the 4th International Conference Proceedings

Transport, Fate and Effects of Silver in the Environment

Madison, Wisconsin
August 25-28, 1996

Editors
Anders W. Andren
University of Wisconsin Sea Grant Institute

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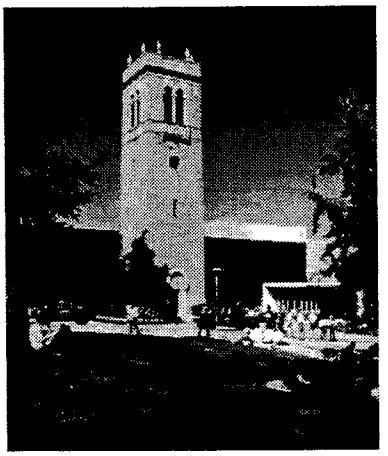
Editors' Note: The extended abstracts included in this document have been printed as submitted by their respective authors and have not been subjected to peer review. The material presented here reflects solely the findings, opinions and conclusions of the individual authors. Questions and answers on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



It is a pleasure to welcome Argentum IV participants to the University of Wisconsin-Madison again, the site of our original two conferences in this series. The UW-Madison enjoys a world-class reputation and has been a pioneer in many academic fields for nearly a century and a half. Those among us who are associated with it take particular pride in our accomplishments in the areas of environmental and multidisciplinary research. Although the majority of environmentally related research is carried out in traditional departments, many such efforts on campus are supported and focused in the Center for Limnology, Water Chemistry Program, Environmental Toxicology Program, the Sea Grant Institute and the Institute for Environmental Studies. These programs are all striving to put the "Wisconsin Idea" into action. That is the idea that the university serves as the research arm of the state, and as such the faculty and staff seek to quickly transfer new information and technologies to a number of user groups. These include federal, state and local governments, industry, advocacy groups and the public.

In the five 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 date 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 fourth conference.

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 University and the many amenities of the Madison area.

Extended Abstracts

Transport, Fate and Effects of Silver in the Environment

Madison, Wisconsin

Session 1

Metal Speciation and Analytical Chemistry of Silver

T. W. Bober
Session Chair

How Does the Speciation of Dissolved Trace Metals Affect Their Bioavailability? A Critical Evaluation of the Free-Ion Model

Peter G.C. Campbell
University of Quebec
Ste.-Foy, Quebec, Canada

When considering the interactions of trace metals with aquatic biota, one can identify three levels of concern: (1) metal speciation in the external environment; (2) metal interactions with the biological membrane separating the organism from its environment; (3) metal partitioning within the organism, and the attendant biological effects. The emphasis in this presentation will be on levels (1) and (2), i.e., links that exist between the speciation of metals in the external environment and their uptake by aquatic organisms. The following points will be addressed:

- Laboratory evidence supporting the idea that the biological response elicited by a dissolved metal is usually a function of the free-metal ion concentration (M^{z+})[1].
- Documented exceptions to the Free-Ion Model (e.g., passive diffusion of neutral lipophilic species; accidental transport of metals with low-molecular-weight metabolites).
- Tests of the Free-Ion Model in the presence of natural organic matter (fulvic/humic acids).

Particular research challenges will be identified, (e.g., determinations of free-metal ion concentrations in systems containing natural dissolved organic matter; identification of lipophilic ML_n° species in natural waters; elucidation of metal speciation at biological interfaces) and the applicability of the Free-Ion Model to silver and its complexes will be discussed.

1. Campbell, P.G.C. 1995. Interactions between trace metals and organisms: critique of the free-ion activity model. In Tessier, A. and Turner, D.R. (Eds.), Metal Speciation and Bioavailability in Aquatic Systems. J. Wiley & Sons, New York, NY, pp. 45-102.

Questions & Answers: How Does the Speciation of Dissolved Trace Metals Affect Their Bioavailability? A Critical Evaluation of the Free-Ion Model.

Q. PETER SANTSCHI (Texas A&M): In your experiments on cadmium accumulation in fish gills, how did you know that the free cadmium concentration was the same in both exposures?

A. We actually measured the free cadmium ion concentration using an equilibrium ion exchange technique — more specifically, two techniques — either dialysis or an ion exchange technique. The cadmium-specific electrode is insufficiently sensitive to work at those levels.

Q. JIM KRAMER (McMaster University): I was thinking, with regard to your discussion on the dissolved organic matter (DOM), with the confusion in results, particularly with regard to the enhancement, one might argue that there are other confounding parameters that are not specified. For example, in the Giese experiments, he used different kinds of waters: different DOM's and other competing ligands, different metals, different pH's. I wonder if you could conclude that there is a clearer picture if you just consider the laboratory studies, where things are better controlled, and that you could argue that a lot of confusion stems from the fact that the field studies are not well-poised in terms of variance.

A. You've come to much the same conclusion I have, actually. The cases of enhanced toxicity, as you point out, are ones which have been done under the least well-controlled conditions. Among other things, you may have noticed that the examples that behaved properly in the presence of DOM, I think in all of them the media also contained a buffer of some sort — either TRIS or HEPES. I have a feeling either that the copper ions behave properly in the presence of the buffer, because you can have greater total concentrations, or that there is something weird going on — interactions of DOM in the presence or absence of buffer— at the biological surface. So I think that the experiments that seem to behave properly all were done in the presence of a buffer and DOM. You could argue that it's really not a natural water, you've got a very large (millimolar) concentration of, say, TRIS or HEPES present. Therefore, I think the jury's still out. The important point to realize is that there are so few examples out there of good experiments done in the presence of DOM, and as Peter [Santschi] just pointed out, almost all of them are dealing with copper, and that's because the only ion-selective electrode that will get down to environmentally realistic levels is the copper electrode. So the other metals are excluded, and it's not by chance that all the examples that I gave you, with the exception of one, deal with copper. The only other example, also from our lab, deals with aluminum.

Q. DALAND JUBERG (Eastman Kodak): Dr. Campbell, you presented some nice examples of responses with individual metals; can you comment, conjecture or surmise what the case might be in terms of mixtures? I don't want to throw mixtures at you, but would you expect the responses to be the same, either enhanced, diminished, or no effect in terms of response to several different metals concurrently?

A. The nice thing about the free ion model or paradigm is that you can, in principle, take into account competition, or interactions at the same site. So if you're talking about interactions at the same site, there already are examples of competition with calcium and the hydrogen ion. In some of Morel's work he's shown interactions between iron and cadmium or manganese and copper, for example. So there are documented examples for binary pairs of metals. But this is dealing with interactions at the biological surface, not with what goes on once the metals are inside the cell. So it's only a partial answer to your question, but I think it's a useful approach to dealing with metal mixtures.

Silver in River and Estuarine Waters of Texas: Evidence for Complexation to Macromolecular Organic Matter *

L.S. Wen, P.H. Santschi, G.A. Gill, C.L. Paternostro and R.D. Lehman
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Concentration and phase speciation of Ag in selected Texas rivers and in the Trinity River estuary were measured in order to establish the major factors which control its fate in the aquatic environment from source to sink. Concentrations of Ag in the filter-passing fractions in Texas rivers ranged from <0.01 to 62 ng/L. In the Trinity River estuary (Galveston Bay), they ranged from 0.4 to 6.4 ng/L and showed a non-conservative estuarine mixing behavior. An example is shown in Figure 1. An internal source of both filter-passing (≤0.45μm) and colloidal (1kDa~0.45μm) Ag was observed in the upper Trinity Bay. Silver, associated with colloidal macromolecular organic matter, which was isolated using cross-flow ultrafiltration techniques, amounted to 15-70 % of the filtered (≤0.45μm) Ag concentration, decreasing with increasing salinity. Such a trend was similar to that of dissolved and colloidal organic carbon. Estuarine distributions of colloidal Ag were also broadly similar to those of suspended particulate matter. The ratio of colloidal Ag to filter-passing Ag was similar to the ratio of colloidal organic carbon to total dissolved organic carbon (Figure 2), suggesting not only that Ag is complexed by organic macromolecules, likely to sulfhydryl groups, but also that these functional groups were evenly distributed over the different molecular weight fractions. Particulate Ag was found associated mainly with a Fe-Mn oxyhydroxide/sulfide phase. The close relation between Ag and Fe in colloidal and particulate phases (Figures 3 and 4) suggests common surface complexes, probably again sulfhydryl groups.

*) To be published as:
Wen, L.S., P.H. Santschi, G. Gill and C. Paternostro. 1996. Colloidal and particulate silver in river and estuarine waters of Texas, Environ. Sci. and Technol., in press.

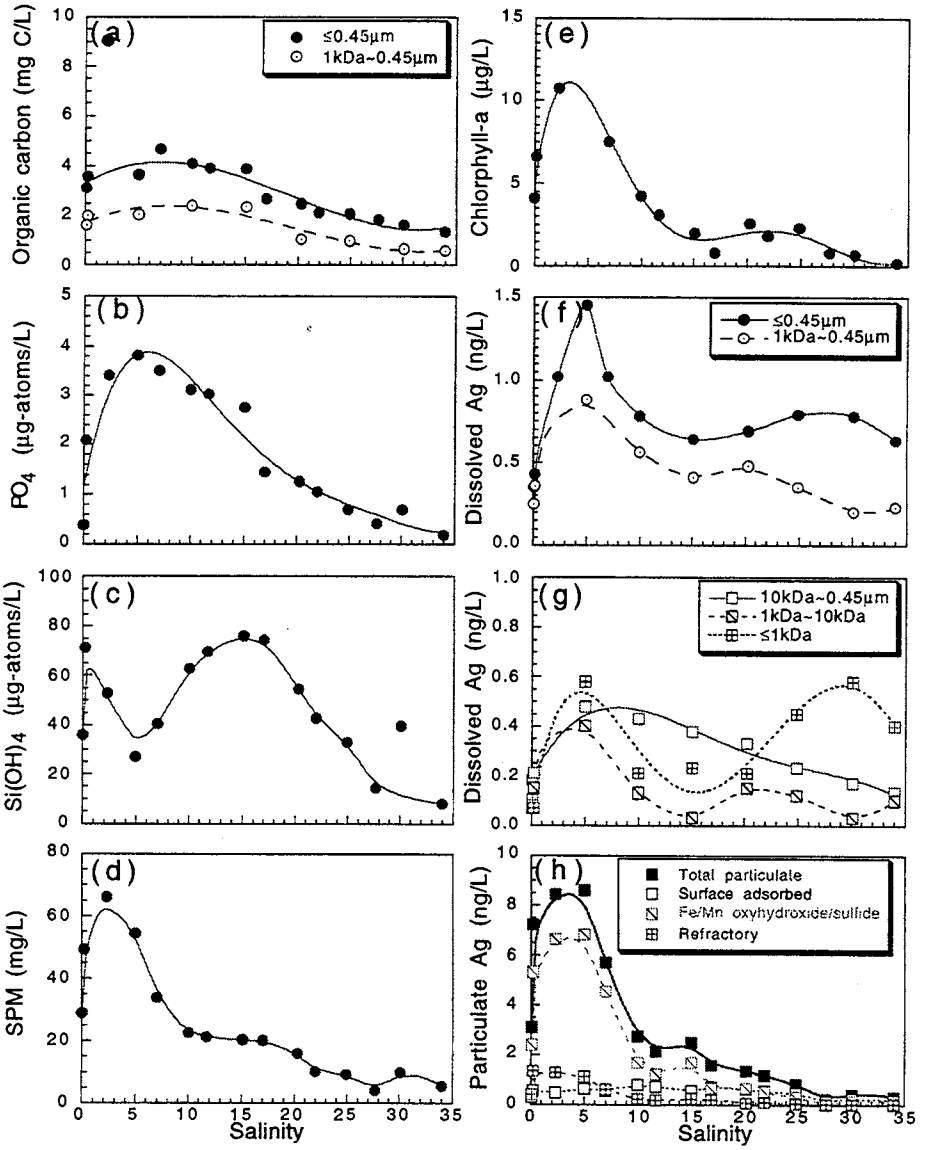

Figure 1. Estuarine distributions of a) dissolved (≤0.45µm) and colloidal (1kDa~0.45µm) organic carbon, b) dissolved (≤0.45µm) PO_4 and c) dissolved (≤0.45µm) $Si(OH)_4$ and, d) suspended particulate matter, SPM (≥0.45µm), e) Chlorophyll-a, f) filter-passing (≤0.45µm) Ag, colloidal (1kDa~0.45µm) Ag, g) low molecular weight colloidal (1~10kDa) Ag, high molecular weight colloidal (10kDa~0.45µm) Ag, truly dissolved (≤1kDa) Ag and h) particulate (≥0.45µm) Ag phases in Galveston Bay, July 1995.

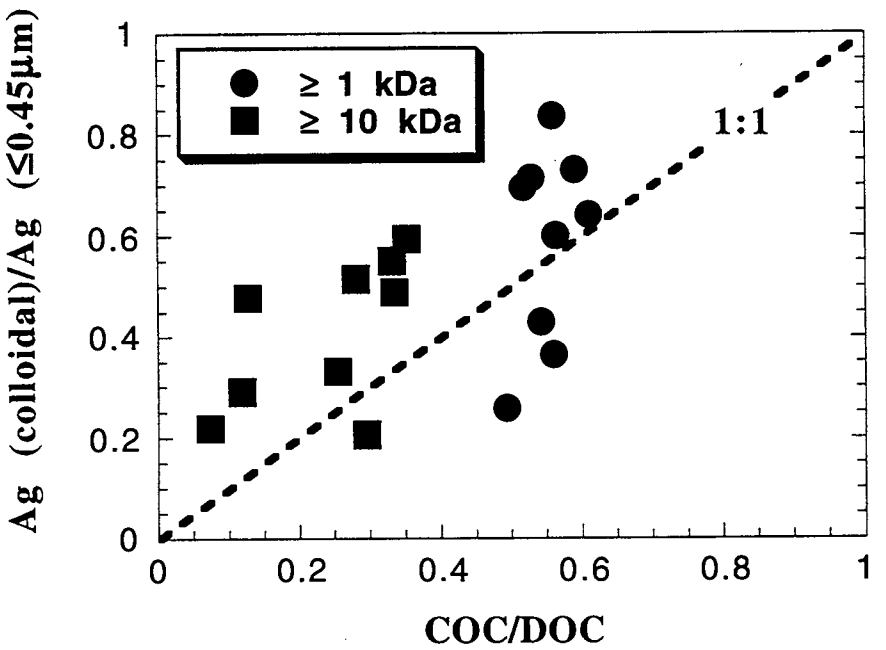

Figure 2. Comparison of the fraction of colloidal Ag, [Ag(c)]/Ag(≤0.45µm)], with the fraction of colloidal carbon, [COC]/[DOC], in the filter-passing fraction.

