

the 5th International Conference Proceedings

**T r a n s p o r t , F a t e a n d E f f e c t s**

**o f S i l v e r**

**i n t h e E n v i r o n m e n t**

Hamilton, Ontario, Canada  
September 28 – October 1, 1997

Editors  
**Anders W. Andren**  
University of Wisconsin Sea Grant Institute

**Thomas W. Bober**  
Eastman Kodak Company

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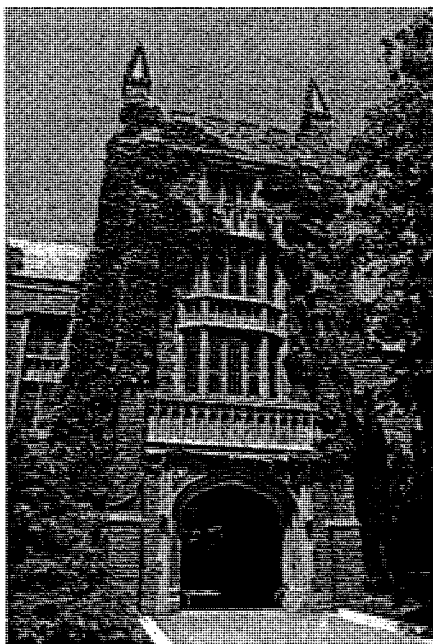
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Editors' Note: The extended abstracts included in this document have been printed as submitted by their respective authors and have not been subjected to peer review. The material presented here reflects solely the findings, opinions and conclusions of the individual authors. Questions and answers from the audience 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.



# Welcome to HAMILTON



We are pleased to welcome Argentum V participants to Hamilton, Ontario, Canada. We have had international attendees at each of our four previous silver conferences, held in Madison, Wisconsin in 1993, 1994 and 1996, and Washington, D.C. in 1995. However, this represents the first such conference to be located beyond the physical boundaries of the United States, making it now truly international. Hamilton is home to several respected institutions, including McMaster University, from whence come a number of our esteemed researchers who have contributed so much valuable and insightful environmental information to our program since its inception.

In the six years since the Argentum conferences were first conceived, great strides have been made in our collective worldwide understanding of silver behavior. Some past laboratory studies had been faulted because they were designed without considering all the variables that truly influence the metal's behavior in nature. When attempts were made to extrapolate results from such laboratory simulations to the more complex natural environment, often the data did not seem to fit with actual tests and measurements made in the field.

Of particular benefit has been the gathering together of scientists from various disciplines who can critique each other's project proposals and data from their own viewpoint, thus bringing valuable cross-disciplinary insight to efforts that otherwise might have been conducted strictly within the confines of a single discipline. This wholistic approach produces better experimental designs that yield more universally acceptable information, often at lower cost and without need to engage in peripheral supporting studies. It has helped eliminate the confusion that resulted from past conflicting data on silver, which made it difficult to adopt reasonable environmental standards. The advent of the new "clean" sampling and monitoring procedures has also greatly revised past thinking regarding ambient concentrations of trace metals in the environment. These successes over the past few years have resulted in better and more reasonable dialogue between researchers, regulators and the regulated community. We hope to continue that process through this fifth conference. This year we have added new features to the agenda: presentations and a panel discussion on risk assessment and life-cycle analyses as applied to silver and silver-containing products.

On behalf of the organizing committee, we welcome new presenters and attendees as well as many colleagues and friends from previous meetings. We hope you will derive benefit from the conference as well as enjoying the autumn color season in lower Canada and the many amenities of Hamilton and the surrounding region.

# Extended Abstracts

*Transport, Fate and Effects of Silver in the Environment*

*Hamilton, Ontario, Canada*



# Session 1

*Metal Speciation and Analytical Chemistry of Silver*

*T. W. Bober  
Session Chair*



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## Analytical Techniques for Determining Metal Speciation in Polluted Waters

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Berkeley, California, USA

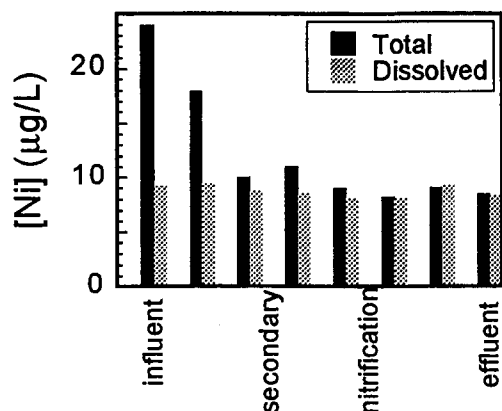
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Speciation is a term used to describe the physical and chemical properties of metals that determine their fate, transport and toxicity. In sediments, speciation techniques have been applied to distinguish between weakly adsorbed metals and metals associated with insoluble metal sulfides (1-3). These measurements are the basis for sediment criteria based upon measurements of acid volatile sulfides. In solution, speciation techniques have been developed to distinguish metal oxidation states and to measure concentrations and apparent stability constants of ligands associated with metals (see 4,5 for reviews). Such measurements provide information that is useful in the prediction of fate, transport and toxicity of metals in engineered and natural systems.

Until recently, the use of analytical techniques for measuring dissolved metal speciation had been limited unpolluted waters where concentrations of interfering substances, such as surface active dissolved organic matter were low. In our laboratory, we have developed and tested several analytical techniques for measuring metal speciation in municipal and industrial wastewater, runoff and polluted surface waters. Results of our analyses provide new information on the role of metal speciation in wastewater treatment. Our results also provide insight into the fate and toxicity of metals discharged by different sources. Although most of our research has focused on nickel and copper, these principles and analytical techniques can be extended to study the speciation of other metals.

To illustrate our approach for analyzing metal speciation in polluted waters we will consider the results of a recent study conducted in South San Francisco Bay (6,7). Anthropogenic sources of metals (*e.g.*, wastewater effluent, surface runoff) to South San Francisco Bay have come under scrutiny because concentrations of copper and nickel in surface waters frequently exceed water quality objectives (*i.e.*, 80 and 140 nM, respectively [8,9]). However, data on concentrations of dissolved and particle associated metals provide little insight into the potential ecological effects of the metals and actions that can be taken to minimize the concentrations of metals entering the ecosystem. In contrast, measurements of metal speciation provide a valuable perspective on these problems.

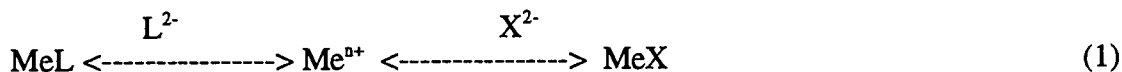
On a mass loading basis, the San Jose/Santa Clara wastewater treatment plant is one of the most important sources of nickel in South San Francisco Bay. At this tertiary treatment facility, particle-associated nickel is removed during primary and secondary treatment while concentrations of dissolved nickel are unaffected by the treatment process (Figure 1).



**Figure 1:** Total and 0.45 µm filtered Ni at the San Jose/Santa Clara POTW (data from 10).

Poor removal of dissolved nickel has been reported at treatment plants around the world. The low tendency of the dissolved nickel to adsorb onto particles is inconsistent with the known behavior of inorganic nickel species (*e.g.*, Ni<sup>2+</sup>). This observation led us to posit the existence of nickel-organic complexes in wastewater.

To test the hypothesis that nickel in wastewater is complexed by organic ligands, speciation measurements were performed using competitive ligand exchange. These techniques rely upon the competitive equilibrium that is established when a metal-complexing ligand is added to a sample:



where:

L<sup>2-</sup> represents the ligand(s) originally present in the sample;  
 M<sup>n+</sup> represents the uncomplexed metal cation;  
 X<sup>2-</sup> represents the added competing ligand.

If MeX can be quantified, the concentration of Me<sup>n+</sup> can be determined using equation 2:

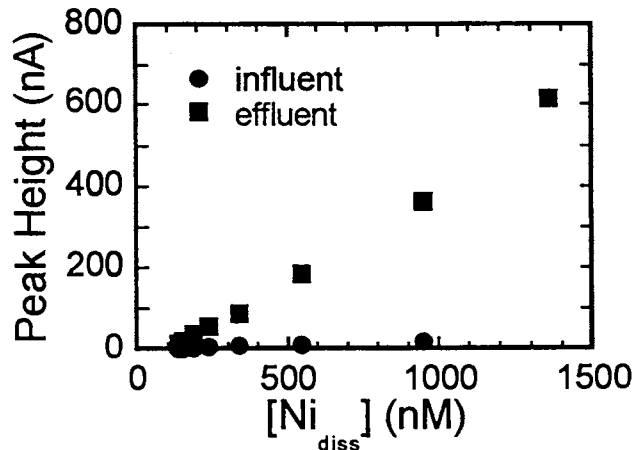
$$[\text{Me}'] = [\text{Me}^{n+}]_{\text{after\_addingX}} = \frac{K_{\text{MeX}}[\text{MeX}]}{[\text{X}^{2-}]} \quad (2)$$

where:

K<sub>MeX</sub> represents the conditional stability constant for the formation of MeX.

By adding increasing amounts of Me to the sample and measuring MeX, the concentration of L and a conditional stability constant for MeL can be determined using linear transformations or nonlinear regression (4,5).

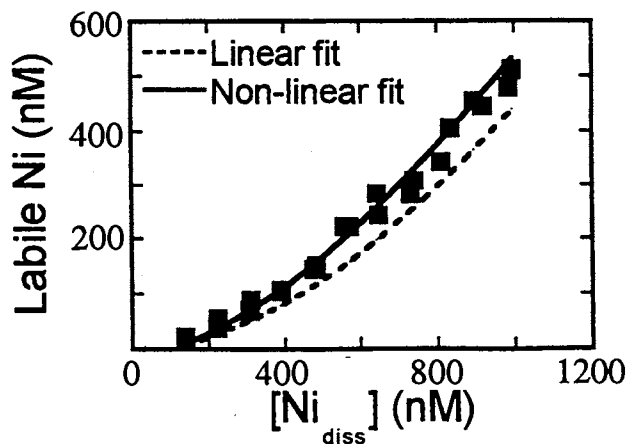
To determine the concentration of nickel associated with strong nickel complexing ligands, cathodic stripping voltammetry (CSV) was employed (11). In this technique, dimethylglyoxime (DMG) is added as a competitive ligand. The Ni(DMG)<sub>2</sub> complex formed after ligand addition is adsorbed onto a hanging mercury drop electrode and quantified by measuring the current produced when the complex is reduced. Measurement of Ni speciation in wastewater samples by CSV indicate that only about 25% of the nickel initially present in the effluent can be converted into Ni(DMG)<sub>2</sub>. Titration of wastewater effluent samples with nickel indicate a linear relationship between added nickel and signal (Figure 2).



**Figure 2:** Typical results from the measurement of wastewater effluent and influent samples by CSV. The lower slope in wastewater influent indicates a decrease in sensitivity attributable to the presence of surface-active compounds.

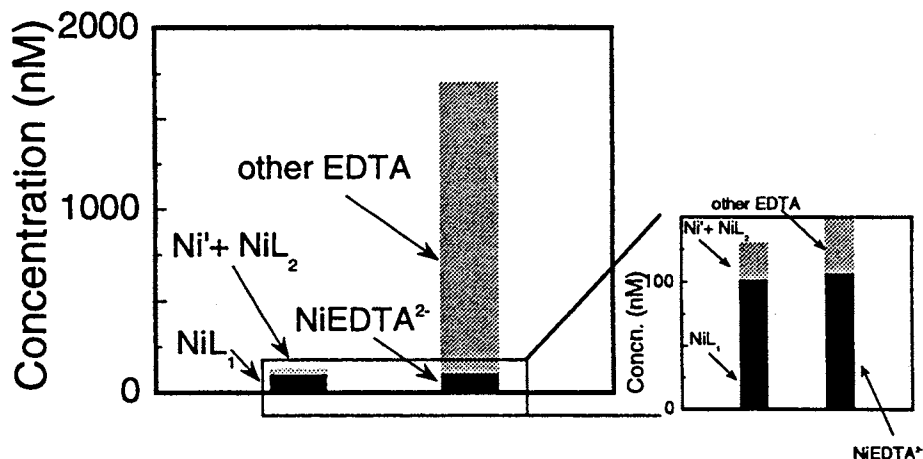
These results suggest that approximately 75% of the nickel in wastewater effluent is strongly complexed and that no excess ligand is present. Measurements of Ni speciation in surface runoff indicate that only about 25% of the nickel in stormwater runoff is strongly complexed.

To determine the concentration of nickel associated with moderately strong nickel complexes, chelating resin columns were used (11). In this technique, metal-complexing ligands immobilized on resin particles are used as the competing ligand. After passing samples through the resin columns, the concentration of nickel associated with the competing ligand is measured by rinsing the column with acid. Measurements of wastewater effluent indicate that most of the nickel is in a form that is not removed by the resin (Figure 3). After subtracting the contribution from the strong nickel complexes characterized by CSV, the concentrations of nickel associated with moderately strong ligands can be calculated. Despite the relatively high concentrations of moderately strong ligands, moderately strong nickel complexes account for only a small fraction (*i.e.*, <10%) of the nickel in wastewater effluents and surface runoff.



**Figure 3:** Analysis of Ni speciation by chelating resin in wastewater effluent collected from the San Jose/Santa Clara POTW. Dashed and solid lines represent predictions made using  $K_{MeL}$  and  $[L]$  calculated by linearization and nonlinear regression, respectively.

Measurements of metal speciation suggest that most of the nickel in wastewater effluent is complexed by a strong ligand while only a small fraction of the nickel in stormwater runoff is strongly complexed. To test the hypothesis that synthetic ligands are responsible for complexation of nickel in wastewater effluent, we reviewed manufacturing data for synthetic chelating agents (12). Our review suggested that EDTA is the synthetic nickel chelating agent most likely to be present in wastewater effluent. Using an analytical technique that is capable of differentiating  $\text{NiEDTA}^{2-}$  from other EDTA complexes (7), we were able to confirm that  $\text{NiEDTA}^{2-}$  was the strong nickel complex present in wastewater effluent (Figure 4).



**Figure 4:** Comparison of nickel speciation measurements with direct measurements of  $\text{NiEDTA}$  complexes.

Preliminary measurements of nickel speciation in San Francisco Bay are consistent with the data presented above: strong nickel complexes in wastewater effluent behave as conservative pollutants. During summer, when inputs of surface runoff are low relative to those of wastewater effluent, high concentrations of dissolved nickel are observed in South San Francisco Bay. Because most of the nickel originates in the wastewater effluent, most of the nickel is strongly complexed. In contrast, less strongly complexed nickel is observed in winter, when surface runoff is an important source of freshwater in San Francisco Bay. Strategies for minimizing potential toxic effects of nickel in South San Francisco Bay therefore should focus on minimizing the bioavailable forms of nickel in stormwater runoff rather than the complexed forms of nickel in wastewater effluents.

#### Acknowledgments

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## Questions & Answers: Analytical Techniques For Determining Metal Speciation in Polluted Waters

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- Q. JIM KRAMER (McMaster University): I have two questions - the first one is very easy to answer. When you use the word dissolved, how was that defined?
- A. In our studies our dissolved has been operationally defined as less than 0.45 microns.
- Q. So that would include all kinds of colloids?
- A. Yes, however we've done some dialysis experiments that have shown that most of the metals were dissolved. In the case of nickel the good agreement between nickel-EDTA and complexed nickel suggests that it's not a colloid.
- Q. I just point this out to the people because last year the whole conference was on colloids and of course we're concerned about them, but more importantly on the ligand computation method. There's one important assumption that you left out and it may be okay, but it's something I think everybody who uses this technique must check, and that is you assume that the ligand only reacts with the soluble phase. You do not assume the ligand absorbs on the solid, or the new metal-ligand complex would completely change your system. I think the very high copper ligand value that Laura Sigg (EAWAG - Switzerland) and Stan van den Berg (Liverpool University, U.K.) are showing is due to that, so I think if we use that technique we should always use a labeled compound on the ligand just to do a mass balance. In your situation you were able to show that's not the case, but in general and particularly again with silver I think that is a problem.
- A. That is a good point, and the other point which I can't emphasize enough is that these metal speciation techniques are also operationally defined in terms of equilibrium. If you remember, in our system we had a lot of EDTA, a lot more than we had of free nickel, and if you actually take these samples and heat them up, more nickel is complexed. That is, I can add 500 nanomoles of nickel to my sample, heat it up for a few hours, and I see more or what appears to be more ligand present. So the other caveat that I would add to using any of these techniques is that not only are you operationally defined in dissolved versus particle associated, but you're also operationally defined in terms of whether you're at equilibrium or what your equilibrium constants are.
- Q. PETER SANTSCHI (Texas A&M University): I just wanted to mention, because you focused on EDTA, that EDTA is not very good ligand to look for silver.
- A. I think that that's something good to add to the audience. From the stability constants for silver, you would not expect silver EDTA complexes to be important in the environment. However, I think the approaches we've taken would be applicable to, for example, organo sulfur-containing ligands or other ligands that can form strong complexes with silver. So that's a good caveat.
- Q. NICK FISHER (State University of New York-Stony Brook): It sounds like there's a lot of EDTA flowing into South San Francisco Bay, which may mean that there's really not very much of a metal problem in that body of water. Is this reflected in lower metal concentrations in organisms there or lower  $K_d$  values in either suspended particles or sediments?
- A. I can speak to the second question and not the first one. I think maybe you're a little bit or Sam Luoma is a little more qualified to speak to that. With respect to  $K_d$  values, people have observed lower than expected  $K_d$  values in the South Bay. It was attributed in a paper by the group at OGI a few years ago. I'm blanking on the name. Anyhow it's been attributed to not having enough time for equilibration or something like that. I think it draws our attention the fact that  $K_d$  values are operationally defined and you really need to know something about metal speciation. But they are lower than expected, or lower than the rest of the bay.
-

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Q. Thank you.

Q.(questioner not identified) Is that an experimental group at OGI?

A. No it's a modeling group.

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## Silver Complexes of Environmental and Related Thiols: Structural Studies

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Sulfur, as inorganic hydrosulfide or in organic derivatives as in mercaptans (thiols), is a powerful complexant of univalent silver ion. A number of mercaptans exist in appreciable concentrations in environmental waters (see Table 1) and, although the concentration ranges are wide, with the higher values referring to mainly pore waters, they are quite sufficient to complex silver ion with log  $K_f$  strengths in the order of 12-14. While in the real environment these mercaptans are most likely associated with either inorganic (eg silica based) or degraded organic particulate matter, silver ion may compete effectively for the sulfur atoms and complex to them, unless there is present an excess of another more strongly binding species such as  $Hg^{2+}$ . Silver can also readily migrate from one sulfur complex to another as illustrated by the important experiment carried out by Adams and Kramer<sup>1</sup> where they noted firstly the sorption of  $Ag^+$  from solution onto FeS particles and then the *re-solution* of  $Ag^+$  by addition of 3-mercaptopropanoic acid (3-MPA). Clearly these mercaptans are important in the speciation and possible movement of silver between solid and solution phases and it is surprising to find that no information has been reported on the solid state structure of *any* environmental mercaptan-silver complex and very little on their solution properties. This paper begins to address this lack of data by examination of two mercapto-silver complexes of compounds related to the amino acid group of environmental mercaptans (cysteine, glutathione, and N-acetylcysteine in Table 1). They are silver-(D)-penicillamine, silver-cysteine ethyl ester, and silver-cysteine complexes. These compounds have been studied by use of x-ray crystallography, proton and carbon-13 Nuclear Magnetic Resonance (NMR) spectroscopy, and ElectroSpray Mass Spectrometry (ESMS). The silver-cysteine complex forms both micro and macro crystalline material, but only an x-ray powder diffraction pattern has been obtained at this point.

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TABLE 1. Environmental mercaptans commonly found in fresh<sup>a</sup> and marine waters<sup>b</sup>

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	<u>CONCENTRATION <math>\mu</math>M</u>
Dimethylsulfoniopropanoate (DMSP)	100 - 200
Carbon disulfide	0.17 - 0.2 nM
Dimethyl sulfide	0.05 - 0.1 nM
Mercaptomethane	0.75 - 1.1 nM
3-Mercaptoglycerol	1.6 - 20
3-Mercaptopropanoic acid (3MPA)	0.02 - 20
2-Mercaptopropanoic acid (2MPA)	0.04 - 5.4
3-Mercaptopyruvic acid (MPV)	0.04 - 20
Mercaptoacetic acid (MAC)	0.04 - 0.6
Glutathione (GSH)	0.04 - 2400
Cysteine (CSH)	0.04 - 12.4
N-Acetylcysteine	0.03 - 0.3

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<sup>a</sup> F. Caron and J.R. Kramer, *Anal. Chem.* **1989**, *61*, 114.

<sup>b</sup> W. MacCrehan and D. Shea (1995). In *Geochemical Transformations of Sedimentary Sulfur*.

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While there are a number of interesting Ag-S structures in the literature with aromatic groups and highly hindering substituted silyl species<sup>2</sup>, those most relevant to environmental mercaptans are the alkyl mercaptans. Work reported by Dance *et al.*<sup>3</sup> in 1991 on the x-ray powder diffraction patterns of butyl, pentyl, hexyl, and heptyl mercaptan-silver complexes was interpreted in terms of a non-molecular layered structure for these materials in the solid state.

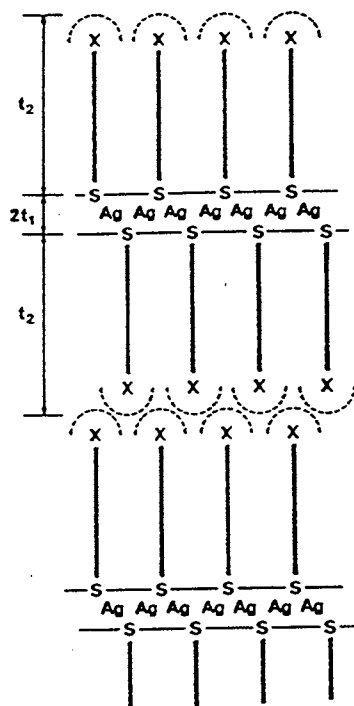


Figure 1. Layered structure of simple alkyl mercapto-silver complexes.

Here a two-dimensional "slab" of Ag-S-Ag-S atoms has the alkyl chains extending from alternating silver ions on either side of the slab with overall dimensions dependent on the length of the alkyl chain. A diagrammatic representation of a side view of the slab is shown in Figure 1, where  $(2t_1 + 2t_2)$  represents the width of the layer and the "X" group at the end of each chain would be a  $\text{CH}_3$ . Additionally, Dance proposed that in an idealized tetragonal unit cell of such a structure, the silver would be tricoordinate and the sulfur tetracoordinate.

The only single crystal structure of a tertiary alkyl mercaptan-silver complex reported to date is that of silver 3-methylpentane 3-thiolate, illustrated in Figure 2.<sup>4</sup> The structure is non-molecular and there are

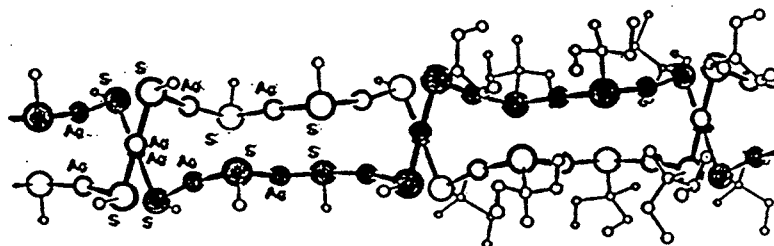
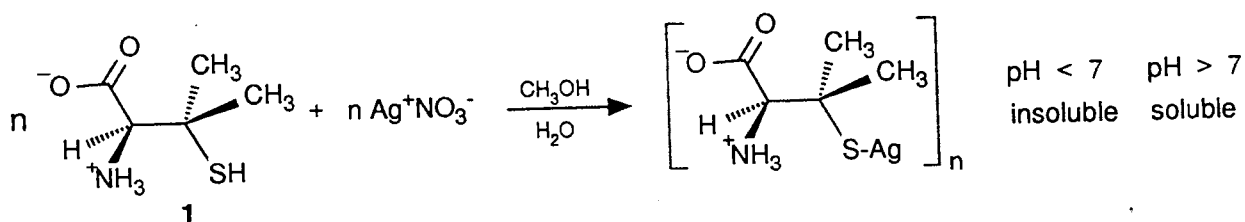


Figure 2. Intertwining double chain structure of silver 3-mercapto-3-methylpentane.

two chains in the unit cell that intertwine about each other at each 4th silver atom as is illustrated by the shaded chain looping, first below and then above, the unshaded chain in Fig. 2. The structure can be described as a double helix and it contains Ag digonally coordinated to two sulfurs, although there are a number of silver-silver close contacts (they are non-bonding) in the 3 Å range.



Scheme 1

**Penicillamine 1:** This amino acid (a 3,3-dimethylcysteine, Scheme 1 above) has not been identified firmly at present in natural waters but is likely to be one of the many unidentified thiols found by Mopper and Taylor.<sup>5</sup> Penicillamine forms a well defined 1:1 complex with  $\text{Ag}^+$  in aqueous or organic solvents and we have been able to grow x-ray quality crystals from methanol/water containing a small quantity of ammonia. The structure is illustrated in Fig. 3 and is non-molecular with two intertwining chains of  $[\text{R-S-Ag}]_n$  forming a double helix in exactly the same manner as for Dance's 3-mercapto-3-methylpentane-silver complex above. Because of the approximately  $90^\circ$  dihedral angle between an Ag-S-Ag unit, each chain progresses in a "zig-zag" manner with a twist at every 4th silver imposed by the packing requirements of the penicillamine fragment. The amino acid moieties lie on the outside of the double helices and there are electrostatic attractions between adjacent helices. Examination of one helix shows strictly digonal S-Ag-S

coordination and the presence of close Ag-Ag contacts between adjacent silvers in an S-Ag-S-Ag-S unit. The close contacts between silver ions are in the range of 3 Å and although they are non-bonding they appear to add extra stabilization to the structure through van der Waals interactions.

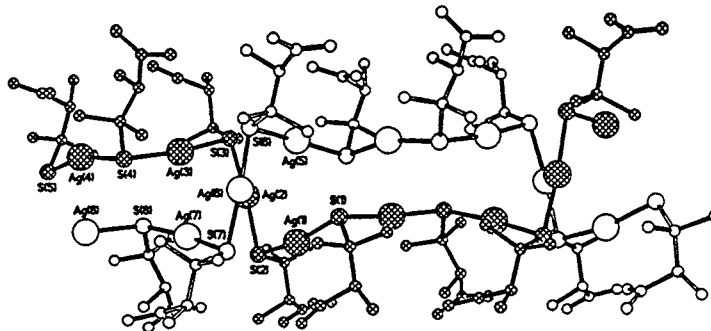


Figure 3. Double helical chain structure of silver-penicillamine complex.

In aqueous solution at pH 9 Ag-penicillamine gave a proton NMR spectrum which showed an upfield shift of 0.45 ppm for the C-H proton and a smaller upfield shift for one of the CH<sub>3</sub> groups of 0.1 ppm. More significantly, the linewidths of the signals were broadened in comparison to the free ligand with the C-H proton for example showing a linewidth at one-half peak height of 5 Hz. Such broadening is indicative of either molecular aggregation or to an intermediate rate exchange process.

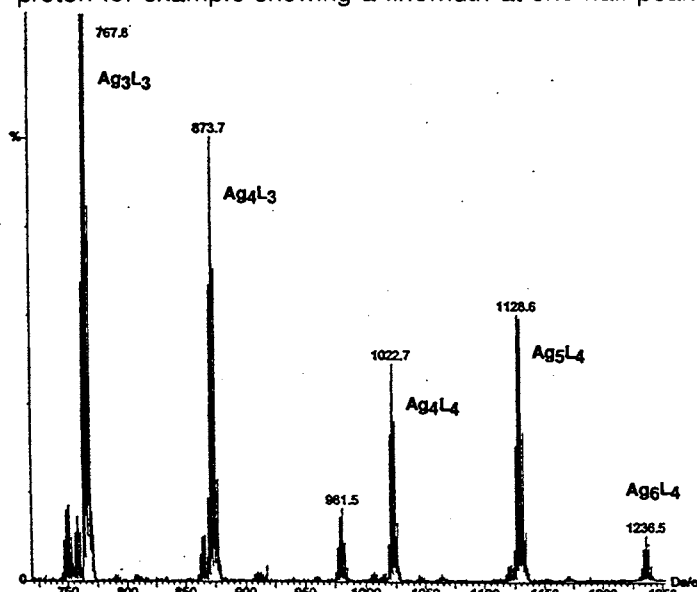
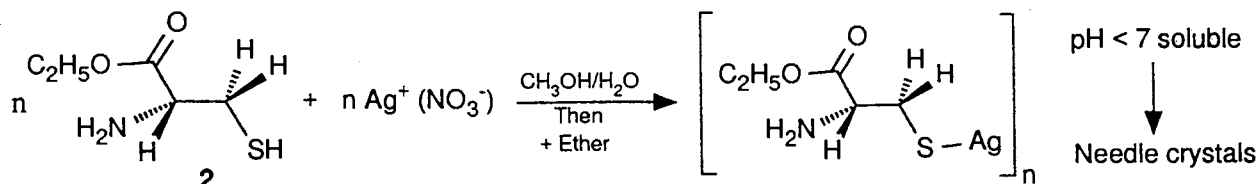


Figure 4. Partial ESMS spectrum of silver-penicillamine.

That the former was the cause of the line broadening was suggested by measurement of the carbon-13 spin lattice relaxation time ( $T_1$ ) of the free penicillamine ligand and the Ag complex, where the C-H carbon in the free ligand showed a  $T_1$  of 2.7 seconds but the complex showed a  $T_1$  of only 0.3 seconds. These data imply that the Ag-penicillamine complex is highly aggregated in solution with a molecular mass in the 4 to 7 kDa range.

Investigation of the ESMS spectra of very dilute solutions of Ag-penicillamine (<1 μM) in water-acetonitrile, in negative ion mode, gave further evidence for aggregation and a partial spectrum is shown in Fig. 4. Because of the approximate 50% natural occurrence of each of 107 and 109 isotopes of silver, the presence of multiple silvers is readily recognized by the isotope

pattern in ESMS spectra. Thus, if one Ag is present a doublet is observed, if two a triplet, and if three a quartet. The ion at 255 (the molecular weight of Ag-penicillamine) showed only one line and therefore contained no Ag, but ions centred about 1128.6 in Fig. 4 for example showed a distinct sextet structure and therefore contained 5 Ag atoms. The major current carrying ion containing Ag corresponded to Ag<sub>3</sub>L<sub>3</sub> and, as may be noted from Fig. 5, species as high as Ag<sub>6</sub>L<sub>4</sub> are clearly discernable. Evidently there are aggregated species present even in very dilute solutions and further MS-MS experiments should shed light on how these aggregates fragment in the spectrometer.



Scheme 2.

**Cysteine ethyl ester 2:** When cysteine ethyl ester was treated with  $\text{Ag}^+$  as shown in Scheme 2, an acid soluble Ag-complex was obtained which deposited small plate-like crystals from water. The structure of this crystalline material was determined by x-ray crystallography and found to be very unusual. Firstly in having an empirical formula of  $[\text{Ag}_2(\text{ethyl cysteinate})]^+\text{NO}_3^-$ , and secondly in having two different types of  $\text{Ag}^+$  present; one bound strongly to S and the other more weakly bound to S and the N atom of cysteine. A side view of the structure is shown in Fig. 5. It is non-molecular and consists of two infinite chains of Ag-S-Ag-S-Ag zig-zags one lying on top of the other and along the sides of this pair of chains are placed a row of  $\text{Ag}^+$  ions that coordinate to the chain sulfurs and each  $\text{NH}_2$  group of the cysteines. These long chain columns are bound on the sides by  $\text{NO}_3^-$  ions and van der Waals interactions between the organic fragments on the top and bottom. Again ESMS spectra (positive ion mode) showed ions from  $\text{Ag}_6\text{L}_5$  species down to  $\text{Ag}_3\text{L}_2$ , suggesting that they are aggregated similar to Ag-penicillamine.

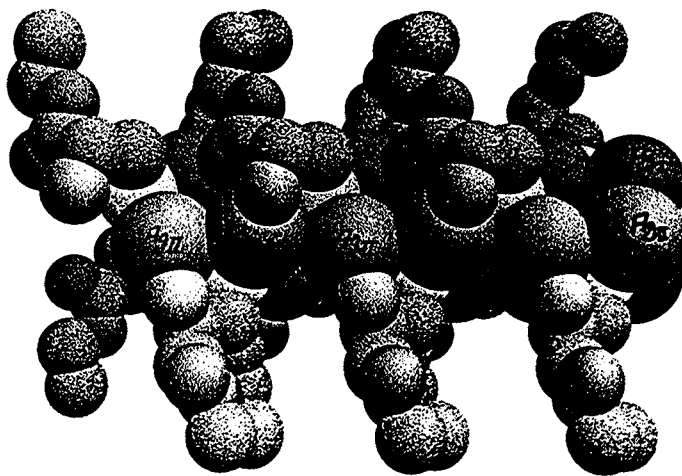
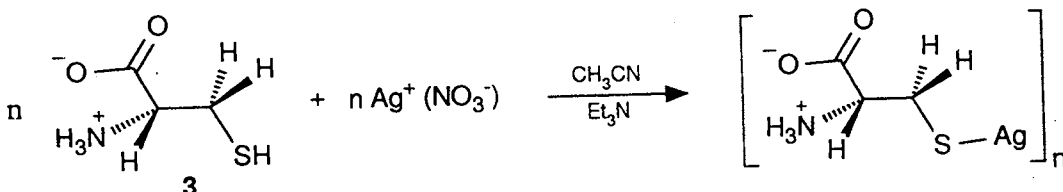


Figure 5. Side view of the silver-ethyl cysteinate complex showing the two types of silver ions. H atoms are omitted for clarity.

**Cysteine 3:** Silver forms a 1:1 complex with cysteine below pH 7 but higher ratios of Ag form above pH 7.<sup>6</sup> We have prepared Ag-cysteine as shown in Scheme 3 and  $\log K_f = 12.7$ , determined by Adams and



Scheme 3.

Kramer,<sup>7</sup> is typical of silver thiolates. Heating amorphous Ag-cysteine in acetonitrile containing 2% triethylamine resulted in the formation of microcrystals which gave a clean x-ray powder diffraction pattern shown in Fig. 6. This pattern is entirely analogous to that reported by Dance<sup>3</sup> for simple Ag-alkyl thiolates and the interlayer spacing of 11.4 Å matches well with the calculated length of a cysteine unit (5.5 to 6.0 Å depending on the conformation). Thus, these data suggest that Ag-cysteine can exist in a layered structure similar to that proposed by Dance for Ag-alkyl thiolates illustrated in Fig. 1.

In a search for larger crystals of Ag-cysteine, we found that amorphous Ag-cysteine recrystallized from warm (<50°C) dilute  $\text{HNO}_3$  (<1.0 M) to give long colourless needles that are insoluble in both water and acetone. Unfortunately these attractive crystals are severely disordered and we have been as yet unable to obtain a good single crystal x-ray diffraction data set. Preliminary data suggests that the crystals are non-molecular and contain long columns of Ag-S units very similar to those found for Ag-ethyl cysteinate discussed above. The proton NMR spectrum of Ag-cysteine in basic solution showed similar chemical shift changes and line broadening as noted for penicillamine, and the  $^{13}\text{C}$  spectrum a severe shortening of the spin-spin lattice relaxation time ( $T_1$ ). These data are again indicative of aggregation in solution and are consistent with observations made by Andersson in 1972 from ultracentrifuge sedimentation data where he suggested the aggregates had molecular weights ranging from 4000 to 7000 Daltons.<sup>6</sup> ESMS spectra were

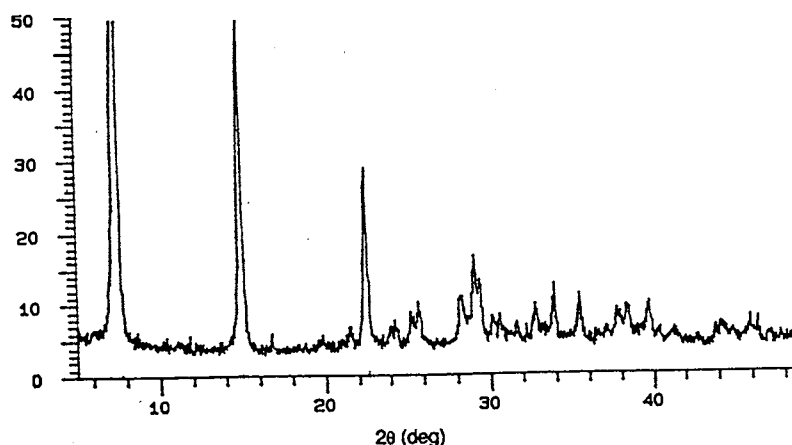


Figure 6. X-ray powder diffraction pattern of silver-cysteine complex.

also consistent with Ag-cysteine aggregation and we observed ions from species  $\text{Ag}_5\text{L}_4$  (at  $m/z$  1017) down to  $\text{AgL}_2$  (at 349) with the most intense ion being  $\text{Ag}_3\text{L}_3$  (at 684).

### CONCLUSIONS

Structures of silver complexes of the environmental amino acid cysteine and two related compounds, cysteine ethyl ester and penicillamine (a dimethyl cysteine) have been investigated.  $[\text{Cysteine-Ag}]_n$  can exist as a layered structure when converted to a microcrystalline form by heating in acetonitrile. This layered structure contains a two-dimensional core of Ag-S and is similar to that adopted by simple alkyl mercapto-silver complexes.  $[\text{Cysteine-Ag}]_n$  may also be crystallized from dilute  $\text{HNO}_3$  giving colourless needles that possibly exist as pairs of long zig-zag chains of Ag-S-Ag-S units, although no good single crystal x-ray data set has been obtained yet for this form of  $[\text{Cysteine-Ag}]_n$ .  $[\text{Penicillamine-Ag}]_n$  forms non-molecular crystals that contain two intertwining chains of Ag-S-Ag-S units making a double helix. This structure is similar to that formed by silver 3-mercapto-3-methylpentane reported by Dance in 1991.<sup>3</sup> Silver forms a crystalline complex with ethyl cysteinolate with molecular formula  $[(\text{Ethyl cysteinolate-Ag}_2)^+(\text{NO}_3^-)]_n$ .

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4. I.G. Dance, L.J. Fitzpatrick, A.D. Rae, and M.L. Scudder. *Inorg. Chem.* 1983, 22, 3785.
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6. L.-O. Andersson. *J. Polymer Sci. Part A.* 1972, 10, 1963.
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## Questions & Answers: Silver Complexes of Environmental and Related Thiols: Structural Studies

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- Q. KEN ROBILLARD (Eastman Kodak): Russ, from your observations with the silver penicillamine and the oligomeric nature that you discern from NMR, do you feel at this point that that may be representative of what's happening with some of the silver complexes in actual environmental samples - that we will discover that they are more oligomeric than monomeric in nature?
- A. Yes. What we don't know is where the monomolecular wall is. Is it possible in an aqueous solution actually to have a monomeric species? I feel at this point that no, that they probably are oligomers, but they get smaller as you go to lower concentration. Part of my rationale is the mass spectrometry experiment. Even though it's liable to artifacts, the time that that one micromolar solution goes through the nozzle, gets charged and the tiny particles get evaporated and then recorded, is on the order of a microsecond. So either a monomer is aggregating very quickly or it's already an aggregate even at that low concentration. So I suspect a lot of them, if they're out there, will be aggregates. Now they may be very mobile aggregates, and would therefore be breaking up and continually interchanging.
- Q. CHUCK CHRIST (Eastman Kodak): Can you discern anything in your NMR studies about temperature dependence or viscosity dependence? Can we determine anything about the nature of the aggregation of these species from NMR?
- A. We have done some carbon 13 experiments and you can measure the correlation time. That's the time that it takes for a molecule to rotate one radian. A normal small molecule would be around  $10^{-11}$  seconds. These other molecules' times are much slower than that. Now unfortunately there isn't a direct relationship between correlation time and molecular size. But certainly we're going to push this and probably with some spherical models, we may be able to say "okay, this spherical model has correlation time of such and such, so our silver species are a bit bigger or a bit smaller." A bit like ultra-centrifugation, where again you use the model. So we can make some guesses, but not super-good guesses.



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## X-ray Absorption Spectroscopy Study of Model Silver Compounds

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X-ray absorption spectroscopy (XAS) analysis provides information on the nearest-neighbor and next-nearest neighbor interactions of almost all elements of the periodic table. XAS is becoming an important tool in environmental chemistry because it describes the electronic environment of elements in both crystalline and poorly crystalline solids such as soil clay minerals, and on the coordination environment of soluble and surface sorbed species. Important features of XAS include:

- XAS provides information about the average local structure and environment of most elements; it is an especially useful technique for studying the composition of complex materials.
- Synchrotron-based XAS has sufficient intensity to study low concentrations (ppm) of most elements in gaseous, solid, or liquid states. Furthermore, the analyses can be carried out under relatively extreme conditions, including high or low pressure or temperature, and in fluids such as water.
- Because of the short electron mean free path in most materials, XAS provides short-range structural information, within about 0.6 nm of the absorbing atom.

In our study, transmission XAFS (x-ray absorption fine structure) measurements of the following samples were made at the National Synchrotron Light Source (NSLS) in February, 1997:

Fixer-amended sludge	Ag TMT sludge powder
Ag <sub>2</sub> O	Ag <sub>2</sub> S
Ag foil	AgNO <sub>3</sub> solution
Ag adsorbed on hydrated ferric oxide	Ag albumin solution

The two sludge samples and one Ag<sub>2</sub>S sample were provided by Kodak; the remaining samples were purchased or prepared from reagent-grade chemicals. These samples were chosen to provide a broad initial basis set to compare with more complex samples. Measurements were made over an energy range spanning 25.5 KeV, the Ag K-absorption edge. A summary of some of the results from these measurements is provided below.

An example of how XAS can be used to readily distinguish between different electronic environments for the target element can be seen in Figure 1, where transmission spectra for  $\text{Ag}_2\text{S}$  and  $\text{Ag}_2\text{O}$  reference samples are compared. To compile this figure, the background signal that occurs before the Ag absorption edge (25.5 KeV) was subtracted from the raw data and the resulting spectra were plotted on the same figure. In this figure, the relative signal amplitude is not important. The shape of the spectra above the edge, however, contain the fine structure information that makes it possible to see differences in the coordination environment for Ag in these two samples. Higher frequency oscillations above the edge indicate a longer nearest neighbor distance for  $\text{Ag}_2\text{S}$  relative to  $\text{Ag}_2\text{O}$ .

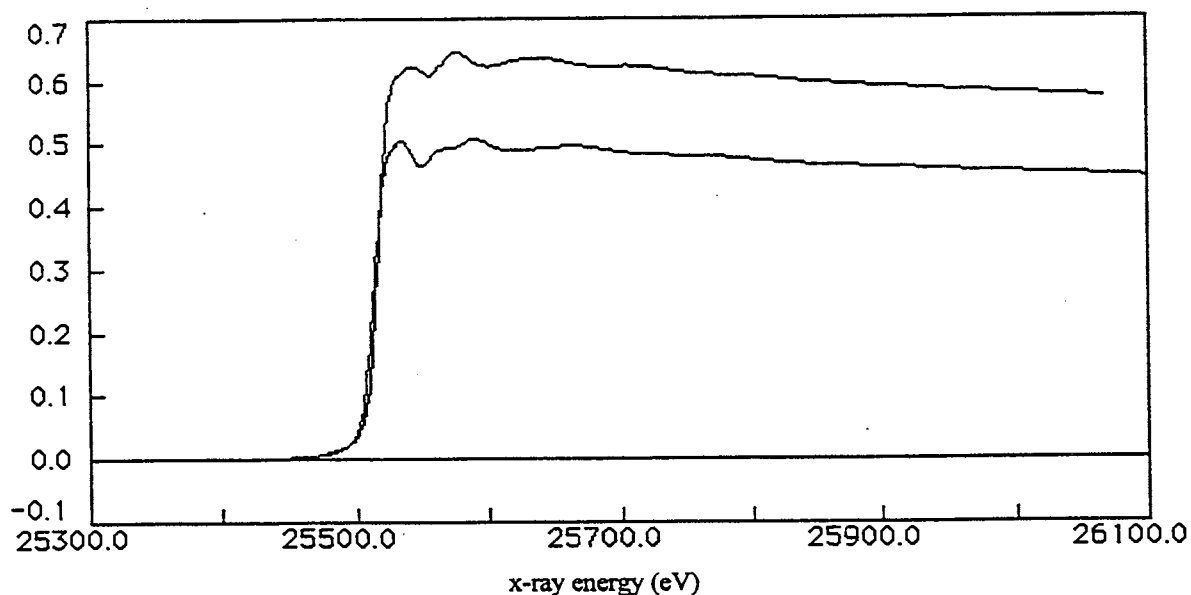


Figure 1

Once reference spectra are defined for the basis set, this information can be used to identify unknown samples. For example, transmission spectra for the fixer-amended sludge are qualitatively similar to  $\text{Ag}_2\text{S}$  (Figure 2). A more quantitative comparison of the spectra can be seen in Figure 3, where a regression analysis was used in an attempt to match one signal to another. In this type of analysis, any residual outside the noise indicates physical differences in the samples' structures. In this case, oscillations for the  $\text{Ag}_2\text{S}$  signal show a slight lead in phase relative to the fixer-amended sludge, indicating a slightly longer average nearest neighbor distance in  $\text{Ag}_2\text{S}$ . A similar comparison between  $\text{Ag}_2\text{S}$  and the Ag TMT sludge (Figure 3) suggests even greater structural differences.

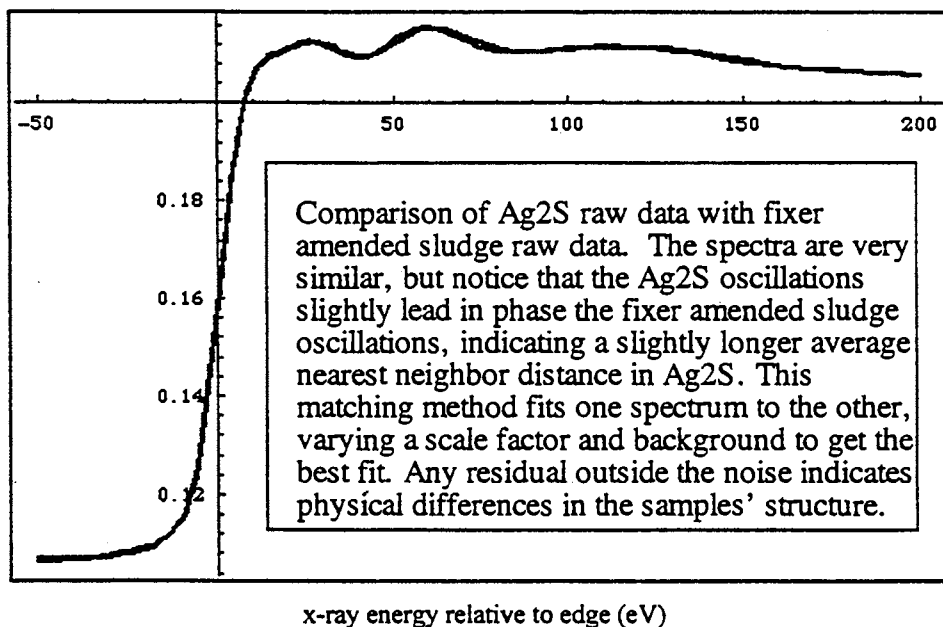
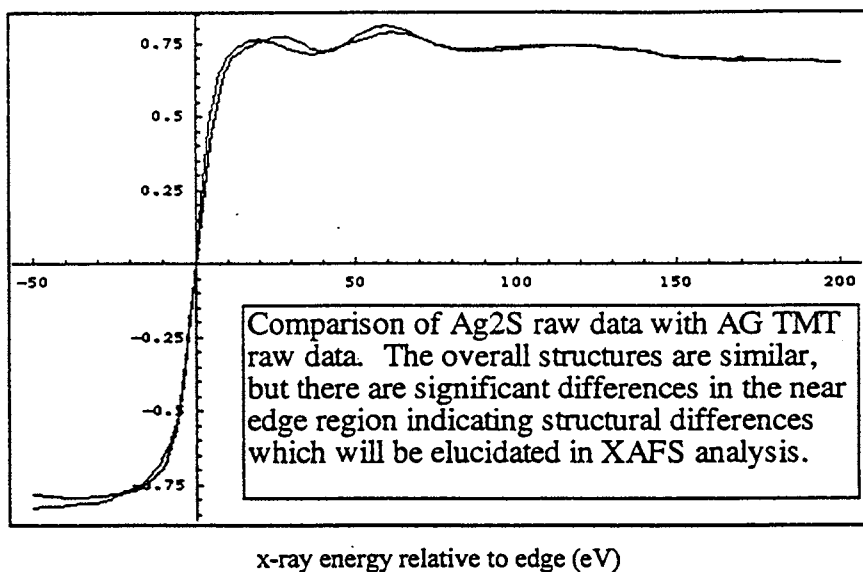


Figure 2



**Figure 3**

A more quantitative description of these structural differences can be obtained for analysis of the Fourier transform of the spectra. The Fourier transform includes two parts: The modulus, which describes the structure, and the phase, which describes the atomic number. For simplicity the figures included in this progress report show only the modulus. Figure 4 compares the Fourier transform of  $\text{Ag}_2\text{S}$  with the fixer-amended sludge.

Peaks in the Fourier transforms represent groups of atoms at approximately the same radial distance from the Ag atoms. The results suggest that although the elemental composition and structure of these two samples are similar, the nearest neighbor and second neighbor distance is slightly shorter for the sludge relative to  $\text{Ag}_2\text{S}$ . Quantitative fitting of these and the remaining sample spectra is in progress.

The peak area is approximately proportional to the average amplitude of the single shell EXAFS over the transform range, and the peak position is approximately equal to the average slope of the phase. The radial coordinate is essentially the radial distance, except the transform peaks are shifted approximately  $0.5 \text{ \AA}$  to shorter distance due to the negative slope of the central atom phaseshift. The fourier transforms are an intermediate step in the analysis; quantitative analysis is done by nonlinear least squares fitting or other methods.

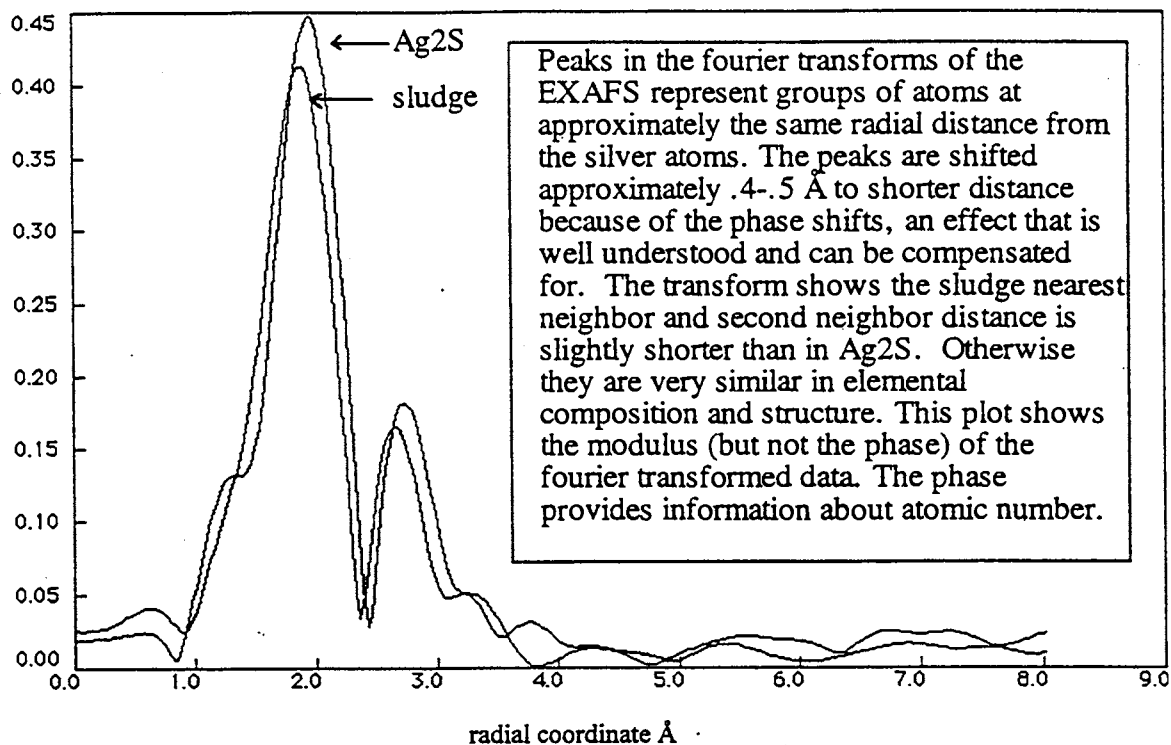


Figure 4

The initial measurements at NSLS demonstrate the feasibility of this approach for resolving Ag speciation in complex samples. Bend magnet beamlines at NSLS, however, are not well-suited for measuring the relatively high energy Ag absorption edge. We are currently planning future XAS measurements for the Advanced Photon Source (APS) at Argonne National Laboratory. Not only is the APS conveniently accessible from IIT, but its higher energy characteristics and higher photon flux provide a much better signal-to-noise ratio and make it possible to examine samples with lower Ag concentrations. The Materials Research Collaborative Access Team beamline we plan to use for the analysis is presently being commissioned and should be available for experiments in summer '97.

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## Questions & Answers: X-ray Absorption Spectroscopy Study of Model Silver Compounds

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- Q. ANDERS ANDREN (University of Wisconsin-Madison): What sort of concentration levels of solids do you need to have in order to see the different forms?
- A. The samples that we looked at were very concentrated, but we believe we can get down to the ppm range, and that's solid, liquid or gas phase, if that is of interest.
- Q. PETER SANTSCHI (Texas A&M University): When you say ppm range what do you mean? Do you mean ppm silver in a solid, or do you mean ppm of solid in a liquid?
- A. I mean in the total mass of the sample that you are looking at, which could be sludge or could be liquid. I mean parts per million in that total mass.

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## **Silver and Sulfide Downstream from a Municipal Wastewater Treatment Plant**

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### **Introduction**

- Silver is toxic to lower aquatic organisms. For example, the freshwater cladoceran, *Simocephalus* sp., exhibits reduced egg production at chronic exposures of 500 pico-molar silver (Fisher and Hook, 1997).
- Silver forms strong complexes with sulfur(II-) ligands ( $\log K \approx 13$ ). If sulfur(II-) ligand concentrations are in excess of silver concentrations, silver-sulfur(II-) complexes should be the predominate silver species. Sulfur(II-) includes both inorganic sulfide ( $\text{HS}^-$ , colloidal FeS) and organic sulfide (thiols).

### **Objectives**

- To study the distribution of silver amongst the particulate ( $> 0.45 \mu\text{m}$ ), colloidal ( $0.45 \mu\text{m} - 10 \text{KDa}$ ) and dissolved phase ( $< 10 \text{KDa}$ ) in municipal wastewater treatment plant (WTP) effluents and surface waters.
- To examine silver concentrations in relation to inorganic sulfide and organic sulfide (thiols) concentrations in the particulate, colloidal and dissolved phase.

### **Methodology**

- Wastewater and surface water samples were collected during the spring and summer of 1997 at the Burlington and Dundas WTP (Ontario, Canada), using clean handling techniques.
- Sub-samples were filtered through  $0.45 \mu\text{m}$  syringe filters and 10 KDa centrifugal filters.
- Silver analyses were performed by ICPMS.
- Inorganic sulfide was determined by the Methylene Blue method.
- Thiols were determined by HPLC using a pre-column fluorimetric derivatization and a C-18 column (Mopper and Delmas, 1984).

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## Findings

- Inorganic sulfide is present in municipal wastewater effluents and oxic freshwaters at 0.01-0.5  $\mu\text{M}$  levels.
- Thiols were non-detectable in wastewater and shallow surface waters (detection limit = 1 nM).
- Inorganic sulfide (probably colloidal FeS) concentrations are at least 100 times greater than thiol concentrations; however, these analyses do not include concentrations of thiolic sites in high molecular weight compounds (e.g. fulvics and humics).
- Inorganic sulfide concentrations are always in excess of silver (100 times minimum).
- With sulfur(II-) in excess, we infer silver in municipal wastewater effluents and freshwaters is likely complexed to sulfur(2-).
- Silver in the dissolved (< 10 KDa) and colloidal (0.45  $\mu\text{m}$  - 10 KDa) phase appears relatively stable, and may be transported conservatively over distances of at least 0.5 km.
- Particulate silver decreased by 90% (on average) over the same 0.5 km.
- Any measure of dissolved silver which uses filtration will likely not provide a determination of the free metal ion concentration. This study demonstrates significant complexation capacity for silver even in the < 10 KDa filter fraction.

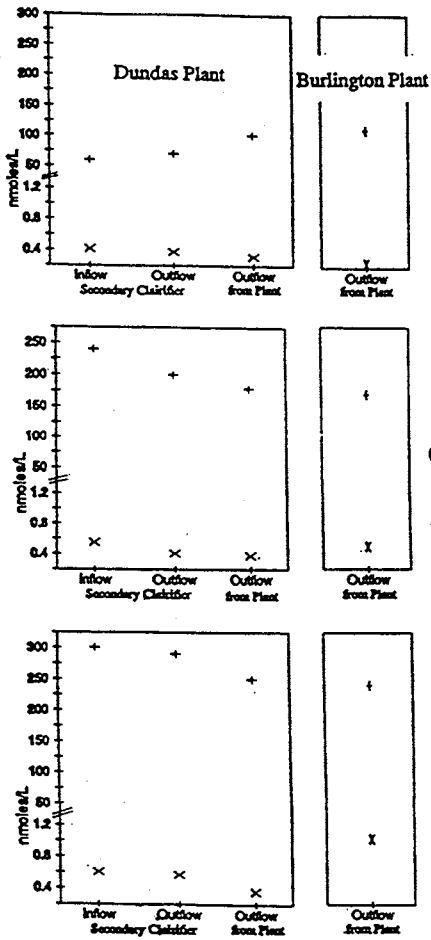
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## Acknowledgement

- Kodak Canada, Eastman Kodak Company and NSERC Canada.





Dissolved (< 10 KDa)

Colloidal (0.45  $\mu\text{m}$  - 10 KDa)

Particulate (> 0.45  $\mu\text{m}$ )

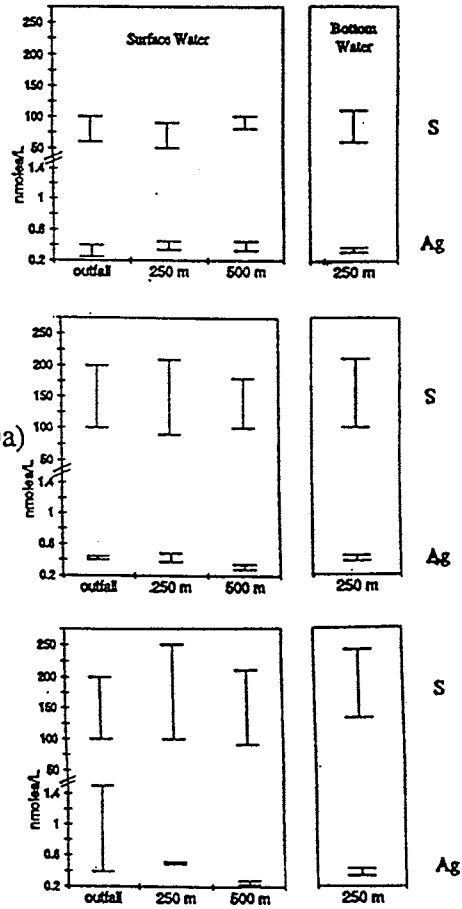


Figure 1. Silver and inorganic sulfide concentrations in the Dundas and Burlington WTP effluents. Sampled in June, 1997.

Figure 2. Silver and inorganic sulfide concentrations in surface waters downstream from the Dundas WTP. Maximum and minimum concentrations for spring and summer, 1997.

x - Silver      + - Inorganic sulfide

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## Questions & Answers: Silver and Sulfide Downstream from a Municipal Wastewater Treatment Plant

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Q. PETER SANTSCI (Texas A&M University): What were the conditions which you used to run your HPLC?

A. We used a C-18 column and fluorescence detector, where we pre-column derivatized the thiols.

Q. JIM KRAMER (McMaster University): I'll bring up a question that we've discussed and put you on the spot. The apparent soluble 10 kilodalton silver values are relatively high, 20-ish to 30-ish nanograms per liter, compared to the background levels that we've been measuring at Wisconsin at 1 or so. The question we have discussed is, considering what you know about these silver compounds and what Russ Bell and his people have said; the question about monomers and oligomers, etc. - is 10 kilo daltons a proper cutoff for defining so-called truly soluble silver, or are we still looking at a lot of colloidal material? I think that's pretty important to a lot of people.

A. Yes. I think it's clear, if you look at both the results from the Des Jardines Canal as well as the Dundas treatment plant, that if the silver in the 10 kilodalton fraction was truly dissolved, then you would see it being taken up. I don't think you would see those constant levels that we're seeing, so I would suggest that the silver is probably in a non-reactive form or at least a meta-stable form, so it may be bound up by organic ligands. I suspect that probably more is bound to colloidal iron sulfide, and that's definitely something that we're going to continue looking at.

Q. DOMINIC DI TORO (Manhattan College): Nick, the sulfide measurements that you're making, Cline spectrophotometric, those are dissolved sulfides you would think, right?  $\text{HS}^-$ ?

A. No, because the way we do that measurement is we add the Cline reagent, which is in an acidic form. It's got both sulfuric and hydrochloric acid, so you do actually get a digestion of any reactive sulfur or sulfide.

Q. Right, because I was thinking with all that sulfide around, all the silver would be there as inorganic silver sulfide complexes, and that would be the end of it.

A. Yes, I would imagine that it's probably either a discrete silver sulfide colloid or cluster, or else a mixed metal sulfide with iron. That's what I'm imagining.

Q. Have you thought of using an electrode to measure the sulfide? I think it may be able to detect those concentrations.

A. That is definitely a possibility.

Q. CHRIS WOOD (McMaster University): Kind of a naive question from a fish guy, not a chemist, but if in fact you've got all that sulfide there, why don't you get silver sulfide precipitating?

A. Well, I imagine that it's probably an issue of size. You may have silver sulfide precipitates but they're so small that they're still carried in the water column, or they're actually still soluble because of their size.

Q. It's a question of the size definition then?

A. Yes.

Q. Would you say that most of your silver there is speciated onto dissolved matter, or is it as a sulfide?

A. I believe that indirect evidence indicates it's there as a sulfide.

Q. Thanks.

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## **The Potential Role of Reduced Sulfur in the Dissolved Speciation of Ag in Fully Oxygenated Waters**

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The chemical form of dissolved heavy metals controls their toxicity and bioavailability in aquatic ecosystems. Depending on its speciation, a metal like Cu can be either toxic, a sufficient micronutrient, or limiting to the growth of plankton and other organisms. Metal bioavailability depends on free ion activity (Hare and Tessier 1996), which is controlled by the abundance and strength of dissolved ligands that can bind the metal. Past research in oxic fresh water environments has focused on complexation by natural organic matter and inorganic anions, while silver-sulfide complexes have been overlooked, even though mere traces of sulfides should dominate speciation of this heavy metal, according to thermodynamic modeling. Interestingly, sulfides do occur at nano- and picomolar concentrations in the ocean (Ciglenecki and Cosovic 1996; Kuwabara and Luther 1993; Luther et al. 1991; Luther and Tsamakis 1989; Millero 1991; Walsh et al. 1994). No equivalent data exist for rivers, where multiple potential sources, large binding constants, and slow oxidation rates could facilitate the persistence of metal-sulfide complexes. Reported here are voltammetric measurements documenting the common occurrence of metal-sulfur species at nanomolar concentrations in natural oxygenated river waters. At the measured levels, the sulfur complexes strongly influence trace metal speciation and should dominate Ag. Presumably, binding by sulfides renders the metals unavailable and nontoxic to aquatic organisms (Walsh et al. 1994), just as acid volatile sulfides in sediments afford protection to benthic organisms (Di Toro et al. 1990).

Our research was designed to identify and quantify metal-sulfur species in oxic river water. Several rivers in Connecticut, USA, were analyzed for sulfide complexes by two independent means. Cathodic stripping square wave voltammetry (Luther and Tsamakis 1989) with a rotating disc electrode identified and quantified metal-sulfide complexes, while coprecipitation/methylene blue spectrophotometry measured total sulfides less thiols [Amer. Public Health Assoc., 1992 #2395] and verified the sulfide mass balance. These methods were augmented by separations consisting of: 1) ultrafiltration (3,000 MW cut-off) for size fractionation into colloidal and truly dissolved forms, and 2) resin chromatography (DEAE) to partition species into organic and inorganic fractions. Clean techniques were used for parallel trace metal measurements (Ag, Cu, Fe, Mn, Pb, and Zn) on all size fractions.

Our results indicate that in oxic surface waters total dissolved sulfides are commonly present in low nanomolar quantities, equal to or greater than the metals with which they interact.

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The persistence of sulfides in these oxygen-saturated rivers is probably aided by the formation of metal-sulfide complexes, especially CuS. The sulfide complexes were quantified based on calibration curves created with laboratory-generated metal-sulfide standards, and a comparison was made with the corresponding total trace metal concentrations to obtain the percentage of total metal bound to sulfides (Table 1). In general, this proportion followed the trend Cu, Pb > Zn, similar to the order of the stability of their sulfide solids. This sequence may also be influenced by the high concentrations of dissolved Zn relative to that of sulfides in the water. For Cu and Pb, most of the total dissolved metal was bound to sulfides, on average.

TABLE 1: Trace metal and sulfide data for four Connecticut rivers in February 1997

RIVER	Cu						Pb					
	Dissolved		S-Complexes		Complexed		Dissolved		S-Complexes		Complexed	
	< 0.45 $\mu\text{M}$	< 3000 MW	< 0.45 $\mu\text{M}$	< 3000 MW	< 0.45 $\mu\text{M}$	< 3000 MW	< 0.45 $\mu\text{M}$	< 3000 MW	< 0.45 $\mu\text{M}$	< 3000 MW	< 0.45 $\mu\text{M}$	< 3000 MW
	(nM)	(nM)	(nM)	(nM)	%	%	(nM)	(nM)	(nM)	(nM)	%	%
HAMMONASSET												
Low development	5.1	2.1	2.8	1.1	55	52	0.2	0.1	n.d.	n.d.	-	-
PAWCATUCK												
Headwaters	9.4	3.1	5.1	1.8	54	58	0.5	0.2	0.2	n.d.	38	-
Medium developm	7.5	3	5.2	2.2	70	80	0.6	0.4	0.4	n.d.	68	-
QUINNIPIAC												
Headwaters	14.3	8.3	9.2	4.2	64	50	0.6	0.4	0.3	0.28	57	70
High development	32	15	13.3	2.1	41	15	0.8	0.5	0.5	0.36	62	72
NAUGATUCK												
Headwaters	6.2	3	1.5	0.6	24	20	0.3	0.18	0.1	0.1	26	66
High development	50.0	24.0	8.2	1.2	16	5	0.5	0.3	0.3	0.2	60	67

Voltammetry shows that three main species classes tend to dominate sulfide complexation: weakly bound transition metal-bisulfides, thiols, and strongly bound Zn and Cu sulfides. Cu and Zn sulfide complexes were found with stoichiometries of both MS and  $\text{M}_2\text{S}_3^{2-}$ . The metal-bisulfides, whose weak stability is evidenced by electrochemical lability even under diffusion-limited conditions, are probably dominated by Fe (and Mn) complexes with stoichiometries of  $\text{M}(\text{SH})^+$ . A low abundance sulfide species, identified as  $\text{PbSH}^+$ , was not labile under diffusion-limited conditions.

All complexes, with the exception of the metal bisulfides, were removed to a significant extent by both DEAE columns and ultrafiltration. This surprising result suggests that some metal-sulfides are present as large, possibly organic molecules. Perhaps they form ternary complexes with natural dissolved organic matter, which is moderately abundant in all these waters (Table 2). Another possibility is that amorphous or crystalline metal sulfides make up this colloidal size fraction.

The majority of dissolved reduced sulfur is believed to be bound to relatively abundant iron as bisulfides and thiols, while most of the trace metals are present as complexes with reduced sulfur. This can occur because, in the waters tested, the concentrations of dissolved substances follow the order  $\text{Fe} > \text{Mn} > \text{S}(\text{II-}), \text{Zn} > \text{Cu} > \text{Pb}$ . The larger binding constants of the less

abundant trace metals ensure a high degree of complexation of them by sulfide. Though not measured directly, this should be especially true of Ag.  $\text{MSH}^+$  (Fe, Mn, Pb) and  $\text{M}_2\text{S}_3^{2-}$  (Cu, Zn) were present in all measured river waters; however, high thiol concentrations sometimes masked detection of the  $\text{PbSH}^+$  complex (due to the close proximity of their stripping potentials). The abundance of specific complexes of trace metals and reduced sulfur varied from site to site and was correlated with ambient conditions.

Presumably, complex stability helps to prevent rapid oxidation of the sulfide despite full oxygen saturation of the water. In a laboratory test, naturally occurring sulfides in a fresh river water sample were monitored in a stirred beaker that was left open to the atmosphere. Sulfides declined over time, but were still measurable up to 200 hr after the experiment was started. The loss rate did not follow any simple kinetic pattern, but decreased most rapidly in the first 25 hr and much more gradually thereafter. The presence of truly dissolved sulfides in the rivers (defined as passing a 3000 MW ultrafilter) also suggests that the thermodynamically favorable precipitation of metal sulfide minerals is kinetically hindered during the residence time of the waters (1 - 2 d). Additional factors such as background chemistry and extent of watershed development influence the size fractionation and composition of sulfide complexes.

Ag-S complexes in natural waters were too low to be measured directly by the stripping square wave technique. This is almost certainly a consequence of the low level of dissolved Ag in the waters we measured, rather than the absence of such complexes. Thermodynamic modeling indicates that all of the dissolved Ag in these rivers should be present in the form of complexes with reduced sulfur. Although metal-sulfide thermodynamic data are notoriously unreliable, Ag-S stability constants would have to be lower than published values by many orders of magnitude for sulfides not to dominate Ag speciation at equilibrium under the conditions we measured.

Formation of metal sulfide complexes reduces the concentration of the free dissolved form of heavy metals. Since free ionic metal is the bioavailable form (Hare and Tessier 1996; Morel and Morel-Laurens 1983), sulfide complexation should reduce dissolved metal toxicity. Sulfides in the water column thus may serve the same protective function as do acid volatile sulfides (AVS) in sediments (Ankley et al. 1994a; Ankley et al. 1991; Ankley et al. 1994b; Carlson et al. 1991; Casas and Crecelius 1994; Di Toro et al. 1990; Leonard et al. 1995; Pesch et al. 1995). Our findings suggest that a rethinking of trace metal speciation may be necessary, since previously ignored sulfide complexes may exert a key influence on trace metal cycling in many oxic riverine ecosystems.

TABLE 2: Ancillary water chemistry

RIVER	pH	DOC (mgC/l)	DO (mM)	Total Sulfides (nM)
HAMMONASSET				
Low development	6.7	3.5	497	38
PAWCATUCK				
Headwaters	5.2	4.7	466	121
Medium development	6.3	3.8	441	128
QUINNIPIAC				
Headwaters	7.3	2.6	475	156
High development	7.2	3.3	463	259
NAUGATUCK				
Headwaters	7.3	1.8	494	53
High development	7.0	2.1	478	190

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## Questions & Answers: The Potential Role of Reduced Sulfur in the Dissolved Speciation of Ag in Fully Oxygenated Waters

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- Q. JEAN-FRANCOIS GAILLARD (Northwestern University): When you do the voltammetry, do you have to remove the oxygen one way or another?
- A. Yes we do.
- Q. Okay, so you can lower substantially the concentration of sulfide because it's volatile, and it depends on the pH of the solution you start with. Do you buffer the solution in some sense?
- A. Yes. You're going to lose  $H_2S$  but you're not going to lose metal sulfides.
- Q. The colloidal, the small particles...
- A. Well, whatever it is, they are not going to be dissociating on a time scale that's rapid enough in terms of the bulk solution, in order to lose them. Well - it's possible that indeed sulfide levels are even higher than this, but these would be a lower limit. Let's put it that way.
- Q. Okay, and do you buffer the solution when you do the assay?
- A. Yes, but I've forgotten which buffer we settled on in the end.
- Q. Okay. Because if you don't you go to very high pH, then it's going to be pretty bad for the whole system.
- A. Well, as you might have seen on that earlier slide, depending on what pH you're at, a given peak could represent different metals, so we actually titrate these across pH.
- Q. I was really puzzled by the first graph that you mentioned, of pH range and the potential. But we need to talk about that later.
- A. Okay.
- Q. PETER SANTSCHI (Texas A&M University): I was surprised you didn't mention iron. When you have higher amounts of iron, wouldn't you expect that iron would also be bound to some of those sulfides or thiols?
- A. Yes. I'm sorry, I don't know why we didn't have that up there. We do measure iron. Iron levels are quite high and there are iron bisulfide compounds present that we measure as well. Insignificant amounts. But they're somewhat less interesting to me.
- Q. NICK FISHER (SUNY-Stony Brook): In the presence of particles -- of course there are particles in natural waters -- you typically see, for example for silver and for some of these other metals as well, fairly high partition coefficients, or  $K_d$  values. For silver in marine systems it's on the order of  $10^5$  or so. If all this silver is speciated as the sulfide, I don't understand then how you would get so much of the silver on particulate matter, unless you're actually measuring silver sulfide on your particulate.
- A. Well, it may well be that there are silver sulfides or iron sulfides with silver on them in solid phase, and I've taken this in isolation in some sense. We haven't measured on the particle phase what sulfides are present and so we filter that out at the beginning. And even if we have a lot of silver sulfide, the total amount of silver present in the dissolved fraction is still quite small, so we're not going to get nanomolar silver sulfide. We're still getting only low picomolar amounts.
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- Q. Okay, let me understand: if the silver is speciated as the sulfide, is the silver that's complexed that way available for sorption onto suspended particles, phytoplankton, or abiotic particles?
- A. I have no idea.
- Q. DOMINIC DI TORO (Manhattan College): I had the same kind of question. You're detecting electrochemically free sulfide, right? So there's no ambiguity about particulate clusters, magic, whatever. Is that true?
- A. No, I wouldn't say that.
- Q. Oh, too bad. I thought we'd finally get to the bottom of this. At long last.
- A. The metal sulfides are reacting at the mercury film.
- Q. So they're electrochemically labile, to use the "weasel words" that the electrochemists use. Okay, so when you make your equilibrium calculations, I presume you assume that what is electrochemically labile can be rearranged in the equilibrium computation. Is that what you do?
- A. Yes.
- Q. And under that set of assumptions, 100 percent of the silver should be complexed to sulfide species all of the time.
- A. That's what I would expect, yes.
- Q. That's what comes out. The further conclusion, then, would be that, if silver in silver sulfide isn't bioavailable in some way...
- A. Well, wait a minute, there's one caveat there. We're not measuring silver sulfide every time we're measuring, say, copper sulfide. Indeed, the silver should be able to outcompete the copper for the sulfide, but the question is, when will it do that?
- Q. Yes, in fact a subsidiary comment would be that it would be interesting just to add some silver to the solution and go through your drill again and see if what you expect to happen actually happens. But, if then we go one step further, which is that silver sulfide ought not to be biologically available or toxic. One would conclude, based on your calculations, that at least in these waters silver is biologically inert.
- A. Yes.
- Q. Thank you.
- A. So this is sort of an AVS of the water column.
- Q. That's exactly where I was going, Gabe – that's what I was going to say. *(laughter)*
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## Silver Partitioning in Effluents of Waste Water Treatment Plants

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We investigated the removal and partitioning of silver in five waste water treatment plants. Comparisons were made with several other metals. Removal was calculated from measurements of influent and effluent metal concentrations. Partitioning among particulate, colloidal, and filtrable fractions was measured using micro filtration and ultrafiltration techniques. Removal by the treatment plants was higher for silver (> 94%) than the other metals measured. Among metals, removal efficiency (%) was related directly to the sorption partition coefficient ( $K_d$ ), indicating that removal efficiencies were controlled mainly by metal sorption to particles removed by settling and/or filtration within the treatment plant. Within plant measurements confirmed the removal of silver during key stages of treatment. The percentage removal of total silver tended to be independent of influent concentration, consistent with a linear partitioning model. Thus, higher influent concentrations were associated with correspondingly higher effluent concentrations of silver, and effluent silver concentration (0.06 to 2.6  $\mu\text{g L}^{-1}$ ) were orders of magnitude higher than typical "background" silver concentrations in streams (< 5  $\text{ng L}^{-1}$ ). Suspended particulate matter concentrations in effluents tended to be relatively low (2 to 15  $\text{mg L}^{-1}$ ), and a substantial fraction (about 25 to 70%) of silver in effluents was contained in the filtrable (< 1.0  $\mu\text{m}$ ) fraction. Similarly, a high proportion (40 to 85%) of filtrable silver (< 1.0  $\mu\text{m}$ ) was associated with sub-micron particles and colloids, and the fraction of "dissolved" silver (< 0.05  $\mu\text{m}$ ) was relatively small. Ultrafiltration of one effluent showed that only 2% of the total silver was in the < 10K nominal molecular weight range. Furthermore, the percentage of "dissolved" silver (< 0.05  $\mu\text{m}$ ) was related directly to the concentration of dissolved organic carbon (DOC), suggesting that "dissolved" silver was associated significantly with organic ligands. Sorption partition coefficient ( $K_d$ ) values for silver ( $\log K_d = 5.6$  to  $6.7$ ) were higher than for other metals, consistent with the high removal efficiencies observed for silver. Silver partition coefficients in effluents tended to be higher for larger particles than for sub-micron particles and colloids. However, in streams where effluent dilution was substantial, this trend was reversed. In these cases, stream colloids exhibited a higher affinity for silver than shown by larger particles.

We conclude that silver is efficiently removed by waste water treatment plants and thereby concentrated in the sludge generated by treatment. Effluent silver concentrations are substantially higher than "natural" silver levels. However, association of effluent silver with

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particles, colloids, and "dissolved" organic ligands likely reduces the bioavailability of effluent silver. Furthermore, silver levels in receiving streams are reduced by incorporation into stream sediments as well as by dilution. Although operational  $K_d$  values for modeling silver partitioning to particles and colloids have been obtained, additional work is needed to characterize the properties of particles and colloids controlling the binding of silver. Similarly, the nature of organic ligands (DOC) which apparently bind "dissolved" silver remains uncertain. Details of this investigation are reported in "Removal, Partitioning, and Fate of Silver and Other Metals in Waste Water Treatment Plants and Effluent-Receiving Streams" by M.M. Shafer, J.T. Overdier, and D.E. Armstrong. Environ. Toxicol. Chem.(accepted). 1997.

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## Questions & Answers: Silver Partitioning in Effluents of Waste Water Treatment Plants

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Q. PETER SANTACHI (Texas A&M University): You showed percent removal for different metals as a function of  $\log K_d$ , correct?

A. Yes.

Q. You would expect a highly non-linear relationship but yet you just drew a straight line. Just looking at the data, a non-linear curve is what you would expect.

A. It might be.

Q. RUSS BELL (McMaster University): This is sort of a hypothetical question, David. If you were to take, say, 100 mL of your 10 kilodalton fraction and lyophilize it, say down to 1 mL, would you expect more aggregation of your particles?

A. I think it's certainly likely, if you have colloids in suspension, that as you concentrate the system, the tendency for those to cluster is going to be greater. When we did the ultrafiltration experiments, we tried to keep the fraction by which we reduced the concentration relatively low, in order to at least try to minimize the possibility that we were forming colloids by concentrating.

Q. DAVID SEDLAK (University of California-Berkeley): Could your correlation's that you observed between DOC and removal of silver in the effluent possibly be related to how the treatment plant was operating that day, and could there be a correlation between DOC and sulfide coming out of the plant? Do you have any evidence for that?

A. We don't have any data on sulfides. Certainly one of the questions that we have in our own minds is whether the relationship is either to inorganic or organic sulfur. We didn't see a lot of variability between different times in the plant, so whatever is going on seems to be fairly consistent and not highly sensitive to how the plant was operating on a particular day. But we have a limited number of visits to these plants to base that on.

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## **Complexation of Silver by Macromolecular Organic Sulfur Complexes in Estuarine Waters of Galveston Bay**

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### **Abstract:**

Studies are currently being conducted which have the objectives of gaining a better understanding of the degree of organic complexation of Ag in fresh and estuarine waters by relating the ambient concentration of stable Ag in colloidal organic matter (COM) to the concentration of reduced S groups in macromolecular organic matter. Here, we report preliminary results from experimental studies using Trinity River and Galveston Bay colloids isolated using cross flow ultrafiltration. Natural organic sulfur compounds, to which small amounts of Ag and a fluorescent tag had been added, were separated by HPLC and eluent fractions were collected. S was determined by fluorescence detection, while Ag in the same fractions was determined by GF-AAS. Ag was found in a single well defined peak in the more hydrophobic region of the HPLC spectrum coinciding with a relatively minor thiol peak. No Ag was found in the more hydrophilic fractions, indicating that all Ag was complexed by ligands of a small fraction of hydrophobic natural organic matter. Since thiols in COM from Galveston Bay are not oxidized for at least one day, and since they are considerably more stable towards oxidation by air-O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> or UV than low molecular weight thiols, further laboratory tests to study the binding of Ag to natural thiols should be promising.

### **Introduction**

Complexation of Ag with macromolecular organic matter reduces the free ion concentration of silver in the water, and thus, its potential toxicity and bioavailability. It is therefore important to better understand organic complexation of Ag which can be expected to be related to the concentration of different reduced sulfur (e.g., thiol) and other potential electron donor groups in colloidal organic matter, COM.

In estuarine waters of Galveston Bay, we previously showed that a large fraction of Ag is bound to macromolecular organic matter >1kDa (COM<sub>1</sub>) with sizes larger than 1nm

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(Wen et al., 1997a), which makes it susceptible to coagulation (Wen et al., 1997b) on time scales of hours to days. Relationships of colloidal Ag with Fe suggested that they both were controlled by the concentrations of reduced sulfur compounds (Wen et al., 1997a).

We report here some initial results from experiments where the concentrations of reduced sulfur compounds in COM<sub>1</sub> were determined by voltammetry (Ciglonecki and Cosovic, 1996), different thiol compounds separated by HPLC after adding a fluorescent tag and which allowed them to be quantified by a fluorescence detector, and the resulting spectrum compared to that of Ag (added at the beginning to the same COM<sub>1</sub> fraction) measured by GF-AAS.

### **Experimental approach & analytical procedures.**

(1) 12 mg (~5mM of C and 5.7 pmol of Ag) of COM<sub>1</sub> was dissolved in 1000 µl of 0.1 M borate buffer (pH=9.5); (2) 100 µl of this buffered sample was transferred into a 1 ml vial; (3) 300 µL of 0.1M borate buffer, 100 µl of 1.5 M NaOH, and 100 µl of SBD-F conjugate (fluorescent tag) reagent were added to the sample vial; (4) sample was incubated at 60°C, until a yellow color developed; (5) samples were transferred into an autosampler, (6) samples for later Ag analysis by GF-AAS were hand collected. Note that 170 fractions were collected in a 35 min run, resulting in 5 vials/min.

The HPLC we used consisted of the following components. (1) Waters 510 pump, (2) Waters 717<sup>+</sup> autosampler, (3) Waters 474 scanning fluorescence detector, (4) Phenomenex Ultracarb chromatography column, (5) Phenomenex C18 presaturator (guard) column, (6) Mobile phase: 0.36 M acetate buffer, pH 3.8 with 1% methanol, and (7) cysteine, homocysteine, albumin, glutathione, and penicillamine were used as standard compounds.

Oxidation rate experiments with 2µM Na<sub>2</sub>S, low molecular weight (LMW) thiols (e.g., 3-mercaptopropionic acid, l-cysteine) and natural colloids preconcentrated by cross flow ultrafiltration, were carried out with UV irradiation (1mM of organic molecules or colloidal concentrates in teflon bottles were irradiated for 24 hours) and 0.2 mM of H<sub>2</sub>O<sub>2</sub>. Reduced sulfur concentrations were determined by square wave stripping voltammetric analysis (Ciglonecki and Cosovic, 1996).

### **Results and Discussion**

Initial results from HPLC separations are shown in Figures 1-2. They show that most thiol compounds in natural colloids were found in the more hydrophobic region of the

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chromatogram. Retention times for S in natural COM did not coincide with any of the standard compounds used except, at times, for homocysteine.

Figure 3 shows the Ag concentrations measured in the same eluent fractions as the thiols shown in Figures 1-2. Ag was found in a single well defined peak in the more hydrophobic region of the HPLC chromatogram, coinciding (in terms of retention time) in one of the samples (Figure 1, GB-Colloids) with a relatively minor thiol peak. No ionic Ag was found in the more hydrophilic fractions. A comparison of Ag added to the experimental solution (0.57 pmol) to the total recovered Ag in the main peak regions (0.51 and 0.09 pmol) indicates that all Ag added to the experimental solution was recovered, and found to be complexed by, most likely, strongly chelating ligands present in the more hydrophobic fraction of the macromolecular organic matter. While 0.57 pmol of the Ag in the peak region was present, ~7 nmol of S was contained in the small thiol peak with the same retention time as that of the Ag, indicating that only 1 in  $10^4$  S molecules was complexed with Ag. Furthermore, the HPLC results of these rehydrated freeze-dried colloids suggest a higher thiol concentration than we obtained by voltammetric methods (see below), i.e., a C:S ratio of about  $10^3$ . Given this ratio, Galveston Bay's waters should contain total thiol concentrations in COM of about 200 nM.

Concentrations of total organically bound sulfur in COM isolated from Galveston Bay's waters using cross-flow ultrafiltration followed by diafiltration and  $\text{SO}_4$  correction, are close to 1 wt % (C:S of ~300), similar to concentrations of S in humic compounds (Guo and Santschi, 1997), suggesting a total organic S concentration of up to 0.5-1  $\mu\text{M}$ . Determination of reduced sulfur compounds using voltammetry (square wave stripping analysis, Ciglenecki and Cosovic, 1996) resulted in a total reduced sulfur concentration of only about 10-20 nM, which is a few % of the value obtained by HPLC. Regardless of the methodology, however, this would indicate that most S in  $\text{COM}_1$  is present in oxidized forms.

Oxidation experiments to test the partial oxidation of reduced organic sulfur species using UV irradiation and  $\text{H}_2\text{O}_2$  revealed that reduced S in natural colloids, as measured by square wave stripping voltammetric analysis, is more resistant to oxidation than low molecular weight thiols such as 3-mercaptopropionic acid. UV irradiation totally oxidized 3-mercaptopropionic acid within 24 hours, while during the same time, the voltammetric sulfur peak of natural colloidal matter just became broader with time (indicating the production of new species), but the peak integral remained the same. Similarly, oxidation rates by 0.2 mM  $\text{H}_2\text{O}_2$ , produced a sequence of decreasing oxidation rates  $\text{HS}^- > \text{LMW thiols (e.g., 3-mercaptopropionic acid, l-cysteine)} > \text{natural colloidal thiols}$ . Furthermore,

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reduced S in solution is stabilized by transition and B metal ions. After one day, there was less than a 10% change in peak height of reduced S of 100nM of 3-mercaptopropionic acid with 100 nM of ZnCl<sub>2</sub> in 0.5 mM NaCl solution after the sample was opened to air, and no change for DL penicillamine.

### Summary and Conclusions

We were able to clearly show that different thiol compounds are important components of COM in Galveston Bay water. Using HPLC methodology, we found that thiols constitute about 20-40% of all organic sulfur in the water column. While it was possible to clearly separate organically bound Ag, added to the isolated COM<sub>1</sub>, and match its position in the spectrum (i.e., retention time) to one of the thiol fractions, more work remains to be done to unequivocally relate the binding of Ag to thiol groups in macromolecular organic matter.

Results from experiments testing the tendency of reduced S groups to oxidize by air-O<sub>2</sub>, H<sub>2</sub>O<sub>2</sub> or UV radiation showed that it decreases in the order HS<sup>-</sup> > LMW thiols; most of the thiols in COM from Galveston Bay did not oxidize after 24 hours of UV irradiation, suggesting that the thiol groups of COM are quite robust. Furthermore, reduced S in solution is stabilized by transition and B metal ions.

### Acknowledgements

This work was, in parts, supported by grants from the Photographic and Imaging Manufacturers, Inc., PIMA, the Office of Naval Research, ONR, and the Texas Institute of Oceanography, TIO.

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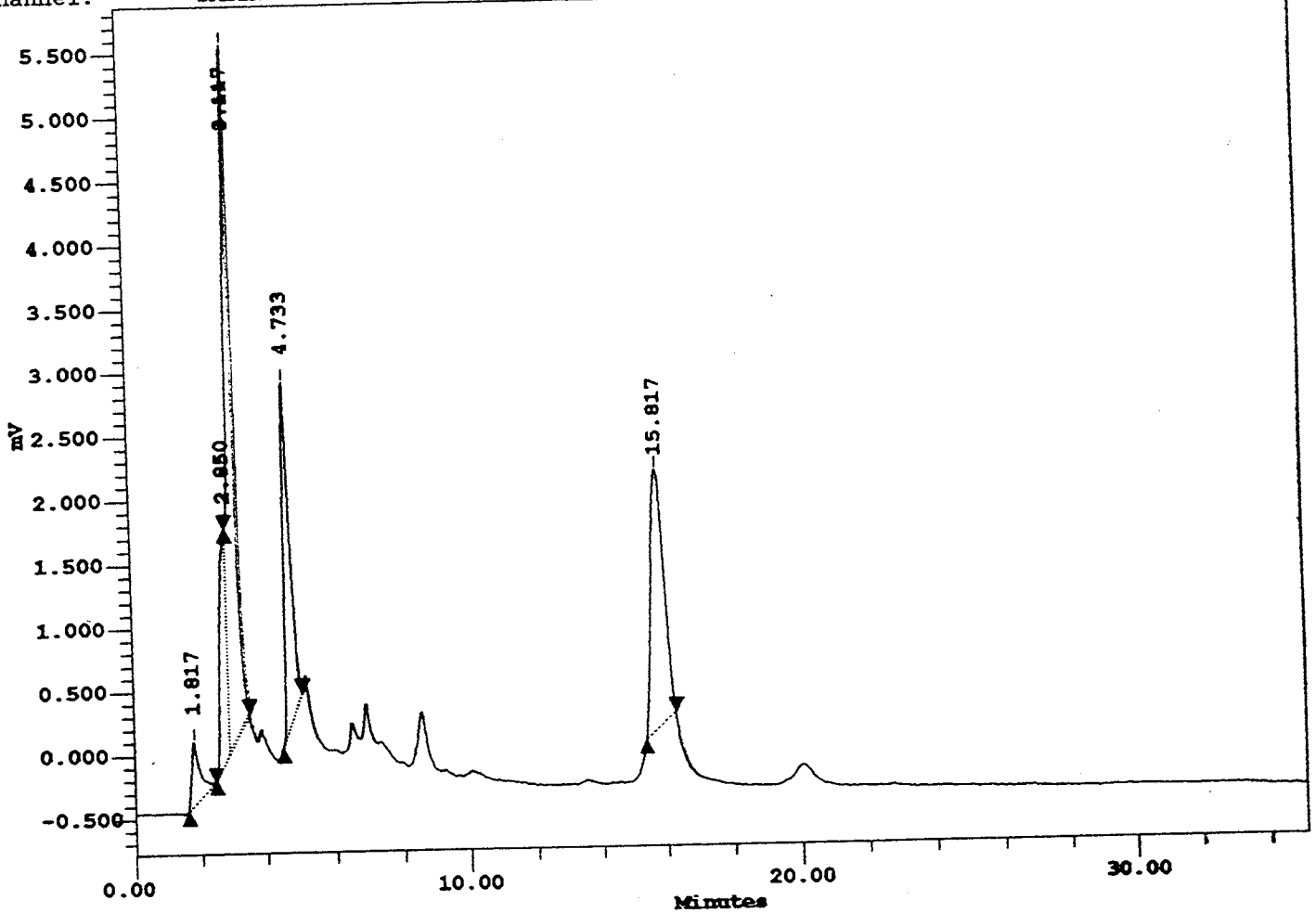
**Figures**

- Figure 1. HPLC Chromatogram of thiols in COM collected from the Trinity River mouth, Hwy 10 bridge, in Houston.
- Figure 2. HPLC Chromatogram of thiols in COM collected from Galveston Bay, off the Texas City Dyke.
- Figure 3. HPLC Chromatogram of Ag in COM collected from both Galveston Bay and Trinity River. 170 vials = 35 minutes.

Spectracell Sample Information

Project Name: HCY\_SEPT2  
 Sample Name: GB-Colloids  
 PatientName:  
 Vial: 13  
 Injection: 1  
 Channel: SATIN

Set Name:  
 Sample Type: Unknown  
 Volume: 100.00  
 Run Time: 35.0 min  
 Date Processed: 09/21/97 01:38:56 PM  
 Date Acquired: 09/21/97 01:03:40 PM



*Peak Results*

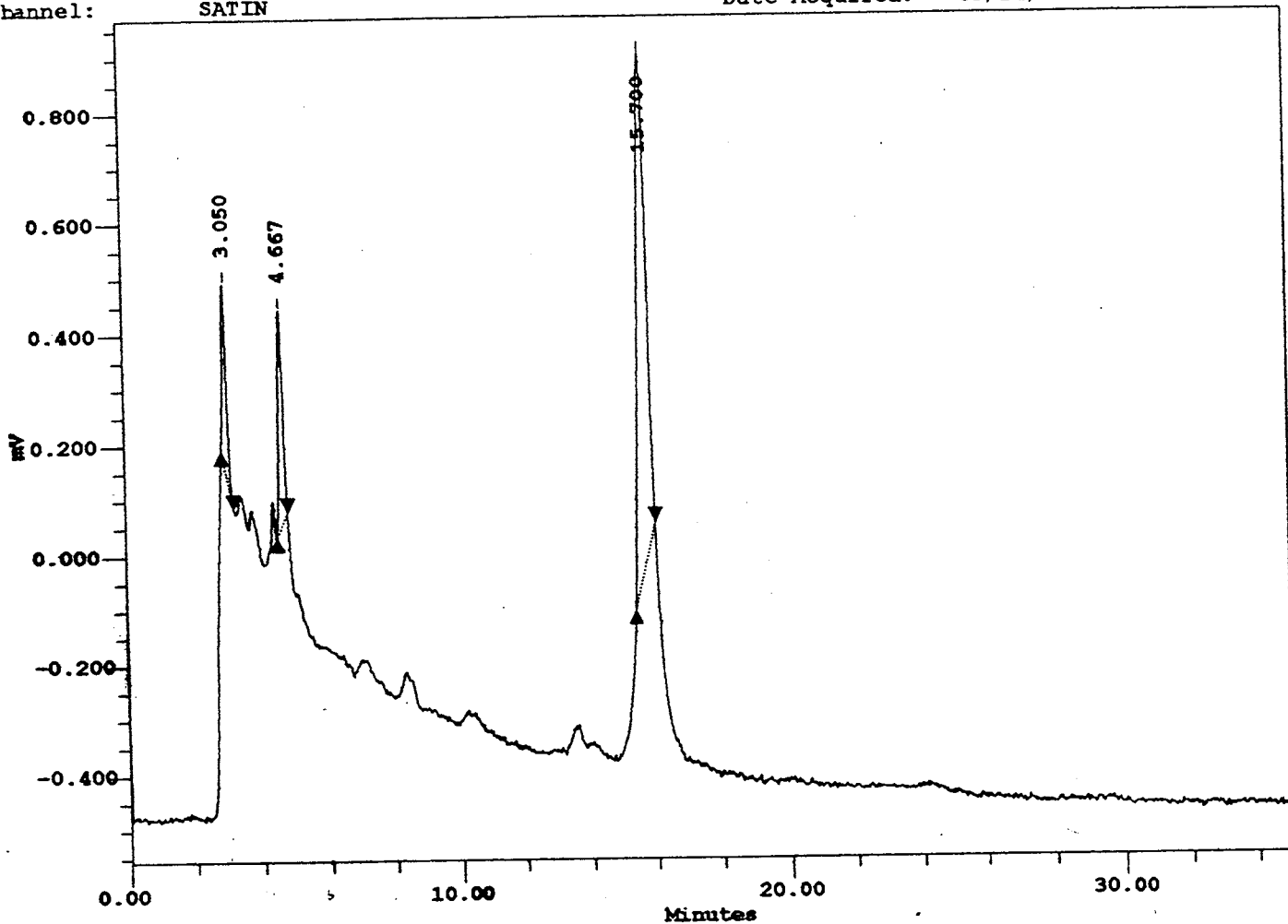
#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	cysteine	4.350				Missing
2	homocysteine	5.900				Missing
3	cys-gly	6.600				Missing

Figure 1

Spectracell Sample Information

Project Name: HCY\_SEPT2  
 Sample Name: TR-Colloids  
 PatientName:  
 Vial: 11  
 Injection: 2  
 Channel: SATIN

Set Name:  
 Sample Type: Unknown  
 Volume: 20.00  
 Run Time: 35.0 min  
 Date Processed: 09/21/97 11:44:44 AM  
 Date Acquired: 09/21/97 11:09:26 AM



*Peak Results*

#	Name	Ret Time (min)	Area (uV*sec)	Height (uV)	Amount	Int Type
1	cysteine	4.350				Missing
2	homocysteine	5.900				Missing
3	cys-gly	6.600				Missing

Figure 2

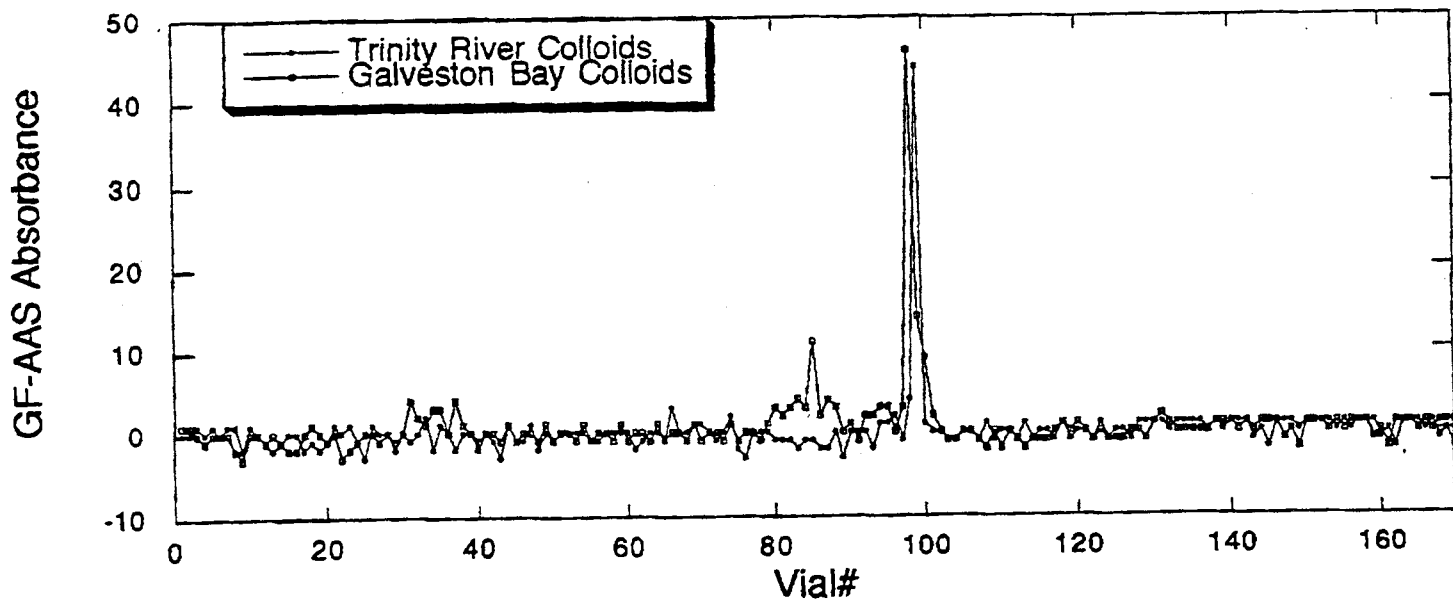


Figure 3

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## Questions & Answers: Complexation of Silver by Macromolecular Organic Sulfur Complexes in Estuarine Waters of Galveston Bay

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- Q. DOMINIC DI TORO (Manhattan College): Peter, you found something very interesting. As you pointed out, the silver all came out in one sort of minor peak on the HPLC, which is somewhat counterintuitive. I'm sure you didn't expect that, because you've got a lot of sulfhydryl groups all mixed up along the whole scan, and one would have expected, with the stories we've been hearing about the importance of sulfhydryl groups and so forth, that it should have been tagging right along the whole scan. But it didn't do that.
- A. Yes.
- Q. Which is very puzzling. Yes?
- A. Which is puzzling. And of course, we are just at the beginning of it, so the silver was added before the compounds were tagged. So you would think it should have distributed over all the ligands, but yet we found there was just one ligand which got it all.
- Q. Yes, it's quite remarkable.
- Q. KEN ROBILLARD (Eastman Kodak): Peter, two quick questions. First of all, how much influence do you think this silver has on the HPLC chromatography, and could we be maybe jumping to some conclusions there with regard to the narrow dispersity of the silver-organosulfur compounds? Secondly, assuming that the dispersity is very narrow, how does that relate to, or how do you justify that with regards to, the ASV work, which suggests a polydispersity of the silver-organothiol compounds?
- A. I start from the back. I don't know what you mean by the polydispersity of this.
- Q. The broadness you mentioned...
- A. Could you clarify the question? I'm not sure, because the thiols were polydisperse, whereas with the silver we don't really know.
- Q. When you showed the ASV results, you pointed out that the breadth of the peak was due to the fact that you had different constituents.
- A. Oh, you mean the voltammetry, yes. Well, the spectra which I showed you included peaks where you had just sulfides, polysulfides and thiols. But when those groups are bound to metals, the peaks shift to the left on the spectrum, which I showed you, and you get basically broad peaks. But the metals there are not silver. The metals there are iron, copper, what have you. They are much more abundant and they will have more influence. And so it has to be something which complexes a major fraction of the thiols, and as I showed you, even when you add silver to the mixture, the ambient silver concentrations are very low. So when you add picograms of silver, you still have a sulfur - to - silver ratio on the order of  $10^4$ , so it wouldn't show up there. And also the voltammetry methods are not sensitive enough to measure any silver.

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## Silver – DOM Interactions in Natural Waters: Preliminary Results

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### Introduction

Growth in concern regarding the ecotoxic effects of metals in aquatic systems has been paralleled by research indicating that their effective toxicity is strongly affected by their chemical forms. Different chemical species of a given metal can in addition have very different transport properties in surface water and groundwater. Silver (Ag) is no exception, and recent work (Shafer et al. 1996, Wen et al. 1996) has shown the importance of particles and colloids in Ag speciation. An aspect of Ag speciation of potential importance, but about which little is known, is the effect of dissolved organic matter (DOM) on its speciation. This project will attempt to characterize interactions of Ag with DOM, using competitive ligand techniques.

In competitive ligand experiments designed to investigate Ag speciation, a known amount of a (usually organic) ligand with a well-defined complexation constant with Ag is added to a natural water sample. The sample is then titrated with Ag, and the added ligand, including that which has formed a complex with Ag, is separated from all other forms of Ag. This separation can be performed electrochemically, via solvent extraction, or by filtration if the competing ligand is a functional group on an ion-exchange resin. If the titration curve is a straight line, representing a simple standard addition with Ag, this indicates that no natural ligand associates strongly enough with Ag to compete with the added ligand. If the slope of the titration curve is zero, then natural ligands are out-competing the added ligand for Ag. If the curve has a nonzero slope and is concave upward, this indicates that the added ligand is competing with the natural ligands. From such curves, inferences can be made regarding the Ag complexation constants and concentrations of natural ligands (Miller and Bruland 1995).

Of the three methods mentioned above for Ag speciation determination, solvent extraction and electrochemical methods have been used most extensively. Problems are associated with each of these techniques when they are applied to determination of Ag speciation. Solvent extraction techniques require a competing ligand that can be almost completely extracted into the organic phase, while none of the naturally present ligands can be extractable. Such a ligand-solvent pair can be difficult to find. In addition the techniques are time-intensive, and difficult to perform via clean techniques. The principal variety of electrochemical technique used to perform competing ligand experiments is adsorptive cathodic stripping voltammetry (ACSV). This technique has been used for a variety of metals other than Ag (Donat et al 1994). During a concentration step, the added ligand-metal complex sorbs to a mercury-based electrode. A cathodic voltage is then applied; this reduces the metal and the ensuing current is measured. This technique is unfortunately quite difficult to apply to Ag, since the difference between the reduction potentials of Ag and Hg are very similar and reduction of the Hg electrode masks the Ag signal. It is difficult to find a suitable electrode for ACSV analysis of Ag that provides a sufficiently low limit of detection. Resin techniques offer many advantages that make them potentially useful for Ag speciation studies. Several available resins strongly bind Ag; contaminating metals can be cleaned from resins using strong acids; resins can be cleanly separated from solutions by filtration and rinsing; and resin-associated metals can be desorbed for analysis using strong acids. Based upon these considerations, we are investigating the feasibility of techniques using the functional group on ion

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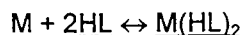
exchange or chelating resins as the competing ligand in studies of this variety. Our initial work has involved use of Chelex resin, which contains an iminodiacetate (IDA) functionality.

### Theory: Resin Chelation

Two mechanisms are possible for the association of metals by iminodiacetate groups in Chelex resin. Metals can form chelates, as in the reaction



or by complexation, as in the reaction



where L is the IDA group bound to the resin, the resin is initially in the hydrogen form, and the charge on the ligand-metal chelate or complex depends upon the valence of the metal ion. At pH 5, copper and nickel are sorbed to Chelex through the chelation mechanism, calcium is sorbed through complexation, and zinc and cadmium are sorbed through a combination of the two. Complexes may form more readily in a resin system than in an aqueous solution of a monomer containing an iminodiacetate functionality; the functional groups are so concentrated in the pore space of a resin that formation of complexes with 1:2 molar compositions may be promoted (Pesavento et al. 1993). The difference between chelation and complexation in terms of molecular structure is shown in Figure 1. Since Ag has been found to form only chelates in aqueous solutions of monomers with the iminodiacetate functionality (Israeli and Pettit 1975), and has an extremely low stability constant with an acetate functionality (Martell et al 1995), it is likely that it will behave more like copper than calcium in its sorption to Chelex. The possibility of complex formation, however, cannot be discounted.

Chelex resin contains pores 1.5 nm in diameter (Apte and Batley 1995), so the extent to which it sorbs metals will be affected by Donnan exclusion effects (Helfferich 1962). Donnan exclusion predicts that ion exchange at a surface will be hindered by a charge imbalance created by the fact that the anionic iminodiacetate group is fixed to the resin surface; the negatively charged resin creates a potential that opposes anion (and as a result anion-cation pair) diffusion into the pore space. The diffusion driving force that causes a net migration of metal ions into the pore space must therefore be balanced against electroneutrality requirements within the pore space, and extent of metal sorption may therefore be lower than solution equilibrium considerations would predict.

### Experimental

Experiments performed to date have addressed three principal questions: (1) which currently available resins have the best characteristics for competing ligand experiments? (2) how can the solution be kept at the sample pH without disturbing Ag speciation in the sample? and (3) do speciation modeling results, based on iminodiacetate-containing monomers in solution, agree with experimental results involving the resin?

#### (1) Resin Selection

The behavior of three resins has been explored. Amberlite IR-118H (Rohm and Haas, Inc.) contains a sulfite ( $R-SO_3^-$ ) functionality, the same functionality as Dowex resin. Chelex 100 (Bio-Rad, Inc.) contains an iminodiacetate ( $R-CH_2N(CH_2COO^-)_2$ ) functionality. And SR-4 (Sybron Chemicals, Inc.) contains a thiol ( $R-S^-$ ) functionality. Both the sulfite and iminodiacetate functional groups have Ag complexation constants in a range that may be useful for competitive ligand

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experiments; their log  $K_1$  values for association with Ag are 5.6 and 4.3, respectively (Martell et al. 1995). Thiol-Ag association may be too strong for use as a competing ligand (log  $K_1 = 13.6$ ). Chelex has been chosen for initial experiments since, compared with Dowex, it has good selectivity for Ag as compared to Ca, which is often present at high concentrations in natural systems. Use of a Dowex-type resin has not been abandoned, however, since its sorption characteristics are less strongly affected by pH than those of Chelex (Apte and Batley, 1995).

## (2) pH Considerations

It is important to consider the effects of pH and pH buffers in performing speciation experiments. Changes in sample pH during the experiment can change the concentrations of species of interest, but pH buffers must also be chosen so that they do not affect speciation; for example, the first stability constant for the Ag-TRIS complex (log  $K_1 = 3.14$ ) is only an order of magnitude less than that of the Ag-iminodiacetate chelate (Martell et al. 1995). Acetate and bicarbonate buffers, however, do not form even moderately strong complexes with Ag. An attempt was made to buffer Chelex, in the Na form, with a sodium acetate/acetic acid buffer before the resin was contacted with a sample solution, similar to the technique of Buckley (1985). Poorly-buffered ( $[\text{NaHCO}_3] = 73 \mu\text{M}$ ) solutions, however, increased from pH 6.8 to pH 9.4-10.3 during a 12-hour contact with Chelex resin. When Chelex was pre-buffered with  $\text{NaHCO}_3$  and solution  $\text{NaHCO}_3$  concentration was increased to 6.3 mM, pH only changed from 8.5 to 8.8 under similar experimental conditions. It appears that  $\text{NaHCO}_3$  will be a suitable buffer for samples in the range of pH from 8 to 9.

## (3) Comparison of Experimental Results to Speciation Modeling

Using well-defined solutions prepared in the laboratory, experiments were performed to determine if speciation in a solution to which Chelex had been added would be similar to chemical speciation modeling using stability constants for iminodiacetate groups attached to alkyl chains. Using the chemical speciation model MINEQL, the effect of increasing Cl concentration on Ag-iminodiacetate chelation was modeled. Experiments were then performed, with solutions identical to those modeled ( $[\text{Ag}] = 93 \text{ nM}$ ,  $[\text{NaHCO}_3] = 6.3 \text{ mM}$ ), and at NaCl concentrations of 0.28 mM and 5.15 mM. Modeling results, as well as the results of GFAAS analysis of acid eluants of Chelex columns are shown in Figure 2. Column eluants contain very high concentrations of Na, and this Na appears to interfere with GFAAS analysis of Ag; as a result only approximate, standard addition analyses could be made, and T-tests comparing the means of the samples at the two different Cl concentrations indicated no significant difference. It is therefore unclear how closely experimental results matched modeling results, and this analytical difficulty will have to be resolved before further experiments are performed.

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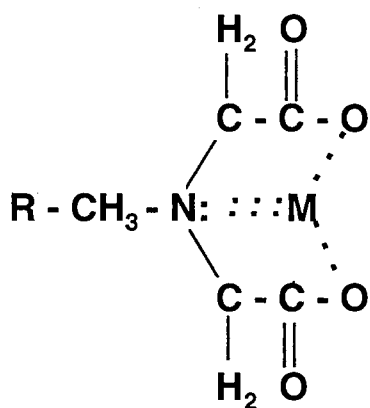
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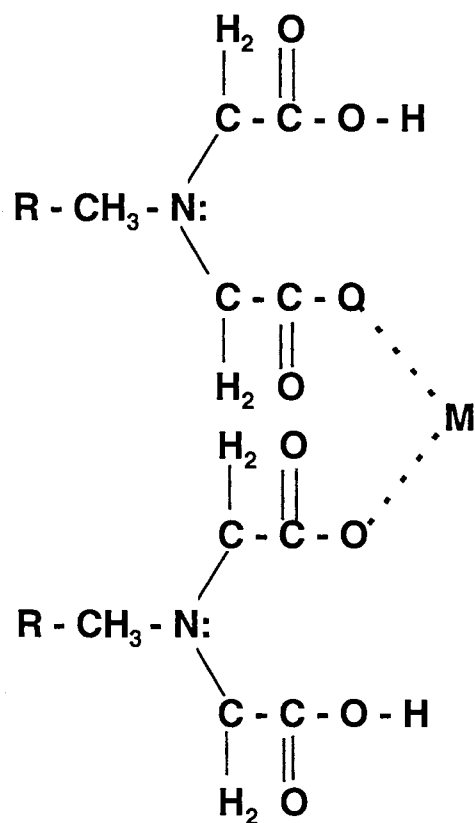
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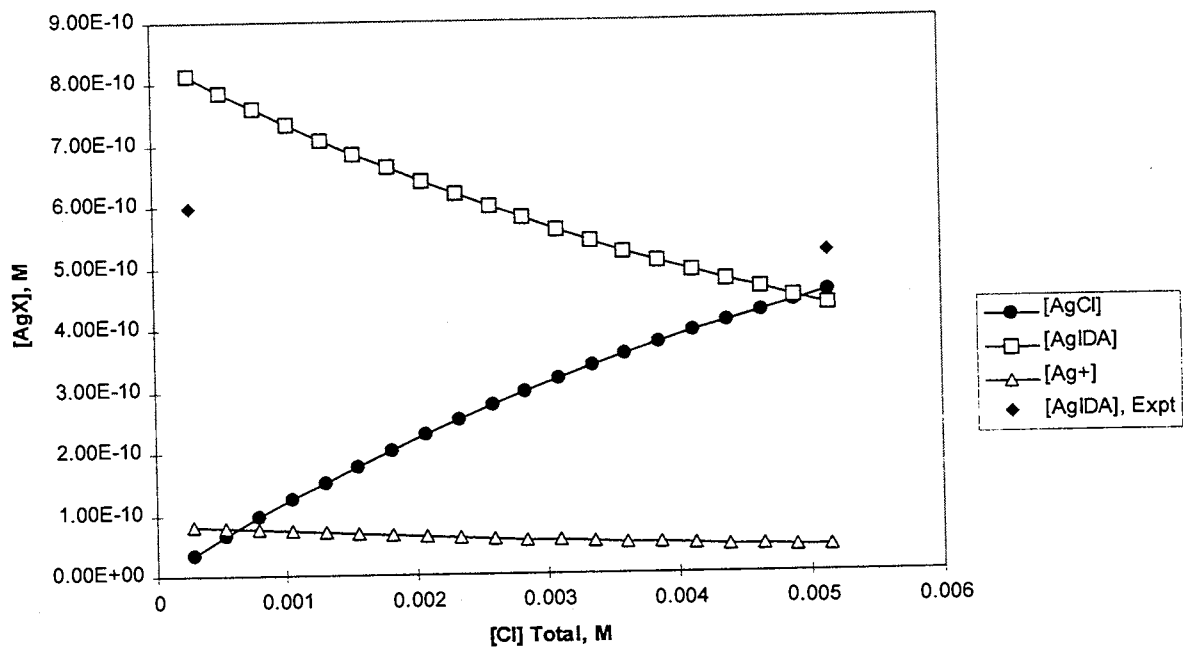


(a)



(b)

**Figure 1:** (a) Chelation of a metal, M, by an iminodiacetate group. (b) Complexation of a metal by two iminodiacetate groups.



**Figure 2:** Comparison of modeling and experimental results, for a system containing Ag, NaCl and Chelex, and buffered with sodium bicarbonate.

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## Questions & Answers: Silver – DOM Interactions in Natural Waters: Preliminary Results

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- Q. LIANG-SAW "CAT" WEN (Texas A&M University): In essence, you are repeating the work of Dr. John Donat from UC Santa Cruz, are you not?
- A. Not exactly. You mean the Donat, Lao and Bruland paper for example?
- Q. Yes.
- A. He was using a technique that I alluded to where he depended on the slow kinetics of metal-DOM interactions and passing samples through resin columns at a very fast flow rate, and assuming that what was coming out of the column were these metal-DOM complexes that were kinetically too slow to dissociate. I'm proposing using more of an equilibrium system, where you would actually allow the entire system to come to equilibrium. You would leave the Chelex in contact with the sample for as long as days. And one of the reasons I propose using this for silver is that a very nice control to use in these sorts of experiments is a photo-oxidized sample. You can pass that through a resin, and then pass a non-photo-oxidized sample through a resin and assume that the differences are due to dissolved organic matter. I'm as yet unconvinced, silver being the photoactive metal that it is, that you can UV-photo oxidize it and expect the speciation to stay the same, or for that matter even expect all the silver to be in the ionic form any more. So I feel I need to find a different method for this that doesn't require that particular control for the results.
- Q. Yes, just for your batch experiments. I think the batch experiment with silver on the Chelex was done in the 1970's by a group at Liverpool University. I think you might want to check the reference on that.
- A. Okay.
- Q. And also there are a couple of papers in this year's literature you might want to check. Considering that your paper deals with interactions of DOM with silver, I think there are two papers in there you might want to check too.
- A. Okay. Thank you.
- Q. JIM KRAMER (McMaster University): I like your idea in principle, but I wonder if you've thought about, given these oligomers and colloids that we've been talking about, when you get to the real stuff, how are you going to mix it? Will you stir it, or roll it softly? Because the actual mixing with the Chelex could change the nature of these complexes.
- A. What I've done to date is used a gentle shaking on a shaker table. I can't say that I've done any work to see for sure, but I pretty firmly believe the Chelex will stand up to that sort of treatment.
- Q. No, I'm referring to when you go to a real sample. It's been shown, certainly with other metals, that you would change the nature of the speciation. So you have to consider the physical condition, I think, of the mixing system.
- A. Yes.
- Q. GARY GILL (Texas A&M University): You alluded to the fact that you have both silver-iminodiacetic acid one-to-one complex and a one-to-two complex. In order to be able to carry out these studies in batch equilibration, which I assume is what you're describing predominantly, you have to be able to model that. If you have the possibility of both of these interactions taking place on your resin, what thought do you have as to how you're going to separate these out so that you can appropriately model that?
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- A. I had a couple of slides with equations developed by Pesavento and Biesuz where they did that exact sort of modeling. When I first got into this I thought I was perhaps going to be lucky enough to have these effects actually be minor enough that I would just be able to look at it as an equilibrium speciation with free iminodiacetate groups. As I say, it's not looking like that anymore. But these sorts of resin group interactions have been studied at some length and there are models out there. At the moment this Pesavento and Biesuz model looks particularly useful.
- Q. I guess what I'm getting at is that you can model this if you know exactly what the ratio is in your interactions, what percentage is in one form versus the other. But my question really is, how are you going to determine that on the resin? What percentage of the silver goes into the one-to-one complex and what goes into the one-to-two?
- A. I can't remember off the top of my head how that was handled. It's folded into the equations that Pesavento and Biesuz use. It is partially derived from the solution equilibrium constants, but I may have to verify their result analytically, based on the fact that I know the number of functional groups on the resin per gram of resin. That is a step that I'll have to go through eventually.
- Q. PETER SANTSCHI (Texas A&M University): Before, in your response to a previous question, you indicated that UV-oxidized samples would not provide a useful control for determining DOM speciation of silver. Have you seen our poster, which shows that we were unable to recover all of the silver from our samples until we exposed them to ultraviolet light?
- A. I did see your poster, and I agree that UV oxidation may be necessary for recovering all the silver from a water sample. My concern, though, is that exposing a sample to UV may change the speciation of the silver by changing its redox state. Still, I am very interested in looking into using UV oxidation to determine silver concentrations in whole water samples or samples that have already been separated using Chelex resin.
- Q. Our work would indicate you will have to do that to recover all of the silver in a natural water sample.

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## Using Spectroscopy and Voltammetry to Evaluate Silver Activity in Aquatic Toxicity Evaluations

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Discharges from industrial, commercial, and domestic sources containing silver have caused regulatory concern regarding silver entering aquatic ecosystems. The present silver water quality criterion uses a water hardness based model (45 F.R. 79318, November 28, 1980) which does not consider silver bioavailability at the variety of dissolved organic carbon (DOC) and chloride (Cl<sup>-</sup>) concentrations that may be found in the environment. A flow-through diluter system was used to determine 96 hour LC<sub>50</sub>s for rainbow trout (*O. mykiss*). Six nominal silver concentrations (0, 2.5, 5, 10, 20, and 40 µg/L) were used while maintaining hardness (30 mg/L CaCO<sub>3</sub>) and varying DOC (0, 2.5, 5 mg/L) and Cl<sup>-</sup> (0, 3, 20, 40 mg/L) concentrations. Each test parameter was measured, and the highest and lowest concentrations of DOC and Cl<sup>-</sup> were combined (5 mg/L DOC:40 mg/L Cl<sup>-</sup> and 2.5 mg/L DOC:3 mg/L Cl<sup>-</sup>). An additional test with a hardness of 70 mg/L CaCO<sub>3</sub> and no DOC or Cl<sup>-</sup> was performed. Hardness was increased from 30 mg/L to 70 mg/L by co-varying the concentrations of MgSO<sub>4</sub> and CaSO<sub>4</sub>•2H<sub>2</sub>O in the stock solution. Total silver concentrations were confirmed by graphite furnace atomic absorbance spectroscopy (GFAA) at time=0 and time=96 hours during toxicity testing. Samples were filtered through either 0.45 µm or 0.2 µm Acrodisk® to approximate dissolved silver (bioavailable) concentrations. Filtration was performed before and after acidification to determine the effect of acidity on filterable silver. Additional analysis of silver was conducted by Osteryoung Square Wave Stripping Voltammetry (OSWSV).

The effect of sample acidification before analysis was noticeable. These tests were performed when there was no DOC or chloride in the test system. Filterable silver concentrations increased when samples were acidified to pH≈2 (Figure 1). The measured silver concentrations in samples that had been acidified and filtered closely approximated ionic silver concentrations, (Ag<sup>+</sup>), that were calculated using MINTEQA2 software.

