolved:total silver ratio, with dissolved silver being less prevalent at Day 4. It appears that humic material is preventing nucleation of silver containing particles at day 0 and is keeping silver in suspended particles or soluble macromolecules during the four day study period. Similar results have been observed in solutions containing more combinations of water quality parameters (Jeffers et al. 1995).

Silver behavior in the presence of humic acids implies that silver-humic complexation occurs, but the formation of the humic-silver complex has the same charge as the humic material alone. Complexation of silver cations by humic acids without loss of charge apparently does not lower solubility of the humic-silver complex to the point that precipitation occurs. Association of several of these complexes could form a species large enough to be captured by 0.45 μm filters. This phenomenon is consistent with existing theory describing metal binding with organic sulfhydryls. Sulfhydryl groups are not sufficiently acidic ($pK_a \approx 15$) to become deprotonated in aqueous solution (Peters, Heiftje, and Hayes, 1976; Carey and Sundburg, 1977). At a pH=7 the fraction of sulfhydryl groups deprotonated would be on the order of 10^{-8}. Thus sulfhydryl groups will not contribute appreciably to the overall charge density of humic materials.

Aqueous cation complexation by sulfhydryl groups occurs with concomitant release of a proton from the sulfhydryl. In the case of monovalent silver, this process will occur without altering the overall charge of the humic material. Metal binding to sulfhydryl groups is often thought to involve metal binding to a deprotonated sulfide. However, loss of the proton is not required before a sulfhydryl binds silver or other metals. Sulfur bonded to organic substituents has low lying d molecular orbitals as the lowest unoccupied molecular orbitals (LUMOs). These orbitals are known to strengthen metal-sulfur binding, and LUMOs of sulfur have also been shown to initiate metal ligand binding (Huheey, 1978).

Physical chemical data are essential for understanding metal solubility and speciation. Toxicological responses of aquatic organisms are being evaluated in a collaborative research

project (Klaine et al., this publication) and appear to be explained by soluble silver as described above. Measured silver concentrations were evaluated with toxicity results for aquatic organisms and the effect of DOC on silver solubility appeared to protect aquatic organisms from silver toxicosis.

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Peters D.G., G.M Heiftje, and J.M. Hayes. 1976. A Brief introduction to Modern Chemical Analysis. Saunders Press: New York, NY.

Aqueous Silver Concentrations
3 ppm Cl; 50 ppm hardness; 0 ppm DOC

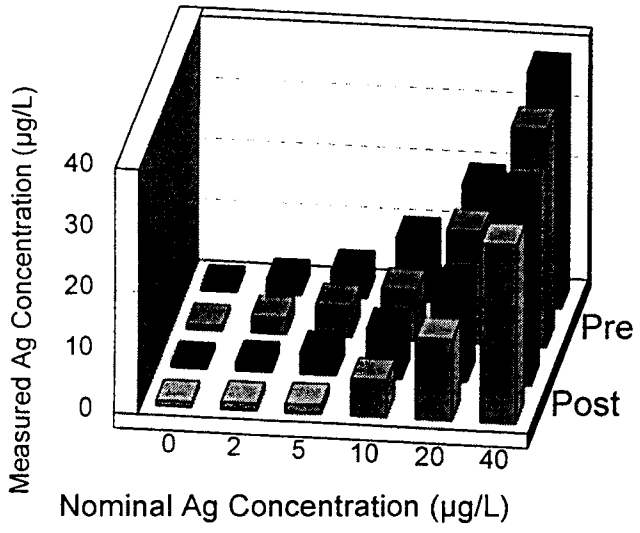

Figure 1. Aqueous silver concentrations in filtered (light shading) and unfiltered (dark shading) samples taken from solutions of low chloride, low hardness and low dissolved organic matter during a 96 hour period.

Aqueous Silver Concentrations
3 ppm Cl; 200 ppm Hardness; 0 ppm DOC

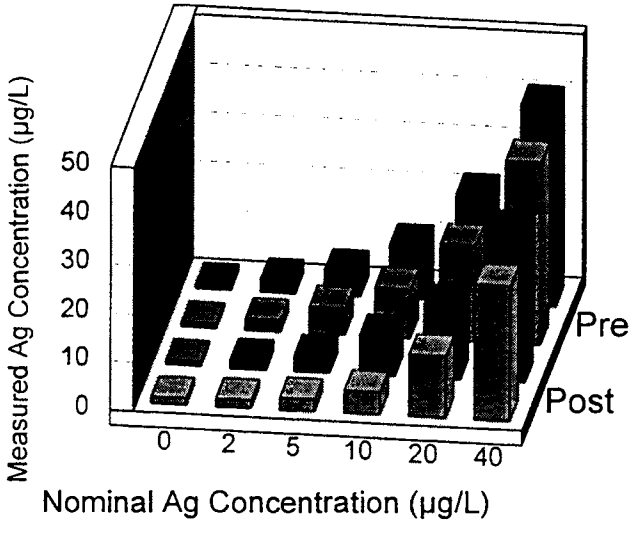

Figure 2. Aqueous silver concentrations in filtered (light shading) and unfiltered (dark shading) samples containing low chloride, high hardness and low dissolved organic matter.

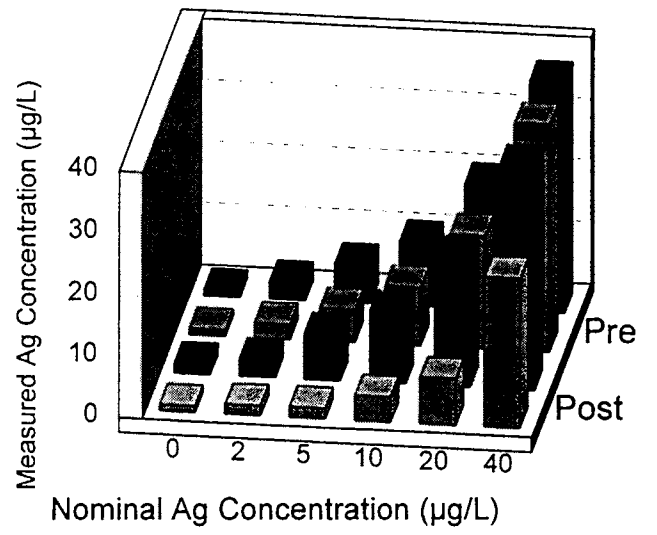

Figure 3. Aqueous silver concentrations in filtered (light shading) and unfiltered (dark shading) samples taken from solutions of low chloride, high hardness and high dissolved organic matter during a 96 hour interval.

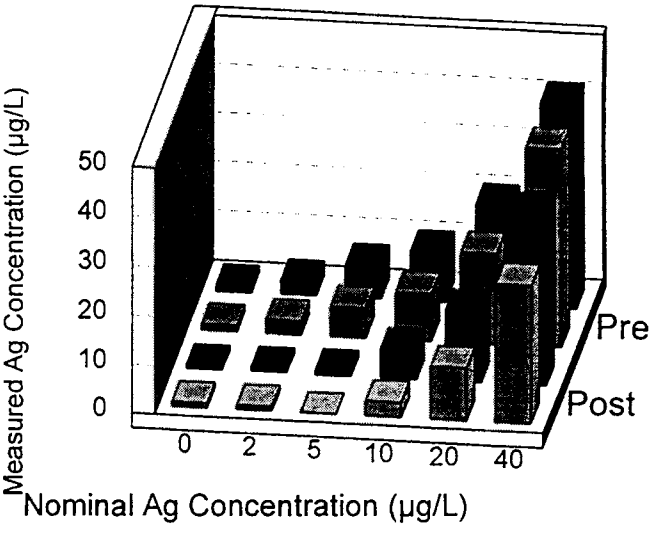

Figure 4. Aqueous silver concentrations in filtered (light shading) and unfiltered (dark shading) samples taken from solutions of high chloride, low hardness and low dissolved organic matter during a 96 hour interval.

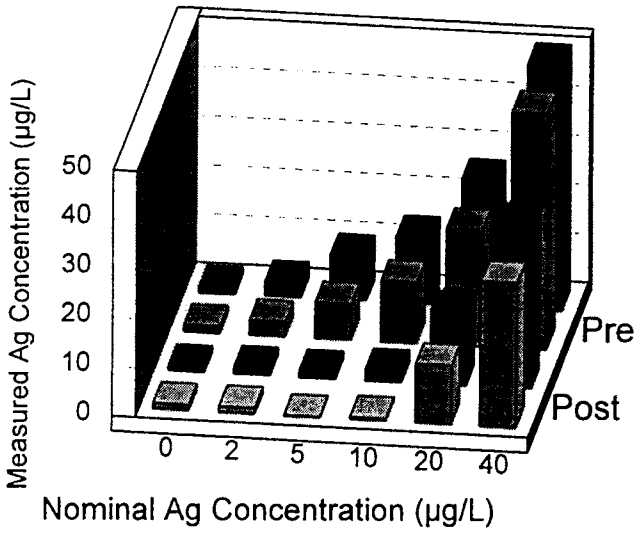

Figure 5. Aqueous silver concentrations in filtered (light shading) and unfiltered (dark shading) samples taken from solutions of high chloride, high hardness and low dissolved organic matter during a 96 hour interval.

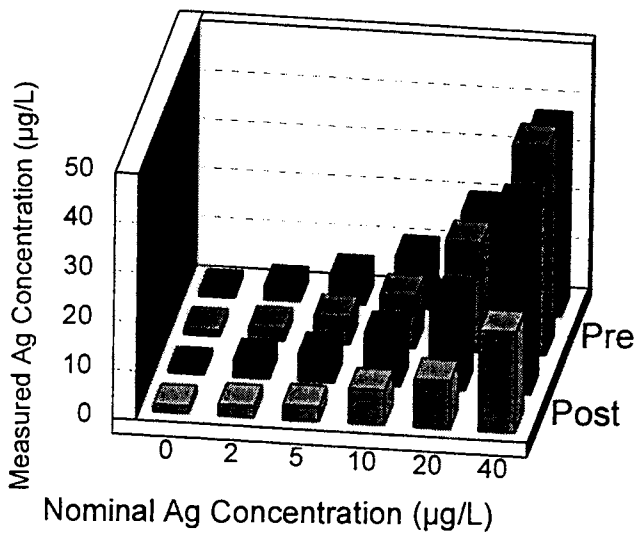

Figure 6. Aqueous silver concentrations in filtered (light shading) and unfiltered (dark shading) samples taken from solutions of high chloride, high hardness and high dissolved organic matter during a 96 hour interval.

Questions & Answers: Effect of Water Quality Parameters on Silver Availability

Tape malfunctioned during the questions & answers session.

Aqueous Silver in the Environment: Conceptual

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Summary:

1. Ag_2S shows a maximum and minimum solubility as a function of total soluble sulfide, $[S]_T$. The minimum $[Ag]_T$ is found for $[S]_T \sim 10^{13}$ M.
2. Cl forms important Ag complexes and affects its mobility only at very low $[S]_T$. Cl concentrations similar to sea water (0.5 M) would influence Ag_2S mobilization only for very low $[S]_T$ = less than 10^{-13} M.
3. When $[S]_T$ is limiting ($[S]_T < [M]_T$), Ag will not be affected by competition from other sulfide forming metals except Hg (II) and Cu(I) and Cu(II). Thus the stoichiometric condition, $[S_T] \geq [Hg] + [Ag] + [Cu]$ is optimum for binding of Ag and for a minimum $[Ag]_T$. Thus any micro-molal detection of $[S_T]$ infers that Ag is bound as Ag_2S.
4. Ag_t concentrations from about 3 - 100 ng/L can be expected in anoxic waters elevated in total S_t and in equilibrium with FeS.

Introduction:

Every study so far shows that Ag is transported in the environment predominantly in the solid state. Ag partitioning between suspended (oxic) sediment and water has a distribution coefficient, log K_d, of about 5, showing the strong association with sediment.

One question that has arisen is, what are the constraints on the aqueous concentration of Ag? Literature data show that Ag and associated metals bind very strongly to inorganic/organic reduced sulfur. A simplistic estimate of a K_d for Ag-S would be nearer to log K_d of 10 rather than 5. The reasons for the apparent weaker binding are many. Ag-S binding in oxic environments may be modified by the competition of other metals: Fe, Cu, Zn ... due to the paucity of total soluble sulfide, $[S]_t$. The measured log K_d of ~5 may reflect a paucity of S sites and this competition. Alternately Ag may associate with more abundant weaker binding sites (e.g. carboxyl on humics) which have log K_d's of 4-6. Finally there may be strong aqueous complexes that keep Ag in solution and decrease the K_d. In reducing sediments, Ag adsorption/solubility is affected by total reduced sulfur because soluble Ag-S complexes are formed. For example, dissolved Ag is predominantly $AgHS^0$ in reducing environments where S_t is prevalent. There is, in addition, the question of Ag-chloro species. Recent studies by Wingert-Runge and Andren (1995) suggest that chloride solutions are quite aggressive in the mobilization of Ag in sediments.

In this paper, calculations are made regarding the above points, assuming that the solid, Ag_2S, is in equilibrium with the aqueous phase at a pH of 8 and 25°C. The assumption of the solid phase and equilibrium are arbitrary in that the calculations are meant to estimate the relative changes in soluble silver and the percentages of different species. An assumption of a pH of 8 mimics the value of many surface water systems in U.S.

Cl⁻ and HS⁻ species and interactions in natural waters:

Cl⁻ may mobilize Ag. Ag also forms very strong complexes with S (e.g. HS⁻)
At a pH greater than 7 and less than 11 or so, the predominant sulfide species are: HS^- and $Ag(HS)^0$. At high pHs with elevated total sulfide, S_T, polysulfides, S_n^{2-}, and polynuclear species become important. Ag^+ forms $AgCl^0$, $AgCl_2^-$, $AgCl_3^{2-}$ and $AgCl_4^{3-}$ in an increasing salinity gradient from freshwater levels (ca. 10^{-3}M) to seawater (≥ 0.5 M). From Sillen and Martel, best* conditional stability constants appropriate for fresh water ionic strength for $AgCl^0$ and for sea water (I = 0.5 M) for the other chloro species are:

$$\frac{[AgCl^0]}{[Ag^+][Cl^-]} = 10^{3.5}, \qquad \frac{[AgCl_2^-]}{[Ag^+][Cl^-]^2} = 10^{5.4} \qquad \text{(1a, 1b)}$$

$$\frac{[AgCl_3^{2-}]}{[Ag^+][Cl^-]^3} = 10^{5.63}, \qquad \frac{[AgCl_4^{3-}]}{[Ag^+][Cl^-]^4} = 10^{5.23} \qquad \text{(1c, 1d)}$$

which can be summarized for the molal chloride concentrations ([Cl⁻]) when the two adjacent species (e.g. $[AgCl^0] = [AgCl_2^-]$ are equal:

$$Ag^+ \overset{10^{-3.5}}{\underset{0.3\ mM}{\rightleftharpoons}} AgCl^0 \overset{10^{-1.9}}{\underset{13\ mM}{\rightleftharpoons}} AgCl_2^- \overset{10^{-0.23}}{\underset{0.6\ M}{\rightleftharpoons}} AgCl_3^{2-} \overset{10^{0.4}}{\underset{2.5\ M}{\rightleftharpoons}} AgCl_4^{3-} \qquad (2)$$

We see that $AgCl^0$ would be the predominant Cl species for most freshwaters, and $AgCl_3^{2-}$ would be the predominant forms for sea water (Cl = 0.5 M for 35 o/oo normal seawater).

* The literature data are quite variable (MINEQL+ is incorrect and inconsistent) for $AgCl_m^{m-1}$ species. A review of Sillen and Martel, ( ) shows values of log K_1 from 3.30 to 3.53 with 3.5 being a best value; log K_2 from 1.81 to 1.97 with 1.9 being a best value; log K_3 from 0.32 to -0.05 with 0.23 being a best value; and log K_4 from 0.86 to -0.40 with -0.40 being the best value. These best values correspond very closely to the estimates made by Kratohvil and Težak (1954).

$AgHS^0$ is the predominant form of Ag^+ for most total sulfide ($[S_T]$) concentrations greater than about 10^{-13} M. The stability is given by:

$$Ag^+ + HS^- \rightleftharpoons AgHS^0, \qquad K = \frac{[AgHS^0]}{[Ag^+][HS^-]} = 10^{13.3} \qquad (3)$$

We can estimate what the level of $[S_t] \sim [HS^-]$ would be for sea water concentration of Cl⁻ (0.5 M) for the condition, $[AgHS^0] = [AgCl_3^{2-}]$. A similar calculation can be done for freshwater (e.g. 35 mg/l (10^{-3} M) ~ L. Erie concentration) for $[AgHS^0] = [AgCl^0]$ from:

$$AgCl_3^{2-} + HS^- \rightleftharpoons AgHS^0 + 3\ Cl^-, \quad K_1 \qquad (4a)$$
$$AgCl^0 + HS^- \rightleftharpoons AgHS^0 + Cl^-, \quad K_2 \qquad (4b)$$

Combining equations (1d) and (3) and equations (1a) and (3) gives the K_1 and K_2 for equations (4a) and (4b) of $10^{8.67}$ and $10^{9.8}$. Substituting in the values of $[Cl^-]$ of 0.5 and 0.001 M to equations (4a) and (4b) gives values of $[HS^-]$ of ~ $10^{-9.6}$ and ~10^{-13} for the $[HS^-] \sim [S_T]$ concentrations for the equivalence of $AgHS^0$ and $AgCl_3^{2-}$ in seawater and $AgCl^0$ in fresh water. Thus the most abundant Ag-chloride species in sea water, $AgCl_3^{2-}$, would be as predominant as $Ag(HS)^0$ for $[S_T]$ between 10^{-9} and 10^{-13} molal. The value for $[S_T]$ between 10^{-9} to 10^{-13} M is very small and would probably only be found in oxidizing waters. Thus chloride ion should only affect silver chemistry through the formation of aqueous silver chloride complexes in oxidizing waters.

Solubility of Ag_2S at pH of 8 and varying total sulfide, S_T:

The solubility of Ag_2S has been studied quite extensively and quite carefully by Schwarzenbach and Widmer (1966). Over a wide range of pH and total sulfide concentrations, $[S_T]$, they defined the system as follows:

$H_2S(aq) = H^+ + HS^-$	$\log K_{s1} = -6.68$
$HS^- = H^+ + S^{2-}$	$\log K_{s2} = -14.0$
$Ag_2S\ (s) = 2\ Ag^+ + S^{2-}$	$\log K_{so} = -49.7$
$Ag^+ + HS^- = AgHS^0$	$\log K_1 = 13.3$
$AgHS^0 + HS^- = Ag(HS)_2^-$	$\log K_2 = 3.87$
$Ag_2S + 2\ HS^- = HSAgSAgSH^{2-}$	$\log K_3 = 3.2$

The system can be broken down into simpler segments. Both the polynuclear species, $HSAgSAgSH^{2-}$, and the species, $Ag(HS)_2^-$, are significant only at high S_T concentrations. At pHs less than about 12, $[HS^-] >> [S^{2-}]$, and for a pH between about 7.5 to 10, only $[HS^-]$ would be the predominant species of S. Most natural waters are within this pH range.

As a first approximation, total soluble silver, $[Ag]_T$, would be:

$$[Ag]_T = [AgSH^0] + \{ [Ag(HS)_2^-] + 2\ [HSAgSAgSH^{2-}] \}$$

with free silver ion, $[Ag^+]$ being negligible and the species in braces ({ }) being significant only at high S_T.

Total soluble sulfide, $[S]_T$, in the pH range of about 7.5 - 10, is given by:

$$[S]_T = [HS^-] + \{ [AgHS^0] + [Ag(HS)_2^-] + 2\ [HSAgSAgSH^{2-}] \}$$

with $[H_2S]$ and $[S^{2-}]$ being negligible compared to $[HS^-]$ and the terms in braces ({ }) only being significant for low values of $[S]_T$ and when $[Ag]_T > [S]_T$.

The equations are substituted and readily solved using the above approximations. Figure 1 shows the solubility of Ag_2S, represented by total aqueous silver concentration, $[Ag]_T$, as a function of total aqueous sulfide, $[S]_T$ for a pH of 8. The distributions of the other Ag species are also shown. The hatching shows the concentrations of total soluble silver often

measured in the environment. The range of $[S]_T$ from 10^{-2} to 10^{-7} M, reflect reduced sulfur and thiol values determined by Shea and MacCrehan (1988) in Chesapeake Bay. A fairly oxidizing pE value of between -1 to 1 would be coincident with a $[S]_T$ of about 10^{-21}.

The minimum Ag_T concentration is calculated for an S_T of about 10^{-13} M. At values greater than 10^{-13}, the major aqueous species is $AgHS^0$. At very low levels, the controlling species is free soluble Ag, Ag^+.

Silver chloride species effect on total soluble silver:

Recalling the above discussion regarding the importance of $AgHS^0$ vs $AgCl_m^{(1-m)}$, the limiting concentration level for the predominance of $AgCl_m^{(1-m)}$ was between $[S]_T = 10^{-9}$ to 10^{-13} M. We can modify Figure 1 to include the effect of different aqueous silver chloride species. This is accomplished by redefining total soluble silver, Ag_t, to include $AgCl_m^{1-m}$ species. In this case $AgHS^0$ will be constant and is defined by total soluble sulfide, $[S]_T$, which is approximately equal to $[HS^-]$. Thus,

$$Ag_t = [Ag^+] + [AgHS^0] + \Sigma[AgCl_m^{1-m}] \sim [AgHS^0] + \Sigma[AgCl_m^{1-m}]$$

Figure 2 is a plot showing the total soluble silver concentration as a function of Cl^- concentration for an assumption of $[S]_T = 10^{-9}$, and Figure 3 is for the assumption, $[S]_T = 10^{-13}$ M. Note that $[S]_T = 10^{-13}$ M is coincident with the minimum Ag_2S solubility in Figure 1. One sees from Figure 2 that the total soluble Ag, $[Ag]_t$, is affected by chloride complexes only at concentration values near those of normal seawater. In addition, the effect is small. When $[S]_T = 10^{-13}$ M, however, the Ag-chloride complexes control the total soluble silver, $[Ag]_T$. At even lower, levels of $[S]_T$, the effect on $[Ag]_t$ by $AgCl_m^{1-m}$ is very pronounced. This is as one would expect since the Σ $[AgCl_m^{1-m}$ species are much greater than $AgHS^0$.

Caution, must be used, however, when interpreting these two figures. It would be a most unusual situation to find $[S]_T$ to be extremely small when [Cl] is very large, even in an oxidizing environment and especially in urban environments where significant DOC and micro-reducing environments would be abundant. Thus one should anticipate only very low $[S]_T$ when $[Cl^-]$ species are quite small.

Deficient total soluble sulfide, $[S]_T$, re Metals:

The question then becomes, what is the limiting value of $[S]_T$ for silver sulfide formation. In what environments might this value of $[S]_T$ not be achieved. Two questions are posed: What is the effect of other metals that bind with sulfide when $[S]_T$ is limiting? And secondly, can one anticipate environments when all of the Ag can not be associated with sulfide.

The minimum amount of S_T for Ag_2S formation (saturation) can be estimated by equating to the total Ag, $[Ag]_T$, in Figure 1. This would be the limiting concentration of S_T if no other sulfide reacting metals are present. We can readily assess the "interference" of other metals by comparing the stability of two different metal sulfides:

$$Ag_2S + M^{2+} = M_2S + 2\,Ag^+$$

$$K = \frac{[Ag^+]^2}{[M^{2+}]} = \frac{K_{Ag_2S}}{K_{MS}}$$

concentration at a pH of 8. Note that the species $AgHS^0$ and Ag^+ define the total soluble silver concentration, Ag_t

or, $$[M]_{limit} = K\,[Ag^+]^2$$

where $[M]_{limit}$ is the maximum limiting concentration of the other metal which will not suppress silver sulfide formation. Table 1 compiles the solubility products (K_{sp}) for a number of metal sulfides. There is also a simple calculation for the limiting metal concentration ($[M_{limit}]$) when $[Ag]_T = 10$ ng/L $= 10^{-10}$ M.

Table 1. **Competition of other metals for sulfide when $[S]_T$ is less than the total reacting metals.** The column, $[M]_{limit}$ is the molal concentration of the metal at which it will begin to interfere with Ag_2S formation for limiting conditions of $[S]_T$ and $[Ag^+] = 10^{-10}$ M.

Metal	-log K_{sp}	log $[M_{limit}]$ (M)
Ag(I)	49.7	see fig 1
Cu(I)	48.5	-9.4
Cu(II)	36.1	-6.4
Hg(II)	51.0	-21.3
Fe(II)	18.1	11.6
Zn(II)	24.4	5.3
Pb(II)	27.5	2.2
Cd(II)	25.8	3.9
Co(II)	21.3	8.7
Ni(II)	19.4	10.3

It is apparent from examination of the third column in Table 1 that only Hg (II), Cu(I) and Cu(II) would be at high enough concentrations for most natural waters to compete with the formation of Ag_2S. One might also speculate that Ag, Hg, Cu(I) and Cu(II) would form insoluble sulfides in nearly the same chemical environments as for Ag_2S.

FeS and Ag_2S in equilibrium:

If we imagine FeS in excess and in equilibrium with Ag_2S, then we can calculate the S_t and Ag_t concentrations given the $Fe(II)_t$ concentration in solution. The logic is that the major metal is Fe(II) and thus the major "source" of sulfide for other metals is from equilibration with FeS which is not as stable a metal as Ag-sulfides etc. Thus the Fe and S_T

concentrations are regulated by the equilibration with FeS, and the concentration of the S_T sets the equilibrium total soluble silver value (i.e. Figure 1)

As noted in Figure 1, S_T in Chesapeake Bay ranges from about 10^{-6} to 10^{-2} M.

Recently, Yao and Millero (1995) studied in some detail the S_t and Fe(II) - Mn concentrations within a Norwegian anoxic fjord. In the anoxic layer, S_t increases to very high values (~ $10^{-2.2}$ M) whereas Fe(II) increases and then decreases due to the formation of FeS. The supply of S is virtually unlimited due to the large amount of SO_4^{2-}; thus the formation of S_t depends upon the microbial degradation of SO_4 - S. On the other hand, Fe(II) is less abundant in the water column.

Figure 4 shows a vertical profile of S_t and Fe_t as well as the concentration product $[Fe^{2+}][S^{2-}]$. The $[Fe^{2+}][S^{2-}]$ product is virtually constant ($10^{-16.5}$ to 10^{-17}) even though S_t increases more than two orders of magnitude and Fe_t decreases almost two orders of magnitude with increasing depth. The constant ion product strongly suggests that FeS is at equilibrium over this interval. It is noteworthy that the concentration product is 10 to 40 times greater than the equilibrium product given in table 1. This difference easily can be accounted for by the effect of activity coefficients ratio $\gamma_{Fe} \cdot \gamma_S$. For example, using the Gűntelberg expression for ion activities, gives a product of 35 compared to the range of 10 to 40 at 25°C. In addition, the calculations were carried out at 25°C since there are no established thermodynamic data for other temperatures.

Thus the S_t range from about $10^{-2.2}$ to $10^{-4.8}$ M reflects expected concentrations for FeS equilibration in an anoxic environment. Using this range and the information used to construct Figure 1 gives an expected Ag_t concentration of 3 - 100+ ng/L.

This calculation then confirms that FeS is saturated in the water column certainly at levels greater than $10^{-6.6}$ M (0.2 μM) S_T (e.g. AVS). Furthermore we should anticipate levels of Ag_T between 3-100$^+$ ng/L, assuming the predominance of the $Ag(HS)^0$ species in waters rich in S_T. It remains to confirm this information by direct measurment.

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List of Figures:

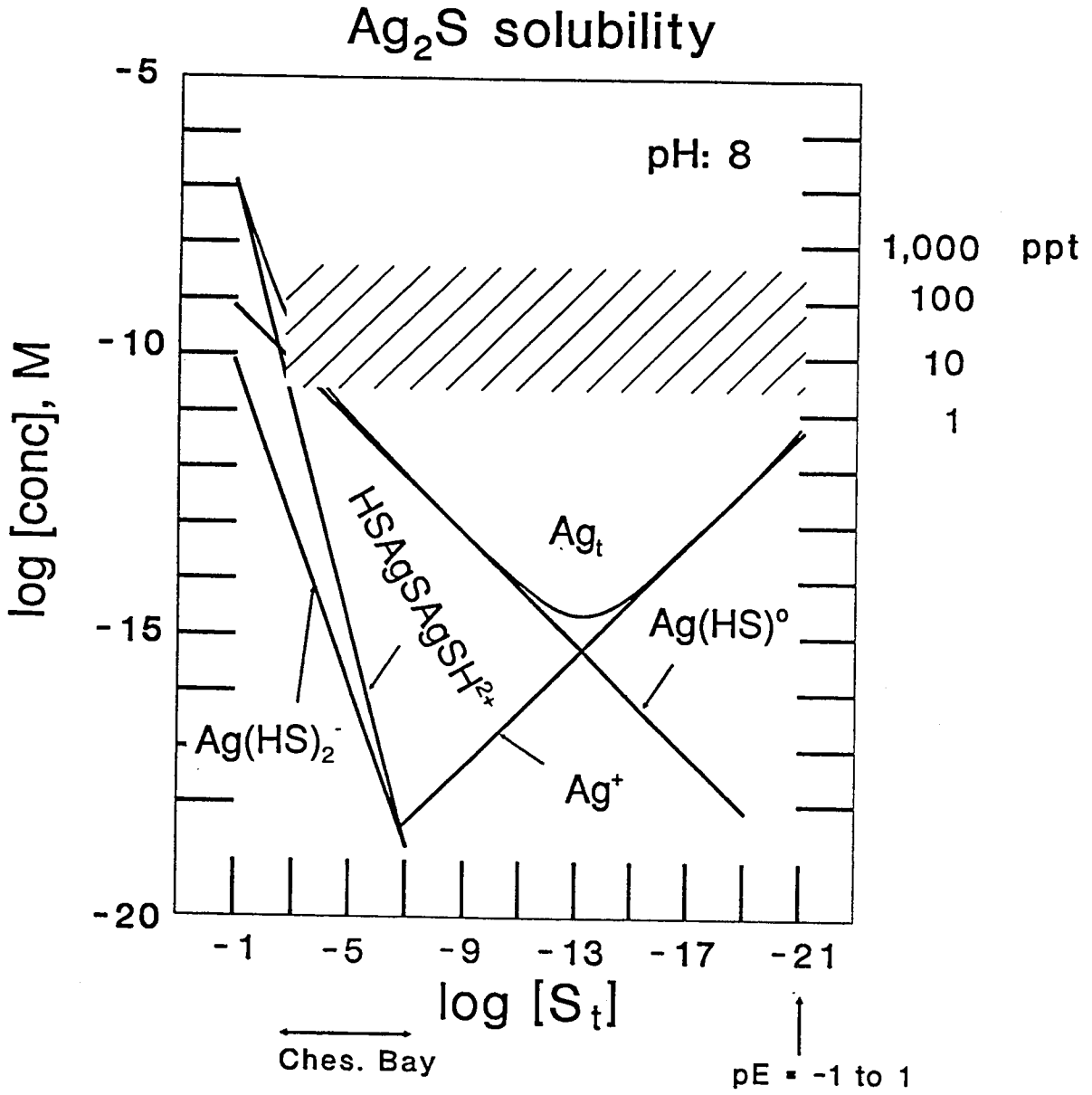
Ag_2S solubility
pH: 8
1,000 ppt
100
10
1
log [conc], M
-5
-10
-15
-20
Ag_t
$HSAgSAgSH^{2+}$
$Ag(HS)^\circ$
$Ag(HS)_2^-$
Ag^+
-1 -5 -9 -13 -17 -21
log [S_t]
Ches. Bay
pE = -1 to 1

Figure 1

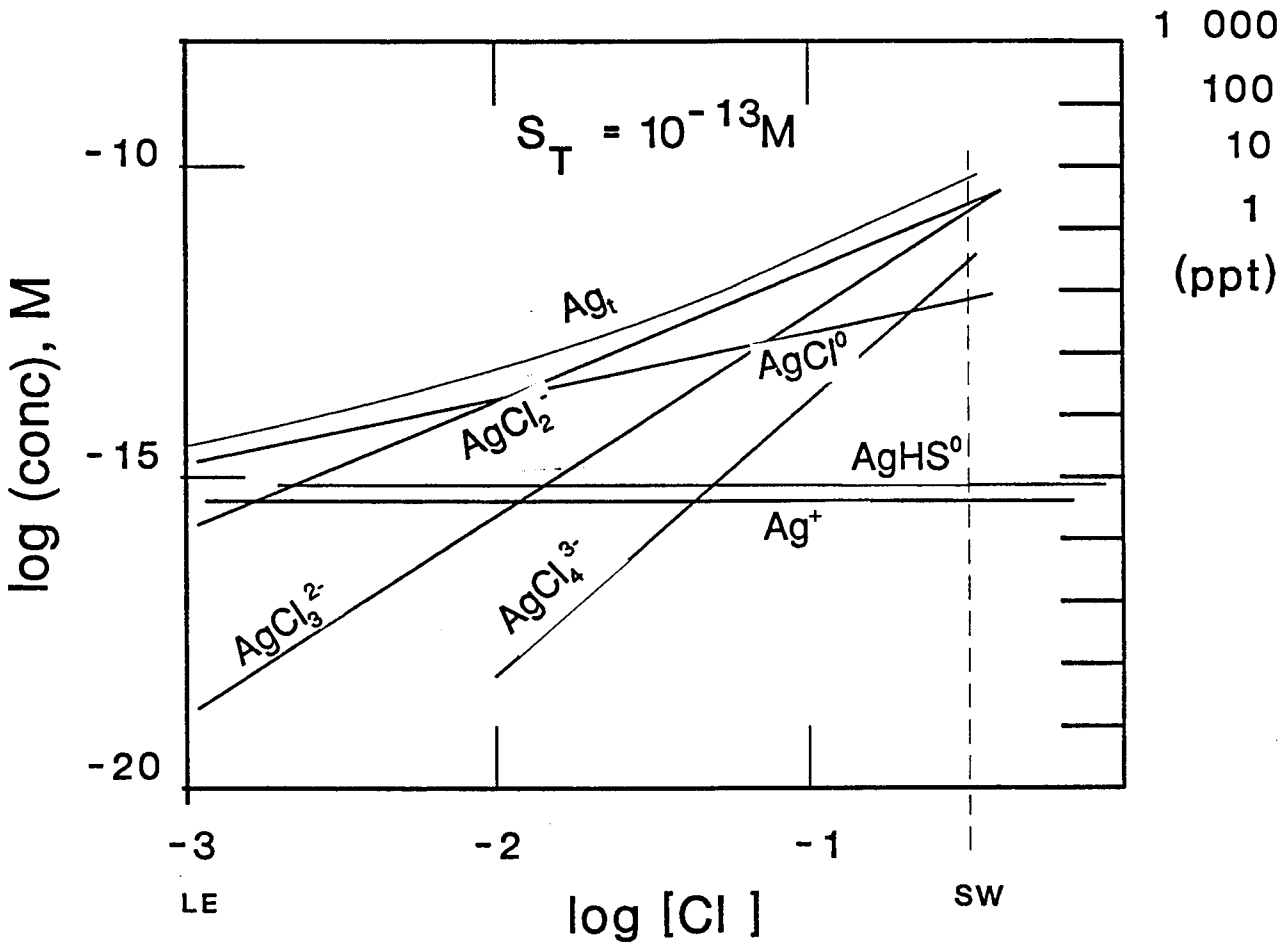
1 000
100
10
1
(ppt)
$S_T = 10^{-13}M$
-10
-15
-20
log (conc), M
Ag_t
$AgCl^0$
$AgCl_2^-$
$AgHS^0$
Ag^+
$AgCl_3^{2-}$
$AgCl_4^{3-}$
-3
-2
-1
LE
SW
log [Cl]

Figure 2

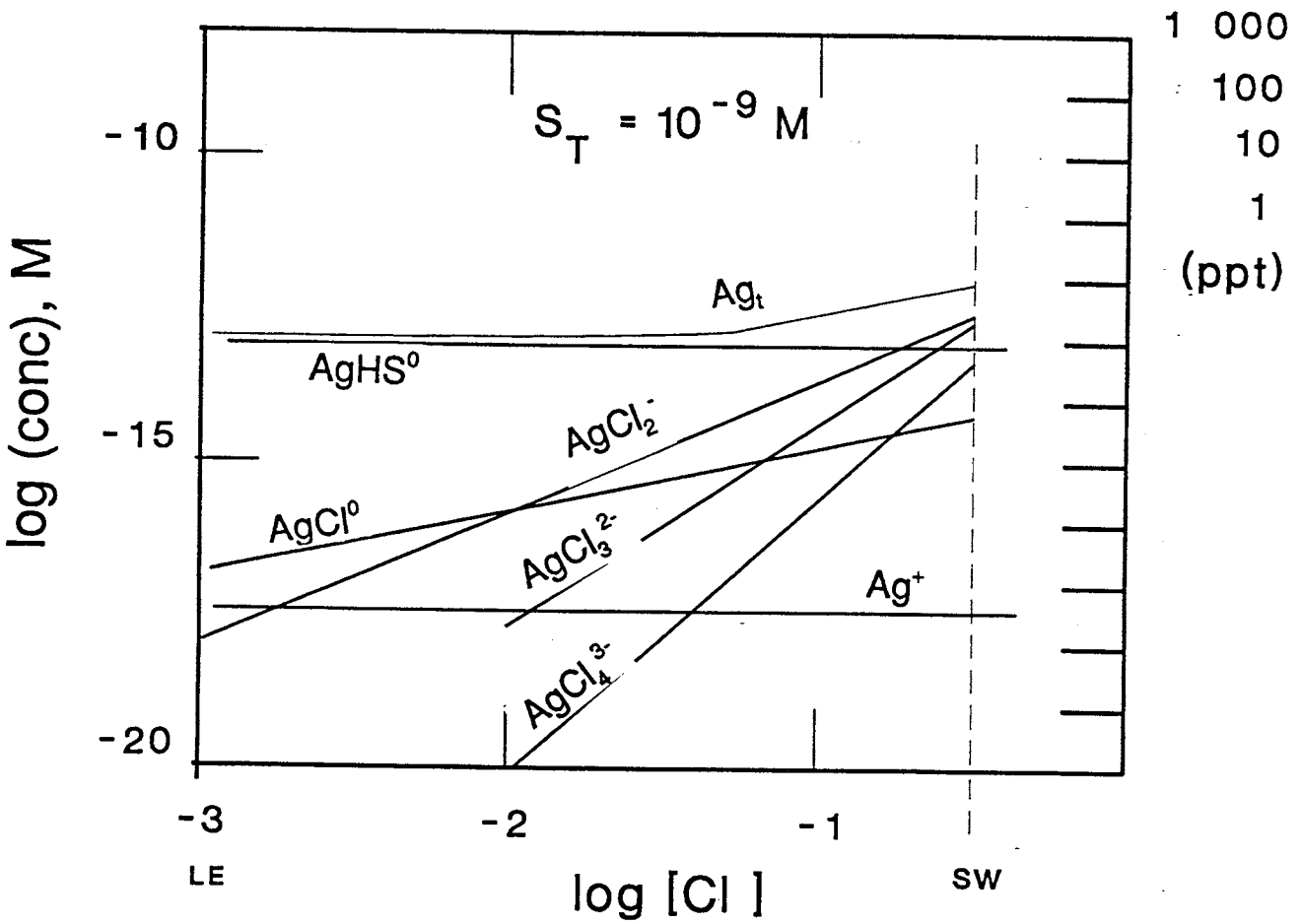
$S_T = 10^{-9}$ M
-10
-15
-20
log (conc), M
Ag_t
$AgHS^0$
$AgCl_2^-$
$AgCl^0$
$AgCl_3^{2-}$
Ag^+
$AgCl_4^{3-}$
-3
-2
-1
LE
SW
log [Cl]
1 000
100
10
1
(ppt)

Figure 3

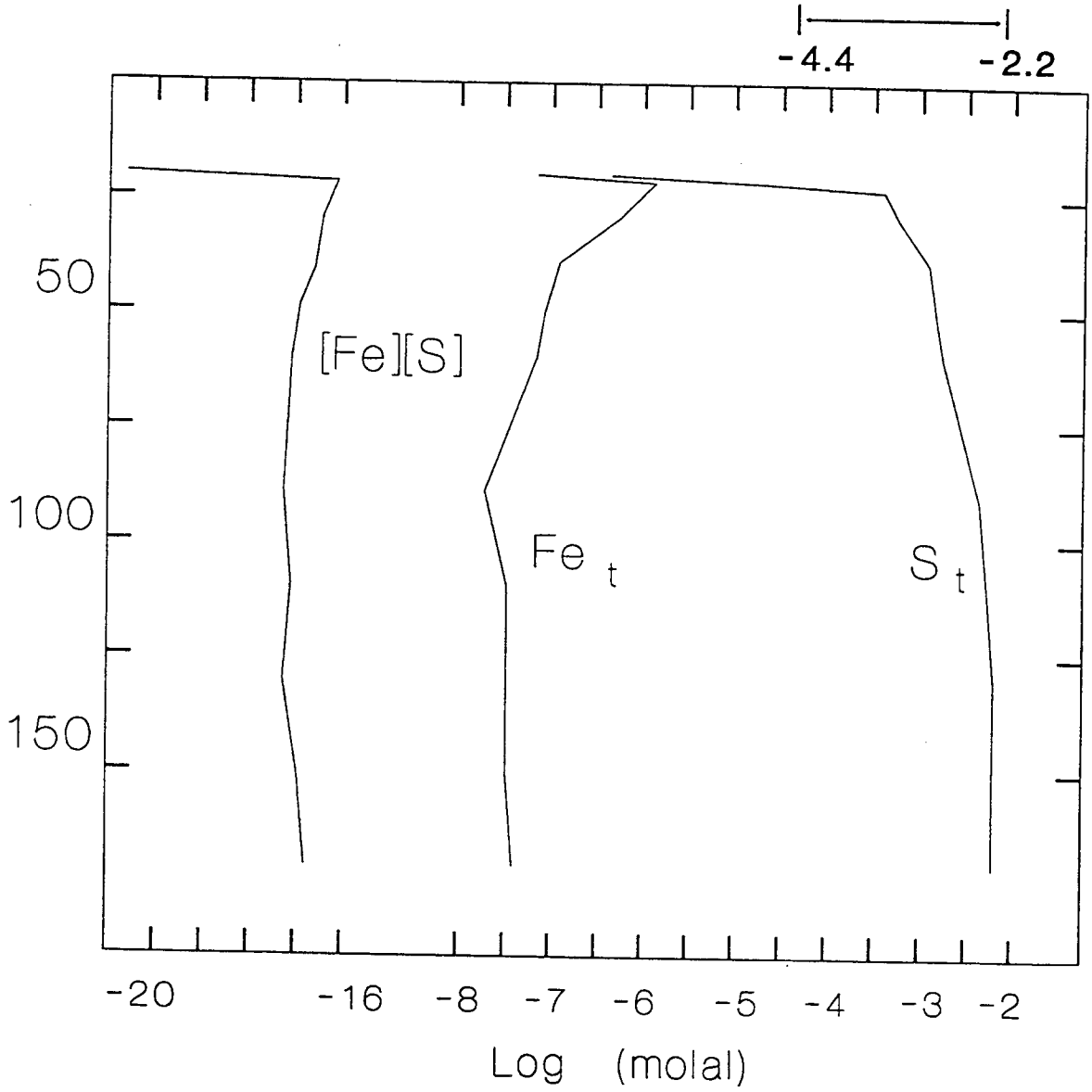

Figure 4

Questions & Answers: Aqueous Silver in the Environment: Conceptual

Tape malfunctioned during the questions & answers session.

Silver Sorption by Manganese Oxide

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Abstract

Silver-manganese oxide ores are present in western United States, Mexico, South America, and Sumatra. The amount of silver varies between a few parts per million (ppm) to thousands of ppm. However, these ores are not compatible with conventional metallurgical extraction of silver. In parts of Colorado silver-cryptomelane ore bodies have up to a maximum of 1 wt% of silver in them. This research is a study of the methods for the nature of silver binding in cryptomelane as a model manganese oxide phase with tunnel structures. In order to better understand the nature of silver binding in cryptomelane, synthetic samples of cryptomelane were prepared, characterized and silver sorption experiments were carried out. The silver uptake appears to be a function of the solution chemistry conditions, pH and potassium nitrate concentration. The sorption of silver was found to increase with decreasing pH and at a fixed pH value, the sorption density was higher at a lower potassium nitrate concentration. Kinetics and equilibrium sorption data with solutions containing lithium and sodium nitrate as supporting electrolyte demonstrate three main results: firstly, most of the uptake of silver is compensated by release of potassium; secondly, the exchange for silver with protons and potassium ions is almost stoichiometric; and lastly, the tunnel sites play a major role in the sorption reaction in cryptomelane.

Introduction

Silver-manganese oxide ore bodies are of secondary origin, where silver is transported from its source to being locked up in the manganese oxide matrix [1]. In fresh water streams, the concentration of silver is around 0.2 part per billion, in streams (near silver bearing source) are typically in the order of one part per million (ppm), and the relative concentration in manganese oxide varies anywhere from 0.3 to 300 ppm [2] and in some rich deposits as high as 1000 ppm. Deposits of silver-manganese oxide are present in Colorado, Nevada, New Mexico, Arizona, Mexico, parts of South America and Sumatra. In Treasure Hill, Nevada, the concentration of silver has been reported to be as high as 7.5% (of Ag_2O) in aurorite and 3.9% (of Ag_2O) in argentain todorokite [3] and in Silver Cliff, Colorado, between 0.5 to 1% silver in argentain cryptomelane [4]. Hilderbrand [5] in his study of samples of argentain cryptomelane from Silver Cliff, Colorado, observed that a high silver content of 10000 ppm did not produce any structural change of the oxide phase. But, these ore bodies do not respond to the conventional metallurgical procedure used to extract silver and therefore require specialized extraction techniques.

The reagent consumption for the extraction of silver from silver- manganese oxide ore bodies has been high, thereby making the process expensive. Both Pesic and Wey [6] and Scheiner and coworkers [7] obtained recovery of silver higher than 80%, but their reagent consumptions were rather high. Clevenger [8] in his summary report on the various chemical treatments showed that most processes to make silver recovery amenable start by reducing MnO_2 to MnO. This seems to indicate that silver is bound strongly in the manganese oxide structure. It is therefore necessary to study how silver interacts with manganese oxide to better understand the chemistry of the system.

The model oxide system for this research is cryptomelane. A thorough investigation of the factors affecting the sorption could possibly lead to a fundamental understanding of the formation of these ore bodies.

Manganese oxides exist in different phases, which according to Burns [9] can be combined into to three broad categories: chain, tunnel and layered structures. For the different geometries, the smallest building unit is a $[MnO_6]^{8-}$ octahedron. Cryptomelane has a tunnel structure where the octahedra form a double chain, and share corner oxygen atoms with another double chain, to produce a three dimensional network, potassium ions occupying the cavity sites. The structural formula for cryptomelane is $K_xMn_8O_{16}$ ($1<x<2$), with manganese having variable oxidation states.

Manganese oxides are present in soils, rocks and deep-sea nodules. In all these environments, manganese oxides act as very good ion exchangers/scavengers to other metal ions. There are two main factors that contribute to this behavior. Firstly, the surface chemistry of the oxides in solution plays an important role. At different hydrogen ion concentrations in solution, the oxide surface has different affinities for hydroxide ions and protons. For a pH value equal to the point of zero charge (PZC) of the oxide, the affinity is equal. For pH < PZC, the oxide surface is positively charged due to accumulation of hydrogen ions, and for pH > PZC, the oxide surface is negatively charged due to hydroxide ions. Secondly, the presence of tunnel sites and vacancies in their structure enables metal ions to occupy these positions.

Experimental

Synthesis and characterization of cryptomelane

When concentrated HCl are added dropwise to a well-stirred aqueous solution of $KMnO_4$ at temperature 363 K, birnessite (another phase of MnO_2) is precipitated by the following chemical reaction [10], [11] :

$$2\,KMnO_4\ (\text{excess}) + 8\,HCl = 2\,MnO_2 + 2\,KCl + 3\,Cl_2 + 4\,H_2O \qquad (1)$$

Excess $KMnO_4$ is used to ensure oxidizing conditions [12]. After the completion of the reaction, the mixture is boiled for ten minutes, and the precipitate is filtered and washed to remove any adhering KCl. When ignited at 673 K for 60 hours, birnessite prepared by the foregoing procedure is transformed to cryptomelane [10].

Birnessite samples were prepared in a batch mode and all the batches were mixed prior to the phase transformation step. The cryptomelane samples were then washed in perchloric acid, sodium hydroxide and distilled water for a total of sixty six times to remove adsorbed impurities resulting from precipitation. The samples were then dried at 318 K.

X-Ray (see Table 1) and electron diffraction was used to verify the crystal phase. A bright field image of the particles is shown in Figure 1. Composition analysis of the washed sample performed at the Microchemical Laboratory at University of California, Berkeley showed that the material contained 57.2% manganese and 7.0% potassium on a weight percent basis. The surface area of the oxide powder obtained by nitrogen gas adsorption at 77 K is 23.2 $m^2\,g^{-1}$.

By electrophoresis, the isoelectric point (IEP) of the starting sample of cryptomelane before washing occured at pH 3.1 and after washing at pH 5.1.

Silver uptake by cryptomelane

All silver sorption experiments were carried out at 293 K and solid samples of 100 milligrams were used in a 125 ml polypropylene bottle. All solutions were prepared with reagent grade chemicals and distilled water free of carbon dioxide. All silver-containing solutions were stored/equilibrated in amber bottles, and the addition of reagents, pH measurements were carried out in the presence of red light to prevent the photo reduction of silver. The pH adjustments were made using a microburette with either nitric acid or alkali hydroxide (Li, Na, K). The sequence of addition of chemicals was water, supporting electrolyte, solids, silver nitrate and pH adjusters. Silver concentrations were measured using atomic absorption spectroscopy and potassium ion concentration was directly measured using an ion-selective electrode. Argon gas was used to inert the system to prevent any further absorption of carbon dioxide. All containers used for the experiments were cleaned with

hydroxylamine hydrochloride (reducing agent to dissolve manganese oxide), washed in water and then treated with non-chromix sulfuric acid, rinsed with water and dried at 318 K in an oven. The solid-liquid separations were either carried out using a simple filtration method or in a temperature-controlled centrifuge. Kinetics experiment were carried out at each of the chosen pH values, to determine the equilibration time for the sorption experiments. Figure 2 shows the results obtained at pH 5 and similar results were obtained at other pH's tested. It is clear that the silver uptake attains equilibrium value 6 hours, but we chose 24 hours equilibration time for all sorption experiments.

Since cryptomelane has potassium in the tunnel sites, the influence of potassium ions from solution on the sorption behavior was investigated. Two different potassium concentrations, 0.01 M and 0.1 M, were chosen. Sorption isotherms were performed at two pH values above the IEP and two below. After the addition of silver, the pH drops due to the exchange of silver ions with protons on the surface of the oxide. Base additions are made to bring the pH to the chosen value. Representative results obtained at pH 6, 5 and 4 are shown respectively in Figures 3, 4 and 5. Sorption isotherms are plotted by calculating the total amount of silver picked up by the oxide against the equilibrium concentration of silver. With change in potassium concentrations and pH, the maximum uptake of silver is changed (see Table 2).

To study the possible exchange reaction of potassium in the tunnel sites with silver ions, sodium and lithium nitrate were chosen as the indifferent electrolyte. Maintaining the same solution conditions as used in the potassium electrolyte case. The kinetics result for the uptake of silver and the release of potassium are shown in Figure 6. The proton release was estimated by the amount of 0.1N base required to reach the set solution pH. From equilibrium sorption measurements, the total exchange for silver with potassium and protons is tabulated in Table 3.

Discussion of results

Since the oxide has two different kinds of sites for sorption, the tunnel and the surface hydroxylated sites, the uptake of silver is represented on a mole per mass basis. From the shape of the isotherms shown in Figures 3, 4, 5, it can be clearly seen that silver exhibits strong binding to cryptomelane. The initial rise of the sorption density is rather steep and at higher silver equilibrium concentrations a saturation sorption capacity is achieved. The maximum sorption density was found to occur at pH 7, with a decreasing trend at lower pH values. The oxide surface has an isoelectric point of pH 5.1 and therefore at pH values above 5.1, the surface is negatively charged and at pH values less than 5.1, the oxide surface is positively charged. As silver is a positively charged, monovalent ion and the surface is negatively charged at pH 7, maximum sorption is obtained. However, at pH values lower than 5.1, the interaction is now between a positively charged ion and a similar charged surface, there is still appreciable sorption taking place. This seems to suggest that the tunnel sites indeed play an important role in sorption through an ion-exchange reaction mechanism rather than a relative accumulation of silver ions at the surface.

The effect of potassium ion concentration in solution on the sorption of silver was studied, namely, 0.01M and 0.1M. These concentrations of potassium in solution are at least an order of magnitude higher than the amount present in the solid. This therefore ensures that the potassium concentration in solution is almost a constant. The isotherms indicate that the silver sorption capacity by cryptomelane is higher in a 0.01 M KNO_3 background electrolyte solution than in a 0.1 M solution. For an ion-exchange reaction of the type:

$$K\text{-}MnO_2 + Ag^+ = Ag\text{-}MnO_2 + K^+ \quad (2)$$

with increasing concentrations of potassium in the solution, the equilibrium is shifted to the reactant side due to a common ion effect, thereby making the formation of a silver-manganese oxide more difficult. This trend is consistent at all pH values studied.

In order to validate the ion exchange reaction mechanism, sorption was carried out with lithium and sodium as indifferent background electrolyte. The kinetics of the uptake of silver

and the release of potassium is shown in Figure 6. The only source of potassium in solution is from the an ion exchange reaction. As the silver concentration reaches its equilibrium value, the potassium release also attains a saturation value. Table 3 represents the total exchange for silver obtained under equilibrium conditions. The ratio of potassium released to silver sorbed is between 0.7 and 0.8, indicating the extent to which the tunnel sites take part in the uptake reaction. The total molar exchange for silver within the limits of experimental accuracy, with surface protons and tunnel potassium ions, is pretty close to stoichiometric.

For cryptomelane, the maximum amount of potassium corresponds to the case where the stoichiometry is 2. The samples used for the sorption experiments contain 7 wt% potassium and a maximum possible would correspond to a 10.08 wt% . Therefore, for a 0.1 g sample as used in all experiments (in a total solution of 100 ml, the solid loading ratio is 1 kg m^{-3}), the potassium concentration is 179.5 micromoles and the maximum possible value is 258.4 micromoles. Therefore 2.584 moles kg^{-1} would correspond to a maximum silver loading when all the tunnel sites in the crystal is occupied by silver. From the exchange reactions performed in lithium and sodium nitrate solutions, assuming that all the silver goes to the tunnel sites, both silver and potassium now occupy on an average 80% of the total available tunnel sites.

Conclusions

The sorption behavior was found to be dependent on the solution chemistry conditions. With increasing pH, the sorption density was found to be higher. At constant pH and varying potassium electrolyte concentration, the sorbed quantity of silver onto cryptomelane is higher at lower potassium levels in solution. Both lithium and sodium behave as indifferent electrolytes and the sorption densities for silver were found to be similar. Since silver exchange is predominantly taking place with the tunnel sites, monitoring the release of potassium gives an estimation of the extent of replacement in the structure of manganese oxide.

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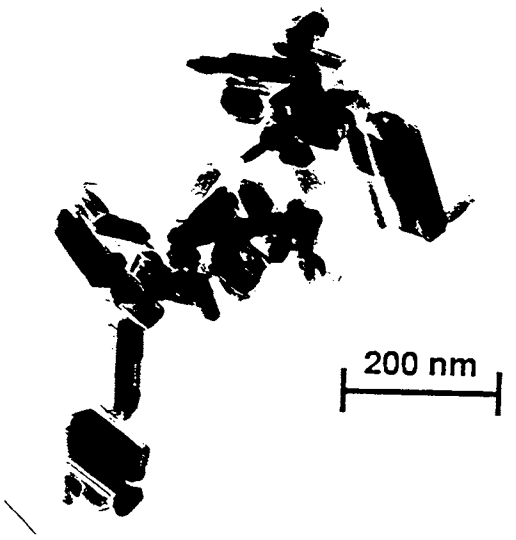

Figure 1. Bright field image of cryptomelane particles.

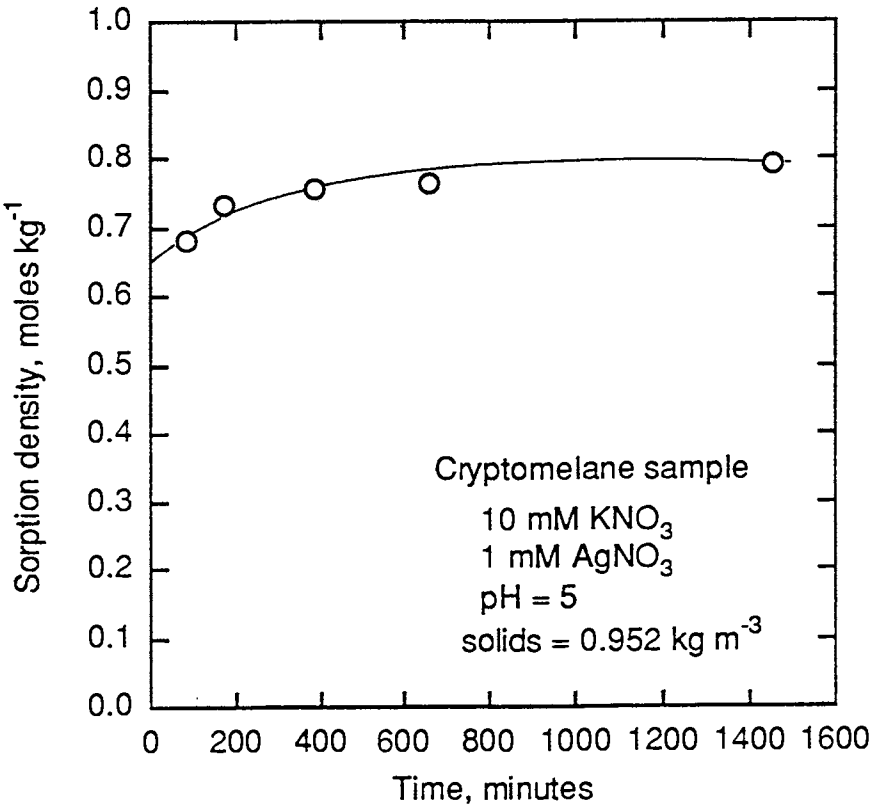

Figure 2. Kinetics of silver sorption at pH 5.

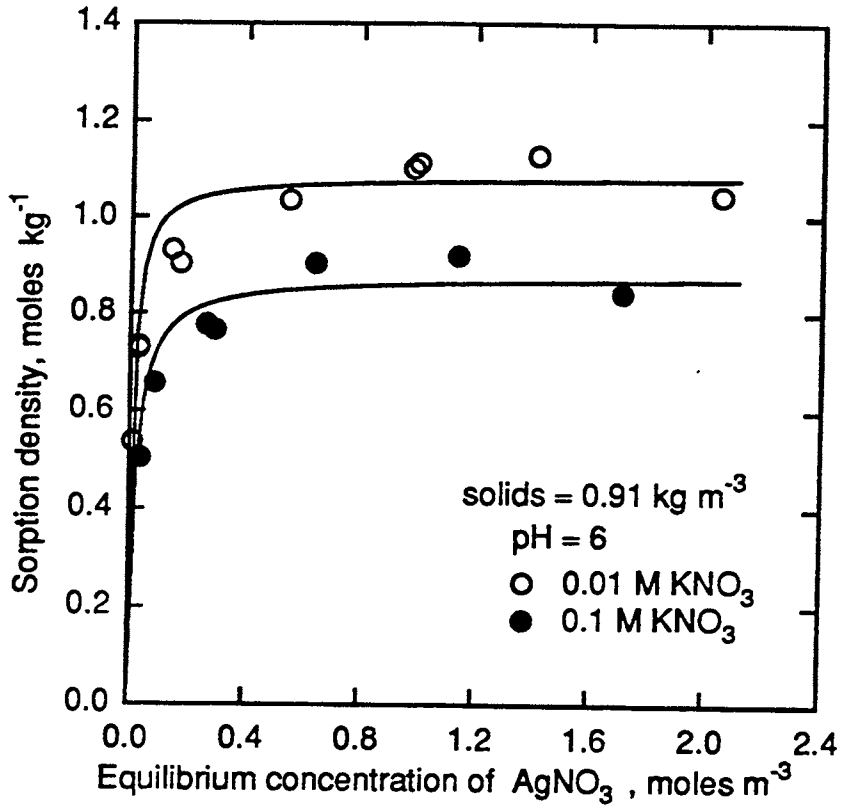

Figure 3. Sorption isotherms at pH 6 ± 0.15.

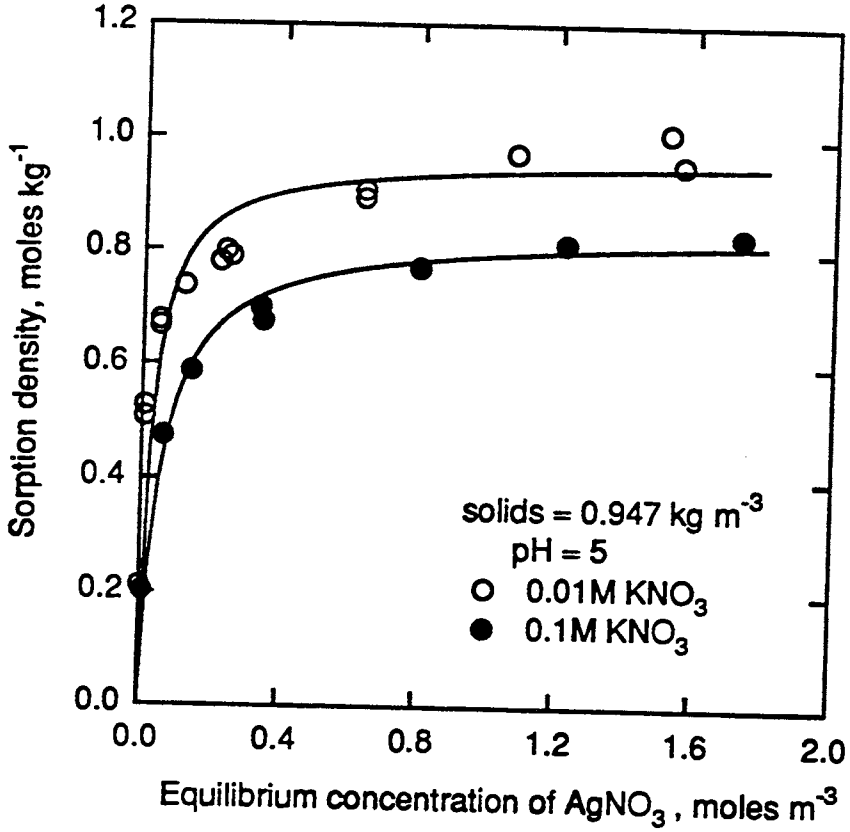

Figure 4. Sorption isotherms at pH 5.0 ± 0.1.

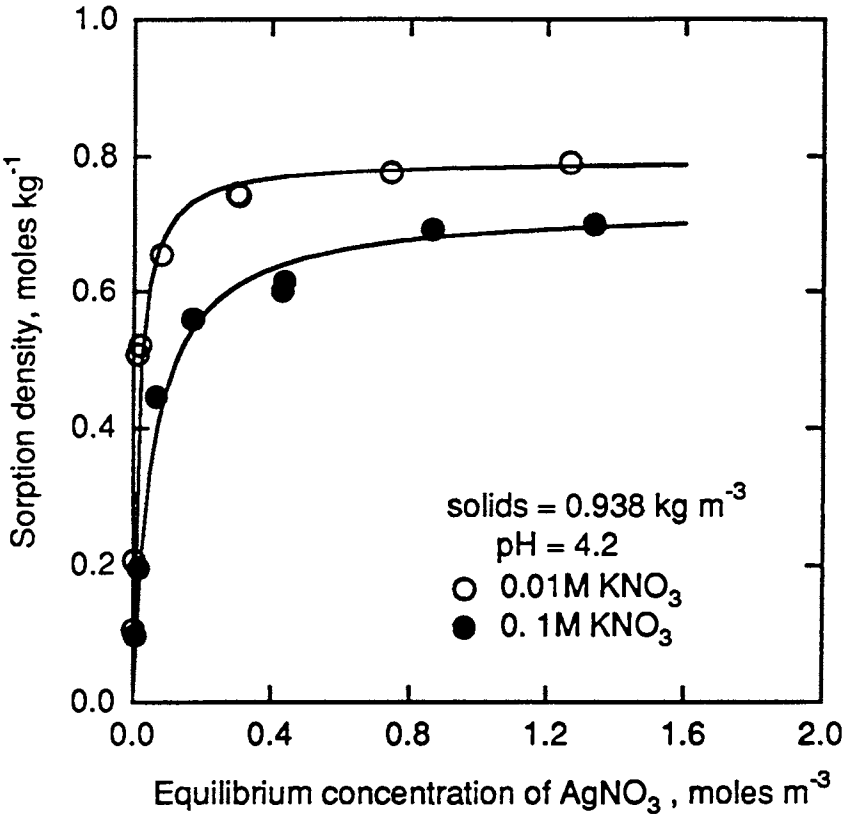

Figure 5. Sorption isotherms at pH 4.2 ± 0.05.

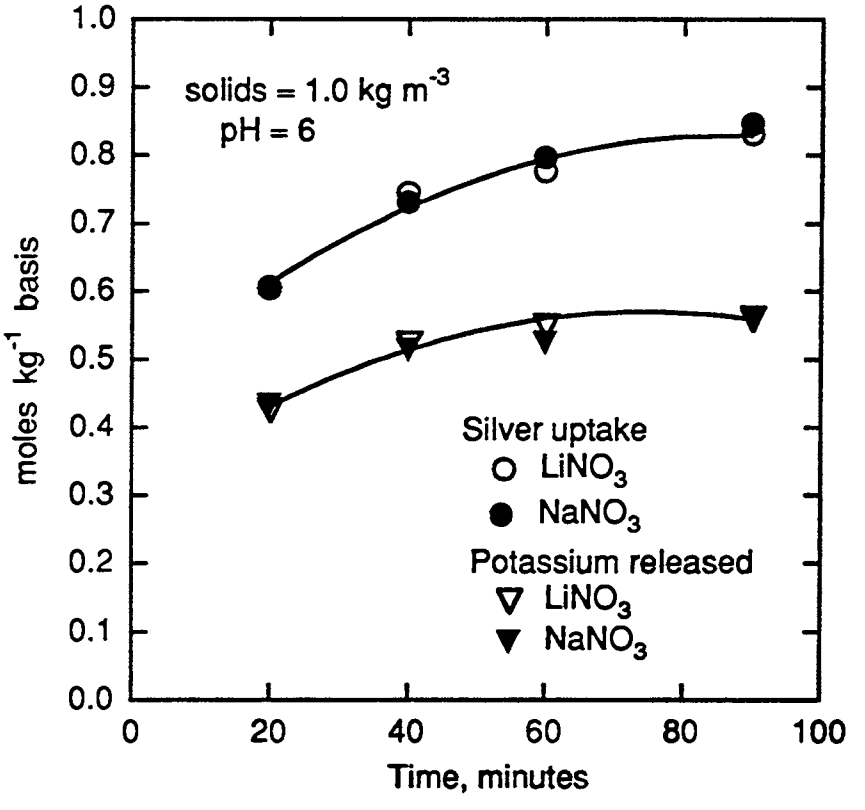

Figure 6. Kinetics for the uptake of silver and release of potassium with initial concentrations of 10^{-3} M for $AgNO_3$ and 10^{-2} M for $LiNO_3$ and $NaNO_3$.

Table 1: X-Ray diffraction data for cryptomelane

(hkl)	d(hkl)-observed, nm	d(hkl)-actual, nm
110	0.6967	0.6940
200	0.4924	0.4908
310	0.3118	0.3104
211	0.2399	0.2389
301	0.2156	0.2148
411	0.1834	0.1826

Table 2: Maximum sorption capacity for silver as a function of pH and KNO_3 concentration in moles kg^{-1}

pH	KNO_3 0.01 M	KNO_3 0.1 M
7	1.11	1.02
6	1.09	0.88
5	1.00	0.85
4	0.80	0.73
3	0.72	0.52

Table 3: Molar balance for the uptake of silver and exchange with potassium ions and protons in lithium and sodium nitrate solutions
(same weight basis of 0.1 g in 100 ml of solution)

Salt, M	pH	H^+ exchange µmoles	K^+ exchange µmoles	Total ($H^+ + K^+$) µmoles	silver sorbed µmoles
$LiNO_3$					
0.01	6	17.0	68.1	85.1	95.5
0.10	6	17.6	68.1	85.7	95.5
0.01	6	30.0	81.0	111.0	117.0
0.01	5	8.0	64.9	72.9	89.4
0.10	5	9.0	65.4	74.4	88.9
0.01	5	21.0	74.2	95.2	107.3
$NaNO_3$					
0.01	6	16.0	74.4	90.4	96.2
0.10	6	18.0	71.5	89.5	95.5
0.01	6	30.0	83.7	113.7	114.4
0.01	5	8.0	70.0	78.0	88.8
0.10	5	9.0	69.4	78.4	88.6
0.01	5	12.0	84.7	96.7	108.3

Questions & Answers: Silver Sorption by Manganese Oxide

Tape malfunctioned during the questions & answers session.

Silver Binding to Mixed Metal Sulfides

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Abstract

A series of experiments were developed to examine Ag binding with fine-grained iron sulfides, which are a common constituent of anoxic sediments. The effects of complexing ligands (Cl^- and thiols), organic matter and competing trace metals were examined with respect to Ag solubility. Co-precipitations of Ag,Fe were also performed to determine whether differences might be seen compared to contacting pre-aged FeS with Ag solutions. Various extractions were performed on the FeS solids to assess Ag-FeS binding strength. The results of this study showed that silver is rapidly removed from solution in the presence of FeS. The complexing agents (Cl^- and 3-MPA) used in this study appear to lower "soluble" Ag concentrations, possibly through enhancing coagulation of the colloidal FeS, which has associated with it a large fraction of the total dissolved Ag.

Introduction

Silver partitioning between water and (oxic) sediments has a log K of -5 as shown by measurements on particulate and aqueous fractions in streams and lakes. Binding of Ag to inorganic/organic sulfides can have log K as high as 13. The nature of silver binding in the solid phase is not known. Silver may occur as an inorganic sulfide. In urban sediments and wastes, silver may be associated with the sulfide fraction of sediments as a discrete Ag_2S precipitate or as a coprecipitate with other more abundant metal sulfides. Jacobs (1984) has suggested dissolved metal concentrations are not at equilibrium with pure metal sulfides, as shown from studies of trace metal geochemistry in anoxic waters. However, more recent work by Tessier (1995) has shown that for many transition group metals, metal sulfide solubility products can closely predict dissolved metal concentrations for lakes of near neutral pH. At this time, there is very little information as to what is, in fact, controlling silver concentrations in anoxic waters.

Iron sulfide is undoubtedly the main sulfide host in sediments. Amorphous FeS and very fine-grained greigite and mackinawite are the major iron sulfides in sediments (Berner 1967). We have set up a number of controlled laboratory studies to investigate what happens to dissolved Ag concentrations in the presence of an FeS host. This simplified system allows us to examine the influence of factors such as the presence of complexing ligands and/or organic matter. The obvious starting point was to look at what affect silver chloro complexes

might play in increasing dissolved Ag concentrations. Thiol (or sulphydryl) groups are also thought to play an important role in increasing the solubility of transition metals. Therefore, in this study we have used 3-mercaptopropionic acid (3-MPA) as a model thiol compound, since many researchers have reported 3-MPA at μM concentrations in porewater samples. To investigate the effect of organic matter on silver-FeS interactions, we have used the well-characterized Suwanee River fulvic acid. We also have explored the role of competing trace metals using Cu as the competing metal. Finally, we have coprecipitated Ag and Fe to see whether any differences in silver solubility could be observed compared to a silver solution interacting with an pre-aged FeS solid.

Experimental

Precipitations

FeS was precipitated from 1 M $Na_2S \cdot 9H_2O$, standardized by the methylene blue colorimetry method, and 1 M $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ at pH 8 and 25°C under purified argon. Solutions were added in equal amounts at a rate of 1.5 ml/min to a 500 ml three-hole round-bottom flask. The system was set up in a similar fashion to that outlined in a recent paper by Arakaki and Morse (1993), using a pH and double-junction reference electrode and autotitrator for maintaining constant pH throughout the precipitation. Ag_2S and a Ag,Fe coprecipitate (molar ratio 1:100) were precipitated using the same procedure, using $AgNO_3$ as the source of Ag.

Characterization of Precipitates

The FeS and Ag_2S precipitates were analysed by x-ray diffraction. The iron sulfide precipitate was found to be amorphous by this techique while the silver sulfide was shown to be ~40-50% acanthite (Figure 1). A K_{sp} of $\sim 10^{-18}$ for the FeS precipitate was determined by MB colorimetric analysis of sulfide and determination of Fe by ICPMS. Sulfide in equilibrium with the Ag_2S precipitate is well below the detection limit of the MB method and so a K_{sp} was not determined. A detailed study of the coprecipitate has not yet been carried out.

Time Studies and Extractions

0.5 g samples of FeS (15% dry/wet) were overlayed with 120 ml Ag solutions (Table 1) in darkened 125 ml teflon bottles. Ten milli-litre samples were taken with 0.2μm millipore syringe filters after ten minutes and one hour of continous stirring under a flow of O_2-free nitrogen. After sampling at one hour, the remaining suspensions were poured into two 50 ml teflon centrifuge tubes and centrifuged at 3000 rpm for 30 minutes. The supernatant was replaced with 50 ml of extractant (H_2O, 0.01 M KNO_3, 0.01 M KCl, or 0.001 M 3-MPA). Samples were rolled for 30 minutes and sampled using the 0.2μm filter. Centrifuge tubes were then recapped, allowing air to fill the headspace above each sample. These samples

were rolled for 30 minutes and left 24 hours before taking a final sample. All samples were acidified with 2 N HNO_3 and analyzed by ICPMS (using standard addition methods to account for differences in matrices between samples).