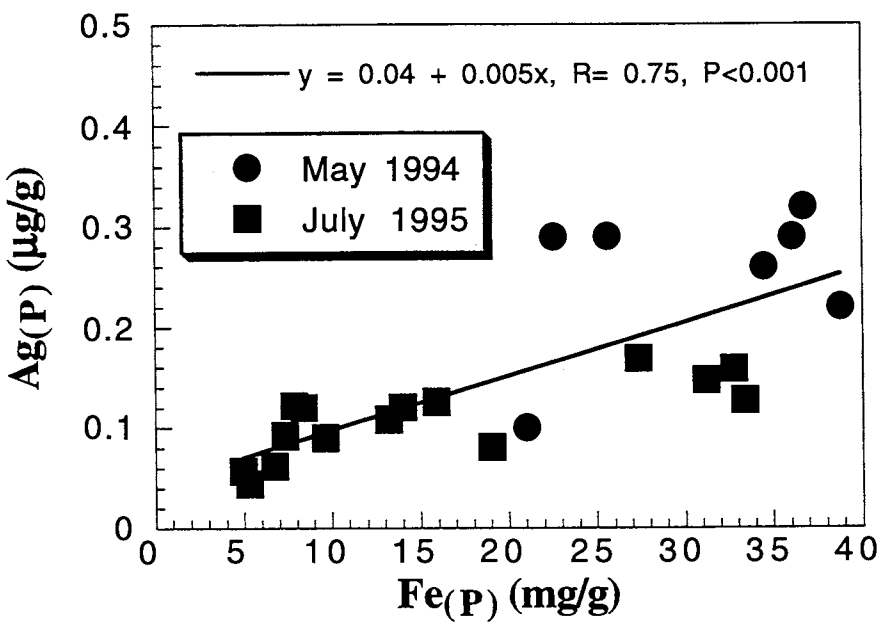

Fig. 3. Comparison between the concentrations of Ag and Fe (in µg/g) in suspended particulate matter from Galveston Bay.

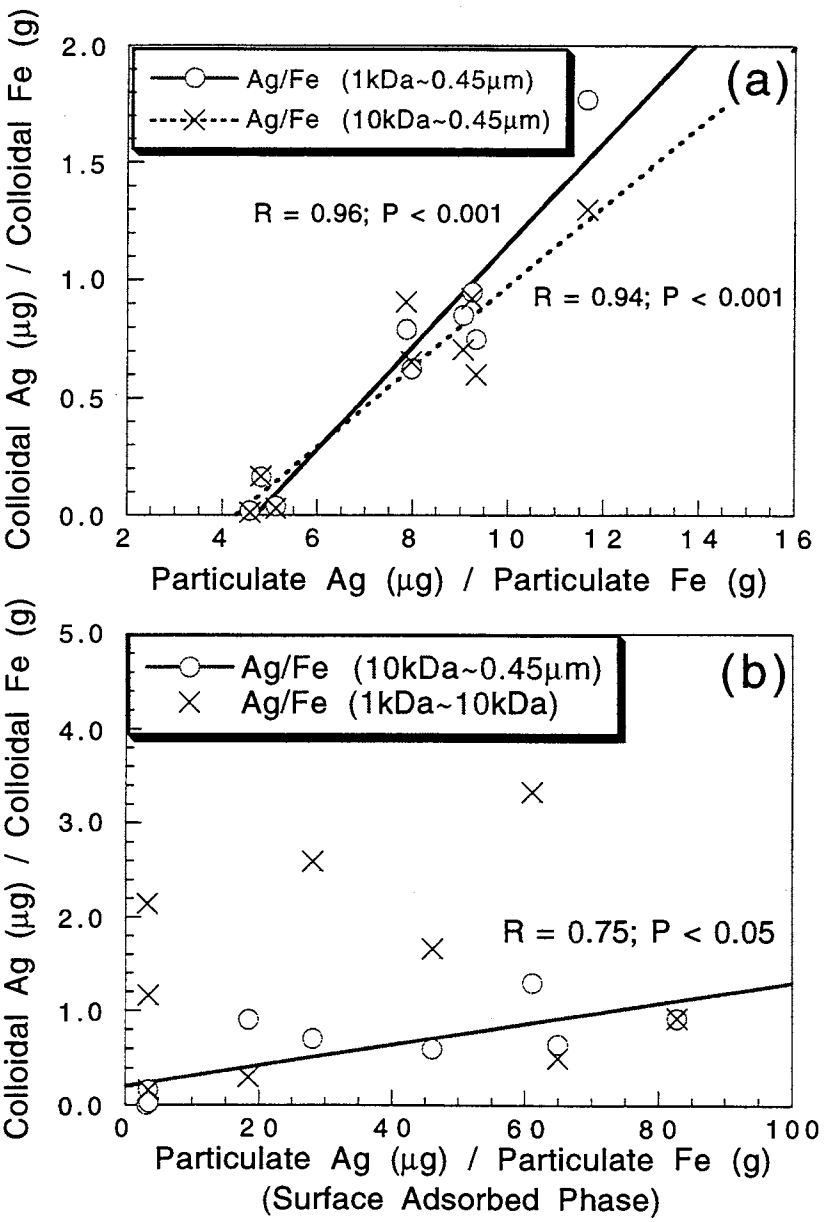

Figure 4. Correlations of the Ag/Fe ratio (in µg/g units) between a) the particulate phase and the colloidal phase (in µg/g units) b) particulate, surface adsorbed phase (1st leachate) and colloidal phase from the July 1995 samples of Galveston Bay.

Questions & Answers: Silver In River and Estuarine Waters of Texas: Evidence for Complexation to Macromolecular Organic Matter

Q. JIM KRAMER (McMaster University): Peter, do you see any indications, when you look at the analysis of the dissolved size fraction, that there's any metal uptake by the medium that would give you a false (lower) value?

A. I've shown you the mass balances and they add up, on the average, to 98 percent. So we are missing a few percent. For most of the metals, we didn't have problems when we cleaned the cartridges properly. We had low recoveries for iron and aluminum, for example, but you spend more time cleaning your cartridges than using them for samples. If you don't do that you get biofouling, you get all sorts of artifacts and the results are not usable. That's also the reason that our earlier results are not as good and we couldn't use them with the same level of confidence.

Q. NICHOLAS FISHER (SUNY-Stony Brook): Peter, have you considered the possibility of silver binding to viruses? They would fall into the colloidal size fraction, would have protein coats (and therefore lots of sulfur to which the silver might bind), and viral particle densities in coastal waters around the U.S. — I imagine certainly in Galveston Bay — would be extremely high. I think Kurt Settle has probably been measuring those in Texas estuaries. I think that might be a likely possibility for at least some of the silver binding to the colloidal fraction. Possibly bacteria as well; the 0.4-micron filter will not exclude all bacteria.

A. As a matter of fact, we got quite a significant difference between 0.4 and 0.1 micron filters in fresh waters. We didn't do the same kind of study in estuarine waters, but that might show up there also. I agree with you that we have to look for proteins and protein carriers which contain the sulfur groups, if you want to look for silver binding. I would love to be able to isolate the viruses separately and look at the binding; from what I know, people still apply some size-specific size fractionation that is not chemically or biologically specific, but one would have to further purify the fraction. That is certainly an avenue that one should pursue.

Preliminary Observations on the Distribution of Ag and other Trace Metals in the Colloidal Size Fractions of Streams and POTW Effluents

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The significance of colloidal bound trace metals to the total load of metals in surface waters and effluents is being studied through the use of trace metal clean micro-filtration and ultra-filtration techniques. The trace metals (V, Cr, Mn, Co, Ni, Cu, Zn, As, Mo, Ag, Cd, Tl, Pb, U) and indicators of major associative phases (DOC, Al, Fe, SPM), in particles (>1.0 μm) sub-micron particles (0.1/0.2 - 1 μm), large colloids (100K - 0.2 μm), small colloids (10K - 100K), and "dissolved" fractions (<10K) are isolated in the field and metals quantified by ICP-MS. The six background surface water systems under study encompass a range of watershed geochemistries - including those dominated by wetlands, glacio-lacustrine clays, and highly permeable sands.

Method Evaluation

Our recent studies have emphasized the analytical aspects of ultrafiltration methodology, exploring potential artifacts and problems in applying this technology to ultra-trace levels of Ag and other trace metals in natural waters. Demonstrating that credible and repeatable data could be obtained from rivers with very low to moderate ionic strength, and at DOC levels ranging from 2 to over 30 mg L^{-1} , was the primary goal of the method evaluation.

The specific areas which we addressed in our method evaluation studies were:

I. Blank contributions and development of cleaning protocol.
II. Selectivity and efficiency of the membrane.
III. Sorption of DOC and metals to the membrane.
IV. Ion rejection by the membrane.
V. Fouling / performance changes of the membrane.

Two spiral wound membrane materials were evaluated, polyethersulfone and regenerated cellulose, in both 10K and 100K nominal molecular weight cut-offs. The influence of ionic strength on membrane efficiency, DOC/metal sorption, and ion rejection was studied in both field and laboratory experimental designs. Experiments were set-up so that a mass balance approach (permeate time series, retentate, rinses [MQ, base, acid],

and feed) could be used to interpret the results. A Teflon diaphragm pump was used to produce cross flow rates of 1000 ml min^{-1}, and permeate production rates of 100-150 ml min^{-1} (100K) and 30-50 ml min^{-1} (10K). Concentration factors were in the range of 5 - 12.

■ BLANKS: Field ultrafiltration (UF) method blanks for Ag averaged 0.15 ng L^{-1} (±0.08), giving a method detection level of 0.26 ng L^{-1}, acceptable for most surface waters. UF method detection levels for other metals were generally less than 10 ng L^{-1}, the exceptions were Cr and Zn with method detection levels averaging 40-50 ng L^{-1}.

■ SELECTIVITY: Membranes were challenged with macromolecules of defined molecular weight under both low and high ionic strength conditions. Data from the 10K polysulfone membrane showed excellent performance with <3% of 1.4K cyanocobalamin retained by the membrane, and >92% of 18K β-lactoglobulin recovered. The 100K polysulfone membrane performed slightly poorer (29% of 67K bovine albumin retained, 85% of 150K alcohol dehydrogenase recovered). Time sequence measurements of molecular weight markers in the permeate indicated some sorption of marker to the membrane very early in the ultrafiltration process, however no sorption was detected beyond that point, and the mass represented by the early sorption was very small.

■ DOC SORPTION: Sorptive loss of natural DOC to the membranes was evaluated by first processing samples through a 10K membrane, and then challenging the 100K membranes with these permeates. Both polysulfone and cellulose membranes performed well, with less than 2% of DOC lost. A small loss of DOC to the membrane was seen very early in the UF process, as was observed in the molecular weight marker experiments. Sorption of DOC was also evaluated with un-altered natural samples. At low ionic strength sites, direct field comparisons of polysulfone and cellulose membranes showed that greater retention, and significant loss of DOC, occurred on the polysulfone membrane. With both membrane types, permeate DOC levels increased as UF processing proceeded, although the trend was less pronounced on cellulose membranes. At higher ionic-strength sites, mass balances indicated that both membrane types performed similarly with very minor DOC sorption.

■ METAL SORPTION: Metal sorption from natural samples onto UF membranes was studied using similar experimental designs as described for DOC sorption. Sorption was also evaluated in lab-experiments, in synthetic media, in the absence of DOC (see ION REJECTION) below. Loss of metal from 10K processed natural water onto 100K polysulfone membranes in both low and high ionic strength systems was generally small. Typically >85% of metal mass was found associated with the permeate fractions.

Exceptions included the oxyanions Mo, V, and U, and also Pb where significant retention by the polysulfone membrane was observed. Sorption of Ag to the membrane was minimal, except in experimental solutions with elevated chloride levels, where major losses of Ag to the membrane occurred. Metal mass balances in un-altered high DOC, low ionic strength waters on both 100K and 10K polysulfone membranes were generally acceptable. Typically 80-105% of metal mass (including Ag) in the feed sample was accounted for among the fractions collected, however, up to 30% of Cu and U appeared to be lost to the membrane. A significant colloidal metal association is observed in these test samples, therefore a mass balance based performance criterium is valid.

■ ION REJECTION: Ion rejection studies were conducted in high purity Ca/Na nitrate solutions at specific conductance levels of 35, 110, 250, 600, and 1200 μS. Tests were run under both neutral and acidic pH conditions. Rejection of Ag was <u>not</u> observed on either membrane at any of the conductance levels evaluated, although ~5% of Ag was <u>sorbed</u> to the polysulfone membrane. Metal ion rejection was significant for many of the divalent cations under low ionic strength conditions on the polysulfone membrane (Fig. 1a). However, little or no rejection was observed on the cellulose membrane (Fig. 1b). Significant sorption of the oxyanions (Mo, U, V, Cr) and Cu and Al to the polysulfone membrane, and to a lesser degree to the cellulose membrane, was observed. Increasing ionic strength reduced rejection for all metals, and also decreased sorption for selected metals.

Though all method evaluation studies have not yet been completed, it appears that ultrafiltration can be a useful tool for phase speciation for Ag and many other metals if strict protocols of trace metals clean techniques are coupled with appropriate cleaning and maintenance of the membranes. Researchers must verify that ion rejection and metal/DOC sorption are suppressed under the specific geochemical conditions of the system for each metal under study. In addition frequent performance checks such as routine mass balance measurements and blank evaluations must be carried out.

Preliminary Field Results

From our initial survey of background study sites we can conclude that:

1. Most Ag is associated with particulate and large colloid fractions - similar in distribution to Fe and Pb. (See Fig. 2 and Fig. 3).

2. Colloidal (0.2 μm - 10K) Ag represents from 10-40% of <u>total</u> Ag and typically ~40% of the <u>$\leq$1.0 μm</u> fraction. The percentage of colloidally bound Cu and Zn is significantly smaller. (See Fig. 2).

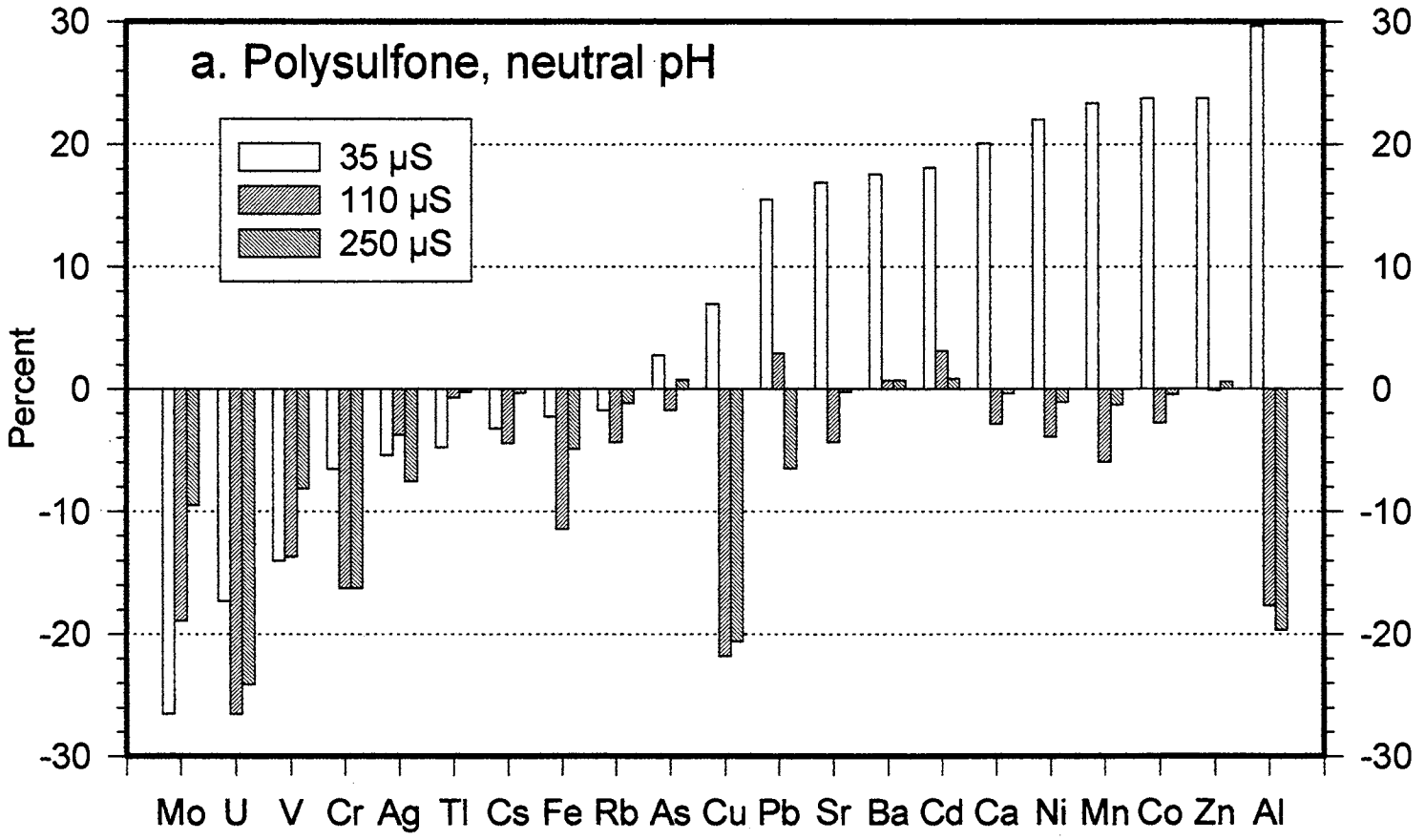
3. Colloidal metals (including Ag) were related to the colloidal organic carbon content of the river water, which in turn may be associated with colloidal iron. (See Fig. 4).