Silver, chloride, DOC, and hardness data were analyzed in a best subsets regression model to predict the influence of each component on predicting soluble silver (at the 0.45 and 0.2 µm levels). Three separate analyses were performed. Varying hardness in the absence of Cl<sup>-</sup> and DOC showed that total silver (Ag<sub>t</sub>) and hardness (Ca) both had an effect on the concentration of silver in the 0.45 µm filtrates (Ag<sub>0.45</sub>) and 0.2 µm filtrates (Ag<sub>0.2</sub>):

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$$Ag_{0.2}=0.16+\mathbf{0.93*Ag_t}+\mathbf{0.03*Ca}$$

$$Ag_{0.45}=6.44+\mathbf{0.99*Ag_t}+\mathbf{0.04*Ca}$$

The bolded terms are significant to the regression at  $p<0.01$  and underlined terms significant to the regression at  $p<0.05$ . When the data set was expanded to include systems with increased chloride and DOC the total data set contained nine experiments. In this expanded data set, hardness was no longer a significant factor in predicting soluble silver. Chloride (Cl) was a significant factor and dissolved organic carbon (DOC) was not (Bold terms indicate terms significant to the regression at  $p<0.01$ ).

$$Ag_{0.2}=1.52+\mathbf{0.87*Ag_t}-0.03*Ca-\mathbf{0.063*Cl}+0.05*DOC$$

$$Ag_{0.45}=1.49+\mathbf{0.91*Ag_t}-0.02*Ca-\mathbf{0.04*Cl}-0.1*DOC$$

Results from previous fathead minnow (*P. promelas*) toxicity tests conducted in our laboratories were used to further expand the data set. In the expanded data set, which contained fifty experiments, chloride, DOC, and total silver concentration were all significant factors in predicting 0.45  $\mu\text{m}$  filtrates.

$$Ag_{0.45}=0.30+\mathbf{0.85*Ag_t}-\mathbf{0.02*Cl}-0.001*Ca-\mathbf{0.12*DOC}$$

In this case, 0.2  $\mu\text{m}$  filtration could not be evaluated as the data were not routinely collected during the fathead minnow tests. Soluble silver, silver that passes through a 0.45  $\mu\text{m}$  filter, approximates the toxic fraction of silver. Our data suggests that hardness, alone, does not have a significant role in predicting filterable silver from total silver concentration. The lack of hardness effect would suggest that there might be some problems with the current EPA guidelines at the silver concentrations reported in this test.

Anodic Stripping Voltammetry (ASV) was also used to determining active silver in aqueous samples from the toxicity bioassays. Aqueous samples were acidified to  $\text{pH}\approx 2$  and then measured by ASV. During the toxicity tests, voltammograms were poorly defined when humic acid was present. After the toxicity tests were complete, a series of tests was performed to evaluate electrode performance. Synthetic water samples were tested at  $\text{pH}\approx 8.5$  and  $\text{pH}\approx 2$ , while using humic acid and silver concentrations that approximated those used during the toxicity tests.

Figures 2 and 3 show the effect of acidification on electrode response in the presence of Aldrich humic acid at concentrations of 14 mg/L and 7 mg/L (for the medium and low respectively) were used. For each graph, first three points are 5 µg/L silver standards, the following five points are either acidified or non-acidified humic acid solutions that have been spiked with approximately 5 µg/L AgNO<sub>3</sub>. With the acidified samples three times more current was produced at the potential for silver stripping from the carbon paste electrode, suggesting that more silver was present at the electrode surface. However, measurements of the non-acidified samples showed an opposite effect. Less current was produced when silver was stripped from the electrode, suggesting that the presence of humic acid decreases “active” silver concentrations. This decrease in active silver correlates with the increase in LC<sub>50</sub> values that was seen in similar test waters.

Equations were derived to relate soluble silver to total silver. In these equations, chloride and DOC have a greater effect on the prediction of soluble silver than hardness. Voltammetry was useful in determining “active” silver concentrations in non-acidified samples and worked best in the absence of DOC or in the presence of less than 2.0 mg/L DOC. Chloride and humic acid affect “active” silver measurements. Decreased “active” silver measurements correspond to increased estimated LC<sub>50</sub> values.

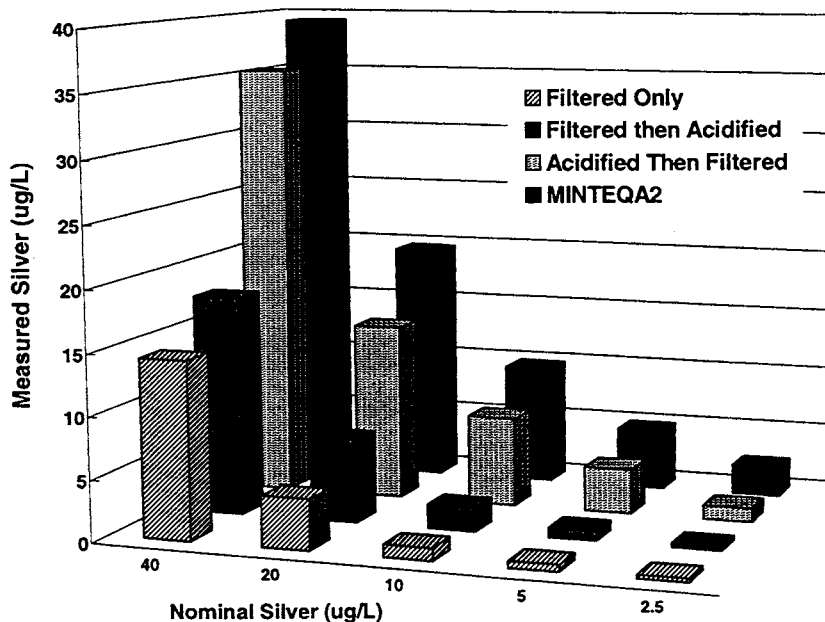


Figure 1 Silver concentrations at 24 hours for high hardness test, effect of acidification on soluble silver.



**Medium Humic Acid and 5 ug/L Silver  
Acidified and Non-acidified samples**

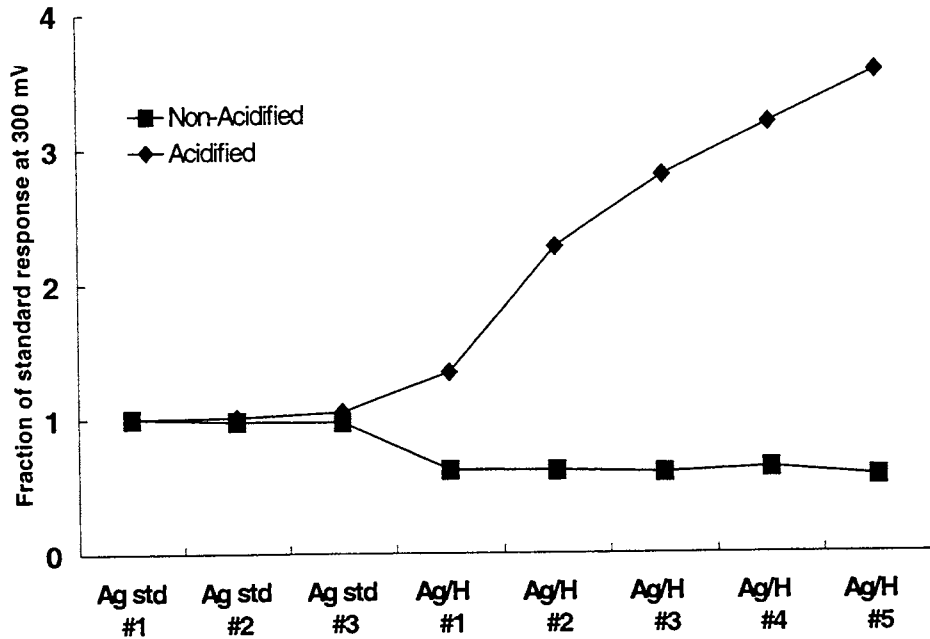


Figure 2 Change in electrode performance with acidification of the sample matrix when 14 mg/L of humic acid is present. Humic acid increases the response greater than 3 fold from baseline with acidified samples. The standard response goes down in the non-acidified samples, indicating a decrease in "active" silver.

**Low Humic Acid and 5 ug/L Ag  
Acidified vs Non-Acidified Samples**

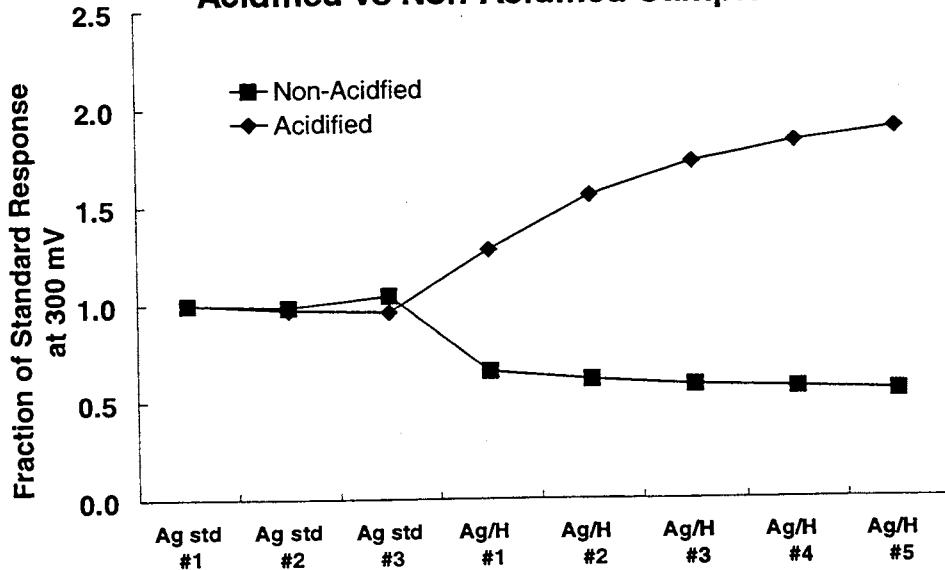


Figure 3 Change in electrode performance with acidification of the sample matrix when 7 mg/L of humic acid is present. Humic acid increases the response almost twice that of baseline with acidified samples. The standard response decreases in the non-acidified samples, indicating a decrease in "active" silver.

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## Questions & Answers: Using Spectroscopy and Voltammetry to Evaluate Silver Activity in Aquatic Toxicity Evaluations

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- Q. DOMINIC DI TORO (Manhattan College): The data you showed was in micrograms per liter. Have you tried doing anything at environmental concentrations of nanograms per liter or lower?
- A. So far we have not. The ASV method does have detection down to those levels, but we are still getting used to using the equipment.
- Q. ARUN MUKHERJEE (University of Helsinki): Can you tell me why, in the U.S. EPA's equation for limits on silver which they have published, they use both hardness and total silver concentration to determine the toxicity?
- A. That's a really good question, and one of the things that I think we're trying to suggest to the EPA is that they need to take a good look and possibly revise those standards for determining toxicity. I don't think that this is the only evidence; I think there's some other evidence that's beginning to suggest that they need to revise that model.
- Q. Because we're the scientists, and if we cannot convince the bureaucrats or politicians how to make these rules, then I think that is a big problem in our society.

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## **CCD + Rotating Anode = Fast Molecular Structures from Very Small Crystals**

**James F. Britten**  
McMaster University  
Hamilton, Ontario, Canada

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Recently, CCD (Charge Coupled Device) area detectors for molybdenum X-rays have appeared on the market. These 2D cameras provide better quality data at a much faster rate than one dimensional scans with a zero-dimensional scintillation counter. They provide better resolution and improved signal-to-noise in a fraction of the time. This allows for routine structure determination of larger, small molecules (~400 nonhydrogen atoms) and of small crystals (~10 micron).

At McMaster, we have combined this CCD with a rotating anode X-ray source. This provides more powerful X-rays than the conventional sealed-tube (18kW vs. 3kW) and allows us to determine structures using crystals which would have previously been discarded. We will present the latest definition of a single crystal and provide a brief look at the operation of this instrument, using some interesting Ag complexes as examples.

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**Questions & Answers: CCD + Rotating Anode = Fast Molecular Structures from Very Small Crystals**

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No questions.

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## Trace Silver Measurement Utilizing a Silicon-Based Sensing Electrode

Oliver Chyan, Jin-Jian Chen, Xu Fei  
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### I. Introduction

It is well known that the heavy metals (like Ag) could be the most toxic and persistent pollutants in surface water systems. A great deal of research effort has been dedicated to determine the sources, transport, and fate of the silver in the various aquatic environments. Silver pollutants in the natural waters are well known to distribute among different chemical and physical forms (e.g. truly dissolved free  $\text{Ag}^+$ , adsorbed in organic or inorganic particulates, colloids). It was further recognized that the bioavailability or toxicity of Ag containing species were also closely related to the chemical forms of silver rather than the total recoverable silver concentration. For example, Woods and co-workers<sup>1</sup> have investigated the silver toxicity in the freshwater rainbow trout system. They concluded that the free silver ion,  $\text{Ag}^+$ , is near 10,000 folds more toxic than  $\text{AgCl}$  and  $\text{Ag}(\text{S}_2\text{O}_3)_n$ . Therefore, it is highly desirable to measure trace level of free silver ions in the natural water system. In this paper presentation, we will discuss a novel silicon-based sensor assembly which was shown to sensitively detect free silver ion (below 50 ppt) in several water samples.

### II. Survey of Trace Silver Detection Methodology

Table I lists several trace silver detection methodologies and their corresponding limit of detection (LOD). In the spectroscopic approaches, the graphite furnace atomic absorption spectroscopy(GFASS) and inductively coupled plasma mass spectroscopy (ICP/MS) are the two most frequently utilized analytical instrumentations. With liquid-liquid

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preconcentration using dithiocarbamate organic chelating agents (like APDC and DDDC), the LOD of less than 1 pM Ag has been reported using GFASS.<sup>2</sup> However, extensive sample preparation requirements<sup>3</sup> and high capital investment could prevent the spectroscopic approaches to be used effectively in the field applications. As also listed in Table I, the electrochemical silver detection approaches have a great advantage over spectroscopic techniques since they can cause minimal perturbation of sample matrixes during the measurement. The solid state Ag<sup>+</sup> ion-selective electrode (ISE) which functions very similar to a pH electrode can be an ideal Ag detection method with very low cost. However, the currently available Ag<sup>+</sup> ISE has a relatively high LOD (10 ppb) which is not suitable for the most environmental sampling applications.

### **III. Silicon-based Sensing Electrode for Silver Detection**

Recently, we have demonstrated that the direct open circuit potential ( $V_{oc}$ ) measurements on silicon-based sensing electrodes provide a sensitive method for detecting ppt level of Ag<sup>+</sup> in acidic aqueous solutions.<sup>4</sup> As illustrated in the scheme I, the silicon sensor surface, upon contacting with free silver ions, will be rapidly covered by the segregated nanometer size of silver metal deposits. We believe the metal deposition acts to extract surface electrons from the silicon sensing electrode and results in more positive shift of the open circuit potential than is expected by the Nerstian equilibrium. Scheme II illustrates the schematic energy level diagram of the n-silicon/solution interface. Unlike a metallic electrode/solution interface where the potential gradient is mainly distributed across a very thin electrical double layer extending from the metal electrode surface into the solution, in the semiconductor/solution junction, most of the potential gradient is retained in a "space-charge region" within the semiconductor surface. In the present open circuit potentiometric measurements, the hole injection of strong oxidizing Ag<sup>+</sup> ions into the valence band of silicon help to deplete electrons near the surface space-charge region and consequently shift  $V_{oc}$  more positive to achieve ppt detection sensitivity.

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Figure 1 shows the performance of a silicon-based sensor operated in dilute HF solutions prepared from the standard reference water SLRS-3 (National Research Council of Canada). Various  $\text{Ag}^+$  ion concentrations (100 ppt to 1 ppm, independently calibrated by GFAAS) were added to the solution *b* to solution *g*. As depicted in Figure 1, the  $V_{oc}$  of the silicon electrode were found to shift increasingly positive as the concentration of  $\text{Ag}^+$  ions in the HF/SLRS-S solutions increased. A linear calibration curve of  $\log [\text{Ag}^+]$  versus the silicon  $V_{oc}$  potentials can be established from Figure 1. The calibration curve data indicate that the  $\text{Ag}^+$  ions in the standard reference water SLRS-3 should not exceed 36 ppt. It is well known that  $\text{Ag}^+$  ions concentration in the SLRS-3 water sample is far smaller than 36 ppt. However, the experimental data in Figure 1 did provide a useful indication about the limit of  $\text{Ag}^+$  detection based on the new silicon based sensing methodology.

#### IV. Potential Applications

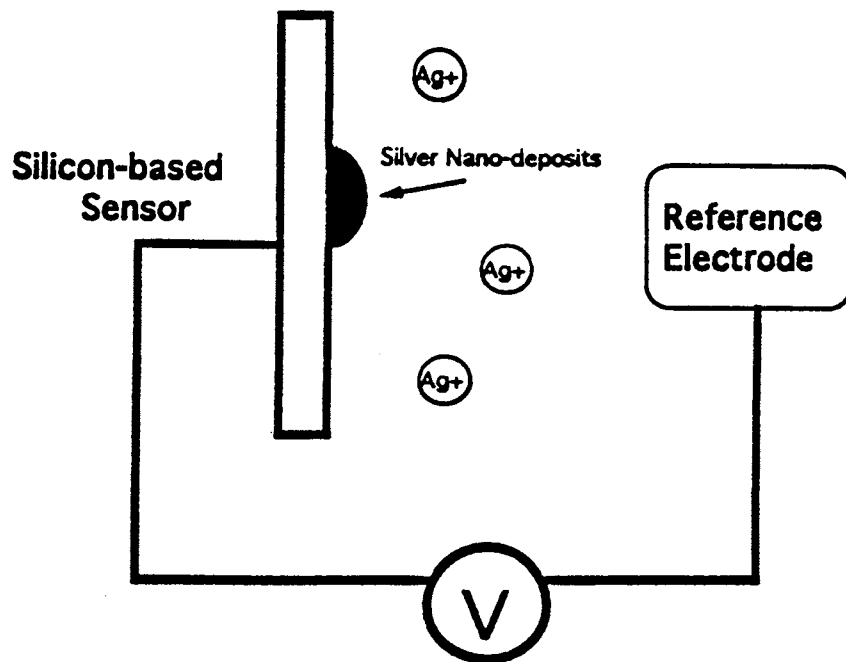
Although more works are required ahead to critically evaluate the sensor's performance and its limitations. Our silicon-based sensing methodology could have important impacts in several areas. One example is to assist in establishing a more technically sound regulation for silver. Our experimental data have demonstrated that the silicon-based sensor selectively detect the most toxic free ionic silver which can be suppressed by different chemical environments (complex or precipitate formation) existing in the nature water conditions. We anticipate the silicon-based sensor will not only detect the existent level of free ionic  $\text{Ag}^+$  but also provide data regarding the natural  $\text{Ag}^+$  remediation capacity in rivers and lakes. The obtained data can be useful in determining the bioavailability of silver in nature environments and assisting in silver ion discharge regulations.

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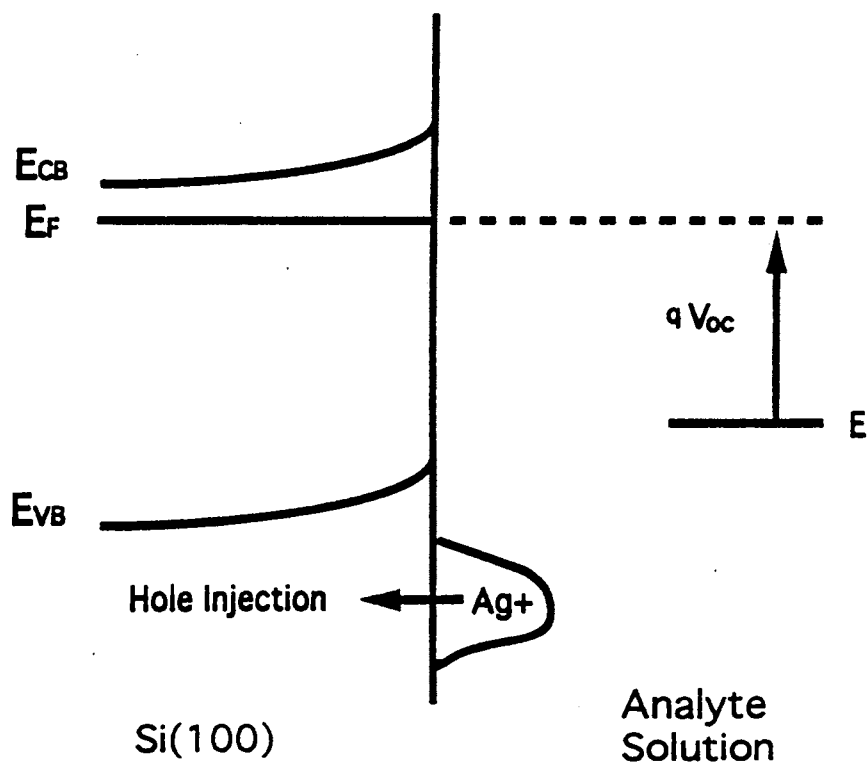
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Scheme I



Scheme II

**Table I. Trace Silver Detection Methods**

Methodology	Limit of Detection (LOD, silver)
<b>I. Spectroscopic approaches</b>	
• GFAAS, ICP-MS	~ 10 ppt
• Liquid-liquid preconcentration with organic chelators (APDC and DDDC) + GFAAS	~ 0.1 ppt (1 pM)
<b>II. Electrochemical approaches</b>	
• Ion Selective Electrode (ISE)	≥ 10 ppb
• Anodic Stripping Analysis (ASA)	≥ 0.1 ppb (1 nM)
• Silicon-based Sensor	≥ 50 ppt (500 pM)

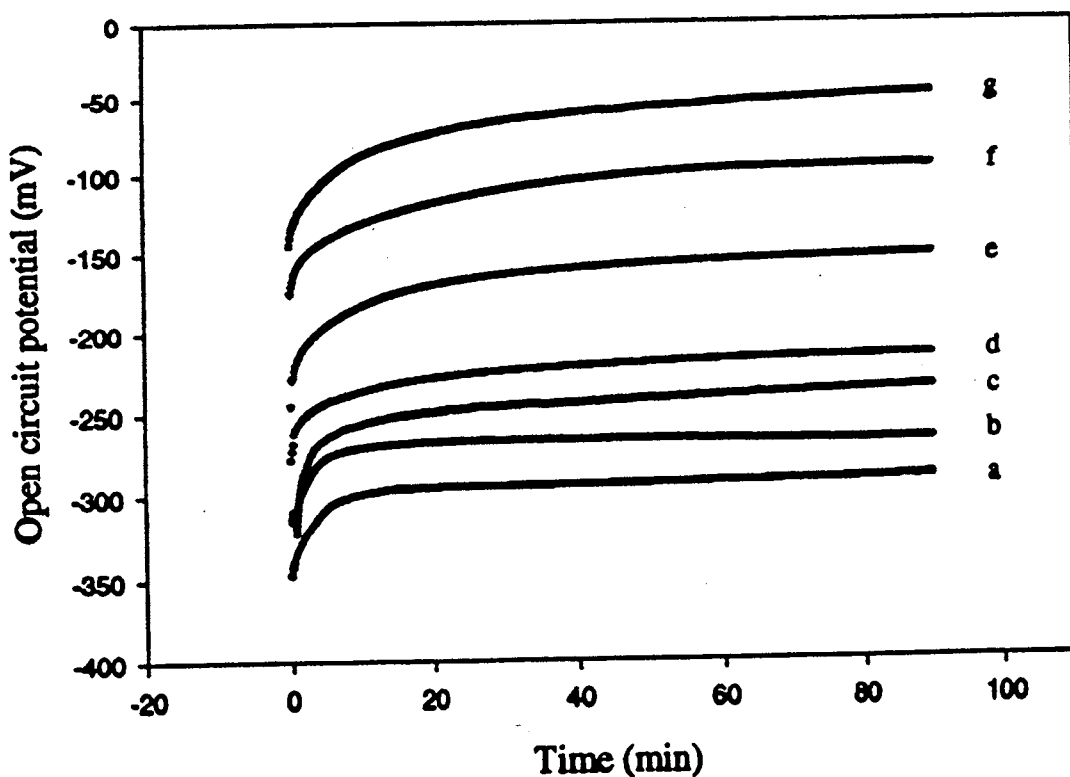


Figure 1, silicon-based sensor performance in SLRS-3 water samples with different silver ion concentrations. (a: blank, b: 100ppt, c: 500 ppt, d: 1ppb, e:10 ppb, f: 100 ppb, g: 1 ppm)

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## Questions & Answers: Trace Silver Measurement Utilizing a Silicon-Based Sensing Electrode

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(NOTE: In Oliver Chyan's absence, his talk was presented and questions were answered by Chuck Christ, Eastman Kodak)

Q. TOM BOBER (Eastman Kodak): I guess I have a question or comment on a point you touched on right at the very end: This is intended to be a disposable one-time-use electrode, isn't it? Not regenerable?

A. That's right.

Q. CHRIS WOOD (McMaster University): This is really quite remarkable. It could be incredibly useful to many people. Is it available?

A. Well, it depends on what you mean by available. This is really preliminary work. There are major problems with every technique, and I am sure that they will surface as we begin to work with this. But by the same token it might offer you the possibility to make those kind of measurements. Now, Oliver would probably be willing to work with whoever was interested. He's convinced, on account of we pushed him on this early, that this would be a very simple, easy-to-use technique and that's kind of what makes it attractive. Now, my understanding is that for some of these electrochemical techniques, you've got to basically get a PhD in the technique before you can use it. What he's telling me is that he thinks that it's, "dip it, measure it, throw it away, get another one." And because the surface of silicon is so well defined, he thinks these can be made very reproducibly, and so as a result it may actually be as simple as that. Now you'd have to work with him to try to get those chips and make that happen, but I think he would be amenable to looking at that.

Q. One additional question, do you know the selectivity relative to copper?

A. It's probably a little less selective than it is in situations where iron is present, and really I think it's related to the ability of the metal to inject holes into the semiconductor surface and what the potential is for that. Now, we have discussed this a little bit and he believes that he can make some modifications to the surface that might allow you to differentiate better between those two than would the electrodes he's showing here.

Q. JIM KRAMER (McMaster University): I'm very intrigued by the 150 mV per decade slope. How do we view this in an electron transfer, do you know?

A. Again, that telephone number for Oliver is ... *(laughter)*.

Q. Because there is a little, to my simple mind, a little mysticism at the moment.

A. Yes, I kind of agree and also you probably didn't notice it, but there are cases when it's not exactly 150 mV, so there may be interactions with other things in the solution that reduce that below the 150 mV per decade sensitivity. So I think that there's a little hand-waving going on in describing exactly why this occurs in this particular system.

Q. Do you know offhand if there is a proposed half-cell reaction, or whatever the electron is? Because you need something like a 1/3 of an electron per equivalent of silver to get 150 mV.

A. I'm sorry, I'd ask the same question of Oliver.

Q. PETER SANTSCHI (Texas A&M University): You mentioned something about pre-treatment which was important, because otherwise you've got matrix effects. But you never mentioned what it actually involved.

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A. The matrix I was talking about specifically was in making measurements in photoprocessing solutions. These are solutions that are concentrated in many different things and one of those is thiosulfate. So in order to remove the matrix effect he oxidizes the thiosulfate. I'm talking about, I don't know, probably a little less than tenth-molar thiosulfate, so it's not like an environmental sample, and in those cases he was using peroxide to oxidize the thiosulfate. So you don't have to do that pretreatment in these other samples. That wasn't the case in these other samples.

# Session 2

*Environmental Cycling, Assessments and Distribution of Silver*

*J. R. Kramer  
Session Chair*



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## Biogeochemical Aspects of Metal-Microbes Interactions

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### INTRODUCTION

To a large extent, the speciation of metals in the aquatic environment is under the control of biological processes. If higher organisms present defense mechanisms at various cellular levels, they are usually less adaptable to metal stress than unicellular organisms. Microbes can efficiently cope with toxic metals because of various specific defense mechanisms that regulate the free ion concentration inside the cell. As an example of this adaptative strategy, one can point to microorganisms that mobilize heavy metals, *e.g.*, arsenic and mercury, to concentration levels that cause acute toxicity to eukaryotes. These defense mechanisms affect not only the metal inside the cell, but also control its extracellular speciation. The overarching paradigm that we suggest in this paper is that microorganisms play a central role in controlling the speciation of metals in natural waters. We first review briefly the various detoxification mechanisms that microbes display to reduce metal toxicity, and then we present results from two field studies that exemplify the role of microbes in metal speciation.

### METAL SPECIATION AND MICROBIAL PROCESSES

The definition of the speciation of a metal in the aquatic environment relies primarily on analytical techniques that allows one to characterize different chemical compounds. These techniques are usually based on operational methods, *e.g.*, filtration to distinguish between the dissolved and the particulate phases, combined with analytical schemes that probe the chemical state of the metal in solution, *e.g.*, voltammetry and ion exchange chromatography. In a seminal monograph, Buffle (1988) has discussed in depth the distribution of chemical species in aquatic systems that is schematically presented in Fig. 1. On the one hand, we have a good knowledge of the dissolved inorganic ligands present in natural waters. On the other hand, the stoichiometry, structure, and reactivity of dissolved metal complexes with organic macromolecules remain equivocal. The information on suspended particles is even more ambiguous, primarily because it is most often based on particle size. Thermodynamics requires that each solid phase be considered independently; this clearly can not be achieved by means of filtration. In essence, bulk particle measurements provide only averaged chemical information that

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can mask the detailed nature of individual particles, and consequently obliterate our investigation of the relevant biogeochemical processes responsible of metal speciation. Therefore microscopic techniques of investigation are required to analyze particles.

Among the living organisms, and small "particles", microbes continuously exchange chemical species with their surroundings. Because microbes depends on nutrients, they have evolved sophisticated membrane transport systems for the ions commonly present in the environment. These systems are related to corresponding genes and proteins that govern the movements of the anions and the cations in and outside of the cell. Since these transport systems are not always species specific, toxic metals can enter the bacterial cell. In response, microbes have developed metal resistance mechanisms (Silver, 1996). Various types of microbial resistance mechanisms to toxic metals have been elucidated using pure cultures subjected to metal stress, primarily under aerobic conditions. Often, these mechanisms are present on plasmids, *i.e.*, small circular genetic elements that reproduce autonomously, and that can be easily moved from one cell to the other. In most instances, these resistance mechanisms involve an efflux pump that remove the metal ion that has entered the cell passively. In other cases, enzymatic detoxification, based primarily on redox chemistry, converts the toxic metal species to a less toxic form.

Living organisms have also developed specific ligands that can strongly bind strongly metals. The biological functions of these ligands can vary from metal sequestration, *i.e.*, metallothioneins and phytochelatins, to metal extraction/concentration, *i.e.*, siderophores. Metallothioneins are found in nearly all organisms and have high affinity for the metals in Group IB and IIB ( $\text{Ag}^+$ ,  $\text{Cd}^{2+}$ ,  $\text{Cu}^+$ ,  $\text{Hg}^{2+}$ , and  $\text{Zn}^{2+}$ ,...). These proteins have a high cysteine content and a repetitive *cys* structure that allows the preferential binding of soft metal ions. The metals are usually bound in a tetrahedral symmetry to four thiolate groups. The structural study of these metal-protein entities, as revealed by NMR and X-Ray crystallography, shows two types of clusters (Fig. 2):

- the  $\beta$ -domain, 3 metals bridged by 3 thiolates forming a 6-membered ring,
- the  $\alpha$ -domain, 4 metals bridged by 5 thiolates forming a bicyclic 9-membered ring.

Stability constants for metal-metallothioneins are in the range of  $10^{12} \leq K_f \leq 10^{31}$ .

It has also been suggested that exopolysaccharides (EPS) produced by bacteria can play an important role in metal resistance (Pirog, 1997). The excretion of soluble microbial products (SMP) also provides ligands of moderate strength, *i.e.*, similar to monomeric organic ligands such as acetate with  $10^4 \leq K_f \leq 10^6$ . In these latter cases, little is known on the coordination of the metal.

The last defense mechanism used by microbes is the precipitation of metal phases, *e.g.*, sulfides or phosphates, either inside the cell or at the cell wall. When the precipitation is intracellular, it is generally promoted by an organic framework that presents a selective surface site matrix or cluster. The saturation index of the mineral phase is therefore controlled locally and internally by the microbe. On the other hand, if the precipitation is extracellular it requires that a chemical supersaturation exists in the external aquatic medium. In that circumstance, the microbe cell wall can be viewed as a catalytic surface where the nucleation of the mineral phase occurs.

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## BACTERIAL MANGANESE OXIDATION AND COBALT CYCLING

The geochemical behavior of Co in aquatic systems has often been related to the presence and oxidation of manganese (*e.g.*, Ballestrieri et al., 1992). The oxidation of Mn(II) to Mn(III-IV) oxides, or MnO<sub>x</sub>, is autocatalytic but also considered to be primarily mediated by micro-organisms (Diem and Stumm, 1984; Nealson et al., 1988). To obtain direct evidence of the association of Co and Mn, we have conducted a water column study on a small lake (Paul Lake, MI). In this lake, the chemical signatures are intensified at a semi-permanent oxic/anoxic transition that develops because of the presence of a biogenic meromixis. We have analyzed concurrently water column samples and individual micro-particles. The results of this investigation are reported briefly below, and in details in Lienemann et al. (1997).

The concentrations of dissolved Mn and Co increase sharply across the oxic/anoxic transition. The concentration of Co rises from the detection limit, 0.4 nM where O<sub>2</sub> is still present, to 6.8 nM at depth, while Mn augments from the detection limit to 3 μM. In the 4.5 to 6 m suboxic layer, numerous bacteria covered with electron-dense inorganic deposits were encountered. Transmission Electron Microscopy - Energy Dispersive Spectrometry (TEM-EDS) analyses revealed that these micro-particles are Mn-rich and that they contain significant amount of Co (Fig. 3). The deconvolution of the EDS spectra allowed us to clearly demonstrate that Co and Fe were associated with the Mn-rich bacterial overgrowth. Using a standardless analysis (Reed, 1993) of the Co (K<sub>α</sub>) and Mn (K<sub>α</sub>) transitions, one obtains Co:Mn =  $(2.0 \pm 0.7)10^{-2}$  (n = 9).

The depth distributions of dissolved Co and Mn observed in Paul Lake (MI) demonstrate that their biogeochemical cycles are coupled. The inspection of the particulate material by TEM, and its analysis by EDS, show that bacteria covered by Mn-oxide overgrowths are responsible for the scavenging of Co in the water column. A schematic diagram of the coupled cycles of Co and Mn at the oxic/anoxic interface that develops in Paul Lake is shown in Fig. 4. One aspect to note is that Co(II), which is the stable form of dissolved Co, is likely converted to Co(III) after its sorption by the bacterial manganese oxide overgrowths. This solid-state redox chemistry involves the reduction of Mn(III) to Mn(II) with the concomitant oxidation of Co(II) to Co(III) (Mañeau et al., 1997).

### ZN SPECIATION IN LAKE DEPUE PORE WATERS

The sediments of Lake Depue (IL) have been heavily contaminated by the operation of a zinc smelter and a fertilizer plant. Although the lake water contains relatively high concentrations of total Zn, the primary productivity of the lake remains relatively high during the summer months. This suggests that most of the Zn, and associated heavy metals, are complexed in this aquatic environment. To study the fate of Zn in Lake Depue sediments, we have extracted pore waters by reverse centrifugation and analyzed the samples using differential pulse anodic stripping voltammetry (DPASV). Fig. 5 compares two voltammograms: one obtained using a ligands free solution reflecting the total concentration of Zn in the pore waters as determined by graphite furnace atomic absorption spectrometry (GFAAS), and another acquired from a pore water aliquot. The absence of electroactive Zn in the pore water sample demonstrates that most of the Zn in Lake Depue pore waters is bound to a strong ligand. Since

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most of the biogeochemical transformations occurring during early diagenesis of surficial sediments are mediated by bacteria, and because of the high concentration of Zn in this environment, one can conclude that microbes have produced specific ligands that are effectively binding the metals. We are currently conducting a more detailed study that aims at characterizing the ligand(s) in parallel with microbial investigations to determine the key players in metal resistance in this environment.

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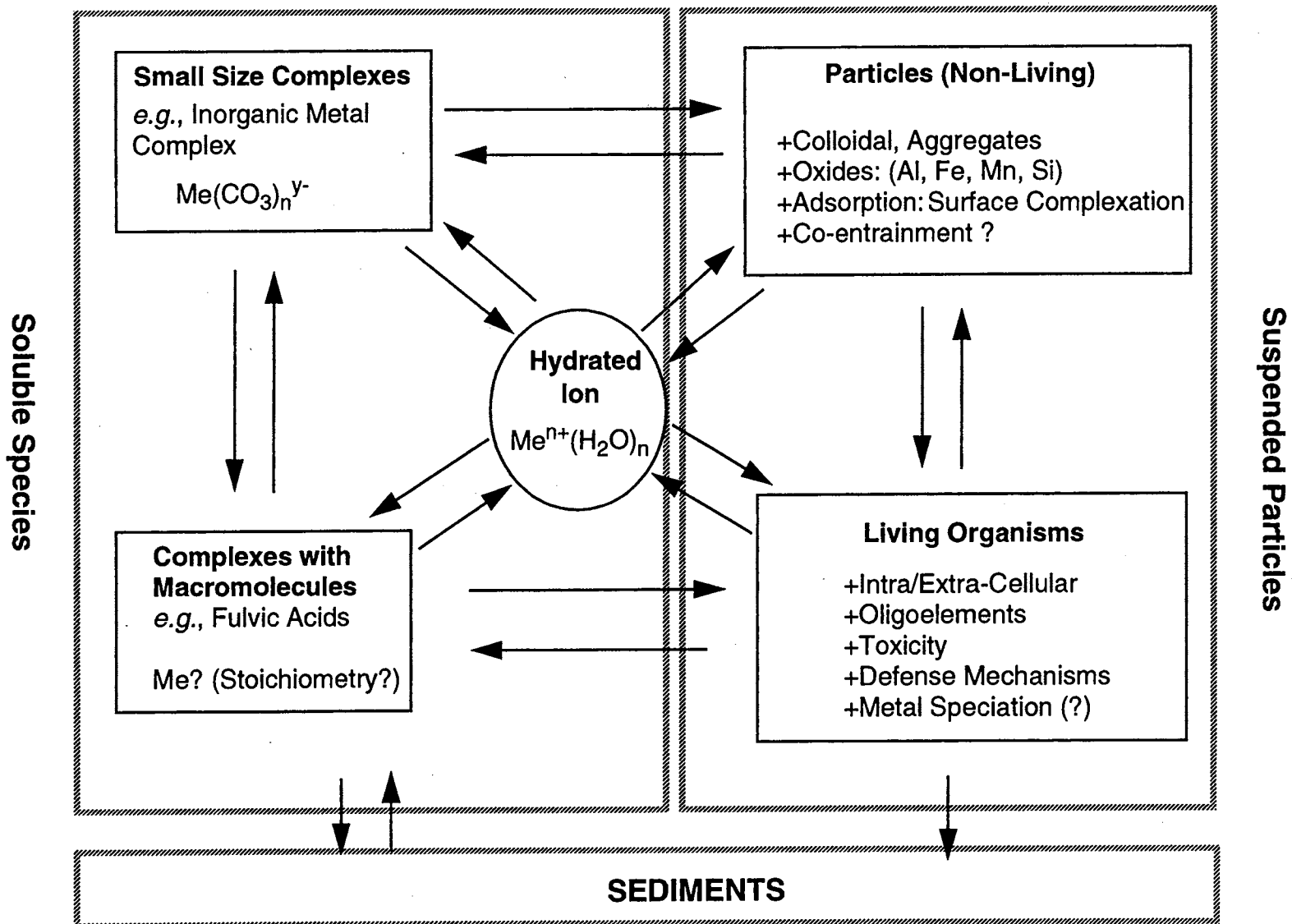
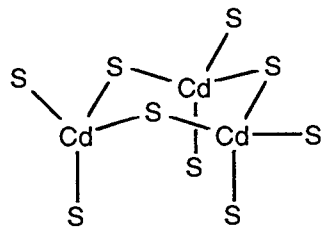
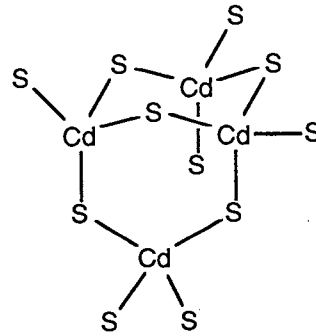


FIGURE 1: SCHEMATIC REPRESENTATION OF THE SPECIATION AND REACTIONS OF A METAL ION IN THE AQUATIC ENVIRONMENT. MODIFIED FROM BUFFLE (1988).



$\beta$



$\alpha$

**Figure 2: The  $\beta$  and  $\alpha$  domain structure in metallothioneins**

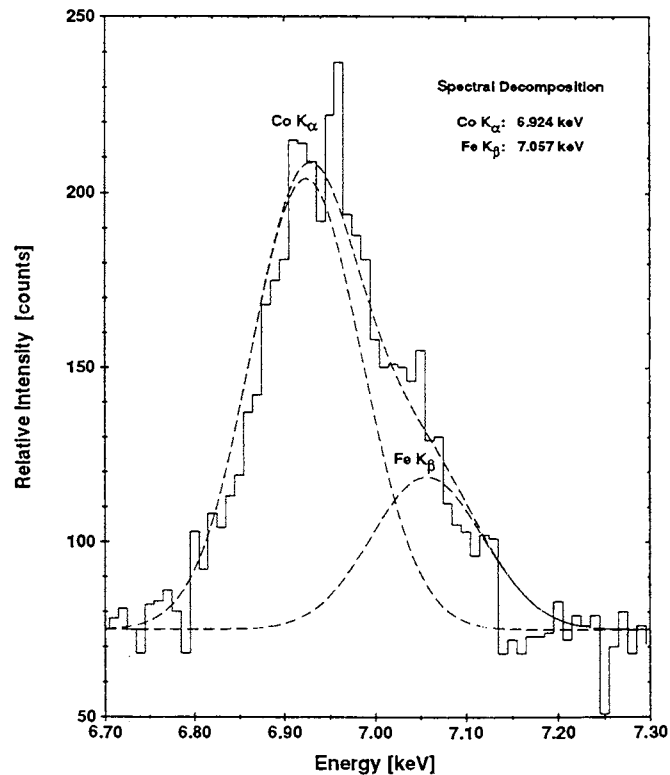
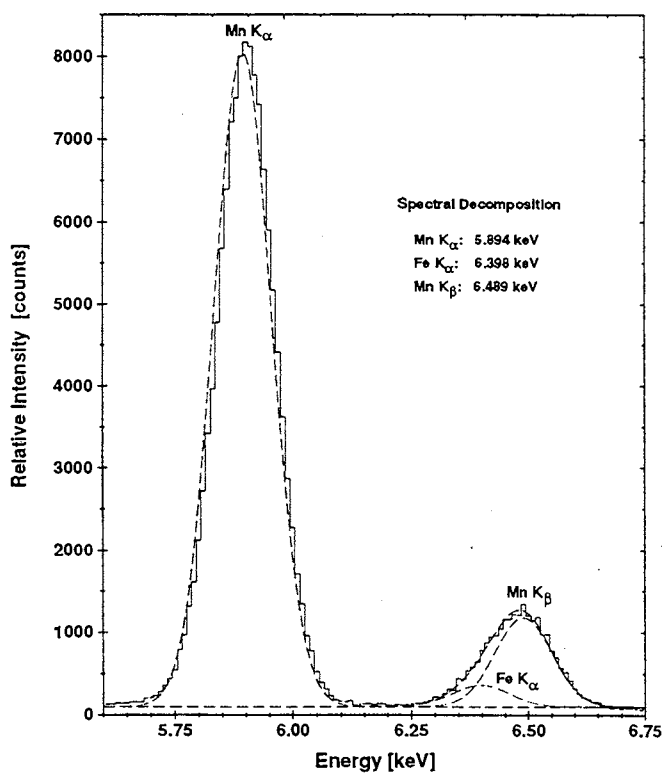
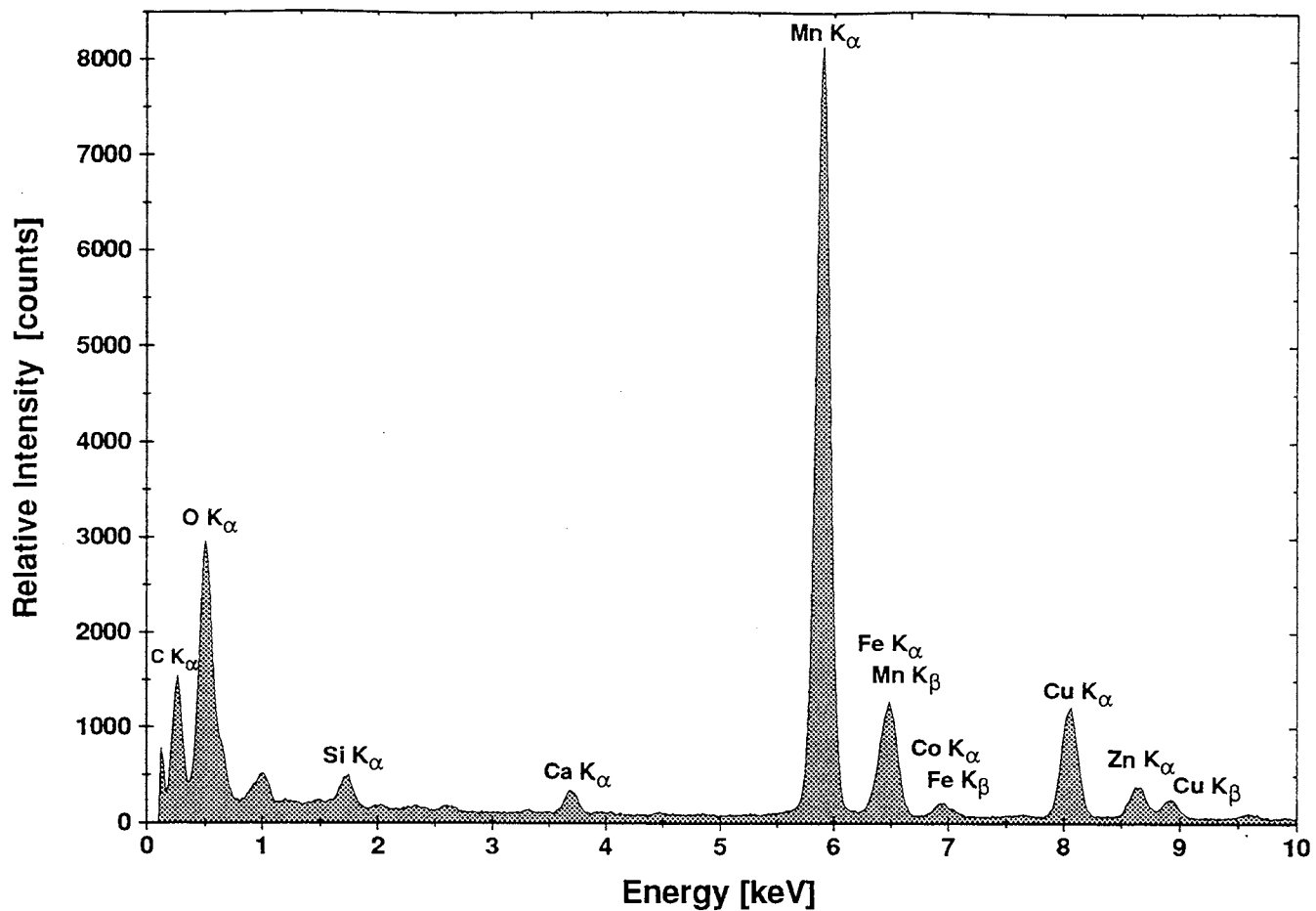


Figure 3: EDS spectrum of a Mn-rich bacterial overgrowth showing the presence of Co at around 2%

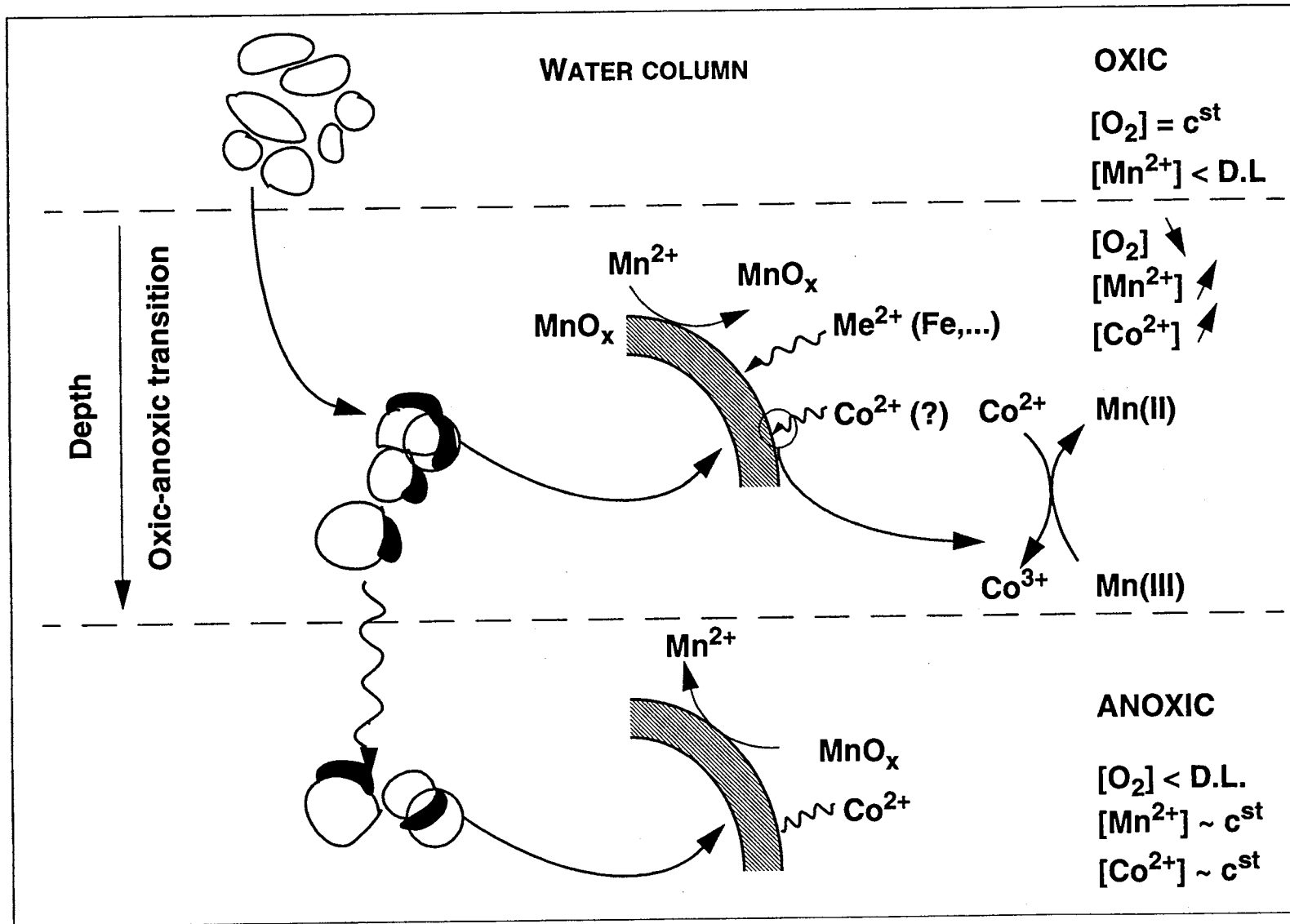


FIGURE 4: THE BIOGEOCHEMICAL CYCLE OF COBALT AS MEDIATED BY MANGANESE OXIDIZING BACTERIA

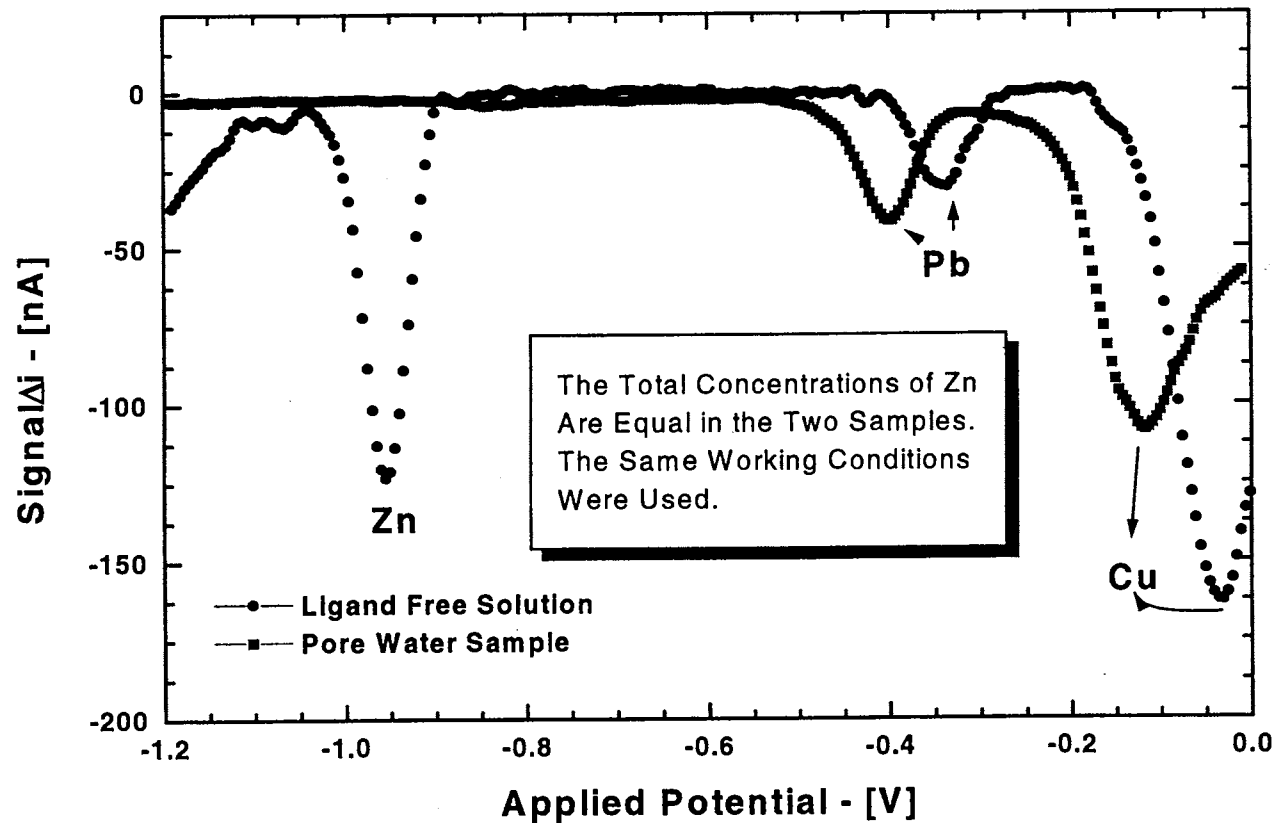


Figure 5: Evidence of the binding of Zn in pore waters of Lake Depue as revealed by voltammetry (DPASV). The background electrolyte is 0.1 M KCl and the pH is adjusted to ca. 7.0 using HEPES.

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## Questions & Answers: Biogeochemical Aspects of Metal-Microbes Interactions

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- Q. PAUL ANDERSON (Illinois Institute of Technology): Why does cobalt correlate so well with manganese? Many transition metals have a strong affinity for manganese dioxide, yet the other metals in your data followed ferric hydroxide and not manganese dioxide. Perhaps the presence of cobalt forms an  $Mn_xCo_{x-1}O_2$  solid solution that modifies the surface chemistry.
- A. It's been shown that cobalt (II) is oxidized to cobalt (III) after its sorption on manganese oxides. The details of this redox process have been elucidated recently by Manceau et al. where they show that Mn (III) is reduced to Mn (II), whereas Co (II) is oxidized to Co (III). Because of the reactivity of  $MnO_x$  one observes an accumulation effect for cobalt in this case, since Co (III) fits perfectly in the manganese oxide lattice. Also one has to remember that with EPS one can only determine elemental concentrations above 0.1%. Other elements can be sorbed but are not detectable. It is likely that the Co redox chemistry will affect the reactivity of  $MnO_x$ . Defining a solid solution with these non-stoichiometric manganese oxides will be a real challenge.
- Q. ALINA KABATA-PENDIAS (IUNG, Pulawy, Poland): The relationship between manganese and cobalt has been studied for many years in soils. It has been shown that manganese oxides fixed cobalt so strongly that it became unavailable to plants, and created a secondary deficiency of cobalt in animals that consumed the plants in this area. So we've known this for years. An explanation has not been clear yet, but it's related to oxic/anoxic transitions on manganese and an especially strong chemical affinity of cobalt. Based on your results, is it possible to relate these processes to microbial effects in the soil, not on oxic/anoxic transitions?
- A. Oh, in the soil?
- Q. Yes.
- A. Yes. I think that - you mentioned these oxic/anoxic transitions - I think it is going to be exactly the same thing. At the oxic/anoxic transition you're going to have the formation of \_\_\_\_\_ manganese oxide, and the manganese oxide will have mixed valence state with respect to the manganese. Now since the group of Manceau and Drits from Russia have found out that actually it is Mn (III), which is a reactive one, then you have the key. You only form Mn (III) when you form these manganese oxides, so it's going to be at this oxic/anoxic transition where you will have maybe a major effect in terms of cobalt scavenging. That other could be a nice explanation; however, I think that's not it, though.
- A. Thank you.
- NICK FISHER (SUNY- Stony Brook): The same microorganisms that are involved in the manganese redox reaction can also participate in cobalt redox.
- A. Tebo and collaborators have shown that bacterial spores, which are manganese oxidizers, are also involved in cobalt redox.

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## The Role of AVS and Organic Carbon in Partitioning of Silver to Sediments

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### Introduction

The application of the  $\Sigma$  SEM/AVS methodology to silver in sediments has been previously reported [1,2,3,4]. The results show an equilibration time for the reaction of silver with either pure amorphous FeS or sediment AVS of approximately 24 hr. However, when the added  $\text{Ag}^+$  exceeds the FeS or sediment AVS by a molar factor of 2, the dissolved  $\text{Ag}^+$  corresponds to the expected value only when pure phase FeS is used. In sediment experiments when SEM- $\text{Ag}^+$  exceeds AVS by more than a molar factor of 2 the dissolved concentration is considerably lower than the expected value, suggesting the presence of an additional binding phase as has been shown for other heavy metals [5].

Modifications to the experimental procedures for measuring SEM-  $\text{Ag}^+$  and AVS are necessary because of the insolubility of AgCl. The study of these modifications requires the preparation of  $\text{Ag}_2\text{S}$  at various degrees of crystallinity. These can also be used to identify the probable form of the  $\text{Ag}_2\text{S}$  (and other heavy metal sulfides) in sediments. It is then possible to study the stability of  $\text{Ag}_2\text{S}$  in sediments especially with respect to oxidation when bioturbation or resuspension occurs.

### Measurement of SEM- $\text{Ag}^+$ and AVS

In contrast to other heavy metals (with the possible exception of lead at SEM > 3mg/L), silver forms insoluble AgCl, making the direct measurement of SEM  $\text{Ag}^+$  impossible. Alternatives involve using either an acid other than HCl or extraction of the silver from the AgCl precipitate after reaction of the sediment with HCl is complete. The only other acid that can be used instead of HCl is  $\text{HNO}_3$ . While  $\text{HNO}_3$  is an oxidizing agent, at low concentrations, the rate of its oxidizing properties is somewhat reduced. ( $\text{H}_2\text{SO}_4$  is not a possible alternative because of the low solubility and extraction resistance of  $\text{Ag}_2\text{SO}_4$ ). The results of a comparison of AVS measurements on several sediments consistently show a 3 to 7% lower AVS value when 0.3M  $\text{HNO}_3$  is used instead of 1M HCl. It should be noted that the AVS reaction is not quantitative for any of the metal sulfides, studied compared to an  $\text{Na}_2\text{S}$  control. Silver sulfide like the sulfides of Hg(II), Cu(II) and Ni(II) is virtually unreactive to either acid. The results are shown in Figure 1. It should be noted that this in no way affects the validity of the SEM/AVS approach to heavy metal bioavailability since for these sulfides only unbound or weakly bound metals are released in the AVS procedure. Because bioavailability correlates to pore water activity, when SEM/AVS < 1, 1M HCl or 0.3M  $\text{HNO}_3$  unreactive as well as reactive metal sulfides can not provide soluble metal ion activity beyond the limit of the solubility product constant.

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As indicated above, if 1M HCl is used in the AVS procedure for silver in sediments, then an extraction method must be used that selectively removes silver from AgCl. A pH=10 extraction using NH<sub>4</sub>OH is incomplete when pure phase Ag<sub>2</sub>S is subjected to the HCl-AVS procedure at equivalent concentrations of 1.5 and 30 μmole/L. (Surprisingly an AgCl control at 1.5 μmole/L equivalent concentration was only 66.6% extractable by this technique). The results are shown in Figure 3. Because acidification of the extract is not possible (AgCl would reprecipitate) atomic emission or absorption analysis of the metal was difficult. The use of thiosulfate as an extractant presented similar problems when the silver is sorbed to the sediment and its reaction with Ag<sub>2</sub>S is uncertain. Figure 3 shows only 20% recovery for a spike-recovery experiment using a sediment of known AVS. Thus, for the determination of SEM-Ag<sup>+</sup> and AVS a choice must be made: only partial AVS values when 0.3M HNO<sub>3</sub> is used, or only partial SEM-Ag<sup>+</sup> values that are concentration dependent when 1M HCl is used.

### **Preparation and Extraction Techniques for Metal Sulfide Oxidation Experiments**

In our work, the heavy metal sulfides have been characterized in terms of their degree of crystallization into three classes related to the temperature at which they are prepared or aged: (1) low temperature (LTMS), (2) intermediate temperature (ITMS), and (3) high temperature (HTMS). The first two are prepared from equimolar amounts of sodium sulfide and the metal nitrate (twice equimolar in the case of Ag<sub>2</sub>S) using equal volumes of 0.1M solutions of each (0.2M in the case of silver nitrate). All solutions are deaerated and all procedures are carried out in a glove box. The solutions are combined with a 0.1M solution of potassium acid phthalate buffer (pH = 4.0) to give a final concentration of 0.03M. The resulting metal sulfides are aged at 20°C or 70°C for 8 days depending on whether LTMS or ITMS is being prepared. The precipitates are then filtered using a 3μm membrane filter, rinsed with dilute deaerated HCl and dried - all under a nitrogen atmosphere. This method is taken from the work of Y.Xu, M.A.A. Schoonen, and D.R. Strongin [6].

The HTMS powders were used as received from Alfa Aesar or Noah Technologies Corp. They are prepared from the pure metal and sulfur at high temperatures. Their purity is reported as >99.9% in most cases and is determined by the purity of the metal used.

The metal sulfides described above were used in some of the studies described here as well as those related to their oxidation which will be presented in a subsequent paper by Dominic M. DiToro.

### **The Partitioning of Silver to Sediment Organic Carbon**

As was previously pointed out, the aqueous silver concentration is far less than expected when Ag<sup>+</sup> is titrated with a sediment rather than pure phase FeS to a stoichiometric excess. This suggests the presence of an additional binding phase for silver, and based on our previous work [5], sediment organic carbon was considered as a reasonable possibility.

The experimental apparatus used is shown in Figure 4. It consists of a silver/sulfide ion selective electrode (Orion-941600), a double junction reference electrode (Orion #900200), connected to a millivolt meter (Orion #SA720), and a standard pH electrode and meter. The electrodes are immersed in a 10 g/L suspension of sediment. The system is kept anaerobic by the continuous introduction of nitrogen gas. The system is titrated with a AgNO<sub>3</sub> solution. Dissolved Ag<sup>+</sup>

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activity is measured by the electrode. From this, the known AVS of the sediment, and the total silver added, the sorbed silver can be determined, assuming that the total aqueous silver is not significantly different from the electrode value. Typical results are shown in Figures 5 and 6. The isotherm shown in Figure 5 is typical of Langmuirian behavior. Figure 6 shows the data in linearized form. Calculated values for the Langmuir constants as well as for the partition coefficient,  $K_d$ , and the organic carbon normalized partition coefficient,  $K_{oc} = K_d/f_{oc}$ , where  $f_{oc}$  is the fraction organic carbon, are also shown. In Figure 7 the results of experiments on four different sediments of varying organic carbon content, as well as Aldrich humic acid are summarized. From these limited results it is possible to calculate an average value of  $K_{oc}$  for silver. This value is shown as  $1.82 \times 10^5$  L/kg  $oc$ .

In Figure 8, this result is presented with those of our previous work (2). Using sediment quality criteria (SQC) values, AVS,  $K_{oc}$ , and  $f_{oc}$ , a value for  $C_{SQC} - AVS$  can be calculated. This is then correlated with  $f_{oc}$  at a given pH. The proposed  $C_{SQC}$  for silver is probably unrealistically low (0.12  $\mu\text{g/L}$ ). Even at that level, for a sediment with 5% organic carbon the sorbed silver in the sediment would have to exceed 1ppm to yield pore water concentrations in excess of the criterion. If the criterion were set at 1.2  $\mu\text{g/L}$ , similar to that for cadmium, this same sediment would have to have 10 ppm of sorbed silver before its pore water would exceed the criterion. All of this is in addition to any silver that was AVS bound.

Additional work will involve measuring total dissolved silver as well as silver ion activity in the carbon sorption experiments. Toxicology studies correlating pore water silver concentration with the acute and chronic effects of silver on benthic organisms and the establishment of a valid water quality criterion for silver must also be undertaken. Most importantly, determining the oxidation kinetics for  $\text{Ag}_2\text{S}$  in sediments must be completed for an adequate fate and transport model to be established.

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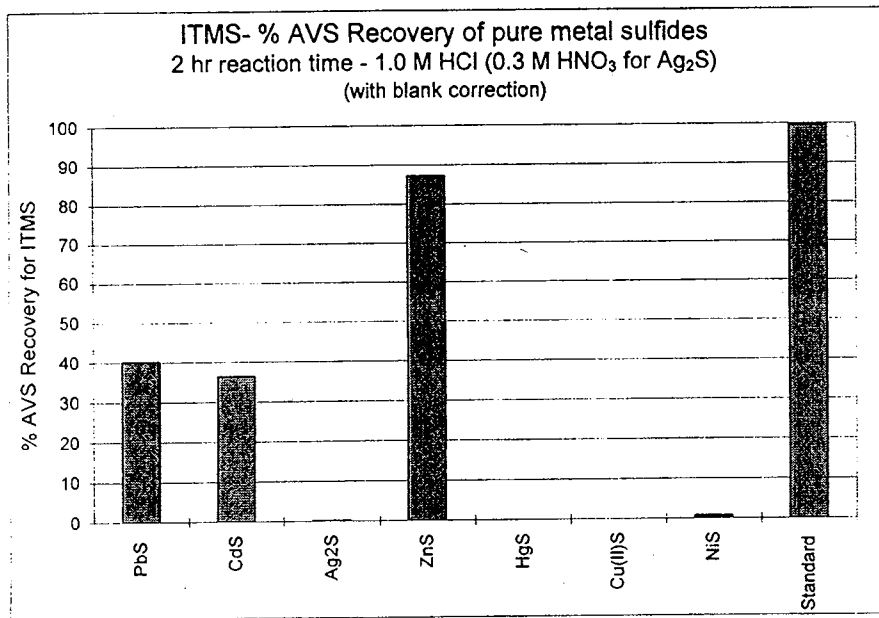


Figure 1

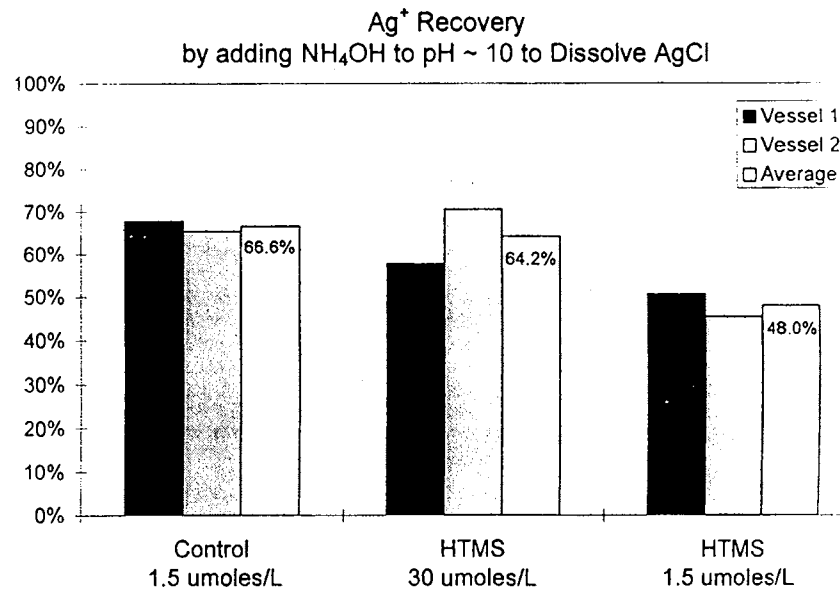


Figure 2

### West Bearskin, Silver Thiosulfate Extraction

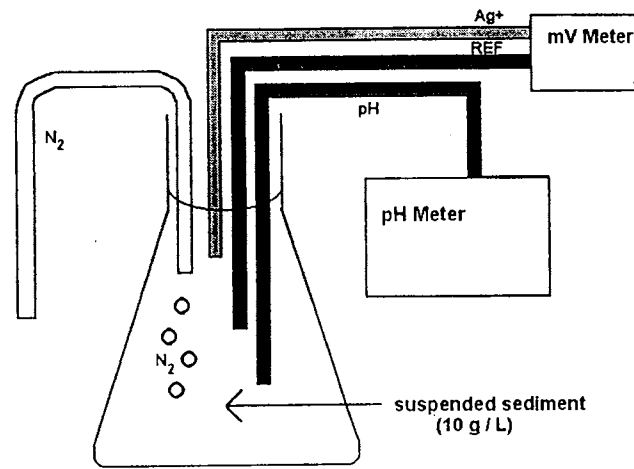
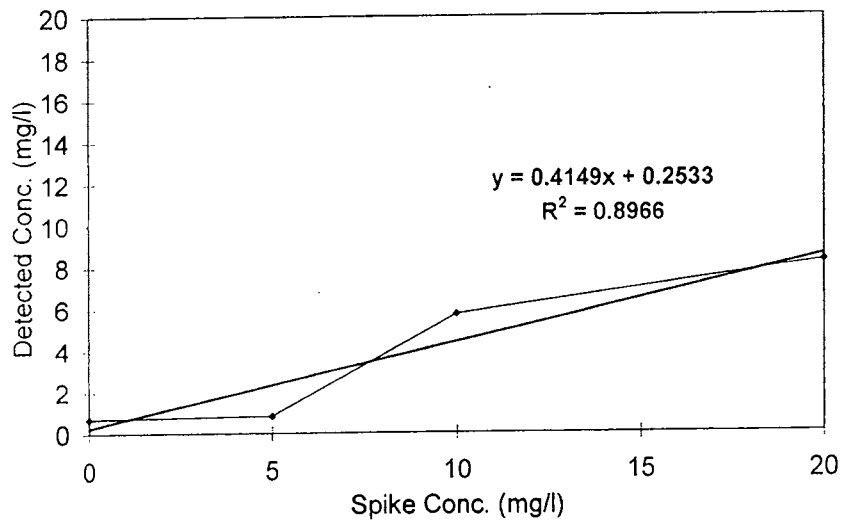


Figure 4

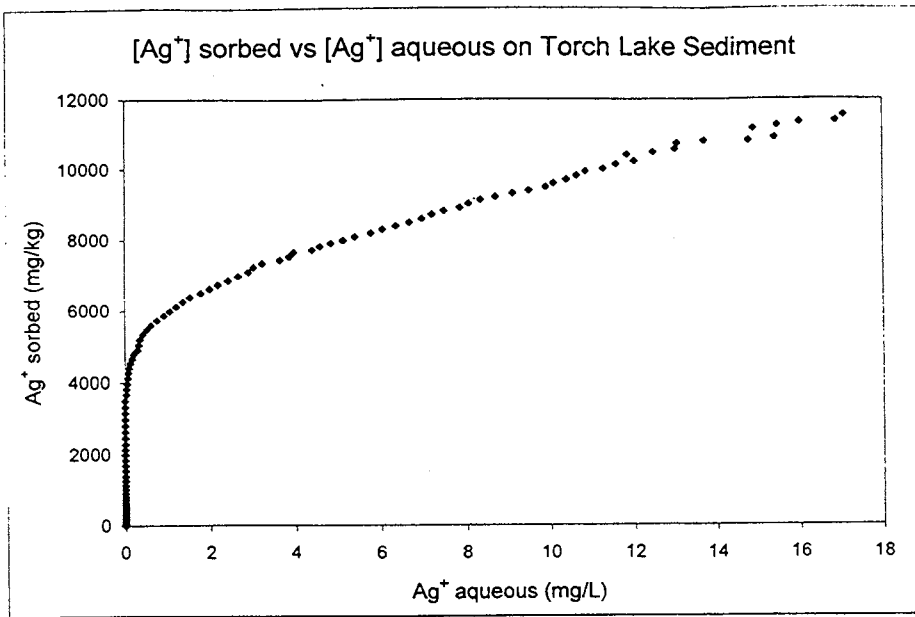


Figure 5

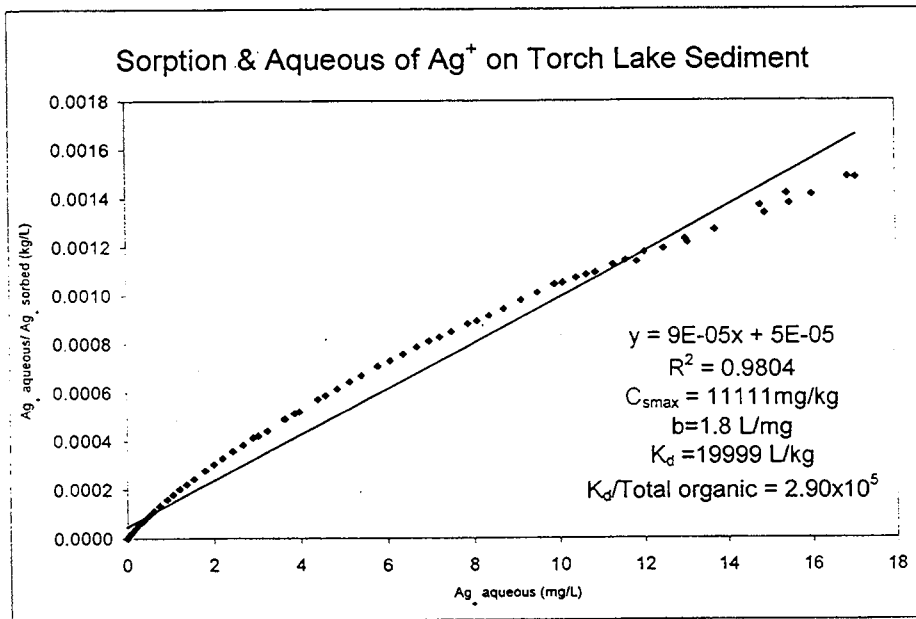


Figure 6

**SUMMARY TABLE FOR SILVER ELECTRODE EXPERIMENTS**

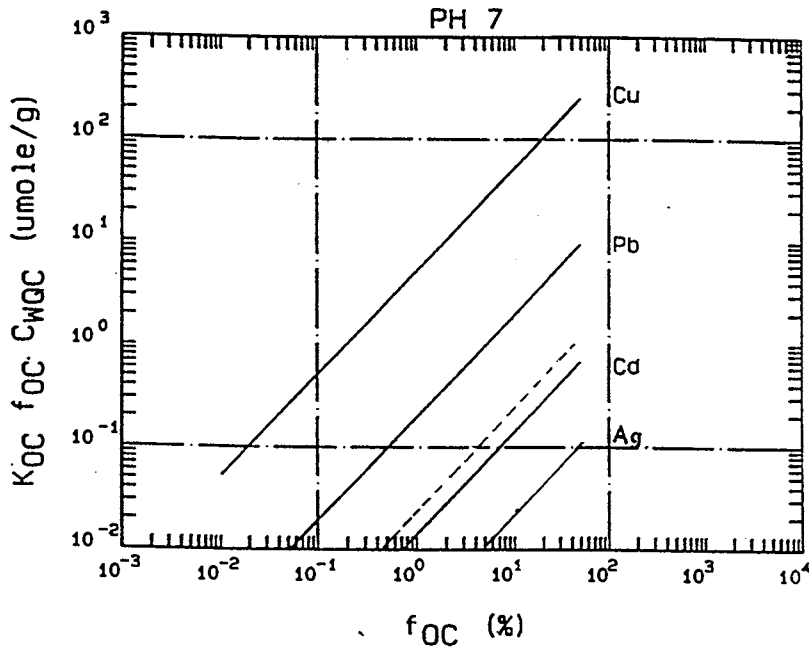
SAMPLE	DATE RUN	f <sub>oc</sub> , %	K <sub>D</sub> / f <sub>OC</sub>
DEFOE #1	6/3/97	1.47	136000
COLE CREEK	6/13/97	3.11	166000
WEST BEARSKIN	4/15/97	5.69	176000
TORCH LAKE	4/23/97	6.89	290000
ALDRICH HUMIC ACID	4/22/97	38.64	143000

AVERAGE 182000

Figure 7

$$SQC = AVS + K_d C_{WQC}$$

$$SQC - AVS = K_d C_{WQC} = K_{oc} f_{oc} C_{WQC}$$



$C_{WQC}$  = Fresh Water Chronic Criteria at Hardness =  
100 mg/L as  $CaCO_3$

Cadmium WQC = 1.1  $\mu g/L$   
Copper WQC = 12.0  $\mu g/L$   
Lead WQC = 3.2  $\mu g/L$   
Silver WQC = 0.12  $\mu g/L$

Dashed horizontal lines show range of AVS  
Dashed vertical lines show range of  $f_{oc}$

Figure 8

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## Questions & Answers: The Role of AVS and Organic Carbon in Partitioning of Silver to Sediments

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- Q. DAN CALL (University of Wisconsin-Lake Superior Research Laboratory), I was just curious: for this West Bearskin Lake sediment, then, how does the organic carbon compare to the AVS for binding silver?
- A. Well, in terms of chemistry the silver will form the sulfide long before it will undergo sorption, so the organic carbon is really an added kind of binding phase. Does that answer your question? So if you have no organic carbon, then the data that I presented on the y-axis essentially would be how much silver could be in the sediment before you'd exceed the water quality criteria.
- Q. ANDERS ANDREN (University of Wisconsin-Madison): I have a question. I got a little confused about the room-temperature experiment and the low-temperature experiment. One was prepared at room temperature the other one at 70 degrees Fahrenheit.
- A. They all were prepared at room temperature. It was the aging temperature that varied. The LTMS was simply aged at room temperature; the ITMS was aged at 70 degrees.
- Q. What's the difference?
- A. Well, the procedure is...
- Q. I mean in terms of temperature. 70 degrees is room temperature.
- A. 70 degrees Celsius?
- Q. Oh, you had Fahrenheit there, I'm sorry.
- A. Did I? Oh, I beg your pardon.
- Q. Okay, then that's all right, thank you.
- A. There were a lot of typos, I apologize for that. These slides were put together as usual at the last minute.
- Q. ARUN MUKHERJEE (University of Helsinki): Sorry, I am a little bit confused. When you show the data that the chronic criteria of silver is 0.12 for at 100 milligram per liter hardness. Is it correct?
- A. No, this is what was in the chart that I have which listed sediment quality criteria. However, I've since learned that 0.12 was a proposed but never accepted chronic criteria for silver. So that was just put on because I was taking the data from this chart that I had. Probably there will be a different chronic criteria, and it will be higher than 0.12.
- Q. Thank you very much.

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## **The Relationship Between Silver Binding in Sediments and Acid Volatile Sulfide (AVS)**

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The purpose of the study was to demonstrate that sediment containing acid volatile sulfides (AVS) can bind silver, presumably as silver sulfide. We examined the stability of the "silver-sulfide" phase in sediment samples that were resuspended in oxic water, simulating sediment resuspension.

### **Experimental Methods**

Surface sediment (0 to 5 cm) samples were collected from several lakes and estuaries. The samples were stored at 4°C, sealed in glass jars with minimal head space. The AVS and simultaneously extracted metal (SEM) concentrations were determined by the method of Allen (1990) using 1N HCl for 30 min at 20°C. The concentration of silver in water and SEM extracts was determined by inductively coupled plasma mass spectrometry (ICP-MS). For total silver determination, sediment was digested with a mixture of HCl and HNO<sub>3</sub> acid before ICP-MS analysis, according to the method in Daskalakis et al. (1997), which prevents the precipitation of silver chloride in marine sediment. The grain size was determined by wet sieving through a 62- $\mu$ m screen, using the term "mud" as material that passed this screen. Total volatile solid was determined by loss on ignition at 550°C.

After the initial AVS concentrations of the sediment samples were determined, 10-g aliquots of wet sediment were spiked with silver nitrate to produce nominal silver concentrations of 0.5, 1, and 2 times the equivalents of AVS. The sediment with the silver spike was diluted with 10 mL freshwater or seawater, mixed for 30 min, then centrifuged. A subsample of the spiked sediment was analyzed by AVS and SEM. One week later, an aliquot of the same spiked sediment was resuspended in either river water or seawater (0.1 g wet sediment in 50 mL water), shaken for an hour, then passed through a 0.45  $\mu$ m filter. The filtrate was acidified to pH <2 and analyzed by ICP-MS for silver.

### **Results**

The characteristics of the marine and freshwater lake sediment samples are listed in Table 1. The mud content ranged from 2.6% to 95%, TVS ranged from 2.8% to 19%, and AVS ranged from 0.4 to 48  $\mu$ mol/g dry weight. The AVS concentrations were determined in sediment spiked with various ratios of silver to AVS.

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In general, after spiking sediment with silver equal to the AVS molar equivalent concentration and reanalyzing for AVS, only approximately 15% of the original AVS was detectable. This indicates the silver spike formed a stable silver sulfide phase that prevented most of the sulfide from being detected during the subsequent AVS analysis.

The concentrations of silver in the spiked sediment that were prepared for resuspended contained silver at half the AVS concentration, on an equivalent basis. Therefore, all the silver could be present in the sediment as silver sulfide, a highly insoluble compound. Presumably the silver sulfide can slowly oxidize and dissolve, but the rate of oxidation was not investigated. The concentrations of silver in the spiked sediment were very high (ranging from 43 to 5200  $\mu\text{g/g}$  dry wt) compared with the concentrations of silver in industrial harbors.

When unspiked and spiked sediment were suspended in natural water, very little silver was released from the sediment. The concentrations of dissolved (0.45  $\mu\text{m}$  filter) silver, copper, and zinc were generally not much higher in the water when silver-spiked sediment was suspended compared with concentrations when unspiked sediment was resuspended.

Based on these results, we do not expect significant concentrations of silver to be released from resuspended sediment in urban/industrial harbors. Further investigation is needed to determine the longer-term stability, solubility, and bioavailability of silver in sediment.

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**TABLE 1.**

**Marine Sediment Characteristics**

<b>Site</b>	<b>% Mud</b>	<b>% TVS</b>	<b>% AVS <math>\mu\text{mol/g}</math></b>
Oakland	95	3.4	6.1
New York	61	8.0	48
Sequim	31	13	36

**Lake Sediment Characteristics**

<b>Site</b>	<b>% Mud</b>	<b>% TVS</b>	<b>% AVS <math>\mu\text{mol/g}</math></b>
Crescent	59	13	0.4
Green	41	19	20
Union	2.6	2.8	8.2
Ballard	45	12	21

**TABLE 2**

**Concentration of Dissolved Metal  
When Marine Sediment Resuspended  
(spike 0.5 x AVS)**

Sediment	$\mu\text{g/L}$		
	Ag	Cu	Zn
Oakland Unspiked	<0.1	1	5
Oakland Spiked	0.6	3	6
New York Unspiked	0.2	3	6
New York Spiked	1.1	2	3
Sequim Unspiked	0.2	1	10
Sequim Spiked	0.2	3	6

**Concentration of Dissolved Metal  
When Lake Sediment Resuspended  
(spike 0.5 x AVS)**

Sediment	$\mu\text{g/L}$		
	Ag	Cu	Zn
Crescent Unspiked	<0.05	1	6
Crescent Spiked	0.14	1	19
Green Unspiked	0.05	1	17
Green Spiked	0.85	1	11
Union Unspiked	<0.05	1	5
Union Spiked	0.57	1	11
Ballard Unspiked	<0.05	1	42
Ballard Spiked	1.5	1	41

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## Questions & Answers: The Relationship Between Silver Binding in Sediments and Acid Volatile Sulfide (AVS)

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Q. JOHN MAHONY (Manhattan College): I didn't quite get your SEM procedure. You said that you used aqua regia to extract the vessel?

A. We used aqua regia in attempts to recover the silver that we'd spiked in sediment, on a different aliquot than the SEM. SEM was one normal hydrochloric acid.

Q. You used one-molar hydrochloric acid. So if any silver was released, it would precipitate as silver chloride.

A. Well, you can see on these x-y plots, we normally recovered from 10-50% of the silver with the hydrochloric acid by the first standard, as standard SEM method. So we're recovering a fair amount of the silver we're not precipitating out. Otherwise, we should be measuring about 1 ppb silver or a few ppb silver. So we were measuring here hundreds to thousands of parts per billion of soluble silver.

Q. In the presence of HCl?

A. In HCl.

Q. That's surprising.

A. Yes, it doesn't all make sense.

Q. WALTER BERRY: Eric, I didn't catch how long you re-suspended your sediments for, and do you consider that a sort of worst-case simulation of a dredging event or something wonderful like that?

A. Yes, we just re-suspended them on a shaker for a few hours, and the silver sediment had been aged for a few weeks. When we re-suspended them for a few hours, it would simulate like a dredging activity or prop wash from a boat, so it's just a short-term re-suspension and the water was filtered and analyzed.

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## The Oxidation of Silver Sulfide and Other Heavy Metal Sulfides in Sediments

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### Introduction

The oxidation of silver sulfide is a critical process in the mobilization of silver from sediments. Experiments are being conducted in order to quantify the kinetics of the oxidation process and the environmental variables that influence the rate and extent. Both pure phases and spiked sediment are being employed. In addition various oxidants are being examined in order to gain insight into the mechanism of the oxidation.

A model has been developed for the flux of cadmium from sediments [1]. This model will be employed to analyze the results of silver oxidation experiments employing sediment cores. The results have important consequences with respect to silver sediment quality criteria since the ultimate reservoir of silver is the sediments and the rate of its mobilization is critical component in its eventual fate and toxicity.

### pH Titrations

The nature of the metal sulfide phase in sediments is important in determining what phase to employ in oxidation experiments. A method we have employed is to titrate both pure phases and contaminated sediments with acid and record the metal released. The idea is: if the release profiles are similar for a particular synthetic metal sulfide and the sediment, then that suggests that the sediment phase is similar to the pure phase. Fig.1A presents the release profile obtained for zinc sulfide synthesized at low (20 °C) temperature (LTMS) and intermediate (80 °C) temperature (ITMS). Fig. 1B presents the release profile obtained from a contaminated sediment. Note that the sediment release profile is similar to the low temperature metal sulfide (LTMS). Fig. 1C presents initial results for a silver spiked sediment aged for 1 year, and the two silver metal sulfides. It appears that the sediment and the ITMS have similar release profiles.

We are also employing x-ray powder diffraction to examine the degree of crystallinity of the metal sulfide phases. Fig. 2 illustrates the results for the low, intermediate and high temperature synthesized ZnS (Fig. 2A) and Ag<sub>2</sub>S (Fig.2B). There are marked differences in the degree of crystallinity for the zinc sulfides which correspond to the difference in the pH release profiles (Fig.1A). The LTMS has the least crystalline pattern and it releases metal at the highest pH. The differences are not so marked for the three Ag<sub>2</sub>S minerals which suggests that the crystallization is complete even for the low temperature sulfide. The pH release profiles (Fig. 1C) suggests there is a small difference between the high temperature (HTMS) and intermediate temperature (ITMS) silver sulfide and that the sediment more closely resembles the ITMS. More refined diffraction profiles should delineate the differences more clearly.

### Oxidation Rate

Oxidation experiments for suspensions of pure phase metal sulfides have been conducted. Fig. 3A,B present the results for low temperature copper and silver sulfide (LTMS). Note the difference in oxidation rate: Cu concentrations exceed 100 mg/L within the first 200-400 hrs. whereas the maximum silver concentration is approximately 0.1 mg/L after 1000 hrs.

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The oxidation of sediments are illustrated in Fig.4A,B. The copper sediment is a field collected contaminated sediment. The silver sediment is a spiked sediment that has aged over one year at 4°C. Note that for both Cu and Ag, the oxidation rate is much faster in the sediment than as a pure phase. The maximum oxidation times in Fig.3 for the pure phases is ~ 1200 hr. ~ 50 days. The maximum oxidation times in Fig. 4 for the sediments is 200 – 300 hrs = ~ 8 – 12 days. Both experiments released approximately the same quantity of copper and a 10× greater amount of silver. Thus the sediments released considerably more Cu and much more Ag than the pure phase metal sulfide suspensions.

We have also employed hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) as an oxidant for the pure phase metal sulfides. The oxidation kinetics of H<sub>2</sub>S have been examined using both O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> [2] so these experiments parallel those results. Fig.5 presents the results for CdS and Ag<sub>2</sub>S. as linear (A) and logarithmic (B) plots. The time scales of these experiments are ~ 4 days. Note the rapid release of silver and compare this to the release of silver for the ~ 50 day experiment using O<sub>2</sub> as the oxidant (Fig.3B). It is clear that oxidation using H<sub>2</sub>O<sub>2</sub> is much more rapid.

The oxidation rates observed in the low temperature silver sulfide (Fig.3B), the spiked sediment (Fig.4B), an estimate of the rate of oxidation from a sediment core experiment reported at last years Argentum conference, and the hydrogen peroxide oxidation (Fig.5A) are compared in Fig.5C. The data are expressed as the rate at which silver is released per unit silver in the suspension

$$\frac{\text{Rate of Ag released}}{\text{Mass of Ag present}} = \frac{\text{mg Ag}}{\text{g Ag} \cdot \text{day}}$$

The oxidation of the LTMS is approximately 30 times slower that the rate of release of the spiked sediment and the sediment core. The rate of release using hydrogen peroxide is faster still.

Since the oxidation rate of H<sub>2</sub>S using H<sub>2</sub>O<sub>2</sub> is much faster than with O<sub>2</sub> it was expected that the same should be found with the metal sulfides. However the rapid rate found for the sediments relative to the pure phase was quite unexpected. For silver, the pH titration profiles are reasonably similar for the pure phase and sediment suggesting that the phases have similar characteristics. It suggests that there is a different oxidation mechanism operating in the sediment suspension.

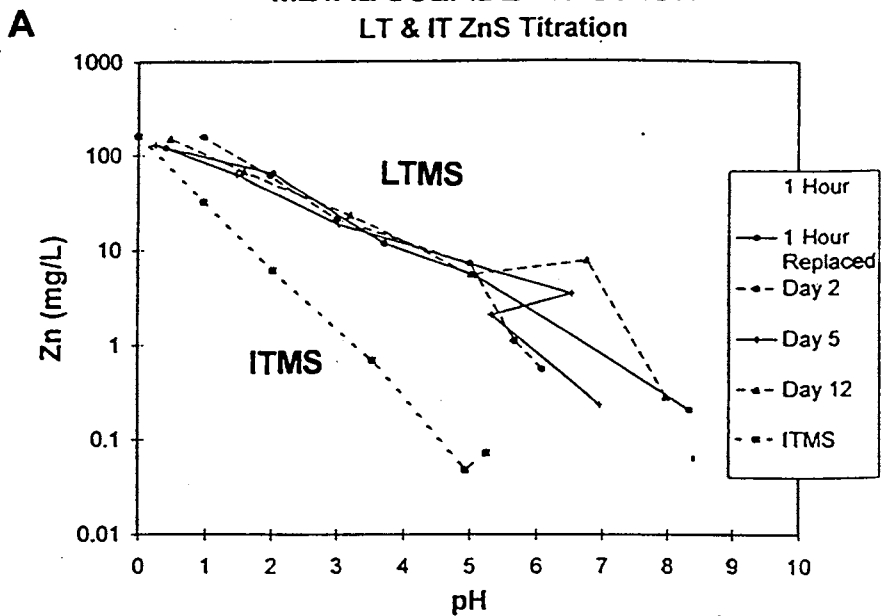
One possibility is that the initial electron acceptor not oxygen in the sediment but rather iron. This mechanism has been suggested for the oxidation of pyrite [3]. The idea is that the oxidant is Fe(III) which is reduced to Fe(II) as the sulfide is oxidized. Then Fe(II) oxidizes to Fe(III) using O<sub>2</sub> as the electron acceptor. The iron is an intermediate which is cyclically reduced and oxidized. Since no iron is available for the pure phase oxidation, oxygen must react directly with the silver sulfide which, presumably, is a slower process. This hypothesis will be checked experimentally.

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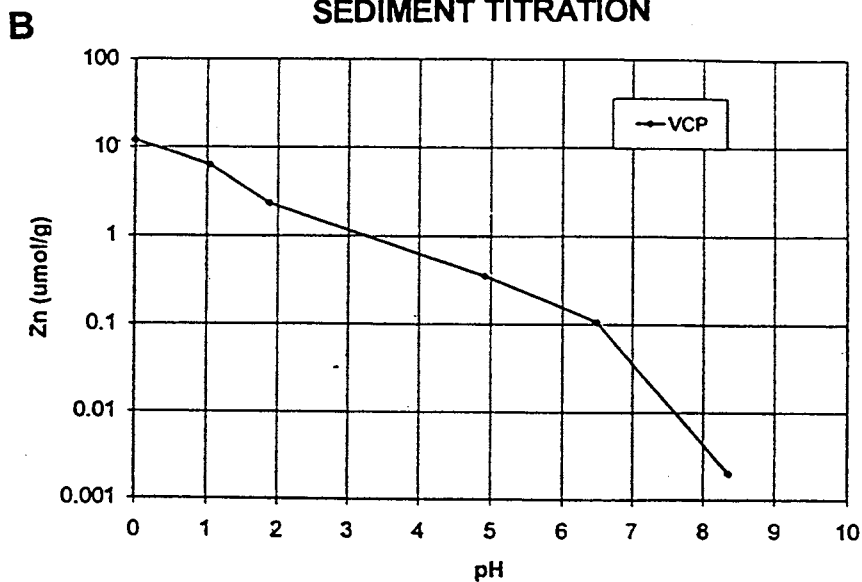
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# METAL SULFIDE TITRATION

LT & IT ZnS Titration



# SEDIMENT TITRATION



# Ag CONCENTRATION vs. pH

(ITMS, HTMS, EPA)

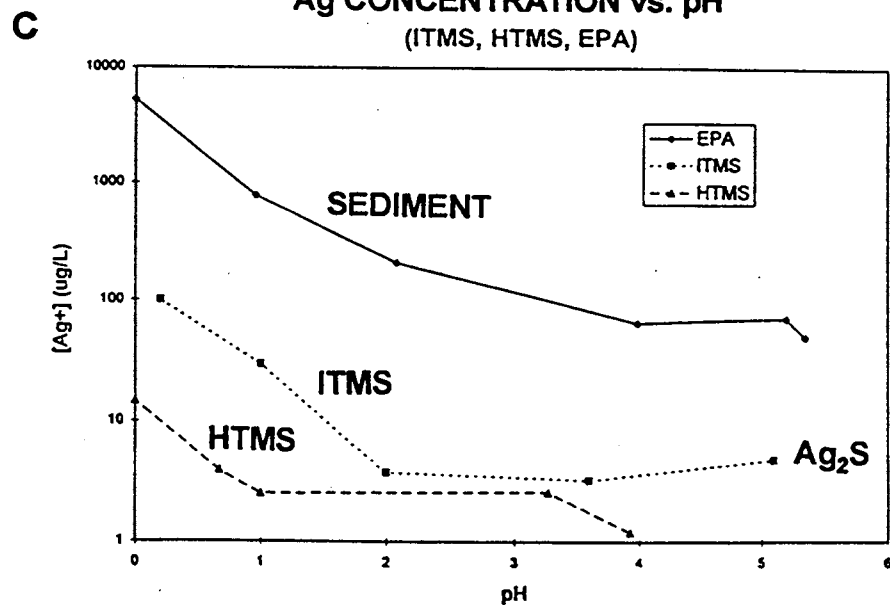


Figure 1

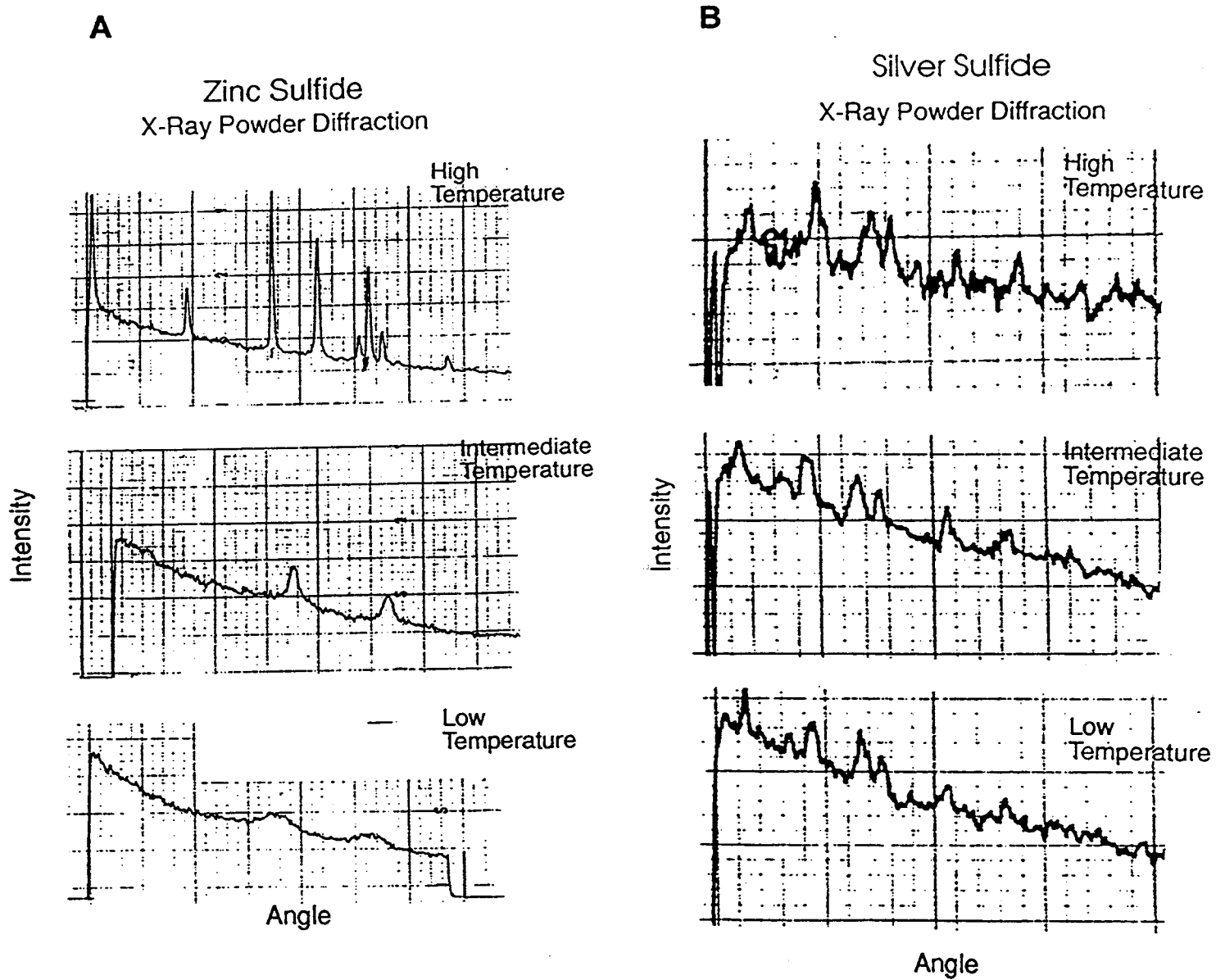


Figure 2



**METAL SULFIDE OXIDATION**

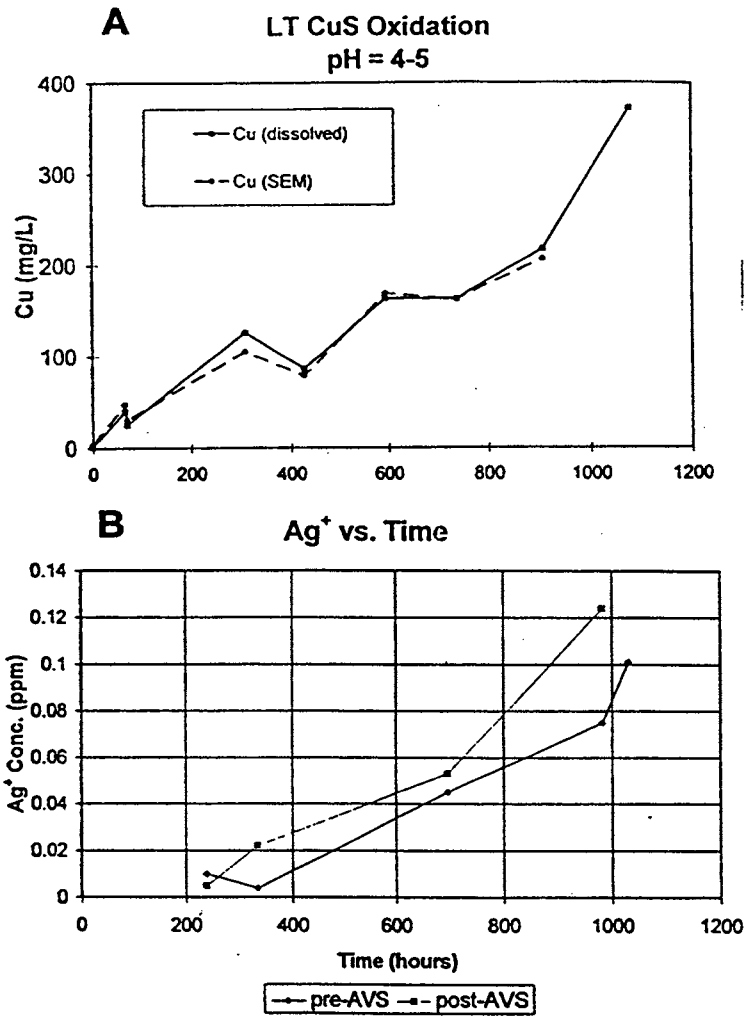


Figure 3

**SEDIMENT SUSPENSION**

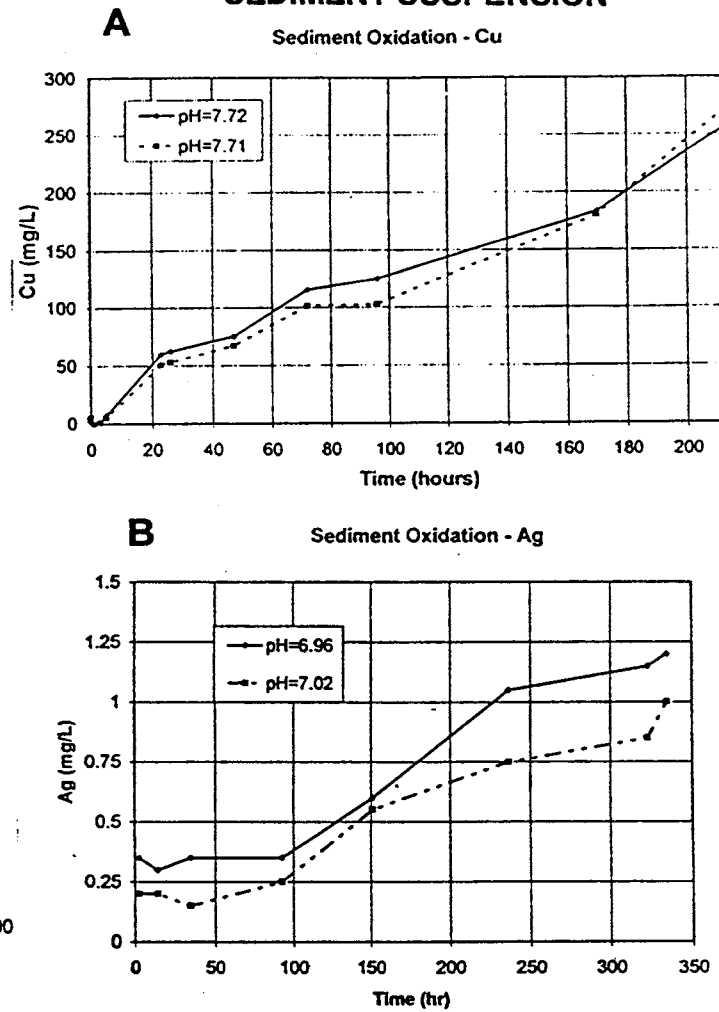
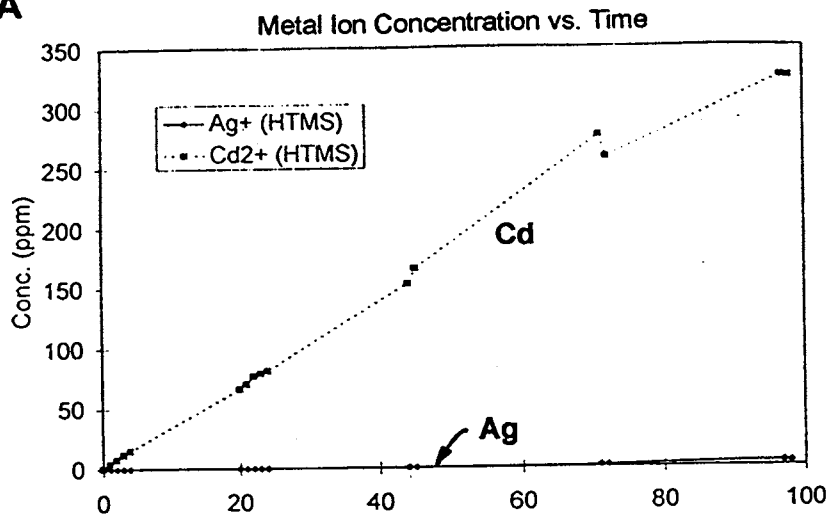


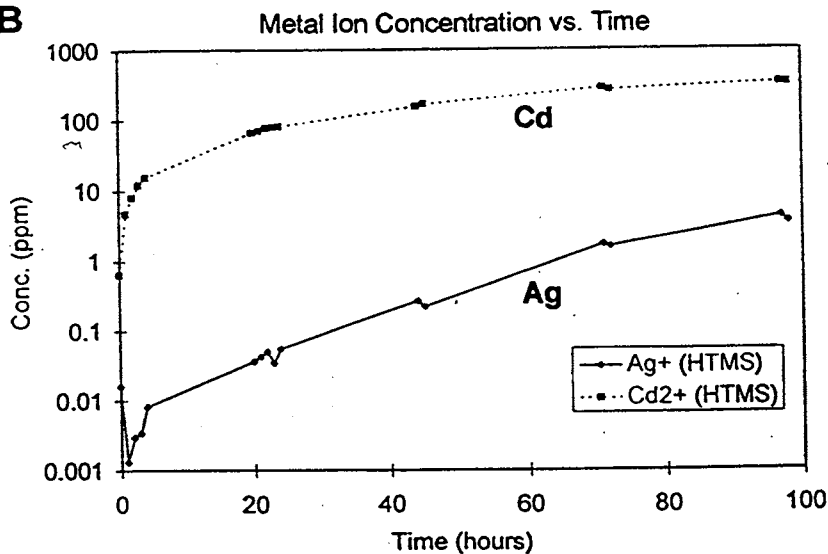
Figure 4

# METAL SULFIDE & HYDROGEN PEROXIDE

**A**



**B**



**C**

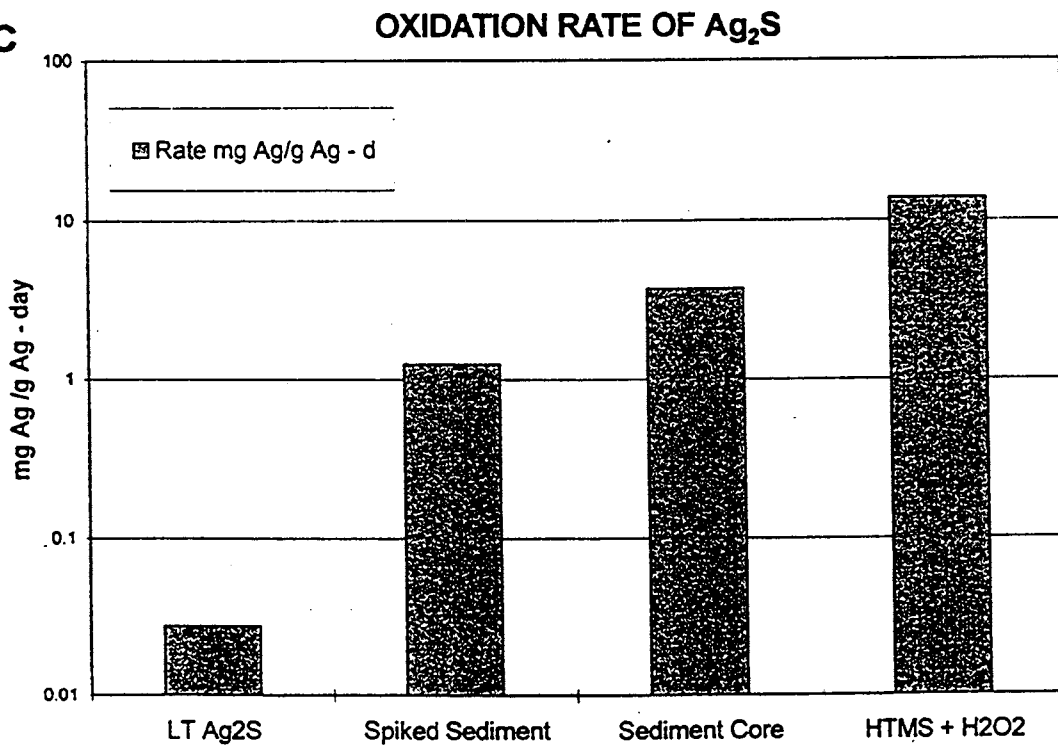


Figure 5

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## Questions & Answers: The Oxidation of Silver Sulfide and Other Heavy Metal Sulfides in Sediments

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- Q. DAVID ARMSTRONG (University of Wisconsin-Madison): In the way that you do the oxidation in the sediments, is it possible that the silver sulfide might be oxidized and the silver re-adsorbed, so you would underestimate how much might actually be oxidized?
- A. Yes, we worried about that. We actually do an extraction, as John mentioned. We played around with that for awhile to make sure with spike recoveries that we could get everything back that we added in simulations. Our early data was just a nightmare, and until we corrected for the re-absorption we didn't have anything worth talking about.
- Q. JIM KRAMER (McMaster University): It's a good hypothesis. I guess you have to know what's in your sediment, though, to carry it out.
- A. It would help.
- Q. But maybe a simpler thing would be to just work with the pure-phase silver sulfide.
- A. That's right.
- Q. But put ferric iron in.
- A. Well, we were thinking about that for the next experiment.
- Q. I don't know if you were here a couple years ago, when Nick showed the mobilization against pure silver sulfide and iron had virtually no effect.
- A. Yes, but with an oxidation it might. In fact, that's exactly our next experiment. Add iron sulfide to this thing. One of the problems is it's not so easy to do because the iron (II)-iron (III) couple here may depend on a redox boundary.
- Q. That's right.
- A. So you get cycling. Whereas here, this is simply a suspension.
- Q. And it is known that these are organic-rich, humic-rich, materials.
- A. They are.
- Q. You can get electron transfer right on the humic matter.
- A. That's another possibility. That the electron transfer is happening right on the particles.
- Q. ANDERS ANDREN (University of Wisconsin-Madison): Dominic, when you titrate your particles, is that the initial pH that you use or is it the final measured pH?
- A. Final measured pH.
- Q. Because otherwise you might have a lot of things happening to the pH as you approach. . .
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A. Absolutely.

Q. Unbuffered, right.

A. We titrated to a pH and then let it sit there, and if the pH is wandering we keep adjusting it until we get the pH to stabilize and then measure it.

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## Assessment of the AVS Method and "Cline's" Method with Respect to Reactive Sulfide

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Reactive sulfide is often measured in reducing and oxidizing environments to estimate the metal speciation and the amount of trace metals, such as Fe(II), Ni, Cu, Zn, Ag, Cd Au and Hg bound as sulfides. Direct measurement of reactive sulfide in aqueous samples, using Cline's (1969) procedure, is often carried out for marine samples. The acid volatile sulfide (AVS) method has been characterized (Allen et al. 1993) to estimate reactive sulfide in sediments. Reactive sulfide in AVS is generally defined by that sulfide that reacts with N HCl. The resulting H<sub>2</sub>S (g) is purged into a basic solution which is then often determined using Cline's procedure.

This paper considers the species of reduced sulfur that react with Cline's procedure and the AVS method. First we review the forms of reduced sulfur found in the environment and their approximate concentrations. Then we assess the forms that are determined by Cline's method and the AVS procedure.

### Forms of reduced sulfur in the environment:

Reduced sulfur species in the aqueous phase include simple monomeric species such as H<sub>2</sub>S, HS<sup>-</sup> and S<sup>2-</sup>, polysulfides (S<sub>n</sub><sup>2-</sup>, HS<sub>n</sub><sup>-</sup>) organo-sulfides (thiols), and in the solid phase metal sulfides such as amorphous FeS, mackinawite, greigite, pyrite, NiS, CuS, Cu<sub>2</sub>S, ZnS, Ag<sub>2</sub>S, CdS, and HgS. These metals (M) may form simple monomeric complexes such as MHS, MS, polymeric species (M<sub>m</sub>S<sub>n</sub>, M<sub>m</sub>HS<sub>n</sub>), thiol complexes (MR-S<sub>n</sub>) and polymeric thiol complexes (M<sub>m</sub>R-S<sub>n</sub>). These complexes may be true aquo ions. Perhaps more often, they may be found bound to colloidal (organic) substrates, such as natural organic matter (NOM) or occur as thiol compounds. Table 1 summarizes the occurrence and chemical predominance environment for some of these species.

Silver and other metals may bind in solution as simple and polymeric inorganic and organic metal sulfides. Silver probably binds as the simple complex, AgHS<sup>0</sup> in open waters and as polymeric species in pore waters where the sulfide and metal concentrations may be elevated. Metal sulfide complexes may sorb on particles and other sulfides. For example, dissolved silver sorbs very strongly on amorphous FeS (Adams 1996). In addition, polymeric metal sulfides form complex solid and aqueous structures. Refer to Bell (this issue) for detailed discussion of Ag-thiol compounds. Table 2 summarizes some of the metal polysulfides that have been determined for aqueous and solid phases reported in the literature.

Table 1. Predominance of different reduced sulfur species. NOM - natural organic matter.

<u>Form</u>	<u>Species</u>	<u>Comments</u>
Monomers:	$H_2S, HS^-, S^{2-}, Ag(HS)^0$	$HS^-$ predominate above pH of 6; $S^{2-}$ predominate at pH > 15(?); predominate at low $\Sigma [S(II)] \leq 1$ mM $Ag(HS)^0$ predominate Ag species, sorbed to FeS
Thiols:	3 mercaptopropanoic acid (3-MPA) methanethiol monothioglycerol ethanethiol + 4-10 major unknowns Ag-thiols common	form in high biol. productivity; $\Sigma [S(II)] \geq 0.1$ mM thiols mobilize Ag Binding constant $\geq 10^{12}$ Many Ag-thiols are probably polynuclear Ag-thiol bound to NOM
Polysulfides (i S atoms with one $S^{2-} \rightarrow S_iS^{2-}$ ): $iS^0 + HS^- \rightarrow S_iS^{2-} + H^+$		$S_iS^{2-}$ increases as pH and $\Sigma[S(II)]$ increase Polysulfides predominate at $\Sigma [S(II)] \geq 0.01$ M $HS_5^-$ stable at pH ~8; $S_2^{2-}$ stable at pH ~17 (?) for marine waters: $S_6^{2-} - S_2^{2-}$ ; $HS_5^-$ , $HS_4^-$ (?)

Table 2. Metal - (thiol) - polysulfides

	<u>Complex</u>	<u>Reference</u>
Aqueous:	$Ag(S_4)_2^{3-}, AgS_5S_4^{3-}, Ag(HS)S_4^{2-}$ $Cu(S_4)_2^{3-}, CuS_4S_5^{3-}, [Cu_3(S_4)_3]^{3-}, [Cu_4(S_{13-15})]^{2-}$	Cloke, 1963 Helz (1993)
Rings Mostly:	$[Ag(S_9)S]_8^-$ , $[Ag_2(S_8)_2]_2^-$ , $(PPh_4)_4[Ag_2S_{20}]S_8$ $[Cd(S_8)_2]^{2-}$ $[Hg(S_8)_2]^{2-}$ , $[Hg(S_4)_2]^{2-}$ $[Zn(S_8)_2]^{2-}$ $[Au_2S_8]^{2-}$	Müller et al. (1984, 1985)
	"Cage" complexation may encapsulate and stabilize complex in oxidizing environment (?) e.g. $[Pd_2S_{28}]^{4+}$	

In summary, we may anticipate relatively simple inorganic/organic metal complexes in dilute sulfide and metal solutions (oxidized fresh waters) with the organic complexes predominant in areas of high biological productivity. Polymeric inorganic/organic metal sulfides will predominate in pore waters, especially in marine environments where total sulfur is elevated. Colloidal Ag complexes may occur metastably in oxidizing waters and be predominant in sediments.

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Assessment of Cline's and AVS methods:

Cline's (1969) method for reactive sulfide consists of reaction of N,N-dimethyl-p-phenylenediamine sulfate with Fe(III) in HCl and measurement of the resultant solution against an Na<sub>2</sub>S standard at 670 nm. The response is linear up to 1 μM S(II-) and can readily be used up to 10 μM. Response of various sulfide species was compared to a 10 μM concentration of Na<sub>2</sub>S. If a 10 μM sulfide concentration did not respond, a 1,000 μM concentration was tested. We also tested the pH effect as defined by varying the concentration of the NaOH trap solution in the AVS method.

We also scanned the absorption spectra of the sulfide complex with Cline's solution from 350-805 nm. Interestingly the absorbance at 740 nm is more sensitive than the suggested wavelength of 670 nm. We also confirmed that the lower the concentration of NaOH in the AVS trapping solution, the more sensitive the analysis. The suggested concentration of 0.5 N NaOH for the trapping solution in the AVS method was determined to be acceptable, but not optimal.

The reactivity of different sulfides to Cline's method compared to Na<sub>2</sub>S is summarized as follows:

Thiol compounds at 10 and 1,000 μM (Glutathione, 3-MPA, L-cysteine, (CH <sub>3</sub> ) <sub>2</sub> S, C <sub>2</sub> H <sub>6</sub> SH	no recovery (max 0.2% for L-cysteine)
Oxidized sulfur: (thiosulfate, sulfite)	no recovery
Polysulfide (Na <sub>2</sub> S <sub>4</sub> )	~80% re one S

The AVS method (Allen et al., 1993) considers reaction of a sediment slurry in HCl generally for 60 min, purging and recovery in a 0.5 N NaOH. The resultant solution is analyzed for reduced sulfur by different methods, but Cline's method is more often used. Cornwell and Morse (1987) originally proposed this method to differentiate between metastable forms of FeS and pyrite. Allen et al. showed that (commercial) CuS did not react to the specified protocol. This assessment considered the effect of the time of reaction and the concentration of the HCl solution. All original tests were run with N HCl; if the metal sulfide did not react or reacted slowly, 6N HCl was used. All test materials were added to produce a 10 μM S solution in the NaOH trap.

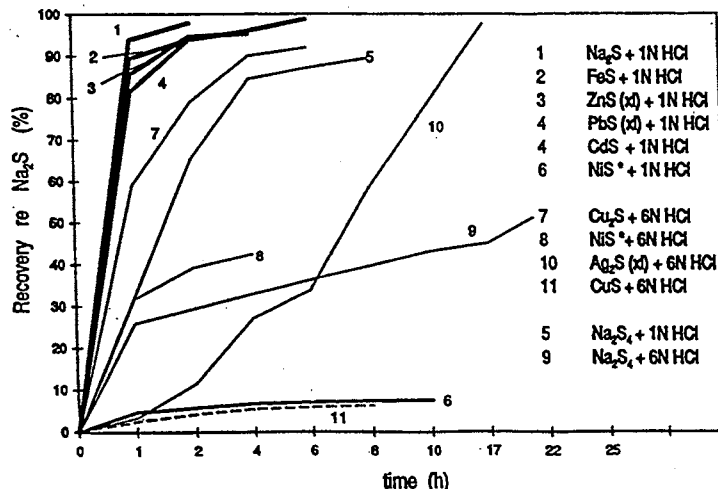
We synthesized all of our metal sulfides and determined their crystallinity by powder X-ray diffraction. If no recognizable pattern resulted, the sample was described as amorphous. Summary of the metal sulfides as to their crystallinity is:

<u>Crystalline</u>	<u>Amorphous</u>
Ag <sub>2</sub> S (acanthite)	FeS
PbS (galena)	CdS
ZnS (sphalerite)	CuS
	NiS

Ag<sub>2</sub>S is unusual in that it develops a very sharp XRD pattern very quickly whereas PbS and ZnS develop broad peaks, perhaps due to small crystal size. Various recipes for the formation of NiS were tried due to its anomalous behavior as discussed below. All NiS solids were XRD amorphous, and all gave the same results with respect to the AVS test.

Figure 1 summarizes the reactivity of various solid sulfides and  $\text{Na}_2\text{S}_4$  over time and for 1 and 6 N HCl. The results confirm previous work of Allen and Morse with some exceptions. Amorphous FeS, ZnS and PbS (2,3,4) react completely or nearly completely within 2 hrs. About 70 percent of the CdS (5) reacts in 1 N HCl and nearly all reacts in 6 N HCl (not shown).  $\text{Ag}_2\text{S}$  reacts nearly completely only in 6 N HCl and after 10 hrs. NiS (6 vs 8) reacts incompletely in 6 N HCl, and CuS (11) does not respond in 6 N HCl.  $\text{Ag}_2\text{S}$  may respond better than CuS and NiS due to the formation of chloro complexes. The polysulfide solution reacts less in the stronger HCl solution, due to the formation of colloidal sulfur at low pH. The polysulfide response is calculated for one sulfur atom.