Table 1 120 ml Silver Solutions used in time studies

20 µg/l $AgNO_3$
1000 µg/l $AgNO_3$
1000 µg/l $AgNO_3$, 0.01 M KCl
20 µg/l $AgNO_3$, 0.001 M 3-MPA
20 µg/l $AgNO_3$, 15 ppm Suwanee River fulvic acid
20 µg/l $AgNO_3$, 80µg/l $Cu(NO_3)_2$

NB 0.5g co-precipitate, 120 ml milli-q H_2O

Results and Conclusions

1. Silver is rapidly removed from solution in the presence of FeS. Figure 2 shows 95% removal for a 20 µg/l Ag solution after only ten minutes. For a solution initially containing 1000 µg/l $AgNO_3$, only 0.5% remains at ten minutes. There is no significant change between ten minutes and one hour. Inspite of this dramatic removal of Ag, the remaining concentration is well above what is predicted by Ag_2S solubility calculations and two orders of magnitude higher than what is typically measured in nature waters.

2. The addition of complexing ligands, Cl^- and 3-MPA, result in lower filtered Ag concentrations than for all other conditions (Figure 3). The presence of Cu as a competing trace metal results in the highest Ag solubility. In these systems, AgCl does not precipitate and there is excess sulfide under all conditions.

3. Extractions performed on the FeS after exposure to a 20 µg/l $AgNO_3$ solution, produce a result that is contrary to expected effects of mobilizing ligands (Figure 4). In fact 3-MPA, which forms a stronger complex with Ag than chloride, extracted the smallest amount of silver from the FeS solid. 0.01 M KCl extractions on the conditioned FeS solids (Figure 5), produced results supporting the findings from the time study. The exceptions for this were the Cu-containing solution, which went from displaying the highest solubility to the lowest extractable Ag, and the thiol-containing solution, which produced the second highest extractable Ag.

4. Figure 6 shows the results of exposure of FeS suspensions to oxidizing conditions. While silver is certainly mobilized due to the oxidation of the FeS, there continues to be a suppression effect by the KCl even after significant oxidation of the FeS.

Discussion

Elevated silver concentrations in the time study compared with Ag_2S solubility predictions and field study measurements may be explained by the very fine-grained nature of the FeS precipitates. Estimations of the grain size from the x-ray diffraction data point towards colloidal particles of < 0.1 μm. This would result in substantial contributions from colloidal Ag in 0.2μm filtered samples. The apparent decrease in "soluble" Ag in the presence of chloride may be explained by this observation. Charge neutralization at the surface of the FeS particles would result in coagulation of particles and therefore a reduction of colloidal material passing through the 0.2 μm filter. The effect of 3-MPA on measured Ag concentrations is less certain, but could possibly be explained by similar particle size effects. It appears that this colloidal fraction of Ag makes up such a large portion of the total dissolved Ag that it far outweighs any effect due to the presence of complexing ligands.

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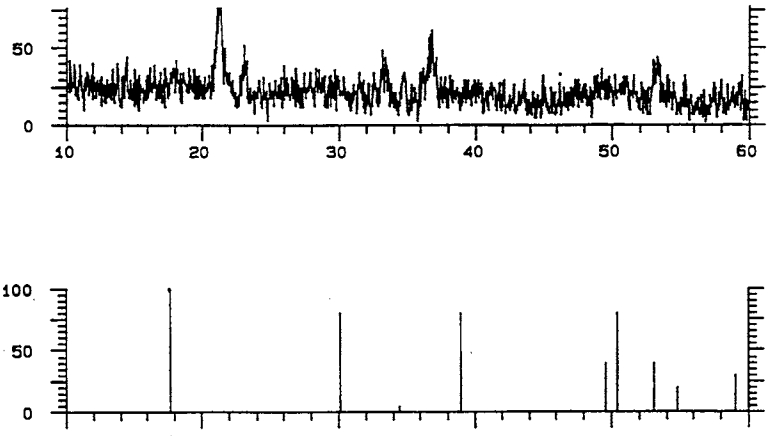

Figure 1a. XRD spectrum of FeS precipitate compared to Mackinawite standard

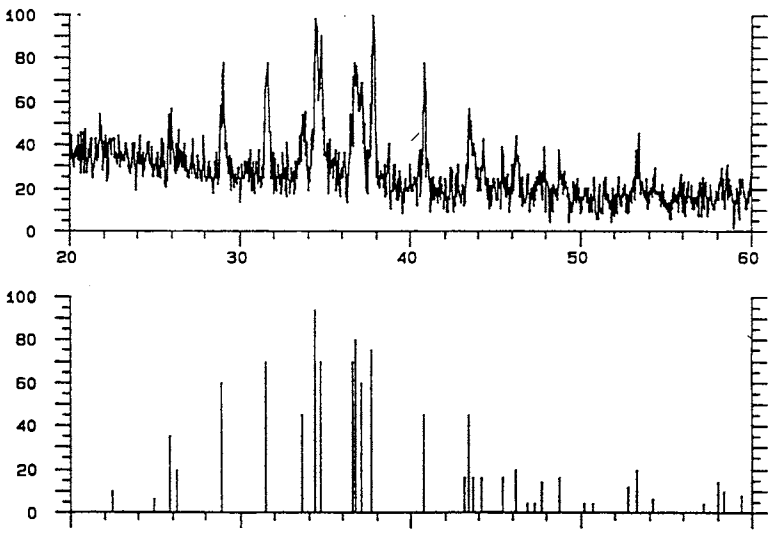

Figure 1b. XRD spectrum of Ag_2S precipitate compared to Acanthite standard

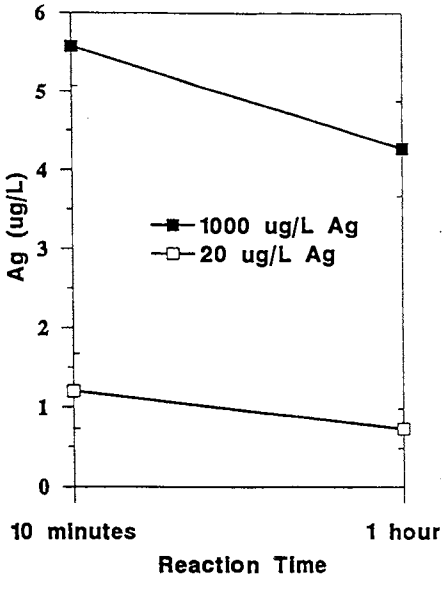

Figure 2. Removal of Ag(I) for $AgNO_3$ solutions in contact with FeS

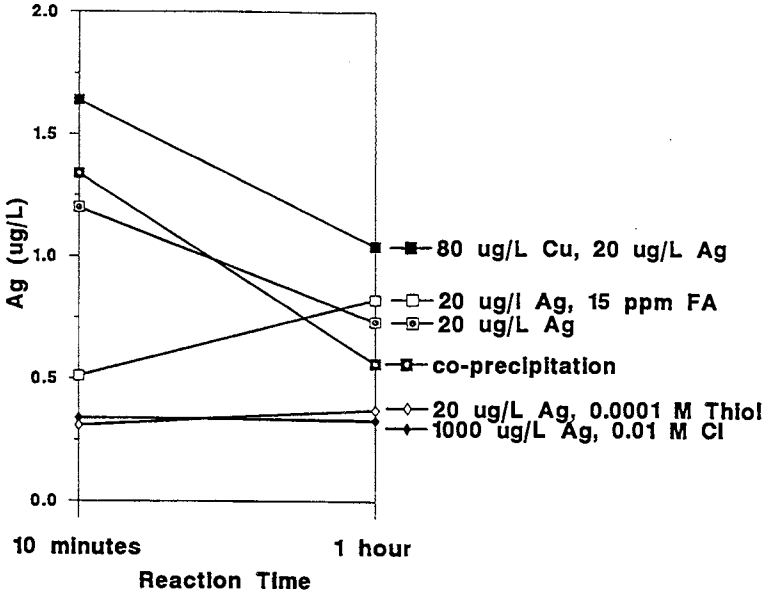

Figure 3. Silver solutions in contact with FeS

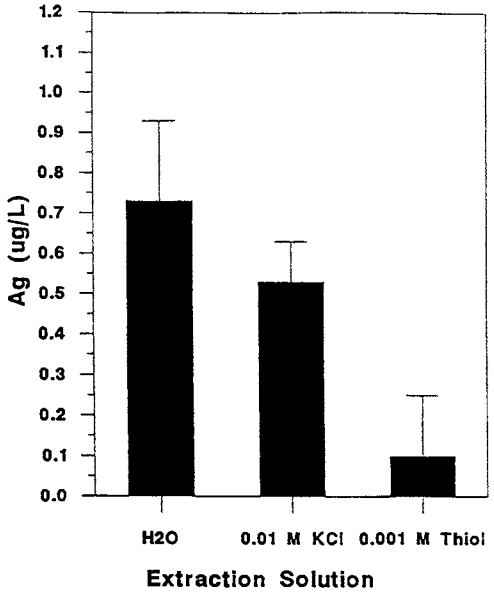

Figure 4. Extractions of 1 ppm silver in FeS

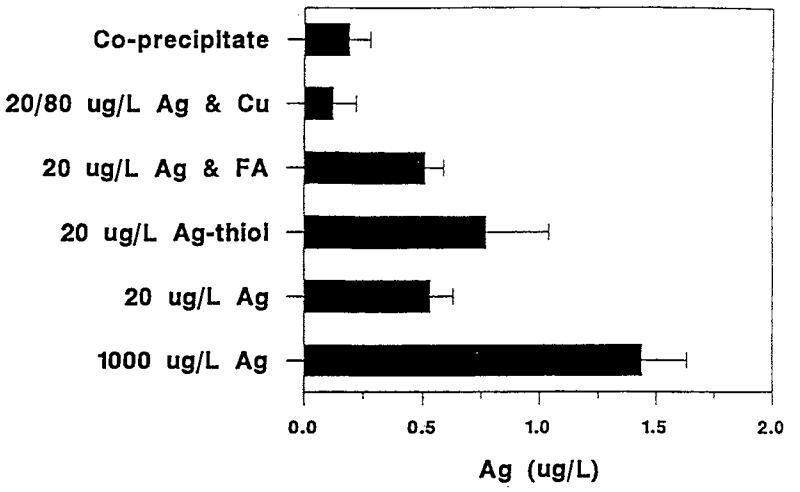

Figure 5. 0.01 M KCl extractions of silver from FeS solids

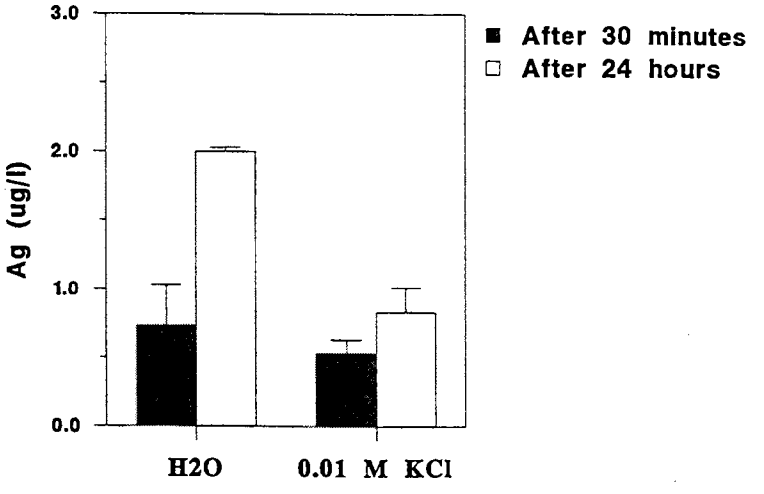

Figure 6. Silver remobilizaion due to low level exposure to oxygen

Questions & Answers: Silver Binding to Mixed Metal Sulfides

Tape malfunctioned during the questions & answers session.

AG ARGENTUM
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Session 2

A. Sodergren
Session Chair

The Toxicity of Silver in Fresh and Marine Waters

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University of Kentucky, Lexington, Kentucky, USA

In Fig. 1, we have summarized the results of approximately 50 studies to show the approximate ranges reported for acute and chronic toxicity of $AgNO_3$ to aquatic animals in freshwater and in seawater. The summary includes data from both the peer review and non-peer review literature, as well as a number of unpublished studies. The summary is both selective and critical, and reflects our opinion on certain issues. For example, we have interpreted tests where the endpoints were negative effects on growth, development or gametes as more indicative of chronic than acute toxicity. Furthermore, by simply lumping all freshwater and all seawater data into two groups, we are ignoring the important influence of water chemistry variation within each environment (see below) on toxicity. This summary should therefore be viewed as broadly indicative, rather than quantitatively precise. Nevertheless, several conclusions are clear. First, silver (tested as $AgNO_3$) is far less toxic in seawater than in freshwater, on both an acute and chronic basis. Second, each acute and chronic range is greater than one order of magnitude, and up to two orders, attesting to variation from both test water chemistry differences and interspecific differences in sensitivity. As a result, there is considerable overlap of the chronic and acute ranges, especially in freshwater. Third, while the relationship between current U.S. EPA criteria (1980) for silver in freshwater and actual toxicity is problematical (see below), the present U.S. EPA acute criterion for seawater (2.3 $ug.l^{-1}$) would appear well chosen to protect against chronic as well as acute toxicity to most marine organisms.

In freshwater, there is currently no chronic EPA criterion, while the acute criterion is allowed to vary solely as a function of water hardness, expressed in $CaCO_3$ equivalents, as illustrated in Fig. 2. Earlier (Galvez and Wood, 1994), we have questioned the validity of this relationship based on literature data, speciation modelling, and our own research on juvenile rainbow trout which indicates that Cl^-, rather than Ca^{2+} ("hardness") is the true protective agent. Fig. 2, using LC50 data from both trout and fathead minnow (the latter presented by Brooke *et al.*, 1994) suggests that the EPA "hardness" equation places undue reliance on hardness, such that it is underprotective at high hardness levels yet unnecessarily conservative at low hardness levels. We suggest that there is a need to critically re-examine the relationship, and to incorporate other variables such as Cl^- and possibly DOC concentrations.

Our physiological research on silver toxicity to freshwater fish (rainbow trout) has identified negative effects on ionoregulation as the key toxic action (Wood *et al.*, 1993, 1994). Despite living in an ion-poor environment, freshwater animals maintain major electrolytes such

as Na^+ and Cl^- in their body fluids at levels similar to those of mammals. This is accomplished by powerful transport systems on the gills which actively transport electrolytes from the dilute water to the concentrated blood plasma, thereby compensating for the simultaneous passive losses of ions to the water by simple diffusion. The acute toxicity of Ag (at levels close to the 7 day LC50 - *eg.* 10 µg/l as $AgNO_3$) to freshwater fish appears entirely due to the presence of free ionic Ag^+; the presence of excess anionic ligands such as chloride and thiosulphate essentially eliminates acute toxicity. Acute toxicity of Ag^+ is manifested as a progressive fall in plasma Na^+ and Cl^- levels, which in turn induces internal shifts in fluid volume distribution, circulatory failure, and death. Our recent experiments demonstrate that these effects are due almost entirely to a highly potent blockade of the active uptake mechanisms for Na^+ and Cl^- at the gills; effects on passive diffusive loss rates are minimal (Morgan *et al.*, 1995). Qualitatively similar effects on ionic regulation are seen in fish chronically exposed to much lower levels of $AgNO_3$ (Fig. 3). Ag also readily enters the fish in these exposures and accumulates in the liver, but a potent induction of metallothionein appears to serve as an internal detoxification mechanism (Hogstrand *et al.*, 1993; Galvez *et al.*, 1995).

Despite living in an ion-rich environment, seawater animals maintain Na^+ and Cl^- in their body fluids at levels very close to those of freshwater animals. In marine fish, this is accomplished by actively pumping Na^+ and Cl^- out of the blood plasma into the concentrated seawater, thereby compensating for the simultaneous passive uptake of these electrolytes from the water by simple diffusion - essentially the mirror image of the situation for freshwater fish. In addition, marine fish incur an additional Na^+ and Cl^- burden by continually drinking seawater to compensate for osmotic losses of H_2O across the gills. The acute toxicity of $AgNO_3$ in seawater varies with chlorinity, but in general is 1 to 2 orders of magnitude lower than in freshwater (Fig. 1; see also Ferguson *et al.*, 1995). This difference would be anticipated given the fact that the concentration of free Ag^+ (the toxic moiety in freshwater) is negligible due to the presence of high levels of chloride in seawater; negatively charged Ag-chloro-complexes predominate in solution, and their relative contributions vary with chlorinity. Nevertheless, higher levels of $AgNO_3$ are toxic in seawater despite the absence of Ag^+, and field and laboratory studies on invertebrates indicate that Ag-chloro-complexes are bioavailable (Luoma, 1994).

At present, almost nothing is known about the mechanism of Ag toxicity to fish in seawater. Therefore using starry flounder exposed to 250 $\mu g.l^{-1}$ Ag (added as $AgNO_3$) in full strength seawater for 6 days, we recently investigated whether ionoregulatory disturbance occurs (Hogstrand *et al.*, 1995) - *ie.* by analogy to the freshwater situation, a progressive increase in plasma Na^+ and Cl^- levels might be expected. A range of other blood and tissue parameters similar to those of our freshwater studies were also monitored. Plasma Na^+ and Cl^- levels were not affected, suggesting that the toxic mechanism differs from that in freshwater. Other parameters (blood gases, acid-base status, hematology) also exhibited negligible response. However, plasma ammonia levels increased markedly and a substantial uptake of Ag into internal organs occurred, especially the liver. Mortality did not occur. More work will be required to evaluate the significance of these findings in terms of toxicity.

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Figure 1. Approximate ranges of acute and chronic toxicity reported for total silver, added as silver nitrate, in freshwater and seawater. The current U.S. EPA (1980) water quality criterion for acute toxicity in seawater is indicated.

Figure 2. A comparison of the current U.S. EPA (1980) acute criterion for total silver in freshwater, as calculated by the "hardness" equation, with 4 day LC 50 values determined for fathead minnow (from Brooke *et al.*, 1994) and rainbow trout as a function of water hardness. Silver was added as silver nitrate in these tests, and hardness is expressed in calcium carbonate equivalents.

Figure 3. The influence of chronic exposure to 2.0 $\mu g.l^{-1}$ of total silver, added to freshwater as silver nitrate on a flow-through basis, on plasma sodium and chloride regulation in juvenile rainbow trout. Means $\pm$ 1 SEM (N = 9 - 10 at each point). Asterisks indicate significant differences ($P < 0.05$).

Toxicity of $AgNO_3$ in Freshwater and Seawater

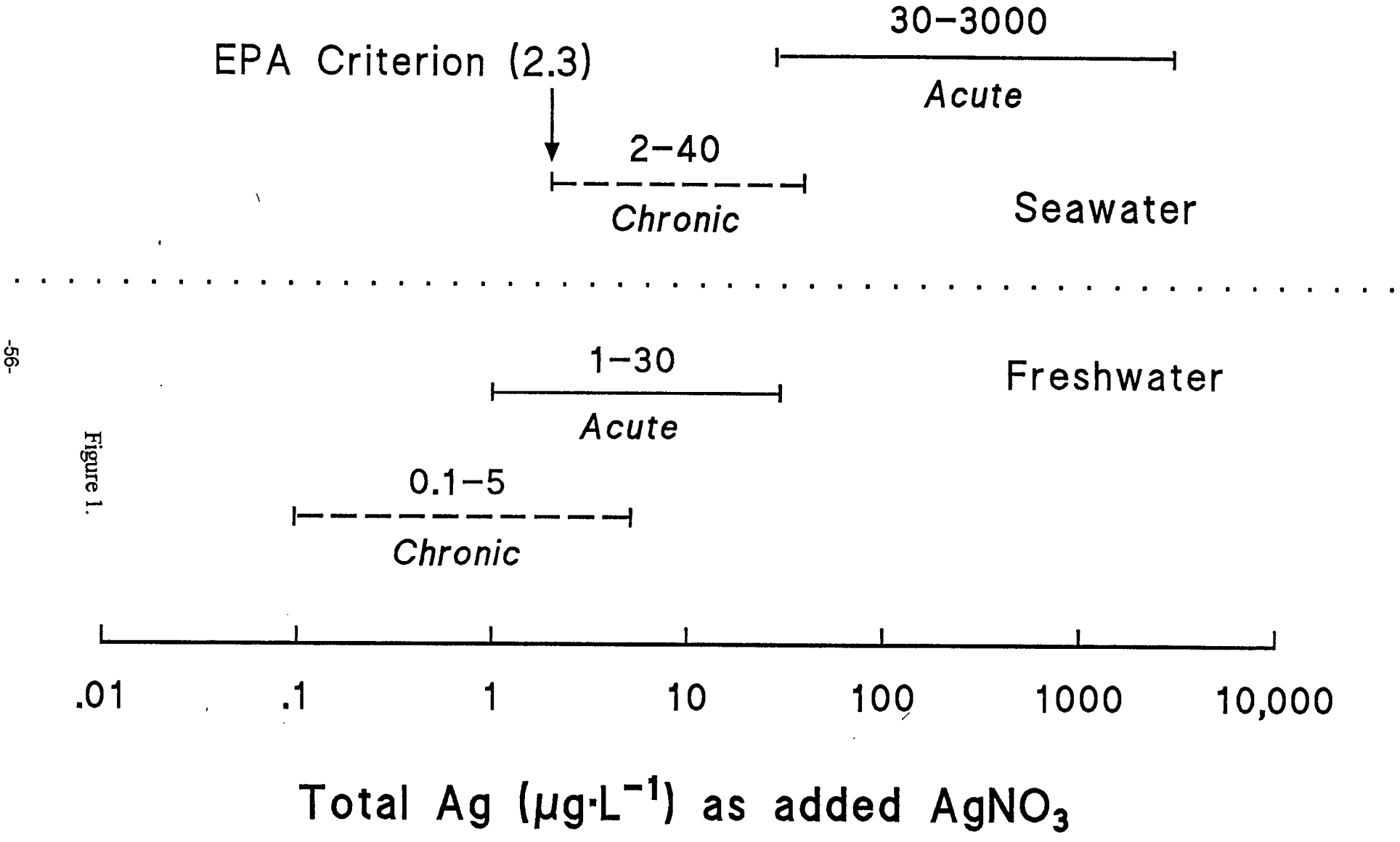

Figure 1.

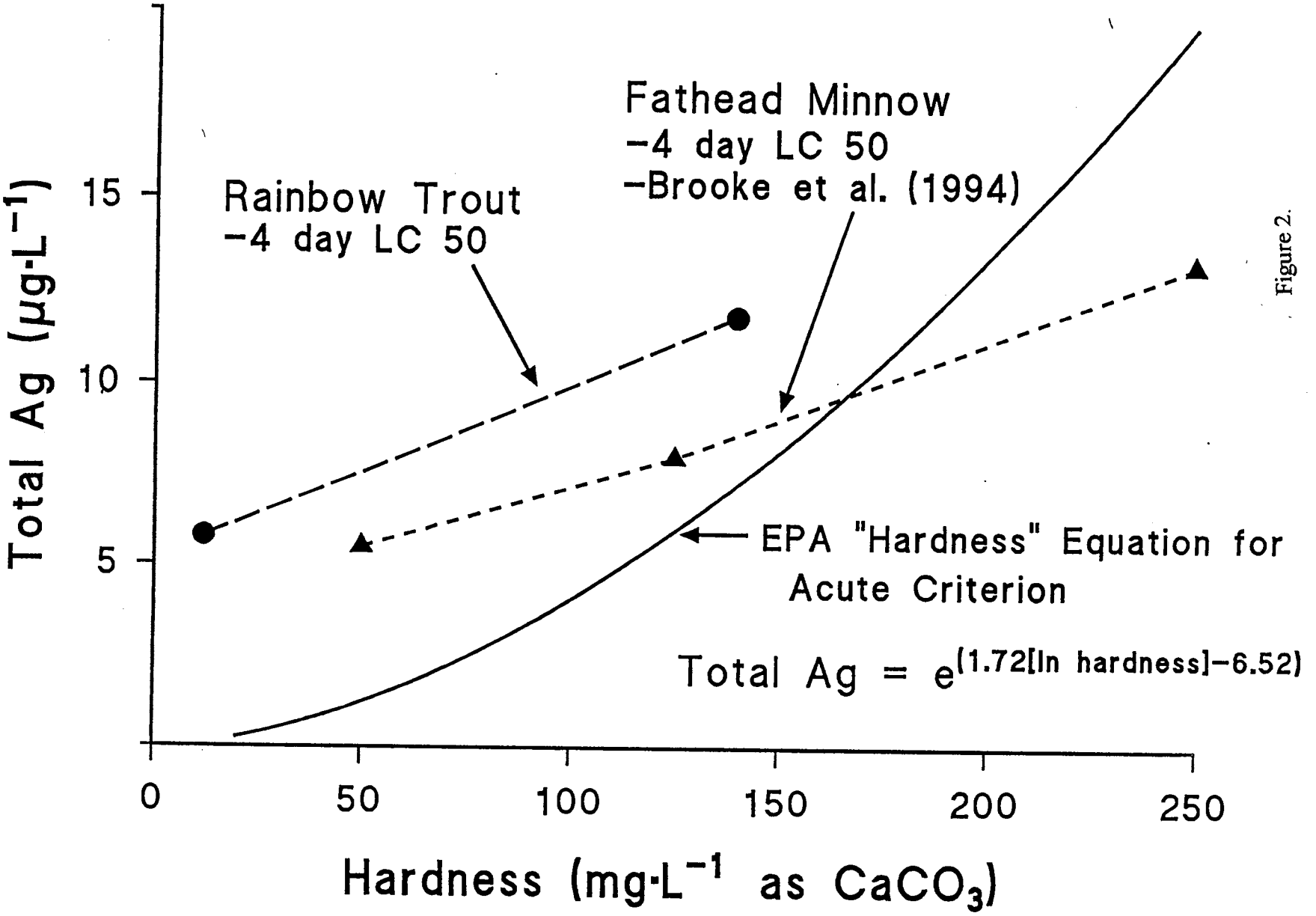
Fathead Minnow
-4 day LC 50
-Brooke et al. (1994)
Rainbow Trout
-4 day LC 50
EPA "Hardness" Equation for
Acute Criterion
Total Ag = e(1.72[ln hardness]-6.52)
Total Ag (µg·L-1)
15
10
5
0
50
100
150
200
250
Hardness (mg·L-1 as CaCO3)

Figure 2.

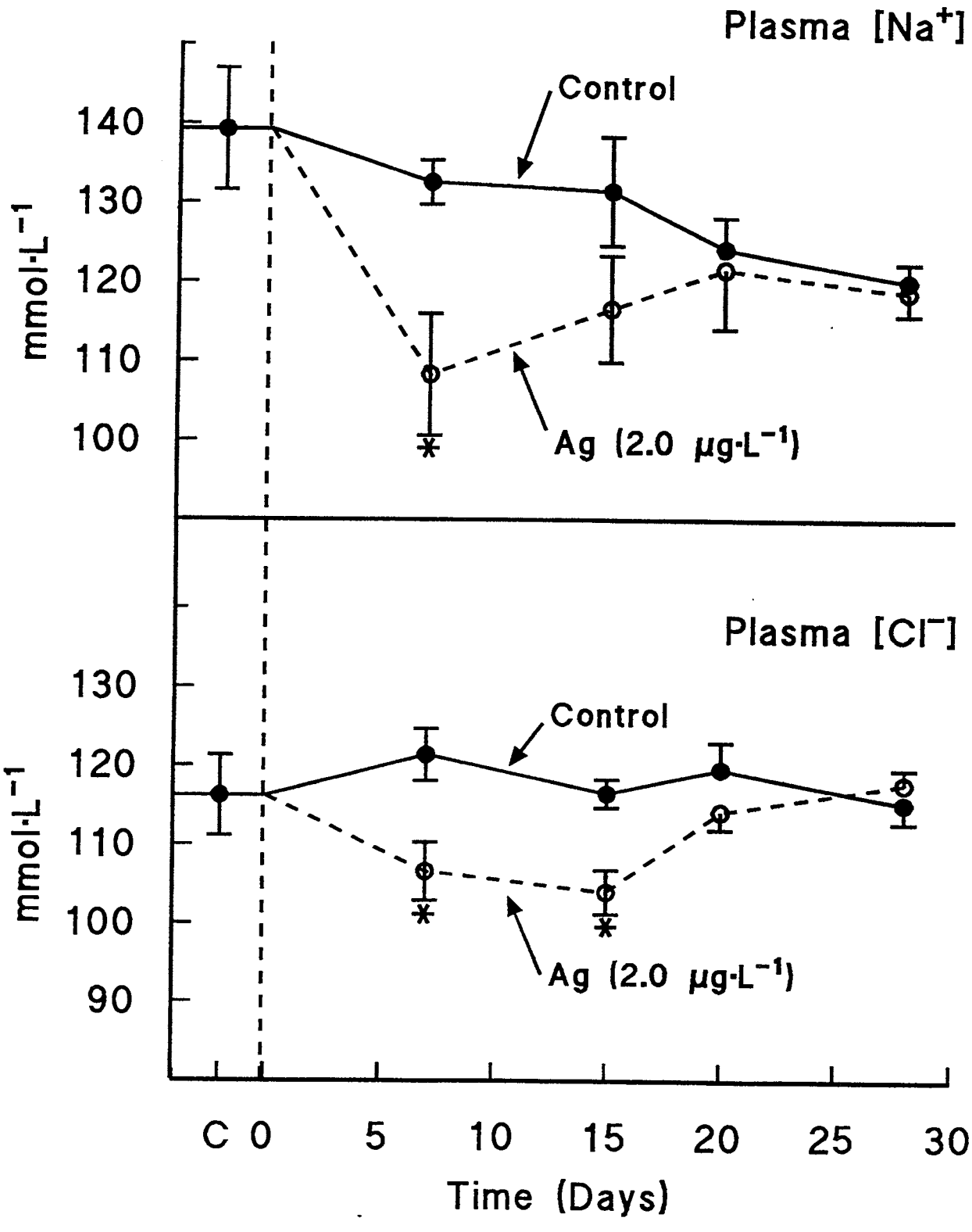
Plasma [Na+]
Control
Ag (2.0 µg·L-1)
mmol·L-1
140
130
120
110
100
Plasma [Cl-]
Control
Ag (2.0 µg·L-1)
mmol·L-1
130
120
110
100
90
C 0
5
10
15
20
25
30
Time (Days)

Figure 3.

Questions & Answers: The Toxicity of Silver in Fresh and Marine Waters

Q. NICK FISHER (SUNY-Stony Brook): You have nicely shown the potential impact of dissolved silver to marine and freshwater fish. However, fish may well be exposed to silver through ingestion of contaminated food. Have you examined the toxicity of ingested silver to fish?

A. It's a good question. Almost nothing is known about this. As silver may end up in sediments, and, thereby, enter the food chain via benthic detritivores, it should be looked at. So far, we've made several attempts to generate silver-contaminated food for freshwater rainbow trout. We've learned that it's not as easy as it sounds — you can't just grind silver salts into the food. In nature, the silver would be biologically incorporated into prey organisms. We've now succeeded in getting biologically incorporated silver by exposing fish to high levels of waterborne silver thiosulfate, which readily enters, and then using these fish as the basis of a pellet diet for trout. We hope to do a preliminary experiment feeding this diet to freshwater rainbow trout over the next couple of months.

Another aspect to this is the fact that seawater fish drink the seawater, and this could be a significant route of uptake. Recently, Christer Hogstrand and I found that marine starry flounder exposed to waterborne silver has high levels of silver in the wall of the intestine.

Q. NICK ADAMS (McMaster Univ., Hamilton): Is there any way you could separate uptake through the gills from uptake through the gut in the seawater fish, perhaps by using a divided chamber?

A. It would be difficult because drinking in seawater fish is known to be stimulated by stress, and these sorts of approaches can be quite stressful.

Q. ARUN MUKHERJEE (Univ. of Helsinki): Do you have any information on "safe" levels of silver in food?

A. Well, as I said, almost nothing is known in fish. In mammals, I think a fair amount of studies have been done feeding various silver salts into the stomach. My impression is that the overall conclusion is that orally ingested silver is not very toxic relative to many other metals. I'm sure Daland Juberg could answer your question better than I — perhaps we'll hear more information on this issue in his upcoming talk.

Q. JOHN MAHONY (Manhattan College, NY): Have you done any speciation calculations to determine the free silver ion concentration in the silver thiosulfate exposures to compare with that in the silver nitrate exposures?

A. Yes, we have. In the silver thiosulfate exposures, the concentration of free silver ion is vanishingly small — several orders of magnitude lower than in the silver nitrate exposure, despite the vast amount of total silver present.

Q. (unidentified): Does silver ion have the same effects on ion regulation at lower, more environmentally realistic concentrations?

A. We think so. We've done chronic exposures at concentrations as low as 0.5 µg/L total silver (added as silver nitrate to the water) and we have seen sublethal effects on plasma sodium and chloride levels in juvenile freshwater rainbow trout.

Silver, Copper, Cadmium, Dissolved Organic Carbon and Fish

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Environment Canada, Burlington, Ontario, Canada

Previous work of ours has shown that dissolved organic carbon (DOC) protects against silver, lead, copper, cadmium, and cobalt binding to gills of fish. A ligand exchange method, which uses chemicals of known metal binding ability, has been used by us to calculate conditional metal-gill and metal-DOC binding constants for these metals (Janes and Playle 1995; Playle et al. 1993a,b; Richards and Playle unpublished; Kuehn and Playle unpublished).

Thiosulphate ($S_2O_3^{=}$) binds Ag with a conditional equilibrium binding constant of log $K_{Ag\text{-}S2O3}$ = 8.8. In experiments with $S_2O_3^{=}$, Ag, and rainbow trout (*Oncorhynchus mykiss*, 1-3 g), the Ag-gill binding constant was calculated as log $K_{Ag\text{-}gill}$ = 10.0, and the Ag-DOC binding constant was log $K_{Ag\text{-}DOC}$ = 9.0 (Janes and Playle 1995). Sensitivity analyses using these values, plus binding constants for Ca, Na, and H^+ interactions at Ag binding sites on fish gills, suggest that DOC concentration is a critical factor in determining Ag binding at fish gills. Sensitivity analyses were run after inserting the experimentally determined equilibrium binding constants into an aquatic chemisty program, MINEQL$^+$ (Schecher and McAvoy 1992).

Equilibrium binding constants for Cu- and Cd-gill interactions are log $K_{Cu\text{-}gill}$ = 7.4 and log $K_{Cd\text{-}gill}$ = 8.6 (Playle et al. 1993a, b). Binding constants for Cu- and Cd-DOC interactions are log $K_{Cu\text{-}DOC}$ = 9.1 and log $K_{Cd\text{-}DOC}$ = 7.4 (Playle et al. 1993b). From the differences between the respective Ag, Cu, and Cd-gill and Ag, Cu, and Cd-DOC binding constants, DOC protects best against Cu accumulation on fish gills, gives intermediate protection against Ag, and protects least well against Cd accumulation on gills.

We have investigated whether source of the DOC or age of the metal-DOC complex influences metal accumulation on fish gills. Playle et al. (1993a) demonstrated that Cu was kept off fish gills at DOC concentrations $\geq$ 4.8 mg C·L^{-1} DOC, independent of source or size fraction of the DOC. Current work of ours has shown that freshly mixed Cu (0.5 μM) and DOC (5 mg C·L^{-1}) solutions keep Cu off trout gills as well as Cu-DOC solutions mixed two weeks earlier. Neither fresh nor aged Cd-DOC mixtures kept Cd (0.15 μM) off trout gills in our exposures.

Although the protective effects of dissolved organic carbon against metal toxicity are well established, the possibility of physiological effects of DOC itself on

fish has not been investigated. Our approach is to cannulate adult rainbow trout (~300 g) to allow repetitive blood sampling during week long exposures in flowing soft water, in the presence or absence of 5 mg $C \cdot L^{-1}$ DOC with and without an added metal mixture. The DOC was concentrated from Luther Marsh, near Grand Valley, Ontario, by reverse osmosis, and was passed through a cation exchange resin to remove Cu contamination of the DOC. The test metal mixture was about 0.3 μM Cu and 0.06 μM Cd, and was used to assess the protective effects of DOC against losses of Na (effects of Cu and probably Ag) and Ca (effect of Cd) from trout.

Results to date indicate no physiological effects of 5 mg $C \cdot L^{-1}$ DOC on cannulated rainbow trout. The DOC reduced Cu binding to trout gills and may have reduced Cu entry into fish plasma, but did not reduce Cd binding to the gills. Using higher concentrations of DOC (0, 20, and 40 mg $C \cdot L^{-1}$), Cu (0.7 μM), and Cd (0.15 μM) in static renewal exposures, we demonstrated reduced metal toxicity in the presence of DOC. Once again, Cd was not kept off the gills but Cu was, and there were no adverse effects of even these very high DOC concentrations.

In summary, DOC is able to keep metals such as Ag, Cu, and Cd off fish gills, if in high enough concentration relative to the metal. The age of metal-DOC complexes and DOC source or size fraction are not important in determining protective effects of DOC against metal binding at fish gills. No physiological effects of DOC alone were observed. That is, DOC appears solely protective in its actions. Specific questions regarding protective effects of DOC against Ag accumulation of fish gills, and resultant toxicity, still need to be addressed.

This work was funded by grants to R. Playle from the Canadian Network of Toxicology Centres and from the Natural Sciences and Engineering Research Council of Canada.

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Questions & Answers: Silver, Copper, Cadmium, Dissolved Organic Carbon and Fish

(Initial part of Q&A not recorded due to tape failure.)

Q. ANDERS ANDREN (Univ. of Wisconsin): Ken Bruland has worked with copper a lot. He has titrated copper with dissolved organic carbon (DOC) in seawater, and what you find is that dissolved organic matter possesses a distribution of complexation sites. If you really were to be very careful, what you're looking at is some sort of average complexation site. Similar observations have been made for solid surfaces. What that tells us then is that the stability constant is very much concentration dependent for silver as well as for other cations. Have you done titration of silver versus your DOC to look at the complexation capacity — wouldn't that be a much better way of doing it than increasing the DOC?

A. I see your point. It could be, but I guess what I wanted was a practical answer that applies to the concentrations of DOC that are realistic.

Q. I think my point is that the quantity of DOC is not that important. What is important is the complexation sites on the DOC, which will vary, so that it's probably much more important to know the complexation capacity of the DOC. If you can have a relationship between that and total DOC that would be nice.

A. Okay, I might still not be getting the point but these are high affinity sites that we're seeing on the DOC. If you pack more and more silver, or copper, onto the DOC, that equilibrium binding constant between the metal and the DOC would get lower and lower. But again, what I'm trying to look at is useful values for relatively common DOC concentrations and again working with that relatively low concentration of silver.

Q. IAN MORGAN (McMaster Univ.): I would imagine that metal binding sites on the gills have a number of types, some of which might exhibit toxicity and some might not. My question has two parts: One is, is there any correlation between strength of binding and toxicity, and secondly, is it possible to use connected binding studies to differentiate between metal binding sites that might exhibit toxic effects and those which don't?

A. My answer to the first question is, yes, there is a correlation between strength in binding and toxicity. For example, using my data cobalt is not very toxic at all and had the lowest value; cadmium is pretty toxic, it has a pretty high value. There is a direct correlation. The second question was?

Q. Whether you could differentiate between general binding sites on the gills and those which, in a way, are directly related with metal physiology.

A. Well, that would be the ultimate goal. Some people have done it with radio tracers, for example, cadmium. They tried it for mercury, and we should do it for silver, too.

Q. GABOURY BENOIT (Univ. of Connecticut): I have an open question to you and anybody else at the conference. If you look at the stability constants for the common functional groups that attribute to the effect of binding by DOC, the carboxyl and phenolic groups, you expect them to be much stronger for other metals than silver, whereas in your data and other data we see silver is bound quite effectively by dissolved organic carbon. I'm wondering whether the effect might be attributed to the small amount of sulfur that's included in the DOC or exactly to what kind of sites of interaction with the sulfur in the DOC?

A. Good question. As with carboxylic and phenolic groups, silver could easily be interactive with sulfuric groups. That sort of goes along with Anders' question because when you titrate it you get, for example, sulfido groups. Once you fill those up then you go to phenolic groups, probably, and then you saturate all of the carboxyl groups. So that might be a way of approaching it.

Q. JIM KRAMER (McMaster Univ.): I have a slightly different question. First of all, the Luther-March DOC work, it does have a lot of sulfur in it. Sort of along the line of Anders' question, which you already commented on, is what the nature of the sites on the gills are. I'd like to make a comment. I just happen to have some metal-sulfide stability constants. Except for copper, which is completely anomalous, they are quantitatively the sequence you've showed us. Do you have any thoughts about the nature of the binding to the gill?

A. It's probably not carboxyl groups because they would be too weak for binding. They are probably quite likely sulfido groups. If silver is taken up through sodium channels there'll be some kinds of sulfido groups on the inside of the channel, like on some proteins. So it might be very difficult to get ahold of those.

Q. FERNANDO GALVEZ (McMaster Univ.): This is a comment to what Ian actually asked. We did some work a couple of years back and we showed that high concentrations of calcium did not affect zinc binding. However, it is known that calcium does affect zinc uptake and also affects zinc toxicity; the higher the calcium concentrations the lower is the zinc toxicity. With the chloride stuff we did last year, we showed when you increase chloride you decrease silver toxicity. Could you comment on that?

A. Well, as far as chloride and silver are concerned — in spite of what I might have said — certainly, yes, when you put chloride in the water you will protect against silver toxicity to some degree. Though it won't protect as well as you might think if you just look at the water chemistry because you've got to account for the relative complexation strength of chloride and silver, and the gills and silver. The other question I think I can answer by analogy to cadmium. We've done calcium work with cadmium and we can reduce the toxicity of cadmium with calcium, but calcium does not keep cadmium off the gills very well. The reason the toxicity is reduced is because the electrochemical gradient for calcium to leak out of the fish is reduced if you increase the calcium. There's not that much calcium in the fish — it's about 2 mmol — so you don't have to increase the calcium in the fish and the calcium in the water too much before you can protect against hypocalcimia that's caused by cadmium. So that might have something to do with the zinc, too. I'm not sure exactly if zinc is toxic like that.

Influence of Water Quality Parameters on Silver Toxicity: Preliminary Result

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INTRODUCTION

The fate and effects of silver in aquatic ecosystems has been of concern to the silver industry and to environmental regulatory agencies. Water quality criteria for metals in general and silver in particular have been generated using a water hardness based model (45 F.R. 79318, November 28, 1980). Using this model, water quality criteria for silver ranges from 1.2 ug/L at 50 mg/L hardness (as mg CaCO3) to 13.0 ug/L at 200 mg/L hardness. Unfortunately, water hardness is not the only water quality characteristic known to play a critical role in metal bioavailability. It is, however, the only parameter around which a sufficient database has been generated in order to facilitate its use in determining water quality criteria.

Other water quality characteristics of interest include chloride concentrations, dissolved organic carbon (DOC), alkalinity and pH. This research was initiated in order to generate a data set that accurately reflected the influence of both single and combinations of water quality parameters. The water quality charateristics examined in this research included hardness, DOC and chloride. The ultimate goal of this research was to generate a response surface for *Daphnia magna* (Water Fleas) and *Pimephales promelas* (Fathead Minnows) exposed to silver in waters containing ranges of hardness, DOC, and chloride. This paper presents results of range-finding tests that were critical in determining the final experimental design for both species. In addition, the first half of the definitive bioassay results with *P. promelas* are presented and preliminary conclusions developed.

METHODS

All research presented was conducted under Good Laboratory Practices and all phases have been audited by the Quality Assurance Unit of The Institute of Wildlife and Environmental Toxicology (TIWET), Clemson University.

Organism Culture. *D. magna* were cultured in our laboratory in reconstituted, moderately hard water following proceedures described by USEPA (EPA/600/4-90/027F), "Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms." The water hardness was maintained at 150 mg/L as CaCO3. Organisms were fed a diet of green algae (*Selenastrum capricornutum* at 120,000 cells/ml/day) and yeast and trout chow (20 ug/ml/day). *P. promelas* were obtained from Aquatic Research Organisms, Inc., Hampton, NH. Specimens arrived as <24 h old newly hatched fish. These were maintained in reconstituted, moderately hard water for 4 days prior to initiation of the bioassays.

Bioassay methods. Static, non-renewal *D. magna* bioassays were conducted for 48 hr at 22 C under a 16:8 light: dark photoperiod with a light intensity between 10 and 20 uE/m^2/s. Daphnids and fish were both fed immediately prior to the initiation of the test and were not fed during the bioassay. Each test chamber consisted of a 50 ml polypropylene beaker containing 40 ml test solution and 5 daphnids. There were six replicate chambers per treatment. Endpoint for this bioassay was immobility. Static, non-renewal *P. promelas* bioassays were conducted for 96 hr at 22 C under a 16:8 light: dark photoperiod with a light intensity between 10 and 20 uE/m^2/s. Organisms were fed *Artemia* nauplii prior to the initiation of the bioassay. Each test chamber consisted of a 600 ml polypropylene beaker containing 500 ml test solution and 10 fish. There were three replicate chambers per

treatment. Endpoint for this bioassay was mortality. Reagent grade chemicals were used throughout the bioassays. Aldrich humic acid was used as the source of dissolved organic carbon.

Experimental design. Several range-finding tests were conducted with each organism. The outcome of these tests was an experimental design that would encompass not only the values of hardness, DOC and chloride of interest, but also contain a wide enough range of silver concentrations to document any toxicity reduction due to water quality changes. The final experimental designs were as follows:

D. magna: hardness, 100, 200 mg/L as CaCO3
chloride, 3, 20, 40, 60 mg/L
DOC, 0, 2, 5, 10 mg/L
silver, 0, 0.5, 1, 2, 3.5, 5 ug/L

P. promelas: hardness, 50, 100, 200 mg/L as CaCO3
chloride, 3, 20, 40, 60 mg/L
DOC, 0, 5, 10 mg/L
silver, 0, 2, 5, 10, 20, 40 ug/L

A complete factorial design was conducted for each organism and four separate tests were run blocking on chloride. Silver concentrations were confirmed using graphite furnace atomic absorption spetrophotometry; chloride concentrations were determined on an ion chromatograph; and, DOC was measured using a carbon analyzer. All other water quality measurements including pH, alkalinity, and hardness were measured using standard methods.

PRELIMINARY RESULTS

At the time of the Argentium 3rd International Conference (Silver Conference) none of the daphnid definitive bioassays had been completed and only 2 of the 4 fish definitive bioassays were completed. Hence the following is a **very preliminary** presentation of the fish data.

Steep dose-reponse curves were observed for P. promelas exposed to silver under a variety of water quality conditions (Figures 1-4). These data suggest that once the toxicity threshold is achieved, a small increase in silver results in a dramatic increase in mortality. In addition, the difference in response surfaces between 3 and 20 mg/L chloride is not evident (compare Figure 1 with 2 and Figure 3 with 4).

The 96h LC50 values were computed for each treatment (Table 1). The numbers, and the 95% confidence intervals indicate that hardness does not significantly influence silver toxicity over the range used in this study (Figures 5 and 6). The addition of DOC, however, significantly reduced toxicity (Figures 7 and 8).

While these results are preliminary, they suggest that the hardness-based water quality criteria for silver is not appropriate. Other water quality parameters such as chloride and DOC must be considered in the generation of site-specific water quality criteria for silver.

Table 1. Summary of acute toxicity of silver to larval fathead minnows in various water qualities.

Chloride (mg/L)	Hardness (mg/L $CaCO_3$)	DOC (mg/L)	96-hr LC50 (ug/L Ag)	Confidence Interval (CI)
3	50	0	2.76	2.29-3.33
3	100	0	2.38	1.86-3.04
3	200	0	3.12	2.60-3.73
3	50	5	6.25	5.36-7.30
3	100	5	5.10	4.30-6.04
3	200	5	7.38	6.42-8.48
3	50	10	8.88	7.23-10.91
3	100	10	6.42	5.13-8.04
3	200	10	8.94	6.93-11.52
20	50	0	3.42	2.89-4.05
20	100	0	2.13	1.55-2.93
20	200	0	4.70	3.94-5.62
20	50	5	6.91	5.66-8.43
20	100	5	6.28	5.13-7.70
20	200	5	7.05	5.74-8.67
20	50	10	8.50	7.12-10.14
20	100	10	8.13	6.73-9.82
20	200	10	8.05	6.82-9.51

Figure 1. Effects of DOC on Silver Toxicity to Larval Fathead Minnows (Hardness = 50 mg/L, Chloride = 3 mg/L)

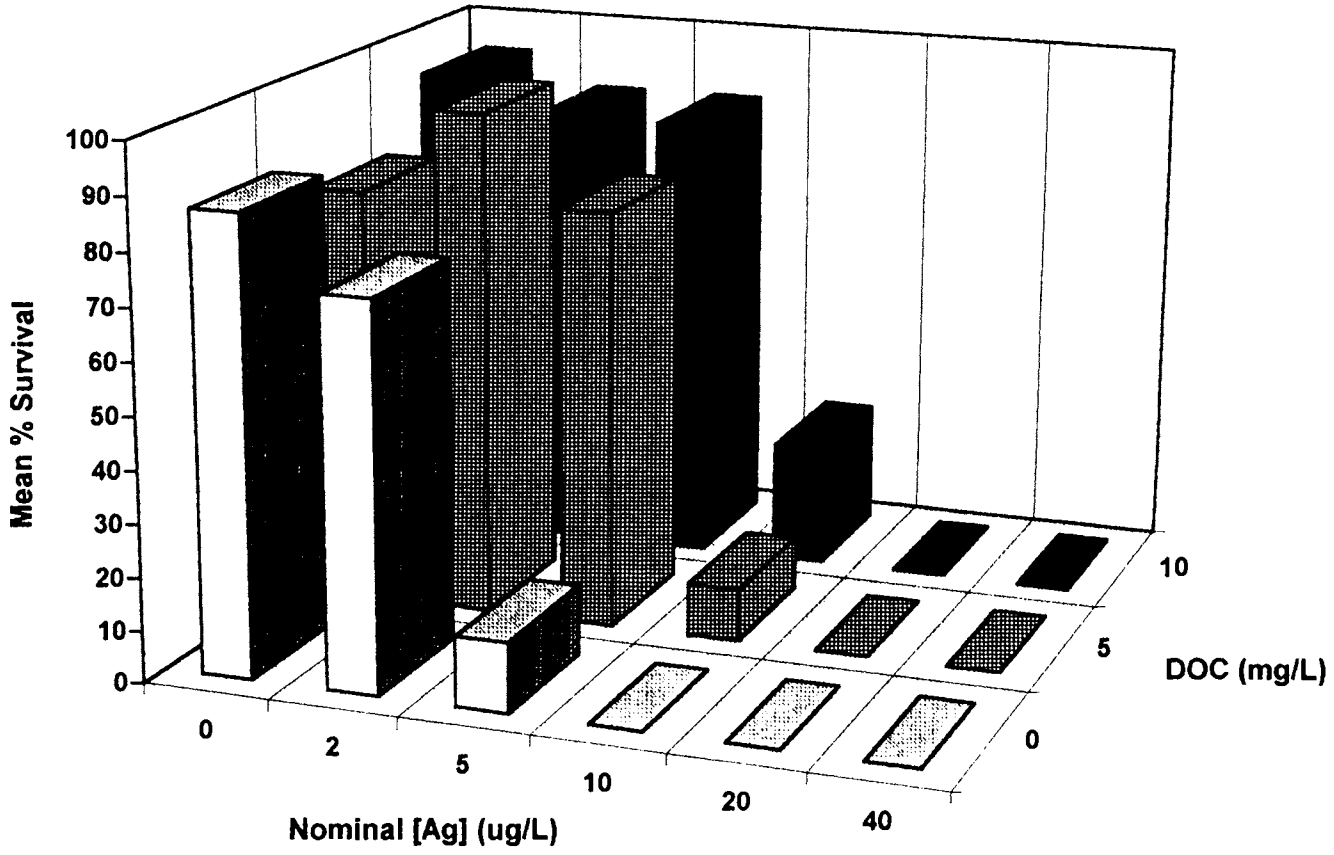


Figure 2. Effects of Hardness on Silver Toxicity to Larval Fathead Minnows (DOC = 0 mg/L, Chloride = 3 mg/L)

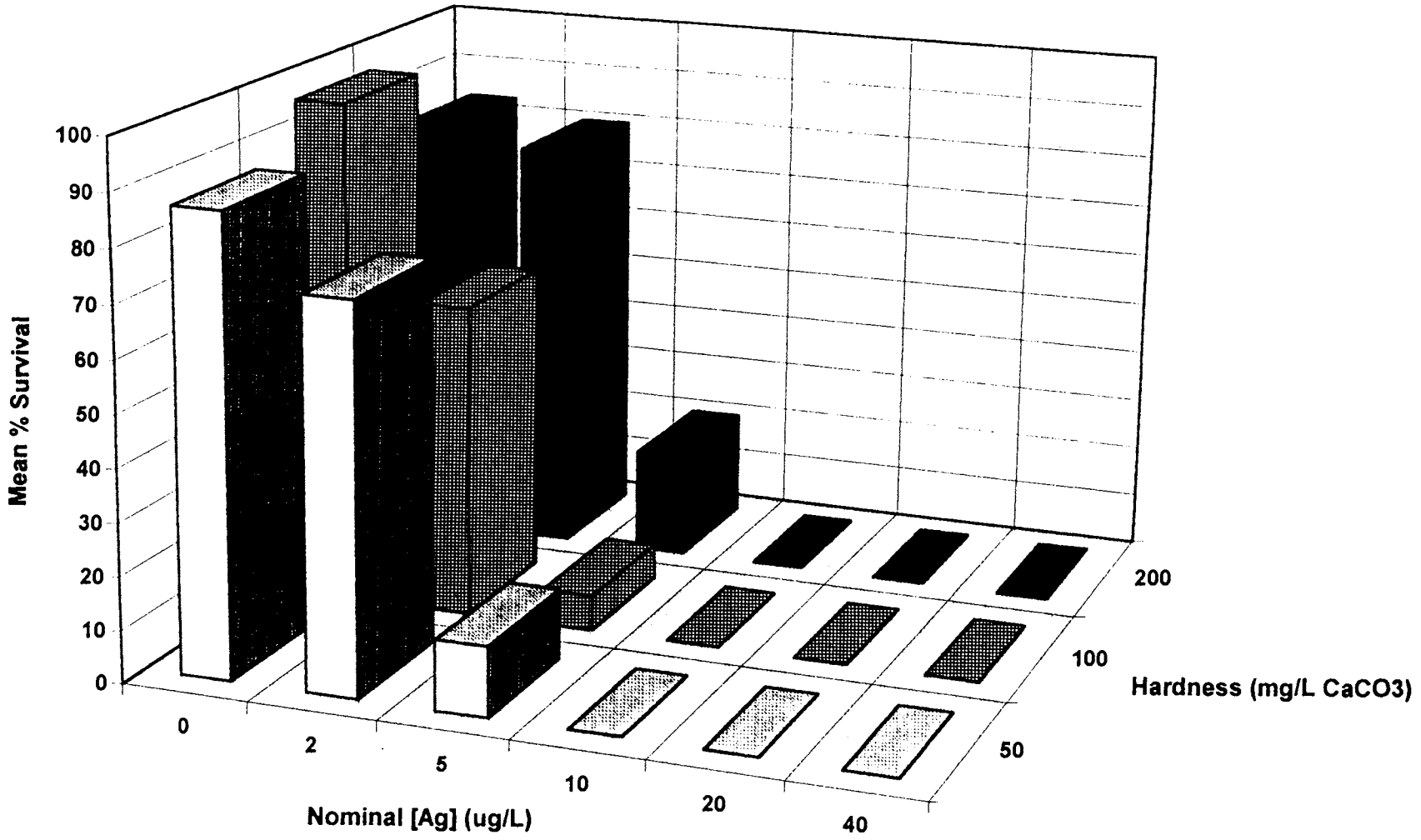


Figure 4. Effects of Hardness on Silver Toxicity to Larval Fathead Minnows (DOC = 10 mg/L, Chloride = 20 mg/L)

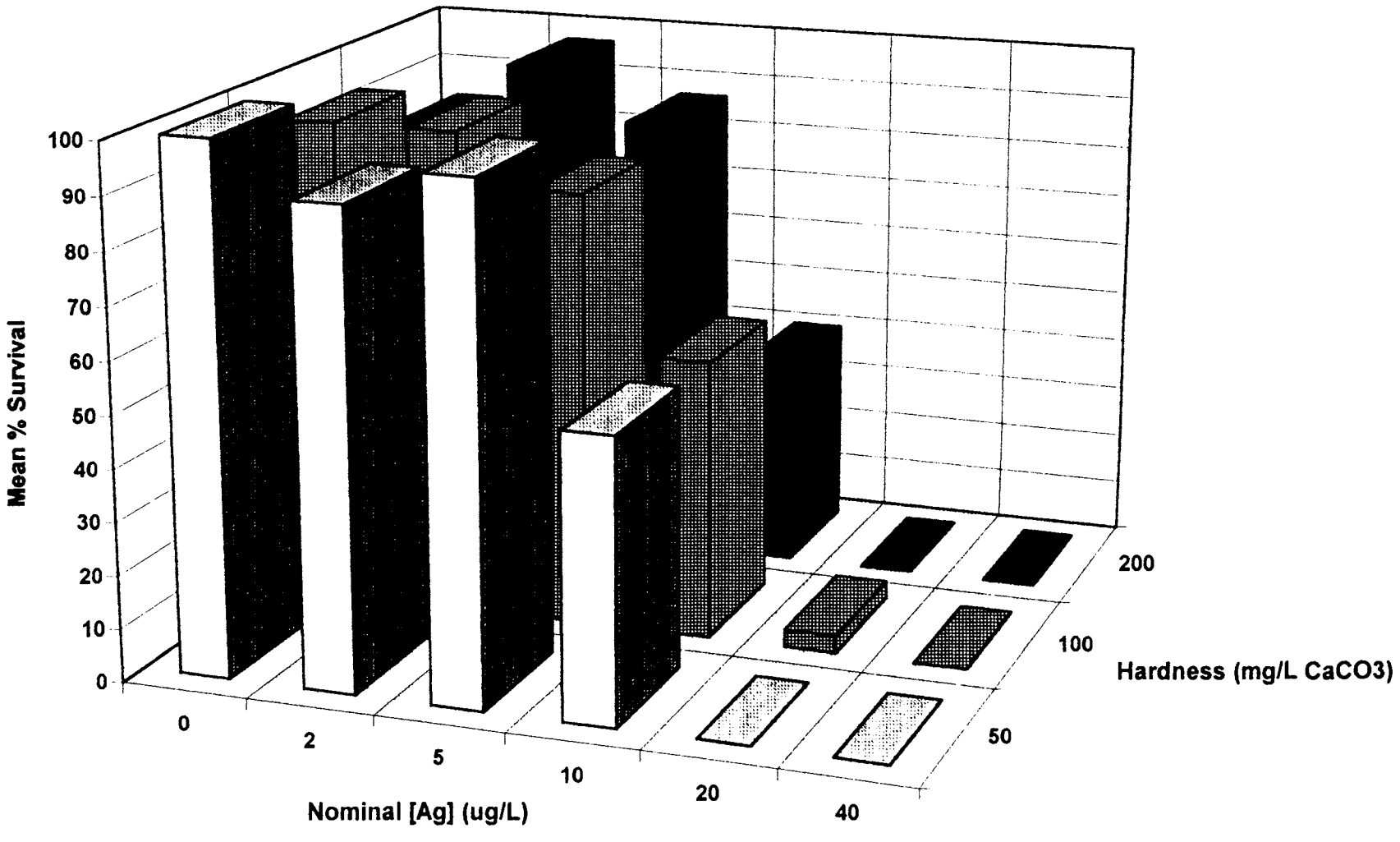


Figure 5. Effect of Hardness on Acute Silver Toxicity to Larval Fathead Minnows, at 3 Levels of DOC and 3 mg/L Chloride.

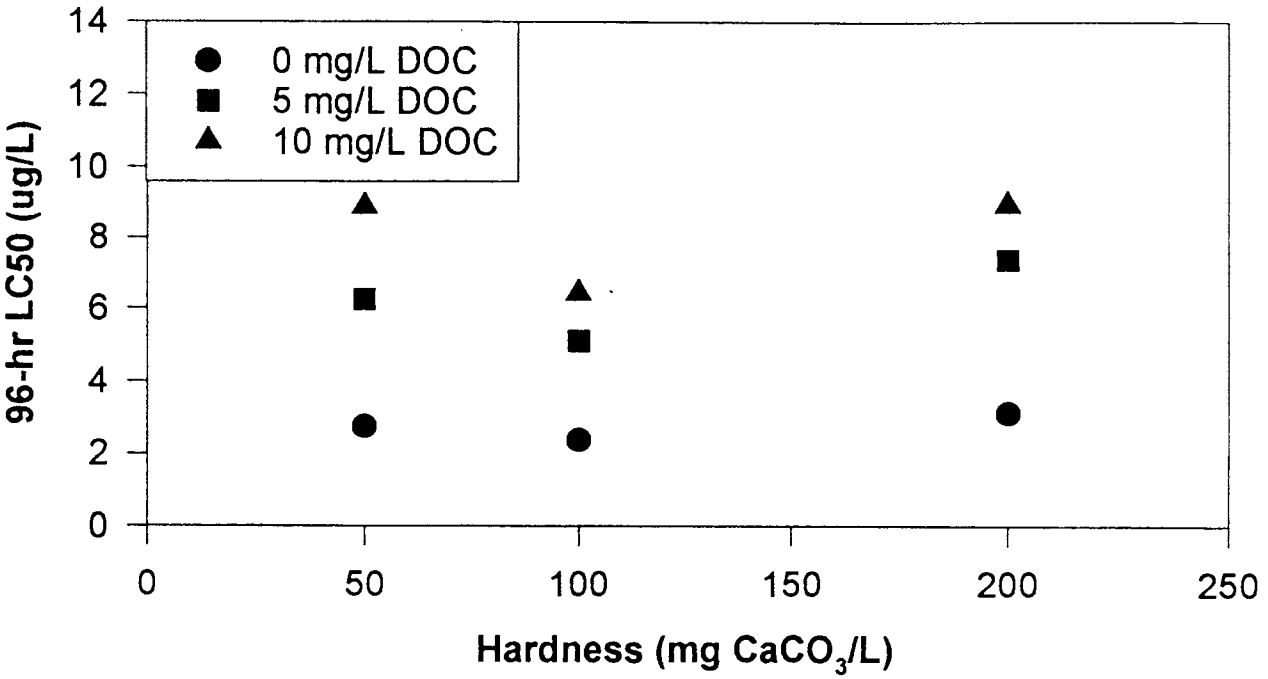


Figure 6. Effect of Hardness on Acute Silver Toxicity to Larval Fathead Minnows, at 3 Levels of DOC and 20 mg/L Chloride.

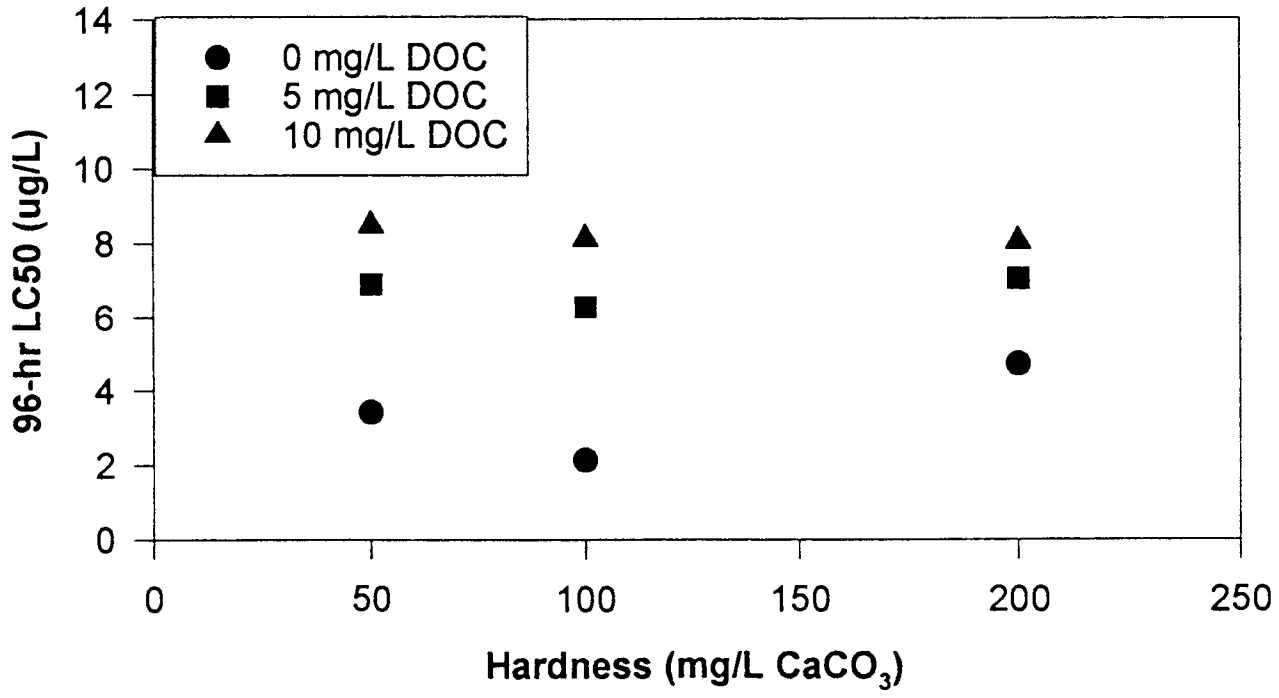


Figure 7. Effect of DOC on Acute Silver Toxicity to Larval Fathead Minnows, at 3 Levels of Hardness and 3 mg/L Chloride.

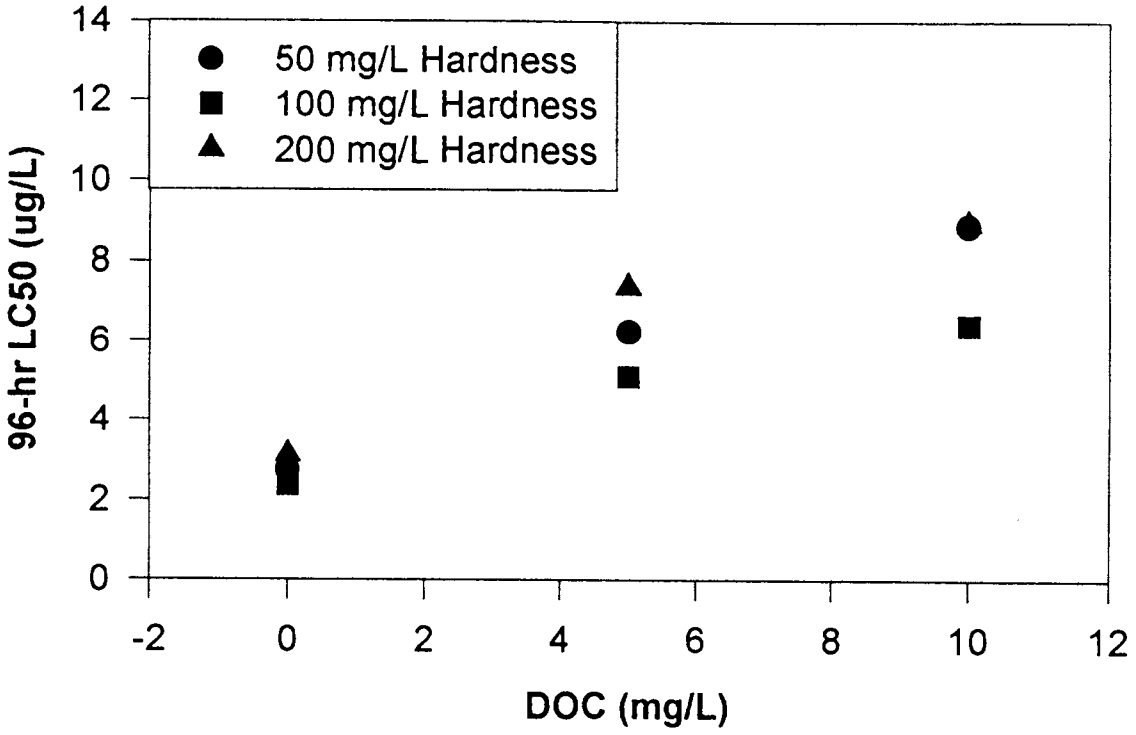
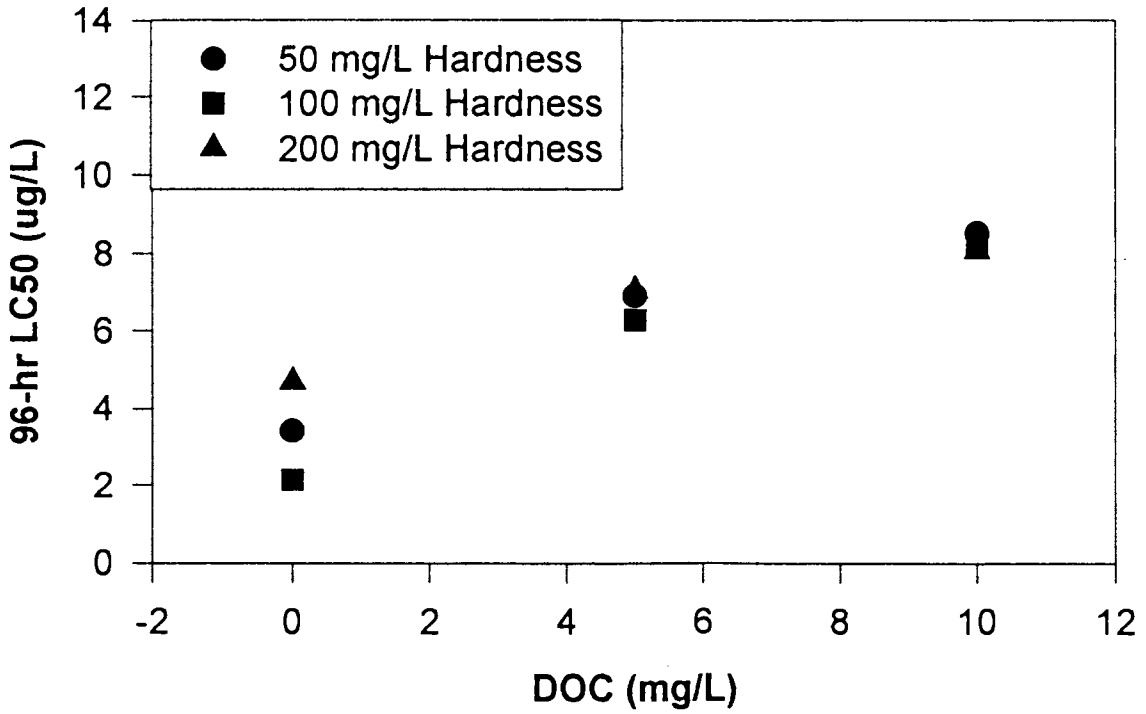


Figure 8. Effect of DOC on Acute Silver Toxicity to Larval Fathead Minnows, at 3 Levels of Hardness and 20 mg/L Chloride.



Questions & Answers: Influence of Water Quality Parameters on Silver Toxicity: Preliminary Result

Q. JIM KRAMER (McMaster Univ.): What was the make-up of your water? Was it just distilled water, or did you have some DOC or particles or other components?

A. It was the same water that we cultured the organisms in. It was the EPA carbon model adjusted for hardness, so it's a synthetic reconstituted water.

Q. RICHARD PLAYLE: In these past presentations we saw there is quite a loss of silver and the filterable fraction changed. Do your toxicity analyses correct for what numbers to use, do you use total added, do you use filtered fraction, do you make these corrections in the beginning or at the end of your experiment?

A. I forgot to mention the computation for the LC_{50}s was done with a nominal value just as was presented earlier. The actual measured values were pretty close to the nominal values at the beginning. So, we felt comfortable using the nominals at this point because we didn't have the silver analysis completed when we made these analyses. The final product of this will incorporate the measured values.

Q. How did you measure values changing over time? Do you use some sort of averaging procedure?

A. I think at first we look at the initial values and then look at the change in the concentration over time, mediating computed beginning and end value. I think we'll have to deal with that somehow and it was significant proof of a loss, so we haven't proposed that yet. The silver analysis is pretty recent.

Q. I think you should move to higher chloride levels because it seems quite a significant loss of silver.

A. It's definitely something we have to deal with in terms of quantitating numbers.

Q. PHAT DAO (Eastman Kodak Co.): In the conclusion, you didn't mention anything about the effect of chloride. I wonder if you have any results about chloride variation.

A. As I said, we only have the data for the 3 mg/L chloride run and the 20 mg/L chloride run, so we didn't feel comfortable putting out a slide that actually showed any variation effects. The difference didn't seem to be significant. It may be a different phenomenon when we're looking at the 40 and the 60 mg/L chloride runs.

Q. PETER SANTSCHI: A question regarding the losses which you have observed. Did you feed the fish during the experiment? Perhaps the losses are due to adsorption on the food or walls. Or where did you lose it?

A. The fish were not fed during the experiments. If you think about what we were using, we were only using 3- to 5-day old organisms. They are extremely tiny fish. My suspicion is that any loss of silver in this situation would be physical in terms of adsorption onto the sides of the containers, or some sort of chemical reaction that I don't think a fish or biota are taking part in.

GEORGE COBB (Clemson Univ.): You can't account for loss of silver in this experiment by adsorption to the surface of the fish. They are just not large enough. There is not enough mass of fish.

Q. ANDERS ANDREN (Univ. of Wisconsin): I think with these types of experiments we're still faced with a fundamental problem that surfaced today; that is, if we are to really get to the bottom of this, we have to better understand the speciation. What you have presented, I think, is a nice step forward, but we still don't really know how much of that silver is complexed with DOC or what the speciation distribution is. When we are in nature, we know that aging of organic matter, particulate matter, changes the distribution or, perhaps, the availability of the form of metals in general. So you have this material, you add DOC and with time — I've done those experiments a long time ago — you can take DOC, dissolve it in water, you can filter it through a 5,000 molecular weight filter, wait two days, filter the filtrate again and you collect organic material every time you do it. The point is, we still have to try to interpret these values with respect to what happens at the moment of the speciation. Do you have some comments on that?