4. Consistent trends among colloidal metals were observed across the spectrum of study sites.

The majority of Ag, Pb, Fe, and many other metals in POTW effluents is associated with particles (>1 μm). Among the colloidal fractions, a large component (>80%) of the metal is bound to large colloids (0.1-1.0 μm). However, colloidal particles in the effluents do not appear to be enriched in Ag relative to particles >1 μm.

Partition coefficients based upon 0.4 or 1.0 μm separations do not reflect a thermodynamic partitioning process and must be re-examined to reflect the colloidal component of most metals.

Fig. 1. Ultrafiltration Ion Rejection: Percent of Metal Rejected



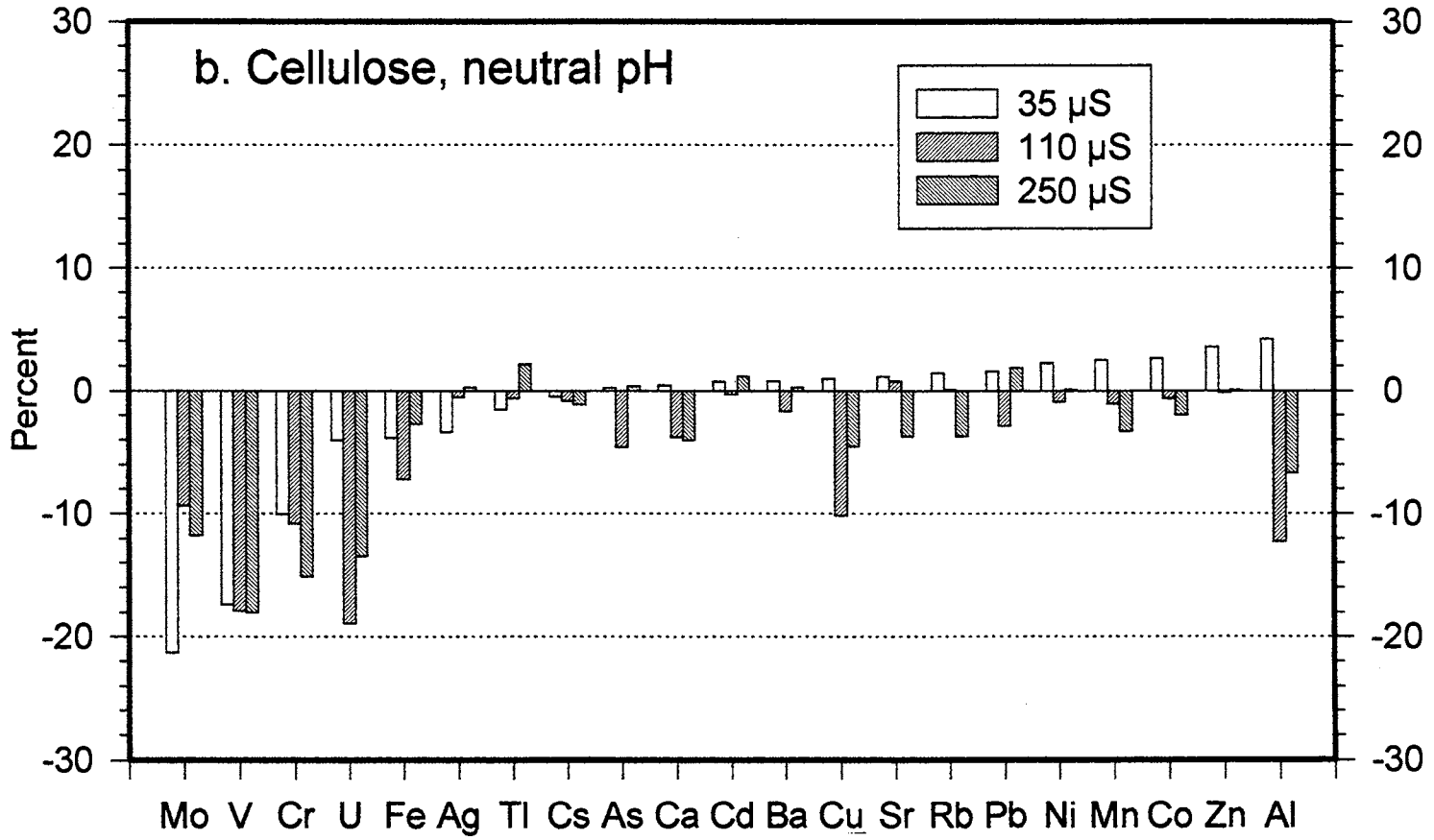


Figure 2. Silver size-association in Wisconsin rivers.

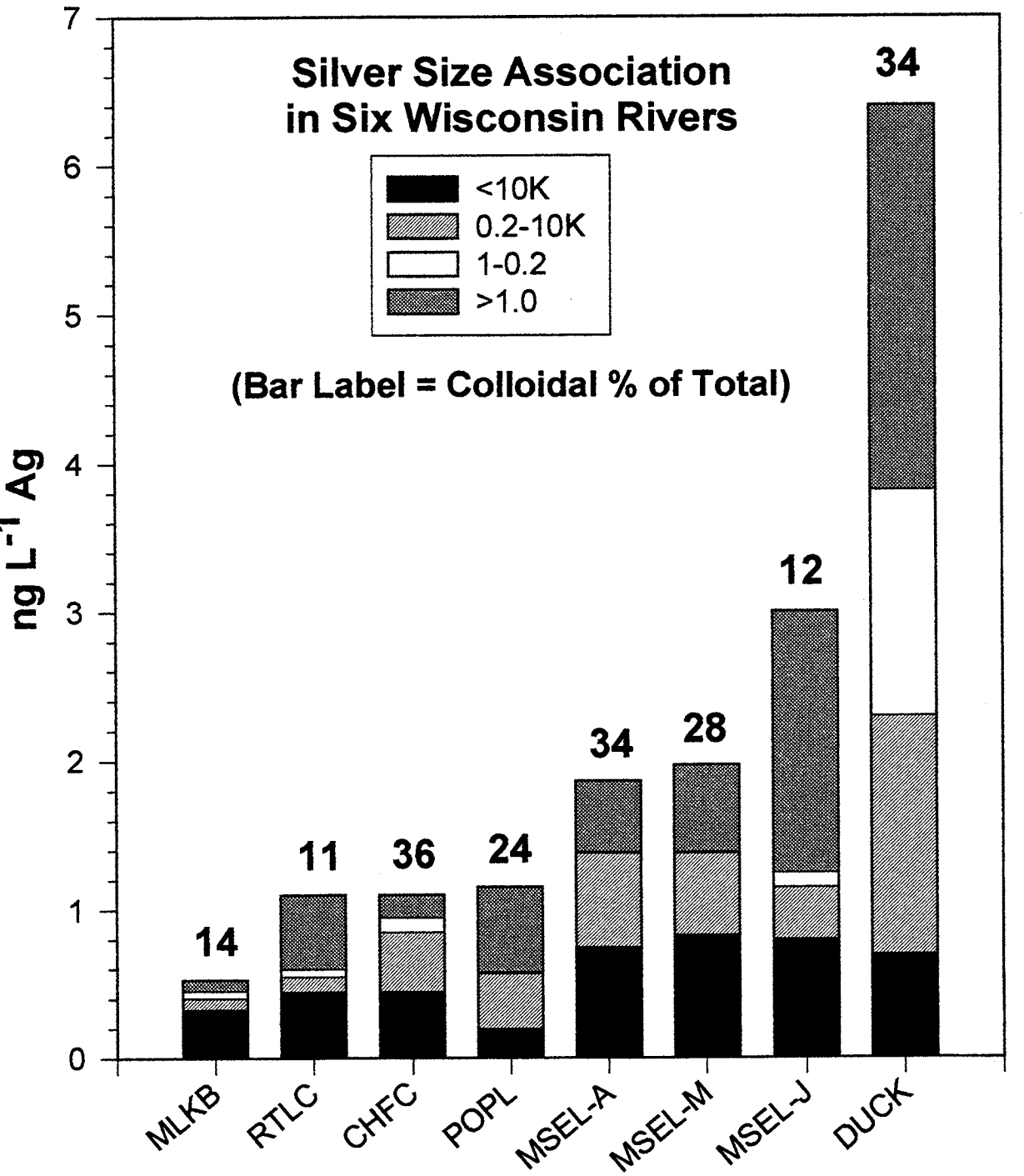
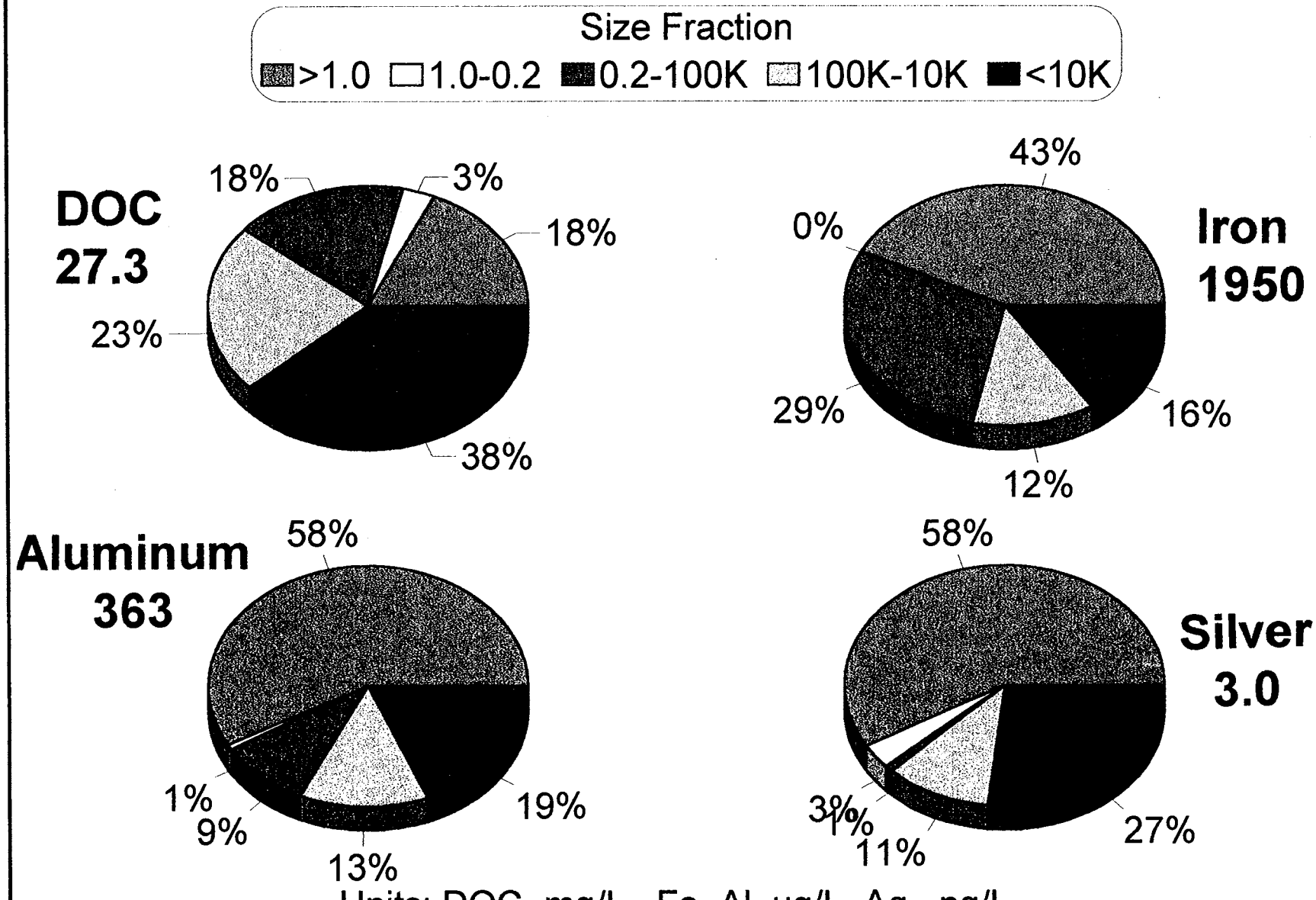


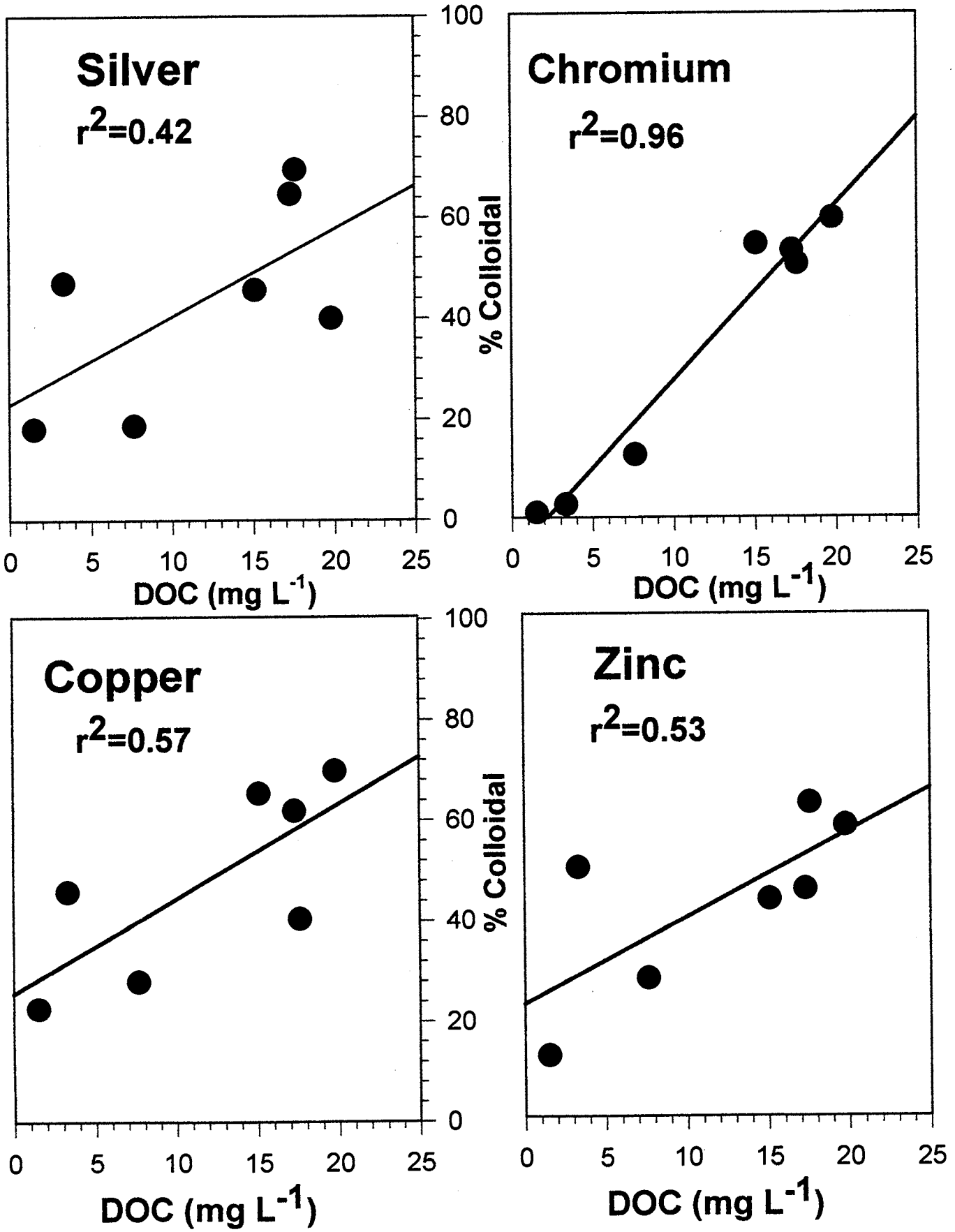
Figure 3. Moose River: (06-26-96)

Trace Metal and DOC Size Distribution



Units: DOC, mg/L. Fe, Al, ug/L. Ag, ng/L

Figure 4. Metal (percent colloidal) vs Colloidal Carbon



Questions & Answers: Preliminary Observations on the Distribution of Ag and Other Trace Metals in the Colloidal Size Fractions of Streams and POTW Effluents

Q. PETER SANTSCHI (Texas A&M): Did you try to relate the colloidal particulate fraction to that of iron in your system?

A. We plan to do that, but it's all very fresh data which we're just starting to look at. We're not quite where we planned to be. Obviously, we'll be doing that.