Figure 1. AVS reaction time for metal sulfides and polysulfides ( $\text{S}_4^{2-}$ )



#### What does this mean?

The previous work of Siegal (1965) on reactivity of thiols to Cline's method is confirmed as well as the AVS sensitivity to amorphous FeS and non-reactivity of CuS. Thus the AVS protocol and Cline's method do not detect thiols. Where thiols would be significant (POTW outfalls, marine environment, generally high biological activity), another method should be employed. Inclusion of thiols is important due to their stronger metal binding constants and their metastability in oxidizing environments compared to simple sulfides. In high  $\text{S}(-\text{II})$  concentration solutions as may be found in interstitial waters, polysulfides may be predominant, and other methods of detection are needed. On the other hand, where the predominant metal sulfide is FeS, the AVS method is valid. Furthermore the AVS method would be applicable to calculations involving some metal sulfides (Fe, Zn) which occur at elevated concentrations (micro-molal), but the calculation may be in error for more dilute metal concentrations (nano-molal and pico-molal) where thiols complexes of metals may be strong and predominate. Furthermore some of the thiol complexes may persist metastably in an oxidizing environment.

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## Questions & Answers: Assessment of the AVS Method and "Cline's" Method with Respect to Reactive Sulfide

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- Q. DOMINIC DI TORO (Manhattan College): Jim, just a comment. It's a good opportunity to comment on the number of people who are noticing that the AVS/SEM extraction doesn't take out all of the particulate sulfides. The trick is to realize that if it doesn't dissolve in the extraction, you neither measure sulfide, the sulfur, nor do you measure the metal. And therefore in the subtraction, which is what you use to decide whether you have a problem or not, those terms don't show up on either side of the equation. It's really not a problem with respect to understanding the toxicological consequences. In fact, you really don't want to extract the  $\text{Cu}_2\text{S}$  because that fouls up your molar assumptions that everything is Cu metal sulfide in the...
- A. Well. . .
- Q. . .so in fact we got lucky. The extraction really leaves behind some of the sulfides, which would actually complicate your interpretation.
- A. The only dilemma with all of this, Dominic, is for silver. Because silver is around in such trace amounts, it can react with anything in there. Including the thiols you aren't measuring. And we do know that it reacts of course with the FeS, but we also do know we can mobilize it. So...
- Q. The comment with the thiols is well taken.
- A. I'm just saying that's an unknown black box. The other thing is, I think in terms of toxicity, we have to get to the question of what kind of bacteria cleave these things, if any.
- Q. And whether the thiol complex is...
- A. Right.
- Q. Yes. A, if you cleave, and B, does that have any toxicological significance.
- A. If silver were at the concentration of iron or copper, then I'd say go with AVS and forget all these other details.
- Q. TOM BOBER (Eastman Kodak): Jim, I was going to make a tongue-in-cheek comment this morning when Russ Bell showed the double helix structure of the silver compounds, and he mentioned something about RNA and DNA. And now you've brought up the same subject referring back to this other paper. Is this just a coincidence, or is there really something possibly behind this?
- A. Oh no. This is very important. And this was made by a very distinguished professor at Wisconsin, so it has to be true. (laughter)



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## Specific Chemical Interactions Between Silver(I) and Sludge Particulates: Effects of pH and Dissolved Organic Matter (DOM)

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### ABSTRACT

Concern over the toxicity of silver to aquatic organisms has prompted research that will aid in predicting the levels and chemical forms of silver discharged from wastewater treatment plants. The aim of this research project is to increase understanding of the rates and equilibria of silver ion interactions with sludge particulates and dissolved organic matter (DOM) in the wastewater treatment process. The role of AgCl and Ag<sub>2</sub>S precipitations in this process must be taken into account. To date equilibrium experiments have been performed that involve adding silver ion (as silver nitrate) at various concentrations to wastewater sludges at various pH values. Experiments were done with both unwashed sludges and sludges washed to remove chloride and sulfide. Results of experiments with unwashed sludges suggest that AgCl and Ag<sub>2</sub>S precipitations were in large part responsible for the high percent removal of silver (from the supernatant phase to the solid phase) observed. Experiments with washed sludges suggest that silver ion has a high affinity for DOM, and that silver binding to particulates decreases with decreasing pH. It is proposed that the particulate surface sites that bind silver are anionic, weak Brønsted bases that become protonated with decreasing pH, and thus unavailable for silver ion binding.

### INTRODUCTION

There is concern about the toxicity of silver to aquatic organisms. Free silver ion is the form of silver most toxic to organisms, while Ag<sub>2</sub>S<sub>2</sub>O<sub>3</sub> (silver thiosulfate), soluble AgCl complexes, and Ag<sub>2</sub>S are the least toxic forms (Petering and McClain, 1991). Therefore it is important to know and be able to predict the levels and chemical forms of silver being discharged from wastewater treatment plants. This requires understanding the rates of silver interactions, and how silver distributes between the aqueous and solid phases in the wastewater treatment system. The aim of this research project is to study the rates and equilibria of silver ion (Ag<sup>+</sup>) interactions in municipal wastewater treatment systems.

The specific objectives of this research project are to 1) determine rate equations and rate constants for silver ion uptake by wastewater particulates, and 2) determine equilibrium expressions and equilibrium constants for silver ion uptake by wastewater particulates. In addition, the equilibrium between silver bound to sludge particulates, silver bound to DOM (dissolved organic matter), and free Ag<sup>+</sup> must be characterized. Four different types of wastewater will be studied: primary sludge, secondary sludge or mixed liquor, aerobic sludge, and anaerobic sludge. Sludge was taken from five different wastewater treatment plants: Wilmington Wastewater Treatment Plant, Northeast Wastewater Treatment Plant (Philadelphia, PA), Back River Wastewater Treatment Plant (Baltimore, MD), Blue Plains Wastewater Treatment Plant (Washington, D.C.), and Seneca Wastewater Treatment Plant (Seneca, Maryland).

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## METHODS

The objectives of this research project will be accomplished by performing both rate and equilibrium experiments for silver interaction with sludge particulates and DOM. To date equilibrium experiments have been the focus of the work.

### Silver Equilibrium experiment

Batch equilibrium experiments were done with six different total silver concentrations (ranging from 5 to 300  $\mu\text{M}$ ) at eight different pH values (3 through 10). The equilibrium experiment consists of the following main steps:

- 1) Add silver ion (in the form of  $\text{AgNO}_3$  stock solution) to sludge samples in polyethylene bottles.
- 2) Adjust pH of samples with 1.0 N  $\text{HNO}_3$  or 1.0 N  $\text{NaOH}$ .
- 3) Shake bottles for 4 hours in a mechanical shaker.
- 4) Use a portion of each sample to determine the final pH.
- 5) Centrifuge each sample at 19,000 g for 10 minutes to obtain a supernatant.
- 6) Measure silver content of supernatant by flame atomic absorption (AA).
- 7) Measure COD (chemical oxygen demand) of supernatant as a measure of the DOM content (by standard methods - closed reflux colorimetric method).

The amount of silver in the solid phase was determined by subtracting the amount in the supernatant from the total silver added.

Silver forms strong complexes and stable precipitates with both sulfide ( $K_{sp}=1 \times 10^{-29}$ ) and chloride ( $K_{sp}=1.8 \times 10^{-10}$ ). For both primary and secondary sludges, chloride measurements typically ranged from 100 to 300 mg/L. Sulfide concentrations typically ranged from 0.02 to 0.1 ppm for primary sludge, and  $\leq 0.002$  mg/L for secondary sludge. Because the silver complexes with sulfide are much stronger than those with chloride, silver ion added up to the stoichiometric amount of sulfide is expected to bind sulfide. Silver ion added in excess of this amount may bind to chloride, sludge particulates, or DOM.

When  $\text{AgNO}_3$  is added to the sludge,  $\text{Ag}_2\text{S}$  and  $\text{AgCl}$  precipitates formed will end up in the solid phase of the sludge suspension. Thus, in an equilibrium experiment with unwashed sludge, the silver in the solid phase will consist of  $\text{Ag}_2\text{S}$  and  $\text{AgCl}$  precipitates and silver adsorbed to sludge particulates. In order to determine the nature of silver interaction with sludge particulates without the confounding factor of  $\text{Ag}_2\text{S}$  and  $\text{AgCl}$  precipitations, it was necessary to wash the sludge to remove sulfide and chloride before the equilibrium experiment. Equilibrium experiments were conducted with both washed and unwashed sludges. The procedure for washing involved adding de-ionized water to sludge, allowing the solids to settle for 20 minutes, and then draining off the supernatant. Each wash was equivalent to a five-fold dilution and the process was repeated six times. The chloride content of the washed sludges was measured by a mercuric nitrate method (Hach PermaChem Chloride Reagent Set and Digital Titrator), which has a chloride detection limit of  $10^{-5}$  M. All washed sludges had a chloride content below  $10^{-5}$  M. The sulfide content of washed sludges was not measured, but this will be done in future experiments.

The flame atomic absorption method has an error of  $\pm 0.002$  absorbance units, which results in an error of  $\pm 0.25$   $\mu\text{M}$  silver. Silver nitrate calibration curves for atomic absorption had R values greater than 0.999. In measuring silver concentrations greater than 50  $\mu\text{M}$ , dilution of the samples was necessary. Dilution typically resulted in an error of 3 percent.

The potassium hydrogen phthalate calibration curves for COD measurement had R values greater than 0.99. COD measurements typically had an error of  $\pm 20$  mg/L for high range COD and  $\pm 5$  mg/L for low range COD.

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## RESULTS

### Equilibrium Experiments with Unwashed Sludges

Equilibrium experiments were done with the following unwashed sludges: Philadelphia primary sludge, Philadelphia secondary sludge, Washington secondary mixed liquor, Wilmington secondary mixed liquor, and Seneca aerobically digested sludge. Figures 1 show graphs of percent silver removal (percent of silver added that ended up in the solid phase) as a function of pH for these sludges. The legends indicate the total silver concentrations for the different curves. All showed a common pattern of very high percent removal at all pH values, with a slight decrease in percent removal at the highest pH values. Figure 2 shows COD as a function of pH for several of these sludges. It can be seen that increasing the pH above 6 was associated with an increase in the DOM content, especially at pH values above 8. Also, there was a slight increase in COD when pH was lowered below 6. This pattern in COD values was common to all experiments with washed and unwashed sludges.

In the experiment with unwashed Wilmington secondary mixed liquor, the chloride concentration decreased from 3.6 mM to 3.5 mM when 263  $\mu\text{M}$  of total silver was added.

### Equilibrium Experiments with Washed Sludges

Equilibrium experiments with washed sludges showed a much different pattern of percent removal than the unwashed sludges. Figure 3(a) shows percent removal as a function of pH for Baltimore secondary mixed liquor, using a TSS concentration of 2.69 g/L. Between pH 4 and 6, the percent removal was very high (near 100%). But at pH values above 6, there was a dramatic decrease in percent silver removal. In the high pH range (pH 7 to 9), there was a pattern of lower percent removal the lower the total silver concentration. For the lowest total silver concentration, 10  $\mu\text{M}$ , the percent silver removal decreased almost to zero at alkaline pH.

Because maximum (near 100 percent) percent removal was observed in the intermediate pH region with Baltimore secondary mixed liquor, it was decided to use small TSS concentrations for the following equilibrium experiments. Figure 3(b) shows percent silver removal as a function of pH for Wilmington secondary mixed liquor, using a TSS concentration of 0.18 g/L. From pH 3 to 7 percent removal increased with pH. Also in this pH range percent removal decreased with increasing total silver concentration. In the alkaline pH range, percent removal decreased with pH, except for the two highest total silver concentrations (150 and 300  $\mu\text{M}$ ). In the same pH region, it was observed that percent removal increased with increasing total silver concentration, with the exception of the two highest total silver concentrations.

Figure 3(c) shows percent removal as a function of pH for Seneca aerobically digested sludge, using a TSS concentration of 0.20 g/L. The percent removal behavior was similar to that observed for Wilmington secondary mixed liquor. Figure 3(d) shows the total amount of silver bound per gram of TSS ( $\mu\text{mol Ag}^+/\text{g TSS}$ ) as a function of pH for Seneca aerobically digested sludge. As pH increased, the amount of silver uptake increased. Also, the higher the total silver concentration, the greater was the extent of uptake. Figure 4 shows COD as a function of pH for washed Wilmington secondary mixed liquor and washed Seneca aerobically digested sludge.

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## DISCUSSION

### Silver Equilibrium Experiments with Unwashed Sludges

A common pattern was observed in all experiments (for both washed and unwashed sludges) of increasing DOM with an increase in pH above 6, with a pronounced increase at pH values above 8. It is hypothesized that this increase in DOM at alkaline pH was due to base-mediated hydrolysis of particulate organic matter, resulting in creation of DOM.

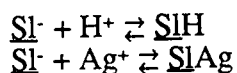
Because the chloride concentration decreased from 3.6 mM to 3.5 mM when 263  $\mu\text{M}$  total silver was added to Wilmington secondary mixed liquor, a significant portion of the silver added must have formed AgCl precipitate. Although chloride measurements were not done for the other experiments with unwashed sludge, this result suggest that at high total silver concentrations a significant portion of silver added to unwashed sludge will form AgCl precipitate. Because the solubility product of Ag<sub>2</sub>S is much lower than that of AgCl, it is reasonable to conclude that if AgCl formed in the experiments with unwashed sludge, all of the sulfide present must have formed Ag<sub>2</sub>S precipitate.

The formation of AgCl precipitate is most likely responsible for the differences in percent removal (at similar loadings) between unwashed sludges and the same sludges when they are washed. When comparing an unwashed sludge with the same sludge when it is washed, one must compare similar loadings ( $\mu\text{mol}$  silver per gram TSS) because the TSS concentrations may be different. For example, in comparing the results for unwashed Wilmington secondary mixed liquor (figure 1(d)) with washed Wilmington secondary mixed liquor (figure 3(b)), a total silver of 263  $\mu\text{M}$  for the unwashed sludge (loading = 100  $\mu\text{mol/g}$  TSS) may be compared to the total silver of 20  $\mu\text{M}$  for the washed sludge (loading = 111  $\mu\text{mol/g}$  TSS). In comparing these two curves, one can see that at alkaline pH, the percent removal for the washed sludge is significantly lower than that of the unwashed sludge. Also, at around pH 3 the percent removal for washed sludge is slightly below 100 percent, whereas for the unwashed sludge, the percent removal is at 100 percent. Similar differences can be found when comparing equivalent loadings in washed and unwashed Seneca aerobically digested sludge (figures 1(e) and 3(c)). The reason the percent removal is lower for washed sludge at some pH values is most likely the formation of AgCl precipitate in the unwashed sludge.

### Silver Equilibrium Experiments with Washed Sludges

Results from equilibrium experiments with sludges that were washed to remove chloride and sulfide suggest several things about the interaction of Ag<sup>+</sup> with sludge particulates and DOM (Figure 3). The decrease in percent silver removal at alkaline pH is thought to be due to Ag<sup>+</sup> binding to DOM which is not adsorbable to the sludge. As shown in Figure 3(a), percent silver removal was almost zero at alkaline pH for the total silver concentration of 10  $\mu\text{M}$ . This suggests that Ag<sup>+</sup> has a high affinity for the DOM. It was also observed that at alkaline pH, the higher the initial silver concentration, the higher the percent removal. To explain this, one must consider that the amount of DOM present in the supernatant at any given pH is finite. It is hypothesized that at alkaline pH if the concentration of silver is increased, the capacity of the DOM to bind Ag<sup>+</sup> is exceeded, and the remaining Ag<sup>+</sup> can bind to sludge particulates. This explains why at high pH, a higher percent removal was observed the higher the total silver concentration.

The experiments with Wilmington secondary mixed liquor and Seneca aerobically digested sludge (figures 3(b) and (c)) had a relatively low TSS concentration (around 0.2 g/L) and results show a decrease in percent removal at acidic pH. It is thought that the particulate binding sites for silver are anionic and can act as weak Brönsted bases:



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where  $Sl$  = Sludge particulate binding site. If pH is decreased (and thus  $H^+$  concentration increases), then some of these anionic sites bind to  $H^+$  and are no longer available for  $Ag^+$  to bind. With less binding sites available at low pH, less silver binds to the particulates, resulting in lower percent removal. Figure 3(d) demonstrates how dramatically the binding capacity of the particulates changed with pH. At pH 3 there was very little difference in the amount of silver bound per gram TSS for the different initial silver concentrations; all but the two lowest total silver concentrations resulted in silver binding at about  $50 \mu\text{mol } Ag^+/g$  TSS. Increasing total silver above  $40 \mu\text{M}$  did not result in any significant increase in binding. This implies that the binding capacity of the particulates was reached for the four highest initial silver concentrations at pH 3. Thus the total binding capacity of this sludge at pH 3 must have been around  $50 \mu\text{mol } Ag^+/g$  TSS. Observing the line for a total silver concentration of  $300 \mu\text{M}$ , one can see how the amount of silver bound per gram of TSS increased from around  $50 \mu\text{mol } Ag^+/g$  TSS at pH 3 to around  $1000 \mu\text{mol } Ag^+/g$  TSS at pH 9. Because substantially less silver was bound per gram TSS at pH 9 for  $150 \mu\text{M}$ , it is safe to conclude that the total binding capacity of the sludge at pH 9 had not been reached by the initial silver concentration of  $300 \mu\text{M}$ .

It was observed in Figure 3(b) and (c) that at low pH, the higher the total silver concentration, the lower the percent removal. To explain this, one must first consider that there are a finite number of binding sites on the particulates. If at a given pH the silver concentration is increased, the total amount of silver bound to particulates increases until it reaches the maximum binding capacity at that pH (Figure 3(d)). When the binding capacity is approached, further increases in silver concentration do not result in significant increases in the amount of silver bound. Thus the ratio of the amount of silver bound to the total silver added becomes smaller as the silver concentration increases. This ratio is the same as the percent removal. This explains why at low pH the percent removal decreased with increasing total silver concentration.

## CONCLUSIONS

In equilibrium experiments with unwashed sludge more than eighty percent of the silver ion added ended up in the solid phase for the entire pH range tested (pH 3 to 10). The silver in this solid phase is thought to be a combination of  $AgCl$  and  $Ag_2S$  precipitates, and silver bound to sludge particulates. Results of equilibrium experiments with washed sludges led to two major conclusions about the nature of silver ion interactions with sludge particulates and DOM. First, the experiments indicate that silver ion has a high affinity for DOM. With increasing DOM content of the supernatant (due to alkaline pH), more silver stayed in the supernatant and less bound to sludge solids. Second, with decreasing pH less silver ion bound to sludge solids. This is thought to be due to increasing protonation of anionic binding sites with decreasing pH, resulting in less sites available for silver ion to bind.

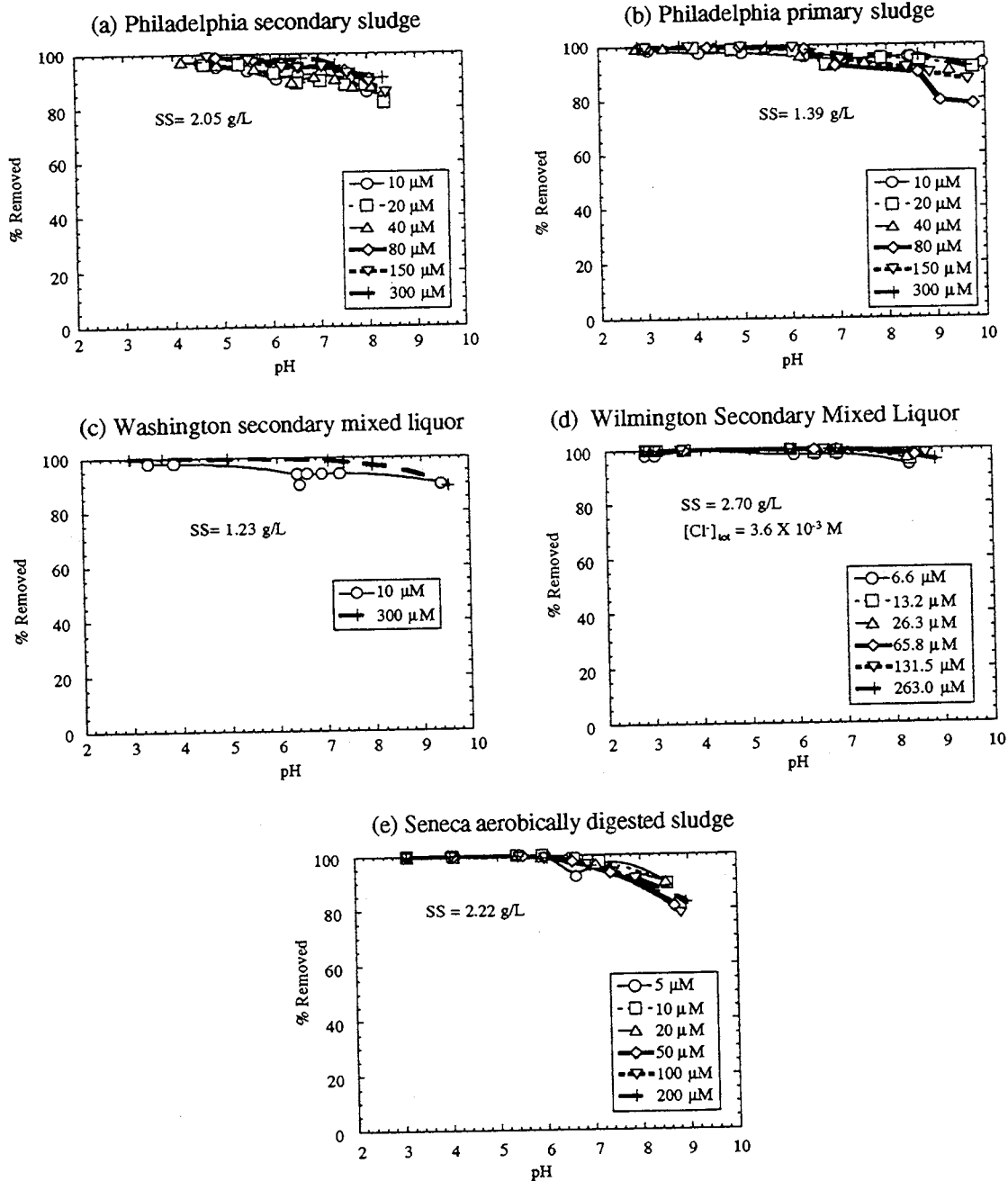


Figure 1. Silver removal by unwashed sludges  
 (a) Philadelphia secondary sludge  
 (b) Philadelphia primary sludge  
 (c) Washington secondary mixed liquor  
 (d) Wilmington secondary mixed liquor  
 (e) Seneca aerobically digested sludge



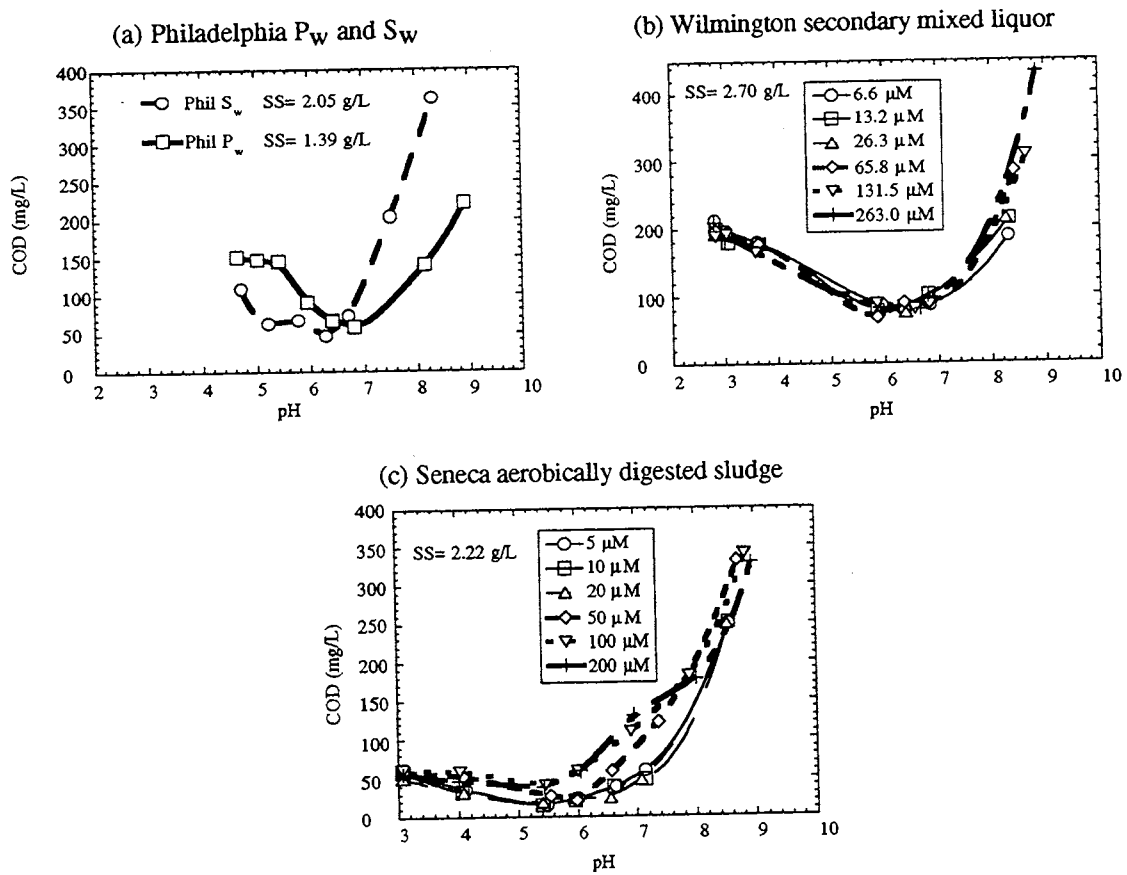


Figure 2. COD for silver equilibrium experiments with unwashed sludges  
 (a) Philadelphia primary and secondary sludges, in the presence of Ag(I) at  $10 \mu\text{M}$   
 (b) Wilmington secondary mixed liquor in the presence of Ag(I) at various concentrations  
 (c) Seneca aerobically digested sludge in the presence of Ag(I) at various concentrations

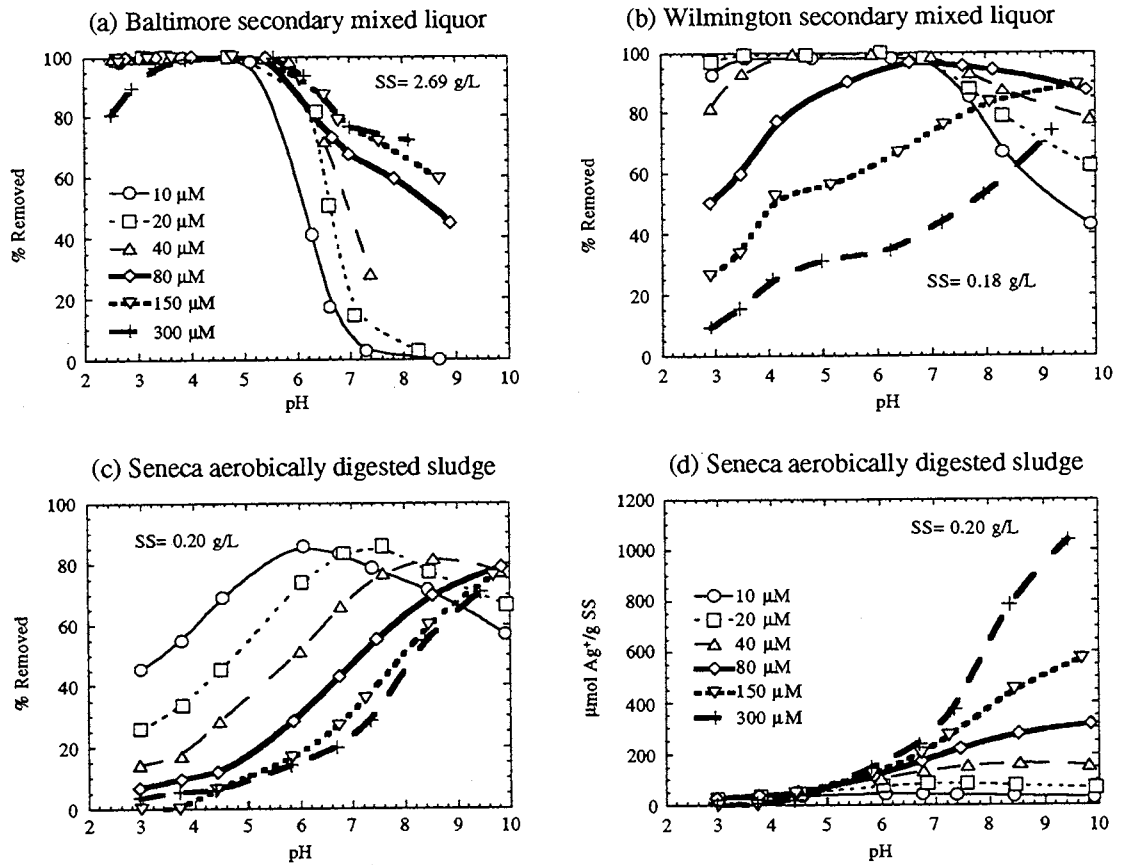


Figure 3. Silver removal by washed sludges  
 (a) Baltimore secondary mixed liquor  
 (b) Wilmington secondary mixed liquor  
 (c) Seneca aerobically digested sludge, percent removal vs. pH  
 (d) Seneca aerobically digested sludge,  $\mu\text{mol Ag}^*/\text{g TSS}$  vs. pH

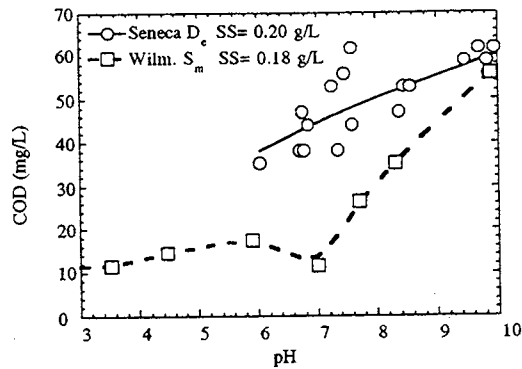


Figure 4. COD vs. pH for washed Wilmington secondary mixed liquor and Seneca aerobically digested sludge

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**Questions & Answers: Specific Chemical Interactions Between Silver(I) and Sludge Particulates:  
Effects of pH and Dissolved Organic Matter (DOM)**

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- Q. JIM KRAMER (McMaster University): Another way of looking at the recovery - it's very high in the first set you showed us - is what is the residual concentration in solution, not for the very high loadings, but say for the small ones. Can you give me a ballpark estimate of what the residual solution concentration is, and relative to what size?
- A. I can't remember exactly, offhand. They were in the micromolar range, I would say. Even at low loadings like say 20 micromolar, that was when I had a total silver of 20 micromolars, the soluble silver was still maybe 1 or 2 micromolar.

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## Distribution and Early Diagenesis of Ag in the Sediments of the St. Lawrence River and Estuary

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The following summarizes the results on the distribution of Ag, acid-volatile sulfides (AVS), Mn and  $^{210}\text{Pb}$  in two sediments cores extracted, one in the St. Lawrence River, the second largest river in North America, at a depth of 24 m in the vicinity of Lake Ontario, and another in its Estuary, at a depth of 350 m in the Laurentian Trough (Fig.1). These results are the first to be reported concerning Ag in those environments, which are already known for their contamination by other metals (Carignan et al., 1994 ; Gobeil et al., 1995).

### Methods

The sediment cores were collected using an Ocean Instrument Mark II box-corer. The cores were subsampled into horizontal layers in a specially designed glove-box and the sediments were kept frozen in polyethylene bottles. Back to the laboratory, sediment aliquots were freeze-dried, homogenized by grinding, and digested in a microwave oven with a mixture of nitric, hydrochloric, fluoric, and perchloric acids (Nakashima et al., 1988). The digestates were then analyzed by atomic absorption spectroscopy, using a pyrolytic graphite furnace equipped with a L'vov platform for Ag and an air-acetylene flame for Mn. The overall analytical procedure was verified with the reference sediment BCSS-1 (National Research Council of Canada). For the two metals, the precision was  $\pm 4\%$ , expressed as the coefficient of variation of replicate analyses ( $n=6$ ) of the reference material. The accuracy was within 9%. Acid volatile sulfides (AVS) were determined on wet sediment using the protocol of Allen et al. (1993). The detection limit was  $0.01\ \mu\text{mol g}^{-1}$ , defined as twice the standard deviation of the blank. The analytical precision was  $\pm 8\%$  at concentrations above  $0.2\ \mu\text{mol g}^{-1}$ . Finally,  $^{210}\text{Pb}$  was determined following the procedure described by Eakins and Morrison (1978) with analytical precision on the order of  $\pm 14\%$ .

### Results

**Ag.** The concentration of Ag in the sediments reaches  $1.1\ \mu\text{g g}^{-1}$ , in core 865 collected in the vicinity of Lake Ontario, and  $0.20\ \mu\text{g g}^{-1}$ , in core 23A collected in the Laurentian Trough (Figs. 2a). The sedimentary records present a subsurface maximum at 4 and 7 cm depth in cores 865 and 23A, respectively. The subsurface Ag peaks exceed the sediment surface value by 35% in core 865, but is not as pronounced in core 23A, exceeding the surface value by only 15%. Below these peaks, Ag decreases to values close to its abundance in continental crust ( $0.07\ \mu\text{g g}^{-1}$ ).

**AVS and Mn.** The sedimentary profiles of AVS are characterized by strong concentration gradients (Figs. 2b). In cores 865, AVS raise sharply from  $2.5\ \mu\text{mol g}^{-1}$  at the sediment surface to

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15  $\mu\text{mol g}^{-1}$  at 5 cm depth and then slightly decrease downward. There is no surface layer enriched in Mn in this core, where the molar concentration of AVS at the sediment surface is already 300 times higher than the total molar concentration of Ag ( $0.01 \mu\text{mol g}^{-1}$ ). In core 23A, however, AVS are below detection limit ( $0.01 \mu\text{mol g}^{-1}$ ) down to 2 cm depth, but then also increase downward, reaching  $28 \mu\text{mol.g}^{-1}$  at 15 cm depth. There is a well defined surface layer enriched in Mn in this core.

<sup>210</sup>Pb. Profiles of excess <sup>210</sup>Pb activities, determined assuming that <sup>210</sup>Pb supported by <sup>226</sup>Ra is given by the values obtained from the deepest samples in the cores, are showed on Figs. 2c. The two profiles are characterized by a surface mixed layer, below which the excess <sup>210</sup>Pb activity decreases exponentially with depth. Sedimentation rates, calculated using the results below the mixed layer (Robins and Edgington, 1975), are  $0.14 \text{ g cm}^{-2} \text{ yr}^{-1}$  and  $0.33 \text{ g cm}^{-2} \text{ yr}^{-1}$  in cores 865 and 23A, respectively.

### Discussion

Sites of net sediment deposition being infrequent in the St. Lawrence River, the vertical profile of Ag in core 865 may be considered atypical. It nonetheless demonstrates a significant anthropogenic Ag load in the River, for the Ag concentrations in the core exceeds crustal abundance by factors of up to 15. The wide dispersion of Ag in the region under investigation is further evidenced by an enrichment factor of about 3 in core 23A, extracted 1500 km away from core 865. In view of the fact that the Great Lakes and the St. Lawrence River drain highly urbanized regions, municipal effluents are a probable major source of anthropogenic Ag in the St-Lawrence, as they are in other areas (e. g. Sanudo-Wilhelmy and Flegal, 1992 ; Ravizza and Bothner, 1996).

The coincidental increases of the Ag and AVS concentrations just below the sediment surface in the two cores (Fig. 3) suggest a diagenetic influence on the sedimentary distribution of Ag. AVS are formed during the oxidation of the organic matter by sulfate reducing bacteria. The main reaction product of this reaction is hydrogen sulfide, which reacts with iron, abundant in porewater, as well as other metals, to form the AVS. Also, the presence of AVS in close proximity to the sediment-water interface in our cores suggests that dissolved Ag, originating either from the water column or from its remobilization in the oxic surface layer, diffuses downward into the sediments and subsequently precipitates as a sulfide, by reaction with hydrogen sulfide or, according to recent model (Di Toro et al., 1992), through the displacement of the Fe in iron sulfide.

The importance of the downward diffusive flux of Ag into the sediments can be estimated at our station 23A, where the AVS horizon occurs at 2-3 cm, assuming that Ag is remobilized in the oxic surface layer, as concluded by Rivera-Duarte and Flegal (1997) in a recent work on sediments of San Francisco Bay. Under steady state, this is done by using the Fick's law ( $J = -\phi^2 D_s \partial[C]/\partial z$ ), where J is the diffusive flux,  $\phi$  the porosity,  $D_s$  the whole sediment diffusion coefficient, which is assumed to be equal to  $\phi^2 D_o$  for high porosity sediments (Ullman and Aller, 1982),  $D_o$  being the molecular diffusion coefficient at the *in situ* temperature (Li and Gregory, 1974), and  $\partial[C]/\partial z$  the Ag concentration gradient as a function of depth z. The concentration gradient of Ag supporting a downward flux in the sediments has been set at  $0.12 \text{ pmol cm}^{-4}$ , after the work of Rivera-Duarte and Flegal (1997) who found an average Ag concentration of 120 pM

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for 55 porewater samples from 5 different cores, excluding anomalously high values.

Based on this crude assumption, the downward diffusive fluxes of Ag is  $0.003 \mu\text{g cm}^{-2} \text{yr}^{-1}$  at station 23A. This flux represents 5% of the present deposition rate of Ag at the sediment surface, estimated by multiplying the Ag concentration in the surficial sediment ( $0.18 \mu\text{g g}^{-1}$ ) by the sedimentation rate ( $0.33 \text{g cm}^{-2} \text{yr}^{-1}$ ). This is a small fraction, but our estimate is very conservative because it does not take into account the biorrigation, which can increase the flux by a factor of 5 at the study site (Silverberg et al., 1987). If we apply this factor, then the downward flux becomes more than sufficient to contribute to the formation of a subsurface peak, which is at station 23A 15% higher than the surface value. Due to the occurrence of AVS and the virtual absence of Mn enrichment at the sediment surface at station 865, the downward diffusive flux of Ag may be higher at this site because dissolved Ag has to diffuse through a shorter distance before it precipitates.

### Conclusion

The above results reveal the wide dispersion of anthropogenic Ag in the St Lawrence River and Estuary. The coincidental increases of the Ag and AVS concentrations just below the sediment surface in the two cores, as well as flux calculations based on crude assumptions suggest that the sedimentary accumulation of Ag through downward diffusion of dissolved Ag is not negligible. Such a mechanism can contribute to the formation of a subsurface maximum in the sedimentary record of Ag.

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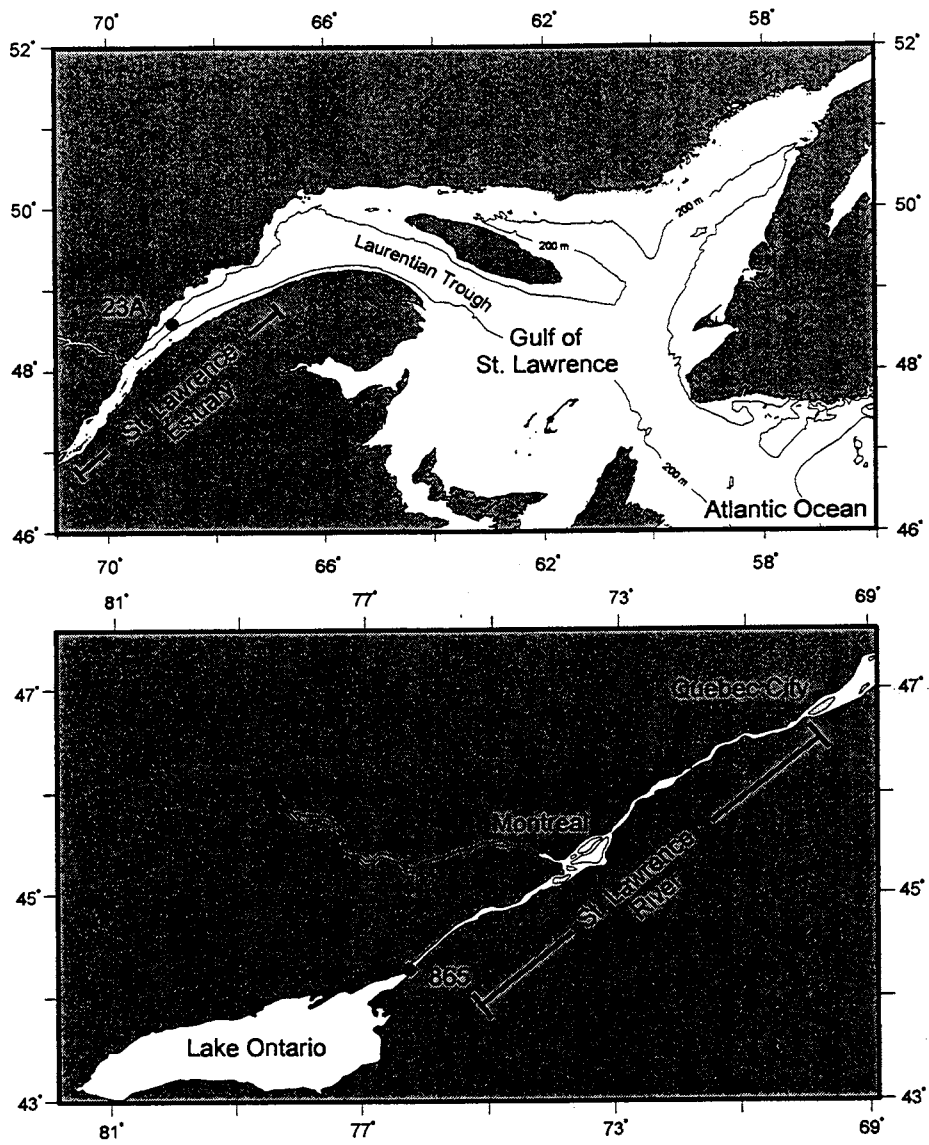


Figure 1. Sampling locations in the St. Lawrence River and Estuary.



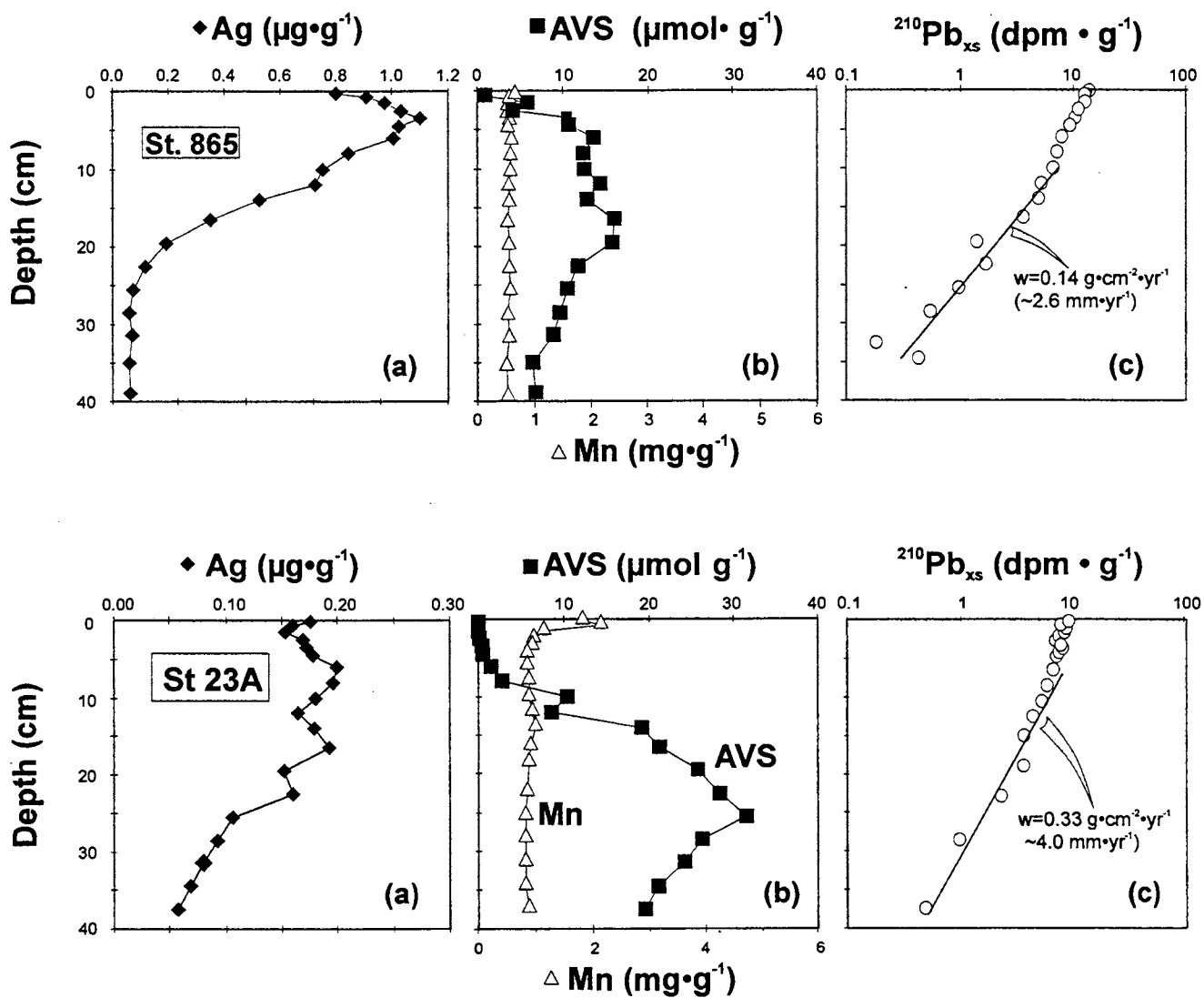
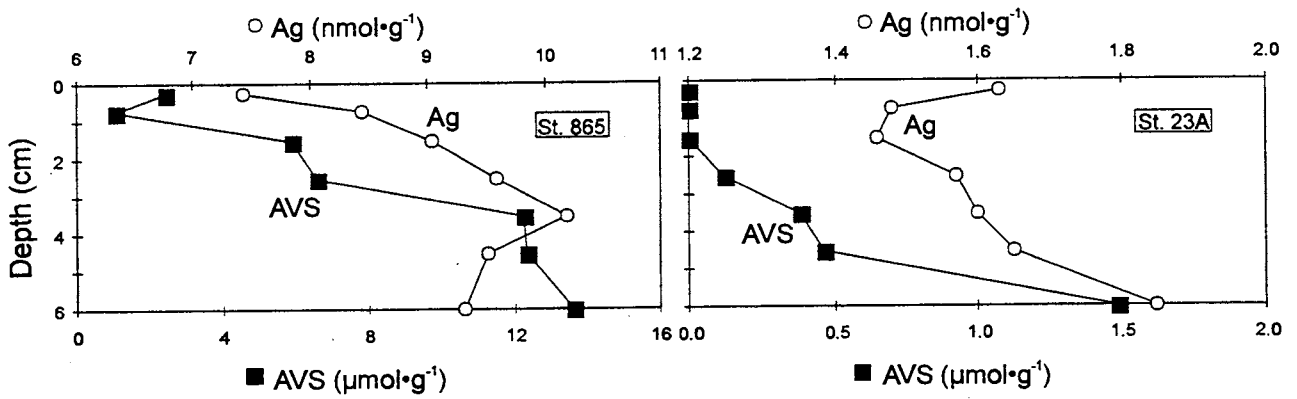


Figure 2. Sedimentary records of Ag (a), acid-volatile sulfides (AVS) and Mn (b), and  $^{210}\text{Pb}$  (c) at the two sampling sites.



**Figure 3.** Vertical profiles of Ag and acid-volatile sulfides (AVS) over the top 6 cm of the sediments at the two sampling locations.

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## Questions & Answers: Distribution and Early Diagenesis of Ag in the Sediments of the St. Lawrence River and Estuary

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- Q. DOMINIC DI TORO (Manhattan College): It certainly is interesting, the silver and the lead coincidence, but I'm not sure I believe your explanation. If you've got lead moving around as much as you need it to move around in order to explain your results, then I would think that the lead-210 dating procedures just wouldn't work. And they've been calibrated against pollen deposition and other stratigraphies. So, on the one hand it helps with your silver story, but it more or less destroys the power of lead-210?
- A. I don't feel I have all the answers at the moment. But it's hard to pretend that the lead-210 dating technique does not work because it has been used in so many places and it's so very, very useful. But at the same time we seem to have some sort of indication here that lead is maybe a little bit more mobile than we thought before.
- Q. Right. Have you thought of just looking at the production figures for silver and lead? Maybe it's just a coincidence?
- A. It may be just a coincidence. But we don't have the proof.
- Q. I mean, you know the amount of silver that's manufactured. I'm sure there are people in this room that can tell you the silver mobilization.
- A. Yes, yes. But in the core from the Arctic Ocean it's hard to believe that all these things are simply coincidence.
- Q. Yes, it is peculiar.

ANDERS ANDREN: Actually, just a comment, Dominic. It's probably true these days that lead-210 dating of cores really work only in a few places. People who are real involved in lead-210 dating these days are sort of searching for perfect cores, and they really use their lead-210 record as much to explain a lot of post-depositional phenomena as anything, if you talk to the Edgington's and the Robbins'.

DOMINIC DI TORO: This is their latest revisionist history of lead-210? I don't know.

PETER SANTSCHI: Sorry, I believe it's well documented that lead-210 doesn't work in seasonally anoxic lakes. There are a number of us who have shown in Swiss lakes that there is a problem with post-depositional mobility. So the record is there. Those papers have been published. There's one by a radiochemist named von Gulton. We published a paper in the '80's and there are a number other papers which have been published concerning when you have seasonally anoxic conditions. Once it's stable, lead-210 seems to work, but when it varies seasonally and when you have anoxic conditions in the summer, oxic conditions in the winter, it doesn't seem to work.

DOMINIC DI TORO: Unfortunately, the St. Lawrence is not anoxic in the summer.

A. No, no.

DOMINIC DI TORO: Nor is that fjord, I would think. So we've got a problem.

PETER SANTSCHI: Well, the redox clime is changing in that region.

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A. Yes.

DOMINIC DI TORO: But the redox climate must change everywhere. Lake Ontario, anyplace where there's a seasonal phytoplankton bloom. I don't know, it's a bit too unique.

ANDERS ANDREN: Let's just underscore what I said before.

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## Silver Mobility in the Presence of Iron Sulfides Under Oxidizing Conditions

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As a type-B metal cation, silver ( $\text{Ag}^+$ ) binds strongly to sulphide and consequently finds a sink in the anoxic portion of the sediment zone where it is immobilized (Stumm and Morgan, 1996). Upon exposure of sediments to oxygen through activities such as dredging or bioturbation however, the fate of sediment-bound silver has not been determined. The oxidative dissolution of sulphides could result in the release of free silver into the water column where uptake by biota is possible. This study examined the effect of molecular oxygen ( $\text{O}_2$ ) on silver in the presence of synthetic iron monosulphide ( $\text{FeS}$ ) (a common constituent of anoxic sediments) as a function of time.

### Instrumentation

The glass apparatus illustrated in Fig. 1 represents the reaction vessel that was used to study the effect of  $\text{O}_2$  on sediment-bound silver. It consists of a closed cylinder with a gas inlet system attached to the bottom and a smaller inner cylinder that splits and directs the flow of the incoming gas. Consequently, the water contained in the vessel was pneumatically mixed either by nitrogen gas or air or a mixture of both. Water running through the outer jacket encompassing the outer cylinder controlled the temperature of the water in the apparatus. A thermometer, an oxygen probe, a pH electrode and a burette tube were placed inside the reaction vessel through the inlet ports occurring at the top of the vessel. Oxygen concentrations and pH were monitored via a computer and periodically recorded. When changes in the pH were detected, an appropriate amount of base ( $\text{NaOH}$ ) was titrated into the water by the burette to maintain a constant pH level. Regulation of the gas flow allowed for a constant flow of oxygen to enter the system. This experimental design allowed for temperature, pH, and incoming oxygen concentration to be controlled carefully.

### Methodology

A fresh sample of  $\text{FeS}$  was precipitated under a nitrogen atmosphere and filtered through a  $0.45 \mu\text{m}$  Millipore filter. 5 g (0.057 mol) of  $\text{FeS}$  were suspended in 2.5 L of deoxygenated-deionized water. Mixing was accomplished via a nitrogen gas stream (passed through an oxygen scrubber). The temperature was maintained at  $21^\circ\text{C}$  while the pH was adjusted approximately to 8. Once a homogeneous suspension was obtained,  $\text{AgNO}_3$  was added to attain a silver concentration of 4  $\mu\text{g/L}$  (0.037  $\text{nmol/L}$ ). A number of

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samples (filter through 0.45  $\mu\text{m}$  and 10 kD filters) were taken over a 24 h period and analyzed for silver and total iron concentrations (ICP-MS) as well as sulphide concentrations. At the end of the 24 h period, the nitrogen gas stream was replaced with

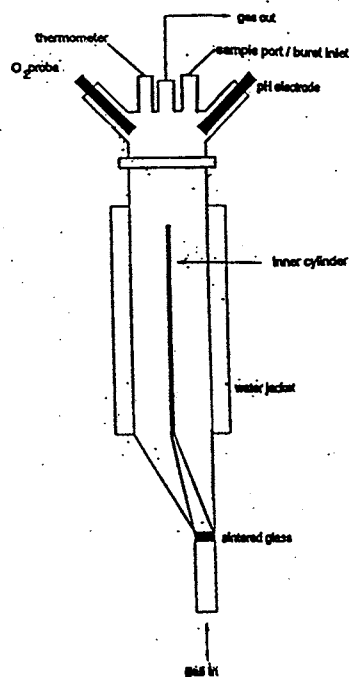


Fig. 1: Reaction vessel (diagram modified from Nicholson and Sharer, 1994).

air and the oxygen content of the water was allowed to reach saturation. FeS oxidation proceeded for 48 h and samples were again taken periodically to measure silver, total iron and sulphide concentrations.

### Results and Future Work

Under deoxygenated conditions, the silver was found to be immediately scavenged from solution by the FeS and increases in the silver concentration were not detected over the 24 h period. This is in accordance with the previous results reported by Adams and Kramer (1996). Under oxygenated conditions, formation of lepidocrocite ( $\gamma\text{-FeOOH}$ ) and elemental sulphur were detected by XRD analysis. Furthermore, the results suggested that slight increases in silver concentrations may have occurred over time but more experiments are necessary to verify these findings. In future work, the effect of organosulphur ligands on silver mobilization and stabilization of "dissolved" silver through complex formation will also be examined.

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**Questions & Answers: Silver Mobility in the Presence of Iron Sulfides Under Oxidizing Conditions**

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No Questions.



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## **Fate of Metals Downstream of a Domestic Wastewater Treatment Plant**

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Tallahassee, Florida, USA

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### **Introduction**

The Florida Department of Environmental Protection (FDEP) regulates trace metals discharged to surface waters based on measurement of the total recoverable fraction. Recent recommendations from EPA suggest that states adopt surface water criteria for metals based on the dissolved fraction because that fraction is thought to be more readily available to aquatic organisms. An understanding of the transformations between the particulate-bound and dissolved states of trace metals discharged to surface waters is important for the protection of aquatic life. The FDEP Bureau of Laboratories undertook a study to examine the fate of particulate-bound and dissolved metals discharged from a wastewater treatment plant to a freshwater stream. Silver was of particular importance in the study because the State's surface water criteria (0.07 µg Ag/L measured as total recoverable silver) often required that stringent controls be placed on pre-treatment programs by publicly owned wastewater plants. In order to successfully carry out such a study, it was necessary to locate a wastewater treatment facility that a) had readily measurable amounts of silver in the discharge and b) discharged to a small stream such that discharged metals could be tracked well downstream. With regard to silver, the wastewater treatment plant (WWTP) in Lake Butler, Florida, was found to be an ideal candidate (refer to Figure 1). Results are presented that describe the fate of silver and other metals discharged from the treatment facility at Lake Butler. Transformations of metals among particulate fractions are evident only after the effects of dilution are taken into consideration. The changes in particulate-bound and 'dissolved' fractions of metals downstream from the discharge are presented.

### **Study Design**

The Lake Butler WWTP discharges approximately 0.4 million gallons of treated waste to Silver Run Creek daily. Studies of metal distributions in Silver Run Creek were conducted in January 1996 (Study 1) and October 1996 (Study 2). Study 1 corresponded with low-flow conditions whereas Study 2 was conducted during high flow conditions in the stream. In each study, Rhodamine WT dye was used to determine travel times of effluent 'slugs' as they moved downstream from the point of discharge. Sampling stations were established along the stream and discrete effluent 'slugs' were followed downstream and sampled. Samples were collected representing whole water (unfiltered), 0.45 µm filtered, and 0.2 µm filtered samples (0.2 µm filtration was only performed in Study 2). Water column concentrations of discharged metals in Silver Run Creek were dominated by dilution. Investigating the fate of discharged metals required that dilution be accurately measured in order to separate dilution effects from losses to the stream bed and to evaluate transformations among various particulate fractions. In Study 1, trace levels of rhodamine dye were used to measure dilution of the effluent during the study. Concentrations of conservative ions were also measured in the effluent and in background streams and dilution calculated from those measurements correlated well with dilution based on rhodamine dye concentrations. During Study 2, rhodamine dye was not used to measure dilution during sampling because of concerns that the dye might affect metal fractionation or speciation. Instead, six conservative anion and cation markers within the effluent identified from Study 1 were monitored and compared to dye dilution measurements taken before and immediately after the sampling exercise.

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## Methodology

*Equipment:* For the analysis of major cations and anions and other gross chemical parameters, new polyethylene plastic bottles were purchased for sample collection. For trace metal sampling, virgin Teflon bottles and Teflon tubing were cleaned and prepared for field use in a Class 1000 clean laboratory with Class 100 clean benches. Teflon bottles were rinsed 5 times with 18 Mohm deionized water and acid leached with aqua regia (10% HNO<sub>3</sub> and 30% HCl) for 48 hours. After leaching, the bottles were again rinsed 5 times with deionized water, refilled with deionized water and placed inside double amber plastic zip-lock bags. Teflon tubing and silicon C-Flex pump tubing was acid rinsed with aqua regia and flushed with copious amounts of deionized water prior to packing inside double zip-lock plastic bags. All sampling equipment was packed inside clean coolers for transport to the field.

*Field Methods:* Before sampling, fluorometric dye studies were conducted using Rhodamine-WT dye slugs to determine time-of-travel to selected stream sites. In addition, dye-labeled effluent was used to determine nominal effluent dilution at those sites. Using clean hands/dirty hands sampling techniques (U.S. EPA Method 1669), samples were collected at the discharge point and at locations downstream. Sample collection was timed to follow a single discharge 'slug' downstream. Samples were collected through new, precleaned Teflon tubing using peristaltic pumps and, where appropriate, through in-line filters (0.45 and 0.2  $\mu\text{m}$ ) into new, precleaned Teflon bottles. New tubing and filters were used at every sample site. PVC booms were rigged to support the Teflon tubing, allowing sample collection to take place with sampling staff away from the stream edge. The booms also allowed the Teflon tubing intake to be securely supported at the mid-depth of the stream. Samples were collected for metals analysis, anion and cation analysis, dissolved organic carbon (DOC) and total suspended solids (TSS). Conductivity and pH were measured at the sampling sites. Samples were preserved as appropriate on site, placed on ice, and transported to the FDEP Central Laboratory in Tallahassee, FL, for analysis.

All filtered samples were collected after 0.45- $\mu\text{m}$  filtration in the field using in-line QED™ filters. Additional 0.2- $\mu\text{m}$  filtration (Gelman) and subsequent preservation was performed in a clean laboratory immediately following the sampling event in Study 2. To investigate the effects of transportation and storage on metal fractions, 0.2- $\mu\text{m}$  filtration was performed both in the field and in the clean laboratory on triplicate samples at the first and last stations in the study. Data indicate that there were no differences in the results between the two 0.2- $\mu\text{m}$  filtration protocols. The Gelman filters used to perform the 0.2- $\mu\text{m}$  filtration in Study 2 were found to release significant levels of copper relative to what was found in stream water in that fraction. Consequently, the copper results from the 0.2- $\mu\text{m}$  filtered fraction were useless.

*Analytical Methods:* Samples were taken directly to the laboratory after collection. Samples collected for trace metals were removed from the double zip-lock plastic bags for preservation in a Class 1000 clean room. One milliliter of quadruple-distilled HCl was used to preserve 250-mL trace metals samples prior to analysis. Unfiltered samples collected for total metals were digested in aqua regia prior to analysis. Digestions were carried out in Teflon vessels on a heating block at 95 °C. Trace metal samples were analyzed on an ELAN 6000 ICPMS fitted with an ultrasonic nebulizer. Peak hopping mode was utilized to further improve sensitivity. Major cations and anions were analyzed in filtered samples to measure dilution of the effluent as it traveled downstream. Cations were analyzed using a Thermo Jarrell Ash ICP optical emission spectrometer. Anion concentrations were determined using a Dionex ion chromatography system. Organic carbon measurements were made on a Shimadzu 5050 TOC analyzer.

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## Discussion

Following discrete slugs of effluent downstream from the point of discharge required multiple sampling teams at stations near the discharge where times between stations were relatively short. During Study 1, one slug was followed downstream and samples of that slug were collected at each station. The high-flow conditions during Study 2 necessitated that two different slugs be followed due to time constraints. The first effluent slug was followed to the 15-minute station while the second slug was followed from the 30-minute station to the 90-minute station (see Figure 1). Initial samples from both slugs were taken at the point of discharge. Conservative dilution curves were calculated using the initial effluent metals concentrations measured in each effluent slug. Natural, conservative tracers in the effluent (Cl, SO<sub>4</sub>, Ca, Mg, Na, Sr) were also used to calculate dilution. Dilution modeled using natural tracers within the effluent correlated well with direct rhodamine dye measurements. Concerns that rhodamine dye might affect the distribution of metals during the studies, prompted elimination of the dye as a direct measure of dilution during Study 2. As a check, dye-determined dilution was measured in Study 2 immediately following sampling.

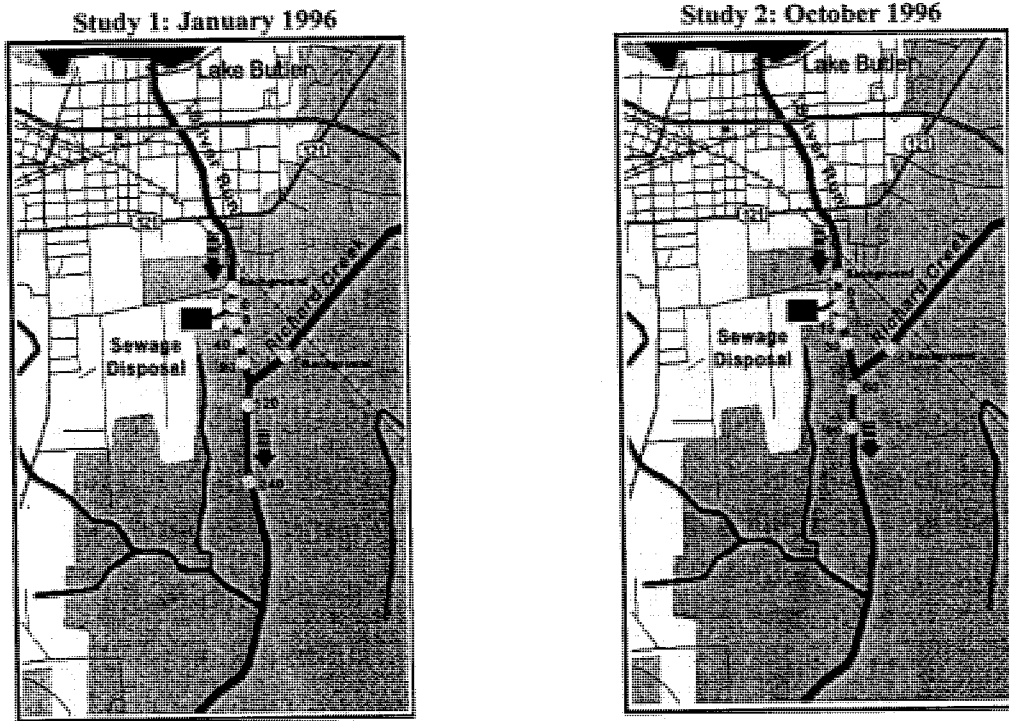
An evaluation of dilution-corrected metal concentrations revealed that total silver, cadmium, copper, nickel and lead behaved nearly conservatively in both studies for up to 4 hours of flow time below the discharge point at the Lake Butler WWTP (refer to Figure 2a & 2b for silver results). Even through relatively-quiet stretches of the stream, metals were not removed from the water column through settling of particulate matter. However, a dramatic increase in the "dissolved" fraction of silver (0.45  $\mu\text{m}$  filtered) was observed during Study 1 just upstream from the confluence of Silver Run and Richard Creek (see Figures 3a & 3b). A similar but less pronounced increase in the fraction of "dissolved" copper was also observed at that site but the phenomena was not observed for cadmium. Similar effects were observed during Study 2 (Figures 4a & 4b). In fact, Figure 4b reveals that, although only about 10% of the silver in the stream at the discharge point was associated with the <0.2  $\mu\text{m}$  fraction, nearly 75% of the silver was in that fraction after 90 minutes of flow. When the various fractions of silver in the stream were corrected for dilution effects, there were clear additions to the "dissolved" fractions (0.45 and 0.2  $\mu\text{m}$  filtration) of silver (Figure 5). The fact that there was no observed increase in metals associated with the 0.2 - 0.45  $\mu\text{m}$  particulate fraction downstream of the discharge and the observation that 'dissolved' metal fractions continued to increase in relatively quiet stretches of the stream below Richard Creek suggest that water quality changes may play a greater role in the distribution of metals than does the physical breakup of larger discharged particles.

## Conclusions

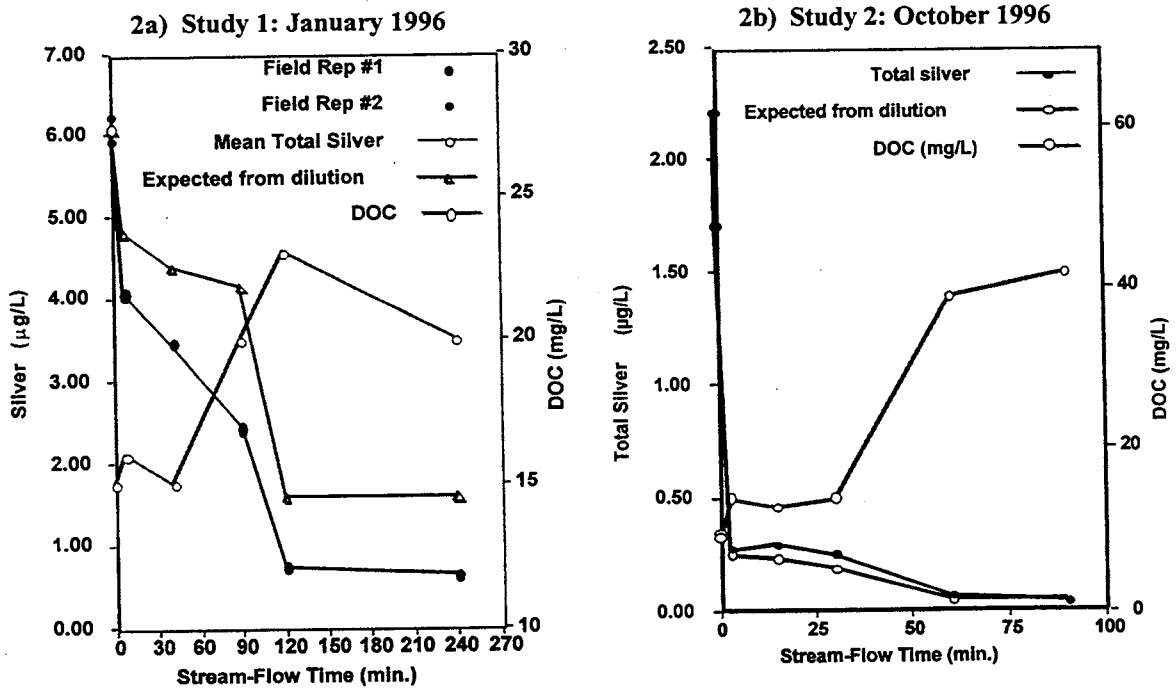
Particulate-bound metal fractions appeared to undergo transformations downstream of the discharge as water quality changed. For silver, dilution of stream waters with water from a tributary containing higher dissolved organic carbon and lower pH appeared to cause a dramatic increase in silver associated with the fraction containing particles < 0.2  $\mu\text{m}$ , and a corresponding decrease in silver associated with all fractions containing particles > 0.2  $\mu\text{m}$ . Similar but less dramatic trends were observed for some other metals. The mobility of metals downstream of the wastewater treatment plant appeared to be driven by the migration of metals to smaller particulates or to the dissolved state. Mobility and transformations of metals between particulate and operationally defined dissolved states far downstream from the point of discharge need to be considered to protect watersheds from acute and chronic toxicity effects. The toxicity and bioavailability of metals in fractions containing particles less than 0.45- $\mu\text{m}$  in size should be further investigated.

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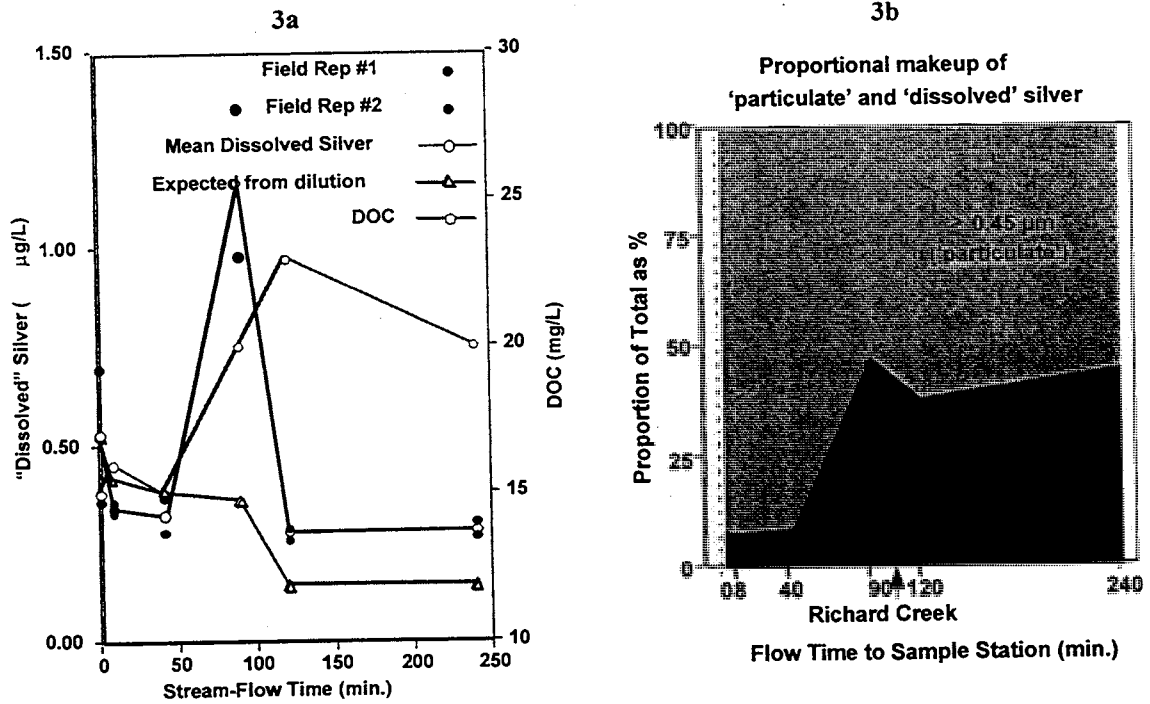
**Figure 1: Study Area Around the Lake Butler Wastewater Treatment Plant**



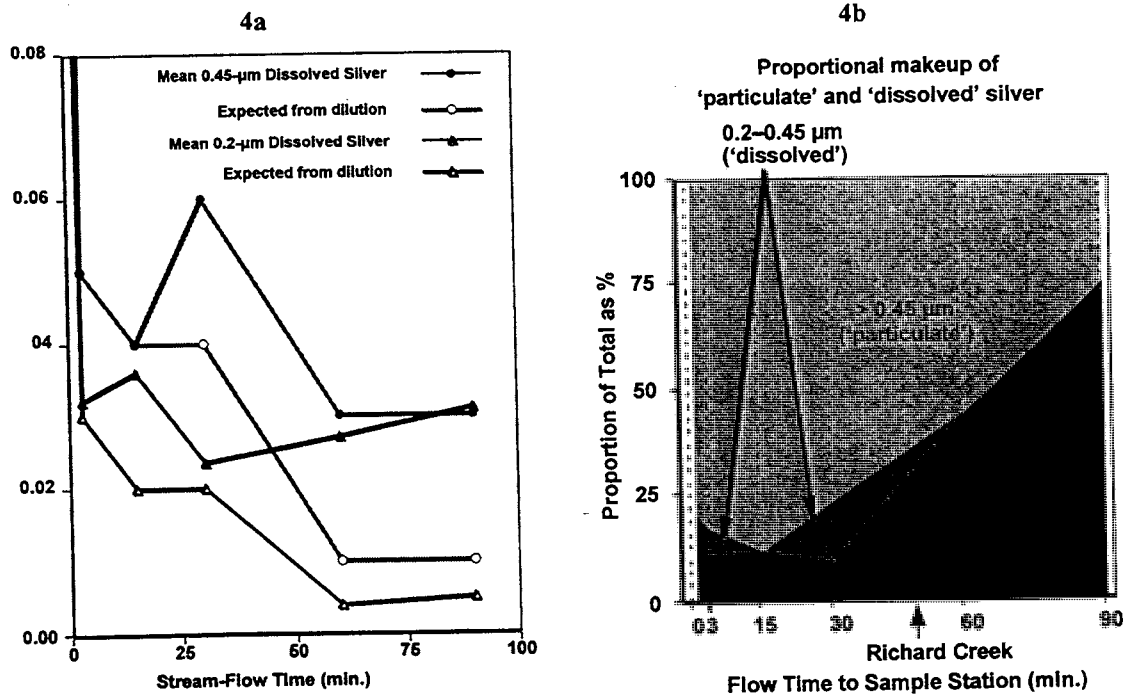
**Figure 2a and 2b: Total Silver Measured in Silver Run Creek and Expected Concentrations Based on Conservative Dilution Calculations**



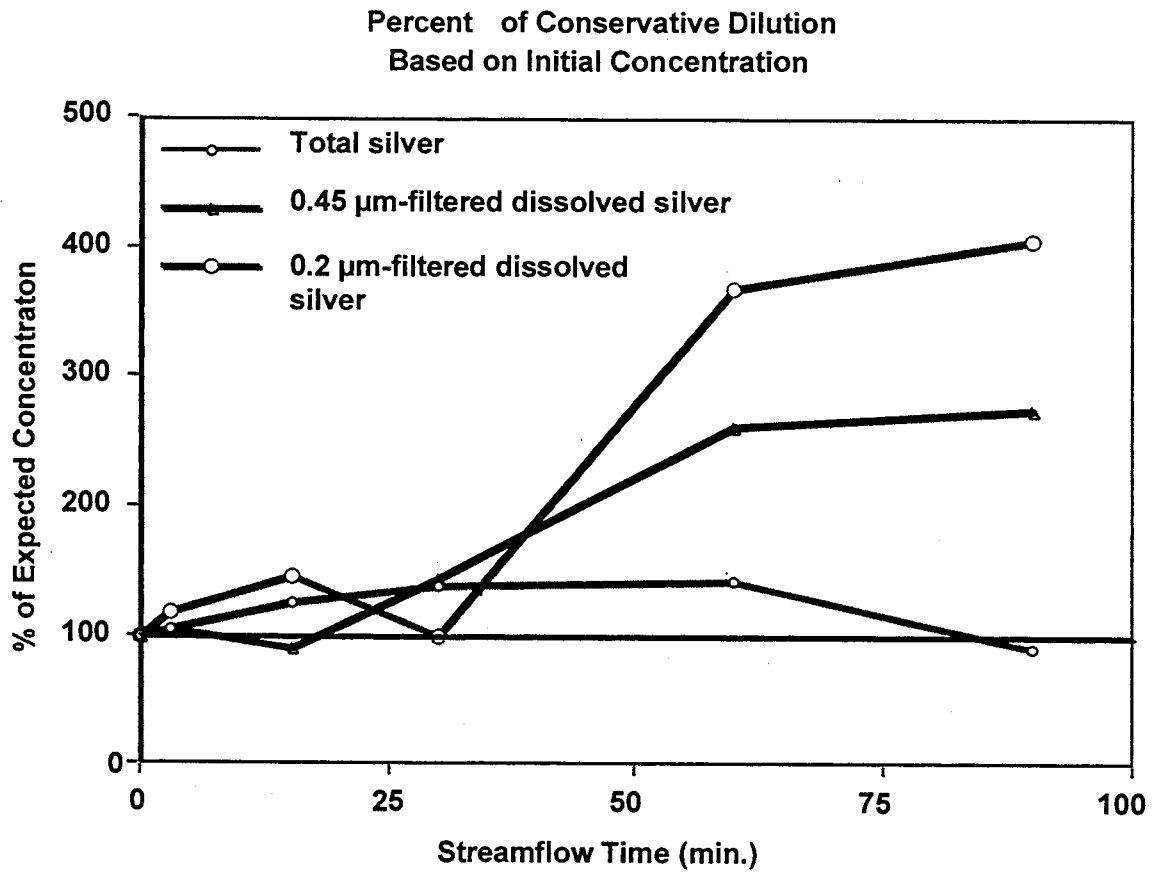
**Figure 3a and 3b: Dissolved Silver (0.45  $\mu\text{m}$  Filtration) in Silver Run Creek During Study 1 (January 1996)**



**Figure 4a and 4b: Dissolved Silver (0.45 and 0.2  $\mu\text{m}$  Filtration) in Silver Run Creek During Study 2 (October 1996)**



**Figure 5: Silver Concentrations in Silver Run Creek as a Percentage of Concentrations Expected From Conservative Dilution - Study 2 (October 1996)**



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## Questions & Answers: Fate of Metals Downstream of a Domestic Wastewater Treatment Plant

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- Q. Gabe Benoit (Yale University): A very interesting result, that the dissolved concentrations are actually increasing downstream, and you have a sort of natural experiment or convecting factor involved there where that stream is coming in with very high level of DOC. I was wondering if you had done any bench scale mixing experiments, perhaps to see how important that was in influencing the increase in dissolved metals?
- A. No, unfortunately we haven't done any laboratory experiments. The reason being our agency funds us for applied projects only, and once we answer a question to their satisfaction they're not willing to fork out any more money.

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## Useful Phytoindicator (Dandelion) for Trace Metal Pollution

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**Introduction.** Bioindicators are organisms with a relatively high ability to accumulate several or specific chemical elements. Among phytoindicators, dandelion (*Taraxacum officinale*, Web.) fulfills most of the requirements as a useful plant in the investigation of trace metal pollution. It is a widespread perennial herb in various ecosystems and under differentiated climatic conditions. Simultaneous collection of leaf and root samples gives a possibility of distinguishing atmospheric from soil-borne metals.

Several studies carried out with various phytomonitors have indicated a great feasibility of using the common dandelion plant around the world (1, 2, 5, 9). The Coefficient of Specific Relative Accumulation (CSRA) proposed by Lambert et al. (7) and modified by Kabata-Pendias and Krakowiak (6) was adopted for the assessment of the accumulation of trace metals by different plants (Table 1). CSRA values clearly indicate significantly greater uptake of Cd, Cu, Mn, Pb, Zn, and Fe by dandelion than by other candidate plants.

Table 1. Coefficient of Specific Relative Accumulation (CSRA)<sup>a</sup> of the Elements by Plants Grown under the Same Conditions (after Kabata-Pendias, 2)

Plant	Cd	Zn	Pb	Cu	Mn	Fe
Dandelion <i>Taraxacum officinale</i> Web.	3.42	6.80	4.04	2.60	8.43	2.72
Plantain <i>Plantago major</i> L.	0.93	0.54	0.57	0.89	0.23	0.85
Bromegrass <i>Bromus uniloides</i> L.	0.11	0.09	0.26	0.52	0.06	0.49
Acacia <i>Robinia pseudoacacia</i> L.	1.49	0.25	1.33	1.02	0.49	0.76
Horse Bean <i>Vicia faba minor</i> L.	0.23	0.45	0.27	1.49	0.82	0.71

<sup>a</sup> CSRA value is calculated as a ratio of the content of an element in a given plant to the average content of this element in all other plants grown in the same site (or pot)

Although *Taraxacum officinale* is known to exist in a number of microspecies, and is an apomixis plant, the research done by Djingova and Kuleff (1) suggests that the variation in metal uptake is negligible. This is also supported by the similarity of background metal concentrations calculated for dandelion leaves in Bulgaria, Poland, and USA (1, 4, 8).



**Materials and Methods.** Several studies were carried out with dandelion (2-6). In the last project, dandelion plants (leaves and roots) were collected in 1993 and 1994 from the whole territory of Poland, at the same stage of development, during two weeks of May. Air-dried plants were ashed at 450° C, dissolved in HCl (C=6 mol/L) and analyzed for metals by AAS spectroscopy. Cd and Pb were measured after organic extraction with APDC/MIBK. Analytical errors estimated, using reference materials and multiple analyses, varied from 5-20% depending upon the metal.

**Results and Discussion.** Plants grown in the southwestern (SW) industrial region contained significantly higher amounts of metals than average values calculated for the whole country, and more than plants grown in the other regions (Table 2). Especially, values of the Relative Deviation to Constant (RDC) calculated against the average metal concentrations in dandelion from three countries (Bulgaria, Poland and USA) show increased levels of metals in dandelion grown in the SW region of Poland. The ratio of metals in leaves to roots, however, is a more sensitive indicator and clearly indicates areas where there is an increased atmospheric deposition of trace metals (6). This phenomena is also observed for light metals; e.g., Li average content in dandelion leaves in the industrial region (SW) are 6.3 mg/kg, and ratio of leaves to roots is 5.0 mg/kg, whereas these values for the rural region (NE) are respectively 0.7 mg/kg and 0.8 mg/kg (3).

Table 2. Metals in Leaves of Dandelion Grown in Different Regions of Poland (mg/kg dry weight)  
(N = Number of samples taken)

Metal <sup>a</sup>	Country N=780	All Regions N=60	Region SW N=60	Region SE N=70	Region NW N=60	Region NE N=50
Cd	0.5 -20	0.6 0	1.2 50	0.6 0	0.5 -20	0.4 -50
Cu	9.4 -27	9.4 -22	13.4 10	10 -20	8.4 -42	7 -71
Cr	0.8 25	0.8 25	0.7 14	1.3 53	0.7 14	0.4 -50
Mn	60 0	65 7	74 18	103 41	69 13	42 -18
Ni	3.4 50	1.3 -53	4.2 52	6.4 68	3.1 35	1.9 -5
Pb	1.1 -81	1.2 -66	3 33	1 -100	1 -100	0.5 -300
Zn	45 0	50 10	72 37	67 32	35 -28	40 -12
Fe	241 64	261 67	525 83	526 83	218 61	103 17

<sup>a</sup> Upper values for metals are geometric means, and lower values are RDC - Relative Deviation to Constant Values,  $RDC = \{[AM - RF] / RF\} \times 100$ .

RF - Reference Contents (mg/kg d.w.): Cd 0.6, Cr 0.6, Cu 12, Mn 60, Ni 2, Pb 2, Zn 45, Fe 85.

The dandelion plant was used to observe trace metal pollution in Warsaw. Metal concentrations in leaves of dandelion grown in the area of Warsaw were lower than the reference constant value (RF) accepted for the "world" dandelion (Table 3). Only Pb was significantly increased in the tops, also when compared to its content in the roots. The other metals, e.g., Mn, Ni, Zn, and Fe, were also at higher concentrations in the tops than in the roots of the dandelion. This may indicate both physiological effects, as probably in the cases of Mn and Fe, and the impact of pollution, as in the cases of Pb, Zn, and Ni.

Table 3. Trace Metals in Leaves of Dandelion Grown in the City of Warsaw and Surroundings (mg/kg dry wt.)

Metal	Parameter <sup>a</sup>	Whole Area N=22	Surroundings N=9	City N=13
Cd	AM	0.42	0.48	0.38
	RDC	-42	-42	-58
	L/R	0.60	0.46	0.73
Cr	AM	0.69	0.51	0.81
	RDC	13	-17	25
	L/R	0.60	0.47	0.70
Cu	AM	10.4	8.5	11.4
	RDC	-15	-41	-5
	L/R	0.58	0.64	0.54
Mn	AM	56	77	41
	RDC	-6	22	-46
	L/R	1.38	1.04	1.63
Ni	AM	4.2	3.5	4.3
	RDC	52	42	53
	L/R	1.05	1.00	1.08
Pb	AM	1.7	1.61	2.4
	RDC	-18	-25	16
	L/R	0.94	0.49	1.26
Zn	AM	58	57	59
	RDC	22	21	23
	L/R	0.87	0.49	1.26
Fe	AM	150	125	325
	RDC	43	32	73
	L/R	0.85	0.95	1.34

<sup>a</sup> Parameters:

AM - arithmetic mean

RDC - relative deviation to constant values,  $RDC = \{[AM - RF] / RF\} \times 100$

L/R - leaves-to-roots ratio.

The data for Cd, Cr, Cu, Mn, Ni, Pb, Zn and Fe in dandelions growing in different areas of the country show a significant variation. The highest concentrations (geometric mean, mg/kg d.w.) of some metals (Cd 1.2, Ni

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4.2 and Pb 3.0) were in leaves of plants from the SW region (industrialized) and the lowest ones (Cd 0.6, Ni 1.7, Pb 0.9) in plants from the NE region (agricultural). This trend is also observed in the distribution of metals in plant roots. A relative accumulation, as compared to the reference plant, of all the metals is higher in roots than in leaves of plants from the SW area. However, leaves of plants from the NE area contain less metals, by a factor from 10 to 100%, than the reference values and than the average values for the whole country. It is an evidence that in the SW area dandelions take up trace metals from both sources, air and soil, while in the NE area the main source of metals is soil.

In the city of Warsaw a predomination of airborne over soil-borne Pb and Zn was observed. The highest relative concentration of Cd in dandelion leaves versus soil Cd, as compared to other metals, was also noted.

The chemical status of the dandelion plant is influenced by a number of biotic and abiotic factors, and therefore it is not simple to assess environmental pollution based on the one bioindicator. However, multifunctional analyses, e.g., of dandelion leaves, roots and soils, are potentially the most useful in environmental studies.

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## Questions & Answers: Useful Phytoindicator (Dandelion) for Trace Metal Pollution

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- Q. TOM BOBER (Eastman Kodak): I have two questions. First of all, did you make any measurements at all of silver? I know you didn't do it across the matrix, but did you encounter silver at all in your measurements? And secondly, is there any land application of sludge used in any of these areas that you were talking about - of sewage sludge from sewage treatment plants?
- A. Well, I haven't done measurements in this project for silver, but silver was measured in soil and in bottom sediments, river sediments, in my country within a project which was funded by The Geological Survey. So I can tell you that in soil, silver was a little bit increased but only about 1 ppm in soil. And in this area, southwestern Poland, there are a number of copper mines, lead and zinc mines and smelter operations. So there is a visible increase in soil silver but much more visible increase, up to 100 ppm at one site, in bottom sediments. So bottom sediments seem to be an indicator for special differentiation of silver in soil. You ask about sewage sludge? Do we use or do we distribute sewage sludge?
- Q. Yes.
- A. Yes, we do, and if you want to hear some results maybe tomorrow I will mention them. But yes, we do. But we have some regulations for that.
- Q. ARUN MUKHERJEE (University of Helsinki-Finland): Professor Kabata-Pendias, I'm very astonished that you didn't mention anything about a moss indicator. Do you think that your dendrite indicated metals better than moss? Because here in your indicator you can find out metals from the atmosphere as well as from the soil. But moss has no roots, and in the moss I can only find out, at most, the atmospheric presence of a trace element. Do you have some comments on this?
- A. Yes, I do have. And I do believe that the dandelion is a better phytoindicator worldwide than moss. And I will tell you, it reminds me of a previous discussion I had with Germund Tyler from Lund in Sweden, who did very broad studies with moss and was surprised that I did not. Yes, I had such a project, and when I went down to my country, close to industrialized regions, there were not mosses and there were not lichens! So how I could make a study of bioindication? And people even from the country you are now from, I mean from Finland yes, but I mean all Scandinavian countries, they can hardly believe that there are some other environments in the world where moss and lichens do not exist! (*laughter*) So that's my explanation.

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## Silver in San Francisco Bay\*

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Santa Cruz, California

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The relationship between elevated concentrations of silver in surface waters and benthic sediments in the neritic zone was substantiated by analyses of the distribution of silver in two semi-enclosed embayments (Flegal and Sanudo-Wilhelmy 1993). Comparable levels of contamination were observed in surface waters of San Diego Bay and South San Francisco Bay, where dissolved silver concentrations ranged up to 300 pM. Those elevated concentrations occurred in the back reaches of the two embayments, which are not systematically flushed by natural fresh water discharges.

The elevated concentrations corresponded with elevated silver concentrations in benthic sediments of the most contaminated regions of the two embayments. For example, silver concentrations (dry weight) of sediments within San Francisco Bay were  $< 0.5 \mu\text{g/g}$  in its relatively pristine northern reach and 0.5 to  $0.7 \mu\text{g/g}$  in its relatively contaminated South Bay in 1970 (Luoma and Phillips 1988).

Benthic sediments appear to be an important source of silver contamination in both embayments. Essentially all of the excess silver within San Diego Bay waters is attributed to the diagenetic remobilization of silver from its sediments, because all point source discharges to San Diego Bay were terminated more than three decades ago (Flegal and Sanudo-Wilhelmy 1993). This suggests that a large percentage of the excess silver in South San Francisco Bay appears to be derived from diagenetic processes, even though waste water outfalls continue to discharge relatively large amounts of silver into that area.

The relative importance of inputs of silver from contaminated sediments to overlaying waters in those embayments was substantiated by mass balance calculations of silver fluxes in the San Francisco Bay estuary (Smith and Flegal 1993). These indicated that the benthic flux of silver from diagenetic remobilization from contaminated sediments in South San Francisco Bay was  $\approx 1.7 \mu\text{g m}^{-2} \text{d}^{-1}$  and the benthic flux of dissolved silver throughout the entire estuary was  $\approx 1.2 \mu\text{g m}^{-2} \text{d}^{-1}$ . Those initial estimates were further substantiated by two other independent calculations of benthic fluxes in the estuary (Rivera-Duarte and Flegal 1996). Both indicated that the total benthic flux of dissolved silver from contaminated sediments in South San Francisco Bay were as high as  $8,800 \text{ kg yr}^{-1}$ , which is nearly three orders of magnitude greater than the fluvial flux of dissolved silver to the estuary ( $12 \text{ kg yr}^{-1}$ ; Smith and Flegal 1993) and comparable to the total anthropogenic flux of both dissolved and particulate silver from all municipal and industrial point source discharges to the estuary ( $2,700$  to  $7,200 \text{ kg yr}^{-1}$ ; Davis et al. 1991).

Those initial measurements were substantiated by more extensive analyses of dissolved silver concentrations in San Francisco Bay estuary (Smith and Flegal 1993) and in six Texas estuaries (Benoit et al. 1994). While the average dissolved ( $< 0.4 \mu\text{M}$ ) silver concentration in the Texas estuaries was commonly below the detection limit of  $1.0 \text{ ng/kg}$  ( $9 \text{ pM}$ ) during the fall, the average ( $x \pm \text{SE}$ ) concentrations were markedly higher in the Sabine ( $65 \pm$

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\*Extracted from Flegal et al. 1996. Silver contamination in aquatic environments. Reviews in Environmental Chemistry and Toxicology 148:139-162. The oral presentation at the 1997 Silver Conference also included data subsequently published in: Rivera-Duarte and Flegal 1997. Benthic fluxes of silver in San Francisco Bay. Marine Chemistry 56:15-26.

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10 pM), Galveston ( $72 \pm 9$  pM), San Antonio ( $37 \pm 6$  pM), and Corpus Christi ( $39 \pm 8$  pM) estuaries during the summer. These seasonal increases were also consistent with the seasonal increases of silver concentrations in San Francisco Bay, where the highest concentrations occurred in the summer month of low fresh water discharge (fresh water flushing) and high benthic activity (diagenetic remobilization).

Based on comparisons with ambient concentrations in oceanic surface waters, those analyses indicate that concentrations of silver in contaminated estuaries are at least two orders of magnitude greater than the ambient levels of silver in oceanic surface waters.

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## Questions & Answers: Silver in San Francisco Bay

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- Q. GABE BENOIT (Yale University): You distinguish between the benthic flux and the current anthropogenic flux quite well there. But isn't ultimately most of the metal that you see in the sediments that's coming out, and cycling probably also, from people? So ultimately in the long term that also was anthropogenic flux?
- A. Yes, the sins of the fathers will be revisited on their sons. The natural baseline concentration of silver based on the cores we did going back 700 years is 0.01 ppm, which is somewhere around one hundred times or 500 times less than the concentration we see there. And so what we have is exactly as you said, the silver goes in - I mean this is what I believe - the silver goes in from anthropogenic sources, biogenic processes bring it up into the water column and release it, then it is brought back down and it's added to the silver coming in from contemporary sources. So what we have essentially is 100 years of anthropogenic silver discharge concentrated into that area.
- Q. PAUL ANDERSON (Illinois Institute of Technology): If I understood your benthic flux model, it hinged on silver being released from reduction of iron in manganese oxides. But I thought that most of the silver was complexed. I don't know how that complex reacts with sulfur complexes and things. How does that fit in?
- A. Yes. Well, we don't know what is scavenging the silver. I'm sure Fisher would say it's all the phytoplankton, and Santschi would say it's all the iron and manganese hydroxides. In San Francisco Bay we don't find - and Ken Bruland actually did this, we thought that a lot of the silver would be organically complexed. But he didn't find that it was organically complexed. Then we looked at the distribution of silver in the different fractions, including the colloidal and truly dissolved. Most of the silver in San Francisco Bay we found was in the truly dissolved, so when it's in the water column it does exist as a cation and so it would go onto these clays as a cation. Then in terms of what's happening in the sediments, this is actually delineated by F. Fitzgerald with his work on pore waters in the New York Bight. And silver does actually come back up into solution or into the water column. In fact, Crecelius did the thermodynamics on that with Jenne.
- Q. JIM KRAMER (McMaster University): Following up on this, I think it's a very important point, what makes this stuff come down and what makes it come up? Your comment about silver being scavenged by oxyhydroxides is pretty contrary to everything else that other people have found. What is your fundamental evidence for this? I mean, that's a nice model for other metals, but I personally can't buy that for silver. What is your fundamental field evidence that that is the mechanism that's going on?
- A. Well, you spent too much time thinking about your question and not listening to my answer. What I said was that we had not determined the form of the silver in the sediments or what was scavenging it. What we do find is that essentially all of the silver comes off with a half-normal HCl leach. A half-normal HCl leach will take off freshly formed iron-manganese oxyhydroxides. This stuff coincides, it follows the spring bloom, so you have a bunch of phytoplankton, they come down and they decompose and so it's a very complex system, but we don't know the form of the silver in the sediments. We do know that, as I showed you, the concentration of the silver in the pore water goes up markedly at that interface where you do have this reduction going on.
- Q. So basically your evidence - the work you had done from your weak hydrochloric acid leaches - would be the fundamental evidence you're going on at the moment?
- A. I have no evidence. I'm just talking about - we have a co-variance between silver concentrations and iron/manganese concentrations in the pore waters, and that's it.
- Q. TOM BOBER (Eastman Kodak): Russ, a couple of years ago when you gave your paper, you mentioned in passing that there had been a cinnabar mine and a mercury smelter on the SE corner of the bay. Have you noticed any variation, any covariance between silver and mercury, for example - have you studied that effect?

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- A. Well, Gary Gill has done all the mercury work. The co-variance is not as strong as we thought. I'm going back to Jim's question, we thought we'd see a high co-variance between silver and dissolved organic carbon, and we don't see that either. The cinnabar drained from the New Almaden Mine is a different source than the silver. So our closest correlation's of silver are with lead. Both in multi-varied analysis and just geochemical profiles.
- Q. PETER SANTSCHI (Texas A&M University): I just wanted to mention, because you mentioned my name, if you do adsorption experiments with silver onto iron or manganese oxide, it doesn't sorb. It doesn't sorb very much. So the answer has to be something else.



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## Silver in Colorado Watersheds

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### Introduction

Surface river water samples and municipal and industrial discharge effluents were collected using ultra-clean sampling protocols at 5 sites in the State of Colorado. River water samples were obtained above and below a municipal or industrial discharge point, for characterization of the silver discharge on the receiving water body. The main purpose of this study was to provide reliable silver measurements in conjunction with water chemistry parameters for monitoring and regulatory purposes.

### Methods

The sampling locations monitored for this study were the Cache-la-Poudre River at the Kodak Colorado Facility in the city of Windsor, and also at the Mulberry and Drake Water Treatment Facilities in Fort Collins. The Upper Big Thompson River was sampled at the Estes Park Wastewater Treatment Facility in Estes Park. Sampling of Fountain Creek in Colorado Springs was conducted at the Colorado Springs Wastewater Treatment Facility and the #4 Diversion Canal. In addition, monitoring of Cyprus Climax Metal Company's Henderson mine near Empire Colorado was conducted upstream of the Upper Urad Reservoir, the URAD 004 outfall, the Henderson mine effluent and at U2, downstream of the Reservoir Spillway.

Surface river water samples and municipal and industrial discharge effluents were collected using ultra-clean sampling protocols. Both filtered and unfiltered samples were collected using a peristaltic pump system equipped with Teflon<sup>®</sup> tubing inlets and outlets. Filtered samples were obtained by attaching either a 0.45 or 0.1  $\mu\text{m}$  acid-cleaned polyethylene membrane cartridge (MSI, Micron Separations Inc.) to the pump outlet and dispensing the water directly into a Teflon<sup>®</sup> sample bottle. Particulate silver samples were collected by pumping approximately 300 to 500 mL of water through a preweighed Teflon<sup>®</sup> filter (0.45  $\mu\text{m}$ ) held in a Teflon<sup>®</sup> filter holder.

All samples for silver determinations were analyzed for total (unfiltered collections), dissolved (0.1 and 0.4  $\mu\text{m}$  filtration), and particulate (particles on 0.45  $\mu\text{m}$  filters) content using APDC/DDC organic extraction under class-100 clean laboratory conditions and graphite furnace atomic absorption spectrometry for sample quantification. 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).

## Results and Discussion

Silver measurements in unfiltered samples spanned more than four orders of magnitude, from a high of 33,400 ng/L at the KCD effluent (Kodak Colorado) to a low of 1.8 ng/L at Outfall 004 (Henderson Mine) (Table 1). In general, upstream unfiltered silver concentrations fell in a fairly narrow range, 3.1 to 21 ng/L. Downstream unfiltered silver concentrations were more broad, 2.8 to 1107 ng/L, with the higher downstream concentrations being associated with higher Ag levels in discharge effluents.

**Table 1.**  
**Silver Concentrations and Particle-Water Partition Coefficients in Colorado Watersheds**

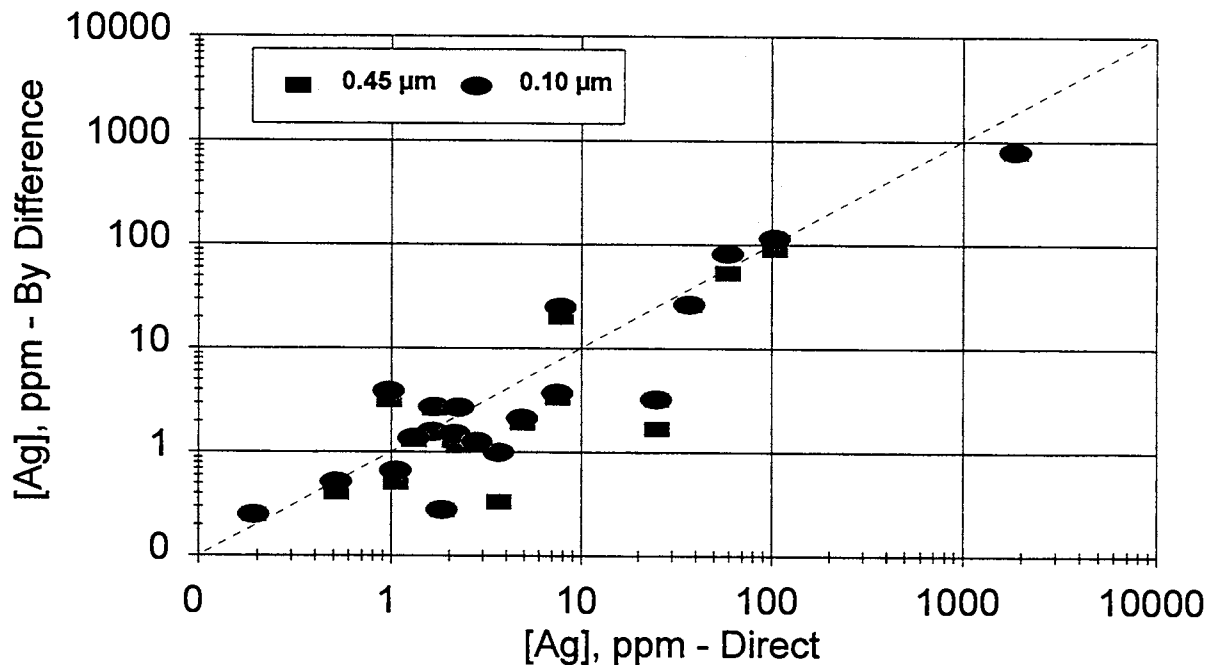
<b>Sampling Sites</b>	<b>Unfiltered (ng/L)</b>	<b>Filtered 0.45 µm (ng/L)</b>	<b>Filtered 0.10 µm (ng/L)</b>	<b>Particulate &gt;0.45 µm (ppm)</b>	<b>Log K<sub>d</sub></b>
<b>Estes Park</b>					
Upstream	10.3	4.8	4.6	1.6	5.53
Downstream	11.9	6.1	5.1	2.1	5.54
Plant	55	20	16.2	7.5	5.23
<b>Henderson Mine</b>					
Upstream	3.1	1	0.9	1.3	6.12
Outfall 001	1.9	1.7	1.2	1.8	6.03
Outfall 004	1.8	1.7	1.5	3.7	6.33
Downstream	2.8	1.7	1.4	0.5	5.48
<b>Colorado Springs</b>					
Upstream	13.3	0.4	0.3	0.2	5.68
Downstream	60.1	15.9	12	2.8	5.25
Plant	74.1	64.1	55.1	25.1	5.59
Channel #4	70.2	22.1	16.6	4.9	5.34
<b>Kodak Colorado</b>					
Upstream	5.4	2.5	1.6	1.1	5.62
Downstream	1,107	292	69.9	104	5.55
KCD Effluent	33,400	2,355	1,880	1,860	5.9
Windsor Effluent	32.9	3.7	3.5	37	7
<b>Fort Collins</b>					
Upstream MWRf	6.3	0.5	0.3	1.7	6.53
MWRf Effluent	537	327	212	59	5.26
Downstream of MWRf	50.2	14	4.9	7.8	5.74
Upstream of DWRF	21.1	16.7	10.8	2.2	5.13
DWRF Effluent	340	116			
Downstream of DWRF	30.4	11	6.7	1	4.94
Average					5.71
Std. Dev.					0.48

Filtered samples also had a wide range in silver concentration, which in general mirrored that observed for the unfiltered samples, but at lower overall concentration

levels. In all cases, the concentration of silver observed in the 0.1  $\mu\text{m}$  filtered fraction was less than that observed in the 0.45  $\mu\text{m}$  filtered fraction.

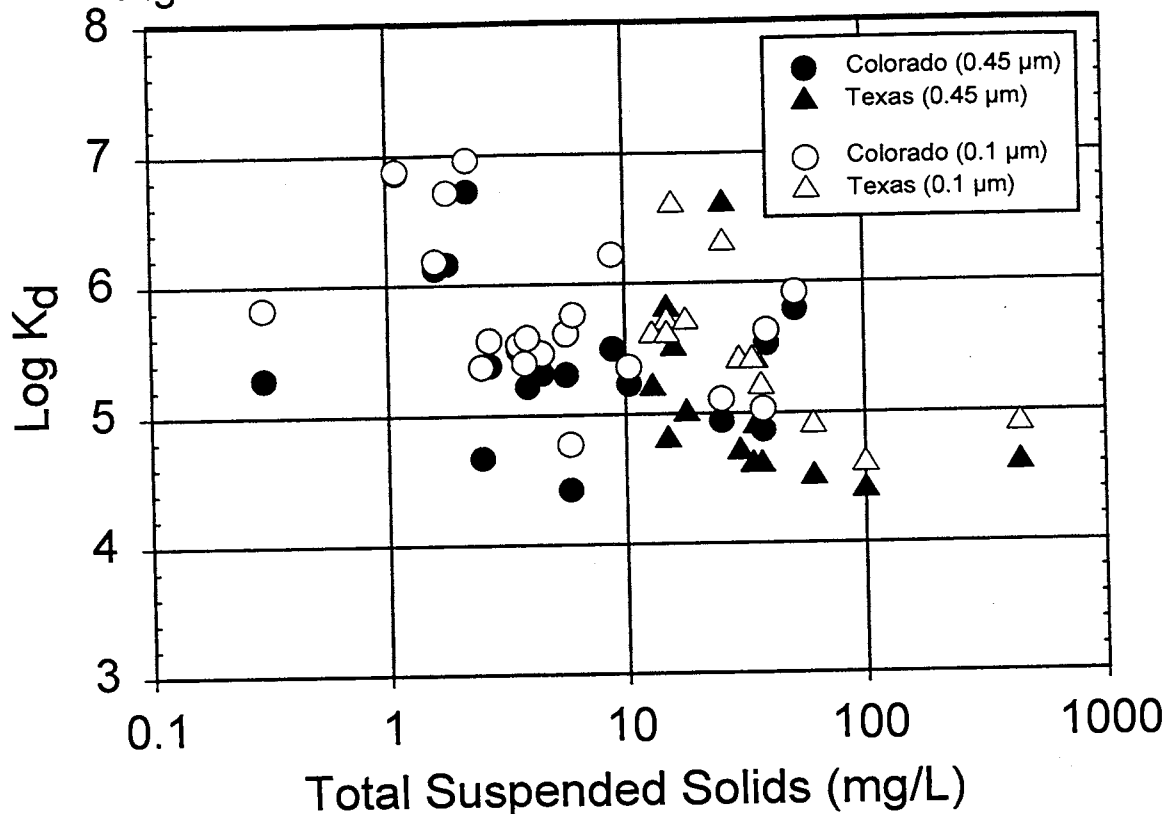
Direct determinations of particulate silver also had a wide range in concentration reflecting pristine background conditions to Ag laden discharge effluents. Upstream particulate silver concentrations, as for all the other silver fractions, fell within a narrow range, 0.2 to 1.7  $\mu\text{g/g}$  silver. Downstream of effluents the range was higher, 0.5 to 104  $\mu\text{g/g}$ , again reflecting the effluent discharge silver level. Direct determinations of particulate silver on particles agreed quite well with particulate silver determined by the difference between unfiltered and filtered determinations combined with separate measurements of total suspended solids (Figure 1). The average log particle-water partition coefficient ( $K_d$ ) for all the sites, using the 0.1  $\mu\text{m}$  filtered Ag concentrations and direct particulate Ag measurements was  $5.7 \pm 0.5$ . These values agreed well with previous measurements in Texas and also suggest a "particle concentration effect" (Figure 2).

Figure 1. Comparison of Methods for the Determination of Particulate Silver



Thermodynamic and phase speciation modeling efforts show that particulate matter and chloride are dominant controls on the phase and inorganic solution speciation of silver (Figure 3a-e). Free silver ion concentrations varied from a high of 72% of the total silver present (1.8 ng/L), when both total suspended solids and chloride ion content were low (Outfall 4 at Henderson Mine), to a minimum of 0.4%, where chloride and TSS were both abundant (KCD Effluent at Kodak Colorado).

Figure 2. Silver Particle-Water Partition Coefficient



For the waters studied, the most important control on the solution speciation of silver appears to be the TSS content as evidenced by the high percentages of total silver present in the particulate fraction at most sites. Chloride species of silver are most important in the effluent waters where maximal levels of chloride ion concentration were observed. In general, the chloride levels in background waters in Colorado are low, resulting in only a marginal influence on the solution speciation of silver.

Figure 3a. Estes Park

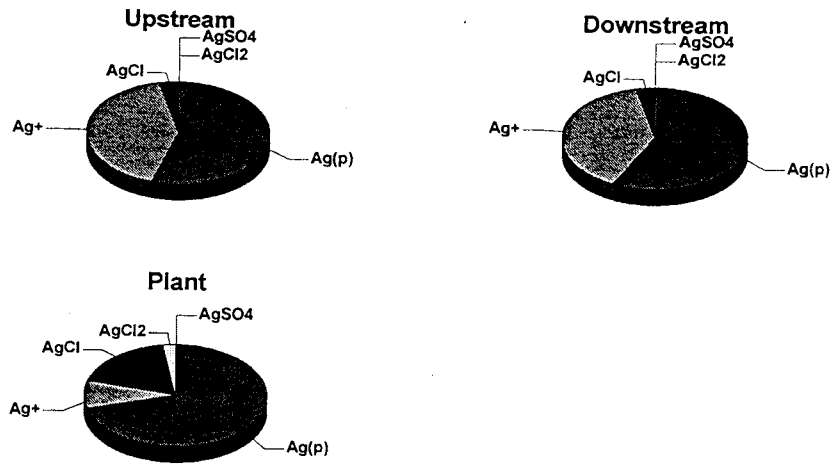


Figure 3b. Henderson Mine

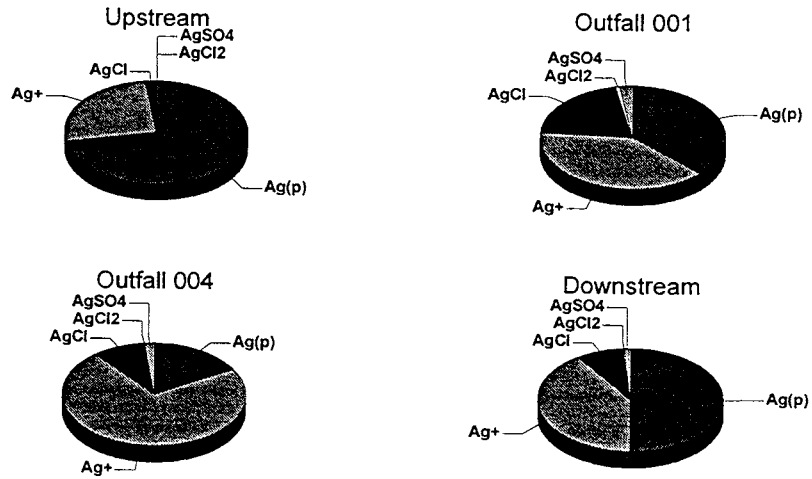


Figure 3c. Colorado Springs

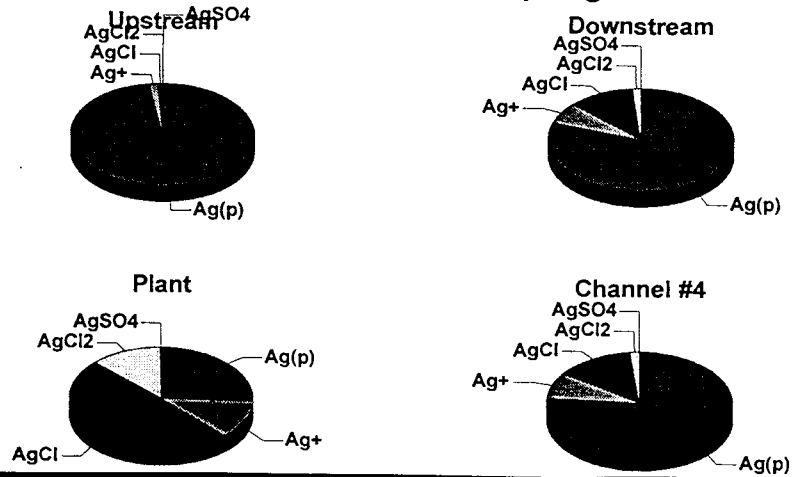


Figure 3d. Kodak Colorado

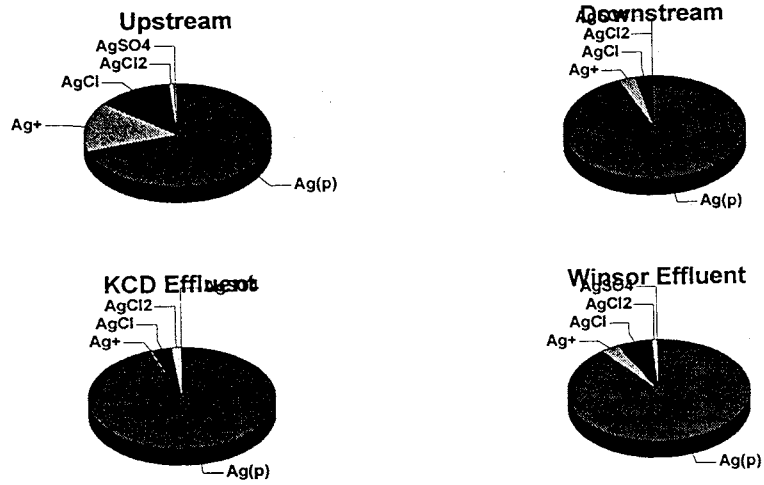
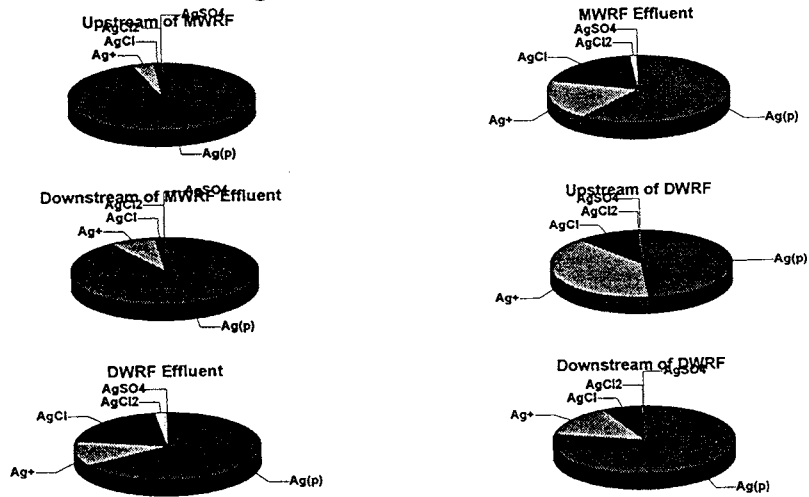


Figure 3e. Fort Collins



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## Questions & Answers: Silver in Colorado Watersheds

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- Q. TOM BOBER (Eastman Kodak): Gary, you show the Henderson mine site had a very high percentage of free silver ion. Do you know if that is primarily a gold mining site, and do they use a cyanide extraction process?
- A. I didn't go there, but I believe that it was a molybdenum mine.
- Q. JOE GORSUCH (Eastman Kodak): Gary, I've had an opportunity to review the report several times, and a couple of things you may want to emphasize is that even though the dissolved or the ionic fraction of the silver runs from about 0.4 to 72%, the actual concentrations of ionic silver run from 0.004 nanograms to approximately 7 nanograms in the effluent. So it is quite small. So even though you're dealing with large percentages, the actual ionic concentrations are small, running about 1-2 ppm in the actual water, not the effluent. The other thing is that I don't recall hearing you say that this model did not take into consideration the reduced sulfur, nor the fact that it doesn't take in consideration the organic complexes.
- A. If I didn't say the organic complexes I apologize. I did mean to say that, but you're right. I did not mention the fact that we did not determine reduced silver, so that was not incorporated into the model as well. It was basically the major ion chemistry, pH, hydroxide and chloride that was used to make those calculations, as well as the particle/water partition co-efficient.

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## Silver in the North Atlantic Ocean\*

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(Presented by A.R. Flegal)

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### Abstract

Silver concentrations (unfiltered) are reported for the second Intergovernmental Oceanographic Commission (IOC) Baseline Survey of 1993 in the North Atlantic. These concentrations are oceanographically consistent with those previously reported for the eastern Atlantic, South Pacific and North Pacific oceans. These data also indicate that silver concentrations in surface waters of the North Atlantic are affected by continental inputs, and are (on average) ten-fold larger (0.69 to 4.6 pM) than those considered natural background (0.25 pM) in surface waters of the South Atlantic. Evidence is presented that silver is conservatively advected in deep waters of both the Atlantic and Pacific oceans, whose silver concentrations represent the inputs in both the North Atlantic and Antarctic waters. The relation between silver and silicates for the North Atlantic is different than that for the eastern Atlantic and North Pacific.

### Introduction

There are relatively few measurements of silver concentrations in the world ocean. There are no previously reported measurements of silver concentrations in the higher latitudes of the North Atlantic, and there are only a limited number of reports on the distribution of silver in other oceanic (Murozumi, 1981; Martin et al., 1983; Flegal et al., 1995) or coastal waters (Sañudo-Wilhelmy and Flegal, 1992; Flegal and Sañudo-Wilhelmy, 1993; Smith and Flegal, 1993; Sañudo-Wilhelmy et al., 1996; Wen et al., 1997). Therefore, the following analyses of silver concentrations in the North Atlantic were made, as part of the second Intergovernmental Oceanographic Commission (IOC) Baseline Survey of 1993.

### Materials and Methods

Samples from the North Atlantic area between Nova Scotia and Iceland were collected as part of the second IOC cruise in August, 1993. Trace metal casts using Go-Flo bottles, Kevlar line and Teflon messengers, were conducted in nine stations (2, 3, 4, 5, 7, 11, 12, 13 and 14) (Figure 1, Table 1). Unfiltered subsamples were collected in acid-cleaned 1L polyethylene bottles, using trace-metal clean techniques. Ancillary information from the IOC'93 cruise was provided by Chris Measures.

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\* This preliminary report is based on the draft of a manuscript for the IOC volume in Deep-Sea Research.



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The silver in the oceanic water was preconcentrated and analyzed by graphite furnace atomic absorption spectrometry (GFAAS). The samples were acidified to pH < 2 with sub-boiling (20) quartz distilled HCl for six months prior to the preconcentration. The latter consisted in an organic extraction with ammonium 1-pyrrolidine dithiocarbamate / diethylammonium diethyldithiocarbamate (APDC/DDDC), as detailed by Bruland et al. (1985). The concentration of silver in the extracts was then measured by GFAAS, using stabilized temperature platform furnace and standard addition techniques. Procedure blanks ( $\bar{x}$  (s)) were 0.025 (0.007 ng (n = 24)). Analyses of the seawater reference materials for trace metals, NASS2 and NASS4, from the National Research Council of Canada gave concentrations of 6.3 (1.5 pM (n = 4)) and 12 (1.5 pM (n = 4)) respectively. It should be noted that these reference materials are not certified for silver.

## Results and Discussion

### *Comparability of data*

Trace metal-clean sampling and analytical techniques were used in this study. Similar preconcentration with APDC/DDDC and measurement with GFAAS were also used in other previous studies in oceanic (Martin et al., 1983; Flegal et al., 1995), neritic (Sañudo-Wilhelmy and Flegal, 1992) and estuarine waters (Flegal and Sañudo-Wilhelmy, 1993; Smith and Flegal, 1993; Sañudo-Wilhelmy et al., 1996; Wen et al., 1997).

In oceanic waters silver has low affinity to particles. Analysis of filtered and unfiltered samples in the Pacific Ocean (Martin et al., 1983) indicate that most (>80%) of the silver is in the filtered fraction. In coastal waters silver is found to be either mostly  $\approx 100\%$  (10 kDa; Sañudo-Wilhelmy et al., 1996) or preferentially (57 to 80% (1 kDa; Wen et al., 1997) in the "truly dissolved" fraction. The lack of affinity of silver for particles have been theoretically explained as the result of the formation of the neutral silver-chloro complex ( $\text{AgCl}^0$ ) in saline waters (Cowan et al., 1985; Jenne et al., 1978; Flegal et al., 1997).

### *Oceanographic consistency*

The concentrations of silver for the North Atlantic are consistent with previously reported oceanographic distributions. The range in concentration (0.69 to 7.2 pM) and the mean ( $\bar{x}$   $\pm$  s) concentration ( $3.6 \pm 1.3$ ) are comparable to the range (0.24 to 9.6 pM) and average ( $3.6 \pm 2.3$  pM) measured for the eastern Atlantic ocean (Flegal et al., 1995). Both distributions have the same general trend with depth as those from the Pacific Ocean (Murozumi, 1981; Martin et al., 1983), with lower concentrations in surface waters and relatively higher concentration in deep waters. These concentration profiles indicate that silver has a nutrient-type regeneration cycle in deep waters (Broecker and Peng, 1982; Flegal et al., 1995). This type of regeneration is also attested for by the simple linear relationship between silver and silicate concentrations in oceanic waters (Flegal et al., 1995) as discussed in the following section.

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Silver concentrations in the North Atlantic are affected by continental inputs. In spite of their general agreement, there are some discrepancies in the distributions of silver in the eastern and North Atlantic. The top <200 m of the surface waters of the North Atlantic is relatively enriched in silver (0.69 to 4.6 pM,  $2.8 \pm 0.91$  pM) than the eastern Atlantic (0.24 to 1.1 pM,  $0.52 \pm 0.37$  pM). Excess concentrations have also been observed for other trace elements (Yeats and Campbell, 1983; Véron et al., 1994, 1997) and anthropogenic tracers (Sy et al., 1997) in the North Atlantic. The excess in silver is attributed to its input from continental sources (aeolian, streamflow, surface runoff, benthic remobilization) to the waters of the North Atlantic. Following the assumption of Flegal et al. (1995) that the background levels of silver in surface waters are those of the equatorial and South Atlantic (0.25 pM), these concentrations indicate that in average surface waters of the North Atlantic have ten times more silver than background levels. There are evidences that silver behaves conservatively during the advection of deep water. By comparing the distributions of silver in the eastern Atlantic with those the North Pacific Flegal et al. (1995) suggest a systematic enrichment of silver in deep oceanic waters. However, similar concentrations of silver are observed in either the deep waters of the North and eastern Atlantic, or the deep waters of the South and North Pacific. In contrast to the similitude in silver concentrations between the North and eastern Atlantic deep waters, silicate concentrations increased by about four times in the same transect (from 25 to 90  $\mu$ M; Flegal et al, 1995; Measures, 1995; Yeats and Measures, 1997). Silver has essentially the same concentration in deep waters of the South (Murozumi, 1981) and North Pacific (Martin et al., 1983) oceans (Figure AgSiAl22.spw); but these are up to four times higher (35 pM in South Pacific; Murozumi, 1981) than in the Atlantic Ocean. These evidences indicate that silver enters the deep ocean in the North Atlantic, and is advected conservatively through the Atlantic Ocean, and that the high silver concentrations in the Pacific are the result of intermediate silver inputs most probably in Antarctic waters.