A. Again, the purpose of this work was to study the phenomena and try and get a quantitative handle on the influence observed. As regards the mechanisms, we need some work done on the interactions between silver and DOC. My suspicion is that as you change DOC and the age of the DOC source, you will change the protective nature of DOC against silver toxicity. The important thing is not that DOC is protective but, rather, what is it about DOC that is protective? For once you know that, you'll be able to extrapolate from one source of organic carbon to another. And you'll also be able to see how that phenomenon changes as you age DOC. I certainly agree with your point, as anybody doing that kind of research will.

Q. ANDERS SODERGREN (Lund Univ., Sweden): In order to balance all these fish studies, I wonder if you just can disclose some results from your *Daphnia* studies.

A. The reason I did not discuss the *Daphnia* results is that we're not very far into the factorial experiment for the daphnia. So, unfortunately, you'll have to wait for next year for that. It will certainly be finished next year — it should be finished actually within the next month.

Q. JIM KRAMER (McMaster Univ.): In the experiment, I'm not quite sure exactly what went on from a chemical point of view. So you start with your fatheads in a certain water — is that the water where the defining ions, chloride, DOC, everything is equilibrated or conditioned except for the silver?

A. Yes.

Q. So whatever reaction went on, did you try to make other combinations? In other words, you have new sites on these little guys and then the silver reacts in a different way than if one of the parameters were missing.

A. Logistically, this could have been a nightmare; actually my students told me that it was a nightmare. The way that the experiments were run is that all water quality parameters were made up ahead of time in the lab. For example, for one of the runs, like the 3 mg/L chloride, there were nine different sets of water quality parameters that were distributed, and silver added and mixed in the containers. Then the fish were placed in those containers. The goal was to place the fish into the containers as quickly as possible after the silver was placed into the containers. Obviously, that was necessary to avoid problems with silver concentration changes before the fish experienced the solution.

Q. FERNANDO GALVEZ (McMaster Univ.): When we did our study looking at the effects of chloride, the concentrations we used were 50, 225 and around 730 µmolar. Most of the effects that we saw with chloride was actually seen between 50 and 225. Between 225 and 730 the effect was less pronounced. The concentrations you did were about what, 100 µmolar?

A. 3 mg/L.

Q. Which is about 100 µmolar. I'm just wondering, if you tried a chloride concentration below that, maybe somewhere around the one we did or lower, what effect you might see.

A. So you are suggesting a low concentration?

Q. Basically, when we saw our effects it was between 50 and 225 µmol/L — somewhere between there we see a variation. We're not exactly sure at what point and, certainly, if you see a little effect going from 3 mg/L to 20 mg/L it might be consistent with what we're seeing. For 225 µmol/L chloride the LC_{50} for silver was around 7 µg/L, whereas at 730 µmol/L it was in the order of 9 µg/L. That's a small step compared with 50 µmol/L where the LC_{50} was around 3.2 µg/L.

A. I don't have much comment on that, only that for silver we didn't see any effect between the 3 mg/L and the 20 mg/L chloride concentration so we wanted to go higher. Seems to contradict with what you have. I really don't know.

The Comparative Toxicity of Silver to Aquatic Biota

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Silver studies were initiated as a toxicological component in evaluating the leachability and potential environmental effects of coal and coal wastes. The initial effort was devoted to developing a flow-through model system for analyzing leachates. Thirty-three inorganic elements traceable to coal toxicology were detectable in different waters perfused through fly ash and other coal products (Birge, 1978; Birge *et al.*, 1978; 1979). The leaching chamber, developed for use with solid wastes, was adjustable for water to solid ratio, general water quality parameters, flow rate, and the collection of settleable solids. Continuous leachability of toxic metals from coal fly ash was evident for more than 80 days.

Chemical analytical data were used in initial assessments of the impact potential of complex ash effluents but comparisons with U.S. EPA aquatic criteria and other data were complicated by lack of pertinent information. Consequently, laboratory studies were initiated 1) to characterize the toxicity of inorganic elements detected in coal leachates and 2) to apply direct toxicological testing to untreated and treated coal effluents. Independent laboratory toxicity data were used 1) to prioritize coal elements as to impact potential and 2) to categorize animal test species according to sensitivity, including the selection of surrogate species. Each inorganic element was evaluated with at least three aquatic species and with up to 14 animal species for metals considered to be more problematic.

The application and modeling of independent laboratory toxicity data, together with the chemical effluent monitoring results, facilitated identification of the most problematic elements. However, this traditional approach was not fully effective in quantifying the biological activity resulting from the chemical milieu contained in complex coal effluents, or in evaluating effluent treatability and/or sediment sorption of water column metals. Therefore, direct toxicological testing (*i.e.* biomonitoring) with continuous coal effluent was performed with early life stages of fish and amphibian species. Good dose response data were obtained for effluent dilutions using embryo-larval stages, and biomonitoring consistently provided more reliable results than aquatic life criteria for assessing the biological impact of coal effluents. The results of this early investigation formed the basis of our long-term effort in effluent biomonitoring, and led to the conclusion that leachability, bioavailability and/or transport of priority pollutants cannot necessarily be predicted accurately with the use of U.S. EPA aquatic criteria or conventional laboratory toxicity data.

Laboratory toxicity tests with silver were conducted with six species of amphibians and four species of fish. Test organisms were maintained in reconstituted water of 100 to 200 hardness (mg $CaCO_3$/L) using twelve-hour static renewal procedures (Birge *et al.*, 1985a; Weber *et al.*, 1989). Organisms and test parameters were monitored once to twice daily (*e.g.* pH, dissolved oxygen, conductivity, hardness, alkalinity). Sample size varied from 100 to 150 organisms, except for *A. opacum* (n = 35). Exposure was maintained from fertilization through four days posthatching, and overall treatment ranged from six to eight days for amphibians up to twenty-eight days for rainbow trout. Results were based on mortality and gross terata of embryos and larvae. These responses were combined and threshold values (LC_1, LC_{10}) and median lethal concentrations (LC_{50}) were determined by probit analyses (Table 1). The leopard frog (*Rana pipiens*) and rainbow trout (*Oncorhynchus mykiss*) were the most sensitive species, with LC_{10} values of 0.7 to 0.8 µg/L, and an LC_{50} value of 10 µg/L. The LC_{10} values for the other species ranged from 1 to 30 µg/L and the LC_{50} values varied from 10 to 240 µg/L. The probit LC_{50} and threshold (LC_1, LC_{10}) values were comparable to MATC and chronic values determined in various chronic tests (*e.g.* life cycle studies) by other investigators (Davies *et al.*, 1978; U. S. EPA, 1980; Nebeker *et al.*, 1983; Eisler, 1995).

Toxic effects of silver also were investigated using *Ceriodaphnia dubia* and the three-brood procedure. The NOEC values were 5 and 4 µg/L and the LOEC values were 10 and 8 µg/L in two independent experiments conducted using U.S. EPA methods (Weber *et al.*, 1989). The chronic values were 7.1 and 5.7 µg/L and the IC_{10}, IC_{25} and IC_{50} values were 5.8 and 4.5 µg/L, 7.3 and 5.2 µg/L, and 9.8 and 6.4 µg/L, respectively. The above studies revealed the LC_{10} to be a more reliable indicator of threshold effects than the IC_{25} suggested by U.S. EPA. By comparison, Nebeker *et al.* (1983) reported similar values obtained in three 21 day life cycle tests with *Daphnia magna*. They determined a mean LC_{50} of 3.5 µg/L based on survival, and LOECs of 4.1 µg/L and 10.1 µg/L based on survival and reproduction, respectively. Thus, there was remarkably little variation among results for both cladoceran species.

In fish and amphibian tests, silver was among the more toxic metals evaluated (*i.e.* LC_{50}). In an attempt to further characterize biological effects of silver, data were combined for different combinations of sensitive and tolerant species to give mean toxicity indices. Results for amphibian species are given in Table 2. The leopard frog (*Rana pipiens*), pickerel frog (*Rana palustris*) and narrow-mouthed toad (*Gastrophryne carolinensis*) were the most sensitive amphibians, with LC_{10} values of 0.7 to 2 µg/L and an LC_{50} value of 10 µg/L. Combining responses for these three species, the LC_{10} was 1.0 µg/L and the LC_{50} remained 10 µg/L. For the more tolerant species, the individual LC_{10} values ranged from 3 to 34 µg/L and the LC_{50} values varied from 20 to 240 µg/L. The combined LC_{10} and LC_{50} values were 3 and 90 µg/L, respectively.

Silver nitrate, one of the more soluble forms of silver, was used in the above investigations. Based on calculations with the aquatic equilibrium program MINEQL$^+$ (Schecher and McAvoy, 1991), it was concluded that the silver nitrate used in these studies was highly dissociated and that the observed effects were attributable to the "free" silver ion. Predictably, other forms of silver (eg. silver chloride, silver sulfide) would be less soluble and results based on total recoverable metal concentrations would reflect proportionally less toxicity (Le Blanc *et al.*, 1984; Wood *et al.*, 1995). Considering either single-species or combined data (Tables 1, 2), silver was remarkable in providing less diversity of response among test organisms than did mercury or most other metals. However, in further studies of coal toxicity silver was deprioritized because of its more limited leachability and/or bioavailability.

Numerous investigations have demonstrated that the embryo-larval procedure used in these studies is sufficiently sensitive to give reliable predictions of the chronic toxic effects of single compounds or complex effluents, and that test results correlate well with life cycle studies used to develop aquatic criterion values (Birge *et al.*, 1981; 1985b; Birge and Black, 1990; Weber *et al.*, 1989). It should be noted, however, that more soluble forms of silver have been used in most laboratory investigations, and that test procedures and/or the use of reconstituted water likely exacerbate stresses to test organisms and optimize bioavailability of the free silver ion. Consequently, laboratory test results may overestimate silver toxicity in the field. In general, caution should be used in applying laboratory toxicity data to assessments of ecological impact.

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Table 1. Silver Toxicity Values for Early Life Stages of Fish and Amphibians

	mg/L[b]			
Species[a]	LC_{50}	LC_{25}	LC_{10}	LC_{1}
R. pipiens	0.01	0.004	0.0007	0.0001
O. mykiss	0.01	0.005	0.0008	0.0001
R. palustris	0.01	0.007	0.001	0.0001
I. punctatus	0.01	0.007	0.002	0.0003
G. carolinensis	0.01	0.007	0.002	0.0006
R. catesbeiana	0.02	0.01	0.003	0.0005
C. auratus	0.02	0.01	0.004	0.001
M. salmoides	0.11	0.07	0.018	0.004
B. fowleri	0.23	0.07	0.004	0.0001
A. opacum	0.24	0.13	0.03	0.007
Geometric Mean	0.03	0.02	0.003	0.0004
Arithmetic Mean	0.07	0.03	0.007	0.001

[a] Organisms were maintained through four days posthatching.
[b] Probit values were calculated using the EPASTATS program.

Table 2. Combined Silver Toxicity Values for Amphibians

Species	mg/L[a] LC_{50}	LC_{25}	LC_{10}	LC_{1}
R. pipiens	0.01 (0.007-0.015)	0.004	0.0007 (0.0003-0.0013)	0.0001 (0.0000-0.0002)
R. palustris	0.01 (0.003-0.050)	0.007	0.001 (0.0000-0.0040)	0.0001 (0.0000-0.0009)
G. carolinensis	0.01 (0.004-0.030)	0.007	0.002 (0.0001-0.0060)	0.0006 (0.0000-0.0023)
R. catesbeiana	0.02 (0.017-0.032)	0.012	0.003 (0.0010-0.0040)	0.0005 (0.0002-0.0009)
B. fowleri	0.23 (0.150-0.350)	0.073	0.004 (0.0020-0.0080)	0.0001 (0.0000-0.0004)
A. opacum	0.24 (0.150-0.360)	0.130	0.034 (0.0120-0.0640)	0.007 (0.0010-0.0180)
All Species	0.03 (0.019-0.052)	0.012	0.001 (0.0004-0.0028)	0.0001 (0.0000-0.0003)
Geometric Mean	0.03	0.017	0.003	0.0004
Arithmetic Mean	0.09	0.039	0.007	0.001
More Sensitive Species				
R. pipiens *R. palustris* *G. carolinensis*	0.01 (0.009-0.015)	0.007	0.001 (0.0008-0.0020)	0.0002 (0.0001-0.0003)
Geometric Mean	0.001	0.006	0.001	0.0002
Arithmetic Mean	0.01	0.006	0.001	0.0003
More Tolerant Species				
R. catesbeiana *B. fowleri* *A. opacum*	0.09 (0.020-0.340)	0.033	0.003 (0.000-0.0070)	0.0002 (0.000-0.0019)
Geometric Mean	0.10	0.048	0.007	0.001
Arithmetic Mean	0.16	0.072	0.013	0.003

[a] Probit values were calculated using the EPASTATS program.

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Questions & Answers: The Comparative Toxicity of Silver to Aquatic Biota

Q. ARUN MUKHERJEE (Univ. of Helsinki): I understand that you worked with coal and analyzed the ashes that yield when you burn the coal at high temperature. For example, if you take one pound of coal and know what is the amount of silver within the coal and you know the efficiency of your precipitator, how much silver would go into ashes and how much would go into the atmosphere?

A. You're talking about the precipitator for the ash?

Q. Yes. Could you tell me how much would go into the atmosphere?

A. The only thing I can tell you is what we analyze in the air before we start the leachate. Normally, we're in the range of 40 ppb and, after some time, as much as 600 ppb, rarely maybe even 700 ppb. Normally, it's in the 40-60 ppb range if the precipitator works with chloride.

Q. Ppb. That means, 2 ppb is 0.002 ppm. Am I correct?

A. Yes. So we have 0.04 mg/kg.

Q. 0.04 mg/kg, good. And how much will go out into the atmosphere?

A. How much goes into the atmosphere? Actually, that's a difficult question, really, to answer. What happens there, at the lead-high temperature, is that some of the elements are not sorbed to particulates. But, then again, in the gas phase they do sorb, and then, if you look at the leachate part for sorption effects, you find that some do and some don't sorb. So what's going up secondarily, a lot of it is probably collected on particulates also and sent out into the atmosphere and then deposited.

Q. Do you have any experience with domestic waste?

A. We don't look only at sewage waste. We have several other studies around where we're looking at, or examining what comes out from power plants. But there, about the only elements that turn up in soils around the site of the plants would be in much higher concentration than in coal. So we're normally looking at aluminum and iron, copper, zinc. I don't recall actually seeing major silver amounts.

Q. ANDERS ANDREN (Univ. of Wisconsin): You presented an incredible amount of data, so there are lots of questions, but I'll probably just limit myself to two. The first one, when you characterize your leachate; did you filter your material or is that total concentration of metals, or what?

A. We were doing total metal, full recovery metal.

Q. I defy anyone to make any sense out those data, then, in terms of ascribing any effects to any particular metal.

A. That's an interesting point to debate that we're heading at here, the dissolved versus the solid argument, depending on the filter. I hope we have the time to do it. But remember, a great amount of subtoxic and almost all the toxicity data on metals were done for total metal concentrations, and with these data were given the bioavailability and response level. I think that this may give problems for some other scientists, but I'd be happy to discuss this.

Q. The other question deals with your LC_{50}s, etc., how can you, — as I don't have much biology background — how can you do an LC_1?

A. Well, with the photo systems we were using, you can't really do this with all the available photo systems out there. We were using a special process and, basically, you have the opportunity to get a read-out anywhere from 1 to 99.

Q. But you have to determine the concentrations at that level?

A. It would depend on the inhomogeneity of your animal response data and water flea data. We realized that they really don't vary so much. The variability within these tests isn't great. A lot of these animals are adapted to toxicity tests; that reduces mortality and the ability of a chemical to get a critical life support mechanism knocked down. The opportunity for more individual variation is less here. When we're looking at embryonic development; that is, the period when the DNA is doing its thing. That's when there is more regulation, more control and more reproduction in the lab, at least for the organisms we looked at. Many of you have studied embryonic development, you know what kind of reproducibility is involved. An enormously complex major event would be needed to take influence and they all would change in the same way. That can be taken advantage of in various toxicity tests.

And we can show that when you do a normal batch test you often get less variability in your data than you expect, but it leads to a better opportunity for further analysis. With a lot of chemicals we can't do an LC_1 with reasonable confidence, but that's when we back off and we do the LC_{10} and we think it works out pretty well. We do all kind of other kinds, not just the LC_1.

Q. What concentrations of DOC, chloride, and particulate matter do you have in your bioassays?

A. In these particular assays? I have information on that but I didn't bring it with me. I have a list over there. We don't have a lot of the usual things as effect level variability and normal water quality and a lot of these parameters. It's more homogeneous with effect to a lot of the nonmetal constituents that you can find in different forms. I have some information on that I can show you later. Anything else? The question that I want to leave you with is, why don't we see a more varied response of silver during the early life stages? A lot is depending on the life stage, for example, the LC_{50} we studied with adult fish, they tend to be different, and the main thing for this is inhomogeneous response. There's got to be a reason for this. You can either be looking at some universal receptors that are out there, or it is a matter of membrane composition and individual toxic patterns, but with the same level of sensitivity as for the first (that is, the universal receptors). Thank you.

The Effects of Salinity on the Acute Toxicity of Silver to Marine Fish

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Previous studies on the toxicity of silver to aquatic organisms have primarily focused on freshwater species (Nebeker, *et al.*, 1983, and LeBlanc *et al.*, 1984). Results from these freshwater studies have indicated that the free silver ion is the most toxic silver species. Analysis of silver chloride speciation by MINEQL+ (Schecher, 1991) indicates that the presence of the toxic free silver ion is diminished as chloride concentration is increased. In fact the free silver ion concentration is reduced to a negligible amount at Cl^- concentrations equivalent to that of sea water. Therefore as water salinity is increased there should be a shift in the prevalent species of $AgCl_n$ away from the free Ag^+ ion and the small uncharged AgCl(aq) species toward the larger charged species. It seems plausible that silver complexes of different size and charge may have different toxicological properties.

This study explores the acute toxicity of silver to two sea water living organisms, the rainbow trout (*Oncorhynchus mykiss*) and the tidepool sculpin (*Oligocottus maculosus*). Use of the rainbow trout for marine studies has several advantages. The freshwater rainbow trout is diadromous and should acclimate easily to sea water. Farmed rainbow trout also show a high degree of genetic homogeneity making it an excellent test species in that a difference in response can less easily be discounted as individual variance. Finally, data from sea water toxicity tests can easily be compared to freshwater data already published on this species (Wood *et al.*, 1994). Toxicity data obtained for the rainbow trout are complemented by data obtained from toxicity studies on the tidepool sculpin. This hardy fish is tolerant to environmental variation (i.e. temperature and salinity) making it a very suitable candidate for toxicity studies. The small size (30 mg - 1 g) of the tidepool sculpin is especially useful in toxicity testing.

Results from 96 hour acute toxicity tests on rainbow trout and tidepool sculpins are shown in Table 1. The tidepool sculpins were tested at two salinities, 25 ppt and 32 ppt, while the rainbow trout were tested at 25 ppt only. As expected the toxicity of silver to rainbow trout in sea water is much less than that in freshwater. Hogstrand *et al.* (1995) reported a 96 hour LC_{50} of 12 μg $Ag \cdot L^{-1}$ (111 nM: added as $AgNO_3$) for juvenile freshwater rainbow trout. The 96 hour LC_{50} to Ag in 25 ppt salinity was almost 35 times greater than that reported for freshwater. The sculpin data indicated an LC_{50} at 96 hours, very close to that of the rainbow trout. A comparison of the tidepool sculpin 96 hour acute toxicity tests at 25 ppt and 32 ppt shows yet another increase in the LC_{50} at the higher salinity. Likely, this is produced by differential toxicity of different dissolved silver chloride complexes. A comparison of the results of the 96 hour and 168 hour toxicity tests on the tidepool sculpin are shown in Table 2.

Table 1. Results of 96 hour acute toxicity test on rainbow trout and tidepool sculpins

Salinity	Rainbow Trout - LC_{50}	Tidepool Sculpin - LC_{50}
freshwater	12 $\mu g \cdot L^{-1}$ (Hogstrand et al, 1995)	not available
25 ppt	401.5 $\mu g \cdot L^{-1}$	409.2 $\mu g \cdot L^{-1}$
32 ppt	not available	660.9 $\mu g \cdot L^{-1}$

Table 2. Results of the 96 hour and 168 hour acute toxicity tests on tidepool sculpins

Salinity	96 hour test - LC_{50}	168 hour test - LC_{50}
25 ppt	409.2 $\mu g \cdot L^{-1}$	241.0 $\mu g \cdot L^{-1}$
32 ppt	660.9 $\mu g \cdot L^{-1}$	518.9 $\mu g \cdot L^{-1}$

From these data it appears that the LC_{50} of silver is indeed markedly higher in sea water than freshwater. These differences may be indicative of a different mechanism of silver toxicity in sea water. Overall, we have concluded that as far as the acute toxicity is concerned silver does not pose a great problem at current environmentally realistic levels.

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Questions & Answers: The Effects of Salinity on the Acute Toxicity of Silver to Marine Fish

Q. PHAT DAO (Eastman Kodak Co.): You mentioned ionic silver, that there's no ionic silver in seawater?

A. No free ionic silver.

Q. Do you measure that by any technique, or do you just have it from literature?

A. We have that from the MINEQL speciation. We do not have any hard data.

Q. ANDERS SODERGREN (Lund Univ.): It would seem good to me if we have organisms that can be used both in fresh and in salt water to study mechanisms. You suggested that there are different kinds of mechanisms operating in fresh and salt water. Because mostly the kind of mechanisms we have described here frequently are the fresh water mechanisms.

A. Yes.

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Session 3

Environmental Cycling, Distribution and Analytical Chemistry

E.A. Crecelius
Session Chair

Chemical Interactions of Toxic Metals with Sedimentary Sulfide Minerals Near the Sediment-Water Interface of Anoxic Marine Sediments

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The speciation and chemical reactivity of metals in natural aquatic environments plays a major role in controlling their availability and toxicity to biota. The behavior of toxic metals in sediments near the sediment-water interface is of special interest because the dynamic chemical processes occurring within this environment cause major transformations in the speciation of metals that may act to mobilize or sequester them from organisms. There is growing observational and experimental evidence that interactions between toxic metals and authigenic sulfide minerals in anoxic sediments may play a major role in controlling the bioavailability of these metals.

Although there are occasional exceptions, a general speciation pattern has been observed in which As, Hg and Mo are more extensively pyritized than iron. Transition metals usually have a similar extent of pyritization to that of iron, and class B metals (e.g., Cd2+, Pb2+, Zn2+) undergo lesser pyritization than iron. A major portion (generally >60%) of the nonsilicate-bound fraction of toxic metals, such as As, Cu and Hg, is commonly coprecipitated with pyrite near the sediment-water interface. New data indicate that Ag is also strongly partitioned into authigenic pyrite during early diagenesis. Consequently, for many toxic metals of interest, their major sink in anoxic marine sediments appears to be in authigenic pyrite rather in the ephemeral AVS fraction which has received the most attention recently.

Traditional methods (e.g., 1N HCl or citrate dithionite extractions) for determining the concentration of potentially bioavailable metals in sediments do not include the pyrite-bound fraction. However, pyritized metals can be potentially bioavailable if the pyrite is oxidized. Pyrite (and AVS) oxidation commonly occurs during sediment resuspension, seasonal migration of the redoxocline in sediments and when dredge spoils are dumped on land. Experimental measurements of metal release from pyrite, in initially anoxic sediments exposed to oxic seawater, indicate that a major portion (20% to over 90%) of the pyrite-bound metals can be released in a day or less. The As, Cu and Hg released from pyrite usually exceeds the concentration of their potentially bioavailable fraction determined by traditional methods. Consequently, the pyritization-depyritization of toxic metals is probably an important process in controlling the bioavailability of many important trace metals.

Questions & Answers: Chemical Interactions of Toxic Metals with Sedimentary Sulfide Minerals Near the Sediment-Water Interface of Anoxic Marine Sediments

Q. PETER SANTSCHI (Texas A&M Univ.): When you just looked at the sediments, do you really see release into the water? Can you say that? What you see is just a smaller degree of pyritization but you don't know where it's going. I mean, the metal can be less in the pyrite phase and you say that it's being released, but basically, it's just what we account for in iron oxides, or in some other phase.

A. That's a good point I guess I didn't make clear. We've actually done quite a few experiments where we put metals in that are bound with iron or whatever. When we put the oxidized metal in, we never see — except in one case for cadmium — the metals going up in the water when you resuspend sediments. Because you've got so much particulate matter in the water column, its maybe released into the water but it's almost immediately readsorbed onto another phase. The point is, with the pyrite there's movement out of that phase into one maybe adsorbed on oxides or something but much more labile. But that's a very good point, Peter.

Q. JOHN MAHONY (Manhattan College): We've been studying the oxidation rates of what we presume were the results of a combination of acid volatile sulfides (AVS) with toxic heavy metals, silver, copper, cadmium, zinc, lead. We found very slow oxidation rates for some and far slower oxidation rates for all of them compared with iron sulfide, either amorphous ones or macchanite. I was wondering, especially as we're noticing that the oxidation rate of silver sulfide is extremely slow, would you expect that would be due to the pyrite or rapid pyritization of silver or is it an inherent property of, I think it was, amorphous silver sulfide?

A. That's a very interesting question. Like, if silver makes its own sulfide, silver sulfide, and cadmium makes cadmium sulfide, it's going to behave quite differently than if that metal is associated with one of the iron sulfides either through adsorption or coprecipitation. In which case, its release is going to be largely dominated by the rate of oxidation of the iron sulfide. We're starting to look into that same thing. Question is if you get a more rapid release of the coprecipitated than if you just put in a pure metal sulfide. It's not inconceivable as we learn more about the oxidation kinetics that rate constants may to some extent — this has been observed at least in other systems — be somewhat proportional at least to the solubility of the metal sulfide. I think you have a good point there: if you take pure silver sulfide you may have a very low oxidation rate versus if it's coprecipitated with one of the iron compounds.

Q. It seems though that the form in which the metal is actually taken in the sediment is that of the metal sulfide and not of the sorbed metal on the surface of the iron sulfide, wouldn't you say?

A I wouldn't say it that universally. Certainly, the behavior of copper and mercury, from the data we showed here, are suggesting that with the oxidation of the iron sulfide the metal is coming out. We see a relationship to that. As a matter of fact, when you look at copper and mercury here from these sediments, they seem to be released preferentially from the pyrite phase, which would suggest that they might have higher concentration on the surface.

Q. But when you treat, for example, the sediment amended with silver less than the AVS value, and you attempt to do AVS extraction on it with hydrochloric acid at 1 mol/L, or even with nitric acid at 0.3 mol/L, you see no AVS occur. So that sort of indicates that if you amended with so much less silver than sulfide then you will see the residual as AVS. If you amended at the exact stoichiometric amount of the sulfide then you will see no AVS.

A. I'm not sure I totally follow.

Q I'm sorry. If you take 10 µmoles of acid-volatile sulfide per gram and you amend it with 20 µmoles silver, in that case, when you again make the AVS measurement on that sediment you get no result. Whereas, if you amended it at, let's say, 15 µmoles of silver for 10 µmoles of AVS, you would see an AVS but it would only be what would be left of the iron sulfide that had not reacted with the silver.

A. So, what you're basically saying is, you put the silver in and it displaces the iron and grabs the sulfide.

Q I think, at least, the experimental evidence indicates this.

TOM BOBER (Eastman Kodak Co.): Copper does that, too.

Q RICHARD PLAYLE (Wilfrid Laurel Univ.): Earlier on you showed a slide of salt water and freshwater effects, so I'd like to hear a little more about that, whether there are many substantial differences of salt water versus freshwater. Or whether it might be temperature effects. In Galveston it maybe doesn't get that cold in the winter when the sediments are being resuspended, but there might be cold spots.

A. Okay, in our collection studies we saw distinct, but not large, differences between sea water and distilled water or freshwater. For these options, the cobalt and the nickel that we looked at, there is an effect that looks like it's fairly small. That's probably because the main site competition is with ions like calcium and magnesium in seawater and that the minerals have very high adsorption coefficients, and there's much more calcium there to displace the metals. A number of models have been made up from these things that react fairly weakly at the surface, but there are a lot of them there. Some of them have a very high affinity. It's likely then, it strongly decreases the effect of freshwater versus salt water. I think that's probably seen there.

On the temperature. We haven't done much work on the temperature. It's a minor aspect on the matrix of many variables. We do expect some temperature effect in the colder waters in winter. One of the biggest effects of temperature for us, I think, that's of interest is that in the more Northern climates the biodegradation slows down tremendously during the winter. There has been a lot of work on it — you tend to build up the sulfides during the winter, and the organic matter and the food in the sediment, and then when it warms up the organisms all un-hibernate, or whatever, and start spreading. You get a really large decrease in the sediments, the pH can go all the way down to inhibit the oxidation of the sulfides. One might worry at that point if you're dealing with metal sulfides or if some of these trace metals there, if this event might not lead to a pulse in the spring when the water warms up. Maybe you get this rapid breakdown in the sulfides. I don't think anybody has really looked at this in detail as it takes a tremendous amount of work.

One of the big problems that we're realizing now is trying to look at time series in the natural world. Everybody is used to the sediments as being relatively homogeneous and this is not always by a long shot true, even in environments where you think it might be so. What we've done now, we can use global positioning to get back within a few feet, hopefully. And what you have to do is you take multiple cores, like five cores for every different site each time, and analyze them and try to see what the certain natural spatial distribution in the part is. So that you can potentially fool yourself thinking you're looking at a time variability when you're just looking at spatial variabilities, a very different thing. All this work on sediment types really doesn't want to think about this lateral inhomogeneity. But it's definitely there; in part it has to do with hatching organisms and stuff.

Q. ANDERS ANDREN (Univ. of Wisconsin): Could you just comment on what you think the role of strong organic chelators are vis-a-vis incorporation into the pyrite AVS fraction or perhaps even in the kinetics of dissolution?

A. One would like to think that they have a very major effect. The problem is, if we take copper as example of a metal that everybody agrees likes chelating with organics and makes very strongly bound compounds — if we look at organic-rich sites like in Chesapeake Bay, what we find is that copper, in spite of lots of organic matter, is, in fact, almost entirely in the reactive phase, and eventually ends up in the sulfide. There's a lot of work, I think, that can be done looking at reaction paths, lots of experimental work, but it appears that it's still able to pull it into the sulfides, perhaps because its affinity for the sulfides is even greater than that of organic matter. I just don't have the data out yet and we're still looking at them, but this is why my co-workers and I are working on these sites on dissolved copper. They are doing some fairly beautiful electrochemical measurements of copper speciation in the pore water, one kind of copper fluxing in and another kind fluxing out at the same time and reacting on the sulfides in the sediments. So we're trying to address this question but it's excessively messy.

Sampling and Analytical Techniques for Silver in Natural Waters

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A complete re-evaluation of trace element levels in freshwaters has occurred over the past 5 years as a succession of studies have demonstrated previously published data to be erroneous. Severe contamination during sampling and processing, and inadequate analytical sensitivity plague historic data (see Table 1). Recently, several published studies have applied "standard" oceanographic analytical techniques, developed over the past 10-15 years, with the latest in "clean" techniques to accurately determine natural metal levels. In general, these methods rely on a preconcentration step, either liquid phase chelation/extraction or a solid phase extraction to provide the necessary enrichment for standard instrumental methods. Our group has been developing and applying to natural waters, techniques that allow quantification of many metals at natural levels without a non-instrumental preconcentration step. This paper outlines our field and analytical methods for the quantification of Ag and a select group of other trace elements at low ng L^{-1} levels.

Table 1. Historic Metal Level Comparison Lake Michigan ($\mu g\ L^{-1}$, Unfiltered)

Metal	Copeland & Ayers 1972	Rossman 1984	Shafer & Armstrong 1995
Ag	0.3	0.057	0.0004
Cd	0.42*	0.044	0.009
Cr	1.7	0.68	0.5
Cu	5	0.39	0.4
Hg	0.027	0.045	0.0003
Pb	4.8*	0.25	0.02-0.08
Zn	16	0.59	0.5

*Gara and Hawley (1974)

As analytical techniques have improved and contamination brought under control, measured levels of metals have markedly declined. This is particularly true for the heavier trace metals, Ag, Cd, Hg, and Pb, whose reported levels have dropped nearly an order-of-magnitude in each of the past three decades. By the mid-eighties, however, reasonably accurate levels of Cr, Cu, and Zn were being published for Lake Michigan.

Three inter-related areas must be satisfactorily addressed to produce high quality trace element data: (I) contamination control, (II) representative sampling, and (III) analytical sensitivity.

I. CONTAMINATION CONTROL - FIELD and LAB

The application of "clean" techniques to control contamination is an essential prerequisite for accurate quantification in all modern methods. Personnel must develop a "clean ethic", carefully evaluating all actions in terms of trace metal compatibility and contamination potential. The outline below lists some of the required steps and procedures followed in obtaining un-biased samples.

SAMPLERS

- Non-metallic (preferably all Teflon), simple (e.g. direct sampling into bottle).
- Robust; functional in a wide range of environmental conditions.
- Scrupulously acid-cleaned, blanked.
- Open and close underwater to avoid surface microlayers.
- Isokinetic for those studies that require an integrated sample.
- Easy to clean and keep clean.

SAMPLE CONTAINERS

- Teflon bottles (FEP, PFA).
- Rigorously acid-cleaned (50% HCl, 50% HNO_3, 1% High Purity HNO_3).
- Prepped and double-bagged in clean-room.

SAMPLING PLATFORM

- For small rivers, grab sampling or integrated sampling (e.g. all Teflon DH-81) by careful wading has been proven acceptable. The operator, wearing waders, clean Tyvek suit, and arm-length gloves places the sampler upstream and away from any disturbance he/she may create. The sampler is kept double-bagged in plastic right up to the point of placing it into the river. Handling of the sample bottle is strictly controlled using "clean-hands dirty-hands" techniques. Pumping through Teflon tubing to a platform on shore is also acceptable where cross-sectionally integrated samples are not required, however, great care must be taken to keep the tubing clean.

■ For large rivers and small lakes, the preferred sampling platform is an all plastic (e.g. fiberglass Boston Whaler) boat from which either grab samples or depth/cross-sectionally integrated samples can be obtained. An electric trolling motor propels the boat and is removed from the water at the sampling site/s. The boat is kept clean by transporting in a sealed canvas "cocoon", and scrubbing down with water after each use. A dedicated acrylic stage, which mates with the gunwales of the boat, is used to hold pumping apparatus and filter cartridges. Unfiltered and filtered samples are obtained by pumping through Teflon tubing, weighed with a heavy all Teflon intake unit, and held away from the boat on a polyethylene boom. A short length of scrupulously acid-cleaned C-flex tubing is used in a plastic wrapped peristaltic pump.

■ In large lakes where transport to the sampling site involves a large ship, one must remove oneself from the dirty environment of the vessel. We typically use Zodiac inflatables (for stability) rowed or trolling motored several 100 meters upwind of the mother ship. Sampling on the Zodiac can be conducted in an identical manner to that described for the whaler.

FILTRATION - PHASE SEPARATION

- All Teflon holders, scrupulously acid-cleaned.
- Holders pre-loaded in clean-lab, double-bagged.
- Extensively acid-leached filters (polycarbonate track-etched or all polypropylene).
- In-line or in field glove-box.
- Minimal surface contact - only filter holder/column - directly into Teflon filtrate bottle.
- Separations performed at time of collection.

PRESERVATION

- Teflon vials, double-bagged in clean-room.
- Pre-dosed in clean-lab with 50% Ultrex HNO_3.
- Acidification acid spiked with surrogate metals.

SAMPLE HANDLING

- Tyvek coveralls and poly gloves to minimize contamination from personnel.
- "Clean-Hands" - "Dirty-Hands" Technique. Frequent glove changes.
- Ultra-high purity reagents.
- All supplies double-bagged in plastic after prep in Clean-Lab.
- Maximize preparation and pre-packaging in Clean-Lab; minimize field handling.

FIELD QC PLAN

■A meaningful field quality assurance program is essential for the demonstration, maintenance and documentation of data quality. In addition to the measures described above, the following categories of samples are obtained to track performance.

TYPE	FREQUENCY OF COLLECTION
Bottle Blanks	20%
Filtration Blanks	20%
Analyte Spikes	15%
Complete Replicate	25%
Recovery Surrogates	100%

ENVIRONMENT

■Clean-lab processing, Class 100 or better environment (>150 air changes per hour), Class 10-100 clean benches for critical handling and additional scrubbing of clean-room air. Clean-lab dedicated high purity water system, and construction or elimination of all metallic surfaces.

Scrupulously cleaned double-bagged Teflon sampling apparatus, gloved and garmented personnel, and "clean-hands" - "dirty-hands" techniques are standard elements of clean field protocols. Particulate contamination in equipment preparation and sample processing is controlled by working under clean-room environments.

II. REPRESENTATIVE SAMPLING

The ability to accurately determine trace metal concentrations, fluxes and yields depends not only on the collection of un-contaminated samples but also on obtaining representative samples as well. We approach this complex problem through the use of various clean compositing techniques (large rivers), or with direct compositing iso-kinetic samplers (wadable systems). Issues such as contamination potential and logistics must be carefully weighed against the potential improvement in representiveness in moving from grab samples to more complex collection approaches.

In most of the wadable, non-point source impacted systems, where we have compared trace metal levels in grab samples with cross-sectionally integrated, isokinetically collected samples, little difference was observed. However, in point source impacted systems it is essential to collect representative integrated samples. In large rivers our evaluation of cross-sectional and depth variability in filtrable and particulate metal phases, particle partitioning, and sampling precision has yielded the following general conclusions:

■ Variability of metal levels between discrete, unfiltered quarter-point collected samples, was on the order of 5-20%, compared with 2-10% in sequential triplicate collections at a given point. Metals strongly partitioned to particles showed the greatest variation; filtrable levels were more consistent.

■ Much of the variability was in the vertical (depth), not cross-sectional direction, and because of this and other factors (see below) we typically employ a sampling strategy of direct compositing into the collection bottle of water obtained from 0.2 and 0.8 x the river depth at the center of flow.

■ While we were able to cleanly and accurately devise composite sampling schemes for quarter-point sampling of metals, the time, detailed attention, and cost involved were unacceptable when potential benefits were considered.

■ One of the compositing techniques evaluated, an epoxy coated USGS D77 sampler, despite clean handling, showed consistently higher metal levels. We therefore caution that the contamination potential of this device may be unacceptable in certain conditions.

III. ANALYTICAL METHODS

Levels of Ag in aqueous systems, generally accepted as reliable, are given in Table 2. Instrumental methods must be evaluated carefully in view of these extremely low levels. Most of the values in Table 2 were determined by graphite furnace atomic absorption on samples pre-concentrated using trace metal clean chelation/extraction (e.g. APDC/DDDC). For analytical detection we have pursued two distinct approaches: Graphite Furnace Atomic Absorption (GFAA) methods for Ag levels >10 ng L^{-1}, and Inductively-Coupled Plasma Mass Spectrometry (ICP-MS) for Ag levels down to 0.5 ng L^{-1}. Automated multiple-pipetting is applied to increase sensitivity in GFAA, and a simplified matrix modification protocol controls interferences. GFAA operating conditions are summarized below.

Table 2. Silver Levels in Aqueous Systems

System	ng L^{-1}	pM	log K_d
Open Ocean	**0.1-0.2**	**1-2**	**4-5.5**
Coastal	**0.3-2.0**	**3-18**	**-----**
Estuarine	**0.1-32**	**1-300**	**4.2-6.2**
Midwest Rivers: Total	**0.5-50**	**5-450**	**4.7-6.0**
: Filtrable	**0.2-5**	**2-45**	
Texas Rivers: Total	**0.2-100**	**2-900**	**4.4-6.6**
: Filtrable	**0.1-60**	**1-550**	
Lake Michigan	**0.2-0.5**	**2-5**	**5.8**

GFAA OPERATING CONDITIONS

PE 5100Z Spectrophotometer
AS400 Furnace
AS40 Autosampler

Source Lamp:	Hollow Cathode, 12 mA
Wavelength:	328.1 nm
Matrix Modifier:	High purity $NH_4H_2PO_4$ (400 μg in 20 μL)
Char Temperature:	750 °C
Atom. Temperature:	1800 °C
Sample Volume:	320 μL (8 x 40 μL pipettings)
Furnace Tube:	L'vov platform in pyrolized tube
Purge Gas:	Argon, grade 5

Table 3 summarizes figures of merit for GFAA when operated under specified conditions.

TABLE 3. Silver: Multiple-Pipetting GFAA. Analytical Figures of Merit

Criterium	Value
Typical Sensitivity (A•s/ppb)	0.7
Typical "Noise" (A•s)	0.003
S/N	250
Typical Blank Standard Deviation ng L^{-1}	2.5
IDL ng L^{-1}	7.5 (5 - 10)

IDL = 3 σ of 7 blank replicates

Ultrasonic nebulization coupled with a high efficiency interface and modern mass spectrometer provide a nearly 15 fold improvement in detection capability over multiple-pipetting GFAA methods. When Mo and Zr spectral interferences, and background noise, are monitored and controlled, the ICP-MS can accurately quantify Ag abundances and isotope ratios of most natural waters. Samples are run in batches of 12-15 samples along with an equivalent number of QA/QC samples. Sensitivity is monitored with three internal standards; Ga, In, and Bi spiked at a level of 2 μg L^{-1}. Surrogate recoveries of; Y, Ho, Yb, and Th are evaluated on every sample. Oxide/hydroxide formation is addressed by monitoring parent and both oxide and hydroxide masses of Y, Ce, and Th. ICP-MS operating conditions are summarized below:

ICP-MS OPERATING CONDITIONS

VG Plasmaquad II STE
Cetac Ultrasonic Nebulizer 5100AT

Analyte Masses:	107, 109
Other Masses:	90, 95, 98, 99, 101, 104, 105, 106, 108
Solid State RF:	27.12 MHZ
Forward Power:	1350 W
Reflected Power:	<2 W
Argon Cool Gas:	13 L/min.
Argon Auxiliary Gas:	1.2 L/min.
Argon Nebulizer Gas:	0.8 L/min.

Sample Uptake:	1.5 - 2.5 mL/min.
Operating Vacuum:	1.2 x 10^{-6} mbar
Quad Mode:	Peak Jump
Peak Dwell Time:	200 msec.
Points per Peak:	3
Acquisition Time:	90 sec., 5 cycles
Rinse Time:	480 sec.
Cones:	1 mm Nickel
USN:	140 °C heat, 2 °C cool
Replicates Acquisitions:	4

Table 4 summarizes figures of merit for ICP-MS when operated under specified conditions.

TABLE 4. Silver: ICP-MS. Analytical Figures of Merit (mass 107 or 109).

Criterium	Ultrasonic Nebulization	Pneumatic Nebulization
Sensitivity (cps/ppb)	250,000 - 300,000	25,000 - 35,000
"Noise" (cps)	150 - 250	40 - 60
S/N	1400	600
Blank STD ng L^{-1}	0.15	0.4
IDL ng L^{-1}	0.45 (0.2 - 0.8)	1.2 (1 - 2)

IDL = 3 σ of 7 blank replicates

In both analytical techniques, filtrate samples are introduced to the instrument with no pre-treatment or pre-concentration. Total samples are taken through an in-bottle (original Teflon sample bottle) digestion at 60°C for 12 hours with added Ultrex HNO_3 (1.6%), before instrumental analysis. This approach eliminates the contamination and recovery problems inherent in most pre-concentration schemes. The only surfaces the sample contacts prior to uptake into the instrument are the original Teflon sample bottle and polypropylene autosampler vial/tube.

Table 5 outlines the performance of the ICP-MS technique and associated field methods on a set of 49 river samples collected in Spring of 1993.

TABLE 5. ICP-MS Performance Evaluation: Silver

Sample Type	Value
Field Method Blanks (ng L^{-1}) MQ water through complete filtration process	mean = 0.74, n = 31 STD = 0.15 (USN); STD = 0.5 (PNU)
Replicate Precision (Relative % Difference)	mean = 10.1, STD = 9.8, n = 52
Analyte Matrix Spike Recovery (%)	mean = 88.7, STD = 8.3, n = 16
Silver 107/109 Isotope Ratio	mean = 1.059, STD = 0.081, n = 166 accepted = 1.056

No aqueous, environmental matrix, Standard Reference Material is certified for Ag at low ng L^{-1} levels. Accuracy of the Ag analyses is evaluated with dilutions of higher level certified SRM's, and with other internal checks. The Canadian NRC/IERT SRM SLRS-3 is run three times during a typical batch sequence of 15 samples, and serves well for most trace elements, however, Ag is not certified.

Table 6 presents a comparison of silver method detection limits.

Table 6. Silver Method Detection Limits

Method	Preconcentration	MDL (ng L^{-1})
ICP-MS (USN)	none	0.2-0.4
ICP-MS (PNU)	none	1-2
GFAAS	multi-inject	5-15
GFAAS	APDC/DDDC	0.02-0.2

It has been our experience that the trace metal clean field procedures outlined in this paper can be effectively carried out by well-trained field crews following carefully developed guidelines and documentation. This is only possible, however, if clean lab facilities are available to prepare and package the required field equipment and supplies. Silver quantification in all natural waters requires an extremely sensitive technique such as the latest generation of ICP-MS instrumention, or a pre-concentration procedure followed by either ICP-MS or GFAAS analysis. The pre-concentration technique will place additional requirements on the trace metal chemist in terms of contamination control and recovery quantification. For many natural waters, given the state-of-the-art in analytical instrumentation, a pre-concentration step will be required, especially for "dissolved" phases, and as research progresses toward further speciating Ag, clean methods of pre-concentration and matrix removal will become even more essential.

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Q. PHAT DAO (Eastman Kodak Co.): I'm very impressed with the facility that you have for sampling, for collecting samples. I just wonder if you have some kind of collaboration with NIST to try to use your facility to provide reference river or lake water for people like us, who might use it as reference water for measurements?

A. I think the Canadians do a good job in that respect. The National Water Quality Group has several reference materials that are available, but not certified, for silver that I'm aware of. But there are environmental matrices certified at realistic levels that one can use. But we haven't been in the business of creating reference materials at this point.

Q. TIM FITZPATRICK (Florida Dept. of Environ. Prot.): Two questions, I guess, at this point. My first question is, you showed a slide that indicated apparent silver concentrations dropping from 1972 from about 300 ng/L to the current levels of less than 1 ng/L. How much would you attribute that to clean collection techniques in the field and how much might be improvements in laboratory technology, background correction techniques, sample handling, and the higher purity ranges in the laboratory? My second question is related to the polysulfone filters. We found, for mercury anyway, when we're doing trace level work at 1 ppt or less that if the filters are acid-washed, we have seen some cases where we see losses of certain metals with sulfone filters, which is one of the reasons we switched to the nylon filters. Have you any experience regarding various filters at very low levels?

A. Yes, we've certainly done that with the suite of trace metals that we registered. The mercury data are being produced as we speak. We did have some suspicious mercury data with the polysulfone filters. It did not fit in with what we expected in terms of fractionation with sites or some of the other filter materials that we were using. So, while we certainly did the blanks, we probably did not do the adsorption experiments with mercury at that point before using them. We should have done that in the process, but the other metals haven't demonstrated a significant loss at the levels that we had spiked. As far as silver is concerned, a lot of that has to be attributed to techniques that were available. It mostly has to do with reagents, laboratory handling, maybe not so much for silver than the field component of it. The silver data that were produced there, some of it was chelation extraction. I can't see if that was clean and the laboratory environment wasn't clean. I don't think that some of the field problems that we have experienced with zinc and cadmium would be so severe for silver as for the other metals.

Q. KEN ROBILLARD (Eastman Kodak Co.): Two questions. First of all, when you do your QC samples, what types of recovery do you typically get, and if you see less than 100 percent do you correct your data for those results? And secondly, have you done any studies with information that would suggest what you gain by wearing the elaborate suits and gloves — does that really make a substantial difference in the results?

A. Your first question, for silver specifically, I think you're interested in?

Q. Yes.

A. For recoveries I had a slide, I just didn't show it. Average for that long survey with 25 field spikes was above 88 percent between 10 and 20 ng/L, that's with what we spiked it. We did not correct any of the

data for the low recovery. The other metals were typically in that range. I don't think we have a recovery problem with the filtrates or the total digestion that we do. The clean suits, the gloves are just for the mercury part of the field work; it's essential to use the gloves for handling. Other metals, I would take the gloves, too. The clean suits, I think, are mostly there out of caution: only once or twice in 20 samples might something get into the sample, but you just don't want it to happen. So I think you had better wear them on site to be careful rather than risking that occasional bad metal getting into your samples.

ERIC CRECELIUS (Battelle): Could there be a lot more concern for zinc and lead and some other elements you might carry on your clothes?

A. Certainly. It doesn't take but a microfiber of anything getting into the sample to completely destroy it.

Q. ARUN MUKHERJEE (Univ. of Helsinki): I think I have two questions. One question: The technology which you showed us — is it possible that I can apply this thing with industry? Other question: When you think of production and consumption of a metal, for example, silver, it has gone quite high in the world but you have found quite low values as I saw in your one slide. Is this due to the new technology in your lab where you use all these white suits and gloves and all these things? Or how do you regard this situation? I have seen in a sewage plant how the workers take a sample, and if I tell him that you have this type of suit and gloves and things he might just go to kill me. Because it's really expensive.

A. I don't think so; the field aspect of the sample is really not that expensive. It's more of an attitude that you get into: you have to think before you do something. The gloves, the suits, they're all very inexpensive, they are disposable. The samplers, admittedly, we now buy off the shelf, but they could be made for a few hundred dollars. The bottles are, admittedly, expensive, but they are probably a small component of the total cost of the whole analysis. I don't think it's very difficult or that it would be that difficult expensewise in total. In fact, we have it formatted so that very large projects for mass balances for the Great Lakes (Lake Michigan) have several state agencies using just these techniques. And they didn't kill us yet. They may in the future but they do that routinely, you know. I think after they've seen the results of the day, they recognized that it's important to do that.

Q. JIM KRAMER (McMaster Univ.): One of your steps that is very important is adding your spikes, as you pointed out, and carrying them through the whole procedure. Have you had any problems in particular with silver spikes of that level with high purity? We had those for a few years, we used them earlier and we had a lot of problems with that.

A. As I said, our approach has been to generally minimize adding anything at all to the sample, avoiding preconcentration steps if possible. We use for blanks Ultrex-cleaned acid verification, and that, typically, has levels of silver that contribute maybe 5-6 percent or 5-10 percent at max at the very low end — that would be at best 100 ng/L. The internal standards that we add to the sample, the ones that we use, we get them from High Purity Standards, a company on the East Coast here. They are proven to be very clean. For iron, they are only less than 1 ppb, so we're only adding 1 ppb and the potential problems are minimized there.

Q. GABOURY BENOIT (Yale Environ. Studies): I'm also a great believer in simplification in any of these procedures because that's one of the best ways to avoid contamination, as you mentioned as well. My question refers to the filtration step. It seems that you showed several different techniques which you've used there, and you also mentioned that it is one of the more problematic parts of the entire sampling-analysis chain. We've worked a lot with in-line filtration while we sampled. It's very simple. I'm wondering what your experience has been in that regard.

A. I might comment that the type of filter we're using, even if they were graded around 0.4 or 0.45 μm, can become clogged as well. The depth cartridges that we use, which have triple capacity, routinely gave much higher filtrable levels for lead and silver than do the polycarbonate trackage filters and that's not too logical. Most of the mercury data that we produced, and a fair portion of the metal data, again, were actually done in-line. But we've done limited comparisons of in-line filtration, and then versus the same sample that we collected, the silver sample we've collected with the grab sampler and then filtered in a glove box with the same type of filter, and I don't see large differences. Differences of what, 20 percent maybe at max — I'm not quite sure what you're after.

Q. I think that mainly answered my question. With the large sealed capsule that filters, you're saying you get high levels of dissolved metals. Is it merely coming from inability to completely clean those?

A. No, there's something about the physics of the collection of particles of those cartridges versus the membrane filter. For those metals that are more colloid-associated, we suspect that the behavior is different. So I'm saying filtration can give almost any answer you want, depending on what filter you use and how you load it.

Q. I guess I might rephrase the question. Going to the glove box and all of that in the field seems to me to require quite a bit of additional labor. I'm wondering under what circumstances that is desired compared to the filtration with Teflon tubing and Teflon holder and just doing it on-line.

A. The trouble with the in-line filter is that it's more difficult to collect a representative sample. You have to wade across the stream and collect; one can't really do that very easily with just an in-line filtration. But what we've been doing more recently, we collected an integrated sample and we filter that in-line through the Teflon tubing and the filters. The glove box technique was developed because we're doing a lot of grab samples, and once you have a grab sample, it's just a simple filtering in the glove box.

Q. TIM FITZPATRICK (Florida Dept. of Environ. Prot.): You didn't talk much about analytical schemes. Do you look at the recoverable fraction or do you HF digest it, or what?

A. What we call acid-labile, or unfiltered samples, are in bottles where we projected the acid concentration up to close to two percent. We cook them in an oven for almost a day, at least 12 hours — that's what we're using as our unfiltered levels. We have taken filters that were from the same collections and brought them through a Teflon bottle complete HF digestion, and, for the most part, the recoveries of the involved digestion are very close. There were sometimes some discrepancies for mercury but you should have expected that. You have to balance some of the clean aspects with the digestion technique.

Matrix Effects on the Measurement of Active Silver by Anodic Stripping Voltammetry

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Introduction

This study is a continuation of measurements of "active" silver in surface waters by Osteryoung square wave stripping voltammetry (OSWSV) (1). We define "active" silver as free hydrated silver ion and/or silver (+1) that exists as a labile complex in solution. In our previous work we determined the optimum experimental conditions for the silver measurements and estimated the method precision for the analysis of samples that contained between 0.2 and 5 ng/mL silver. We observed that day-to-day variability and the renewal of the electrode surface contributed significantly to the variability of our results. We also noticed that the stripping peaks changed depending on water quality standards.

The purpose of this study was to investigate matrix effects on the determination of silver, study recovery of silver upon spiking different kinds of samples, and gather more information on the "active" silver content of representative surface natural waters.

Background

OSWSV is an electroanalytical technique that combines high sensitivity (detection limit ca. 5×10^{-11} M), multielement measurement capability, and speciation of metal ions with speed (2). 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 that causes dissolution of the deposited metal.

In OSWSV, 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 (or the peak area) 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 concentrated on the electrode surface to a considerably higher concentration than it exists in solution. This technique has been successfully used and widely applied for trace metal measurements in a variety of aquatic samples.

Experimental

A detailed description of the experimental setup and the measurement procedure were provided before (1). Silver standard solutions were prepared daily by appropriate dilutions of a Spex Industries (1000 µg/mL) silver nitrate standard with Milli-Q and a 9:1 mixture of moderately hard synthetic water (3) to 0.05 M potassium hydrogen phthalate (primary standard ACS, 99.5-100.5% GFS Chemicals) buffer (pH 4.5). The resulting solutions had a silver concentration between 2 to 0.2 ng/mL and were protected from room light. Moderately hard synthetic water has a pH between 7.9 and 8.3, its water hardness is 80-100 (expressed as mg $CaCO_3$/L), and its quality alkalinity is between 60-70. An NIST (#1643c) trace element fresh water standard with a certified silver concentration of 2.21 +/- 0.3 ng/mL and SLRS-3 (National Research Council of Canada) river reference water were also used for calibration and testing. All lake and river water samples were collected in polyethylene bottles. They were analyzed "as is" (non-acidified) by ASV as soon as received after dilution with supporting electrolyte (9:1) for ionic silver. Total silver in the same samples (acidified to pH 2 for preservation) was measured by inductively coupled plasma atomic emission spectroscopy (ICP-AES) without acid digestion. All samples contained 1.0×10^{-6} M (ethylenedinitrilo) tetraacetic acid dipotassium salt (EDTA) for minimizing interferences from other metals. Samples were analyzed with and without filtering. A 0.1 µm filter (Millipore) was used for the filtered samples.

The deposition of silver took place at -0.400 V vs SCE at a rotation rate of 3000 rpm. The stripping peak was measured at a frequency of 50 Hz, an amplitude of 50 mV, and a step height of 2 mV. The deposition time was 10 minutes. After each stripping step the electrode potential was kept at +0.800 V vs SCE for five minutes to assure total dissolution of all silver deposited.

Results

Silver gives rise to one major stripping peak. There is a smaller secondary peak that may also be observed at less positive stripping potentials. The current and potential of these peaks depend on the concentration of "active" silver and the sample matrix used. Our previous studies showed that the electrolyte used has a strong influence on the efficiency of the deposition and stripping of silver, which has been related to coverage of the electrode surface based on the size of the counter ions neutralizing the charge on Ag^+ (3).

Calibration curves were obtained in three different matrices to be able to analyze surface water samples coming from different sources (Figure 1, Table 1). It was found that peak charge (integrated area under the stripping(peak) provided a more linear curve and a y-intercept that is closer to zero than peak current as a function of silver concentration. Since the peak shape and the presence or absence of the secondary smaller peak depend on the sample matrix, we feel the calibration curves based on the total integration of the area under the stripping peaks are more reliable for quantitative analysis.

The silver response in both the SLRS-3 and the moderately hard synthetic water were similar with almost the same slope for the calibration curve (Figure 1). However, the response obtained for a NIST 1643c fresh water reference sample was smaller. We believe this difference is because of the high acidity of the NIST 1643c sample and the absence of ions that are normally present in natural surface waters. NIST 1643c is an artificially prepared reference material (4), and contains a significantly higher concentration of additional metal ions. We believe SLRS-3 matrix can be used as a reference matrix for measuring ionic silver in natural water samples, i.e., river or lake water. However, if there is a need for adjusting parameters such as hardness or complexing capability, synthetic water can be used to mimic natural waters using different amounts of $CaCO_3$, Cl^-, and/ or humic acid.

Lake and river water samples were analyzed both for their total silver content by ICP-AES and their "active" silver concentration by OSWSV (Tables 2 and 3). The water samples were collected on different days at different locations in polyethylene bottles. Total silver concentrations in the undigested and unfiltered surface natural waters ranged from 0.3 to 2.8 ng/mL Ag. The samples were also spiked with a known concentration of Ag^+. The percent Ag^+ recovery was between 68 to 94%. Less than complete recovery in undigested and unfiltered samples by ICP-AES measurement suggests that some spiked silver adsorbs onto undissolved particulates. There was no detectable "active" silver in the same samples. Percent recovery for the OSWSV measurements of silver spiked river waters was significantly lower than the values obtained by ICP-AES (between 5 to 76%), and the percent recovery changed with respect to location and date. This may suggest that in addition to adsorbing onto particulates, silver ions may also form non-labile complexes with ligands present in river water. It was observed that lake water provided the most stable matrix (recovery of spiked silver was between 71-76%) (Table 3).

Filtered silver spiked lake water resulted in higher peak current and charge compared to silver spiked unfiltered samples. This gave a higher Ag^+ recovery, 84% vs 71% (Table 4). We believe this behavior consistent with our theory that spiked silver adsorbs onto undissolved particulate material and thus not freely available. Silver recovery in spiked natural waters was found to decrease

with time (Figure 2). This observation may indicate that silver slowly adsorbs onto particulate material, and/or forms non-labile complexes and its "active" concentration reaches an equilibrium between 4-8 hours.

The effect of $CaCO_3$, chloride and humic acid on the "active" silver was studied using a 1.0 ng/mL silver spiked Milli-Q water sample. Both the peak currents and peak areas were measured. Changing the concentration of $CaCO_3$ from 100 to 200 mg/mL did not cause any significant change in the current and potential of the stripping peak. However, when increasing the chloride concentration from zero to 40 mg/mL, the peak current increased and the potential shifted to more negative values. In addition, the stripping peak became more symmetrical and narrow. Integrated area under the stripping peaks was approximately the same (Figure 3). The shift towards more negative potentials is consistent with silver complexing with chloride, thus the stripping is becoming easier. The narrow peak shape may indicate formation of surface insoluble AgCl. Toxicity studies (5) indicated that there was no significant difference between the toxicity of silver under these experimental conditions. However, when soft and hard water samples were compared, there appeared to be 30% less "active" silver available when the water hardness and the level of chloride and humic acid were increased. These results may indicate that in hard waters and high concentration of undissolved particulate materials the bioavailable silver concentration may be reduced, thus making the surface waters under such conditions less toxic.

Conclusions

Based on the experimental observations, we can draw the following conclusions:

1. Sample matrix strongly affects the characteristics of an anodic stripping voltammogram of silver (both the stripping potential and the peak shape are affected).

2. $CaCO_3$ and Cl^- (between 50 to 200 mg/L $CaCO_3$ and between 0 to 40 mg/L Cl^-) does not appear to have an effect on the charge passed when their levels were varied, keeping the silver concentration constant.

3. Presence of humic acid (above 2 mg/L) decreases the amount of silver measured and also gives a large background signal.

4. Upon filtration the spiked silver recovery improves.

5. SLRS-3 appears to be a good reference material that closely mimics surface waters.
6. There is no "active" silver (>0.2 ng/mL) found in any of the lake or river samples analyzed.

7. Unfiltered, silver spiked surface water samples have decreasing silver signal over time.

8. It is recommended that for any quantitative work, the charge (i.e., the area under the stripping peak(s)) should be measured as a function of concentration instead of using the peak currents.

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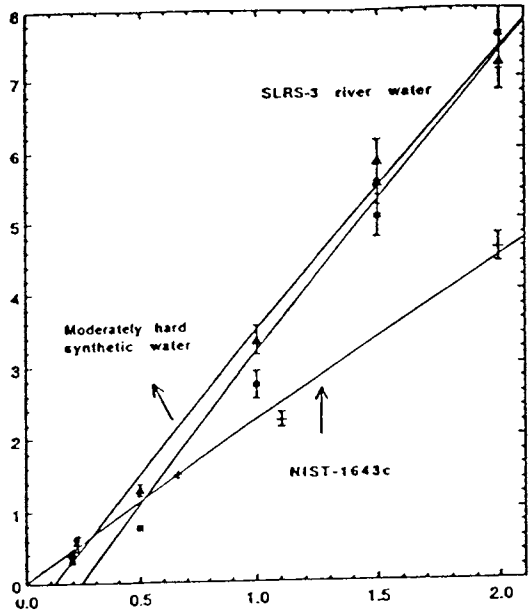
Figure 1. Comparison of calibration curves in different sample matrices.



SLRS-3
Peak current =-1.03 + 4.2 [Ag^+] r^2 = 0.98
Moderately hard synthetic water
Peak current = -0.49 + 4.0 [Ag^+] r^2 = 0.99
NIST-1643c
Peak current = 0.009 + 2.2 [Ag^+] r^2 = 0.99

Table 1. Sample matrix effect on measured peak current and potential

Sample Matrix	Peak Potential (mV vs SCE)	Peak Current (µA)	pH
Milli-Q water	276 +/- 6	5.5 +/- 0.2	4.5
NIST 1643 c	221 +/- 2	4.6 +/- 0.2	0
Moderately hard synthetic water	246 +/- 5	7.3 +/- 0.3	4.5
SLRS-3	245 +/- 3	7.7 +/- 0.5	1.6

Table 2. Silver measurements in lake water by ICP-AES and OSWSV.

	Total Silver Measured by ICP			Ionic Silver Measured by ASV		
Sample	Ag^+ spike (ng/mL)	Ag^+ found (ng/mL)	Recovery (%)	Ag^+ spike (ng/mL)	Ag^+ found (ng/mL)	Recovery (%)
Lake water #1	0	0.8	-	0	<0.2	
	5.0	4.5	74	2.0	1.5	73
#2	0	0.6	-	0	<0.2	
	5.0	4.0	68	2.0	1.4	71
#3	0	0.5	-	0	<0.2	
	5.0	5.2	94	2.0	1.5	76

Table 3. Silver measurements in river water, location B by ICP-AES and OSWSV.

	Total Silver Measured by ICP			Ionic Silver Measured by ASV		
Sample	Ag^+ spike (ng/mL)	Ag^+ found (ng/mL)	Recovery (%)	Ag^+ spike (ng/mL)	Ag^+ found (ng/mL)	Recovery (%)
River water location B #1	0	0.3	-	0	<0.2	-
	5.0	4.9	94	5.0	1.7	33
#2	0	2.8	-	0	<0.2	-
	5.0	6.6	76	5.0	1.2	24
#3	0	1.0	-	0	<0.2	-
	5.0	5.3	86	5.0	0.3	7.0

Table 4. Spiked silver analysis results, effect of filtering.

Sample	Spiked $[Ag^+]$ (ng/mL)	Peak potential (mV vs SCE)	Peak current (μA)	Charge (10e-7C)	$[Ag^+]$ found (ng/mL)	Ag^+ recovery (%)
Lake water unfiltered	2.0	208 +/- 5	4.9 +/- 0.2	2.9 +/- 0.1	1.4 +/- 0.1	71
Lake water 0.1 μm filtered	2.0	206 +/- 5	6.0 +/- 0.3	3.5 +/- 0.2	3.5 +/- 0.2	85

Figure 2. Change in the silver concentration as a function of time analyzed by ICP-AES and OSWSV.

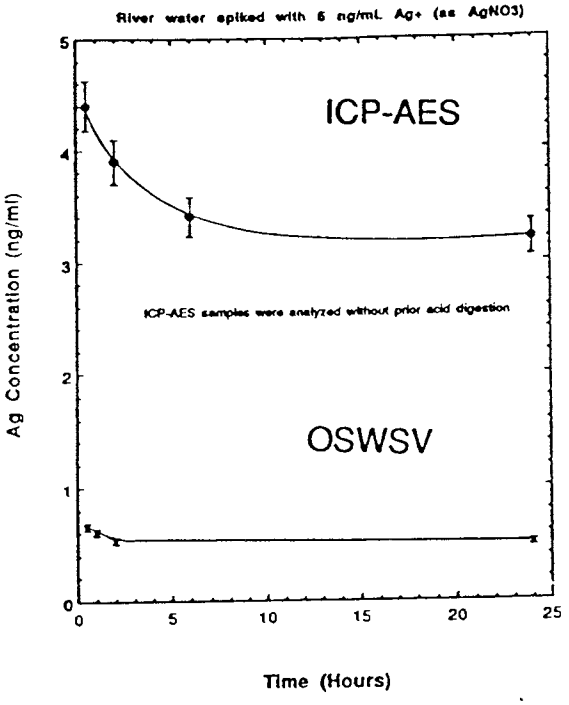
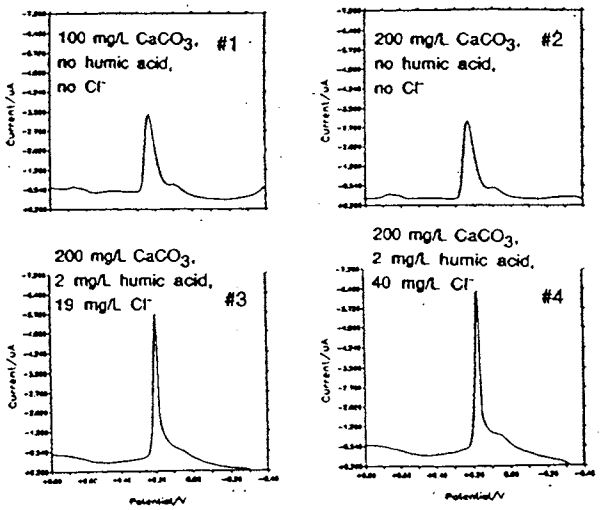


Figure 3. Effect of varying CaCO3 and Cl in synthetic water on silver analysis by OSWSV.



Sample	Peak Potential (mV)	Peak Current (μA)	Peak Charge (10e-7 C)
#1	233	2.8	2.5
#2	236	3.0	2.5
#3	198	5.2	2.6
#4	176	6.0	2.6

Questions & Answers: Matrix Effects on the Measurement of Active Silver by Anodic Stripping Voltammetry

Q. TIM FITZPATRICK (Florida Dept. of Environ. Prot.): Have you attempted to correlate any of your results for a hydrated silver ion with those of any thermodynamic models for your synthetic waters?

A. You mean trying to model?

Q. You showed some results for some of the synthetic waters that you formulated in the laboratory. If you perform thermodynamic modeling calculation on those metals you can arrive at a hydrated silver ion concentration. How do those correlate with your results from the anodic stripping experiments? [end of tape; and malfunction of recorder; answer and last question missing]

Preconcentration and Voltammetric Measurement of Silver Ion (I) at a Chemically Modified Carbon Paste Electrode

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Speciation of silver in water samples is an important environmental subject [1-3]. Anodic stripping voltammetry with an inert solid electrode is a means to determine hydrated silver (Ag^+) concentration, the most toxic form of silver, in environmental water samples down to sub-ng/mL levels [1-3]. A carbon paste (a mixture of graphite powder with a pasting liquid) electrode offers advantages of low background current, better precision, and easy renewal of the electrode surface in comparison to other electrodes such as glassy carbon, platinum or gold. During the last few years, chemically modified carbon paste electrodes have become an active research area [4] and may provide increased selectivity and sensitivity for metal speciation analysis. A chemically modified carbon paste electrode can be prepared by mixing immobilized reagents into a carbon paste matrix. Accumulation of an analyte can be achieved via ion-exchange, complexation, etc. [4-8] at chemically modified carbon paste electrodes. For example, researchers have utilized zeolite [5] and ploy vinylpridine [6] as ion-exchange modifiers, and employed 2,9-dichloro-1,10-phenathroline [7] and thiacrown compounds [8] as complexing agents to study the accumulation and stripping voltammetric behavior of silver with chemically modified carbon paste electrodes.

In this work, chemically modified carbon paste electrodes were prepared by incorporating a perfluorosulfonate ionomer (Nafion, a registered trade mark of the E. I. Du Pont de Nemours & Co.) directly into the carbon paste matrix. Silver can be preconcentrated with a Nafion-modified carbon paste electrode at a potential of -0.4 V (vs. SCE). The deposited silver was then stripped by a square-wave voltage (amplitude, 50 mV; step height, 2 mV; and frequency, 60 Hz) scan from -0.4 V to +0.8 V, and a stripping voltammogram was recorded. Electrochemical measurements were performed with a PARC Model 273 potentiostat and Model 270 electrochemical software; and a Pine Instrument rotating disk unit. The electrode was rotating at a rate of 3000 rpm during the deposition procecess while stopped rotation mode was used at the stripping step.

The content of Nafion in the carbon paste influences the stripping signal; the best concentration was obtained with 5% (w/w) of Nafion in the paste (alcohols in the Nafion solution was evaporated at 60 °C after it was mixed with graphite powder and mineral oil); that is, the stripping peak current was the largest with the lowest background. A comparison of square-wave anodic stripping voltammograms of a Nafion-modified carbon

paste electrode and a non-modified carbon paste electrode was performed in a 9:1 (by volume) Saint Lawrence Riverine (SLR) water to pH 4.5 potassium hydrogen phthalate (KHP) solution spiked with 2.0 ng/mL silver standard, as shown in Figure 1 curve a and b, respectively. The peak current obtained at the Nafion-modified carbon paste electrode (curve a) is approximately three times greater than that obtained at the carbon paste electrode (curve b). A decreased stripping peak was, however, observed when the Nafion-chemically modified carbon paste electrode was immersed in a 9:1 moderately hard water (dilution of the Perrier Mineral water to 20% with the Milli-Q deionized water) to pH 4.5 KHP solution containing silver standard. We presently do not have an explanation for this trend.

Reproducibility of the same Nafion-modified carbon paste electrode was studied in a 9:1 SLR water to pH 4.5 KHP solution spiked with 2.0 ng/mL silver standard. Five measurements resulted in peak currents of 3.1, 3.2, 3.3, 3.4 and 3.5 (μA) with a relative standard deviation of 4.8 %. Square-wave anodic stripping voltammograms of a Nafion-modified carbon paste electrode with preconcentration time of 10 minutes in a 9:1 SLR water to pH 4.5 KHP solution spiked with 0.0, 0.2, 0.5 , 1.0 and 2.0 ng/mL silver standard were obtained. The corresponding calibration plot is constructed with peak area (μC) vs. concentration, as shown in Figure 2. Each data point was taken as the average of two measurements. The slope of the calibration curve for a Nafion-modified carbon paste electrode for 0.2-2.0 ng/mL silver in a 9:1 SLR water to pH 4.5 KHP solution is approximately twice that of a carbon paste electrode in a 9:1 moderately hard water to pH 4.5 KHP solution.

Based upon the ion exchange property of Nafion, a possible mechanism of the electrode process is

Preconcentration step	$nAg^+ + \text{Nafion} \rightarrow nAg^+\text{—Nafion}$
	$nAg^+\text{—Nafion} + ne^- \rightarrow nAg^0 + \text{Nafion}$
Stripping step	$nAg^0 \rightarrow nAg^+ + ne^-$

In the preconcentration step, a reaction between silver ion and Nafion forms a complex via ion exchange. Subsequently, the silver ion complex with Nafion (nAg^+—Nafion) is reduced to metal silver (Ag^0), which is reoxidized to silver ion (Ag^+) during the stripping process.

In conclusion, the Nafion-modified carbon paste electrode offers increased sensitivity over a carbon paste electrode for the speciation of silver in the SLR water sample. The Nafion-modified carbon paste electrode may have potential use for the speciation analysis of silver in other water samples.