Silver Complexation with Sulfur Organics

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McMaster University
Hamilton, Ontario, Canada

Sulfur is one of the main group elements that strongly coordinates silver in geometries from linear to octahedral. Inorganic sulfide is a common coordinant of environmental silver but organosulfur compounds, particularly mercaptans (thiols), are present in significant concentrations and may coordinate silver. This presentation is a discussion of the structural aspects of silver complexes with mercaptans. Some mention will be made of silver thio-ether complexes. Structural knowledge of silver complexes with environmental mercaptans[1] such as mercaptoethanol, cysteine, glutathione, 3-mercaptoglycerol, and 3-mercaptopropanoic acid is sparse. A brief review of our present understanding of the structures and coordination of silver with simple mercaptans is presented.

Low molecular weight mercaptans, such as mercaptoethane, form very insoluble, amorphous precipitates when treated with an equivalent of silver ion. The complexes were considered to be polymeric in nature, followed an equation of the type

$$n\ CH_3CH_2SH + n\ Ag^+ \rightarrow [CH_3CH_2SAg]_n + n\ H^+$$

and possessed linear chain structures where the silver was digonally coordinate as illustrated in Fig. 1. Formation constants lay in the range log K_f = 12 - 14.

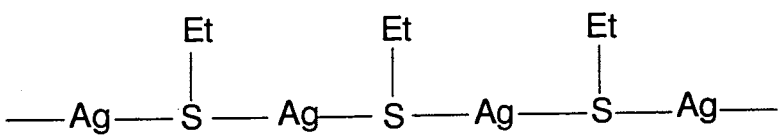

Figure 1. Proposed chain structure of alkyl mercapto-silver complexes illustrated by ethyl (Et) mercaptan.

Going to sterically larger alkyl chains, the solubility of the mercapto-silver complexes increases in organic solvents. Akerström[2] determined the degree of association of some tertiary and secondary mercapto-silver complexes by cryoscopic and ebulloscopic means in benzene and found tertiary mercaptans to have association values of 8 and secondary mercaptans 12.

Solid state structures, as deduced from single crystal X-ray crystallographic data, were first presented by Hesse in 1975 for silver-cyclohexyl mercaptan[3], which he proposed to be a linear chain. The data were re-interpreted by Dance[4] in 1977 and were suggested to better fit a cyclic structure containing 12 units of Ag-S-cyclohexyl as illustrated in Fig. 2. In this large 24 membered ring there were two intra-ring Ag-S contacts (shown by circled atoms in Fig. 2) with Ag-S distances of 2.75 Å which represent "long" Ag-S bonds (digonal Ag-S bonds are 2.37 Å and trigonal, 2.55 Å). The circled Ag atoms are thus close to being

trigonal and the S atoms an unusual tetragonal coordination.

In 1991 Dance *et al.*[5] obtained microcrystalline samples of primary alkyl mercapto-silver complexes, $[RSAg]_n$, for R = n-propyl, n-butyl, n-hexyl, and n-heptyl. The structures were non-molecular but gave X-ray powder diffraction patterns consistent with a layered structure, illustrated in Fig. 3. For example, the distance t_2 increased as the alkyl chain length was increased whereas the distance t_1 remained relatively constant. The Ag and S atoms form a central slab from which the alkyl chains project in alternate vertical directions as shown in Fig. 4. Here all the Ag atoms are trigonal and all the S atoms are in the unusual tetra-coordinate state.

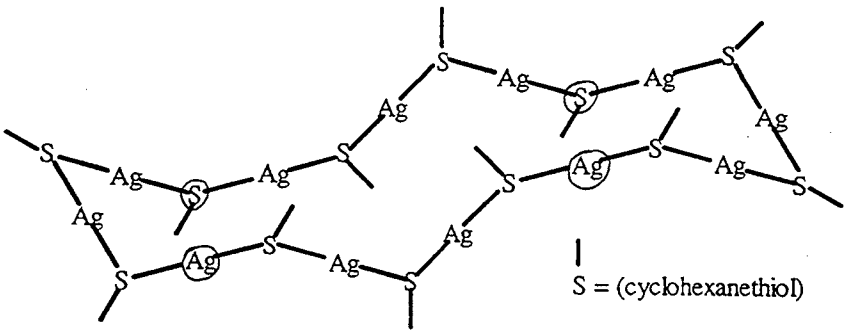

Figure 2. Solid state structure of silver mercaptocyclohexane.

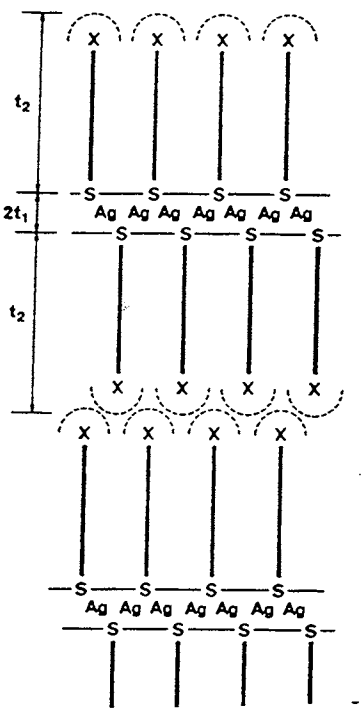

Figure 3. Layered structure of simple alkyl mercapto-silver complexes.

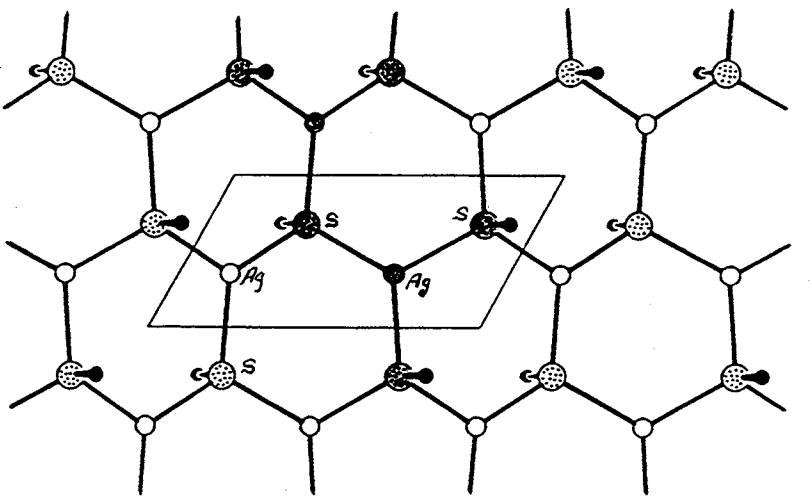

Figure 4. Perpendicular view of Ag and S atoms in simple alkyl mercapto-silver complexes.

When very bulky tertiary mercaptans are used, such as *tris*(trimethylsilyl)methyl mercaptan, crystallization is more facile and is governed by the large alkyl group rather than the Ag and S atoms. Because of the size of the alkyl group the slab structure is no longer feasible and an 8 membered ring is formed as shown in Fig. 5.

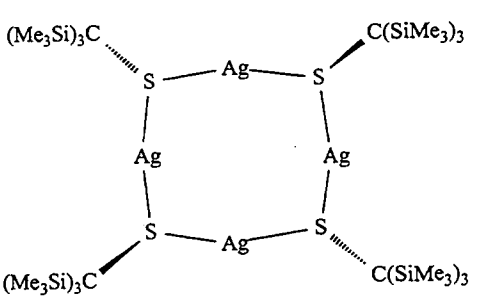

Figure 5. Structure of $[AgS\text{-}C(Si(CH_3)_3)_3]_4$.

The intermediate sized 3-mercapto-3-methylpentane gives a silver complex which crystallizes in the originally suggested linear chain structure but with a subtle difference[5]. There are *two* chains in the unit cell that intertwine about each other at each 5th silver atom as is illustrated by the shaded chain looping, first below and then above the unshaded chain in Fig. 6. The structure contains digonal Ag and represents a bridge between simple linear chains and a cyclic structure.

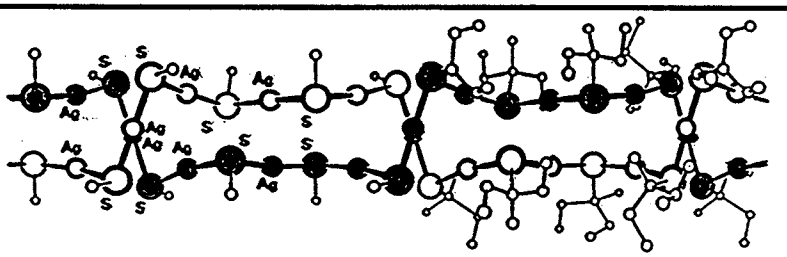

Figure 6. Intertwining double chain structure of 3-mercapto-3-methylpentane.

Thioethers readily form complexes with silver, albeit of lower stability than with the corresponding mercaptans. An interesting structure which is pertinent to the present discussion is that reported by Black *et al.*[6] for silver-1,3-*di*-thiomethylpropane, $[Ag_n(CH_3S(CH_2)_3SCH_3)_n]^{n+}$ (see Fig. 7). The dithioethers are held together by trigonally coordinate silver forming long chains and, intriguingly, every second sulfur atom is tetracoordinate (shown by S(1) in Fig. 7), a situation similar to that proposed by Dance for the Ag-S core "slab" of simple alkyl mercapto-silver complexes noted above.

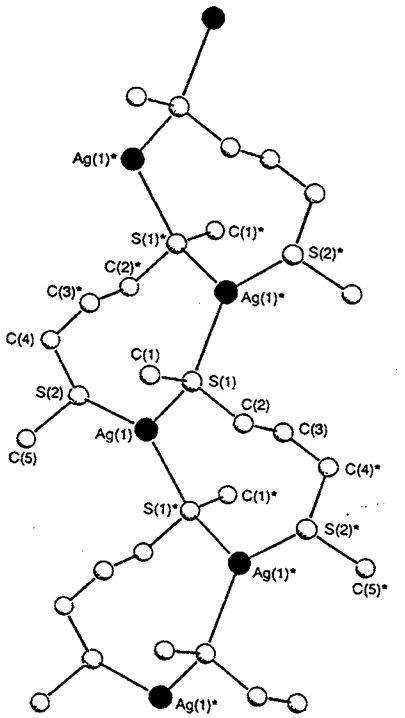

Figure 7. Solid state structure of $[Ag_n(CH_3S(CH_2)_3SCH_3)_n]^{n+}$

There are a number of mercaptans in cells whose duties are to handle transition metals like copper and zinc and as expected silver will likewise interact strongly with these compounds. The materials all contain the amino acid cysteine as the mercapto-containing unit and the two important ones in mammalian cells are glutathione (γ-Glutamine-Cysteine-Glycine) and metallothionein ($[Cys\text{-}X\text{-}Cys]_{n/2}$-Lys-Lys-$[Cys\text{-}X\text{-}Cys]_{n/2}$ where X = another amino acid and the value of n = 10 - 16). In plants metallothionein is replaced by phytochelatins[7] which are oligomers based on $[\gamma\text{-Glu-Cys}]_n$-Gly where n = 2 - 11.
It is well known that amino acids like cysteine, methionine, and penicillamine and tripeptides like glutathione form 1:1 complexes with silver with log K_f values ranging from a low of 3.15 for the thioether methionine to 13.1 for cysteine. Hard structural data about these complexes is however astonishingly sparse with no recorded crystal structures for silver complexes of the environmentally important cysteine or glutathione yet reported. Kojima *et al.* reported[8] in 1983 a crystal structure for silver cyclo(L-methionyl-L-methionyl) which showed a spiral chain two units of which are illustrated in Fig. 8. The silver is digonally coordinate and interestingly the ^1H NMR of the complex in hot D_2O showed the CH_3 group resonance to have moved to lower field (2.37 ppm) from the ligand shift (2.12 ppm). A 66:44 mix of ligand to complex showed an averaged shift at 2.29 ppm demonstrating that in these D_2O solutions the silver was in fast exchange between ligands.

While there is no experimental evidence for the type of coordination used by silver in the 12:1 complex (Ag:ligand) formed by metallothionein, it has been proposed to be mainly trigonal since metallothionein consists of two cysteine rich domains separated by two lysines and each domain contains 10 cysteines and only 6 silver atoms are maximally bound[9]. The complex is remarkably stable for the silver cannot be removed by excess mercaptoethanol but is displaceable by metals such as mercury.

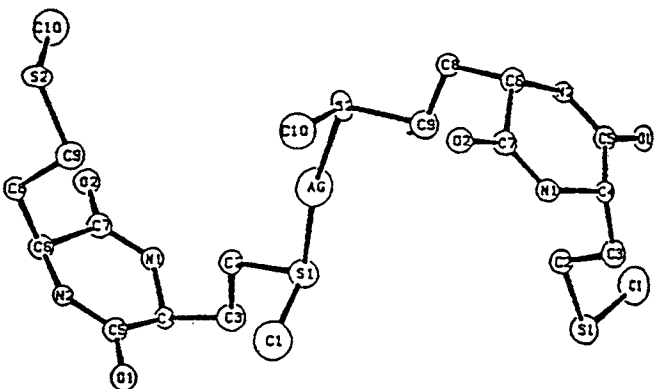

Figure 8. Two units of the spiral chain of silver cyclo(L-Met-L-Met).

In recent research at McMaster we have focused on examining the reaction of silver with model mercapto-peptides. The rationale for this is to explore the possibility of simple tripeptides forming S-Ag-S chelates that are more stable than linear structures. In anaerobic media degrading proteins can produce short peptides that could strongly complex silver and potentially provide a means for its transport. As an example of this approach, we have synthesized mercaptoacetyl-histidine-(S-benzyl)cysteine (Fig. 9) and reacted it with silver tetrafluoroborate in acetonitrile. The product is completely soluble in acetonitrile and has slight solubility in water and a possible chelate structure is illustrated in Fig 10.