### *Covariance of silver and silicate*

In the North Atlantic there is a simple direct correlation between silver and silicates, which is different to those in other oceanic waters. The covariance for the North Atlantic is characterized by the following simple linear correlations:

$$\text{Ag (pM)} = 2.309 + 0.167 * \text{H4SiO4 } (\mu\text{M}) \quad (\text{R} = 0.680) \text{ all samples using averages, and}$$

$$\text{Ag (pM)} = 2.449 + 0.162 * \text{H4SiO4 } (\mu\text{M}) \quad (\text{R} = 0.733) \text{ excluding two outliers}$$

As previously reported (Flegal et al., 1995), the following simple linear correlations apply for both the eastern Atlantic and the North Pacific, respectively

$$\text{Ag (pM)} = 0.685 + 0.107 * \text{H4SiO4 } (\mu\text{M}) \quad (\text{R} = 0.916), \text{ and}$$

$$\text{Ag (pM)} = -0.691 + 0.111 * \text{H4SiO4 } (\mu\text{M}) \quad (\text{R} = 0.897)$$

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The slopes for the three relationships are essentially the same.

The difference in the intercepts indicates the excess silver with respect to silicates in the North Atlantic. This relative excess in silver is because the concentrations of silicates in the North Atlantic are at least four times lower than in the eastern Atlantic. As mentioned in the previous section, once silver is introduced to the North Atlantic deep waters it seems to remain relatively conservative, while silicate is being released from biotic and abiotic particles, and increases in concentration as is advected to the South. These different biogeochemical behaviors are shown by the Ag / H<sub>4</sub>SiO<sub>4</sub> ratios (pM / μM) in different oceanic water masses. The distribution with depth indicates larger Ag / H<sub>4</sub>SiO<sub>4</sub> ratios in deeper waters of the North Atlantic than in the eastern Atlantic (Flegal et al., 1995), while surface water ratios have similar ranges. The latter is tentatively attributed to the relative low concentrations of silicate in both of those oceanic surface waters.

### *Covariance of silver and aluminum*

The covariance of silver and aluminum in surface waters has been previously noted (Flegal et al., 1995). The presence of such a relationship in the North Atlantic is now being determined. This will, presumably, provide a measure of the relative contribution of silver from natural and anthropogenic sources.

### *Silver as tracer of oceanic circulation*

Systematic enrichment of silver concentrations along the main advective flow of deep water from the North Atlantic to the North Pacific suggests that this element could be used as a tracer of oceanic circulation (Flegal et al., 1995). Preliminary results indicate that the geochemistry of silver in subsurface waters is relatively conservative. For example, a comparison of potential temperature-salinity (T-S plot) vs. silver concentrations Vs salinity (Ag-S plot) in the northeast Pacific indicates that different water masses could be identified by their silver content. The temperature-salinity profile shows three distinct water masses (subtropical surface, subtropical subsurface and Antarctic intermediate water or AAIW) which are also clearly identified in the silver-salinity relationship. Therefore, the validity of that hypothesis is being assessed with these new data.

### **Summary**

In summary, these initial data from the North Atlantic appear to be consistent with previous measurements of silver in oceanic waters. Analyses are now being made to determine the extent of the element's oceanographic consistency, the relative contribution and natural and anthropogenic silver to the world's ocean, and the validity of hypotheses on the biogeochemical cycling of silver in the world ocean.

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### **Acknowledgments**

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## Questions & Answers: Silver in the North Atlantic Ocean

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(NOTE: In Ignazio Rivera-Duarte's absence, the paper was presented and questions answered by Arthur R. Flegal)

Q. ARUN MUKHERJEE (University of Helsinki-Finland): Most of my colleagues here speak about the Atlantic, Pacific Ocean or San Francisco Bay. But can you say something about the Baltic Sea? It is very vulnerable and changing the water all the time. It varies very much, maybe water stays one day in some parts near Denmark, but in the upper side it stays 3 months. So how can silver be in the sediment or in the water? How will it differ from your results in San Francisco Bay, the Atlantic, or Pacific Ocean?

A. How will it be different?

Q. Yes.

A. Well, in the South Bay the silver just stays there. It never leaves. It goes into the sediments and out of the sediments. In the Baltic what you form is the deep water for the entire world's ocean. I didn't go into this but in these profiles when we looked at the metal concentrations we also looked at lead concentrations co-varying with silver, and when we did the isotopic composition of the lead we could actually fingerprint the industrial sources of lead in the western, central, and eastern parts of that polar region. It's all anthropogenic, and it's all rapidly advected with the water masses into the deep water that forms the North Atlantic deep water and then carried out. So it's a much more dynamic system but all of the lead, 99% of that lead, is industrial and we showed the composition. It's either of North American or European origins. The silver co-varies with the lead, so we believe that those elevated levels of silver are in fact anthropogenic, but they are carried out of the system.

Q. NICK FISHER (SUNY-Stony Brook): Are there reliable sediment trap measurements of fluxes of silver out of surface waters, either in the Atlantic or Pacific?

A. John Martin covered that in *Nature*.

Q. Nothing in the Atlantic?

A. No.

Q. Is it tightly coupled to flux of organic carbon? Do you recall?

A. You've seen all the data there is. It's pathetic; that's all there is.

Q. Associated with that, have there been estimates of residence times of silver in surface water? Do you know what they are offhand?

A. Yes. We've done residence time calculations for San Francisco Bay where they are the same as lead, which is about two weeks. And we've done residence time calculations in the Southern California Bight - coastal waters off Southern California - and there the residence times are between 7 days and 2 months, depending on algal bloom.

Q. And open ocean, did Martin do those? Were they like weeks to months, that kind of thing?

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- A. Well, what you typically do is you just take it and double it. (*Discussion among audience members*) This is our way - what if I'm wrong? The residence time of lead in surface waters of the central Pacific is maybe a year, and so silver which co-varies with lead in estuarine waters has the same  $K_d$  and coastal waters should be about the same. In fact, Nick, it fits with your scavenging model.
- Q. Yes, I realize. We make estimates of what the residence times ought to be. But we would like to have some real-world data to compare it with.
- A. We substantiated our estimates based on your estimates. (*laughter*)
- Q. Okay.

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## Transfer of Cadmium, Zinc and Lead from Soils to Plants

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**Introduction.** A major exposure route of trace metals to humans is via consumption of plants. Soil is a main source of these elements in crop plants. Uptake of trace metals by plants depends on several factors of which soil parameters, plant characteristics, and also biogeochemical properties of an element play the most important roles. Understanding of some principles governing trace metal uptake by plants is fundamental in the evaluation of the dietary risk of trace metal pollution of soils.

**Materials and Methods.** Field, lysimeter and pot experiments were carried out with different crop and herb plants: (a) field experiments: Zn-Pb smelter flue dust was incorporated into the plough layer of the soil, (b) lysimeter experiments: sewage sludge was incorporated into the plough layer of the soil, and (c) pot experiments: technical salts of metals were mixed with potted soil. In all experiments a similar soil was used: Podsoluvisols, light loamy sand, SOM - 0.6 - 0.8%, pH (KCl) 6 - 7. Flue dust contained, in %: Cd 0.4, Zn 45, Pb 20, of which dominant forms, in % of the total elemental content, were oxides: Cd 47, Zn 90, Pb 81. Sewage sludge contained, in mg/kg: Cd 7, Zn 6100, Pb 228. Dominant forms, in % of the total elemental content, were: Cd oxides 70, Zn oxides 72, Pb organic 42, and Pb residual 44.

The crop plants were grown in soils containing the flue dust in the following rotation: (a) spring barley (*Hordeum vulgare* L.), (b) clover (*Trifolium pratense* L.), (c) grass (*Poa pratensis* L.), (d) lupine (*Lupinus sativus* L.), and (e) potatoes (*Solanum tuberosum* L.). In the experiment with sewage sludge, oats (*Avena sativa* L.) and spring barley were grown. In the experiment with technical salts, corn (*Zea mais* L.) was grown.

Total metal contents were measured in air-dried soil samples by AAS spectroscopy after digestion in concentrated HCl/HNO<sub>3</sub> acids. Sequential extractions of metals were done using Tessier's method (8). Air-dried plants were ashed at 450° C, dissolved in HCl (C=6 mol/L), and analyzed for metals by AAS spectroscopy. Cd and Pb were measured after organic extraction with APDC/MIBK. Analytical error estimates, using reference materials and multiple analyses, varied from 5-20% depending upon the metal.

**Results and Discussion.** Metals usually enter the plant through root uptake and are concentrated most often in the root (3). In general, trace metal contents decrease in the order of roots > stems > leaves > fruits > seeds (4). Although metal uptake by plant is a function of the contents of the soil, it is also affected by a plant's inherent affinity for a given metal, which is highly controlled by plant variety and type of cultivar.

Variable responses of plants grown in soils contaminated by various sources of metals were observed during the experiments. Depending upon a source of metals, there were different changes in metal species in soils. The addition of Zn-Pb flue dust at a level of about 500 mg Zn/kg affected the increase of Zn fractions in the soil (in



ratio to control content or percent of total values) as follows: exchangeable 14 mg/kg, or 5%; carbonates 28 mg/kg, or 63%; and oxides 8 mg/kg, or 32%. Adding sewage sludge (under conditions similar to the flue dust experiment), the Zn fractions increased as follows: exchangeable 56 mg/kg, or 25%; carbonate 18 mg/kg, or 40%; and oxides 8 mg/kg, or 33%. The addition of zinc sulfate affected mainly the carbonate fraction: 16 mg/kg, or 32%; and oxide fraction 10 mg/kg, or 36%. The other fractions of metals, organic and residual, were not changed significantly.

Similar trends in changes of metal species (forms) in soil were also observed for Cd and Pb. The variation in metal species affected their availability to plants (Table 1). The addition of the sulfates of cadmium and zinc

Table 1. The Relative Effects on Plants (Calculated as a Ratio of Experimental Values to the Control) of Metal Species from Various Sources in Soils: Metal Accumulation Factor (MAF)\*

Metal and Treatment	Plant	Soil – Total	Soil – Exchange	Plant
Cd – flue dust	Barley	230	12	
	straw			7
Cd – sulfate	grain	16	150	6
	Corn			150
	leaves			250
Zn – sewage sludge	stems	7	2	
	Oats			1.8
Zn – flue dust	young shoots	169	120	
	Barley			4
	straw			2
Zn – sulfate	grain	23	120	
	Corn			10
	young leaves			70
Pb – flue dust	stems	322	60	
	Barley			1.8
Pb – sulfate	straw	18	37	5.5
	grain			2.5
	Corn			1.5
	young leaves			
	stems			

\*Metal Accumulation Factor (MAF) is defined as the direct ratio of metal concentration in a given plant grown in contaminated soil to the concentration in the same plant grown in the same, but noncontaminated, reference soil. The metals content of the reference soil is Cd – 1.5, Zn – 48 and Pb – 26 mg/kg (see "Materials and Methods")

increased primarily the exchangeable forms of these metals but not that of lead. The response of plants to increased exchangeable forms of Cd and Zn was manifested mainly by the corn plant, although there was a very small effect on the Pb concentration in corn. Regardless of the source, Cd was the most intensively absorbed metal. This is also confirmed by the values calculated for the MAF (Metal Accumulation Factor) index for different crop plants (Table 2). Trace metal concentrations in plants grown on the soil most polluted with Zn-Pb flue dust demonstrated that potato tubers had the greatest ability to accumulate both Cd and Pb, especially intact tubers (well washed). This observation is supported by our previous results (7) and also by other authors (1, 6).

Table 2. MAF Values for Cd, Zn and Pb Taken Up By Crop Plants, Grown in Soils Contaminated with Cd-Zn-Pb Flue Dust at the Levels (in mg/kg) of Cd 100, Zn 15000, Pb 6000.

Plant	Cd	Zn	Pb
Barley, straw	8	4	2
grain	6	2	6
Potato tubers, intact	21	7	27
peeled	10	2	5
Clover	6	4	4
Grass	6	6	8

The MAF values for crop plants grown in soils loaded with flue dust are smaller than that index calculated for corn grown in soil amended with metal sulfates (Table 1). Calculations showed that a weed, convolvulus (*Convolvulus arvensis* L.), which grows widely in heavily polluted soil, had the highest rate of metal uptake from soils amended with flue dust. The MAF values for this weed were: Cd 12, Zn 10 and Pb 11. Somewhat similar rates of metal uptake were seen for grass, lupine, and clover, and exceeded the MAF values for barley straw and grain.

Clover and grass grown for two years in soil with flue dust additions revealed variable responses depending upon the time of the year. Both plants accumulated slightly smaller amounts of Cd, Zn and Pb during the first year than in the second. In both years of the experiment, higher concentrations of metals were found in the last harvest cut (August). This is clearly explained by three factors: (a) higher concentration due to a lower yield of the third cut, (b) weather impact, and (c) increased pool of the exchangeable forms of the metals. A noticeable overall increase of metal content was observed in clover at the different levels of a particular metal in the soil (in mg/kg), e.g., Cd 3, Zn 900, Pb 6000. An increased Pb content in clover was observed only in the third harvest cuts in both years. The response of bluegrass was smaller than of clover, and was observed mainly in the second and third cuts, at the same metal levels and in the same soils used for clover.

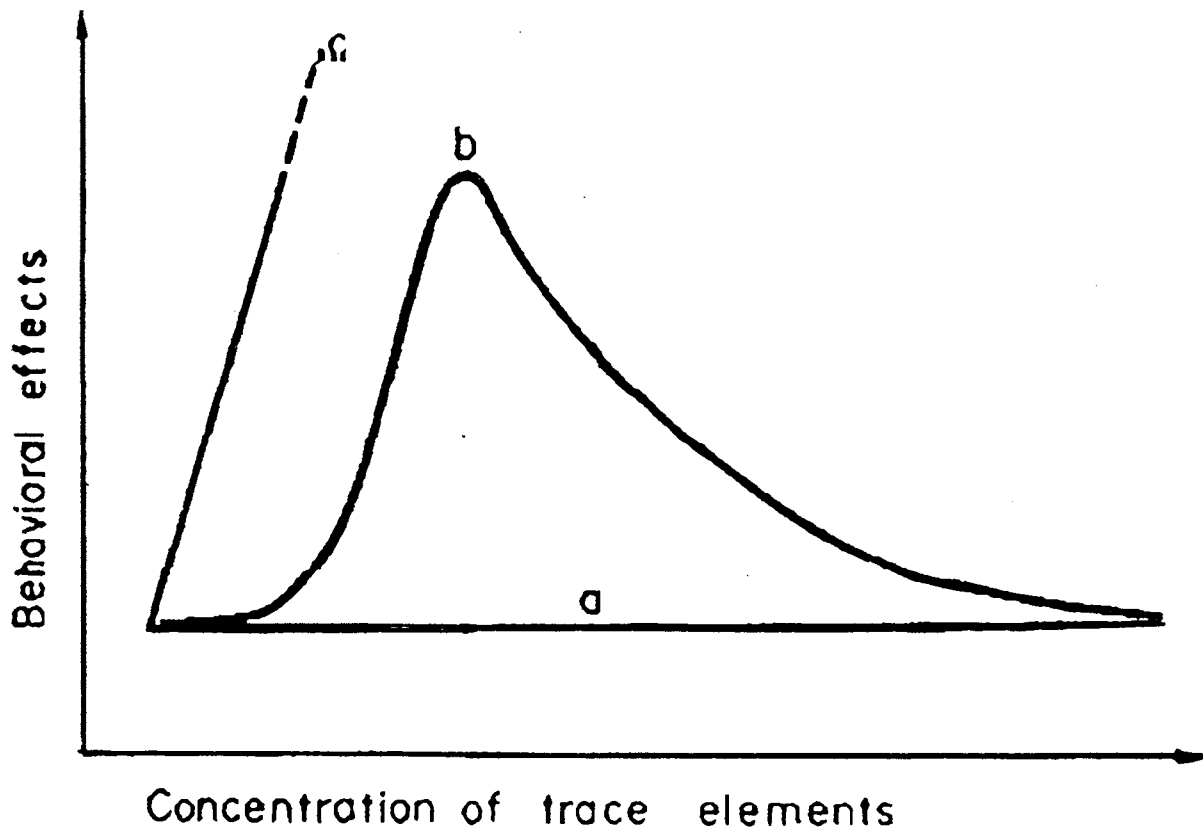
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Potatoes and cereal grains are significant exposure routes for metals transfer from soils to humans in several countries. In particular, there is a potential risk of easy Cd uptake from light acid soils (7), on which potatoes and some cereals are preferably cultivated. The results of all the experiments with soil loaded with different sources of Cd, Zn and Pb showed that the Cd (3 mg/kg) and Zn (700 mg/kg) threshold MAC (Maximum Allowable Concentration) values for light acid soils are very reasonable (5). The MAC value for Pb is established in most countries at 100-500 mg Pb/kg (2); however, it could be revised to a higher level from the point of view of dietary risk from trace metal pollution of crop plants.

The results of these experiments confirmed earlier conclusions, that Cd is the trace metal most easily taken up by crop plants and especially by the potato (1, 6, 7). Thus, a serious dietary risk of soil pollution with metals is related mainly to increased Cd levels.

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**Figure 1**

Schematic diagram of behavioral plasticity of plants under chemical stress.

a) no behavioral change in entirely tolerant species, b) development of behavioral tolerance, c) reaction of nontolerant species leading to damage of organisms followed by no recovery.

Source: Kabata-Pendias, A. and H. Pendias, 1992. Trace Elements in Soils and Plants, 2nd ed. CRC Press, Boca Raton, 365 pp.

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## Questions & Answers: Transfer of Cadmium, Zinc and Lead from Soils to Plants

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- Q. JIM KRAMER (McMaster University): Relative to the key point you made that it's the absorbability of the plant and the nature of the metal, I'm wondering if you found that if the timing of addition, particularly of the doping of the soluble metal, was important relative to the stage of growth? Years ago we did work on aluminum, and we found that uptake just as the tap root developed was the very key, and later on the uptake was much less. Does anything like this work for the metals you studied?
- A. Well, to some extent. The manner you mentioned is not quite obvious from a soil chemistry point of view. It is a kind of competition. When you add metal at the time of root development, the root can uptake it very easily because it is in a mobile form or it is next to the root surface, because competition between the root surface and soil particles owes this phenomena. With time soil particles fix or adsorb the metal stronger, and the root does not have the capability to mobilize it.
- Q. Well, what we found were all the ligands in the root system were maximum right at that first initiation of growth.
- A. Well, but is a kind of competition plants have to develop. Root plant has a great capability and especially with time certain plants, most of the plants, develop microrhiza at the root surface. Again, the capability to uptake metal is increased. So I think it's not - well this relationship is not a readily predictable one, I would say.
- Q. TOM BOBER (Eastman Kodak): Alina, I just wanted to know, has Poland established any regulatory standard, for example, for lead in food products? In your last bullet there you said that you felt the regulatory standard could be increased. So there is a regulatory standard?
- A. Yes. We have one. And it can be increased for soil, but not for food. It is developed for soil, because we have certain guidelines established but we still want to correct this and find out what is happening. So since lead is, as we know, really very unmobile and not available to most plants, I think this target value or standard for lead could be increased. And in the condition of soil in my country or in the area, my area of Europe, we are very willing now to decrease this target value for cadmium. Especially that cadmium is so very nicely and easily taken up by the very important food crop, which is potatoes.
- Q. ARUN MUKHERJEE (Helsinki University-Finland): Alina, you mentioned that lead is not mobile, but when there is an acidic condition, don't you think it is very mobile or can be taken up by plant species?
- A. Well, you know mobility is always relative, let's put it that way. When I said it's not very mobile, it's not very mobile compared to cadmium and zinc.
- Q. That I agree with you.
- A. And if I would make a diagram or scale, this would be mobility of cadmium at it's very, very lowest place. Here are other trace metals and here is lead. So in most soil conditions, I would not say in all, but in most of the soil conditions lead would be always the least mobile, what we mean is the least available metal to plants, compared to all other trace metals we think of or we deal with.
- Q. Thank you.
- A. Excuse me, with one exception: it is possible that it can be taken up and concentrated in the root system, but not transported.
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## **Is It Possible To Use Silver As Sewage Tracer In Coastal Waters Of The Adriatic Sea?**

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The northern Adriatic Sea is a relatively poorly flushed body of water that is heavily impacted by man's activities. The most visible and best-studied environmental problem in this region is hypereutrophication, due principally to the discharge of enormous quantities of nutrients flowing into coastal waters. From northern Italy alone, extensive nitrogen and phosphorus inputs into the Adriatic via several rivers, most notably the Po, can account for a significant fraction of the total budgets of these nutrient elements in the Adriatic (Table 1). Additionally, there are many major municipal sewage treatment plants located on or near the coast, and these discharge effluents are also rich in N and P. The impact of the excessive nutrient loading into the Adriatic has been most evident in terms of nuisance blooms of toxic and non-toxic algal species (red tides) (Degobbis et al., 1995; Sellner and Fonda Umani, in press). Severe effects caused by hypoxia and anoxia, particularly during summer months, have also been related to these nutrient inputs (Marchetti et al., 1989).

Recent studies of the East and West Coasts of the US have shown that silver can be an excellent chemical tracer of sewage effluents in coastal waters (Sanudo-Wilhelmy and Flegal, 1992; Bothner et al., 1994; Ravizza and Bothner, 1996). Silver may also prove to be useful as a tracer of sewage input into the Adriatic as well, particularly because there are no known natural sources of silver in the Adriatic basin, so the background silver concentrations would be expected to be very low relative to anthropogenic inputs. The riverine input of diverse metals and organic contaminants has been recorded (Table 1), but no studies have systematically measured the input and distribution of silver in the Adriatic. We therefore propose that an initial investigation be launched to evaluate the fluxes and distributions of silver in the northern Adriatic. We further suggest that silver could be examined as a potential tracer of sewage effluent, as has been demonstrated in coastal U.S. waters. Silver is also of interest in because it is highly concentrated out of seawater or prey by diverse marine organisms (IAEA, 1985; Fisher and Reinfelder, 1995) and can be toxic at nanomolar levels (Eisle, 1996). Because silver is very particle-reactive in aquatic systems, with  $K_d$  values or bioconcentration factors typically in the  $10^5$  range (IAEA, 1985; Fisher and Reinfelder, 1995), its distribution will be strongly affected by suspended particulate matter, including biogenic particles (Lee and Fisher, 1993). Thus, the production of biological particles via plankton dynamics, which is related to

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current patterns (Fonda Umani, 1996), and the subsequent sinking of biogenic debris, which has been well described in the Adriatic (Giordani et al., 1992), would strongly influence the fluxes and residence times of silver in the Adriatic water column.

**Table 1.** Riverine input of organic matter, nutrients and metals (F.A., 1990).

	O. M.		Nutrients			Metals			
	TOC 10 <sup>3</sup> t/yr	COD 10 <sup>3</sup> t/yr	TKN 10 <sup>3</sup> t/yr	P-PO <sub>4</sub> 10 <sup>3</sup> t/yr	TP 10 <sup>3</sup> t/yr	Cd t/yr	Pb t/yr	Cr t/yr	Cu t/yr
<b>Po</b>	217.5	700.1	81.8	3.5	10.9	2.3	16.2	34.8	30.1
<b>Adige</b>	19.6	67.5	7.7	0.4	1.2	0.4	1.7	4.4	4.6
<b>Tevere</b>	39.2	157.4	20.2	1.9	3.2	0.6	2.9	7.0	3.6
<b>Arno</b>	12.7	58.2	4.6	1.0	1.8	0.2	0.3	2.4	1.0
<b>Total</b>	298.0	983.2	114.3	6.8	17.1	3.5	21.1	48.6	39.3

O.M.= Organic matter; TKN= Total nitrogen according to Kjeldahl method.

Further the most important geographic features of the Adriatic Sea have to be considered. The Adriatic Sea is a semi - enclosed and elongated basin, stretching roughly SE to NW for 800 km from the Straits of Otranto to the Gulf of Venice (Fig.1). The elongate shape and the presence of the great Dalmatian Archipelago create an extremely long coastline. The eastern coasts are high, rocky, and articulated. The western (Italian) coasts generally are sandy, flat and alluvial with the exception of the Gargano Peninsula and the Apulian coast in the southern part. Extensive lagoons characterized the northern part and the areas of Po River Delta. Based on the bathymetry and different oceanographic properties it is possible to distinguish three distinct areas: North, Mid and South Adriatic. The North Adriatic is the shallowest (max. depth 100 m) of the three basins. Its northern end is even shallower and gently sloping, with an average bottom depth of about 35 m. Along the Italian shore the sea bottom is characterized by pelitic sediments of terrigenous supplies. Sand predominates in the central and southern part of the shelf. The Mid and South Adriatic are deeper basins (max. depth 270 m and 1200 m respectively); they are separated by the Pelagosa sill (160 m). Another sill (800 m) situated in the Channel of Otranto separates the South Adriatic from the Ionian Sea. The basin bottom is mainly composed of pelitic sediments (Brambati, 1992).

The whole Adriatic basin is affected by a strong seasonal variability in both the circulation and the ecosystem due to the main forcing functions (e.g. atmospheric forcing determining and annual average heat loss of about 20

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$W/m^2$ ; river runoff affecting the circulation through buoyancy input and introducing large amount of nutrients (Zavatarelli et al., in press).

The circulation is generally cyclonic, with two main cyclonic gyres in the middle and southern Adriatic and an autumn cyclonic gyre limited to the northern basin. Along the western coast a strong coastal current is observed only in the northern basin in winter and along the entire coastline in the other seasons. Along the eastern coast a northwards current associated with LIW (Levantine Intermediate Water), occurs, being particularly strong in autumn (Artegiani et al., 1996a-b).

The main source of fresh waters and consequently of nutrients is the Po river located on the Italian coast south of Venice which drains a watershed of 75,000  $Km^2$  encompassing heavily populated northern Italy (15.5 million inhabitants) (Harding et al., in press). The mean annual rate of 1,470  $m^3/sec$  results in a discharge of about  $46.4 \times 10^9 m^3/yr$ . The total inorganic nitrogen (TIN) load from the Po is estimated to be  $115 \times 10^3 t/yr$ , and total nitrogen (TN) is  $190 \times 10^3 t/yr$ , while total phosphorus (TP) currently is about  $8.5 \times 10^3 t/yr$ . Point sources account for some 40% of TN in the Po basin which are mainly due to inhabitants reflecting the lack of waste water treatment (Seagle et al., in press). An estimated 43% of the N loadings and 35% of P loadings into the Adriatic are attributable to wastewater discharge (Amato et al., 1989).

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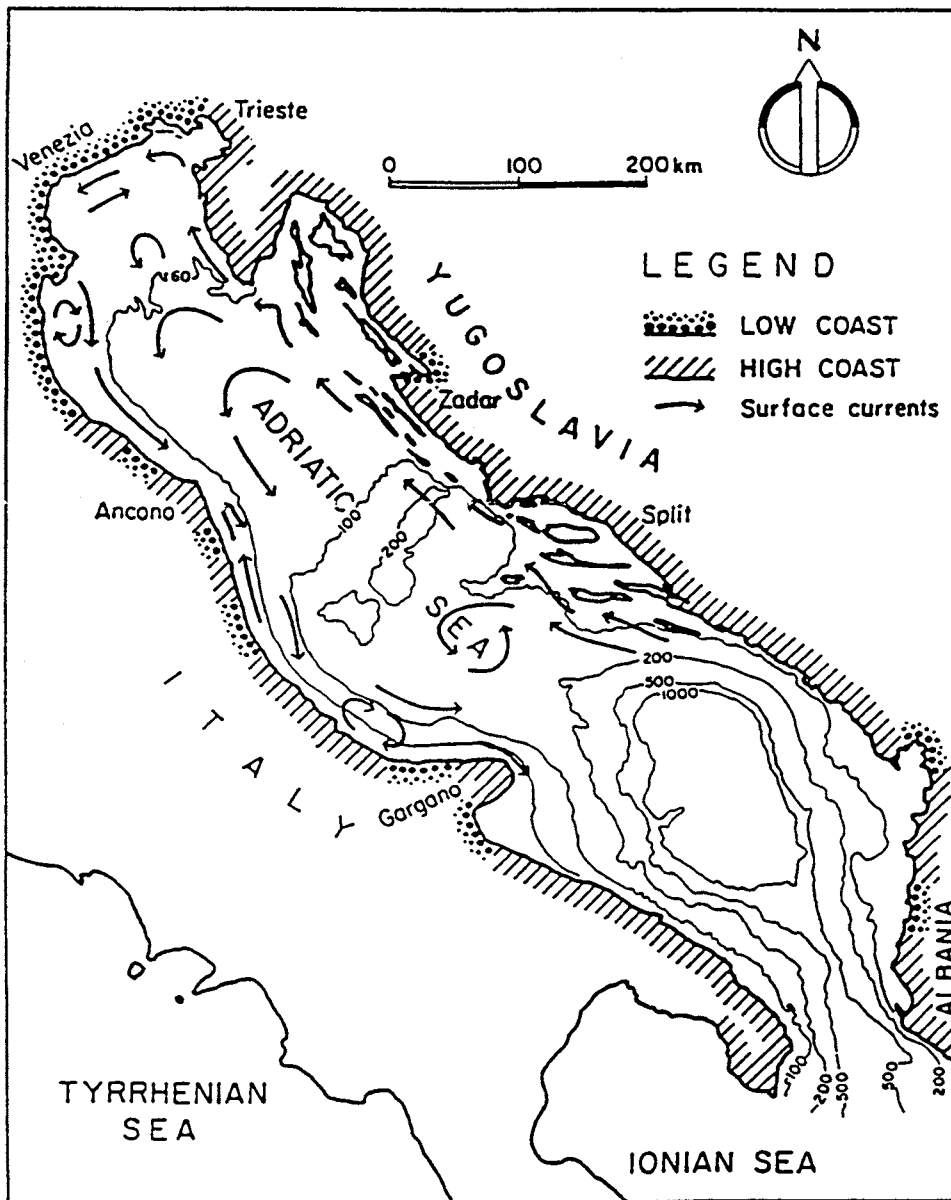


Fig. 1. The Adriatic Sea.

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**Questions & Answers: Is it Possible to Use Silver as Sewage Tracer in Coastal Waters of the Adriatic Sea?**

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Q. RUSS FLEGAL (University of California-Santa Cruz): Wasn't the Roman Empire funded by silver mined around Trieste from galena deposits?

A. Silver mines? No, there are many mercury mines, just mercury...

Q. Aren't there a lot of galena deposits surrounding the Adriatic?

A. No, there are not. But there are some on the Mediterranean Sea.

Q. OK.

Q. JIM KRAMER (McMaster University): Serena, I have a question. Except that we're at a silver conference, how about the other elevated metals, for example that are in the coastal waters? I'm sure there's data on them for the Adriatic and surrounding waters. Are they not good indicators? I guess my question is, why silver versus the other?

A. That is a fair question. Particularly in the coastal area closer to the metropolitan areas, we have high residual pollution of other metals, which means that our input into the system in terms of non-point sources is really strong. But silver from these sources is low and is mostly associated with municipal discharge. I think that the point sources can pose a lot of problems. It seems very strange, but big cities like Milan are still lacking any waste treatments. So you can imagine what the situation is.

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## Health Risk Assessment of Environmental Silver

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Presently, there is a perceived disparity among U.S. regulatory initiatives and viewpoints concerning the hazard potential and subsequent health risk that silver in the environment poses to humans. The existence of a U.S. Environmental Protection Agency (EPA) Reference Dose (RfD - a daily exposure level deemed to be acceptable for humans) for silver has largely been responsible for its inclusion on State or Federal lists of contaminants generally recognized as hazardous to either human health or the environment. To illustrate this point, the Agency for Toxic Substances and Disease Registry (ATSDR) developed a Toxicological Profile for silver based on Congressional mandates to "prepare toxicological profiles for hazardous substances which are most commonly found at facilities on the CERCLA National Priorities List and which pose the most significant potential threat to human health" (ATSDR, 1990). Similarly, the EPA's Office of Solid Waste (EPA, 1997) and RCRA Program (EPA, 1996), respectively, have included silver amongst other hazardous environmental chemicals based on its 'potential' to cause adverse effects in humans. However, in 1991 the EPA reevaluated the database for silver and concluded that silver is not associated with chronic adverse health effects in humans. Consequently, the Maximum Contaminant Level Goal (MCL) for silver in drinking water was deleted (Federal Register, 1991). With this background, the following discussion is intended to show that (a) the inclusion of silver on lists of environmental contaminants that pose risks to human health should be revisited in light of our knowledge concerning the toxicity of, and relative exposure to silver, and (b) that chronic lifetime exposure to trace amounts of silver in the environment does not pose a human health risk.

Silver has not been associated with adverse health effects or chronic toxicity in humans and there is no evidence for carcinogenicity, neurotoxicity, or reproductive/developmental toxicity resulting from exposure to silver. The current RfD for silver is based on argyria, a cosmetic pigmentation of the skin, mucous membranes, and eyes (primarily conjunctiva), that results from tissue deposition of a silver-protein complex or its metabolized product (silver sulfide or silver selenide) following long-term exposure (absorbed silver > 1 g) to silver or silver-containing compounds. It is important to note that argyria, the only known effect resulting from chronic overexposure to silver, is not associated with adverse health effects and this was the determining factor in the EPA's decision to delete the MCL in 1991. More relevant to this discussion, argyria has not been reported for, and is not expected to occur as a result of, environmental exposures. A comparison of environmental metals shows that silver is the only one with an RfD based on an effect that is

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considered a cosmetic effect in contrast to numerous other significantly more toxic metals with endpoints of concern, which include carcinogenicity and neurotoxicity (Table 1). Based on our current knowledge of the toxicological properties of silver, there is little basis for concern over the hazards that silver presents, one of the critical determinants in the assessment of health risk.

Exposure is the other critical component in assessing health risk, and in contrast to some other environmental metals, silver is among the least encountered in terms of environmental presence (Table 2). Recently, the EPA reviewed data for Toxicity Characteristic (TC) metals detected at non-hazardous waste management unit sites, and while this review was limited in its scope, it does provide a perspective on the relative presence of metals at certain waste sites, and perhaps in the ambient environment in general (EPA, 1996). As can be seen from Table 2, among those metals detected at non-hazardous waste management unit sites, silver is relatively low, both in frequency of occurrence and concentration in soil. In addition, silver is typically found within environmental regulatory limits, in contrast to other toxic metals that are frequently detected at concentrations significantly above Federal or State standards (Table 2).

It has been estimated that the majority of human exposure to silver is through dietary sources with ingestion of water contributing a lesser amount and air contributing very minor amounts to overall exposure (Table 3). In order to evaluate human health risk to environmental silver, it is necessary to compare estimates of daily and lifetime exposure to the endpoint on which the RfD is based, namely argyria. A simple quantitative assessment of potential exposure to silver from food, water, and air sources reveals that the hazard index ( $HI = \text{exposure}/RfD$ ) for silver is below one, thus indicating that environmental silver exposure does not pose a human health risk (Figure 1). It is important to point out that this example is considered to be a high-end exposure scenario and uses EPA data that assumes high-end ingestion estimates as well as ingestion of two liters of silver-containing water per day. The conclusion from this simple, yet reasonable worst-case assessment, is that exposure to silver from environmental sources is not expected to result in argyria. In support of this assessment, the EPA conducted an exposure and risk assessment for silver in 1981 and noted that "adverse effects are not likely among humans because the silver concentrations in drinking water, food, and air are low relative to the concentrations required to cause effects" (EPA, 1981). In summarizing the exposure and risk assessment, the EPA concluded that "the risk to humans of adverse effects resulting from exposure to silver is believed to be quite low."

In summary, silver and silver compounds are not associated with adverse effects or toxicity in humans and current regulatory initiatives and exposure limits are based on argyria, a cosmetic effect that is known to occur principally following chronic occupational overexposure or ingestion of silver-containing medicinal products. Importantly, argyria has not been associated with exposure to silver from environmental media or sources. EPA exposure assessments, as well as recent environmental analyses (Shafer et al., 1997; Wen et al., 1997), confirm that silver is present in environmental media at concentrations much lower than other, more toxic metals. In fact, silver is one of the least prevalent metals in the environment based on a recent EPA assessment of metals at non-hazardous waste management unit sites (EPA, 1996). Quantitative risk estimates using high-end chronic exposure scenarios illustrate that argyria is not expected to occur from

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environmental exposures to silver. Thus, silver in the environment does not pose a risk to human health.

Based on the absence of known health effects associated with silver, coupled with the low environmental levels and subsequent exposure potential to humans, the policy of including silver on various regulatory lists because of perceived health risks should be revisited. At a minimum, silver should not be included on lists of chemicals of concern based on human health effects. Harmonization of regulatory efforts should be pursued to develop consistency among agencies in recognizing that silver in the environment is not a human health concern. Recognition of this fact will enable regulatory officials to concentrate their attention and efforts on those metals in the environment that do possess known toxicity and pose real health risks to humans.

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**Table 1. A Comparison of RfDs for Environmental Metals**

<b>Metal</b>	<b>EPA RfD (mg/kg/day)</b>	<b>Basis for RfD</b>	<b>EPA Carcinogenicity Status</b>
Lead	NA	Neurotoxicity, developmental and hematological effects	Probable human carcinogen
Mercury	NA	Neurotoxicity	Not classifiable
Arsenic	0.0003	Neurotoxicity and cardiovascular effects	Human carcinogen
Cadmium	0.0005	Kidney toxicity	Probable human carcinogen
Chromium VI	0.005	Kidney toxicity	Human carcinogen
Selenium	0.005	Clinical selenosis	Not classifiable
Silver	0.005	Argyria	Not classifiable
Barium	0.07	Hypertension	Not evaluated

**Table 2. RCRA TC Metals Detected at Non-Hazardous Waste Management Unit Sites**

Metal	TC Level (mg/L)	# Sites with Constituent Detected	# Sites with Constituent Detected Above Federal or State Standards	Range of Federal or State Standards (mg/L)	Average Maximum Detected Concentration (mg/L)	Highest Maximum Detected Concentration (mg/L)	Ratio of Highest Detected to Federal or State Standards
Lead	5	37	22	0.0015 - 0.05	1.3	28	560-18667
Chromium VI	5	36	21	0.01 - 0.1	2.3	58	580-5800
Arsenic	5	29	15	0.005 - 0.05	28.4	595	11900-119000
Cadmium	1	28	17	0.0004 -0.005	0.2	3	600-750
Barium	100	28	11	0.2 - 2	31.1	630	315-3150
Mercury	0.2	19	6	0.0002 - 0.002	0.002	0.007	3.5-35
Selenium	1	18	6	0.01 - 0.05	2.2	27	540-2700
Silver	5	12	3	0.01 - 0.1	0.006	0.01	0.1-1

Reference: Hazardous Waste Characteristic Scoping Study, EPA, 1996.



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**Table 3. Estimation of Silver Exposure from Environmental Sources**

<u>Route of Exposure</u>	<u>Estimated Intake (<math>\mu\text{g}/\text{day}</math>)</u>	<u>Reference</u>
Ingestion - diet	35-88	EPA, 1979
Ingestion - drinking water	0.2-20 <sup>a</sup>	EPA, 1985
Inhalation (urban areas)	0.05-0.19 <sup>b</sup>	EPA, 1981

<sup>a</sup> Assume ingestion of 2 liters water/day

<sup>b</sup> Assume inhalation rate of 1.8 m<sup>3</sup>/hr and 24 hr exposure

**Figure 1. Calculation of Human Health Risk from Environmental Silver**

- ◆ Hazard Index = Exposure/RfD (0.005 mg/kg/day x 70 kg)
- ◆ High-End Daily Exposure Estimate:  $88 \mu\text{g} (\text{diet}) + 20 \mu\text{g} (\text{water}) / 350 \mu\text{g} (\text{RfD}) < 1$
- ◆ High-End Lifetime Exposure Estimate:  
 $110 \mu\text{g}/\text{day} \times 1 \text{ g}/10^6 \mu\text{g} \times 365 \text{ days}/\text{yr} \times 70 \text{ yrs} = 2.8 \text{ g}$   
 $2.8 \text{ g} \times 10\% \text{ absorption} = 0.28 \text{ g} (\text{benchmark of } 1 \text{ g for argyria})$

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## Questions & Answers: Health Risk Assessment of Environmental Silver

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- Q. NICK FISHER (State University of New York at Stony Brook): What's known about where silver goes in the body once you eat it or drink it, and what is the biological half-life in the different major tissues that it resides in?
- A. It distributes fairly widely, it's not organ-specific. There have been a number of tissue distribution studies and it really localizes pretty much to all tissues. Some of the more prominent ones are spleen, liver, kidney. Half-life is going to be very long. I can't quote an exact half-life, but essentially silver does end up binding with proteins and sulfides and essentially sits around for quite awhile. The half-life in the lungs I think is about on the order of a day or two and in the liver it's about 48-50 days.
- Q. Two questions related to that: first, when EPA considers the hazard, the dose that you could take in per day times your average weight, that takes into consideration the residence time of the silver in the body, is that correct?
- A. It actually does not. It only takes into consideration the absorption, which for silver is fairly low. You really only absorb about 4% of it. So, in the calculation they did, the half-life was not considered.
- Q. The other question is, given that silver has a very strong affinity for sulfur, and you mentioned protein, one might expect based on first principles that it would also bind with enzymes which have typically or often have sulfhydryl groups at the active site. Is there any evidence of silver toxicity to key enzymes in mammalian tissue?
- A. No. Certainly if there are those effects it's a reasonable question, one that could be investigated. Yes, there may be sub-toxic or non-toxic effects that are ongoing. We don't have any evidence of it, but certainly it's a plausible suggestion.
- Q. ARUN MUKHERJEE (University of Helsinki-Finland): Actually, could you give us what should be the concentration of silver in micrograms per cubic meter of air at the working place, and how long a worker should be exposed so that he may not have that - I don't say that disease, but effect?
- A. You're asking about occupational exposure levels. See, I've not done those calculations. But there are OSHA - which is the US Occupational Safety Health & Administration Act - standards. They distinguish silver metal from other forms. I think for silver metal it's 0.01 mg per cubic meter, for other forms it's 0.1 or 100 micrograms per cubic meter, and you would then multiply essentially 10 cubic meters which is basically an 8-hour workday. I've not done those calculations to see what the permissible exposures are, but I think if you did those, they're probably a little bit above what I showed you here, the EPA reference. There are certainly limits and levels for air exposure in occupational settings.
- Q. The other comment is that you mentioned we should separate silver from heavy metals. Then I should say that the definition of heavy metals then should have to change?
- A. Yes, I'd like to see that.
- Q. When you talk about the heavy metals, the heavy metal toxicity, you can say I have taken so much food, I feel very much heavy, I must take a little nap - that is completely different. But when the word 'heavy' is used with 'metal' it indicates it is a toxic. That means in the periodic table there are 70 elements which we can say that it

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is heavy metals. Again, we come to the specific gravity of a metal that is near about 4.9 grams per cubic meter, I don't remember exactly the number but it should be like that. Then we can say it is a heavy metal. Aluminum is not a heavy metal, it is only 2 specific gravity. So I think we should not separate silver from heavy metals or trace elements.

A. Yes, point well taken. I think certainly the terminology could be improved. Maybe we should talk about a toxic metal per se.

Q WALTER BERRY (US EPA): I was just curious as to how silver is regulated in some of the other countries. In contrast to the United States?

A. My knowledge is not very good on that.

JIM KRAMER (McMaster University): Can I interrupt on this? I think there is a panel discussion on exactly this topic tomorrow. Would it be okay, Walter, if we put that off? But maybe you want to say something quickly.

A. No, that was it, Jim.

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## **Chemistry of Silver Bioavailability: A Model of Acute Silver Toxicity to Fish**

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(Presented by Paul Paquin)

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### **INTRODUCTION**

The importance of explicitly considering bioavailability in the development of water and sediment quality criteria for metals has been demonstrated scientifically (Di Toro et al., 1991; Ankley et al., 1994 and 1996; Allen and Hansen, 1996) and is being recognized by regulatory authorities (Renner, 1997; Bergman and Dorward-King, 1997). In addition, significant advances have been made during recent years in developing modeling frameworks for use in assessing the environmental fate and effects of metals, including silver, in aquatic systems. However, a unified framework which incorporates the sub-models needed to complete a detailed assessment for metals is not currently available. Such a framework is being developed as part of ongoing efforts for the Silver Council and the International Copper Association. It will include the following sub-models: a Monte Carlo model to simulate time variable inputs; a fate and transport model to predict water column and sediment concentrations over time and space; a metal speciation/complexation model; and an acute toxicity model.

This paper will focus on the metal speciation/complexation and acute toxicity sub-models in order to demonstrate their utility. The discussion begins with a description of the modeling framework and an illustration of how metal accumulation at the site of action is related to acute effects. The framework is then applied to two datasets, one for copper and one for silver, as examples of the modeling approach.

### **DESCRIPTION OF MODEL FRAMEWORK**

The conceptual framework for the chemical speciation and toxicity sub-models is an adaptation of the Gill Site Interaction Model (GSIM) originally proposed by Pagenkopf (1983). The general framework, illustrated for silver on Figure 1, is applicable to other metals including copper, cadmium and zinc. This model is based on the hypothesis that toxicity is not simply related to total metal concentration. Metal complexation and interaction at the site of action of toxicity must be considered as well. The role of complexation is critical, since formation of both organic and inorganic metal complexes renders a significant fraction of the total metal non-bioavailable. As shown on the left side of Figure 1, the dissolved metal exists in solution as free metal ion—which is hypothesized to be the bioavailable species in the free ion activity model (FIAM) of toxicity (Morel, 1983; Campbell, 1995)—and as relatively non-bioavailable metal complexes that result from reactions of the metal with organic and inorganic ligands.

The free metal ion also reacts with negatively charged, physiologically active binding sites at the site of action. For fish this is the surface membrane at the gill. It is imagined to be a biotic ligand to which metals can bind (Figure 1, right side). This results in the disruption of iono-regulatory processes of the organism (e.g., sodium transfer across the gill is restricted), which leads to the expression of acutely toxic effects. The principal feature which distinguishes the biotic ligand model from the FIAM is that in the biotic ligand model the free metal ion competes with other cations (e.g.,  $\text{Ca}^{2+}$ ,  $\text{H}^+$  and  $\text{Na}^+$ ) for binding at physiologically active sites. As a result, the presence of other cations in solution mitigates toxicity, with the degree of mitigation a function of their concentrations

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as well.

The computational framework of Figure 1 may be viewed more generally as a metal-biotic ligand acute toxicity sub-model. Binding of the free metal ion at the site of action of toxicity—the membrane at the gill surface in the case of fish—is analogous to formation of a metal complex in solution. The tissue at the organism site of action is thought of as a “biotic ligand.” The metal-biotic ligand interaction is represented in the same way as any other reaction of a metal with an organic or inorganic ligand. Both the binding site density of the biotic ligand, which is analogous to the concentration of other ligands in the solution, and the conditional stability constant for the metal-biotic ligand complex, and for any other cations with which the biotic ligand reacts, must be specified. Model parameter values determined on the basis of experimental results are currently available for a number of metals, including cadmium and copper (Playle et al., 1993a and 1993b) and silver (Janes and Playle, 1995). Studies are in progress to develop the requisite constants for other metals for both fish and crustaceans.

The speciation computations may be performed with one of several models. The choice is dictated primarily by the availability of requisite model inputs for that metal, and the experience of the analyst. Though Pagenkopf (1983) recognized the ameliorating effect of organic matter on toxicity, the effect of organic matter was neglected in the original model formulation, which was applied to test results using laboratory waters which were low in organic matter content. Where dissolved organic matter is judged to be important, the Windermere Humic Aqueous Model (WHAM; Tipping, 1994 and 1996) provides a relatively detailed basis for the speciation computations, provided that the requisite model inputs are available for the metal of interest. This is the approach followed in the example dataset for copper which is subsequently presented. Alternatively, the CHEMICAL Equilibria in Soils and Solutions model, CHESS (Santore and Driscoll, 1995), or EPA's MINTEQA2 model (Allison et al., 1991; Brown and Allison, 1987) or any one of a number of programs (Westall et al., 1976) can also be used. CHESS is used in the analysis of the dataset for silver which is subsequently presented.

## RELATIONSHIP TO WATER QUALITY CRITERIA

The current EPA water quality criteria for both silver and copper (USEPA, 1980; USEPA, 1984) are functions of hardness, but are independent of other water quality characteristics. Since it is known that water quality characteristics such as pH and organic carbon affect toxicity as well, it is frequently necessary to perform extensive bioassay testing to develop water effect ratios (WERs) which can be used to establish site specific water quality criteria for metals. The model framework described herein provides an alternative means of explicitly evaluating these effects. The conceptual framework is subsequently applied to two data sets, one for copper and one for silver, as examples of how the model can be used to predict the effects of varying water quality on metal toxicity.

## RELATIONSHIP OF METAL ACCUMULATION TO ACUTE TOXICITY

The examples to be presented will draw on data for rainbow trout and fathead minnows, species which have comparable sensitivities to both copper (USEPA, 1985) and silver (USEPA, 1980), particularly when compared to the range of organism sensitivities upon which the current ambient water quality criteria for these metals are based. As indicated previously, the fish gill is the “biotic ligand” of interest for fish. Hence, it is necessary to predict metal accumulation at the surface of the fish gill in order to predict toxicity to fish. Several studies have shown that when juvenile fathead minnows and rainbow trout are exposed to copper, there is a relatively rapid increase above background levels of copper bound to the gill (Playle et al., 1992; MacRae, 1994). This initial

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increase occurs over a time scale of a few hours to a day and is followed by a more gradual, longer term, increase. The rapid initial increase reflects binding of the metal to physiologically active receptor sites at the gill surface, those sites which control the iono-regulatory processes of the fish.

The question is: "Can the short term accumulation of metal on the gill be related to acute effects associated with more extended exposure durations, such as a 96-hour or longer bioassay?" Although the available data are limited at present, the answer appears to be that they are related. This is illustrated on Figure 2, which shows the dose response curve for 120-hour rainbow trout mortality as a function of the 24-hour gill copper accumulation (MacRae, 1994). Note that even though the same total Cu (10 ug/L) was used for all exposures, a wide range of variation in survival was observed. The gill Cu was regulated by adding organic ligands having varying affinities for copper to the test water. As shown on Figure 2, the gill copper LC50 is estimated to be 22 nmol/gram wet weight (nmol/g<sub>w</sub>) and the gill copper concentration associated with the no effect level for rainbow trout is approximately 10 - 12 nmol/g<sub>w</sub>. This compares well with the fathead minnow background gill copper of approximately 12 nmol/g<sub>w</sub> that was measured in the absence of added copper (Playle et al., 1992). Based on these results, the rapidly exchangeable gill Cu LC50, the difference between the gill copper LC50 and the background copper, is approximately 10 nmol/g<sub>w</sub>. An exchangeable gill copper concentration of 10 nmol/g<sub>w</sub> should therefore be indicative of 50% mortality.

#### MODEL APPLICATION FOR COPPER

The first application of the model is to the analysis of larval fathead minnow copper toxicity data (Erickson et al., 1996). The initial group of experimental results to which the model is applied are with variable dissolved organic carbon concentration. The pH and hardness are approximately constant. The upper panel shows that the measured total Cu LC50 data (filled data points and associated trend line) increases with DOC concentration, as is often observed. This is because the DOC forms a relatively non-bioavailable organic complex with the added copper. As a result, as DOC increases more copper is needed to exert the same degree of toxicity. Since it is desirable for risk assessment purposes that a given effect (e.g., 50 percent mortality) be uniquely associated with a single exposure concentration, it is clear that total copper concentration does not satisfy this requirement.

The middle left panel on Figure 3 shows the corresponding free copper (Cu<sup>2+</sup>) LC50s. These LC50s were calculated with WHAM using the measured total Cu LC50's on the upper panel. The results are somewhat variable, but they are approximately independent of DOC concentration. This is consistent with the FIAM of toxicity, where toxicity is directly related to the concentration of free copper. These results also indicate that, at least in this instance, cupric ion activity can be used to predict toxicity over a range of water quality characteristics.

Finally, the bottom left panel of Figure 3 shows the calculated gill copper concentrations associated with the measured total copper LC50 data. The gill copper was calculated using WHAM to compute the metal humic complexes and CHESS to compute the gill accumulation, given measured values for Cu<sup>2+</sup>-gill, Ca<sup>2+</sup>-gill and H<sup>+</sup>-gill conditional stability constants and gill site density (Playle et al., 1993). The exchangeable gill copper LC50 averages slightly less than 5 nmol/g<sub>w</sub>. This level of fathead minnow gill copper accumulation is comparable to the measured exchangeable gill copper LC50 for rainbow trout of 10 nmol/g<sub>w</sub> (Figure 2), as would be expected for fish species having similar sensitivities to copper. The fact that the gill copper LC50 is approximately constant across the DOC range tested indicates that, as was the case with free copper, gill copper can also be used to provide an unambiguous predictor of acute effects.

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The second group of fathead minnow experiments to which the model is applied is shown on the right side of Figure 3. The experiments were performed with DOC and pH constant and with calcium varying. Over the range of experimental conditions of 0.5 to 2.5 meq Ca/L, the ratio of Ca:Mg was approximately 2:1 and the corresponding hardness range was 75 to 375 mg/L as CaCO<sub>3</sub>. The data are displayed as before except that the LC50 results are plotted versus calcium. As was the case with increasing DOC, the measured total Cu LC50 also increases with increasing Ca concentration. This increase in LC50 with hardness is qualitatively consistent with the current WQC for copper, which increases as a function of hardness as well.

Following the same approach as previously, the calculated free Cu LC50s, corresponding to the total copper LC50s on the upper panel, are shown on the middle right panel. In this instance, however, the free copper LC50 now varies over the range of calcium levels tested, so a single free copper concentration is no longer uniquely associated with 50% mortality. Further, in combination with the results from the DOC experiments shown on the left of Figure 3, these results indicate that there is an overall 10 fold range in variation of free copper (0.015 to 0.15 umoles/L) associated with the fathead minnow free copper LC50. These results are clearly not in conformity with the FIAM.

In contrast to the free copper results, the calculated gill Cu accumulation, shown on the bottom right panel, does indicate a relatively consistent level across the range of calcium concentrations tested. The average fathead minnow gill copper of about 12 nmol/g<sub>w</sub> is in reasonably good agreement with the gill copper concentration of slightly less than 5 nmol/g<sub>w</sub> calculated for the DOC experiments (Figure 3, lower left panel) and with the exchangeable gill Cu LC50 of about 10 nmol/g<sub>w</sub> of Figure 2. The reason that the gill copper tends to be relatively constant even as the free copper concentration increases is that the calcium competes with the free copper in solution for binding at the site of action. Hence, a higher free copper level is required to achieve the same gill Cu level, and the same associated effect, as calcium increases.

The reasonable agreement between the estimated rainbow trout exchangeable gill Cu LC50 (10 nmol/g<sub>w</sub>, Figure 2) and the calculated fathead minnow gill Cu LC50 results (averaging 5 and 12 nmol/g<sub>w</sub> for the two sets of experiments) is consistent with recent findings that rainbow trout and fathead minnows accumulate gill Cu in a similar manner, and that binding constants for metal-gill interactions determined for one species can be generalized to other fish species (Hollis et al., 1997).

It is interesting to note that for the calcium experiments the observed increase in total copper LC50 (slightly more than a factor of two over the range of conditions tested) is half as much as would be expected from the hardness correction incorporated in the current copper WQC (4.5-fold, for hardness increasing from 75 to 375 mg/L as CaCO<sub>3</sub>). The model framework provides an explanation for this difference (Meyer, 1997). Briefly, Erickson et al. (1995) conducted the hardness variation experiments at constant alkalinity, by adding calcium in the form of CaSO<sub>4</sub>. Hence, as hardness is increased in these experiments, the only factor which mitigates toxicity is competition between Cu and Ca for physiologically active gill sites. In contrast, in most studies upon which the WQC is based, hardness was adjusted by adding CaCO<sub>3</sub> to the water, and hence alkalinity increased as well. The added CO<sub>3</sub><sup>=</sup> reacts with Cu to form CuCO<sub>3</sub>, a relatively non-bioavailable inorganic complex. Hence, toxicity was mitigated to a greater degree than in the experiments by Erickson et al., and it was therefore necessary to add more copper, for the same increase in hardness, to achieve a corresponding effect on survival.

In summary, the metal-gill model seems to work quite well, with predicted exchangeable gill copper LC50s within about +/- 50% of the measured rainbow trout gill copper LC50, over the range of DOC and hardness levels considered. Whereas the FIAM is of limited applicability with respect to

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hardness variation, the metal-gill model appears to be more robust. The preceding results, as well as ongoing analyses of this same dataset with respect to pH variation, indicate that the proposed model framework has the potential to account for variation in toxicity as a function of not only hardness, but of site specific variations in DOC, pH and alkalinity as well.

### EXAMPLE MODEL APPLICATION FOR SILVER

The second dataset to which the metal speciation/complexation and acute toxicity sub-models are applied is an inter-laboratory comparison involving two EPA laboratories and four commercial laboratories. Each laboratory conducted 96-hour toxicity studies with juvenile fathead minnows, rainbow trout and *Daphnia magna*, with silver added as silver nitrate (Lemke, 1981). Similar results were obtained for both types of fish; rainbow trout results are presented for illustrative purposes.

Measured test results and computed model results are shown on Figure 4 for the flow through tests with rainbow trout. The upper panel compares the observed variation of LC50 on a total silver basis (measured results, filled symbols) and free silver basis (computed results, open symbols). The free silver concentrations were calculated with CHESS (Santore and Driscoll, 1995) using the measured total silver LC50, the reported (or estimated when not available) laboratory water characteristics (pH, chloride and DOC) and conditional stability constants from the MINTEQA2 database. Although the interlaboratory variability is reduced somewhat by the speciation computations, significant residual variability is still evident on a free silver basis.

The middle panel of Figure 4 displays the computed distribution of silver in the test water, expressed as a percent of the total silver LC50 (shown on the upper panel), for each of the six laboratories. As shown, the free silver (corresponding to the predicted free silver LC50s) is 10 to 60 percent of the total silver. The relatively low percentage of free silver for Lab 2 in part reflects the significantly higher chloride level in the test water (0.90 mmol/L, versus 0.05 to 0.35 mmol/L in the other lab waters), which results in the formation of a high percentage of relatively non-bioavailable silver chloride complexes, including both soluble (the primary species included in "others") and insoluble forms. More specifically, it is predicted that the combined effects of the high silver and chloride levels in the Lab 2 water results in the formation of cerargyrite, an insoluble precipitate, AgCl(s), which complexes more than half of the silver. In these computations a significant amount of the dissolved silver is bound to DOC. Unfortunately, Lemke et al. do not report DOC concentrations for the lab waters and a low concentration of 1 mg/L was assumed in all cases. Given the importance of DOC complexation on Ag speciation it is crucial that future studies provide complete information on water chemistry, including measurements of DOC.

The short term (2-3 hour) gill silver accumulation for these tests was calculated using the simulated free silver concentrations (shown on the upper panel of Figure 4), reported laboratory specific calcium and sodium concentrations (Lemke, 1981), and measured rainbow trout silver-gill, sodium-gill and calcium-gill conditional stability constants (Janes and Playle, 1995). The resulting calculated gill silver levels are displayed on the lower panel of Figure 4. As shown, the predicted gill silver levels are relatively uniform across the 6 laboratories tested, ranging from 11.3 to 12.8 nmol/g<sub>w</sub>. These computed short term gill silver levels appear reasonable with respect to longer term (7 day) gill silver measurements, where gill silver levels of surviving rainbow trout were generally in the range of 15 to 30 nmol/g<sub>w</sub> (Hogstrand et al., 1996).

The uniformity in computed gill silver levels shown here is in part a result of the generally low complexation capacity in the test waters (i.e., DOC and chloride levels were low), and also, that levels of competing cations (Ca<sup>2+</sup> and Na<sup>+</sup>) were insufficient to keep Ag<sup>+</sup> from binding at the gill. As a result, the gill silver binding sites associated with short term uptake of silver are nearly saturated

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and relatively uniform levels of gill silver are predicted. This finding is consistent with laboratory measurements of short term accumulation of silver on rainbow trout gills, where much higher levels of complexing ligands, competing cations and sodium than were present in these experiments are required to prevent silver accumulation on rainbow trout gills (Janes and Playle, 1995). Also, to some extent these results can be attributed to the high value for the Ag-gill complexation constant reported by Janes and Playle (1995). A re-evaluation of the Ag-gill binding constant using a state of the art Ag-DOC binding model is planned to confirm these results.

A significant limitation of the analysis presented for silver is that the available water chemistry characterization is incomplete. Hence, in some cases it was necessary to assign the concentration of a key constituent and this can have a significant effect on the calculations. The fact that DOC was not reported for any of the laboratories is a particularly significant limitation, necessitating that a representative DOC of 1 mg/L be assigned to all waters. More complete lab-specific information on water characteristics would lead to a more definitive analysis, especially given the importance of Ag-DOC complexes in these results. Dose response information relating mortality to gill silver levels would also add to the utility of these results.

## SUMMARY

In summary, the modeling results presented herein illustrate how consideration of metal speciation and bioavailability provides an improved understanding of how environmental exposure levels of metals are related to acute effects. The modeling framework which was applied has a number of potential useful applications, including: (1) setting discharge permit limits, (2) development of site specific WQC via a modified Water Effects Ratio (WER) procedure, (3) evaluation of fate and toxicity of metals for use in ecological risk assessments, and (4) development of updated metals water quality criteria where, in addition to hardness, the refined WQC might also be a function of TOC, DOC, pH and other variables which affect the speciation, complexation and toxicity of silver in aquatic systems.

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FIGURE 1. CONCEPTUAL DIAGRAM OF SILVER SPECIATION AND SILVER-GILL MODEL (After Pagenkopf, 1983)

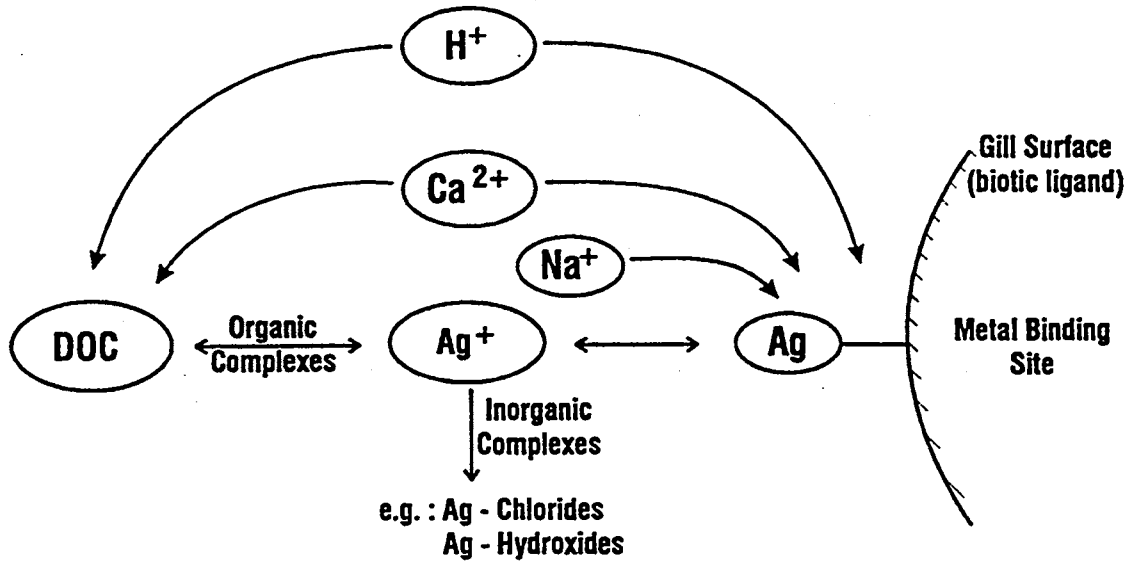
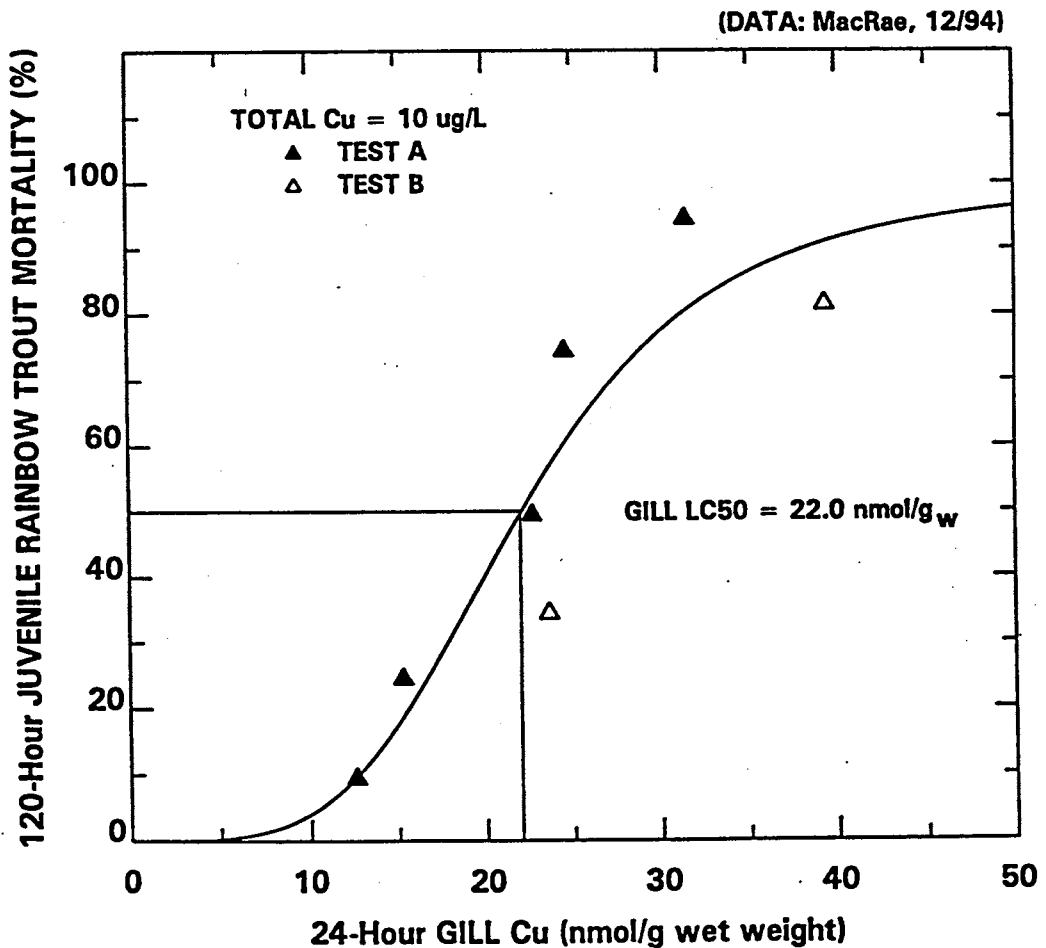
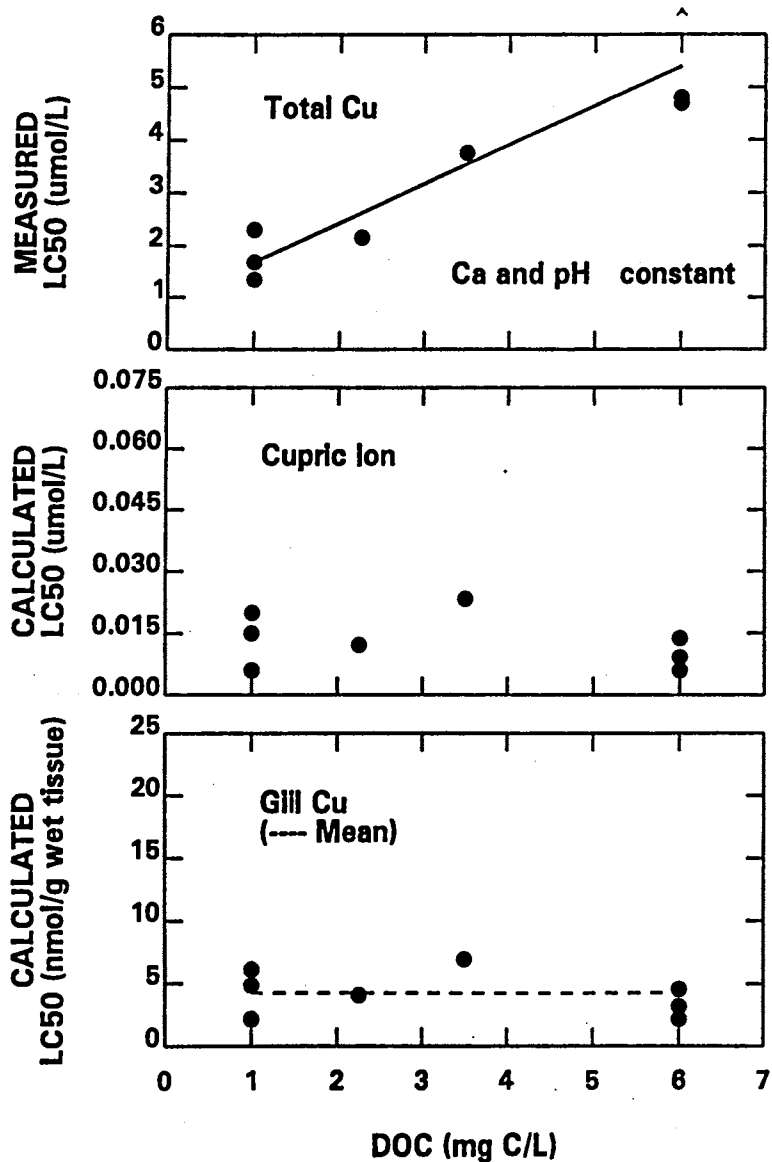


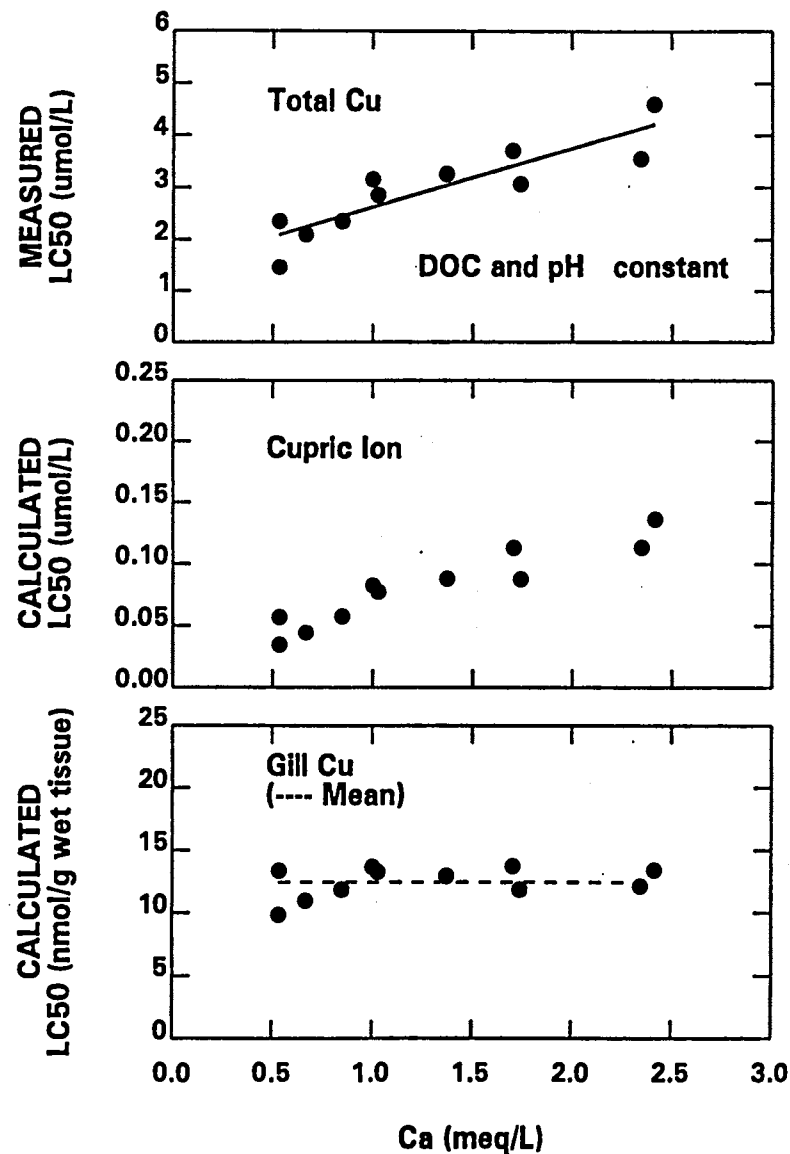
FIGURE 2: DOSE-RESPONSE CURVE FOR RAINBOW TROUT MORTALITY AS A FUNCTION OF GILL COPPER



### EFFECT OF DOC



### EFFECT OF CALCIUM



**FIGURE 3. EFFECT OF DOC AND CALCIUM ON LARVAL FATHEAD MINNOW 96-HOUR COPPER LC50 (Data: Erickson et al., 1996)**

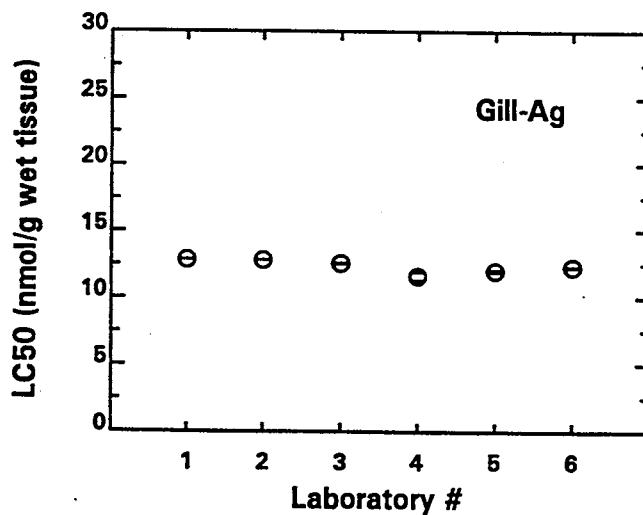
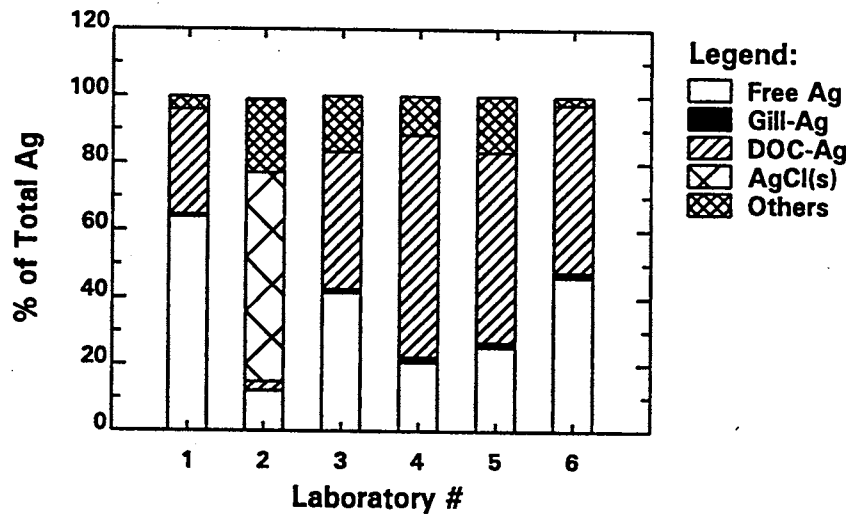
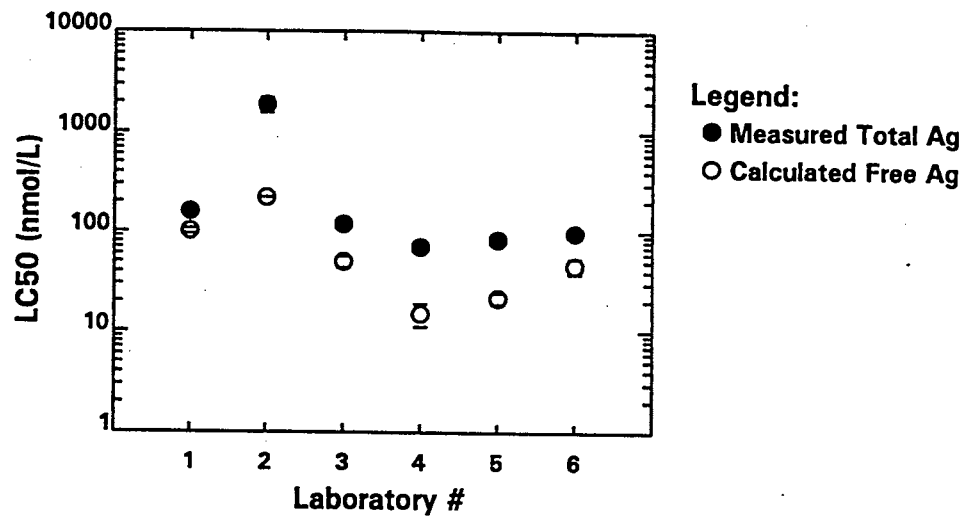


FIGURE 4. COMPARISON OF MODEL RESULTS TO INTERLABORATORY COMPARISON STUDY RESULTS - RAINBOW TROUT FLOW THROUGH TEST (Data: Lemke, 1981)

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## Questions & Answers: Chemistry of Silver Bioavailability: A Model of Acute Silver Toxicity to Fish

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Q. PETER SANTACHI (Texas A&M University): I just wanted to speak up on what you left out about the silver DOC complexation constant. I think the reason why you got some reasonable results is because you worked at a one micromolar level of silver, so there you might not have enough complexation capacity for your DOC. But if you want to work at the environmental levels you will have to take into account the sulfur ligands, and that is not really very well characterized. And so you don't have the proper constants for that.

A. Right. Well, that's an area where probably we need to do some additional experimental work to try and understand that and pin those numbers down.

DOMINIC DI TORO (Manhattan College): It's hard to kill anything at environmental concentrations.

Q. PAUL ANDERSON (Illinois Institute of Technology): Just a quick question. I'm not very familiar with the gill model, so excuse my ignorance. But is the idea, I think, that sodium, calcium and copper sort of compete equally? The total number of the capacity for each one adds up to one, or the capacity for each one is the same?

A. Something like that. There are probably people here who can explain that better to you and I'm sure they will this afternoon, but that's the general idea. I mean, they have different binding strengths and they're also at different levels in the water, so it's the combination of effects that determine how much gets on the gill.

Q. JIM KRAMER (McMaster University): I think it was on your very first overhead that you showed the probability plot. It was bi-modal; it was not Gaussian. I don't know what the different organisms were because you just had X's, but is there an explanation for that? That's quite intriguing.

A. I haven't really ever thought about it and no, I don't know why that would be.

Q. I mean, most people, when they look at those things and they see a break, they think of two mechanisms and so...

A. Right.

# Session 3

*Physiological Effects and Food Chain Transfer of Metals  
in Aquatic and Terrestrial Environments*

*A.W. Andren/E.A. Crecelius  
Session Co-Chairs*





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## Bioavailability, Physiology and Toxicology of Silver in Freshwater Fish: Implications for Water Quality Criteria

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Over the past 5 years, our knowledge about the effects of silver on freshwater fish has increased markedly through a concerted research effort by several groups. This research has been supported by the photographic industry, other silver-related organizations, and government agencies, and greatly stimulated by the annual *Argentum* conference series. Below we summarize our views on the present status of important issues; unless otherwise noted, supporting data and references can be found in Hogstrand & Wood (1997).

**Speciation** Virtually all available evidence supports the view that an understanding of the speciation of silver in the water column is critical to understanding its bioavailability, its acute toxicity, its physiological effects, and in framing sensible environmental regulations. Analyses and approaches based on total silver levels alone are of little value. Aquatic geochemical speciation programs such as MINEQL+ (Schecher & McAvoy, 1994) and MINTEQA2/PRODEFA2 (U.S. EPA, 1991) are extremely useful tools in this regard, and have indicated that free ionic  $\text{Ag}^+$  is the key moiety causing acute toxicity for freshwater fish (see below). A promising new development, based on the original ideas of Pagenkopf (1983), is the incorporation of anionic ligands on or in the fish gill itself into geochemical models (Janes & Playle, 1995; Bergman & Dorward-King, 1997). The idea here is that a particular degree of saturation of "toxic receptor sites" (anionic ligands) on or in the gills by  $\text{Ag}^+$  will cause death, and can be predicted if water chemistry and gill chemistry are known. A limitation of such models is that they do not have kinetic components, but rather assume thermodynamic equilibrium, which may not apply in natural systems, especially when living organisms are involved.

Most laboratory toxicity tests are performed using  $\text{AgNO}_3$ , often in simplified synthetic freshwater. Because  $\text{NO}_3^-$  is an extremely weak ligand ( $\log K = -0.3$ ), substantial amounts of free  $\text{Ag}^+$  will usually be present during such tests. However, in most natural and polluted waters, both geochemical modelling and the very few available measurements indicate that free  $\text{Ag}^+$  levels are extremely small (low ng range), because of the presence of stronger ligands such as chloride ( $\log K = 3.3-5.5$ ), thiosulfate (8.8-14.2), dissolved organic carbon (DOC; 8.0 - 10.0), and sulfide (19.2) in natural waters. There is an urgent need for the development of methods to accurately and directly measure free  $\text{Ag}^+$  concentrations in natural waters; in this regard, the report by Chyan, Chen, and Xei (this symposium) is of considerable interest.

**Bioavailability.** It is now clear that, at least for silver, we must distinguish between

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bioavailability for acute toxicity and bioavailability for bioaccumulation. Depending on speciation, Ag may undergo considerable bioaccumulation in fish without causing acute toxicity, and alternately, Ag may cause acute toxicity without substantial bioaccumulation. This reflects the fact that waterborne  $\text{Ag}^+$  is a "surface-active toxicant" at the gills, doing its damage on or in the branchial epithelium, whereas internal bioaccumulation may occur without apparent damage to these key toxic sites.

**Acute Toxicity.** The literature is in general agreement that silver is extremely toxic to freshwater fish when presented as  $\text{AgNO}_3$ , with most 96h LC50's in the range of 5-70  $\mu\text{g.l}^{-1}$ . However  $\text{AgNO}_3$  yields considerable amounts of free  $\text{Ag}^+$  in solution (see above). When other forms of silver have been tested such as silver chloride, silver thiosulfate, and silver sulfide, acute toxicity is virtually eliminated, with 96h LC50's 3-5 orders of magnitude higher in both fathead minnow (*Pimephales promelas*; Leblanc *et al.*, 1984) and rainbow trout (*Oncorhynchus mykiss*; Hogstrand *et al.*, 1996). Recently, unpublished work from our labs and others (see the report of La Point *et al.*, this symposium) has demonstrated that DOC, which binds  $\text{Ag}^+$  with great avidity, is highly protective against  $\text{AgNO}_3$  toxicity. Hardness, the major modifying factor incorporated into some environmental regulations (see below), exerts only a very modest protective effect (*e.g.* Goettl & Davies, 1978; Davies *et al.*, 1978; Nebeker *et al.*, 1983; Brooke *et al.*, 1994; Klaine *et al.*, 1996; Galvez & Wood, 1997; Bury, Galvez, & Wood, unpubl. results).

To further evaluate the idea that acute toxicity is solely due to free ionic  $\text{Ag}^+$ , we have analyzed the results of tests on juvenile trout performed by Hogstrand *et al.* (1996) and Galvez & Wood (1997) at a range of different chloride concentrations (Fig. 1). As chloride concentration increases, the 7 day LC50 rises markedly from about 30 to 900  $\text{nmol.l}^{-1}$  (3.2 to 97  $\mu\text{g.l}^{-1}$ ) when expressed as total Ag, but stays remarkably constant at 30  $\text{nmol.l}^{-1}$  when expressed as the free  $\text{Ag}^+$  ion. In further confirmation of the idea that  $\text{Ag}^+$  alone is responsible for acute toxicity, Galvez and Wood (1997) performed a toxicity test in which the total concentration of Ag was held constant, but the concentration of  $\text{Ag}^+$  was altered by varying the chloride concentration. Again the 7 day LC50, expressed as free  $[\text{Ag}^+]$ , was about 30  $\text{nmol.l}^{-1}$ .

**Chronic Toxicity.** There have been far fewer studies on the chronic toxicity of silver to fish; the reported thresholds for chronic effects or MATC's (maximum acceptable toxicant concentrations) range from less than 0.1 to about 5.0  $\mu\text{g.l}^{-1}$ . Table 1 summarizes 4 studies (all performed using  $\text{AgNO}_3$ ) which have reported MATC's towards the lower end of this range, and therefore have indicated that silver can be extremely toxic at very low levels. While all appear to be very careful, well-controlled studies, two notes of caution must be made. Firstly, all are based on nominal rather than measured values, because most laboratories have difficulty in measuring such low concentrations of Ag. Secondly, these same laboratories have performed acute tests on the same species; the ACR's (acute-to-chronic ratios) which can be calculated from these data are extremely high (28 to > 100), whereas ACR's for most metals are usually quite low. For example, the ACR value is about 3 for copper (U.S. EPA, 1985), which is thought to have a similar physiological mechanism of action as silver (see below).

**Biocummulation** While the major internal fate of bioaccumulated silver appears to be storage in the liver, considerable amounts may also build up in gills, kidney, and blood plasma (Hogstrand

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*et al.*, 1996; Wood *et al.*, 1996a,b; Webb & Wood, 1997). In contrast to acute toxicity, bioaccumulation may occur from (or as) many different forms of waterborne silver, not just the free ion. Indeed we have found that the greatest internal levels of Ag are seen in trout when the fish are exposed to extremely high levels of silver thiosulphate, which is relatively non-toxic. However, to more adequately compare the potential for bioaccumulation of different species of Ag, we have calculated the rate of accumulation of total Ag in the liver per day *per p.p.b.* ( $\mu\text{g.l}^{-1}$ ) of external Ag concentration in trout exposed for 6-7 days in different water chemistries (Fig. 2). These results, when correlated with speciation analysis, suggest that both the free  $\text{Ag}^+$  ion (which is highly toxic) and the neutral dissolved  $\text{AgCl}_{aq}$  complex (which is not acutely toxic) are readily taken up, whereas silver thiosulfate complexes are not easily absorbed.

At present, we know nothing about the mechanism(s) by which bioaccumulated silver crosses the gills. Potentially  $\text{Ag}^+$  could be "off-loaded" from ligands such as chloride and thiosulfate to stronger anionic ligands on the gills; alternately the silver chloride and silver thiosulfate complexes themselves could enter the fish. Silver may also be bioaccumulated from the diet (Galvez *et al.*, this symposium), but the actual rates per unit concentration are many orders of magnitude lower than from the water. There is no evidence that biomagnification occurs in the food chain. At present, there is also no evidence that bioaccumulated silver is in any way harmful to the fish; the only documented effect is an induction of metallothionein in the gills, kidney, and especially the liver (Hogstrand *et al.*, 1996; Wood *et al.*, 1996a,b; Galvez *et al.*, 1997). The greater the tissue burden of total Ag, the greater appears to be the metallothionein induction, regardless of the source of the silver. This low molecular weight, cysteine-rich protein is thought to be important in the internal immobilization and detoxification of metals. For example, it has been shown to protect an important ion transport enzyme ( $\text{Na}^+, \text{K}^+$ ATPase) against inhibition by Ag (Ferguson *et al.*, 1997; see below)

**Physiological Effects** The physiological responses of rainbow trout to  $\text{AgNO}_3$  at concentrations around the 7 day LC50 level (total Ag  $\sim 10 \mu\text{g.l}^{-1}$ ; ionic  $\text{Ag}^+ \sim 30 \text{nmol.l}^{-1}$ ) are now well documented (Wood *et al.*, 1996a; Morgan *et al.*, 1997; Webb & Wood, 1997). In brief, there is a severe and progressive loss of  $\text{Na}^+$  and  $\text{Cl}^-$  from the blood plasma which sets in motion a complex series of events which eventually kills the fish by circulatory failure. The scenario appears similar to that seen during low pH exposure (Wood, 1989). A severe stress response occurs, manifested in marked increases in plasma concentrations of the stress hormone cortisol, of glucose (likely due to mobilization of other stress hormones, catecholamines), and of ammonia (likely due to increased proteolysis driven by the stress hormones). Osmotic imbalance between the extracellular and intracellular fluid compartments causes a net fluid shift out of the blood plasma into the tissues, and a marked fall in blood volume. Plasma protein and red blood cell (hematocrit) concentrations increase, the latter compounded by discharge of stored erythrocytes from the spleen, leading to a rise in blood viscosity. Cardioacceleratory and vasoconstrictor actions of catecholamines, in addition to the greatly increased blood viscosity, likely cause a large rise in blood pressure at the same time as plasma volume declines to critically low levels. The fish dies of "hypovolemic cardiovascular collapse", but the proximate cause can be traced back to a "surface active" effect of the free ion  $\text{Ag}^+$  on or in the gills. None of these responses occur when silver is presented as silver thiosulfate, even at more than 3 orders of magnitude higher concentration (Wood *et al.*, 1996b).

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This surface action of  $\text{Ag}^+$  is a highly potent blockade of the active uptake "pumping" of  $\text{Na}^+$  and  $\text{Cl}^-$  from the water into the blood plasma by specialized salt transport cells in the gills; passive diffusive effluxes are not substantially altered (Morgan *et al.*, 1997). This active uptake normally slightly exceeds the combined rate of diffusive and urinary effluxes; its elimination results in the progressive net loss of  $\text{Na}^+$  and  $\text{Cl}^-$  from the plasma. Kinetic analyses demonstrate that the inhibition of  $\text{Na}^+$  and  $\text{Cl}^-$  uptake is due to a loss of transport sites rather than a change in affinity of the transport system. This loss of transport sites is due to a potent blockade of the key enzyme,  $\text{Na}^+$ ,  $\text{K}^+$ ATPase, which energizes the  $\text{NaCl}$  "pumping" functions of the gill salt transport cells (Morgan *et al.*, 1997).  $\text{Ag}^+$  competitively inhibits the binding of  $\text{Mg}^{2+}$  to a key activation locus on the enzyme, thereby explaining the loss of transport capacity (Ferguson *et al.*, 1997)

Further evidence that this action is a specific effect of  $\text{Ag}^+$  and not of other forms of silver comes from very recent experiments on trout exposed to sublethal levels of  $\text{AgNO}_3$  (see reports of McGeer, Bury & Wood and Bury, McGeer, & Wood, this symposium). Elevations of water hardness have very little protective effect. However, elevations of water chloride and DOC levels protect against the inhibition of gill  $\text{Na}^+$ ,  $\text{K}^+$ ATPase, branchial  $\text{Na}^+$  uptake, and plasma  $\text{NaCl}$  regulation. These protective effects can be related in a concentration-dependent fashion to the reduction in the level of free  $\text{Ag}^+$  in the exposure water, and *not* to the total amount of  $\text{Ag}$  on the gills. The latter reflects the fact that silver may accumulate on the gills to high levels in a non-toxic form under certain conditions - *e.g.* when high levels of  $\text{AgCl}_{aq}$  are present. We recommend therefore that future modelling efforts (*e.g.* Janes & Playle, 1995; Bergman & Dorward-King, 1997) employ  $\text{Ag}^+$  inhibition of gill  $\text{Na}^+$ ,  $\text{K}^+$ ATPase (*i.e.* saturation of "toxic receptor sites"), rather than simply total gill  $\text{Ag}$  burden, as the appropriate endpoint.

**Implications for Water Quality Criteria.** The implications from this newly acquired knowledge are, most importantly, that an understanding of the geochemical speciation of silver in the water is critical to sensible environmental regulation. Clearly  $\text{Ag}^+$  is the agent of concern, at least for acute toxicity, and it is possible to predict its concentration using geochemical models if local water chemistry is known, thereby allowing more accurate and cost-effective site-specific criteria rather than "blanket criteria". An additional refinement for future consideration is to incorporate the chemistry of anionic gill ligands (*i.e.* "toxic receptor sites" as quantified by inhibition of  $\text{Na}^+$ ,  $\text{K}^+$ ATPase) into such models. The development of selective electrodes capable of accurately measuring  $\text{Ag}^+$  at environmental levels is urgently required, and should provide independent validation for this approach.

At present, there does not appear to be sufficient information on which to construct reliable chronic criteria. There is an urgent need for new studies in which total  $\text{Ag}$  levels are directly measured and water chemistry is fully characterized, thereby allowing speciation analysis. If such studies confirm the toxicity of silver at extremely low levels, as suggested by Table 1, then chronic criteria should be developed.

Current guidelines and criteria vary considerably between jurisdictions, but none appear to recognize the current state of knowledge in the field. Most are based on total  $\text{Ag}$  concentration, some greatly (and probably erroneously) emphasize the modifying role of water "hardness", and many appear to be idiosyncratic. For example, the national guideline in Canada is that "the concentration of total silver should not exceed  $0.1 \mu\text{g.l}^{-1}$ " (CCME, 1995). However this same document states (probably wrongly) that "surface waters in non-industrialized areas

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contain an estimated  $0.3 \mu\text{g Ag.l}^{-1}$ ! British Columbia has implemented criteria which incorporate a role for water hardness, with a remarkable breakpoint at a hardness of  $100 \text{ mg.l}^{-1}$  (in  $\text{CaCO}_3$  equivalents). The acute criterion ("maximum allowable concentration") increases 30-fold from  $0.1 \mu\text{g.l}^{-1}$  below this hardness to  $3.0 \mu\text{g.l}^{-1}$  above this hardness. Similarly the chronic criterion ("30 day average") increases 30-fold from  $0.05$  to  $1.5 \mu\text{g.l}^{-1}$  at this same breakpoint (B.C. MOELP, 1995). This belief as to the marked effect of water hardness is probably founded in U.S. regulations for silver in the aquatic environment. At present there is no national chronic criterion in the United States, but since 1980, the acute criterion has been expressed in terms of a "hardness equation" (U.S. EPA, 1980). This logarithmic function, depicted in Fig. 3, gives the maximum allowable acute concentration expressed in terms of total Ag, although since 1995 a "translation" to the dissolved component (based on  $0.45 \mu\text{m}$  filtration) has been allowed. The relationship attributes a major, exponentially increasing, protective effect to water hardness.

Our own work and that of others (cited above) indicates that hardness exerts at best a only a modest protective effect against acute  $\text{Ag}^+$  toxicity. Comparison of several acute data sets for rainbow trout (RBT) and fathead minnow (FHM) with the output of the hardness equation suggests that it may be underprotective at high hardness and overprotective at low hardness (Fig. 3). We have therefore explored the origin of the hardness equation. In the original document (U.S. EPA, 1980), those available data which indicated only a small role for hardness were explicitly excluded, and the final derivation of the hardness equation was strongly influenced by a data set compiled by Lemke (1981). Our own reanalysis of the Lemke data set suggests that chloride rather than hardness itself (*i.e.* calcium) was the effective protective agent (Hogstrand *et al.*, 1996; Galvez & Wood, 1997). We suggest that an important first step in reforming water quality regulations for silver in light of current knowledge will be to reformulate this equation. In addition to hardness, more important and environmentally relevant variables should be incorporated, particularly chloride, DOC, and sulfide.

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## Legends

**Table 1.** A compilation of several data sets which indicate that the threshold for chronic toxicity of silver (tested as  $\text{AgNO}_3$ ) to freshwater fish is extremely low. MATC = maximum acceptable toxicant concentration; ACR = acute-to-chronic ratio.

**Figure 1.** Seven day LC50 values for juvenile rainbow trout measured at different freshwater chloride concentrations, expressed both in terms of total Ag, and in terms of free ionic  $\text{Ag}^+$  as calculated by MINEQL+ (Schecher & McAvoy, 1994) based on measured water chemistry.

**Figure 2.** A comparison of total accumulation rates of silver in the liver of rainbow trout exposed to various species of Ag for 6-7 days, as achieved by using different freshwater chemistries. Concentrations of different species of Ag (in  $\mu\text{g.l}^{-1}$ ) in the exposure water were calculated by MINEQL+ (Schecher & McAvoy, 1994) based on measured water chemistry. To facilitate comparison, rates are expressed per p.p.b. ( $\mu\text{g.l}^{-1}$ ) of external Ag concentration.

**Figure 3.** A comparison of the current U.S. EPA (1980) acute criterion for total silver in freshwater as a function of hardness, as calculated by the "hardness equation", with several 96h LC50 data sets on fathead minnow (FHM) and rainbow trout (RBT) performed at different values of hardness. Silver was added as  $\text{AgNO}_3$  in these tests, and hardness is expressed in  $\text{CaCO}_3$  equivalents.



# Chronic Ag Toxicity

			MATC	ACR	
Rainbow Trout (eggs - adult)	18 months	mortality growth	0.09 - 0.17	~54	Davies <i>et al</i> , 78
Rainbow Trout (eggs - swimup)	60 days	growth	< 0.10	>100	Nebeker <i>et al</i> , 83
Rainbow Trout (juveniles)	28 days	ionic status feeding	< 0.50	> 24	Galvez <i>et al</i> , 97
Fathead Minnow (embryo-larval)	32 days	mortality	0.37 - 0.65	~28	Holcombe <i>et al</i> , 83

All MATC's ( $\mu\text{g Ag /L}$ ) are nominal, not measured

ACR's (acute-to-chronic ratios) appear unusually high for metals. *e.g.* Cu ~3

TABLE 1

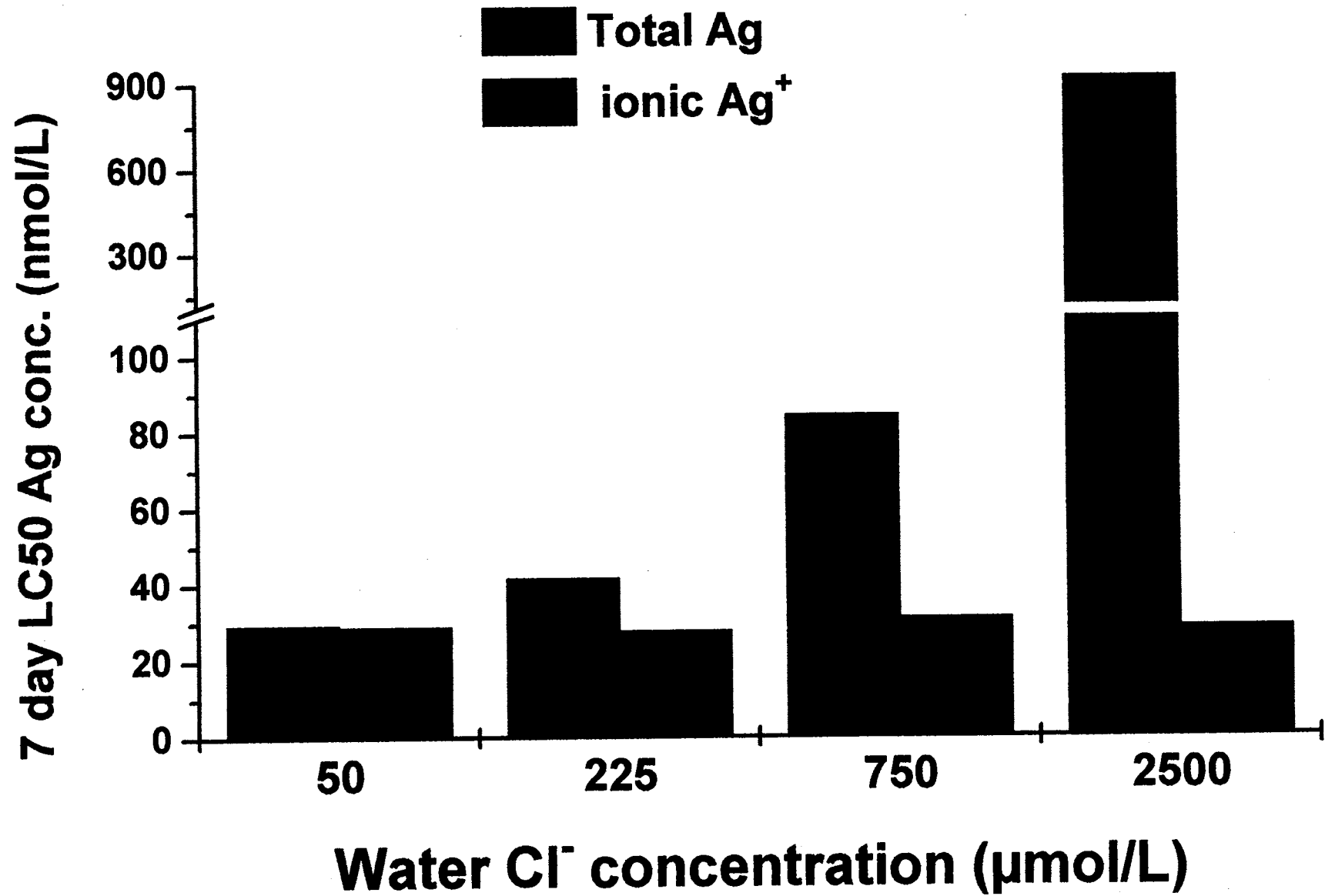
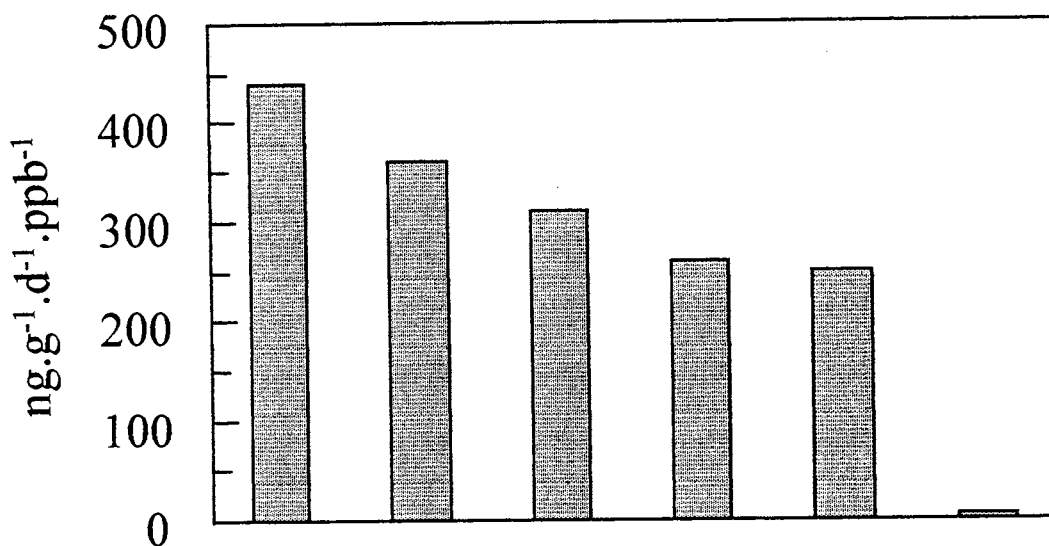


FIGURE 1

from: Galvez and Wood 1997  
& Hogstrand *et al.* 1996

# Total Ag Accumulation Rate in Liver of Juvenile Trout per unit of Ag Concentration



Ag <sup>+</sup>	5.0	5.0	2.5	3.2	0.1	0.0
AgCl <sub>aq</sub>	0.5	2.3	4.3	5.6	16.8	0.0
AgCl <sub>2</sub> <sup>-</sup>	0.0	0.0	0.4	0.5	91.2	0.0
Ag(S <sub>2</sub> O <sub>3</sub> ) <sub>2</sub> <sup>3-</sup>	0.0	0.0	0.0	0.0	0.0	2943.0
AgS <sub>2</sub> O <sub>3</sub> <sup>-</sup>	0.0	0.0	0.0	0.0	0.0	879.0

Hogstrand et al. (1996)  
Wood et al. (1996)  
Galvez & Wood (1997)

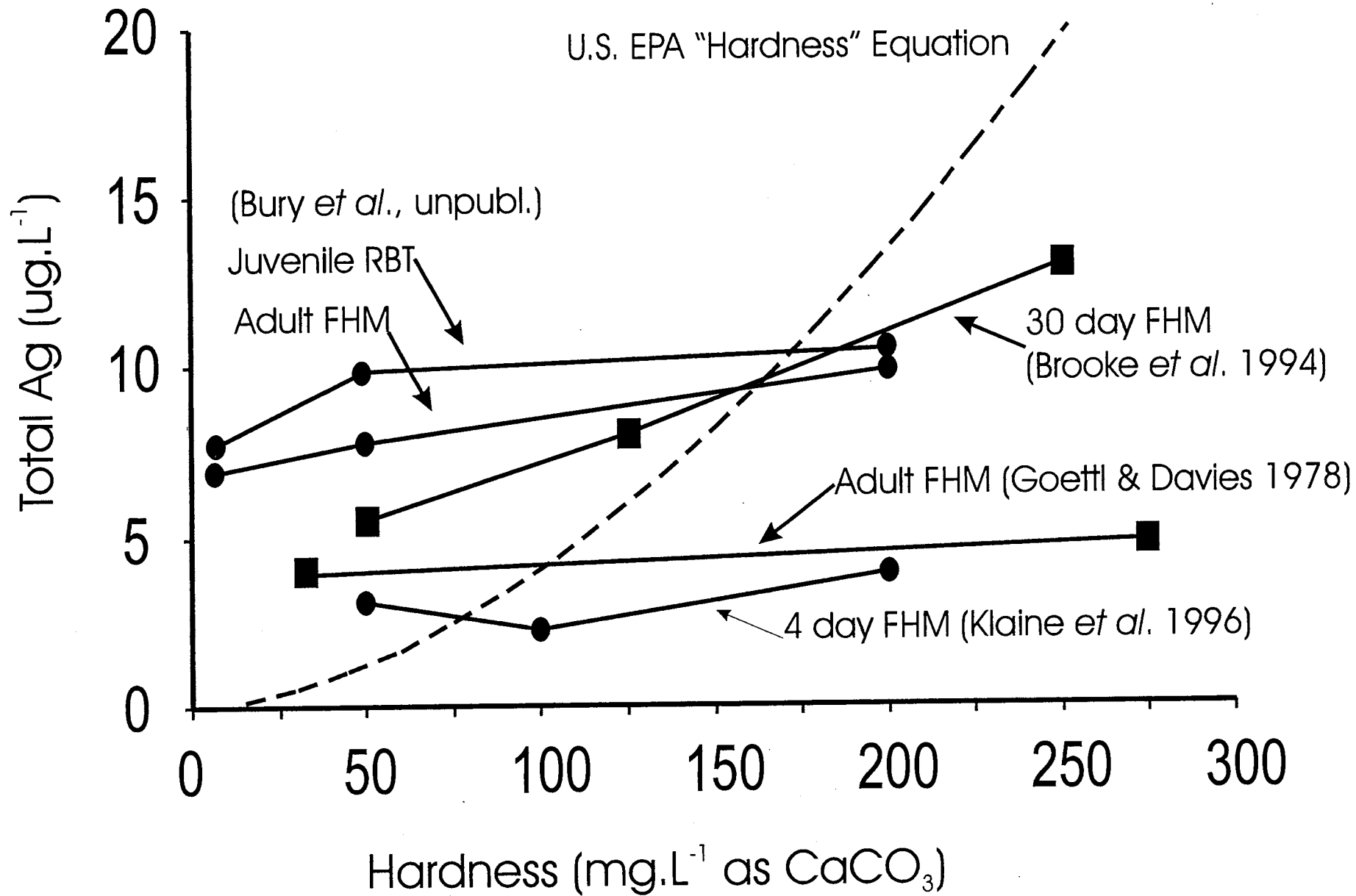


FIGURE 3

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## Questions & Answers: Bioavailability, Physiology and Toxicology of Silver in Freshwater Fish: Implications for Water Quality Criteria

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- Q. DOMINIC DI TORO: (Manhattan College): Chris, that's a very nice job. It's very illuminating, but you haven't told us why the rainbow trout are so different than the fathead minnows. I was just waiting to find out, since the ATP-ase is presumably the same in both species?
- A. I think that may be the answer.
- Q. Is that it's not the same?
- A. It could be some relationship between the free silver ion and the inhibition of the ATP-ase differing in the two species. That's what, if anything, the data would suggest. But we have done no physiological work as yet on fathead minnows.
- Q. Is it hard to do them? Is it hard to do it, because it's a smaller animal?
- A. They're small, they're really small.
- Q. Do you have any small graduate students? (*laughter*)
- Q. TIM FITZPATRICK (Florida Dept. of Environmental Protection): Chris, I was a little puzzled in that for your acute test you didn't see any effect of competition on the gill for chloride. For instance, you saw essentially only an effect with free silver ion. Yet for the bioaccumulation, you saw that some accumulation occurred with chloride species, suggesting that there's some competition occurring there. I wonder how you might explain that?
- A. Well, what we believe - and this is perhaps pre-empting a little bit what Nick Bury's going to say - we believe that neutral silver chloride does go on in and through the gill really quite readily, and it's not toxic. We think in fact, the key is that it is only about 5% of the gill cells that really count. Those are the salt-transporting cells. As the silver ion hits those cells, lots of silver can go on the gill as a silver chloride, and perhaps other forms as well, through cells that really don't matter in terms of toxic mechanisms.
- Q. ARUN MUKHERJEE (University of Helsinki-Finland): What do you think if you do some experiments with selenium?
- A. Well, I guess I would not anticipate that there should be any interaction between selenium and silver.
- Q. Why doesn't it follow the interaction between selenium and mercury?
- A. And mercury? Well, it turns out that that's a very complicated story, and selenium is not the magic bullet for mercury that we used to think it was. I really cannot say, but I mean there's nothing about the chemistry of selenium and the chemistry of silver that to me would say we should have similar effects. Where there are similarities in the chemistry of selenium and the chemistry of mercury are in essential enzymes, for example.

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## How Water Chemistry Influences The Na<sup>+</sup> Losses Associated With Silver Exposure in Rainbow Trout

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### Abstract

The influence of water Cl<sup>-</sup> (50-600 μM), Ca<sup>2+</sup> (50-1500 μM), Na<sup>+</sup> (50-1500 μM) and dissolved organic carbon (DOC, 0.31-5 mg l<sup>-1</sup>) concentrations on silver induced physiological perturbations to Na<sup>+</sup> transport of rainbow trout were investigated. Trout were initially acclimated to softwater (50 μM; Cl<sup>-</sup>, Ca<sup>2+</sup> and Na<sup>+</sup>), and then exposed to 3.7 μg Ag l<sup>-1</sup> (as AgNO<sub>3</sub>) for 6h. This exposure resulted in a reduction Na<sup>+</sup> influx, an inhibition of gill Na<sup>+</sup>/K<sup>+</sup>-ATPase and an increase in gill Ag levels. Increasing the water [Cl<sup>-</sup>] or DOC levels mitigated the silver toxicity. However, increasing water [Ca<sup>2+</sup>] or [Na<sup>+</sup>] did not reduce the silver induced Na losses. There was a negative correlation between the "free" Ag<sup>+</sup> concentration (calculated by the computer program MINEQL+) and both Na<sup>+</sup> influx and gill Na<sup>+</sup>/K<sup>+</sup>-ATPase. However, there was no correlation between gill Ag levels and either Na<sup>+</sup> influx or Na<sup>+</sup>/K<sup>+</sup>-ATPase activity. These results support the notion that it is the "free" Ag<sup>+</sup> ion concentration that is of major importance when assessing silver toxicity in fish, and that this should be taken into account in regulatory strategies for silver in the natural environment.

### Introduction

Silver, when present as AgNO<sub>3</sub>, is one of the most toxic metals to aquatic organisms with an LC50 to freshwater fish in the range of 6.5-70 μg Ag l<sup>-1</sup>. The free ion, Ag<sup>+</sup>, is thought to be the toxic moiety (reviewed by Hogstrand and Wood, 1997). The mechanism of *acute* toxicity of Ag is disruption to branchial ion transport, primarily inhibition of Na<sup>+</sup> and Cl<sup>-</sup> influx (Morgan *et al.*, 1997). Death ultimately occurs due to secondary consequences of ionoregulatory failure (Wood *et al.* 1996).

The toxicity of metals to freshwater fish can be affected by various physiochemical parameters, such as water hardness, pH, alkalinity, Cl<sup>-</sup> or dissolved organic matter (Pagenhoff, 1983, Morel and Hering, Erickson *et al.*, 1996). The influence of these parameters on toxicity can be explained by either rendering the metal inactive by complexation or by direct competition between the ligand and the metal at the site of toxic action i.e., the gill (Janes and Playle, 1995). In the case of Ag, complexation is probably the major factor influencing toxicity (Hogstrand and Wood, 1997).

This study aims to determine the effects of changing water chemistry (Cl<sup>-</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> and DOC) on Ag-induced inhibition of Na<sup>+</sup> influx and gill Na<sup>+</sup>/K<sup>+</sup>-ATPase activity, as well as gill Ag accumulation.

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## Materials and Methods

Juvenile rainbow trout were obtained from Humber Springs Hatchery, Orangeville, Ontario, Canada and were acclimated to soft water (in  $\mu\text{mol l}^{-1}$ ,  $\text{Na}^+$ , 50;  $\text{Cl}^-$ , 50; and  $\text{Ca}^{2+}$ , 50 and DOC, 0.1-0.3  $\text{mg l}^{-1}$ ).

Eight fish (3.2-31g) were transferred to black perspex boxes containing 2.5l of soft water 24 h prior to each experiment. On commencement of the experiment the flow to the boxes was stopped and water spiked with the appropriate salts (for  $\text{Cl}^-$ ,  $\text{Ca}^{2+}$ ,  $\text{Na}^+$  the salts were KCl,  $\text{CaNO}_3$ ,  $\text{Na}_2\text{SO}_4$ , respectively) or dissolved organic carbon (DOC), added as humic acid (Aldrich Ltd). Silver was added as  $\text{AgNO}_3$  to give a concentration of 3.7  $\mu\text{g Ag l}^{-1}$ . The flow was then turned on and the concentration of salts, DOC or  $\text{AgNO}_3$  maintained by the addition of a concentrated salt solution to the inflow via a peristaltic pump. After 4h of exposure the flow was stopped and the fish remained exposed for a further 2h in static condition, following which they were sacrificed.

Unidirectional  $\text{Na}^+$  uptake from the water (" $\text{Na}^+$  influx") was measured radioisotopically ( $^{22}\text{Na}^+$ ) by methods of Wood (1992). Gill  $\text{Na}^+/\text{K}^+$ -ATPase activity was measured by the methods of Bonting *et al* (1961). Gill were digested by methods of Janes & Playle (1995) and Ag concentration determined using graphite furnace atomic absorption spectrophotometry (Varian 1275 fitted with a GTA-95 atomizer).

## Results

Increasing concentrations of water  $\text{Cl}^-$  reduced the degree of inhibition of  $\text{Na}^+$  influx induced by Ag exposure.  $\text{Na}^+$  influx rates returned to control levels in fish exposed to 300 $\mu\text{M Cl}^-$  and 3.7 $\mu\text{g Ag l}^{-1}$  (Fig 1A). However, these  $\text{Cl}^-$  concentrations did not prevent Ag from accumulating on the gills (Fig. 1B). Increasing concentration of DOC reduced the inhibition of Ag induced  $\text{Na}^+$  losses from the fish (Fig 2A). At a level of 2.5  $\text{mg DOC l}^{-1}$  the  $\text{Na}^+$  influx rates were restored to control levels. DOC kept Ag off the gills of fish in a dose-dependent manner (Fig 2B). Both chloride and DOC prevented the inhibition of gill  $\text{Na}^+/\text{K}^+$ -ATPase activity in a dose dependent manner (Fig 3A and B). Increasing the  $\text{Ca}^{2+}$  concentration to 1500  $\mu\text{M}$  had no effect on the inhibition of  $\text{Na}^+$  influx by Ag. This level of  $\text{Ca}^{2+}$  did not keep Ag off the gills. Increasing the concentration of external  $\text{Na}^+$  increased the  $\text{Na}^+$  influx rate. However, it did not effect the inhibition induced by Ag. Increasing  $\text{Na}^+$  levels did not prevent Ag from accumulating on the gill.

### Correlation analysis

The "free"  $\text{Ag}^+$  levels (calculated from the computer program MINEQL+, Schecher and McAvoy, 1992) negatively correlated with gill  $\text{Na}^+/\text{K}^+$ -ATPase activity and whole body  $\text{Na}^+$  influx (data not shown,  $R=-0.64$ ;  $p=0.018$ ). There was no correlation between the "free"  $\text{Ag}^+$  levels and the gill Ag levels. Similarly, there was no correlation between gill Ag levels and gill  $\text{Na}^+/\text{K}^+$ -ATPase activity nor  $\text{Na}^+$  influx (data not shown).

## Discussion

Our results show that chloride and DOC prevent Ag-induced  $\text{Na}^+$  losses from rainbow trout. However, increasing water calcium or sodium levels did not affect the Ag-induced perturbations to  $\text{Na}^+$  uptake. Both chloride and DOC, have also been shown to ameliorate Ag toxicity in rainbow trout (reviewed by Hogstrand and Wood, 1997). The present results from this

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study suggest that this reduction in toxicity is a result of a reduction in the Ag-induced inhibition of branchial ion transport. However, the mechanisms by which chloride and DOC prevent this inhibition may differ. DOC reduces the degree of Ag-induced inhibition of  $\text{Na}^+$  influx and prevents Ag from accumulating on the gill. This is probably due to the formation of DOC-Ag complexes (Janes & Playle, 1995). In the case of chloride, Ag still accumulates on the gill as water  $[\text{Cl}^-]$  increase (coinciding with an increase in concentration of neutral aqueous complexes of  $\text{AgCl}$ ), but there is a reduction in the inhibition of ion transport. These results suggest that it is not the total Ag accumulating on the gill which is important to toxicity, but perhaps where and how it accumulates.

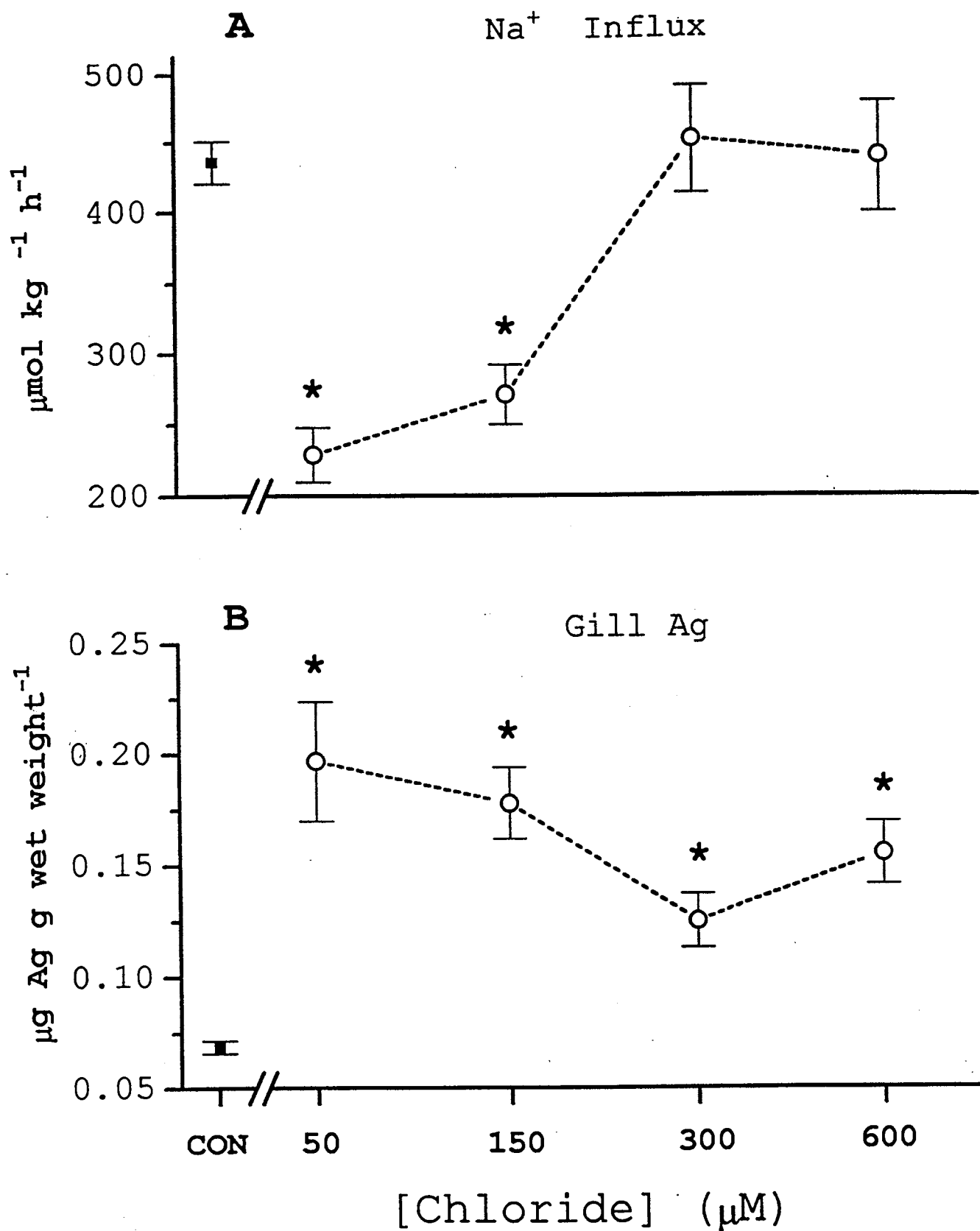
There are strong negative correlations between the "free" water  $[\text{Ag}^+]$  (as calculated from MINEQL+, Schecher & McAvoy, 1992) and both  $\text{Na}^+$  influx and gill  $\text{Na}^+/\text{K}^+$ -ATPase activity. Therefore, the use of geochemical models to predict water metal ion concentration maybe a good indicator of physiological disturbances seen in fish. However, there was no correlation between gill Ag levels and these parameters. Which shows that the gill Ag levels may not a good indicator of acute Ag toxicity. It is proposed that when developing gill models as a predictor of water metal toxicity (e.g. Janes and Playle, 1995) that the relationship between the water metal ion levels and the site of toxic action, i.e. the gill  $\text{Na}^+/\text{K}^+$ -ATPase activity should be considered.

Supported by grants from NSERC (Canada) Industrially Oriented Research Program and Kodak Canada.