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Acknowledgment

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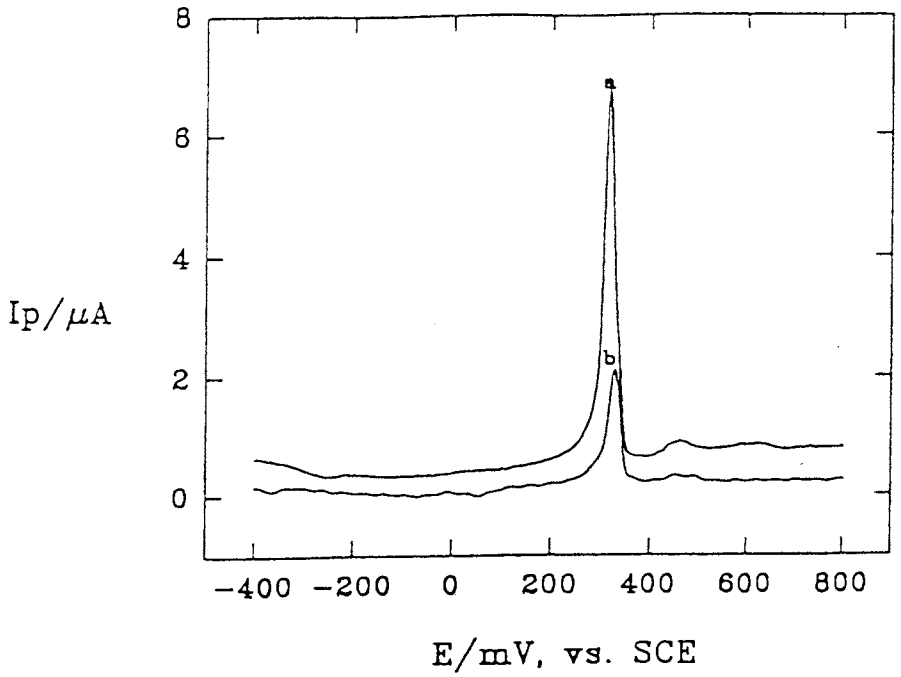

Figure 1 Square-wave anodic stripping voltammograms of silver at a Nafion-modified carbon paste electrode (a) and a carbon paste electrode (b)

Solution: 9:1 SLR water to pH 4.5 KHP solution spiked with 2.0 ng/L silver. Deposition potential, -0.4 V vs. SCE; Deposition time, 5 min.

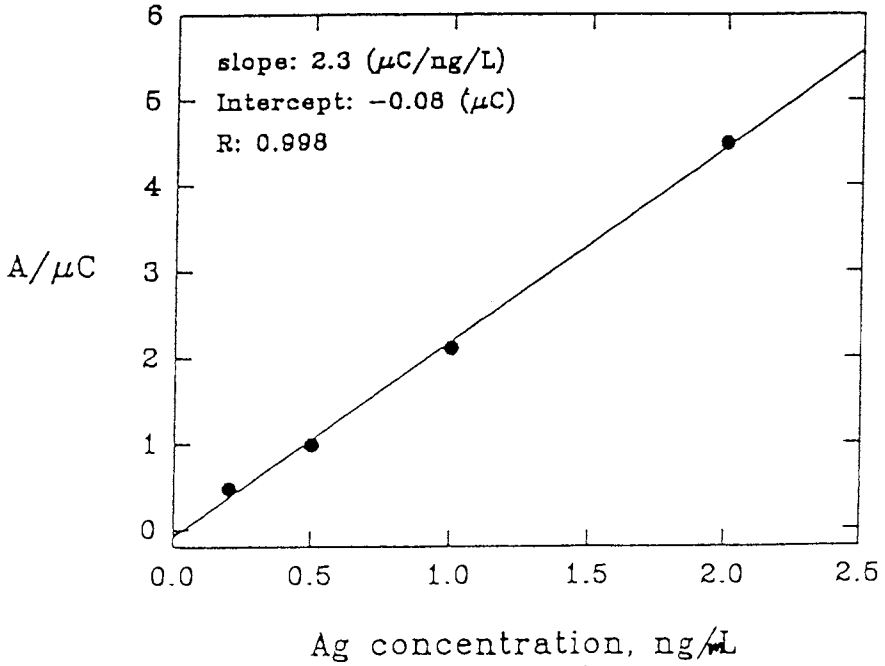

Figure 2 Calibration curve of Ag^+ in 9:1 SLR water to pH 4.5 solution

Questions & Answers: Preconcentration and Voltammetric Measurement of Silver Ion (I) at a Chemically Modified Carbon Paste Electrode

Q. NORMAN NEWMAN (3M): What I see happening here is a rather complex set of equilibria between silver that you have in solution and silver that gets adsorbed to the Nafion. In addition, the intent of this method is to discriminate between the species of silver. Have you done any work which is actually showing that the method is discriminating between the different species of silver by putting in mixed species of silver, hydrated silver ion versus some of the complexed forms, and showing that the method is actually discriminating between them?

A. Well, actually I haven't done the whole work, but I guess because the electrodes in this method are relatively positive or, rather, positive in their potential for most metals. Sure, because we deposit a silver complex — they deposit at more negative potentials — so at 0.4 V mostly you just deposit silver or plate out silver. Is that clear?

Q. I see your argument, but the problem is that your 0.4 V is sufficient to deposit silver from the Nafion complex and that's what you're actually electroplating out. If you have complexes of silver in solution in which the equilibrium is to move to form the complex with the Nafion, then any complex in solution in which the direction is to form a Nafion complex will also be picked up at your 0.4 V, and the only ones that won't would be such complexes that are forming a stronger complex such that they don't form complexes with Nafion.

A. I guess I see your point. Actually the complex between Nafion and silver is not that stable. I don't know what the complex constant is, but I'm sure it's not just an ion exchange complex. Most silver complexes like silver sulfate and silver thiosulfate are very stable complexes so they may not complex with Nafion.

PHAT DAO (Eastman Kodak Co.): I'd just like to add some comment on the silver measurement by ASV [anodic stripping voltammetry]. In my case, I'm just using carbon paste, but from my presentation you see that there is evidence that silver comes from different silver complexes — in my case, the silver standard is coming from solid silver nitrate. When I add the silver standard you can also see from my data that I have silver that's complexed with chloride, and right away you see the big potential comes out differently: it shifts to negative potential. So this technique does give you an indication of different types of silver that can be measured, that come from different types of silver complexes. That helps you to understand more about silver.

RICHARD PLAYLE (Wilfrid Laurel Univ.): I'll step in on the comments. It would appear that the method you're using does measure something a little bit more than just free ionic silver from what I can tell. That could be turned to an advantage if we're looking at biological silver, for from my work with fish gills it's obvious that silver binds fairly strongly, free silver, free ionic silver may get connected biologically. If this method more or less approximates reactions of silver with the biological membrane, that could be very useful, which I think it can.

NORMAN NEWMAN (3M): I'm only concerned about being able to distinguish between the different forms of silver.

RICHARD PLAYLE (Wilfrid Laurel Univ.): Yes, we'd better look at use of thiosulfate in these solutions and then see whether you can keep all you get. Certainly, it could be qualified fairly easily, it would appear, but it looks like that really needs to be done. But it could be, you know, that it depends on measuring more than ionic silver, free ionic silver, and that can certainly be turned to an advantage as opposed to sometimes a disadvantage.

A Review of Toxicity and Epidemiological Data for Silver in Animals and Humans

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While regulatory efforts to control the release and transport of silver in the environment are initially aimed at protection of aquatic life and ecosystem health, such efforts are also intended for the protection of human health. Therefore, it is important to consider available mammalian toxicity and human epidemiology data for silver when assessing and prioritizing potential hazards to public health. This abstract will review (1) toxicity data for silver in animals, focusing primarily on oral studies using soluble forms of silver, (2) epidemiological studies involving occupational exposures to silver, and (3) the derivation of the U.S. Environmental Protection Agency's (EPA) Secondary Maximum Contaminant Level (SMCL) for silver in drinking water.

Acute oral LD_{50} studies using silver nitrate, silver oxide, silver fluoride, and silver chloride in various animal species have reported LD_{50} values in dose ranges that are considered indicative of slight to moderate toxicity. In subchronic drinking water studies with animals given soluble forms of silver (primarily silver nitrate), effects reported following exposure to 0.015 to 0.02% silver included deposition of silver grains in the conjunctiva (rat) and kidneys (rat), and hypoactivity (mice). It should be noted that these effects have not been consistently reported or observed in other animal studies. Additionally, the lowest observed effect level tested in animals (0.015%) from these studies is 1500 times greater than the current SMCL for silver in drinking water. Although silver deposits in various organs in animals, it is not associated with target organ toxicity.

In humans, acute occupational exposure to silver nitrate has been associated with skin, eye, and respiratory irritation, most notably at high airborne concentrations. Chronic silver exposure in humans has not been associated with target organ toxicity and no

significant hematological or hepatic effects have been reported in two occupational studies involving exposure to both soluble and insoluble silver. Epidemiological studies typically evaluate chronic exposures and are useful and considered more relevant than animal data when evaluating potential health effects in humans. For silver, there are several adequate epidemiology studies. The available studies have involved exposure to numerous silver species (silver nitrate, silver oxide, metallic silver, insoluble silver halides) at estimated air concentrations ranging from 0.001 to 0.378 mg/m^3 as 8-hr time weighted averages. Duration of exposure in terms of years of employment ranged from 5 to 20 years. Our experience involving silver reclamation employees has shown increased levels of silver in blood, feces, and hair with no evidence of adverse health effects. In addition, respiratory function and clinical chemistry (hematology indices, hepatic enzyme levels) analyses were not different from referent groups. The principal effect observed in our study and in other epidemiological investigations is argyria, a condition characterized by bluish-gray pigmentation of the skin, mucous membranes, and eyes (primarily conjunctiva).

Argyria results from tissue deposition of a silver-protein complex or its metabolized product (silver sulfide or silver selenide) following long-term exposure (absorbed amounts in excess of 1 g) to silver or silver-containing compounds. Argyrosis is a term that refers to ocular silver deposition while argyria describes the systemic distribution of silver that manifests itself as pigmentation of those sites most exposed to sunlight. Argyria occurs most commonly following airborne exposure in occupational settings; the only cases of argyria resulting from ingestion of silver-containing compounds occurred in individuals, usually with compromised health status, taking oral medications containing high concentrations of silver. Argyria or argyrosis have not been reported as a result of exposure to silver in the environment. Most importantly, argyria and argyrosis have not been associated with adverse health effects or compromised health status and are considered cosmetic effects by the EPA.

Silver is not extensively metabolized in mammalian species and this may contribute to its low degree of toxicity in animals and humans. Silver is associated with low absorption (less than 10% of administered or ingested dose in animals) although the presence and extent of silver-binding proteins and the solubility of the particular silver species are important modifiers of absorption. Once absorbed, silver passes through the liver and spleen and if not eliminated, is then systemically distributed. Elimination of silver from the body is primarily (> 90%) through fecal excretion with urinary excretion only a minor

factor in clearance of silver from the body. The half-life of silver in the lungs and liver is approximately 1 day and 50 days, respectively.

In 1991, EPA deleted the Primary Drinking Water Standard of 50 µg/L silver and replaced it with a SMCL of 100 µg/L based on the endpoint of argyria. The derivation of this number included the following assumptions: (a) the development of argyria in the most sensitive individual could occur following absorption of 1 g of silver (based on clinical case reports); (b) the oral absorption rate is 4%; (c) the exposure period is 70 years; and (d) the body weight is 70 kg. In addition, it is assumed that 100% of a person's silver intake is from drinking water and that the average person ingests 2 L of water per day. A cosmetic reference dose (safe exposure level) is generated, which includes a safety factor of 3. Finally, an adjustment (subtraction from that amount permitted in drinking water) is included for the presence, and assumed ingestion, of silver in food. In addition to EPA, both the American Conference of Governmental Industrial Hygienists and the Occupational Safety and Health Administration have established permissible air exposure limits for silver based on the development of argyria as the endpoint of concern.

In summary, although some species of silver are irritating, e.g., silver nitrate, the only significant effect from exposure to silver is argyria, a cosmetic effect, which does not impair the functioning of the body. A number of occupational studies of employees exposed to silver have clearly indicated that this cosmetic effect is limited to the skin and mucous membranes without evidence of health impairment. Our recent epidemiology study in silver reclamation employees confirmed these findings. Regulatory and standard setting organizations have used argyria as the endpoint for establishing acceptable exposure levels for both occupationally exposed employees and the general public.

Q. ERIC CRECELIUS (Battelle): Do you have any speculation on why silver seems to be that way?

A. I guess the answer to that question is, I've thought about it. Certainly the insoluble nature of many silver compounds, combined with the fact that it does not seem to target a particular organ, may be keys to its lack of human toxicity. It's certain that some of the other metals do target specific organs, such as lead — it's a soft tissue toxicant, a liver toxicant. Cadmium is a known kidney toxicant and tends to bind there and exert effects, although a lot of it may still be insoluble. With silver, we're not adsorbing enough and it just doesn't exert clear toxicity.

Q. CHRIS WOOD (McMaster Univ.): Is there any information of exactly how it's carried? You just said it is not excreted at all in the urine, so does that mean it is bound to proteins, or is it in cells? What form is it in the blood in occupationally exposed people?

A. That's a good question, Chris. I don't know the answer to that. It is carried by proteins, so it must be in cytoplasm. As to the form, I don't know. That's clearly an important system to look at and why some of those occupational studies have focused in on that and say it's a site of potential toxicity. Again, only solubilized silver can enter the bloodstream.

Q. JIM KRAMER (McMaster Univ.): Maybe you said that and I missed it. In going through the logic of studying the drinking water, food, and so on, is that the logic that assumes all the silver — or is that the total silver level or any silver in particles, wherever it is — will be reactive?

A. You mean, is this all of the silver a person will be exposed to?

Q. Yes. In going through the calculation you showed us, that assumes all of the silver will be effective?

A. Certainly, right.

Factors Affecting Silver Accumulation and Metallothionein Induction in Freshwater Fish

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Although significant advances have recently been made to elucidate the mechanism(s) of acute Ag toxicity in fish, the physiological effects of elevated tissue Ag concentrations are less well understood. Previous studies have shown that Ag is readily accumulated in both gills and livers of fish, resulting in a significant induction of metallothionein in these tissues. This low molecular weight protein is believed to play an integral role in internal metal homeostasis and detoxification processes.

Current studies are being performed to assess factors influencing the accumulation and tissue-specific distribution of Ag during acute exposure to $AgNO_3$, $AgCl$, and Ag_2S. In addition, the effects of these Ag exposures on MT synthesis are being assessed. The objective is to analyze Ag and MT levels in gills, livers, kidneys and white muscle of juvenile and adult rainbow trout.

(Supported by a grant from the National Association of Photographic Manufacturers/Silver Coalition.)

Questions & Answers: Factors Affecting Silver Accumulation and Metallothionein Induction in Freshwater Fish

Q. GEORGE COBB (Clemson Univ.): In your gill uptake of silver it looks like there is a possibility that you have a classical uptake depuration-type phenomenon occurring, and that's also approximately the position of the range where you saw a lot of noise in your plasma silver concentrations. Is there a possibility that there are some site-dependent or age-dependent differences in your physiology?

A. Which one, the plasma?

Q. The tissues above, it looks like in the middle of the weight range there's something.

A. I'm wondering whether there is something going on there with the fish, if at that period of time it is going through some stage which would affect accumulation. I really can't say, but it does appear now that around 15 g there's a definite change in the data, despite the trend it does appear to be quite noisy. So it might suggest that something's going on to affect that accumulation.

Interaction of Silver and Metal Chelators on *Ceriodaphnia dubia* Survival and Reproduction

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INTRODUCTION

Silver is a metal used in many different processes. Some of these, such as photographic development, combine silver with a metal-binding chelator. Improper disposal could result in these compounds entering the aquatic environment. To understand the how aquatic life is affected by silver and chelator combinations, a set of tests were run using two common metal-binding Chelators, ethylenediaminetetraacetic acid (EDTA) and nitrilotriacetic acid (NTA), with silver in *Ceriodaphnia dubia* chronic toxicity tests.

MATERIALS AND METHODS

Test organisms (*Ceriodaphnia dubia*) were raised at the University of Kentucky from a single daphnid obtained from the U.S. EPA Environmental Monitoring and Support Laboratory in Newtown, Ohio. They were kept in an environmental chamber that had a constant temperature of 25 ± 0.5 °C and a photoperiod of 16:8, light:dark. The animals were cultured and tested using standard EPA methods (1) with some modifications. The water was ultrapure water that was reconstituted to a medium-hard hardness (80-100 mg/L $CaCO_3$) with 2 µg/L selenium added as sodium selenate. During both the culturing and the testing, *C. dubia* were fed the YTC (yeast-Tetramin fish flakes-cerophyll) mixture and a green algae, *Selenastrum capricornutum* (6 x 10^5 cells/mL). Culture water and test solutions were changed daily.

The silver nitrate was obtained from Aldrich, the disodium EDTA was purchased from Fisher while the NTA was acquired from ICN Pharmaceuticals. The tests conducted were the 3-brood *Ceriodaphnia dubia* survival and reproduction short-chronic test (1). Increasing amounts of chelator was added to a silver solution and allowed to equilibrate for thirty minutes prior to being used. The concentrations of silver utilized were based on the IC_{50} concentration obtained from silver tests using a dilution series performed prior to the chelator/silver experiments. The concentrations of EDTA and NTA were tested without silver to determine if the chelator itself was causing any toxicity. Also the ionic forms of the silver and the chelators were assessed using the MINEQL+ Chemical Equilibrium Modeling System program.

RESULTS AND DISCUSSION

The tests using EDTA indicated that this chelator increased the toxicity of silver by decreasing survival time (Fig. 1 and 2). Although a decrease in reproduction was observed, this decrease was due to a decrease in survival rather than a decrease in brood size. In contrast, the effect of NTA on silver toxicity resulted in an increased survival time for *C. dubia* (Fig. 3). However, like EDTA, no real effect was observed on the depressed reproduction caused by silver. In all tests, the concentrations of the chelators alone were found to be nontoxic.

The results of the NTA with silver tests seemed to indicate that NTA was binding with the silver, thus limiting its availability to the daphnids and increasing survival. Since NTA did not reduce the reproductive toxicity, it is probably a weak chelator of silver. This is supported by the MINEQL+ program that predicted that NTA would bind with less than 10% of the silver in solution, but that some chelation would occur.

Among the preconceived possibilities for the action of a chelator on metal effects was a reduction in toxicity as seen with NTA and silver. Another expected possibility was the chelator would have no effect on silver toxicity. However, EDTA increased the toxicity and reduced the survival of the *Ceriodaphnia*. Why this happened could be explained by several different hypotheses.

The first hypothesis was suggested by individuals who knew that EDTA was used in culture media for mammalian cell lines and algae. This is that EDTA increased the cell permeability to certain metals. This idea was not supported by any evidence in the literature for a membrane receptor with which EDTA could bind and cause a channel to open for silver ion entry.

A second hypothesis is that EDTA is remobilizing the silver that is attached to sides of the test chambers. This also is not likely in that EDTA does not have a high affinity for silver. In fact the stability constant for EDTA with silver (logK = 7.32) is less than that for copper II (logK = 18.4), zinc (logK = 16.2), calcium (logK = 10.6) and magnesium (logK = 8.69) (2). With both calcium and magnesium in relatively large quantities compared to silver, EDTA probably wouldn't interact with the silver. In addition, when the ion concentrations were assessed using MINEQL+, the model showed that none of the silver was likely to have bound with EDTA.

A more probable hypothesis is that EDTA is binding with a metal that is essential for allowing the cells to fight silver toxicity. Metallothionein is a metal-binding protein that is strongly induced by silver (3) and should bind with silver (4). But either copper or zinc is required for transcription of the metallothionein gene to occur (5, 6). Therefore, since EDTA binds rather strongly with both zinc and copper, induction of metallothionein may not be possible.

A final hypothesis is based on the fact that silver is antagonistic to copper metabolism (7). In this scenario, EDTA binding to copper would act synergistically with silver to upset copper metabolism which decreased the survival time for the daphnids. Since induction of metallothionein takes several days to weeks (8) and the effects of EDTA addition are seen in two to three days, this last hypothesis seems to be the most probable.

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Fig. 1 Effect of EDTA on the Lethal Time-50% (LT50) of Silver (8.00 μg/L) with Ceriodaphnia dubia.

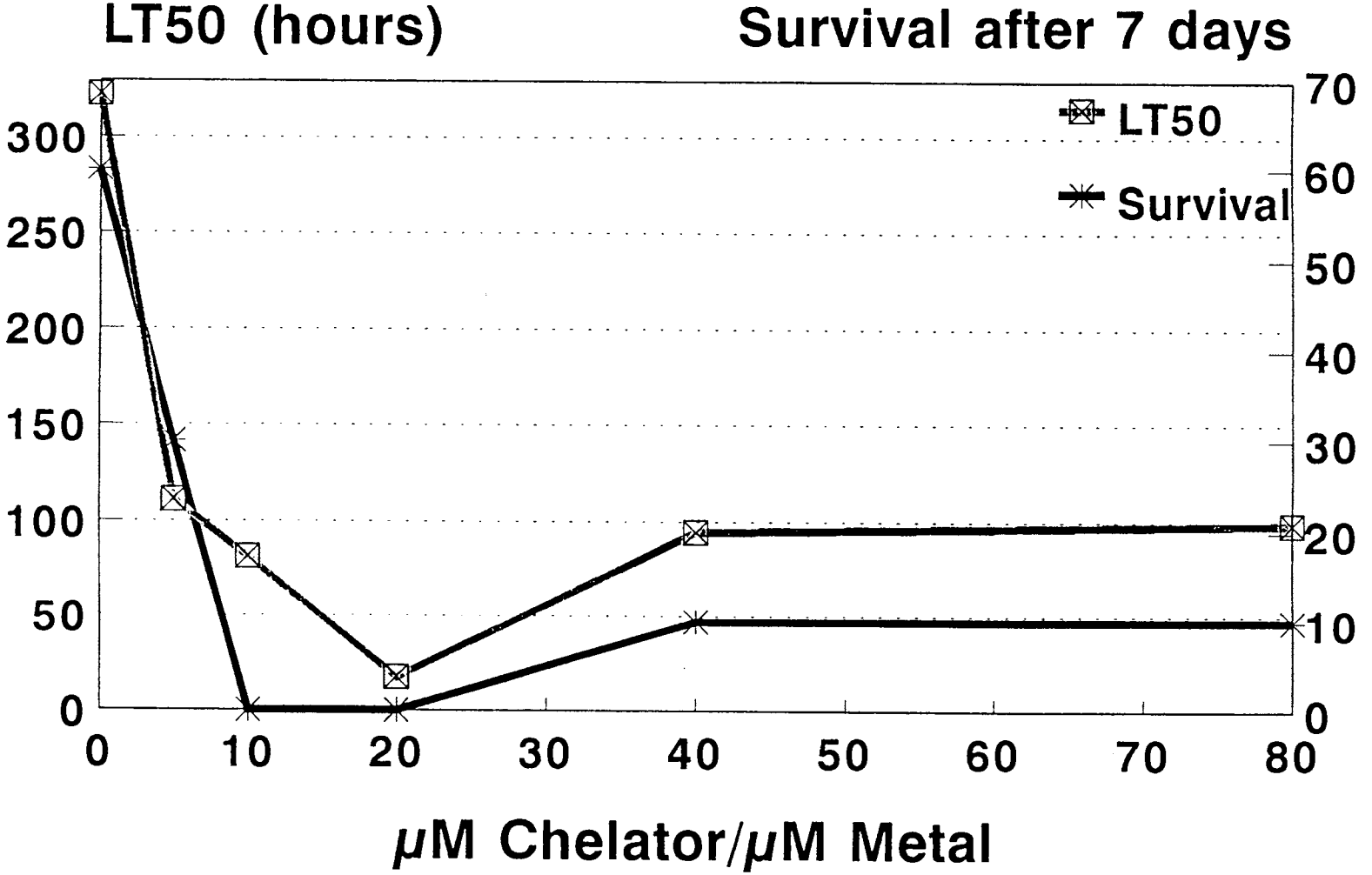
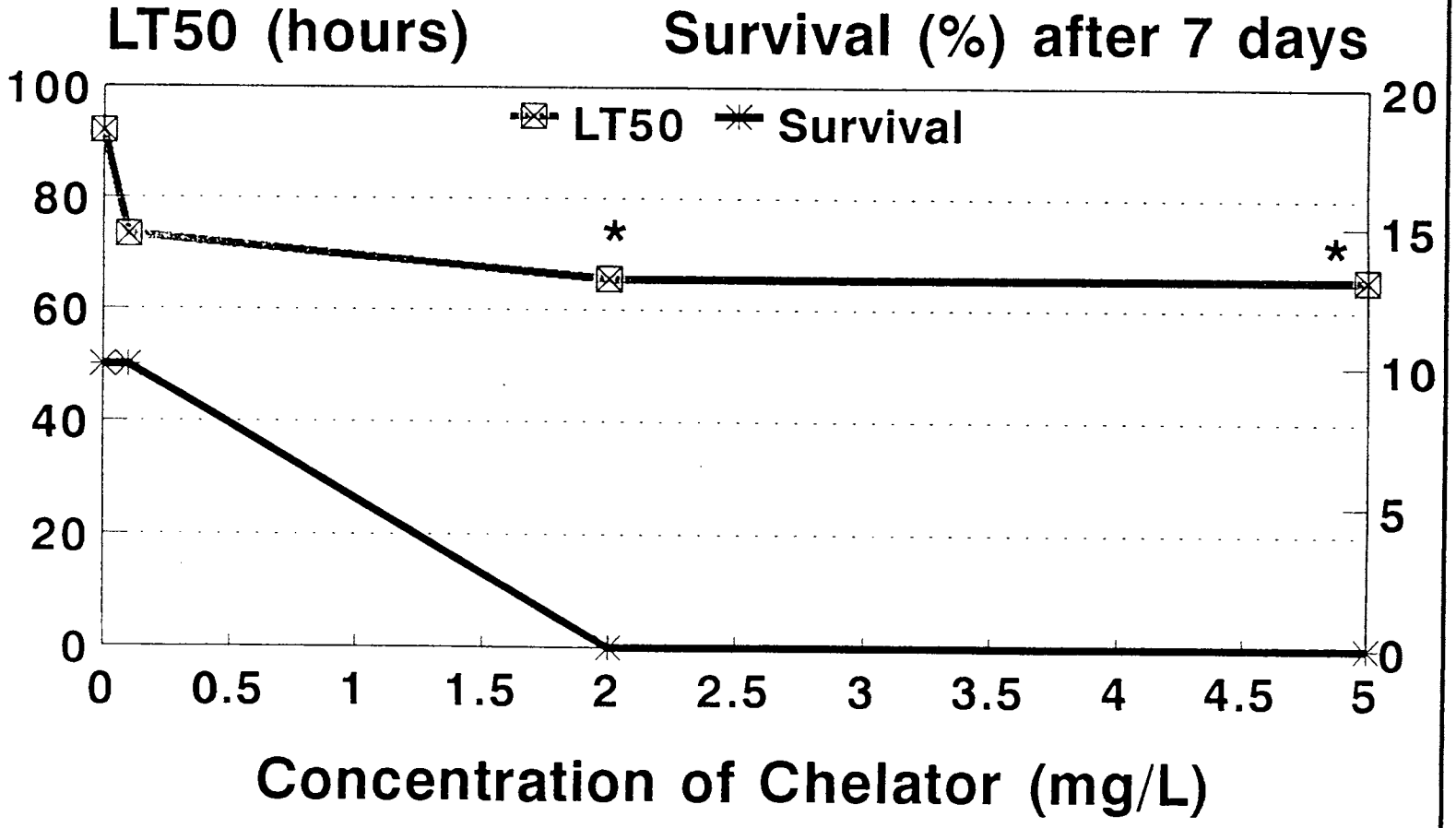
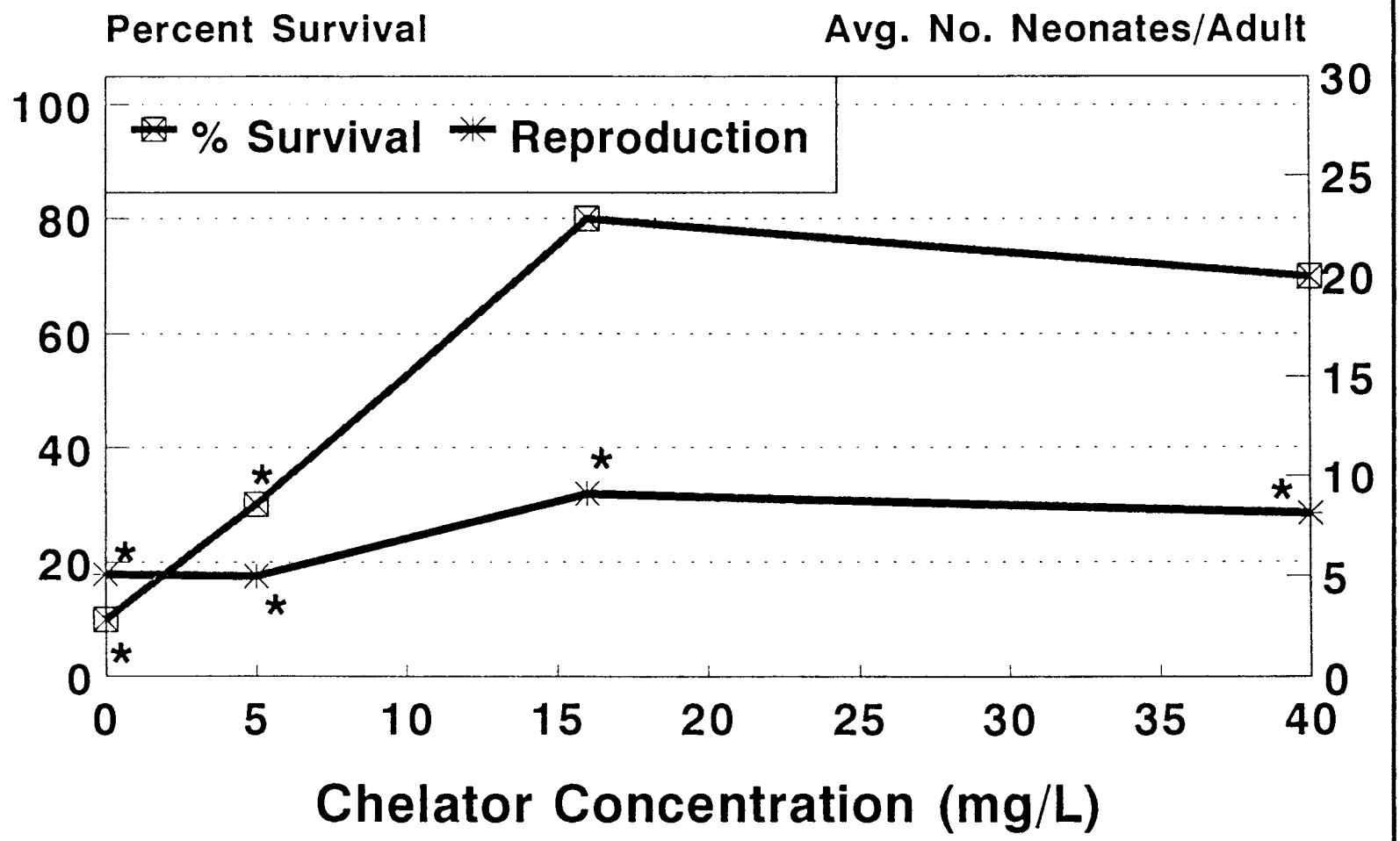


Fig. 2 Effect of EDTA on the Lethal Time-50% (LT50) of Silver (18 μg/L) with Ceriodaphnia dubia.



* - LT50 confidence intervals fall outside that for silver alone

Fig. 3 Effect of NTA on the Chronic Toxicity of Silver (18 μg/L) to Ceriodaphnia dubia.



* Significantly different ($p<0.05$) from control values (90%; 27.2 neonates)

Questions & Answers: Interaction of Silver and Metal Chelators on *Ceriodaphnia dubia* Survival and Reproduction

Q. CHRISTER HOGSTRAND (Univ. of Kentucky): Have you done any speciation modeling of your media?

A. No, sir.

Q. Because regarding why you see the EDTA concentrations that you do and the silver concentration that you do, I'm almost sure that you only will have marginal changes in the concentration of silver ion in the media but you will have a massive change in the concentrations of magnesium and calcium. And I'd say that is the reason why you see the effects you do.

JOHN MAHONY (Manhattan College): Considering the binding constants for a metal with EDTA, the ability of a metal to bind with EDTA is very pH dependent. For example, magnesium and calcium and aluminum are 10 and the binding constant of silver is less than magnesium. So I think the problem is simply pH, which will enable silver to bind to EDTA at the pH of the study. I don't have a quantitative analysis chemistry book with me, unfortunately, to look up what it is, but I think that should be checked.

Q. GEORGE HELZ (Univ. of Maryland): My question is really on the same point as these other two: did you show that the toxicity you have at high EDTA occurred only in the presence of silver? In other words, all your mechanisms that you proposed suggested that there was some sort of an interaction on EDTA with the silver antagonist or silver receptor, but it seems to me that in these huge amounts of EDTA you just get trace element or magnesium deficiency problems that would cause the toxicity. Did you show — if so, I missed it — that you had the EDTA toxicity only when silver was present and not when it was absent?

A. The EDTA load in that caused toxicity, at those levels?

Q. At these high levels.

A. Right. They are not that high, I mean, five ppm is not that high for EDTA. That's on a chronic test. They use that routinely for the toxicity classification work done by the EPA so that shouldn't be a problem, and, in fact, with the very first studies with the µmolar comparisons you actually had levels that were about 0.1 ppm for EDTA, so the EDTA wasn't exerting the toxicity alone. With other metals that I tested it did not seem to have an effect, but mostly other metals that it binds to fairly well that we tested, such as cadmium, copper, zinc.

RICHARD PLAYLE (Wilfrid Laurel Univ.): EDTA binds silver better than NTA, so NTA may work better in your system. But it's not because it binds silver better; that was because it binds silver and everything else less well. I just worked with nickel. I was trying to determine a nickel-gill binding constant using that ligand exchange method, and it didn't work because I needed such really high concentrations of NTA and EDTA that eventually the NTA and EDTA were toxic to the fish. But if you added nickel you detoxed the NTA and EDTA by tying it up. So I think, I guess it's theory 3: you're probably stripping calcium, magnesium off the animals and they are dying of regulatory distress. That would be my guess.

Q. ARUN MUKHERJEE (Univ. of Helsinki): I heard about the one reference on antagonistic reactions. Do you have any citations on the synergistic or antagonistic effects of silver with other metals in the environment?

A. The papers I was talking about, I think, were older papers that were talking about silver in diets of chicken, and it reduced copper absorption. They were more about free ion compounds.

Q. WESLEY BIRGE (Univ. of Kentucky): Jeff, before you did any calculation work, did you not characterize the drug effects of EDTA on ceriodaphnia?

A. Yes.

Q. And what were those levels compared to the treatment levels that you gained from your work?

A. The beginning of effects, which were on reproduction only, occurred starting at about 7 ppm and the effects on mortality in total got up to 30-50 ppm.

Physiological Effects of Silver to Seawater-Acclimated Rainbow Trout

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The free Ag^+ is one of the most toxic metals to fish in freshwater (Nebeker, *et al.*, 1983; LeBlanc, *et al.*, 1984; Hogstrand, *et al.*, 1995). The toxicity of Ag to fish is very much dependent upon its chemical form and in freshwater and brackish water systems the Cl^- concentration of the water has a dramatic effect on the acute toxicity (Galvez *et al.*, 1994; Hogstrand *et al.*, 1995). By increasing the Cl^- concentration of the water from 0.7 mM to 50 mM, the 168-h LC_{50} for Ag to rainbow trout (*Oncorhynchus mykiss*) can be reduced more than 10,000 times (Hogstrand *et al.*, 1995). This protective effect of Cl^- against Ag toxicity is most likely caused by formation of $AgCl_n$ complexes, in particular the insoluble form of AgCl known as cerargyrite. In sea water little or no cerargyrite is formed (at realistic concentrations of Ag) and virtually all waterborne Ag^+ is present in the form of negatively charged $AgCl_n$ complexes (Schecher, 1991). These dissolved $AgCl_n$ species are much less toxic than the free Ag^+, but there is documentation of moderate toxicity of Ag to fish in sea water (Ferguson *et al.*, 1995).

Toxicity of Ag^+ in freshwater is caused by a specific blockade of the branchial uptake of Na^+ and Cl^-, which leads to osmotic imbalance, a fluid shift from the blood plasma to the tissues, and a subsequent circulatory collaps (Wood *et al.*, 1995a). The same mechanism is unlikely to be responsible for Ag toxicity in seawater, because the concentrations of Na^+ and Cl^- in the body fluids of seawater teleost fish are below that of the surrounding water. Thus, if the osmoregulatory systems are blocked in seawater teleost fish, the concentrations of these ions in the plasma would increase. The objective of the present study was to diagnose the physiological mechanism for acute silver toxicity in sea water.

The experimental approach was similar to that of Wood *et al.* (1995a,b). Starry flounder (*Platichtys stellatus*; 450-1,200 g) were fitted with chronic indwelling catheter in the caudal portion of the dorsal aorta 36 - 48 h prior to experimentation. The catheter allowed repetitive blood sampling without disturbance of the animal. After surgery, the fish were placed in individual plastic tubs. The tubs were aerated and supplied with a constant flow-through (350 ml/min) of fresh 32 ppt sea-water with a temperature of 13°C. To avoid visual disturbance, the tubs were covered with a plastic mesh. The exposure system consisted of a header tank which delivered a constant flow (3.0 l/min) of aerated sea water to a 20-l vigorously aerated mixing chamber. Silver nitrate, dissolved in distilled water, was dispensed from a stock solution into the mixing chamber by a peristaltic pump at a rate of 1.0 ml/min. The silver stock solution was renewed every 48 h. The silver concentration, 250 µg Ag/l (added as $AgNO_3$), was choosen based on the toxicity data obtained for tidepool sculpins in identical water quality (Ferguson *et al.*, 1995). The selected silver concentration was 50% of the lowest concentration that caused mortalities within 96 h in the bioassays with sculpins. Blood samples were withdrawn

from experimental fish and controls 24 h before the start of the exposure and then 12, 24, 48, 96, 144 h after onset of exposure. The blood samples were analyzed for plasma electrolytes (Na^+ and Cl^-), plasma glucose, plasma ammonia, plasma protein, blood gasses (P_{O2} and P_{CO2}), pH, hematocrit, hemoglobin, and lactate. After the final sampling, the animals were perfused with heparinized saline and various tissues dissected out for analysis of silver content.

Respiratory and ionoregulatory functions of starry flounder gills were little affected by the 250 µg/l Ag exposure. Thus, the mechanism for Ag toxicity in seawater fish may fundamentally differ from that occurring in freshwater living fish (Wood *et al.*, 1995a). The only measured variable that showed a notable difference between Ag exposed flounders and controls was the plasma ammonia concentration (Fig. 1). The ammonia concentration in starry flounder plasma increased markedly during the first two days of Ag exposure. Later in the experiment, Days 4 and 6, there was a partial recovery of the plasma ammonia level, but the concentration of plasma ammonia in the experimental group remained significantly elevated compared with the control group throughout the rest of the exposure period. Copper, which is chemically related to Ag, has been shown to increase the plasma ammonia level in seawater acclimated rainbow trout (Wilson and Taylor, 1993a). Interestingly, the physiological mechanism for Cu toxicity in freshwater acclimated rainbow trout is also similar to that for Ag in freshwater rainbow trout (*i.e.* blockage of Na^+ and Cl^- uptake; Laurén and McDonald, 1986; Wilson and Taylor, 1993b). Thus, Ag and Cu may have similar toxicological properties to fish in both freshwater and sea water.

The increase in plasma ammonia level during silver exposure could be caused by blockage of the branchial ammonia excretion or an increased ammonia production. To separate these two possibilities we studied the effect of Ag on the excretion rate of ammonia in another fish species, the tidepool sculpin (*Oligocottus maculosus*). The small size of this species (1.8 - 4.1g) made it ideal for such experimentation. Essentially the same exposure system was used as described above for the experiment with starry flounders. The sculpins were housed in individual 30-ml polypropylene syringes supplied with a water flow of 20 ml/min. The exposure level was set to 500 µg Ag/l and the experiment was continued for 58 h.. In this experiment with we found no evidence of decreased ammonia excretion during Ag exposure. Indeed, after 58 h of the experiment, the Ag exposed fish had a higher ammonia excretion rate than the simultaneous control. These results suggest that Ag exposure causes an increased ammonia production in tidepool sculpins while the ammonia excreetion is unaffected. Further studies are required to conclude whether or not an increased ammonia production is the ultimate cause of acute Ag toxicity to marine fish.

Analysis of Ag in liver, kidney, gill epithelium, and intestinal mucosa of starry flounders showed that Ag was taken up from the water accumulated in the fish (Fig. 2). The background levels of Ag in the examined tissues were very low, suggesting little exposure to Ag prior to the experiments. Of the examined tissues, the highest levels of Ag were found in intestinal mucosa from exposed fish. The intestine was thoroughly flushed with saline directly after the disection, but it still cannot be determined whether the silver was adsorbed to the mucosa or actually absorbed by the tissue. However, the markedly elevated concentration of Ag in intestinal mucosa suggest that absorption of Ag from

ingested water may be an important route for Ag uptake in marine fish. Among the internal organs (*i.e.* liver and kidney), the liver of exposed fish showed the highest concentrations of Ag. These results are in accordance with previous studies, which have depicted the liver as a primary accumulatory organ for Ag in fish (Pentreath, 1977; Wood *et al.*, 1995a,b).

(Supported by a grant from the Silver Coalition)

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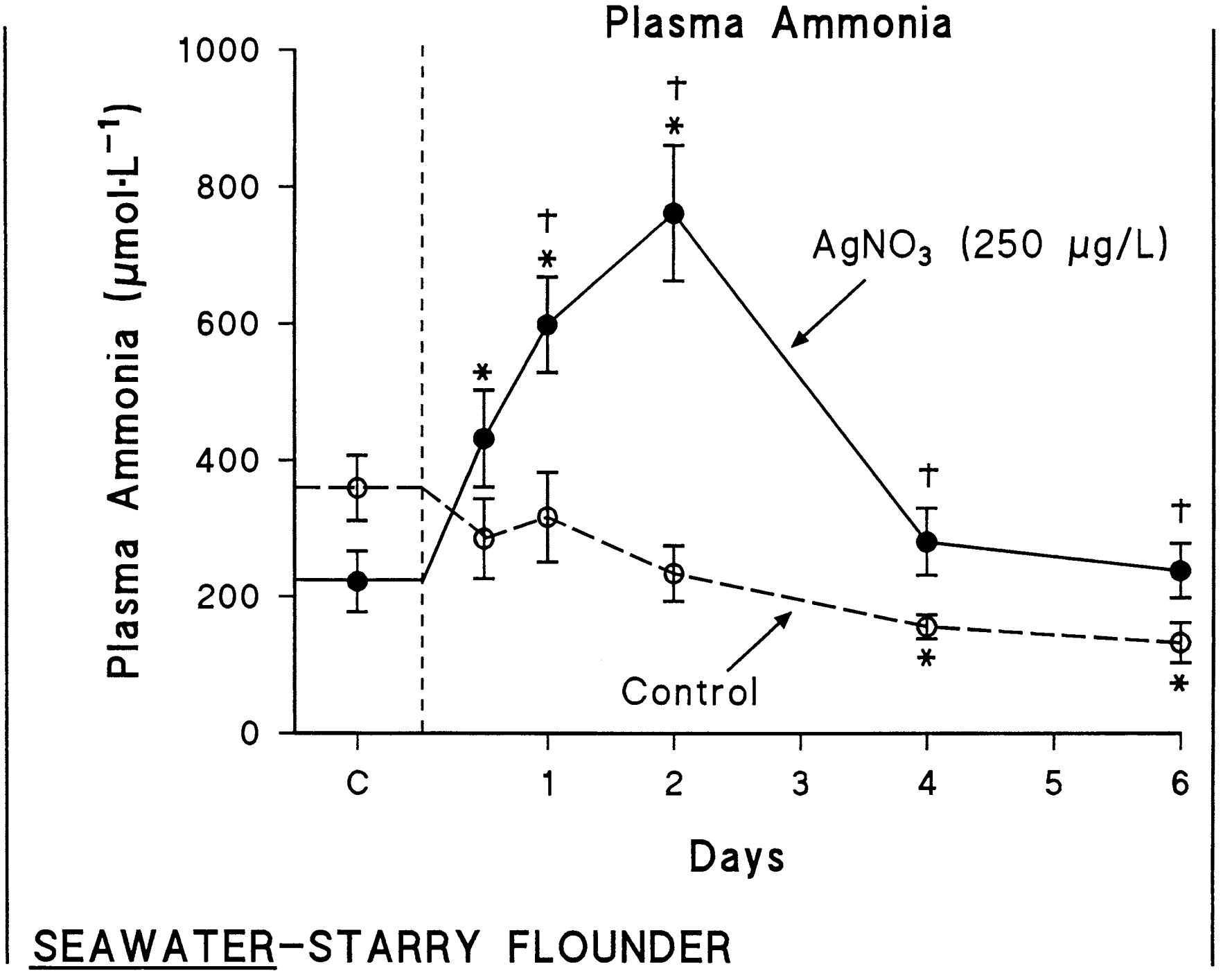
Figure legends

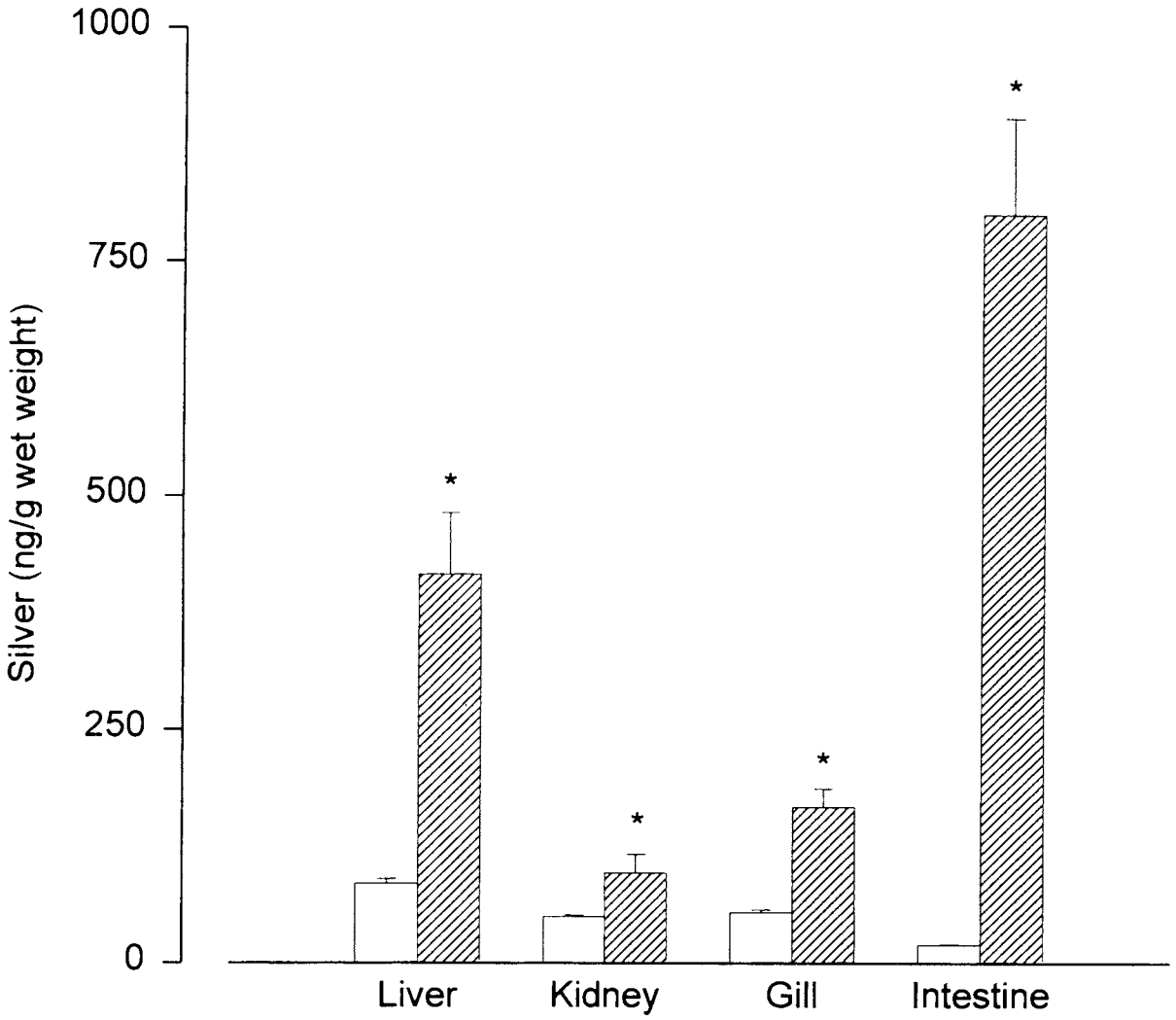
Figure 1. Plasma ammonia concentration in starry flounders during exposure to 250 μg Ag/l or during control conditions. Means ± SE (N=8). Asterisks indicate means significantly different (Bonferroni-Student t-test; $p<0.05$) from the pre-exposure value. Experimental means were significantly different from simultaneous control means from Day 1 onwards as denoted by daggers.

Figure 2. Silver concentrations in liver, kidney, gill epithelium, and intestinal mucosa of starry flounder exposed for six days to 250 μg Ag/l or held during control conditions. Values are shown as mean ± SE (N=8). Asterisks indicate means significantly different from the control.



SEAWATER–STARRY FLOUNDER

Tissue Silver Levels



Questions & Answers: Physiological Effects of Silver to Seawater-Acclimated Rainbow Trout

Q. IAN MORGAN (McMaster Univ.): Would you care to speculate, perhaps, on how silver might increase ammonia production in the fish? Any ideas?

A. I said that because I don't want to speculate. I really don't know.

Q. RICHARD PLAYLE (Wilfrid Laurier Univ.): Interesting, that this really nice observation might include two mechanisms. You might have an answer already in your respirometers — did you measure carbon dioxide production? Or did you measure oxygen consumption?

A. We measured oxygen consumption.

Q. But not carbon dioxide?

A. Right.

Q. Okay, because my speculation is, if I've got it correctly, about the respiratory quotient, if it goes down then that suggests a shift to protein catabolism which might indicate that silver has an effect, especially where it might inhibit carbohydrate metabolism.

A. We have a problem there, and that is that these are very small fish and they probably will very rapidly be void of whatever they have in the guts. And as time goes by during an experiment you would expect a difference in the ammonia excretion.

Q. JIM KRAMER (McMaster Univ.): I wonder, for the increase in ammonia, if it's really silver. You also added nitrate. Is it possible that the nitrate somehow got reduced to increase your ammonia?

A. Let me see now. The difficulties would be that we have different redox conditions. I have to make the calculations, really, to figure out if that is true, but I wouldn't think so, really not. I think the levels are too small.

Q. You think the nitrate that's added with the silver is similar to what nitrate is already in the system?

A. I think the nitrate in the surrounding system is much lower than what is in the fish.

Q. NICK FISHER (SUNY-Stony Brook): As far as I know, most dissolved silver concentrations in seawater are in the ng/L range and even in fairly contaminated, fairly stagnant waters they rarely exceed or even reach 1 µg/L. Have you done any studies examining the toxicity of more realistic — environmentally realistic — silver concentrations to these fish?

A. This is the starting point, now, that we are working on. The same as we did in freshwater, we wanted first to establish what kind of effects are there, and as you saw, we had difficulties even finding effects at these high concentrations of silver. But if it turns out now that to that fraction we encountered a toxic mechanism of ammonia, then of course the next step is to get down in concentrations to see if we see similar things. I would doubt that we really see any effects at the water-relevant concentrations.

ARGENTUM

AN INTERNATIONAL CONFERENCE

Session 4

J.R. Kramer
Session Chair

Determination of Thermodynamic Stability Constants of Metals with Natural Ligands

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Determination of the thermodynamic stability constants of natural ligands isolated from organisms is often hampered because only milligram or submilligram quantities can normally be isolated. We report a voltammetric method using square wave voltammetry at a hanging mercury electrode for the determination of thermodynamic stability constants. The voltammetric method detects individual complexes of a metal with the ligand and has sufficiently low detection limits to work with small quantities of ligand material. The method has been applied to Fe(III) and Zn(II) complexes.

Thermodynamic stability constants have been estimated for the complexation of iron(III) with catecholate-type siderophores isolated from the marine bacterium *Alteromonas luteoviolacea*, from the marine cyanobacterium *Synechococcus* sp. PCC 7002 (Lewis et al 1995a) and from the edible mussel *Mytilis edulis* (Taylor et al. 1994) in 0.1 M NaCl and 5 mM bis-tris at pH 7. Thermodynamic stability constants were determined utilizing the "chelate scale" approach (e.g., Taylor et al., 1994). The scale is based upon a linear relationship between the reduction potentials [Fe(III) to Fe(II)] and the pH-independent log thermodynamic stability constants for known iron(III) complexes. The Fe(III) chelate scale relates the reduction potentials of selected iron(III) complexes with known stability constants (Smith and Martell, 1989), based upon eq. (1):

$$E'_p \approx E_p - 2.303\ (RT/nF)\ \log K_{ox}/K_{red} \qquad (1)$$

where E_p and E'_p are the reduction potentials of the free metal ion and the complex, n is the number of electrons involved in the process (one for the Fe(III)/(II) redox couple), and K_{ox} and K_{red} are the pH-independent stability constants of the oxidized and reduced forms of the metal-ligand complex. The assumptions inherent to the method are: (a) There are no ion pairing or electrolyte effects; (b) There is no solvolysis of either Fe(III) or Fe(II) at high pH due to complexation by bis-tris in the media; (c) The diffusion coefficients for Fe(III)L and Fe(II)L are equivalent, and the Fe(II)L/Fe(III)L redox couple is reversible. We observed a linear relationship ($r^2 = 0.98$) between E'_p and Log K_{ox} for known iron(III) complexes from Smith and Martell (1989). The reason for the independence of Log K_{red} values is unclear. One possibility is that K_{red} values are similar for all complexes which is true for enterobactin and CDTA Fe(II) complexes such that eq. (1) can be simplified to eq. (2), and K_{red} can be incorporated into the intercept:

$$E'_p \approx (E_p + 2.303\ (RT/nF)\ \log K_{red}) - 2.303\ (RT/nF)\ \log K_{ox} \qquad (2)$$

Fig. 1 shows representative data for known and natural ligands. The binding strengths

of the iron(III) complexes examined in this study are quite high, indicating that catecholate siderophores play a role in the solubilization and biological uptake of iron in the marine environment. UV-VIS spectrophotometry is used to verify the stoichiometry of the number of catecholate functional groups per molecule binding to Fe(III). Our data indicates that the chelate scale for Fe(III) works well for molecules containing one or more catechol groups.

For Zn(II) complexes (Lewis et al, 1995b), all Zn(II) complexes reduce to zinc-amalgam (Zn/Hg) at the mercury electrode and eq. (1) reduces to eq. (3).

$$E'_p \approx E_p - 2.303\ (RT/nF)\ \log K_{ox} \qquad (3)$$

We observed a linear relationship ($r^2 = 0.965$) between E'_p and Log K_{ox} for known Zn(II) complexes from Smith and Martell (1989). The Zn "chelate scale" was calibrated in seawater (I = 0.7; pH = 8.1) in order to measure the constants for unknown natural ligands binding to Zn(II) in seawater. In order to determine the constants at nanomolar levels in seawater, pseudopolarograms using square wave anodic stripping voltammetry (SWASV) were performed on model compound complexes and natural samples. Fig. 2 shows data for known and natural ligands in seawater. The method indicates that several natural ligands can be determined in a seawater sample simultaneously in contrast to competitive equilibrium or titration approaches which are commonly employed for low level determination of organic complexation with Zn(II) and which measure conditional not thermodynamic stability constants.

We are applying the method to other metals which reduce to form amalgams like Zn(II) or have high positive reduction potentials that can be shifted to negative potentials on complexation like Fe(III).

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Figure 1. Data for known organic complexes with Fe(III) (#1-7; o) and natural ligands isolated from marine organisms (#8-12;▾). Dashed lines are the 95 % confidence limits.
(1) $[FeCDTA]^-$; (2) $[FeNTAtiron]^{4-}$; (3) $[FeNTAcat]^{2-}$; (4) $[Fecat_2]^-$; (5) $[Fe(4Ncat)_3]^{3-}$; (6) $[Fecat_3]^{3-}$; (7) $[Feent]^{3-}$; (8) Fe[alterobactin-B]$_2$ (pH=6); (9) Fe[alterobactin-B]$_2$ (pH=8.2); (10) Fe[*Synechoccous* isolate No.1]; (11) Fe[*Synechoccous* isolate No.3]; (12) Fe[*Mytilus edulis* foot protein 1]. The peptides from *Mytilus edulis* lie between 10 and 12 and are FeL_2 complexes.
CDTA = cis-1,2-cyclohexylenedinitrilotetracetate; NTA = nitriloacetate; tiron = 4,5-dihydroxy-1,3-benzene disulfonic acid; cat = catechol; 4Ncat = 4nitrocatechol; ent = enterobactin.

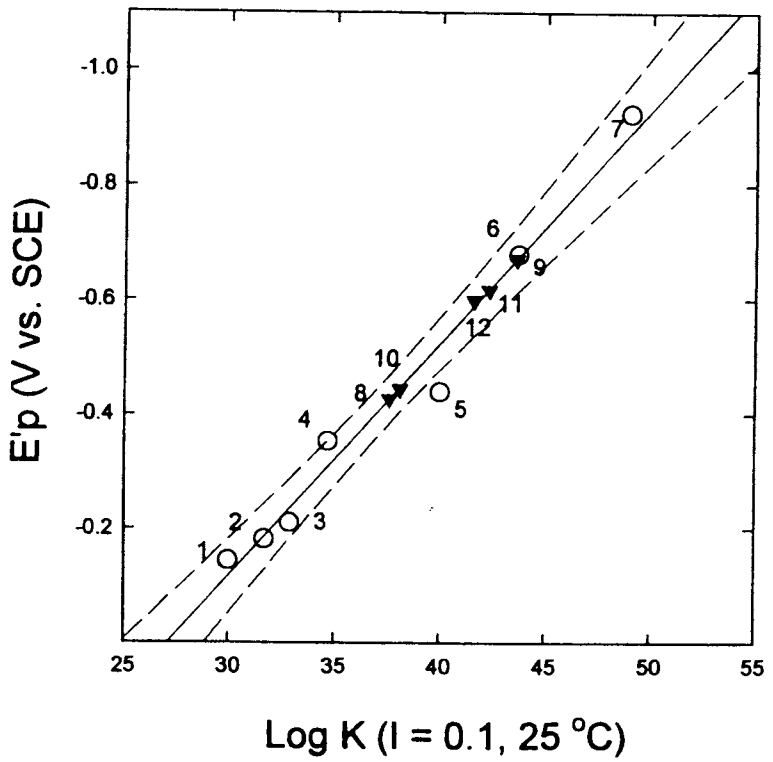
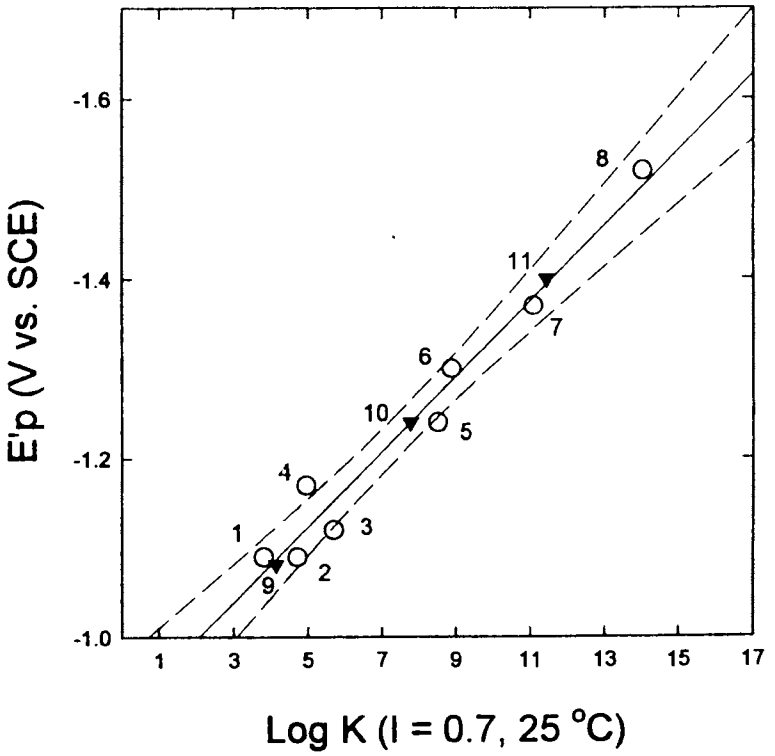


Figure 2. Data for known organic complexes with Zn(II) (#1-8; o) and natural ligands present in a coastal seawater sample (#9-11; ▾). Dashed lines are the 95 % confidence limits.

(1) [Zn(ox)]; (2) [Zn(CTP)]; (3) $[Zn(en)]^{2+}$; (4) $[Zn(gly)]^{+}$; (5) $[Zn(8HQ)]^{+}$; (6) [Zn(impa)] (7) [Zn(EDDA)]; (8) $[Zn(NTA)_2]^{2-}$; (9), (10) and (11) are unknown organic ligands in seawater with weak, moderate and strong binding abilities, respectively.

ox = oxalate; CTP = cytidine-5'-(tetrahydrogentriphosphate); en = ethylenediammine; gly = glycine; 8HQ = 8-hydroxyquinoline; impa = iminobis(methylenephosphoric acid); EDDA = ethylenediamminediacetate; NTA = nitriloacetate.



Questions & Answers: Determination of Thermodynamic Stability Constants of Metals with Natural Ligands

Q. NICK FISHER (SUNY-Stony Brook): Have you attempted to assess complexing capacity for any ion in seawater related to the presence or absence of plankton blooms or decomposition of planktonic material?

A. I would say that we haven't gone out specifically in blooms, but when we've done titrations they indicate that it's entirely complexed. When we used a rotating disc electrode (Rotel) we found something rather novel. On one of the slides I showed you, with adsorption with Fe(III), certain organic ligands were adsorbed right to the surface of the electrode. And what we observed is that with zinc, if rotating we have a larger sensitivity in the square wave mode. What we see initially is a free zinc peak, but if we keep on doing the experiment for replicates, we see the zinc peak shift and all of a sudden the complex indicating there's organic adsorption. That's why I like to sort of argue that we saw the free zinc peak. So yes, we've done those so-called complexation capacities, but I'm not sure I believe them based on the adsorption phenomena of some organics. So I'm a little bit leery about saying certain types of things. I should point out that in theory, because of that one curve we have where we did three different peaks, you should be able to add those three up for the total zinc. They don't always do that, and that's again because, due to adsorption, the organic complex is giving different types of peak heights.

Q. PETER SANTSCHI (Texas A&M Univ.): You said that whatever you did with iron and zinc at micromolar and nanomolar levels should be applied to silver which is at picomolar levels, and that it should be possible — what made you so confident that you can operate at picomolar levels?

A. Well, I know we can do it in the laboratory. We make solutions at this level. I'm not sure we can do, perhaps, picomolar level in seawater. It's possible that you can do it if you can use the square wave mode. Most people aren't using the square wave mode in the marine community, other than myself. And the square wave mode is at least an order of magnitude better than differential pulse. So, for example, I know I can get for zinc down to 0.1 nmol/L without too much hassle and with Rotel I can get another order of magnitude. So it's conceivable that with silver we might be able to do something like that. If you have a series of complexes you throw out one point and you will be able to set up a scale like that in a very straightforward manner. That's a good question. We were wondering about that.

Q. ANDERS ANDREN (Univ. of Wisconsin): What do you think is the fate of these very interesting complexes?

A. Basically we know about charge-transfer complexes, and under UV visible conditions normally seen in the field, what can happen is that electrons from the catechol can reduce the Fe(III) to Fe(II) and then you have some of that iron leaking out. So what happens is that now you have Fe(II) available which may be even better for uptake by an organism than the Fe(III) initially. In the case of, say, certain bacteria and cyanobacteria, some of these chelates were actually excreted for a whole community. But there are other organisms that actually have these membrane chelates. So it's possible that they can take up quite a bit of surface area around so they can acquire an essential metal like zinc or iron. Iron is a key for electron transport carriers in the photosynthetic chain for phytoplankton, and zinc is involved in certain mechanisms in hydrate systems. That's the reason why we chose these two specific ones — in addition to the fact that they're primary metals for funding, perhaps.

About silver? I don't know, nobody's asked me to try for silver. I think we're going to summarize a proposal with several metals and shop around with it. I think it's the industrial community that's going to drive silver research, would you agree?

Phase Partitioning of Silver in River and Estuarine Waters of Texas

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The normal range of total dissolved (≤0.4μm) silver concentrations in Texas surface waters is low, typically 1-10 ng/L. In the vicinity of major sewage treatment plant effluents, values of 10-100 ng/L are found. Particulate silver concentrations were high, and truly dissolved concentrations of silver were much lower than these values (Gill et al., 1994). We report here results from further experiments studying the methodology of colloidal silver measurements in river and estuarine waters of Texas, as well as new results on the distribution of colloidal silver, as a function of salinity, in the Trinity River estuary and Galveston Bay. The aim of this ongoing study is to find possible controls of the solid/colloid/solution partitioning of silver and other trace metals, in relation to the solution concentrations of dissolved organic carbon, DOC, and colloidal organic carbon, COC, and organic carbon or Fe content in the particle phase. In order to better understand the sorption behavior of silver to suspended particles and colloidal particulate matter, accurate measurements of the colloidal fraction of Ag are needed.

Our previous results on the phase speciation of silver in these waters indicate that a large fraction of the "dissolved" Ag occurs in a colloidal form. The colloidal fraction of silver, determined after total digestion of filters of different pore sizes, is of high molecular weight (i.e., is in the fraction between 0.4μm and 0.1μm), and amounts to 33-89% of the total dissolved fraction.

Colloidal silver, isolated using cross-flow ultrafiltration techniques, amounted to 15-70 % of the total dissolved silver concentration, decreasing with increasing salinity, similar to dissolved and colloidal organic carbon. The Ag concentration in colloids were broadly similar to those in suspended particulate matter (in μg/g), and were found in similar proportions of the total filter-passing Ag as colloidal organic matter was partitioning to DOC.

INTRODUCTION

The fate of trace metals such as silver in aquatic environments is dependent, to a large extent, on the energetics of heterogeneous reactions (e.g., Santschi et al., 1995). Solution species are often transformed by reactions on heterogeneous phases, such as the oxyhydroxides of Fe and Mn, clay minerals, carbonates, and organic matter of sizes ranging from nanometers to tens of micrometers in diameter, which act as sites for sorption/desorption, proton or electron exchange, or photochemical reactions. In most cases, the bioavailability and toxicity of trace metals is enhanced when metals exist as true solution phase species. However, the difference between beneficial and toxic concentrations of trace metals in aquatic environments is often very small. In most instances, trace metals are bioavailable or toxic to organisms only in low-molecular weight forms. Many environmental regulations are written in terms of the "dissolved" forms of trace metals; however, the currently accepted operational definition of "dissolved" includes colloidal and macromolecular species, which are filter-passing.

Because of the associations of many trace metals with macromolecular organic matter, metal concentrations cannot be accurately predicted from thermodynamic models because of a dearth of data on metal - natural organic matter, NOM, interactions. One approach is to measure the trace metal in different operationally defined fractions, i.e., to carry out phase speciation experiments. One of those methods, cross-flow ultrafiltration, CFUF, offers the possibility to extract colloidal species from large volumes of water. Because silver in aquatic systems normally occurs at the pM level, water samples require large preconcentration factors. CFUF offers the advantage of preconcentration from large volumes within a few hours (e.g., Wen et al., 1995a).

Silver in aquatic systems is often removed to the sediments at a fairly rapid rate (e.g., Santschi, 1988). Silver measurements in estuaries are few, and can show non-conservative behavior (e.g., Santschi, 1988; Benoit et al., 1994, Flegal et al., 1991). Galveston Bay receives Ag from river inputs through the Trinity and San Jacinto River, which themselves showed Ag concentrations of the order of 10s of ng/L near large cities, and rapidly declining concentrations below (Gill et al., 1994). In addition, Galveston Bay also receives direct point source inputs from direct industrial and municipal discharges. Since 70% of the freshwater input comes from the Trinity River, a salinity transect can reveal sources and sinks across the bay, i.e., the degree of non-conservative behavior of silver.

We report here results of silver speciation measurements from transects across a salinity gradient in the Trinity River estuary and Galveston Bay. Silver was measured in particulate (i.e., ≥0.45μm), colloidal (i.e., ≤0.45μm and ≥1kDa cut-off), and truly dissolved (≤1kDa) fractions. Results are compared to those of other trace metals, such as Pb, Cu, Cd, Ni (Wen et al., 1995b), and Hg (Stordal et al., 1995).

METHODOLOGY

Sampling sites

Galveston Bay is one of the largest estuaries on the US coastline. It receives river inputs from the Trinity and the San Jacinto Rivers. The annual fresh water inflow into Galveston Bay is about 1.24×10^{10} m^3 (Armstrong, 1982), with ~70% of the total freshwater inflow from the Trinity River. Sampling sites during the May 1994 cruise are depicted in Figure 1.

Sampling methods.

Extraordinary precautions must be taken to prevent contamination of water column trace metal samples. Samples were collected using ultra-clean sampling protocols. Both filtered and unfiltered samples were collected using a peristaltic pump system equipped with teflon tubing inlets and outlets (Flegal et al., 1991). For sample collection, the tubing was attached to a non-metallic pole and the tubing inlet was oriented into the current to obtain water untouched by the sampling apparatus. Unfiltered samples were drawn directly into acid-cleaned Teflon bottles using ultra-clean sample handling protocols. Filtered samples were obtained by attaching an acid-cleaned polyethylene membrane cartridge to the pump outlet and dispensing the water directly into the Teflon sample bottle. Sample bottles are transported to the field in double plastic bags (inner bag acid-cleaned), filled with 1% triple distilled HNO_3 as a final wash/storage solution. Samples were handled in the field only by personnel wearing clean room plastic gloved. Samples for silver determination were acidified with triply-distilled HNO_3 to a final pH of approximately 1.5 within 12 hours of collection under the protection of a clean air bench. Filters were unloaded from their sample holders in a clean bench and transferred to acid-cleaned 30 ml Teflon screw-cap vials for further analysis (Landing and Lewis, 1991). Leaching agents included 1) 4.5N Q-HAc (Acetic Acid, for weakly adsorbed trace metals), 2) 2N HCl and 1N HNO_3 (for trace metals associated with Fe-Mn oxyhydroxide and some sulphidic phases), and 3) conc. HNO_3, 6N HCl and HF (for trace metals associated with refractory phases).

Parallel filtration was carried out to collect suspended particulate matter, SPM, by drawing water through an acid cleaned 47 mm diameter Nuclepore 0.45 μm polycarbonate membranes loaded with TEFZEL filter assemblies (Savillex). The sampling units were prepared and processed in a Class-100 trace metal clean laboratory, using triple distilled, sub-boiling quartz distilled reagents. Separate samples were also collected for the determination of pH, alkalinity, major ions and nutrients according to standard methods, and dissolved organic carbon according to Guo et al. (1994).

Experiments to isolate colloidal Silver from water samples.

General procedures for isolating colloidal organic carbon and trace metals are described in Guo et al., 1994, Guo and Santschi, 1995, and Wen et al., 1995a. Approximately 20L of sample water was prefiltered through 0.4μm cartridge filters and then ultrafiltered through 1 kDa crossflow ultrafiltration (CFUF) cartridges (Amicon). The prefiltration was conducted using the ultra-clean peristaltic pump collection system described above. The filtered water was stored in 20L Teflon Bag-in-Bottle containers (Berghoff) and kept on ice and in the dark

prior to ultrafiltration. Test experiments to check for DOC mass balance and trace element contamination during the whole procedure were performed. Agreement to within 5% of the expected values were indicated from test experiments of DOC and trace metal mass balance from individual fractions. For Ag, mass balance was 95%.

Determination of silver.

Samples were preconcentrated under class-100 clean laboratory conditions for total, dissolved (0.1 and 0.4μm), and colloidal (1kDa cut-off) silver using a modified version of the ammonium 1- pyrrolidine dithiocarbamate/diethyldithiocarbamate (APDC/DDC) organic extraction method described by Bruland et al. (1979, 1985). Prior to preconcentration, all samples were irradiated with a bank of ultra-violet lamps (120W) to photo-oxidize organic matter present in the sample which may complex silver and interfere with the extraction. Filtered water samples were thus processed in their original container using the preservation acid by first ultrasonification for 60 min at 60°C and then UV-irradiation for 24 hrs. Silver measurements were conducted using a Perkin Elmer 5100 graphite furnace atomic absorption spectrophotometer (GFAAS) equipped with Zeeman background correction. Determinations were conducted utilizing the method of standard additions to further correct for sample matrix interferences. Sample blanks and spiked samples were run with each set of samples extracted to determine yield correction factors and detection limit capabilities. We estimate our detection limit for silver measurements, based on three times the standard deviation of a blank signal combined with a sample preconcentration factor of fifty-fold, at 0.1 ng/L (0.93 pM). Spike additions of silver to sea and fresh water samples yielded recoveries of 91% after UV digestion of the samples and APDC/DDDC extraction.