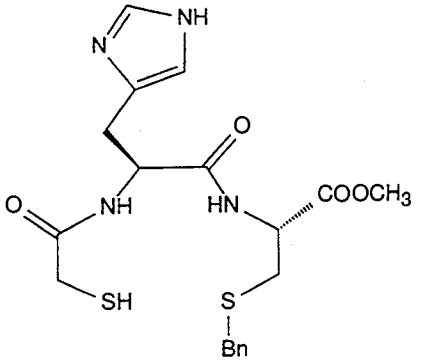

Figure 9. Mercaptoacetyl-His-(S-benzyl)Cys

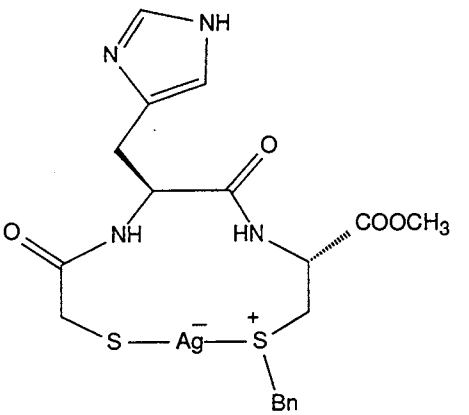

Figure 10. Possible silver chelate of mercaptoacetyl-His-(S-benzyl)Cys

The ^{1}H NMR spectrum of the silver complex (in acetonitrile) showed broad lines at room temperature that sharpen upon heating, a property that clearly demonstrated the presence of more than one complex and that these complexes were in intermediated exchange with each other on the NMR time scale. This observation is consistent with Kojima's work that showed that in solution silver can readily migrate from one thio-ligand to another.

CONCLUSIONS

There is now a reasonable understanding of the solid state structures of simple alkyl mercapto-silver complexes and the coordination of silver in such structures. For primary alkyl the $[R(prim)SAg]_n$ give layered structures with Ag and S tetragonal while a secondary mercaptan like cyclohexyl mercaptan gives a large ring structure $[Cyclohexyl\text{-}SAg]_{12}$ with mainly digonal Ag and some trigonal Ag and very bulky tertiary alkyl give small ring structures with Ag digonal. Intermediate sized tertiary alkyl groups give linear intertwining chains with Ag digonal. Large secondary and tertiary alkyl mercapto-silver complexes, $[R(sec)SAg]_n$ and $[R(tert)SAg]_n$ are soluble in organic solvents and associate with n = 12 and 8 respectively.

There is very little information on the solid state structures of silver and environmentally important mercaptans like cysteine, glutathione, and 3-mercaptopropanoic acid and much remains to be done to fully characterize these materials. A model mercapto-thioether peptide, mercaptoacetyl-histidine-(S-benzyl)cysteine, proposed to form a cyclic chelate with Ag, gives an Ag complex that in solution showed the presence of multiple species that were in intermediate exchange on the ^{1}H NMR time scale.

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Questions & Answers: Silver Complexation with Sulfur Organics

No questions.

Interaction of Silver Ion with Thiol Ligands in the Presence of FeS

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Introduction

Studies to date suggest that silver is transported predominantly in the colloidal phase, and is rapidly scavenged by suspended sediment. Iron sulfides are important for trace metal sequestering in reducing waters (Luther et al., 1996). Since silver sulfides are very insoluble ($K_{sp}(Ag_2S)/K_{sp}(FeS) \sim 10^{-32}$), the interaction of silver with FeS is considered important in the removal of silver from the water column. However, the nature of this interaction is unclear. Models based on dissolved metal concentrations in equilibrium with a discrete metal sulfide phase may be incorrect. Studies indicate that dissolved silver concentrations are often super-saturated with respect to the formation of a discrete acanthite phase (Ag_2S) (Cowan et al., 1985). The discrepancy between calculated dissolved silver concentrations and observed values may be attributable to how silver exists in the solid phase.

Another possibility that would account for the discrepancy is that there may be significant dissolved silver species present in nature not accounted for in existing equilibrium models. The observation that silver binds strongly to organosulfur (thiol) ligands, commonly found in reducing environments (Vairavamurthy and Mopper, 1987; Shea and MacCrehan, 1988) suggests that such an explanation is possible.

Here we summarize results for an experimental investigation of silver interactions with iron sulfide (See Adams and Kramer (1997) for details.) In this study, we look at the effect of complexation of silver by thiol ligands, 3-mercaptopropanoic acid (3-MPA) and cysteine. As well, we report on the effect of competition by Cu^{2+}, complexation by Cl^-, and interferences caused by dissolved organic matter on silver reactivity with FeS. The results are compared to equilibrium calculations for reference.

Methods

Amorphous FeS was precipitated under reducing conditions, and collected on a 0.45 μm Millipore filter.

For each experiment, 5.0 g of freshly precipitated FeS was weighed into a darkened FEP bottle. One litre of silver containing solution was carefully poured into the bottle so as not to disturb the FeS. Solutions were mixed for the duration of the experiment in a glove-bag flushed continuously with N_2. 0.2 μm and 10 kDalton filtered samples were taken at 10 minutes, 1 hour and 2 hours. Analyses were performed by ICPMS. The different experiments are given in table 1.

Table 1. Experiments performed on saturated FeS system, at pH 8.0 , 25°C, and Ag_T = 3000 ng/L.

1. Ag added to FeS
1a. Mobilize Ag from FeS with 1.8 x 10^{-3} M 3-MPA thiol
2. Ag added to FeS with a one hour equilibration time between additions (0, 1, 5, 10, 25, 35 µg/L Ag)
3. Add Ag + thiol to FeS suspension
   a. Ag and 1.8 x 10^{-3} M 3-MPA
   b. Ag and 1.8 x 10^{-4} M 3-MPA
   c. Ag and 9.2 x 10^{-4} cysteine
4. Add Ag + 15 mg/L fulvic acid (coloured waters)
5. Add Ag + 15 mg/L fulvic acid + 1.8 x 10^{-3} M 3-MPA
6. Add Ag + 50 µg/L Cu^{2+}
7. Add Ag + 50 µg/L Cu^{2+} + 1.8 x 10^{-3} M 3-MPA
8. Add Ag + 10^{-3} M KCl (freshwater)
9. Add Ag + 10^{-3} M KCl + 1.8 x 10^{-3} M 3-MPA

Results

Previous work involving amorphous FeS has shown it to be extremely fine-grained. Iron concentrations for both the 0.2 µm and 10 kD filtrate solutions were compared as a measure of colloidal FeS. In all cases, no significant variations in Fe concentrations were observed. We conclude therefore that colloidal FeS did not interfere with the determination of dissolved silver concentrations.

Points connected by solid lines in figure 1 show experimental results for uptake of dissolved Ag concentrations in the presence of FeS. The removal of dissolved Ag to the solid phase is very rapid. Samples taken after one and two hours showed no appreciable change, indicating that equilibrium for the Ag-FeS system is achieved quickly. As seen in figure 1, thermodynamic calculations based on the formation of a Ag_2S mineral phase predicted a similar equilibrium value to what was found experimentally. Since most metal sulfide minerals form rather slowly, we precipitated Ag_2S to investigate the possibility that acanthite could form under highly super-saturated conditions. The resulting XRD pattern for the precipitate indicated at least 50% acanthite. This provides evidence that XRD crystalline acanthite forms rapidly in elevated Ag^+ solutions.

As well, dissolved silver concentrations were determined over a range of total silver concentrations from 1 to 35 µg/L. The results presented in figure 2 show no increase in dissolved silver levels with increasing total silver. The results suggest dissolved Ag is controlled by Ag-S solubility, and demonstrate that under conditions where FeS is in excess, dissolved silver will be at the low ng/L level.

Inorder to make some predictions about what factors might be important in the

partitioning of silver between aqueous and solid phases, we have investigated the effect of silver complexation by organosulfur (thiol) compounds. Our calculations show that under reducing conditions (sediment interface, high DOC, etc.), silver complexation with thiol compounds could potentially explain the disparity between observed and predicted dissolved silver levels found in many contaminated waters. Our calculations also show that in organically rich systems, including many marine environments, silver thiol complexes will dominate over all other dissolved silver species. To test this prediction using our model system, we first looked at dissolved silver in the presence of two thiols found in porewaters at μM levels, 3-MPA and cysteine. The effect of thiol concentration was also examined. Figure 3 shows dissolved silver compared with calculated values. As seen with the Ag-FeS system, reactions occur rapidly. At the low thiol concentration level, results compare closely to predictions. However, at higher thiol concentrations, thermodynamic calculations predict two to three times higher dissolved silver. To investigate the potential for remobilizing Ag with thiols after reaction with the FeS, we performed experiments where an equilibrated Ag-FeS system was spiked with 3-MPA. The dashed portion of the plot in figure 1 clearly shows that Ag can be remobilized by thiols.

As shown in table 1, Ag interactions with FeS were investigated for Cu^{2+}, Cl^- and DOC in presence and absence of the 3-MPA thiol ligand. Figure 4 shows these results. Comparisons made for the 2 hour sample data showed that in the absence of 3-MPA, none of the three factors significantly increased $[Ag]_T$ ($p < 0.05$, one-tailed t-test) above the control conditions, the Ag-FeS experiment shown as the solid line in figure 1. However, 3-MPA gave a significantly higher result in all three cases, comparable to what was found for 3-MPA in the absence of these factors.

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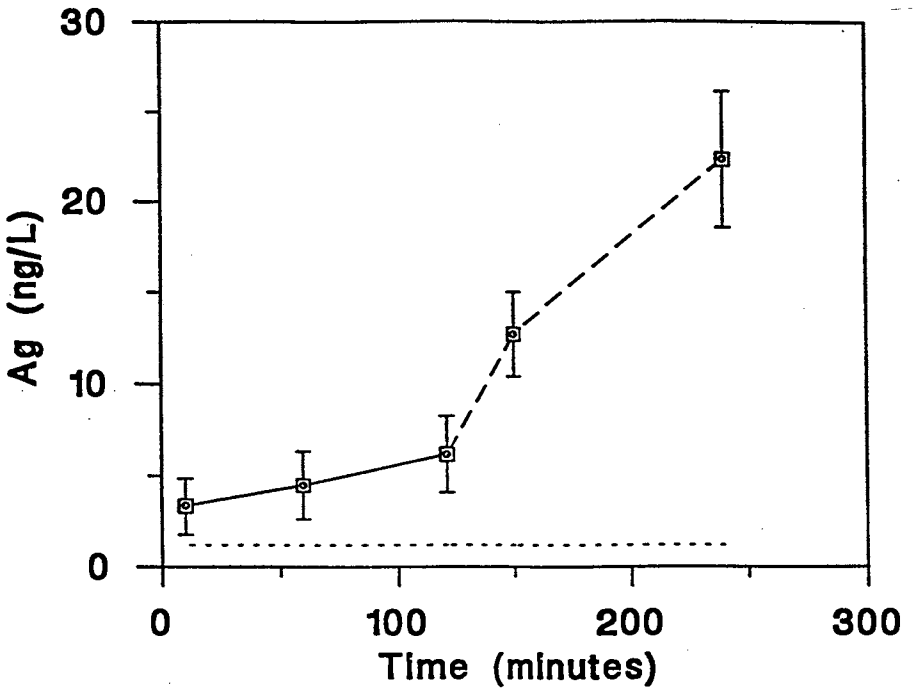

Figure 1. Ag_T uptake in the presence of FeS at pH 8.0. Solid line: uptake of dissolved Ag in the presence of FeS; dashed line: remobilized Ag with 1.8×10^{-3} M 3-MPA; dotted line: calculated Ag_T for Ag_2S equilibrium. Error bars represent ± 1 SD.

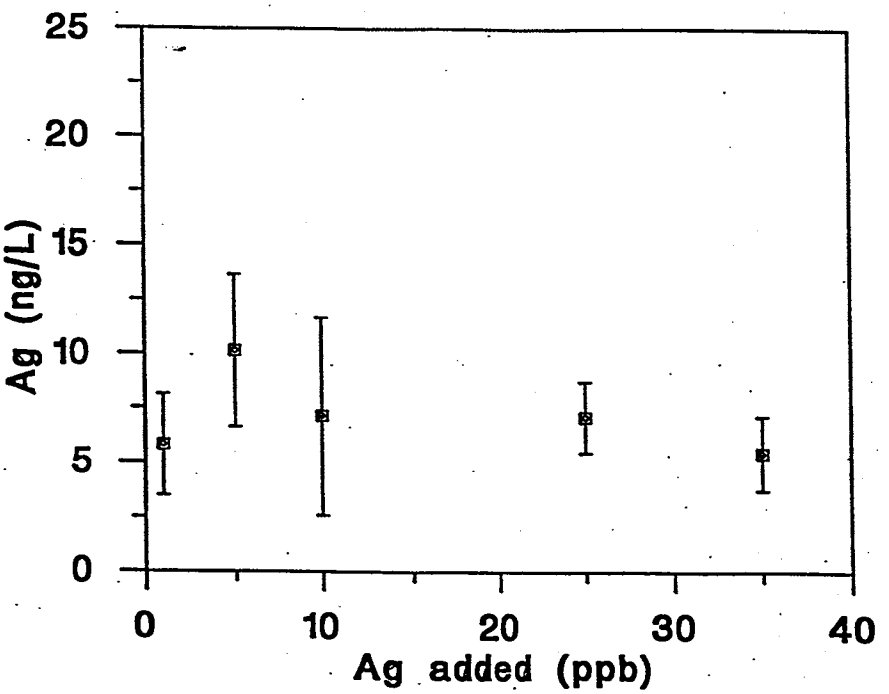

Figure 2. Uptake of Ag_T in the presence of FeS at pH 8.0. Error bars represent ± 1 SD.

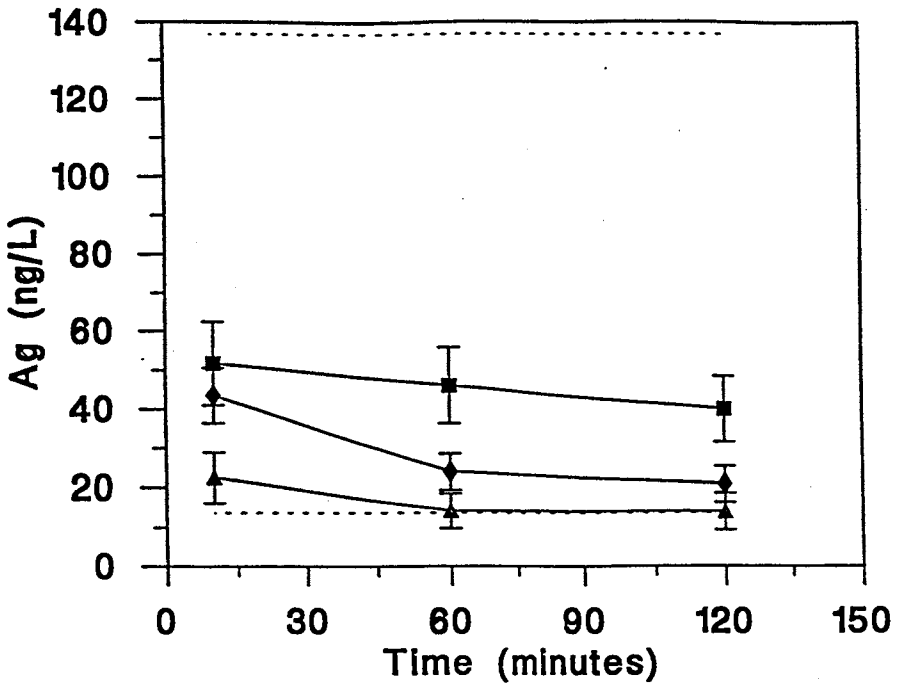

Figure 3. The effect of thiol ligand on dissolved Ag in the presence of FeS. Error bars represent ± 1 SD. Dashed lines: calculated Ag_T for Ag_2S equilbrium with 1.8 x 10^{-4} - 10^{-3} M 3-MPA. —♦—1.8x10^{-3} M 3-MPA; —▲—1.8x10^{-4} M 3-MPA; —■—9.2x10^{-4} M cysteine.