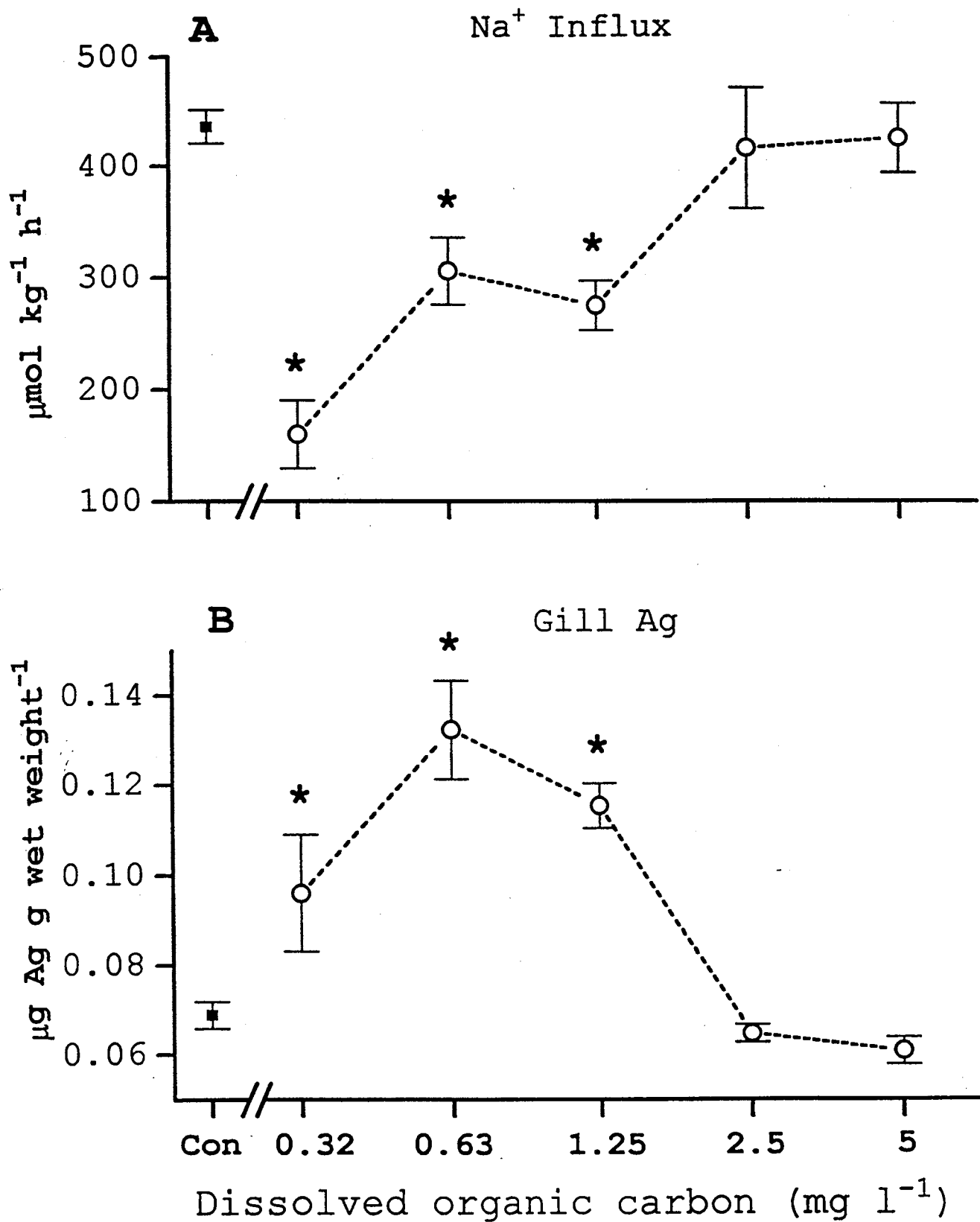
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**Figure 1.**  $\text{Na}^+$  influx rates (A) and gill Ag levels (B) of rainbow trout exposed to  $3.7 \mu\text{g Ag l}^{-1}$  (presented as  $\text{AgNO}_3$ ) at different chloride concentrations. Values are mean  $\pm$  sem, \* indicates significant difference from controls (t-test,  $p < 0.05$ ).



**Figure 2.**  $\text{Na}^+$  influx rates (A) and gill Ag levels (B) of rainbow trout exposed to  $3.7 \mu\text{g Ag l}^{-1}$  (presented as  $\text{AgNO}_3$ ) at different dissolved organic carbon concentrations (presented as humic acid, Aldrich Ltd). Values are mean  $\pm$  sem, \* indicates significant difference from controls (t-test,  $p < 0.05$ ).

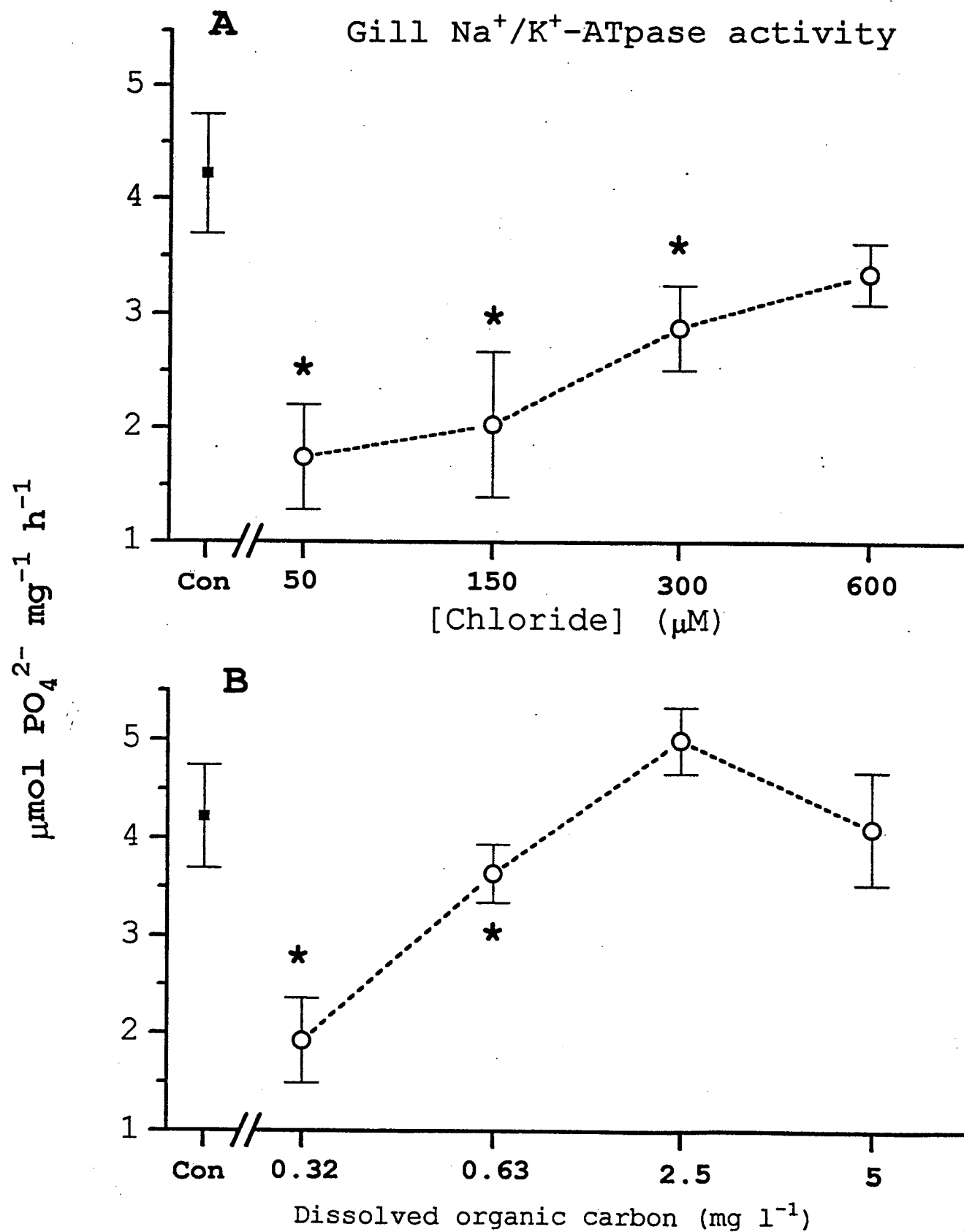


Figure 3. Gill Na<sup>+</sup>-K<sup>+</sup>-ATPase activity of rainbow trout exposed to 3.7 μg Ag l<sup>-1</sup> (presented as AgNO<sub>3</sub>) at different water (A) chloride and (B) dissolved organic carbon concentrations. Values are mean ± sem, \* indicates significant difference from controls (t-test, p < 0.05).

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## Questions & Answers: How Water Chemistry Influences The Na<sup>+</sup> Losses Associated With Silver Exposure in Rainbow Trout

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Q. JOHN MAHONY (Manhattan College): Just a quick question concerning the hardness that you're referring to. Natural waters contain approximately 1/4 to 1/3 of their hardness as magnesium ions. And in view of the fact that the site that was suggested in the earlier talk for the silver action with sodium-potassium ATP-ase was the magnesium site, might it not be better to include magnesium ions in some of your studies as well as calcium ions?

A. Yes, a simple answer is yes, we need to have a look at that. I don't know if someone from Christer's lab has been looking at that, I'm not too sure if that will be presented later.

CHRISTER HOGSTRAND (University of Kentucky) We actually did that just for that reason. We were anticipating very nice results but it turned out that we didn't get at all what we thought we would get. The data set is not completely analyzed, so I can't really tell you what it's going to look like. But one thing I can tell you, it's not as simple as increased magnesium in the water protecting the gill from sodium uptake - or sorry, the other way around.

Q. BRUCE WALKER (Michigan Dept. of Envir. Quality): I've just got an observation. One of the posters on the wall in our dining area talks about - I think it was Nancy that did that - was talking about how 20 mgs of DOC really didn't protect against the plasma changes. And you had 2.5 mgs of DOC keeping enough silver off the gill that you didn't get your sodium effect, so there is basically an order of magnitude difference there. Do you have any comment on that?

A. You see, I have to discuss this slightly with Rick as well because I obviously noticed that, and we do see differences actually between the labs. What's actually going on, to be honest, I don't really know why there is such a big difference. I can pass the buck again. I don't know if Rick can make a comment on that. Sorry, Rick.

RICK PLAYLE (Wilfrid Laurier University-Waterloo, Canada): Yes, we were talking about it, and we don't really know some of the differences. We used much higher concentrations of silver in our experiments. I think that's the quick answer.

Q. RICK PLAYLE: But now that I'm up here, Nick, if I may ask a question? Nice work, but I'm curious, how long, when you measured the gill silver, how long were you exposing the fish? Was that after a few hours, a couple of days, or what?

A. Six hours.

Q. That's interesting. Because what I found with some mercury work that we've done recently is that it's a fast accumulation. Like 4 hours really correlates nicely with toxicity, but like a 24-hour amount of mercury on the gills doesn't correlate as well. If you wait 48 hours the correlation is even worse. So what I'd like to suggest is maybe a fast silver accumulation on the gills, maybe at 2 hours, might correlate very nicely with the sodium potassium ATP-ase, but a later accumulation, and even the difference between 2 and 6 hours, might be enough to throw off the correlation. But nice work.

A. Yes - that's quite logical.

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## The Effects of Salinity on $^{110m}\text{Ag}$ Uptake and Distribution in European Eel (*Anguilla anguilla*)

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### Abstract

Euryhaline, European eels (*Anguilla anguilla*) were acclimated to seawater, brackish water, hard fresh water and soft fresh water for at least 30 days prior to  $^{110m}\text{Ag}$  exposure ([Cl<sup>-</sup>] 314, 136, 1.26, 0.06 mM, respectively). Toxicity tests revealed the European eel to be highly tolerant to waterborne Ag (added as AgNO<sub>3</sub>) with 24h LC50 of ~ 750  $\mu\text{g Ag}\cdot\text{l}^{-1}$  and ~ 100000  $\mu\text{g Ag}\cdot\text{l}^{-1}$  in soft and hard fresh water, respectively. The kinetics of  $^{110m}\text{Ag}$  accumulation were measured in gill filaments, blood, liver, kidney, esophagus, intestine and bile during 72 hours of static exposure to 20  $\mu\text{g Ag}\cdot\text{l}^{-1}$ ; 31  $\mu\text{Ci}\cdot\text{l}^{-1}$ . Branchial  $^{110m}\text{Ag}$  accumulation in soft and hard freshwater (~8 and ~10  $\mu\text{g Ag}\cdot\text{g gill filament}^{-1}$  dry weight, respectively) did not reflect the observed differences in tolerance. Branchial  $^{110m}\text{Ag}$  accumulation in brackish and sea water was, however, 100 times lower than in both types of freshwater. The eels tended to accumulate slightly more  $^{110m}\text{Ag}$  in the blood, kidney and liver in hard fresh water compared to soft fresh water, but brackish- and seawater clearly reduced  $^{110m}\text{Ag}$  accumulation. The plasma accounted for 71-94 % of blood  $^{110m}\text{Ag}$  after 24 hours of exposure. Exposure in seawater resulted in the highest  $^{110m}\text{Ag}$  accumulation in the intestine indicating that intestinal Ag uptake could be important in sea water fish.

### Introduction

To our knowledge there have been no reports on the effects of salinity, ranging from soft freshwater to seawater, on Ag uptake and internal distribution among tissues in fish. The European eel can be found at all salinities and is distributed throughout Europe and is thus well suited for such a study.

The objective of the present study was to compare Ag uptake and internal fate in the euryhaline European eel in soft freshwater, hard freshwater, brackish water and seawater during exposure to 20  $\mu\text{g Ag}\cdot\text{l}^{-1}$ .

### Materials and Methods

European eels (*Anguilla anguilla*) were caught in fyke nets in Roskilde fjord (a brackish water system ranging from freshwater to full strength seawater) during October 1996. Mean weight was 58.5 g (range 28.7-95.2 g) and all fish were yellow stage eels. The eels were acclimated to laboratory condition for at least one month in flow through, aerated, hard freshwater prior to salinity acclimation. Commencement of feeding by the eels was used as sign of acclimation. The eels were fed mussels three times a week.

Prior to Ag exposure eels were acclimated at 15°C to soft freshwater (SFW) and hard freshwater (HFW) under flow-through conditions in 300 l cylindrical PVC tanks and to recirculated brackish (10 ppt) (BW) and seawater (25 ppt) (SW) (400 l Biological filter, with a weekly 50% water renewal) for at least 30 days. See Table 1, for water chemistry.

For 24 h LC50 bioassays, eels were placed in a number of 25 l all glass tanks (8 fish per tank) and

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were allowed to acclimate to these conditions for 24 hours with a flow-through of  $0.2 \text{ l} \cdot \text{min}^{-1}$  at which point the flow was stopped and Ag was added as  $\text{AgNO}_3$ . During the bioassay, the water was static with a 12 h renewal. The tanks were placed in a waterbath to maintain a constant temperature at  $15^\circ\text{C}$ .

Prior (15 h) to  $^{110\text{m}}\text{Ag}$  exposure eels that had been acclimated to SFW, HFW, BW and SW were transferred to a total of 24 all glass tanks containing 25 l water that was identical to the respective holding tanks. (5 fish $\cdot$ tank $^{-1}$  in SFW, HFW and SW, 3 fish $\cdot$ tank $^{-1}$  in BW).

Ag exposure was initiated by adding radiolabeled  $\text{AgNO}_3$  to achieve a nominal concentration 20  $\mu\text{g Ag}\cdot\text{l}^{-1}$ ; 31  $\mu\text{Ci}\cdot\text{l}^{-1}$ ). A reference water sample for  $^{110\text{m}}\text{Ag}$  counting was taken from each tank 30 minutes after addition of the  $^{110\text{m}}\text{Ag}$  labelled silver and at the end of the exposure. After 1, 2, 4, 8, 24 and 72 hours of exposure, eels were sampled from the exposure tanks and were rinsed once in clean water of the same composition and anesthetized in urethane ( $10 \text{ g}\cdot\text{l}^{-1}$ ). A bloodsample was taken from the caudal vessel with a heparinized syringe and the content of the gall bladder was obtained by a syringe. Subsequently gill filaments, liver, kidney, esophagus and part of the intestine were sampled. The  $^{110\text{m}}\text{Ag}$   $\beta$ -radio activity was assayed (3 $\cdot$ 5 min) directly in dried homogenized tissue samples as well as evaporated water, blood and bile samples using two low-background five-sample Geiger type multi-counters (Bøtter-Jensen & Nielsen, 1989).

The Ag accumulation in the respective tissues was calculated as described by Grosell *et al.*, (1996).

The silver accumulation at the different salinities were compared by a two way ANOVA, with exposure time and salinity as the main variable factors. Groups were considered significantly different at  $P < 0.05$ .

## Results and Discussion

The 24 hours LC50 was 0.75 and 100  $\text{mg Ag}\cdot\text{l}^{-1}$  in SFW and HFW, respectively, which is extremely high when compared to values reported for rainbow trout and fathead minnows (Hogstrand *et al.*, 1996; Leblanc *et al.*, 1984). The high level of Ag tolerance in the freshwater eels could be related to their very low ionic turnover compared to a number of other species including the rainbow trout (Perry *et al.*, 1992a;b). This low turnover probably makes them less sensitive to any impairment of branchial ion uptake - the key target of silver toxicity (Wood *et al.*, 1996a; 1996b). Secretion of mucus could be another protective mechanism during metal exposure and elevated mucus production was observed in fish exposed to high Ag concentrations.

Branchial [Ag] was around 100 times higher in the eels sampled from the two types of freshwater compared to the eels sampled from BW and SW. Branchial [Ag] showed a saturation pattern reaching a "steady state" after only a few hours of exposure (Fig. 1). A similar pattern was observed in brackish water but not in seawater eels where branchial [Ag] seemed to increase over time in a linear fashion. The observed difference in Ag toxicity between HFW and SFW was not reflected in the branchial Ag accumulation. This suggests that both the  $\text{Ag}^+$  ion which is the most toxic form of silver and the less toxic  $\text{AgCl}$  complexes may be accumulated by the fish gills.

Except for the gills in freshwater eels, the liver accumulated the highest Ag concentration of all investigated tissues. This is in agreement with findings recently reviewed by Hogstrand & Wood, (1997). The eels accumulated Ag in the livers independently of external salinity in an exponential fashion during the 72 hours of exposure (Fig. 3). While there seemed to be some correlation between branchial [Ag] and hepatic [Ag] in the freshwater eels, this was not the case in either brackish water or seawater eels. This could indicate either that Ag can be transported across the gill

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epithelium in BW and SW eels without actually being accumulated in the gills or that Ag may be taken up by an entirely different route in these fish. The Ag speciation in BW and SW does not support branchial Ag uptake without accumulation in the gills since  $\text{AgCl}_2^{-1-a}$  complexes predominate. These can not be expected to be readily transported across the gill epithelium. Fish in a hyperosmotic environment counteracts the water loss to the surroundings by drinking and Ag uptake by the gastrointestinal tract could thus play a role in the whole body Ag accumulation in seawater fish. Our findings support this hypothesis because the intestine in the seawater eels accumulates more Ag than the BW, SFW and HFW fish (Fig. 6). This means that the intestine of seawater fish could be involved in Ag uptake during a waterborne exposure and also that the intestinal active iontransport processes could be a target for Ag toxicity in seawater fish. Overall eels accumulated less silver with increasing ionic strength of the surrounding environment. Based on the 72 hour data, the relative contribution of the investigated tissues to the total Ag accumulation changed from the gills being the tissues that accumulated most Ag in freshwater to the liver and the gastro-intestinal tract to be the tissues with the highest Ag concentration in the seawater fish (Fig. 2).

Some silver accumulated in the bile, indicating a potential hepatobiliary Ag elimination. However, comparing the plasma Ag concentration with the biliary Ag concentration reveals no Ag-gradient between these two compartments. This is different from Cu that is accumulated in bile against a gradient of 10-100 in both eels and rainbow trout during waterborne Cu exposure (Grosell *et al.*, 1997; 1998).

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### Legends

Fig. 1. Branchial Ag accumulation in gill filaments of European eel (*Anguilla anguilla*) Mean ± SEM. N=5 for HFW,SFW,SW and n=3 for BW

Fig. 7. Distribution of Ag among all investigated tissues (normalized to a 1 kg fish) in European eels (*Anguilla anguilla*) after 72 hours of exposure.



Table 1

	[Ca <sup>2+</sup> ] mmol·l <sup>-1</sup>	[Mg <sup>2+</sup> ] mmol·l <sup>-1</sup>	[Na <sup>+</sup> ] mmol·l <sup>-1</sup>	[K <sup>+</sup> ] mmol·l <sup>-1</sup>	[HCO <sub>3</sub> <sup>2-</sup> ] mmol·l <sup>-1</sup>	[Cl <sup>-</sup> ] mmol·l <sup>-1</sup>	[SO <sub>4</sub> <sup>2-</sup> ] mmol·l <sup>-1</sup>	pH	tot [Ag] <sup>+</sup> μg·l <sup>-1</sup>	[Ag] <sup>+</sup> ** μg·l <sup>-1</sup>	24 h LCSO* mg·Ag·l <sup>-1</sup>
SFW	0.12	0.12	0.05	0.004	<0.33	0.06	0.0004	5.92	20	17.6	0.75
HFW	3.22	0.62	0.74	0.1	4.92	1.24	1.04	8.22	20	4.92	100
BW	4.72	12.7	104.4	2.07	5.16	121.3	6.35	8.43	20	0	-
SW	6.96	30.1	252.3	6.14	5.97	310.3	15.61	8.33	20	0	-

\* nominal concentration  
 \*\* according to MINEQL+  
 Ag speciation according to MINEQL+

Fig. 1

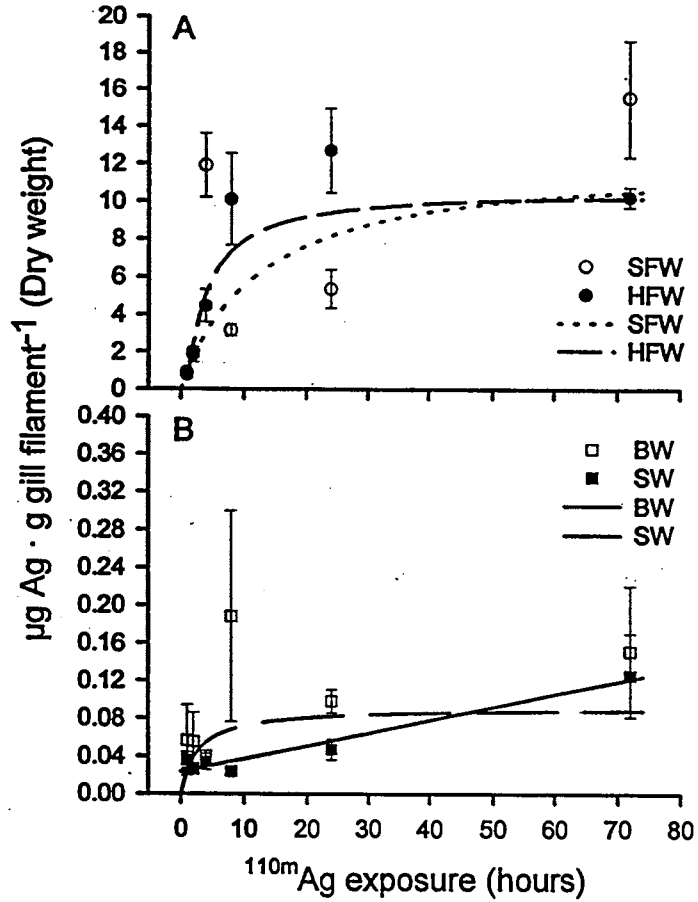
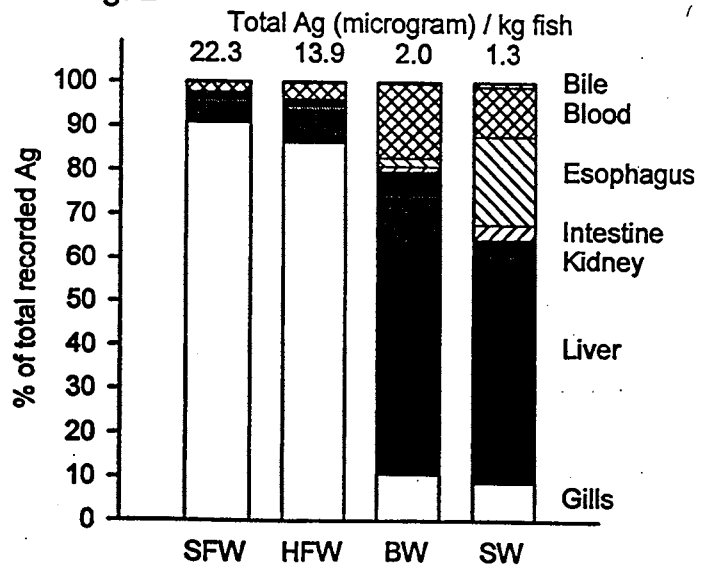


Fig. 2



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**Questions & Answers: The Effects of Salinity on  $^{110m}$  Ag Uptake and Distribution in European Eel (*Anguilla anguilla*)**

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- Q. BRUCE WALKER: You had showed that your gill uptake in the freshwater fish basically kind of plateaued after about 24 hours, but your liver concentration continued to increase. Is there any long-term or physiological implications in that implied to you, or all that you know about because of the plasma work that's been done?
- A. Well, I can't really say anything about that based on this study, but it seems that the silver concentration in the liver is not reflecting toxicity at all, not even in chronic studies. So I would expect that, well, the fish can cope with a very high silver concentration in the liver. It's going to be either protein bound and that way de-toxified, or yes, just stored in an unavailable form. You could expect some interference with other metals, some of the essential metals like zinc and copper maybe, assuming that silver would replace those metals at various mining sites, but to my knowledge those haven't been shown.

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# Long Range Atmospheric Transport of Silver in Northern Europe

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## INTRODUCTION

Very few data for the concentration of silver in air and precipitation exist in the literature so far. Nriagu and Pacyna (1988) did not even include Ag in their general assessment of worldwide contamination of air, water and soils by trace metals. The main reason for this is obviously that the environmental concentration levels of this element are generally too low to be determined reliably by most existing methods for trace element analysis.

In the author's laboratory the atmospheric deposition of a wide variety of trace elements has been studied regularly over the last 20 years (Steinnes 1977, 1980; Steinnes et al. 1992, 1994; Berg et al. 1994, 1995; Berg and Steinnes 1997). Occasionally similar trace element studies of aerosols have been carried out at specific sampling stations run by the Norwegian Institute for Air Research (e.g. Amundsen et al. 1992). In some of the early studies where instrumental neutron activation analysis (INAA) was employed, analytical data for Ag were recorded, but these data were discussed only to a very limited extent (Amundsen et al. 1992, Steinnes et al. 1992). Recently (Berg and Steinnes 1997) apparently reliable data for Ag were also obtained by inductive-coupled plasma mass spectrometry (ICP-MS). Altogether these data for Ag allow some conclusions to be drawn concerning the atmospheric transport and deposition of this element, in particular when discussed in relation to similar data for other heavy metals studied simultaneously.

## LONG RANGE ATMOSPHERIC TRANSPORT OF POLLUTANTS IN NORTHERN EUROPE

The aspect of long range atmospheric transport of pollutants (LRTP) in Northern Europe most frequently focused on in public discussion as well as in the scientific literature is the continuous supply of many chemical substances to the southern parts of Scandinavia from other, more densely populated and strongly industrialized parts of Europe. The extinction of fish in thousands of lakes and rivers strongly affected by atmospheric deposition of sulfur and nitrogen compounds from LRTP is clearly the most serious result of this transboundary pollution (Overrein et al. 1981). However, the polluted air masses arriving to Scandinavia also contain appreciable amounts of other pollutants such as ozone, heavy metals, and persistent organic pollutants. At episodes with southerly winds the pollutants

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from other European countries are frequently deposited particularly in southern Norway and south-western Sweden by orographic precipitation.

The atmospheric deposition of selected heavy metals has been followed regularly over the last 20 years at the Birkenes station in southernmost Norway. More recently this monitoring has been gradually extended to other stations (Berg et al. 1994). Every 5 years the atmospheric deposition of a large number of trace elements is monitored in a network of about 500 sites by collection and analysis of naturally growing moss (e.g. Steinnes et al. 1992), a method originally introduced by Swedish scientists (Rühling and Tyler 1973). Air concentrations of 20 elements were studied on a daily basis at Birkenes by air filter sampling during two periods in 1978-79 and 1985-86 (Amundsen et al. 1992). Altogether these data have documented that elements such as Pb, Zn, Cd, V, As, and Sb are supplied to southern Norway almost exclusively by LRTP.

## THE PLACE OF SILVER IN LONG RANGE ATMOSPHERIC TRANSPORT

Concentrations of Ag in moss were registered by INAA in 1976 at about 40 sites (Steinnes 1977) and in 1977 at about 500 sites (Steinnes et al. 1992) all over Norway. Silver data by INAA also exist from the 1985-86 aerosol sampling at Birkenes (Amundsen et al. 1992). In a moss survey in 1995 (Berg and Steinnes 1997), data for Ag were obtained by ICP-MS. Unfortunately it has not succeeded so far to determine Ag in precipitation samples from the Norwegian monitoring network (Berg et al. 1994). Nevertheless the data now available for Ag, when combined with similar data for other elements associated with LRTP, allow some conclusions to be drawn with respect to concentration levels in air and atmospheric deposition, as shown in the following.

### Air concentrations

Diurnal aerosol samples from the 1978-79 and 1985-86 campaigns at Birkenes were assigned to sectors representing different source areas by using trajectory data (Amundsen et al. 1992). Eight sectors of equal size were defined. The highest concentrations of elements predominantly supplied by LRTP were observed in sectors 4, 5, and 6, representing mainly source areas in eastern Europe, central/western Europe, and Great Britain, respectively. Average median values (in  $\text{ng m}^{-3}$ ) for V(2.3), Zn(21), As(0.69), Se(0.54), Cd(0.12), Sb(0.47), and Pb(11) in these sectors were generally 5-10 times higher than those representing air from sectors 1 (Norway) and 8 (North Atlantic). The 1985-86 values for Ag followed the same sector distribution with maximum concentrations in air derived from sectors 4-6, which clearly places this element among those associated with LRTP. The average median value however was only **0.035  $\text{ng m}^{-3}$** , *i.e.* much lower than for the other elements.

### Atmospheric deposition.

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Isopleths for Ag from the national surveys in 1977 (Steinnes et al. 1992) and 1995 (Berg and Steinnes 1997) both show a geographic distribution in the moss where the southernmost part of Norway receives 5-10 times higher deposition than the northern part of the country, similar to that of the other LRTP-derived elements. The 1995 level in southernmost Norway is about 40 % of that observed in 1977, which indicates a reduced deposition similar to that evident for As, Cd and Sb, but less than that of Pb, during this period of time.

Sampling of bulk precipitation at six Norwegian background stations in 1989-1990 (Berg et al. 1994), using clean techniques for sampling and analysis, made it possible to determine bulk deposition fluxes at these sites. Even though Ag concentrations in precipitation were too low to be determined, it is possible to estimate the deposition of Ag at Birkenes, assuming that the ratio between Ag and other LRTP elements in the aerosol is the same as in bulk deposition, and accounting for differences in deposition trends of different elements between 1985 and 1990 as indicated by the moss analysis. Thus, the bulk deposition of Ag at Birkenes in 1990 is estimated to be  $32 \pm 11 \mu\text{g}/\text{m}^2\text{y}$ , which corresponds to an average concentration in precipitation of  $23 \pm 8 \text{ ng L}^{-1}$ .

#### Natural surface soils

Large-scale investigations of organic-rich surface soils in Norway have shown that the topsoil in southern Norway is strongly contaminated with elements such as Pb, Cd, As, and Sb, derived from LRTP (Allen and Steinnes 1980; Steinnes et al. 1989; Page and Steinnes 1990; Steinnes et al. 1997). Samples from a nationwide program in 1995, taken from the upper 3 cm of the soil, were analyzed recently (Nygård et al. 1997) with respect to Ag, which was not included in any of the previous soil surveys. Generally values are of the order of 0.4 - 0.8 ppm, and the geographical distribution does not indicate any significant contribution from LRTP. One trend, however, appears to be very pronounced: soils from areas along the western coast generally show much lower concentration levels than elsewhere in the country, often 0.2 ppm or less. Apparently the higher input of airborne seasalt components in these coastal areas lead to weaker binding of Ag in the humus layer, possibly due to the formation of the AgCl complex in the soil.

## CONCLUSIONS

The main conclusions from the present work may be formulated as follows:

1. Long range atmospheric transport from other parts of Europe is a primary source of airborne silver in Scandinavia.
2. Atmospheric deposition of silver in southern Norway has declined by over a factor of 2 over the last 20 years.

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3. Current concentrations of silver in precipitation falling in different parts of Norway can be estimated to be of the order of 2-20 ng L<sup>-1</sup>.
  4. Airborne silver is not a major source to contamination of soils and surface waters except perhaps near strong point sources of air pollution.
  5. Components of marine aerosols appear to affect the chemistry of silver in organic topsoils.

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## Questions & Answers: Long Range Atmospheric Transport of Silver in Northern Europe

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- Q. GARY GILL (Texas A&M University): You showed a relationship - I believe it was for lead - between deposition and the levels of lead that were in the moss. I'm curious whether that correlation is a relationship between wet deposition or if it also includes some dry deposition component? Then sort of a corollary question: Is the moss reflecting predominantly a wet or dry component, or a compilation of both?
- A. Well, the situation in Norway is that lead deposition is mainly wet deposition. And what we used there was bulk deposition collectors which collect all wet deposition and only part of the dry deposition. We don't know so well how good this moss reflects the dry deposition, but in our case it's not so important.
- Q. ANDERS ANDREN (UW-Madison): Eiliv, I'm not sure I really understand your argument about the marine influence part. Are you saying that silver comes in the precipitation as a silver chloride, and it doesn't adsorb as effectively as that silver which might come from other sources?
- A. No. What I'm saying is that whatever the source of silver is in the surface soil, that the binding of silver is affected by the marine ions coming in.
- Q. Oh, I see what you mean. So you have the anions in the soil already.
- A. Yes.
- Q. ALINA KABATA-PENDIAS (IUNG-Poland): The data you present for your country for silver in surface soils are very much comparable with data which we have for our kind of soil in our countries, which are not necessarily enriched in humus. Most of our surface soils are very poor in humus. But anyway, results are pretty comparable, there is 1 ppm silver in soil. But what we could see is a kind of a halo phenomenon, if you know what I mean by halo, around any larger city. This 1 ppm of silver doesn't exist very much elsewhere, but is quite visible around each city. And I hadn't seen this on the map showing distribution for your country. Do you observe such an influence of municipal activity on silver levels in surface soils?
- A. I think that depends on how dense your network is.
- (Balance of answer and follow-up question lost due to tape malfunction)*
- Q. ARUN MUKHERJEE (University of Helsinki-Finland): Is it possible that we can calculate or find out the load of silver in the Baltic Sea? I know that would not have very much data on silver loading to the whole Scandinavian area, but we know now mercury, lead, we know very much about cadmium on the Baltic Sea, but is it possible that in the near future we can do something on the load of silver in the Baltic Sea?
- A. If you mean the anthropogenic load over all times, I think that would be difficult. If it was to calculate the load at a certain time I think you could use, for example, measured lead deposition data and assume a certain ratio. But calculating the load over all time, I think would be very difficult. I think that's better done by taking sediment samples.



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## Predicting the Toxicity of Silver-Spiked Sediments Using Interstitial Water Metal and Acid Volatile Sulfide Normalizations

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Numerous studies have shown the utility of interstitial metals concentrations and metals:acid volatile sulfide (AVS) relationships in explaining the bioavailability of sediment-associated metals to benthic organisms in both the laboratory and the field (Ankley et al., 1996; Berry et al. 1996; Hansen et al., 1996). To date the use of AVS in predicting metals bioavailability in sediments has been limited to the divalent cationic metals which form sulfides: cadmium, copper, lead, nickel, and zinc. However, AVS should also be useful for other metals which form sulfides, such as silver. Silver cannot be treated exactly like these other metals for a number of reasons. (1) Silver is monovalent for the most part, therefore each mole of sulfide binds two moles of silver. (2) The formation of highly insoluble silver chloride complicates saltwater sediment spiking and analysis. (3) Equilibration times can be very long: one fine-grained, silver-spiked sediment did not equilibrate until after 70 days. (4) Silver is not quantified in the conventional AVS extraction.

In this study two saltwater sediments were spiked with a series of concentrations of silver, such that the nominal ratio of silver/AVS ranged from 0.0 to 20.0. One sediment was coarse-grained with low AVS (Ninigret Pond), and one was fine-grained with higher AVS (Pojac Point). The amphipod, *Ampelisca abdita*, was then exposed to the sediments in ten-day toxicity tests.

Dose-response relationships were sediment-specific when amphipod mortality was plotted against dry weight silver (Figure 1), although mortality increased with added silver in both sediments, more silver was required on a dry weight basis in Pojac Point sediment in order for the sediment to be toxic. For both Ninigret Pond and Pojac Point sediments, sediments with a nominal excess of AVS relative to silver had interstitial water toxic units (IWTU) <0.5, and were generally not toxic (Figures 2 and 3). Sediments with a nominal excess of silver relative to AVS had measurable silver present, no measurable AVS, silver IWTU >0.5, and were generally toxic (Figures 2 and 3). No sediments in which measurable AVS was present were toxic. These results indicate that silver:AVS relationships and IWTU can provide valuable insight into the role of silver in the toxicity of saltwater sediments.

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One anomalous result was recorded: there was higher mortality at one of the intermediate concentrations in the Pojac Point sediments than there was at the next higher concentration in the same experiment. This result was repeated in a separate spiking with sediment from the same site, and appears to be due to an increase in unionized ammonia in the interstitial water in that sediment treatment. These data have been omitted from Figures 2 and 3 because they were probably due to an experimental artifact, resulting from the addition of the nitrate salt of silver.

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## Amphipod Mortality vs. Silver (Dry Wt)

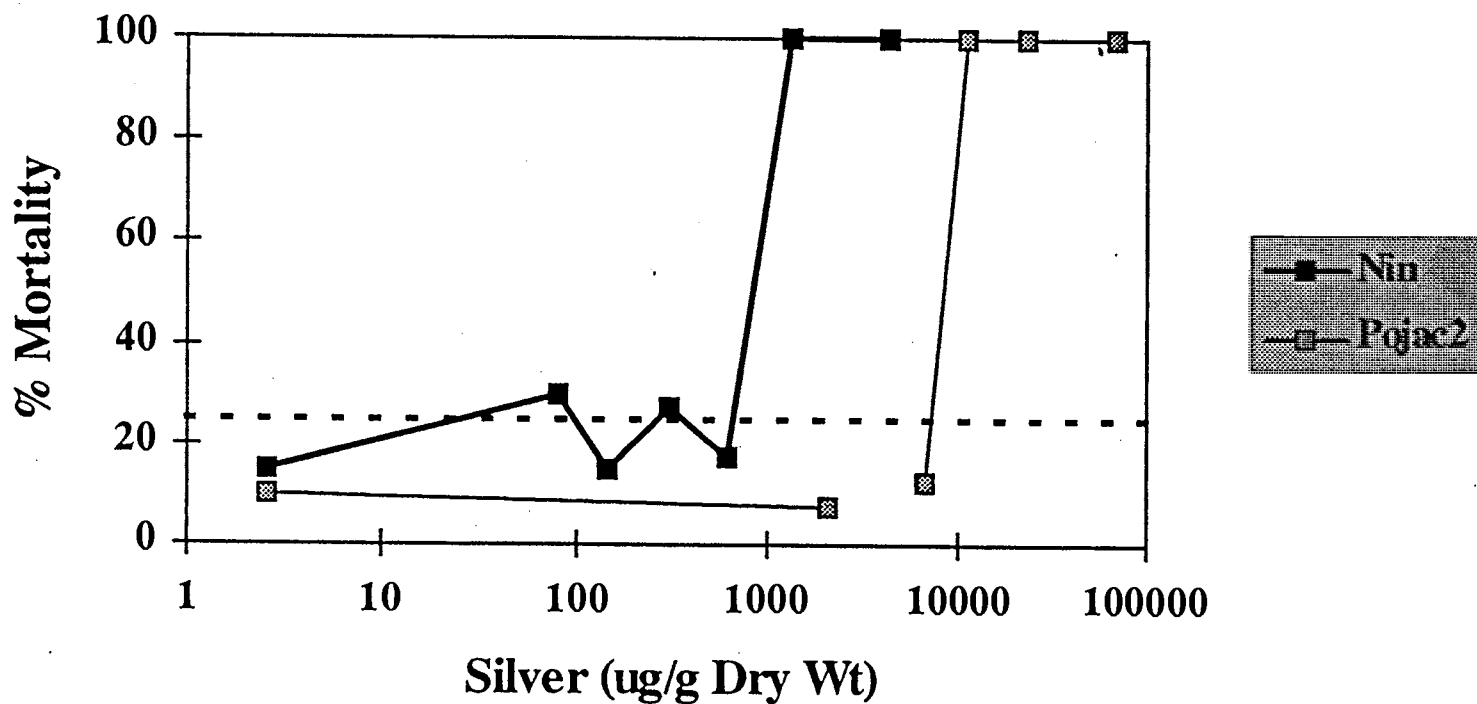


Figure 1: Amphipod mortality vs. dry weight of silver. Nin = Ninigret Pond, Pojac2 = second Pojac Point Experiment. Gap in Pojac2 line indicates data not shown because mortality was assumed to be due to high IW ammonia.

## Amphipod Mortality vs. Nominal Silver/AVS

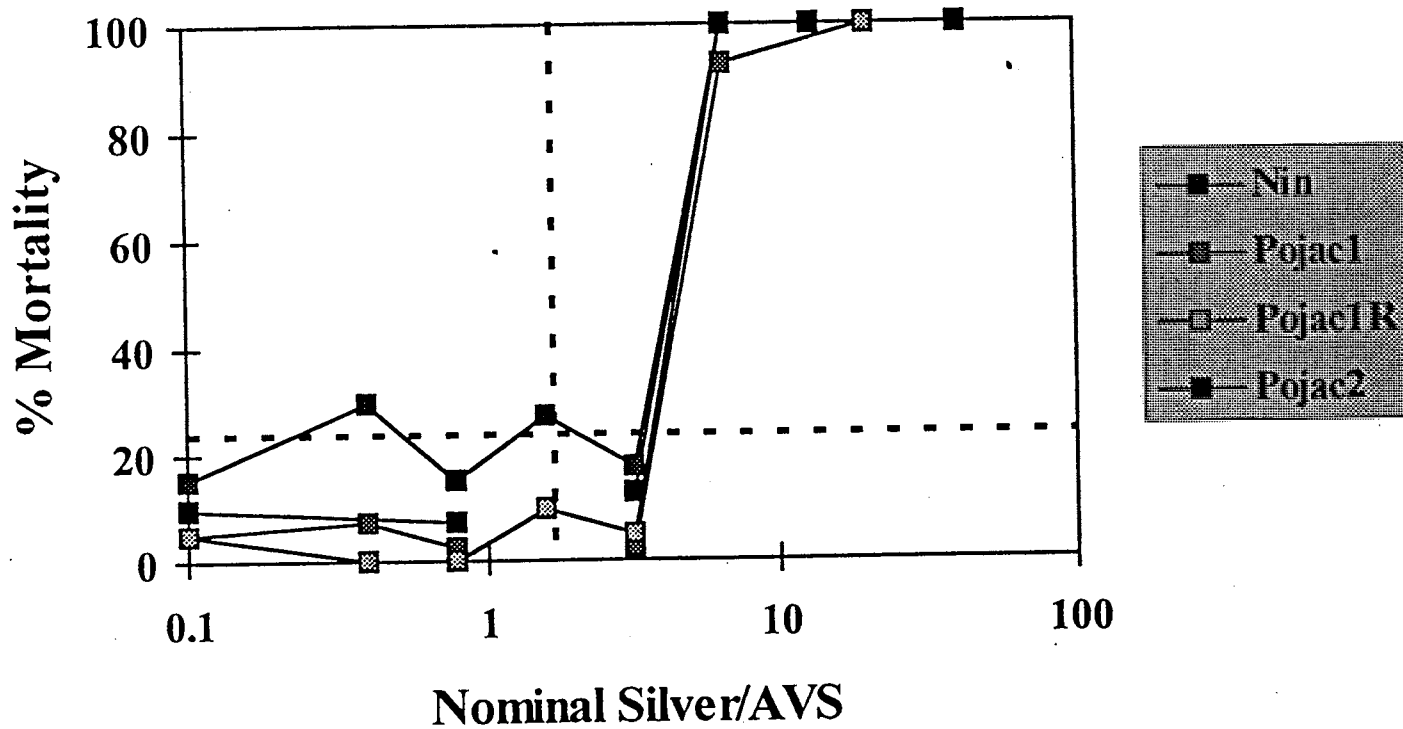


Figure 2: Amphipod mortality vs. nominal silver/AVS. Nin = Ninigret Pond, Pojac = Pojac Point. Gaps in Pojac1 and 2 lines indicate data not shown because mortality was assumed to be due to high IW ammonia.

### Amphipod Mortality vs. IWTU

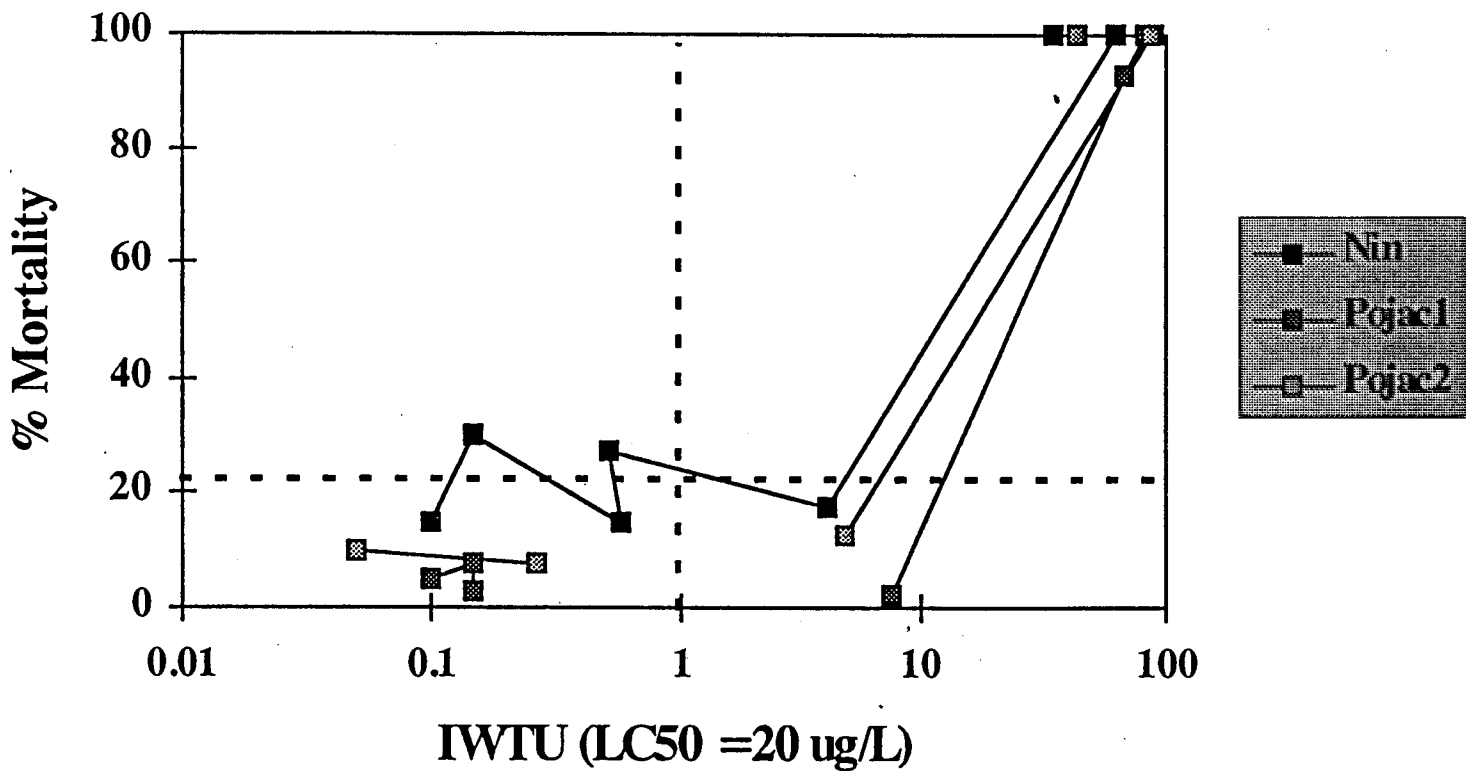


Figure 3: Amphipod mortality vs. silver interstitial water toxic units (IWTU). Nin = Ninigret Pond, Pojac = Pojac Point. Gaps in Pojac1 and 2 lines indicate data not shown because mortality was assumed to be due to high IW ammonia.

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**Questions & Answers: Predicting the Toxicity of Silver-Spiked Sediments Using Interstitial Water Metal and Acid Volatile Sulfide Normalizations**

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- Q. DAN CALL (University of Wisconsin-Lake Superior Research Laboratory): Walter, you added quite a bit of silver nitrate into the sediments. Do you think that the toxicity you saw was due to silver, or to other displaced metals?
- A. We did in fact measure the metals that were displaced, and there was a slight elevation in the SEM and in the interstitial water, but nothing that would be responsible for mortality.

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## Bioavailability and Toxicity of Silver to *Chironomus tentans* in Water and Sediments

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### Introduction

Silver and other metals in the aquatic environment may exist in various forms in the water and sediment. The freely dissolved, ionic form is considered to be the most readily available and toxic form to aquatic biota. For benthic species of animals, the controlling factors that determine metal toxicity are those that influence metal distribution between the solid phase sediment and pore water (Pesch *et al.*, 1995). Acid volatile sulfide (AVS) has been recognized as one of the important binding sites of sediment that affects metal bioavailability (see e.g., DiToro *et al.*, 1990; Ankley *et al.*, 1996). Other binding sites present in the sediment, including organic carbon, may also play important roles in mediating toxicity (Ankley *et al.*, 1996; Mahony *et al.*, 1996). Most sediment studies to date have focused upon the divalent metals - Zn, Ni, Cu, Pb and Cd. Comparatively few studies have been conducted that address the effects of silver-contaminated sediments on benthic species.

*Chironomus tentans* is a Dipteran insect with an obligate benthic larval stage, placing it in close contact with sediment and pore water during this life stage. The present study was conducted first to determine the acute toxicity of silver to *C. tentans* larvae in a toxicity test without sediment present, secondly to determine the total binding capacity of a sediment for silver upon addition of AgNO<sub>3</sub> to the sediment, and thirdly to conduct an acute toxicity test with *C. tentans* in sediments amended with a series of AgNO<sub>3</sub> concentrations, some of which would exceed the total binding capacity of the sediment for silver.

### Experimental

Second and third instar *C. tentans* larvae were exposed to five concentrations of AgNO<sub>3</sub> plus a control in duplicate, in a 10-day toxicity test in water without sediment present. Ten larvae per replicate were exposed in 300 mL screened high-form beakers placed in aquaria within a 23° C water bath. Silver nitrate solutions were prepared in 18.9 L polyethylene containers, and were pumped directly into the screened exposure beakers contained within aquaria. Test solutions were renewed continuously over the 10-day exposure period, with the dilution water being dechlorinated laboratory water

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with a Cl<sup>-</sup> concentration of approximately 4 mg/L.

Ag concentrations were measured on days 0, 3, 6 and 10 as total, dissolved and free silver. Total silver concentrations were determined by acidifying an unfiltered water sample to 0.1% nitric acid prior to analysis by flame atomic absorption spectrophotometry. Dissolved silver was operationally defined as the silver that passed through a 0.2  $\mu\text{m}$  pore size polyethersulfone membrane. Free silver was measured as the silver that passed through the membrane and was measured with a specific ion electrode.

West Bearskin Lake (Lake County, MN) sediment was used in studies of total silver binding capacity and in a toxicity test with AgNO<sub>3</sub>-amended sediment. This sediment contained approximately 1.1  $\mu\text{mol/g}$  of AVS and 1% total organic carbon (TOC). Mean initial simultaneously extracted metal (SEM) concentrations were 0.004, 0.102, 0.118, 0.067 and 0.488  $\mu\text{mol/g}$  for Cd, Cu, Ni, Pb and Zn, respectively. The Ag SEM concentration was <0.008  $\mu\text{mol/g}$ , and the mean  $\Sigma\text{SEM}$  was 0.777  $\mu\text{mol/g}$ .

In sediment binding and equilibration studies, AgNO<sub>3</sub> solutions were stirred into homogenized sediment contained within 2 L polyethylene containers maintained under a nitrogen atmosphere. Upon stirring, an acrylic peeper containing two 6-mL chambers covered with a 0.2  $\mu\text{m}$  pore size polyethersulfone membrane was placed into the sediment. The peeper chambers contained deaerated deionized water. The sediment was kept in cold storage at 4° C, and was removed at weekly intervals for analysis of pore water within the peeper chambers for Ag and other metals. AgNO<sub>3</sub> was incorporated into the sediment in a series of experiments at levels that resulted in concentrations ranging from 0.1 to 12.8 g AgNO<sub>3</sub>/kg wet sediment.

Based upon observed toxicity of Ag to *C. tentans* in the AgNO<sub>3</sub> toxicity test without sediment present, and the peeper chamber pore water concentrations of Ag and other metals observed in sediment spiked with AgNO<sub>3</sub>, a series of AgNO<sub>3</sub> spiking concentrations was selected for a 10-day sediment toxicity test. The test consisted of nominal bulk sediment concentrations of 1.6, 2.1, 2.8, 3.8, 5.1, 6.8 and 9.0 g AgNO<sub>3</sub>/kg wet sediment, plus sediment and sand performance controls.

For the sediment toxicity test, approximately 100 mL of AgNO<sub>3</sub>-amended sediment was placed into 300 mL high-form screened beakers. The test was performed in a system based upon that of Benoit *et al.* (1993). This system provided for renewal of overlying water to the aquaria at a rate equivalent to approximately eight volume exchanges daily. The beakers were placed into aquaria, which were housed in a 23° C water bath. Three replicate beakers were used for biological testing at each exposure level, and four beakers were used for chemical measurements. The chemistry beakers each received a two-chambered peeper with a 0.2  $\mu\text{m}$  polyethersulfone membrane, positioned to a depth within the sediment such that the lower chamber was buried below the surface of sediment and the upper chamber was in the overlying water. The beakers containing sediment were placed into the toxicity test system for 7 days prior to the introduction of test animals.

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Ten second and third instar *C. tentans* larvae were randomly added to each beaker including the chemistry beakers. Each beaker was fed the equivalent of 6.0 mg of dry solids daily, as in the water-only test. Chemistry beakers were sampled on days 0, 5 and 10 of the toxicity test. Peepers were removed, and the peeper chamber water was analyzed for pH, Ag, Zn, Ni, Pb and Cu. Some samples were also analyzed for concentrations of Fe, ammonia and dissolved organic carbon.

On day 10, the biology test beakers were sieved, survivors counted, and then placed into a drying oven for dry weight determinations. Survival and weight data were statistically analyzed.

## Results

The 10-day LC50 values for *C. tentans* exposed to AgNO<sub>3</sub> in the absence of sediment were 0.063, 0.057 and 0.035 mg/L for total, dissolved and free silver, respectively. Dry weights of surviving larvae were less than half of control larval weights at the two highest exposures, where the mean free silver concentrations were 0.041 and 0.110 mg/L. Free silver was below detection (0.012 mg/L) at the three lowest exposures and the control.

Initial studies of time for Ag to equilibrate in peeper chambers using polyethersulfone membranes and AgNO<sub>3</sub> solutions at various concentrations in both deionized water (no Cl<sup>-</sup>) and laboratory water (4 mg/L Cl<sup>-</sup>) showed poorer equilibration in laboratory water than in deionized water, presumably due to the formation of AgCl precipitate which did not pass through the membrane. Equilibration was improved in laboratory water at high AgNO<sub>3</sub> concentrations (i.e., > 12.2 mg/L) where approximately all of the Cl<sup>-</sup> ions in laboratory water had been precipitated as AgCl, and free Ag<sup>+</sup> was able to pass through the membrane.

The incorporation of AgNO<sub>3</sub> into West Bearskin Lake sediment caused the pore water pH to drop as AgNO<sub>3</sub> concentration increased. Zn, Ni and Pb were displaced in the sediment by Ag, and increased in concentration in the pore water with increased AgNO<sub>3</sub> addition (Table 1). Cu was displaced to a lesser extent. Concentrations of these displaced metals followed the order Zn > Ni > Pb > Cu. Large quantities of Fe were also released from the sediments, with a visible rusty orange precipitate developing in the four highest exposures. Pore water within the peeper chambers contained Fe at concentrations as high as 558 mg/L at the 9.0 g AgNO<sub>3</sub>/kg sediment exposure. The displaced metals and Ag also were present in the overlying water.

Mean survival levels of *C. tentans* larvae were 80.0, 76.7, 63.3, 10.0, 46.7, 26.7, 33.3 and 40.0 percent for the control and AgNO<sub>3</sub>-spiked sediments containing 1.6, 2.1, 2.8, 3.8, 5.1, 6.8 and 9.0 g AgNO<sub>3</sub>/kg wet sediment, respectively. The LC50 was 2.59 g of AgNO<sub>3</sub> added per kg of wet sediment. It was not possible to calculate LC50 values based upon pore water concentrations of silver due to the nature of the

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relationship between Ag concentrations and toxicity. Dry weights of surviving larvae generally decreased with increasing AgNO<sub>3</sub> concentrations up to 5.1 g/kg sediment. However, the weights of survivors at 6.8 and 9.0 g/kg increased relative to the 5.1 g/kg sediment, a pattern that was similar to the pattern for percent survival (Figs. 1 and 2). The observed toxicity was not likely due to elevated ammonia concentrations (high of 15.2 mg/L), but may have been due to an additive effect of the metals displaced from the sediment to the pore water, plus pore water Ag at the higher exposures, in combination with other stressors such as reduced pH.

It appears that West Bearskin Lake sediment has several binding sites for Ag that effectively hold Ag in association with the particulate or undissolved phases. *C. tentans* larvae exposed to 1.6 g AgNO<sub>3</sub>/kg sediment were similar to controls in their survival and dry weights following a 10-day exposure. This concentration of Ag in the sediment is two to three orders of magnitude higher than concentrations typically measured in aquatic sediments near cities (Eisler, 1996).

## References

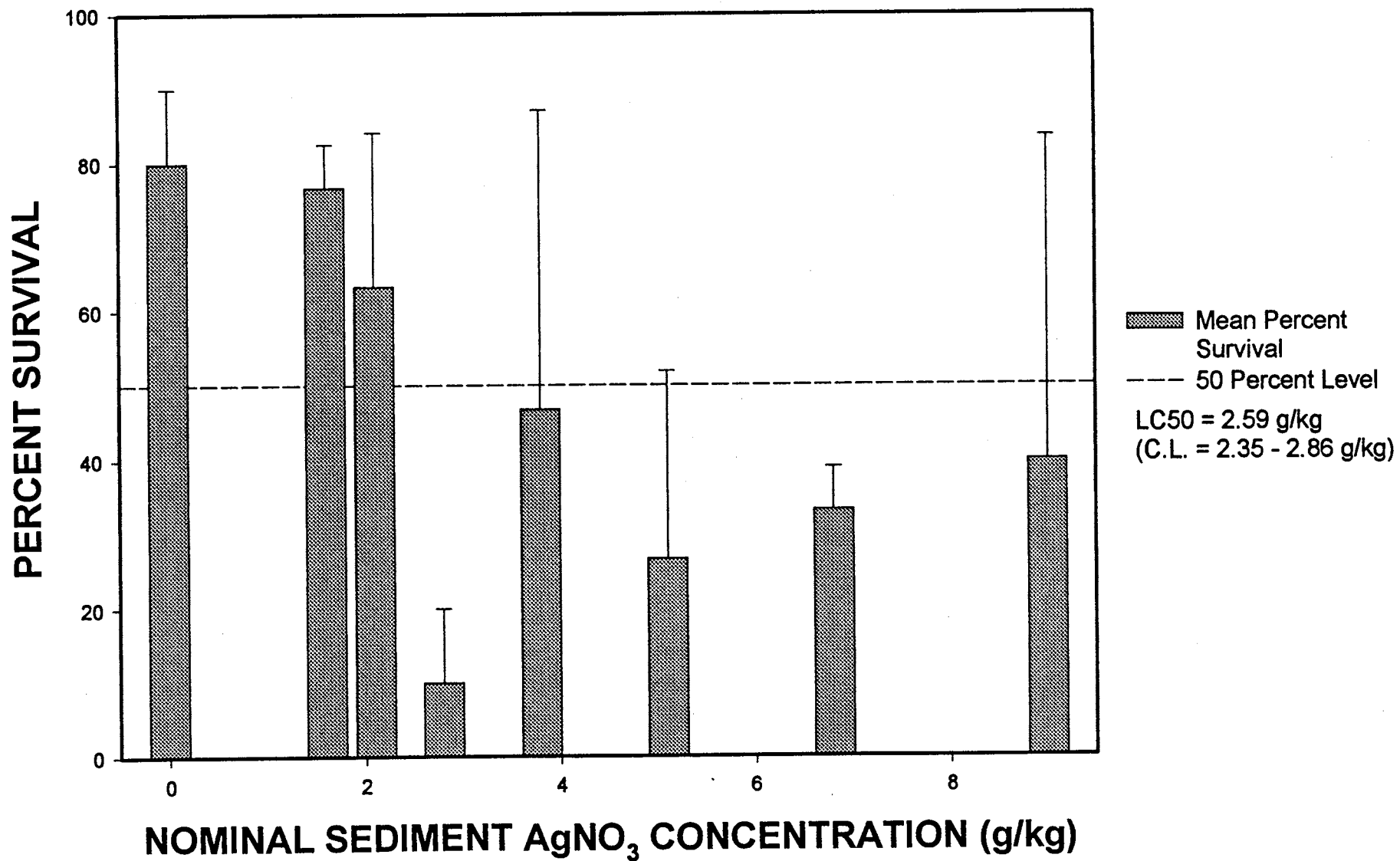
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**Table 1.** Mean concentrations of chemical parameters measured from peeper chambers during a 10-day toxicity test with *C.tentans* exposed to silver nitrate spiked sediment. OW refers to overlying water, PW refers to pore water.

Spiking Conc. (g/kg wet sed)	pH	Ag (ppm)	Zn (ppm)	Ni (ppm)	Cu (ppm)	Pb (ppm)	NH <sub>3</sub> (ppm)	NPDOC <sup>1</sup> (ppm)
Control OW	7.67	<0.005	<0.005	<0.022	<0.006	<0.018	1.72	2.59
Control PW	7.07	<0.005	<0.005	<0.022	<0.006	<0.018	4.32	17.08
1.6 OW	7.78	<0.005	<0.005	<0.022	<0.006	<0.018	-	2.07
1.6 PW	6.68	<0.005	0.005	<0.022	<0.006	<0.018	-	5.01
2.1 OW	7.62	0.006	<0.005	<0.022	<0.006	<0.018	-	-
2.1 PW	6.44	<0.005	0.023	0.026	<0.006	0.022	-	-
2.8 OW	7.27	0.158	0.007	<0.022	<0.006	<0.018	2.31	-
2.8 PW	6.16	0.299	0.080	0.077	<0.006	0.037	7.81	-
3.8 OW	7.39	0.031	<0.005	<0.022	<0.006	<0.018	-	1.54
3.8 PW	6.02	<0.005	0.139	0.118	<0.006	0.043	-	5.67
5.1 OW	6.72	0.011	0.027	0.024	<0.006	<0.018	-	-
5.1 PW	5.46	0.042	0.438	0.281	<0.006	0.049	-	-
6.8 OW	6.32	0.057	0.123	0.070	<0.006	<0.018	2.01	-
6.8 PW	5.09	0.010	1.23	0.665	0.011	0.067	14.13	-
9.0 OW	5.76	0.209	0.374	0.177	0.044	0.049	-	3.21
9.0 PW	4.26	0.059	3.94	1.64	0.090	0.076	-	10.17

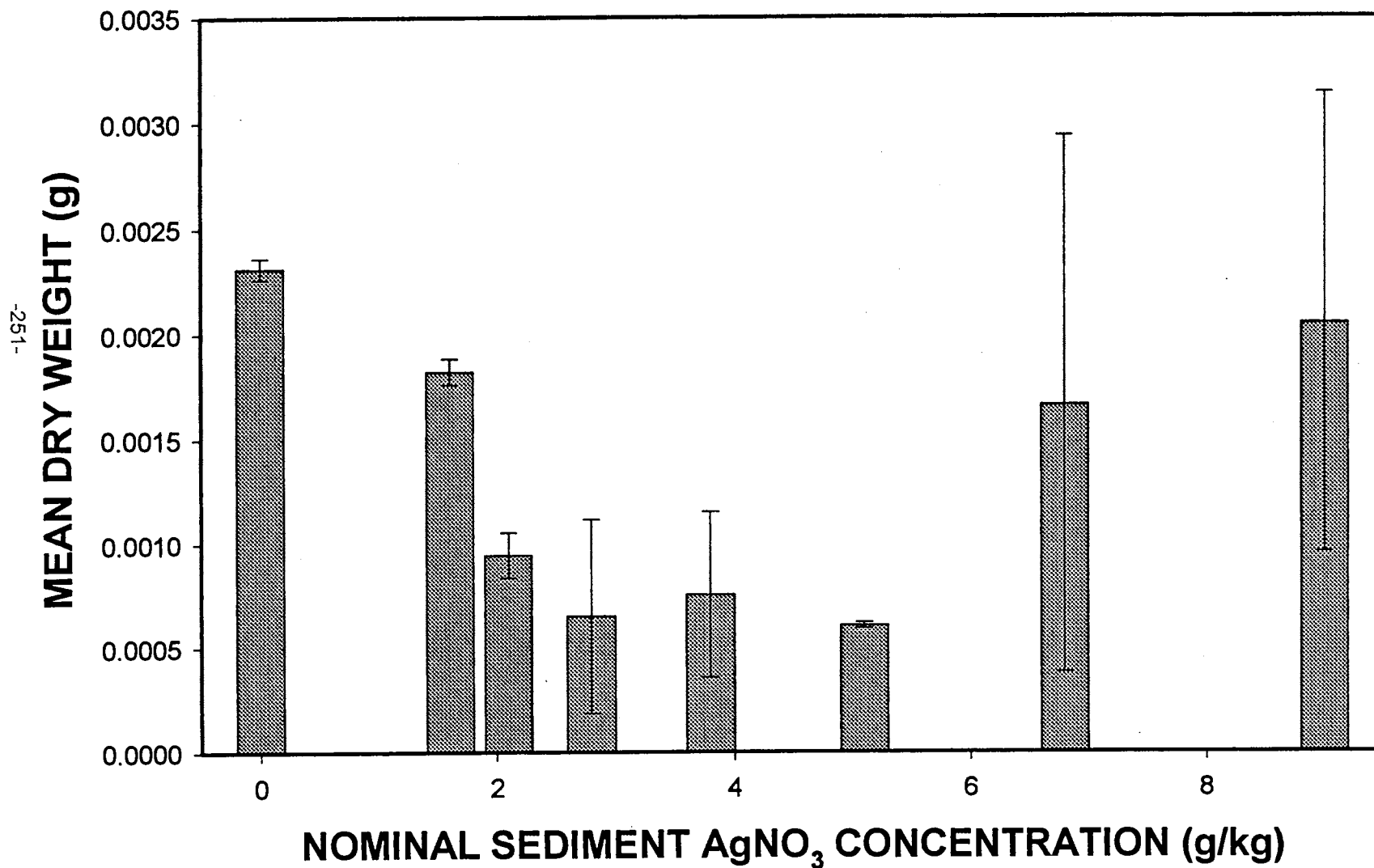
<sup>1</sup> NPDOC refers to Non-Purgeable Dissolved Organic Carbon

**Figure 1. CHIRONOMUS TENTANS SURVIVAL IN AgNO<sub>3</sub> SPIKED SEDIMENT**



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**Figure 2. CHIRONOMUS TENTANS MEAN DRY WEIGHT IN 10-DAY TOXICITY TEST WITH  $\text{AgNO}_3$  - SPIKED SEDIMENTS**



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**Questions & Answers: Bioavailability and Toxicity of Silver to *Chironomus tentans* in Water and Sediments**

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No questions.

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## Physiological Effects of Dietary Silver Exposure: Biologically Incorporated Silver versus Silver Sulphide

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University of Kentucky, Lexington, Kentucky, USA

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### Introduction

The toxic mechanism of acute waterborne exposure to  $\text{AgNO}_3$  has been characterized as a severe branchial ionoregulatory disturbance (Morgan *et al*, 1997) leading to a dramatic decline of both plasma  $\text{Na}^+$  and  $\text{Cl}^-$  concentrations (Wood *et al*, 1996). As a consequence of this disturbance, a secondary hemoconcentration effect arises eventually leading to toxicity. In addition to this acutely toxic effect of silver, previous studies have also demonstrated that silver is readily bioaccumulated during waterborne exposures. Hepatic Ag levels as high as 900  $\mu\text{g Ag/g}$  (~1000-fold increase above controls) were observed in juvenile trout following acute waterborne silver thiosulphate exposure (Hogstrand *et al*, 1997). At present, the physiological effects of bioaccumulated silver in aquatic animals have not been adequately investigated. Furthermore, virtually nothing is known concerning possible deleterious effects of silver as it is passed along the food chain. Consequently, the objective of the first study was to determine the physiological effects of dietary exposure to biologically incorporated silver. Secondly, the physiological effects of dietary silver sulphide exposure were investigated; silver sulphide being the primary form which silver is found in the environment.

### Materials and Methods

Biologically-incorporated silver feeding study. A silver enriched diet (3.10  $\mu\text{g Ag/g}$ ) was prepared from the carcasses of rainbow trout previously exposed to waterborne silver thiosulphate (0.93 mmol/L) (Ag diet) for one week. This silver thiosulphate concentration was achieved by exposing the fish to 0.93 mmol/L  $\text{AgCl}$  and 3.71 mmol/L sodium thiosulphate in dechlorinated Hamilton tapwater. In addition, control diets (~0.05  $\mu\text{g Ag/g}$ ) were formulated using fish held in either Hamilton dechlorinated water (control diet) or Hamilton water containing 3.71 mmol/L sodium thiosulphate alone (thiosulphate diet). A third control produced from commercially manufactured herring meal (herring diet) was used to test the quality of the other diets. In each case (except for herring diet), fish carcasses were dried, ground into a fine powder, and mixed with other ingredients to yield nutritionally-balanced diets. Diet formulations were steam-pelleted to produce commercial-grade food pellets.

Juvenile rainbow trout maintained under ambient conditions (5°C to 11°C) were fed experimental diets over a four month period. Two groups were fed either the control or Ag diet to satiety daily. The additional three groups were given either the control, thiosulphate or herring diet at the same consumption rate on a per fish basis as the Ag treatment (pair-fed controls).

Silver sulphide feeding study. Metal-laden diets were prepared by adding varying amounts of

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silver sulphide to pulverized commercial trout pellets to yield Ag concentrations ranging between 3.0 and 3000  $\mu\text{g Ag/g}$ ; no silver was added to the control diet. Each formulation was repelleted prior to it being administered to fish. Juvenile rainbow trout were fed to satiation once daily over a two month period.

## Results and Discussion

Recap of results to date. At Argentum IV (Madison, Wisconsin; August 25-28, 1996) we reported that no discernable adverse physiological effects were seen in juvenile trout fed dietary silver when presented in a biologically-incorporated form of the metal (Galvez *et al*, 1996). In short, food consumption and growth rates of fish fed the Ag diet were not significantly different from the rates of their simultaneous controls. Moreover, branchial  $\text{Na}^+$  uptake rates, as well as plasma  $\text{Na}^+$  and  $\text{Cl}^-$  levels, remained constant between all treatments. These results contrast with *in vitro* studies by Morgan *et al* (1997) which showed a decrease in  $\text{Na}^+/\text{K}^+$  ATPase activity following waterborne  $\text{AgNO}_3$  exposure and an acute inhibition of  $\text{Na}^+$  uptake. In agreement with this lack of ionoregulatory disturbance, hematological parameters were not affected. It was previously shown that waterborne Ag exposure resulted in significant increases to plasma protein, hematocrit and whole hemoglobin concentrations (Wood *et al*, 1996) as secondary consequences of ionoregulatory impairment. Furthermore, oxygen consumption, and ammonia and urea excretion rates were not altered by dietary Ag exposure. These parameters are considered sensitive indicators of stress in fish, and in addition can yield information concerning preferential energy-utilization of fish under aerobic conditions.

Metal accumulation. Hepatic Ag levels of fish fed the Ag diet were elevated approximately 12-fold above control values following 91 days of exposure (Table 1a). Surprisingly, metallothionein levels (Table 1b) were not significantly different between control and Ag-diet fed fish despite a modest accumulation of Ag in the liver. In comparison, elevated hepatic metallothionein concentrations (~ 30% increase) were produced after only 6 to 7 days of waterborne Ag exposure (Hogstrand *et al*, 1996) during which Ag levels appeared to stabilize at around 20  $\mu\text{g Ag/g}$ . These results suggest that the rate of Ag accumulation may be important in dictating whether metallothionein is induced in response to an elevated metal burden.

Since metallothionein is a cytosolic protein shown to be essential in the homeostasis of metals such as Cu and Zn, these metals were subsequently measured to test whether accumulated Ag was altering their regulation. Interestingly, hepatic Cu levels were significantly lower in Ag-diet fed fish on days 16 and 91 (Table 1c), whereas Zn levels were unaffected by the exposure (Table 1d). It is possible that accumulated Ag displaces Cu from intracellular stores.

Dietary Ag exposure also produced elevated levels of Ag in the remaining tissues analyzed, although the rate of accumulation was much slower than observed for the liver. Gill and kidney Ag levels were each over 2-fold higher in the Ag-diet fed fish at days 91 and 122 when compared to controls (Table 1e, f). This apparent accumulation of Ag in these tissues is partly due to a gradual decrease in Ag levels of control fish over time. Although delivery of Ag to the gills (no accumulation via the water) and kidney is expected to occur via the plasma, plasma Ag concentrations are not elevated in the Ag-diet fed fish until day 122. By the end of the experiment, Ag levels in the plasma were increased approximately 4-fold (2.0  $\mu\text{mol/L}$ ) over control concentrations. In comparison, Wood *et al* (1996) noted that plasma levels reached

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equilibrium at approximately 2.0  $\mu\text{mol/L}$  Ag after only 2 to 3 days of acute waterborne  $\text{AgNO}_3$  exposure.

#### Preliminary results from silver sulphide feeding study

Preliminary results from the silver sulphide feeding study suggest that levels as high as 3000  $\mu\text{g/g}$  Ag (as  $\text{Ag}_2\text{S}$ ) in the diet are benign to juvenile trout over a two month exposure. There were no significant differences between the growth rates of any of the Ag-diet treatments and their simultaneous control. In addition, no toxicity was associated with this elevated silver burden in the diet. The only observed effect was a dose-dependent decrease in food consumption with increased levels of silver in the diet. This decrease in food consumption was approximately 26% for fish fed the 3000  $\mu\text{g/g}$  Ag diet. It is presently unclear whether this result represents a sublethal effect of the dietary silver to juvenile trout, or possibly an effect produced by decreased palatability of the silver-laden food.

Acknowledgements: This work was supported by grants from Kodak Canada and the NSERC Industrially Oriented Research Program.

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**Table 1.** (a) Hepatic silver, (b) hepatic metallothionein, (c) hepatic copper, (d) hepatic zinc, (e) gill silver and (f) kidney silver concentrations of juvenile rainbow trout fed either a control or silver-enriched diet. For each tissue, control levels are given in the top rows (unshaded), whereas the silver treatment values are shown in the bottom rows (shaded). Mean values  $\pm$  SE are expressed in  $\mu\text{g/g}$  weight units ( $n=10$ ) for fish sampled on days 0, 16, 39, 91 and 122.  
\*  $p<0.05$

	<b>Tissue</b>	<b>Day 0</b>	<b>Day 16</b>	<b>Day 39</b>	<b>Day 91</b>	<b>Day 122</b>
<b>(a)</b>	<b>Hepatic Silver</b>	0.19 $\pm$ 0.02	0.24 $\pm$ 0.03	0.30 $\pm$ 0.04	0.24 $\pm$ 0.04	
		0.18 $\pm$ 0.02	0.27 $\pm$ 0.04	1.57 $\pm$ 0.17 *	4.92 $\pm$ 0.60 *	
<b>(b)</b>	<b>Hepatic Metallothionein</b>	86.1 $\pm$ 15.4	97.8 $\pm$ 16.9	84.8 $\pm$ 11.2	96.6 $\pm$ 21.0	60.9 $\pm$ 16.0
		51.3 $\pm$ 16.2	84.5 $\pm$ 8.9	69.8 $\pm$ 7.6	99.2 $\pm$ 20.3	55.2 $\pm$ 8.7
<b>(c)</b>	<b>Hepatic Copper</b>	12.9 $\pm$ 1.2	21.9 $\pm$ 1.0	20.2 $\pm$ 1.1	35.8 $\pm$ 2.7	26.6 $\pm$ 3.3
		14.0 $\pm$ 2.1	14.0 $\pm$ 1.4 *	19.3 $\pm$ 2.7	21.2 $\pm$ 1.7 *	33.1 $\pm$ 8.0
<b>(d)</b>	<b>Hepatic Zinc</b>	12.2 $\pm$ 0.6	15.3 $\pm$ 0.4	21.5 $\pm$ 0.6	24.1 $\pm$ 1.2	
		12.3 $\pm$ 0.8	15.5 $\pm$ 0.8	21.0 $\pm$ 1.5	23.0 $\pm$ 1.6	
<b>(e)</b>	<b>Gill Silver</b>	7.4 $\pm$ 1.3	11.3 $\pm$ 2.2	7.7 $\pm$ 1.2	2.2 $\pm$ 0.7	5.7 $\pm$ 1.1
		10.0 $\pm$ 1.7	8.9 $\pm$ 2.3	7.6 $\pm$ 1.0	9.1 $\pm$ 2.3 *	15.9 $\pm$ 1.9 *
<b>(f)</b>	<b>Kidney Silver</b>	150.4 $\pm$ 31.3	106.4 $\pm$ 37.4	90.4 $\pm$ 32.1	32.9 $\pm$ 2.4	28.8 $\pm$ 5.2
		146.3 $\pm$ 24.8	171.9 $\pm$ 49.5	105.8 $\pm$ 16.7	93.7 $\pm$ 15.5 *	61.0 $\pm$ 13.3 *

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**Questions & Answers: Physiological Effects of Dietary Silver Exposure: Biologically Incorporated Silver Versus Silver Sulphide**

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- Q. JEAN-FRANCOIS GAILLARD (Northwestern University): Do you know what type of metallothionein you were forming in your fish?
- A. We did not actually separate the metallothioneins out. The RIA basically determines both isoforms; it measures both isoforms of the metallothionein.
- Q. But you know they are metallothioneins?
- A. Yes. Because we measured via the RIA which is sensitive to metallothionein.
- Q. Thank you.

## Uptake, Accumulation and Distribution of Silver in Juvenile Rainbow Trout

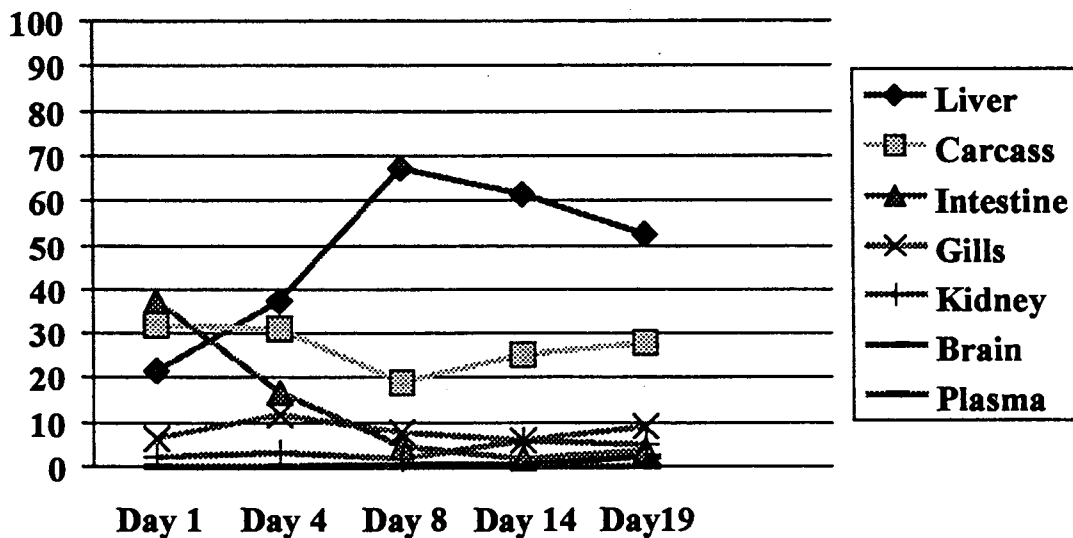
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University of Kentucky, Lexington, Kentucky, USA  
McMaster University, Hamilton, Ontario, Canada

There is a growing concern with pollutants in our environment. Silver, in various complexed forms, is a component of industrial effluent from photomanufacturing and developing processes. As such, silver has gained a following of research that is dedicated to understanding its geochemistry, transport, and fate. Just as it is important to know how and where pollutants reside in the environment, it is also important to understand how these toxicants invade and compartmentalize in affected organisms.

To study Ag uptake and distribution, we subjected juvenile rainbow trout to a two-day radioactive "pulse" of  $2.0\mu\text{g}$  silver as  $^{110\text{m}}\text{Ag}$  followed by a subsequent exposure of  $0.5\mu\text{g Ag/L}$  (as  $\text{AgNO}_3$ ). Samples were then analyzed for the distribution of labeled Ag within the organism. This study was subdivided into three parts, including distribution of silver between; 1) tissues, 2) subcellular compartments, and 3) cytosolic proteins.

For part one of this study, fish were sampled on days 1, 4, 8, 14, and 19 after the initial two-day radioactive "pulse". Gills, liver, kidney, brain, intestine, plasma, and the remaining carcass were dissected and analyzed for  $^{110\text{m}}\text{Ag}$  levels by gamma ray detection. After day 1 of the pulse, the gills were responsible for only a small portion of the total body burden of  $^{110\text{m}}\text{Ag}$ . Early in the experiment, the intestine showed a high activity of  $^{110\text{m}}\text{Ag}$ . However,  $^{110\text{m}}\text{Ag}$  levels in the intestine quickly diminished and were presumably redistributed to other tissues. With the exception of the liver and remaining carcass, other sampled tissues had relatively low  $^{110\text{m}}\text{Ag}$  burdens.

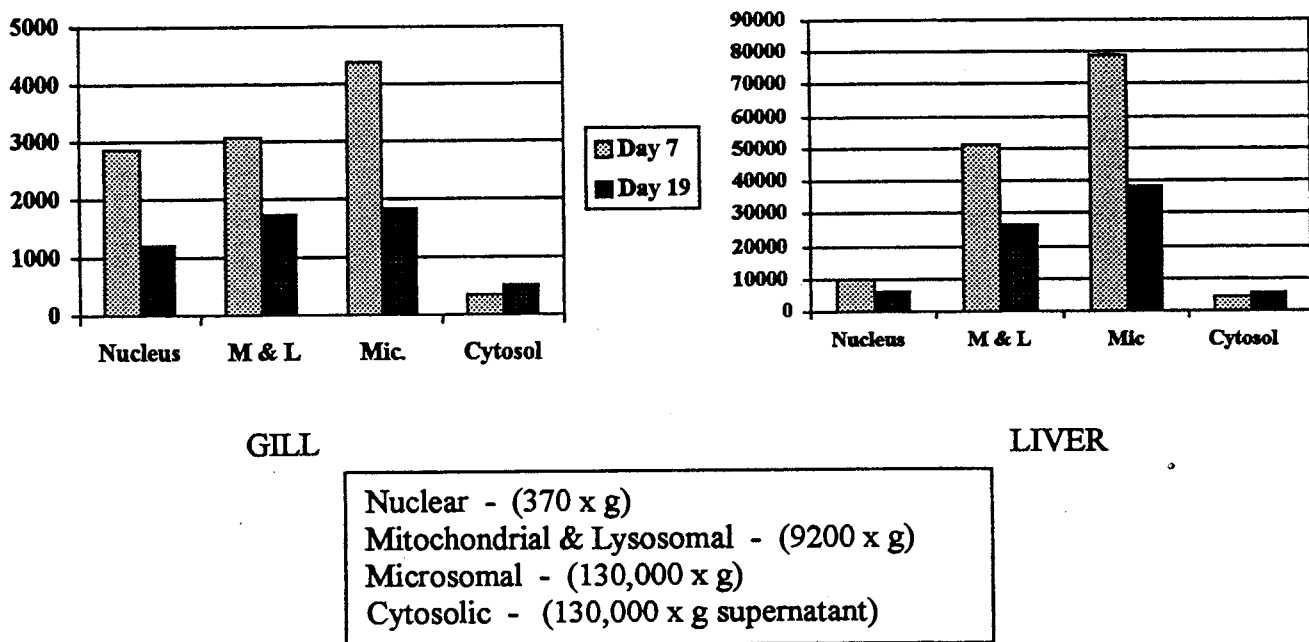
Fig. 1



These findings suggest that Ag enters the gill, is quickly expelled into the bloodstream, and is transiently accumulated in the intestine. From the intestine, Ag is redistributed to, and accumulated in the liver. Brain silver concentrations were virtually undetectable which contradicts previous findings (1).

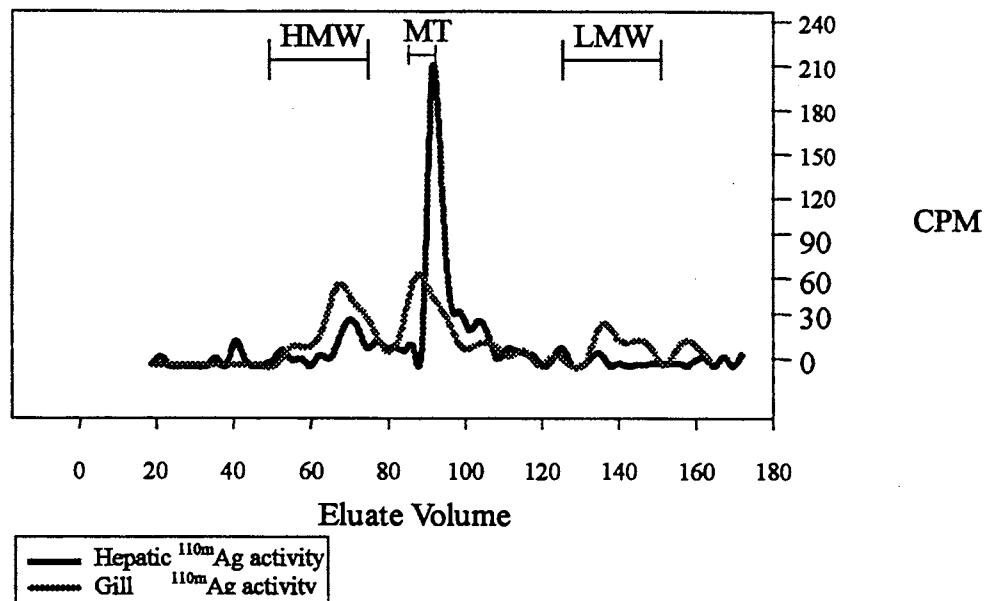
Two key tissues in the uptake, and accumulation of silver were further examined for subcellular distribution of silver. Gills and liver from trout in the same exposure tanks were collected on days 7 and 19 of the study. Tissue homogenates were separated by differential centrifugation, and the resultant fractions were analyzed for  $^{110m}\text{Ag}$  content. Both liver and gills showed an insignificant redistribution of silver to the cytosol from day 7 to day 19. Because of the behavior of other metals (Cu, Zn, Cd) a majority of silver was expected to be in the cytosolic fraction of the cell (2). Contrary to these predictions, a majority of silver was located in the microsomal compartment of the cell. Reasons for the localization of silver in this fraction are presently undetermined. However, a possible explanation could be allied to the incorporation of silver into sulfur-rich inclusion bodies.

Fig. 2



Further separation of the cytosolic component of the gills and livers was performed utilizing size exclusion chromatography. The prepared cytosolic fractions were separated using Superdex-75 media (Pharmacia) on a 60cm x 1.6cm gel filtration column. Fractions were then analyzed for  $^{110m}\text{Ag}$  content in conjunction with their respective eluate volumes. A majority of  $^{110m}\text{Ag}$  content in liver cytosol was eluted concomitantly with metallothionein (MT).  $^{110m}\text{Ag}$  in the gill cytosol was eluted in the MT fraction as well as with high and low molecular weight proteins, as demonstrated by the following representative chromatograph.

Fig. 3



The right X-axis corresponds to the <sup>110m</sup>Ag concentrations in both the hepatic and gill traces. Overhead bars denote regions corresponding to eluates containing high molecular weight proteins (HMW), metallothionein (MT), and low molecular weight proteins (LMW).

The gill is thought to be the primary site for uptake of waterborne metals in freshwater dwelling fish, since these fish do not drink. This model is preserved by comparison of influx amounts of <sup>110m</sup>Ag relative to calculated hypothetical drinking rates. The relatively low levels of accumulated silver in the gills indicate that this element is rapidly eliminated and redistributed to other tissues. Likewise, silver accumulation in the intestine is transient. The liver presents itself as the principal storage compartment for silver, as evidenced by its steady accumulation and relatively high level of <sup>110m</sup>Ag. Presumably, as <sup>110m</sup>Ag levels decline in the liver, silver is being eliminated from the organism. Subcellular distribution studies showed that silver is predominant in the mitochondrial & lysosomal and microsomal fractions of the cell. Size exclusion chromatography indicated that the small amount of cytosolic silver was primarily bound to MT in the liver, while in the gill silver was bound to a range of proteins including MT.

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## Questions & Answers: Uptake, Accumulation and Distribution of Silver in Juvenile Rainbow Trout

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- Q. RUSSELL BELL (McMaster University): Do you have any suggestions as to what species might be migrating out of the gill into the blood stream and then off into the liver and kidney?
- A. No. I would assume free silver ion. We don't know exactly how the silver is pumped through the gill. We assume it's getting out through one of the transporters, possibly the sodium bi-chloride transporter. I would think it might be in an ionic form there, but I would think once it does reach the blood stream it's probably quickly complexed to the serum proteins.
- Q. May I make a suggestion here?
- A. Absolutely.
- Q. My suggestion would be that once your silver ion, be it complexed chloride or amine or carboxylate, gets into your gill, it's going to be tied up with that glutathione like crazy. Because there's a very large amount of glutathione normally in cells.
- A. Yes, we saw that on the chromatograph with the low molecular weight compounds.
- Q. Yes. So I would suggest perhaps even that it's the glutathione species that gets transported out, or one of your other proteins that will have sulfhydryl groups on them.
- A. Okay, thank you.
- Q. MARTIN GROSELL (Risoe National Laboratory, Denmark): As a comment on the question of how silver transports from the gill to, say, the liver, I'm convinced that it will be protein bound. If it behaves any way like, say for instance copper, it will be bound to maybe amino acids, maybe glutathione, for the transport through the organism. We don't know that for sure but that's my bet. And I have a question on the intestine data: how did you compare your samples for counting? Did you dissect out the spleen or is that included in your counting?
- A. No. Actually this included no spleen and no stomach compartment. They're actually snipped and actually scraped. The lumen was scraped.
- Q. JIM KRAMER (McMaster University): Can you give me a ballpark picture of what the value is for the high molecular weight protein material?
- A. The value meaning...?
- Q. How many kilodaltons?
- A. Oh, yes. Very high. We saw that for metallothionein. It came out at 66 or 6.6 K<sub>d</sub>, so above that - well, 10 - 20,000 and above.
- Q. WALTER BERRY (EPA Narragansett): This may be showing my ignorance but if you had low silver in the gill, very low silver in the plasma, higher silver in the intestine, and then finally in the liver: is there a possibility that it's coming in through the intestine first and then going to the liver from there?

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- A. Very good question. Actually, if you remember in freshwater fish, freshwater fish don't drink. These fish had no diet while they were in the 2-day pulse with the radioactive silver, and once we changed the water there was really no radioactive silver in the water, as analyzed by water samples. If the fish were stressed and they did gulp water, maybe drink a little bit, they would have to drink so much water, well it would be impossible for them to drink enough water to actually incorporate this first into the intestine and have it accumulate that way.
- Q. FERNANDO GALVEZ (McMaster University): Just a comment. We did some initial calculations, and what we calculated was drinking rate, a very liberal drinking rate of 10 ml per kilogram per hour, which is threefold higher than seawater fish would ever drink, I believe. It would only account for about 5% of the total count, so since we didn't feed the fish it would mean that the radioactivity is getting through the gills.
- A. Thanks, Fernando.
- Q. NICK BURY (McMaster University): Did you measure the cold silver in your samples?
- A. We did. Actually, they were very much in line with what Fernando measured from the feeding. We did it by AA, and they were proportionately in line with what we saw in the hot silver. I have the numbers, but I don't know them offhand.
- Q. When you look at the tissues you need to look at specific activity between the hot silver and the cold silver to get the true distribution.
- A. Right, absolutely. But what I was saying was, percentage-wise they were right in line with the cold silver distribution.



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## Silver Accumulation and Toxicity in Marine and Freshwater Zooplankton

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If toxicity tests are to accurately predict the impact of a contaminant, contaminants must be tested at environmentally realistic concentrations using all possible routes of exposure. We have conducted a preliminary series of bioaccumulation and toxicity experiments in which we have assessed the lethal and sublethal toxicity of Ag obtained via dissolved and ingested pathways to marine and freshwater zooplankton. Marine copepods *Acartia tonsa* and *A. hudsonica* and the freshwater cladoceran *Simocephalus* sp. were exposed to silver via the dissolved phase and through phytoplankton food. Total Ag body burdens were determined using the gamma-emitting radioisotope  $^{110m}\text{Ag}$  and radiotracer methodology. Copepods were exposed to solutions with Ag concentrations of 0, 0.5 nM, 1 nM, 2 nM and 5 nM, added as  $\text{AgNO}_3$ . Additionally, copepods were fed phytoplankton cells grown in solutions with these same silver concentrations. Cladocerans and their algal prey were exposed to Ag at 0, 125 pM, 250 pM, 500 pM and 1 nM. Sublethal toxic parameters including egg production rate, growth and survival of juveniles, and response to environmental stimuli were measured.

We determined the accumulation of Ag in phytoplankton cells (for freshwater, the chlorophyte *Chlorella vulgaris*, for seawater *C. autotrophica*) and found Ag concentration factors of about  $10^5$ , comparable to earlier reports (Fisher et al., 1984; Fisher and Reinfelder, 1995). We also determined that the assimilation efficiency of ingested Ag from algal food in both copepods and cladocerans was 16%, and that these animals grazed on phytoplankton at a rate approximately equal to their own body weight per day, comparable to findings of previous studies (Reinfelder and Fisher, 1991; Wang and Fisher, in press; Fisher and Wang, in press). By combining the findings with the Ag concentrations in the algal food, it is possible to express metal toxicity as a function of the Ag body burdens in the animals. This also enables a meaningful body burden-based comparison with Ag toxicity when Ag is obtained from the dissolved phase. And finally, this enables us to relate Ag toxicity with typical background Ag concentrations (approximately  $0.1 \mu\text{g g}^{-1}$  dry wt) in these zooplankton in natural waters.

The uptake pathway of Ag greatly affected the sublethal toxicological response. Egg production was the most sensitive indicator of sublethal toxicity of Ag, but this was only evident when Ag was obtained through the zooplankton's food. When cladocerans were fed algae which had been exposed to Ag at concentrations ranging from 0 to 1000 pM, egg production declined significantly when the cladoceran Ag concentration was  $0.172 \mu\text{g g}^{-1}$  dry wt, or about 1.7 times natural background levels. When the Ag concentration in the cladocerans reached  $0.24 \mu\text{g g}^{-1}$  dry wt (or 2.4 times background levels), egg production by females decreased by >70% (Fig. 1). By contrast, Ag concentrations in cladocerans as high as  $1.45 \mu\text{g g}^{-1}$  dry wt had no effect on egg

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production when the Ag was obtained solely from the dissolved phase (Fig. 1). Similarly, egg production by female copepods decreased significantly when Ag body burdens were  $0.39 \mu\text{g g}^{-1}$  dry wt or higher, but only if the Ag was obtained from food. When the Ag was obtained from the dissolved phase, there was no reduction in egg production in copepods. The toxicity of Ag as determined by the hatching rate of produced copepod eggs indicated that only the Ag obtained from food was toxic; no change in hatching rate was observed when parents or eggs were exposed to dissolved Ag (Fig. 2). Standard  $\text{LC}_{50}$  values using dissolved Ag indicated acutely toxic levels to be  $\geq 1 \mu\text{M}$  for marine water, and  $\geq 100 \text{ nM}$  for freshwater (Fig. 3), 3-4 orders of magnitude above sublethal concentrations. However, it is very difficult to evaluate such tests because many individuals starve after food deprivation, so there was substantial mortality even in control cultures. Preliminary experiments indicated that behavioral responses and larval development were not impacted by Ag exposure, regardless of the route of exposure, but additional experiments are underway to examine this further.

It is clear from these experiments that low levels of Ag, when ingested, can be toxic to both marine and freshwater zooplankton, and further that consideration of only the dissolved source term of Ag is inadequate for protection of these species. In the cases considered here, application of "safety factors" toward  $\text{LC}_{50}$  values, particularly those based on dissolved Ag concentrations, can lead to very misleading conclusions. By reducing egg production by females, Ag could have profound effects on zooplankton populations without having any detectable effects on growth rate or survival of juvenile or adult stage animals. We propose that by considering the routes of Ag uptake and the body burden of Ag (rather than ambient dissolved Ag concentration), a more realistic appraisal of Ag toxicity, including sublethal effects, becomes feasible.

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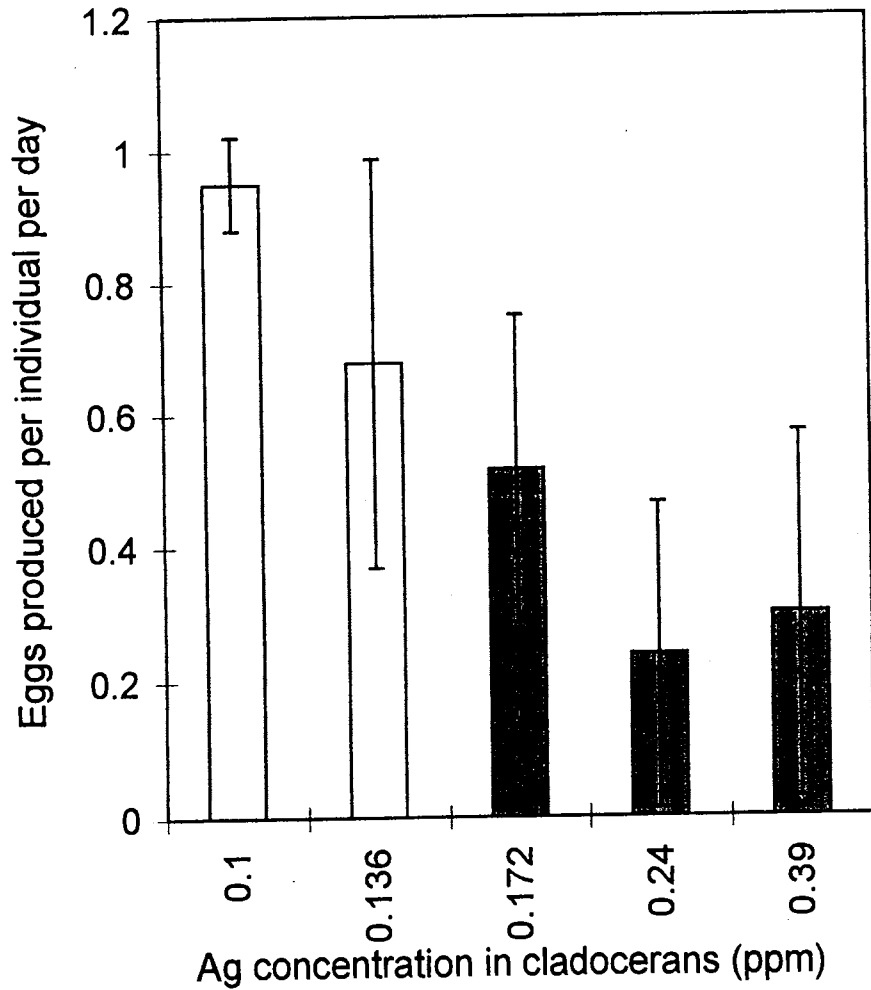
## Figure Legends

Fig. 1. Effects of different Ag concentrations on egg production by the freshwater cladoceran *Simocephalus* sp. The cladocerans were exposed to Ag via consumption of Ag-containing *Chlorella vulgaris* (food pathway) or via exposure to dissolved Ag. Egg production is shown as a function of the body burden of Ag in the adults. Data are means of five replicates  $\pm$  1 standard deviation. Shaded bars are significantly different ( $P < 0.05$ ) from other treatments.

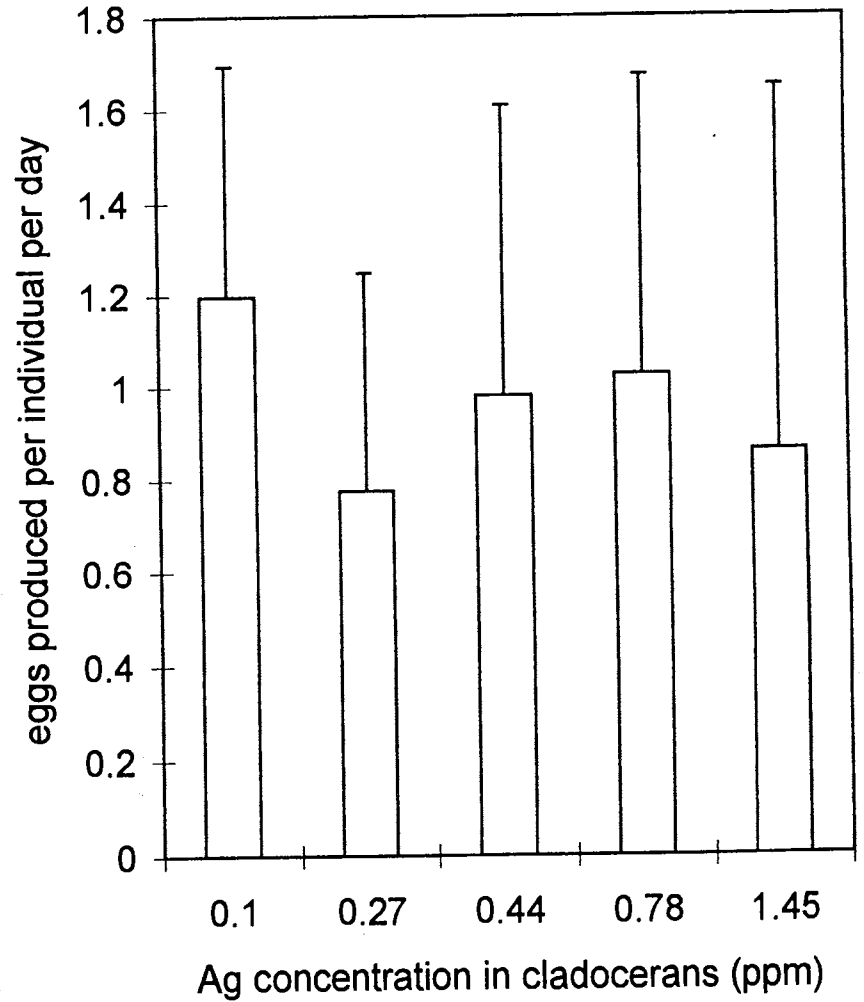
Fig. 2. Effects of different Ag concentrations on the frequency of hatching of produced eggs by the marine copepods *Acartia tonsa* and *A. hudsonica*. The copepods were exposed to Ag via consumption of Ag-containing *Chlorella autotrophica*; values shown are as a function of Ag body burden in the copepods. Also shown are hatching rates for eggs exposed to dissolved Ag. Shaded bars are significantly different ( $P < 0.05$ ) from other treatments.

Fig. 3. Effects of different dissolved Ag concentrations on mortality of cladocerans (*Simocephalus* sp.) and copepods (*Acartia tonsa* and *A. hudsonica*) for 1, 2, 3, and 4-day exposures (copepods) and 1 or 2-day exposures (cladocerans). Data are means of three replicates  $\pm$  1 standard deviation.

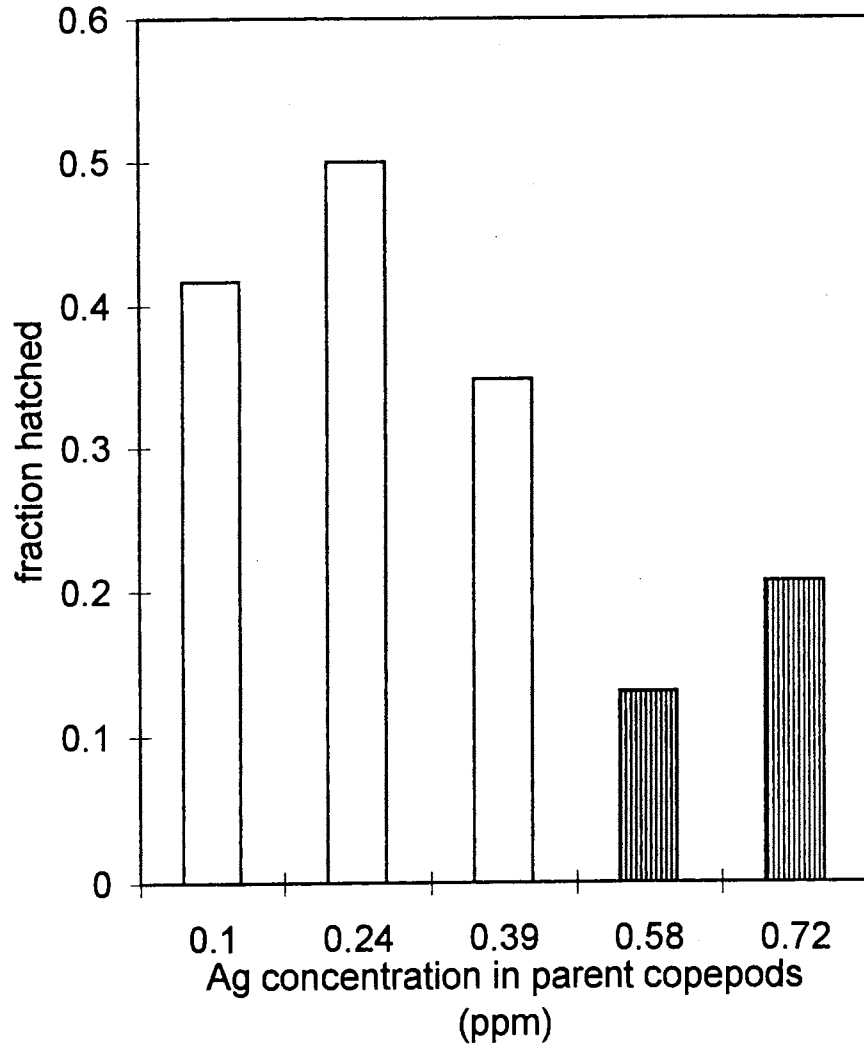
Eggs produced by cladocerans exposed to Ag via food



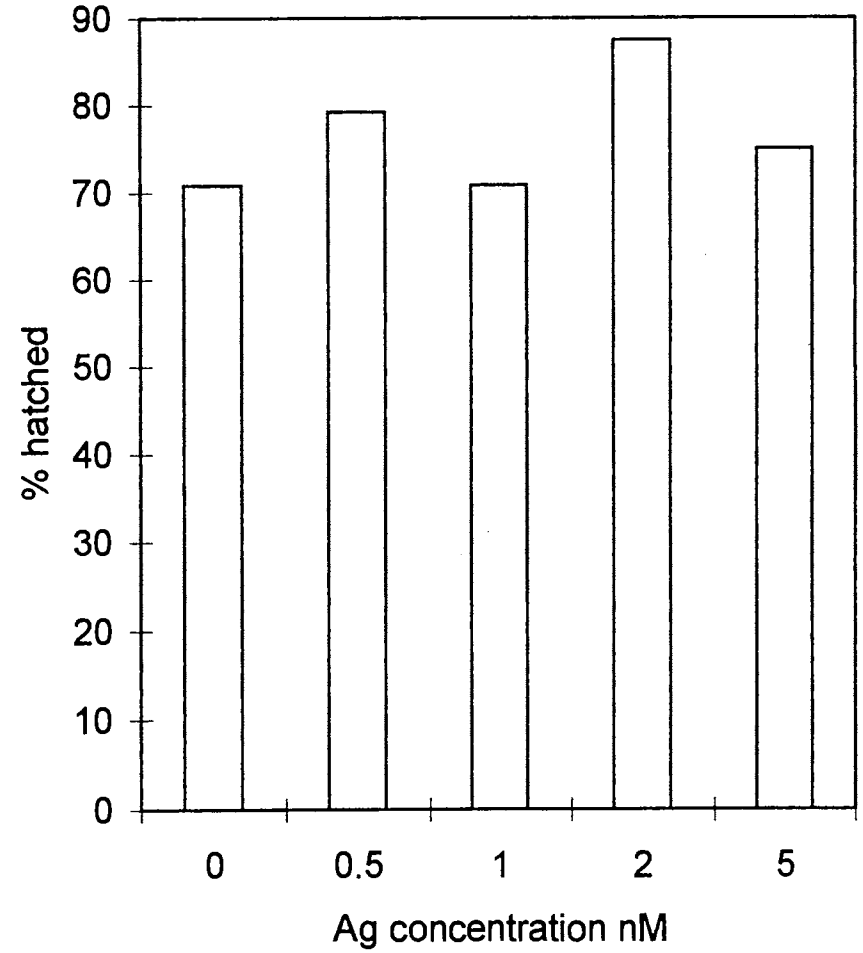
Egg production in cladocerans exposed to dissolved Ag



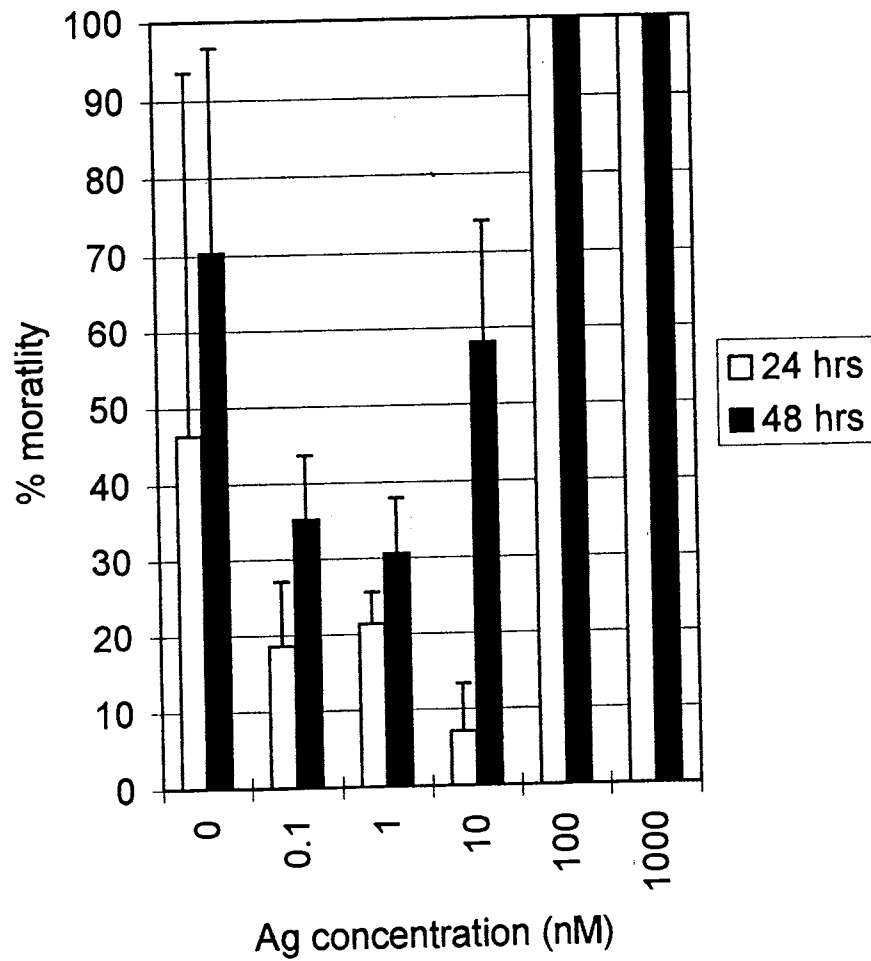
### Hatching rate per silver dose



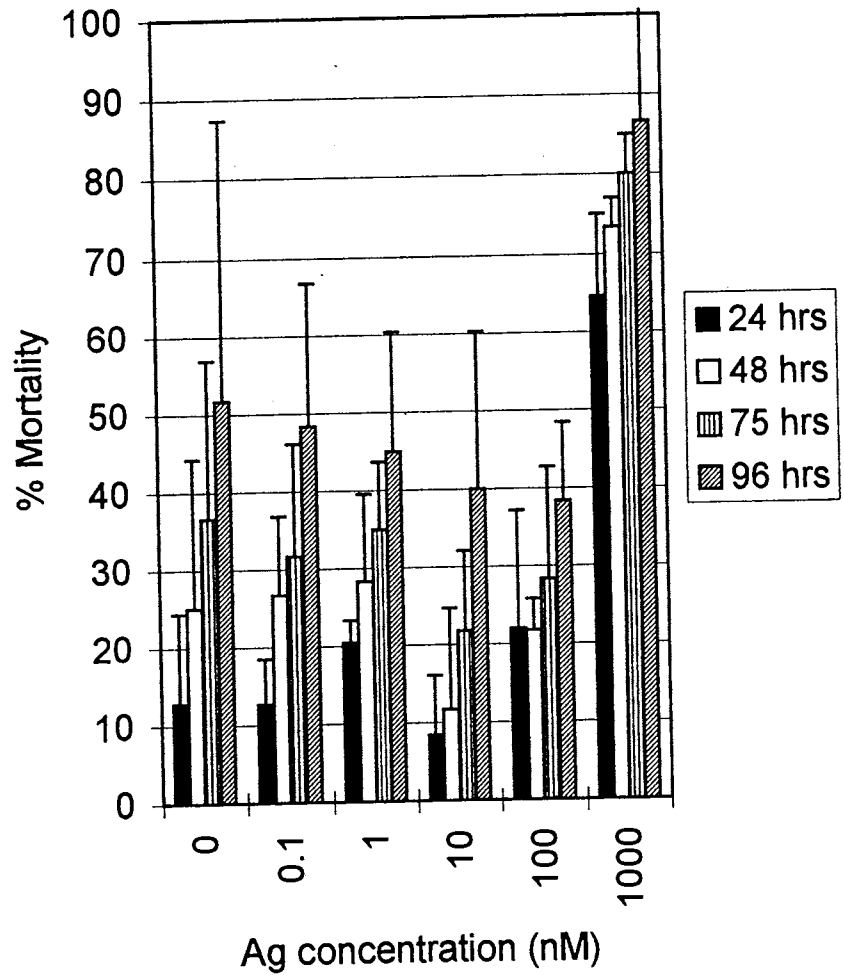
### Hatching rate in eggs exposed to dissolved Ag



% mortality of cladocerans exposed to dissolved Ag



% mortality of copepods exposed to dissolved Ag



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## Questions & Answers: Silver Accumulation and Toxicity in Marine and Freshwater Zooplankton

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- Q. CHRISTER HOGSTRAND (University of Kentucky): Do you have any idea what the difference in handling is? Why did the silver from the water end up in the exoskeleton, and the dietary silver end up elsewhere?
- A. We're just starting to essentially try to discern, using radio-labeled silver, where the silver goes. The dissolved silver goes primarily on the exoskeleton which is chitinous - lots of amino groups there. There may be some sulfur as well, I don't know. But almost all the dissolved silver does go on the chitinous exoskeleton. Almost all of the ingested silver, in our experiments, goes in the "soft" parts of the animal, virtually all of that in the polar fraction. There's virtually none in the non-polar fraction. That's as far as we've characterized it so far. When we do the feeding experiments, the experiments are conducted in such a way that we tried to present the silver to the animals only in the food. But even though we keep the feeding exposure short, there's a certain amount of bleeding of the silver off the food into the dissolved phase during that feeding period. We monitor that, so we know exactly how much of the silver the copepods are getting from the dissolved phase versus from food. It's about 80-90% from food, but there is some that goes onto the exoskeleton following exposure from food. That's because of the release from the dissolved phase and sorption onto the exoskeleton. But what particular metabolic pathways are being impacted, we have no idea. This is like 3-4 week old data, most of it.
- Q. As you know, one problem of looking at chronic toxicity or chronic effects of any contaminant on an animal is to know where to look, and I think you found a very nice system there with the egg production. It really would be interesting to know what the silver is doing in these animals so perhaps we can start to look for the same kind of targets in other animals.
- A. Yes, I agree and this will be the subject of Sharon Hook's PhD thesis.
- Q. DOMINIC DI TORO (Manhattan College): I think you're on to something, Nick. I was calculating in my head 200 picomolar or 2/10 of a nanomolar - 20 micrograms per litre, atomic weight of 100. Twenty micrograms per litre is around the acute marine water quality criteria if memory serves, right Walter, something like that?
- A. WALTER BERRY (EPA Narragansett): 2.3.
- Q. DOMINIC DI TORO: Oh, 2.3 is the chronic marine?
- A. WALTER BERRY: There is no chronic marine.
- Q. DOMINIC DI TORO: There is no chronic marine? Okay, so you're within a factor of 10 of the acute. All right, but you haven't got a dose response yet; those concentrations are moving by decades, right?
- A. Yes. See, our feeling is that, because of all the complications we heard about in the first day, complications with silver speciation, you measure the ambient silver concentration and you don't know how much of that is actually going to end up in the organism.
- Q. Don't confuse yourself with all of that.
- A. No, I'm not (*laughter*). What I'm saying is that if you assess the silver toxicity as a function of the silver in the animal, that's the key thing. And I'm saying that if the uptake is from the dissolved phase, no matter what the concentration, we just don't really see an effect. But if the uptake is from food, if you increase the silver concentration in the animal, about double to a factor of 5, you get a depressed egg production.
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- Q. Sure. But I mean the way I thought about it was this: I mean that's interesting, but, in trying to assess the applicability to real situations, you ask yourself, what is the concentration in the sea water which then gets the concentration in the algae to the point where it gets the concentration in the copepods to the point where they're getting sick? And that number in your experiment was 10 parts per billion, yes?
- A. No. 200 picomolar which is ..
- Q. Multiply it by 100 and that gives 0.2 - that gives you 20 micrograms per liter. No, I mean that's 20 nanograms per liter.
- A. Right. 20 nanograms per liter, that's 20 parts per trillion.
- Q. 20 nanograms per liter? You're sitting here telling me that 20 nanograms per liter...
- A. Yes. And I think this is why we're concerned. I mean, it's very nice to do studies and it's certainly easy to measure impacts when you're working with a thousandfold higher concentration.
- Q. Oh sure, that's why I popped up.
- A. Right. But in fact, the concentrations in the real world are in the typically one to 10's of picomolar.
- Q. In your real world, but in the polluted real world...
- A. No, no, no. In the real world that the chemists go out and measure.
- Q. Yes, I understand. But I mean, if you take a look... I'm trying to put this in perspective for you, to point out that you're on to something I think is pretty hot. You're seeing an effect at concentrations that are much lower than anybody has seen effects at.
- A. Yes.
- Q. Is that true?
- A. Yes.
- Q. Well then, I think that's something we ought to focus on, because this is an order of magnitude kind of game.
- A. Yes, that's right.
- Q. Very good, it's very nice.
- A. Thank you.
- Q. JIM KRAMER (McMaster University): I want to make things complicated. Maybe you've seen this paper from the September issue of *Science* - it says disulfide perturbation increases cysteine and glutathione secretion. And of course the minute we think of cysteine and glutathione we think of good binds for silver. This is at a cellular level. Nice piece of work but my question is, perhaps it's very important in these kinds of studies to specify the redox conditions in general in some global term. Because there may be other factors in terms of accommodation pathways. So my question is Nick, what generally can you tell us about this? Generally, are these all oxic experiments?
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- A. Yes. Oxygenated sea water. The sea water was collected 8 km offshore, surface Atlantic water, which was filtered two times through a 0.2 micron polycarbonate membrane and not amended with anything. Typically collected fairly freshly, that is within a week of the experiments. The fresh water was a model fresh water, it was oxic as well, WLC1 medium.
- Q. Okay. But do you agree that we should be looking at lower redox conditions, at least for the sediment interface?
- A. No question.
- Q. My guess is that we're going to see a lot of different ...
- A. I totally agree with that. However, these animals live up in surface waters for the most part.
- Q. CHRIS WOOD (McMaster University): I think this is just remarkable. What can the oceanographers tell us about the concentrations of silver in algae or phytoplankton in the sea?
- A. That's a great question. There's essentially one good number and that was, believe it or not, collected by John Martin 24 years ago. We have recently conducted a series of cruises, collected water and phytoplankton and zooplankton samples, and those are currently being measured now. I don't recall offhand what the concentrations are of the silver in the phytoplankton. I guess I can tell you that the concentration factors - that may be an easy way to do it - are on the order of  $10^5$  on a volume - volume basis, or about 3 or 4 times  $10^5$  on a dry weight basis. So, if you know what the dissolved concentration is in the water, the concentration factors are not very sensitive to a range of dissolved concentrations at environmentally realistic levels. So you can calculate concentration factors. I don't remember offhand what they are. But there's really only been one number out there to date, and so we're attempting to produce more numbers. How good they'll be I don't know. It's very difficult using trace metal clean techniques to get a perfect sample. We've spent a lot of time cleaning up our lab and sampling apparatus, and the data are being analyzed as we speak. Yes, it's a good question. We're trying to get that number right now.

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## Influence of Water Quality Parameters on Silver Toxicity to Rainbow Trout, *Oncorhynchus mykiss*

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Effluent from anthropogenic sources often contains silver thiosulfate and silver chloride complexes. Low silver chloride levels are found in POTW effluent whereas silver thiosulfate complexes are converted to silver sulfide resulting in little bioavailable silver. Previous silver toxicity bioassays have indicated ionic silver, typically dosed as silver nitrate, to be highly toxic to aquatic organisms like rainbow trout (*Oncorhynchus mykiss*), fathead minnows (*Pimephales promelas*), and daphnids (*Daphnia magna*). We have quantified various conditions under which silver is toxic to rainbow trout (*O. mykiss*). Such conditions include low chloride, low pH, and low DOC.