RESULTS AND DISCUSSION

DOC and SiO_2 concentrations as a function of salinity show a simple, classical conservative mixing behavior (Figure 2a,b), while river-borne suspended matter (SPM) is removed in Trinity Bay near the river mouth (Figures 1, 2c). Dissolved and colloidal Ag concentrations in the salinity transect in Galveston Bay show evidence for a substantial internal source in Trinity Bay (Figure 3a), while particulate Ag is again removed near the river mouth, similar to the behavior of SPM (Figure 3b). The concentration increase of Ag in Trinity Bay occurs at the same site where Cu (Wen et al., 1995b) and often phosphorus (Santschi, 1995) also show concentration maxima. (but not Hg: Stordal et al., 1995; and Pb: Wen et al., 1995). We believe that Ag is remobilized from sediments at this location, but we cannot exclude external inputs from cooling water discharges directly into Trinity Bay. However, Ag concentrations in suspended matter (in μg/g) are not enriched at this location, as they show constant concentrations throughout the estuary (Figure 4), and are similar in concentrations which have been described previously (Benoit et al., 1994). A substantial Ag enrichment in suspended matter is only indicated in the water of the Trinity River (Figure 4). Interestingly, this apparent remobilization of Ag from sediments in Trinity Bay (Figure 3a) is not linked to the cycling of dissolved silica (Figure 2b) as in the open ocean, or to that of DOC (Figure 2a). Ag concentrations in colloidal matter (in μg/g, assuming 50% C in colloids) are broadly similar to Ag concentrations in particulate matter (Figure 5), indicating similar complexation capacities. Colloidal silver, as a fraction of the total dissolved concentration, partitions similarly to colloidal organic matter to DOC (Figure 6); 15 to 70% of Ag, and 30 to 70% of organic carbon are in this found in the colloidal fraction. Such a similarity of partitioning would be expected if Ag (along with other trace metals) is trapped in colloidal aggregates (Mackay and Zirino, 1994; Santschi et al., 1995), rather than bound in highly specific ligands of low concentrations. Other trace metals such as Cu show similar colloidal features (Wen et al., 1995b). Average particle-water distribution coefficients for estuarine waters were 1×10^5 (L/kg) at SPM concentrations of about 10-60 mg/L, similar to values reported earlier (Benoit et al., 1994; Gill et al., 1994).

CONCLUSIONS

Silver concentrations in colloids were measured using established cross-flow ultrafiltration procedures, followed by UV digestion and solvent extraction procedures. Chelex-100

extraction procedures for the determination dissolved Ag concentrations were shown to be seriously affected by the UV digestion procedures employed, and cannot be used in estuarine waters.

Silver concentrations in the Trinity River estuary show non-conservative behavior, with strong sinks for particulate Ag, and an internal source for dissolved and colloidal Ag. Ag concentrations in colloids appear to be similar to those in suspended matter. "Dissolved" Ag appears to partition to colloids in ways which are broadly similar to those of organic carbon, which would be expected if Ag is trapped in colloidal aggregates rather than bound to highly specific ligands present at low concentrations.

Even though these preliminary data are encouraging, trace metal behavior in estuaries is complex, and firm conclusions can only be drawn after more data is available.

ACKNOWLEDGMENTS

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FIGURES

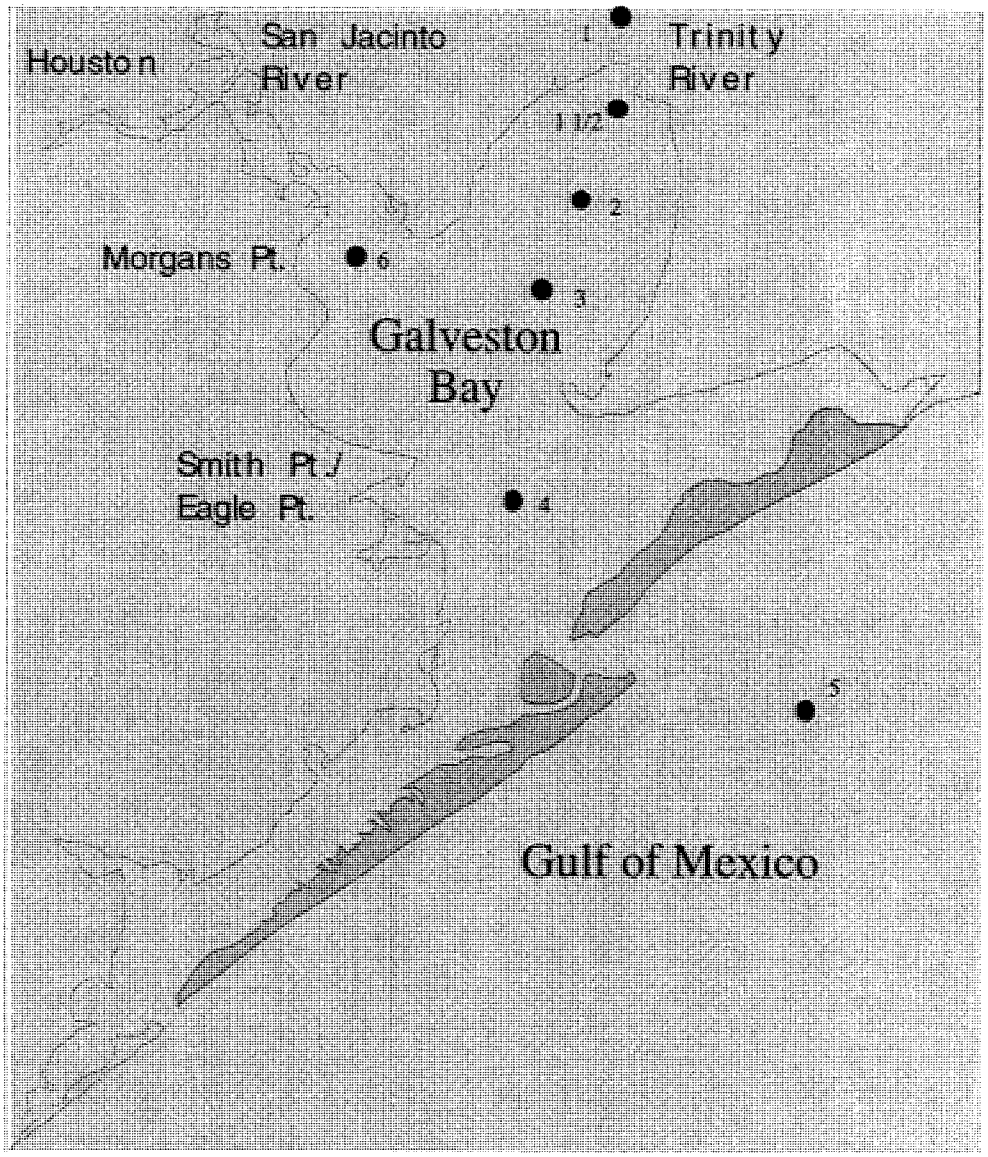

Figure 1. Locations of sampling stations in Galveston Bay. Sampling Stations: 1. Trinity River (0.1 salinity); 11/2. Trinity River mouth (0.1 salinity); 2. Trinity Bay (3.6 salinity); 3. Trinity Bay (9.3 salinity); 4. Smith Point/Eagle Point (15.2 salinity); 5. Gulf of Mexico (19.9 salinity); 6. Morgan's Point (4.8 salinity).

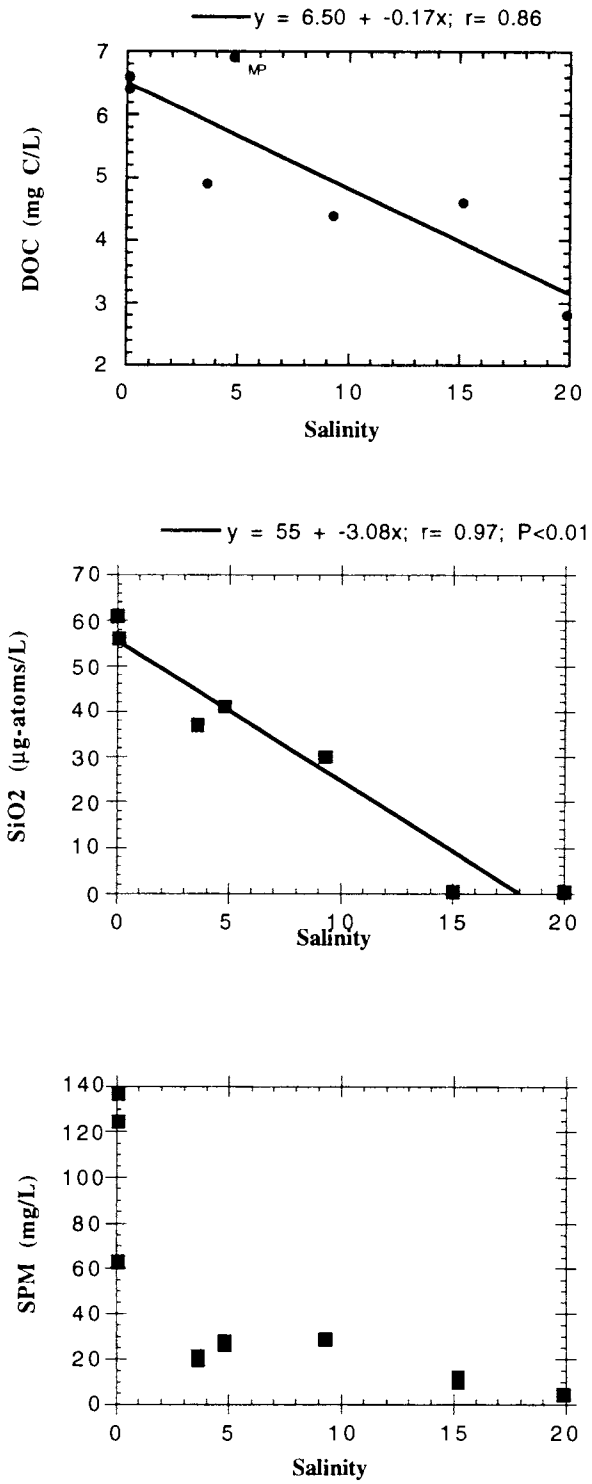

Figure 2. a) Dissolved organic carbon, DOC, b) dissolved SiO_2 and c) suspended particulate matter, SPM, across a salinity transect in Galveston Bay, taken in May 1994. MP indicates a sample from Morgan's Point (see Figure 1).

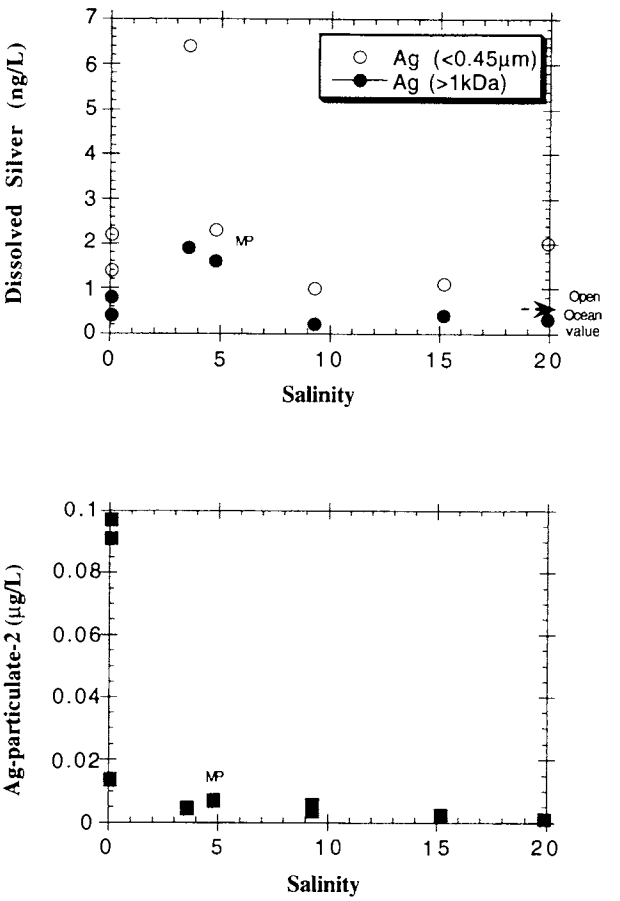

Figure 3. A) Total dissolved (≤0.45µm), colloidal (≥1kDa) and B) particulate Ag concentrations across a salinity transect in Galveston Bay, taken in May 1994. MP indicates samples from Morgan's Point (see Figure 1).

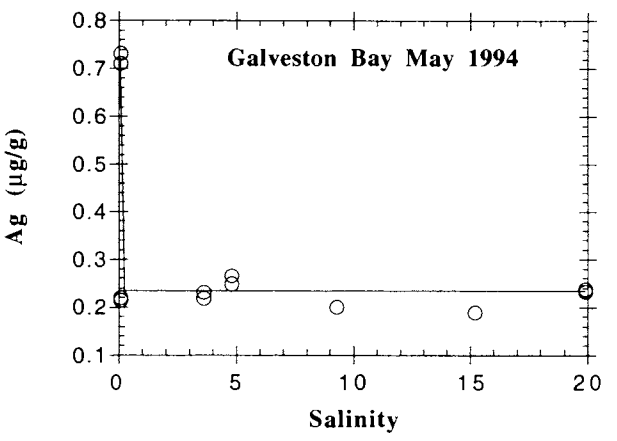

Figure 4. Ag concentrations in suspended matter (Ag(p), in µg/g), as a function of salinity.

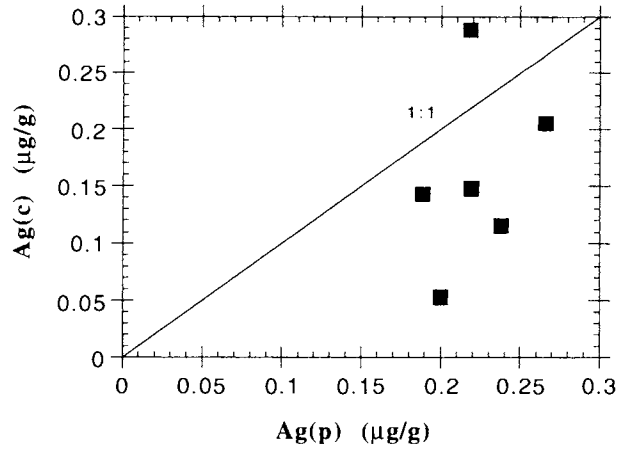

Figure 5. Ag concentrations in colloidal matter (Ag(c)) plotted as a function of Ag concentrations in suspended matter (Ag(p)).

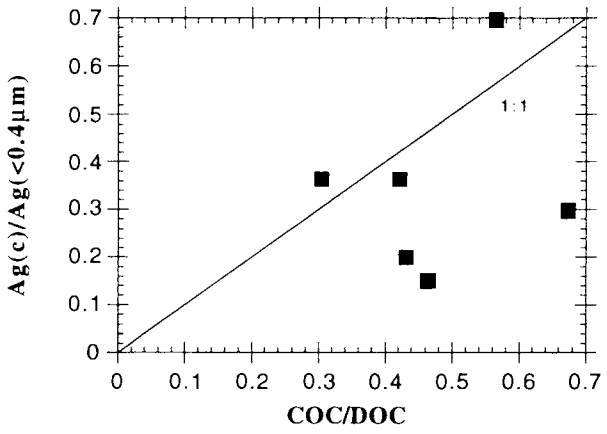

Figure 6. Fraction of colloidal silver, [Ag(c)]/[Ag(≤0.4µm)], as a function of the fraction of colloidal carbon, [COC]/[DOC].

Questions & Answers: Phase Partitioning of Silver in River and Estuarine Waters of Texas

Q. GEORGE HELZ (Univ. of Maryland): I'm not sure if this is the burning question, but I'm interested. When you reoxidized you said the silver went away. Where did it go, presumably if it became inaccessible to chelates? Did you try strong acids, how far away did it get, how inaccessible was it?

A. That's a good question — I don't have a good answer. It basically wasn't caught by Purex, zero, there was nothing, no detected point, we have detection limits of 0.1 ng/L. But you would expect that if you photo reduce silver, you have atomic, elementary silver, it's hydrophobic. It wouldn't be taken up by minerals, so wherever it got hung up it could have gone to the walls because the walls are Teflon, but it couldn't go anywhere. It just wasn't there. It was in acid but it didn't show. We basically gave it up as it looked like it's too much work. We have a procedure which works, it's just time consuming, we didn't like to use it if we didn't have to. But once you get results which don't work and you have another procedure to fall back on where you know you get good results, that's all we were able to do.

Silver Levels and Partitioning in Effluent-Receiving Streams, and a Preliminary Mass Balance for Silver in the Lake Michigan Basin

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Lake Michigan Silver Mass Balance

Levels and fluxes of Ag were determined in key compartments of the Lake Michigan basin, and compiled to construct the first reported mass balance for Ag in Lake Michigan. Concentrations of Ag in sectioned bottom sediment cores, pelagic waters, sedimenting particles, and tributary waters were determined and associated with measured vector rates to compute Ag fluxes. Atmospheric inputs were estimated indirectly using two independent methods as described below.

Burial Rate:

Sub-cores of box cores obtained from the central basin of southern Lake Michigan were sectioned into ½ cm intervals (0-5cm) and 1 cm intervals (>5cm). Ag levels in the upper 2 cm were uniform at 0.35 $\mu g\ g^{-1}$, and declined progressively to "background" levels of 0.13 $\mu g\ g^{-1}$ at 5 cm depth (ca 100 years). Dated (^{210}Pb) cores at this site indicate a mass sedimentation rate of 150 $g\ m^{-2}\ yr^{-1}$, which compares with a basin-wide average rate of 69 $g\ m^{-2}\ yr^{-1}$. Using a focusing factor of 2.17 we calculate a Ag burial rate (recent) of 24.2 $\mu g\ m^{-2}\ yr^{-1}$, and a historic burial rate of 9.0 $\mu g\ m^{-2}\ yr^{-1}$. Sediment profiles of Ag closely mimicked those of Zn.

Pelagic Waters:

Open water Ag levels are extremely low, and until very recently no reliable measurements were available. We obtained samples for Ag determination using trace metal clean techniques on several cruises to the central southern basin during 1995. In each case, profiles of epilimnetic water (<20m) were taken from a Zodiac craft maneuvered several 100 meters upwind of the mother ship. Samples collected in January showed average unfiltered Ag levels of 0.49 $ng\ L^{-1}$. In May Ag levels averaged 0.34 $ng\ L^{-1}$. Analysis of samples obtained in July have not been completed. For this preliminary study, we averaged all data points to arrive at a mean unfiltered Ag level of 0.42 ± 0.09 $ng\ L^{-1}$ (3-4 pM), a level comparable to open ocean concentrations. An areal concentration of 34 $\mu g\ m^{-2}$ is calculated using a mean depth of 82m. To estimate an average Ag partition coefficient, we used typical Ag levels in suspended Lake Michigan particles (see below) and buried sediment (see above), 0.15 $\mu g\ g^{-1}$, and typical suspended mass levels (0.5-1.0 $mg\ L^{-1}$) to calculate an average particulate Ag level of 0.05-0.15 $ng\ L^{-1}$. Partition coefficients in the range of 400,000-600,000 $L\ Kg^{-1}$ ($Log\ K_d$ = 5.6-5.8) are obtained.

Depositional Flux:

A vertical array of sediment traps was maintained in the center of the southern basin of Lake Michigan (160m water column) for a period of one year. Traps were positioned at eight depths, 4 near bottom to resolve resuspension flux, 3 in the hypolimnion, and one just below the base of the seasonal thermocline. Samples were retrieved at intervals of between two and four weeks, for a total of 15 deployments.

Silver levels in two size-fractions of the trapped material are shown in Figure 1 for stratified period deployments. Levels of Ag are consistently greater in the 6-1 μm fraction compared with the 70-20 μm fraction except during late August and early September when $CaCO_3$ precipitation influences the complete spectrum of particle sizes. The preference of Ag for the small size-fraction indicates a selective association with allochthonous components (clays, hydrous oxides, etc.) of the particulate matter over diatoms and other larger algal forms which dominate the intermediate size classes. A general decline in Ag levels in the trapped material is seen from late spring through summer, reflecting gradual removal of the enriched phase from the water column and dilution by authochthonous phases. Figure 2 shows Ag deposition ($\mu g\ m^{-2}$) and flux ($\mu g\ m^{-2}\ day^{-1}$) for each deployment interval. Nearly 55% of the deposition occurs from early June through mid-July associated with sedimentation of the spring algal bloom and resuspended sediments as the water column stratifies and stabilizes. Little net flux is evident during the mixed period. The total annual Ag flux, summing each deployment interval, is calculated to be 32.8 μg m-2. Annual regeneration of recently deposited Ag at the sediment surface can be estimated from the difference between deposition (sediment traps = 32.8) and burial (sediment core = 24.2) = 8.6 $\mu g\ m^{-2}$. The regeneration flux represents 25% of depositional flux.

Tributary Loading:

Eleven tributaries to Lake Michigan, representing 55% of the drainage basin area, are under study to assess loadings of contaminants to Lake Michigan over the period April 1994 - October 1995. Modern trace-metal-clean techniques are being used to collect representative composite unfiltered and filtered samples near river mouths. Acoustic velocity meters installed at sampling sites allow for continuous monitoring of discharge. From 20 - 50 samples will be collected at a given river over the study period, dependent upon the discharge variability of that system. Silver data from 1994 site visits were compiled to generate average Ag levels in each of the river systems, and these data are shown in Figure 3. Mean unfiltered levels range from 1.8 ng L^{-1} in the Manistique R. which drains a remote forested watershed in the Upper Peninsula of Michigan, to 44.2 ng L^{-1} in the Grand Calumet Ship Canal which drains the heavily industrialized Gary Indiana region. Mean concentrations of Ag in the largest rivers (Fox, Grand, Kalamazoo, and St. Joseph) fall within a relatively narrow range of 12-17 ng L^{-1}. Mean annual discharges obtained from USGS databases were combined with mean Ag concentrations (Figure 3) to generate annual Ag loads for each river. Loading data is presented in Figure 4. The St. Joseph, Fox, and Grand rivers account for 56% of the tributary loading of Ag to Lake Michigan. The combined loading from six rivers accounts for less than 8% of the total, a consequence of both small hydraulic loading and in certain rivers significantly lower Ag levels. Flux from the unmonitored areas was calculated assuming a Ag concentration of 4 ng L^{-1}. Total tributary loading of Ag amounts to 275 kg yr^{-1}, which normalized to lake surface area results in a flux of 4.76 $\mu g\ m^{-2}\ yr^{-1}$. This quantity of Ag is extremely small if one considers that for metals like Pb, that amount of metal could be delivered in one day during a high flow event.

Atmospheric Flux:

We have not attempted to directly measure Ag deposition from the atmosphere to Lake Michigan, nor are any reliable data available to our knowledge from which an estimate could be made. Therefore we estimated atmospheric inputs by the two independent methods outlined below. For a highly particle reactive and rapidly scavenged element like Ag, stratified period sediment trap fluxes at the base of the epilimnion should closely trace atmospheric input. We therefore took the average of 5 deployments (40m traps) from mid-summer through mid-fall, and obtained a daily Ag flux of 0.0809 $\mu g\ m^{-2}\ d^{-1}$. This flux was annualized to give an atmospheric flux of 29.5 $\mu g\ m^{-2}\ yr^{-1}$. The other approach used published estimates of Pb atmospheric deposition and element ratios in sediment trap particles. Several published estimates of Pb flux are available which agree remarkably well giving annual loadings in the range of 10000-11000 $\mu g\ m^{-2}$. Lead and Ag have similar partition coefficients. Both are highly partitioned to particles. Therefore, we make the assumption that the Ag/Pb ratio in aquatic particles collected in metalimnetic stratified-period traps approximates that of atmospheric aerosols. The average Ag/Pb ratio in summer metalimnetic traps was calculated to be 0.00313 ±0.00073, and multiplying this ratio by the Pb deposition gives an annual Ag flux of 32.9 $\mu g\ m^{-2}\ yr^{-1}$, in close agreement with the first approach.

Outflow:

Mean epilimnetic Ag levels were multiplied by published values of lake water discharge to Lake Huron and diversion through Chicago to arrive at an outflow loss of Ag of 0.34 $\mu g\ m^{-2}\ yr^{-1}$.

Mass Balance:
Sources and sinks of Ag in the Lake Michigan basin are summarized below.

Sources ($\mu g\ m^{-2}$)		Sinks ($\mu g\ m^{-2}$)	
Atmosphere (86%)	31.2	Sedimentation (98%)	24.2
Tributaries (14%)	4.76	Outflow (2%)	0.34
	36.0		24.5

Deposition (Traps)

32.8 $\mu g\ m^{-2}$

Silver inputs are dominated by the atmosphere (86% of loading), whereas, sedimentation is the primary loss mechanism. Agreement between sources and sinks, while not perfect, is quite good considering that this analysis is basically a one year snap-shot. Further confidence in the source loading estimate is given by the close agreement with trap-based deposition (traps) measurements. Additional sources of Ag include both shoreline erosion and river bed sediment transport. However, reliable data are not available to even attempt an estimate of loading from these sources.

The whole lake residence time of Ag with respect to deposition can be calculated by ratioing the water column pool (0.42 $\mu g\ m^{-3}$ * 82m) = 34.4 $\mu g\ m^{-2}$ with the depositional flux = 32.8 $\mu g\ m^{-2}\ yr^{-1}$, giving a residence time on the order of 1.0 years. Considerably shorter epilimnetic residence times are predicted.

Silver Levels and Partitioning in Effluent-Receiving Streams

To examine the fate of Ag through municipal wastewater facilities which are collecting waste from major silver end-users and the impact of the effluent on receiving streams, sampling was conducted at three treatment facilities in 1993 and in more detail at two plants in 1994 and 1995. Results from these studies (Tables 1 and 2) showed that in-plant removal efficiencies for Ag ranged from 92-99%. Variations in influent Ag concentrations and differences in treatment procedures between the facilities had minimal impact on in-plant removal efficiency. Despite 95+% removal efficiencies, effluent Ag concentrations of 1-5 $\mu g\ L^{-1}$ are nearly 3 orders-of-magnitude higher than in the receiving stream.

Very high levels of partitioning of Ag to suspended solids in the plant effluents was observed (particle bound: 100-1000 $\mu g\ g^{-1}$, partition coefficient 450,000-1,200,000 $L\ Kg^{-1}$), suggesting an important role for particles in regulating Ag concentrations. The particle association of Ag was characterized in more detail in the 1994-95 sampling by examining the size-distribution of particle-bound Ag in the effluents and receiving streams. In these systems we consistently find a large (25-35%) portion of the total Ag in small (50-400nm) and large (400-1000nm) colloidal size fractions, with the "dissolved" fraction (<50 nm) representing less than 15% of total Ag. Enrichment of Ag on the particles in the colloid fractions compared with the >1.0 μm particles was not observed except on one date where elevated Ag levels were measured in the small colloid fraction. A large fraction of the total Ag in the receiving stream is similarly associated with colloids: e.g. 10-20% of total Ag in upstream collections is colloidally associated, whereas 25-30m downstream from the effluent discharge colloidally bound percentages are in the range of 25-45%. In contrast with observations in the effluent, colloidal particles in the receiving stream are significantly enriched in Ag compared with large particles.

Silver leaving the plant was rapidly reduced to near background levels by dilution, particle scavenging, and incorporation into stream sediments. Simple mixing models indicate that non-conservative processes can account for 20-60% of this loss, as shown in Figure 5. In spring, under high discharge and high dilution conditions, 19-28% of Ag is lost via non-conservative processes. However in summer when the dilution factor is considerably reduced, non-conservative processes account for 53-56% of the removal. Non-conservative processes affect the loss of Ag in the receiving stream to a greater extent than any of the other metals examined in this study (Figure 6).

Silver Fate Through Jackson POTW*
Ag Levels in μg L-1

Sample	May 1993	June 1994	August 1994	April 1995	June 1995
POTW 24hr Flow (MGD)	0.521	0.498	0.620	1.38	0.600
Inflow Grab	105	73.2	49.9	24.0	53.1
Inflow 24 hr	101	41.2	42.9	------	
Effluent Grab	0.85	5.56	2.58	0.812	0.984
Effluent 24 hr	0.90	2.60	1.28	0.501	
% Removal	99.2	92/94	95/97	96.6	98.1

*Tertiary treatment facility. Alum and polymer added to settling tanks. Sand filtration prior to chlorination. Waste oxidized using rotating biological contactors.

Silver Fate Through Other Studied POTWs

POTW	Brookfield	Fall River	Medford
Type	Tertiary/AS	Lagoon	Tertiary/AS
Chemical Added	Ferric Chloride	none	none
Typical Flow (MGD)	9-11	0.4 - 1.7	1 - 2
Sampling Date	June 1993	July 1993	May 1995
Inflow Ag ($\mu g\ L^{-1}$)	4.27	54.4	1.79
Effluent Ag ($\mu g\ L^{-1}$)	0.079	0.78	0.0279
% Removal	98.1	98.6	98.4

Silver Content of Sedimenting Particles Lake Michigan Southern Basin (ug g^{-1})

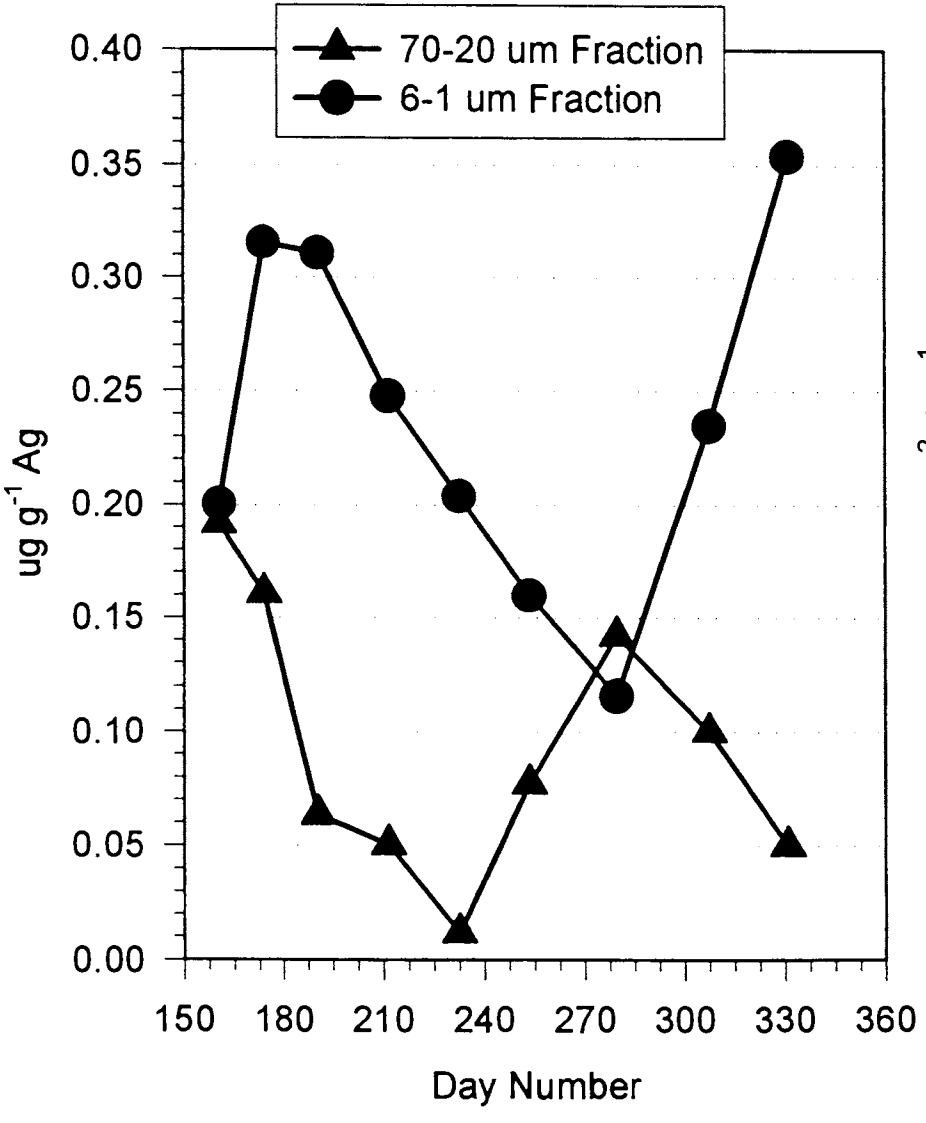


Figure 1.

Primary Flux of Ag To Surface Sediment Lake Michigan Southern Basin

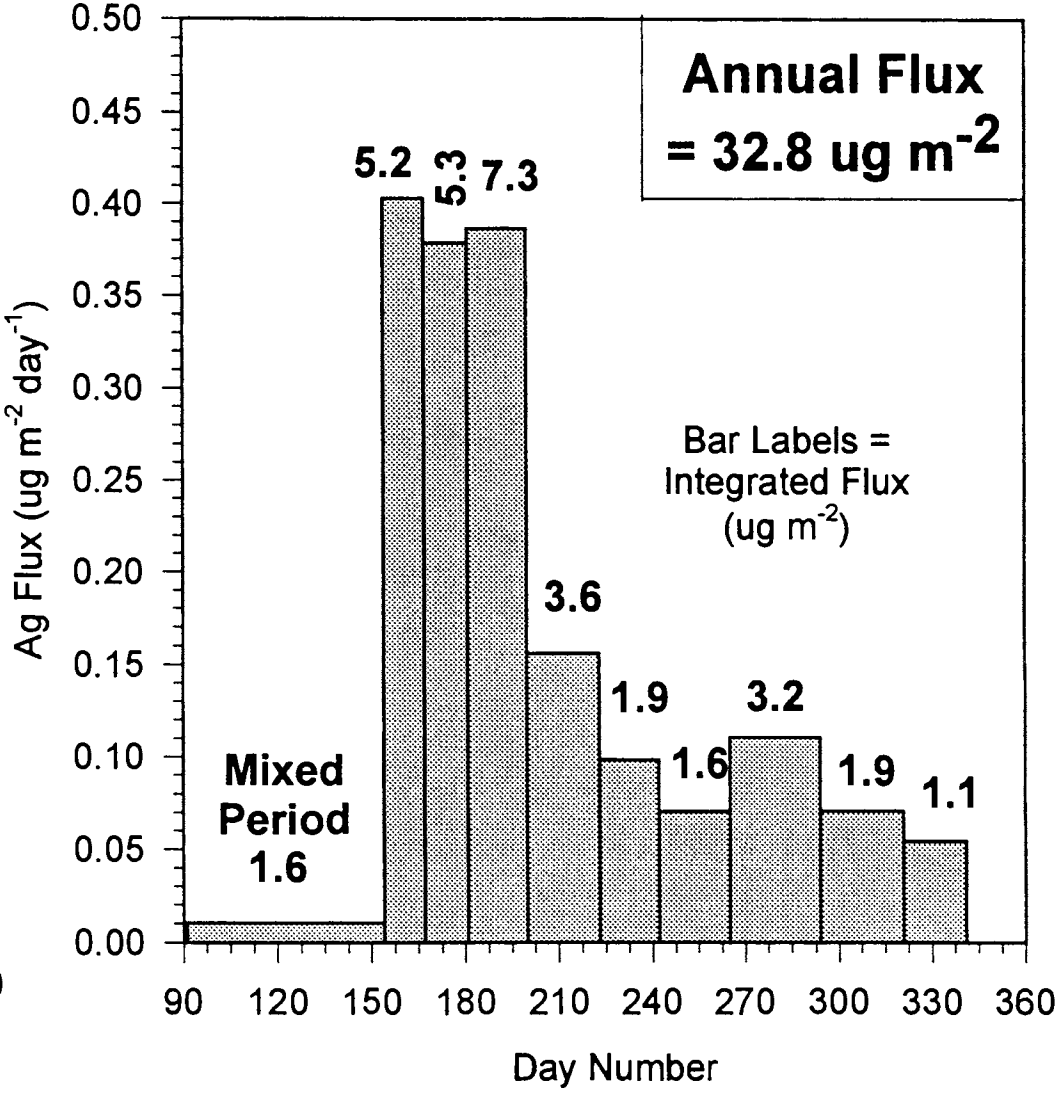


Figure 2.

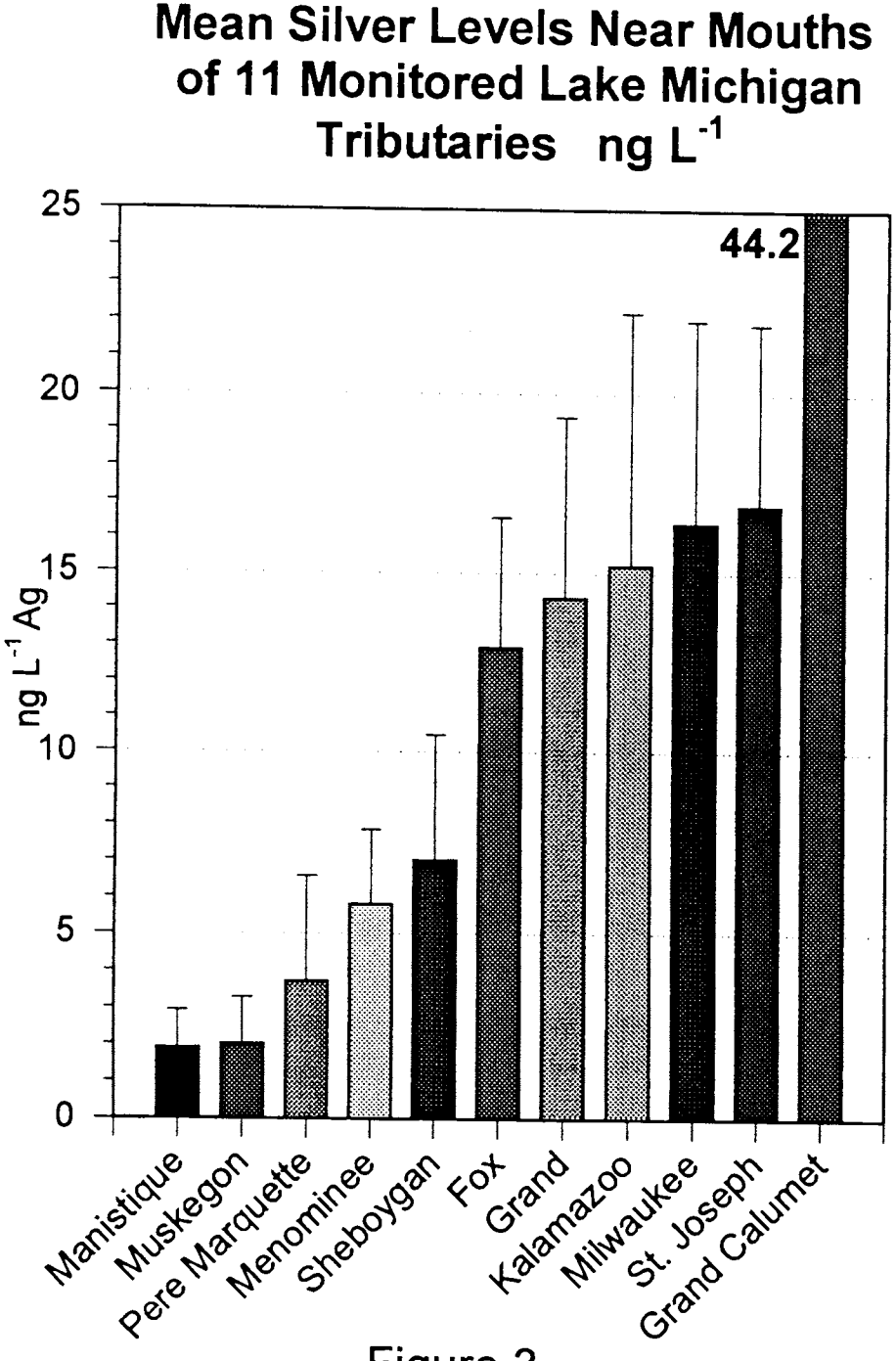


Figure 3

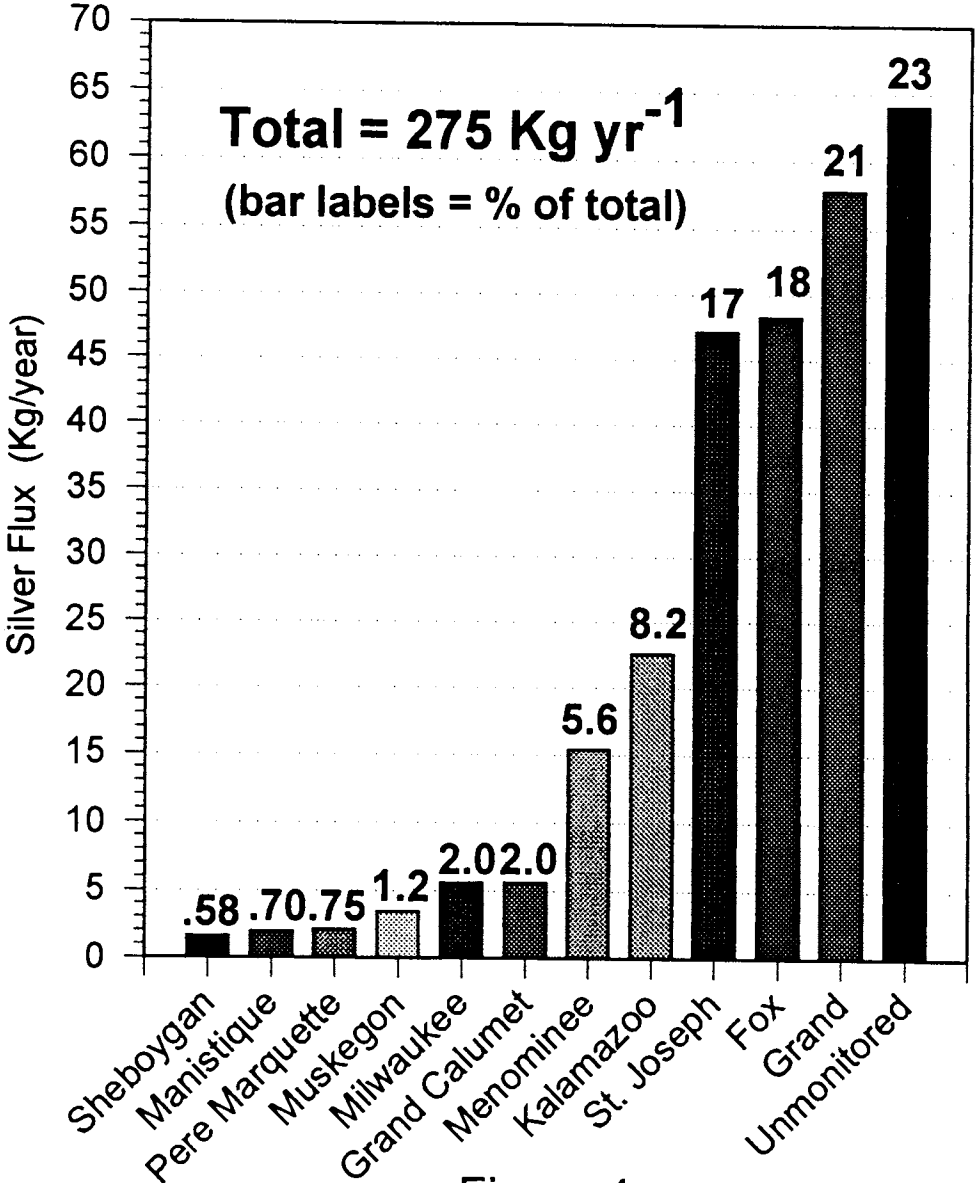


Figure 4

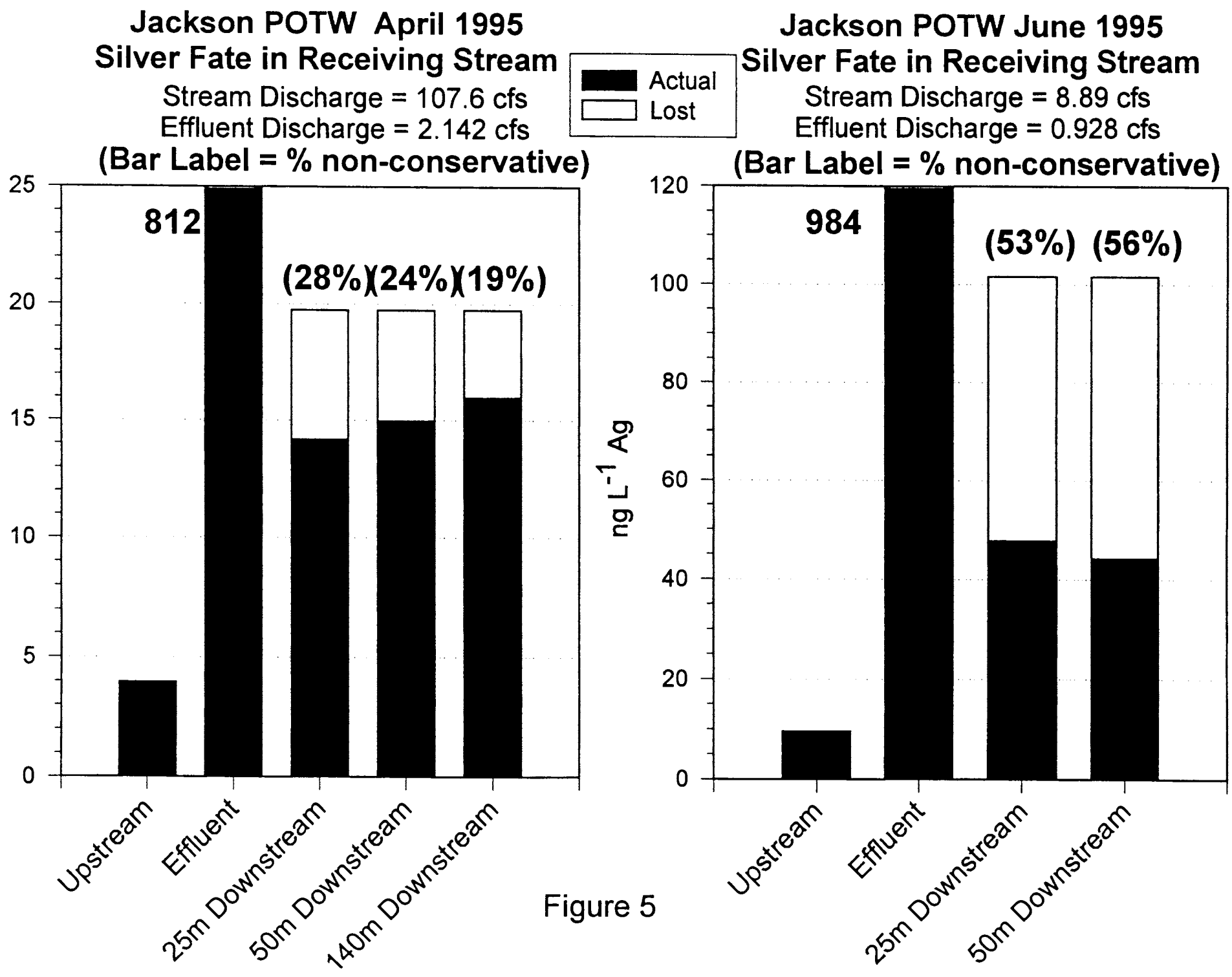
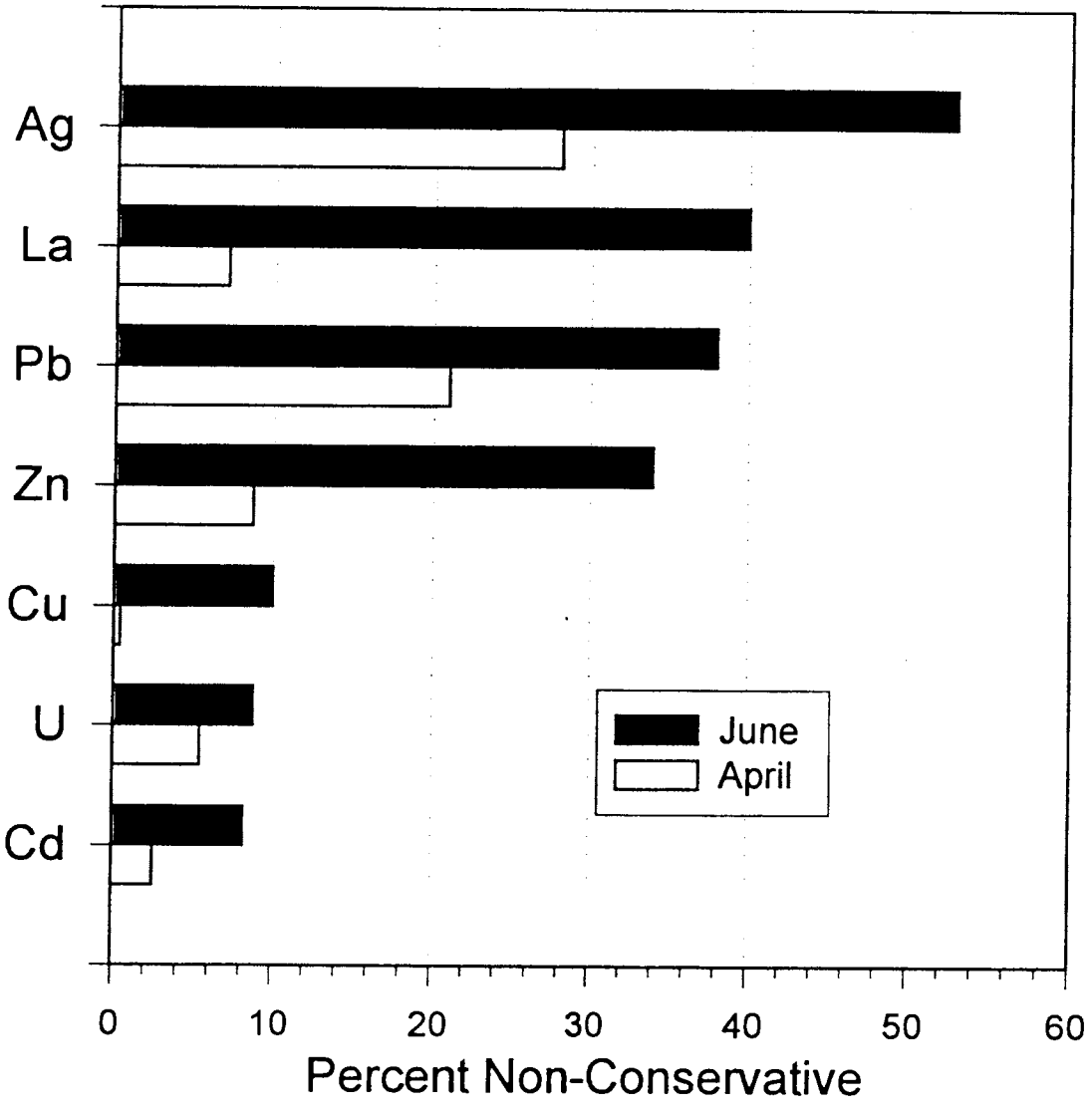

Jackson POTW April 1995
Silver Fate in Receiving Stream
Stream Discharge = 107.6 cfs
Effluent Discharge = 2.142 cfs
(Bar Label = % non-conservative)
Actual
Lost
812
(28%)
(24%)
(19%)
25
20
15
10
5
0
Upstream
Effluent
25m Downstream
50m Downstream
140m Downstream
Jackson POTW June 1995
Silver Fate in Receiving Stream
Stream Discharge = 8.89 cfs
Effluent Discharge = 0.928 cfs
(Bar Label = % non-conservative)
984
(53%)
(56%)
120
100
80
60
40
20
0
ng L-1 Ag
Upstream
Effluent
25m Downstream
50m Downstream

Figure 5

Jackson POTW
Metal Fate in Receiving Stream
Fraction Non-Conservative 25m Below Discharge



Stream - Effluent Discharge Ratio:

June: 9.58

April: 50.2

Figure 6

Questions & Answers: Silver Levels and Partitioning in Effluent-Receiving Streams, and a Preliminary Mass Balance for Silver in the Lake Michigan Basin

Q. TIM FITZPATRICK (Florida Dept. of Envir. Prot.): During your studies looking at the conservative or nonconservative nature of silver downstream, did you actually add a tracer as a marker to dilution or did you just take the streams flow in the effluent flow down as markers to dilution?

A. We used conductivity, and primarily it fits a conductivity balance perfectly, within +/- 2%, so I think that's our best marker. We can, for reliability, go back and look at some things, some other markers, too. I think we've done a pretty good job of collecting appropriate samples.

Q. ANDERS SODERGREN (Lund Univ.): You showed the major part of the input in Lake Michigan was via the air. What kind of sources do you expect?

A. I don't know. I think about distinct offshore emissions of silver but I would presume that there is silver from coal-fired power plants in the area. Lead, cadmium and mercury are almost entirely delivered from the atmosphere, so it might not be so surprising that silver would also be delivered that way. Maybe there's a volatile component of silver that is somehow being ashed to become a part of the atmospheric aerosols and finds its way into the lake. Combustion of some sort could add to the process.

Q. ARUN MUKHERJEE (Univ. of Helsinki): I think my comment would be that, actually, for all contained silver, it depends upon from where it comes and it varies very much in the United States or other places of the world. It seems to me that atmospheric silver over Lake Michigan is not only from industrial sources but also from long-range transport. It may be that silver particles may be so small they can travel hundreds of kilometers and maybe after that there is dry deposition into Lake Michigan.

A. That's undoubtedly true. Much of the mercury input is transported from Missouri to Lake Michigan, which is 800 miles away. Certainly long-range transport is implicated.

Q. JIM KRAMER (McMaster Univ.): I have one question I was wondering if you could comment on. I guess the hydrogeologic regimes in different seasons might have some effect in terms of the distribution downstream. Does that seem to be important or is that not a factor? I'm asking because of the data you showed.

A. It's obvious that it shows something but I'm not quite sure what. It may take a certain amount of colloidal particles from the effluent to get to some critical coagulation level, or just a simple cohesion or a combination of those two to result in the varying degrees of removal that we've seen with seasons.

Silver in Fresh Water: Sources, Transport and Fate in Connecticut Rivers

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Silver is one of the most toxic and persistent contaminants in the environment. Nevertheless, little is know about its baseline concentration, transport, and ultimate fate in riverine systems. In addition, most earlier measured values suffer from contamination artifacts and are unreliable. Using ultra-clean techniques, measurements were made to determine the seasonal variations of the concentration and distribution of silver between filter-retained and filtrate forms for three Connecticut rivers: the Connecticut, the Naugatuck and Quinnipiac.

In each river, silver concentrations follow a similar pattern. Total silver levels are generally under 5 ng/l in undeveloped headwaters, and between 25 and 100 ng/l in areas influenced by sewage effluent, urban runoff, or industrial discharges. Variations are due not only to the size of the input, but also to size of the river and its dilution capacity. In the Quinnipiac River, which supported a large silver plating industry for over a century, silver exceeds 500 ng/l in the water column in one region near its mouth (fig. 1). On average in these rivers, particulate silver (>0.45 μm cut-off) accounts for over 80% of the total silver concentration in sections "contaminated" by sewage effluent.

The Quinnipiac River was studied in more detail to determine mechanisms controlling transport and fate. Located in the central lowlands of Connecticut, the river meanders through glacial deposits and finally empties into Long Island Sound at New Haven. Historically, the watershed of the Quinnipiac has been marked by high levels of industrialization, most notably supporting a now defunct silver plating industry in its central and southern reaches. Today, new sources of metal pollution primarily consist of permitted industrial discharges and sewage treatment plants. However, examination of floodplain and ponded river sediments has revealed a large inventory of deposited silver contamination. Using radiometric dating techniques, peak concentrations were found to have occurred in the 1950's. The concentrations in the southern floodplain deposits ranged up to 30 μg/g. In Hanover Pond, a river impoundment located directly downstream from the former major silver plating industry, silver concentration in the

sediment ranged up to 250 μg/g. The presence of high concentrations of Ag stored in the sediments raises the question of the extent to which past pollution contributes to present day contamination levels of Quinnipiac River waters.

To answer this question, periodic and storm event water column sampling were conducted. Bi-weekly samples were collected from a single location on the Quinnipiac River over a wide range of flow conditions. Suspended particulate matter (SPM) measurements ranged from 1.5 mg/l to 44.2 mg/l on 26 dates collected over a twelve month period between May, 1994 and April, 1995 (fig. 2). Two storm events were sampled in April, 1995 at the same location. Total silver concentrations correlate linearly with SPM, both for periodic measurements over the course of a year and for intensive sampling over storm events. During the storm hydrograph, the silver follows SPM, with peak concentrations of both parameters occurring at the same time. The peak silver concentration resulting from a one inch rainfall was over 800 ng/l (fig. 3). The two sets of data are consistent with the hypothesis that silver is being supplied from resuspension of contaminated sediments and river bank erosion.

To confirm this hypothesis, river bottom sediments, point bar deposits, and river bank soil were analyzed. In the sandy river bottom sediments, total silver is below 3 μg/g, but this value is much higher when normalized to the fine fraction. To corroborate these findings, samples were taken along a transect from the center of the river channel, up a point bar, to the river bank. These results showed an increase in both the fine grain fraction and silver concentration away from the channel Near the river bank, silver concentrations reached 9 μg/g in this transect. Nevertheless, the total amount of silver stored in the river sediments is small compared to Ag in the water column. However, silver concentrations in the river banks ranged from 15 - 30 μg/g, similar to the silver concentrations on SPM. We hypothesize that these deposits were once an important sink for contaminant silver delivered from upstream industrial sources, and are know acting as the main source of silver to the river. This source is likely to remain active for the foreseeable future.

In an effort to quantify present day sources and investigate removal processes, several detailed river profiles were conducted in the vicinity of a secondary sewage outfall. The concentrations in the treated effluent ranges from 120 - 180 ng/l of which over 90% was dissolved silver. The resulting profiles revealed that dissolved silver from treated sewage declines dramatically in a zone extending just 500 m downstream from the outfall. Plotting Ag against the conservative tracer conductivity produces a straight line (fig. 4), showing that the decrease in Ag occurs via conservative mixing. This suggests that non-

conservative scavenging of the metal does not occur on the time scale of the mixing process (<30 min.).

Fig. 1 Silver concentrations in the Quinnipiac River, CT.

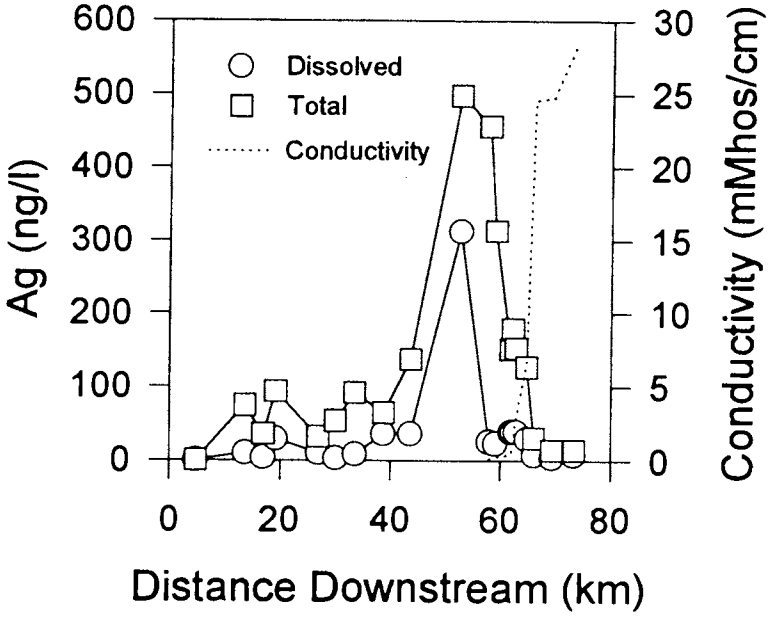


Fig. 2 Silver concentrations versus suspendended particulate matter.

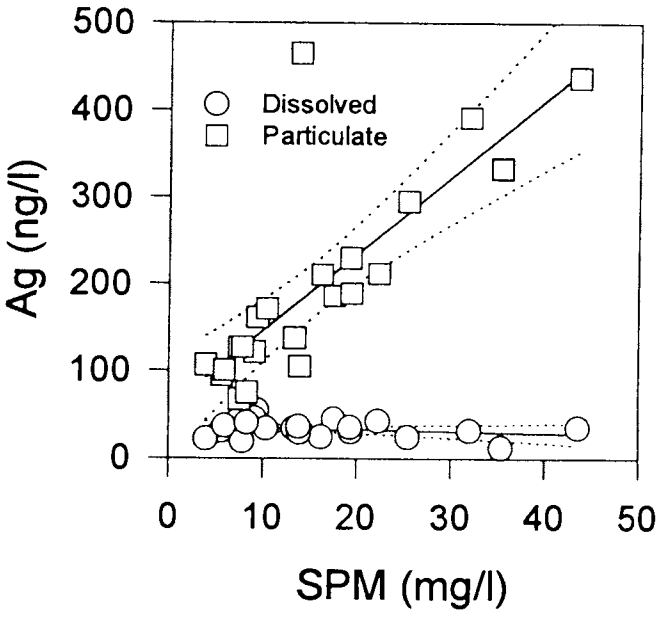


Fig. 3 Silver concentration during a storm hydrograph.

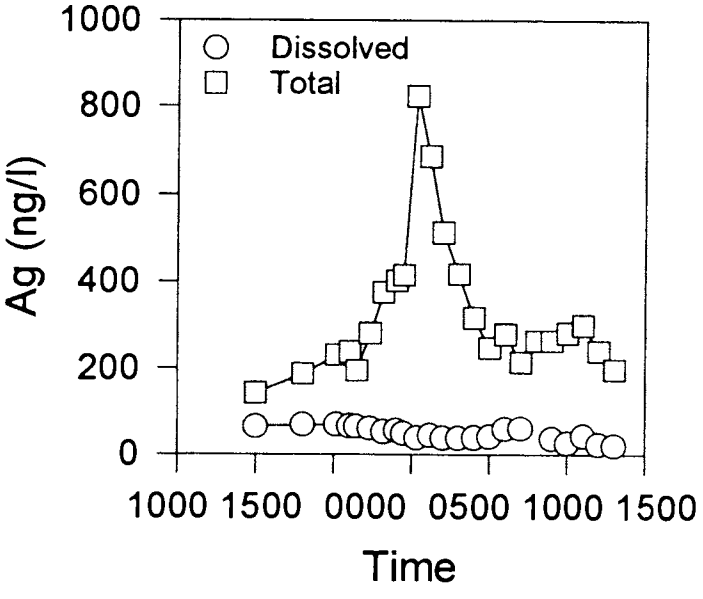
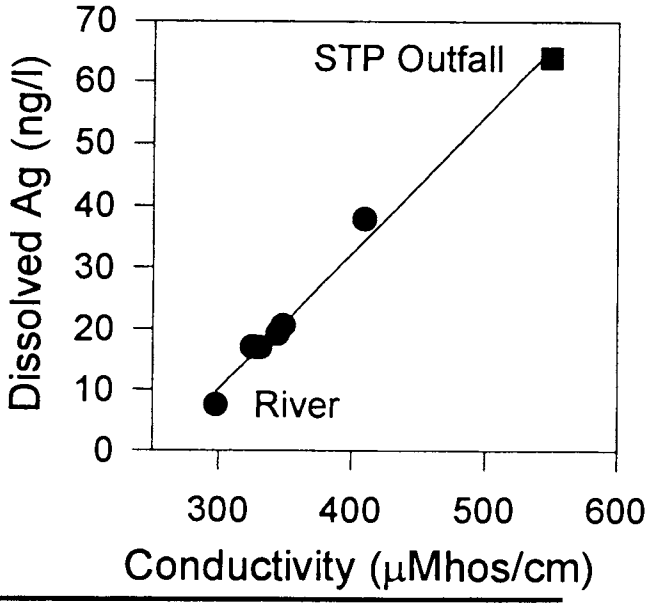


Fig. 4 Dissolved silver versus conductivity downstream from a sewage treatment plant.



Questions & Answers: Silver in Fresh Water: Sources, Transport and Fate in Connecticut Rivers

Q. KEN ROBILLARD (Eastman Kodak Co.): Could you comment at all about the source of silver or might you speculate about the sources of silver in the erosional banks that you tested?

A. The source of silver in the erosional bank is fallout that has been deposited from the silver in the stream when ore was in its heyday. We've dated the floodline deposits using ^{137}Cs and they correspond to a deposition from the 1960s, with a maximum going back to about the 1950s. That also happens to correspond to what we found in the investigated pond where you see that big, huge peak coming out of the sediments. So in this system, it looks like the majority of the silver is coming from what looks like two major sources, the larger one is industrial silver and the smaller one is the silversmith part in another town that's further downstream.

Q. How do you get from the sampling sites to the source?

A. If it's not soil but in the floodline deposits you can follow the river — sometimes it [the silver deposit] is too poor but here there is enough in the floodline.

Q. JIM KRAMER (McMaster Univ.): This is sort of one of the first really complete looks at a major river system. We have a lot of these and I wonder if you could speculate or generalize, given all these old silver deposits: What is the ultimate fate, are they going to just sort of move down or are they fixed there permanently? What would you guess?

A. I actually don't have a guess, but that's what Gabe's going to talk about tomorrow. We actually have continued this investigation throughout the estuary. As part of Gabe's talk tomorrow there's going to be a mass balance that's calculated from what's coming out of the river and what continues down to Long Island Sound. So we should have a good answer to that question if you can wait until tomorrow.

Silver Loadings in Sewage Treatment Plants and in the Natural Aquatic Environment of Finland

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Geological Survey of Finland, Espoo, Finland

Silver is a rare, but naturally occurring element which exists in several oxidation states (0, 1+, 2+ and 3+) in the environment. The oxidation states (0 and 1+) are more common than the 2+ and 3+ states. Its presence in sludge, sediment and water courses is deleterious to aquatic species.

In this study, four sewage treatment plants in the south of Finland were studied with special reference to photoprocessing effluent piped to these plants. Based on the results of total silver in sewage sludge, silver balance for all the sewage treatment plants in Finland was estimated. It was observed that about 5.4 metric tons of silver passed into the sludge of which 58 % is used on arable lands and gardens as fertilizers and the rest is dumped in landfills. On the other hand, about 0.1 tons of silver is discharged through effluent into rivers and the Baltic Sea.

Silver concentration in sediments of headwater streams was also analyzed and the mean value was noted to be 0.11 ppm with the median value of 0.09 ppm. However, the main part of silver found in stream sediments was derived from sulfide minerals. Long range transport of silver pollutants in the southern part of Finland was also documented.

INTRODUCTION

Many heavy metals present in the environment have been reported to be detrimental to man, animals and aquatic species. These heavy metals which include silver are widely distributed in most of the continents of the world. In 1993 about 23 % of the world silver production stemmed from sensitized materials and other sources (The Silver Institute, 1994). In Finland about 38.5 metric tons of silver was recovered in 1994 of which 32 % was from photographic solutions, films, papers, electronic and jewelry scrap. During production, recovery and uses, silver can be expected to enter into the aquatic environment (Lytle, 1984; Sanders and Abbe, 1988). Soils and sediments are also sinks for heavy metals. In sediments, silver can be attached for a long periods and it is estimated that in estuarine and oceanic sediments, the residance time of silver is about 100 years (Boehm *et al.*, 1985). Hence, there are considerable possibilities for bioavailability of silver for marine and freshwater species.

The purpose of this study is to examine the loadings of silver from different sources into sewage treatment plants in Finland and the movement of silver through sludge into arable land and landfills as well as through effluent into rivers and the Baltic Sea. In addition the results are given for analyses of sediments in stream waters carried out through out Finland by the Geological Survey of Finland (GSF) in order to determine the sources

of, and any potential significance of, silver in the Finnish aquatic environment.

MATERIALS AND METHODS

A part of this paper is based on the study of silver in four sewage treatment plants (Helsinki, Espoo, Turku and Lahti) in the south of Finland conducted at the beginning of 1995 (Mukherjee, 1995). Influent, effluent and sludge samples were collected in the month of March-April, 1995. At the sewage treatment plants of Helsinki, Turku and Lahti, sludge samples were collected daily. The samples for each plant were mixed, dried overnight at 105 °C and homogenized in a porcelain mortar with an agate pestle. The samples collected during one month were mixed and one composite sample made for each plant. Consequently, there were three composite sludge samples. Regarding the water samples, each day samples were collected for a certain interval of time from the influent and effluent and stored in a cool place. The sampling period was the same for the Helsinki and Turku whereas water samples from Lahti were collected for a week. The samples collected in each plant were mixed and consequently three master samples from each plant were prepared for total silver analysis. The samples were analyzed by the Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Samples from Espoo were analyzed by the local authority using Atomic Absorption Spectrometry (AAS).

Stream sediment samples were collected in 1990 by the GSF using a scoop net with a mesh diameter of 0.06 mm (Lahermo *et al.*, 1994). A composite sample was collected over a distance of 100 meters by stirring the bottom deposits. The sediments were composed principally of organic material such as animal and plant detritus, humus, and decayed plant fragments, all mixed with various proportions of mineral material. The stream sediment samples were dried, milled and sieved. The <2 mm fraction was leached with conc. HNO_3 in a micro-wave oven. Leachates were analyzed for silver by the ICP-MS method.

RESULTS AND DISCUSSION

Silver in Sensitized Materials in Finland

The sensitized materials are manufactured by different companies. Hence it is difficult to get specific silver concentration value per square meter of film and paper. However, in 1994 about 14 tons of silver were imported through the sensitized materials (7.86×10^6 m^2) in Finland. The detailed calculation is given in the work of Mukherjee (1995). The silver recovery and a silver balance for Finland is depicted in Figure 1.

Silver in Sludge

Details of four Sewage treatment plants in southern Finland and silver in the sludge, influent and effluent for these plants are given in Tables 1 and 2, respectively.

The largest silver concentrations in sludge were noted in the sewage treatment plant in Helsinki and Espoo, respectively. In both cities there are silver recovery plants for photoprocessing wastes. In addition, four other small neighbouring cities of Helsinki piped their effluent to the sewage treatment plant in Helsinki.

Table 1. Capacities of studied sewage treatment plants in four cities in Finland in 1994.

Sampling Site	Effluent flow m^3d^{-1}	Sludge (dry wt) $m^3\ yr^{-1}$
Helsinki	197×10^3	63×10^3
Lahti	14.5×10^3	5.0×10^3
Turku	73.0×10^3	7.9×10^3
Espoo	80.0×10^3	5.0×10^3

Table 2. Total silver in sludge, influent and effluent of selected sewage treatment plants in Finland, 1995.

Site	Month 1995	Influent µg/L	Effluent µg/L	Sludge µg/g (dry wt)	Method	Drinking water µg/L
Helsinki	March	2.62	0.49	90.6	ICP-MS	0.03
Turku	April	2.39	0.12	13.0	ICP-MS	
Lahti	March	?	0.21	7.31	ICP-MS	
Espoo	March	<10.0	<0.05	32	AAS	

In 1992, the total amount of sludge in Finland was 150 000 tons (dry wt) (Ympäristötietokeskus, 1993) and here silver concentration was estimated to be 36 mg kg^{-1} (dry wt) . This means that about 5.4 metric tons of silver had passed to arable and garden soils and landfills through sludge. On the other hand, $1334 \times 10^3\ m^3\ d^{-1}$ of effluent was discharged to rivers and the Baltic Sea from 563 sewage treatment plants in Finland. Based on the silver concentration in effluent at four sites (Table 2), the average silver in the effluent in Finland is 0.22 µg L^{-1}. This means about 0.10 tons of silver entered into the water systems through effluent.

Behavior of Silver in Sewage Treatment Plant

The commomn sources of silver in the sewage treatment plants along with photoprocessing industry are the jewelry shops, electronic and electroplating industry, dental offices, mirror manufacturing and the industries in which silver is present as a trace constituent in raw materials. Different studies indicate that silver from photoprocessing industry enter into the sludge treatment plants as silver thiosulfate complex which will undergo biodegradation into insoluble silver sulfide and metallic silver. The silver from other sources will also enter as soluble and insoluble compounds - a part of which will enter into the sludge. However, it should be kept in mind that a part of the silver from the sewage treatment plant will finish up in the aquatic environment as silver compounds as well as the free silver ion (Ag^+) (Langston and Burt, 1994).

Silver in Stream Sediments

Silver concentrations in stream sediments throughout Finland are given in Table 3. A special ALKEMIA program has been developed by the GSF to produce maps showing the distribution of silver in Finland (Fig. 2).

The highest silver concentrations in stream sediments were noted in the south of Finland and these originate not only from the weathering of sulfide minerals such as sphalerite (ZnS), chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S) but also from industrial sources. In addition there is also the influence of long range transport of silver aerosols to southern Finland. This is evidenced by the measurements of 30 elements from three Finnish EMEP (Cooperative Program for Monitoring and Evaluation of the Long Range Transmission of Air Pollutant in Europe) background stations (Jalkanen and Häsänen, 1994).

Table 3. Total silver concentration ($\mu g\ g^{-1}$ dry wt) in stream sediments in Finland (Unpublished data from Lahermo, 1995).

Number of samples	1164
Maximum Ag concentration	0.65
Mean value	0.11
Median value	0.09

Langston and Burt (1994) observed high silver levels (13.6 maximum and mean 0.041 $\mu g\ g^{-1}$ dry wt) in estuary sediments in the UK (site Gannel, UK) derived from mining activities. Finnish maximum value is quite low, but the mean value is higher than the observed mean value of Langston and Burt (1994).

Conclusion

The occurrence of silver or any other metals in sewage sludge and freshwater sediments indicates the retention of the metal. Silver in sewage sludge is expected to be non-degradable, but its bioaccumulation to terrestrial plant species depends on the pH of the soil solution. It is expected that minute part of the silver will be discharged in the form of free silver ion through effluent into rivers and the Baltic Sea.

Silver in stream sediments may exist as silver sulfide, silver chloride, silver bicarbonate, metallic silver or as active silver. We have not studied in this paper the speciation and bioavailability of silver. It is possible that part of it is bioavailable and part may be suppressed due to the presence of organic compounds and metals such as copper. Hence, further study is necessary on active silver in the aquatic environment.