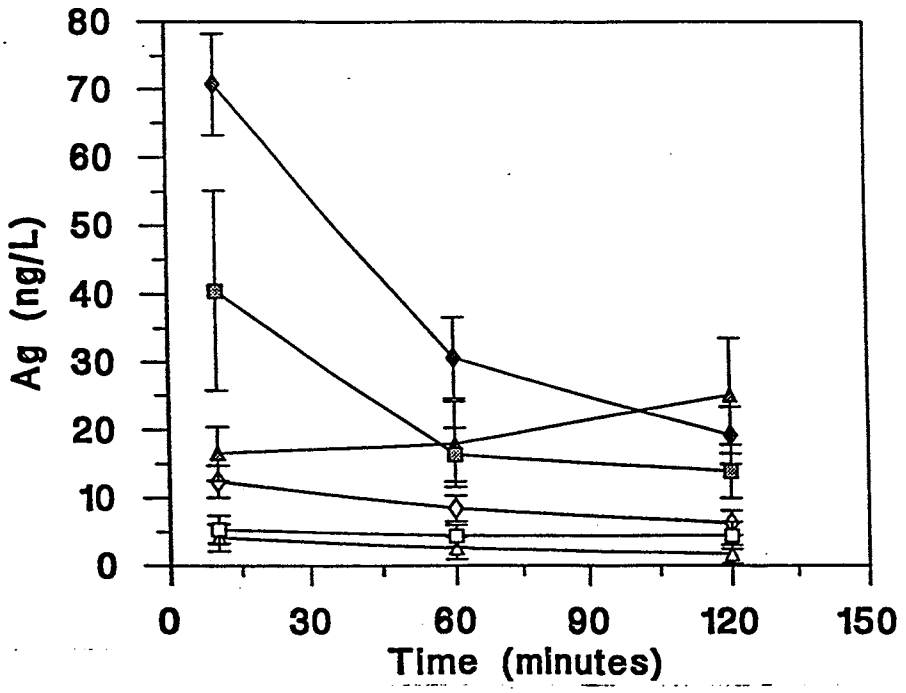

Figure 4. Effect of fulvic acid, Cu and chloride on Ag mobility in the presence of FeS. Error bars represent ± 1 SD. Filled-symbols with 1.8x10^{-3} M 3-MPA, open-symbols without: —◇—15 mg/L fulvic acid; —△—50 µg/L Cu^{2+}; and —□— 10^{-3} M KCl.

Questions & Answers: Interaction of Silver Ion with Thiol Ligands in the Presence of FeS

Q. CHRISTIAN KRAHFORST (University of Massachusetts): These are anoxic conditions you're working with; did you worry about loss of the FeS through oxidation? Did you monitor that at all?

A. We worked pretty hard on developing a system whereby we can maintain anoxic conditions for long periods of time. Visually, the amorphous FeS is very apparent once it becomes oxidized, from the redness of the solution, so you can visually monitor it. When Helen [Manolopoulos] talks to you she'll tell you a little about the work we've been doing to slowly bring up the oxygen levels and see what happens. We're actually monitoring now with an oxygen probe and so on.

Q. CHRISTIAN KRAHFORST: Another aspect that might be important is photo-oxidation, so the role of light may be significant.

A. I didn't mention that; all the experiments were carried out in the dark.

Q. PETER SANTSCHI (Texas A&M): Initially you showed that you reached 5 ng/L within minutes after you added the FeS, but then the concentrations went back up again. Why is that?

A. The increase you are referring to took place after thiols had been added. The difference is only significant after you add in the thiol compounds.

Q. ANDERS ANDREN (University of Wisconsin): It wasn't entirely clear to me; are you postulating that you have adsorption of the silver to the FeS, or do you have precipitation of silver sulfide and that in turn associating with iron sulfide? Presumably the FeS will dissolve somewhat (the amorphous iron sulfide).

A. We don't have results that can discern between adsorbed Ag or a discrete Ag_2S phase. You can get an exchange on the surface of the FeS. There's a lot of sulfide in solution as well, just due to the difference in stabilities between the Ag and FeS. You could potentially be forming Ag_2S before you even saw the surface of the FeS.

Q. DAVID ARMSTRONG (University of Wisconsin): This is partly a clarification; I think you showed you added fulvic acids and didn't get much response. What were the concentrations of the fulvic acid?

A. About 15 ppm.

Q. DAVID ARMSTRONG: Were you able to tell whether the fulvic acid stayed in solution or was adsorbed to the FeS?

A. We didn't check. The only thing I know about fulvic acid is that it doesn't contain any sulfur groups.

Q. RUSSELL BELL (McMaster University): It's known that silver will form intermetal complexes. What do you think of the possibility that your Ag-thiolate may have a bridging sulfur to Fe, and that species may go off into solution?

A. I hadn't really thought about that possibility. I think that, based on the concentration effect we're seeing, when we add higher concentrations of thiols there has to be some interaction occurring between the Ag-thiolate complex and the FeS.

Source Estimates and the Partitioning of Silver and Other Trace Metals in Massachusetts Coastal Waters

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Introduction:

The distribution of silver and other trace metals in coastal New England water samples were collected to evaluate sources to the water column as well as mechanisms that control the partitioning of metals between dissolved (0.4 µm filter-passing) and particulate (0.4 µm filter-retained) phases. Surveys conducted from 1994-1996 were designed to provide, for the first time, distribution patterns of silver from the Gulf of Maine, Boston Harbor and Massachusetts Bay and to assess the potential of silver as a tracer of dispersion and fate of waste effluent in Massachusetts Bay.

Increased awareness of ecosystemic degradation is being manifested in this region by recent efforts to clean up Boston Harbor. These efforts: improved wastewater treatment and the extension of the major outfall pipe to a new site in western Massachusetts Bay, have provided added incentive for a better understanding of the sources of contaminants and their relative contributions to the degradation of coastal water quality. The results of this research are used to estimate sources of silver and other metals to Massachusetts Bay and provide insight to the processes important in governing the fate and transport of trace contaminants in coastal waters.

Physical Setting:

Massachusetts Bay is the body of water that is bounded to the north and east by the Gulf of Maine and in the south by Cape Cod Bay. There is a marked seasonal variation in stratification, from well-mixed conditions in the winter to highly-stratified conditions during the summer. The general circulation during much of the year is a sluggish but persistent counterclockwise flow that may reverse during the fall (Geyer et al., 1992). In general, surface water residence times for this region have been estimated to range between 20-45 days. However, transport processes in the nearshore region of western Massachusetts Bay are more sensitive to the influence of tidal mixing, winds and river inflow and the corresponding surface waters may have much shorter residence times (hours to days). During periods on intense summer stratification, river runoff events are identifiable by decreased near-surface salinity and the establishment of a distinct "coastal current" that significantly impacts salinity distributions in the Gulf of Maine and Massachusetts Bay.

Results:

A preliminary survey was conducted in August 1994 along a 30 km transect out of Boston Harbor to the central eastern boundary of Massachusetts Bay. Particulate and dissolved silver concentrations reached a maximum of 484 and 210 pMol kg^{-1} respectively in the vicinity of the waste effluent outfall in Boston Harbor, to a minimum of 2.9 and 12.7 pMol kg^{-1} respectively in the surface waters offshore. A bay-wide survey of surface waters was conducted in July 1995. The distribution of silver in the surface waters of Massachusetts Bay is shown in Figure 1. The general trend in both particulate and dissolved silver (as well as other metals) shows a marked decrease in concentrations with distance from Boston Harbor. Dissolved metal concentrations in the surface waters of the north and eastern regions of Massachusetts Bay were significantly higher than observed in August 1994.

Particulate and dissolved silver concentrations were determined in a second, more extensive, survey of Boston Harbor in June 1996. Mean silver concentration in the particulate phase was 114 ± 38 pMol Ag kg^{-1} and 86 ± 26 pMol Ag kg^{-1} in the dissolved phase.

Water column profiles of silver and other metals at five stations in the Gulf of Maine were constructed from discrete samples collected in the upper 60 m along a 30 km transect extending off the southernmost portion of Maine. (See Figure 2.) Silver distributions are shown in Figure 3. The fraction of dissolved silver accounted for 82 (± 8) % of the total silver concentrations in the Gulf of Maine.

Discussion of Results:

End-Member Concentrations: Physical oceanographic studies of Massachusetts Bay (Geyer et al., 1992) show that mixing in the western portion of the Bay and Boston Harbor is dominated by tidal forces and may also be sensitive to the influence of wind and river inflow. Surface water residence times in this region are therefore expected to be much less than the mean residence time of 20-45 days for Massachusetts Bay. High correlation of silver and other metal concentrations with salinity from inshore stations was observed for the August 1994 survey (Table I). The most-likely process controlling these distributions was simple two end-member mixing between Boston Harbor and Massachusetts Bay occurring over time scales of hours to days. Extrapolation of regression lines of metal concentration and salinity to 0 PSU provide estimates for "apparent" end-member metal concentration for Boston Harbor (Table II). These apparent end-member concentrations are in good agreement with 1994 measurements of "total recoverable" metals in Boston's municipal waste treatment effluent (Uhler et al., 1994).

The July 1995 bay-wide survey shows a persistent inverse relationship, (lower metal concentration at lower salinity), between metal concentration and salinity. Mixing of higher salinity Boston Harbor water with lower salinity Massachusetts Bay water can be invoked to largely explain these observations. This process

can result in a "fresher" Massachusetts Bay end-member, relative to Boston Harbor.

Massachusetts Bay Mass Balance: Determinations of silver concentrations in water samples collected from the Gulf of Maine in July 1996 indicate that the Gulf of Maine may contribute significantly to the flux of silver into Massachusetts Bay. Based on mean velocity measurements, the near-surface advective transport from the Gulf of Maine to Massachusetts Bay is estimated between 16000 - 18000 $m^3 s^{-1}$ (Geyer et al., 1992). Using the mean concentration of total silver observed in July 1996 (12 pMol kg^{-1}), the corresponding flux of silver from the Gulf of Maine into Massachusetts Bay is 0.2 mMol s^{-1}. The advective flux from the Gulf of Maine when compared to the flux of silver from the discharge of Boston's waste effluent (0.8 mMol s^{-1}) may contribute an additonal 25% of silver entering into Massachusetts Bay.

Particle Composition Effect: Of particular importance in the understanding of the fate of contaminants in the coastal ecosystem is the partitioning of these contaminants between dissolved and particulate phases during transport through the water column. This is traditionally quantified in terms of an empirical partition coefficient:

$$K_D = \frac{\text{mass of filter} - \text{retained metal} \;/\; \text{mass of total suspended matter}}{\text{mass of filter} - \text{passing metal} \;/\; \text{mass of water}}$$

The log K_D's for the 1995 metal data when plotted against total suspended matter (TSM) show marked decreases with increasing suspended particulate concentrations. This pattern is not uncommon (Benoit et al., 1994 and references therein) and has been generally referred to as the "particle concentration effect". For samples containing high amounts of suspended particulate matter (> 5 mg l^{-1}), the particle concentration effect may be related to the presence of colloidal forms of metal, which pass through filters used in filtration and become part of the "dissolved" fraction. If the colloid concentration (and hence the colloidal form of the metal) increases in proportion to the quantity of total suspended matter, then an apparent decrease in K_D with increasing TSM can be observed.

Total suspended matter concentrations in the surface waters of July 1995 ranged from 2.6 mg l^{-1} in Boston Harbor to 0.3 mg l^{-1} in Massachusetts Bay. The apparent decrease in K_D observed for these samples may be more related to the changes in composition of the suspended matter rather than the result of filtration artifacts due to changes in the significance of colloidal forms of the metals. The organic carbon content of suspended particles in these samples increase exponentially with decreasing TSM concentration. In the case of all metals measured (Ag, Zn, Cd, Cu, Fe and Pb), K_D increased by almost an order

of magnitude with increasing fraction of organic carbon in suspended matter (Figure 4). The composition of particles in the water column at the TSM concentrations prevalent in most coastal waters is an important variable controlling the partitioning and speciation of metals in the marine ecosystem.

Summary:

1. Silver can be used as an effective tracer for mixing of Boston Harbor and Massachusetts Bay water over short time scales (hours to days) in the immediate nearshore region and perhaps over the longer time scales (days to weeks) of mixing in the Massachusetts Bays.

2. Elevated 1995 dissolved metal concentrations relative to 1994 reflect changes in the relative significance of inputs (e.g., waste water effluent, river runoff) relative to removal processes (e.g., particle scavenging, advection).

3. Total silver concentrations determined from Gulf of Maine water samples suggest that advective transport to Massachusetts Bay is a minor but significant source of silver to the waters of coastal Massachusetts.

4. Partitioning of trace metals between dissolved and particulate phases in Massachusetts Bay show a distinct dependence on the fraction of organic carbon associated with suspended particles. Compositional differences in suspended matter, rather than the "particle concentration effect", can explain the observed decrease in K_D with increasing suspended matter concentration.

References:

Benoit, G., S. Oktay, A. Cantu, M.E. Hood, C. Coleman, O. Corapcioglu and P. H. Santchi. 1994. Partioning of Cu, Pb, Ag, Zn, Fe, Al, and Mn between filter-retained particles, colloids and solution in six Texas estuaries. Mar. Chem. **45/4: 307-336.**

Geyer, W.R, G.B. Gardner, W.S. Brown, J. Irish, B. Butman, T. Loder, R. Signell. 1992. Physical oceanographic investigation of Massachusetts and Cape Cod Bays. Report to the Massachusetts Bays Program. **MBP-92-03.**

Uhler, A.D., D.E. West and C.D. Hunt. 1994. Deer Island effluent characterization June-November, 1993. MWRA Enviro. Qual. Dept. Tech. Rep. Series 94-4. Massachusetts Water Resource Authority, Boston, MA .