A flow-through bioassay system (Figure One) was modified to assess the modulation of various parameters on silver toxicity to rainbow trout (swim-up fry) after 96 hour exposures. The diluter modifications included a first stage, which allowed mixing of two influent water sources. The second stage serially diluted the test chemical so approximately 40, 20, 10, 5, and 2.5  $\mu\text{g AgNO}_3/\text{L}$  were delivered, with a control, to two replicate test chambers. Alkalinity and hardness were maintained, whereas chloride and dissolved organic carbon levels were manipulated to estimate their amelioratory affects. Nominal concentrations of 3, 20, and 40 mg  $\text{Cl}^-/\text{L}$  and 2.5 and 5.0 mg  $\text{DOC}/\text{L}$  (as percent carbon) were used. Graphite Furnace Atomic Adsorption Spectroscopy (GF-AAS) was used to determine total silver concentrations. Titrametric methods were used to determine alkalinity and hardness. Dissolved oxygen, pH, and temperature were measured with appropriate probes. Chloride and DOC concentrations were measured with Ion Chromatography (IC) and thermal oxidation (Shimadzu TOC-5000), respectively. Measured estimates of water quality parameters are summarized with 95% confidence intervals in Table One.

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Twenty day old rainbow trout (approximately) were obtained from three local hatcheries (Walhalla, SC; Erwin, TN, Brevard, NC). On average, age differences of each batch of fish were within three days. Nominal testing conditions remained constant, with a 16:8 light cycle, constant temperature (10-12 °C), constant alkalinity (140 mg/L), DO (8.5 mg/L), pH (9.0), and hardness (30 mg/L, except for elevated hardness test) over the 96 hour test period.

Our 96 hour toxicity bioassays, summarized in Figure Two, indicate that at measured Cl<sup>-</sup> concentrations of 3.82 mg/L, 20.18 mg/L, and 45.27 mg/L and measured DOC concentrations of 2.57 mg/L and 4.6 mg/L, LC<sub>50</sub> estimates are 3.39 µg/L, 2.42 µg/L, 3.76 µg/L, and 5.57 µg/L and 9.46 µg/L, respectively, compared to the (positive) control LC<sub>50</sub> estimate of 1.48 µg/L. Estimates of LC<sub>50</sub>s for two mixture bioassays of chloride and DOC (3.25 mg/L chloride and 2.53 mg/L DOC; 47.74 mg/L chloride and 6.27 mg/L DOC) are 17.07 µg/L and 28.42 µg/L respectively. An LC<sub>50</sub> estimate of 3.58 µg/L for elevated hardness (60.39 mg CaCO<sub>3</sub>/L) is also protective. These estimates are summarized with 95% confidence intervals in Table Two.

Our toxicity assays also show there is a significant mortality decrease in each test after 24 hours. This trend becomes apparent for several tests, in which there is no change in measured toxicity at 48, 72, and 96 hour intervals. Significance comparisons were made using 95% confidence intervals, summarized with respective point estimates in Table Two.

Filtration prior to GF-AAS analysis allowed us to assess the effects of three operationally defined dissolved silver concentrations for any given test. These results, no filtration, filtration with a 0.45 µm Acrodisk, and filtration with a 0.20 µm Acrodisk, are summarized in Figure Three.

A three dimensional surface plot (Figure Four) of mortality, chloride, and DOC also shows the amelioratory effects of chloride and DOC on silver toxicity. Regression analyses of the relationships between mortality vs. DOC and mortality vs. chloride show the enhanced protective effects of DOC over chloride. This is also demonstrated with the graded change in color moving along (increasingly) the X-axis when chloride is absent compared to the absence of any graded color change moving along the Y-axis when DOC is absent. Dissolved organic carbon appears to

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be critical in lessening total silver bioavailability to trout, while Cl<sup>-</sup> and hardness have only a minor protective effect. However, Cl<sup>-</sup>/DOC mixtures show a synergistic protective effect. Our results indicate that environmentally realistic concentrations of aqueous silver pose no problem to larval trout.

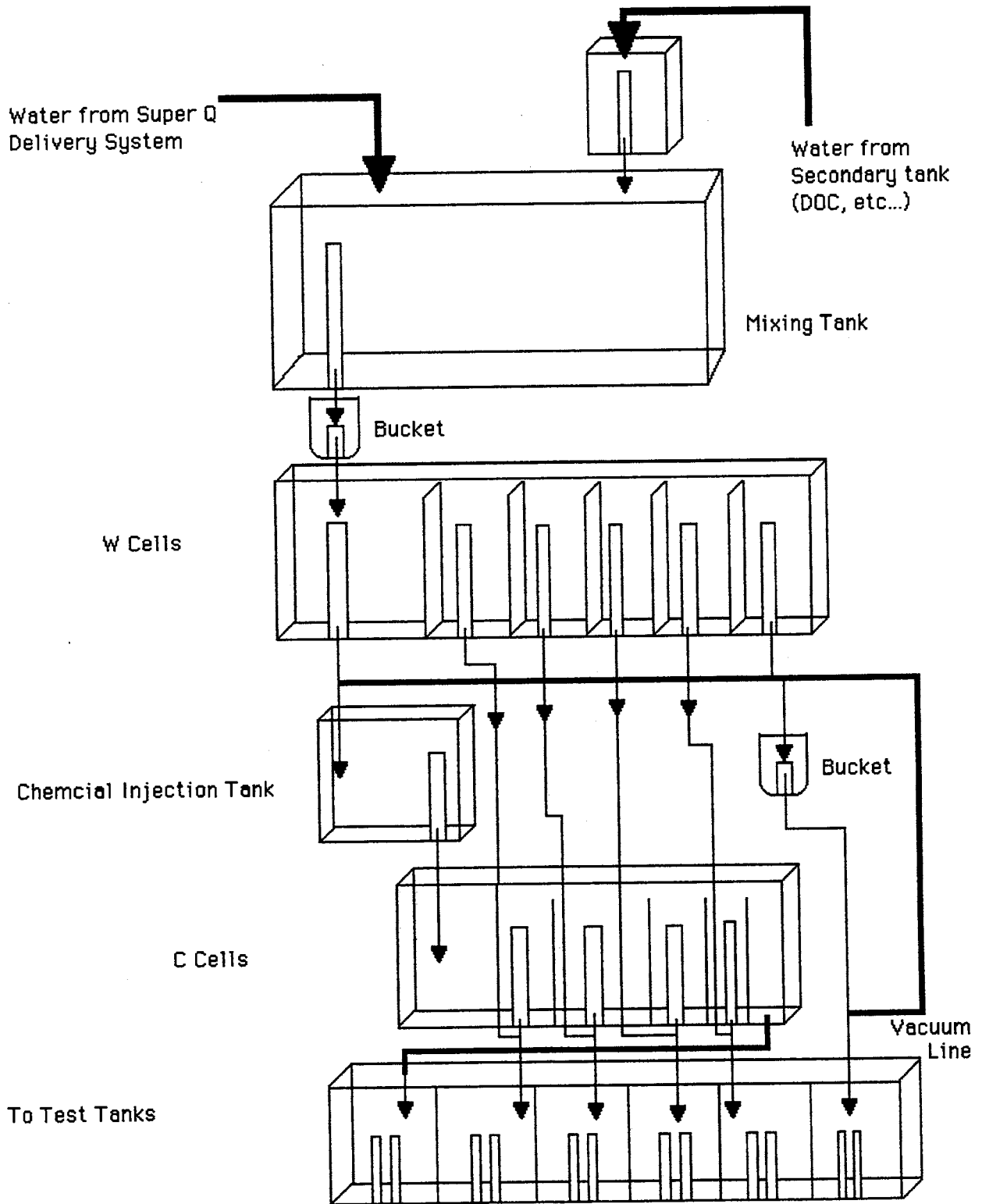
Test	Parameter	Mean	95% LCL	95% UCL
CONTROL	DO	9.603	9.538388	9.667612
	pH	9.312	9.294176	9.329824
	Temp	13.192	12.34536	14.03864
	Alkalinity	159	157.4003	160.5997
	Hardness	27.72	27.72	27.72
	DOC	0.552917	0.137585	0.968249
	Cl <sup>-</sup>	-	-	-
HIGH HARDNESS	DO	8.5417	8.490699	8.592701
	pH	8.8383	8.816048	8.860552
	Temp	13.3417	13.15679	13.52661
	Alkalinity	146	144.2655	147.7345
	Hardness	60.39	59.2381	61.5419
	DOC	0	0	0
	Cl <sup>-</sup>	0	0	0
2.5 mg/L DOC	DO	8.792	8.749668	8.834332
	pH	9.289	9.271176	9.306824
	Temp	10.892	10.71153	11.07247
	Alkalinity	185.333	180.5094	190.1566
	Hardness	43.56	42.02491	45.09509
	DOC	2.568667	1.74301	3.297353
	Cl <sup>-</sup>	-	-	-
5.0 mg/L DOC	DO	8.808	8.750072	8.865928
	pH	9.135	9.121632	9.148368
	Temp	13.042	12.97293	13.11107
	Alkalinity	169.667	167.947	171.387
	Hardness	36.96	34.97708	38.94292
	DOC	4.599167	4.501367	4.696967
	Cl <sup>-</sup>	0.56583	0.250416	0.881244

3 mg/L Cl-	DO	9.042	8.990756	9.093244
	pH	9.052	9.04086	9.06314
	Temp	13.1	12.99306	13.20694
	Alkalinity	164.667	163.6666	165.6674
	Hardness	31.68	31.68	31.68
	DOC	0	0	0
	Cl <sup>-</sup>	3.8175	3.630586	4.004414
20 mg/L Cl-	DO	8.808	8.790176	8.825824
	pH	9.23	9.216632	9.243368
	Temp	9.467	9.259796	9.674204
	Alkalinity	158	153.544	162.456
	Hardness	29.04	27.78564	30.29436
	DOC	0	0	0
	Cl <sup>-</sup>	20.17917	19.55426	20.80408
40 mg/L Cl-	DO	8.742	8.699668	8.784332
	pH	8.998	8.93116	9.004684
	Temp	10.675	10.49453	10.85547
	Alkalinity	161	159.837	162.163
	Hardness	32.67	31.51812	33.82188
	DOC	0	0	0
	Cl <sup>-</sup>	45.27417	43.07722	47.47112
2.5 DOC 3.0 Cl- mix	DO	8.617	8.569808	8.664192
	pH	8.958	8.938568	8.977432
	Temp	12.367	11.9506	12.7834
	Alkalinity	185.333	176.4193	194.2467
	Hardness	42.9	41.06784	44.73216
	DOC	2.882167	2.36468	3.399654
	Cl <sup>-</sup>	3.25333	3.181774	3.324886
5.0 DOC 40 Cl- mix	DO	9.008	8.938932	9.077068
	pH	9.146	9.137088	9.23512
	Temp	12.45	12.29404	12.60596
	Alkalinity	190.0	187.674	192.326
	Hardness	46.53	45.37812	47.68188
	DOC	6.266667	6.151744	6.38159
	Cl <sup>-</sup>	47.74083	45.86962	49.61204

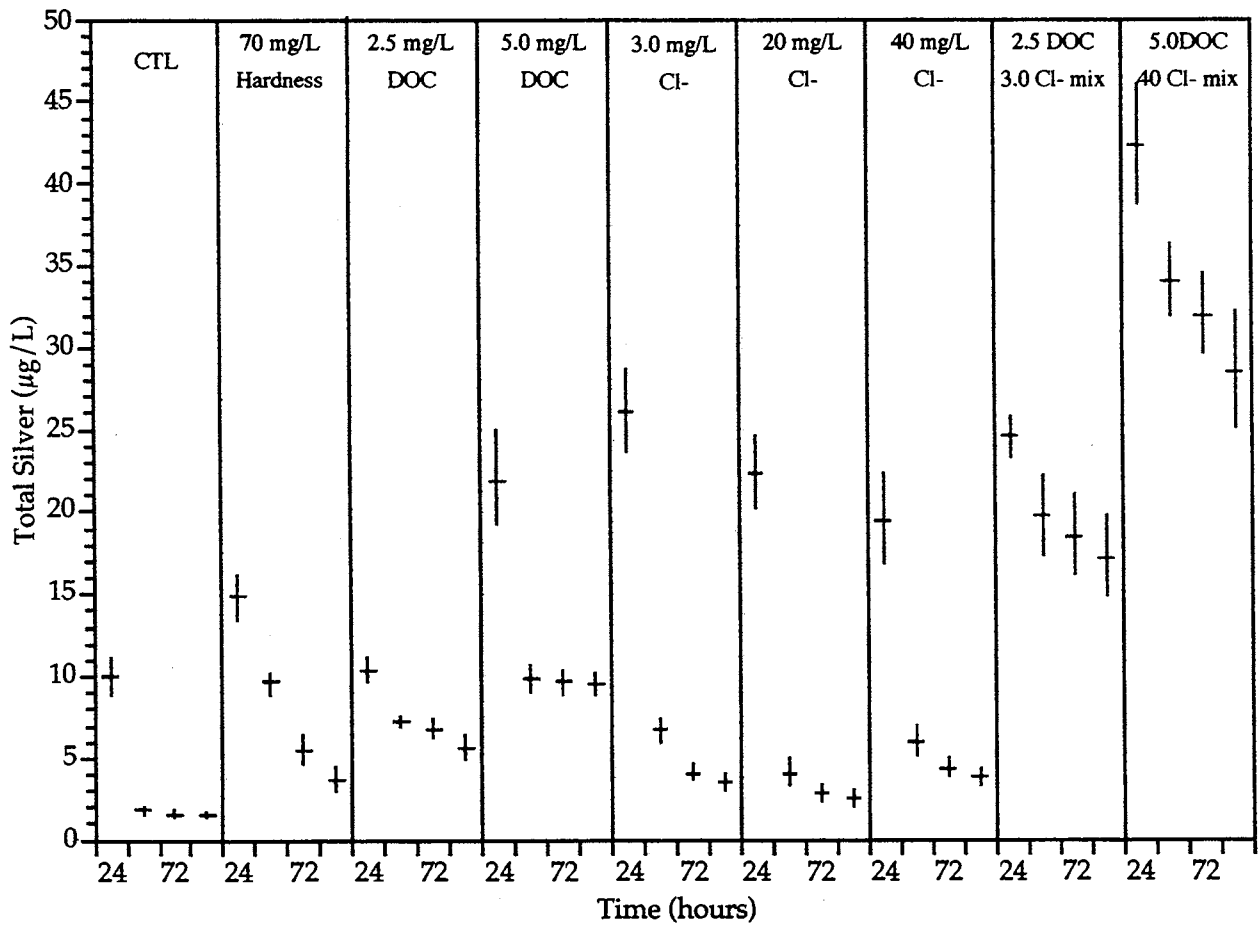
**TABLE ONE** - Measured water quality parameters with 95% confidence intervals for all bioassays. Units are: DO (mg/L), Temp (C), Alkalinity (mg/L), Hardness (mg/L), DOC (mg Carbon/L), Cl<sup>-</sup> (mg/L). Zeros indicate no measurable amount. Dashes indicate unavailable data.

Test	Time (hrs)	LC <sub>50</sub> (ppb)	UCL (ppb)	LCL (ppb)
CONTROL	24	9.939	11.07	8.92
	48	1.767	2.016	1.548
	72	1.553	1.738	1.387
	96	1.48	1.637	1.339
HIGH HARDNESS	24	14.71	16.13	13.42
	48	9.527	10.17	8.921
	72	5.371	6.352	4.541
	96	3.579	4.363	2.936
2.5 mg/L DOC	24	10.29	11.12	9.52
	48	7.156	7.505	6.823
	72	6.711	7.289	6.179
	96	5.571	6.428	4.828
5.0 mg/L DOC	24	21.92	24.92	19.27
	48	9.775	10.53	9.073
	72	9.602	10.32	8.939
	96	9.462	10.16	8.811
3 mg/L Cl <sup>-</sup>	24	26.03	28.78	23.54
	48	6.667	7.399	6.008
	72	4.022	4.574	3.536
	96	3.39	3.836	2.995
20 mg/L Cl <sup>-</sup>	24	22.32	24.59	20.25
	48	4.014	4.826	3.338
	72	2.787	3.369	2.305
	96	2.421	2.907	2.017
40 mg/L DOC	24	19.35	22.29	16.8
	48	5.907	6.793	5.137
	72	4.258	4.862	3.728
	96	3.764	4.264	3.322
2.5 DOC 3.0 Cl <sup>-</sup> mix	24	24.5	25.81	23.25
	48	19.62	22.21	17.33
	72	18.38	21.02	16.08
	96	17.07	19.6	14.87
5.0 DOC 40 Cl <sup>-</sup> mix	24	42.24	46.1	38.71
	48	34.02	36.26	31.91
	72	31.87	34.39	29.53
	96	28.42	32.19	25.09

**TABLE TWO**-Daily LC<sub>50</sub> estimates and 95% confidence intervals for each 96 hour test. These data correspond to Figure One.

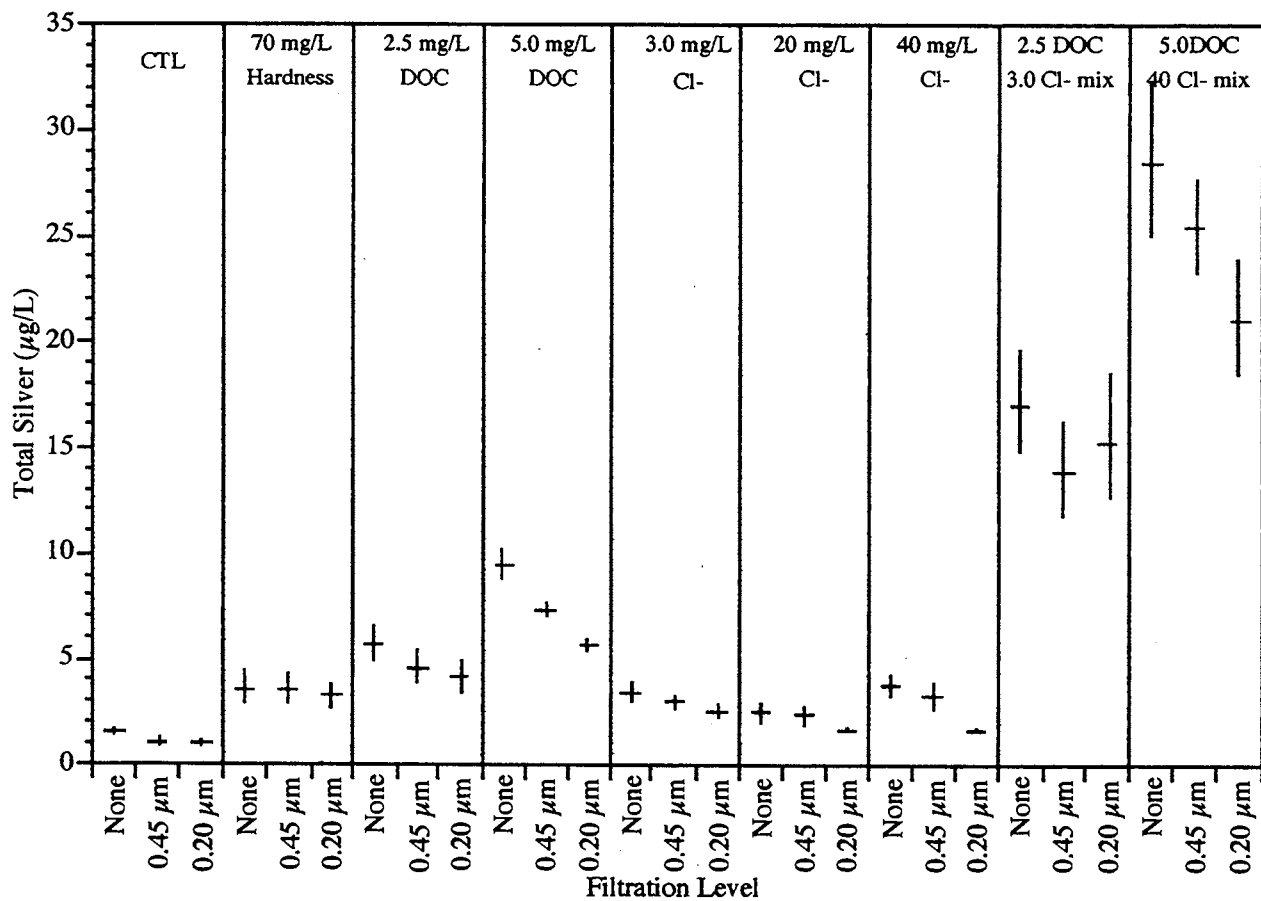


**FIGURE ONE** - Schematic of modifications made to the diluter system. The first stage consists of the mixing tank and small stock tank. The second stage includes the W, C, and F cells.

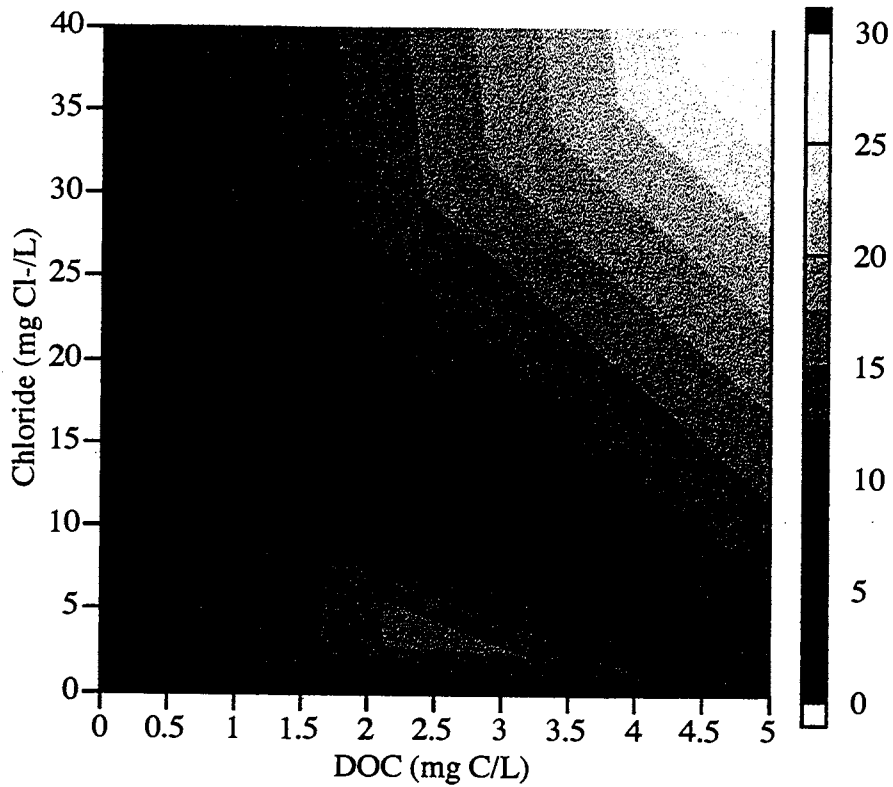


**FIGURE TWO**-Hourly plot of LC<sub>50</sub>s, in µg/L of total silver, with 95% confidence intervals, as estimated by trimmed Spearman-Kärber methods. Groups of four are categorically separated.





**FIGURE THREE**- $LC_{50}$  values with 95% confidence intervals calculated by trimmed Spearman-Kärber using silver concentrations determined following different filtration methods.



**FIGURE FOUR**-Three dimensional plot of DOC, chloride, and estimated 96 hour  $LC_{50}$ s. Silver concentration, and thus the 96 hour  $LC_{50}$ s, increases as color lightens (black to white).

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**Questions & Answers: Influence of Water Quality Parameters on Silver Toxicity to Rainbow Trout, *Oncorhynchus mykiss***

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Q. DAN CALL (University of Wisconsin-Superior): I think with regard to chloride, the fact that the effect wasn't too dramatic might be that the species itself is quite sensitive, and if you had a less sensitive species where the LC<sub>50</sub>'s would have been up probably in the 50 and 100 micrograms per liter you might have a more dramatic chloride effect.

A. We were also using a very young stage of fish, so I'm sure that had an effect as well. It's a good point.

Q. ANDERS ANDREN (University of Wisconsin-Madison): Have you made the calculations that show you are below silver chloride precipitation concentrations in all instances?

A. We had to do that with our setup because, if you remember when I showed you the first stage of the diluter, we were using very concentrated stock solutions to mix and dilute it out, and so that was all made beforehand.

Q. The other question was, you tried to make these experiments so that, everything else being equal, by just changing DOC chloride and alkalinity, how did you keep the pH constant if you changed the alkalinity?

A. We didn't change the alkalinity. The alkalinity was maintained throughout the test at about 140 mg per liter of calcium-carbonate. Oh wait I'm sorry. The other ions we added as calcium sulfate and magnesium carbonate, magnesium sulfate. We took daily measurements to see if the hardness or the alkalinity changed for one of the tests, and in fact it didn't over a 96-hour period, measured every 24 hours. This stuff was made up in batches and then added to our system, so in that sense we tried to maintain the conditions. The pH was a little high, it was around 9 for every test, for each time point.

Q. JIM KRAMER (McMaster University): I was intrigued as you pointed out the fairly rapid change in the LC<sub>50</sub>'s going from 24 hours to longer times. Is it possible that the organism is actually secreting protective ligands?

A. Or under stress. Maybe a mucus layer, as we've seen from some of the work that's been presented, that under stress you might actually see acute toxic effects from an overproduction of mucus cells.

Q. Do you plan to follow this up? I think this is rather important.

A. It would be very interesting to do that. Right now I don't have any plans to do that, but it may evolve to something like that.

Q. CHRIS WOOD (McMaster University): I couldn't quite hear, but you did not change alkalinity, right?

A. We tried to maintain it.

Q. You did not add carbonate, you added sulfates when you added your hardness cations?

A. We added magnesium. We added the sulfates. We added magnesium, we added calcium sulfate.

Q. Sulfates?

A. Yes.

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- Q. It makes a big difference. And the other thing is, have you tried to calculate the silver ion using standard speciation programs, or estimate the free ion?
- A. We didn't do that for this set of data. Dave tried to do some of that with his presentation, to look at how the speciation and the different filtration levels correlate with each other. It would be interesting to go back and look at the calculated levels and see what the mortality was, given those calculated levels. Compare that to the three levels of filtration that we used. It's a good point.
- Q. ELIZABETH FERGUSON (University of Kentucky): Back to hardness again. When you change your hardness are you co-varying the calcium and magnesium or just increasing and decreasing your calcium?
- A. It was the calcium and the magnesium that we changed.
- Q. So you co-vary.
- A. Yes.

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## Bioavailability, Physiology and Toxicology of Silver in Seawater Fish: Implications for Water Quality Criteria

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### Getting the Perspective

Through research largely carried out over recent years, the effects of silver on freshwater fish have been characterized in some detail (Wood and Hogstrand, 1997; Hogstrand and Wood, 1998). In contrast, our knowledge about how silver can affect seawater-living fish is much more limited (Hogstrand and Wood, 1998). To protect marine environments from silver toxicity in a meaningful way, it has been recognized that a better understanding is needed in this area. Lately, research efforts have therefore been directed towards studies of silver effects on marine organisms. This overview, which is largely an excerpt from our review article covering the topic (Hogstrand and Wood, 1998), will summarize available data on bioavailability, physiology, and toxicology of silver in seawater fish.

Silver concentrations in marine environments are very low, ranging from 0.1 – 0.2 ng/L in open oceans to 0.1 – 30 ng/L in estuaries (Shafer, 1995). In general, silver is markedly less toxic to fish in seawater than in freshwater. Hence, documented silver concentrations in marine environments are at least 70 times lower than the U.S.EPA criterion for silver in seawater (2.3 µg/L; U.S.EPA, 1980) and at least 100 times below levels that have been indicated to have effects on fish (Shaw et al., 1997; Shaw et al., 1998; see also below). The basis for this difference is that the free silver ion,  $Ag^+$ , which is the most toxic form of silver, is practically not present in the marine environment. Instead, silver chlorides, silver organothiols, and silver sulfides dominate speciation of silver in seawater (Adams and Kramer, 1996; Ferguson and Hogstrand, 1998).

### Acute Toxicity

In Table 1, we have listed all acute toxicity data for marine fish that are available in the primary scientific literature. To our knowledge, peer-reviewed acute toxicity values for silver exist only for five species: mummichog (*Fundulus heteroclitus*; Dorfman, 1977), sheepshead minnow (*Cyprinodon variegatus*; U.S. EPA, 1993), tidepool sculpin (*Oligocottus maculosus*; Shaw et al., 1998), and rainbow trout (*Oncorhynchus mykiss*; Ferguson and Hogstrand, 1998). We have also included data on shiner perch (*Cymatogaster aggregata*), English sole (*Parophrys vetulus*), and coho salmon (*Oncorhynchus kisutch*), which all come from an internal report of a carefully conducted study by Dinnel et al. (1983). The 96h LC50 values for silver (tested via additions of

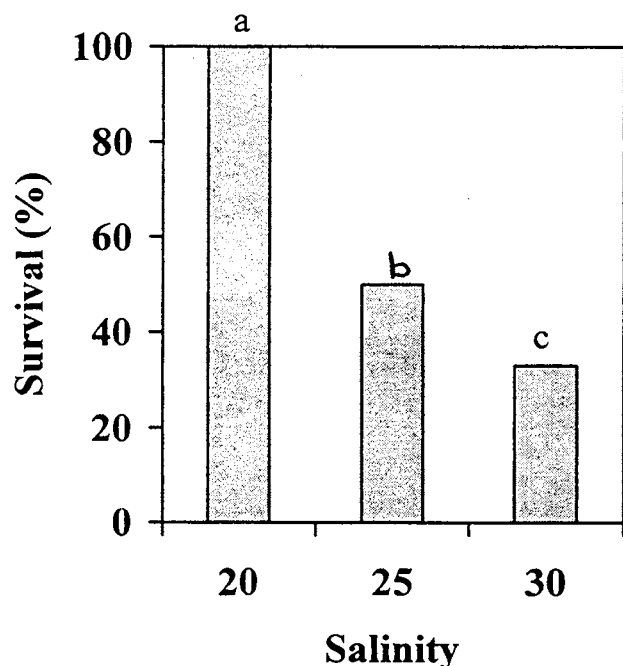
Table 1. Acute toxicity of silver to marine fish.

Species	Life stage	Salinity (‰)	96-h LC50 (µg/L)	168-h LC50 (µg/L)	Reference
Mummichog ( <i>Fundulus heteroclitus</i> )	adult	24	2,700	-	Dorfman, 1977
Sheepshead minnow ( <i>Cyprinodon variegatus</i> )	juvenile	28	1,170	-	U.S.EPA, 1980
Shiner perch ( <i>Cymatogaster aggregata</i> )	adult	29-30	356	-	Dinnel et al., 1983
English sole ( <i>Parophrys vetulus</i> )	adult	29-30	800	-	Dinnel et al., 1983
Tidepool sculpin ( <i>Oligocottus maculosus</i> )	juvenile	25	330	119	Shaw et al., 1998
	juvenile	32	657	472	
Coho salmon ( <i>Oncorhynchus kisutch</i> )	smolt	29-30	487	-	Dinnel et al., 1983
Rainbow trout ( <i>Oncorhynchus mykiss</i> )	smolt	25	401	-	Ferguson & Hogstrand, 1998

AgNO<sub>3</sub>) to these juvenile and adult fish in seawater range from 330 µg Ag/L for the tidepool sculpin (Shaw et al., 1998) to 2,700 µg Ag/L for mummichog (Dorfman, 1977). These values are two orders of magnitude above the current U.S. EPA (1980) seawater acute limit of 2.3 µg Ag/L. The lowest 96h LC50 value for silver among the listed species is more than four times above the highest value obtained with freshwater fish. During the past year, acute toxicity values have been generated with juveniles of three additional marine species (*Atherinops affinis*, *Menidia berylina*, and *Paralichthys dentatus*; Shaw et al., 1997). The 96h LC50 value for one of these species, the topsmelt (*Atherinops affinis*), extended the acute toxicity range for seawater fish down to 183 µg/L.

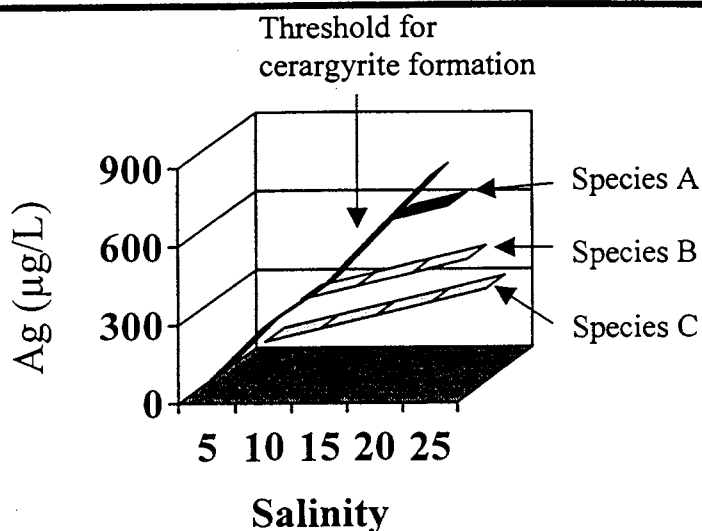
Salinity and concentrations of ammonia and organothiols in the water seem to be important modifiers of acute silver toxicity to marine fish (Shaw et al., 1997, 1998; Ferguson and Hogstrand, 1998). Of these factors, the presence of organothiols may be the most significant. In acute toxicity tests, the addition of 3-mercaptopropionic acid (3-MPA), in equimolar concentrations to silver, almost completely ameliorated mortality at concentrations of silver that without the organothiol killed 50% or more of the animals (Shaw et al., 1997). Organothiols, such as 3-MPA, are believed to be present in sufficiently high concentrations in the water column to be protective (Adams and Kramer, 1996). One of the physiological effects of silver exposure is an elevated plasma concentration of ammonia (Hogstrand and Wood, 1998; see below). Ammonia is toxic itself and, thus, any environmental condition that retards ammonia excretion (*i.e.* high pH, elevated water [NH<sub>3</sub>]) can exacerbate silver toxicity (Shaw et al., 1997, 1998). There are at least three ways by which salinity may affect toxicity of silver. The most universal

effect within the seawater range (17 – 36 ‰) is probably a decreased toxicity with an increase in salinity (Shaw et al., 1997,1998). A second way by which salinity can modify toxicity to silver relates to trout and possibly other freshwater fish that migrate to marine environments. At the time they migrate to seawater their ability to osmoregulate in seawater is often not complete and higher salinities pose a considerable osmotic stress on the animals. If a high level of silver exposure is superimposed on this osmotic stress acute



*Figure 1. Salinity-dependence of silver toxicity for rainbow trout in seawater. Smolt rainbow trout were exposed to 400 µg Ag/L added as AgNO<sub>3</sub> during 96 h. Survival decrease with increasing salinities because of a combined stress of silver and the hyperosmotic environment. Data from Ferguson & Hogstrand (1998).*

toxicity may occur, whereas the same silver concentration might be tolerated at lower salinities (Fig. 1; Ferguson and Hogstrand, 1998). In this case, the intrinsic toxicity of silver is not altered by the salinity change, but the osmotic stress from the seawater is added to the stress from silver exposure. Thirdly, if in brackish water or in seawater the silver concentration is gradually increased, there will be a point at which the insoluble silver chloride complex, cerargyrite, will form and precipitate silver out of solution. The threshold for cerargyrite formation is salinity dependent such that the silver concentration required for its initiation increases with increasing salinities (Fig. 2; Ferguson and Hogstrand, 1998). Cerargyrite is essentially non-toxic, which means that acute silver toxicity can actually be attenuated by raising the silver concentration above the threshold for cerargyrite formation (Shaw et al., 1997, 1998). In brackish water, this threshold occurs at silver concentrations low enough to prevent acute silver toxicity to most, if not all, fish species. The cerargyrite effect is, however, a laboratory phenomenon because environmental silver concentrations are far below the cerargyrite threshold at any salinity (Shafer, 1995). Thus, three strong modifiers of silver toxicity have so far been identified. The present water quality criterion for silver in seawater does not take into account any physiochemical modifier.



**Figure 2.** Relationship between salinity and silver toxicity to most marine fish. Dissolved silver is generally less toxic at higher salinities than at lower as shown for three hypothetical fish species, A, B, and C. If the silver concentration is raised gradually in brackish- or sea-water the level will eventually be reached at which the insoluble silver chloride complex, cerargyrite, forms. The threshold for cerargyrite formation is salinity dependent as shown. Since cerargyrite is, in practical terms, not acutely toxic to fish there is a lower salinity limit at which acute toxicity can occur. This limit is the salinity at which the tolerance curve intercepts with the cerargyrite threshold. The intercept will occur at higher salinities for more tolerant species (e.g., species A).

### Chronic Toxicity

To the best of our knowledge, there is no published information on standardized chronic toxicity endpoints (i.e., full life-cycle tests, partial life-cycle tests, and extended early life-stage tests) for silver on seawater fish. Only very recently attention has been paid to this deficiency and a 28-day early life-stage toxicity test has now been completed with sheepshead minnow (Shaw et al., 1997). In addition, 18-day embryolarval toxicity tests were conducted with sheepshead minnow, topsmelt, and inland silverside. Results from these chronic and sub-chronic tests are presented in this volume (Shaw et al., 1997). Earlier work by Voyer et al. (1982) and Klein-MacPhee et al. (1984) described short early life stage tests for silver on the winter flounder. Voyer et al. (1982) found no adverse effect on survival and successful hatching of embryos at concentrations up to 174 µg Ag/L. When tested in combination with cadmium, silver actually reduced embryonic toxicity of the former in a dose-dependent manner. A more extended exposure (18 days) to silver alone resulted in effects at much lower silver concentrations (Klein-MacPhee et al. 1984). Silver caused effects including reduced hatching frequency, time to hatch and larvae size, and increased larvae mortality and malformations. The highest tested concentration with no effect was 54 µg/L and the 18-day sub-chronic effect level was calculated to 70 µg/L.

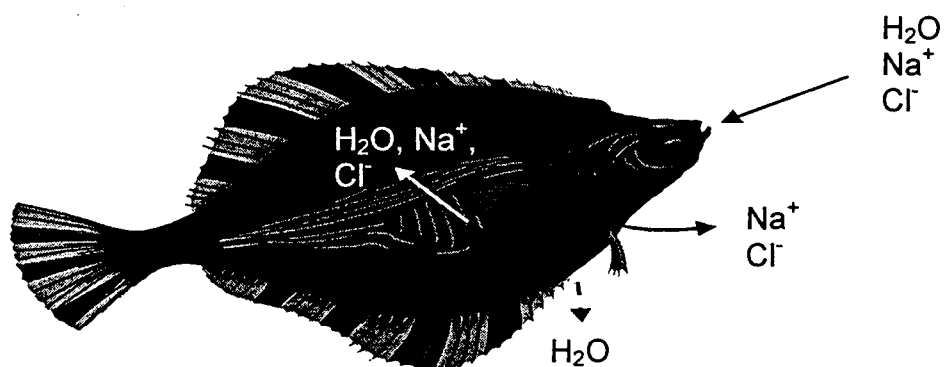
Sub-lethal effects during short and extended exposures to silver have been reported for a few fish species (reviewed by Hogstrand and Wood, 1998). Most notably, exposure of winter flounder to 10 µg Ag/L for 60 days was found to reduce the activity of aminotransferase, a family of enzymes involved in nitrogen metabolism (Calabrese et al., 1977). Jackim et al. (1970) measured the effects of a 96h exposure to 30 - 40 µg/L of silver on liver enzymes in mummichog (*Fundulus heteroclitus*) and found that 4 out of the 5 enzymes assayed displayed reduced activities. The most pronounced effects observed were for catalase (32 % reduction) and xanthine oxidase (27 % reduction).



From the limited data available on sub-chronic silver effects on marine fish it can be concluded that the threshold for disturbances of embryo-larvae life stages in winter flounder is approximately 70  $\mu\text{g/L}$  of dissolved silver. Effect on aminotransferase in the same species was observed during chronic exposure to only 10  $\mu\text{g/L}$  of dissolved silver. There is an urgent need to expand the database to better define both acute and chronic criteria for silver in seawater. Since the most severe early life-stage effects of silver seem to occur post-hatch it can be speculated that species with short embryonic development (*i.e.*, larvae hatch underdeveloped) might be especially sensitive to silver exposure.

### Uptake

Before we discuss uptake of silver in marine fish there are some considerations that have to be made concerning their physiology. Seawater teleost fish live in an hyperosmotic environment and, therefore, the fish tend to lose water to the environment and gain ions by diffusion (Fig. 3). To maintain osmotic balance, marine fish drink water (in contrast to freshwater fish) and excrete ions. Drinking opens a second uptake route for waterborne silver in addition to the gills. Furthermore, some common organothiols can specifically be absorbed across the intestinal epithelium. Therefore, it seems quite possible that organothiol-bound silver could be effectively absorbed through the same pathway. While the gut becomes an important uptake route for silver in marine teleost fish, silver uptake across the gills is low because of complexation of silver to chlorides and other ligands. This relationship has been nicely illustrated in the European eel (*Anguilla anguilla*; Grosell and Hansen, 1997). The eel was found to accumulate 100 times more silver in gill tissue when exposed in freshwater than they did in seawater. In contrast, much less silver accumulated in the intestine of freshwater eels than in eels exposed to silver in seawater. Overall, however, uptake of silver from seawater is markedly slower than that from freshwater (Hogstrand and Wood, 1997; Shaw et al., 1997). It should also be noted that food has been indicated to be a more important source than water for silver uptake in seawater fish (Pentreath, 1977).



**Figure 3.** Osmoregulation in marine fish. Diffusive water losses are replaced by drinking. Water is absorbed from the intestinal lumen by passive diffusion driven by  $\text{NaCl}$  pumping from the intestine to the body. Excess  $\text{Na}^+$  and  $\text{Cl}^-$  are excreted across the gills.

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## Physiological Effects

As for freshwater fish, acute toxicity of silver to teleost fish in seawater is caused by an osmoregulatory disturbance. However, there are several important differences between the effects of silver in the two media. Most importantly, seawater teleost fish are hypoosmotic relative to the environment whereas freshwater fish are hyperosmotic. The consequence of this difference is that a disturbance of the osmoregulation result in opposite effects in freshwater and seawater fish. Thus, the pivotal physiological net effect of acutely toxic silver exposure to a marine teleost is an accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$ ; in freshwater fish the key physiological disturbance of silver is a loss of the same ions (Wood et al., 1996; Wood and Hogstrand, 1997).

In a recent study, we exposed starry flounders (*Platichthys stellatus*) to two different concentrations of silver (250 and 1,000  $\mu\text{g/L}$  dosed as  $\text{AgNO}_3$ ) in the water. Blood was sampled repeatedly via a chronically indwelling catheter in the dorsal aorta. Of a large number of blood chemistry variables measured, the increase in plasma concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  was the only effect that could be tied to mortality. Moribound fish displayed plasma  $\text{Cl}^-$  concentrations exceeding 200 mM, compared with an average of 143 mM before the start of the exposure. This osmotic breakdown was manifested by a markedly elevated plasma glucose concentration.

Before we take a more detailed look at the possible mechanisms involved in osmoregulatory failure during silver exposure it is important that we understand the strategy of osmoregulation in marine teleosts (Fig. 3). To compensate for the diffusive water loss, seawater teleosts drink water, which obviously contains high concentrations of  $\text{Na}^+$ ,  $\text{Cl}^-$ , and other inorganic ions. Water is absorbed in the intestine by the active pumping of  $\text{NaCl}$  from the lumen across the intestinal epithelium. By this action, the concentrations of  $\text{Na}^+$  and  $\text{Cl}^-$  on the intestinal lumen are lowered below that of the blood. Consequently, water diffuses from the gut to the surrounding tissues. The excess  $\text{Na}^+$  and  $\text{Cl}^-$  that are gained though the water absorption process are excreted across the gills.

The water transport across the intestinal epithelium is indirectly driven by the  $\text{Na}^+/\text{K}^+$ -ATPase, which is the target for acute silver toxicity in the gills of freshwater fish (Ferguson et al., 1997; Morgan et al., 1997). We measured the  $\text{Na}^+/\text{K}^+$ -ATPase activity in both intestine and gill of silver exposed starry flounders but found no inhibition of this enzyme in either tissue. Therefore, there must be a different target for silver toxicity in seawater fish and there are reasons to believe that the direct target is one or several of the other transporters involved in  $\text{Na}^+$  and  $\text{Cl}^-$  transfer across the intestinal epithelium. In addition to any such ion transporter blockage, silver has been found to inhibit drinking. A lowered drinking rate likely contributes to the increase in plasma electrolyte concentrations by reducing the volume of the blood plasma. Thus, we suggest the following etiology of acute silver toxicity to seawater fish. Silver inhibits drinking and intestinal  $\text{Na}^+$  and  $\text{Cl}^-$  uptake. These effects act in concert to decrease water uptake, which in turn leads to an increased plasma osmolarity. When the plasma osmolarity rises water is osmotically pulled out from the tissues with cell dehydration. Death due to dehydration in the final result.

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**Questions & Answers: Bioavailability, Physiology and Toxicity of Silver in Seawater Fish: Implications for Water Quality Criteria**

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- Q. ARUN MUKHERJEE (University of Helsinki, Finland): Actually I am neither a biologist nor a toxicologist, but perhaps you know that in the world population they eat more fish than meat. For the last two days we have discussed here quite a lot about silver accumulation in fish, in different sectors of the fish and its toxicity, and then what happens in the marine and freshwater. But nobody has talked about what should be the limit of silver in the fish, and if one eats that fish, if that limit goes very high, what happens to that human being. Will they have some kind of toxic effect like that from mercury in fish? We know if mercury in fish is more than 0.5 to 1 ppm then we say don't eat fish more than twice a week. But can you tell us that if the silver content in the fish is more than x ppm, then we should not eat that fish?
- A. I think that Daland Juberg is probably more suited to answer that question than I am, and I think he addressed that question yesterday in his talk. Silver in general and also in seawater is probably more of a concern for the species that live there than it is for human health. That's what we firmly believe. Also, we do show in these experiments, and with industrial or extreme concentrations of silver, that we get high levels of silver accumulation in these animals, but again by using much higher concentrations that you find in the environment. With that I don't mean that there is no silver in fish. There is silver in fish, but actually in the marine fish species that we have analyzed there seems to be a lot less silver in the animals than we find in the freshwater fish species that we analyzed. Isn't that a correct statement, Chris? I think that's true. There are other animals that can show quite remarkable accumulations of silver, which probably has to do with fish consumption. Beluga whales, for example, I think have the highest concentration of silver in the liver than do other animals. They probably top the list for concentrated silver. Being fish eating animals mainly, that's probably where they're getting it from. So it's probably possible to accumulate silver from fish, but I haven't heard of any human health effects from silver due to eating fish.

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## Protective Effects of Water Cl<sup>-</sup> on Physiological Responses to Waterborne Silver in Rainbow Trout

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### Introduction

In studies of the acute toxicity of Ag (presented as AgNO<sub>3</sub>) it is clear that water chemistry greatly influences the response of freshwater rainbow trout to waterborne Ag (reviewed Hogstrand and Wood 1997). This is likely due to complexation of Ag with ligands such as thiosulphate, organic material and Cl<sup>-</sup>. The recent work of Galvez and Wood (1997) showed that water Cl<sup>-</sup> content had a dramatic protective effect on Ag toxicity and that ionic Ag<sup>+</sup> was directly correlated with toxicity.

The primary physiological complication arising from exposure to AgNO<sub>3</sub> is a loss of plasma ions, particularly Na<sup>+</sup> and Cl<sup>-</sup> (Wood et al 1996). Morgan *et al* (1997) showed that this disruption of internal Na<sup>+</sup> and Cl<sup>-</sup> balance in rainbow trout was due to an inhibition of the ion uptake mechanisms in the gill, particularly Na<sup>+</sup>/K<sup>+</sup> ATPase.

The experiments described here were designed to establish if the protective effect that water Cl has on acute toxicity of Ag exposure in rainbow trout occurs at sublethal Ag concentrations. The focus of the study was on Na<sup>+</sup> balance.

### Material and Methods

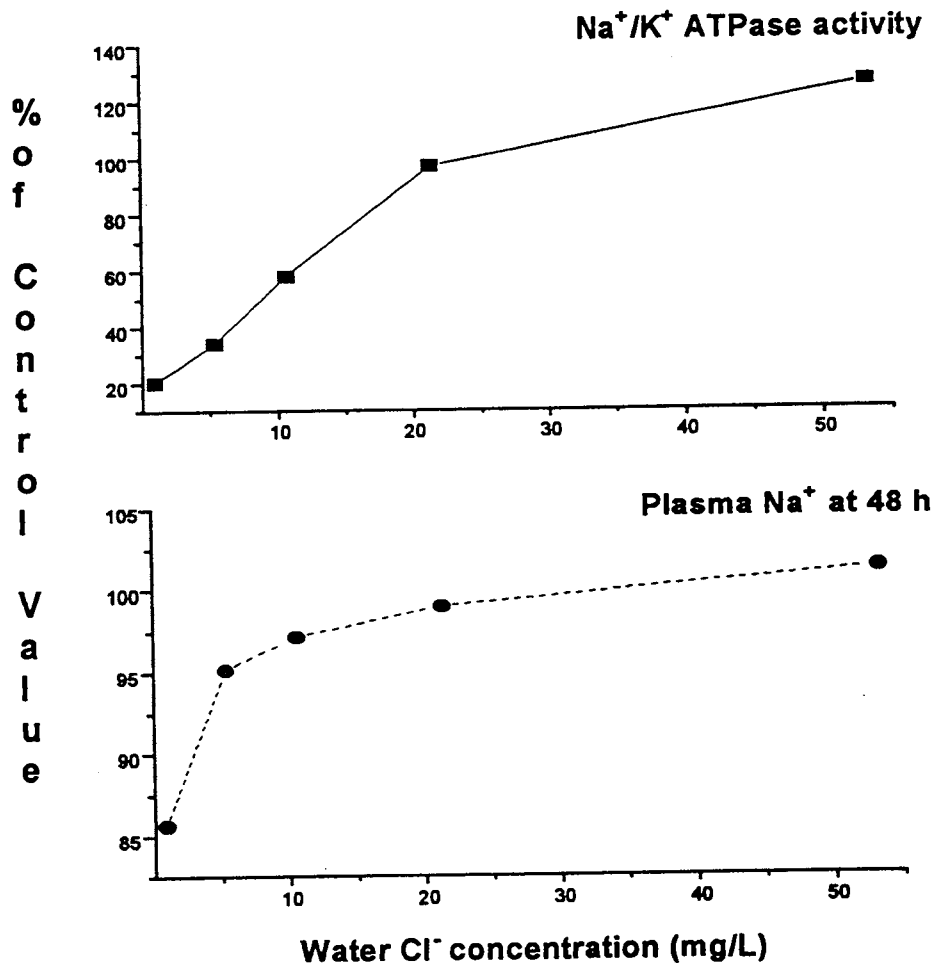
Rainbow trout were obtained from a local commercial trout farm and acclimated to soft water (ionic content: Na<sup>+</sup> 0.04 mmol/L; Ca<sup>++</sup>, 0.04 mmol/L; Cl<sup>-</sup>; 0.04 mmol/L; pH 7.0). Fish were exposed to waterborne Ag 3.2 µg/L (as AgNO<sub>3</sub>) via a flow-through system that included a header tank into which concentrated AgNO<sub>3</sub> and/or KCl solutions were metered and then mixed with soft water by vigorous aeration before distribution to fish boxes. Water Cl concentration ranged from 1 to 50 mg/L. The water Cl treatments used produced a series of treatments in which ionic Ag<sup>+</sup> levels ranged from 90% to 20% of total Ag (3.2 µg/L).

Plasma Na<sup>+</sup> concentrations were determined by atomic absorption spectrophotometry (AA1275 Varian). Gill filament samples were assayed for Na<sup>+</sup>/K<sup>+</sup> ATPase activity using the methods of Holliday (1985).

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## Results

Exposure to 3.2  $\mu\text{g/L}$  Ag resulted in a significant disruption in  $\text{Na}^+$  balance in rainbow trout, the effects of which were reduced with increasing water Cl content. Fish exposed to Ag at the low Cl concentration of 0.8 mg/L for 48 h experienced a 15% reduction in plasma  $\text{Na}^+$  content and with elevations in water Cl the loss of plasma  $\text{Na}^+$  was reduced. The  $\text{Ag}^+$  induced loss of plasma  $\text{Na}^+$  was correlated with an inhibition of  $\text{Na}^+/\text{K}^+$  ATPase in gill tissue. In water of low Cl content,  $\text{Na}^+/\text{K}^+$  ATPase activity was severely inhibited and as Cl increased this inhibition was alleviated.



**Figure 1.** Relative  $\text{Na}^+/\text{K}^+$  ATPase activity of gill tissue (top graph) and plasma  $\text{Na}^+$  content (bottom graph) from rainbow trout exposed to 3.2  $\mu\text{g/L}$  Ag for 48 h with 5 different water Cl concentrations. Data is expressed as the % change from control fish similarly sampled but not exposed to Ag. Control  $\text{Na}^+/\text{K}^+$  ATPase activity was  $1.6 \pm 0.29$   $\mu\text{mol PO}_4/\text{mg protein/h}$  and control plasma  $\text{Na}^+$  content was  $138 \pm 4.3$   $\text{mmol/L}$  (mean  $\pm$  SEM).

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## Discussion

These experiments support the previous work showing that water Cl has a protective effect on Ag toxicity (Galvez and Wood 1997) and provides evidence on the mechanism of protection. Water Cl alleviates the internal  $\text{Na}^+$  imbalance caused by Ag exposure. This protective effect of water Cl appears to be via protection against the inhibitory effect which  $\text{Ag}^+$  has on  $\text{Na}^+/\text{K}^+$  ATPase. Thus, in the water column, waterborne Cl complexes with the toxic  $\text{Ag}^+$  ion and as a result, the inhibition of  $\text{Na}^+/\text{K}^+$  ATPase is reduced and the disruption of  $\text{Na}^+$  balance in the fish is minimized.

This study provides more evidence for the suggestion that speciation should be considered when determining the site specific toxicity of waterborne Ag. It also illustrates that water Cl concentration has protective effect on Ag toxicity, as a result of reductions in  $\text{Ag}^+$  concentrations.  $\text{Ag}^+$  is very effective in obstructing  $\text{Na}^+$  uptake in rainbow trout through an inhibition of salt transport mechanisms in the gill.

## Acknowledgements

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**Questions & Answers: Protective Effects of Water Cl<sup>-</sup> on Physiological Responses to Waterborne Silver in Rainbow Trout**

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No Questions.

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## **An Overview of the ISO 14040 Life Cycle Assessment Approach and an Industrial Case Study**

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### ISO Life-Cycle Assessment Standards

A life cycle assessment (LCA) is a technique for evaluating the environmental aspects and potential impacts associated with a product from raw material acquisition through production, use and end-of-life management. Life cycle studies have been performed using various techniques for over thirty years. Most of these studies have been inventories of energy usage and emissions across various stages of a product or material's life.

In the late 1980's and early 1990's several LCA were conducted which received international attention, such as the Procter and Gamble study comparing the environmental emissions associated with disposable and reusable diapers (Disposable versus Reusable Diapers Health, Environmental and Economical Comparisons, Arthur D. Little, Inc., 1990). The publicity and controversy over these studies highlighted both the promise and the limitations of the life cycle approach. Limitations include the lack of available data, how to assess the impacts of environmental emissions, and the cost of completing a study. Despite these limitations, there continues to be a desire by industry, governmental groups, and others to use LCA thinking in decision making and marketing. However, without a standard and verifiable approach, LCA results can be mis-leading and result in poor decisions.

Currently, the International Standards Organization (ISO) is working to prepare a comprehensive set of standards for conducting life cycle assessments. ISO 14040, which was recently finalized, lays out a framework for conducting and reporting LCA studies, and includes certain minimum requirements. The overall approach consists of the following three phases:

1. Life cycle inventory analysis - the phase of the LCA involving the compilation and quantification of inputs and outputs, for a given product system throughout its life cycle (ISO 14041)
2. Life cycle impact assessment - the phase of the LCA aimed at understanding and evaluating the magnitude and significance of the potential environmental impacts of a product system (ISO 14042)
3. Life cycle interpretation - the phase of the LCA in which the findings of either the inventory analysis or the impact assessment, or both, are combined consistent with the defined goal and scope in order to reach conclusions and recommendations. (ISO 14043)

It is expected that the inventory analysis standard will be finalized in early 1998 with the impact assessment and interpretation standards following in late 1998 or 1999.

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## Eastman Kodak Silver Recovery Life Cycle Inventory Case Study

In 1991, Eastman Kodak Company contracted Franklin Associates to complete a life cycle inventory comparing three different silver recovery operations for photoprocessing. While this study did not follow ISO-type standard procedures, it provided an opportunity to learn about the benefits and limitations of the inventory phase of a life cycle assessment.

Three types of silver recovery processes were examined in the study. Comparisons made in the study were based on the treatment of the wastewater generated from the processing of 10,000 rolls of film. The specific silver recovery processes examined are listed below:

- Electrolytic cell recovery only
- Electrolytic cell recovery followed by chemical replacement cartridge
- Electrolytic cell recovery followed by in-situ ion exchange

Undertaking this study with a company experienced with the LCA process was an excellent learning opportunity for Kodak. In general, we gained the following insights about life cycle inventories:

- Data are not always available. For example, data for the typical performance of an electrolytic cell recovery system in the real world were not available because the performance of that system is insufficient to meet most silver discharge limits.
- Data collection is a lengthy process. Even when data are available, it is very time-consuming to obtain the information across the life cycle of the product system. Often, the data are hard to access because they are maintained by outside agencies and companies in a form not directly applicable to the life cycle inventory.
- Life cycle inventories reveal "hidden" environmental emissions. For example, silver recovery operations are usually evaluated based on their ability to remove silver from the wastewater. However, in the process of removing more silver, systems may use more energy (which creates more atmospheric emissions) and may create more wastewater emissions due to the chemicals used to achieve that improved silver recovery performance.
- While the data generated in a life cycle inventory can be very useful, an impact assessment is needed to compare the relative impacts of different systems studied. For example, how do you compare the relative impacts of lower silver in an effluent with higher atmospheric emissions generated from power generation?
- Life cycle assessment data in isolation are not sufficient for driving decisions. Rather, the LCA data augment other essential information used in the decision-making process, such as regulatory requirements, life cycle costs, and customer preferences.

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## Conclusion

The future of life cycle assessment is at a crossroads. The desire by industry, governmental agencies and non-governmental organizations for a practical, comprehensive tool such as LCA to improve their decision making processes continues to grow. The completion of the ISO LCA standards over the next couple of years will establish common practices for LCA studies. Other standards activities, such as the life cycle inventory format being developed by the Society for the Promotion of Lifecycle Development, will make data more accessible. However, it is unknown if the ISO standards will overcome the existing limitations and meet the needs of the various interested parties.

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## Questions & Answers: An Overview of the ISO 14040 Life Cycle Assessment Approach and an Industrial Case Study

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- Q. WALTER BERRY (EPA Narragansett): I don't think that there's any question that we need to use this kind of framework to look for hidden diseconomies and all that sort of stuff. And I don't think that there are any. I've grown a lot during this conference. One thing I'm curious about, though, was what kind of thought has been given to how you could generate an index, comparing something like the difference between increased solid waste due to reusable diapers versus the increased use of pesticide with cotton diapers, or you know. The apples and oranges thing is not a big problem to a regulator. They have to make those kinds of decisions all the time. But as scientific types, how you come up with an index for that I really can't imagine. I wonder if you have any insight on that? I also have a personal question: has a life-cycle assessment been done on single-use cameras?
- A. Actually, Agfa's done some work on single-use cameras, and Kodak has done some work on looking at the life-cycle of single-use cameras, to answer your question. But nothing that's been done has been published. We've done some work internally to help drive design improvements, which is one of the major uses of life-cycle assessments internally. It's to help you identify where you have improvement opportunities so that you can develop your product. For example, one of the things we do is we bring the single-use cameras back to various manufacturing facilities for remanufacturing, and so by doing not a quantitative but a qualitative study, you can find out where you have opportunities for improvement. We have done that, but we haven't published the results.

I don't know that I can answer your first question. That is the real crux of the problem, and that is where the real controversy stands today around life-cycle assessments: how to do that impact assessment. And how to take all that different information and condense it down into a couple of impact indicators. I think there's fairly good agreement around some impact indicators. For example, for global warming, you can look at the emissions and lump those together and say well, what's its relative impact on global warming or relative impact on ozone depletion. But there's not much agreement on other indicators like human health impact, because you don't have the data from the inventory that allow you to do a risk assessment. There's no understanding, for example, of the consequences of concentrations, there's no understanding of the locations, so before you can do a risk assessment you need to know all that. It's going to be this concentration over this date and to this receiving body of water with this type of fish species, and you don't have that so there's no agreement on how to do it. I don't think that really answers your question, except for you to understand where the problem is. It will be interesting to see how that plays out over the next couple of years. And hopefully it will play out, because it could be a great tool to have if everyone would agree on those impact indicators.

- Q. ARUN MUKHERJEE (University of Helsinki, Finland): You didn't show any data in your presentation. For example, you started with a product like paper, film manufacturing, then the process, and then you come to the energy and so on. But have you used some program by which you can find out how much energy you are putting in, or it is more or less, or how much energy it takes to bring the silver from the mines to the Eastman Kodak Company? Do you have any data on this subject, or any computer program which you have used for your life-cycle assessment?
- A. We have not done a life-cycle assessment on film manufacturing for example, which is what you're asking. So that data are not available. It would be a mammoth undertaking. This one study we did which seems pretty small, and it was fairly small, ended up taking us about 9 months and ended up costing probably \$40,000. This was just looking at those three little silver recovery techniques, and not paying attention to all the film and paper and chemistry manufacturing. So no, we don't have the information. The study hasn't been done. There are some models being developed that would allow you to plug information in, but I think again, what you'd find if you wanted to look at a complicated system like film manufacturing, the data wouldn't exist. So you couldn't plug them in to do the inventory because you wouldn't have the information.

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## Protective Effects of Dissolved Organic Matter Against the Physiological and Toxicological Effects of Silver and Other Metals on Rainbow Trout

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Work done in my laboratory and by others has shown that dissolved organic matter can protect against the adverse effects of metals on fish. Dissolved organic matter (DOM) reduces the effects of metals by complexing them, reducing the amount of metal available to interact with sensitive gills (Playle et al. 1993a, b; Janes and Playle 1995; Hollis et al. 1996, 1997; Playle 1997; Figure 1). The protective effects of DOM can be incorporated into simple models of metal interactions at fish gills (Figure 2), which can calculate the amount of metal actually accumulating on or in the gills (dark bars in Figure 1). However, models generated in my laboratory have been based on one DOM source, and therefore do not take into account possible differences in DOM source.

Work with inorganic Hg in my laboratory demonstrated the protective effects of Hg accumulation on trout gills. The system is dominated by DOM, meaning that DOM concentration is the most important factor in determining Hg accumulation by trout gills (Hughes and Playle, unpublished). When we tested our Hg model using natural lakewaters the fit was poor, and the model could only be made better by increasing some Hg-DOM binding strengths ten fold (e.g. by one log  $K$  unit) or by increasing the number of binding sites per mg C ten fold for some of the natural waters. This result was the strongest indication yet in our laboratory that DOM source is important when modelling the protective effects afforded by DOM.

To test the protective effects of DOM from different sources, we ran a complicated six metal, three DOM source experiment. As test metals we chose Ag, Cd, Cu, Hg, Pb, and Co, in a mixture at concentrations of  $\sim 0.1$  to  $3.5 \mu\text{M}$  in soft water. The three DOM samples were from Luther Marsh, Beverly Swamp, and a pond on Point Pelee, all in southern Ontario. The DOM samples were supplied by Dr. Kent Burnison, National Water Research Institute, Environment Canada, Burlington, Ontario. We predicted that if a metal binds strongly to DOM in relation to gill binding, then DOM source would not matter as much as if a metal binds more strongly to the gills than to DOM.

For Ag, Point Pelee DOM protected best, with  $9.5 \text{ mg C}\cdot\text{L}^{-1}$  DOM keeping Ag off the gills (Figure 3). In contrast, Point Pelee DOM kept Pb off the gills less well than did Luther Marsh or Beverly Swamp DOM, although all protected equally well at  $9.5 \text{ mg C}\cdot\text{L}^{-1}$  (Figure 4). Overall, the Luther Marsh DOM protected best against the mixed metal solution, as judged by toxicity of the metal mixture over three days, and the Point Pelee DOM protected least well. For more results from this experiment see Richards et al., this volume. Thus, DOM source does matter in

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metal binding to fish gills, depending on the metal and the concentration of DOM, but the mechanisms for these differences need to be worked out.

Previously in our laboratory we demonstrated the protective effects of  $\sim 5$  mg C·L<sup>-1</sup> DOM against the respiratory and ionoregulatory effects of Cu and Cd in fish (Richards and Playle, in preparation). We have continued this work to examine the protective effects of DOM against the respiratory and ionoregulatory effects of Ag on fish in synthetic soft water, through repetitive blood sampling of adult rainbow trout (*Oncorhynchus mykiss*).

In soft water exposures with 0.04 to 0.06  $\mu$ M Ag added as AgNO<sub>3</sub> the addition of 8 mg C·L<sup>-1</sup> Luther Marsh DOM partially reduced the entry of Ag into trout plasma, whereas the addition of 5 mg C·L<sup>-1</sup> DOM did not (Figures 5, 6). However, in these relatively low Ag concentration experiments there were no respiratory or ionoregulatory effects of Ag in the fish. In another experiment with higher concentrations of Ag (0.15  $\mu$ M Ag) there was still not a large protective effect of 20 mg C·L<sup>-1</sup> Aldrich DOM against the respiratory and ionoregulatory effects of Ag. See Rose-Janes et al., this volume, for more details of these experiments.

Dissolved organic matter does protect against the toxicological and physiological effects of Ag, but in our relatively harsh, soft water exposures to a high Ag concentration an even higher concentration of DOM is likely needed to protect the fish. In short, approximately three hour exposures to Ag in soft water, it took about 24 mg C·L<sup>-1</sup> DOM to keep Ag off the gills of small trout (Figure 1) We estimate that 30 to 40 mg C·L<sup>-1</sup> is the likely amount of DOM that will be needed to protect against the physiological effects of Ag in adult fish during a five day exposure in soft water.

In summary, it appears that DOM source does matter, to varying degrees, in determining the protection afforded by DOM against metal deposition at fish gills. However, the metal-DOM relationships are not simple and need further elucidation. In our soft water system the concentration of DOM needed to protect against the physiological effects of  $\sim 0.1$   $\mu$ M Ag in soft water is  $> 20$  mg C·L<sup>-1</sup>, and is likely 30 to 40 mg C·L<sup>-1</sup>. This range in concentration of DOM is similar to that determined previously by us in short exposures of small trout to Ag.

### Acknowledgements

Nancy Rose-Janes, Jeff Richards, Chris Hughes, Lydia Hollis, and Dr. Kent Burnison are thanked for their laboratory help and collaboration. I thank Kodak Canada Inc. for a research grant, which has allowed us to purchase a Total Organic Carbon Analyzer for our DOM and Ag work. This research was also supported by NSERC and by Wilfrid Laurier University.

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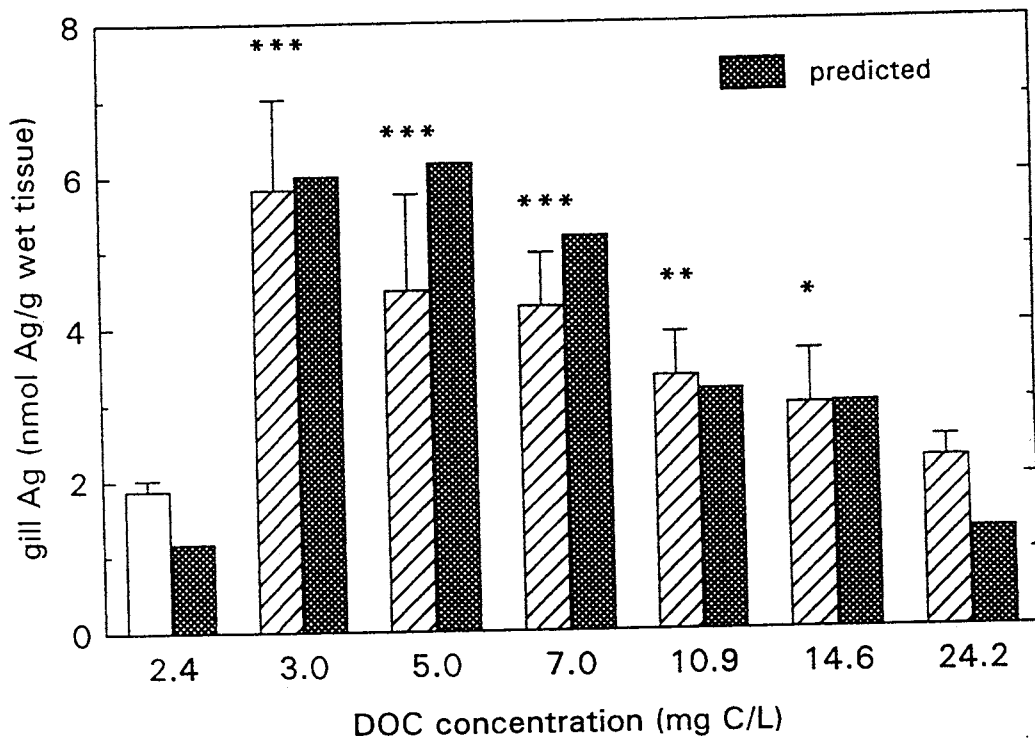
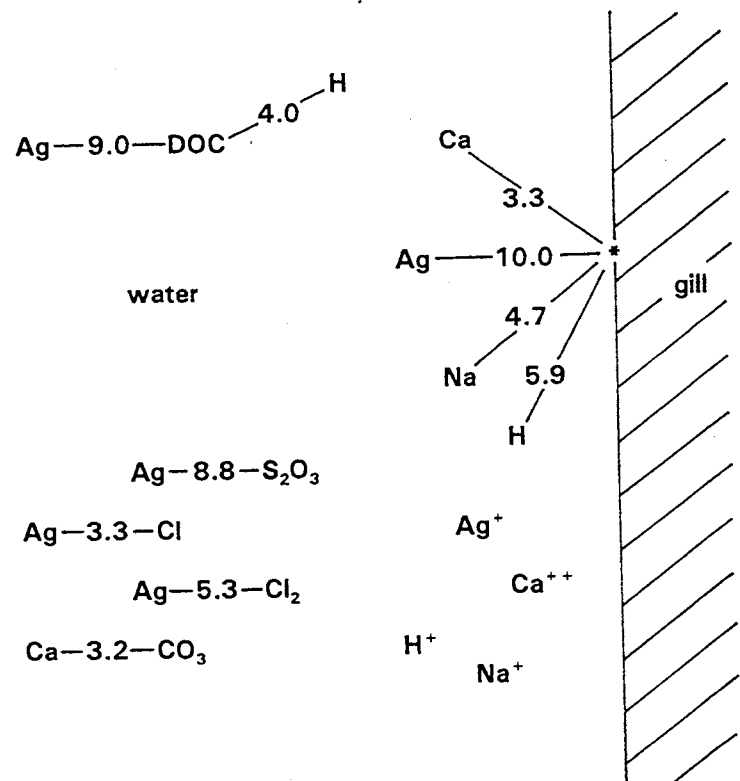


Figure 1. Observed and predicted gill Ag concentrations in the presence of 3 to 24 mg C·L<sup>-1</sup> DOM. Striped bars represent gill Ag concentrations from fish exposed to 0.17  $\mu$ M Ag for 2 to 4 h, six fish per bar. From Janes and Playle (1995).

Figure 2.

The most important parameters in the Ag-gill model. Conditional stability constants (log *K* values) are indicated in the Figure. From Janes and Playle (1995).



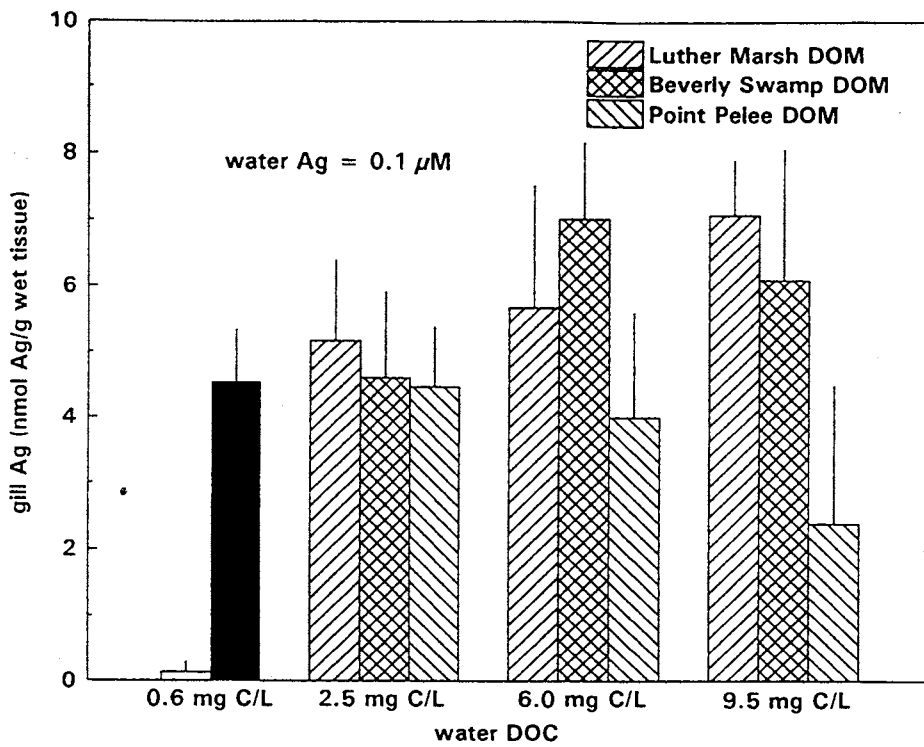


Figure 3. Amount of Ag on or in the gills of rainbow trout held in the six metal, three DOM solutions for 4 h. The only apparent protective effect of DOM was for Point Pelee DOM at 9.5 mg C·L<sup>-1</sup>.

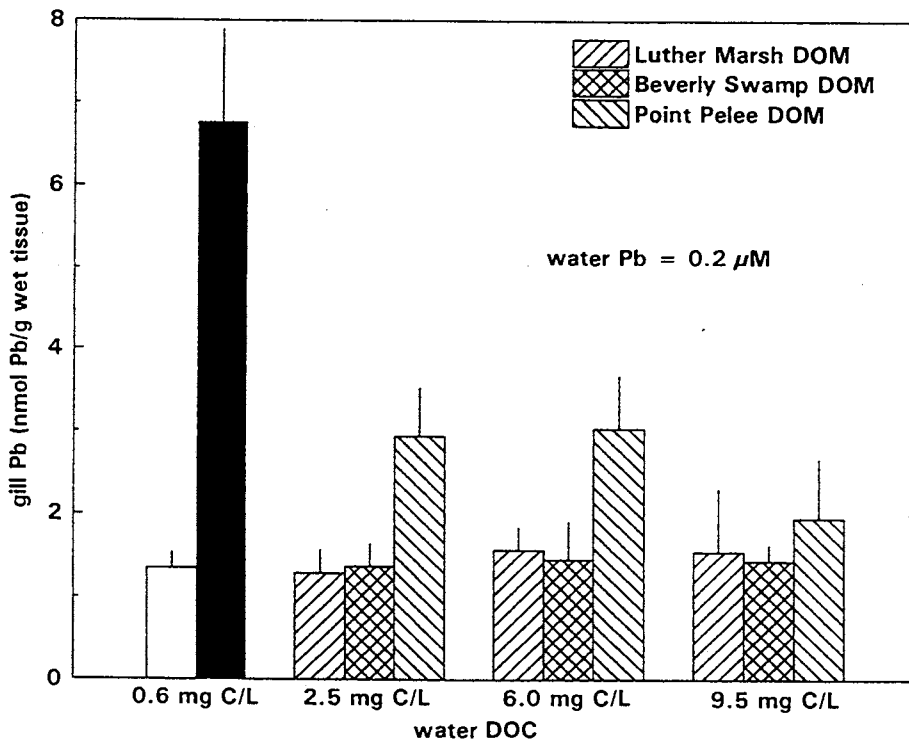


Figure 4. Amount of Pb on or in the gills of rainbow trout held in the six metal, three DOM solutions for 4 h. Point Pelee DOM protected least well.

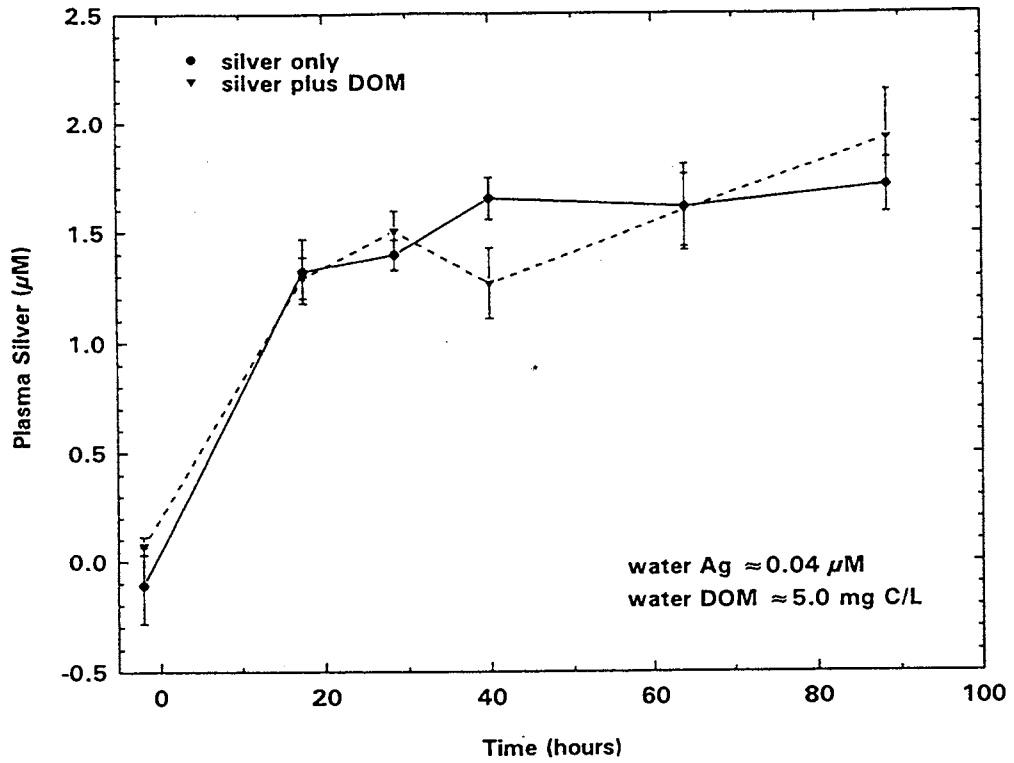


Figure 5. The amount of Ag entering adult trout plasma during exposures to  $0.04 \mu\text{M}$  Ag in synthetic soft water was the same with 0 or  $5 \text{ mg C}\cdot\text{L}^{-1}$  DOM.

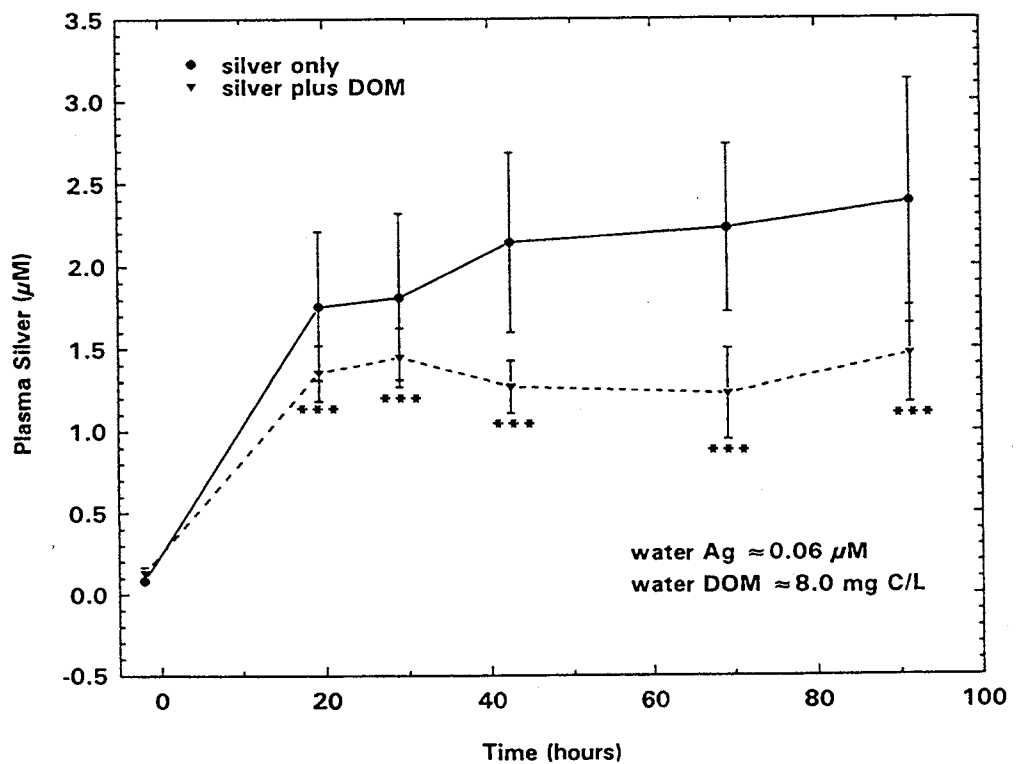


Figure 6. The amount of Ag entering trout plasma during exposures to  $0.06 \mu\text{M}$  Ag in synthetic soft water was slightly less in the presence of  $8 \text{ mg C}\cdot\text{L}^{-1}$  DOM.

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**Questions & Answers: Protective Effects of Dissolved Organic Matter Against the Physiological and Toxicological Effects of Silver and Other Metals on Rainbow Trout**

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- Q. PAUL ANDERSON (Illinois Institute of Technology): Do you think all metals compete for the same sites on the gill, and do you think the toxicity mechanism of each metal is the same?
- A. No, I think they are different sites and I'd say the mechanisms are different. We've seen some very nice work from Christer Hogstrand's group and Chris Wood's group on the actual mechanism of silver, for example, which is on the sodium-potassium ATP-ase and specifically right on the magnesium group there, which is really great. Copper seems to affect sodium uptake, but I'm not sure it's been worked out whether it does it in an identical way. I'm sure some metals work in similar ways and some work in other ways.
- Q. JIM KRAMER (McMaster University): That's a real nice piece of work because I think it's pointing us more to the kinds of things we have to ask, particularly with DOM and DOC. It's not a black box that you can use all over the place. I think your interpretation is good, the idea of looking to protein and so on and also even in your first slide, the well water, which would have more sulfhydryl groups. There's only one conundrum, and that is mercury in general, everything else being equal, will bind more strongly to the sulfur than silver. And yet you've got the opposite effect. So I guess there's still something more to know?
- A. Very good point. I would perhaps suggest it might be related to the first question, that maybe mercury interacts with different groups on the gills than silver does, which confuses the question perhaps.

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## Effects of Silver Sulfide on the Terrestrial Earthworm

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(Presented by Charles J. Ruffing)

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The effects of silver compounds in the aquatic environment have been well studied, and previous conferences in this series have provided much insight into the extent and nature of these effects. There is less information available on terrestrial species, and this study has investigated the effects of silver sulfide, a predominant product of the treatment of silver bearing waste by wastewater treatment plants (WWTP), on the earthworm.

Silver present in photographic film and paper is removed during processing by chelation of the silver with thiosulfate. Because of the intrinsic value of silver and regulations controlling its discharge, waste solutions containing silver are treated using a variety of silver-recovery processes. After treatment to remove most of the silver, small amounts of silver thiosulfate are discharged to sewer systems. Silver thiosulfate is converted at wastewater treatment plants to silver metal and silver sulfide. Field measurements have shown that greater than 90% of influent silver is removed at a treatment plant. The silver and silver sulfide formed in wastewater treatment is strongly associated with the sludge (biomass), because of the very low water solubility of both species. In the U.S., about 30% of the waste sludge is applied to land as fertilizer. In this application, there is potential for silver exposure to terrestrial organisms.

The earthworm (*Lumbricus terrestris*) is an important species in the terrestrial community. It contributes to soil community health by aerating the soil and serving as a food source for other organisms. In this experiment, a synthetic sandy loam was prepared and two grams of silver sulfide (as silver) was added per kilogram of soil dry weight. After an acclimation period to the laboratory, earthworms were exposed to this limit concentration or to control soil with no silver. The worms were not fed any additional food aside from nutrients contained in the soil, and were observed at 7 and 14 days (test end).

Analysis of the soil for silver content showed a mean concentration of 1595 mg of silver per kg of dry soil in the exposure vessels. There was no mortality observed in the study, nor any effects in burrowing time, appearance or weight. These results show that exposure to this level of silver does not affect survival and there was no change in several important sub-lethal parameters compared to the controls.

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Most silver from photoprocessing operations is recovered. However, a secondary pathway for silver fate is through a WWTP to land. Silver concentration in wastewater treatment plant sludge is generally < 500 mg/kg, therefore, this limit concentration study suggests that such applications are not a risk to the terrestrial earthworm.

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## Questions & Answers: Effects of Silver Sulfide on the Terrestrial Earthworm

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- Q. CHRIS WOOD (McMaster University): I've got two totally unrelated questions. The first one is, was there any uptake of silver at all by the organisms?
- A. We actually didn't study that in this project, Chris. In our next study after this, which isn't complete, we generated some silver in sludge by a laboratory processing unit. Then we applied that sludge to soil and then measured the toxicity and the concentrations there. The data are rather fresh, there is more silver in the tested organisms than in the control. I think the controls had about 0.2 micrograms per gram of dry weight of the worm and the silver in the test organisms was about 0.7. I don't know if that's statistically significant yet. We haven't measured that, but it's 0.7 micrograms in the worm compared to 0.2 grams in the medium, so the accumulation factor will be quite low.
- Q. The other one is, what happens to the other 70% of sludge that doesn't get land applied? Where does it go?
- A. A lot of it is incinerated. In fact, in the document that I took that citation from, mass doesn't add up to 100%. 30% was "as being applied to lands; 10 or 15% being incinerated, and the rest was kind of "black box".
- Q. We ate it or something?
- A. Yes. Exactly. A comment was made it could go to landfills.
- TOM BOBER: I think I can answer your question. Most of it comes back up to Canada, in recycled ash and sludge. *(laughter)*

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## The Acute and Chronic Toxicity of Silver to Marine Fish

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### Introduction

The free silver ion ( $\text{Ag}^+$ ) is extremely toxic to fish. However, it only is present in freshwater systems. Typical 96-h  $\text{LC}_{50}$  values for silver in freshwater systems range from 7 to 50  $\mu\text{g Ag.L}^{-1}$ , with 30-40% present as  $\text{Ag}^+$  (Hogstrand and Wood, 1998). While the toxicity of  $\text{Ag}^+$  has been well documented, little is known about the toxicity of dissolved silver chloride ( $\text{AgCl}_n$ ) species. In seawater  $\text{Ag}^+$  is diminished to insignificant concentrations and  $\text{AgCl}_n$  species predominate. In addition, several parameters have been identified that influence silver toxicity in marine environments. However, few published studies have focused on the effects of silver in seawater-dwelling fish.

Marine fish living in estuarine environments tolerate and frequently encounter wide ranges of water  $[\text{Cl}^-]$  and, when silver is present, different  $\text{AgCl}_n$  species. Modeling of  $\text{AgCl}_n$  speciation indicates that as salinity increases from brackish systems to full strength seawater the activity of the dissolved neutral  $\text{AgCl}$  (aq) is reduced and negatively charged  $\text{AgCl}_n$  species ( $\text{AgCl}_2^-$ ,  $\text{AgCl}_3^{2-}$ ,  $\text{AgCl}_4^{3-}$ ) dominate (Ferguson and Hogstrand, 1998). However, little attention has been given to the toxicity of different  $\text{AgCl}_n$  species.

Water column ammonia may increase silver toxicity. Silver causes an immediate and dramatic increase in plasma ammonia concentrations (Hogstrand and Wood, 1997). As water column concentrations of ammonia are increased, transbranchial diffusion gradients for excretion (e.g. fish to water) are reduced or even reversed. This could exacerbate silver toxicity. For marine fish, this effect applies not only to un-ionized ammonia but also to the charged ammonium ion,  $\text{NH}_4^+$  (Wilson and Taylor, 1992).

Organosulfur (e.g. thiol) ligands have been implicated as modulators of silver concentrations in the water column and sediments. Thiols readily bind with silver to form dissolved complexes. When present, these silver-thiol compounds will dominate over all other dissolved silver species in many marine systems. In fact, thiols can remobilize silver from solid phases,  $\text{Ag-FeS}$  (Adams and Kramer, 1997). Three-mercaptopropionic acid (3-MPA) is a prominent environmental thiol, which is typically found in micromolar concentrations in reducing marine environments (e.g. porewater; Vairavamurthy and Mopper, 1987). However, the effects of silver-thiol complexes on toxicity and accumulation are not known.

Thus, investigations were conducted to elucidate the effects of silver to seawater-dwelling fish. The primary objectives of the present study were to:

- 1) determine the acute toxicity of a suite of marine fish species to waterborne silver;
- 2) define parameters that influence silver toxicity in seawater; and
- 3) detail the effects of chronic silver exposure on marine fish during early development.

### Methods

A three-tiered approach to toxicity testing was developed to study the relative sensitivities of several marine fish species to waterborne silver. Initially, static renewal 96-h acute toxicity tests were conducted on five fish species (Tier I). In addition, the effects of ammonia, salinity, and silver-

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thiol complexes on silver toxicity were examined. Acute toxicity tests were conducted according to EPA/600/4-90/027F (U.S.EPA, 1993a). Following acute toxicity tests, short-term chronic embryolarval procedures were performed according to EPA/600/4-91/003 (U.S. EPA, 1993b) on three fish species (Tier II). Embryolarval toxicity tests were carried 4 days post hatch. The long-term effects of silver on the sheepshead minnow were evaluated using 28-d early life-stage toxicity tests (Tier III). Early life-stage tests were conducted according to EPA/500/9-86/003 (U.S. EPA, 1986a). For all tests except those conducted with the tidepool sculpin, seawater was reconstituted Forty Fathoms® bioassay grade salt. Natural seawater was used for sculpin toxicity tests. All tests were conducted under a 16:8-h photoperiod at  $25\pm 1^\circ\text{C}$  for inland silversides (*Menidia berylina*) and sheepshead minnows (*Cyprinodon variegatus*);  $18\pm 1^\circ\text{C}$  for summer flounder (*Paralictys denatus*) and topsmelt (*Atherinops affinis*); and  $10\pm 1^\circ\text{C}$  for tidepool sculpins (*Oligocottus maculosus*).

## Results and discussion

### Tier I

The acute toxicity of silver was determined for five marine fish species. The 96-h  $\text{LC}_{50}$  values ranged from 183 (95% confidence limits: 157-216)  $\mu\text{g Ag.L}^{-1}$  for the topsmelt to 1065 (1008-1119)  $\mu\text{g Ag.L}^{-1}$  for the sheepshead minnow (Table 1). When silver was tested in combination with ammonia, toxicity was enhanced and the onset of mortality hastened. In tests conducted with the tidepool sculpin, mortality progressively increased at 6.35  $\mu\text{mol Ag.L}^{-1}$  from 55 to 100% in the presence of total ammonia ( $\text{amm}_\text{T}$ ) concentrations ranging from 0 to 12.6  $\text{mmol amm}_\text{T.L}^{-1}$ . Conversely, the  $\text{LT}_{50}$  estimated at this level of silver exposure dropped in a dose-dependent fashion from 5730 to 1180 minutes over the same range of ammonia concentrations (Shaw *et al.*, 1998). The effects of salinity were varied. Cerargyrite,  $\text{AgCl(s)}$ , was not acutely toxic. If mortality was not observed at silver concentrations lower than the threshold for cerargyrite precipitation, toxicity did not occur at higher silver concentrations (Fig. 1, upper). When toxicity was observed, 96-h  $\text{LC}_{50}$  values increased with salinity (Fig. 1, lower). In tests conducted with the sheepshead minnow (Fig. 2), no mortality occurred at exposure concentrations up to 2000  $\mu\text{g Ag.L}^{-1}$  at 20 and 24‰ salinity. However, toxicity was observed (96-h  $\text{LC}_{50}$ : 1065  $\mu\text{g Ag.L}^{-1}$ ) at 28‰ salinity, following a shift of only 4‰. As salinity was increased to 32‰, toxicity was reduced (96-h  $\text{LC}_{50}$ : 1173  $\mu\text{g Ag.L}^{-1}$ ). Thiols reduced acute silver toxicity (Fig. 3). Survival approached that of controls, when silver and 3-MPA were present in equimolar concentrations, for all species tested. However, we do not know the effect of Ag-thiol complexes on bioaccumulation and chronic toxicity.

### Tier II

The short-term embryolarval effects of silver were evaluated for three fish species. The  $\text{LC}_{50}$  values ranged from 85 (73-98)  $\mu\text{g Ag.L}^{-1}$  for the inland silverside to 2674 (2305-3100)  $\mu\text{g Ag.L}^{-1}$  for the sheepshead minnow (Table 2). While toxicity was observed, there were few embryological responses (e.g. mortality, terata). Thus, most chronic effects occurred post hatch.

### Tier III

The effects of chronic silver exposure during early development were evaluated with the sheepshead minnow. The 28-d  $\text{LC}_{50}$  was 1095 (960-1215)  $\mu\text{g Ag.L}^{-1}$  (Table 3). This value was roughly half the value obtained from short-term embryolarval procedures.

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## Conclusions

Collectively, these studies provide results useful for the development of water quality criteria. The range of acute and chronic toxicity was in accordance with literature values (Hogstrand and Wood, 1998). It was several orders of magnitude greater than measured silver concentrations in marine environments, 0.1-32 ng Ag.L<sup>-1</sup> (Shaffer, 1996), and the U.S. EPA acute criterion for silver in seawater, 2.3µg Ag.L<sup>-1</sup> (U.S. EPA, 1986b). In addition, ammonia, salinity, and 3-MPA were identified as parameters that greatly influence silver toxicity. However, current regulatory strategies do not include any physio-chemical modulators. Results from these studies indicate that water quality criteria that do not incorporate such modifiers cannot accurately predict toxicity.

## Acknowledgement

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**Table 1. Results from 96-h Toxicity Tests**

Species	Salinity (%)	LC <sub>10</sub> (95% C.I.)		LC <sub>50</sub> (95% C.I.)	
		µg Ag. L <sup>-1</sup>	µM	µg Ag. L <sup>-1</sup>	µM
Topsmelt	28	99(42-127)	0.92	183(157-216)	1.7
	32	188(124-228)	1.74	259(208-306)	2.40
Inland silverside	24	171(133-198)	1.59	260(236-280)	2.41
Tidepool sculpin	25	229(125-288)	2.12	331(254-429)	3.07
	32	483(441-516)	4.48	664(635-696)	6.16
Summer flounder	29	175(85-265)	1.62	565(430-700)	5.23
Sheepshead minnow	20	> 2000	>18.5	> 2000	>18.5
	24	> 2000	>18.5	> 2000	>18.5
	28	820(738-882)	7.59	1065(1008-1119)	9.87
	32	959(863-1026)	8.89	1173(1112-1231)	10.9

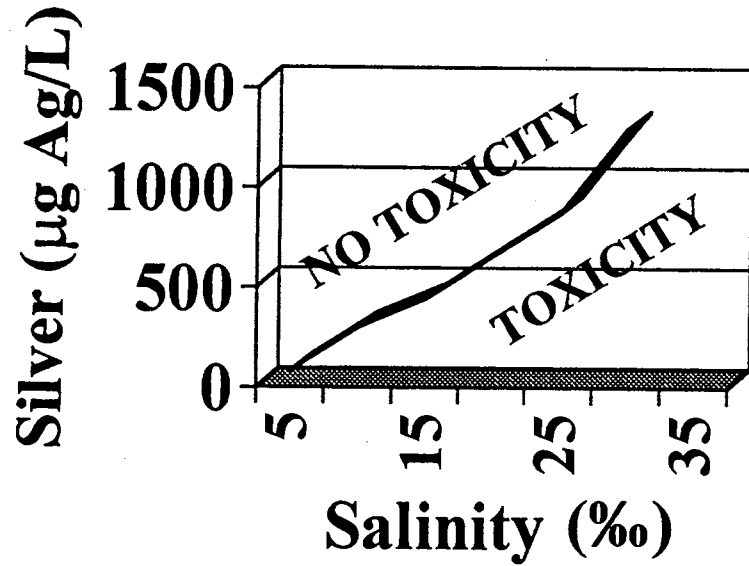
**Table 2. Results from Short-Term Chronic Embryolarval Toxicity Tests**

Species	Life-stage	Salinity (%)	LC <sub>50</sub> (µg Ag/L)
Inland Silverside	early organogenesis	24	85(73-98)
Topsmelt	gastrulation	28	416(362-476)
Sheepshead Minnow	early organogenesis	24	2674(2305-3100)

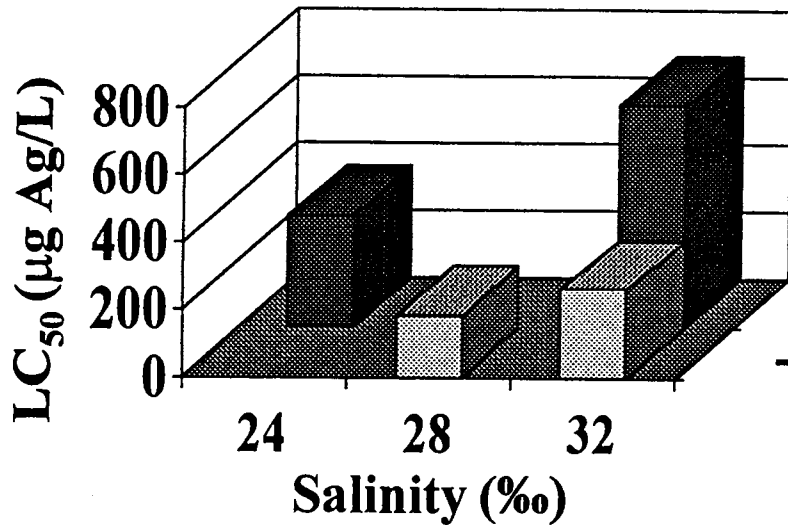
**Table 3. Results from 28-d Early Life-Stage Toxicity Tests**

Species	Life-stage	Salinity (%)	LC <sub>50</sub> (µg Ag/L)
Sheepshead Minnow	early organogenesis	24	1095(961-1210)

**Figure 1. The Effects of Salinity on Silver Toxicity**



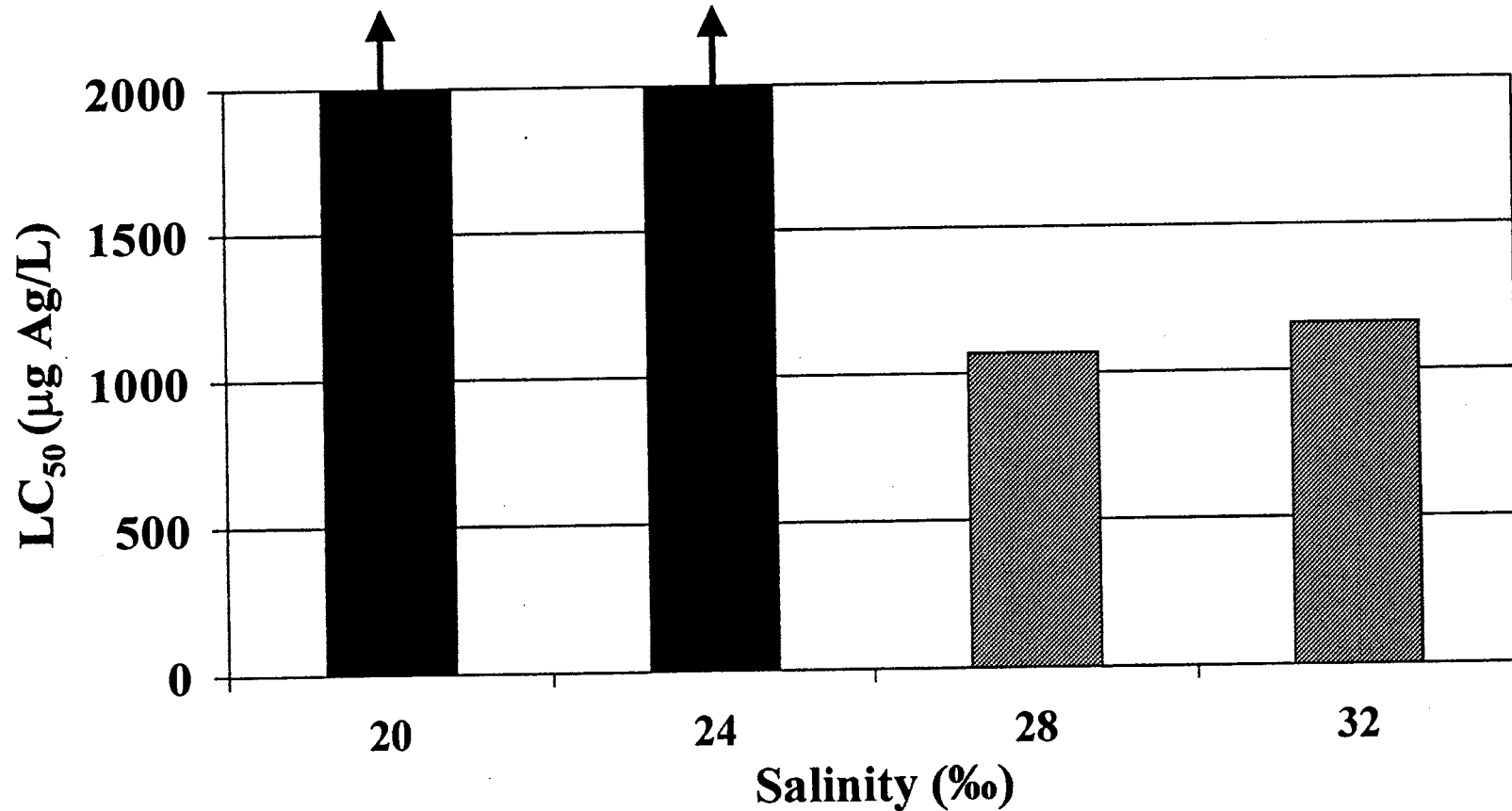
**Case I**  
 If tolerance was higher than the threshold silver concentration for cerargyrite formation, then no toxicity was observed



**Case II**  
 Toxicity was reduced with increasing salinity.

- Tidepool Sculpin  
 - Topsmelt

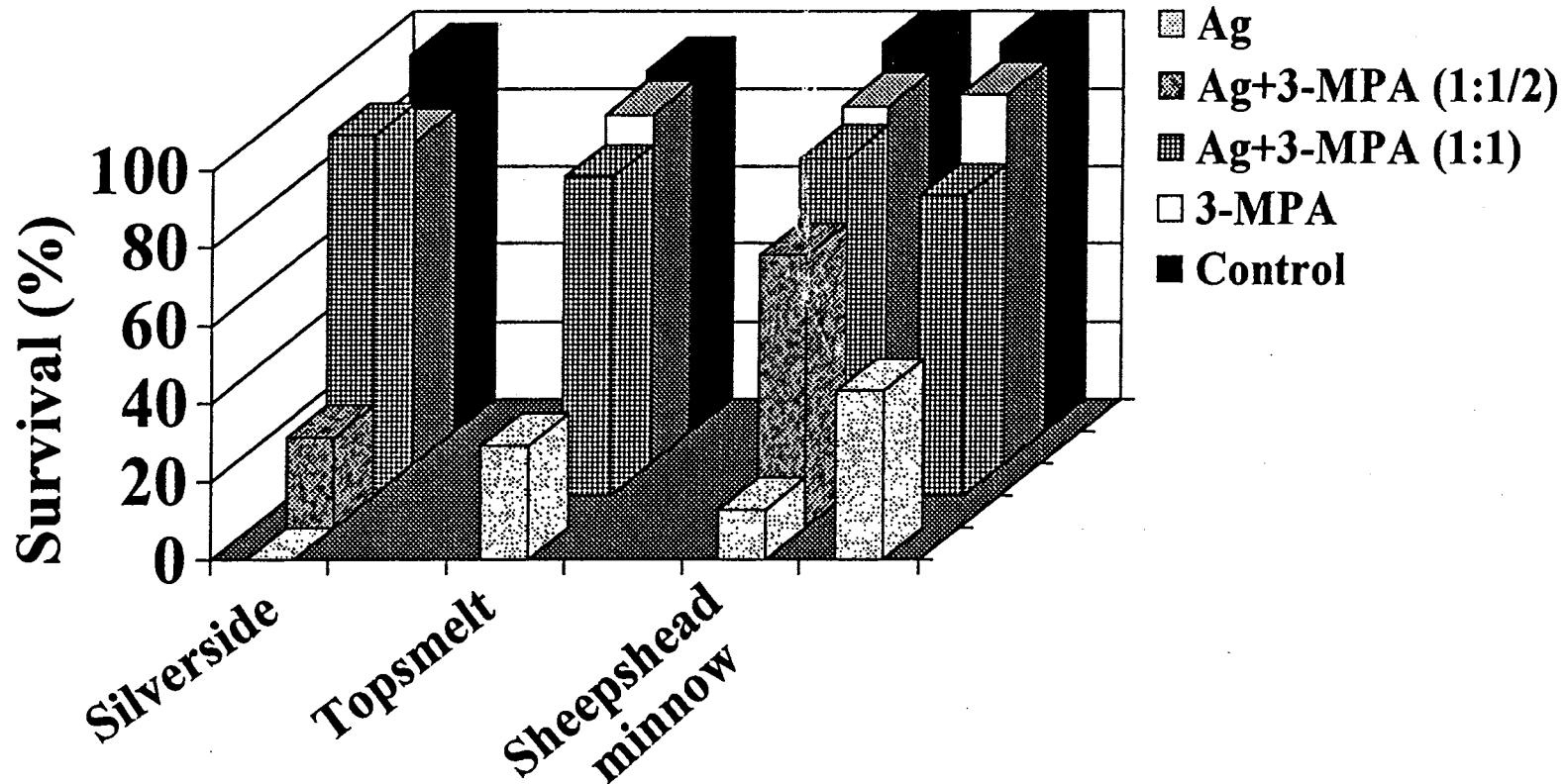
**Figure 2. Salinity and Sheepshead Minnow Toxicity**



**Case I: At 20 and 24‰, cerargyrite precipitation occurs before lethal concentrations of dissolved silver species are reached.**

**Case II: At 28 and 32‰, toxicity decreases with increasing salinity.**

Figure 3. Effects of Thiols on Silver Toxicity



### CAUTION

We do not know the effects of 3-MPA on silver accumulation and chronic toxicity.

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## Questions & Answers: The Acute and Chronic Toxicity of Silver to Marine Fish

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- Q. RUSSELL BELL (McMaster University): I'm very happy to see you making use of some silver thiolates. It's a nice piece of work, and I think you were quite smart in saying be careful with the data here, because I think it's entirely possible that even although these may be quite large molecules in aqueous solution...
- A. They may actually increase uptake?
- Q. Well, no, they may not necessarily increase uptake, but you may still get uptake simply because silver is actually mobile between one thiol and another. So if you have a thiol on the surface of your gill, then this big thing can come up and actually do a displacement reaction, and you do a swap, and you'll actually get a silver going on to the thiol on the gill. So you might well get some slow uptake of silver. A very nice experiment.
- A. That's an interesting comment.
- Q. WALTER BERRY (EPA Narragansett): I can't tell you how refreshing it is to see a salt water fish other than the rainbow trout (*laughter*). I know everybody doesn't have the luxury of having a view of Narragansett Bay from their window, but I'm concerned about the use of 40 fathoms in an experiment like this. Over the last couple of days we've heard about how fussy silver is about ligands and all sorts of things, and I wonder how, what sorts of methods do you use to characterize the salts, looking for something sneaky in there that might be doing something we're not sure about?
- A. Well, apparently we're not doing much on that. But I would like to add that we do plan on repeating some of these toxicity tests with some natural sea water to see if we actually see any different effects.
- Q. JIM KRAMER (McMaster University): I think I was the culprit that suggested the use of 3-MPA, and again I think I would back your comment as well as Russell's about being cautious. Not only the swap but the other aspect that I think you need to think about to make these correct, is that probably all these thiols are not in solution. They're all bound, and that was a point I made and I wanted to re-emphasize - that they may be cleaved by bacteria, but they're on a substrate. And that I think will be an important aspect too, and maybe we can talk via e-mail or something about how you might rig an experiment to look at a bound versus a free thiol.
- A. I might like to add, with regard to that experiment - which will help some of the geochemists - it was carried out at a pH of about 8.3 and it was in 90% saturated sea water.

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## Seawater Performance by Rainbow Trout Following Long-Term Silver Exposure in Freshwater

Elizabeth A. Ferguson and Christer Hogstrand

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Silver has been shown to be a potent and specific inhibitor of the ionoregulatory enzyme,  $\text{Na}^+/\text{K}^+$ -ATPase (Morgan et al., 1997; Ferguson et al., 1996). This enzyme is key to survival as it is involved in numerous tasks that aid cells in maintaining ionic and osmotic balance. In freshwater fish, Ag has been shown to specifically target the  $\text{Na}^+/\text{K}^+$ -ATPase in the gill epithelium (Morgan et al., 1997). In fact, blockage of this enzyme is believed to be the key mechanism causing freshwater Ag toxicity in fish. Even at sub-lethal  $\text{Ag}^+$  concentrations (0.5  $\mu\text{g}/\text{L}$  and up), disturbances have been noted in the ionic composition ( $\text{Na}^+$  and  $\text{Cl}^-$ ) of plasma samples taken from exposed fish (Wood et al., 1996, Galvez et al., 1997). This type of sub-lethal effect on ionoregulation could become quite significant during the sensitive stages of development (parr and smolt stages) in which the anadromous fish move from a freshwater habitat to seawater. During this transition, termed smoltification, the fish must quickly adapt to a hyperosmotic environment employing completely opposite ionic and osmotic strategies than those used in freshwater.

In order to determine the effect of freshwater Ag exposure on the ability to smolt, 24-h seawater challenge tests were performed throughout the parr-smolt transformation period. This test has a long history of use in aquaculture as a means for determining the maturity of parr and smolt stage fish beyond looking for typical changes in coloration, morphology, and behavior. In the laboratory, the test has been used to look at physical and physiological factors that might influence marine survival of anadromous fish during smoltification including exposure to pollutants such as cadmium and arsenic (Haux, et al., 1987; Larsson et al., 1981). For our study, hatchery raised, parr stage ( $\approx 50$  g) rainbow trout were obtained in December and randomly sorted into three tanks. The exposure conditions included a control, 0 Ag, 0.51  $\mu\text{g}/\text{L}$ , and 2.54  $\mu\text{g}/\text{L}$  of Ag added as  $\text{AgNO}_3$ . The Ag concentrations were chosen such that the free silver ion,  $\text{Ag}^+$ , concentrations were 0.2  $\mu\text{g}/\text{L}$  and 1.0  $\mu\text{g}/\text{L}$  respectively as calculated using computer modeling with MINEQL+ applying ionic parameters of carbon filtered Lexington tap water. Ag exposures were started in late December and maintained for five months through late May. Water temperatures were allowed to fluctuate naturally. Photoperiod was adjusted biweekly to closely match natural light/dark cycles. Ten fish were removed from each exposure condition and placed directly into aerated and chilled, 25 ppt reconstituted seawater at 60, 99, 120, and 148 days (February, March, April, and May respectively) into the exposure. The seawater challenge was performed for 24 h after



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which, a plasma sample was taken from each fish for analysis of ionic composition and cortisol levels. After 152 d of Ag exposure, ten fish from each exposure condition were sampled directly from freshwater to provide an unchallenged baseline for comparison. Gill, liver, and the remaining carcass were also collected for digestion and analysis of Ag content.

Results indicate slight differences in ionic composition between the control group and the two exposure groups in March and April. The trout in the control group showed a decrease in plasma  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Mg}^{2+}$  concentrations in March that clearly indicated an increase in hypoosmoregulatory capacity, a "symptom" of the onset of smoltification in these fish (Figure 1A, 2A, and 3A). In fact, the plasma ion concentrations from the seawater challenged fish in March were not significantly different from those of the freshwater baseline fish sampled in May. The fish exposed to 0.51  $\mu\text{g/L}$  Ag showed what appears to be a delay or extension of the smoltification period (Figure 1B, 2B, and 3B). Plasma  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Mg}^{2+}$  concentrations were decreased in both the March and April samples; however, they did not decrease to control or freshwater fish concentrations. Similarly, the measured plasma ion concentrations from the high Ag exposure, 2.54  $\mu\text{g/L}$ , began to decrease in March (Figure 1C, 2C, and 3C). In April, the concentrations of plasma  $\text{Na}^+$  and  $\text{Cl}^-$  rebounded slightly while the  $\text{Mg}^{2+}$  ionoregulatory capacity increased (Figure 3C). Plasma cortisol concentrations were predictably higher in all seawater challenged fish than those of freshwater (Figure 4A-C). The increase in plasma cortisol is known to be an adaptive response used by fish to increase proliferation of ion transporting cells necessary in a hyperosmotic environment. It was noted as interesting that measurements of the plasma cortisol in all of the experimental groups closely mimicked the trends of the  $\text{Mg}^{2+}$  concentrations. The significance of this finding are yet to be determined. The wet body weights of the fish were recorded at the time of sampling for each group of fish. The results showed a trend of increased body weight throughout the experimental period in all exposure groups with no significant difference noted within a sample.

The present study suggests that Ag may have a slight effect on the concentrations of the plasma ions of rainbow trout during the period in which they are preparing to smolt. There is a trend of higher plasma  $\text{Na}^+$ ,  $\text{Cl}^-$ , and  $\text{Mg}^{2+}$  concentrations in the Ag exposed fish compared to control fish during the period of smoltification. There was also some evidence for a change in the timing of the seawater adaptation. It should be noted, however, that the effects observed in this study were very subtle and not totally consistent between exposure groups. This opens the possibility that the differences seen were the result of natural variation. The Ag concentrations used in this study were chosen such that they would border on the high end of environmentally realistic and yet be in the range of physiological impact. Use of such low Ag concentrations in this study did not result in clear cut effects on which to base a solid conclusion. Further work at higher Ag concentrations would have to be performed in order to determine if the apparent extension or delay in smoltification was indeed a true effect of Ag and whether these changes would cause a harmful effect on rainbow trout.