ACKNOWLEDGEMENTS

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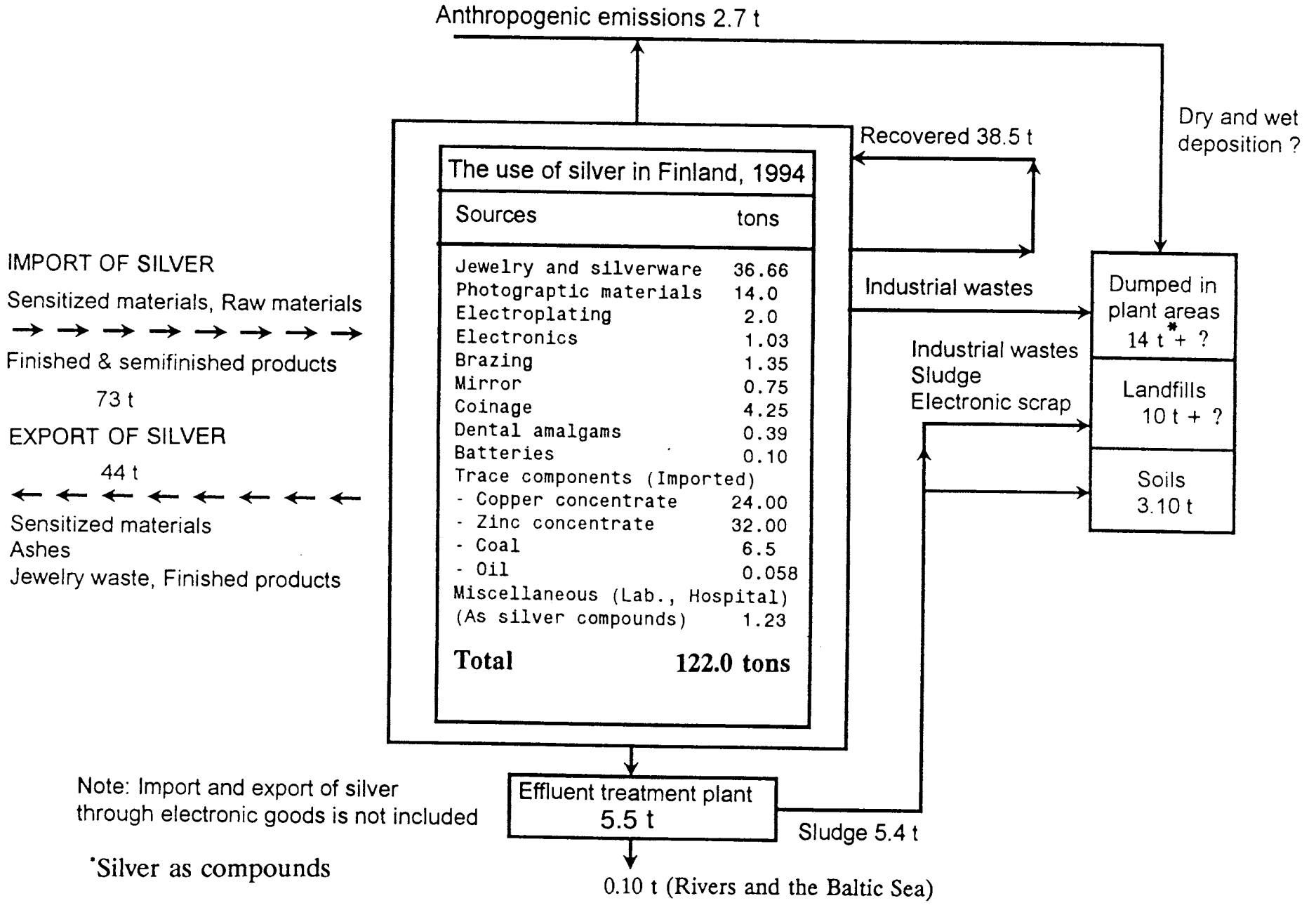

Figure 1. Silver balance for Finland, 1994.

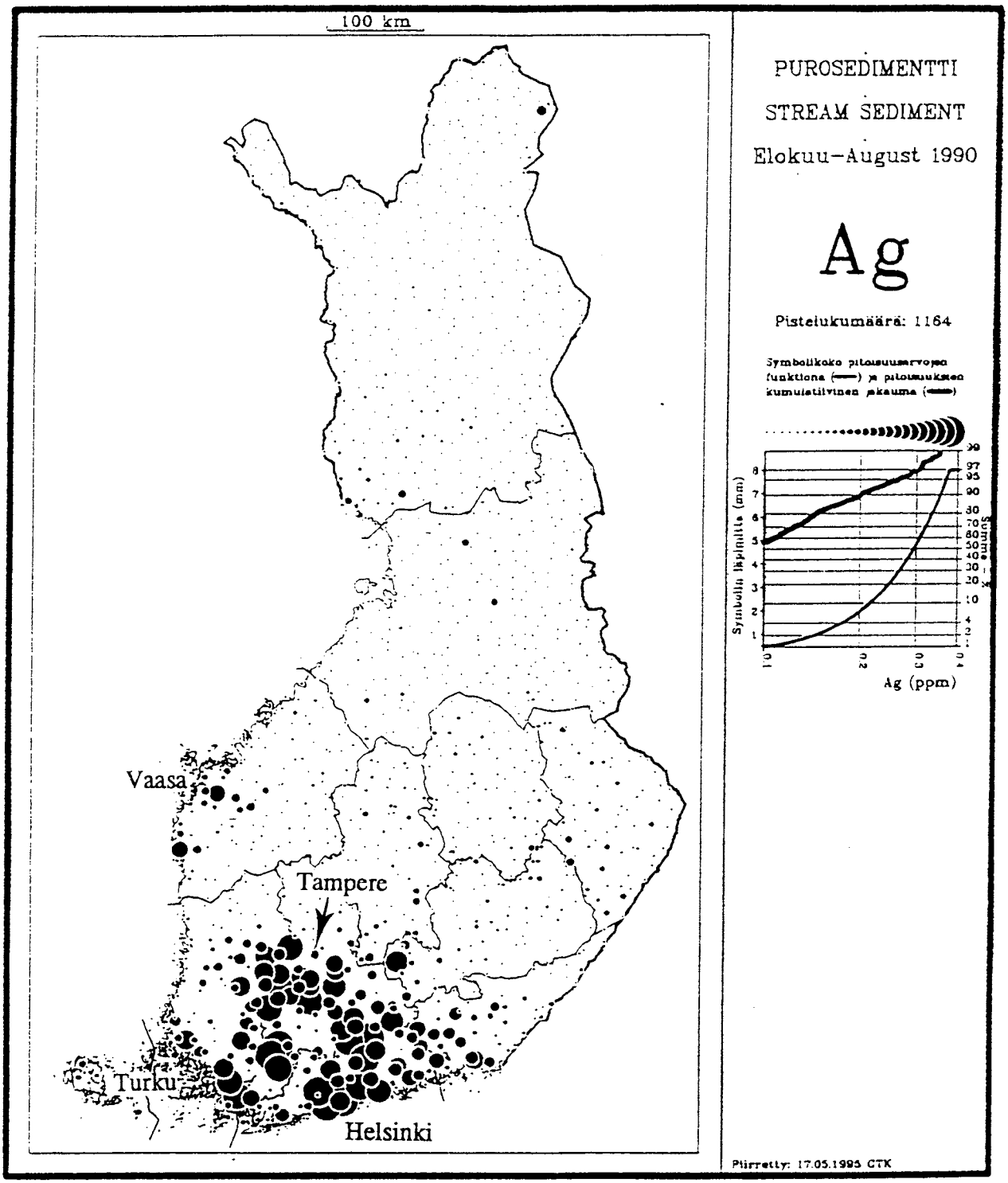

Figure 2. **Silver concentration in stream sediments. Most of the elevated values in southern Finland are presumed to be of anthropogenic and mineralogic sources. In Vaasa and Tampere mineralogical sources are also possible. The reliable detection limit is 0.2 ppm (Lahermo *et al.*, 1994).**

Questions & Answers: Silver Loadings in Sewage Treatment Plants and in the Natural Aquatic Environment of Finland

No questions.

Comparison of Laboratory and Field Partitioning of Silver in Natural Waters

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The determination of silver partitioning coefficients (K_D) is not only of interest for experimental work, but has also been a topic for several recent field studies. Results for field data obtained in Texas rivers and estuaries [1], [2], and Wisconsin rivers [3] were taken for a comparison to partitioning observed in experimental studies [4], to investigate similarities and differences, correlations between silver distribution and environmental parameters, and possible consequences for future laboratory studies and calculation attempts.

In our laboratory work, we studied silver adsorption and desorption in simulated natural waters from fresh to marine type for different solids, and derived partitioning coefficients and parameters for modeling with MINTEQA2, a geochemical equilibrium speciation program [5]. Experiments were carried out under light exclusion (to avoid enhanced adsorption, s. [6]), and with ultraclean techniques developed for field sampling (acid-cleaned labware, high purity grade reagents & solvents, etc.). A known sediment amount was added to the adsorption solution, the suspension was shaken on an incubator/shaker at 25°C for a day to achieve uniform distribution. Aqueous $AgNO_3$ solution was added and shaking was continued for 2 days to ensure full adsorption equilibrium. The suspension was then filtered through precleaned, preweighed, 0.4 µm pore size polycarbonate membranes. After this, either the silver loaded sediment and container were subjected to acid extraction, or the sediment was resuspended in the respective desorption solution and again equilibrated for a defined time period, followed by sediment separation and extraction as described above. All obtained solutions were analyzed for their silver content with Zeeman-background corrected GFAA. Finally, silver and sediment mass balances were calculated from the results.

Compared to "real", environmental samples, the experimental set-up represents a simplified, closed, equilibrated system, where many important parameters can be controlled directly. We worked with a known, high silver concentration (in most cases 10^{-7} mol/L, which is ≥1-2 orders of magnitude above field concentrations), and a relatively short contact time between silver and sediment. Whereas field samples are subject to a variety of influences: e.g. chemical and physical characteristics of the water body, competition with other heavy metals like copper or mercury; presence of complexing agents (esp. dissolved organic matter); composition and particle size of suspended material and bottom sediment and interactions between them.

Experimental K_D were derived using the conditional equilibrium constant approach developed by Oakley et al. [7]. So-called "single point" partition coefficients were determined for cases where no full isotherms were available, by taking the ratio of the adsorption density (particle-bound silver concentration/sediment mass) to the dissolved silver concentration. Averaged results for a simulated lake water solution (pH 8, salinity 0.2 °/$_{oo}$)

are summarized in the following table. Deviations in K_D values were less than 5% for sediments and bentonite, up to 10% for the amorphous oxides.

Adsorber 100 mg/L	K_D (single point) in L/g	K_D (isotherm derived) in L/g
Estuarine Sediment	1300	n/a
Lake Sediment	1200	440
Bentonite	340	250
amorph. MnO_2	270	210
amorph. $Fe(OH)_3$	19	17
Goethite	6	11

Depending on the substrate, K_D values can vary over several orders of magnitude. Possible reasons for these differences have been discussed elsewhere in detail [4]. The measured isotherms showed mostly Langmuir type behavior. When adsorption is considered mainly as a surface reaction between free silver cations and adsorption sites, the sediment surface charge (determined by the "Zero Point of Charge" of the substance, [8]) plays a very significant role. At the experimentally used pH 8, the sediments, bentonite, and MnO_2 should be negatively charged, and therefore have the highest silver adsorption capacity. Additional mechanisms like ion exchange, binding to organic surface compounds, precipitation, etc., can enhance the adsorption (esp. for the sediments).

The coefficients for the most efficient adsorbers (sediments and bentonite) agree very well with results from environmental samples, as can be seen in the following table:

	log K_D(L/kg)
Exp. data for sediments	6.1
Exp. data for bentonite	4.6 - 5.5
Texas field data (Benoit et al., [1])	
• 0.4 µm filter pore size	5.14 (4.4 - 6.6)
• 0.1 µm filter pore size	5.48 (4.6 - 6.6)
Texas field data (Gill, Santschi et al., [2])	
• 0.4 µm filter pore size	5.0
• 0.1 µm filter pore size	5.4
WI field data (Shafer et al., [3])	
• 0.4 µm filter pore size	5.7 - 5.9
• 0.1 µm filter pore size	5.7 - 6.2

Coefficient values can span several orders of magnitude for both field and experimental data, depending (among other influences) on the amount and particle size of the suspended material present.

One important aspect investigated in field studies lately is the high affinity of silver towards colloidal material. Apparently, the amount of dissolved silver measured is determined by the separation method for the sediment. Using a smaller filter pore size, between 30% to over 80% of the "dissolved" silver was detected to be associated with colloids (leading to higher values for the partitioning coefficients). When the obtained K_D are plotted against sediment concentration, an inverse correlation between these values is found: the so-called "particle concentration effect", for which the colloid-bound silver fraction seems to be responsible. No such correlation was found for the experimentally obtained coefficients. In fact, most of our laboratory studies (with 0.4 µm pore size filter membranes) showed no evidence for colloid formation. Practically all of the added sediment was recovered at the end of the experiment (loss was usually less than 1-2%). But: Colloid association might be an explanation for the aforementioned K_D deviations for the amorphous manganese and iron oxides. Another hint was found in long-term desorption studies where after 4 weeks the amount of released silver started to increase moderately (by 3-4%), and not all of the sediment could be recovered. This will be an interesting topic to investigate in future studies.

For both field and experimental data, the molar concentration of particulate silver (Ag-S) was found to increase with increasing sediment concentration (SPM), whereas the silver content of sediment calculated on a per gram basis decreases with growing SPM. Obviously, adsorption does not increase linearly - particulate silver is "diluted" by unoccupied sediment for regions of high SPM.

Solution salinity, esp. chloride concentration plays an important role for silver partitioning, too. A clear (non-linear) dependence of dissolved silver concentration on the chloride concentration was found for the experimental data. The same could be observed for the field data, though the correlation was not as distinct. High chloride concentration is thought to favor the formation of negatively charged silver polychloro complexes which are repelled by sediment surfaces and therefore decrease the amount of adsorbed silver. Experimentally, a direct connection between pH and Ag-S was found, as decreased pH leads to neutral or even positively charged sediment surfaces and in turn to lower silver adsorption. It was even possible to combine effects: At a given SPM, silver adsorption could be decreased by 10% when the pH was lowered from 8 to 5 and the chloride concentration increased from 16 mg/L to 160 mg/L. No pH dependence was found for any of the field data sets - here the influence of other parameters was predominant.

As long as it is not possible to identify all the different silver species and determine their concentrations experimentally, the prediction of silver partitioning for different environmental settings remains a valuable tool for the estimation of silver bioavailability, residence times, sediment loads etc. We used our measured Langmuir type adsorption isotherms to derive the calculation parameters necessary for modeling with MINTEQA2 (see [4] for details). Results for lake sediments in various solutions have been presented earlier [4]. Here, we

performed calculations for silver adsorption to different amounts of bentonite in simulated lake water solution (pH 8, 16 mg/L Cl^-):

Exp. results:	50 mg/L SPM	100 mg/L SPM	200 mg/L SPM
Ag diss.	58.1%	18.3%	6.0%
Ag-S	41.9%	81.7%	94.0%

MINTEQA2 calculation results with parameters log K_L^{act} = 6.9 M^{-1}, $[S_T]$ = 9.7x10^{-7} M:

species	50 mg/L SPM	100 mg/L SPM	200 mg/L SPM
Ag^+_f	41.6%	12.7%	5.5%
$AgCl^\circ$	18.6%	5.7%	2.4%
Ag-S	38.9%	81.3%	92.0%

The prediction agrees very well with the experimentally observed silver partitioning.

The same modeling was applied to a large set of field data, with calculation parameters obtained for sediments (closer to actual composition of suspended material in streams). As examples, the results for two sites with relatively high particle and chloride amount are given:

Exp. results	Site a: 230 mg/L Cl^-, 176 mg/L SPM	Site b: 4.5 g/L Cl^-, 296 mg/L SPM
Ag diss.	63.2%	30.9%
Ag-S	36.8%	69.1%

MINTEQA2 calculation results with parameters log K_L^{act} = 7.4 (M^{-1}), $[S_T]$ = 1.0x10^{-6} M:

species	Site a	Site b
Ag^+_f	4.2%	-
$AgCl^\circ$	47.0%	1.3%
$AgCl_2^-$	33.2%	18.4%
$AgCl_3^{2-}$	-	3.5%
$AgCl_4^{3-}$	-	2.2%
Ag-S	15.2%	74.5%

Here, the calculation gives satisfying results for site b only. An interesting aspect for this site is that all dissolved silver is expected to be present in form of polychloro complexes. The predicted absence of free silver cations is a general finding for high sediment loadings and high chloride concentrations.

For calculations with field sample values, we found average deviations of 15 to 20% between predicted and measured particulate silver. A main reason for this is the use of a very simple adsorption reaction between a free silver cation and an adsorption site in the MINTEQA2 program. While this modeling works well for a simple system, in reality there is not only one kind of adsorption site on a uniform sediment but a distribution of sites with differing affinities and accessibilities for silver. The calculation cannot account for variations in particle size and the heterogeneous composition of the solid matter. On the other hand, development of more sophisticated models will require details about environmental samples exceeding the current knowledge.

From this comparison, we can conclude that the high particle affinity of silver was confirmed experimentally and that silver partitioning can be satisfyingly simulated by laboratory experiments with highly adsorbing materials. Influences of single parameters like chloride concentration, pH etc. are best studied in laboratory experiments (elimination of interfering factors, less data scattering). The high complexity of "real" water systems makes predictions of silver partitioning with relatively simple modeling programs like MINTEQA2 difficult, but can still yield reasonable results. The influence of colloids and SPM particle-size distribution on silver adsorption are interesting subjects for future laboratory studies and calculations on silver partitioning and environmental mobility.

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Questions & Answers: Comparison of Laboratory and Field Partitioning of Silver in Natural Waters

Q. KEN ROBILLARD (Eastman Kodak Co.): At the beginning of your talk you mentioned that care was taken to exclude light from the studies. Do you have any data that suggest that if you do this in the presence of light you will get different results?

A. David Sedlak presented a paper last year where he studied the influence of light-induced adsorption to bentonite, and you can see that adsorption of silver is distinctly enhanced in the presence of light.

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Session 5

Behavior of Silver in Sediments

A.W. Andren
Session Chair

Reactions of Trace Metals in Lake Sediments

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A large body of measurements indicates that an important fraction of trace elements introduced into the aquatic environment is found associated with suspended or bottom sediments. The processes that govern the scavenging of trace elements by particulate matter and their release to the ambient water when the environmental conditions are changed must be understood if the impacts of trace elements on the environment are to be predicted. In this report, we discuss briefly several of the processes occurring in lakes.

Transport of metals. Field measurements in circumneutral lakes show that sinking particles (especially plankton) scavenge trace metals from the water column (e.g. Sigg et al., 1987). In acid lakes, however, dissolved metal concentrations in the overlying waters are typically high and decrease sharply below the sediment-water interface, as shown for zinc in Figure 1a (Carignan and Nriagu, 1985; Carignan and Tessier, 1985; Tessier *et al.*, 1989). The steepness of the profiles near the sediment-water interface tends to increase with decreasing pH. The steep metal concentration gradients observed in acid lakes suggest that downward diffusion across the sediment-water interface plays an important role in the accumulation of trace metals in sediments. It can be assumed that

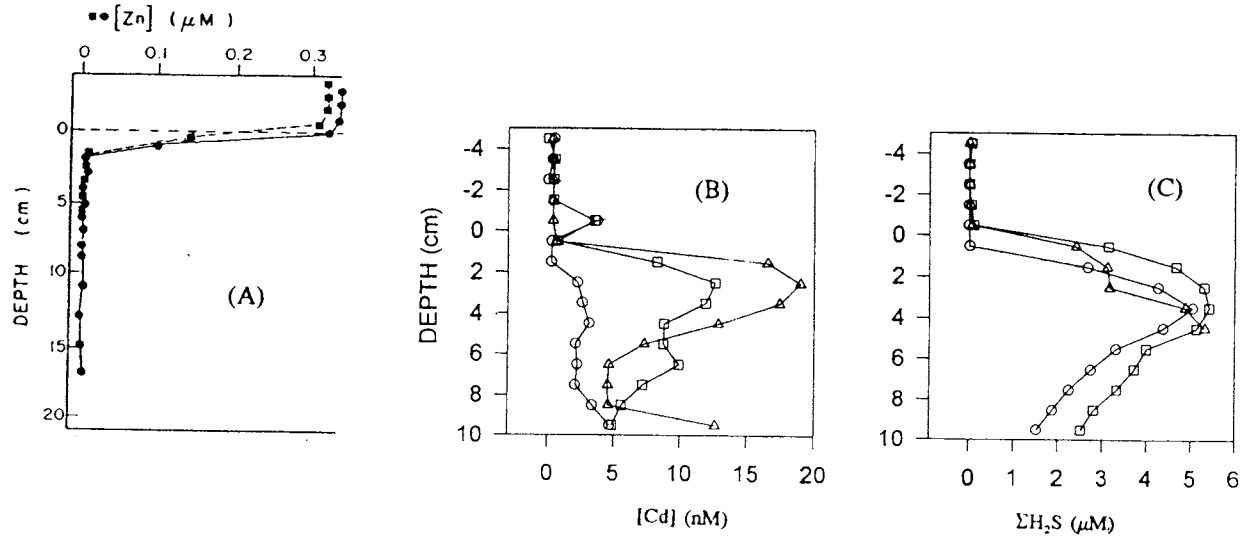

Figure 1. Porewater profiles in acid lakes. Zn in Clearwater L. (A); Cd (B) and ΣH_2S (C) in Tantare L.

transport of trace metals to bottom sediments is by sinking particles or diffusion across the sediment-water interface to a sink located below the interface. For two acid lakes, diffusive contributions were estimated to represent 52-76% for Zn (Clearwater L., pH 4.5 and Tantare L, pH 5.3), 24-52% for Cu and 76-161% for Ni (Carignan and Nriagu, 1985; Carignan and Tessier, 1985). The relative importance of the processes participating in the regulation of trace metals should vary among lakes and trace metals; variables to consider in a systematic study of their relative importance should include pH, sedimentation rate, depth, flushing rate and sediment characteristics.

Solubility equilibrium. Solubility calculations performed on lake porewaters show saturation indexes (log IAP/K_s) close to 0 for Fe (mackinawite, greigite), Cd (amorphous CdS), Co and Zn (amorphous ZnS) sulfides in the anoxic layers of sediments, where sulfate is reduced (Huerta-Diaz *et al.*, 1995). Inference that these solid compounds are formed and control dissolved metal concentrations are essentially based on saturation indexes; more direct evidence is needed. For class B metals, concentration profiles indicate a remobilization in the zone of dissolved sulfide production, as shown in Figure 1b, 1c for cadmium (Huerta-Diaz *et al.*, 1995; Warren, L. unpublished results); this figure shows that dissolved Cd is correlated with dissolved sulfide. The shape of the Cd profiles (but not the absolute Cd values) can be simulated by assuming: i) solubility equilibrium with CdS(s), and ii) speciation of dissolved Cd is dominated by the complex $Cd(HS)_2$. A better knowledge of the stoichiometry and formation constants of sulfide complexes, particularly for those prevailing at the low dissolved sulfide concentrations (micromolar) typical of many aquatic systems would improve greatly our predicting power.

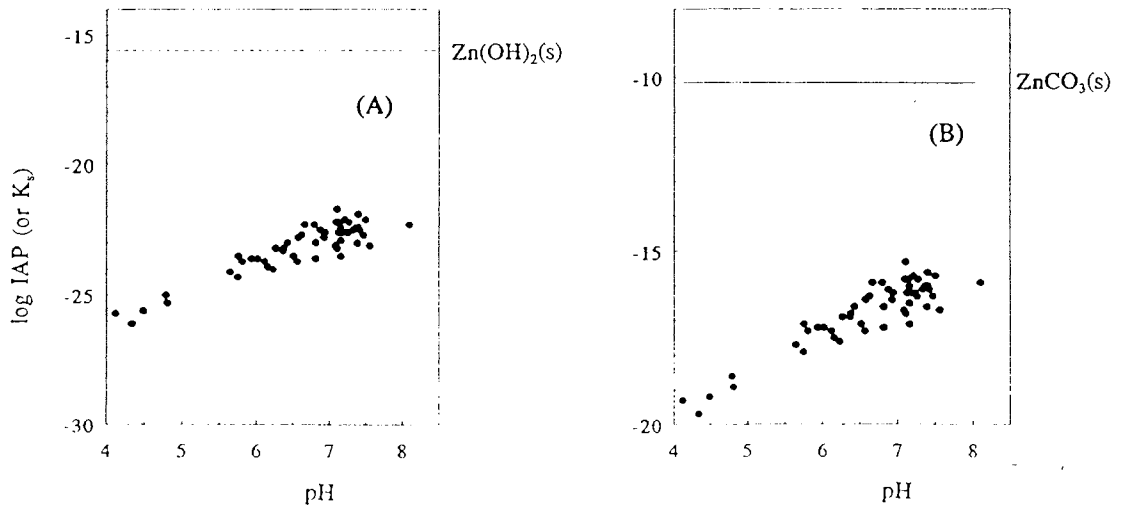

Figure 2. Saturation state of lake waters with respect to $Zn(OH)_2(s)$ (A) and $ZnCO_3(s)$ (B). IAP is the ion activity product. The horizontal lines correspond to the solubility products.

In contrast to anoxic porewaters, oxic overlying waters do not appear to be saturated with respect to known pure solid phases of trace metals, as illustrated for Zn in Figure 2. Similar undersaturations are observed for Cd, Cu, Ni and Pb.

Adsorption on sedimentary Fe and Mn oxyhydroxides. Iron and manganese are commonly recycled in lake sediments. Diagenetic iron and manganese oxyhydroxides which form in the upper oxic layers of the sediments can be isolated *in situ* by vertically inserting inert collectors into the sediments. Conditional constants for the adsorption of trace metals, M, on these diagenetic iron ($K_{Fe\text{-}M}$) or manganese ($K_{Mn\text{-}M}$) oxyhydroxides can be determined using the concentrations of trace metals associated with the Fe and Mn rich material and the measured dissolved concentrations of these metals Tessier, unpublished data). These *in situ* derived conditional constants are correlated with the hydrolysis constants of the metals (Fig 3) and with laboratory derived intrinsic surface complexation constants obtained for adsorption of these metals on well-characterized Fe and Mn oxyhydroxides (Fig. 4). For a circumneutral lake, the values of the conditional constants $K_{Fe\text{-}M}$ and $K_{Mn\text{-}M}$ can be reasonably well predicted with consistent sets of intrinsic surface complexation constants and the other surface characteristics required for the calculation. For an acid lake, however, the measured $K_{Fe\text{-}M}$ values are systematically much higher than predicted, presumably due to binding of the metals to organic matter coating the Fe oxyhydroxides.

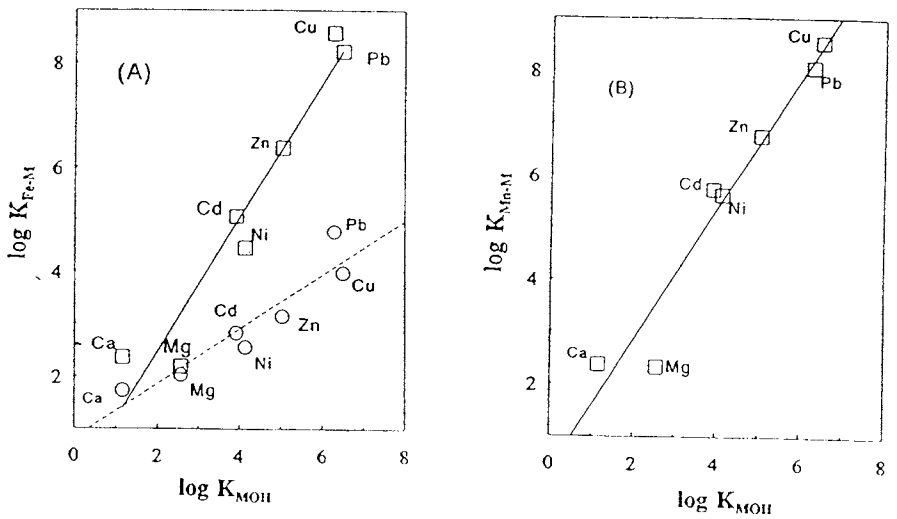

Figure 3. Correlations between log $K_{Fe\text{-}M}$ (A) or log $K_{Mn\text{-}M}$ (B) and log K_{MOH}. (□) and (○) are for McFarlane (pH 7.4) and Clearwater (pH 4.8) lakes, respectively.

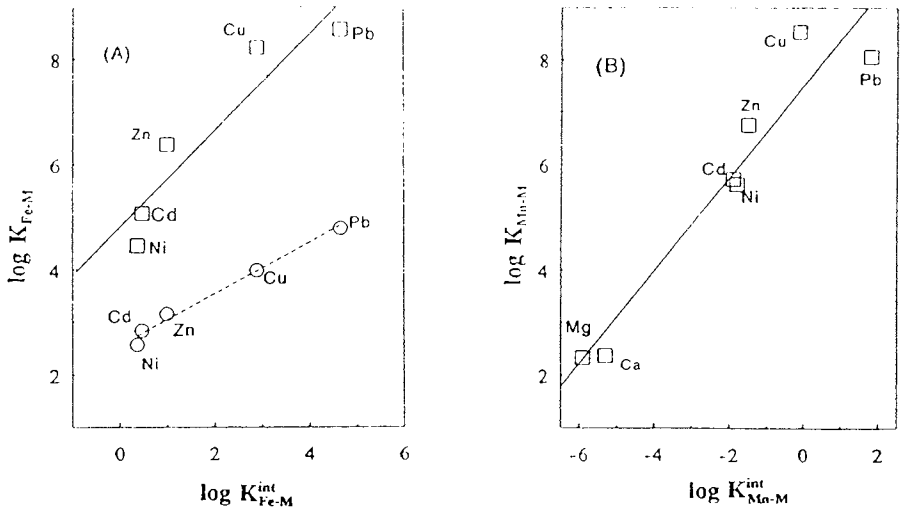

Figure 4. Correlations between log K_{Fe-M} and log K_{Fe-M}^{int} (A) or between log K_{Mn-M} and log K_{Mn-M}^{int} (B). (□) and (○) are for McFarlane (pH 7.4) and Clearwater (pH 4.8) lakes, respectively.

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Questions & Answers: Reactions of Trace Metals in Lake Sediments

Q. PETER SANTSCHI (Texas A&M Univ.): I have a question, actually. You started out showing the zinc profile compared to the lead. This was from a paper which you published in the '80s, I think, and you showed there that you have a very sharp break in the zinc concentration in the layer where the lead content is almost constant. Then you chose to assume that you had particle mixing which smoothes out the lead content, and then you dated the sediments using the lead content deeper down. If you follow that through you could not have the sharp zinc break because with that type of particle mixing zinc would be smoothed out. It's not comparable because you have assumed a very high particle mixing there. So then you chose to ignore that or you said, "Well, maybe I have great lead mobility" — when you do have lead mobility you can't use the lead to date. Now, it's the layman's function to be curious. There was the lead thing, the lead was the one metal which did not agree in your correlations later on, but on the other hand the lead constants were up high. So your experimentally determined lead constants were higher than you would have predicted. So there is only the consistency that the lead is a fascinating element that shows some inconsistencies which you have not addressed. Could you comment?

A. I don't have much comment on that. But that's a fact, that we see that inconsistency even in the adsorption. If you look at the linear adsorptions that are given, and the linear free energy of adsorption that are given, it's that lead deviates there, too. For one, we compare the intrinsic constant we determined against the analysis constant. Lead is the only one, the only outsider in the correlation. If you do the same for the adsorption constants that are given by Smith and Jenne, again lead is an outsider.

Q. What I meant is, if here you have the transport inconsistency, if you say here you have a mixing zone, you can't calculate from the lead content a mixing rate which will be very high if you apply it to the zinc. If, say, you had any kind of sharp input from 1940, it would be smoothed out, it would look like the lead content, almost. You can't have these sharp break-ins in a mixing zone, you can't say that this was produced by particle mixing when you do the actual calculation.

A. You mean for the total zinc?

Q. Yes.

A. It's right, that's why you have this base concentration here, because we suppose it is so without the organisms. The organisms weren't mixed in this high concentration with those at the top so it wouldn't smooth the peak.

Q. But the mixing rates which you calculate from the lead are so high that you can't maintain this sharp break without having a huge flux. When you haven't taken it into account there you should point it out. We have to discuss that later.

But I have another question: You chose the iron as an example and we have heard lately, I think it was on Monday, that silver adsorbs very little on iron oxides. Is your feeling — I don't know, these were probably different iron oxides — is your feeling that silver would adsorb more on the natural iron oxides, which you have looked at, than what Anders was showing on Monday?

A. I'm not sure. You refer to the correlations again, the linear free relationships that I gave, and it looks well that that is valuable for iron and for manganese oxides also. I would guess that it is not that important. It's not strongly bound, though.

Q. JIM KRAMER (McMaster Univ.): I'd like to talk with you a little bit about your invoking the organic coatings for the descriptive data on Clear Water Lake. I have no argument with your reason that at low pH the organics may coagulate. But Clear Water Lake is very, very low in organics. It's not too typical. But I guess my question is, I presumed, first of all, that you may have to add this additional consideration for low-pH lakes where you have, let's say, iron oxides available as a substrate. If that's true, then what about some more typical lakes such as some of the low-pH lakes near Quebec, or some other low-pH lakes?

A. Well, I can show you a peak for that from some other data. You have here the prediction of the metal binding constant against the one that I calculated. This is for various lakes, and you see that these lakes are quite within the applicable pH ranges. They are high-pH lakes. The lower the pH, the less great is the peak.

Q. I think you can make your data fit with those parameters inside an organic model. I guess what I was thinking, and I threw it out during discussion, is that, perhaps, the crystalline form of the iron oxide is different.

A. That's another possibility, of course.

Q. RAMACHANDRAN RAVIKUMAR (Univ. of California-Berkeley): I have two questions. One is: Which piece of manganese oxide are you dealing with? And second question is: You have a point of zero charge for both iron or manganese oxide?

A. Pardon? Your second question?

Q. It's the point of zero charge. From the reactions you chose, you have conditional constants for the pK_1 and pK_2 for the oxides. Do you have the point of zero charge, too?

A. Okay, the main point is, I didn't do the reactions myself, I used the literature data. So the various authors, they use — I suppose — very amorphous manganese oxides. They give various data, but they don't give information about points of zero charge. So that's the data I have used for my calculations. I don't have information about the pH of these solids but I guess it should be around seven or something for iron.

Q. Manganese oxides have points of zero charge usually around 2.8, but it depends largely on the state they are in, that is, amorphous or crystalline, and what kind of crystalline form. That's why I was asking.

A. You're asking for manganese oxides?

Q. Yes.

A. The one I used, the ZPC was supposed to be 2.8.

A Comparison of Sediment, Water and Food As Sources of Silver For Marine Bivalve Molluscs

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Marine bivalves have been employed extensively to monitor the bioavailability of contaminants in coastal regions of the US and other countires for two decades. In the US, this work has proceeded under the auspices of the National Status and Trends Program, which has monitored trace element concentration in mussels and oysters from around the country. Despite an unusually rich data set, it is as yet difficult to unambiguously interpret trace element bioavailability for marine bivalves. Separating the relative contribution of dissolved uptake and particulate ingestion in the overall bioaccumulation of trace elements is important for setting water quality protective criteria, but is difficult to achieve without a complete understanding of the physiological processes controlling trace element bioaccumulation in bivalves.

Once Ag enters the marine environment, it is partitioned between dissolved and particulate phases, which then may become bioavailable to bivalves through adsorption from the dissolved phase and ingestion of contaminated food or sediment. In this study, we employed a simple bioaccumulation model (bioenergetic-based) to delineate Ag uptake pathways. First, we measured the important physiological parameters controlling Ag accumulation in bivalves, including Ag assimilation efficiency from ingested food particles, Ag influx rate from the dissolved phase and Ag efflux rates following either dissolved uptake or particle ingestion. We tested the variability of these physiological parameters under various environmental conditions. We then modeled Ag bioaccumulation in mussels, and compared our model calculations with field tissue concentrations measured in the NS&T Program. We chose San Francisco Bay as an example, because the geochemical behavior of Ag in this system has been well characterized. Finally the relative contribution of dissolved uptake to particle ingestion was modeled.

Several biological and abiotic factors are important in determining Ag assimilation in the mussels: 1) food quantity has an inverse influence on Ag assimilation; 2) different species of algal food also can have a significant influence on Ag assimilation; however, there was no significant relationship between Ag assimilation and carbon assimilation or between Ag assimilation and its cytoplasmic distribution in the algal cells. Gut passage times were found to be an important factor affecting Ag assimilation; 3) temperature has a significant influence on Ag assimilation, where Ag was retained more efficiently at lower temperature, presumably because of the lower protein synthesis/breakdown at lower temperature. However, Ag assimilation efficiencies in mussels (*Mytilus edulis*) in most cases were found to be relatively small, ranging from 5% to 20%, although values as high as 50% have been observed. Mussels ingesting natural phytoplankton assemblages collected during the spring phytoplankton bloom in Long Island Sound had assimilation efficiencies comparable with those obtained using cultured phytoplankton. Assimilation efficiencies of ingested Ag were

44% in the oyster *Crassostrea virginica*, 22-35% in the hard clam *Mercenaria mercenaria*, and 47-60% in the clam *Macoma balthica*.

The influx of dissolved Ag into mussels was determined by 1) dissolved Ag concentration, best described by $I_d = 0.1794\ [C]^{1.066}$, where I_d is the influx rate ($\mu g\ g^{-1}$ dry wt d^{-1}) and C is the dissolved Ag concentration ($\mu g\ l^{-1}$); 2) salinity, where maximum Ag uptake was found at salinities between 15 - 20 ppt. Below or above this salinity range Ag influx rate decreased significantly; 3) Ag influx increased inversely with DOC, although observed DOC effects were small.

The measured Ag efflux rate constants were different when mussels obtained Ag from a dissolved source or a food source, ranging from 0.0189 (dissolved source) to 0.0335 (food source). Duration of exposure was also important in influencing Ag efflux. Mussels displayed comparable efflux rates for Ag (and other metals) when depurating in the laboratory and caged in the field, suggesting that lab studies can be used to estimate efflux rates and metal retention in marine bivalves that would occur in natural waters. Silver is predominantly associated with bivalve shell if accumulated from the dissolved phase and with soft parts if accumulated from ingested food.

The effects of organic content and Fe-oxide levels of sediment on the assimilation of Ag from ingested sediment in mussels and clams were experimentally determined to be 2-12%. Generally, Ag assimilation in mussels was inversely proportional to organic carbon content and increased with montmorillonite and amorphous Fe-oxide content. However, there is an increased bioavailability of Ag to mussels feeding on sediment enriched in bacterial exopolymers. A very high correlation (0.99) was noted between assimilation efficiency and the amount desorbed from fulvic acid-coated particles at pH 5 (simulating the acidic gut of mussels). Similar correlations were observed for other metals (Cd: 0.99, Co: 0.97). Unlike mussels, in the clam *Macoma balthica*, factors such as organic and Fe-oxide content had no pronounced effect on Ag assimilation from sediment, and assimilation efficiencies typically were about 30-35%.

In our modeling effort, Ag partition coefficients in suspended solids (seston) were taken from extensive measurements in San Francisco Bay by Smith and Flegal (1993). Mussel feeding activity was calculated from Bayne et al. (1987) and Bayne (1992). Our model predicted, for a dissolved Ag concentration of 5 - 10 ng l^{-1} (typical concentration in SFB) a Ag concentration in mussels of 0.4 - 0.9 $\mu g\ g^{-1}$, a value very similar to the actual Ag concentration in mussel tissues collected in the NS&T Program (0.4 -0.8 $\mu g\ g^{-1}$ at Dumbarton Bridge). Our model suggested that >70% of Ag was coming from the dissolved phase. The inefficient food ingestion pathway was presumably due to the low Ag assimilation and high efflux rate. Sensitivity analysis indicated that the dissolved Ag concentration is critical in affecting mussel tissue concentration. The partition coefficient was critical in affecting the contribution of dissolved uptake relative to food uptake. In SFB, the log Kd ranges between 4.8 - 5.6 (Smith and Flegal 1993), and the fraction of mussel body burden of Ag deriving from the dissolved phase was calculated at between 60 - 90%. Total suspended solids (TSS) are predicted to have little effect on the Ag concentration in mussels or on the relative contribution of Ag from the dissolved phase.

Questions & Answers: A Comparison of Sediment, Water and Food as Sources of Silver for Marine Bivalve Molluscs

Q. NICK ADAMS (McMaster Univ.): I was just wondering, how much time do the organisms spend in the different feeding modes?

A. Most of our feeding experiments are done for freshwater feeding exposures on the order of a half-hour to an hour to reduce the recycling of the radiotracer during the exposure period. But we have compared assimilation efficiency from long-term exposure up to two weeks versus short-term. We do not see a significant difference. The problem with the long-term is there are a lot more artifacts that one has to control for. I should also point out that we've been worried about the extent to which we can extrapolate lab data to field data. So we have done some experiments. We radio-labeled mussels and divided them in half, and took half of them in a case to depurate in the lab and half of them to depurate caged in the Mediterranean, and we got virtually identical depuration rate constants from the soft parts. But from the shell we found that there was stimulated growth in the field but not in the lab, which retarded the desorption rate from the shell. Nevertheless, one lesson from the lab work is that the field and lab data are in comparable numbers.

Q. I was actually speaking more of suspension feeding versus deposit feeding.

A. We don't have a comparison yet between suspension and deposit feeding. And we think the best organism to address that in the water column is taking the same individual animal. It could go either way. Both experiments are underway now but we have no results yet. So that's an interesting question we've not yet solved.

Q. GEORGE HELZ (Univ. of Maryland): One of the things I wondered about as I've seen studies of this type being done is, to what extent are these different sources independent of one another? If they're not independent, then is this an important question, whether the silver comes from the water or the food or the sediment? In a hypothetical, idealized world where K_D's were constant, all the plankton were related to the water by a fixed constant that was uniform for the environment. But it seems to me it wouldn't matter, you wouldn't need to know whether the food or the water was the most important source. You need to know only one concentration of one of these forms and you would be able to calculate the rest.

A. I think that's right. We do not see that the concentration factor or K_D in phytoplankton varies very appreciably among species for any given element. We do see an enormous variation among the elements ranging from zero to one million, essentially. But within a metal there is less than an order of magnitude variation in concentration factors in phytoplankton, which leads us to believe that species composition is not likely to have a pronounced effect on the extent to which food is a significant source. We were concerned, however, that for some elements the uptake, including zinc and selenium, was clearly uptake through the food chain as opposed to the dissolved phase. That may be significantly influenced by the food concentration and other factors that influence the food. So for elements that are accumulated predominantly from food, we suspect that it does matter whether or not to do these sort of experiments. For elements that are predominantly from the dissolved phase, it looks like food is probably not going to be a significant factor. So it's not influenced by total suspended particulate load. Surprisingly, even though silver is so particle reactive, and that's, in part, because we get lower assimilation efficiencies of ingested silver from higher particle loads. There are a lot of other factors we have to consider here.

The Biogeochemistry of Silver in an Estuarine System

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INTRODUCTION

The Quinnipiac River in central Connecticut has a 18,800 ha watershed that supports a high population density and includes 5 sewage treatment plants. A large and active metal-plating industry that peaked in the mid-1950's caused substantial contamination of downstream floodplains located about 45 km from the mouth of the river. Erosion and resuspension of those deposits continues to contribute high levels ($\approx$ 500 ng/l) of Ag to the river (see companion article this volume). These high levels of Ag drop precipitously (90 - 95%) in the upper estuary where salinity increases from 0.1 to 5 ‰. The salinity gradient of this estuary is located where the tidal river meanders through a large salt marsh. A mass balance was prepared to determine the extent to which removal of Ag could be attributed to filtration by and deposition within the marsh.

STUDY SITE

The salt marsh currently occupies an area of 390 ha, but this space has been much reduced by fill which was added in the course of building railroad yards and a municipal landfill. The estuarine salinity gradient over the range from 0 to 20 ‰ almost always occurs within the borders of the wetland complex, though the exact location within the marsh migrates depending on the tides. The marsh, which has been designated a state wildlife area, includes sections dominated by spartina, phragmites, and mud flats. The marsh runs generally north-south and is bounded to both the west and east by railroad embankments. Within the marsh, the Quinnipiac River meanders such that the thalweg winds 7.6 km in traversing a straight-line distance of 5.3 km. Land uses immediately adjacent to the marsh include residential neighborhoods, rail yards, and commercial and industrial areas. Near its northern edge is located the sewage treatment plant for the town of North Haven, while at its southern terminus is the landfill of the city of New Haven. Several pits have been dug along its western edge to mine glacial clays that underlie the marsh.

METHODS

Water samples were filtered during collection through 0.4 μm Nuclepore filters. Clean techniques were used during all phases of sample collection, pretreatment, and analysis (Benoit

1994). Silver in water samples was measured by graphite furnace atomic absorption spectroscopy after preconcentration by APDC-DDDC chloroform-water extraction (Bruland et al. 1985). Sediment cores were collected by hand with 12.5 cm core tubes that had been pre-sectioned at 1 cm intervals. Sediments were digested with concentrated HNO_3 in high-pressure Teflon bombs in a microwave oven and analyzed by inductively coupled plasma-atomic emission spectroscopy. Standard reference materials were used to check metal recovery. ^{210}Pb and ^{137}Cs were analyzed by non-destructive gamma spectroscopy on dried samples hermetically sealed in 100 cm^3 "tuna can" containers.

RESULTS

Riverine Flux: Average annual discharge of the Quinnipiac River measured at a USGS gauging station near km 40 is 7.6 m^3/s. Bi-weekly measurements of Ag concentration at km 45 (Toelles Rd. station) show a strong correlation with discharge. Based on this regression the mean annual discharge corresponds to a Ag level of 200 ng/l. The product of discharge and Ag concentration is 1.52 mg Ag/s. Maximum Ag levels in the river were actually observed at km 52, downstream from Toelles Rd. At the location of the maximum Ag levels averaged 2.2 × values measured at Toelles Rd. Thus the riverine Ag flux should be close to 3.3 mg/s (106 kg/y). This calculation is a lower limit because discharge is greater at km 52 (the Ag maximum) than at the gauging station (km 40). Also, high discharge events, which carry higher concentrations of Ag, are underrepresented by this method.

Burial Flux: The total marsh area is 390 ha based on planimetry of USGS 7.5' quadrangle maps. The sediment accumulation rate based on ^{137}Cs and ^{210}Pb analysis of two cores analyzed so far is between 0.3 and 0.5 cm/y. This is close to the measured regional relative sea-level rise rate of 0.3 cm/y (Nydick et al. 1995). This latter value seems more reliable for a marsh-wide average, and is used in this calculation. The bulk density of the marsh sediments varies significantly from location to location, and we have assumed an average value of 0.5 g/cm^3. Twenty-seven cores were collected from all portions of the marsh. Surficial Ag concentrations averaged 17.7 ± 6.3 μg/g. Combining all of these values yields a burial flux of 104 kg/y.

Riverbed Inventory: The riverbed within the marsh has a length of 7.6 km and an average width of 74 m. We assume that the bulk density of this sandy material is 1 g/cm^2. Eight measurements of river bottom sediments revealed a Ag concentration averaging 1.7 ± 1.6 μg/g. Based on field observations, we believe that a 1 cm layer of sediment actively exchanges with the water column, but this number is highly uncertain and could be as great as 10 cm or more during unusually high flows. The product of these numbers yields a riverbed sediment inventory of 9.6 kg (based on a 1 cm reworked depth).

DISCUSSION

The flux of Ag from the Quinnipiac River is large. The large flux occurs because levels of Ag are elevated and not because the river's discharge is great. (The Quinnipiac is the fourth largest river in Connecticut.

Comparison of the river's Ag flux ($\geq$ 106 kg/y) to the rate of Ag burial in the marsh (104 kg/y) show that virtually all of the Ag removed during estuarine mixing can be accommodated within the marsh. This calculation does not take into account any Ag supplied to the marsh by atmospheric deposition, but this source is believed to be small. In contrast to the large inventory in marsh deposits, the amount of Ag contained in the upper 1 cm of riverbed sediments is equivalent to that supplied by the river in a period of only 33 d. The riverbed thus does not act as either a long term source or sink for water column Ag. Presumably, any Ag that settles to the bottom is soon resuspended and removed permanently within the marsh itself. The estuary thus acts as an extremely efficient trap of Ag delivered from upstream sources.

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Q. CHRISTIAN KRAWFORST (Univ. of Massachusetts): In your estuary, I was just curious as to what the time scales are for the things that are occurring there. Has there been an estimated residence time for the water in the estuary?

A. You mean that structure in the river from zero to 20 percent salinity?

Q. Right.

A. No, we haven't done that yet. I can tell you that the currents are such that I believe that the travel time of water in the absence of any isolation might be on the order of 4-6 hours. But given the isolation occurring I'd think the waters could be in there for a longer time. Actually, the neat thing is, there is a sewage treatment plant near the head of that zone and they just finished a dye test yesterday, and, hopefully, I'll have some information about what the residence is with a tracer like that. Is there any particular reason you asked me that question?

Q. Yes. Initially, on your first overheads, you showed particulate dissolved silver down the estuary, and you stated that it looked like there was removal for both fractions within the estuary. But if you look at the dissolved part on one slide, one could counter that it is a conservative loss.

A. It's partly because the difference in concentration requires a calibration of the scale, which suppressed some of the dissolved. When you blow that up, I think, and look at our error bars of the measurements, there is clearly a nonconservative behavior. It's not huge though, I agree.

Silver in Oyster Tissue: Relations to Site Selection and Sampling Size

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INTRODUCTION

Contaminant concentrations in the tissues of mollusks have been used for environmental monitoring of coastal waters. Large variability in tissue concentrations have been found among individual mollusks collected at the same site at the same time. This variability is unaccounted for by age, sexual maturity or season. It is important to quantify the variability, especially when composite samples are used, as with many monitoring programs. Previous studies have found the variability to be metal dependent (Gordon et al., 1980; Wright et al., 1985). Depending on the metal, sample sizes of 50-390 individuals were suggested to detect a 10% difference between means in mussels (Gordon et al., 1980). It was the goal of this work to quantify the metal concentration variability among individual oysters, and determine the sample size in pooled samples that will result in variance lower than a predetermined value.

EXPERIMENTAL

Approximately 20 oysters were collected from three sites on the Potomac River; Beacon (BEC), Popes Creek (PC), and Lower Cedar Point (LCP); the sites have increasing salinity, respectively. Oyster shells were scrubbed, rinsed, blotted dry, and opened using a stainless steel knife. Tissue from each oyster was placed in an acid-cleaned low density polyethylene vial and freeze-dried. A Teflon spatula was then used to grind the tissue inside the vial. For Ag analysis, 0.2g of tissue was digested with a mixture of 2.0 ml HNO_3 and 3.5 ml HCl. This stabilizes Ag in solutions as the chloro-complex and avoids dramatically decreased Ag recoveries for oysters with elevated concentration (Crecelius and Daskalakis, 1994). Microwave heating maintained the pressure at 140 psi inside sealed Teflon bombs for 35 min. The digestates were finally diluted to 20 ml, and analyzed using flame atomic absorption .

RESULTS AND DISCUSSION

Concentrations of Ag per individual (C_i) are greater in BEC and PC (lower salinity) oysters than in LCP site (higher salinity) (Fig **1a-1c**). These concentrations are greater than many literature values, and may in part reflect increased Ag recovery due to the improved digestion method used for this study. The greatest variability was

among PC oysters, with a concentration span over a factor of 12. The coefficient of variation (CV) ranges from *ca* 25% for BEC and LCP to 55% for PC.

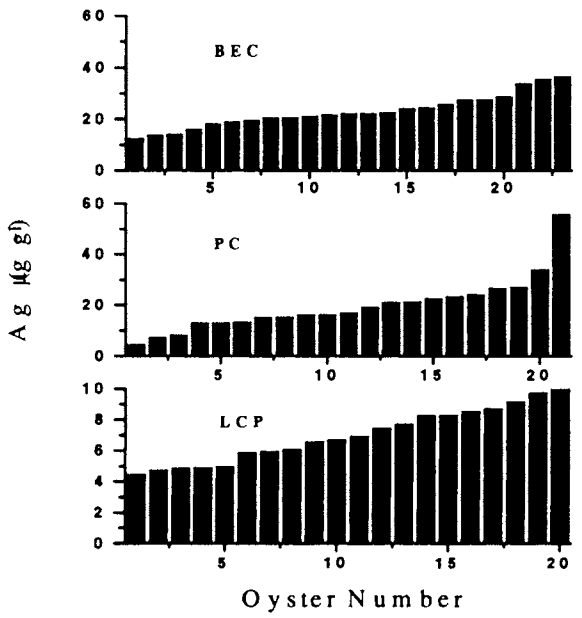

Fig. 1 Silver in Potomac River oysters

It is of interest to many monitoring programs to compare metal concentrations of pooled samples to those of individual animals, and estimate variance as a function of the number of animals combined per pool. To accomplish this, the *bootstrap* method was employed (Efron and Tibshirani, 1991; Fabrizio et al., 1995). Briefly, in the bootstrap method, all calculations are performed by randomly sampling with replacement from the sample distribution, i.e. C_i, which is defined as the population distibution. This technique allows for any sample size by selecting the same oyster from the C_i distribution. To account for mass differences between oysters the mean concentrations ($\overline{M}_n$) of a pooled sample consisting of n number of oysters, can be calculated by random selection of n values from the C_i distribution using eq. **1**:

$$\overline{M}_n = \frac{\sum_{i=1}^{n} C_i m_i}{\sum_{i=1}^{n} m_i} \qquad (1)$$

where C_i and m_i are the concentration and mass of oyster (i), which was randomly selected. In this work, eq. **1** was used for calculations with n=2-50, i.e. simulating pooled oyster samples of 2 to 50 individuals, with 10000 simulations for each n value.

The distributions of pooled mean concentrations $\overline{M}_n$ for the BEC site calculated from eq. **1**, are presented in Fig. **2**, where the 1, 5, 10, 25, 50 (median), 75, 90, 95, and 99th percentiles are plotted as a function of number of animals per pool. The plots in Fig. **2** show that the estimation of median value is poor when a small number of animals per composite sample is used. Precision increases with increasing number of animals per sample.

The relative half confidence interval between means for n animals per pooled sample ($\Delta_{95,n}$) is given by eq. 2:

$$\Delta_{95,n} = 100\frac{\tilde{M}_{95,n} - \tilde{M}_{50,n}}{\tilde{M}_{50,n}} \qquad (2)$$

where $\tilde{M}_{95,n}$ and $\tilde{M}_{50,n}$ are the 95th and 50th percentile of the distribution of pooled means, respectively, calculated from eq. 1. The full interval may be approximated by $\pm\Delta_{95,n}$. Results from eq. 2 are plotted in Fig. 3 as a function of the number of animals per pool. It is seen that there is a near exponential decrease of $\Delta_{95,n}$ with increasing number of animals.

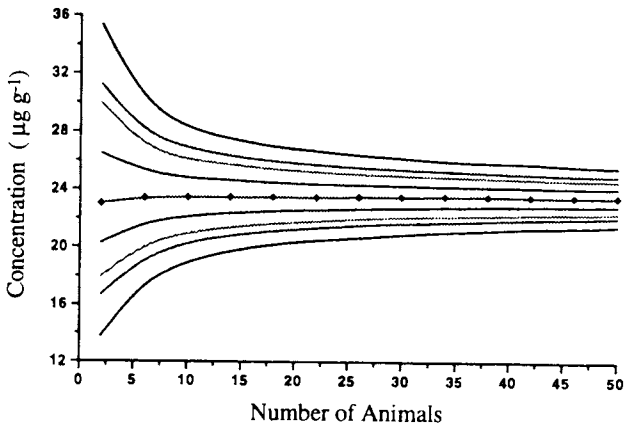

Fig. 2 Distribution of simulated means as a function of number of animals per composite sample. Lines are 1, 5, 10, 25, 50 (circles), 75, 90, 95, and 99th percentile of the distribution of means.

For a typical composite sample of 20 oysters, the confidence interval around the median value $\Delta_{95,n}$ is ±10% for BEC and PC, and ±19% for LCP. These intervals increase to ±15% and ±28% for samples consisting of 10 oysters, and decrease to ±6% and ±12% for samples of 50 oysters. However, population variability should be expected to be somewhat greater than the one calculated here. Collection of 50 oysters instead of 20 would increase the sample collection and preparation cost slightly, while the analytical cost would not be affected. Thus, only in locations with declining oyster populations, would it be impractical to collect pooled samples of 50 or more oysters.

Analyses of individual oysters is the preferable method because it reveals the contaminant distribution, which allows for estimation of population parameters, and detection of outliers, information which is lost during pooling. For example, the Ag concentration in oyster PC21 (Fig. 1) is approximately three times as much as the average concentration in this site. With even more data from this site, PC21 may be considered as a 'hyper-accumulator', i.e. not

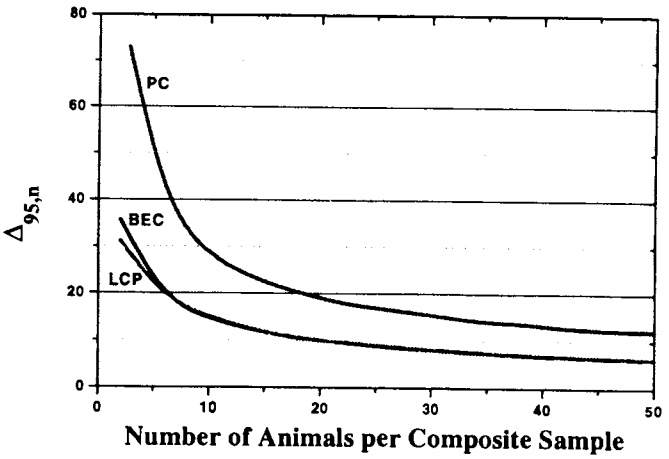

Fig. 3 Half confidence intervals for Ag in Potomac Oysters

representative of the population, and excluded from the statistical analysis.

CONCLUSIONS

For oysters from the Potomac River, significant among-individuals variability of trace metals concentrations in oyster tissue has been found. Silver concentrations are approximately twice as variable at the PC site as the other two sites. Analysis of individuals is the preferred method for environmental monitoring, because important information is lost during sample pooling. However, when it is chosen to analyze pooled samples, bootstrap analysis suggests that 50 or more animals per sample will reduce the uncertainty to less than ±12%.

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Questions & Answers: Silver in Oyster Tissue: Relations to Site Selection and Sampling Size

No questions.

Partitioning and Effects of Silver in Amended Freshwater Sediments

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The fate and effects of silver, amended as silver nitrate, silver chloride, and silver thiosulfate complex, in freshwater sediments with varying characteristics (e.g. diverse sand: silt/clay ratios, percent organic matter, etc.) were studied in static 10-d laboratory experiments. Partitioning of silver to particulates, overlying water and interstitial water was evaluated by measuring dissolved and total acid extractable silver concentrations over a 10 day period. Silver concentrations in the sand and silt/clay fractions were also measured. The results of silver nitrate amended sediments indicate that we can reasonably expect a factor of three difference in the affinity of particulates for silver, but over two orders of magnitude difference in aqueous (< 0.45 μm) silver concentrations were observed. Concentrations of silver in pore water varied widely. Concomitant variations in bioavailability were assessed using the amphipod *Hyalella azteca* Saussure. Ten-day LC_{50}s for *H. azteca* exposed for four sediments amended with silver as silver nitrate ranged from 1.6 to 397.7 mg Ag/kg dry sediment. In experiments with silver chloride amended sediments, *H. azteca* 10-d LC_{50}s were> 2560 mg Ag/kg dry sediment. *H. azteca* was also relatively insensitive to silver as silver thiosulfate complex with 10-d LC_{50}s >569 mg Ag/kg dry sediment. These amendment experiments illustrate three primary principles: 1) sediment amendment procedures are dictated by the intrinsic characteristics (e.g. solubility) of the silver compounds; 2) silver nitrate, silver chloride and silver thiosulfate

complex differ in their affinity for sediments and partitioning in overlying waters and interstitial waters; and 3) bioavailability of silver compounds in sediments varied several orders of magnitude.

INTRODUCTION

As we transfer and transform metals in commerce, medicine and other activities around the globe, questions often arise concerning the consequences of local increases in metal concentrations or alterations of metal forms. Although silver has a predominately lithic biogeochemical cycle (Brookins 1988), the use of water in silver mining and industrial processes serves to alter aqueous and sediment silver concentrations in aquatic systems. Since "free" or bioavailable silver is relatively toxic to aquatic species (USEPA 1980), a clear understanding of the fate and effects of silver is required to accurately predict the potential risks of silver in aquatic systems. Like other metals with an affinity for particulates and sediments (e.g. Cu, Zn), only a fraction of the total silver found in sediment, interstitial or overlying water is bioavailable (LeBlanc *et al.* 1984). For aquatic systems, the presence of a particular concentration of silver in a sediment or water sample may be merely presumptive evidence of a potential problem. Currently, confirmatory evidence is derived from careful studies involving realistic exposures of biota that may be affected. Critical to any accurate risk assessment for silver in aquatic systems will be an understanding of bioavailability at a point in time, and through time. For purposes of this study, bioavailability is operationally defined as the ability of silver (element, compound, etc.) to concentrate in or on an organism and/or to elicit a response (adverse or beneficial)

as a result of exposure.

The laboratory experiments here were designed to accomplish the following objectives: 1) to determine the partitioning of silver introduced in water associated with sediments of varying characteristics (e.g. diverse sand: silt/clay ratios, % organic matter, etc.); and 2) to assess the bioavailability or effects of silver in sediment/water systems on *H. azteca* (a freshwater amphipod).

MATERIALS AND METHODS

Silver Partitioning to Sediments

Sediments for measurement of silver partitioning were selected based upon the following criteria: 1) representative of range in U.S. (pH 5-9, OC<1%-2.5%, Eh neg-pos); 2) characteristics that may be important in silver speciation and bioavailability (e.g.>, redox, pH, OC); 3) accessible: 1-2 h drive or colleague lives near by; 4) representative of both karst topography and granitic (quartz) topography; 5) sufficient volume to obtain pore water for analysis; 6) sediments must be unconfounded with toxicity; and 7) matching some current site with potential silver contamination. Based upon these selection criteria, seven sediments from throughout the US and a formulated sediment (Suedel and Rodgers 1994a, 1994b) were selected for the partitioning study (Table 1). These sediments represented the range of characteristics found in freshwater throughout the USA (Suedel and Rodgers 1991). Sediments were characterized by the methods of Allen *et al.* (1991), Black (1986), and Plumb (1981).

Three silver compounds, silver nitrate, silver chloride, and silver thiosulfate complex, were used for sediment partitioning experiments. Silver nitrate has a solubility of 2500 mg/ml (25°C) (Windholz *et al.* 1983). A stock solution of 10,000 mg Ag/L was prepared using Kodak silver nitrate crystals (173-1082). A series of silver concentrations were prepared by serial dilution from the silver nitrate stock, added to the sediments and homogenized to produce a range of sediment associated silver concentrations. Silver chloride (Sigma Brand silver chloride 7783-90-6) was amended directly to sediments in solid form due to its relatively low aqueous solubility (1.93 mg AgCl/L at 25°C) (Windholz *et al.* 1983). Silver thiosulfate complex is "relatively" insoluble and was also amended directly to sediments. Each sediment was amended with the calculated amount of silver compound to obtain the targeted (nominal) concentration.

Sediment amendment procedures involved homogenizing sediment and amended silver compounds with a spatula until thoroughly mixed. Sediments were amended and analyzed for silver periodically over a 10 day period. The contact period of ten days was chosen as the experimental duration since this would match the duration of the bioavailability experiments (Ingersoll and Nelson 1990; Tomasovic *et al.* 1995). Overlying water, pore water, and sediment samples were collected and analyzed for silver at 0d, 24h, 48h, 96h, 7d and 10d after initial silver amendment. Samples of sediment silt/clay fraction were also collected and analyzed for silver at 0d and 10d.

Silver Bioavailability in Sediment Experiments

Four sediments (Sediments 1, 3, 6 and 7) encompassing a broad range of

characteristics were selected for silver amendment and bioavailability experiments (Table 1). *H. azteca* (1-2 weeks old) were exposed in 250-ml beakers to 70 g of sediment amended with aqueous silver nitrate, solid silver chloride, or solid silver thiosulfate complex. UMBFS filtered pond water was added as the overlying water in a ratio of 4:1 water to sediment volume. Experiments were conducted in light (16 h light/8 h dark) and temperature (21°+/- 1°C) controlled incubators. During the 10 d exposures, water characteristics [temperature (°C), pH, dissolved oxygen (mg/L), alkalinity (mg/L as $CaCO_3$), hardness (mg/L as $CaCO_3$) and conductivity (µmhos/cm)] were measured at the beginning and end of each experiment. Temperature, pH, dissolved oxygen, and conductivity were also measured at 24h, 48h, 96h, and 7 d of the experiment. Experiments were initiated by adding 10 juvenile *H. azteca* to each of 4 replicate beakers per concentration. As a food source *H. azteca* were given 4 leached maple leaf disks (7 mm diameter each) per beaker at the beginning of each test. All organisms were obtained from cultures maintained at the University of Mississippi Department of Biology laboratory (Lawrence 1981).

Sediment toxicity tests were conducted with Sediments 1, 3, 6, and 7 amended with solutions of silver nitrate in a series of silver concentrations and an unamended control (Nebeker *et al.* 1984). Silver chloride was amended directly to Sediments 1, 3, 6 and 7 in a concentration series as in the partitioning experiments described above. Ten day sediment toxicity tests were also conducted by amending these sediments with solid silver thiosulfate complex. A single nominal concentration of 2000 mg Ag (as silver thiosulfate complex)/kg dry sediment was amended to all four sediments with an unamended

sediment sample as a control. The silver thiosulfate complex used in sediment toxicity testing contained 0.598% silver. Two replicates of control and treatment were used for each silver thiosulfate sediment experiment. Prior to the addition of *H. azteca*, the overlying water in beakers containing silver thiosulfate were aerated for a period of 10 minutes to oxidize excess thiosulfate. Samples of sediment, overlying water, and pore water were collected and analyzed for silver concentrations as described below.

Analytical and Statistical Procedures

Overlying water from experimental beakers was collected and acidified to pH <2 with 15-16N redistilled nitric acid. Water samples were then filtered through a 0.45 µm Gelman Metricel membrane filter and analyzed for total acid extractable silver using flame atomic absorption spectrophotometer (AA). Sediment samples were collected from each beaker and centrifuged in a Beckman model J2-21 centrifuge at 10,000 rpm for 10 minutes to extract pore water. Pore water was acidified to pH <2 with 15-16N redistilled nitric acid, filtered (0.45 µm Gelman metricel membrane filter) and analyzed for total acid extractable silver using AA.

Approximately 5g of sediment from each centrifuged sediment sample were dried in aluminum weigh boats for 24 hours at 75°C. Dry sediment (2g) was then acidified with 10 ml of 15-16N redistilled nitric acid and heated for 5h at 200°C to extract sediment associated silver. Samples were cooled and vacuum filtered through a 0.45 µm Gelman Metricel membrane filter. Samples were brought to volume (25ml) with Milli-Q®water and analyzed for total acid extractable silver by flame atomic absorption spectrophotometer.

Additional sediment samples were separated into sand and silt/clay fractions by specific gravity, and analyzed for silver according to the same procedure. Biological endpoints for toxicity experiments included LC50s and 95% confidence intervals (CI) calculated using methods described in Stephan (U.S. Environmental Protection Agency 1985) or the trimmed Spearman-Karber method, as appropriate.

RESULTS AND DISCUSSION

The divergent characteristics of the sediments selected for this research are readily apparent (Table 1). The pH of these sediments ranged form 6.0 to 7.9. Sediments ranged from slightly oxidized (+62 mv) to reduced (-300 mv). Organic carbon content (0.03%-2.54%) spanned a range that would be expected to encompass most sediments in the U.S. Organic matter ranged from 0.29% to 6.05%. Ratios of organic carbon:organic matter ranged from 1.71% to 62.1%. Cation exchange capacity ranged from 0.11 to 14.29 meq/100g. Acid volatile sulfides (AVS) were not detectable in two sediments and were as high as 133.5 umol/g of dry sediment. The bulk density of sediments ranged from 80 to 44% sediment. The coarse (sand) fraction of these sediments constituted 28 to 96% of these samples. The fine fractions (silt and clay) similarly ranged widely with silt ranging from 4 to 37% and clay from nondetectable (<0.01%) to 3.1% of the sample. With this range in characteristics, these sediments should permit a thorough evaluation of the potential for sediments to interact with silver and alter bioavailability.

Partitioning of silver compounds ($AgNO_3$ and AgCl) was evaluated using these

sediments (Figs. 1-6). The test systems were amended with silver compounds as mentioned previously and the concentrations of silver were determined in overlying water, pore water, and particulate fraction or compartments. The solubilities of the silver compounds necessitate adjusting the amendment procedures for the various compounds. Silver nitrate can be amended to sediment in an aqueous solution with a concentration of up to 2500 g/L in the solution. Silver chloride and silver thiosulfate complex have to be amended in solid forms.

For $AgNO_3$ amended sediments, silver concentrations associated with particulates ranged from <50 µg Ag/kg for Sediment 2 to >150 mg Ag/kg for Sediment 4 (Fig.1). After an initial contact period of 10d, the results indicate that we can reasonably expect a factor of 3 difference in the affinity of particulates for silver for sediments. Total silver concentrations in overlying water ranged more widely (Fig. 2). Total silver concentrations in overlying water ranged from <0.25 mg Ag/L to >8 mg Ag/L. The two orders of magnitude difference in total aqueous silver concentration was not readily attributed to a specific sediment or aqueous characteristic. Similarly, silver concentrations range from a low of approximately 9 mg Ag/L, to a high pore water concentration of >35 mg Ag/L in Sediment #5.

Concentrations of silver associated with AgCl amended sediments ranged from about 30 mg Ag/kg to >65 mg Ag/kg. Thus, concentrations in nature might be expected to range by a factor of two. Similarly, the dissolved silver found in the pore water was an order of magnitude less than for $AgNO_3$ amended sediments. Pore water concentrations of silver ranged from 0.025 mg Ag/L to 0.25 mg Ag/L. This order of magnitude range in

pore water concentration of silver should translate into differences in toxicity. The concentration of total silver in samples of the overlying water from AgCl tests ranged from 0.036 mg Ag/l to 0.084 mg Ag/L. These overlying water concentrations of silver are two orders of magnitude less than those observed for $AgNO_3$ amended sediments.

Relatively little change in silver concentrations in the three fractions (particulate associated, pore water, and overlying water) was observed over the ten days of this experiment. Silver concentrations were relatively stable after the initial 2 hours of contact for both $AgNO_3$ and AgCl amended sediments. For both $AgNO_3$ and AgCl amended sediments, an affinity of silver for the silt/clay fraction is apparent. Enrichment of silver concentrations in the silt/clay fraction relative to the sand fraction ranged from two to approximately one hundred fold.

The 10-d LC_{50} values for *H. azteca* sediment toxicity experiments with silver nitrate are shown in Table 2. *H. azteca* tested in Sediment 3 had a 10 d LC_{50} of 1.6 (95% CI 1.5-1.7) mg Ag/kg, whereas organisms in Sediment 7 had a 10-d LC_{50} over two orders of magnitude higher at 397.7 (95% CI 345-417) mg Ag/kg.

In 10d *H. azteca* experiments with silver thiosulfate complex, sediments were amended with a nominal concentration of 2000 mg Ag/kg of silver thiosulfate complex. Analytical measures of silver as silver thiosulfate in sediments were 1126, 648, 569 and 682 mg Ag/kg in sediments 1, 3, 6 and 7, respectively. There was no detectable concentration (detection limit = 13 µg Ag/L) of silver in the overlying water after 24 hours of contact time, or at the end of the 10d experiment. In 10d-exposures of *H. azteca*, survival was 90% or greater in all test sediments. Survival of control organisms was 100%.

The 10-d LC_{50} for Sediment 1 was >1126, for Sediment 3 was >648, Sediment 6 was >569, and Sediment 7 was >682 mg Ag/kg.

Four sediments were amended with silver nitrate and the bioavailability of silver was evaluated using *H. azteca*. A change in sediment characteristics had a dramatic effect on silver partitioning relative to bioavailability with *H. azteca* 10-d LC_{50}s ranging from 1.5 mg Ag/kg to 397.7 mg Ag/kg. Upon examination of individual sediment characteristics, it does not appear that any one parameter is predominantly influencing silver partitioning, but observed partitioning is the result of an interaction of several characteristics that determine the fraction of bioavailable silver. For example, Sediment 3 and Sediment 7 had similar particle size characteristics and redox (Table 1) and yet *H. azteca* in Sediment 3 had a 10-d LC_{50} of 1.5 mg Ag/kg and in Sediment 7 had a 10d LC_{50} of 442 mg Ag/kg. These sediments however, have different pH values, a factor which can alter the speciation of silver. The sediment with the lower pH is predicted to have a higher percentage of free silver ion. An additional parameter that differed between these two sediments was percent organic carbon (and organic matter). Sediment 3 had an order of magnitude less organic carbon and organic matter than Sediment 7. Cation exchange capacity (CEC) also varied by an order of magnitude between the two sediments. Sediment 7 had 0.6% clay and a CEC of 2.4 meq/100 gm dry sediment, which would provide binding sites for silver ions, removing them from the water column. AVS is an another characteristic that was different between the two sediments. Sediment 7 with 2.2 µmols AVS/g dry weight should have had sufficient AVS to sequester most of the amended silver (as silver nitrate) (Allen *et al.* 1993). Indeed, the toxicity of silver amended to this

sediment was significantly less than the toxicity observed for the other sediments.

The data for silver amended Sediments 1 and 6 are somewhat enigmatic. The parameters that are suspected to influence bioavailability and toxicity of silver in sediments (pH, redox, organic carbon, organic matter, cation exchange capacity, and fraction of silt/clay) would indicate that Sediment 1 should be more toxic with the same concentration of silver than Sediment 6. This was not the case; in fact, the observed toxicity did not differ significantly for these two sediments.

CONCLUSIONS

These sediment amendment experiments illustrate three primary principles: (a) sediment amendment procedures must be adjusted based upon the solubility of the silver compounds studied: (b) silver compounds differ greatly in their affinity for sediments and their compartmentalization into pore water and overlying waters; and (c) diverse sediments vary widely in their affinity for silver and their ability to bind silver. This was reflected in differing bioavailabilities of silver amended sediments to *H. azteca* (measured as LC_{50}s).