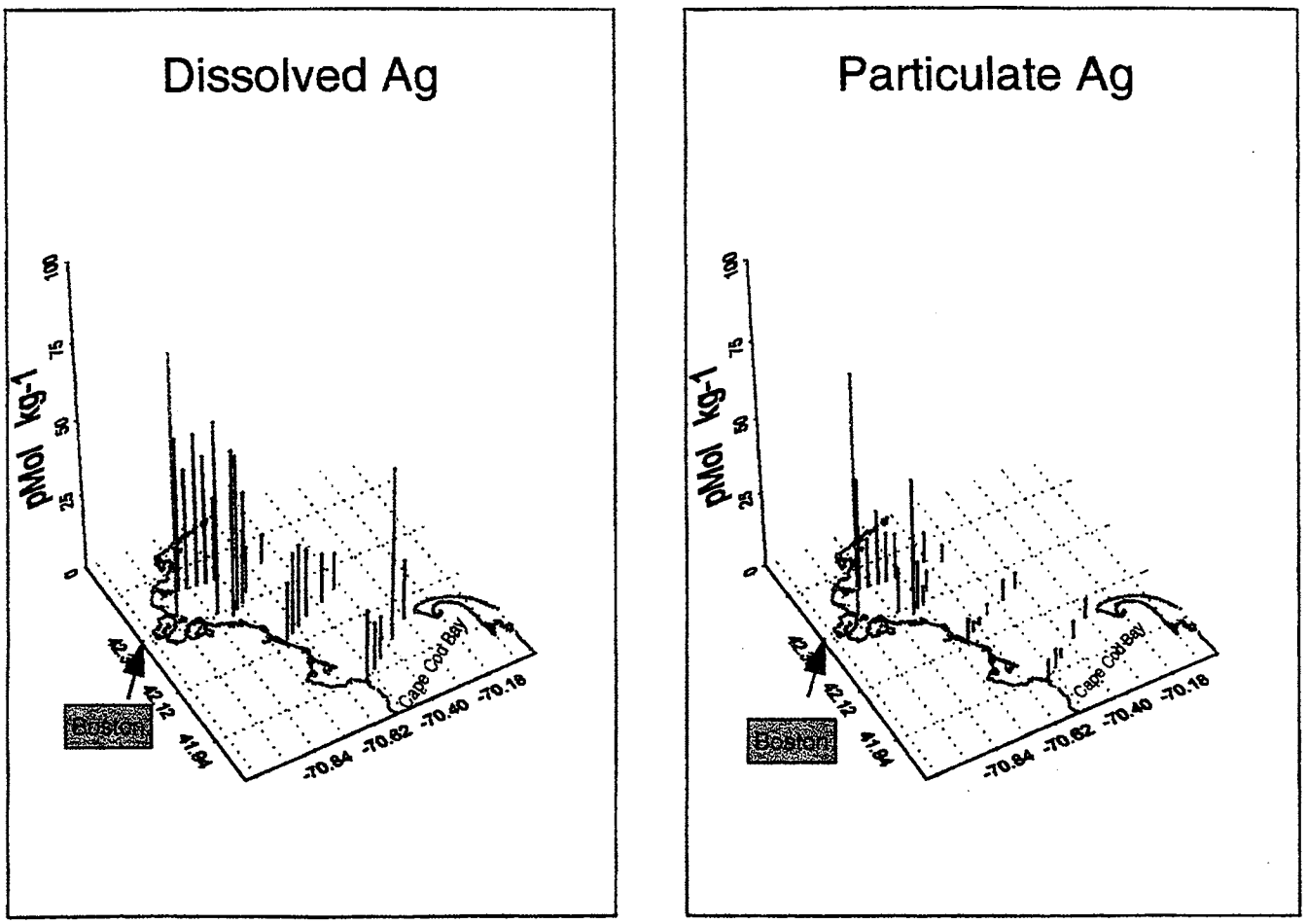

Figure 1. Silver distributions in Boston Harbor and Massachusetts Bay, July 1995.

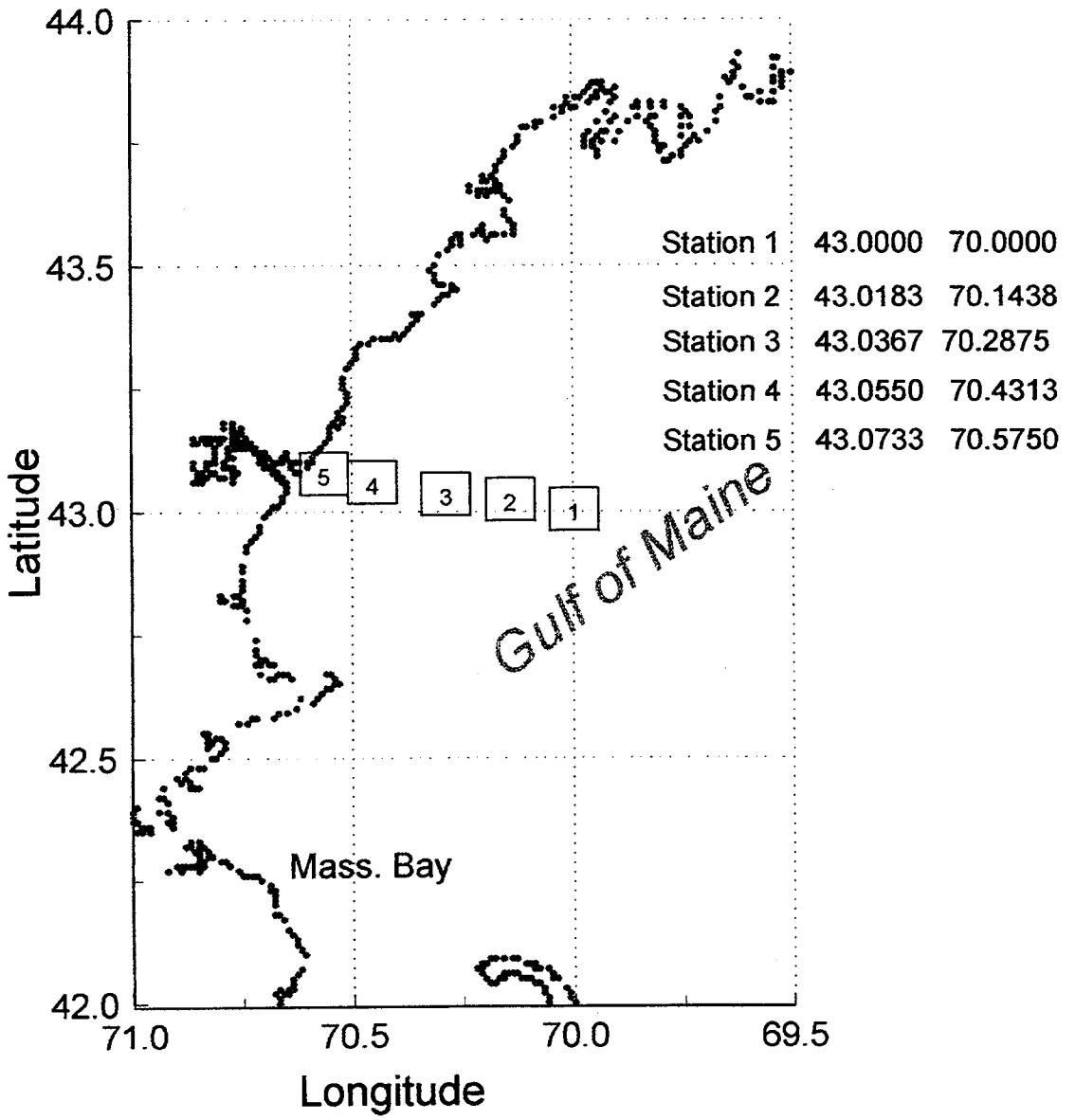

Figure 2. Station locations from 1996 Gulf of Maine transect.

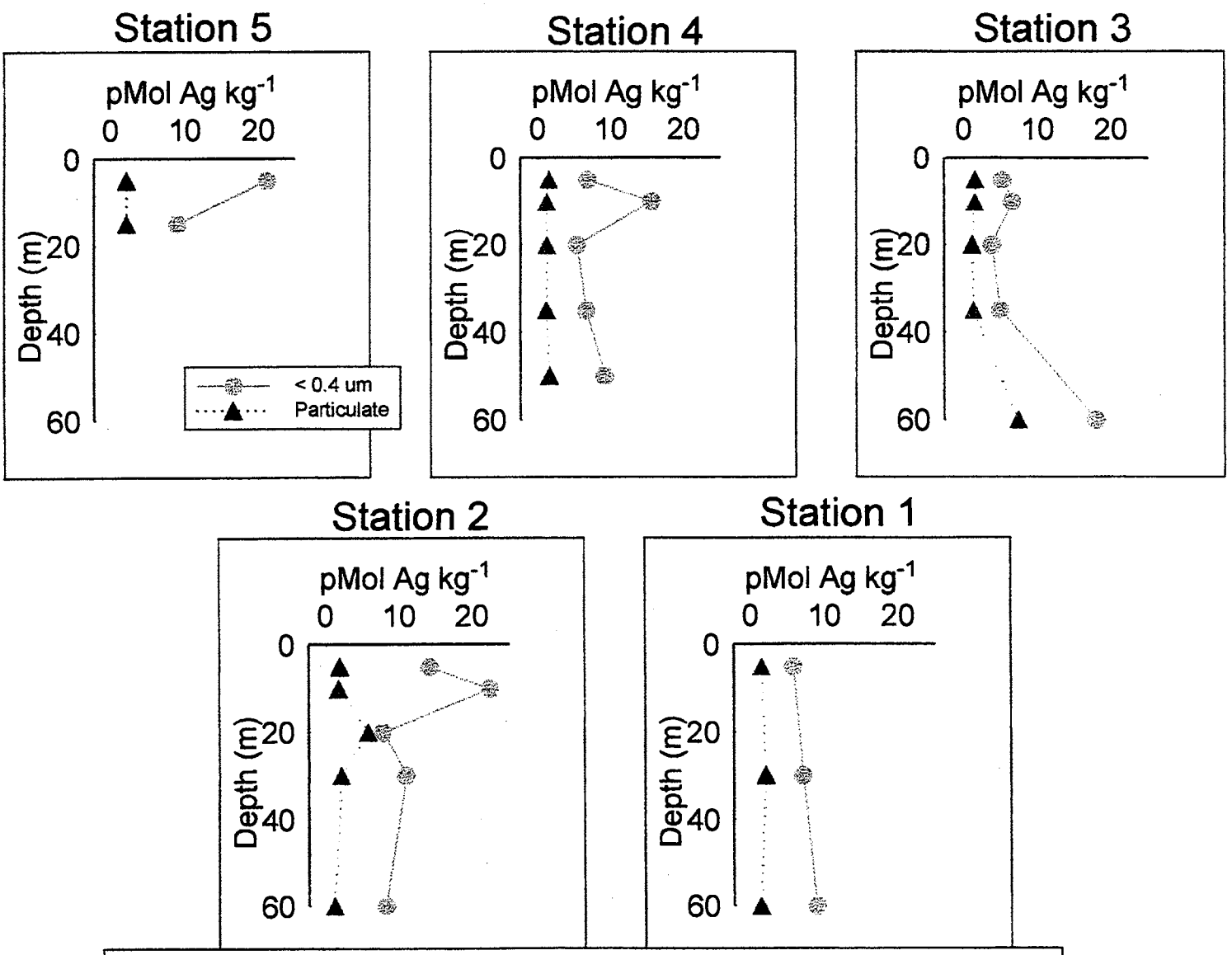

Figure 3. Depth distributions of Silver in the Gulf of Maine, July 1996

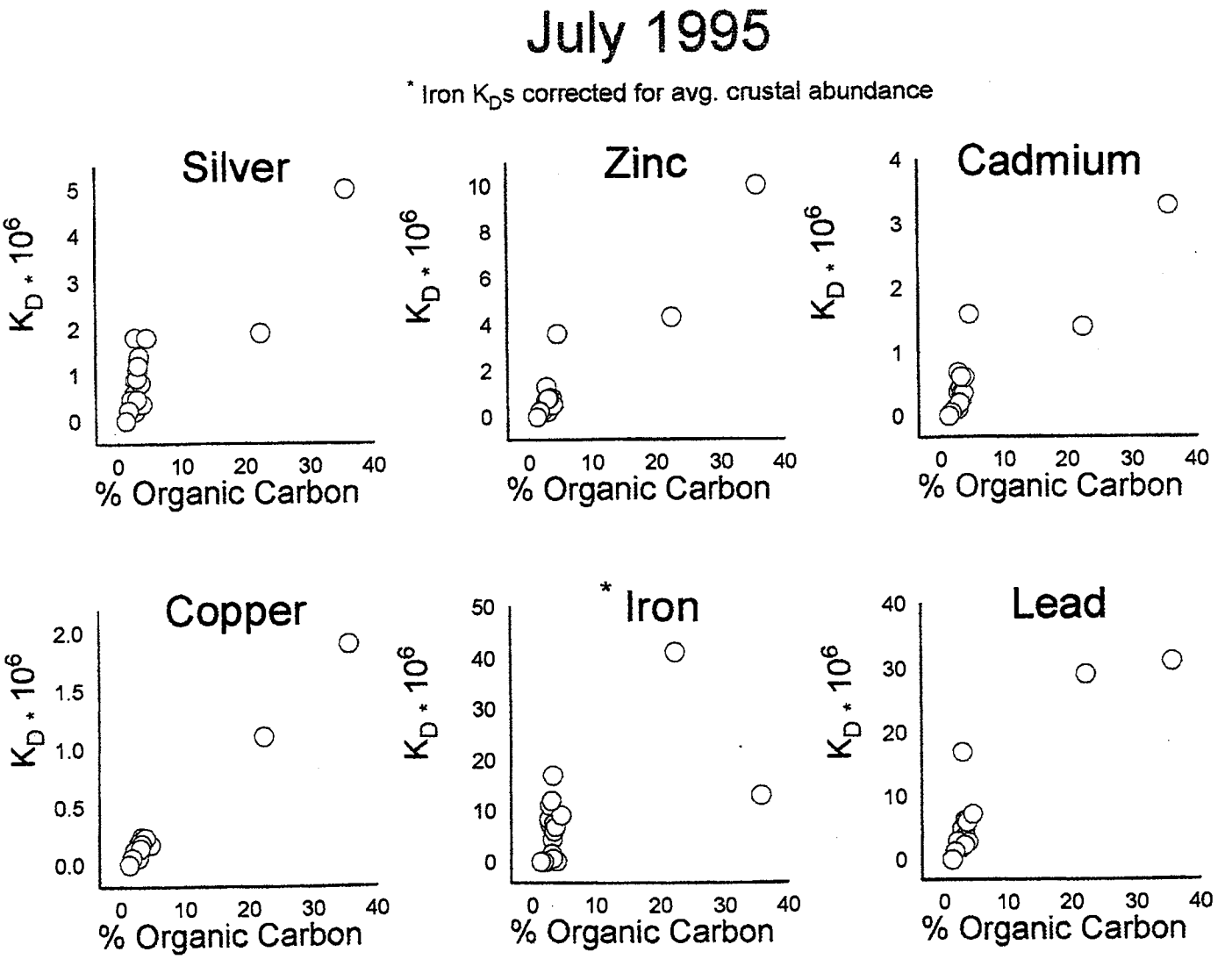

Figure 4. The variation of K_D with percent organic content of suspended particles

Table I Correlations coefficients (r^2) of metal-salinity reationships from 1994 inshore stations.

1994 Regression Coefficients (r^2)	Ag	Pb	Cu	Zn
Dissolved	0.95	0.84	0.87	0.95
Particulate	0.97	0.54	0.96	0.95

Table II Boston Harbor "apparent" end-member concentrations for 1994.

1994 "Apparent End-Members"	Ag nMol kg^{-1}	Pb nMol kg^{-1}	Cu nMol kg^{-1}	Zn nMol kg^{-1}
Dissolved	10	9	860	1600
Particulate	27	10	630	1500
1994 MWRA "Total Recoverable"	48 (±11)	58 (±22)	1200 (±300)	1300 (±300)

Questions & Answers: Source Estimates and the Partitioning of Silver and Other Trace Metals in Massachusetts Coastal Waters

Q. NICHOLAS FISHER (SUNY Stony-Brook): Do you think that the relationship that you had (the higher the organic carbon content of the particles, the higher the K_d) could have had something to do with higher surface areas of particles with more organic matter? Typically, one sees that particles that are more organic-rich have higher specific surface areas. That may have something to do with sulfur content of the particles as well, but it may be a particle surface-area situation.

A. I wonder if that's important in this question, because I don't think any of the sites on the particles are saturated to any extent with respect to complexing with Ag. I think there is an overabundance of these sites. The point that Peter raised to you is, one of the things we want to do is start to look at the sulfur aspects of the particles themselves.