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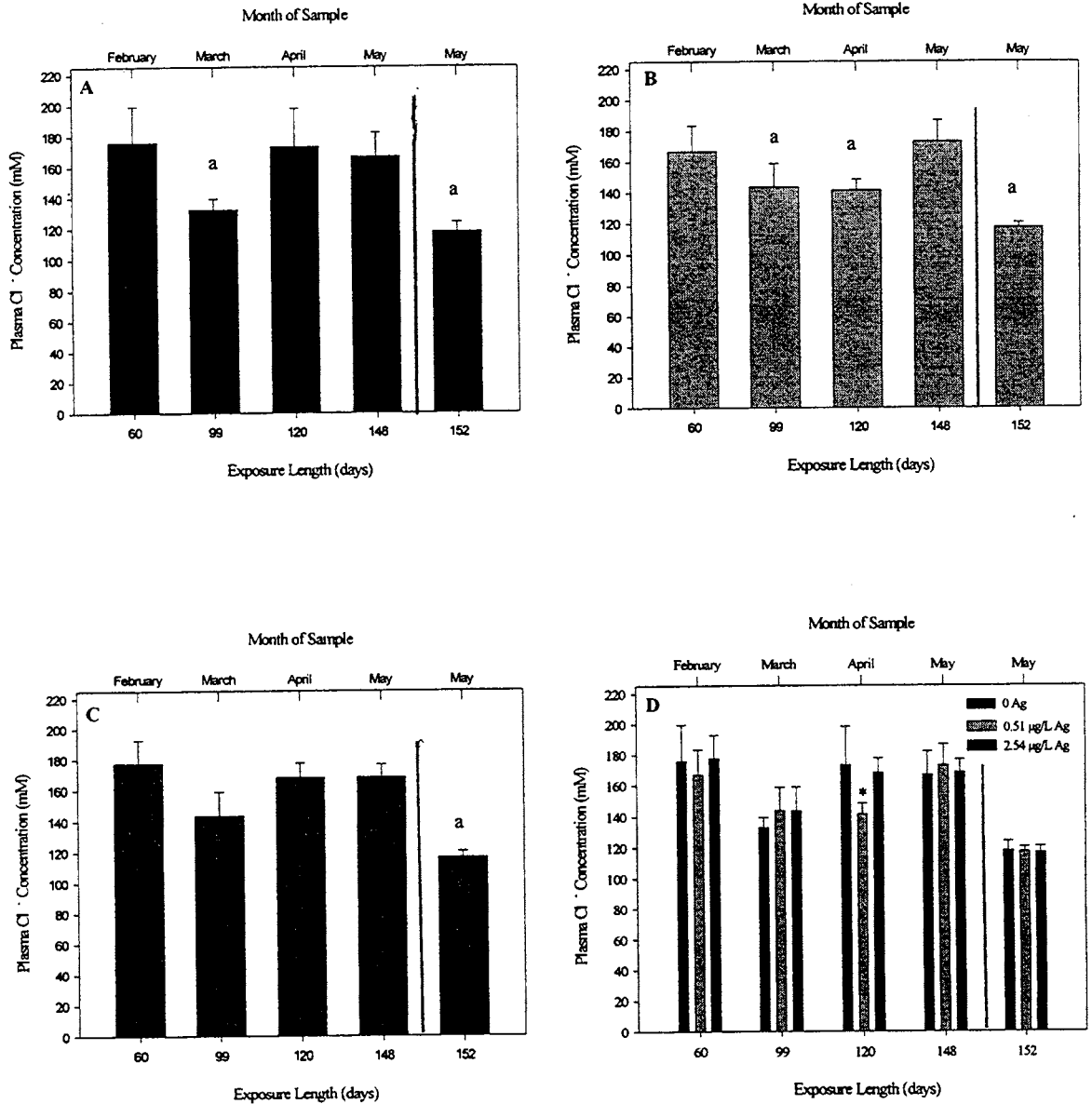


Figure 1. Plasma Cl<sup>-</sup> concentrations of A. 0 Ag, B. 0.51 µg/L Ag, and C. 2.54 µg/L Ag exposed fish after 24-h seawater challenge. Note: the fish in the second May sample (152 d exposure) were not seawater challenged. Statistically significant differences within the exposure level are indicated with a letter. Graph D shows the combined results of the monthly samples. Statistically significant differences within each month's sample groups are indicated with an asterisk (\*)

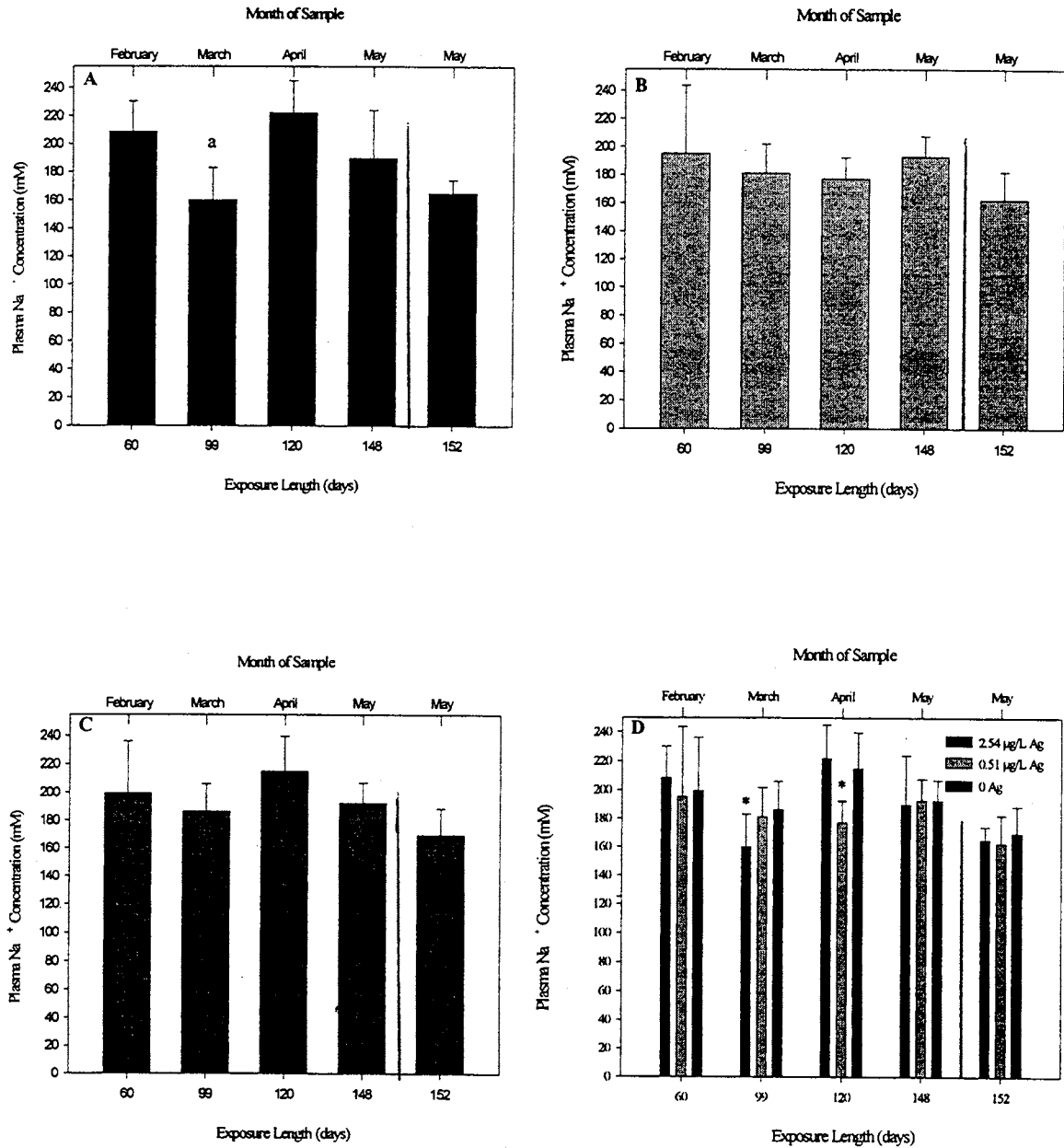


Figure 2. Plasma Na<sup>+</sup> concentrations of A. 0 Ag, B. 0.51 µg/L Ag, and C. 2.54 µg/L Ag exposed fish after 24-h seawater challenge. Note: the fish in the second May sample (152 d exposure) were not seawater challenged. Statistically significant differences within the exposure level are indicated with a letter. Graph D shows the combined results of the monthly samples. Statistically significant differences within each month's sample groups are indicated with an asterisk (\*)

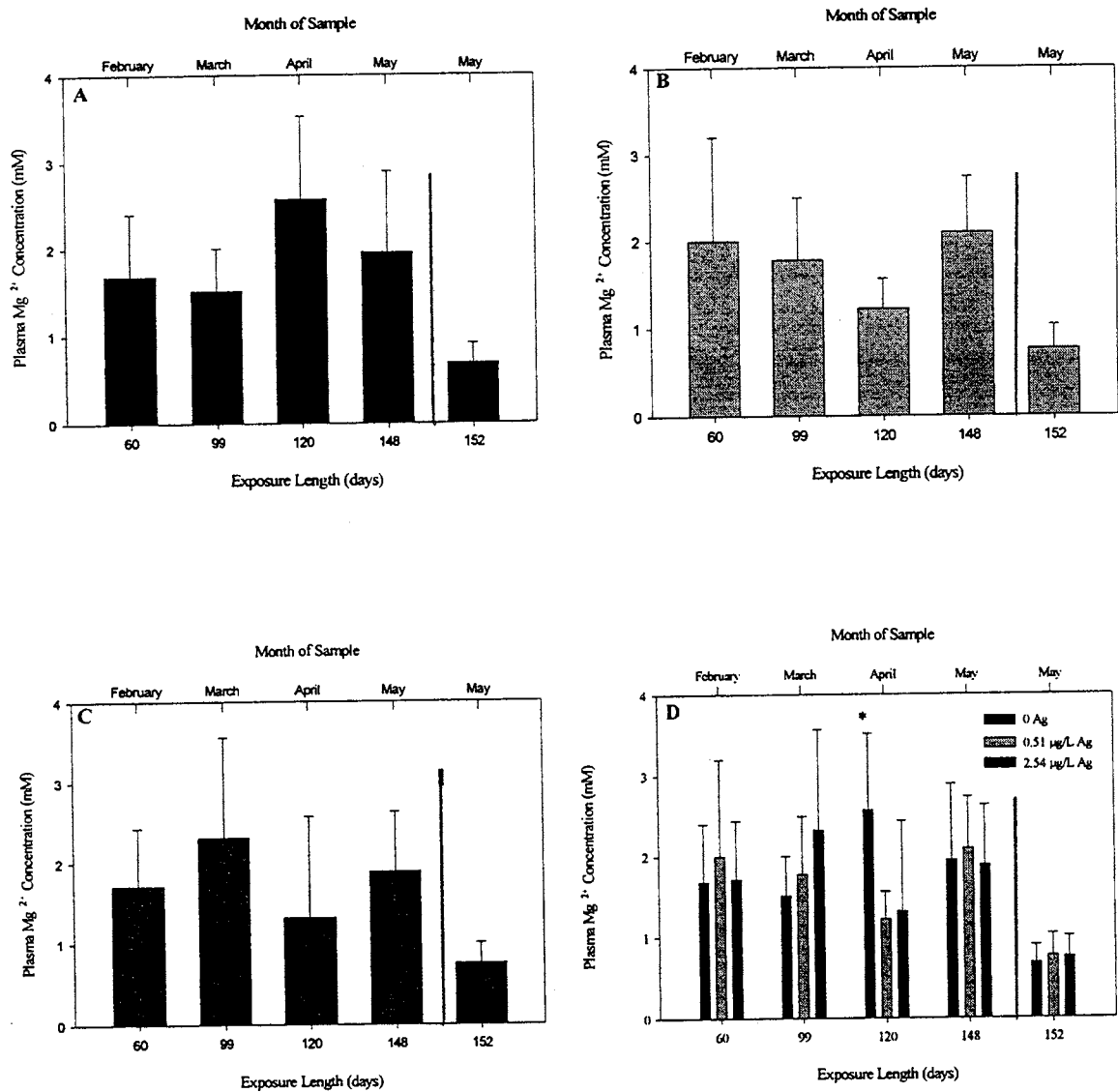


Figure 3. Plasma  $Mg^{2+}$  concentrations of A. 0 Ag, B. 0.51  $\mu g/L$  Ag, and C. 2.54  $\mu g/L$  Ag exposed fish after 24-h seawater challenge. Note: the fish in the second May sample (152 d exposure) were not seawater challenged. Statistically significant differences within the exposure level are indicated with a letter. Graph D shows the combined results of the monthly samples. Statistically significant differences within each month's sample groups are indicated with an asterisk (\*)

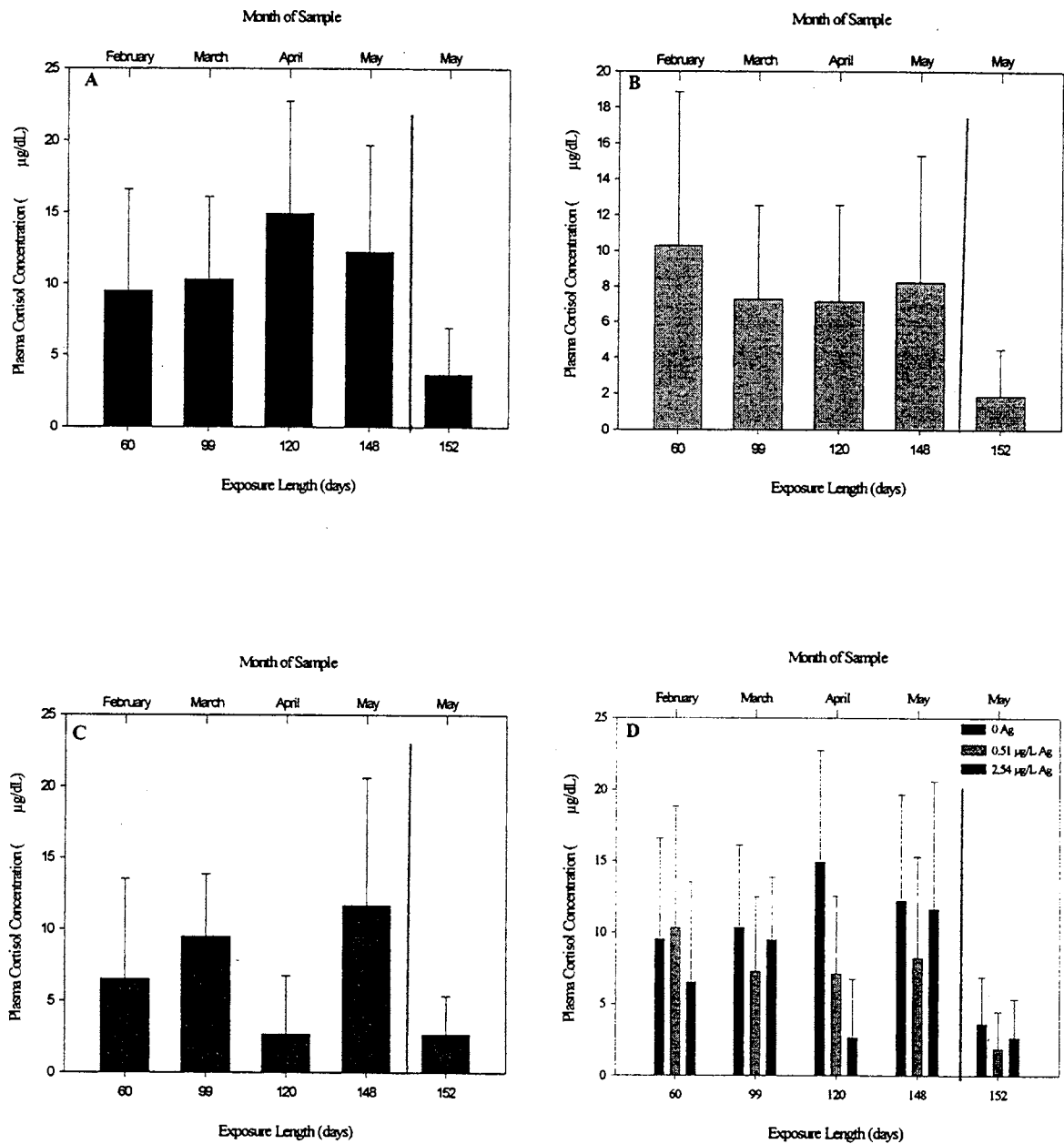


Figure 4. Plasma Cortisol concentrations of A. 0 Ag, B. 0.51 µg/L Ag, and C. 2.54 µg/L Ag exposed fish after 24-h seawater challenge. Note: the fish in the second May sample (152 d exposure) were not seawater challenged. Statistically significant differences within the exposure level are indicated with a letter. Graph D shows the combined results of the monthly samples. Statistically significant differences within each month's sample groups are indicated with an asterisk (\*)

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## Questions & Answers: Seawater Performance by Rainbow Trout Following Long-Term Silver Exposure in Freshwater

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- Q. BRUCE WALKER: This is sort of unrelated to biology, but in one of your test concentrations where you had 0.2 micrograms per liter, you said that is free silver ion. Then you had 0.1, and you had set that up based on calculations through a model. It seems like those are levels that should be able to be measured by some of this voltammetry stuff. Has anybody in this study, or any of the other ones that you're aware of, checked it with any of the available measurement tools - since we don't have the wafer yet - to determine how accurately the model is predicting what we could measure if we would try to measure it?
- A. We haven't measured it, mainly because none of us are good at voltammetry. But it is a great idea. We do need to compare what's modeled to what actually happens, and that's what I'm hoping the geochemists are going to throw at us one day, and say that MINEQUL is either right or wrong, or what our correction factor is on that. But no, we did not measure.

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## Toxicity Response of Freshwater Aquatic Organisms to Bioavailable Silver: A Comparison Among Species and Water Quality Parameters

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Clemson University

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Silver is presently regulated in receiving waters using a hardness-based water quality criterion (U.S. EPA 1980). The formula used to determine site specific limits is:  $\text{Silver} = e^{(1.72[\ln(\text{hardness})]-6.52)}$ . This regression equation was derived using acute toxicity values for aquatic organisms and associated hardness levels. However, a hardness-based water quality criterion for silver in receiving waters may not adequately predict safe levels in natural waters. For example, taking only hardness into account, the resulting criterion for freshwaters with hardness values of 50, 100, and 200 mg/L as CaCO<sub>3</sub> are 1.2, 4.1, and 13 µg/L total recoverable silver, respectively. Data to adequately characterize silver toxicity to freshwater fish for combinations of a variety of water quality parameters have been lacking and are only presently being developed.

Current attempts to extrapolate existing data sets to many sites result in extremely low silver limits. The error associated with this extrapolation is not well characterized; hence these silver limits may be unnecessarily low. Another inherent problem with the current criterion method is the use of total recoverable silver. Most silver present in effluents is in the complexed form, with the relatively nontoxic compounds having low water solubility. It is the free silver ion (Ag<sup>+</sup>) that is of greatest toxicological importance. Thus, it was the objective of this study to generate a silver nitrate (AgNO<sub>3</sub>) toxicity data set for *Daphnia magna* (DM), fathead minnows (*Pimephales promelas*, FHM), and rainbow trout (*Oncorhynchus mykiss*, RBT). The toxicity exposures were designed to account for variations in water quality parameters such as chloride, hardness, and dissolved organic carbon (DOC).

We conducted toxicity bioassays to quantify water quality conditions under which silver, as silver nitrate, is toxic to these freshwater aquatic species. Bioassays for DM and FHM were conducted as static replacement, whereas a flow-through bioassay system was modified and used for RBT. Our studies exposed DM and FHM to silver, as silver nitrate, at chloride concentrations ranging from 3 to 40 mg/L chloride for DM (60 for FHM), up to 200 mg/L hardness as CaCO<sub>3</sub>, and up to 5 or 10 mg/L dissolved organic carbon (DOC) for DM or FHM, respectively (Table 1). For FHM, mortality was 100% at silver concentrations of 20 or 40 µg/L, regardless of water quality parameters. For DM, mortality was generally complete at silver concentrations  $\geq 3.5$  µg/L. For DM and FHM, little protection was afforded by increased CaCO<sub>3</sub> alone, whereas DOC has a major ameliorating influence on measured silver toxicity. Lower concentrations of chloride ( $\leq 20$  mg/L) had little effect on reducing silver toxicity. Results presented here indicate DOC is more important than that of hardness when attempting to predict the toxicity of ionic silver in natural waters. For example, at only 5 mg/L DOC, we measured a significant reduction in acute toxicity of silver nitrate for 4-d FHM. Regression analyses of the relationships between mortality vs. DOC

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and mortality vs. chloride demonstrate the enhanced protectiveness of DOC versus chloride. Similarly, DOC appears to be critical in lessening total silver bioavailability to trout, while  $\text{Cl}^-$  and hardness have only a minor protective effect. However,  $\text{Cl}^-/\text{DOC}$  mixtures show a synergistic protective effect.

Our results indicate that environmentally realistic concentrations of aqueous silver pose no problem to larval rainbow trout. For RBT larvae, at DOC concentrations of 2.5 mg/L and 5 mg/L, the  $\text{LC}_{50}$  were 12.3  $\mu\text{g/L}$  and 22.79  $\mu\text{g/L}$   $\text{Ag}^+$ , respectively, compared to the control  $\text{LC}_{50}$  estimate of 1.62  $\mu\text{g/L}$ . And for DM, only humic acid was shown to significantly reduce silver toxicity ( $p > 0.001$ ). Hardness showed no significant affect on silver toxicity ( $p > 0.5$ ). Whereas hardness, as  $\text{CaCO}_3$  was maintained for RBT, hardness had no significant effect on acute  $\text{Ag}^+$  toxicity to FHM, over the range of 50 to 200 mg  $\text{CaCO}_3/\text{L}$ . Dissolved organic carbon appears to be critical in lessening  $\text{Ag}^+$  bioavailability to trout and minnows. Our results (Table 2) suggest that an organic carbon coefficient be incorporated into the silver criterion equation. The partial correlations in Table 2 express the independent effect of silver, DOC chloride, and  $\text{CaCO}_3$ . It may be seen that silver exerts a strong, negative, effect on survivorship and DOC exerts a positive effect, albeit of borderline statistical significance.

Finally, for all three species, we found  $\text{Ag}^+$  to exert its effect within the first hours (DM) or days (RBT or FHM) of exposure. This concurs with results in the literature (e.g., Hogstrand, et al. 1996; Janes & Playle 1995; Playle, et al. 1993). Given the critical nature of developmental growth at this early stage in life history, variance in survivorship may likely be explained by differences in organism age, even of a few days or hours. The sizes and mass of tested FHM and RBT in our studies are similar (Table 3). This may provide an explanation of their similar toxicity responses, as we measured in our studies and as reported in the literature (Lazorchak & Waller 1993; Rodgers, et al. 1997). The apparent sensitivity of DM to bioavailable silver may stem from its small size, relative to that of the fishes tested. The small size of DM yields a large surface to volume ration, and provides relatively greater surface area for silver to exert its effect on ionic transfer.

Overall, our study conclusions are the following: dissolved silver can be acutely toxic to DM, FHM, and RBT; however, dissolved silver has a high affinity to inorganic and organic matter; hence, using total recoverable silver to establish protective levels of silver in the environment is not warranted.

**Table 1. Tested species and water quality parameters varied during silver bioassays.**

	Time	Hardness	Chloride	Humic Acid	Total Silver
DM	48 hr	100, 200	3, 10, 40, 60	0, 2, 5, 10	0, .5, 1, 2, 3.5, 5
FHM, 4-d	96 hr	50, 100, 200	3, 20, 40, 60	0, 5, 10	0, 2, 5, 10, 20, 40
FHM, 28-d	96 hr	200	3, 20, 40, 60	0, 5, 10	0, 2, 5, 10, 20, 40, 50
RBT	96 hr	50, 100	3, 20, 40	0, 5, 10	0, 2.5, 0.5

**Table 2. Partial correlation analysis of total silver, chloride, DOC, and CaCO<sub>3</sub> hardness versus survivorship in acute toxicity bioassays. See text for further details.**

Species	Dissolved Silver	DOC	Chloride	Hardness
<i>Daphnia magna</i>	-0.64*	0.28	0.04	0.01
<i>Pimephales promelas</i>	-0.73*	0.19	0.03	-0.001
<i>Oncorhynchus mykiss</i>	-0.65*	0.32*	0.01	0.03

**Table 3. Life history characteristics for species used in testing silver toxicity.**

	Size as tested, mm	Mass, g	Time-to-Hatch, d	Life Span, d
DM	2	0.001 - 0.0035	2	60
FHM	7 - 10	0.05 - 0.1	5	900
RBT	10 - 14	0.5	31	2500

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**Questions & Answers: Toxicity Response of Freshwater Aquatic Organisms to Bioavailable Silver:  
A Comparison Among Species and Water Quality Parameters**

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No questions

# *Panel Discussion*

*Silver: Health/Ecological Risk Assessment and Product Life  
Cycle Analyses*

*Moderator:*

*S. Fogler, Eastman Kodak Co., Rochester,  
New York*

*Panelists:*

*J. Gorsuch, Eastman Kodak Co., Rochester,  
New York*

*D. Juberg, Eastman Kodak Co., Rochester,  
New York*

*P. Paquin, HydroQual, Inc., Mahwah, New  
Jersey*

*T. Purcell, Silver Council, Chevy Chase,  
Maryland*

*D. Timmons, Eastman Kodak Co.,  
Rochester, New York*

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## Panel Discussion: Silver: Health/Ecological Risk Assessment and Product Life Cycle Analyses

**Moderator:** Sarah Fogler (Eastman Kodak Co.)

**Panelists:** Joseph Gorsuch (Eastman Kodak Co.)  
Daland Juberg (Eastman Kodak Co.)  
Paul Paquin (HydroQual, Inc.)  
Tom Purcell (Silver Council)  
Donna Timmons (Eastman Kodak Co.)

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SARAH FOGLER (Eastman Kodak): We'd like to welcome everyone to our first panel discussion of the afternoon, which is on health and ecological risk assessment and life cycle analyses issues concerning silver. I'm told we're very short of time and we need to limit this discussion to only 25 minutes, so I'll ask each panel member to make a brief presentation for a few minutes apiece on the areas they've been dealing with, and then hopefully we'll have enough time for some questions from the audience. So now I'll turn it over to our first panel member, Tom Purcell, who is from the Silver Council and holds the position of Senior Vice President for Technical Issues.

THOMAS PURCELL (Silver Council): We're pleased to have the opportunity to make a presentation at this conference, and to start out we'd like to put some sort of perspective on a few of the issues surrounding silver. In terms of the environment, we can attribute the distribution of silver in the ecosystem to a variety of different sources that we've looked at very briefly. The earth's crust generally contains very low quantities of silver, and there it's in the mineralized form to such an extent that it's of no great concern for exposure. There I'm thinking about 0.1 milligrams per kilogram, in that range, is sort of a baseline that you would see if you would look at the earth as a whole and just look at the crustal material. Now there are local areas, of course, where you can mine this stuff, and there you would expect to see about 1,000 milligrams per kilogram, which is typical for a mining area. Quite often it's co-mined with other materials. This has partially to do with economic effects of silver mining and extraction more than anything else, but it's co-mined with gold and a variety of other metals that it's found in common with in these deposits. The deposits aren't widely distributed, though. You see them along the west coast of the United States, Mexico in the Oaxaca area and down the west coast of South America in those types of regions, but again not widely distributed across the surface of the globe. So how does it get to all these other places? Well, it's primarily moved by human activities to all the other spots where we might find exposure.

I think to a large extent what we're looking at are industrial inputs of silver. There you're not only changing the locale of the silver exposure but also the potential exposure to organisms and to the creatures that we've talked about here. But you're also talking about changing the forms of silver, and that depends on the type of industrial uses that we're trying to place the silver to, and there are a number of those. The most common use is as photographic imaging material. In the United States, roughly 50% of all the silver that's used here is incorporated into photographic imaging material. If we were to look at the life cycle analysis of that, then somewhere down the stream it's going through the developing process and almost all the silver is going to come out of there as a thiosulfate complex. That thiosulfate complex, since again a great majority of the photoprocess is devolved into sewage treatment plants, is going to be changed again in the sewage treatment plant before it's finally allowed to move into the environment. So the silver thiosulfate complex is probably of non-interest in environmental exposure. But the other chemical forms that it alters to as it passes through the sewage treatment plant are of interest.

Another large use of silver is in the electronic field and this is continuing to grow. Silver has been used for some time in materials like wire where you want high conductivity. It's been used in silver solders, and now increasingly in hard-wired board materials for a variety of electronic components. A lot of this is incorporated into the product itself and doesn't become an environmental problem until it's disposed of. This is true for something like a silver-based battery as well. You don't have any problem initially because the material is encased in a battery, until

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somebody tosses it out in the trash, and then it moves into the solid waste field. Now, what we find for a lot of these things is that over the last 4 or 5 years, increased recycling and increased reuse of these materials have diminished the amount of silver that's moving actively into the environment. There are some other places where we might find silver uses that have varying degrees of interest from an exposure standpoint. In the past, a lot of silver has been used in coins. That's becoming less common since certainly during the Hunt brothers episode and following that, the silver in the coins became worth more than the value of the coins themselves. I think it was most typified in the case of Mexico where they had silver pesos. The peso itself was worth nothing but the silver peso was worth about \$1 of American money. They used to be melted down and sold rather than spent. In the United States we've gone to bi-metallic coins that have no silver in them at all. So you're not seeing silver in that realm as much as we used to. Silver-bearing materials that are present in tableware are now sort of a relic of the past. We talked to tableware manufacturers and they're not seeing as much demand for that. People are not buying silver any more when they get married, they're not putting that on the list of things they have to have. And you know, normally I wouldn't mention that from an exposure standpoint, except in talking with solid waste landfill people I find that a remarkable amount of silverware is tossed out. It ends up in landfills, along with some silver coins. It's hard to imagine how people would do that. But just as an aside, when my daughter was very young she tossed out almost an entire place setting, because they taught her at school you throw out your plastic ware. She learned her lesson well but she just practiced it at the wrong time. Mirrors typically also use silver; again that's incorporated in a product and unless it's thrown away it's not an issue. Jewelry is another source of silver, somewhat of an issue not only in solid waste but from past manufacturing areas. If we look in the Connecticut area, say, where Gabe Benoit did his work in the Quinnipiac River area, there is a fair amount of silver in the 20cm-30cm depth range where it was disposed not only from just jewelry manufacturing but also plating operations, and we do see a fair amount of silver in that range. Lately, you don't find that as much in industrial discharges. The pressures from the EPA have forced them to clean up. There is less silver going down the drain and as direct discharges from industrial sources. Each source is different. In plating, for example you may have a cyanide-based silver, which is controlled as a separate problem because the cyanide itself is a difficulty. Or you may have particulate silver from a manufacturing process as opposed to any other kind. I think that pretty well encompasses the possibilities for entrance of silver into the environment. On this panel we're talking about risk, and we need to talk about what it does once it gets here.

DALAND JUBERG (Eastman Kodak): As I had mentioned yesterday to you, there are really two components to evaluating human health risks. These are hazard potential and then the subsequent exposure potential. To briefly review, silver with respect to human health is not a very interesting metal. It has very low inherent toxicological properties, and this is partially reflected by its low absorption within the mammalian system, on the order of 5-10% at most. It has no known real activation mechanism. It also has no true metabolism within the body and therefore no activated metabolites, so you have no opportunity then for subsequent interaction with genetic material, DNA and so forth. Finally, we have no evidence of chronic toxicity in humans, and we have some credible epidemiological evidence in occupational settings to back up these types of suggestions. The occupational setting is typically a high-end exposure scenario, so if we saw something in humans we would tend to first see it in occupational settings. So based on those types of considerations, I would submit that silver has low inherent toxicological potential in humans. Combine that with the limited exposure, which Tom introduced, we have negligible inhalation exposure for most humans. Drinking water represents some exposure potential, but again it's fairly limited. Ingestion from dietary sources seems to be the predominant route by which humans are exposed. And to respond briefly to Dr. Mukherjee's question concerning fish advisories and the need for silver to be included on those, or whether or not it should be: Typically fish consumption advisories have been reserved for those materials that are highly lipophilic, such as organomercury compounds. If you tend to protect against those types of material, the inherent assumption is then that you're protecting against any subsequent toxicity that another metal may impart. So those in large part have been based on lipophilic compounds such as PCBs and so forth. Coupled with that, we do not see significant exposure potential to humans from environmental sources. I think all this starts based on the fact that silver is a precious metal as opposed to some other metals out there. So there's some intrinsic economic value to limiting the amount that enters the environment. Be that as it may, EPA has appropriately identified argyria as the end point of concern upon which to limit human exposure. And once you divide exposure by what they consider to be a reasonable limiting exposure, silver exposure is not a risk to human health. I think I demonstrated that yesterday. Let me leave it at that.

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JOSEPH GORSUCH (Eastman Kodak): I have the privilege of introducing the ecological risk assessment, and for those of you that have attended all five Argentum Conferences, that's been the focus of these conferences - ecological data, either environmental fate or environmental effect data. (SLIDE 1) What I have up here is the paradigm. Some people have referred to these as cartoons. Maybe at this point in time we might appreciate a cartoon, but this is the paradigm that EPA suggested in 1992 in their framework document. It's gotten wide acceptance within both the regulatory as well as the industrial area, with a few modifications to this particular paradigm. I want to take you through it quickly. Each compartment has a number of sub-compartments. I'm not going to go through each of those, but just kind of point out some of the highlights and how this might be used for risk assessment for silver. The first compartment involves the problem formulation. One of the critical things about doing an ecological risk assessment is to identify the process by which man has caused some kind of ecological effect. Generally referred to as some kind of unwanted effect, this might be draining of wetlands or introduction of a chemical through a discharge. In the problem formulation, one of the things that has to be clear is what are you going to protect. Let's use as an example, then, water quality issues. Also, let's say we're going to protect the fish, particularly the rainbow trout since we've heard a lot of rainbow trout data. I'm going to actually use that as an example of what we might want to look at. So with that in mind one of the things you need to do is review all the current information. In the past five Argentum conferences we've had somewhere on the order of 180 presentations, many of them from scientific studies. There have been presentations from the same type of study but different components of those studies. We've had more than 25 publications, plus in the April 1998 issue of *Environmental Toxicology & Chemistry* there will be 14 more of our review papers, or actually papers of research from scientists at this conference, being published. So I think there is a wealth of information out there that we can draw upon to begin to do the risk assessment. The other thing is, this data must be reviewed carefully to make sure that Good Laboratory Practices or quality control have been implemented in those studies. The final thing that a risk assessor needs to do is try to keep a balance between the laboratory information and the field information. Once you've reviewed the data, identified what you want to protect at this level, you come up with a concept model that you can move into the next phase, next phase being the analysis. Take all the information you've collected, look at the environmental fate and effect data, keeping in mind with silver as with many other metals that the speciation is critical in this evaluation. As most of you are well aware, that most of the data have been generated using the ionic form of silver. The question is, how does that actually get used in the risk assessment?

Going on down to the third compartment then, with the above information there are some decisions to be made that allow us to make a determination on whether there is a risk either to a certain individual such as rainbow trout, to a population of fish, as an example, or to the entire community or ecosystem. One of the primary differences between human risk assessments and ecological risk assessment is that we aren't only just looking at one species such as man, but we can be looking at populations, communities or entire ecosystems. In the risk assessment characterization phase, there is definitely uncertainty, especially as you go from laboratory data to field data. The other thing you have to make a decision about is, are you going to protect 95% of the organisms, 90%, or what percentage? That determination will be handled in that particular box. Another thing that's critical throughout the entire process, starting way back up with the problem formulation, is that the risk assessor is communicating with a risk manager. Not only that, other critical stakeholders, whether it be the regulators if they're not already involved, or the industry, academics that have expertise in this area, public or interested parties, should really all be involved in the problem identification, so that when we get down to the final end we indeed are in this in a partnership. Also, even though it doesn't show up, after you do the analysis there must be a connection back to the risk manager. That's one of the modifications that I feel is important as well. So in other words, there's communication throughout this particular process. There's also an education process that's often required with the general public. I talked about the EPA paradigm, but what I have not alluded to is other models that are available throughout the world. In Europe, one of the processes that's referred to as ecological risk assessment is where the Predicted No Effect Concentration, or also called PNEC, is compared to the predicted environmental concentration. In other words, what actually goes in. So if you're looking at an effluent going into receiving water you need to determine whether there might be any harm or at least potential harm. That's usually represented by the ratio. As long as the ratio is 1 or less, usually there's not considered to be any harm.

PAUL PAQUIN (Hydroqual, Inc.): I would like to briefly discuss screening-level risk assessments for wildlife. One way a screening level risk assessment is performed is with the Hazard Quotient Analysis, which is fairly simple,

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conceptually. The Hazard Quotient (**SLIDE 1**) is the ratio of the dose, be it either via food or water intake. The intake is the dose of silver that any animal is exposed to, and it's divided by the reference dose, which is the dose causing adverse effects. You have to make estimates of the animal's diet, how much it eats each day and what the silver concentrations are in the food sources available to the animal. If it's home range is wider than the area that's contaminated, you have to take that into account as well. There are a lot of considerations, and this is a very rough estimate. Next, you look at the magnitude of the hazard quotient. Theoretically, if the number is greater than unity, the dose via ingestion exceeds the dose causing an adverse effect and, in theory, a potential problem exists. When these estimates are made, meaningful results are associated with hazard quotients that are tenfold greater than or less than one, because it's such an approximate calculation. For a hazard quotient of 0.8 to 0.9, that's considered borderline and too close to unity to draw a definitive conclusion. So it's a very approximate type of screening level calculation.

Next, I'd like to talk a little bit about both the numerator and denominator of the hazard quotient. The first point I want to make - and there are a lot of points you could make here - but probably a key consideration that we need to start thinking about is bioavailability. I have two examples I'd like to discuss, though not wildlife examples. (**SLIDE 2**) First, a dose-response curve for metal, percent survival versus total cadmium, is shown on the upper panel. Depending on the characteristics of the water the dose-response curve can vary; I'm sure you've all seen this type of data. The same total cadmium concentration (upper graph) can produce very low survival in one water and 100% survival in another, because total cadmium is really not bioavailable under all conditions. If you look at cadmium activity (lower graph), though, these same data converge to a single dose-response curve. I'm sure most of you are familiar with this type of example, if you've been here for the last couple of days. (**SLIDE 3**) In addition to bioavailability in the water column, we've also looked at bioavailability for sediment quality criteria and I think many of you are familiar with these types of data plots as well. If you plot organism mortality in sediments versus bulk sediment, you get a scatter plot of the data, and if you plot organism mortality versus the ratio of SEM to AVS - again, I'm not going to go into the details but I think many of you are familiar with these concepts. When that ratio is less than one, toxicity due to SEM metals is not expected, and when the ratio exceeds one you have the potential for toxicity. The lesson from these examples as it pertains to wildlife is that, in much the same way that bioavailability has been recognized with water and sediments, I think, when we're trying to estimate the dose associated with food intake or water intake by wildlife, we're going to have to start paying attention to bioavailability and how the silver is bound up in the food.

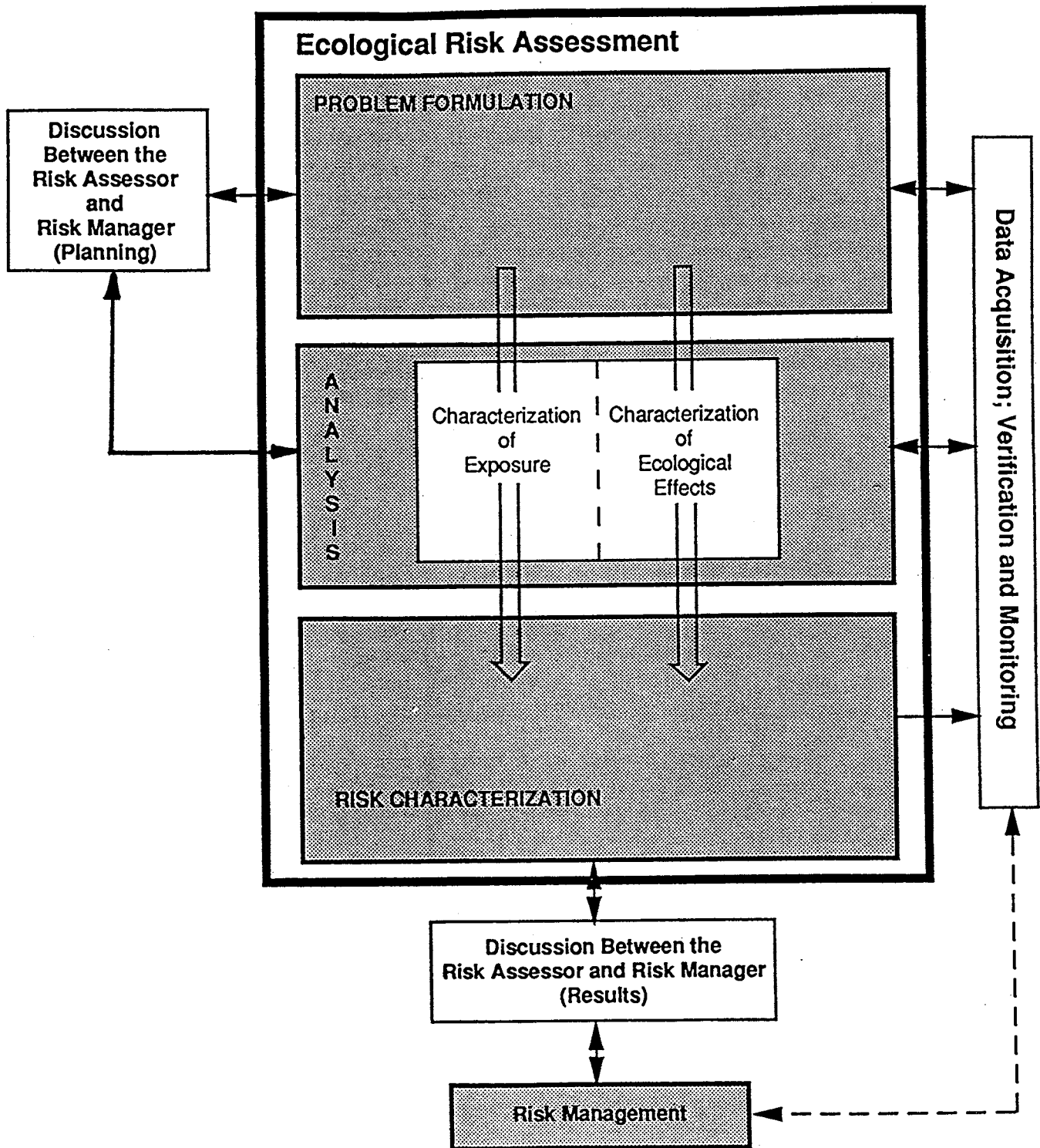
For the denominator of the hazard quotient we need the reference dose. (**SLIDE 4-LEFT SIDE**) Usually you identify a representative indicator species. You can't evaluate all types of wildlife, but you can assess risk for selected indicator species such as a fox, red-tailed hawk, a deer, or perhaps a mallard, representative types of animals that inhabit the site. What you need to do for each of those indicator species, then, is estimate what the dose is via their food consumption, the contamination levels in the food, and then assign a reference dose. You get the reference dose from a review of the literature, and usually there's not much tox data for a fox or a red-tailed hawk, so you use data for a surrogate species. This is what you might be forced to do if you're performing a screening level risk assessment for silver. (**SLIDE 4-RIGHT SIDE**) Let's say for the available tox data for a fox, about the closest you might come is tox data for a white rat. For the red-tailed hawk, you use tox data for a chicken. That's probably the same number you'd use for the mallard, and, of course, a deer is fairly close to a white rat. (*laughter*) That's what you're faced with doing, unfortunately. So there's a real paucity of tox data for wildlife, and I think that's another area we need to pay attention to.

DONNA TIMMONS (Eastman Kodak): (**SLIDE 1**) Well, I've spent the past couple of months being immersed in life-cycle assessments, not necessarily for silver, so coming to this conference has been an eye-opener. I'm part of a group that is trying to develop practical life-cycle assessment-based methods for integration right into our product design process. Our intent is, over the long-term, to improve the environmental performance of our products and concurrently reduce the life-cycle costs of those products. So I've seen all the flavors of life-cycle assessment, life-cycle thinking, and over the past couple of days I've learned a lot about risk assessment. And the thought I come away with - and it's very methodology related, not silver related - is that these two thinking processes are very different means of looking at environmental impact, but they're also very complementary. It's mainly because of the nature of the data that is used to do a life-cycle assessment versus a risk assessment. With a life-cycle assessment you're looking at very aggregated, normalized data - it might be pounds of silver

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emitted per 5"x7" image, for example - and as a result of that you don't have a picture of over what time those emissions may have occurred or even where they occurred. They could be separated by an ocean of distance in some cases. So as a result of that, what you end up with for a life-cycle assessment is a very high-level look at the environmental aspects of the product, and only a brief look at potential impacts. Whereas with a risk assessment, you're looking at a much more specific situation, occurring hopefully at a specified place or at least under specified conditions, so your results are much closer to actual effects. I think, in order to make these two processes effective, there really is a need to tie them together in some way. **(SLIDE 2)** Sarah showed this morning the life-cycle assessment framework, the four-step interrelated processes that you go through to do one of these things, and I think that there's a very natural linkage to risk assessment in the impact assessment phase. Remember, the purpose of an impact assessment is to condense this mass conglomeration of numbers that you have over the life cycle of the product into a smaller set of indicators or numbers that you can get your arms around. As Sarah said, this process is very immature at the moment. It's nowhere near being standardized, but there are some common threads that are starting to emerge, and one of the common threads at least is the name of the impact indicators. **(SLIDE 3)** These are some impact indicators that you'll commonly see, and as you see they're fairly broad. They cover everything from resource depletion to human and ecological health. Most of these can be traced back to some work that was done at the Center for Environmental Sciences and Leyden University in the Netherlands. There is some consensus on how to calculate the first two, the global warming potential and the ozone depletion potential. The others are a different story. There are all kinds of different ways of calculating these things, and as Sarah mentioned it's very controversial. I think the root cause of that is that things like global warming and ozone depletion have a single endpoint. But for things like human toxicity, there are all kinds of possible endpoints: cancer causing effects, reproductive effects, irritation effects, cosmetic effects, for example. How do you condense all that into a single number? I think that may be where risk assessment or some variant of it can come into play. **(SLIDE 4)** I think the question we need to start asking ourselves, either during this panel or more likely afterwards, are when do you use a life-cycle assessment versus a risk assessment, or when do you need both? And what role might risk assessment play in developing the indicators, particularly the human toxicity and ecological health indicators? And then lastly, is there a role potential for life-cycle assessment in identifying which risk assessments need to be done? Kind of a prioritization effort.

*(Because of limited time, there was no opportunity for audience questions to the panel.)*



Framework for Ecological Risk Assessment

(Note: Diagram is a slight modification of EPA's "Framework for Ecological Risk Assessment", Fig. 1, Ref. EPA/630/R-92/001 - Feb. 1992)

$$\text{HQ} = \frac{\text{DOSE VIA INGESTION}}{\text{DOSE CAUSING ADVERSE EFFECT}}$$

DOSE VIA INGESTION:

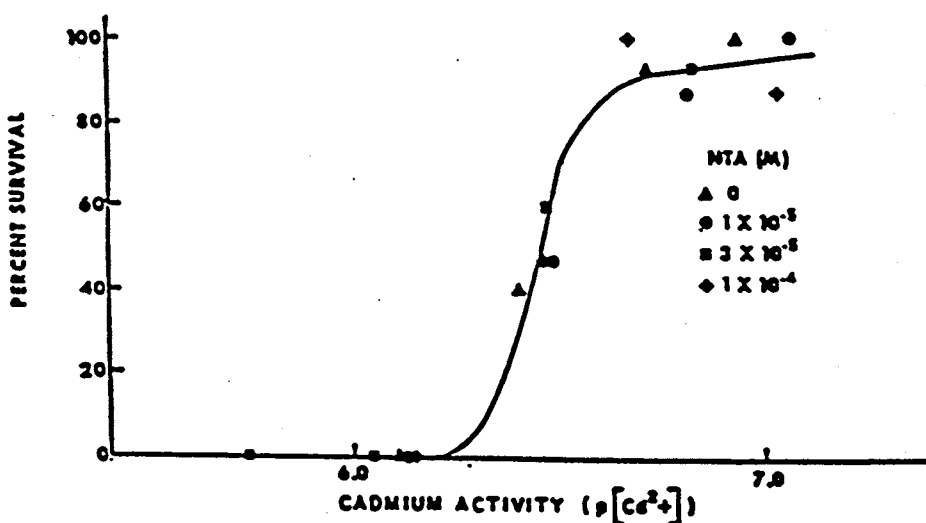
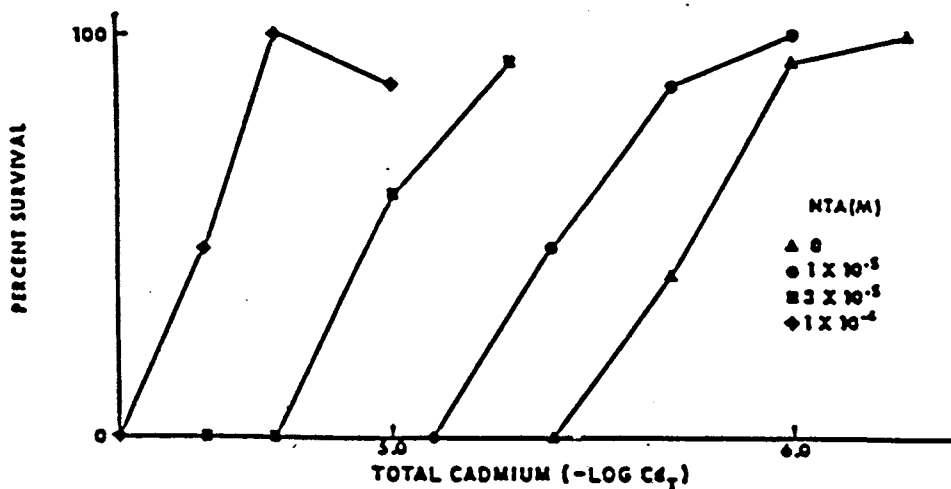
$$\text{DOSE} = \frac{\text{INGESTION RATE OF METAL VIA FOOD AND WATER}}{\text{WEIGHT OF PREDATOR}}$$

DOSE CAUSING ADVERSE EFFECT:

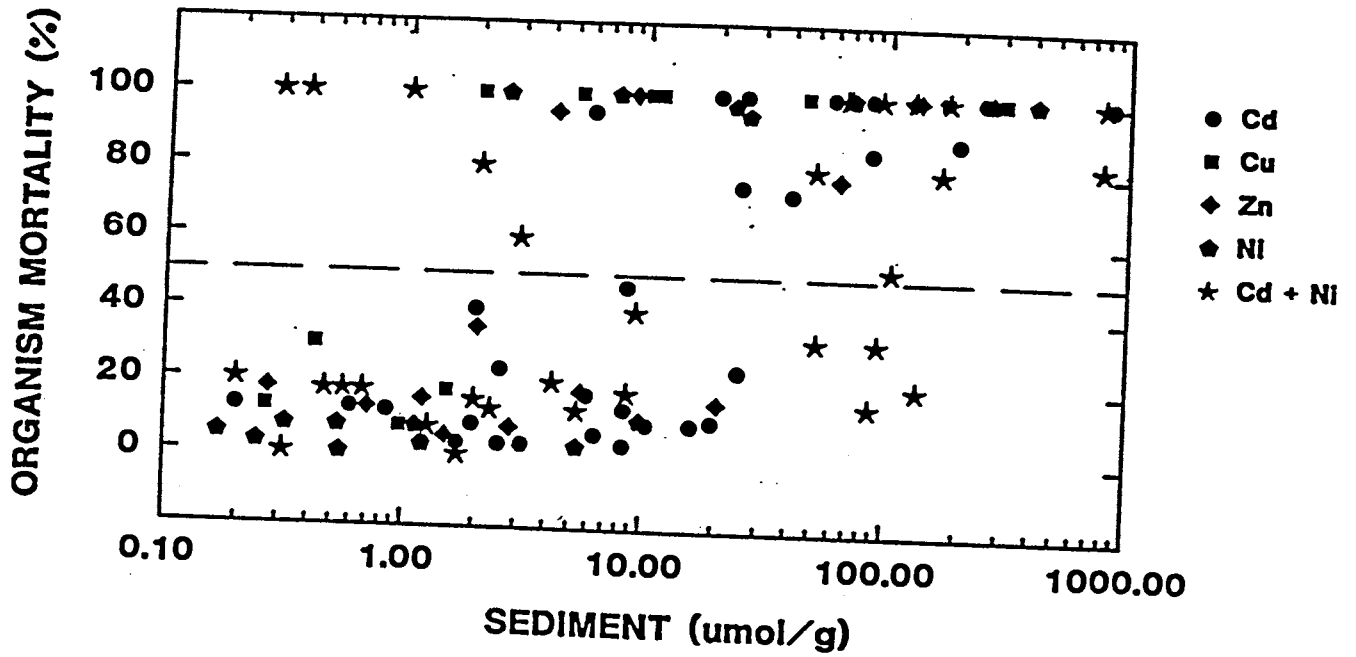
REFERENCE DOSE: MINIMUM EFFECT LEVEL  
DETERMINED FROM A LITERATURE REVIEW

**ACUTE TOXICITY OF CADMIUM TO  
GRASS SHRIMP ( Palaemonetes )  
EFFECT OF NTA COMPLEXATION**

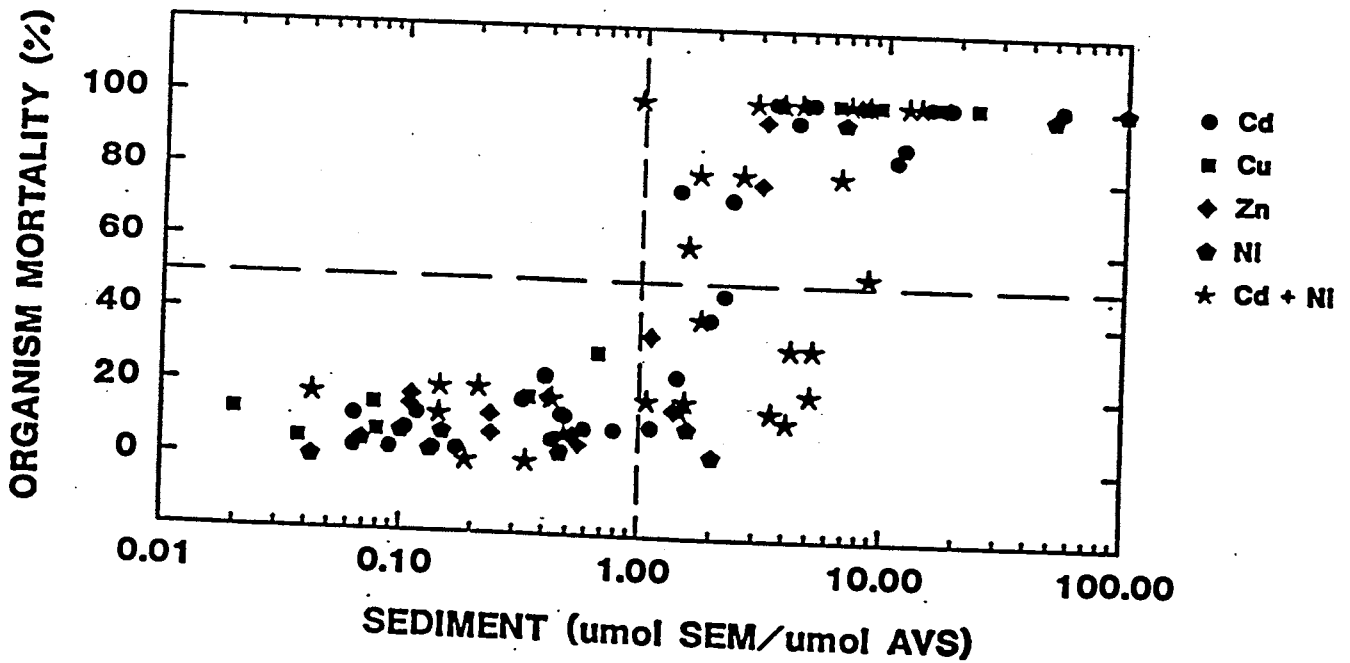
(AFTER W.G. SUNDA et al., 1978)



### ACUTE TOXICITY OF METALS TO AMPHIPODS DRY WEIGHT NORMALIZATION



### ACID VOLATILE SULFIDE NORMALIZATION



**INDICATOR SPECIES  
TO BE EVALUATED FOR  
RISK ASSESSMENT**

**SURROGATE SPECIES  
FOR WHICH TOXICITY  
DATA ARE AVAILABLE:**

FOX

WHITE RAT

RED-TAILED HAWK

CHICKEN

DEER

WHITE RAT

MALLARD

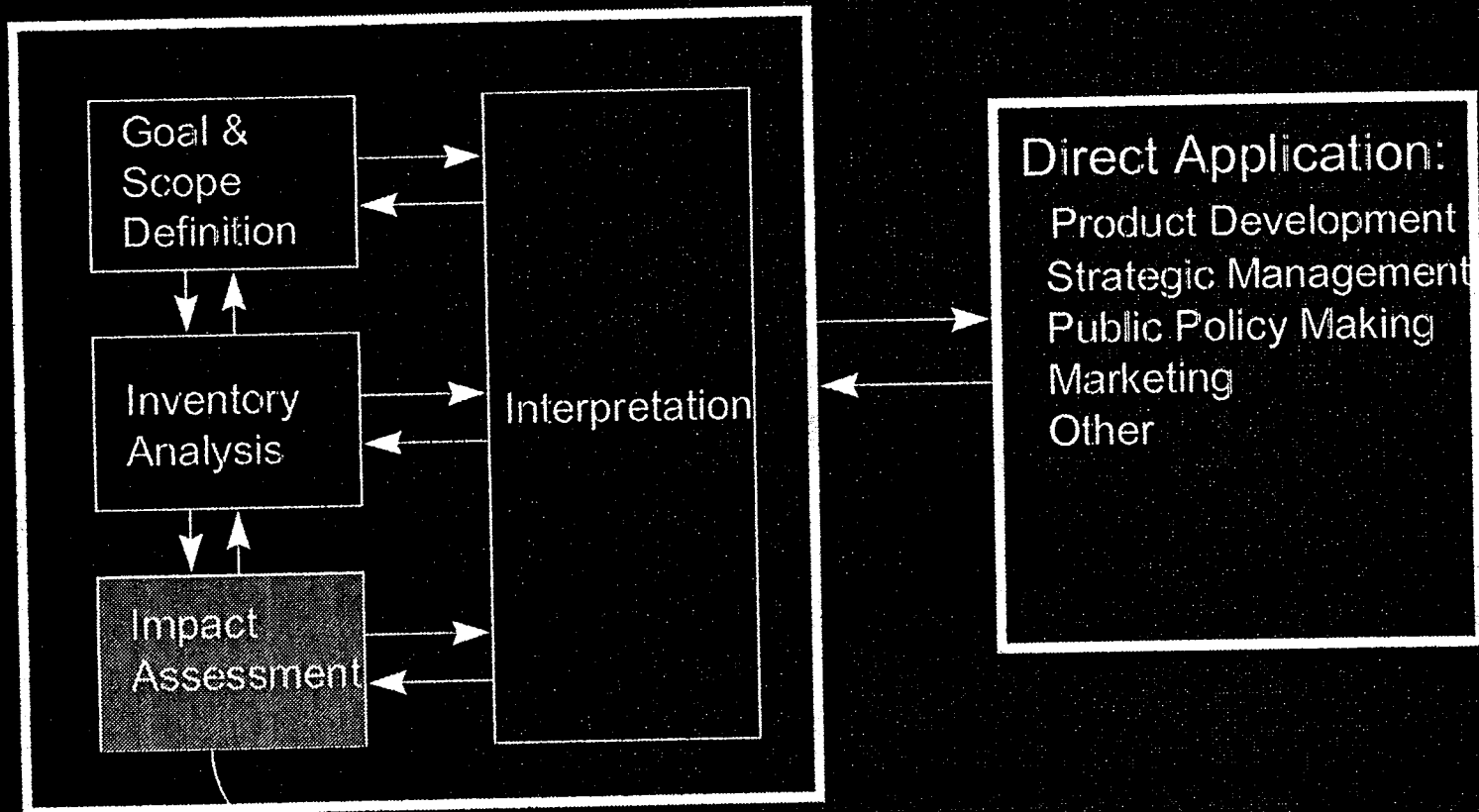
CHICKEN

# Complementary Processes

- ◆ Environmental Life Cycle Assessment
  - Performed on product system, ‘big picture’
  - Identifies *potential* environmental effect(s)
  
- ◆ Environmental Risk Assessment
  - Generally gives specific ‘local picture’
  - Results are closer to *actual* effect(s)



# Life Cycle Assessment Framework



LINKAGE TO RISK ASSESSMENT

# Examples of Life Cycle Impact Indicators

- ◆ Global Warming Potential
- ◆ Ozone Depletion Potential
- ◆ Resource Depletion Index
- ◆ Acidification Potential
- ◆ Smog Formation Potential
- ◆ Solid Waste Generation
- ◆ Hazardous Material Use
- ◆ Eutrophication Potential
- ◆ Human Toxicity Potential
- ◆ Ecotoxicity Potential

## Questions to ponder

- ◆ When to use LCA vs. RA vs. both?
- ◆ What role does RA play in the development of life cycle impact indicators for human toxicity and ecotoxicity?
- ◆ What role does LCA play in prioritizing RA needs?

# Panel Discussion

*Regulations and Environmental Concerns in Europe and Canada  
Regarding Silver and Other Heavy Metals*

*Moderator:*

*J. Kramer, McMaster University, Hamilton,  
Ontario*

*Panelists:*

*I. Boie, AGFA, Leverkusen, Germany*

*B. Ericson, Kodak AB, Jarfalla, Sweden*

*J.-F. Gaillard, Northwestern University,  
Evanston, Illinois*

*T. Gorham, Kodak Canada Ltd., Toronto,  
Ontario*

*M. Grosell, Risoe National Laboratory,  
Roskilde, Denmark*

*A. Kabata-Pendias, Inst. Of Soil Science &  
Plant Cultivation, Pulawy, Poland*

*S.F. Umani, Marine Biology Laboratory,  
Trieste, Italy*

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**Panel Discussion: Regulations and Environmental Concerns in Europe and Canada  
Regarding Silver and Other Heavy Metals**

**Moderator:** James Kramer (McMaster University)

**Panelists:** Immo Boie (AGFA, Leverkusen, Germany)  
Bjorn Ericson (Kodak AB, Jarfalla, Sweden)  
Jean-Francois Gaillard (Northwestern University)  
Thomas Gorham (Kodak Canada Ltd)  
Martin Groseil (Risoe National Laboratory, Roskilde, Denmark)  
Alina Kabata-Pendias (Inst. of Soil Science & Plant Cultivation)  
Serena Fonda Umani (Marine Biology Laboratory, Trieste, Italy)

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JAMES KRAMER (McMaster University): Well, as you've heard we're running very late on time, so I'll ask each panel member to make a brief presentation as the last panel did, and hopefully this time we'll have an opportunity for some questions from the audience afterward. So without further ado I'll turn it over to our first panelist, who is Bjorn Ericson of Kodak Sweden.

BJORN ERICSON (Kodak AB, Sweden): I think the situation in Sweden is best summarized by this statement which was made by the Swedish Chemicals Inspectorate in 1989 (**SLIDE 1**). I will read it in case the people at the back of the room cannot see: "Silver compounds are deemed to be hazardous due to bioaccumulation in plants (phytoplankton), toxicity to aquatic life and to warm-blooded animals, and recorded concentrations in surface waters close to or even above those which cause toxic effects in crustaceans and fish in laboratory tests." Since this statement was made in 1989, we know that much of the information that we have heard at this conference and the previous Argentum conferences, concerning the speciation of silver, was not considered when it was written.

In case you are not familiar with the relationship of Sweden to the rest of Europe, I will show you this map (**SLIDE 2**). You can see Sweden is one of the most northern countries and it is also quite a long country, extending from the Baltic up north to well past the Arctic circle. Most of the industries and the population are concentrated in the southern portion. As you can see from this next slide (**SLIDE 3**), the industrial and commercial sources of silver to the environment are primarily from photographic laboratories and X-ray laboratories, although a large portion comes from unknown sources. We believe that these unknown sources include some of the items that were mentioned by the previous panel, including batteries, electronics, jewelry, mirrors, tableware and other items which are discarded by households or in solid waste from many sources. The next largest source is from coal and oil burning to generate power and supply heat. You will note also that the authorities have established that a certain amount comes from dental fillings and crematories, which I believe may be the first time such items have appeared on a distribution list for silver - at least I have not seen it at this conference before. Most silver is taken out in the sludge from the treatment plant, and only a low percentage is still present in the water discharge.

As you see from this list of restricted metals (**SLIDE 4**), Sweden limits silver discharges to the sewer quite severely, at 0.06 mg/L. This is for total silver. The same restrictions are for chromium, nickel, lead, tin and zinc, and no mercury at all is allowed into the sewer. You will note, however, that silver is also listed on the "observation" list along with boron and cobalt. I believe this is because the Swedish EPA has become aware of the new information that is being developed on silver, and they are watching it closely. For photoprocessing (**SLIDE 5**), if one discharges less than 500 grams per year you do not have to pretreat your effluent before discharging it to the sewer, but if more than 500 grams you must. However, you also must not exceed a given effluent concentration. I show here a slide of how the environmental regulations have reduced metal discharges to the environment since 1985 (**SLIDE 6**). And finally, Sweden is now moving towards the harmonized European

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Union regulations which will uniformly limit the discharge of silver from photographic operations (SLIDE 7). I believe that is perhaps all the time I have. Thank you.

JIM KRAMER: Thank you very much, Bjorn. And now, Immo?

IMMO BOIE (Agfa Corporation, Germany): Thank you. I have a slide to show you of the European regulations, which contains limits from the so-called "black" list and "gray" list. If someone will be so kind as to turn it on? Thank you. I will keep my remarks very short because of the time. First of all we have different legal situations within the European community compared with the countries outside the EC. (SLIDE 1). Second, the EC works out environmental directives and the member states have to transfer these directives into their own water legislation. Third point, the so-called 76 Directive is used with the pollution caused by special heavy metals. This list contains the so-called "black" list and "gray" list. I mentioned it before. And in the "black" list you find only mercury, mercury compounds, cadmium and cadmium compounds, and organic tin compounds. And the only directives the EC has worked out are for these three kinds of metals, mercury, cadmium and tin compounds. Those limit values are legally binding throughout Europe, but only for these three metals.

For the other metals, for instance zinc, copper, nickel, also chromium, lead, arsenic, and silver, we find these on the so-called "gray" list. Each member of the EC has to regulate each of these elements in his own water legislation and has to decide how to manage and how to limit the input of these metals. And so that means even within the EC there are no common limits or values regarding this very important metal we discuss today, silver. Concerning the discharge of hazardous substances into water, the first demand at least in the center of Europe is to apply the "best available" techniques regarding avoidance of use and the treatments of the wastewater. And the consequence of this demand, to apply the best developed techniques, is that the authorities worked out different limits for special regions. These different limit values for wastewater can be given in the form of a concentration limit, milligrams per liter, or can be given based on the quantity of manufactured product. Perhaps, given in the photographic field by milligrams per square meter of processed materials. And so, perhaps especially for Germany, you find all the possibilities. On the bottom here for Germany you find the concentration that is binding for the metal industry and for the galvanizing industry is 0.1 milligram per liter. But in the field of photographic processing we have to limit the load, and so it's not a temptation to solve our wastewater problem by dilution with a lot of other wastewater. And as you can see, the limit values are based on the size of the lab, too - on the use of processing material per year. And we have a similar situation in France and in Austria. I have about 20 copies for European limits, if persons in the audience wish to have one.

JIM KRAMER: Thank you, Immo. There are handouts available up here in front if anyone wants them. And now I'll ask Serena to make some very abbreviated remarks.

SERENA FONDA UMANI (Marine Biology Laboratory, Italy): Very briefly, then, as Immo has stated just now, no overall European law exists about discharging several metals, in particular silver. And in Italy particularly, we have no national limits, even though we have I guess one of the most restrictive laws as regards effluents and drainage for all the other materials. So I hope that the European community wants to face all these kinds of problems and also in this case Italy could have some other problems, because it is next to a restricted marine system connected with the so-called Eastern countries, which in our case means also Albania. I don't know if you have any idea what is happening now in Albania. So I hope that it will be a common effort over all or Europe, mostly Western but Eastern Europe also if possible, to face this very important environmental problem. That's all - thank you.

JIM KRAMER: Thank you, Serena. And now, Alina?

ALINA KABATA-PENDIAS (IUNG, Poland): So I will add to what my colleagues from Europe said already. In my country as in other countries in central and eastern Europe, there are no overall limits yet for silver. For mercury we have some, but silver has not yet been an environmental problem. But it will be and we are aware. So let me highlight what kind of problem do we expect related to increased content of silver in the environment. There are two issues to my understanding, two main issues: concentration of silver in bottom sediments, and concentration of silver in municipal sewage sludge, which concentration will be increasing in sewage sludge with continued

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economic growth. May I have the first slide please?(**SLIDE 1**). There are very general rules that ability of or effect on the biota will depend. I could add here not only pH, high clay granulometric fraction, but also redox. The effect on biota will depend on these three factors, which are very variable in sediments and are again quite variable when sediment is dredged, and when disposed on the land, or soil. And this is what we have to understand and to learn about, especially. May I have the second slide? (**SLIDE 2**). As far as we know from all available data, silver is an element which is taken up passively by plants. It is neither accumulated by plants, nor excluded, but is taken up passively. Up to now we know that the concentration of silver in green plants is a function of silver concentration in the soil. That is the reason why we have to know about and to control this problem. But let me add, I would like to mention that there is one gap in our studies on behavior of silver in the environment. We have been listening here to many interactions of silver with cations and organic ligands and so on, but not with phosphate. And phosphate is the one anthropogenic element of a great importance, especially in bottom sediments now. It is especially important when considering effects on agriculture. I think it is something that has to be included in further studies.

JIM KRAMER: Thank you very much. And that's an important addition. Martin, I'll ask you to say some very terse words.

MARTIN GROSELL (Risoe National Laboratories, Denmark): Well, first of all I'd say that I do not have a lot of experience in silver, silver toxicity, and definitely not in environmental regulation of metals in Europe. But I guess since I'm the only Dane at this conference I'm the most qualified to speak about Denmark, so I guess that explains why I'm up here. I think it's important maybe to explain a little bit about the background for regulation of metals and other chemicals in the environment in Denmark and probably also in a great part of Europe. It's mostly based on what we call a PNEC, Predicted No-Effect Concentration. When the environmental concentration of a given chemical is approaching this concentration or exceeding this concentration is when the environmental authority starts to take action, say towards an industry producing these kinds of chemicals or using these kinds of chemicals. Now the PNEC concentration of a given chemical is derived from all available data on toxicity of that chemical and the frequency of those data. So if this is the concentration - say it could be LC<sub>50</sub> value, 96-hour LC<sub>50</sub> values reported - and this is the frequency in which those concentrations occur, you will, provided you have enough data, have a normal distribution curve like this (**SLIDE 1**). We accept the 5% loss of species in our ecosystems, this is depending of course on the ecosystem. If this is an ecosystem which is worth protecting to a higher degree, then this can be, say, 1% or even less. And it also provides that, say, these 5% of the species are not all, say, primary producers, such as phytoplankton or something like that. On this concentration here a number of safety factors can be applied and will be applied depending on, for one thing, the number of data, and also to take into account that there might a difference in acute toxicity represented by the LC<sub>50</sub> values and chronic toxicity in the environment. One important thing about this curve is that the more data you have on a given chemical, the more slim this distribution, the higher and the more slim it would be, and that would tend to move this concentration upwards and would also tend to reduce the number of safety factors applied to this concentration to reach the PNEC value. Now, a lot of data has been presented for silver in this conference, so I'll just keep my remarks short. To date, there has not been enough data available to establish with confidence a Predicted No-Effect Concentration for silver. (**SLIDE 2**). In a way I guess that's good for the photochemistry or the industry. There are, however, some guidelines - no limits, but guidelines for emission of silver to a sewage plant, and for the maximum total silver concentration in industrial effluent (**SLIDE 3**). That is at, yes, 25 micrograms per liter in water going into the sewage treatment plant and 250 micrograms per liter in industrial effluent. But at this point, no PNEC values.

JIM KRAMER: Okay, thank you very much - sorry to cut you off; but we have to move on. Jean-Francois, s'il vous plait?

JEAN-FRANCOIS GAILLARD (Northwestern University): Can I have Bjorn Ericson's map of Europe just to look at France? OK. A lot of things have been said, and France is part of the European Community so a lot of the rules that ultimately will apply to the European Community will apply to France. To see how a lot of regulations are effected in France, one thing we can look at is the map and see that we have big rivers here, and each of

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these rivers actually are associated with what's called a river basin. Or "water agencies" - since 1964, they just changed the name. So basically, in all of these rivers - let's say the Rhone River here will be one - they will monitor a concentration, and if you're an industry you have to provide your total information in terms of the load that you will propose to give to a river. You have to do this either on a mandatory basis if you are a polluting industry or on a voluntary basis in order to actually show that you are respecting some kind of threshold. The thresholds are actually given in terms of total reject weight say either by the day or year. So dilution won't work, you cannot dilute, you have to go below a certain level. If you do not comply with this, you'll have to pay a fee to this Water Agency. Then the higher your reject load is, the more fee you have to pay. But if you prove that actually you can reduce this load your fee will be reduced. So there is a kind of simple type of incentive for the industry to reduce the load, which is on a fee-reimbursement basis. For some types of different compounds such as chromium, copper, metals, there are some absolute limits set up. There are none for silver, except the one that was mentioned by Immo before. That's in short how it works. I have three slides here summarizing current French policy, and I'll circulate these for whoever wants to see them.

JIM KRAMER: Thank you very much, Jean-Francois. And now for the last "European" country, Canada - Tom Gorham. I'm going to give you an extra minute, because you're the host.

TOM GORHAM (Kodak Canada): OK, thanks! In examining Canadian regulations from an industry standpoint, it's important to recognize that Canada is really made up of five distinct geographic areas. Looking at the BC environment which is mountainous, to the prairies, to Ontario, which is separated from Quebec by language, and on to the Maritimes, and I mean there are five regions here. What this does, it tends to cause the federal government to develop legislation that goes out into a web where each provincial government puts a spin on it to their own requirements, which in turn falls down to the municipal bylaws. I'm really specifically talking about sewer use bylaws and transportation regulations. And in a perfect regulatory world we would want to see legislation being based on good science. But in actual fact we all realize that political forces tend to compromise the science when they're making legislation. So in respect to heavy metals, most regulatory sewer limits can be traced back to the federal Transportation of Dangerous Goods Act. And that's how we really got some standards put into place, and that has been about almost 20 years now in place. The silver limit was originally set at 5 milligrams per liter total silver, and at that time 99% of all the provincial governments and municipal sewer codes adopted that 5 milligrams per liter as the limit. However, although I mentioned that good science isn't always the driving force, we've had a very key exception here in Canada relatively recently, in the past 2 years. The speciation of silver has been recognized by the federal government and much of the work presented through previous Argentum conferences - and that's why it's key to have this work at hand - has led Environment Canada to support a move by Transport Canada to remove silver from their hazardous materials legislation. This does have a cascading effect. From this breakthrough, and recognizing that municipalities want to establish workable regulations, which is rather new with Canadian municipalities (*laughter*), the pressure by key regulators to go after ultra-low limits, which are much lower than 5 milligrams per liter, has been greatly reduced. What this has done is lead to a major initiative to develop and accept pollution prevention programs instead of strict regulations. Now, in the photographic industry we refer to pollution prevention initiatives as "Codes of Practice". And once again working very closely with Environment Canada, the photographic industry was able to participate in the process that developed a national guideline. That's under the Canadian government's Environmental Choice program, which is really a consumer endorsement program that has gained fairly wide acceptance in a very large number of industrial sectors.

So what does the future hold in Canada? Well, we get a good idea of what our next steps are going to be if we take a look at the Ministry of Environment in British Columbia on the west coast. BC Environment recognizes that the current silver parameter in their water quality criteria documentation is extremely and unrealistically low. However, given that fact, they still are not willing to change it at this time. They've issued our industry a challenge: Come up with a dependable, economical method of measuring ionic silver, and they will move to make a change in the water quality documentation, from using a total silver limit to looking at an ionic silver limit. And I think that once we get this move in one province, at least in Canada, we will be able to work at cascading that in all the jurisdictions that we have. So I was very pleased to hear of some work being done in that area. I think it is really



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encouraging; that we are coming to a point in time where we will have a defined test and that we will be able to get this resolved properly. I guess that's about all the time I have, so if you have any questions, feel free to get hold of me at any time and I'll pass along whatever information I have.

JIM KRAMER: Thank you very much, Tom, and thank you very much to all the panel. I think we have three minutes. Is there a pressing comment or question from anybody? Did I cut anybody off who wanted to complete a thought? Hans Beider, did you want to say anything, because you've come so far – did you want to add anything to the European perspective?

HANS BEIDER (DuPont, Germany): Jim, thank you very much, but also I only came as far as Immo Boie came, and certainly I think it's surprising how well the agreement among the Europeans has been here. Because as Immo started saying, and I can only reemphasize this, that there is still quite a lot of differentiation between each country and even between the provinces or states in each country. So in a way it's not much different from what we have been seeing here in the United States. I'm talking regulation of wastewater and silver. I think the effort Serena has requested is a very valid one, that we would now, with the expansion of Europe, include the Eastern European countries and get them also into protecting the environment, as we have been trying so far. Thank you very much.

ALINA KABATA-PENDIAS : May I? I've found the data for one regulation for silver in the water of Poland. It is 50 micrograms per liter for drinkable water. **(SLIDE 3)**. It's a state regulation.

JIM KRAMER: OK, with that, thank you all again, very much.

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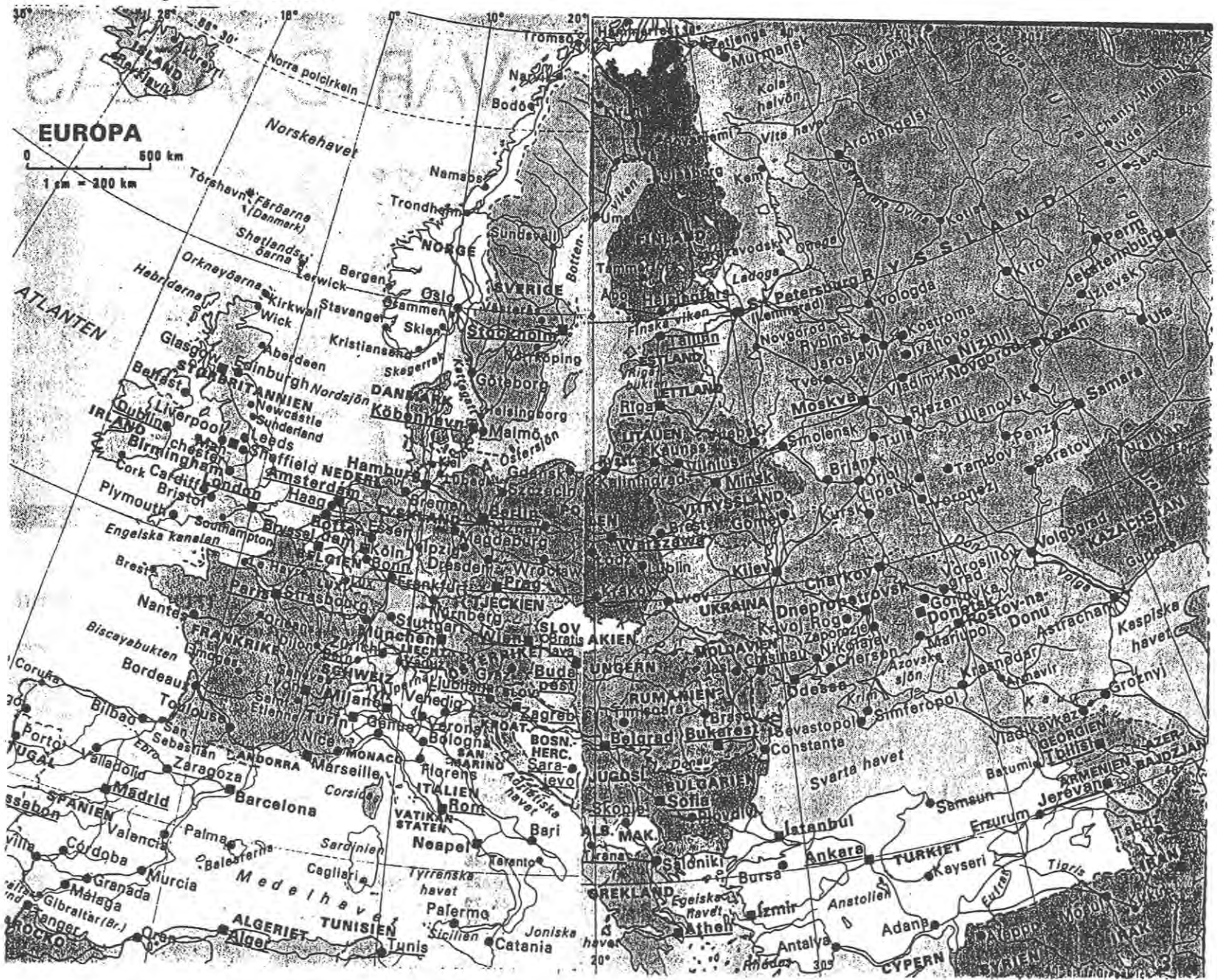
***Slide #1 - Bjorn Ericson***

**Sweden --- Silver**

**"Silver compounds are deemed to be hazardous due to bioaccumulation in plants (phytoplankton), toxicity to aquatic life and to warmblooded animals, recorded concentrations in surface waters close to or even above those which cause toxic effects in crustaceans and fish in laboratory tests."**

**(Swedish Chemical Inspectorate, 1989)**

# Slide #2 - Bjorn Ericson



Slide #3 - Bjorn Ericson

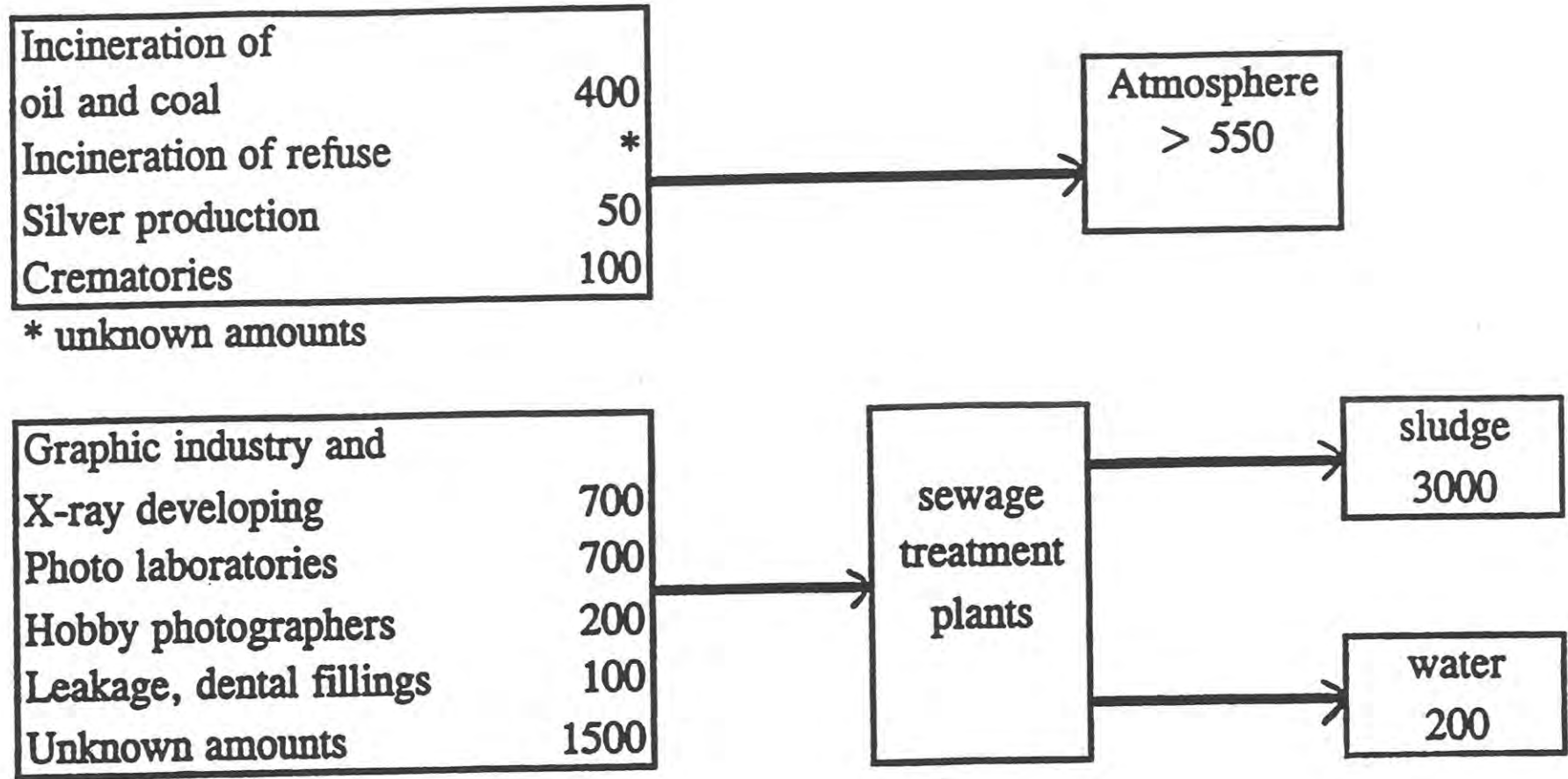


Figure 3. Sources and fate of silver in the Swedish environment, air emissions and water discharges (in kg/year). As the above figures are based on a limited body of data, further conclusions should be drawn with great care.

# Sweden --- Metals

	Restricted Chemicals List	"Observation List"	Sewer Code	POTW Sludge 1998	Land Application 2000
			mg/L	mg/kg	g/ha,year
Ag		X	0,05		
As	X				
B		X			
Be	X				
Cd	X		none	2	0,75
Co		X			
Cr	X		0,05	100	
Cu	X		0,5	600	300
Hg	X		none	2,5	1,5
Ni	X		0,05	50	25
Pb	X		0,05	100	25
Sn			0,05		
Sr	X				
Zn	X		0,5	800	600

## Sweden --- Photoprocessing

Swedish EPA Guide --- Discharge to POTW Sewer Network

### Silver restrictions:

< 500 gram p.a.

no pretreatment required

> 500 gram p.a.

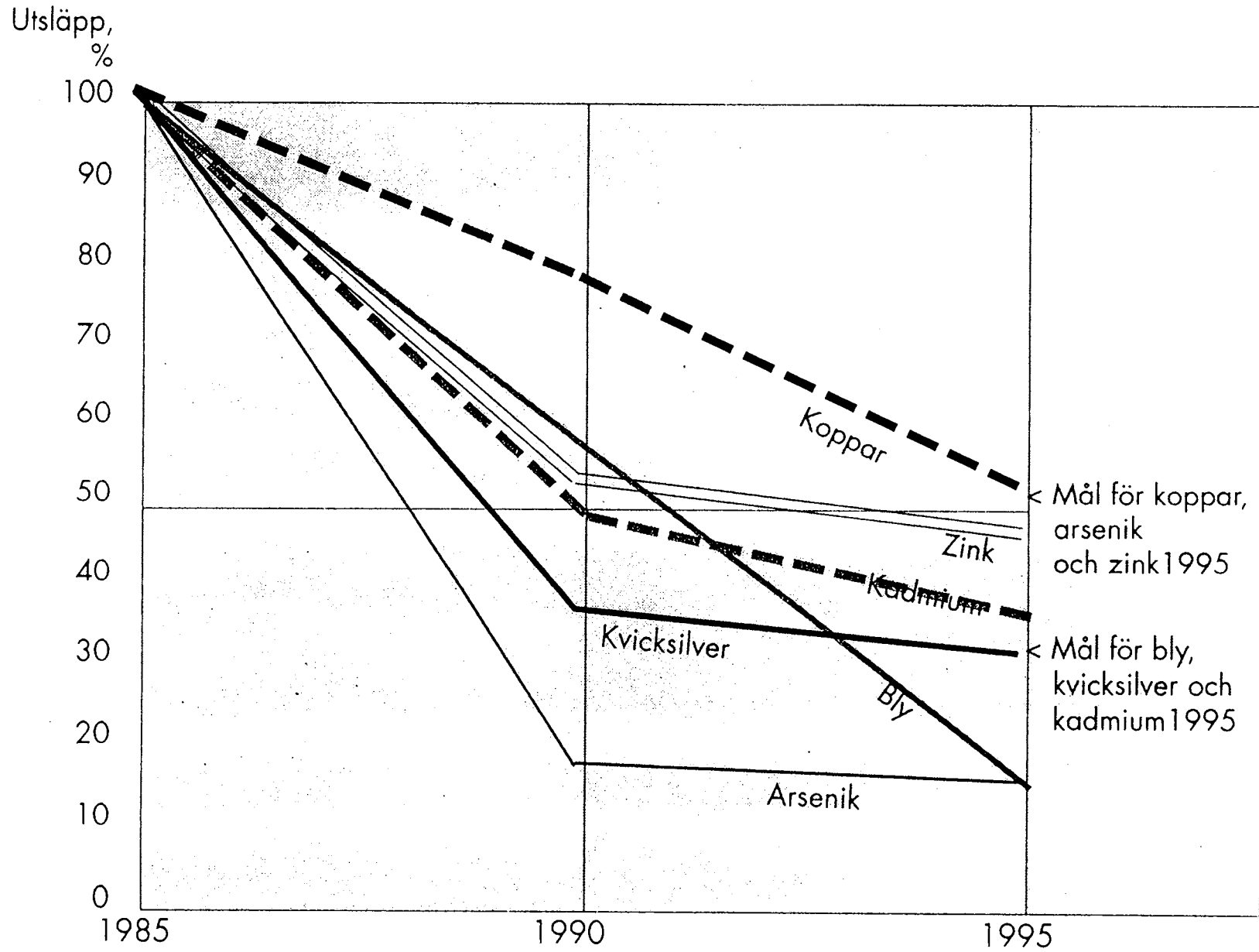
pretreatment required

Pretreatment:

Effluent conc 0,2 - 0,5 g/L

Annual load 10-20 mg/m<sup>2</sup>

# Slide #6 - Bjorn Ericson



# Sweden --- Hazardous Waste

( EU harmonized regulation )

**09                      Waste from photographic industry**

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**090104              Fixing Baths**

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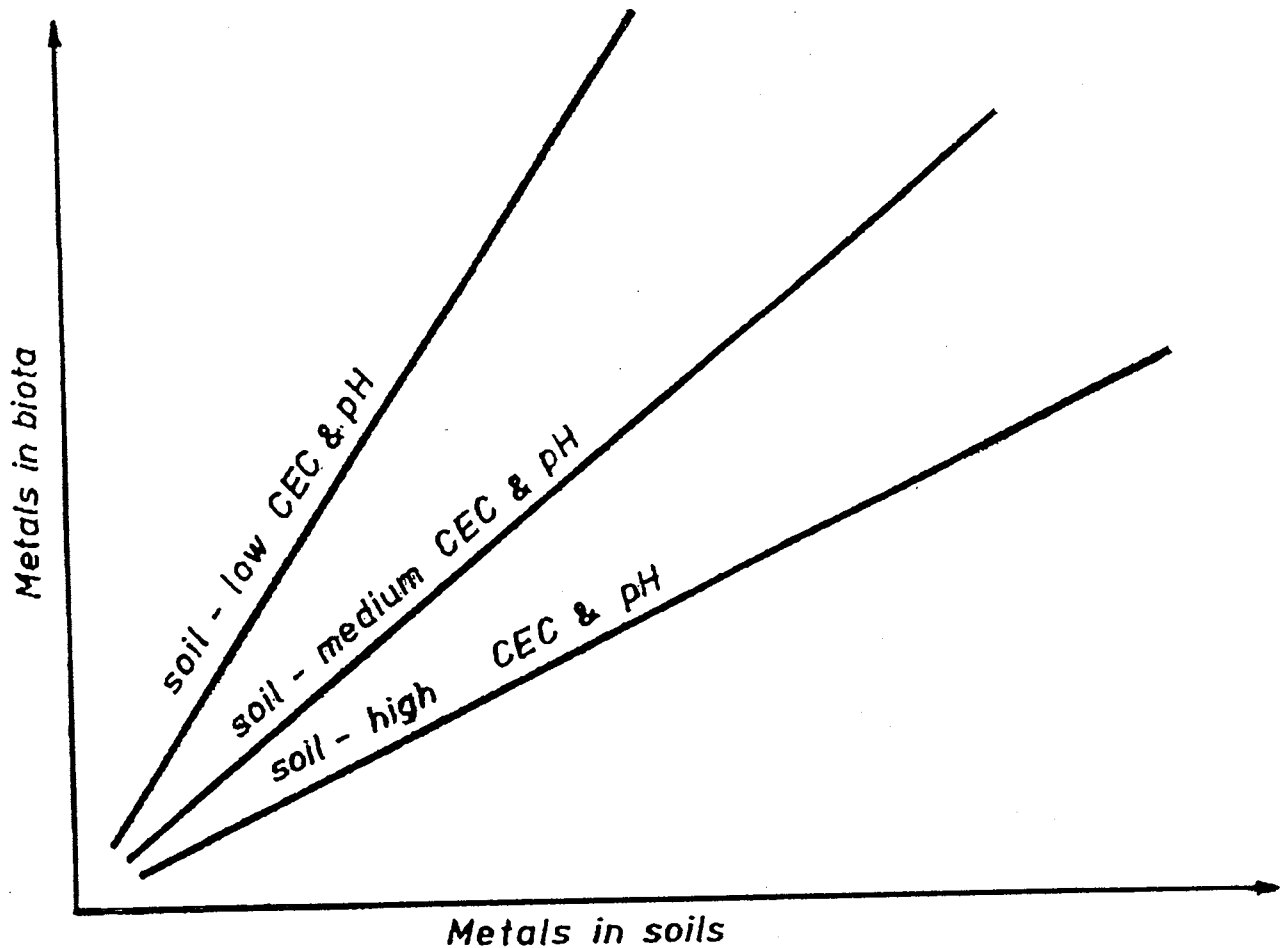
**090106              Silver containing waste from treatment of  
photographic waste on site**

**090107              Photographic film and paper containing  
silver or silver compounds**



country		national limits	local limits
Belgium	EU	1,0 mg/l (graphic industry)	
Germany	EU	<u>load-limit, depending on industrial sector and the amount of processed material:</u> <b>Black&amp;White; X-Ray:</b> 3 000 - 30 000 m <sup>2</sup> /a: 50 mg/m <sup>2</sup> more than 30 000 m <sup>2</sup> /a: 30 mg/m <sup>2</sup> <b>Color:</b> 3 000 - 30 000 m <sup>2</sup> /a: 70 mg/m <sup>2</sup> more than 30 000 m <sup>2</sup> /a: 30 mg/m <sup>2</sup> treatment of fixers: 0,7 mg/l <b>metal industry 0,1 mg/l</b>	
Denmark	EU	-	0,05 - 0,1 mg/l
Finland	EU	-	0,05 - 0,1 mg/l
France	EU	<u>load-limit, depending on industrial sector and the amount of processed material:</u> <b>industrial X-Ray:</b> 2 000 - 8 000 m <sup>2</sup> /a: 150 mg/m <sup>2</sup> 8 000 - 14 000 m <sup>2</sup> /a: 100 mg/m <sup>2</sup> 14 000 - 20 000 m <sup>2</sup> /a: 80 mg/m <sup>2</sup> <b>medical X-Ray; graphic and photographic industry:</b> 5 000 - 20 000 m <sup>2</sup> /a: 150 mg/m <sup>2</sup> 20 000 - 35 000 m <sup>2</sup> /a: 100 mg/m <sup>2</sup> 35 000 - 50 000 m <sup>2</sup> /a: 80 mg/m <sup>2</sup>	-
Greece	EU	5 mg/l if drained to a public sewer system 0,5 mg/l if drained to natural/surface water	-
United Kingdom	EU	-	1 - 10 mg/l
Italy	EU	-	different local limits
Netherlands	EU	1,0 mg/l	national limit can be modified locally
Norway		1,0 mg/l if drained to a public sewer system 0,1 mg/l if drained to natural/surface water	-
Austria	EU	<b>graphic industry:</b> if drained to natural/surface water: 0,1 mg/l if drained to a public sewer system : 1 mg/l <b>photographic industry; X-Ray:</b> if drained to natural/surface water: 0,1 mg/l if drained to a public sewer system: <u>load-limit, depending on the amount of processed material:</u> not more than: 1 000 m <sup>2</sup> /a: 100 mg/m <sup>2</sup> 1 000 - 30 000 m <sup>2</sup> /a: 50 mg/m <sup>2</sup> more than 30 000 m <sup>2</sup> /a: 30 mg/m <sup>2</sup>	-
Poland		<b>No information</b>	
Portugal	EU	<b>No information</b>	
Sweden	EU	-	0,05 - 0,1 mg/l
Switzerland		0,1 mg/l	In most cantons depending on the amount of used fixing bath: up to 1 000 l/a: 50 mg/l more than 1 000 l/a: 5 mg/l
Spain	EU	3 mg/l (3 ppm) if drained to natural/surface water	mostly no limits, if drained to a public sewer-system (Madrid: 0,1 mg/l; 0,1 ppm)
Hungary		the country is divided up into six different water-protection territories; 0,01 - 0,1 mg/l if drained to natural/surface water	national limit can be modified by local authorities
Russia		<b>No information</b>	

**Slide 1 - Alina Kabata-Pendias**

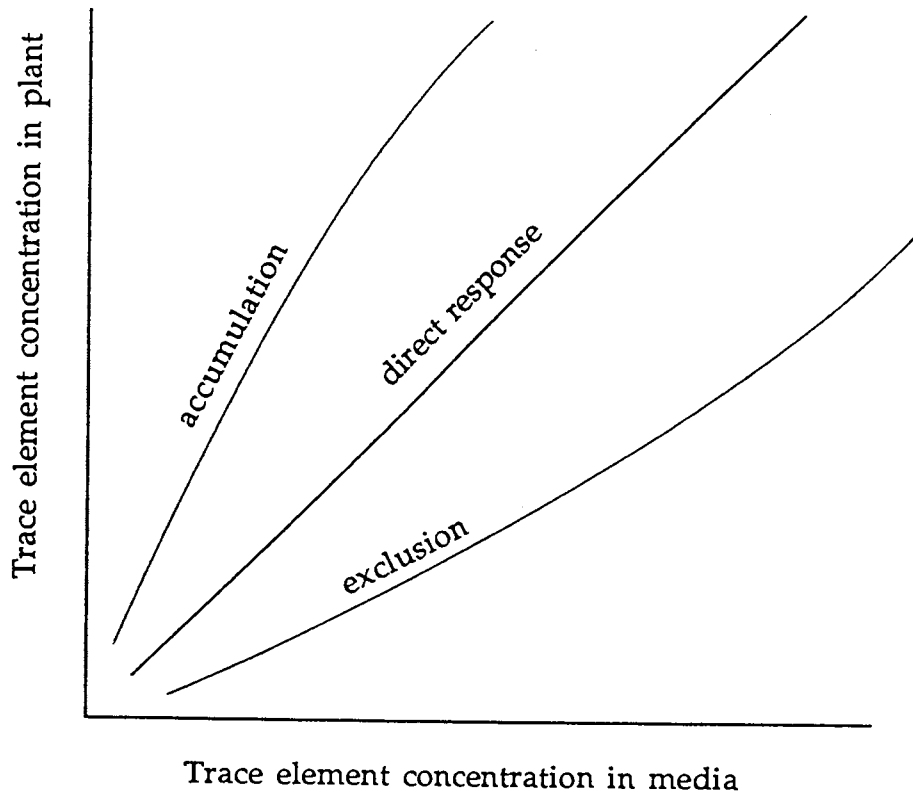


**Figure 1.**

Scheme of ecological effects of metal concentrations in the biota, as influenced by soil properties (cation exchange capacity [CEC] and pH regime)

Source: A. Kabata-Pendias, 1997. Trace metal balance in soils - a current problem in agriculture. In: D.C. Adriano et al., eds., Biogeochemistry of Trace Metals, Science Reviews, Northwood, 173-192

**Slide 2 - Alina Kabata-Pendias**



**Figure 2.**  
Schematic diagram of types of trace element uptake by plants from growth media.

**Slide 3 -  
Alina Kabata-Pendias**

**Data on Silver in Poland**

(after J. Lis and A. Pasieczna, Geochemical Atlas of Poland, Warsaw, 1995)

**SOILS**

Surface samples, N = 10,840

Ag content, ppm (soluble in HCl, ca 5N):

Minimum	<1
Maximum	41
Arithmetic Mean	<1
Geometric Mean	<1
Mean Deviation	<1

Increased concentration is only evident in soils on mineralized dolomites, and around areas where copper and zinc / lead are mined. Highest Ag content found in soils from a copper mine and surrounding a smelter, in ranges of 8-16 and >16 ppm, respectively. More than 90% of the total soil samples contained <1 ppm Ag.

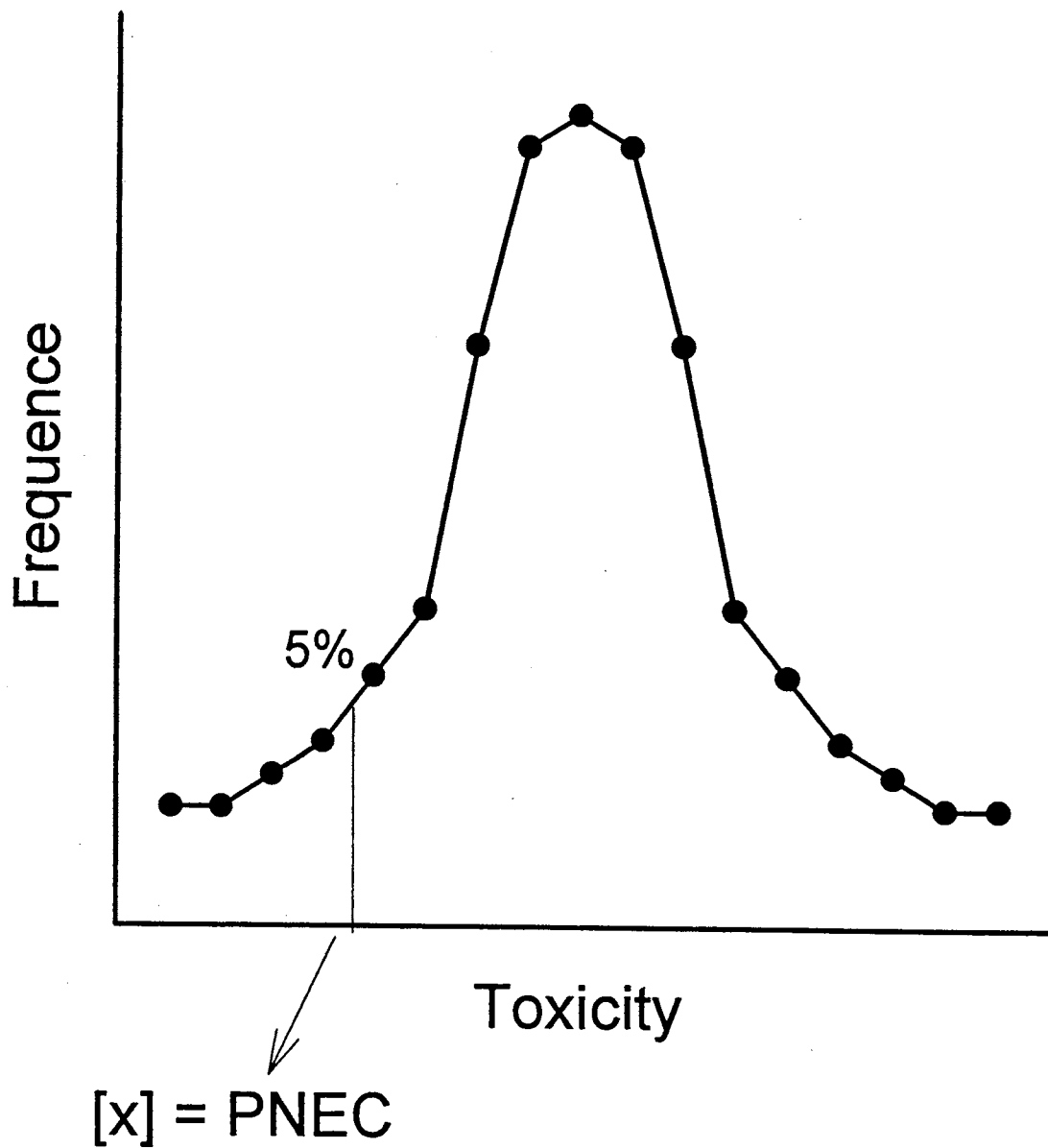
**WATER SEDIMENTS**

Bottom sediment samples, N = 12,778

Ag content, ppm:

Minimum	<1
Maximum	117
Arithmetic Mean	<1
Geometric Mean	<1
Mean Deviation	<1

Increased concentrations and the highest level found (117 ppm) for Ag were in vicinities of mines and smelters for copper and zinc / lead. Increased levels of Ag also observed around almost any communal agglomeration. Near residential towns values ranged from 2 to 8 ppm, whereas around industrial towns values were from 2 to 16 ppm. More than 70% of the total sediment samples contained <1 ppm Ag.



PNEC

Metal	SW	FW
Zn	86	110
Cu	2.9	12
Cr	1	10
Ni	8.6	160
Pb	5.6	3.2
Mg	0.3	1
Cd	2.5	5
Ar	4.0	4
TBT	0.001	0.001
DBT	0.01	0.01
Mo	10	10
Se	1.0	1
Va	1.0	1
Ag	-	-

**Slide 3 - Martin Grosell**

## Guidelines

Metal	To Sewage Treatment Plan	Industrial Effluent
Pb	10	100
Cd	0.3	3
Cr	30	300
Cu	50	500
Hg	0.3	3
Ni	25	250
Zn	300	3000
Ag	25	250

## **Les normes de rejet**

*Source : Arrêté du premier mars 1993 relatif aux prélèvements et à la consommation d'eau ainsi qu'aux rejets de toute nature des installations classées pour la protection de l'environnement soumises à autorisation.*

Les valeurs limites de rejet sont fixées par l'arrêté d'autorisation sur la base de l'emploi des meilleures technologies disponibles à un coût économique acceptable et des caractéristiques particulières du milieu récepteur.

Les valeurs limites doivent être fixées pour :

- le débit des effluents,
- la température et le pH de l'eau rejetée,
- les flux,
- les concentrations des polluants principaux.

Tous les polluants ne présentent pas les mêmes risques car ils n'ont pas la même toxicité.

### **Débit**

L'arrêté d'autorisation fixe le débit maximal journalier.

Lorsque le débit maximal journalier est supérieur à 100 m<sup>3</sup>/jour, l'arrêté fixe également une limite à la moyenne mensuelle du débit journalier.

### **Températures et pH**

La température des effluents doit être inférieure à 30°C et le pH doit être compris entre 5,5 et 8,5 ou 9,5 s'il y a neutralisation à la chaux.

### **Flux journaliers maximaux**

Au-delà d'un certain seuil journalier en polluant, l'exploitant doit réaliser des mesures journalières, voire permanentes.

Lorsque le débit maximal journalier dépasse 100 m<sup>3</sup>, la détermination du débit rejeté doit se faire en continu. Dans les autres cas, le débit devra être déterminé par une mesure journalière ou estimé à partir de la consommation d'eau.



<b>Tableau de seuils de mesures journalières ou permanentes :</b>	
DCO	300 kg/jour
Matières en suspension	100 kg/jour
DBO5	100 kg/jour
Azote global	50 kg/jour
Phosphore total	15 kg/jour
Hydrocarbures totaux	10 kg/jour
Fluor et composés	10 kg/jour
Aluminium et composés	5 kg/jour
Fer et composés	5 kg/jour
Etain et composés	4 kg/jour
Zinc et composés	4 kg/jour
Composés organiques du chlore	2 kg/jour
Manganèse et composés	2 kg/jour
Chrome et composés	1 kg/jour
Cuivre et composés	1 kg/jour
Nickel et composés	1 kg/jour
Plomb et composés	1 kg/jour
Indice phénols	500 g/jour
Arsenic et composés	200 g/jour
Chrome hexavalent	200 g/jour
Cyanures	200 g/jour

Source : arrêté du 1er Mars 1993.

### **Flux annuels maximaux**

Pour certaines substances, un prélèvement continu proportionnel au débit et une mesure journalière doivent être réalisés lorsque le rejet annuel dépasse les valeurs suivantes :

mercure 7,5 kg/an

cadmium 10 kg/an

CCl4 30 kg/an

Lorsque l'exploitant doit mettre en place un tel programme de surveillance de ses rejets, il s'agit

Concentrations maximales dans les rejets des polluants principaux :		
POLLUANTS	Valeurs lim.	Remarques*
Matières en suspension	100 mg/l	flux 10 kg/jour 30 mg/l au-delà
DBO5	100 mg/l	flux 15 kg/jour 40 mg/l au-delà
DCO	300 mg/l	flux 45 kg/jour 120 mg/l au-delà
Azote	30 mg/l	flux 20 kg/jour
Phosphore	10 mg/l	flux 5 kg/jour
Phénols	0,1 mg/l	si flux > 2 g/jour
Chrome hexavalent	0,1 mg/l	si flux > 2 g/jour
Cyanures	0,1 mg/l	si flux > 2g/jour
Arsenic et composés	0,1 mg/l	si flux > 2g/jour (en As)
Plomb et composés	1 mg/l	si flux > 20g/jour (en Pb)
Cuivre et composés	2 mg/l	si flux > 50g/jour (en Cu)
Etain et composés	2 mg/l	si flux > 50g/jour (en Sn)
Manganèse et composés	2 mg/l	si flux > 50g/jour (en Mn)
Chrome et composés	3 mg/l	si flux > 50g/jour (en Cr)
Zinc et composés	5 mg/l	si flux > 100g/jour (en Zn)
Fer et composés	5 mg/l	si flux > 100g/jour (en Fe)
Nickel et composés	5 mg/l	si flux > 100g/jour (en Ni)
Aluminium et composés	5 mg/l	si flux > 100g/jour (en Al)
Hydrocarbures	15 mg/l	si flux > 100g/jour
Fluor et composés	15 mg/l	si flux > 200g/jour (en F)
* d'après le flux journalier maximal autorisé par l'arrêté		

Pour certaines substances (mercure, cadmium...), les valeurs limites de rejet dépendent du secteur d'activité.

# Panel Discussion

## *U.S. Regulation of Silver in Sediments and Water Columns*

*Moderator:*

*M. Reiley, USEPA, Washington, DC*

*Panelists:*

*D. Armstrong, University of Wisconsin-Madison*

*W. Berry, USEPA, Narragansett, Rhode Island*

*D. Di Toro, Manhattan College, Riverdale, New York*

*N. Fisher, State University of New York-Stony Brook*

*T. La Point, Texas Tech University, Lubbock, Texas*

*R. Playle, Wilfred Laurier University, Waterloo, Ontario*

*C. Wood, McMaster University, Hamilton, Ontario*

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## Panel Discussion: U.S. Regulation of Silver in Sediments and Water Columns

**Moderator:** Mary Reiley (USEPA)

**Panelists:** David Armstrong (University of Wisconsin)  
Walter Berry (USEPA)  
Dominic Di Toro (Manhattan College)  
Nicholas Fisher (SUNY Stony Brook)  
Thomas La Point (Texas Tech University)  
Richard Playle (Wilfrid Laurier University)  
Chris Wood (McMaster University)

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MARY REILEY (USEPA, Washington): If we have time left over we'll answer any questions. This is the first opportunity I've gotten to say hello to the entire group. My name is Mary Reiley. I run the Sediment Quality Criteria Research Program at the EPA in Washington, DC. I try to make sure that the research team that I work with is fully funded and all going in the same direction. When you have a crew like I have, that's sometimes a challenge but they are pretty good about it.

We have nine people that you're going to hear from today. I'm going to take a minute or two myself to get a few things started. The first one is, EPA is hiring. We've got seven open positions in my office for aquatic toxicologist, environmental engineers, biostatistician and microbiologist. So if you're interested or know somebody who's interested, my name is in the list of attendees - give me a call or send me an e-mail or something like that. We're going to try to fill those positions in the next six months. They start at a GS-9 and they go to 13 - that's \$50,000 to \$71,000 a year, just in case you're interested. On the side of silver, a couple of things that are happening at EPA right now: RCRA is undergoing a review of the Toxicity Characteristic rule for defining what wastes are considered hazardous. They're doing that in particular for silver at the request of the photographic industry, and Jan Young, who is also listed as one of the attendees here, can help you if you're interested in getting a copy of that report. It's in peer review right now. You can certainly take a look at that. We're also participating with the Silver Council in a research arena for aquatic life, and for another cooperative agreement with the University of Delaware looking at metals in general in aquatic life and sediments. So that's kind of a brief overview of what we're doing, and with that I'm going to turn it over to the panel. Our first person is Chris Wood.

CHRIS WOOD (McMaster University): Thanks, Mary. I'm not really sure what a Canadian is doing on a panel to discuss U.S. regulations. I feel a bit self-conscious about it, but I'm not going to take 3 minutes. All I'm going to say basically is that in real estate they say there are 3 things which are important, and these are location, location, location. I think now in environmental regulation of metals it's speciation, speciation, speciation, and I think that regulations as promulgated in the ambient water quality criteria don't adequately take that into account. That we really need to go back and look at the criteria, we've seen ample evidence of, I think, at this conference. How important, for example, dissolved organic carbon is, chloride is, sulfide is, and that just looking at total metal I don't think makes very much sense anymore.

DAVID ARMSTRONG (University of Wisconsin): Well, I think Chris just took my presentation. I think in a word my feeling on this is also speciation. I think we've seen ample evidence that in many ways it's the activity of silver that is important, not the total concentration, and that ideally what we would like to do is have a regulation based on calculating the free activity of silver based on knowing what the concentrations of the important ligands in the system are. There are still some challenges there to identify some of these ligands and to properly represent what their ability to sequester or associate with silver is, including some of the sulfur-containing ligands, and recognizing that some of these are colloidal materials which we have to figure out how to properly represent. So with that being said, I think speciation is the bottom line. There are also some questions of how we relate free

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silver to its effects on organisms. We've heard some discussion that this may in fact relate partly to how the silver is accumulated by the organism, and we'll probably hear somebody else here say a bit more about that. Thank you.

RICHARD PLAYLE (Wilfred Laurier University): Great – well, I'll just keep on going with this speciation theme. We're three-for-three so far agreeing with this. The way I was thinking about it, a simple way of looking at it is, speciation is composed of three "c's": Concentration, complexation, competition. Concentration sort of drives everything. If you've got such a low concentration that it doesn't matter, then you don't have a problem. If you do have a concentration getting up to some kind of limit then this is the tough part, because this has to take account fate and transport effects downstream, chronic versus acute, it's got to take in life stage, it's got to take in species. This is a little tricky, but if you have some kind of an existing value that if the concentration gets up high enough for any metal you're going to have a problem, then you start looking further. Then you take into account the complexation, dissolved organic matter for example, chloride for silver, carbonate for copper. Colloids are important. Then there's the third c, competition. Calcium perhaps, sodium perhaps, other metals perhaps. Thinking of that, a hardness factor that's in some regulations for silver - in a lot of ways it's not such a dumb idea. It worked for copper. But it doesn't work for silver because people didn't really understand the system. The point is, silver doesn't form very strong complexes of carbonate, and calcium doesn't compete very well. But some kind of a correction factor is the right idea. Again, if dissolved organic matter and chloride were being considered, then that would probably be a not unreasonable way to take into account speciation in a relatively simple way. So the idea is you need to know the mechanisms, especially in the freshwater fish angle. Through advances in the past few years it is just amazing to now know that, for example, where silver interacts on the sodium-potassium ATP-ase on the gills, knowing that it interferes with the magnesium there, is pretty impressive. And then you can back all the way up and base regulations based on the physiology. Having said all that, there have been other advances going on, so the modeling can be done very quickly on computers. I think the framework again is speciation - as I just said, three "c's": concentration, complexation and competition. It can be done by everyone and it's a good framework to at least think about the problems. Not just for silver, but of course for other metals.

WALTER BERRY (USEPA Narragansett): I actually would like to make two points after echoing and certainly agreeing with what my august panelists have said about speciation. One of the main reasons why I came here was to present a sort of proposal that we have for a sediment quality criteria for metals. That's what you see up here (**SLIDE 1**). SEM and AVS are something that you've all heard about before. We have in the works a draft criteria for the 5 metals, copper, cadmium, lead, nickel and zinc, that we've worked with the most. The simplistic and perhaps naïve proposal is just to work silver in among with the rest of that crew. I haven't really heard much of anything from the sediment side during this conference that would make me believe that that was not the way to go. So the proposed criteria is simply that we would suspect that a sediment would not be acutely toxic at least if the SEM minus AVS was less than 0. And we would assume, sort of a check on that also, that even if there was an exceedance of SEM over AVS, that if you looked in the interstitial water and you divided all the metals through by the toxic units and that added up to something less than 1, then you probably still don't have a problem. Obviously, because the interstitial water is not all bioavailable, as we've heard from the first speakers on the panel, even if you have an exceedance of one interstitial toxic unit you may still not have a problem, and you may need to look at what's happening with the speciation in the pore water. This is something that we're thinking about doing and it's out there, certainly I would like to hear comments on it. I know we don't have a lot of time right now, but everybody in EPA basically has this same e-mail address: last name, first name @epa, whatever. But anyway, if you know anybody on EPA's e-mail then you know mine. I'm really very interested in hearing about what people have to say on this proposal. One last point I would like to make, is that I think that in a sense Nick Fisher really threw down a gauntlet for us, to think about other species in addition to simply fish. I think that we have to be careful about how we're regulating silver, obviously. I think we're making great progress, but I think we have to keep looking. Thanks.

THOMAS LAPOINT (Texas Tech University): Well, it's been said that the understanding of a process occurs at a level beneath its importance, and we've certainly seen with the presentations here and beginning with some of the presentations even last year, we're beginning to understand what happens with silver, aqueous silver, and ionic silver. And so I find myself in complete agreement with Rick and with Chris as far as the aqueous exposures go.

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And that's one other component that I think you're getting at, Walter, that exposure scenarios may be different for chronic, and what we've been thinking about in the dietary may be more important. But in terms of getting back to the dissolved components, what's important is understanding how the silver is working, and getting a very good handle on that through Christer's work and Chris Wood's work and others. I think it's time that those components be incorporated into establishing and setting water quality criteria for silver. Thanks.

NICHOLAS FISHER (State University of New York - Stony Brook): I don't have an awful lot to add to what's been said, but a few comments. I'll donate most of my 3 minutes to my friend Dominic Di Toro here. However, I think it is important to recognize, obviously we all are well aware, that there are critters out there other than fish. And even if we're only interested in protecting fish, you can wipe out fish populations without directly affecting the fish; that is, the contaminant could wipe out the food supply, for example, of that fish. You could have other pronounced effects on fish populations as well without any direct effect. I would implore all of the toxicologists here to revisit what the concentrations of silver or any other contaminant are out in the environment, and use concentrations in their toxicity tests that are within the realm of reasonable concentrations. If concentrations are in the picomolar range and if concentrations that elicit toxic effects start in the micromolar range, just for argument sake, that's 6 orders of magnitude difference, and one has to question the relevance. Secondly, I think that there's an awful lot of money, time and effort invested in setting environmental regulations that are based on toxicological testing. And I would hope that from time to time, not just for silver but for any contaminant, that there be an effort made to keep improving toxicological testing methods. And I would implore us to consider, in the case of animals: we have to recall that animals eat, they don't just absorb materials from the dissolved phase. And it is well recognized that there's trophic transfer of contaminants including silver, and that the dietary pathway has to be considered, not just for zooplankton but for any organism.

DOMINIC DI TORO (Manhattan College): Thanks Mary. I would like to suggest a rule. I don't know if I suggested this last year, but I'm going to suggest it again since it's a good rule. It's a way of evaluating whether a regulatory procedure makes any sense or not. And the rule is this: "If the only piece of chemical information that a regulatory procedure uses is the name of the chemical, if that's it, and then there's a number after it, then it's almost surely the case that that regulation is not very scientific". So you remember how the lists go, cadmium, copper, nickel, benzene, chloroform, mucktane, whatever, and then there are a whole bunch of numbers. You can almost certainly be sure that that regulatory procedure is extremely naïve. Because it assumes that the chemical doesn't interact with anything else in the environment and simply exerts it's effect by the very nature of its structure. So that's sort of speciation taken to the ridiculous extreme. Not only are you not considering speciation, you're not considering anything other than the name. In fact, one wonders why toxicology developed along those lines. For years and years that was the distinguishing feature that distinguished chemicals, namely their names. You know, which jar are we going to test today? So that's my rule: sensible regulations have to depend on more than simply the name of a chemical. For example, octanol-water partition coefficient, carbon concentrations, hardness and many things we don't understand yet.

Second comment is, we've concentrated a lot on what I would call the effects concentration, but as you realize in a risk assessment, the exposure concentration is also a difficult game. Rick Playle sort of went through it very quickly and said "once you get the concentration", but there's a long way to figure out what exactly the concentration is that you're going to regulate against, in a stream or an estuary. If you take samples every day, and the concentrations can vary by orders of magnitude throughout the year, how do you deal with that exactly? You can't wave your hand when you're a regulator; you have to come down with something specific. And I think that part of the whole regulatory process was set by engineers many years ago in streams and has persisted to this day, with some modifications, and bears very little relationship to toxicological and biological endpoints. For example, how rapidly does a toxicant react to an organism? The gill clearance data that we saw tends to suggest that silver's a very fast-acting toxicant. On the other hand, it takes awhile for the ionoregulatory imbalance to develop, so maybe it's not so fast. So we're highly focused here on one sort of, albeit very important, issue. I mean, speciation's an order-of-magnitude issue. Factors of 2 issues, by the way, forget about, ignore them; we don't know how to do anything in this business to a factor of 2. But a factor of 10 is worth of thinking about. That's why when Nick showed a factor of 100, I jumped right out of my chair. A factor of 100 is the difference between walking and flying. Driving a car, that's a factor of 10, flying in a jet airplane, that's a factor of 100. So factors of

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100 you should really pay attention to. There is a factor of 10 easily floating around in the exposure concentration area. There may be another factor of 10 floating around in the fluctuating time dependency kind of problem. So those are two areas I think we might want to think about at least, in closing. After we've gotten all the biological stuff straightened out. Thank you.

MARY REILEY: I'd like to open the floor for any questions that you have for me or for any of the other panelists. And also for the panelists to pose questions to each other or rebut a statement that was said. So please feel free.

BRUCE WALKER (Michigan Dept. of Envir. Quality): I've talked to a number of you people here. This is my first silver conference, and I am amazed at the exponential degree of growth and knowledge on silver in the last 5 years. This is quite impressive for this chemical. I guess I'm one that's struggling, as some of you have heard, with trying to take our understanding to apply it to a regulatory mode for dischargers. And one thing I would like to emphasize, that Tom Purcell mentioned, is about British Columbia's challenge, this analytical method on free ionic silver. I think if this wafer thing would work, that's going to be a lot cheaper than doing all kinds of ultrafiltration studies all across the country, and answer a lot of questions relating to redefining appropriate toxicological criteria and also the endpoint. And we'd get to a large extent closer to where we want to be in this game. Appropriate regulation, I think, useful methodology, would also help us biologically validate some of these models. The models predict that everybody should be walking away from this, but we do see toxicity under a variety of conditions from silver. So something is still happening, and if we could somehow, I just think, get biological validation it would increase a lot of people's comfort factor with the modeling of silver. And possibly if we can measure stuff, it may get us closer to developing a simple model that would be more widely applicable to both regulators and permittees towards determining if they have a problem or not. So that maybe we don't need a 26-input model to be able to model and to be able to rely on that model.

I guess given the uncertainty in criteria development and some of the questions we've heard about biological effects, mechanisms and stuff today, I wanted to mention it in the life cycle panel phase but we didn't have time. I think it's also appropriate to consider - I don't know if it's possible, since I'm not the one of the chemists dealing with this, but looking at what we've heard, the photographic industry are users of half the silver and I assume that means half the discharges - possibly improving, coming up with novel ways, or improvements or new ways to pre-treat stuff so that when it goes to a treatment plant, maybe the criteria won't be so much of an issue if you don't have 3 grams going into a treatment plant. Maybe that could be gotten down to some hundreds of micrograms or something, and then the endpoint of the criteria or why we don't have one maybe it might not be as much of a problem. So I would suggest maybe some research on improving treatments, at least for discharges to wastewater treatment plants. We have some non-municipal dischargers of silver in Michigan, and I had mentioned to some people that I haven't seen any studies on free-ion predictions in industrial discharges from, like, a plating or some kind of metal finisher. So, to base our regulations or modeling strictly on the mechanics of going through a municipal treatment plant, I don't feel comfortable applying that conclusion to an industrial discharger without an activated sludge treatment. So I guess three big questions for me that are left from this conference, despite all the wonderful answers we're getting, some new questions have come up. That's what happens, when you answer one question you always raise a few more, as we saw from the data today. We've got some identified differences between species and also between life stages, which seem to raise questions again on developing an appropriate regulation as to what's the appropriate, most sensitive life stage or the species you want to base it on. We've had the reproductive effects, we've talked about early life stages all changing things by orders of magnitude as Dom was talking about. So that's something I think we need to consider in the regulation. And also the whole issue of chronic tox which has been touched on several times, and how little we really know on both the mechanism of that and what we're going to do about it, or what modifies it. So these are from an applied end rather than a strictly research end, but I think the research has gone a long ways towards answering some of these questions, especially compared to where we were, compared to the 1980 Criteria Document which is the official document right now. So, I thank you all for increasing everyone's knowledge on this.

MARY REILEY: Thank you very much. Anybody else that would like to make comments, pose questions?

JIM KRAMER (McMaster University): I want to follow up on Dominic's comments about "don't look at a metal or a symbol or something all by itself". I think it was 28 years ago, it was the early founding of the International Joint

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Commission. We spent 3 days on regulations and we came up the notion that we had to have n-dimensional matrices, very much along the line you had, and that's the only way the Great Lakes Water Quality Agreement would work. It never happened. And the answer was, it was too complicated to regulate. And so I would like a comment from the regulators or from Dominic about that. I mean I agree with you, maybe with computers and everything nowadays it's not as complicated, but that was the engineers' reply.

DOMINIC DI TORO: Yes, anyone who's in the regulatory business wants it simple, neat and clean. And absolutely, I mean if I were in that situation, that's the way I would like it too. Or if not that, I would like it reliable enough so that I could really trust the calculations. The question I would ask a number of people who said they're waiting for measurement technology: how about a computational technology with known error bars? One of the ways of thinking about modern regulations, and the way I think about them, is that no matter how good the regulatory procedure is, what regulations really end up being is concentrations below which you're probably okay. Because you tend to build safety factors into these calculations. Or even if you didn't, you calculate a concentration below which you're pretty well assured you're okay, and calculate another concentration above which you're pretty sure you have a problem. And then you have a range left over in between where things are murky. That's an intelligent way to use almost any scientific state of knowledge. The game is to try and generate enough data so that you can calculate reliably what these uncertainty ranges are. So I would ask you, Bruce, for example, suppose that was the state of the art? That you could measure these five things, you could calculate a concentration and you could say that that concentration was known to within a factor of 3.7 on either side? It would seem to me that a regulatory authority then could use the lower bound and say if you're below that, that's fine, no problem, and if you're above that, a higher range, you probably have a problem and you better do some more measurements and get some site-specific stuff. So that's another way to deal with this problem. It is too complicated to be able to calculate absolutely with high precision in an n-dimensional universe what the activity of anything is. But you can do a much better job than taking the assumption that everything's bioavailable, period, and trying to use that as your upper bound estimate, which is, of course, the extreme upper bound estimate.

MARY REILEY: Anybody like to respond to what Dominic had to say? Anyone else on the panel want to respond? I can say that what Dominic has proposed there is what EPA has proposed in their first set of sediment quality criteria. They were put out for public comment in January of '94 and at that time they were listed as ranges, below which are safe, above which you've got a problem, and somewhere in between you'd better start looking at interstitial waters. And that's the first time the Agency's put out a range as a criterion rather than a single number. So, I'll be curious to see how that actually ends up getting implemented. Going from there to standards is a whole other ball of wax. So, anybody else, questions or comments?

DALAND JUBERG (Eastman Kodak): Actually Dominic, you bring up a good point, and I just wanted to offer some support from the human health standpoint. We've started to see this in terms of National Academy of Sciences and the Presidential Commission on Risk Assessment and Risk Management, and I think it is a very valid point, one that we ought to continue to pursue, and that is the movement away from bright lines. They often don't mean much to us. Risk range is Monte Carlo distributional analysis. All of that I think is going to improve the science, and it may be one of the ways that will help regulatory officials. So, yes, just a voice of support from what we see in terms of cancer regulatory guidelines and so forth.

DOMINIC DI TORO: I remember listening to a talk, this was years ago, at a Human Risk Assessment forum actually, where the risk assessment, the environmental people were trying to learn from the Human Health people, to start the risk assessment applied to environmental problems. And one of the fellows told the following story. It's a long story so I won't tell you the whole thing, but what it amounted to was that two risk assessments were done on a particular problem. And one guy got the number 2 and another guy got the number 20,000. And his point was that that was good enough for risk assessment, because even at 2 that was too much of a problem, even if that was the problem concentration. So a four order-of-magnitude variation in the two estimates really didn't affect his ability to make decisions. And what I thought he was going to say, and what I would have concluded if two people presented me two different analyses that differed by  $10^4$ , was that nobody knows what the answer is. The answer is essentially unknown, and you make the decision based on political grounds or expediency or whatever, but you know, let's not make believe that it's science. So one other thing you can use

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ranges for is to find out how close you really do have a handle on this thing. If it comes back  $10^4$ , you know, fire them. *(laughter)* I mean, don't use it.