Bioavailability to *H.azteca* of silver, as silver nitrate, amended to sediments with varying characteristics (i.e., organic matter and acid volatile sulfide) is not equal from one sediment to another. Sediment characteristics significantly affected the bioavailability of silver nitrate. When the source of silver as a variable is examined by sediment toxicity experiments with *H. azteca*, clearly AgCl when amended to sediments is orders of magnitude less toxic than $AgNO_3$. Sediments amended with silver thiosulfate complex are

similar in toxicity to AgCl amended sediments with 10-d LC_{50}s in excess of 2000 mg Ag/kg. These results have serious and obvious implications for risk assessments of silver in aquatic ecosystems.

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Table 1. Characterization of sediments selected for silver partitioning and bioavailability experiment

Parameters	Sed. 1	Sed. 2	Sed. 3	Sed. 4	Sed. 5	Sed. 6	Sed. 7	Sed. 8
pH	6.00	6.95	6.80	6.87	7.90	7.30	7.48	7.38
Redox	+57.0	+62.0	-232.0	-168.1	-248.5	-297.0	-291.0	-300.0
O.C.	0.38%	0.03%	0.18%	2.54%	0.08%	0.45%	1.02%	ND
O.M.	1.68%	1.75%	0.29%	6.05%	0.47%	3.94%	2.31%	4.56%
CEC	1.50	1.58	0.11	14.29	0.67	7.77	2.40	6.91
AVS	4.63	0. 79	0.36	6.81	NM	NM	70.75	133.52
%Sed.	72.95	80.15	76.70	43.70	78.11	55.40	69.52	51.70
%Sand	75.90	77.05	94.47	28.50	95.59	60.31	95.19	91.38
%Silt	21.40	22.95	5.53	71.50	4.23	36.51	4.25	8.27
%Clay	2.7	NM	NM	NM	0.18	3.18	0.56	ND

Redox (mV), CEC = cation exchange capacity (meg/100g dry sed.), AVS = acid volatile sulfide (µmol/g dry sed.), O.C. = organic carbon, O.M. = organic matter, ND = not determined, NM = not measured (AVS detection limit = 0.01 µmoles/g dry sed, %Clay detection limit = 0.01%)

Table 2. Results of 10-d sediment bioavailability experiments with *H. azteca* in sediments amended with silver nitrate. LC50s are based on total acid extractable silver concentration in sediments (mg Ag/kg).

Sediment Sample	10-d LC50 (95% CI)
Sediment #1	60.7 (55.7- 66.1)
Sediment #3	1.62 (1.5-1.74)
Sediment #6	45.4 (34.1-79.8)
Sediment #7	379.7 (345-417)

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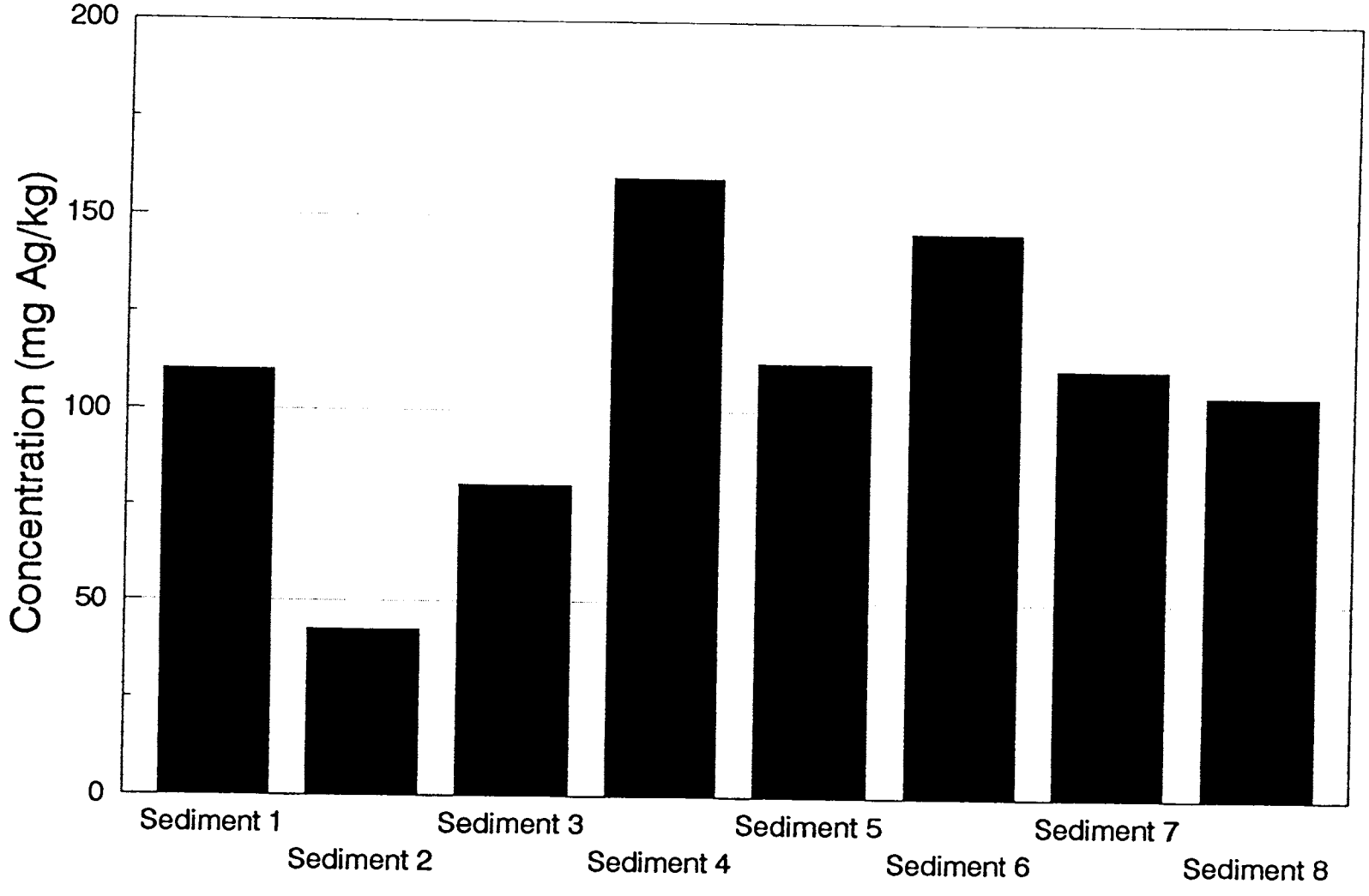

Fig.1 Concentration of acid extractable silver in particulate fraction of sediments. Sediments amended with 320 mg Ag/kg as silver nitrate.

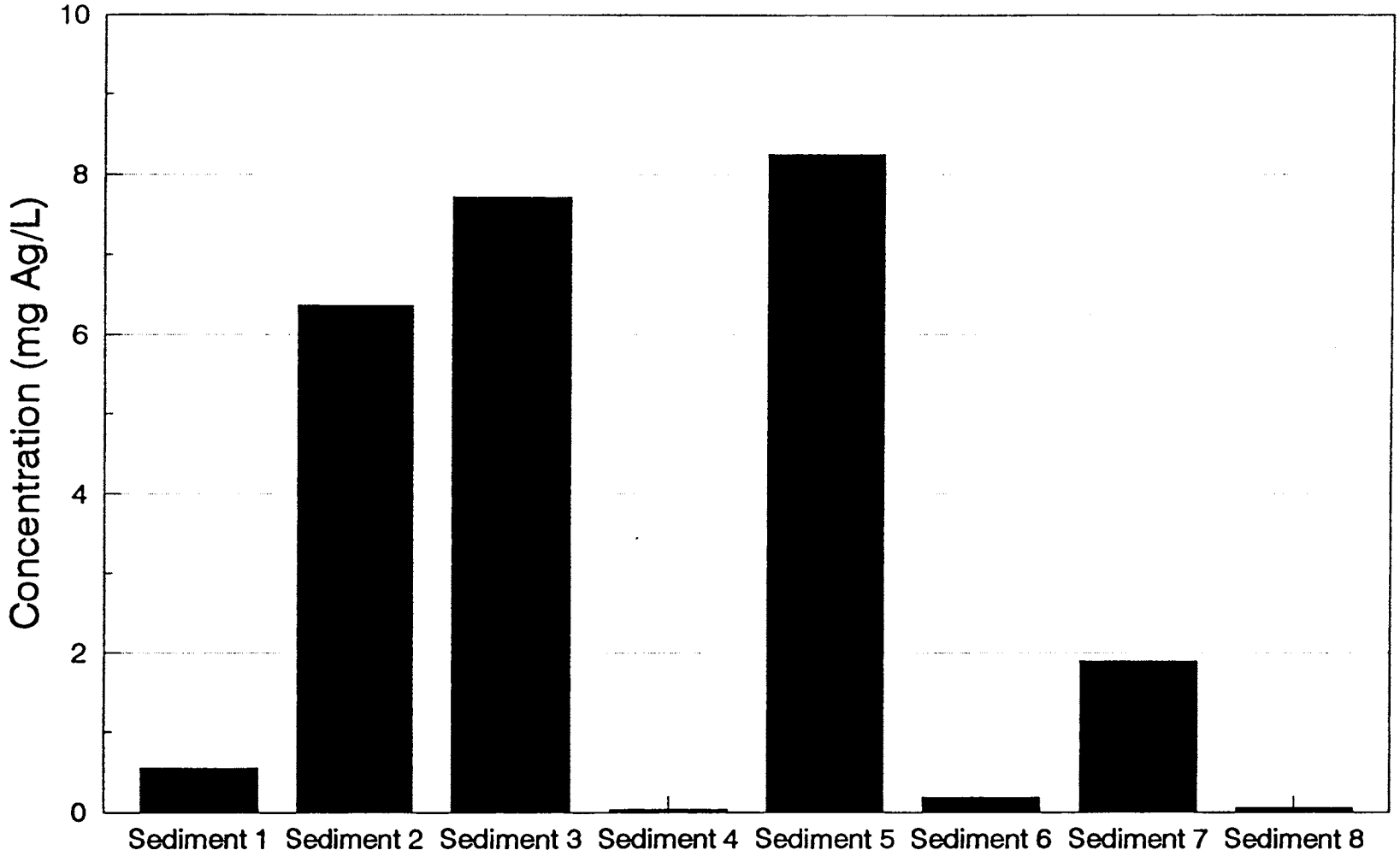

Fig.2 Concentration of dissolved (<0.45 um) silver in overlying water. (Sediments amended with 320 mg Ag/kg as silver nitrate)

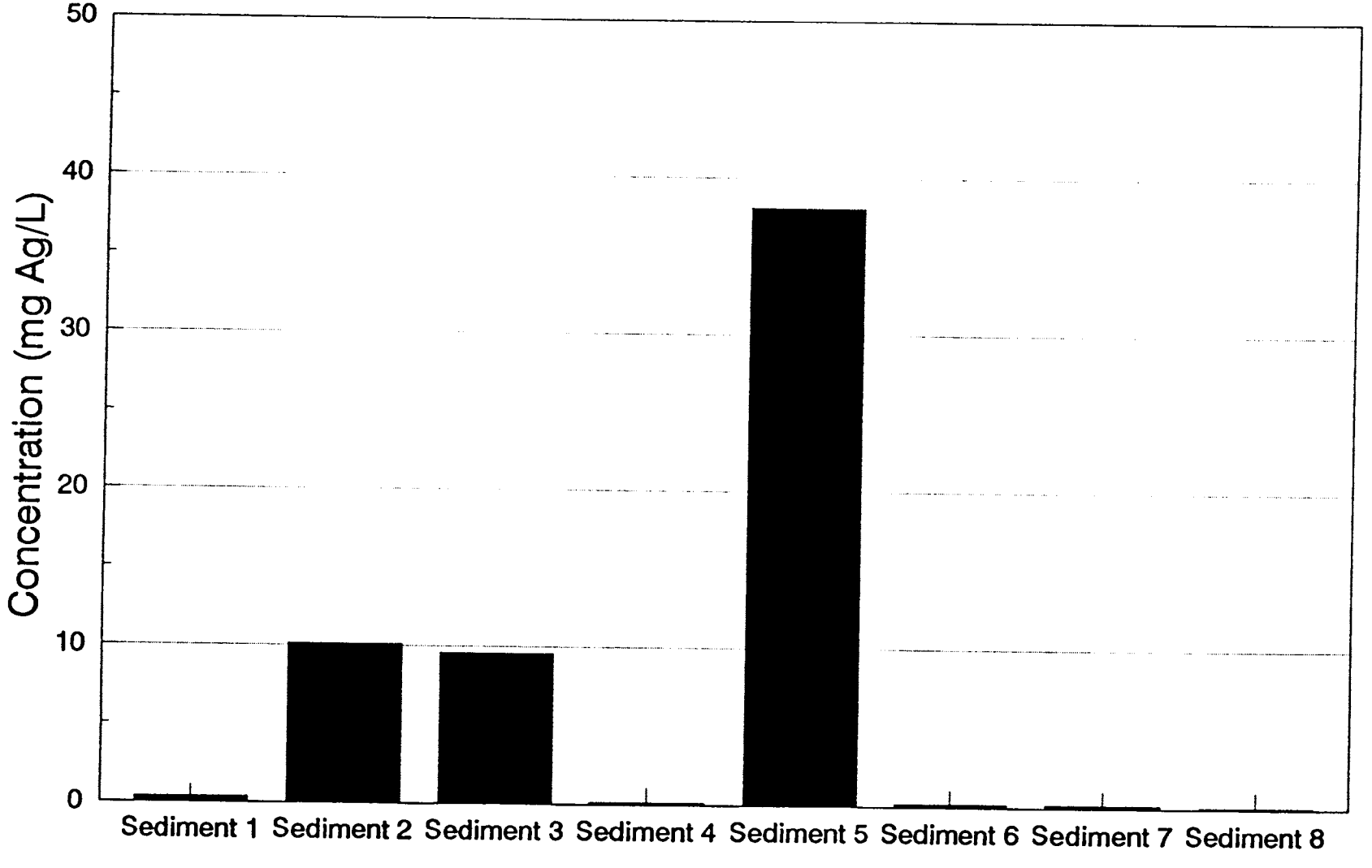

Fig. 3 Concentration of dissolved (<0.45 um) silver in pore water. (Sediments amended with 320 mg Ag/L as silver nitrate)

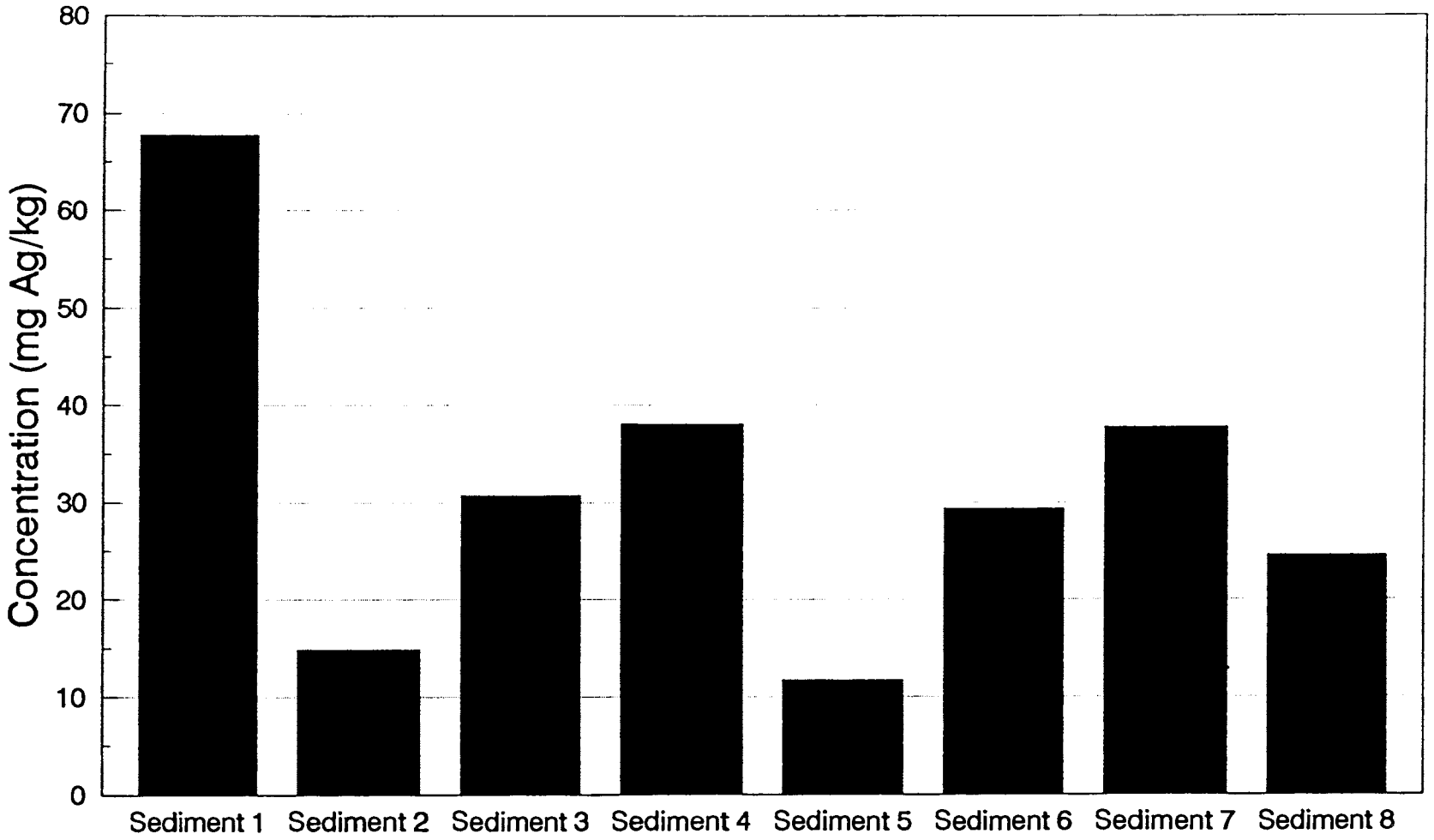

Fig. 4 Concentration of acid extractable silver in particulate fraction of sediments. Sediments amended with 320 mg Ag/kg as silver chloride.

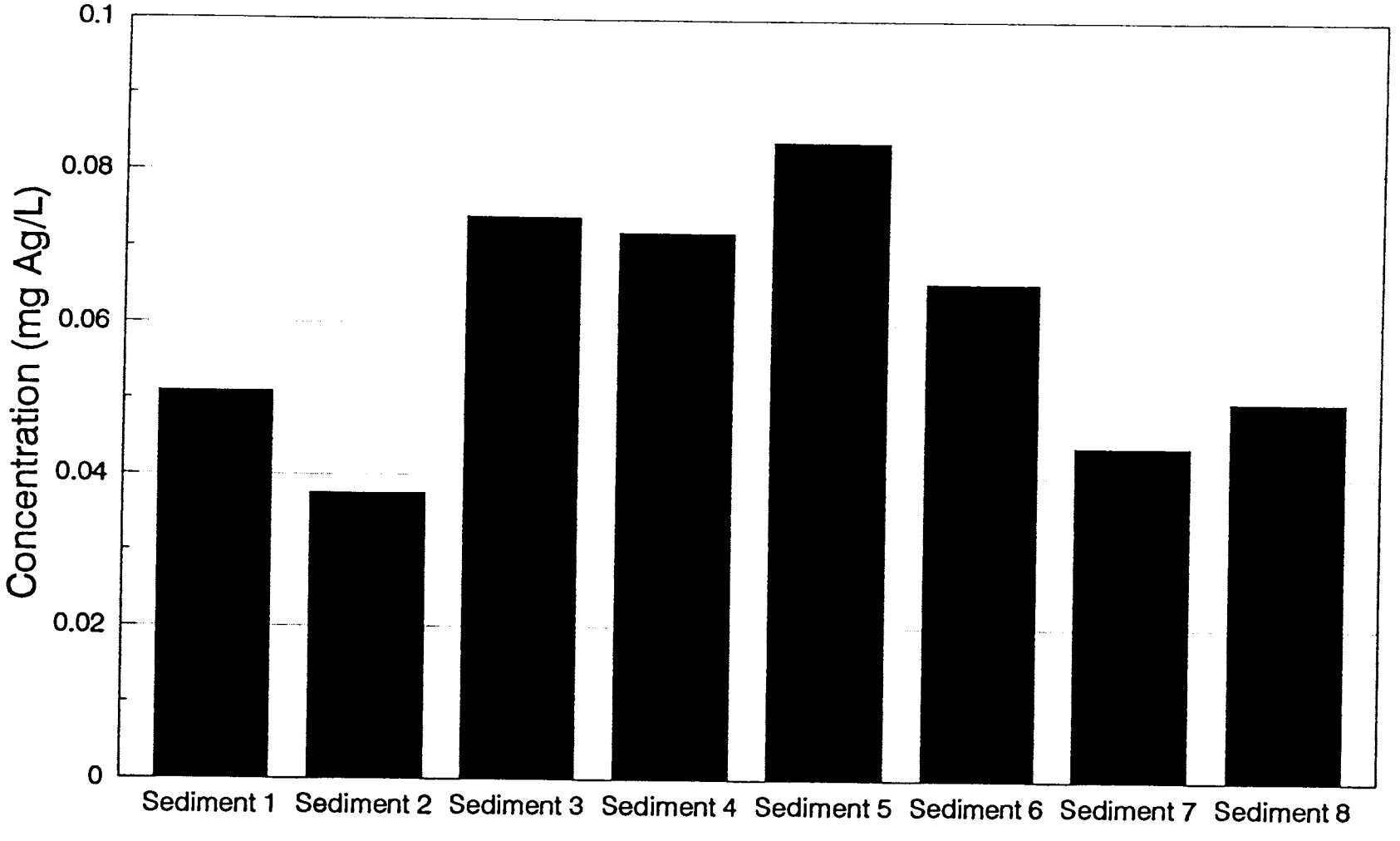

Fig. 5 Concentration of dissolved (<0.45 um) silver in overlying water. (Sediments amended with 320 mg Ag/kg as silver chloride)

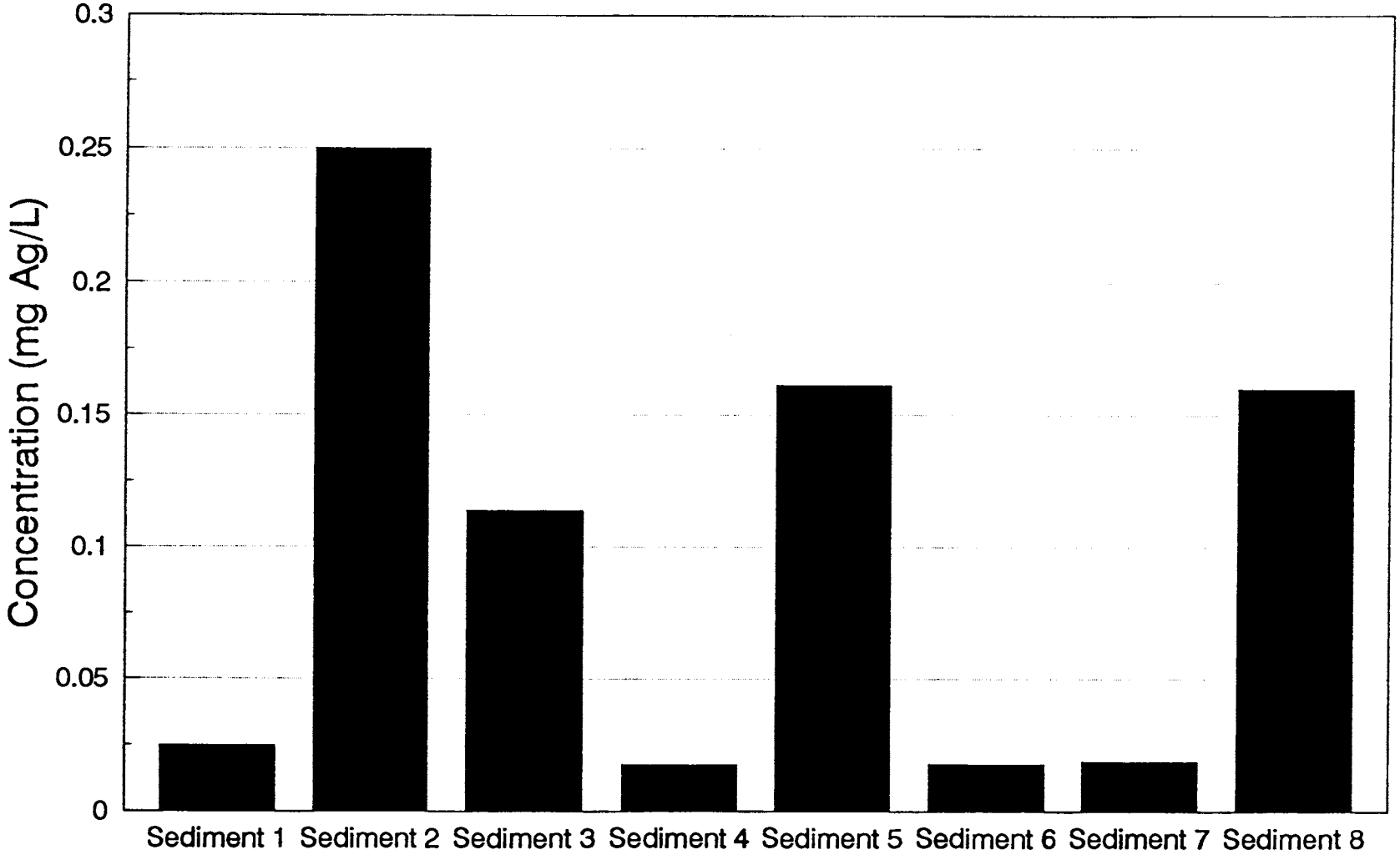

Fig. 6 Concentration of dissolved (<0.45 um) silver in pore water.
(Sediments amended with 320 mg Ag/kg as silver chloride)

Questions & Answers: Partitioning and Effects of Silver in Amended Freshwater Sediments

Q. DOMINIC DI TORO (Manhattan College): I noticed that the acid-volatile sulfide (AVS) in your sediments was quite low. I was wondering if you dried your sediments?

A. No, we took surficial sediments where the organisms are found and one of the things we're interested in is the toxicity of AVS to organisms. We've done a few experiments and we found that they are fairly sensitive to AVS. In other words, they both behaviorly avoid AVS and give up in terms of life when they don't.

Q. Did you show toxicity effects or behavior? We haven't seen that in the sediment quality criteria work that's been going on for five years.

A. Do you find AVS in the areas where the organisms are active?

Q. Absolutely.

A. Well, I guess we could spend hours or even days talking about the sources of volatile sulfides and so on. What we found is that the AVS, when exposed to diatomic oxygen, were all oxidized and at a fairly rapid rate. So these organisms were tolerating AVS where there's no diatomic oxygen. These are aerobes, in sort of loose terms, and they have an absolute requirement for diatomic oxygen. So where you find benthic organisms that are not aerobes, if you will, you then will likely find that the AVS is pretty small.

Q. This is absolutely untrue. The normal set in sediment is that you find obligate aerobes, whatever, and it poses AVS via concentration, that's the normal. If you oxygenate them and deplete the oxygen you are absolutely right, that would leave an anaerobic environment, but it's common finding that obligate aerobes live in there, too. In special areas, of course, but in close proximity to all these sulfidic sediments, and the reason they survive is because they aerate themselves.

A. In terms of the whole of silver required to elicit response in the organism?

Q. Yes, except for the one case where you have, like 1 ppm — was that the sediment with zero AVS by chance?

A. No, that was the sediment with fairly low AVS and fairly low organic carbon.

Q. Right, and did you see any contravention or a notion that the molar ratio of silver to AVS is less than one or less than 0.5 in this case? Did you see any toxicity in that case?

A. As we load these sediments and as we exceed the molar capability of AVS we do see the onset of toxicity, but it's not completely explained by AVS so there are other factors.

Q. I guess the question is the other way, when you have enough AVS have you ever seen toxicity?

A. Yes.

Q. You have seen toxicity in the presence of excess AVS?

A. Yes, and I think the reason is that AVS for a bulk sediment isn't necessarily evenly distributed. Which AVS do you use to even represent one beaker, do you use the AVS for a bulk sample from that sediment and does that represent what the animals actually see?

Q. I will tell you what we do but I'm curious to see that experiment because that would be unusual.

Q. ANDERS ANDREN (Univ. of Wisconsin): From what I understand, as you mix your solid silver chloride, you mix that into the sediment, is that correct?

A. We do that and then allow it to reestablish negative redox and so on.

Q. How do you know that you don't change the AVS?

A. You can measure the AVS before and after and you see similar values. You don't see exactly the same values.

Q. But you mix it in anoxic conditions?

A. No. We mix it under water and it rapidly reestablishes. If it's pretty strongly negative redox, in about 24-48 hours it will reestablish the negative redox.

Q. I know you have some amendments where you have 2,000 mg/kg silver or 3,000 mg/kg. Now, I would suspect that you have solubility product controls at much lower levels than that, so then it wouldn't really matter whether you have 2,000 mg/kg or 3,000 mg/kg as far as the water concentration goes. It seems it would be the same if it's controlled by some sort of the insoluble phase. So then, why should there be a difference in toxicity?

A. In fact, there wasn't in a lot of cases. Once you exceed a certain minimum concentration for the solubility product control you don't see any difference in toxicity. I mean, for a lot of these organisms we've illustrated the need to have the techniques to measure bioavailable metal. For example, in chrome work we can precipitate chrome and allow these animals to build their cases out of chrome, a major part would be made of chrome, essentially, total chrome. So you can take, essentially, pure silver or pure copper or whatever and if it's not bioavailable the animals don't know it's there, and likely, you can digest or biodegrade it and measure it but that measurement does not mean anything.

Q. What do you mean by pure silver? Metallic?

A. Yes. An example I was going to show and hesitated to show to this group because I wasn't sure how it would be received is one showing that 18.3 pounds of metallic silver were deposited at the lower end of the Mississippi River in a sinking ship. In theory, if all of that was bioavailable you would have had mortality for miles of the river. But it's simply not bioavailable.

Trends in Ag Concentrations from Sediment Cores in San Francisco Bay

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This study is an attempt to reconstruct changes in anthropogenic inputs of silver since the onset of industrialization around the San Francisco Bay estuary. The timing of sediment deposition in several cores from Central San Francisco Bay, San Pablo Bay, Grizzly Bay and South Bay is constrained by profiles of the radionuclides ^{210}Pb, ^{137}Cs. Ag in the sediment from these cores was extracted in hot nitric acid. Ag in the acid leachate was quantified by graphite-furnace atomic absorption spectrophotometry with calibration by the method of standard addition.

Concentrations of Ag in the sediment cores range from 0.1-1.4 μg/g with the composition of surface sediment restricted to 0.3-0.4 μg/g. Throughout the bay, deviations from the background value of 0.1 μg/g are limited to the upper part of the sediment cores which contains detectable ^{137}Cs produced by atmospheric testing of nuclear bombs. This shows that anthropogenic inputs of Ag reached a magnitude sufficient to affect the sediments throughout the bay in the 1950s. This contrasts with other metals such as Pb, Cu and Zn, which show detectable enrichments relative to preindustrial background levels a few decades earlier. There is a pronounced subsurface maximum of 1.0-1.4 μg/g Ag in four cores from South and Central San Francisco Bay not seen in cores from North Bay. A spatial and temporal correlation between Ag and Pb in North Bay suggests a lead smelter may have been an important source of Ag, possibly distinct from another source in Central or South Bay. Integrated inventories of Ag in excess of 0.1 μg/g are comparable in the northern and southern reaches of the estuary, however, despite significant differences in circulation, sediment input, and industrial activities.

ERIC CRECELIUS (Battelle): One of the things you might want to be concerned about is that the water samples which you analyzed have been stored for a long period of time. You may lose silver to the walls of the container even in acidified seawater, especially in polyethylene. You lose a significant amount of silver to the walls after several months.

Q. TOM BOBER (Eastman Kodak Co.): You mentioned that industrial discharges were about 26 kg/d. Last year Russ Flegal mentioned that there had been a mercury refinery and a cinnabar mine on the southeastern part of the South Bay, and also there had been extensive mining up the Sacramento River where they used mercury for refining purposes. I wondered if you are taking this into account, as far as the possible amalgamation of silver with mercury and, perhaps, even continuous cycling that way?

A. Well, what I've done, really, is compare the inventory of silver and I'm sure it takes into account and distributes evenly the input over the past 40 years and that happens to match the amount of silver released today. Now, if what we're seeing is true and there is definitely an amount of silver in the sediment, then the implication would be that maybe not all the silver was brought in the sediments but some of it is going out in some way. But the mining, as far as I know, for instance, would have taken place before World War II, and we have no indication whatsoever of any input of silver above the background level until after World War II.

Q. I meant that there are abandoned mining sites, and there are other disruptions of the earth and in the banks, and there's probably development in that area by now. I wonder if this might be contributing.

A. That would be worth looking at.

Q. PETER SANTSCHI (Texas A&M Univ.): I want to find out what kind of resin are you trying to use for the discussed silver?

A. Oh, for that, Peter, I'm just trying to use that hydrophobic resin that I used in the past to preconcentrate copper, nickel, cadmium, and zinc. I ran some preliminary tests for that. The principle is, you add an APDC lab compound with hydrosulfate compounds, which is more water soluble than just APDC itself, in this case. And the resin's hydrophobic, it's an XAD lab resin that I've also manufactured, as well as this divinyl benzene resin, which is much cleaner, and so that's the principle. I eluted it with a weak ethanol solution and, I think, nitric acid.

Q. JIM LEAGAN (Eastman Kodak Co): I was thinking about the earlier information from this conference, that in Lake Michigan over 90 percent silver in the sediment is from aerial deposition. And I look at your numbers, I could see that Lake Michigan has about 127 kg/y of silver depositing in the sediment and you're showing 10,950 kg/y in San Francisco Bay alone, with an estimated value from me of $766,000 a year. I suspect that that would not be coming from industrial sources alone.

A. So what do you think it is coming from?

Q. I was suspecting the amount of silver you estimated at 10,000 kg/y is probably somewhat high. You simply presume an absolute average level of silver across the entire bay. If you look at the rivers in Connecticut, they are showing that you get a much higher concentration of silver deposit in the marsh areas as opposed to the water. I think the distribution is probably not so homogeneous, that binding of silver varies with location.

A. So you say you are questioning — I'm not sure what it is you quoted — are you seeing that as a missing sink or a missing source? I'm not quite sure.

Q. I'm actually talking about the distribution computation that gave 10,000 kg/y deposited in the sediment in the entire San Francisco Bay area, and that number seems just too high even if you have enough sources.

A. So are you questioning — I think I'll better restate that. Do you have problems with this estimate here based on effluent composition on the order of 10-20 kg/d of silver?

Q. I would have probably taken less.

A. Okay, so the problem is that you're questioning both.

Q. Right.

The Role of Colloids in the Transport of Trace Metals in the San Francisco Bay Estuary

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The size distribution of trace metals (Ag, Cd, Cu, Fe and Ni) was examined in surface waters of the San Francisco Bay estuary. Water samples were collected in January 1994 across the whole salinity gradient, and fractionated by tangential-flow ultrafiltration into solution (<10 kD), colloidal (10 kD-0.2 μm) and total dissolved (<0.2 μm) phases. Concentrations of colloidal Fe and Ag accounted for >84 percent of the total dissolved fraction and for 16-18 percent of Cu at the low salinity region of the estuary.

At high salinities, while colloidal Fe was still relatively high (~40 percent of the dissolved), very little colloidal Cu (~1 percent) and no colloidal Ag was detectable. All of the total dissolved Ni and Cd throughout the estuary consisted of small molecular weight (<10 kD) species. The extensive removal of colloidal metals was indicated by highly nonconservative distributions relative to ideal dilution of river water and seawater along the estuary. In contrast, metals in the solution phase (<10 kD) showed a nonconservative excess and appeared to be advected out of the estuary. Partition coefficients (Log K_D) for colloidal particles ranged from 3.5 to 5.3 for Cu and from 4.1 to 6.9 for Fe and were similar to the partition coefficients calculated for large particles. However, the two particle pools appear to have different geochemical cycling along the estuary.

While the positive correlation between the partition coefficients for large particles and salinity suggests coagulation (solution → particle transfer) during estuarine mixing, the inverse relationship between the partition coefficients for colloidal particles and salinity suggests desorption from/or disaggregation of colloids (colloid → solution transfer) within the estuary. Therefore, the nonconservative (sink or excess) behavior of metals along the estuary appears to be determined by the relative contribution of the colloidal phase to the total dissolved fraction, and transport of metals from the estuary to the open ocean appears to be dominated by relatively small (<10 kD) molecular weight species in solution.

Questions & Answers: The Role of Colloids in the Transport of Trace Metals in the San Francisco Bay Estuary

Q. GEORGE LUTHER III (Univ. of Delaware): So you found an interesting comparison between the San Francisco Bay and New York, because the complexes for Fe(III) they showed yesterday would be ranging at 1,000-10,000 molecular weight.

A. It's a really good point because I thought about dimensions. Ken Bruland is analyzing those samples, the samples that we filtered in San Francisco Bay, and he's finding the stability constant in the truly dissolved and the dissolved are the same. So, it's a good point, because the organic computation is in a molecular way. It allows one to determine the colloidal part independently, which is 1,000-2,000 times higher compared to the familiar complexation in that fraction.

PETER SANTSCHI (Texas A&M Univ.): I just would like to point out that you have to be careful with iron and zinc extrapolation because that was at a time when there were very few measurements. When we actually have measurements it doesn't quite work, so you do have to measure the main sort of carbon content, not the colloids.

A. Yes, I would agree to that.

DOMINIC DI TORO (Manhattan College): I haven't really wanted to talk about the carbon concentration of that anymore, but I'm now seeing that the colloid explanation is beginning to fall on hard times. Though it doesn't sound like what everyone's suspicion was, namely that this is purely an artifact. I recommend, by the way, that you take a look at the latest books on that issue, which dismiss the whole thing with a wave of the hand as simply a colloidal effect, and it sounds to me like a good thing, too.

ARGENTUM
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Session 6

J.H. Rodgers, Jr.
Session Chair

Effects of Silver in Sediments

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Abstract not available.

Questions & Answers: Effects of Silver in Sediments

Q. PETER SANTSCHI (Texas A&M Univ.): I'm sure you know that the molecular distribution coefficients of metals are not exactly constants up to the last zero. The metal concentration in the overlaying water, I was just surprised you had it in mg/L and you have high particle concentrations. Could you comment on that?

A. As I said, this was the first experiment which was done, the idea being to kill something and I think it's essentially a proof-of-principle experiment. The concentrations in — you're talking now about the oxidation experiment, yes? — we were wondering what was going to happen, frankly, so we didn't bother to be careful about the ratio of filtration, actually, and all that. It's a fairly rough experiment. Also, we cannot make two experiments the same. We don't have replicates, these are duplicates. This isn't replicates, two different cores; it's not sequential sampling.

Q. JOHN MORSE (Texas A&M Univ.): A comment on your experiment and your model — as they stand, true, but not in the way things work in the real world. The transport in most oxic sediments is dominated quite strongly by bioirrigation. We have lots of microenvironments and, typically, leaps to over an order of magnitude faster transport coefficients. My second comment gets back to copper in which you featured the poisoning by the sulfide.

A. Just let me get the first one first. We have done an experiment where we took a look at the effect of mere bioirrigation in an experiment and we got something which is very interesting. At low levels of animal density we see essentially no effect. You're right absolutely, bioirrigation increases the diffusion coefficient by an order of magnitude. But curiously now, in very high organism numbers it seems to me we actually see a slowdown of the metal flux. We think that the organisms are becoming more anaerobic and keeping the sulfide from oxidizing. But anyway, you're right, I agree that animals are part of this game.

Q. The other thing is, there's been quite a lot of work done on this sulfide toxicity, particularly applying it to plants like seagrasses. What happens is, whether they pump the oxygen in through the brink or through the roots fast enough, it makes a sort of a buffer zone. In plankton this doesn't happen because you get inhibiting matter in the overlaying water or not enough nutrients for the plants. Then the sulfide can build up and you do get this sulfide toxicity. So if people argue about that, you really have got to look at fairly complex ecological situations.

A. Right. By now the time is that people have this notion that there's something like an anaerobic sediment and something called an aerobic sediment and there's a sharp division between the two. My work is intended to argue if that is really the case, if that what you describe is true. The other place for sulfide toxicity is when we have iron sediments.

Q. KOSTAS DASKALAKIS (NOAA): I have a question and a comment. I'm quite happy that I see some change in ideas that were presented some years ago. Now you involve oxidation which was not possible formerly. What you haven't involved yet is complexation of sulfides at least with this method.

A. Correct. We thought that we'll see this actually in the partition coefficients, which basically are modeling in a rough way the amount of metal that is complexed in the sulfides. But we actually do a calculation in the presence of a metal sulfide and an iron sulfide with the standard thermodynamic model which is around. We find that the total dissolved metal including the metal sulfides is very low.

Q. Yes, it may be more for metals bound to organics.

A. I remind you of the tests with the organisms. The toxicity data, I think, we can make theoretical arguments from now to doomsday, but to prove the point is whether the organisms respond "yes" or "no."

Q. Oh, these specific organisms?

A. Well, there are more than a few organisms within the tests. There is no doubt these systems are complex, but I think what that says is that the bioavailable fraction is kept very low by sulfides. I would hazard to guess that aqueous metal sulfide complexes aren't bioavailable. The sites of the organisms where toxicity is exerted weren't accessed by the stuff even when it is within the organisms. I would guess it'd be something of that form. But that's a guess.

Q. JOHN RODGERS (Univ. of Mississippi): Two quick questions. One, as regards the experiments that you did, I think you can't keep the systems completely aerobic. You always have an amount of redox even all the way down the sediments, so that you see reduction effects within a zone. And, like the bioavailability, we've done those kinds of experiments and it doesn't seem reversible, at least not on this planet.

A. Once you form $Cr(OH)_3$, that is the end of it. That's what we seem to see.

Q. The other comment is, if I have it correct, I would bet that was a marine experiment and that, probably, the last one is a compensation experiment?

A. That was a marine experiment, that's right.

Q. You probably need to take a look at the differential toxicity of sulfides to marine organisms versus freshwater, as I pointed out already.

A. I took a look at some work that was published last year, I think, where they looked at the cadmium AVS thing in freshwater sediments, very low concentrations of a few tenths of a µmol/g, in looking at direct toxicity. Such things happen, the AVS in the Great Lakes is typically 1-2 µmol/g, just routinely everywhere.

Q. NICK FISHER (SUNY — Stony Brook): Question into the real world: The experiments with contaminated sediments, the sediments were contaminated with a mixture of metals, not just nickel or just copper?

A. Sure.

Q. I'm wondering if the very clean story that you displayed for AVS relationships to metal toxicity is observed when one works with a mixture of the metals as opposed to an individual metal.

A. The sure answer is, yes, they do, Nick. You can use equal molar mixtures of nickel and cadmium in the sediments, and what you do there is you add up the molar concentrations of the nickel and the cadmium and if that's less than the AVS the silver precedes. We have one experiment where equal molar mixtures of the five metals, cadmium, copper, nickel, lead, and zinc, have been added to the sediments and they follow the rules. So it works very nicely. Also what's interesting, is that they are released in the solubility sequence. So in pore water where the molar concentration exceeds the AVS, you don't find one-fifth of the molar of each of the metals, what you find is nickel. And then as you keep adding the stuff you get zinc and so on.

Q. So it's only with the last amounts you add that you should get mercury?

A. That's right, that's right exactly.

Q. JIM KRAMER (McMaster Univ.): I'm not too familiar with this but I think it's important when we look at silver in this special case from what you just said, and this is a point I made earlier: if we have any AVS there then you can pretty much say that silver is bound to it. Because you don't worry about the nickel and zinc and so on unless there is something in there, some part that is not reversible and that we don't know about. I think in order of importance, you get, basically, silver, you got mercury, maybe copper.

A. You're right. I mean those are the sequences of release, you're absolutely right. But one finds it hard to prove, which is why it's so interesting to find any silver in the environment at all, period. Either it's all coming from sewage treatment plants and that's what everybody is picking up, and once it gets into the sediments it's there forever as an absolutely immobile compound. Or this oxidation that we're picking up is important. Now, you have to understand that we are running a 1,000-fold higher concentrations in our sediments than the ppb that's in normal sediments, because we want to kill something there. So what we're seeing is designed to see something.

Silver in the Waters of Narragansett Bay and New York Harbor

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System wide studies of silver in the waters of Narragansett Bay and New York New/Jersey Harbor were completed in 1985/1986 (Hunt *et al., 1986; Hunt et al., 1987a,b,c; Pilson and Hunt, 1989)* and 1991 (EPA, 1991a,b; Battelle 1992), respectively (Table 1). Stations were located throughout each system and covered the range of salinity characteristic of each system including major rivers discharging to the system. Inputs from sewage treatment plants were also evaluated. Each study determined the dissolved, particulate, or total form of silver using clean metals sampling and measurement protocols, although the studies employed slightly different analytical techniques to determine the forms of the metal (Table 2). Supporting data included salinity and total suspended solids (TSS), measured as the amount of particles retained on 0.4 μm Nuclepore membrane filters.

Table 1. Sample collection summary

CHARACTERISTIC	NARRAGANSETT BAY	NEW YORK HARBOR
Year	1985 (October, November) 1986 (April, May)	1991(January, May, October)
Conditions	Seasonal	High Flow Hudson River Low Flow Hudson River
Stations/System	22 (Baywide)	37 (Baywide) 6 (Tidal Cycle) 18 (Baywide)
Depths/Station	2 to 4	1 (Baywide) 2 (6 stations in salt wedge)

Table 2. Processing and analysis summary	
NARRAGANSETT BAY	**NEW YORK HARBOR**
Shipboard filtration using clean methods	Shipboard filtration using clean methods
Total suspended solids *(0.4 μm membrane)*	Total suspended solids *(0.4 μm membrane)*
Ag- dissolved (Measured) *Co-APDC co-precipitation (pH 3 - 3.5)*	Ag- dissolved (Measured) *APDC-DDDC solvent extraction (pH 1-2); back extract into 2 N HNO_3*
Ag-particulate (Measured) *3N HNO_3, 60°C, sonication*	Ag-particulate (Calculated) *Difference between total and dissolved forms*
Ag-total (Calculated) *Sum dissolved and particulate forms*	Ag-total (Measured) *Evaporative heating to 20% original volume, reconstitution, extraction as dissolved*
Quantification with GFAAS (Standard curve)	Quantification with GFAAS (Standard curve)

Dissolved silver concentrations within each system were generally similar (Table 3). The lowest dissolved concentration measured was 1 ng/L in Narragansett Bay. The highest concentration measured was 0.038 ng/L in May 1986. The highest dissolved silver measured in New York Harbor (NYH) was 28 ng/L. The mean dissolved silver for the seven surveys summarized ranged between 4.9 and 14 ng/L.

Table 3. Minimum, maximum, and mean concentrations of salinity, dissolved and total silver, and total suspended solids for each survey in Narragansett Bay and New York Harbor (NYH). Surveys SB2, SB3, SB4 are from Narragansett Bay. NYH-HF and HYH-LF refer to samples collected during high (January) and low (October) flow conditions of the Hudson River.

SURVEY	SALINITY ‰			TOTAL SILVER □g/L			DISSOLVED SILVER □g/L			TSS mg/L		
	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean	Min	Max	Mean
SB2	2.84	32.31	28.71	0.002	0.082	0.020	0.001	0.033	0.0081	0.57	71.6	3.67
SB3	7.63	32.48	28.91	0.003	0.088	0.025	0.001	0.038	0.0074	0.72	11.3	2.33
SB4	21.4	32.73	30.89	0.003	0.108	0.023	0.001	0.024	0.0049	0.30	14.5	3.68
NYH-HF	0.10	32.70	19.85	0.0004	0.145	0.047	0.003	0.028	0.014	0.61	54.1	12.7
NYH-Tidal	0.09	26.17	16.80	0.005	0.352	0.085	0.0004	0.020	0.007	4.4	409	34.1
NYH-LF	5.0	26.90	19.63	0.007	0.137	0.055	0.002	0.028	0.0091	1.58	25.9	9.9

The range for the lowest total silver concentrations measured was 0.4 to 7 ng/L; the highest total concentration was 352 ng/L measured during the tidal cycle study in NYH. This survey specifically sampled the water column within 1 m of the sediment water interface throughout tidal cycles. Thus, this maximum value and the higher mean total silver concentration from this survey reflect sediment resuspension sampled during this survey. Collection of samples representative of resuspension events was not specifically scheduled during the other surveys. Sediment resuspension during the tidal survey was also evident from the high total suspended solids concentrations measured during this survey relative to the other surveys. Mean total silver concentrations in New York Harbor were about twice those measured in Narragansett Bay and reflect the generally higher TSS concentrations found in New York Harbor (about 10 mg/L) compared to Narragansett Bay (about 3 mg/L).

Dissolve and total silver concentrations generally decreased as the salinity increased in Narragansett Bay (Figure 1), although the specific trends varied between surveys in this Bay. The correspondence between salinity and silver concentrations were particularly evident in the lower salinity regions, and reflect the variable levels of fresh in flow Bay during each survey. Variability in the data was too large to clearly demonstrate either conservative or non conservative behavior of the silver. The data from New York Harbor did not reveal any systematic correspondence to salinity (Figure 1), although the highest concentrations appear to occur at salinities in the 15 to 25 ‰ range and decreased as salinity increased. Concentrations in the fresh water reaches of the Hudson River were generally lower than in New York Harbor. The high silver concentrations in the mid salinity range in the Harbor reflect the input of silver from the 20 plus sewage treatment facilities discharging to the Harbor. The silver concentrations in effluents of these facilities ranged between 500 and 16,000 ng/L and average about 2,000 ng/L. Concentrations in the treatment plants and river in Narragansett Bay were generally not detected above 10,000 ng/L, the detection limit of the instrumentation used to measure the silver in these samples.

Partitioning of silver between the particulate and dissolved phases, determined as a partition coefficient (K_d), displayed a wide range in both systems (Figure 2). Correlation between the K_d and salinity was not apparent for individual samples from either system. Several factors could be responsible for the range in partition coefficients including high analytical variability due to the low concentrations, variable efficiencies in the digestion and extraction procedures, and using the difference between two measured values to estimate one of the terms in the partitioning coefficient. Table 4 presents summary statistics for the K_d estimates for each survey including the mean, standard deviation and standard error. Although there is a wide range in individual estimates of sample K_d, the mean K_d calculated for each survey is similar, ranging between 2.8 and 7.1×10^5 mL/g. The mean K_d for each survey did not vary as a function of mean salinity nor mean TSS observed (Figure 3).

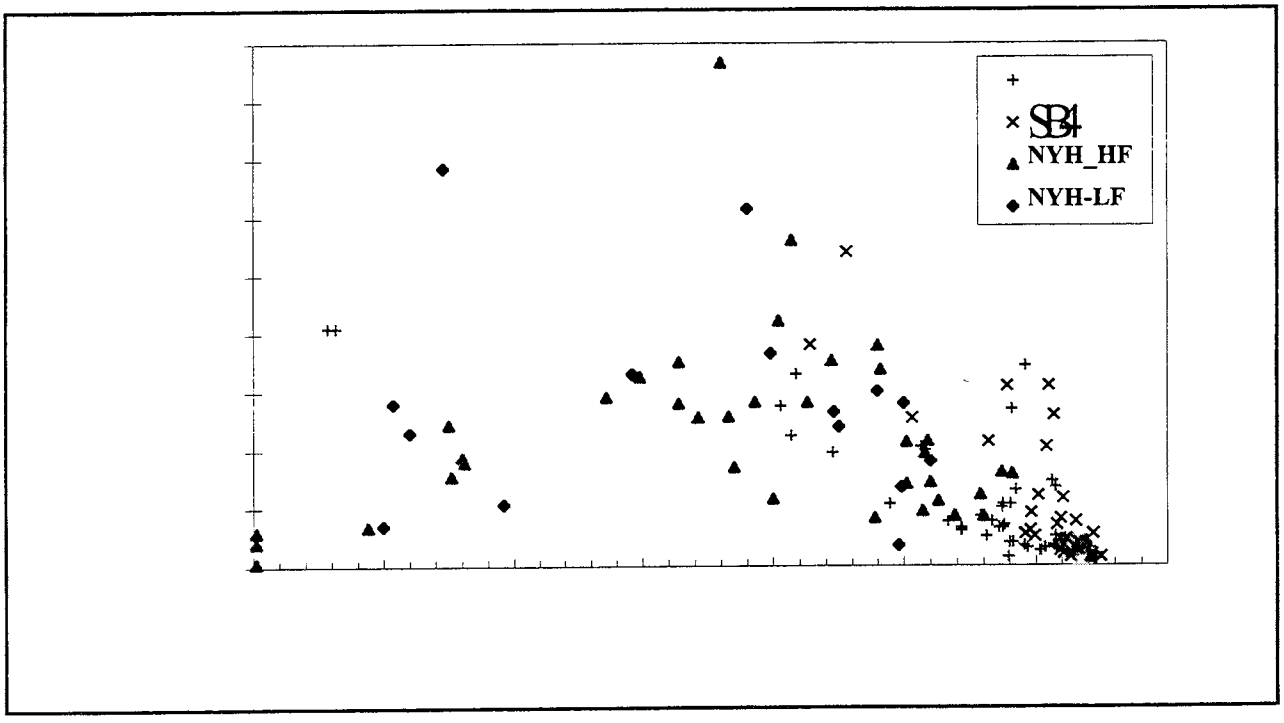

Figure 1. Total Silver in New York Harbor and Narragansett Bay as function of salinity.

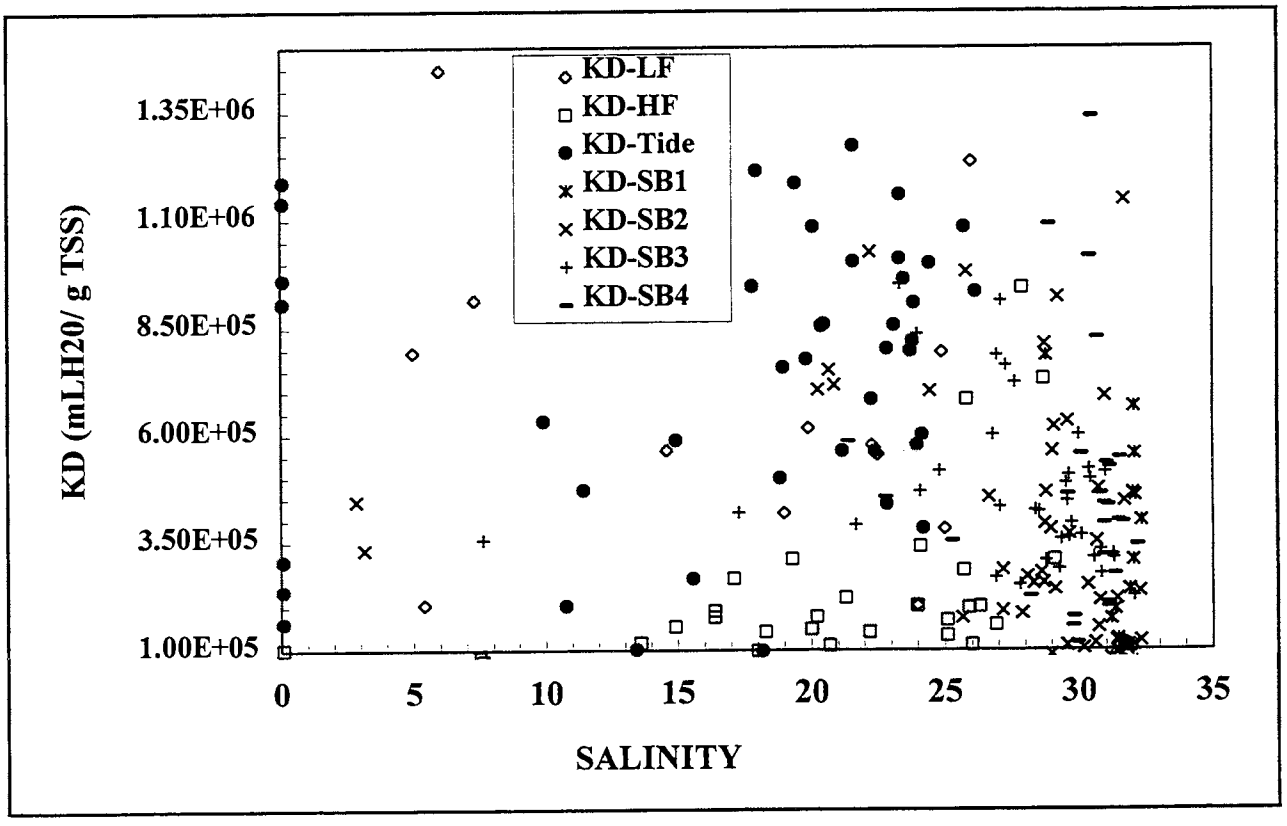

Figure 2. Partitioning coefficient versus the salinity of each sample all surveys.

Table 4. Summary statistics for the silver partition coefficients estimated for Narragansett Bay and New York Harbor.						
Survey	K_D ($\times 10^5$ ml H_2O/g TSS)					N
	Min	Max	Mean	STD	STD Error	
SB2	0.7	11.5	4	2.8	0.42	46
SB3	2.25	22.1	5.2	3.4	0.56	37
SB4	1	13.4	4.4	2.9	0.53	31
NYH-HF	0.56	29.5	2.8	4.8	0.77	39
NYH-Tidal	0.04	12.7	7.1	3.6	0.23	45
NYH-LF	2	14.5	6.7	3.7	1	13

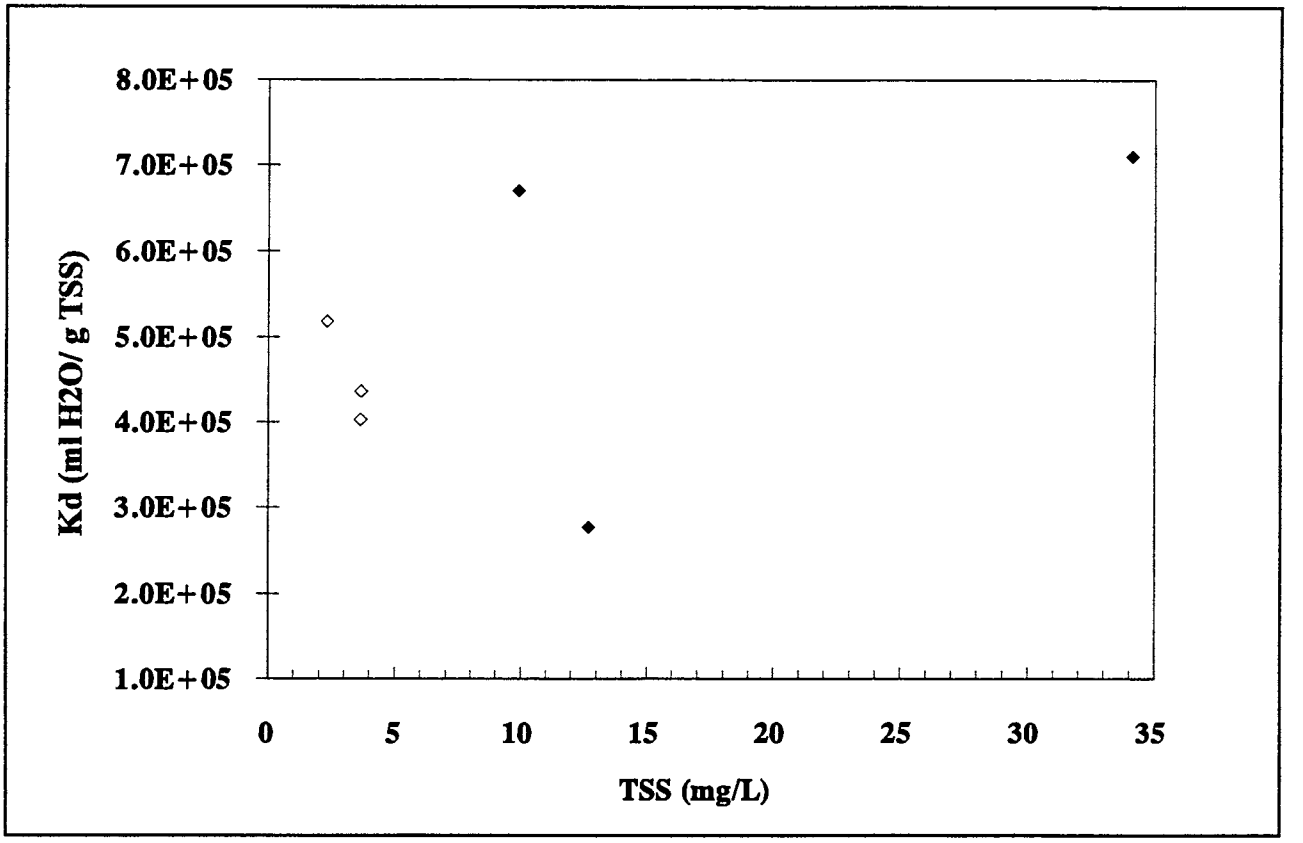

Figure 3. Correspondence between the mean partition coefficients for silver and mean total suspended solids concentrations calculated for each survey. NYH surveys are in closed symbol; Narragansett Bay in the open symbol.

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Questions & Answers: Silver in the Waters of Narragansett Bay and New York Harbor

Q. PETER SANTSCHI (Texas A&M Univ.): You saw this large range of K_D values at levels sustaining higher concentrations spanning about an order of magnitude. Would you want to speculate on that? I mean, is this essentially deposit? We found that impacted systems adsorb usually at higher K_D in sewage, so it may be a higher percentage of sewage derived particles. Is that at all possible?

A. That is a possibility, but if you look even in New York Harbor, again, I think most of the large load of particles coming down from the Hudson River is mostly what's coming out of treatment plants. That could be part of it. A lot of it can be related to the analytical techniques. There is quite a bit of variability, you know, 10-20 percent in some of the numbers; it's a lack in analytical quality. But it could be the sewage plant. One of the things with these two data sets, both have a full range of particulate organic carbon as well as dissolved organic carbon. I didn't have the opportunity to really go in and put all that together to see if it kind of tightens up these relationships.

Uptake and Release of Silver from Marine Sediment

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The relationships among acid volatile sulfide (AVS), simultaneously extracted metals (SEM), and silver concentration in porewater were examined for marine sediment that contained 10 µmoles/g (dry wt) of AVS.

Aliquots of anoxic sediment suspended in oxygenated seawater were spiked with 10 to 250 µmoles/g of silver (1,000 to 27,000 µg/g silver). The seawater in contact with sediment spiked at 100 µmole/g silver (10,800 µg/g) contained 20 µg/L silver. The seawater in contact with sediment spiked with silver at 25 times the molar quantity of AVS (27,000 µg/g silver dry wt) contained approximately 2,000 µg/L silver, which appears to be the solubility of silver in seawater. The release of silver from the spiked sediment samples was determined after resuspending the sediment in seawater for a period of hours to days.

Questions & Answers: Uptake and Release of Silver from Marine Sediment

Q. PETER SANTSCHI (Texas A&M Univ.): I would like to have some clarification about what you said right at the beginning. You said that you measured a flux of silver out of the sediments and that agrees with what Dominic was saying before, but it's sort of contrary. It should have gone into the sediment from the way you describe the transport.

A. Okay, for the benthic flux situation we put a chamber on the bottom and then over time sample all around that chamber. We keep it mixed and with time we see copper, cadmium, and silver, and few of these other metals. Their concentration in that chamber is increasing with time, indicating that one of the things happening here is some of the AVS at the surface sediment is oxidized, leaving free these metals or releasing them to the overlying water. Because in the pore water we don't see those same metals; the metals coming out of the sediment aren't in the pore water. So it seems that what we find in the pore water is insoluble sulfides, but in the overlying water they have a chance to get oxidized up, surface from the sediments and migrate in the overlying water.

Q. But they do. I mean if you simulate the natural conditions you would oxidize it also.

A. I don't mean to say we're changing the natural conditions. What we're doing is just containing that water so we have an opportunity to see the build-up of metal or we can get a flux. I don't know how else to get a flux of metal out of the sediment. We also set up some of the sediment in the lab and resuspend it, and see if we can release metal from the resuspended sediment in oxygenated water.

Q. If we would follow Dominic's model, they should go into the sediments. They should be using sulfur and precipitate as sulfides.

A. In Dominic's model, the way I understand it, are experiments where you add a lot of metals to the AVS, allow that to oxidize with time and you saw a metal coming out of the sediment. Initially coming out, and with time some of it went back in, which were simply the metal oxides that formed as the AVS oxidized.

JOHN RODGERS (Univ. of Mississippi): Dominic, would you care to comment on your experiments?

DOMINIC DI TORO (Manhattan College): Let's see. We do several experiments, and you're talking about the first one and you're talking about the second one. It's a balancing act. I think Eric has it right, the way we visualize it the metal sulfide is oxidized. It diffuses both ways, like the delta function source of the aerobic/anaerobic layer . The stuff that diffuses down gets sucked up by the AVS and so you don't see it in the pore water, that's correct. The stuff that goes up diffuses out by microdiffusion and that's what you're picking up as you measure the flux. If the overlying water concentration, though, were high relative to the source, then what Peter is saying was right. There would be a continual down flux due to the source down, the mass transport down would be larger than with these through oxidation, so it can happen either way. What you guys are seeing is more production basically up than diffusion down. It's interesting that you see silver as well right here. That's surprising.

PETER SANTSCHI: But Dominic, what you are describing was an experiment where you controlled the system. What he was measuring was a natural system. So there was no extra action, just the natural source.

DOMINIC DI TORO: Yes, I understand. But what that experiment says to me is that in the situation he is monitoring there is a net export of metals. Right? And so the surface mass transport down is less than the amount that makes it up through diffusion.

PETER SANTSCHI: But in the steady state, if you look at the natural system, you have your oxic layer, you have your anoxic layer and the net flux should be into the sediment.

DOMINIC DI TORO: Yes, but the net flux down is probably particle settling. So the source of metals to the sediments are particles or colloids or whatever settling. That stuff gets scavenged up into metal sulfides. The metal sulfides are oxidized and part of it is recycled back.

PETER SANTSCHI: And how do you get oxygen?

DOMINIC DI TORO: Because there's oxygen there.

PETER SANTSCHI: But you added it?

Q. RICHARD PLAYLE (Wilfrid Laurier Univ.): You did experiments where you added oxygen, right?

DOMINIC DI TORO: Right, but there's always oxygen.

A. Yes, San Diego Bay is always oxygenated, we put the box down and it maintained oxygen. If it didn't maintain the oxygen, the box would go anoxic in fairly short time because there's a fair amount of oxygen consumption by the sediment. And then we would see the situation of flux going into the interstitial waters. Presumably metals wouldn't come out if the bottom would go anoxic. Manganese and iron would come out and the other metals would stay in the sediment.

Q. JIM KRAMER (McMaster Univ.): Eric, you mentioned, I think, that you used sand in one case. I assume that the others were not sand?

A. Well, the first case was clean marine sand for the reference part.

Q. Can you really compare these two experiments? Can you make any statement on that?

A. Well, in both these situations with the sand and with the marine sediment which contained 10 µmol/g AVS, in both situations we stirred them, so it's basically a suspension.

Q. So the results you gave here were studied side by side with the sand?

A. Yes, of course.

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Panel Discussion

Regulation of Silver in the Water Column

Moderator:
J. Romney, U.S. Environmental Protection Agency, Washington D.C.

Panelists:
D. Armstrong, University of Wisconsin-Madison
R. Cappel, Silver Coalition
E. Crecelius, Battelle Northwest
N. Fisher, State University of New York at Stony Brook
P. Santschi, Texas A&M University at Galveston
A. Sodergren, Lund Institute for Ecotoxicology
C. Wood, McMaster University

Panel Discussion: Regulation of Silver in the Water Column

Panel Chair: **Jackie Romney**
Panel Members: **David E. Armstrong**
Robert C. Cappel
Eric A. Crecelius
Nicholas Fisher
Peter Santschi
Anders Sodergren
Chris M. Wood

ROMNEY: Good afternoon. Today I'd like to present our panel to you. This panel will discuss the regulation of silver in the water column. First, we have on our panel Bob Cappel, who is director of Environmental Affairs for the National Silver Coalition. Bob's educational training is in environmental chemistry and toxicology. The Silver Coalition has been working with EPA and several state environmental agencies for over the past four years on silver regulations, and they have funded much of the scientific work reported at these silver conferences. What we'd like to do is for each of the people on the panel to give a little bit of background information on this issue and discuss their feelings and experience with regulating silver in the water column, and then we'll open it up for questions to the panel. Now, I'd like to start with Bob.

CAPPEL: Thank you, Jackie. I'm here representing the regulated community. A number of years ago there were proposals to omit hardness-based equations and freshwater quality criteria by EPA and proposals to implement chronic water quality standards both for freshwater and salt water, that were more or less based on some of the lowest observed toxic concentrations, lowest observed effects. These proposed standards were adopted from a number of studies as water quality standards. Some states already have standards that were more rigorous than the EPA's. But as these standards began to be incorporated into permits for sewage treatment plants, it soon became apparent to the sewage treatment plant operators they would have problems meeting the permanent limits. If they stopped all the commercial sources of silver from coming into the plants they'd still be out of compliance from the domestic loading of silver. So it became very quickly a problem, not only for industry but for municipalities as well.

At that point in time, a group of trade associations and technical societies and government agencies, all of whom were potentially impacted by silver regulations, got together. They formed a coalition to work with the EPA and state regulatory agencies to try to understand issues around silver regulations, to try to answer the questions that agencies have which were leading to more concern in regulations, to fund studies to answer these questions by the top scientists in the world, and then to try to move silver regulations so that they were based on good sound scientific risk assessment and good sound science.

We had a number of questions — what happens to silver as it is discharged into a sewage treatment plant and that treatment plant discharges silver into receiving waters; what are the forms of silver; what happens to it as far as its fate and transport in natural waters; what are the mechanisms of toxicity of different forms of silver; and how should we regulate silver if you don't like our proposed regulations?

So, the group founded the Silver Coalition. Some of the members, so you get a feel for who is involved, are the American Hospital Association, the American Dental Association, the Printing Industries of America, the Federal Mining Association, the Motion Picture Group, there's also Manufacturing Jewelers and Silversmiths and the Silver

Institute representing some mining companies. So it's a wide range of people. There are also government agencies that are highly involved in the use of silver. Cities and municipalities are members as well as groups like the Department of Defense.

Over the past several years these studies were performed, and I think we've learned a lot more about silver than we knew four years ago when we first met. The studies have been funded through the Silver Coalition as unrestricted grants to university scientists. It's been an excellent relationship between the regulatory agencies, the regulated community and the university scientists. I think that's the type of thing everybody should continue to do, work together. We know a lot more about silver today then we did several years ago, we still have a number of questions and probably will continue to have questions as the years progress. But I think what we need to do is to work together, to incorporate what we do know into regulations so that those regulations are based on science, on the questions and answers that we currently have. We may need to get more conservative, we may be less conservative, but it's important to the industry and the communities that the regulations are not more conservative than they need to be. So, from the standpoint of the coalition as regards funding, the funding has come from a lot of members of the coalition, but largely from photographic manufacturing companies and trade associations. They probably funded over 90 percent of the projects for these studies. So that's where we're coming from the standpoint of the regulated industry. We're not trying to get out of regulations, but we would like to be sure that regulations are based on good sound risk assessments and good science.

ROMNEY: Thank you. The next person on the panel is David Armstrong from the University of Wisconsin-Madison.

ARMSTRONG: Thank you. My area is environmental chemistry, so I'll give a few comments from that perspective. As Bob said, I think, in terms of regulation, what I feel is important is that decisions are made about regulations which are based on a good understanding of the behavior of silver in the environment. Not only from the standpoint of toxicity but also from the standpoint of knowing about its environmental chemistry, the concentrations that are present in various locations, the speciation, the forms of silver present and what the fate of silver is that's discharged into surface waters.

When I first started to give a little more thought to these questions a few years ago, it seemed like there was quite a bit of spread in the information that you find in the literature about the chemistry and fate of silver. I think over the last few years it improved. As reported at this conference today and also at the conferences in the last couple of years, there is emerging a fairly good understanding although we can always pose more questions that we'd like to answer in terms of really understanding all of the answers about the chemistry. But we do know now quite a bit about what levels of silver are present in surface waters of various types and various regions in the country, and, typically, find that these concentrations are pretty low, in the range of a few ng/L up to maybe 100 ng/L or so in various streams in different regions, and that there is a very strong partitioning of silver to particles. The partition coefficients are very high, so that, typically, a very large fraction of the silver is associated with particles. So there is quite a difference between the total silver and the silver that might be in the dissolved form, especially in the free cationic form, which is considered to be the most toxic.

In our own work we saw that there are some cases where there are fairly high levels of silver being discharged in effluents into waste water treatment plants. We've done some work in looking at what the fate of silver is as it goes into the plant and through the plant. Typically, the percentage that is removed or retained in the plant is quite high, in the range of 95-98 percent or something in that region, in a plant that is fairly typical — a secondary, biological treatment plant. Of course, even that two percent that's coming out in the initial discharge from the plant, we have several µg/L in the discharge from the plant. We've also seen that this is fairly rapidly dissipated at a short distance behind the plant, probably through dilution but also through nonconservative behavior where it's incorporated into the stream sediments at a relatively short distance from the plant. So, this is some of the general understanding that I've developed in our own work and in other work that's done. It's actually quite encouraging in

terms of the problems that we might have with silver in the environment that these natural processes are maintaining concentrations so low, especially due to the fact that silver is so particle-reactive and will be removed by particles from the water column. So, I'll pass on to the next person.

ROMNEY: Thank you, David. The next panelist is Chris Wood from McMaster University.

WOOD: My background is physiological toxicology, and I guess I'll make some more comments based on my understanding of mechanistic toxicity and the interaction of water chemistry with silver toxicity. The first comment I'll make is the point I made during my talk, that we need to do a better job in the way we regulate silver in freshwater. I think we have a tool right now that is pretty badly broken, and that is the hardness equation. That equation, if applied at the lower hardness, could be quite conservative and will be no damage for aquatic life — it will, in fact, be overprotective at low hardness. The higher the hardness, I think it will be, in fact, underprotective. I think in order to alter the equation we need take into account much more water chemistry variables, in particular chloride and DOC as well as hardness. And, in fact, we've seen some progress towards that.

Secondly, when it comes to freshwater regulation I think we have to approve that we don't want an equation based on total recoverable metal. It's basic now that recently we have moved to something which is based on dissolved metals and if we start I'm sure we'll get an equation based on ionic metals. I'm sure we could move to a regulation which is based on dissolved. And that's not just for silver, that's for other metals such as copper.

Third point I'll make is that if all is said and done we'll get an equation for the freshwater situation. I think we need freshwater to be protected against acute toxicity. After all I've seen on silver, it won't be long enough in freshwater to be a chronic problem. But I think there are many site-specific circumstances where it is needed to protect against acute toxicity and that's where we need to do a better job, too.

From my more limited knowledge of seawater situation, which I only got into for the last four months or so, it strikes me that the number the EPA has right now is sort of an across-the-board number for seawater, namely 2.3 µg/L. In fact, though it was designed to protect against acute toxicity in seawater, it looks like it would do a pretty good job overall in protecting against chronic toxicity in seawater. We might need to be protective against chronic toxicity in seawater because silver tends to stay around a bit longer in seawater. So, my theme right now is to put a lot of effort into the freshwater.

ROMNEY: Thank you, Chris. Our next panelist is Peter Santschi from Texas A&M University at Galveston.

SANTSCHI: My background is in environmental chemistry, trace metals and regular chemistry. I was involved in a study of Texas watersheds and Texas estuaries where we found with clean methods that silver levels were in the order of ng/L. Only close to the effluents was the total silver in the order of 100 to 150 ng/L, but the dissolved metal was much less than 100 ng/L, around 10 at most. So I feel a need for speciation, for regulation in terms of at least dissolved concentrations.

Our experience brings us to another problem, in that many state labs can't enforce the regulations on trace metals because they don't apply clean methods. In a lot of states, the work in the chemistry lab is often performed by biologists, and they don't take the chemical speciation very seriously and one can't convince state government that it costs money to do these analyses. They are not cheap and it takes a lot of work, and it's not just the lab or the performance of the analyses, it's also the field sampling. I find that with federal agencies and state agencies, often they don't take that seriously enough — they think it's good enough to have clean methods in the lab only. If you don't also apply clean methods in the field, you're wasting your time. Often people just want numbers rather then actually understand what we're measuring. So I feel it's important to have these regulations, but the states need to have chemists in the lab who also have either the freedom to find and to try to understand what they are doing and check which directions are appropriate.

Tomorrow, I'll show some physical speciation balances. We found that silver is, to a substantial fraction, associated with colloids in freshwater and also in seawater in estuaries near Galveston and Houston. Widely, that is due to the importance of sulfur, reduced sulfur and organic sulfur. So if we want to further improve the regulations, if you have the colloid and solved concentration and that is about, well, nanograms per liter to tenths of nanograms per liter, you still feel protected quite likely. What we have found is that the ionic concentration is, again, a lot lower because we have those highly specific ligands which bind the silver, like the thiosulfate which we heard about this morning, and make it nontoxic to organisms. So, if you do want to go to dissolved silver, we do have to try free metal also. Thank you.

ROMNEY: Thank you, Peter. The next panelist is Eric Crecelius of Battelle Northwest.

CRECELIUS: My experience with silver began at Puget Sound. In the early 1980s we constructed a mass balance for several trace metals and heavy metals in Puget Sound, and we included silver in this mass balance.

From that study we realized that most of the silver entering Puget Sound, which is the estuary near Seattle and Tacoma located in Washington state, most of the silver entered through wastewater treatment plants and most of that silver fairly quickly ended up in the fine-grained sediments in the main basin in Puget Sound. So the Sound was a fairly effective silver trap where silver became particle attached and settled out.

I think there are two important questions regarding silver in a marine environment. Are the sediments a significant source for silver back to the water column, and what's the importance or relevance of silver in the sediments to the benthic organisms? Since we're in Puget Sound, I was involved in the National Mussel Watch Program where we were collecting mussels and oysters across the United States for the past nine years. From that study can be concluded that mussels and oysters living near outfalls have relatively high levels of silver. Also, clams in certain coastal areas near outfalls have high levels of silver. What's the significance of that silver as far as biological effects to these organisms — they are accumulating silver, but I'm not sure that anyone has shown it has any relevance as far as the health of the ecosystem.

Two things could be done in the future. One is, EPA is now working on approving clean metal techniques including analysis of silver in freshwater. It put out a draft for that, I think, and is working to get a draft method for doing low-level silver in seawater, especially for dissolved silver, so different laboratories can, hopefully, get similar values for silver in marine water. Also, I encourage EPA to get methods approved for doing analysis of silver in marine sediments, which would help those organizations looking at the fate of silver in sediments.

ROMNEY: Thank you, Eric. The next panelist is Anders Sodergren of Lund Institute for Ecotoxicology.

SODERGREN: I'm an ecotoxicologist, and before arriving at the administrative issues I will try to talk about what we put into the conceptional framework in the ecotoxicology. We usually use what we call a pollution chain. Starting with the sources, then discussing the transport, studying the transformation of the substances, and, based on that, we try to find the exposure situation, which is crucial for the effect on the organisms, or effect assessment. Looking at silver, we should see there are different chains in these particular relationships. They are there, they are weak, some of them are very weak indeed.

Historically, you see when new substances are discussed it's the same pattern which emerges. You start tracing the sources, try to determine them, develop the analytical methods, and back and forth we take a step at defining our findings and progress. Then I think the various sources have been defined and transportation of silver has mostly been studied in the aquatic systems which, I think, is quite right. In that system most of the silver seems to be trapped in sediment. But I would also like to see some discussion of silver in the atmosphere and the transport in the atmosphere. Then as regards the transformation, we have seen numerous efforts and data discussing the various species of silver, which, of course, is very important when arriving at the exclusion of toxicity. Then we

have some toxicological evaluation and effect studies, primarily on fish, and more is to come. I'm sort of saying that we miss the basic part of the system, the primary producers. As an ecotoxicologist, my primary goal is to arrive at the conclusion; what is the effect for the ecosystem, or is this faraway not only for silver, but for many substances we're all studying? But, nevertheless, we can then lower the ambitions to populations or communities.

Today we have seen mostly a discussion on the individual levels and mostly on fish, which is nice, but it's simple. The fish is a very nice and readily available model organism, and looking at what has been discussed here is mostly biochemistry and physiological reactions. This area has, during the last ten years, leaped forward in developing new methods, new instruments, and new techniques, which now are applied not only to silver but also many other substances. What I would also like to see is more studies on the primary producer, the basic part of the ecosystem, and zooplankton and other organisms in the water column, because there we can, perhaps, have other studies that must be directed at the interactive phase, which are crucial for the ecosystem's behavior. That is one thing, behavior.

I would also like to see that we initiate food and behavioral studies not only on fish but also invertebrates exposed in long-term experiments to silver. These experiments are not so exciting and they do not require that kind of instrumentation like the physiologist or biochemist work we've seen today. I'm sort of saying that in the level of evaluating, assessing the population level, we are not making any progress in the methods we are using. They are old-fashioned methods and we are measuring biological diversity, for example, biomasses, functional parameters and so on. I hope in the future we use the new kind of regulation for these levels so they become more exciting for young students. Now there are waste standards, and I would like to see that the laboratory studies which have been presented here and on individual organisms would be sustained by additional studies with, for example, exposure in artificial environments. Like ponds with 1, 5, 10 m^3 of water of which you have several and have exposed singular to the substances in question, and then you study how the various functional levels or structure of the ponds are changing from a control environment. This is a rather nice way although it's quite expensive, but in that way we can study what happens on the lower level of the ecosystem.

So now I'll just add that one point which is very important to the knowledge and the effect studies, which is the reversibility or the irreversibility. We have seen many effects presented here, some reversible, some irreversible. Reversible effects within limits, that is an effect which a system can manage. But irreversible effects that spread over a large area, that might be critical.

ROMNEY: Thank you, Anders. The next panelist is Nick Fisher from the State University of New York at Stony Brook.

FISHER: Thank you. I am pursuing, along with my students and post-docs, studies of the biogeochemical cycling of metals in marine systems. We are focusing, in particular, on interactions of metals, including silver, with organisms primarily at the bottom end of the food chain, phytoplankton, zooplankton and other groups of organisms including bivalves. In particular, we're looking at the bioaccumulation. In recent years, we're looking at bioaccumulations and trophic transfers of metals, again including silver, in the lower end of the food chain, what's the uptake of silver, for example, from seawater in different species to phytoplankton and trophic transfer from phytoplankton to marine bivalves or zooplankton.

Now, going back to first principles, it seems to be good reason that people are concerned and the taxpayer should throw money at these problems that are concerned with potential impact of metals, including silver, to aquatic organisms or to men. But we know that organisms don't really care what's outside, and they only feel what's in them or on them. So it's really critical to present data and explore responsive organisms as a function for the body burden of the silver and efficiency of silver, as opposed to the ambient concentration of silver.

We've now known for many years that there are many species of metals, including silver, that are not biologically available. And the first such studies we've done on copper in marine systems have been extended to accumulative metals, including silver. By measuring total ambient metal concentration exploratory we get a sense on how organisms are going to respond. So we do know that is a much more consistent, coherent self-response of organisms to the body burden of a particular contaminant. It's more complicated with organic contaminants because they can be metabolized, but metals are fairly simple. So we can express the toxicity as a function of body burden, not just ambient concentration. That's one point I would like to address.

Another is that because of that we do need to explore the bioaccumulation of silver and other metals into organisms, not just measure the ambient concentration or study the speciation. In studies I have submitted, studies of speciation should be related to the bioavailability of those species to important parts of the food chain. Interestingly, the bioaccumulation, as well as the form, is important to public health concerns because, as Dr. Crecelius mentioned earlier, we know that certain bivalves species, such as oysters, clams, and mussels, may concentrate silver and other metals to a substantial degree. They may not be impacted at all but organisms, including men that eat those organisms, might be affected. So it's important to know the extent to which silver and other metals are concentrated in key compartments of the food chain.

Let's see what else . . . because silver is very particle-reactive in both marine and freshwater systems, with partition coefficients ranging from 10^5 to 10^6 typically, silver is often associated with particles. We know that animals can accumulate silver or other contaminants both from the dissolved phase as well as by ingesting contaminated food. There needs to be further study on the extent to which contaminants, silver included, are transferred in food chains. It's likely that being such a particle-reactive contaminant that a dominant route of exposure to certain animals is through trophic transfer, not just uptake from the dissolved phase. That had not been done very much although Chris Wood pointed earlier to the fact that such studies are beginning with fish at the moment.

Finally, I would like to add to what Prof. Sodergren has said about importance of exploring the impacts of silver on primary producers and other components of the lower ends of the food web such as zooplankton. There have been studies in marine systems that have examined toxicity of silver both as function of ambient concentration and as function of body burden in plankton, and they have, in fact, shown that those organisms are considerably more sensitive, about 1-2 orders of magnitude more sensitive than are fish to silver. So I agree that these organisms need to be looked at in more detail because the change in the species composition might have significant impact on animal communities, because the animals are very selective in what they ingest and digest. So there can be impact on animal communities indirectly by impacting one food chain. And finally, the 2 µg/L criterion for seawater may be fine for fish since it is at or exceeding levels that are toxic to some of the microorganisms. However, I don't believe 2 µg/L is ever seen in natural marine waters. I think concentrations of silver in natural waters, even fairly contaminated waters, are typically well below that.

ROMNEY: Thank you, Nick. What I'd like to do now is to open up the floor for questions to the panelists and we have two mikes here so please stand behind the mikes and with your questions, please state your name and your affiliation.

KEN ROBILLARD (Eastman Kodak Co.): I guess I'll phrase this as a challenge which any and all of you are welcome to comment on. We learned a lot over the last couple of years. We may never know everything there is to know about the chemistry and environmental toxicology of silver. But really, what do we have to do, in terms of protecting the water column and the organisms that dwell in the water column? When we begin to understand that the concentrations of silver are in a few ng/L and the amount of dissolved silver, which we believe approximates the active form, the biologically active form, is substantially lower than that, are we not climbing a hill that we maybe don't really need to climb at this time? Are the measures that we're currently taking in trying to control releases of silver sufficient, and that additional knowledge and additional engineering or technology won't really add any environmental protection?

CAPPEL: I'm not going to answer that directly but just say that's a very important issue, I think, that Ken brought up. We do have limited resources and we want to put those into the most appropriate studies. Clearly, it seems to me from some of the studies that have been done and looked at the fate of silver that's discharged both in fresh and marine systems that it does not reside for very long, or in very high concentrations in the water column. So I think our emphasis on research now needs to be focused on sediment. I think silver is a sediment issue and some questions that have been raised — are the sediments a source for the release of silver back into the water column or into pore waters? Is silver toxic in sediments to the benthic organisms? Is there transfer in trophic levels or food chain, bottom part of the food chain? So I think those are the most important places to put our research direction in the near future. Clearly, we do have limited resources and we want to put them in the most appropriate places for environmental regulation and protection.

SODERGREN: As long as we haven't the long-term studies proving the fact that silver is not that toxic as you stated, I think the question is difficult to prove.

RON EISLER (National Biological Service): I appreciate the effort that's gone into the marine environment, and generally, the aquatic environment. It seems that all of you gentlemen are representing that phase. But I find it unusual that there isn't a single study on silver fate and effects on avian or mammalian wildlife. There have been some studies on poultry, there have been some on small laboratory mammals and some livestock but nothing on representative species of seabirds or pasturines or any avian wildlife or any mammalian wildlife. How do you, this panel, feel about the priority of studies such as these?

FISHER: I think that's an excellent point. We certainly know that certain birds, for example, diving ducks, feed on clams, and clams, we know, can concentrate silver or any other metal very appreciatively. I agree, I've seen virtually no studies on this. And we know that silver is very toxic because of its strong affinity towards sulfido groups, with affiliation to enzymes and proteins. And because birds will dive down and eat either clams or another mollusk and fly away, they don't even have the chance to depurate in the same way that a fish might. There is every reason to expect that concentrations of food build up to sufficiently high levels in sea birds that may have dangerous impacts, but I've seen those studies nowhere.

SODERGREN: I think this panel reflects the situation in the water today. If you look at the ecotoxicology or at the environmental toxicologists, most of them are dealing with water, with the aquatic system, and these missing results from silver in the wildlife is not unique. This is equally true, we find, for many other metals and environmental studies. The aquatic area is much more studied.

SANTSCHI: I just want to respond to that. I don't want to defend the reason for it, I want to try to explain the situation in terms of what we know. As far as I know the higher the organism the better it can manage toxic compounds and excrete and detox its body. For example, you can biodegrade pills — you take 1 mg of copper today and that's okay. But if you are phytoplankton, 1 mg/L kills you. I mean just as a general rule, so I guess the feeling is that aquatic organisms are more harmed at lower concentrations. Well, I'm not an ecotoxicologist and I would like to know if this is actually right or not.

CHRISTER HOGSTRAND (Univ. of Kentucky): I don't have a question, I just have a comment on the previous comment here about birds and mammals and effects of silver on them. Actually, I'm not aware of any such studies but we're talking about all levels, and I just wanted to bring attention to a recent paper on Beluga whales from Alaska showing that — I could be wrong with the numbers — but the silver accumulation in liver, which is, supposedly, the primary accumulatory organ, is in the range of 100 mg/g wet weight and that is some 300 times more than in Beluga whales that were captured on other sites. Apparently mammals may also accumulate silver.

ARUN MUKHERJEE (Univ. of Helsinki): We heard quite a lot of different topics from the panels and from morning to evening we heard about fish, water toxicity, silver ion and so on. But, actually, when we think the ore materials which we are using to produce metal, in this ore materials silver stays as a trace constituent. I think we are using

more different kinds of raw materials than sensitized materials like photographic products but we are talking all the time about films, photo processing industries, or jewelry waste or electronic waste. We are not going to do anything about the raw materials, like coal, where silver is a trace constituent. So what happens with this silver when at high temperature it goes out, and in which form it goes out, we have done very few experiments to get knowledge about it.

In the 1980s, USEPA wrote some documents on this subject, and after 14 years there is no paper in which form this element goes out and if it goes out where it goes, it goes from the atmosphere to the land or water or to where? How much of it goes to the land, how much to the soil and how much to the other places? I think we do not have any such type of balance like mercury. We know how much mercury goes to the land, how much mercury goes to the ocean, how much mercury goes to the diverse seas. I think it is a high time for us to think about it and do something about silver in the atmosphere, not only in the aquatic environment. I think this panel should think about it that we already discussed about the aquatic environment for the whole conference. There must be some levels also in the atmosphere. Thank you very much.

ROMNEY: Thank you. Would anybody like to comment on that?

CAPPEL: I agree, I haven't seen much analytical data on silver in the atmosphere. The best were done by Davies et al., in the late '70s looking at cloud nucleation, where they used silver iodide for cloud-seeding experiments. And there was quite a bit of work done at that time with some other nucleating agents to see if there was any harmful impact on ecological systems.

But, again, all the work was done in the aquatic environment, and the results of that indicated that there were no apparent adverse effects in the higher order ecological systems. But I don't know how much silver is actually in the atmosphere today, what is transported from incinerators. We do know the main fractions for uses of silver, the most of it is used as a solid, either silver oxide or silver metal. Most of that, at least in the U.S., probably ends up in landfills, and we don't believe silver is very mobile in soils so we don't think there is potential biological impact from that. That's as much as I know about it. I have not seen measurements on silver in the atmosphere or its transport using common techniques.

ARMSTRONG: I have to make one brief comment on that. In the work that we've been doing on the Great Lakes, we're trying to construct a mass balance for silver using recent data available on deposition of silver. Some of them are by Anders Andren, so he might comment on that as he's standing at the microphone there, but when we put that together with the input from major rivers to Lake Michigan it wasn't probably surprising that the atmosphere is a bigger source of total silver that is going into the lake which is rich in sources. The average surface area and generally atmospheric processes are very important so more silver is going into the lake from the atmosphere than from surface waters. We're going to talk about this in a paper that's being presented tomorrow. It has a very short residence time in the lake as well and we're facing a standard problem here. But the atmospheric deposition is estimated, in part, in the work that was done from the composition of particulate material that was falling into the lake from the atmosphere.

WOOD: I've just been thinking quietly about Ken Robillard's questions. I thought I'd come back just to pick up on that. Basically, this question was, "Have we got enough, do we know enough about silver now to change regulations?" My comment is double: We need to know more. If you do a literature search for physiological toxicology, say for silver versus copper, you'll come up with maybe one percent of the scenarios for silver as you will for copper. We're kidding ourselves when we say we know everything about how silver behaves in the water column and how it's toxic to organisms. We do need to know more. I won't think of starting to say no, we know enough, we can change things right then and there. But I think we know enough to know what we do right now in terms of regulation is not adequately good and the regulations can't be approved. Same point I would like to point

out as has been said by a number of people here is that we are now sure that valuable information is in the sediments, like bioamplification and movement to the food chain, all these things are now things that should be considered in research programs, and that what is really needed is funding in that area.

ANDERS ANDREN (Univ. of Wisconsin): Many states based their discharge limits on pipe concentration, that is, concentrations in the pipe. Some states allow mixing zones. What if I could elicit some response from the panelists? What do you think about this practice? We all know that somebody like Di Toro says, "there are no fish in pipes, why do you have to use concentrations for the pipes. It's kind of meaningless." But there are some, however, who are very strict about it. So can I, perhaps, have some reaction as to the practice of using, or not using mixing zones?

CAPPEL: Clearly, some of the mixing zone issues may not make a lot of sense, but usually they start with some dilution factor which takes some conservative flow of the receiving water and the designed flow of the treatment plant and comes up with a dilution factor. If you're in an area of the U.S., like the Southwest where many of the rivers are in severe need of irrigation, you don't get a mixing zone and so you have the more stringent permit limits to meet for water quality standards in areas of limited water. So that's been a great concern to the regulatory community and to a number of sewage treatment plants.

As far as we're concerned, I think the mixing zones are appropriate. I think there are areas of initial discharge where it's safe to even exceed acute criteria as long as you can show that the effluent is not acutely toxic to organisms or passing through the initial zone of discharge. Then there is an area where the acute standards clearly should be met and the chronic standards clearly should apply in the wider range of the terminal water body where reproduction takes place. So, certainly from the regulatory community, we support the mixing zone concept and we think there are some applications that probably should be made from the regulatory standpoint, but they may be more conservative than they need to be for full protection of the aquatic system.

CRECELIUS: I'll comment on that. One of these things that we heard today is importance of DOC, for example, and how that seems to detoxify silver. I think that's important as in the case for cadmium that has a hardness relationship in freshwaters. What we realize is that silver is extremely reactive in the natural waters, there are materials there that obviously detox it to quite some extent, and we should take that into account when setting the standards.

There's no point in taking the in-pipe concentration and then relate that to toxicity in the environment when we know that it's immediately going to be changing its chemical form in the dilution zone, as a matter of hardness, chloride or sulfur compounds, things we don't understand yet. So I think, rather, we should start to understand better this rapid geochemistry, geochemical changes, speciation and so on in the mixing zone and this ought to be applied in regulations. Otherwise, it would well be wasting money, spending more money on treatment in the ecosystems that apparently readily detoxify or reduce the toxic or bioavailable forms of silver.

WOOD: I think I'll quite agree with what Eric said and I think the mixing zone is a very useful concept. For once, I say Di Toro was right, don't try to protect here and help the fish in pipes. I think there are going to be certain instances, however, where total loading should be something that is looked at, loading to the environment. I'm not sure everywhere it's going to be important, but if, in fact, it turns out that the sediment is a limiting factor, that that's the source of the food exposure to higher organisms, then I think we have to work out kind of quickly what the total load discharged into the environment is, rather than the concentration in the particular effluent. And that may be considered in the future years.

GEORGE HELZ (Univ. of Maryland): Another question if we are ready for a new issue. I just wanted to return to a point that Anders made in his talk this morning about how readily silver is reduced to the zero-valent form. I asked him, and I'd like to sort of ask the larger audience and panel, whether we really know very well what the lifetime of

zero-valent silver will be in the environment. If it's longer than a few days then I would suggest that this would be quite important in the biological behavior of silver, for example, and particularly in some of these biological tests, toxicity tests in seawater. Factors such as the illumination of the experiment might be a significant variable in the experiment and we haven't heard much about this type of thing. I was wondering if anybody can shed some light on this. Obviously, if zero-valent silver is very quickly reoxidized then this is not an issue.

FISHER: I think that's a point worth thinking of. I'm sure that there have been studies to look at the longevity of the zero-charged silver, at least in the marine environment, but certainly we know that zero-charged mercurochloride has unique biological features and can cross biological membranes. It is lipopermeable in a way that ionic mercury is not, and I would wonder whether or not zero-charged chlorocomplexes of silver, for example, may behave similarly. There are some allusions to that in the literature but really very little rigorous testing. I think that's an example of a point that I was trying to make earlier, that the speciation studies ought to be related to their interaction with aquatic organisms.

GEORGE HELZ (Univ. of Maryland): Actually, you made an interesting point but it's not the one I was trying to make. $AgCl^{\circ}$, yes, and the analogy you brought, $Hg_2Cl_2^{\circ}$, is certainly one that could be very interesting, but I was thinking of atomic, metallic silver which would have analogy to metallic or atomic mercury. Which, as we know, is very important in terms of the environmental behavior of mercury. And the elemental mercury does persist for an appreciable lifetime to the environment and, therefore, has a significant influence on the behavior of mercury in the environment. Is there an analogy in silver?

NORMAN NEWMAN (3M): May I comment on this? I'm not anywhere involved — my background is actually photographic silver halide chemistry. In that regard, we deal with the singular silver zero-valent state quite a bit. Singular zero-valent silver is extremely reactive. It will react in the presence of simple chlorine atoms or bromine atoms, anything, sulfur, free sulfur, any of these free elements in very low concentrations are sufficient to oxidize silver atoms. Taking an analogy from the formation of the latent image of the photographic process, stability comes when you get to a cluster of about 3-4 silver atoms, so unless you have a cluster of Ag(0) of 3-4 atoms, you have an extremely reactive species. Once you get to a cluster of 3-4 atoms then you have molecular stabilization and the 4-atom state will persist. Based on the physics of that, which I don't think will change much in the environment, it's really less destabling than is a photographic image. So I hope that answers part of your question.

KOSTAS DASKALAKIS (NOAA): I'd like to get back to the bioavailability of silver. Not as silver in the water column, but as silver in the organisms, and let me explain something: I basically work with mollusks so these animals tend to accumulate a lot of silver. It seems to me that the silver they are accumulating doesn't bother them. Some analyses that they did recently gave me probably 50 ppm of silver. Now we know that silver may precipitate as Ag_2S or some type of organic silver. Do you have any comments on how we can test for the silver in the organism and how can we figure out if that will cause any problems? It really doesn't matter how much is in the water column or how much total silver we have in the biological system, but how much of that is active.

FISHER: I can make a few comments on that. Certainly many organisms, including most of marine animals, can produce metallothianines or comparable sources of proteins. Certain metals essentially are there to regulate metal concentrations in the organisms, and silver would be one such metal that it would be regulated by metallothianines because of its affinity for sulfido groups. The organisms that have the ability to produce large quantities of metallothianines can essentially detoxify or sequester the silver to an extent that it's not having an impact on that organism. Similarly, other organisms can detoxify metals by precipitating metal out as a metal sulfide within the organism, so if you measure the metal in the organism you think, "how can this organism be alive?" but, in fact, the organism doesn't even know it's there. It's precipitated out as the sulfide. That has been shown with diverse plants and invertebrates though I'm not sure it's been shown with mollusks, either. And we also know that organisms can develop, there can be genetic strains of organisms which can develop persistence when exposed over long periods of time to high concentrations of metals or other contaminants.

So if they don't initially have the ability, they develop the ability to detoxify metals either by sequestering them in a nontoxic form or regulating them through protein such as metallothianine. I am less concerned myself about the high levels of silver in oysters, for example, in terms of the impact of the silver on the oyster, than I am on the animals including people that eat the oysters. So it may well be that the oyster itself is immune to high concentrations of silver in contaminated areas, but a bird, a mammal, including men, might eat that and they may not have that immunity. Personally, I am more concerned over public health issues than I am over the impact of the silver on the organism itself. At least in a case like bivalves.

KOSTAS DASKALAKIS (NOAA): Maybe that's not the case because you have basically hydrochloric acid in your stomach and now this silver sulfide will have to go through that hydrochloric acid. I don't think you can get much out of this.

FISHER: I'm not aware of studies — I'm sure they exist — of how much silver, for example, we assimilate in food that we eat. I presume that information is known but I'm just not aware of this. I guess I would be more concerned about people consuming very contaminated seafood than I would be on, in some cases, the health of the organisms themselves that were eaten.

CAPPEL: Daland, you want to address that?

DALAND JUBERG (Eastman Kodak Co.): Go ahead, Bob.

CAPPEL: I think that Daland will talk a little bit about that tomorrow. I'm not aware of many studies that indicated silver accumulation argyria from ingestion of compounds or food that contained silver. At least in most cases I'm aware of, argyria seems to be related to inhalation of silver vapors or injection of soluble silver complexes directly into the blood stream. I think it depends on where that silver ends up in the particular organ. At least in men, argyria does not appear to impair organ function in any way, which has led the medical community to classify argyria as a cosmetic effect and not a health effect, and which also support the EPA evaluation of the drinking water standards for silver in 1991. I think on shellfish, again, it depends on where that silver is accumulating. We've been aware for a number of years that shellfish, particularly those around the outfalls of sewage treatment plants, do accumulate some fairly high body burdens of silver and that it's not apparent that this accumulation is resolving in toxicity. But we haven't looked at it to see whether that silver sum is associated with the gut or the gut lining or whether it is getting to some of the other tissues like the reproductive tissues, and whether we see any effect on reproduction. Actually, some of the studies are now involved in looking at that. We're trying to answer that question, whether accumulation is resolving in toxicity or not.

DALAND JUBERG (Eastman Kodak Co.): A couple of comments or, rather, two questions and a comment. Nick, I, hopefully, will be able to provide you with some commentary on the human health significance of silver tomorrow and if those comments don't address your concern please raise it again. Two questions, one for Nick and one for Chris. Nick, you are known for using body burden levels versus water concentrations in terms of regulatory effort. Would you suggest or are you proposing to use adverse effect levels? What was the body burden associated with, an adverse effect level or what — could you comment on that?

FISHER: Yes, an adverse effect level.

DALAND JUBERG (Eastman Kodak Co.): Okay. Secondly, Chris, a question for you. You're proposing that we still need more studies on silver. Would you extrapolate that to say — that goes for Dr. Eisler's comment also — I would wager then for every clam, oyster, mussel, and other aquatic organisms and species out there, that I not only have silver in them but a number of other metals. EPA addressed the issue of mixtures for quite a long time but I think we need a plot before we start doing single compound studies on a number of other species in terms of

what is the relevance of these studies to the real world. Would you advocate looking also at complex mixtures? And not just silver, in terms of relevance to the real world. What is the significance of eating a clam to a bird when, in fact, it has probably got a number of heavy metals in it?

WOOD: Yes, I think that's a good point, Daland. When you actually look at the real world situation we could be very hard pressed to find any discharge site where it is silver alone that is the issue. In fact, if silver is an issue, it's an issue along with other metals. The real problem, however, is that those metal mixture studies either for laboratory or water body exposure are very, very difficult to interpret. And regulations in the end are very difficult to write in terms of multiple exposures. So, what you say directly is true in practical sense but it's going to be very difficult to do it experimentally. The studies that have been done for a couple of mixtures in the past were not all that successful. I have some doubt that we will be able to rephrase regulations in terms of mixtures rather than single toxicants.

ROMNEY: We have time for two more comments or questions.

CHRISTER HOGSTRAND (Univ. of Kentucky): I'm just reflecting — that might be total ignorance from me. The fact that oysters and other bivalves do accumulate a lot of silver and, specifically, that this does not affect the individuals — but so far I've never seen any studies, physiological or biochemical studies on the effects of silver on these animals. I don't doubt them, it's just that I haven't seen them.

GEORGE COBB (Clemson Univ.): Looking at the periodicity of silver and the apparent uptake in bivalves — does anyone have any idea if some of these bivalves use copper as their means of oxygen transport, and does that have any relevance for the uptake mechanism or the accumulation in those organisms?

FISHER: Probably it does. The animals that do use copper also tend to concentrate zinc and silver, I believe, to higher levels than other organisms.

ROMNEY: Does anyone else want to comment on that? (No additional comments.) I'd like to thank our panelists and we all should give them a round of applause to thank them. I'd like to say good-night and I'd like to turn it over to Tom who might have some parting words for you this evening. Thank you.

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Panel Discussion

Regulation of Silver in Sediments

Moderator:
M. Reiley, U.S. Environmental Protection Agency, Washington D.C.

Panelists:
D. Di Toro, Manhattan College
D. Hansen, U.S. Environmental Protection Agency, Narragansett, R.I.
J. Kramer, McMaster University
K. Robillard, Eastman Kodak Company
J. Rodgers, Jr., University of Mississippi
A. Tessier, Universite du Quebec

Panel Discussion: Regulation of Silver in Sediments

Panel Chair: **Mary Reiley**
Panel Members: **Dominic M. Di Toro**
James R. Kramer
Kenneth A. Robillard
John H. Rodgers, Jr.
Andre Tessier

REILEY: Let me start to talk about who I am first, since I looked around and noticed that I'm one of the few EPA people here. My name is Mary Reiley. I'm the coordinator for the sediment contaminant bioavailability program. Our primary product is the sediment quality criteria that I'll show you quite a bit about. I've been with the EPA for 10 years. I started out in the MPS enforcement program, spent the first seven years there and I've been in the sediment program for the last three. My educational background is a master's in environmental biology. I'm going to give each one on the panel, including myself, a couple of minutes to give our thoughts on the regulation of silver, or metals for that matter, in sediments and then open it up for discussion. I encourage all of you to ask questions and make comments not only to the panel, but if you have questions about what's going on at EPA please feel free to ask me as well.

So with that, I take the moderator's prerogative to do my spiel first so that I don't get lost in the science that's going on, on the other side of the room. A couple of things that I want to tell you about have happened recently, in January. The research team that is conducting the investigations for development of the sediment quality criteria went to the Science Advisory Board at EPA, which is a group made of academics, scientists also from industry and multiple fellow agencies. We took to them an approach for assessing bioavailable metals in sediments. Just about one and a half months ago we received back from them their draft review, and the essence of that review was a very strong pat on the back for the science that was conducted, that they thought we had significantly reduced the uncertainties around the sediment assessment methods for metals by using the AVS and SCL approach, of which Dominic had talked about earlier and which a lot of other people have discussed this afternoon. They also stated that for a lot of applications that knowledge is certainly ready for use. They also gave us ideas about what types of research they'd like us to do to further our progress in that. Particularly in the area of researching the chronic effects of metal contamination in sediments and the potential for metals to bioaccumulate, using that as an indicator of bioavailability.

So we're going to spend about the next two years or so following up on those recommendations, conducting the research as necessary in order to answer their questions, further reduce the uncertainties, and also to spend some time trying to figure out how to interweave a sediment metal approach with a water column approach so we can deal with the entire fate and transport of metals at one time, rather than continuing kind of that traditional "more piecemeal" approach. A couple of the other things that I'm going to give you some ideas about is where we stand on what is necessary and why we should do the sediment criteria. It's very important to remember that the sediment quality criteria that we're developing are specifically to be used in a regulatory environment, particularly in the NPDES program, which is the National Pollutant Discharge Elimination System, in case you're unfamiliar with that abbreviation. In particular, that means that we have to be able to deal with a chemical specific number that deals with causality. That when you say that the sediment is toxic because of silver, in this case, when you do the evaluation it actually was toxic due to silver and that you can trace that silver back to a source, either point or nonpoint that can be controlled. We also had to find a way to make that number applicable to a wide variety of

sediments. We didn't want to have to come up with a new number every time we dealt with a different site. And that's why we spent so much time working on the bioavailability and how that can accommodate a variety of different sediments. We also needed a way in which the numbers could be easily expressed and easily understood. That's particularly the reason why we tried to stay away from interstitial water concentrations, which are not so easily sampled, and dealt in the solid phase of the sediments, which are more easily sampled but also more easily destroyed.

We also thought that we needed to start at the beginning, we needed to start with impacts to benthic organisms. And as we learned how to do that we then learned how to deal with transfer of that contamination through benthic organisms to the higher food chain. So bioaccumulation is something that we're just now starting to consider and starting to do research on. We needed to have a criterion that would give us a protection level for most species most of the time — that's pretty much the requirement of our regulation system, to protect 95 percent of tested species. So again, we're trying to pursue that level of protection. For our own satisfaction, or satisfaction of the user community, we needed to make sure that we used an established proven analytical method and that we could take our prediction and have our prediction equal a toxicity test result, what we call a "checking" experiment. And that we address the uncertainties and the differences between what we are predicting and what we are actually seeing, and minimize those uncertainties or find a way to incorporate them into the criteria before they're applied. And finally, we needed a procedure that would allow us to take this nationally applicable approach and give us some site specificity if it was needed, if there was a species of significant concern there or you had an environment that was atypical of the areas which we've studied for establishing the criteria in the first place. And with that, looking at the background and where we came from and what's happening right now in the metals arena — here I'll close up my portion and move on to the fellows' position on regulation of silver or metals in the sediments, and I'm looking forward to some discussion. We'll start with Jim — will you introduce yourself and give us your thoughts?

KRAMER: Okay, I have a list of about six or seven items, and I'll state them in about one sentence and try to be brief. I think in the last couple of years we've learned a lot of things about silver and it seems to matter how you look at it, it comes up with different slight modifications. There are some truths about silver, the business of the forming of sulfides within many sediments. Certainly in many sediments you have AVS, the concentration very largely focusing on silver in the particulate and sediment area. So it seems to me that's the area, that in terms of regulation, we want to make sure we know quite clearly, and make sure that any variables that we have looked at in our simplified lab experiments have been covered. So I should just come to the point here: I think it is important to look at it from the sediment point of view. With regard to recycling, I think the issue is the form of the sulfide — we always mention the AVS, but I don't think it's that easy. I think silver sulfide is very unique; it crystallizes as an acanthite very quickly, and that's totally atypical of all the other metals. So I think we have to know about silver sulfide specifically in context to this. That's point number one. And the whole business of biogenesis and biostability, if there is such a word.

The second point is the synergism or covariance of other metals. A lot of people have brought up other metals, but we sort of looked at them separate from silver. As you may remember, last year the study from the UK showed very strong covariance of silver and copper in sediments and in organisms. There's a lot of crystal chemistry and so I would suggest other combinations. But I think it's important that we look at silver in context of the metals and analyze this clearly in a clever experimental set up.

The third point is the whole question of oxidation. This will take some clever experiments, too. Certainly this involved the variable characteristics of sediments; whether there are biomass solids or whatever you may want to check, like, what is the form of silver and can you mobilize it and reintroduce it back into the system? So, certainly, that is an important situation. We haven't really talked about organosulfides. I know a lot of people who are looking at that, and that gets into other sulfur ligands as well as solids. George Helz mentioned the polysulfides. AVS is too coarse. It may work a lot of the time, but I think scientifically we want to understand this huge amount of sulfur

compounds. It's all at quite high concentrations so I think the other sulfides are important, particularly in that area. And the final point is, and Andre Tessier brought this up: I think now we need to really try to think of clever field experiments, and I emphasize lab experiments, not sampling and analyses, but doing clever things like adding different kinds of sediments, looking at diffusion and so on. I think Eric showed some of his studies were just inching along that way.

REILEY: Okay. Andre?

TESSIER: I am a chemist by background but I have done a little geochemistry and a bit of biology to be able to survive with the biologists. Personally, I don't have much to say because we have other criteria and have not worked with silver so far. But I would like to tell you what I understand of the problems. The metals accumulate in sediments, they constitute a potential danger to the aquatic organisms or the benthics. There are surely immediate actions that are possible. You can think of covering the sediment to remove, to protect. You can think of instituting chemical treatment of the region, or simply reduce the discharge and let the natural processes decrease the contamination. Each of these immediate actions is costly in terms of money and sometimes socially also.
So I think we need to look a little further, we need to be able to predict the existing danger for the organisms and how the remedial actions can ameliorate the situation. Otherwise the risks are either to under protect the organisms, or overprotect the organisms and then waste money. The tools we have presently, the ones we know at least, are laboratory tests or bioassays. But for each of these tests or bioassays with sediments I can see problems. The main one is that when you remove a sediment from the environment you immediately change the sediment; you cannot avoid that. So I think the organisms that are put in the presence of this sediment are not in the same conditions as they would be in the field.

So the conclusions that can be drawn from such experiments might be dangerous. There are all kinds of these experiments. There was one I have heard about, not here but in other meetings, which is the exposition to pore water. They extract the pore water and want to expose the organisms to the pore water. So there are at least two problems: You can try to oxidize the pore water in order to keep your organisms alive, and then you change completely the pore water when you do that. The other action is to try and keep the pore water anoxic but then your organisms will asphyxiate. So I don't know what kind of information you can get from such bioassays.

The next tool they seize upon is field measurement or monitoring. With this, you will measure concentrations in organisms or in sediment or in water, but if you don't have a model to allow you to predict something it wouldn't be very useful. So I think that the way we have to be able to get some predictive power is to get a better understanding of the biological and geochemical processes that are involved, and after that, to incorporate this information into models. It will be useful to predict the biological impacts. I think it is the only way, at least in the mid-term or long-term.

Before I finish I would like to give you a question that I think is very important. I think it's important presently to perform some work on the whole area of exposure of trace metals. We should ask ourselves, can the organisms see the interstitial waters or the anoxic sediments, or are they mostly exposed to the overlaying oxic layer of the sediments? Even if some of the benthic organisms live deeper in the sediments, they usually make cohabitats that do not always resemble the average sediment composition. So I think we should try to answer this question.

REILEY: Okay. John?

RODGERS: Well, for quite a while I was trying to anticipate what the agency's priorities would be. It sounds like you didn't notice, in terms of what I was thinking of, the issues relative to bioaccumulation and then chronic or long-term exposures or actually long-term type experiments where fluxes are issues. But this kind of information is hard to come by, so I'd say they'd take a little while longer. I guess I would have three other things that kind of

stick in my mind in terms of things that need some attention regarding metals, not just silver itself because I would say silver isn't the only thing that we think about. One thing is, there needs to be some sort of system by which we can prioritize and focus concern. If materials or elements or sites are not of concern we need to be able to say that, and if they are of concern we need to be able to say that and we need to have criteria by which we can judge that. I don't just mean water quality criteria or sediment quality criteria. It has to do with the mass level and the size of the issue and so on and so on. And I don't think that's an impossible task.

The second thing I see as I go out in the countryside, is that we need some outreach, the information needs to get out into the heartland and I don't find that. For example, the information we exchange here or at other meetings of this sort gets out very well. You're content to write it into documents or scientific publications, but I guess it needs to be in some more, perhaps, digestible form — spoken as a biologist surrounded by chemists.

The third item would be that I would hate to see us ignore the biocriteria and some of the site-specific guidance that we've learned and developed for the water quality criteria. I think the sediment quality criteria development can learn a lot by the trials and tribulations of water quality criteria and get ultimately developed into some criteria that we live with at a national level. Right now, those biocriteria that stick in my mind, we have some bioaccumulative tests, we have some field tests and there have been some more fields for field testing, I think that too is very important. We've got other things like in situ incubations and so on. So those are the three things that stick in my mind right now.

REILEY: Okay. Ken, you're on.

ROBILLARD: My name is Ken Robillard. I say that because I haven't really had a chance to meet and talk with everyone out there and I'm not that well-known like the other members of the panel here — in fact, I feel rather humble when I think of their credentials. I'd say that I was here to provide, perhaps, some comic relief — but Dominic does such a great job on that. (laughter). I work for Eastman Kodak Company and maybe I'm here to provide a little bit different viewpoint on things, and that's what I'm trying to do right now. On the issue of regulation of metals, particularly silver — in fact I think it's exclusively silver in sediments — I'd like to share a couple of thoughts with you, coming from a bit of a different direction.

During the time I've worked with Kodak, I witnessed a tremendous amount of energy, a tremendous amount of money put into the development of recovery and reuse procedures for silver, reducing the amount of silver that is used in sensitized products, all with the intention, at least in part, of reducing the releases of silver to the environment. We made tremendous progress over the past couple of dozen years in that area, but we will continue to make progress in the years to come. We're witnessing decreases of silver input into the environment. I think we've seen some of that in the data presented today and yesterday and the day before. We know that the silver, the small amount of silver that is released into the environment, we have a much better understanding of what's happening with that silver now.

All in all, it prompts me to ask a question: Do we have a problem? And I guess from my point, I'm not aware that we have a problem. I don't think that anybody has shown definitively that we have a problem in this case. Certainly not of the nature that would require a substantial regulatory effort. And I would go further and say that in terms of utilizing our resources, that applying our resources to continuation of reduced usage of silver, better technologies and treatments of wastewater and reuse and recovery will probably have a much greater impact on environmental quality with regard to silver than will a series of regulations.

The other thing I wanted to mention was, as I listened to the talks, that many of them included a wish list of additional information and I think that's really very good. It shows the scientific inquisitiveness of the people who are working with silver as well as this area in general. Quite probably, a couple of hundred years from now, not we

but others will be studying the environment and processes which take place in it and have their wish list as well. I think we do a good job. Actually, from what I've heard the last three days, we do an absolutely great job designing state-of-the-art experiments. And I compliment all of the people who have shared their thoughts with us on the fine work that they've done.

But I am concerned that while we do pursue and put a lot of energy into pursuing that knowledge, we're not necessarily putting as much energy as we should into trying to develop and utilize that knowledge. We're accumulating information but I'm not sure that we're really developing our ability to put it to use. I remember seeing a sign in some locations, an old sign to the effect that "the purpose of life is not to accumulate knowledge, it's to use it." And I think that we may end up with volumes and volumes and volumes of information but we may not be any further along on how to turn that information into actual decisions and to actions. So I kind of hope there is a challenge that when you design an experiment, you may also want to try and determine exactly how the information is going to be used and present that along with the experimental design.

REILEY: And Dominic.

DI TORO: I'm Dominic Di Toro of the comic relief. (laughter). I'm going to tell you a couple of stories. About 10 years ago we had a conference in Washington and it was the first meeting, really, where EPA decided that they were going to get engaged in sediment quality criteria. The room was filled with, I'd say, one-third biologists and toxicology types, and perhaps one-third chemists and perhaps one-third regulatory, EPA-type people. There was a normal type of squabbling going on about how to do this problem and what we're going to do and an awful lot of whining about how we don't understand what's happening and so on and so forth. And Pat Togan, who was then the director of the Criteria and Standards Division, got up, I guess at the beginning of the second day, and said, "Look, if a few people don't come up with something, I will come up with sediment quality criteria." Pat Togan didn't know anything about science — he was a regulatory guy — or if he did he'd forgotten it. The point was that the regulatory people have to make decisions. They don't have the prerogative, they can't check either column A or column B or column C and wait a while until they understand this better. The art of regulatory science is quite different than the art of academic science or industrial science for that matter. For regulatory science, it's a very hard kind of science because regulatory science has to eventually come down with some sort of workable way of doing a problem that isn't completely embarrassing. That's usually the situation that you're in.

Now, if you take a hard look at some of the regulatory stuff that's out there, I must say a fair quantity of it is embarrassing and there are regulations that make no apparent sense. Why should we clean up a site for eight years so that someone can drink the water, an industrially contaminated site. One wonders where do these things come from. Well, the problem is that when you're on the regulatory side of the business you have to make a decision. And in the lack of solid information or at least semi-solid information on things like risks and effects, if you have to make the decision, what you end up doing is being extremely conservative. That's why regulations always come out conservative, because it's very hard to answer the questions of how things get clean, what is a good way to make regulations in terms of environmental problems across the board. And sediments and metals are no different.

The difference, I think, that is at least currently the most important is something of a change you see at EPA — if EPA, you know, survives the next six months. There is a movement inside the agency that I can detect which had not been there, I would say, 10 years ago, which is much more for concern to try and get the science done. You will never know enough. Down to this and I go down diligently to what all my colleagues laid out, a lot of it is absolutely important, some of it is curiosity driven, some of it caused by another problem. We will never know enough to get it "right." About all you can ever hope to do is get it sort of right most of the time and not be embarrassed. That's about it. Anyway, there is certainly the lottery of wondering about, like, "Gee, I wonder if, you know, some third order or second order or maybe even first order thing is important." But the truth of the problem

is that in the regulatory world you have to be able to make reasonable judgments with some degree of dispatch and with some speed. Otherwise what happens is that bad regulations drive out reasonable regulations. You can't settle down and count on a meeting of the minds who then apply the science that's more or less understood. Come up with a reasonable regulation and what will happen, I assure you, is that Pat Togan will make the decision. And you really don't want that to happen.

So, with that little sermon about regulatory science, I think the issue before us is, what we have got to deal with that is really first order, that we have to get sorted out before we cannot be embarrassed by regulations in sediment for silver or the other metals or, conversely, do we know "nothing?" Are we far enough along so we think we can make reasonable regulations that are better than just the stuff that's out there now, which is pretty much embarrassing? How's that contribution for you?

REILEY: Okay, Dominic is brief. What I would like to do is open it up to questions or comments or to discussion, either from you who have been listening to us lecturing for a while, or between yourselves that are sitting up there on the panel and have questions for each other. So, do I have anybody who wants to take the gun?

DI TORO: I have a question to ask my colleagues. Do you think we know enough to take a correct action at establishing criteria for the metals, the five metals you know, what the situation is for silver in sediments. Do we know enough now to do something sensible, yes or no?

ROBILLARD: Can I answer that question? Just one little part.

REILEY: Simon says, yes.

ROBILLARD: Can we conclude the negative, can we come up with some idea of a lack of a problem, of what constitutes the lack of a problem as opposed to taking one? It might be easier to prove this violation or effect. Could we take that?

DI TORO: In fact, that's what we are doing with the metals. You know the metal criteria are no-effect criteria. If you're below this, don't worry. So go on, that's, in fact, what I wanted, I'll make that a question. What do you guys think? Or what does anybody think?

KRAMER: Actually, I really had that issue on the top of my sheet but I didn't want to go right into it — they were my personal feelings. Mostly with silver, and the thing at the top I've written down, that it's a nonissue globally, but I'm not convinced that there are not specific areas that we should be concerned about. I'm not convinced that we know enough, and I guess that brought in the next point down there, that we perhaps should look around for worst-case situations. The other area that I was not convinced we know enough about is we have not, at least looking at silver — now maybe on the other metals in other areas this is not true, but at least in the discussion here — we have not integrated the main sources of silver, generally speaking, as a collection of treatment plants, and what is their input into our design of model particles. I know there is a lot of work going on, modeling and so on, minimizing particulates and also metals, but I'm not convinced that we've defined all variables. I think we have much but not all. So those are my comments.

JOHN MAHONY (Manhattan College): If, indeed, as Jim says, that on a global scale silver is not a problem but it may be a problem locally in some places, do we need to find a heavily contaminated site for silver, perhaps to test whether or not we have a serious problem anyway? This is what we did with respect to the other metals, we looked at the "supersites" that can really serve as examples for cadmium and nickel. Are there any sites like that for silver? If not, then maybe we don't have to worry about silver at all. If there are, that's fine, we can do all the studies there.

NORMAN NEWMAN (3M): I kind of agree with your statement and this is addressed to Jim, but first, a comment to Dominic. I don't think you were comic relief before, I think you were dead right-on. Jim, you said you brought up the issue of finding the worst-case site. And I would suggest that we might have heard about one today from Benoit in Connecticut. Here is a site that does satisfy that sort of criteria, about which one can look at what's happening there and come to the conclusions, some set of conclusions, as to whether it will still be there for years or it is not a problem.

REILEY: Anybody else?

BOB CAPPEL (Silver Coalition): I think we now have to start with some criteria. I agree that I think we have some sites that we've identified. I think silver today is largely a point source issue. We know there are a few mining sites where we can go through, and I have just found one site that is tending to be a nonpoint source type of silver discharges to sediments. I think generally the others tend to be point sources. I think the information has shown that silver seems very quickly to settle down in sediments. We know in San Francisco Bay, the sewage treatment plants were largely responsible for a lot of the anthropogenic input of silver. However, as they started to meet the water quality standards, which most treatment plants had not met until after 1991, most of them still don't have silver in their NPDES permits. At least in San Francisco Bay we were able to show that when a treatment plant was meeting the water quality standards, silver was not building up in the sediments and was clearly reduced in the plants to what, at least Sam Luoma feels, are acceptable levels.

So I'd like to encourage folks as they do these studies to look at the point sources of silver coming out, modeling as it goes down a gradient to the sediments and evaluate the biological communities in the sediments, and see whether there are, in fact, any problems and maybe the water quality standards of today are sufficient. We liked the AVS approach. One of the things that I think would concern industry is how that gets put into regulations for permits. Do we have to take the lowest AVS, or the last hundred years for the AVS and a lot of things like that. I think the work is really good that's been going on. We certainly learned, I think, an awful lot about silver in the last three years. I think very soon we will certainly have enough information to pull this thing off again.

ANDERS ANDREN (Univ. of Wisconsin): Do we have enough information to set standards that won't embarrass us? I think that we have information for many systems where we would not be embarrassed. And the reason I say this is that I'm somewhat uncomfortable with having just discharge standards without knowing the mass. I think those have to be tied together; in other words, there might be a Mississippi River that might tolerate a little more, or there might be a small rural stream that has problems. And so, I think that sediment standards have to take this into account, so we have to, somehow, record the bulk concentration. It's very difficult to deal with concentrations, because what do you relate them to? If we do a little better in the art of tying the two together, I think we probably can do a pretty good job these days. To paraphrase the old German philosopher Immanuel Kant — I think he said in his "Critique of Pure Reason" — that it is often necessary to make decisions based on information sufficient for action but insufficient to satisfy the intellect. He knew about that a long time ago. We're still faced with that, and I think we will continue to be faced with setting standards based on information sufficient for action, but we've got to leave it to the academics to satisfy the intellect.

REILEY: I have a quick one of my own. One of the things that struck me that I heard played over and over again, is that there is a real strong discussion about assessment tools and the science that's going on in terms of site assessment and determining whether something is contaminated and how contaminated it is. One of the major sides of regulation is preventing contamination in the first place. And so there needs to be a predictive capacity to that, where you can set a standard that keeps you from being in a position of having to go and do a site assessment for the purpose of remediation. You don't want to find out that there's an effect after the effect has happened, you want to keep from having it happen in the first place.

TOM BOBER (Eastman Kodak Co.): For quite a few years there was a lot of discussion about regional standards or river basin standards, which could be based on the assimilative capacity of the water body, and that idea seems to have sort of gone away over the years. Now everything is treated the same way. And I think in view of all the information that has been developed here today, maybe we're going to bring that philosophy back. Because it seems like, if you have some acidic lake or something which doesn't fit the usual conditions, and then you have another situation where you do have a lot of organic matter which should have more sorption capacity, and you're able to look at how many people are discharging and what is the overall use of the water — that certainly seems to make sense all right.

I don't think it's realistic to say that everything has to be pristine. I don't think all watersheds and lakes ever were pristine, even before man started discharging many of these materials into the environment. We certainly have to pay attention to factors like natural erosion, and volcanic action, and all these other natural events that are happening. I don't think we make the right assessment of what happens in nature, even if man were not contributing to a lot of these. A lot of these laboratory simulations are, in fact, very much worst-case situations, using the most extreme conditions that one could anticipate and then trying to base standards on very extreme conditions. That isn't typical of what really happens in nature.

REILEY: You mentioned, Tom, that you had asked what had happened to assimilative capacity and have we abandoned that concept. And really, the "water folk" haven't. The water quality program works in kind of a nutshell, in that a state would determine the number of water basins and watersheds that it has within its jurisdiction and then by individual watershed do what's called the TMDL, the Total Maximum Daily Load. This is an assessment of all of the known and unknown sources of contaminant to that water body, be it air deposition, be it sediment resuspension, be it an effluent discharge from a point source, be it an agricultural well or an urban overflow or something of that type, and determining how much that water body can accept without exceeding the ambient.

It says, what is the assimilative capacity of that water body to not exceed the ambient condition that the state says is the minimum. And then what is done from there is, we go through and determine how many point sources there are, we take that capacity, the TMDL. And we divide that up amongst all of the discharges, the nonpoint sources, the air deposition, the sediment refluxes, and some level for the site for all the things we don't know about, for the future growth, economic growth of the water body we're talking about, and that's called waste load allocation. This is how much each of those facilities or each of those sources over a long period of time can discharge without exceeding the assimilative capacity, the TMDL, of that water body. And then those have been converted into the permit limits that we see on paper that the discharge or the nonpoint source or the CSO has to meet. So, we haven't abandoned the thought of assimilative capacity, we just call it something different now.

Anybody else who has a question to the panel or myself? Or to each other?

RON EISLER (National Biological Service): In our laboratory, we've been finding that many species of mammalian wildlife and water fowl routinely ingest up to 25 percent of their diet as sediment. We've been finding that water fowl in certain rivers that have been contaminated with lead, are coming down with lead poisoning syndromes, which is unusual. Because up to now we thought that nothing other than a solid lead object, like a shot, could cause it but apparently this is not the case. So, since ingestion of dirt is a way of life for many species of mammalian wildlife and for many species of diving birds, storks, swans, mallards, this may present another way of evaluating sediments, metals in sediments or contaminants in sediments.

Specifically, as I mentioned for silver, there is no controlled laboratory study that I know of today on the effects of silver to any species of mammalian wildlife or to any species of avian wildlife. We have lots of data on background concentrations from field collections, we have substantial data on small laboratory mammals and livestock, and some data on poultry, but nothing on mammalian wildlife and certainly nothing on sediments except what's being developed now at our center as a significant route of contaminant exposure.

REILEY: Your statement is one that won't necessarily surprise me. I know that our own wildlife program that we had running at EPA has been, I wouldn't say, eliminated due to budget constraints, but it's certainly been significantly downsized. It's been primarily merged into our integrated criteria program. It's said with the integrated criteria program that the long-term goal is to be able to address contaminants more holistically rather than the piecemeal of having a water quality criterion, a sediment quality criterion, a human health criterion for every chemical that's out there. It should, rather, be looking at the chemical of concern — in your case you've been talking about lead — then looking at the organism which is most likely to be impacted and then determining what kind of criterion needs to be developed to most appropriately address that concern. That could be a sediment criterion, if you're saying that's 25 percent of their diet, or, on the other hand, you could say it would focus on fish tissues concentration because there are species that get the majority of their diet from eating fish, or from aquatic plants, or whatever it might be. So, hopefully, in the long run maybe we'll be addressing this concern, as right now this is kind of a very beginning concept in our minds. Probably the integration will bring us together.

Anybody else have any more comments or questions before we close for the day?

KRAMER: I have a question. And for whoever knows me this is a very naive question. How does EPA regulate the sediments right now, and what is the basis for the laws?

REILEY: Right now, we hope that the water quality criteria is sufficient; that's the aquatic life criteria. I should hesitate to use the term "water quality" alone. Water quality criteria have five components: they are made up of aquatic life criteria, sediment criteria, wildlife criteria, biocriteria, and human health criteria. So right now we're hoping that the aquatic life criteria are sufficient to protect the sediments, and what we're finding as we pursue the sediment researches is that, in some cases, they are, and in many cases, they aren't. So right now, we're kind of leaving it up to luck.

BOB CAPPEL (Silver Coalition): We've heard over the past three years that on silver there was good science reported, and I just like to encourage all researchers to get their data published in journals because we can often not react to it or use it until it's in a published form. So I'd just like to bring out that plea from the industrial sector.

REILEY: Personal correspondence just doesn't cut it, right?

Anybody else? Then I'll turn it over back to the conference. Okay, ladies and gentlemen, let's give the panel a round of applause.

ANDERS ANDREN (Univ. of Wisconsin): Well, I'd like to thank all the participants and especially I'd like to thank Mary for jumping in and helping us moderate the discussion. I'd also like to thank all of the speakers for an excellent set of talks. I think we're making progress at this every year. I'd like to finally thank the scientific organizing committee, and especially, Tom Bober, he's done a lot of work this year. I told him that if we actually held the conference in Washington this year he had to take a little bit more of the burden off my back as I had some very heavy commitments this past year. Thank you very much, Tom, for a really good job. The cosponsors again have come through. I think we all owe them a lot of respect and, particularly, Bob Cappel for his support. His commitment to providing good science is just exceptional — a big thank you for that. And I'd like to thank Delphine Skinner, my assistant at the University of Wisconsin, who is unbelievable at keeping everybody organized; Gloria Gardner for her diligence in proofreading; and Tina Yao for her outstanding artistic interpretations. I'd like also to encourage all speakers who have not given us the extended abstract to try to get them to us as soon as possible so that we can get the proceedings out in a, perhaps, more timely fashion this year, provided that we can decipher the audio tapes again, that is a major drawback every year.

Tom and I are basically talking about publishing all three proceedings in a quotable form for everybody so that it gets a little more quotable. Though I agree with Bob that we all have to publish as much as we can. Tom has also told you that we've been encouraged to hold the fourth meeting in Madison in 1996, and he also showed you what a great place Madison is. Actually, after the article in USA Today appeared here not too long ago saying that Madison was one of the best places to live in, in the United States, right now I travel around telling everyone that that's an outrageous lie, because we all know what happened to this town in Oregon after it got that distinction about four or five years ago. Everybody moved there and now it's 105 on the list or something like that.

I have actually listed a number of points, which I think are the advances that we've made in this topic and also areas where we have to go. But I want to save those for a flyer that Tom and I will be working on very shortly for next year's conference. What we're going to try to do is capture, use a wider net to figure out, really examine what's happening out in the world, try to get researchers from Japan, Korea, and other nations that have been underrepresented here and then start our planning a little earlier. We're going to lean a little heavier on our scientific organizing committee. And we would also welcome if you could send us an e-mail, either to Tom or me, or a note with ideas about how we can make the conference better, more effective.

I think that, if you remember correctly, my idea and our idea is that there are three main purposes here of what this conference does. They are, number one, for scientists from various sectors to get together and provide us with the latest thinking about the research; secondly, for people of various interests — academia, private, government — to take these ideas and apply them, perhaps, to their own sphere of work; and thirdly, as was demonstrated in the last talk here, to see how we can do a little transfer technology and, perhaps, focus our ideas and how to help regulatory agencies a little better. We think about these primary three purposes for this particular conference, and if you can help us to focus our thinking a little better I'm sure we will appreciate that. In the meantime, I thank you again and I hope to see many of you again next year in Madison. Thank you very much.

ARGENTUM

AN INTERNATIONAL CONFERENCE

Poster Session

Transport, Fate and Effects of Silver in the Environment

Washington D.C.

Influence of Temperature and Thiosulphate on Active and Passive Uptake of Silver by Rainbow Trout

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We are currently using silver as a model metal to better clarify the effects of temperature on active and passive metal uptake by fish. Active metal uptake processes at fish gills are temperature dependent since fish metabolic rate changes directly as temperature is increased or decreased. Passive metal uptake such as diffusion through the gills is mostly temperature independent over the range of temperatures tolerated by trout, since passive uptake depends on absolute temperature, °K, not °C. For example, increasing temperature by 10°C causes a doubling or tripling of metabolic rate and therefore an increase in active metal uptake. However, there is only a small change in diffusive flux because absolute temperature increases by only 4%, therefore diffusive flux also increases by 4%.

An increase in fish metabolic rate causes oxygen consumption to increase, making a fish ventilate more water to compensate for the increased O_2 demand. This brings more water with Ag over the gills which can increase the amount of metal uptake at the gills even by passive diffusion (Roch and Maly, 1979). To differentiate changes in active and passive metal uptake, it is necessary to separate metabolic changes from ventilation changes. This can be done by increasing the amount of O_2 in the water as the temperature is increased, allowing the fish to ventilate the same volume of water to meet its increased O_2 demand.

Rainbow trout (*Oncorhynchus mykiss*, ~250 g) were exposed to a nominal concentration of 0.1 μM Ag (as $AgNO_3$) in synthetic soft water at ~14.6°C and ~18°C for 68 h to determine the effects of temperature on active and passive metal uptake. Control fish were also exposed to the same temperatures. Fish were cannulated via dorsal aorta for repetitive blood sampling to monitor blood PO_2, PCO_2, pH, lactate, plasma Na, Cl, and Ca, plasma glucose, and plasma Ag.

We exposed fish to Ag at ~18°C or to Ag at ~14.6°C. Control fish were exposed to water of similar temperatures with no Ag added. Water pH was between 6.1 and 6.6. Water PO_2 was 100-125 torr and water PCO_2 was below 1.6 torr. Arterial PO_2 ranged from 65 torr to 110 torr and arterial PCO_2 was between 2.3 and 2.8 torr. Breath rate for fish held at ~18°C was slightly higher than fish held at ~14.6°C. Blood pH was between 7.5 and 7.9. Water Na was about 300 μM, while water Ca was below 50 μM. Plasma Na was about 143 mM, Cl about 125 mM and plasma Ca ~5 μM.

Active Ag uptake was evident as there was more than 0.1 μM Ag (nominal water concentration) in the plasma of fish exposed to Ag at both temperatures (reached a plateau of ~1.5 μM) than could be explained by passive diffusion alone.

However, no significant difference was found between fish exposed to Ag held at ~14.6°C and ~18°C. This was most likely due to the small temperature difference between test groups.

More Ag was deposited on the gills of trout at ~18°C (~10 nmol Ag/g wet tissue) than at ~14.6°C (~7 nmol Ag/g wet tissue). This may be due to the warmer fish ventilating more frequently thus passing more water containing Ag over the gills. Both groups exposed to Ag were significantly different from the control group.

In a separate experiment fish held at ~16°C were exposed to Ag (nominal concentration of 0.1 μM) or Ag plus thiosulphate (nominal concentrations of 0.1 μM and 2.5 μM, respectively) to determine the effects of thiosulphate on Ag accumulation on gills and in plasma. Our results showed that Ag was more readily taken up in the Ag only exposure than Ag from the Ag plus thiosulphate exposure, contradicting results from Wood *et al.* (1994).

All fish in the second experiment were exposed to Ag, and half of these were also exposed to thiosulphate. Fish were held at ~16°C with water pH between 7.2 and 7.4. Water Na averaged ~775 μM. Water PO_2 was ~110 torr and PCO_2 about 2 torr. Arterial PO_2 was about 90 torr, PCO_2 was between 2.5 and 3.4 torr, and blood pH was below 8.0. Plasma Na was about 135 mM and the fish exposed to Ag only appeared to have a mild ionoregulatory problem. Plasma Cl ranged from 100-120 mM, while plasma Ca was below 1.9. Breath rates were around 100 breaths/min.

Plasma Ag concentrations for both groups were not significantly different initially, but significance was seen at 42 h and 68 h (1.5 μM, Ag only; 0.45 μM, Ag plus thiosulphate). This suggests that there was active uptake of Ag in the Ag only exposure, and a possible uptake of a small amount of free Ag^+ present in the water of the Ag plus thiosulphate exposure. These results do not concur with Wood *et al.* (1994) who found that Ag plus thiosulphate entered the plasma in large amounts. However, Wood *et al.* (1994) used concentrations of Ag plus thiosulphate that were about 48 times greater than the concentrations used in our experiment. The high concentration of plasma Ag in their fish was possibly due to passive diffusion of Ag from the water into the fish.

Gill Ag for the Ag only group (~7 μM nmol Ag/g wet tissue) was not significantly different from the Ag plus thiosulphate group (~3 nmol Ag/g wet tissue), probably due to a small sample size (n=4). However, gill Ag for the Ag plus thiosulphate exposure was reasonably close to the control groups from the previous temperature experiments. It appears that thiosulphate was effective in keeping Ag off the gills, concurring with Janes and Playle (1995).

These results suggest that, to some extent, temperature does have an effect on active metal uptake. However, the temperature difference between the groups was not great enough to see a significant difference. Whether these changes are due to metabolic rate or ventilation changes is still unclear.

In future experiments we intend to differentiate changes in active and passive metal uptake by attempting to isolate metabolic changes from ventilation changes. We will do this by increasing the O_2 content of the water as the temperature is increased, allowing the fish to ventilate the same volume of water to accommodate its increased O_2 demand.

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The Physiological Effects of Acute Silver Exposure in Rainbow Trout (*Oncorhynchus mykiss*)

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Silver nitrate is known to be highly toxic to freshwater fish. Hogstrand *et al.* (1995) found that for juvenile rainbow trout (*Oncorhynchus mykiss*) in dechlorinated Hamilton tapwater, the 7-day LC_{50} of silver (as silver nitrate) was 9.1μg l^{-1} (83nM). Chemical speciation modelling (MINEQL$^+$ 2.1; Schecher, 1991) indicated that only approximately 30% of the total dissolved silver was present as the free silver ion (Ag^+), which is thought to be the most toxic form. Ag^+ is therefore more toxic than Al^{3+}, Cd^{2+}, Cu^{2+}, Hg^+ and Zn^{2+}. However, relatively little is known about the physiological mechanisms of silver toxicity and yet such information is important for sensible regulation of silver discharge.

Previous studies on adult rainbow trout (Wood *et al.*, 1993, 1994) suggested that the principal toxic effect of silver was a severe ionoregulatory disturbance effected at the gills. Fish exposed to 10μg l^{-1} silver (as silver nitrate) showed a dramatic decrease in both plasma sodium and chloride concentrations. In contrast, plasma calcium and potassium concentrations were little affected. This pattern of osmoregulatory disturbance shows some similarity to that incurred in freshwater fish by environmental acidification or by dissolved copper. However, the precise mechanisms of these two toxicants differ in that protons competitively inhibit the sodium carrier on the apical membrane of the ion-transporting cells of the gill and increase diffusive ion losses (Wood, 1989), whereas copper inhibits the Na^+/K^+ ATPase enzyme within the ion-transporting cells which provides energy for ionic uptake (Lauren and McDonald, 1987). The present study was designed to utilise techniques that have been successful in physiological studies of metal toxicity in general, to examine in more detail the mechanistic effects of silver nitrate on branchial ionoregulation in rainbow trout .

Our first experiments measured the rates of movement (fluxes) of sodium and chloride across the gills of rainbow trout. Three types of flux can be quantified: influx (water → fish); efflux (fish → water) and net flux (influx minus efflux). Measurements of such ionic fluxes have been shown to be very sensitive indicators of ionoregulatory disturbance in freshwater fish due to a number of dissolved metals (Wood, 1992). Fluxes of sodium and chloride were measured over three hours in dechlorinated Hamilton tapwater (control) and then at 0, 8 and 72h of exposure to either 2 or 10μg l^{-1} silver (as silver nitrate) added to Hamilton tapwater. This water was used in all experiments, with the exception of the measurement of sodium influx kinetics (see below) and had the following composition: $[Ca^{2+}]$ = 1.0mM; $[Mg^{2+}]$ = 0.2mM; $[Na^+]$ = 0.5mM; $[Cl^-]$ = 0.7mM; pH = 8.0; titratable alkalinity (CO_3^{2-} equiv) = 1.0mM .

Exposure to 10μg l^{-1} silver resulted in dramatic net losses of both sodium and chloride from the fish to the water (Table I). This explains the decreases in plasma sodium and chloride concentrations seen during similar exposures in previous studies (Wood *et al.*, 1993, 1994). Examination of the unidirectional fluxes indicated that the net losses were due primarily to a significant inhibition of both sodium and chloride influx. Influxes were reduced by over 50% immediately and close to 100% by 8h, and showed no signs of recovery by 72h (Table I). In comparison, the effluxes of sodium and chloride were little affected. The effects of exposure to 2μg l^{-1} silver on sodium and chloride transport were similar to those of 10μg l^{-1}, although the inhibition of influx was not as large (Table I).

The effects of silver on the kinetics of sodium uptake were also studied by measuring sodium influx over a range of external sodium concentrations following exposure for 48h to either Hamilton tapwater (control) or Hamilton tapwater plus 2μg l^{-1} silver (as silver nitrate). Michaelis-Menten curves were fitted to the resulting data to obtain estimates of K_m, the affinity of the site of transport for sodium and J_{max}, the maximium sodium transport rate, an index of the absolute number of sodium transport sites. The relative changes in these parameters indicates the nature of the inhibition of sodium influx: either competitive, where the inhibitor (in this case silver) binds to the specific site of sodium uptake; or non-competitive, where the binding of the inhibitor to a second site affects the function of the sodium uptake site itself. Preliminary results showed that the effect of exposure to 2μg l^{-1} silver as silver nitrate on sodium influx kinetics was primarily a reduction in J_{max} ie. a reduction in the number of transport sites. Silver is therefore a non-competitive inhibitor of sodium influx.

Sodium and chloride are known to have separate sites of uptake on the apical membrane of the gill epithelium (Evans, 1981). The simultaneous inhibition of both sodium and chloride influx by silver nitrate, together with the results of the sodium kinetics study suggest that the site of inhibition is unlikely to be at the apical membrane of the ion-transporting cells of the gill epithelium. We therefore studied the effects of silver exposure on the activity of two intracellular branchial enzymes that have a role in ion transport:

A) Carbonic anhydrase (CA). CA occurs in the cytoplasm of the ion-transporting cells and catalyzes the hydration of CO_2 to produce a proton (H^+), for exchange with external sodium, and a bicarbonate ion (HCO_3^-), for exchange with external chloride.

B) Na^+/K^+ ATPase. This occurs on the basolateral membrane of the ion-transporting cells and moves sodium into the plasma against a large concentration gradient. Inhibition of this enzyme with a specific inhibitor, ouabain, reduces both sodium and chloride uptake.

Hence, a poisoning of one or both of these enzymes by silver could result in an inhibition of sodium and/or chloride transport. The gills of rainbow trout were therefore assayed for the two enzymes following exposure for 48h to either 2 or 10μg l^{-1} silver (as silver nitrate).

Branchial carbonic anhydrase activity was not significantly affected by exposure to 2μg l^{-1} silver (Table II) whereas 10μg l^{-1} silver caused a significant inhibition of CA activity of approximately 28%. However, this inhibition was not of sufficient magnitude to fully account for the reduction in sodium and chloride influxes seen in earlier experiments (Table I). The mean Na^+/K^+ ATPase activity at 2μg l^{-1} silver was approximately 50% lower than that under control conditions but the difference was not statistically significant (Table II). However, the ATPase activity at 10μg l^{-1} silver was significantly lower than that of the control by approximately 85% suggesting that inhibition of this enzyme is the primary cause of the ionoregulatory disturbance seen in freshwater fish exposed to silver nitrate. In this respect the mechanisms of silver nitrate toxicity are similar to those documented for dissolved copper.

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Table I. The effects of exposure to 10μg l^{-1} silver (as $AgNO_3$) on the fluxes of sodium and chloride in the rainbow trout (*Oncorhynchus mykiss*). Values are means ± SEM, N = 6-12. *Indicates a value significantly different from the control ($P<0.05$).

Time (h)	SODIUM			CHLORIDE		
	Influx	Efflux	Net flux	Influx	Efflux	Net flux
Control	227 ± 17	-188 ± 31	+39 ± 25	166 ± 23	-171 ± 31	-5 ± 23
0-3	*76 ± 30	-320 ± 43	*-243 ± 20	*73 ± 36	-244 ± 82	*-173 ± 34
8-11	*8 ± 6	-219 ± 57	*-211 ± 61	*0	-212 ± 31	*-212 ± 31
72-75	*0	*-70 ± 26	*-70 ± 26	*3 ± 3	*-69 ± 11	*-66 ± 12

Table II. The effects of 48h exposure to silver (as $AgNO_3$) on the activity of branchial carbonic anhydrase (CA) and Na^+/K^+ ATPase in rainbow trout (*Oncorhynchus mykiss*). Values are means ± SEM, N=6. *Indicates a value significantly different from the control ($P<0.05$).

Treatment	CA activity (μmol CO_2 mg protein^{-1} min-1)	Na^+/K^+ ATPase activity (μmol PO_4^{2-} mg protein h^{-1})
Control	1688 ± 93	1.09 ± 0.22
2μg l^{-1} Ag	1514 ± 82	0.59 ± 0.20
10μg l^{-1} Ag	*1224 ± 54	*0.16 ± 0.08

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