ERIC CRECELIUS (Battelle Marine Laboratory): I had a question, I'm sort of interested in where the fish gill model stands. I know from past years there's been an idea of using the fish gill model for estimating criteria for a wide range of metals in the environment. In the presentations this week there was some reference to the fish gill model, and that maybe with silver it isn't working out as well as hoped. And I was just curious, is the fish gill model still alive, and how is it working with silver as well as other metals?

MARY REILEY: Rick is chomping at the bit to answer that question.

RICK PLAYLE: I'll tell you how I would use the fish gill model right now. Think of the silica wafer to measure free silver. If that had been available here we all would have put in an order for it, without a doubt. Then think of the physiology, and I'm just going to leave it at trout and freshwater for right now, you can see the application. If the toxic mechanism of silver at high doses in soft water is the effect on the sodium-potassium ATP-ase, if some way we could correlate what you can pick up on the wafer with that mechanism, then you've got a powerful simple tool in essence. Where the gill model might fit in is an intermediate way of analyzing. I've talked about this with Nick Bury and Jim McGeer. In essence, correlate a couple hours accumulation of silver on the gills with the sodium-potassium ATP-ase and correlate that with the wafer - there would have to be all kinds of correlation's going on. If they're good enough and tight enough, then you've got a nice analog for toxic effects, acute toxic effects of freshwater fish in water. And if you took the little wafer, stuck it in water and got above a certain value and your alarms went off, it's toxic to fish or close to it, and you can bet it's going to be toxic to other things at the same time. Then maybe you can step back and find out well, that wafer, you know, gives ranges on the machine, for example, green you're okay; yellow, red - alarms go off. I don't know, I think I would say that it's gratifying to see some work done on using that gill model, even if it's just to get people to thinking about the complexation and competition going on. If it gets them using these fairly-easy-to-use computer models then, you know, just use them. Modify them a bit, stick some numbers in, generate some more numbers, see what happens. You can do an awful lot in a day's fiddling around on the computers, you can do a month's hypothetical work, then you go into the lab and do a week's work on some really solid questions, because you've eliminated lots of questions that wouldn't pan out, and I think it's a very powerful tool, a very powerful framework to think in.

CHRIS WOOD: I just hope that the perception was not given that the gill model is not working. I mean, I think by and large the gill model is working. And what we experts in physiology and some of us in geochemistry are talking about is just tweaking the model to get it to work right. Do you look at exactly the gill silver burden, do you look at the inhibition of the ions, I mean that's really a very minor point. The whole point is that you're going to have a tool with which you can make some reasonable predictions with reasonable bounds on them. I think it's probably working best for copper because we know most about copper, and after that I think it's working next best for silver. I think we just need a bit more data, a bit more work, and a bit more fine tuning. But I really do think that it's going to be a useful tool.

MARY REILEY: Anybody else that wants to respond to the gill model question?

DOMINIC DI TORO: At the worst, we'll rename it the biotic ligand model. Which is clearly what's going on anyway. The chemicals are locking into a receptor of some kind, and that receptor has a certain affinity for the free ion and the complexed ions. And at the very worst, it would look like mortality dose-response curves over wide ranges of chemical conditions, where you essentially try and set the model up so that it collapses all the curves into a single dose-response. Even if you don't know what the physiological ligand is. I mean it's got a few holes in it right now, maybe gill body burden won't end up being as useful as we thought it was going to be, based on the copper work. But the notion of the chemical interactions with the receptor ligand is still, I think, alive and well.

BRUCE WALKER: You mentioned one of the holes I guess I'll allude to again. I don't know how this - I haven't run this gill model, I'm not familiar with it, but you touched on it earlier. Maybe this may need to be adjusted for life stages or intraspecies variability also.

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MARY REILEY: Okay, I saw another hand earlier, please.

ARUN MUKERJEE (University of Helsinki-Finland): This is not a question, I'd just like to discuss one point. If you look at the last five silver conferences, most of our colleagues are from North America and from Europe. But if you look at the main countries using silver consumption, you will find Japan. They are producing at about 112.69 million Troy ounces silver. And the photographic industry they are using very much silver too. Near about 52.64 million Troy ounces. But here we are discussing very much about silver ions, the problems and all these things, but we never try to find out how they're thinking about this problem in Japan. And I'm sure the problems which we cannot yet solve, which are still open, maybe we can find some clue from them, and they are quite advanced in technology. So I feel that in the near future we could sometimes ask some scientists from Japan and find out what they are doing in this field of silver ion and its emission or aquatic status, and effects in their fish and all these things. I think this exchange of information from the scientists in Japan and Europe and North America would help quite a lot to solve the problem, as we have today quite a lot of understanding on mercury as a global pollutant, because Japan and Nordic countries, Canada, United States, Brazil, Australia, they have all joined together. And last, I think that since 1990 many problems which we could not understand - the behavior of mercury in the atmosphere - we now understand quite a lot. But still we have to go farther. So I would ask of the council to think of that in the next conference. If you can find some scientists from Japan to discuss what they're doing and what we are doing, we can then put it together, and maybe we can find a better understanding of silver ion in the aquatic or terrestrial system or environment. Thank you.

MARY REILEY: I think we're being asked to expand Argentum to include a little bit more of the world, guys. But I think it's a note well taken. Tom's going to come up and give his two cents.

TOM BOBER (Eastman Kodak): I'll just say that we've repeatedly invited scientists from Japan each year since the beginning, and so far we've not yet convinced them to come to one of these conferences.

MARY REILEY: Maybe some of our fellow participants need to put a little pressure on them. Jim?

JIM KRAMER: I want to go back to regulations. I want to bring up a subject that's sort of been underlying this whole thing in regulation, and I want to go back to something Dominic said. I love to take off after Dominic, we've known each other for such a long time. You talked about, you know, the naughty dumb engineer's - sorry engineers - idea of looking at all these single elements out of context of everything. But we can add something else. Should we use a multiple of species just like we should look at all kinds of species and interactions with chemicals? And how does this work in a logical and acceptable regulatory fashion?

DOMINIC DI TORO: Yes, but one small correction: it wasn't the engineers that used total chemical, it was the toxicologists that did it. Engineers figured out one flow in the stream, that was their contribution to this mess. I think, in fact, that the 1985 - Walter? - the Stefan et al. Water Quality Technical Guidelines Report, which established how the U.S. develops water quality criteria, was in fact an intellectual *tour de force* in some regards because it answered at least that question, or at least it proposed a framework within which that question could be answered. Namely, ranking all of the species tested from high to low, and taking a 5 percentile - which I notice has gotten to Europe as the PNEC cutoff - and so on. That issue of how one deals with different organisms - which is a very perplexing issue - which is the white rat, which is the most sensitive organism. I think they did a really quite intellectually respectable job on that. And what we had intended to do with the more refined models, where we're doing with biotic ligand complexation, is actually do that calculation for the three or four most sensitive organisms. And then calculate when a concentration would knock off the 5% equivalent. So we're proposing, in the copper work anyway, to follow right along in that intellectual path, because I think that's at least a respectable answer to your point, "How does one handle species variability"?

BRUCE WALKER: I think it's really critical to recognize that, you know, a bioassay result with one species is just one point on an index. And so it seems to be pretty obvious that whatever algorithm is used to decide what's the toxic fraction to a species, it still requires testing over a series of species. I mean, I think that's got to be understood. And you're right, I mean that was a valuable input at the time to do that, to take species from different components and put them together.

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WALTER BERRY: I certainly agree with the multi-species aspect in setting the criteria. But there was one other really big problem, I think, with the rather simplistic list of criteria, which of course were generated long before I got into the Agency. *(laughter)* We're much more enlightened now, and one of the things that we are thinking about is that, as silly as it would be to regulate a single chemical on a single organism, it probably is not a good idea either, in many cases at least, to regulate on the basis of a single compound. The sediment quality criteria, most of them which are now being proposed, are in fact mixtures criteria. The metals criteria is a mixtures criteria, the combined pH's criteria is a mixtures criteria. These are based on, if not similar modes of action at least roughly similar, or some similarity in their geochemistry and how they're regulated or at least on how they can be predicted. I think I can sort of throw it out as a challenge to this group, and Jim has alluded to it also as a problem of interactions of other chemicals, is that we have to think of silver as only one of the stressers in the environment. And we need to think about how silver interacts with the other stressers. One of the most interesting examples I think we've had in this conference is silver and ammonia as co-stressers. And if in fact silver affects ammonia in the organism, then it's easy to see why ammonia in the external environment would also be important, and you'd get a feeling for how those two things go together. So, I would sort of like to challenge the researchers in this group as they are figuring out silver by itself, that they not lose sight of the fact that they need to know about silver as just one chunk of the environment.

MARY REILEY: Okay we're coming down to about the last 8 minutes or so. Anybody else that would like to make a comment or put forth a question? Okay Chris.

CHRIS WOOD: I would like to ask a question. What is EPA thinking right now? Is EPA thinking about changing the way they do things? Or is EPA in an information gathering mode? And this is a question to you, Walter, and to you, Mary. Is it a watching brief or an activist brief that you have at present, or what?

WALTER BERRY: Well I can't speak for the Agency, but I certainly can speak for the Office of Research & Development. The ORD I don't think really has much interest anymore in working on a chemical-by-chemical approach. I made my joke about the micro watershed in part because of a frustration that I feel coming from sort of a classical toxicology background, knowing how important even in the watershed the single chemical versus organism approaches are when you go to do a risk assessment, which is a grand thing that takes into account everything all over the place. Still, what it comes down to often is a number that someone generated in a laboratory in a jar with a single chemical and a single organism. That we can't lose sight of. But I don't think that the Agency really knows exactly what to do. I think it knows that it's not happy with where it is and it needs to do things like the mechanistic models that we're coming up with, that it needs to do things with mixtures, that it needs to include things as overall risk assessments. Also, that it needs to regulate on a watershed or a waste load allocation basis as opposed to one single number coming out of the pipe. So, I think it's in a real growth phase at the moment. And I think that the people in this room can really help to affect the direction in which it goes.

MARY REILEY: Walter's very right when he speaks that we're in a real transition period right now in the Agency, in a variety of different areas. In the Office of Water I can certainly tell you what is happening with us, though the entire agency is trying very hard to move towards a risk assessment paradigm. One of the things the Office of Water is doing particularly, and the Office of Science and Technology, is trying to determine how we can incorporate the risk assessment paradigm into the process of developing water quality criteria. When I say water quality criteria I mean all types: aquatic life, sediment, human health, habitat, physical, wildlife and biocriteria. When you say water quality criteria, don't think that you're just thinking water column there. We're talking about the entire aquatic ecosystem that we're trying to address. And so along those lines, we're in the process of trying to design and shift to a paradigm that's going to take us into the next 20-30 years of what we do in the Office of Water. And how do we better incorporate biology, and in-stream assessment, into determining whether or not we are meeting our water quality goals for the nation? And then how do we integrate the science behind all of the different water quality criteria types, so that we can do a more holistic evaluation as to whether or not those goals are being met? So we're pondering these issues right now: we have a couple of different proposals that we're pursuing. The Office of Water has what we call the "criterion standards plan" that is being circulated right now for kind of an "in-house" review, and we need some selected pieces of intelligence from the academic and industrial community to try to help us put some fine tuning on that. It's contemplating how we will use biological criteria in

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order to set our designated uses, which are the determination of how a water body is being used by a state and by its populace. Is it for fishing, or for swimming, or navigation, or industrial water, or irrigation? Those are some of the different types of uses that are out there. And at the same time, we are trying to put together a Pelston workshop that will allow us to discuss how ecological risk assessment is used to solve our current criteria development problems, in order to make it a more integrated process. So we have a whole lot of fingers in different pies, trying to come up with solutions to move us forward in this venue. And we certainly hope that you people will keep an eye out for when those drafts come around and participate in the debate as we ask you to.

Any other questions? I think we're pretty much out of time. I was asked to do one thing, to let you know that the summary table for the European Standard that was discussed in the second panel is sitting here on this center table for anybody who wants to pick one up. And, if there are no further questions, then I'll turn it back over to Anders for our closing arguments.

# Closing Remarks

*Transport, Fate and Effects of Silver in the Environment*

*A.W. Andren/T.W. Bober  
Conference Co-Chairs*



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**CLOSING REMARKS:**      Anders W. Andren and Thomas W. Bober

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ANDERS ANDREN: Tom and I thank you very much for all of your contributions. I think we are continuing to make progress in our understanding of how silver behaves in the environment and I believe that this has been another successful conference.

I hope that Tom and I will be successful in arranging for another conference, if not next year, the following year. We hope to have Proceedings available from this conference in the not too distant future. I hope that our recordings will have proceeded without any technical glitches, at least from the Q & A and Discussion sections. The written summary of these recordings together with extended abstracts should be informative and will, hopefully, reflect some of the new material and ideas that have developed in the past year. I can tell you that the previous Proceedings have been very popular and we have had quite a number of requests. We have made second printings of the earlier workshop proceedings.

Many of you may recall the original intent of these workshops. First, we intended that the workshop would provide a platform for presenting up-to-date information on the behavior and effects of silver in the environment. I believe we have succeeded in attracting some of the world's top-notch researchers to the conference and we have also seen a considerable amount of scientific rigor that has been infused into the field – I hope, in large measure, stimulated by these conferences. Second, we also wanted to make sure that we approached the topic in a multidisciplinary fashion. We felt that too much of the previous work on the biogeochemistry of metals generally, and silver in particular, had been performed without much interaction of ideas from the newest advances in analytical chemistry, chemical speciation, toxicology, etc. I believe that we have accomplished, at least to some extent, the cross-fertilization of new concepts. Perhaps more importantly, we have stimulated several researchers from different disciplines to work together. Third, the original intent of the conference was to assure ourselves that we would talk about scientific and regulatory issues in a nonadversarial way. The conference would provide an important opportunity for technology transfer and, hopefully, some of the new information would find its way into deliberations on environmental regulations and criteria. I hope that we have contributed to this goal to some degree.

And so with that, I would like to thank all of you for your support and for coming to this conference. Again, let me thank the sponsors as well as the presenters. I hope that we all can agree that we are moving forward, at least incrementally, in our understanding of the behavior of silver in the environment. Let me also thank our local hosts, Jim Kramer and his able crew at McMaster University. I would also like to thank Tom Bober for his tireless efforts in dealing with fund raising and meeting logistics. As always, we are also grateful to Delphine Skinner from the University of Wisconsin Sea Grant Institute for her help in coordinating foreign travels arrangements, the meeting logistics, the short abstract and our conference proceedings. Thank you all very much.

TOM BOBER: I think we'd also like to remind everyone to get your extended abstracts in to us as soon as possible. The faster we can get the proceedings out this year, the better chance we'll have for a conference in subsequent years. Thank you again.

# Poster Session

*Transport, Fate and Effects of Silver in the Environment*

*Hamilton, Ontario, Canada*



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## Comparison of the Fate of Dietary Silver in the American Plaice (*Hippoglossoides platessoides*) and the Snow Crab (*Chionoecetes opilio*)

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Maurice Lamontagne Institute, Mont-Joli, Quebec, Canada  
Swedish University of Agricultural Sciences, Uppsala, Sweden

(This paper was delivered by C. Gobeil in the absence of C. Rouleau)

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### Introduction

Most of the anthropogenic Ag released in coastal environments is deposited in sediments, but very little is known on the accumulation of this metal in the organisms that feed and spend their life cycle at or near the sea floor. In order to improve our knowledge on the bioaccumulation of Ag, we have determined the kinetics and distribution of dietary Ag in the American plaice (*Hippoglossoides platessoides*) and the snow crab (*Chionoecetes opilio*), two benthic predators commonly found in the St. Lawrence Gulf and Estuary. The technique used for this study was *in vivo* gamma counting, which allows to monitor over time the amount of radiolabelled Ag contained in single living organisms.

### Material and Methods

Experiments were done at *in situ* temperature and water flow in aquaria was kept high enough to approximate an open system. Groups of 5 or 6 female American plaices (300-400 g) and snow crabs (50-80 g) received a single dietary dose of 5 µg Ag(I), with 37 kBq <sup>110m</sup>Ag(I) as tracer. The animals were then fed twice a week with uncontaminated food.

The Ag content in American plaices and snow crabs was monitored for 42 d and 154 d, respectively, by measuring the activity of the 657-keV gamma ray emission of <sup>110m</sup>Ag. Whole body activity was determined in snow crab, whereas the bigger size of American plaice allowed to measure separately the activity of the caudal muscle tissues and the viscera.

### Results

The temporal evolution of Ag in the viscera of American plaice and in the whole body of snow crab was similar (Fig. 1). While a fraction of the ingested Ag transited through gut and was eliminated with faeces within 3 to 4 days, another fraction, Ag<sub>t</sub>, was retained for a longer period of time by the organisms. <sup>110m</sup>Ag activity was undetectable in the caudal muscle tissues of the American plaice.

The relationship between Ag<sub>t</sub> and time t can be expressed with a simple mono-exponential equation;

$$(1) \quad \ln Ag_t = \ln RE_0 - (0.693 / t_{1/2}) t$$

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where  $t_{1/2}$  is the biological half-life and  $RE_0$  is the retention efficiency ( $0 \leq RE_0 \leq 1$ ). Results of linear regression analysis with eq. (1) (Tab. 1) show a low retention efficiency and a moderately fast elimination of Ag in the American plaice. Conversely, the snow crab retained most of the ingested Ag and the metal was eliminated at a very slow rate.

At the end of experiments, animals were dissected to determine the concentration index of tissues,  $I_C$ , which is defined as:

$$(2) \quad I_C = \frac{\% \text{ of Ag body burden}}{\% \text{ of body weight}} = \frac{[\text{Ag}] \text{ in tissue}}{[\text{Ag}] \text{ whole body}}$$

Values of  $I_C$  for the gut and the rest of body were similar for the two species (Tab. 2).

### Discussion

With our values of  $t_{1/2}$ , we can estimate  $t_{0.95}$ , the time needed to reach 95% of equilibrium between the Ag concentrations in the organisms and their food, using the following equation:

$$(3) \quad t_{0.95} = -(\ln 0.05) / (0.693 / t_{1/2})$$

In the American plaice, the average value of  $t_{0.95}$  is 0.4 y, indicating that the Ag concentrations in the organisms and their food rapidly reach equilibrium. In the snow crab, however, the equilibrium is never reached, since the average value of  $t_{0.95}$  is at least 17 y, which is longer than the average life span of this species (10-12 y). This indicates that snow crabs would continuously accumulate Ag.

We wanted to verify if we could predict *in situ* Ag concentrations in the American plaice and the snow crab,  $[Ag]_t$ , knowing the Ag concentration in the diet,  $C_f$ , the rate of food consumption,  $k_{in}$ , and the values of  $t_{1/2}$ ,  $RE_0$ , and  $I_C$ , with the following equation:

$$(4) \quad [Ag]_t = RE_0 \cdot I_C \cdot C_f \cdot k_{in} \cdot (0.693/t_{1/2})^{-1} (1 - e^{-(0.693/t_{1/2}) \cdot t})$$

We used available data of Ag concentrations in benthic worms ( $0.3 \mu\text{g Ag} \cdot \text{g}^{-1}$  wet weight) from the St. Lawrence Estuary and rate of food consumption values of  $0.01 \text{ g food} \cdot \text{g}^{-1} \text{ body weight} \cdot \text{d}^{-1}$  for the American plaice and  $0.003 \text{ g food} \cdot \text{g}^{-1} \text{ body weight} \cdot \text{d}^{-1}$  for the snow crab. We assumed that there was no growth effect and that Ag uptake occurred entirely through food. Results obtained from the model were compared to Ag concentrations measured in muscle tissues of American plaices and snow crabs from the St. Lawrence Estuary (Gobeil *et al.*, Rapp. Stat. Can. Sci. Halieut. Aquat., no. 1011, 1997). The simulation shows that, over a 10-year period, Ag is continuously accumulated in the snow crab, whereas an equilibrium is reached within a year in the American plaice (Fig. 2). Ag concentrations predicted by the model are similar to values measured in organisms from the St. Lawrence Estuary (shaded areas of Fig. 2).

Table 1: Values of biological half-life ( $t_{1/2}$ ) and retention efficiency ( $RE_0$ ) of Ag(I) given as a single dietary dose to American plaice and snow crab, as determined by linear regression analysis with eq. (1).

	$t_{1/2}$ (d)	$RE_0$	$r^2$
American plaice			
# 1	29	0.103	0.93
# 2	30	0.073	0.93
# 3	101	0.161	0.96
# 4	68	0.042	0.62
# 5	13	0.048	0.98
Snow crab			
# 1	2800	0.672	0.14
# 2	n.s.	0.977	
# 3	n.s.	0.999	
# 4	1030	0.868	0.51
# 5	1390	0.916	0.38
# 6	n.s.	0.890	

n.s. = non significant ( $p > 0.05$ )

Table 2: Values of concentration index,  $I_C$ , of gut and the rest of the body of the American plaice and the snow crab calculated with eq. (2).

	$I_C$	
	Gut	Rest of body
American plaice	$8.8 \pm 4.0$	$0.27 \pm 0.29$
Snow crab	$7.0 \pm 0.9$	$0.42 \pm 0.09$

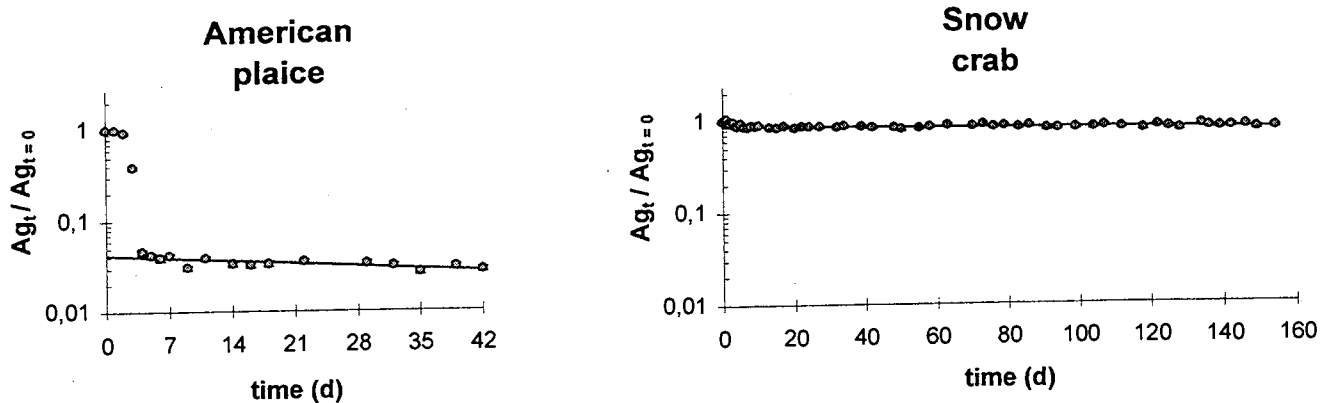


Fig. 1: Typical evolution of Ag content versus time in individual American plaice and snow crab, which received a single dietary dose of metal. Bars represent the sum of positioning and statistical counting error. The line show the result of linear regression analysis.

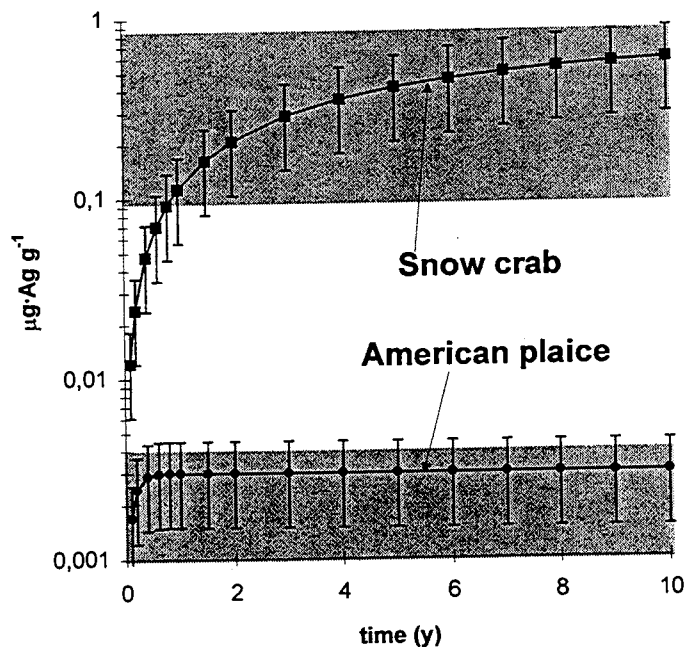


Fig. 2: Simulation of Ag trophic accumulation in the muscle of the American plaice and in the muscle of the snow crab. Shaded areas indicate the range of concentrations measured in the St-Lawrence Estuary. Bars represent interindividual variation, assumed to be  $\pm 50\%$ .

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## Are All Dissolved Organic Matters Equally Protective Against Metal Binding to Fish Gills?

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The gill comprises a surface of critical contact between the internal environment of a fish and its surroundings (Laurén, 1991). Waterborne metal cations can interact and bind to fish gills and disrupt the gill's role in ion regulation, respiratory gas exchange, nitrogenous waste excretion, and the internal acid base balance of the fish (Wood, 1992). Therefore, metal cations must first bind to the gill to elicit a toxic effect, and any process that prevents metal binding to fish gills will prevent metal toxicity.

Dissolved organic matter has been demonstrated to protect against metal toxicity and prevent metal binding to trout gills (e.g. Hollis *et al.* 1997; Janes and Playle, 1995; Playle *et al.* 1993; Richards and Playle, accepted). Dissolved organic matter protects against metal binding to fish gills by forming a complex with the metal cation. Once metal are bound in a metal-DOM complex, metal interactions at fish gills are dependent on the relative equilibrium binding strengths between the metal binding to DOM and metal binding to the gills. Work in our lab with Hg binding to trout gills suggested that not all sources of DOM protect equally against metal binding to fish gills. We used the deposition of Ag and other metals on the gills of trout as a biological assay to determine whether DOM source makes a difference to the protective effect of DOM against metal toxicity to fish.

Juvenile rainbow trout (*Oncorhynchus mykiss*, ~3 g) were acclimated to synthetic soft water for at least two weeks before experiments were run. Acclimated trout were then exposed to a mixed metal solution containing Ag, Cd, Cu, Hg, Pb, and Co in 16 L of soft water in polyethylene buckets. One of three dissolved organic matter sources were added at nominal concentrations of 2.5, 5.0, and 10 mg C·L<sup>-1</sup> DOC. Dissolved organic matters were collected from Luther Marsh, Beverly Swamp, and a pond on Point Pelee, all in southern Ontario. At 4, 24, and 72 h after the start of exposure, six trout were removed from each bucket, stunned with a blow to the head, and both sets of gill arches removed. Gills were digested in five times their wet weight in 1N HNO<sub>3</sub> and analyzed for total metal using graphite furnace atomic absorption spectrophotometry.

Greatest toxicity was observed in trout exposed to the six metal solution without added DOM (Figure 1). Of the trout exposed to the six metals with added DOM, Point Pelee DOM exposed trout experienced the greatest mortality followed by Beverly Swamp and Luther Marsh DOM exposed trout. No mortality was observed in trout exposed to soft water without added metals or DOM.

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Toxicity data related well with Pb accumulation by trout gills which showed the greatest Pb accumulation in the Point Pelee DOM exposure (Figure 2). In contrast, trout exposed to the six metals plus 9.5 mg C · L<sup>-1</sup> Point Pelee DOM for 4h accumulated less Ag on or in their gills compared to the other DOM exposures (Figure 3). In 24 h exposure to the six metals, silver deposition on trout gills was actually enhanced by higher concentrations of Beverly Swamp DOM (Figure 4).

The observed differences in the metal complexation properties of the three DOM sources tested might be due to differences in the concentration of sulphur contained in each DOM. Table 1 shows the protein : carbohydrate ratio for each of the DOM sources used. The higher the protein to carbohydrate content the more abundant are the sulphur groups (e.g. cysteine) contained in the DOM compared to carboxyl groups (COOH) in carbohydrates. Negatively charged sulphur groups can form very strong bonds with Ag, Pb, and Hg, therefore DOM with high sulphur content might be expected to protect better against Ag, Pb, and Hg binding to fish gills. This relationship was noted for Pb (Figure 1) and Hg (data not shown), but not for Ag (Figure 3 and 4).

Clearly, not all DOM protects equally against metal binding to fish gills. The mechanisms underlying the variations in the protective effect of different DOM sources still need to be investigated.

### Acknowledgments

This research was funded by Kodak Canada Inc. and by the Natural Sciences and Engineering Research Council.

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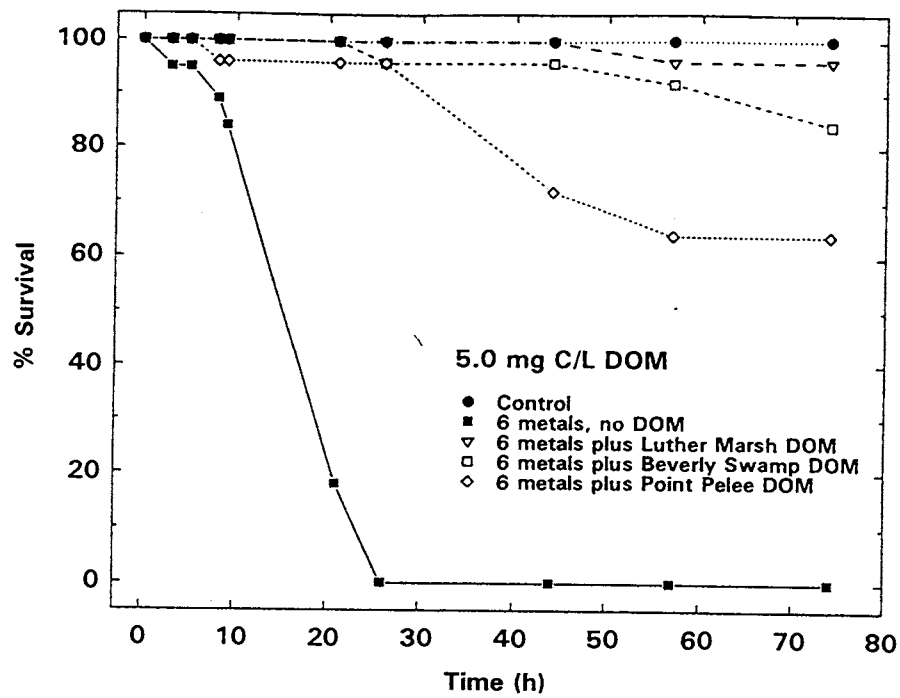


Figure 1. Percent survival of trout exposed to the six metal solution containing 5 mg C · L<sup>-1</sup> DOM.

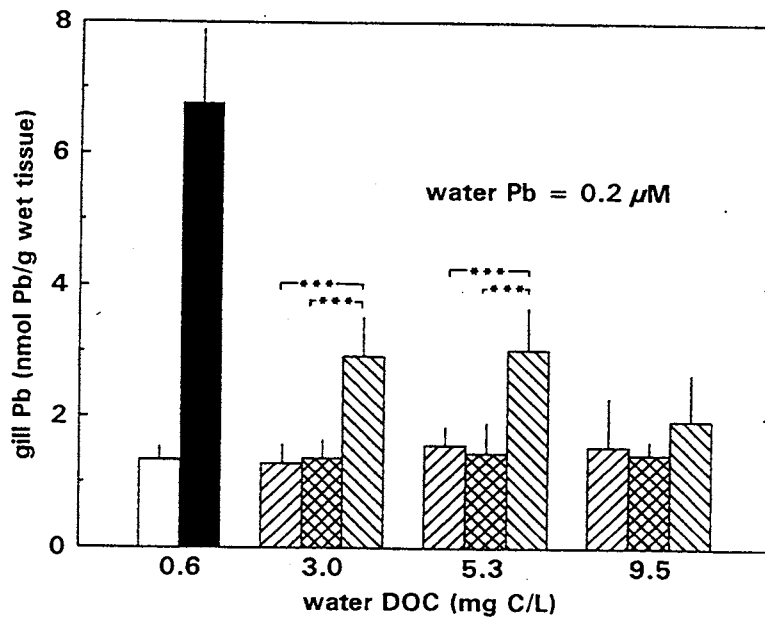


Figure 2. Amount of Pb on or in the gills of rainbow trout held in the six metal, three DOM solutions for 4 h. Data are presented as the mean  $\pm$  95% confidence intervals for six fish. Significance is given between DOM sources at  $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$  (\*, \*\*, \*\*\*, respectively).

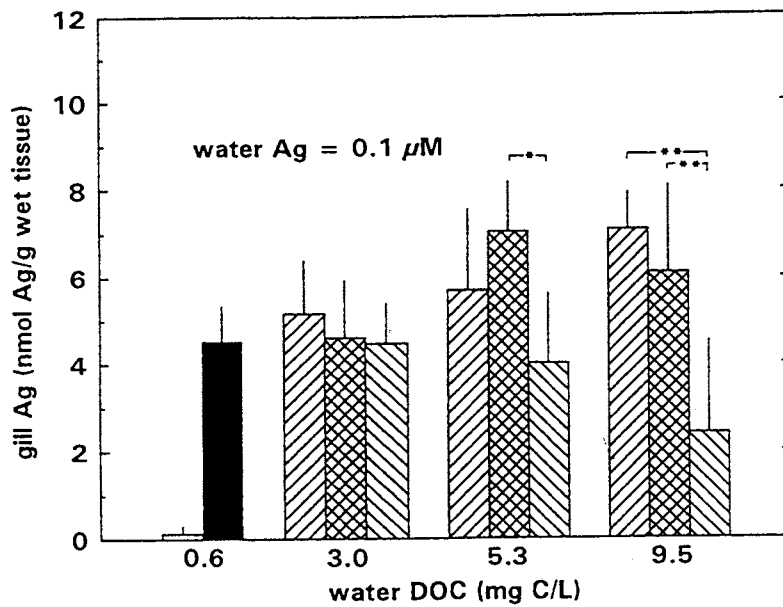


Figure 3. Amount of Ag on or in the gills of rainbow trout held in the six metal, three DOM solutions for 4 h. See Figure caption 2 for more detail.

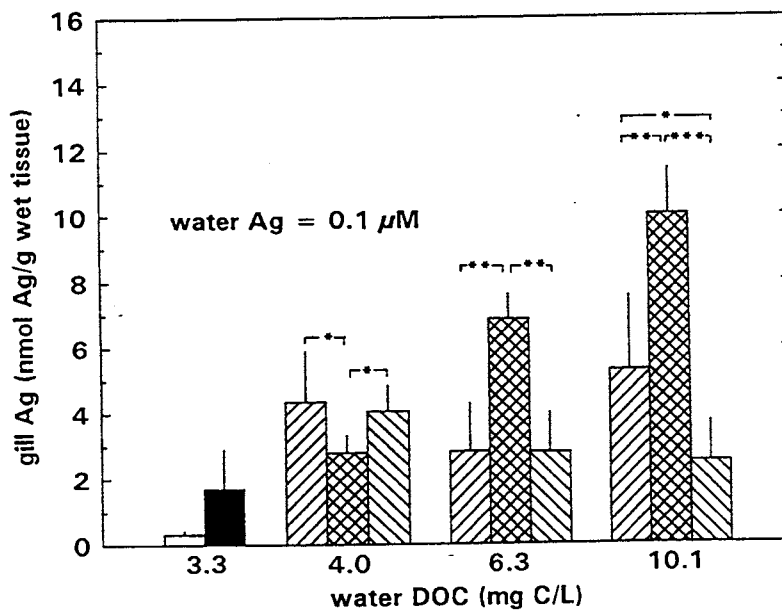


Figure 4. Amount of Ag on or in the gills of rainbow trout held in the six metal, three DOM solutions for 24 h. See Figure caption 2 for more detail.

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Table 1. Protein : carbohydrate ratio for each DOM. The higher the protein to carbohydrate content the more abundant are the sulphur groups contained in the DOM.

DOM	protein/carbohydrate
Luther Marsh	1.08
Beverly Swamp	0.79
Point Pelee	0.47



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## Protective Effects of Dissolved Organic Matter Against the Physiological Disturbances of Waterborne Silver on Rainbow Trout

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Our working theory of how metals interact at the gills are as metal cations. Water systems often contain organic (e.g. dissolved organic matter) and inorganic compounds (e.g. thiosulphate) which are negatively charged and available to complex with metals. These metal-ligand complexes can render a metal less toxic and decrease its interactions at fish gills (Janes and Playle, 1995; Evans, 1987). The ability of a ligand to reduce metal toxicity is dependent not only on the concentration of the complexing ligand in the water, but also on the metal's binding affinity for the ligand compared to its binding affinity for gill binding sites (Giesy *et al.*, 1977).

Previously, we showed that ligands which bind metals (such as thiosulphate binding Ag, and DOM binding Cu and Cd) eliminated the respiratory and ionoregulatory effects of the metals. It was expected that DOM would also protect against the physiological and toxicological effects of silver, through reduced binding of silver at fish gills. Janes and Playle (1995) demonstrated that ~24 mg C/L DOC was required to prevent Ag accumulation by the gills of juvenile trout exposed to ~0.17  $\mu\text{M}$  Ag. From these experiments, conditional equilibrium binding constants were calculated for Ag binding to the gill ( $\log K = 10.0$ ) and Ag binding to DOM ( $\log K = 9.0$ ).

Adult rainbow trout (*Oncorhynchus mykiss*, ~250 g) were cannulated, under MS222 anesthetic, *via* the dorsal aorta for repetitive blood sampling and allowed to recover in dark fish boxes for ~36 h. The boxes were supplied with 50 mL/min. of synthetic soft water with a calcium supplement during treatment exposures. Arterial and water  $PO_2$  and  $PCO_2$  were measured using a Cameron blood gas meter. Plasma Ag concentrations were measured using graphite furnace AAS after a 10x dilution in E-Pure water. Gill lamellae samples were removed at the completion of an experiment (~96 h) and digested in 5x their wet weight of ultrapure 1N  $HNO_3$  for 3 h at 80 °C. The resulting digest was diluted 10x with E-pure water and analyzed for Ag using graphite furnace AAS. Plasma Cl was assayed using Sigma reagents. Ventilation rates were measured visually over 30 seconds. Water DOM was measured using a Shimadzu Total Carbon Analyzer.

In the first experiment, trout were exposed to ~0.06  $\mu\text{M}$  Ag (as  $AgNO_3$ ) in the presence or absence of ~8.0 mg C/L natural DOC isolated from a marsh. Trout in the second experiment were exposed to ~0.15  $\mu\text{M}$  Ag in the presence or absence of ~20 mg C/L DOC added as a terrestrial humic acid (Sigma-Aldrich). Fish were held at ~12 °C with water pH between 7.1 and 7.4. Water  $PO_2$  was ~125 torr and water  $PCO_2$  was ~1.0 torr.

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Fish exposed to Ag alone had a slightly greater concentration of Ag in the plasma compared to trout exposed to Ag and 8 mg C/L natural DOC (figure 1A). However, these results were not significantly different. When 20 mg C/L commercial DOC was added to water containing Ag, plasma Ag concentrations from the two exposure groups closely tracked each other (figure 1B). Again, while these plasma concentrations were significantly greater than pre-exposure levels, there was no difference between the groups.

Previous experiments of ours have shown that Ag exposed fish experience a decrease in arterial  $PO_2$  compared to groups not exposed to Ag or groups exposed to Ag plus thiosulphate, both of which experience a gradual rise in arterial  $PO_2$  over the course of an experiment (Rose-Janes, M.Sc. thesis). In the present research, Ag only exposed fish did not show a decrease in arterial  $PO_2$  (figure 2A and 2B). Arterial  $PO_2$  did not differ between the Ag only and Ag plus 8 mg C/L DOC exposures in the first experiment (figure 2A). In the second experiment (figure 2B), the trend was Ag plus DOM fish having a slightly higher arterial  $PO_2$  than the Ag only exposed group, but there was no significant difference between the two groups. Arterial  $PCO_2$  did not differ between groups in either experiment (data not shown).

Breath rate data indicate that neither the Ag only nor Ag plus DOM exposed fish were having ventilation problems in the first experiment (data not shown). This was further indicated by the coughing rate which did not differ between the groups (0-2 coughs in 30 seconds for both groups). Wood *et al.* (1996a) noted that fish exposed to Ag tended to respire more deeply. This was observed in our second experiment where Ag only exposed fish tended to respire less frequently than the Ag plus 20 mg C/L DOC fish (66-81 and 77-87 breaths/minute, respectively). Coughing rates were also greater in Ag only exposed fish (0-6 coughs for Ag only exposed fish compared to 0-1 cough per 30 seconds for Ag plus DOM exposed fish). This suggests that Ag was irritating the gills of the Ag only exposed fish, causing an increase in mucus production at the gills which the fish attempted to remove by coughing.

Another problem fish face when exposed to a metal toxicant is an ionoregulatory problem. Silver is known to interfere with Na and Cl regulation at fish gills (Janes and Playle, 1995; Hogstrand *et al.*, 1996; Wood *et al.*, 1996a; Rose-Janes, M.Sc. thesis). Plasma Cl results from the first experiment indicated that Ag only exposed trout were not having ionoregulatory problems (figure 3A). In the second experiment, Ag only exposed trout did experience a large decrease in plasma Cl whereas the Ag plus 20 mg C/L DOC exposed fish did not (figure 3B).

Gill Ag results were expected to be similar to previous experiments where trout were exposed to Ag alone and Ag plus thiosulphate, where thiosulphate (a complexing ligand) prevented a significant accumulation of Ag onto the gills (Janes and Playle, 1995; Rose-Janes, M.Sc. thesis). However, neither the addition of 8.0 mg C/L DOC nor 20.0 mg C/L DOC was effective in preventing Ag from binding to the gills of trout

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(figure 4A and 4B).

Our results indicate that the concentration of DOM required to prevent Ag from binding to the gills and entering the plasma exceeds the environmentally realistic concentration (8.0 mg C/L DOC) and is greater than the 20 mg C/L DOC. These results agree with earlier results, where 24 mg C/L DOC was needed to keep Ag off the gills (Janes and Playle, 1995).

In a future experiment we intend to use a DOM concentration of at least 30 mg C/L DOC as a terrestrial humic acid to determine the concentration of DOM needed to prevent Ag from adhering to the gills and entering the plasma of fish exposed to Ag in very soft water.

### Acknowledgements

This work was funded by Kodak Canada Inc. and by NSERC.

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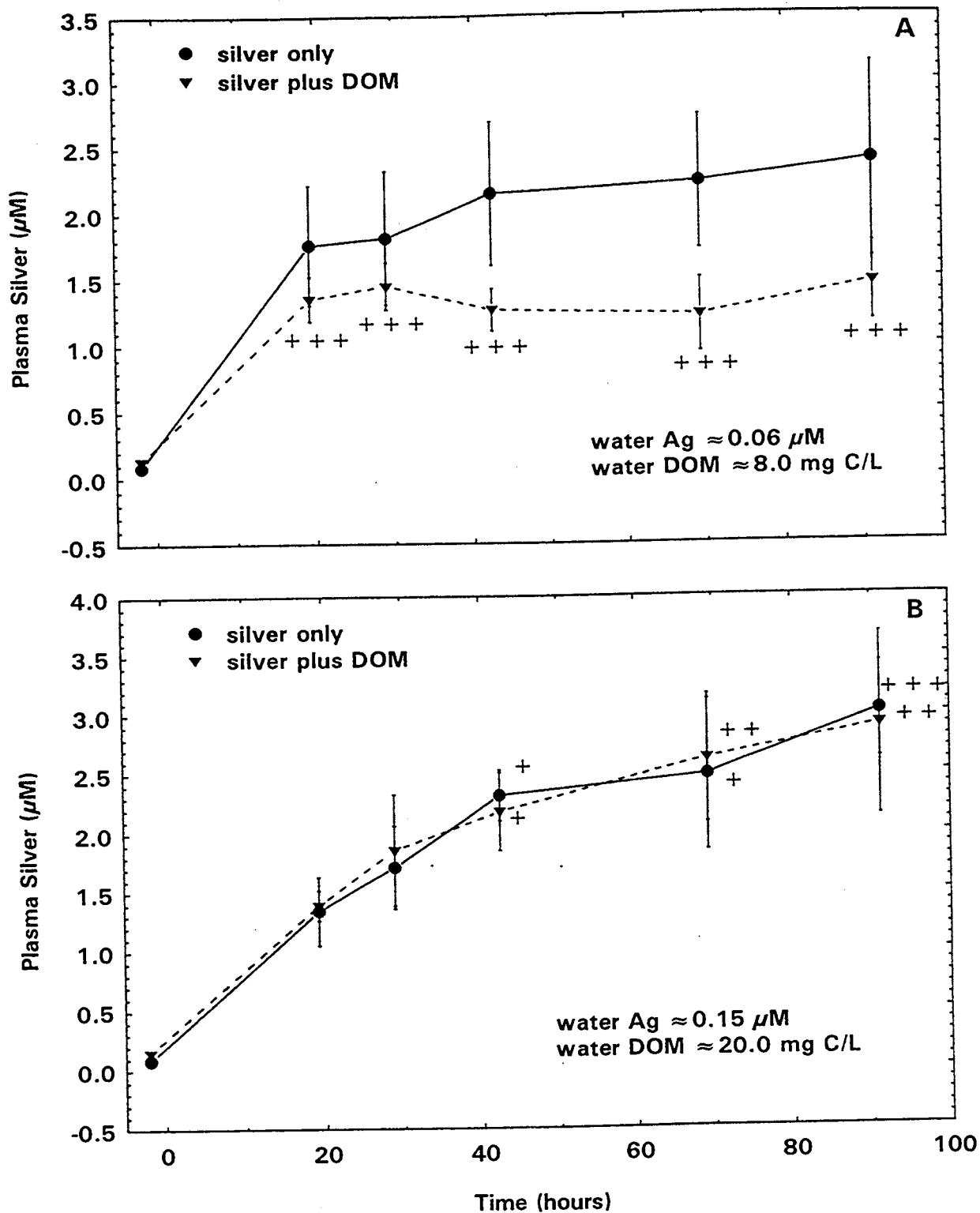


Figure 1. The amount of Ag entering adult trout during exposures to 0.06 µM Ag plus 8.0 mg C/L was slightly lower compared to Ag exposure alone (A). Plasma Ag concentrations of Ag plus DOM exposed trout closely tracked plasma Ag concentrations of Ag only exposed trout in the second experiment (0.15 µM Ag exposure in the absence or presence of 20.0 mg C/L, figure B). Crosses indicate within group differences over pre-exposure values.

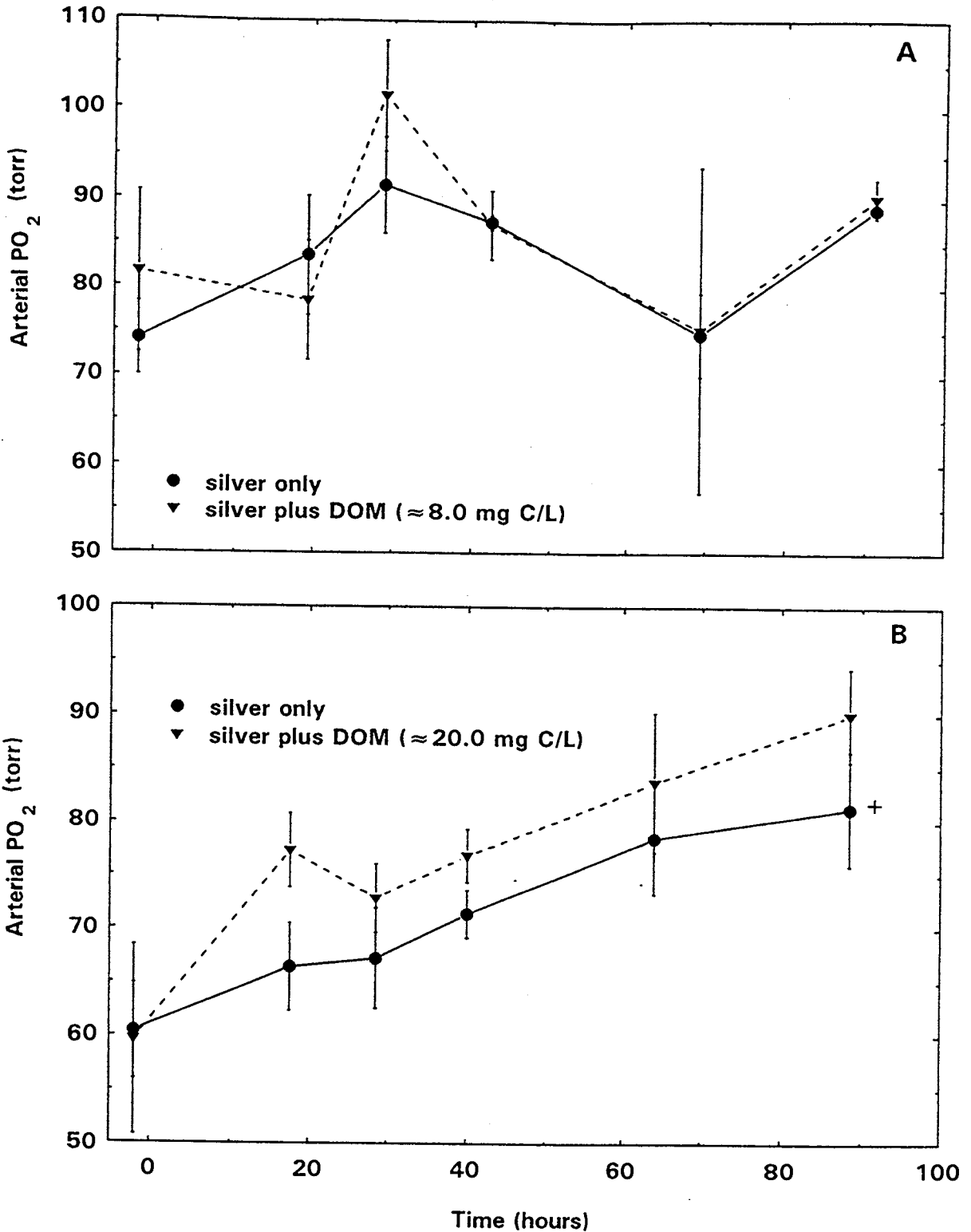


Figure 2. Arterial oxygen tension did not differ between Ag only exposed fish and Ag plus DOM exposed fish in the first experiment (A). In the second experiment (B), Ag plus DOM exposed fish had slightly higher arterial oxygen tensions, but these were not significantly greater than Ag only exposed fish.

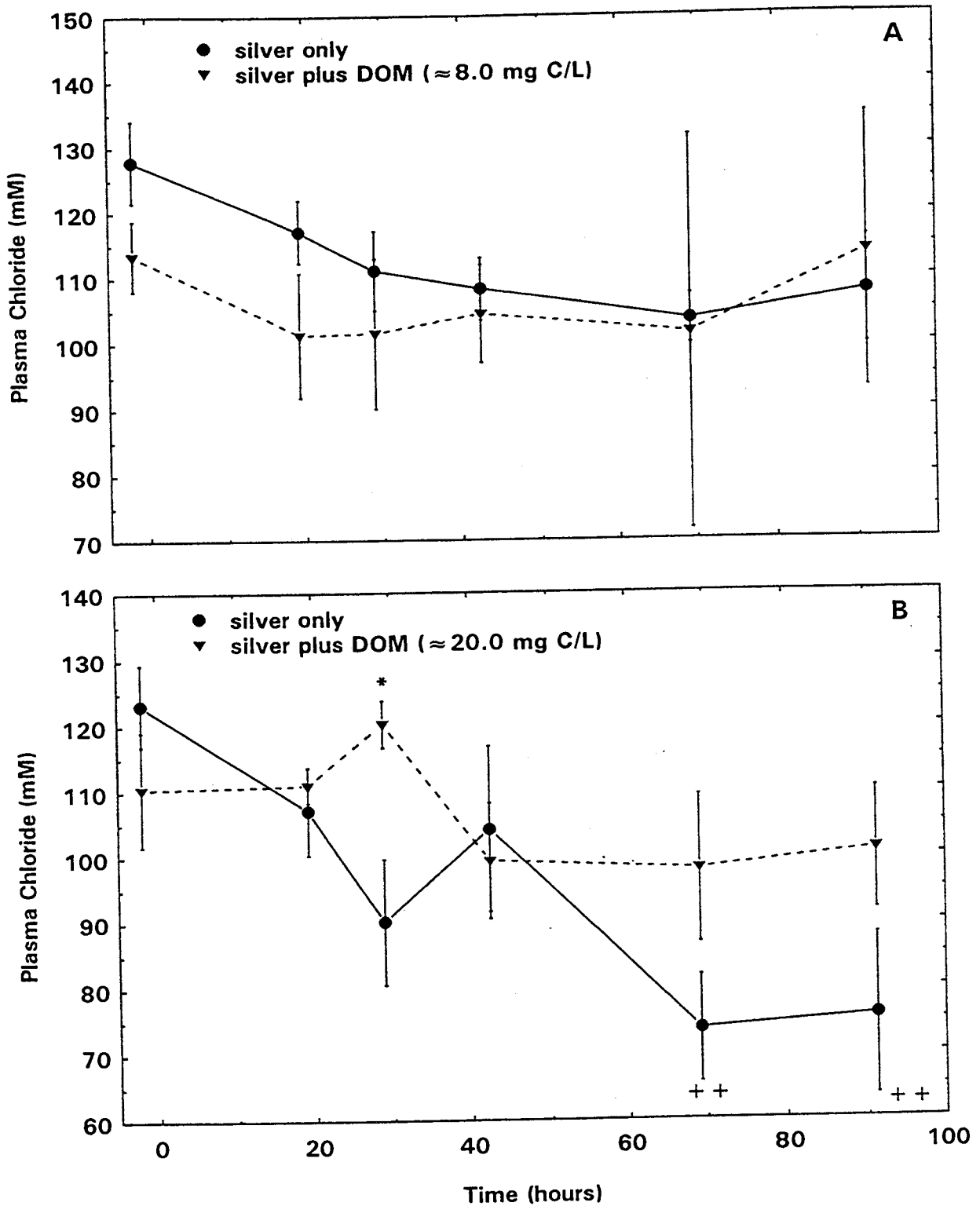


Figure 3. Plasma Cl concentrations did not differ between Ag only and Ag plus DOM exposed groups in the first experiment (A). In the second experiment (B), Ag only exposed fish experienced a significant decrease in plasma Cl that was not evident in the Ag plus DOM exposed fish. Crosses indicate within group differences over pre-exposure values, asterisks indicate differences between groups.

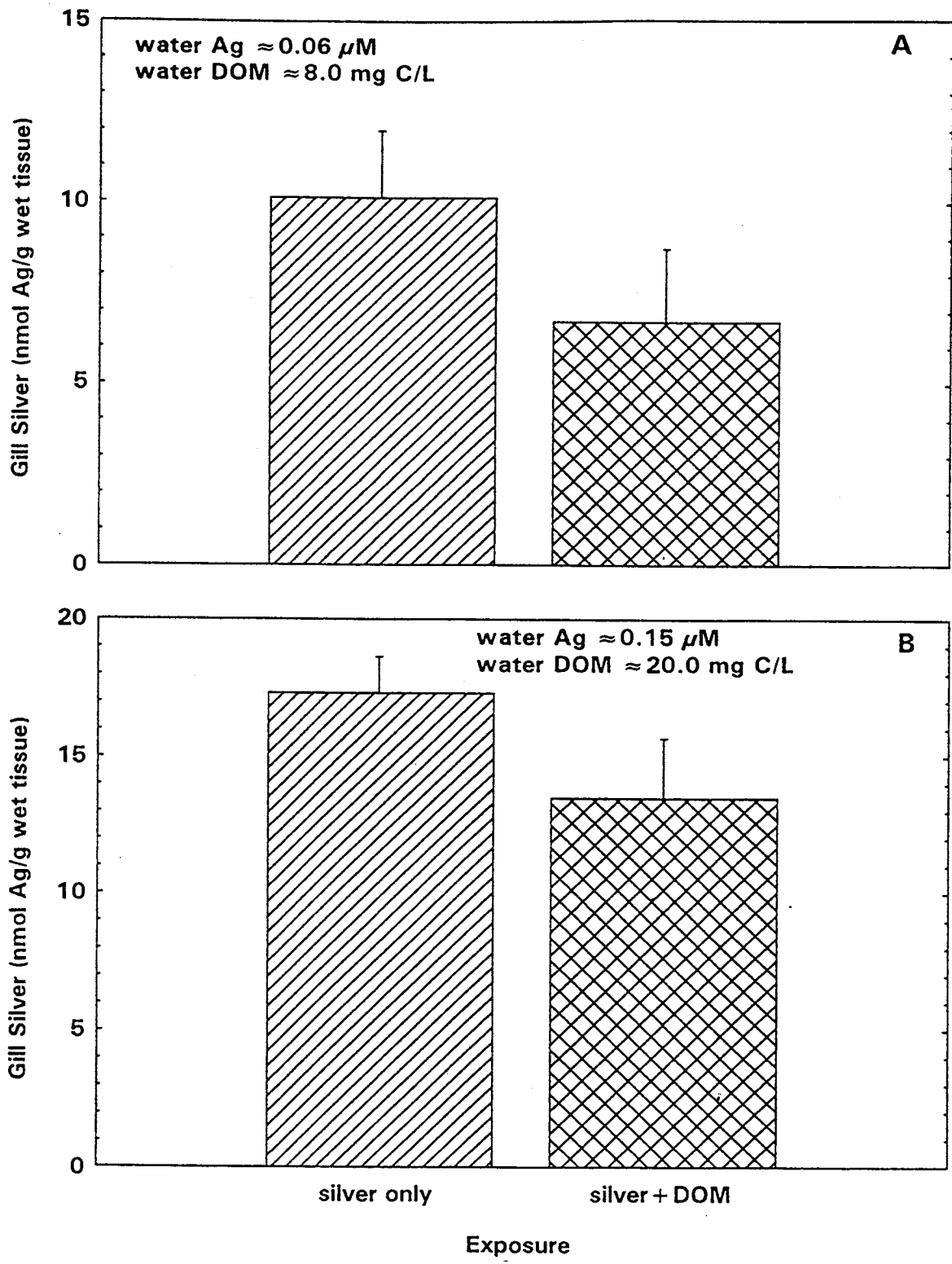


Figure 4. Gill Ag data indicated no significant differences between Ag only and Ag plus DOM exposed trout in either experiment.

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## Dissolved and Colloidal Ag in Natural Waters – Analytical Aspects

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### Abstract

A series of laboratory experiments were conducted to better understand and illustrate the possible occurrence of artifacts during water sampling, sample storage, sample digestion, and preconcentration. Silver in solution tended to sorb onto the walls of sample bottles regardless of the bottle composition even when utilizing a conventional sample acidification (2 mL HNO<sub>3</sub>/L of water) method. However, using a combination of sonification and UV-irradiation, sorbed Ag could be released back into the water. Such a digestion procedure was also a necessary step for colloidal samples before extraction. We also found that more than 30% of the conventionally defined “dissolved” Ag ( $\leq 0.45\mu\text{m}$ ) in fresh and marine waters was retained by a 0.1 $\mu\text{m}$  filter.

### Introduction

Chemical and phase speciation of trace elements is key to understanding the factors controlling their biogeochemical cycling within natural aquatic systems. The fate of trace metals, such as Ag, in aquatic environments is dependent, to a large extent, on the energetics of heterogeneous reactions. Bioavailable solution species often react on surfaces of heterogeneous particulate phases, which include aggregates of organic matter, the oxyhydroxides or sulfides of Fe and Mn, clay minerals, or carbonates, of diameters ranging from nanometers to tens of micrometers. Each of these phases act as sites for sorption/desorption, proton and electron exchange, or photochemical reactions. In most cases, the bioavailability and toxicity of trace metals are enhanced when metals exist predominately as true solution phase species. However, the difference between beneficial and toxic concentrations even, of required trace metals in aquatic environments is often very small.

Silver is often introduced into the aquatic environment from municipal and industrial water treatment plants receiving liquid wastes from the photographic industry. Thereafter, Ag has the potential to be a major urban pollution indicator. In here, a series of experiments were conducted to better understand the proper methods for sampling, storing and analyzing of water samples for “dissolved” Ag.

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## Methodologies and Approaches

“State-of-the-art” ultraclean techniques were used during all stages of sample collection, transport, handling, processing and analysis (Wen et al, 1996). Two ultraclean CFUF systems (AMICON, S10N1 and Miniplate-3) were used for Ag phase speciation studies. A specially constructed UV-irradiation chamber was used for the digestion of all samples. Radiotracer ( $^{110m}\text{Ag}$ ) and a well-type high purity Ge detector interfaced with a Canberra Series 100 multi-channel analyzer were used for the sample storage experiments. A modified solvent extraction procedure (Bruland et al., 1985) was used for preconcentration, while a PE5100 Perkin Elmer GF-AAS with Zeeman correction were used for the analysis of Ag in total dissolved, ultrafiltrate and retentate samples.

For the bottle adsorption and storage experiments, radioactive  $^{110m}\text{Ag}$  was added to filtered ( $\leq 0.45\mu\text{m}$ ) sample waters (DDW, tapwater, unacidified and acidified river water) contained in a Teflon bottle. During a two-month storage period, time series subsamples were taken and gamma counted. After storage, the unacidified samples were acidified (2ml conc.  $\text{HNO}_3$  per 1 L of sample) and subsamples gamma counted, after which time all samples were ultrasonicated for 1 hour at  $60^\circ\text{C}$  and gamma counted again, and finally UV irradiated for 24 hours and gamma counted. For the storage experiment, with natural filtered water samples ( $< 0.45\mu\text{m}$ ) collected from a Colorado river site down stream from a waste water effluent had been used for the adsorption study. First, two samples were extracted immediately after collection. Second, two samples were acidified in the field immediately after collection, and extracted in the laboratory. Third, two samples were acidified and extracted in the laboratory after 3 months. Fourth, 4 samples collected in a 2 L TEFLON bottles were ultrasonicated and UV digested in the laboratory after 3 months, and extracted. Fifth, 4 samples collected in a 2 L TEFLON bottle were acidified, ultrasonicated, and UV digested in the laboratory only after 3 months and then extracted. Two different types of colloidal solutions ( $\geq 1\text{kDa}$  CFUF retentate), Trinity river ( $S=0$ ) and Galveston Bay water ( $S=30$ ) were used for a test study of the importance of a proper digestion procedure before sample preconcentration. The Trinity River filtered ( $\leq 0.45\mu\text{m}$ ) water had a colloidal organic carbon (COC) concentration of 3.4 mg/L at that time, while the surface water sample from the Gulf of Mexico had a COC content of 1.1 mg/L.

## Results and Discussions

Results of sample storage experiments, for both radioactive and stable Ag, are shown in

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Figure 1 and 2. Results indicate that all samples waters with  $^{110m}\text{Ag}$  spiked without acidification sustained a great loss to the bottle walls in less than 24 hours, however even with acidified samples, some  $^{110m}\text{Ag}$  still adsorbed onto the bottle walls (Figure 1). After the sample waters were acidified again, there was no significant desorption of previously sorbed  $^{110m}\text{Ag}$  back into the solution, even after a sonification procedure was applied. However, after UV irradiation of the sample water, 100% recovery was consistently achieved (Figure 1).

For the natural water storage experiment, we had found that in-situ extraction or in-situ acidification resulted in identical Ag concentrations (Figure 2). Even if samples were not acidified immediately, after three months, all the Ag can be recovered with a proper acidification and UV-irradiation method (Figure 2).

For the proposed digestion protocol for colloidal sample waters, samples processed without a UV-digestion procedure usually exhibited considerably lower recovery even with acidification and sonification (Figure 3). These results clearly demonstrate that UV-irradiation is the most effective and convenient water sample digestion method.

Using ultraclean cross flow ultrafiltration, we were previously able to demonstrate that 15~70% of the conventionally defined “dissolved ( $\leq 0.45\mu\text{m}$ )” Ag was actually bound to a colloidal phase in river waters and estuarine waters of Texas (Wen et al., 1997). We found that this was also true for river waters of Colorado. Only 10 to 40 % of the “dissolved( $\leq 0.45\mu\text{m}$ )” Ag was in fractions smaller than 3kDa. A major fraction of the Ag was bound to colloidal organic matter.

## Conclusions

Results from sample storage experiments using both radioactive and stable Ag indicate that even with acidified samples, some Ag will be adsorbed onto Teflon bottle walls. However, by UV irradiating the sample water, 100% recovery was consistently achieved. Also, we found that the UV-irradiation digestion method was required for Ag analysis in concentrated colloidal solutions. In natural waters from widely different geographical regions, we found that 5~90% of the conventionally defined “dissolved” Ag is actually bound to a colloidal phase, and not present in ionic forms. Thus, the use of CFUF is important to reveal the phase speciation of Ag in natural waters.

## Acknowledgements

This work was, in parts; supported by grants from the Photographic and Imaging Manufacturers, Inc., PIMA, the Office of Naval Research, ONR, and the Texas Institute of Oceanography, TIO.

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**Figure 1**

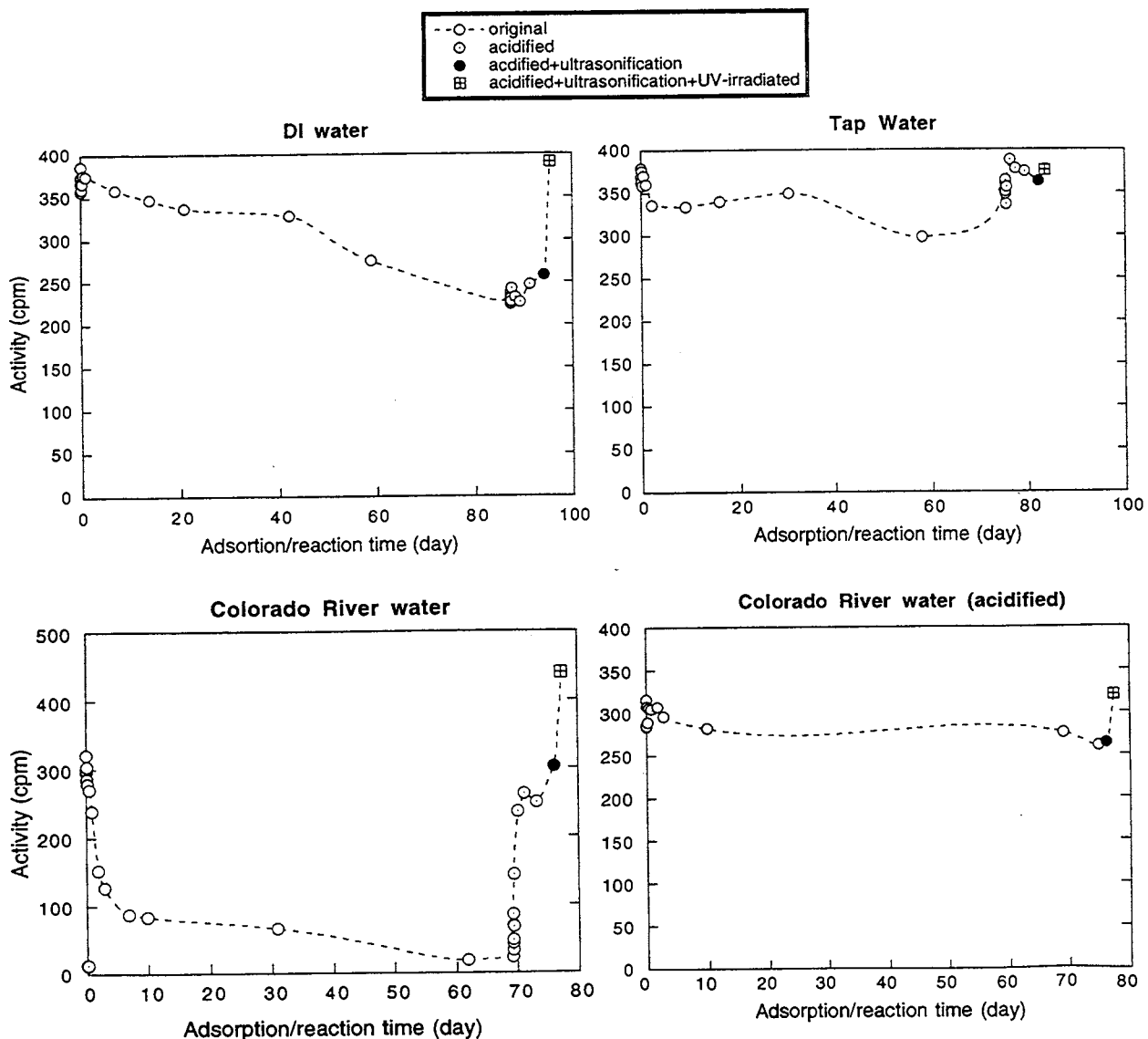


Figure 2

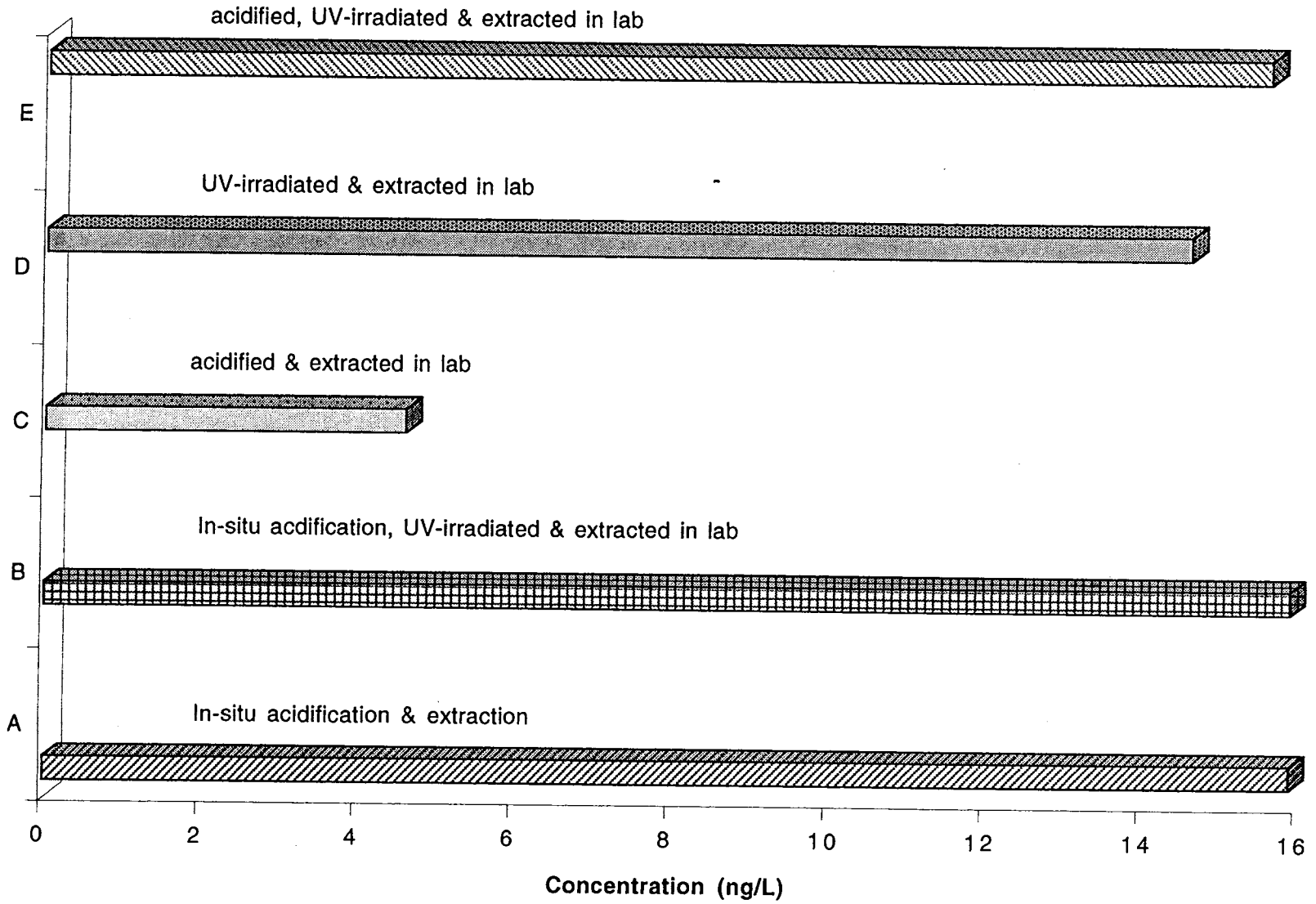
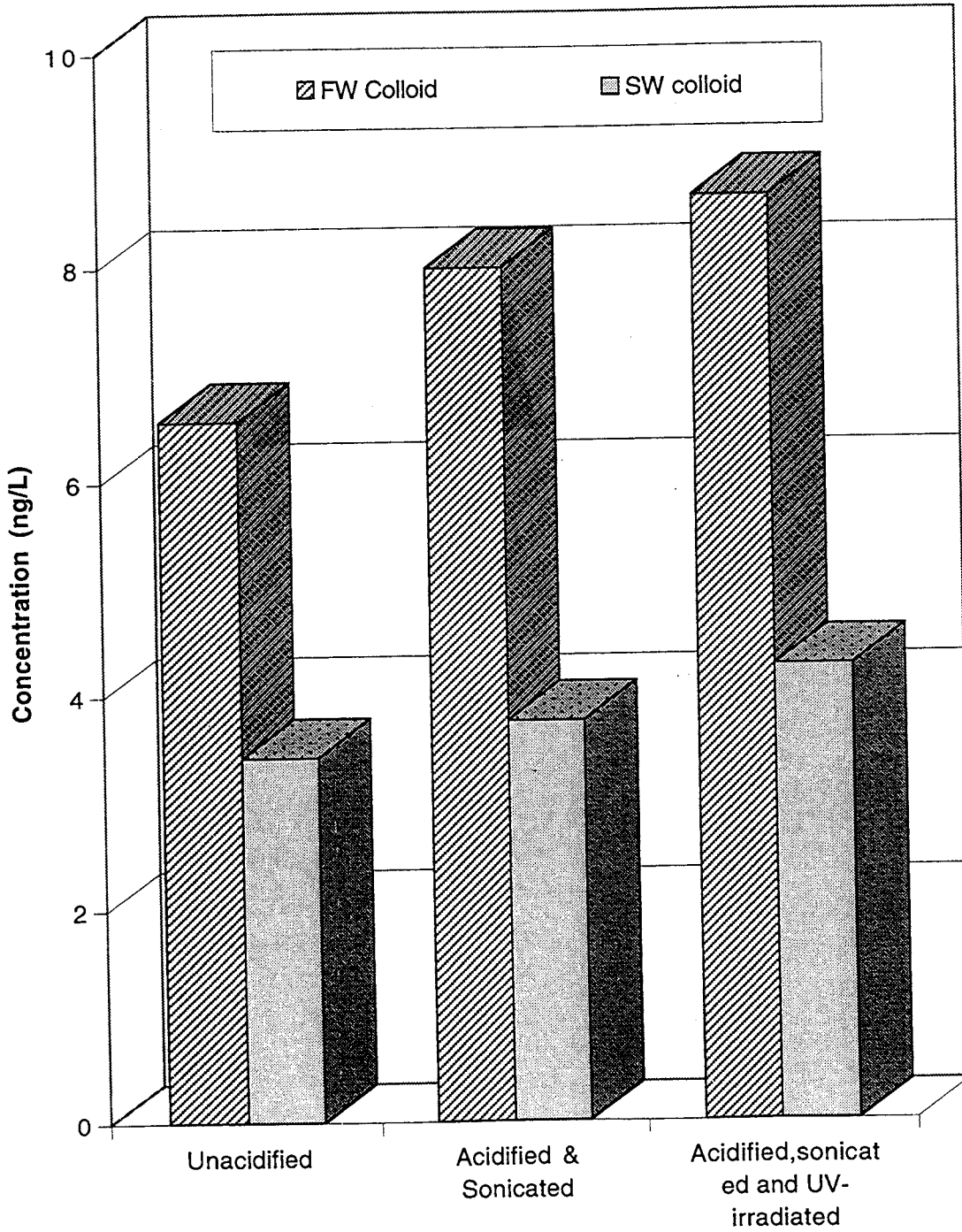


Figure 3



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## Sensitivity of the Spiny Dogfish (*Squalus acanthias*) to Waterborne Silver

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### Introduction

Although only limited information concerning silver toxicity in the marine environment is available, silver toxicity is probably a lot lower for marine vertebrates compared to freshwater vertebrates, with 96h LC50 values ranging from 330 to 2700 µg/l for teleost fish. However, virtually nothing is known about the effects of silver on marine elasmobranchs. Therefore, a study was conducted to evaluate the effects of waterborne silver exposure on the survival and physiology of the spiny dogfish, *Squalus acanthias*, at 3 different silver concentrations.

### Materials and Methods

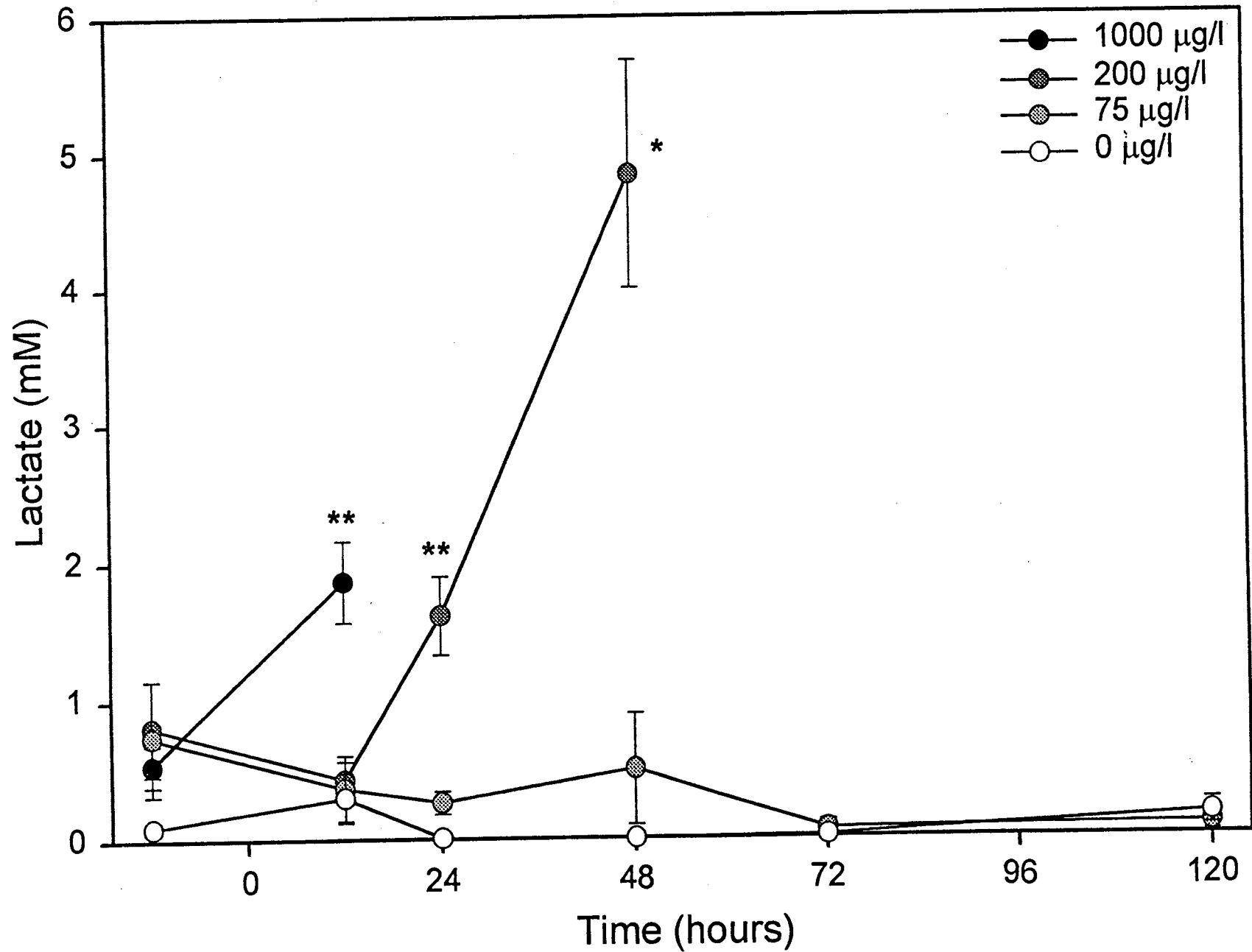
Dogfish of 1 to 3 kg were provided with a cannula in the caudal artery, and allowed to recover from surgery overnight. They were kept in wooden fish boxes for the entire duration of the exposure. Blood samples were taken 12 hours before exposure (control) and after 12, 24, 48, 72 and 120 hours of exposure. Silver exposure to 1000, 200, 75 and 0 µg of silver was static and boxes were flushed with new silver containing sea water every 12 hours. Water samples for determination of silver, ammonia, urea and excreted base content were taken before and after each 12-hour period of the exposure. At the end of each exposure, fish were quickly killed and tissues were taken for determination of silver content, Na<sup>+</sup>/K<sup>+</sup> ATPase activity and morphology.

### Results and Discussion

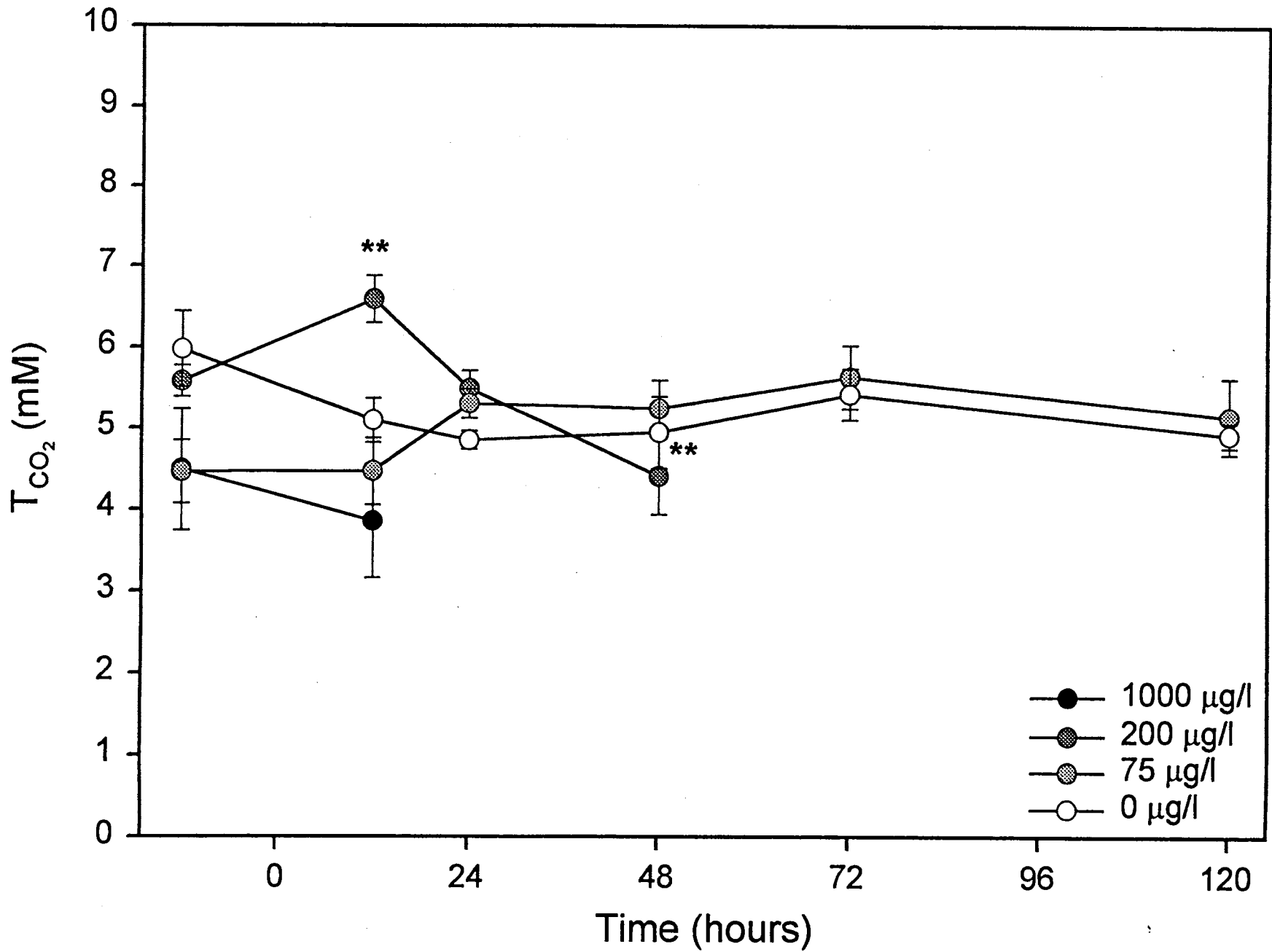
At a silver concentration of 1000 µg/l, all dogfish died within the first 24 hours of exposure. Causes of death appeared to be respiratory as well as osmoregulatory, with arterial blood PO<sub>2</sub> dropping below 20 Torr and plasma chloride levels increasing by almost 40 percent. A blood acidosis occurred, which was at least partially metabolic since lactate levels increased. Urea excretion increased dramatically, and plasma urea concentrations dropped from +340 mM to ±225 mM, which may have challenged the dogfish with an even more demanding osmoregulatory problem as the increased Cl<sup>-</sup> concentrations. At 200 µg/l, all fish died between 24 and 72 hours of silver exposure, and basically the same physiological events occurred but with a small time delay. At 75 µg/l, physiological effects of silver toxicity were much less severe, although even at this concentration mortality occurred. Instead of a blood acidosis, an alkalosis occurred, probably due to hyperventilation indicated by increased blood PO<sub>2</sub> levels after 48 hours of exposure. Plasma Cl<sup>-</sup> concentrations were increased after 72 hours of exposure with a further upward trend at 120 hours, whereas plasma urea concentrations remained stable.

Additional analysis of acid-base regulation, and of gill tissue Na<sup>+</sup>/K<sup>+</sup> ATPase activity and morphology should give further information about the mechanisms of silver toxicity in the spiny dogfish, but these preliminary results clearly indicate that elasmobranchs are more sensitive to silver intoxication than marine teleosts.

# PLASMA LACTATE

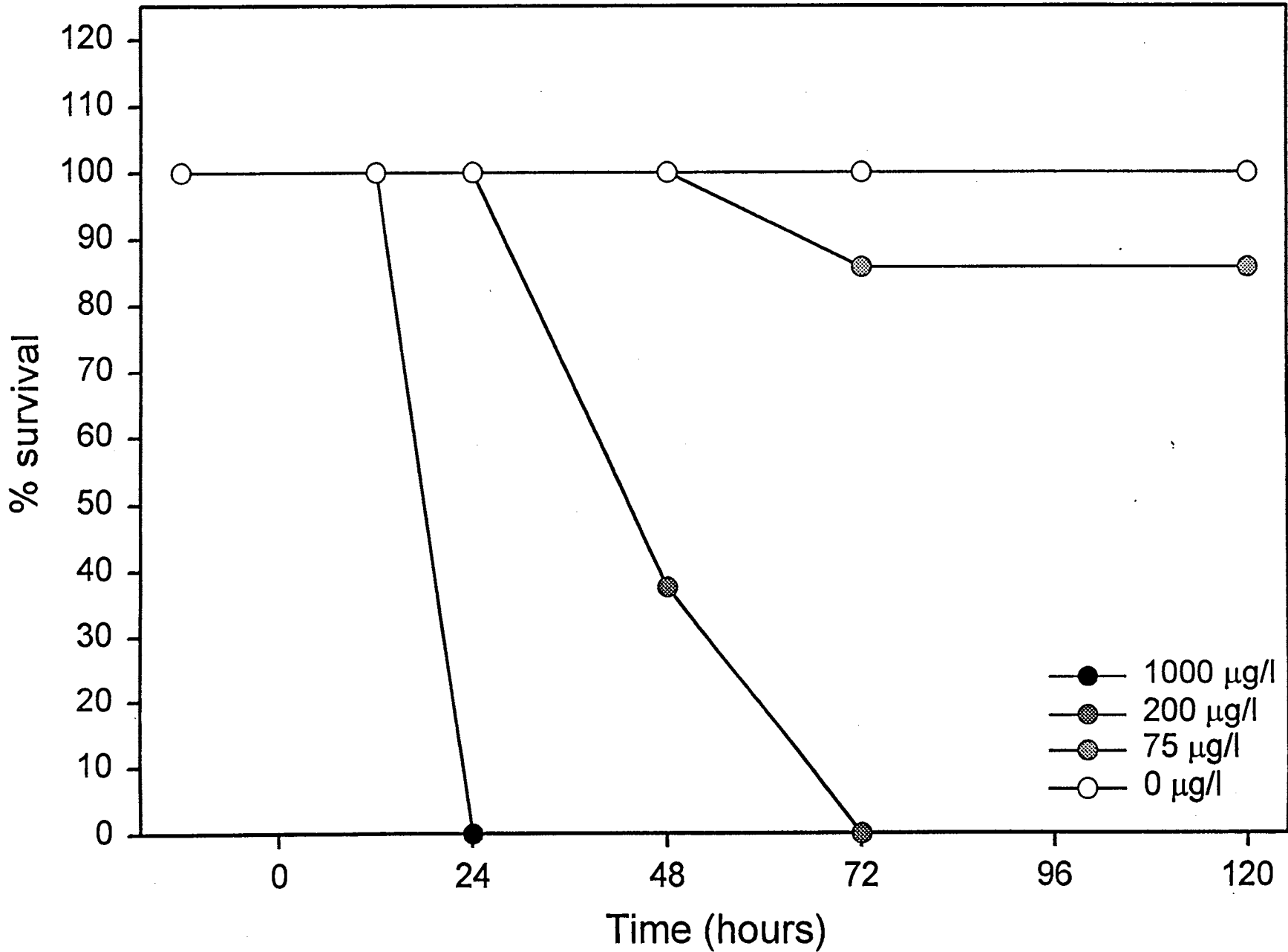


# ARTERIAL TOTAL CO<sub>2</sub>

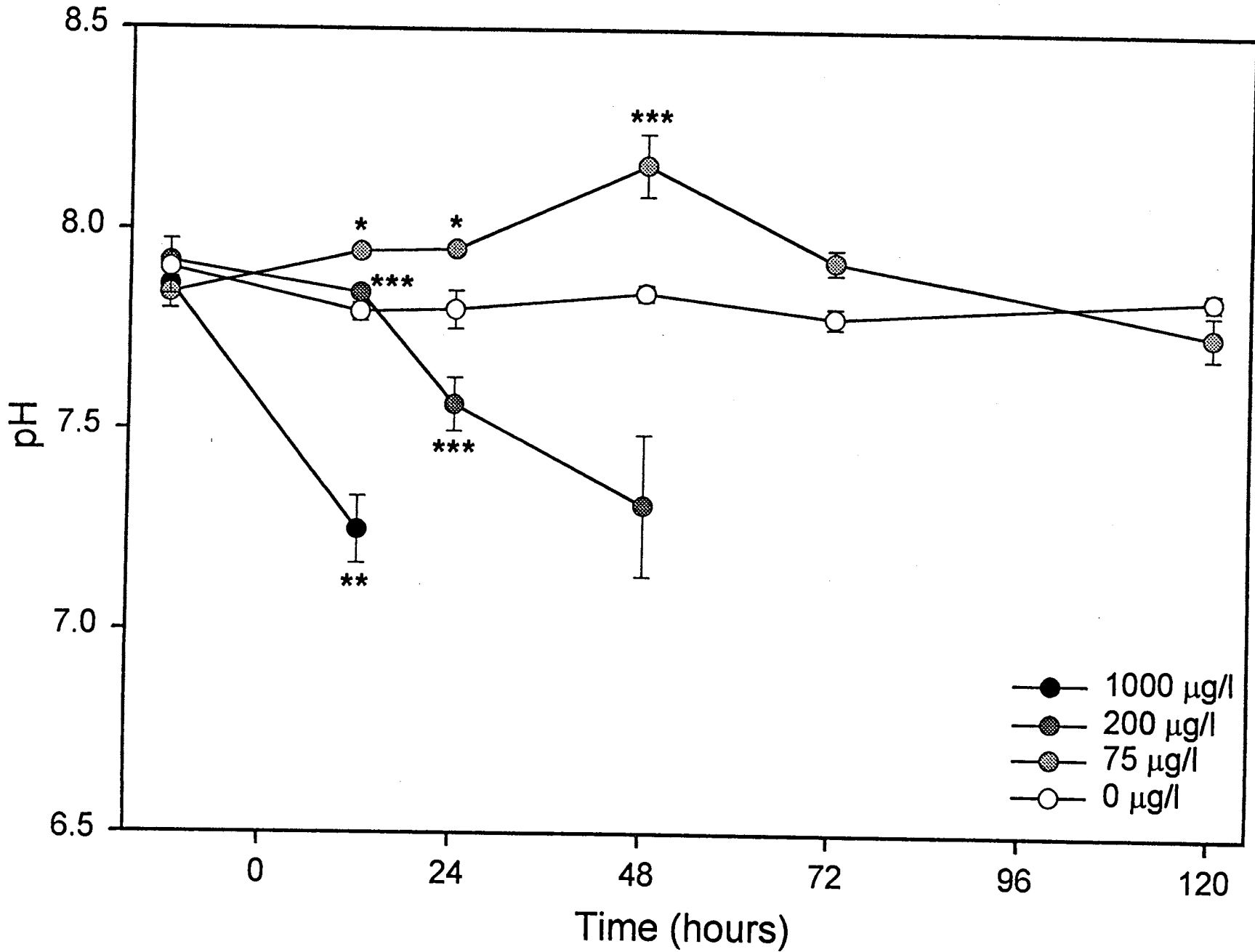




# SURVIVAL RATE

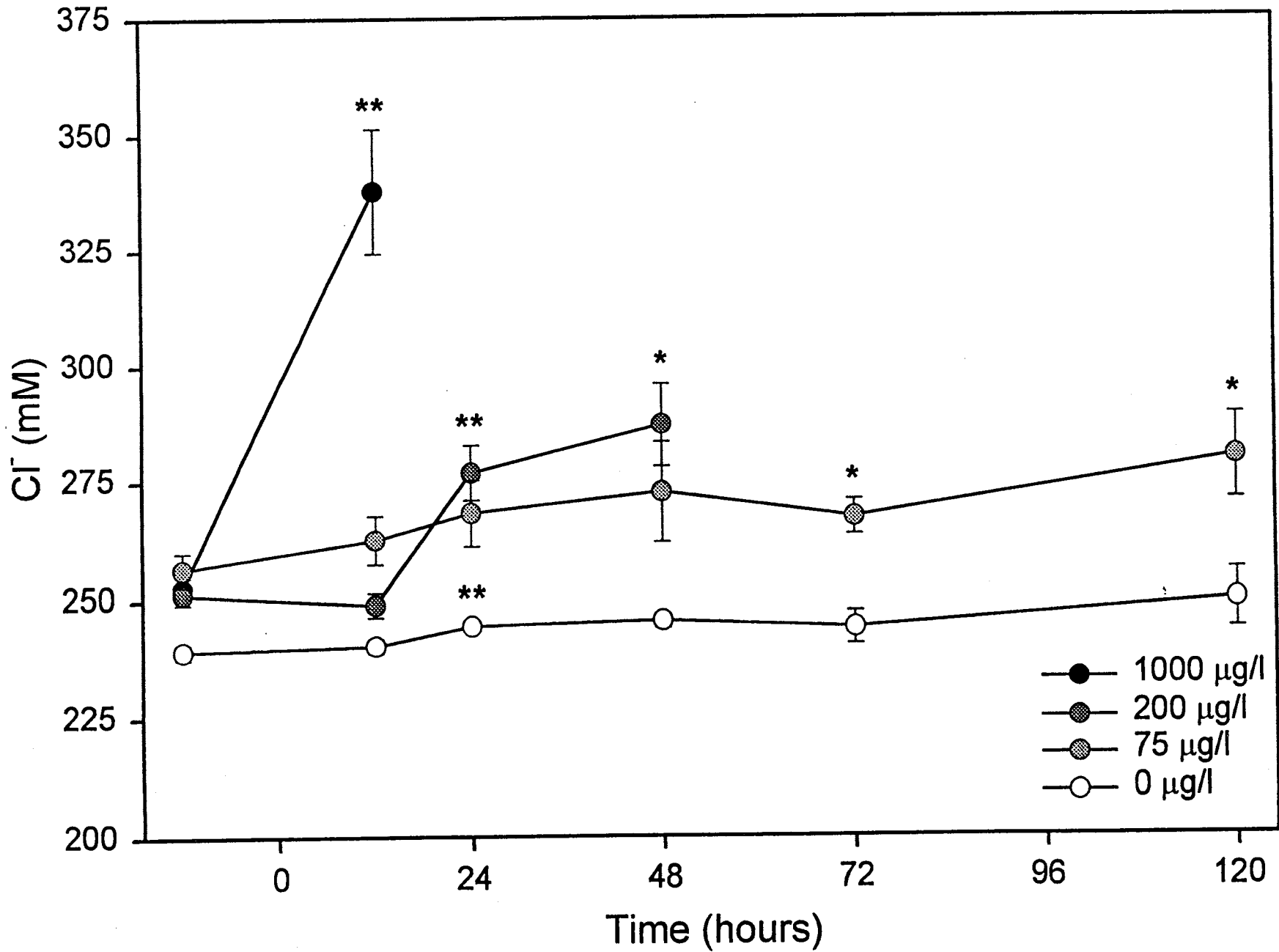


# ARTERIAL pH

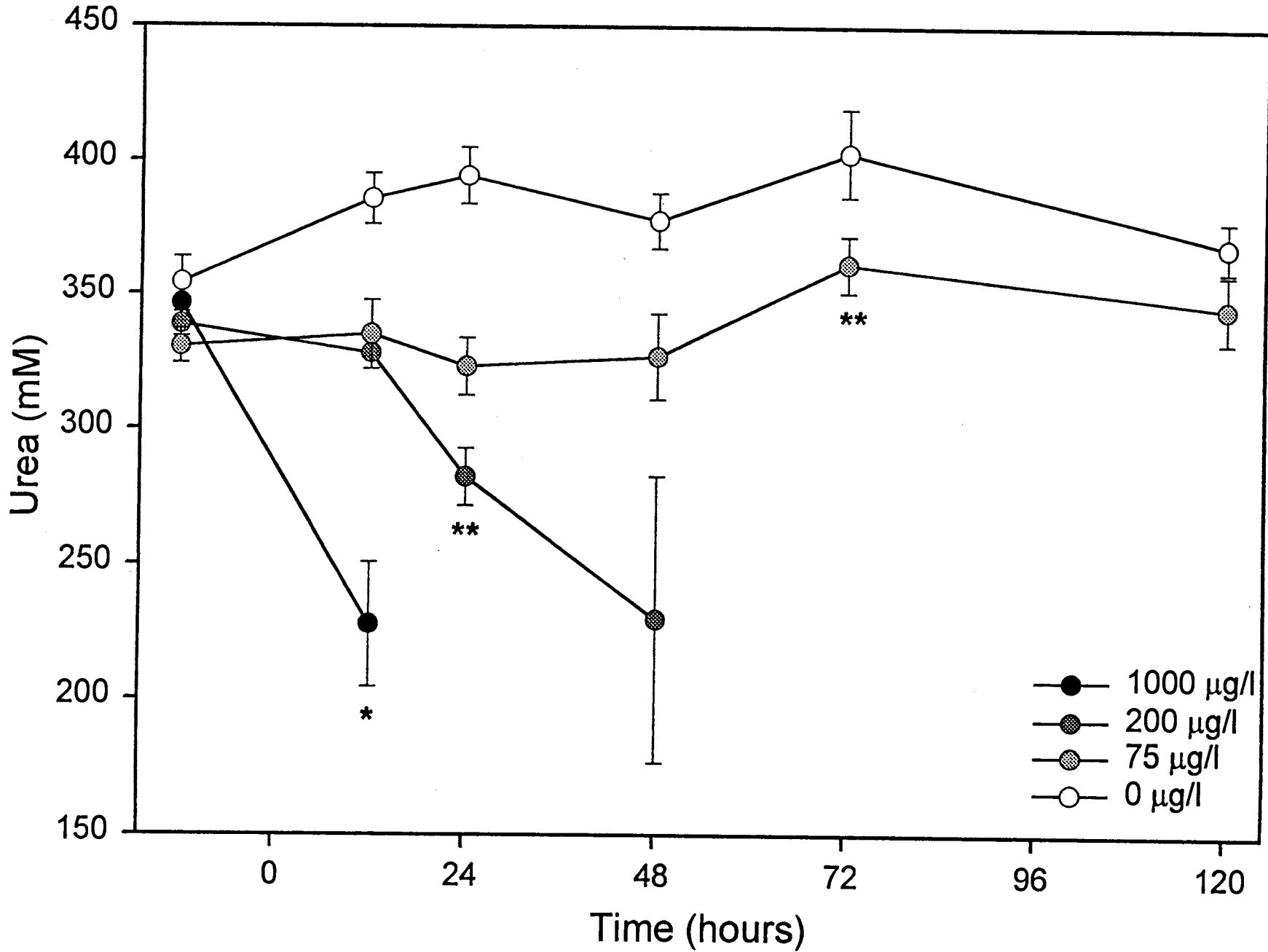


-425-

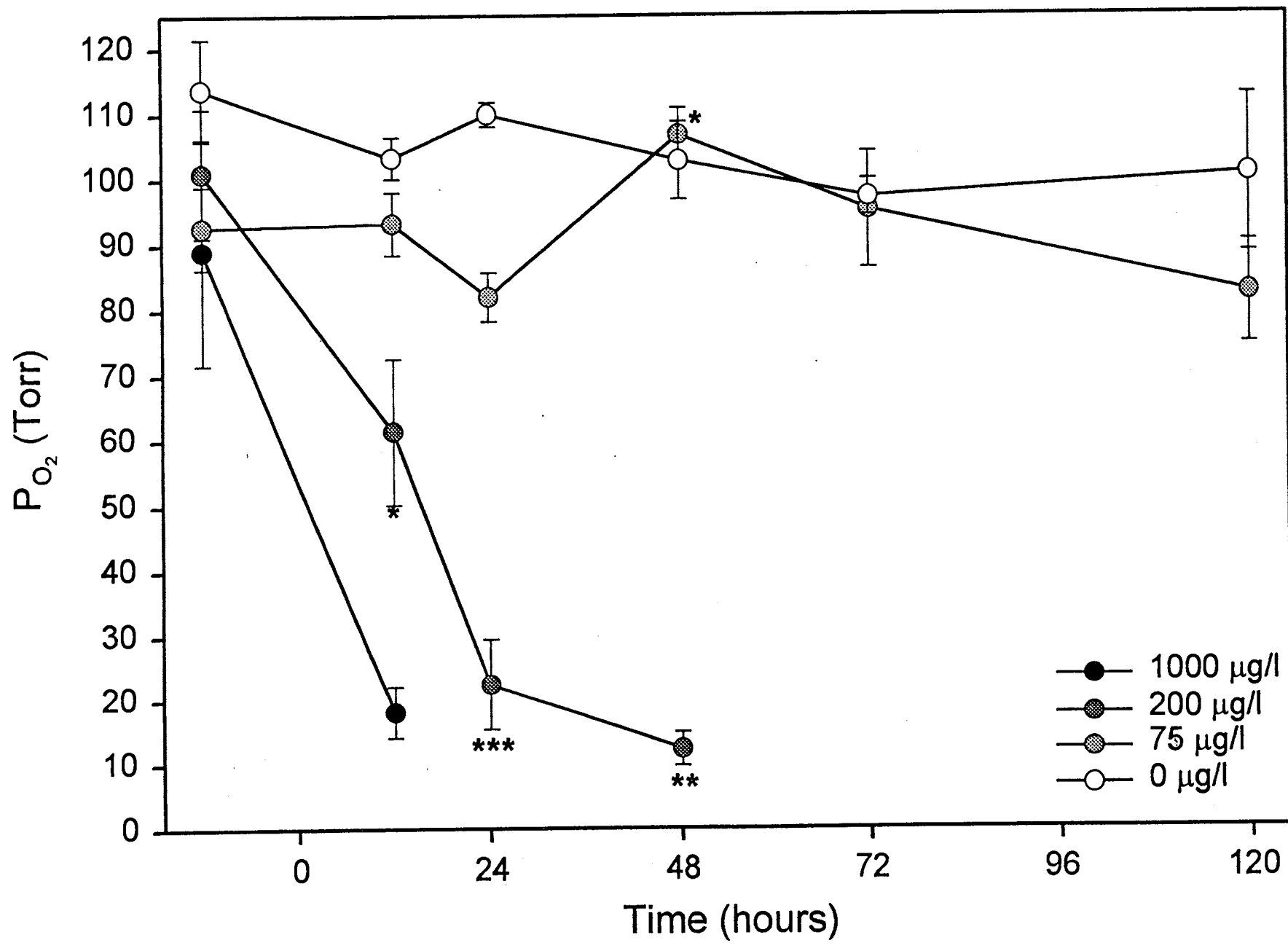
# PLASMA Cl<sup>-</sup>



# PLASMA UREA



# ARTERIAL P<sub>O</sub><sub>2</sub>



# Silver Species in the Photographic Environment

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## Introduction

One of the major uses of silver is in photography, where it is usually employed as a halide salt in a gelatin matrix. Silver halide has certain chemical and physical properties that make it an ideal image capture medium. While these properties may be found in other chemical systems, silver halide combines them all in a single, unique material. These properties include sensitivity to light on an atomic scale to form a latent image, an inherent ability to amplify the image so formed, and the ability to undergo controlled reactions with developing agents to produce a visually discernible image with selected attributes.

Silver sensitivity of silver halide can be modified to suit a specific purpose by changing or combining the halide ion type(s), crystal size, shape and distribution, and by the addition of other materials that alter the crystal structure or surface to make it more sensitive to light overall or to different regions of the visible spectrum. By careful control of these parameters, silver halides can be made that are suitable for many varied applications.

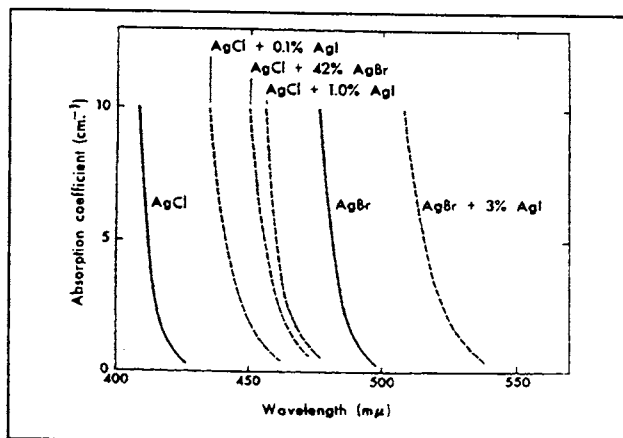
Once the silver halide has been exposed, the image is amplified by processing it. This treatment renders the image visible to the eye. Modifications to this process and including dye forming compounds adjacent to the silver halide in the gelatin matrix allow colour images to be produced.

This extended abstract is only able to touch the surface of the subject. To get a better understanding of the photographic system other works may be consulted<sup>1,2,3</sup>.

## The Effect of Halide Type

The halide type influences the light absorption and therefore the intrinsic sensitivity of the silver halide. Mixed halides have unexpected absorptions that do not fall in intermediate positions of the constituent silver halides. Silver iodobromide absorbs the most light and at longer wavelengths, as can be seen from Figure 1<sup>4</sup>. To some extent this intrinsic activity is modified by dye sensitisation but the underlying absorption is always retained.

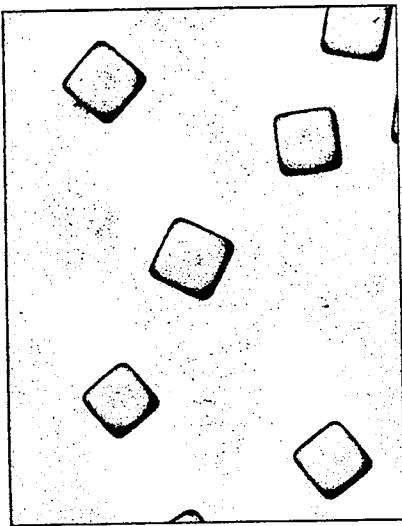
Figure 1 The Light Absorptions of Different Silver Halides



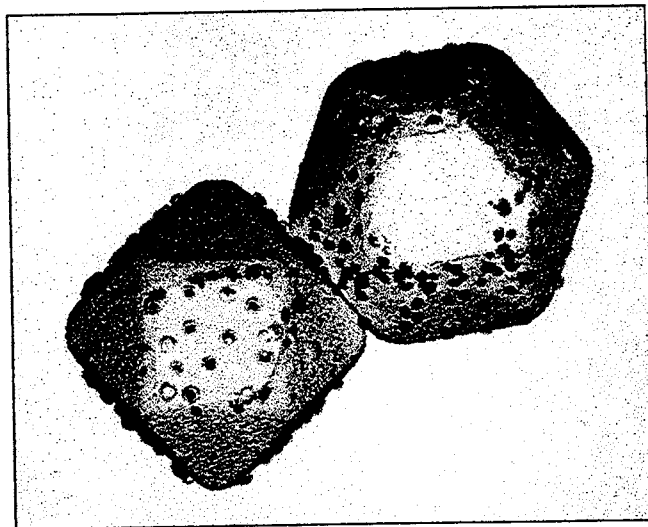
## Silver Halide Crystal Habit

The sensitivity of the silver halide is influenced by the size and shape of the microcrystals or grains. In general the larger the grain the more sensitive the emulsion will be, as each grain will have a better chance of receiving enough quanta of light to render the grain developable. The shape determines the number of low energy sites in the silver halide grain and also influences the amount of sensitising dye that may be adsorbed to the grain surface, which in turn influences the photographic sensitivity. The shape is determined by the way in which the silver halide is precipitated in a gelatin solution. Examples of two grain structures are shown in Figures 2 and 3.

**Figure 2** Silver Chloride  
Cubic Emulsion,  
Magnification ~10,000x



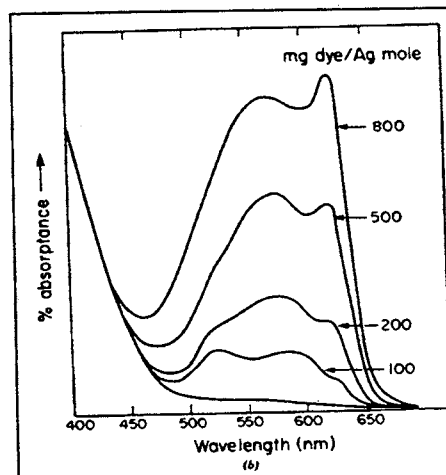
**Figure 3** Silver Iodobromide Cubic Octahedra  
from a Slow Speed Colour Emulsion,  
Magnification ~ 20,000x



## Making the Silver Halide Colour Sensitive

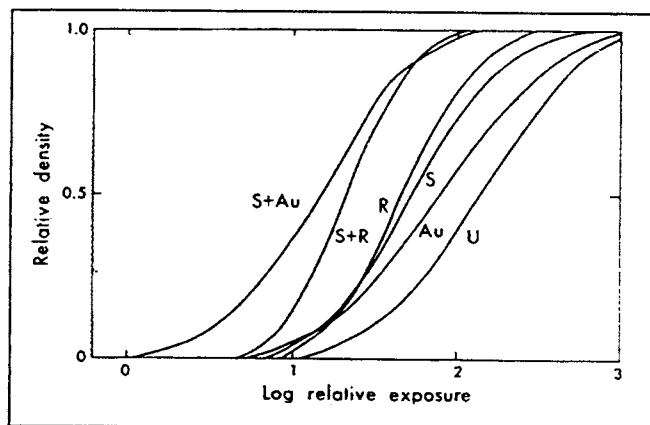
The silver halide is rendered sensitive to a particular wavelength of light by adsorbing a coloured dye onto the surface. The effect on the silver halide light adsorption of adsorbing a magenta dye, is shown in Figure 4<sup>5</sup>. The dye absorbs light and become excited. An electron is then pushed from the dye into the silver halide conduction band where it finds its way to a low energy site and forms a silver atom. A number of such atoms are formed at this site to produce a developable latent image.

**Figure 4** Effect of Adsorbing a  
Dye to Silver Halide



## Improving the Light Sensitivity by Chemical Sensitisation

**Figure 5** Different Types of Chemical Sensitisation of a Silver Iodobromide Emulsion



The effect of some different types of chemicals on sensitivity are shown in Figure 5<sup>6</sup>. The sensitivity of the silver halide (U) can be improved by treating the silver halide with small amounts of sulphur (S) and/or gold (Au) compounds to form sites on the crystal surface where the latent image can grow. Sensitivity can also be improved by treating the grains with a small amount of a reducing agent (R) to form small clusters of silver that are smaller than the latent image but act as growth centres for the latent image.

## Amplifying the Latent Image (Processing)

Once the silver halide has been exposed, the image is revealed by processing. Treatment with a mild reducing agent "develops" those halide crystals that have been exposed to light into metallic silver grains, leaving the unwanted crystals intact and producing an amplification of the original captured image. In colour processing the oxidised developing agent produced at developing grains reacts with colour forming compounds known as couplers, to form a dye of the appropriate colour. In colour materials the developed silver halide is converted back to silver bromide, in a process known as bleaching, by oxidising the silver metal formed in the presence of a soluble bromide. Undeveloped silver halide crystals and silver halide that has been formed in the bleach are removed by dissolving them out in a thiosulphate solution, creating a soluble silver thiosulphate species from which the silver may be recovered for reuse.

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- <sup>3</sup> T.Tani, *Photographic Sensitization*
- <sup>4</sup> T.H.H.James, C.E.K.Mees, *The Theory of the Photographic Process*, 3<sup>rd</sup> edition, 23
- <sup>5</sup> W.Simson, *Photogr.Sci.Eng.*,**18**,302 (1974)
- <sup>6</sup> H.E.Spencer, *Photog.Sci.Eng.*,**15**, 468 (1971)



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## An Overview of Silver in India

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Silver is a member of Group 1B of the Periodic Table. Its use by human civilization has known for more than 6,000 years. Recently, India has ranked as the third largest country in the world for the use of silver ( $3,030 \text{ t yr}^{-1}$  i.e. 97.49 M Toz). There are no silver mines in India, but silver is generally recovered as a by-product from the nonferrous metallurgical industry as well as from photographic effluent, electronic scrap, and scrapped jewelry and ornaments. In 1995, mine production contributed only 42.3 metric tonnes (1.36 M Toz) of silver in India. As domestic production of silver falls short of market demand, bulk quantities are imported from Europe and the Gulf States (The Silver Institute 1996) (Fig. 1).

Due to its versatility, silver is used in numerous applications including: electrical, electronic, brazing and soldering alloys, catalysis, photographic, water purification, jewelry and silverware and many others. Sixty percent of the silver in India is used in jewelry and silverware, whereas only 0.62 % is used in the photographic industry (Table 1). It is a tradition in India for families to acquire silver and gold ornaments, irrespective of their class.

The increased use of silver in the photographic sector in developed countries raises questions regarding its fate and form when discharged to fresh and sea waters. Silver from this source, is present in the form of silver thiosulfate complex  $\text{Ag}(\text{S}_2\text{O}_3)_n$ . This compound is highly stable (log K values 8.8, 13.7 and 14.2 for the mono-, di-, and tri-thiosulfate complexes, respectively) within the photographic solution, but quickly breaks down to silver sulphide ( $\text{Ag}_2\text{S}$ ) within secondary treatment plants and natural waters, due to oxidation and bacterial action (Morel and Hering 1995, Shafer *et al.*, 1993, Bober *et al.* 1992, Dagon 1991).

Silver's toxicity depends on its chemical form, concentration, and the salinity, redox potential and pH of the receiving water. There is no doubt that it is a contaminant discharged from industrial sources. A very recent study indicates that  $\text{Cl}^-$  in water reduced acute toxicity of silver to fish in freshwater and brackish water systems by forming  $\text{AgCl}$  complexes (Hogstrand *et al.* 1996, Galvez and Wood 1994).

There is no quantitative information available, for facilities in India, on silver losses and its effects on the terrestrial and aquatic environment.

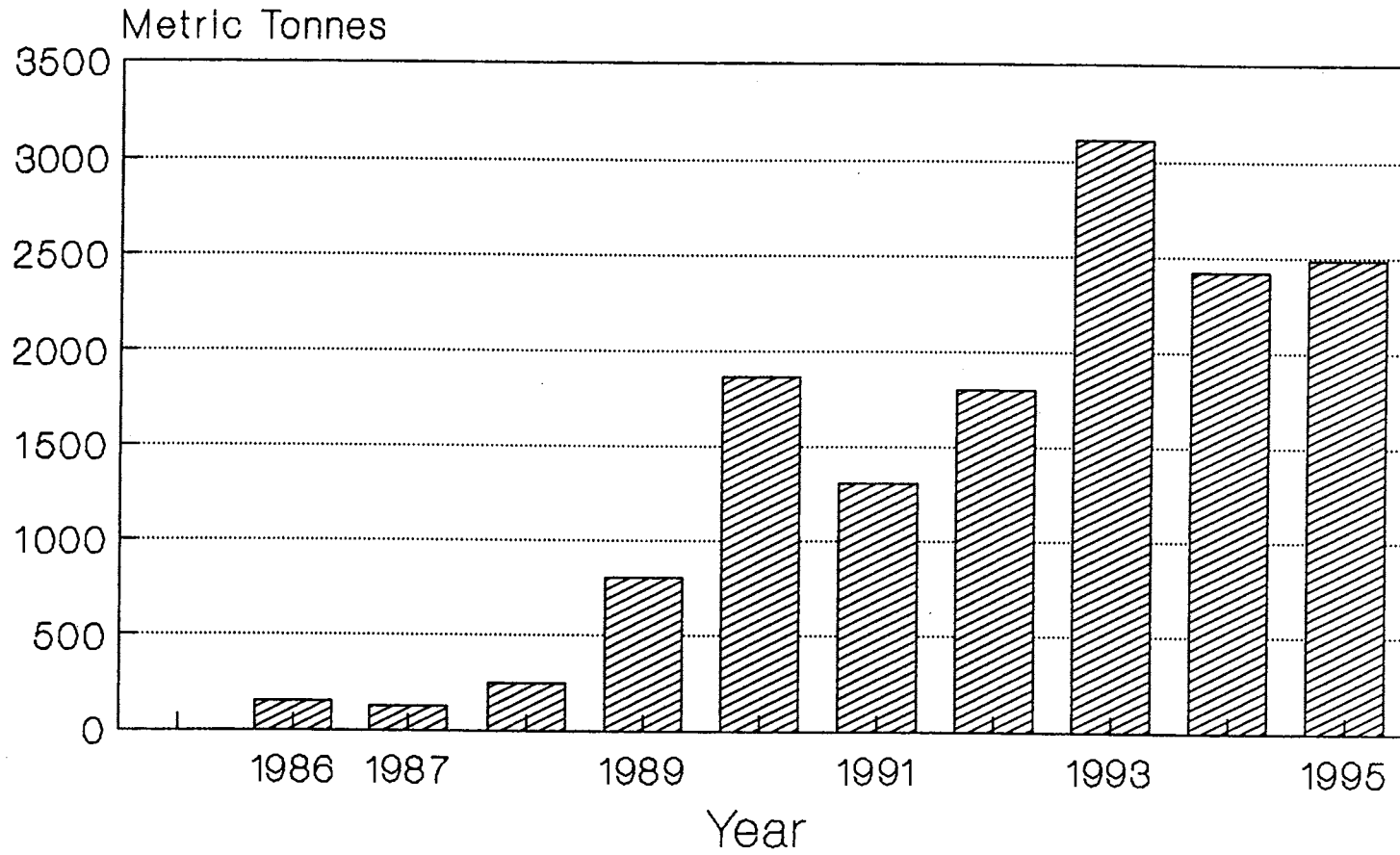
The toxicity of ionic silver and its compounds has been studied on the laboratory scale in the developed countries, but still the study of silver behaviour on natural systems needs to be carried out in greater depth.

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## References

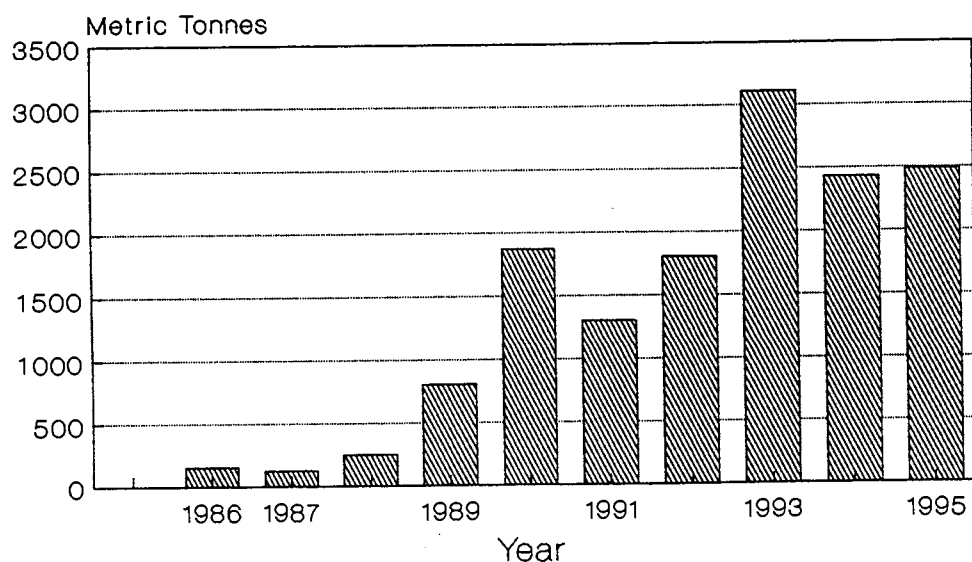
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- To be published as: Mukherjee, A. B. and A. P. Mukherjee 1997. An Overview of Silver in India. International Journal of Surface Mining, Reclamation and Environment (in press).**



**Figure 1. Import of Silver to India, 1986-1995 (The Silver Institute 1996).**

Table 1. Global verses Indian use pattern of silver, 1995.

Use	World (tons)	%	India	%
Industrial & decorative uses	9058	32.12	1062	33.40
Jewelry & Silver-ware	7097	25.17	1950	61.24
Photography	6908	24.50	20	0.62
Electronics	3208	11.38	92	2.90
Brazing alloys & Solders	1210	4.29	60	1.88
Coins & Medals	711	2.52	n.a.	n.a
<b>Total</b>	<b>28,192</b>	<b>99.98</b>	<b>3,184</b>	<b>100</b>



# Participants

*Transport, Fate and Effects of Silver in the Environment*

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