Q. NICHOLAS FISHER: But even when you don't have saturated sites, you typically see higher K_d values or concentration factors on phytoplankton for higher surface-area-to-volume ratio particles, and that would be consistent with your observations. It might be worth trying to attempt to determine surface areas of your particles.

A. Okay.

Oxidation of Silver-Bearing Iron Sulfides: A Preliminary Study

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The sediment zone acts as a sink for many trace metals due its large metal sulfide content. Ag_2S is highly insoluble ($K = 10^{-50.1}$) and forms readily when sulfide ion is present. Laboratory studies have shown that in the presence of authigenic iron sulfide, the concentration of residual dissolved silver occurs at ultra-trace levels (Adams and Kramer, 1996). However, the effects of changes in redox conditions on dissolved silver concentrations have not been examined in a systematic study. The intent of this study is to examine the extent of mobilization of silver bound to iron monosulfide (FeS) as a function of molecular oxygen concentration as well as time.

Careful consideration had to be given to the experimental design to ensure that the system was not being contaminated with oxygen. It was important to avoid the occurrence of oxidation effects prior to the onset of the experiment, as well as to be able to maintain the oxygen concentration at a desired level throughout the experiment. Consequently, all experiments including the precipitation of iron monosulfide, were conducted in a glove bag under an oxygen-free nitrogen atmosphere, where the oxygen level was constantly monitored. Oxygen and pH levels were monitored throughout the experiment and maintained at the required levels with the addition of air and base respectively by a computer controlled system.

The following procedure was used for a preliminary experiment:

- water used in all parts of the experiment was degassed Milli-Q water (degassing was done by bubbling deoxygentaed nitrogen through it)
- fresh FeS was precipitated and filtered through a 0.45 μm filter to eliminate colloidal material

- added ~5g of FeS to 1.5 L of degassed water
- spiked the system with 1 mL of Ag^+ (~ 3 ppb)
- allowed the suspended FeS to settle and the system to equilibrate at which time a blank sample was taken
- increased O_2 level from 0.0 ppm to 0.3 ppm
- samples were collected after 0.5, 1, 2, 3, 4, 5, and 24 h
- all samples were filtered through a 0.2 μm filter and a few were also filtered through a 10 kD
- samples were subjected to ICP-MS analysis for silver detection

Quantitative results have not yet been obtained to determine the effect of molecular oxygen on silver mobilization. However, even at the low oxygen level of 0.3 ppm, visible signs of oxidation occurred. It is possible that under oxic conditions the bound silver becomes mobile as the metal sulfides are dissociated. FeS is readily oxidized by molecular oxygen to form ferrous and sulphate ions. Ag_2S might be oxidized in a similar manner to release Ag^+ and sulphate ions. The oxidation though may prove to be so slow, due to the sulphide's high stability, that no changes in silver concentration are noted at the minute levels at which silver occurs in the environment. More experiments and analyses will be conducted to elucidate the effects of molecular oxygen on silver mobilization.

References:

Adams, N.W.H. and Kramer J.R. (1996) Reactivity of Ag^+ ion with thiol ligands in the presence of iron sulfide, *Croatica Chimica Acta.* (submitted)

Smith, R.M. and Martell, A.E. (1976) *Critical stability constants V.4, Inorganic Complexes*, Plenum Press, New York.

Questions & Answers: Oxidation of Silver-Bearing Iron Sulfides: A Preliminary Study

Q. THOMAS BOBER (Eastman Kodak): In the past we've tried to use commercial-grade FeS for silver recovery, and we found some really complex substances like ferric oxide united with ferrous sulfide in various proportions. Oxysulfides is what we called them. If you've ever taken a piece of natural marcasite and broken it freshly, you see that it changes color very rapidly in atmospheric oxygen. Have you considered those as possibilities?

A. No, we haven't really given any thought to that yet. We haven't had any results to work with, and we haven't thought about it too much, but it's something to consider. What pH levels were you working with? (Tom Bober answers: This was at around 7.) We've just considered the hydroxides at this point.

Q. PETER SANTSCHI (Texas A&M): We did some experiments with radioactive silver just to look at uptake on iron hydroxides, and it's very low. There is some, but it's 1,000 to 10,000 times lower than the average natural particle.

A. Yes, I caught that in your talk; you said there is not much sorption that occurs between iron hydroxide and silver.

Sources and Sinks of Silver in an Urban Estuary: A Mass Balance Approach

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Though heavy metals are among of the most toxic and persistent contaminants of estuaries, we lack a quantitative understanding of their sources, transport, distribution, and fate. For estuarine systems located in industrialized areas, such as urban harbors, this lack of knowledge is confounded by numerous inputs from point and non-point sources pollution, including sewage treatment plant (STP) effluent, industrial discharge, atmospheric inputs, tidal exchange, polluted rivers, urban runoff, and combined sewer overflows (CSO). In order to determine the relative contribution of each of these sources of heavy metals to an urban estuary, a mass balance of Ag, Cd, Cu and Pb was constructed for the Quinnipiac River/New Haven Harbor system.

New Haven Harbor is an embayment in the central part of the north shore of Long Island Sound and is the most active port in Connecticut. It is located at the mouth of three rivers: the Quinnipiac, Mill, and West. The City of New Haven has a long history of industrial activity, including metal fabrication and finishing, brass manufacturing, and arms production. Its waste water treatment system still employs combined sewers in over 30% of the city, which covers 1,600 ha. The largest river, the Quinnipiac, also has a highly industrialized watershed, and thus is potentially a significant source of metal pollution.

Constructing the mass balance involved determining the magnitude of the various input and output fluxes of metals for the harbor. The difference between 1) the sum of the these various fluxes, and 2) the measured change in storage ΔS, represents any unmeasured production or removal processes, such as ground water exchange:

$$\Delta S = \text{Atm. Dep.} + \text{Rivers} + \text{Industry} + \text{STP} + \text{CSO} - \text{Burial} \pm \text{LIS} \pm \text{Unk} \qquad (1)$$

Where:

ΔS	$\equiv$ change in standing stock
Atm. Dep.	$\equiv$ atmospheric deposition
Burial	$\equiv$ burial in sediments below the zone of active exchange with the water column
Industry	$\equiv$ permitted industrial discharges
LIS	$\equiv$ tidal exchange with Long Island Sound
CSO	$\equiv$ combined sewer overflows
Unk	$\equiv$ unknown sources or sinks, calculated by difference

In this formulation, the upper mixed layer of sediments that actively exchanges with the water column (as defined by short lived radionuclide transport) is define as part of the harbor, while the metals in the deeper sediments are considered to fall outside the boundaries of the system. This allows for the exchange between the water column and sediments to be considered as an internal cycling term.

The surficial sediment layer contains nearly all of the standing stock of metals in the harbor (i.e. when compared to the water column) and can continue to exchange metals with overlying water. This layer is defined as that zone undergoing rapid mixing and can be determined from radionuclide analysis. This technique identifies the rapidly mixed layer on the basis of the penetration of short-lived radionuclides, like ^{7}Be, or an abrupt change in slope of the profile of a long-lived radionuclide, like ^{210}Pb.

Solving for the Unk term required integrating each source and sink term, taking into account variations over a specific period of time. For continuous discharges, such as rivers and STP effluent, rating curves needed to be developed to account for the variations in metal concentrations with discharge. For intermittent discharges like CSOs, a categorical method was established to characterize the different ranges of metal concentrations depending on seasonal and daily variations.

Trace metals occur at very different concentrations in fresh waters, salt waters, waste streams, and sediments, such that each medium requires its own sampling techniques and analyses. While contaminated sediments contain metals in the parts-per-million (ppm) range, typical concentrations of metals in water fall in the parts-per-trillion (ppt) range. Thus, to avoid contamination artifacts, all water sampling was conducted following strict clean protocol. In the case of CSO effluent measurement, initial sampling using clean protocols revealed metal concentrations in the high parts-per-billion (ppb) range. Therefore, to facilitate time-series sampling, all subsequent CSO collections were carried out using an ISCO model 3700 portable autosampler and analyzed using standard clean protocols.

The heavy metal mass balance for New Haven Harbor was constructed covering the period from March 1995 to March 1996. For each source/sink term, a magnitude and associated uncertainty were determined (Figure 1). To increase the confidence level associated with predictability of each term in the mass balance equation, the sampling program used for model development sampled across the full range of natural variability, including seasonal and storm based events. In the case of STP effluent, sampling a wide range of discharges revealed a bi-modal distribution between low and high flows. This is due to a capacity limitation of the sewage treatment plant, which by-passing secondary treatment at high flows.

Several significant relations among the source and sink terms have been documented for an urbanized estuarine system. In New Haven Harbor:

- The Quinnipiac River dominates metal sources terms, and large storms and snow melt accounts for 50% of the total yearly metal flux of the river.
- The Quinnipiac River marsh system removes 25% of the metal flux from the river.
- STP effluent and CSO discharge are minor contributors to the overall metal balance, but may be important for the Ag balance in other systems.
- Ag from STP effluent is clearly evident on the ebbing tide, unlike other metals studied.

While the results from this study revealed that the largest contributor of Ag to the estuarine system is riverine sources, this high Ag flux is probably due to erosion and resuspension of previously contaminated sediments from a defunct upstream defunct silver industry. Using baseflow Ag concentrations, a riverine flux can be calculated which minimizes the effect of industrial Ag contamination. This flux is approximately 20 - 30 % lower and is

more similar to the Ag flux from the STP effluent. In addition, the industrial discharge term is insignificant for New Haven Harbor, but may prove more important for other urban systems.

Being able to quantitatively establish the relative importance of each source and sink term is an important step in establishing management strategies for estuarine systems. In today's budget conscious environment, decision makers need to be able know how their management strategies will affect the overall contaminant balance. For example, it is clear from this analysis that the effects to reduce Ag levels in treated sewage would have little effect on the environmental quality of the Quinnipiac River.

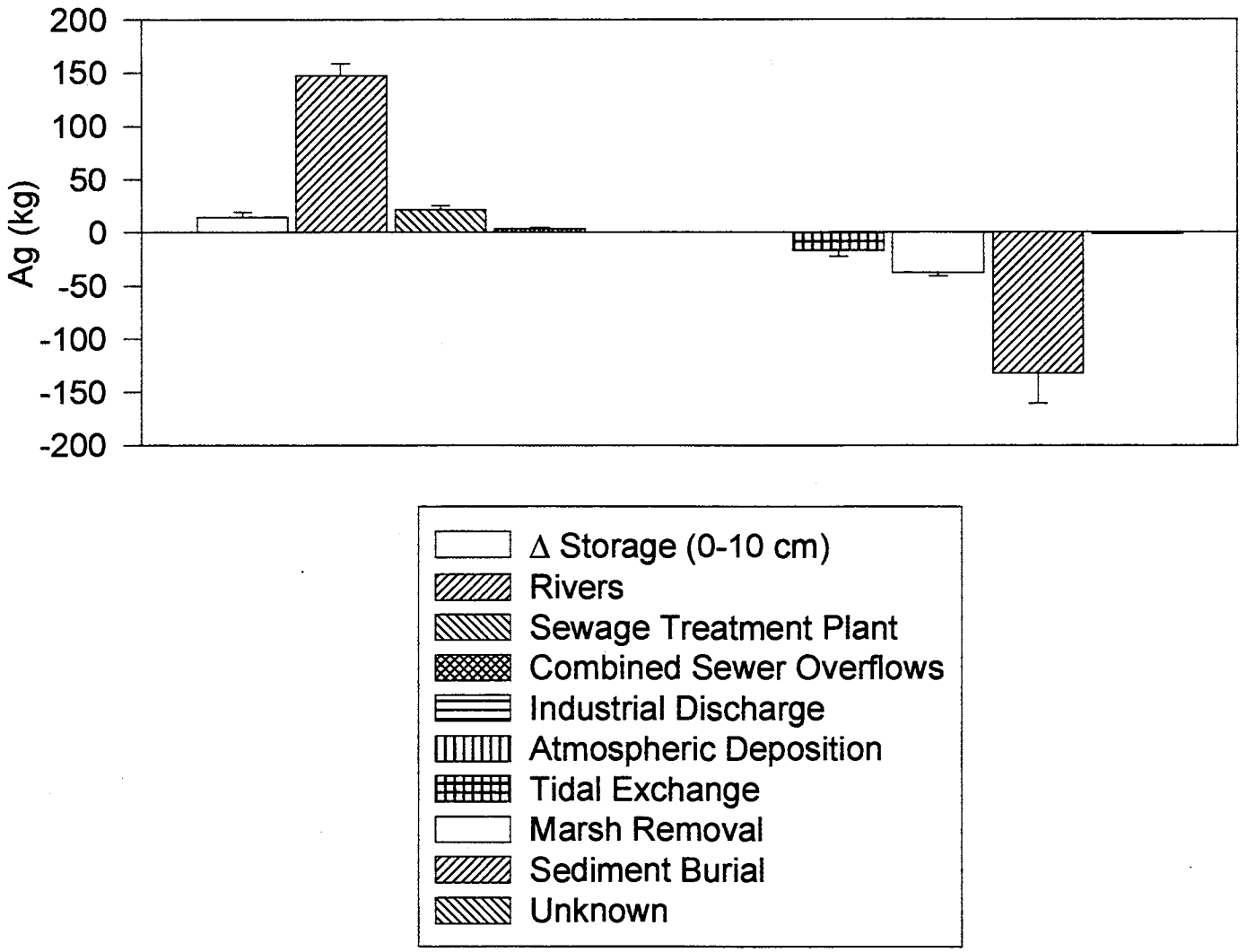

Figure 1. New Haven Harbor silver mass balance for 1995-1996

Questions & Answers: Sources and Sinks of Silver in an Urban Estuary: A Mass Balance Approach

No questions.

Session 2

Environmental Cycling and Distribution of Silver

J. R. Kramer
Session Chair

Application of Radiotracers for Studying Trace Element Accumulation in Mussels

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U.S. Geological Survey, Menlo Park, California, USA

A series of experiments using gamma-emitting radioisotopes has been conducted to quantitatively measure the assimilation efficiencies (AE) of ingested trace metals, the uptake rate of metals from the dissolved phase, and the efflux rate of metals in the mussel, *Mytilus edulis*. AEs ranged between 4-34% for ^{110m}Ag, 1-6% for ^{241}Am, 11-40% for ^{109}Cd, 14-43% for ^{57}Co, 12-72% for ^{75}Se, and 16-48% for ^{65}Zn in mussels ingesting diverse phytoplankton food and natural seston. AEs of all trace elements decreased inversely with mussel ingestion rate. AEs of Cd, Se and Zn were directly correlated with ^{14}C assimilation. AEs of Am, Co and Se also increased with elemental penetration into the algal cytoplasm. Differences of up to 38% in diatom protein content had no major influence on metal AE. Cd assimilation increased with increasing Cd concentration in diatom cells, whereas Zn assimilation was inversely related to Zn concentration in diatom cells.

The calculated dissolved uptake rate constant was greatest for Ag, followed by Zn > Am ≈ Cd > Co > Se. The efflux rate constants for all elements ranged from 0.01 to 0.03 day^{-1}. Metal efflux rates were comparable when measured under laboratory and field conditions. The route of trace element uptake (food vs. dissolved), the duration of exposure to dissolved trace elements (12 h vs. 6 d), and the depuration period did not significantly influence trace element efflux rates.

We have developed a bioenergetic-based kinetic model which uses lab-derived parameters of AE, efflux, and uptake rates from the dissolved phase.

$$C_{ss} = \frac{k_u * C_w}{k_{ew} + g} + \frac{\sum_{j=1}^{n}(AE * IR * C_w * Kd)}{k_{ef} + g}$$

C_{ss} = trace element concentration in mussel soft tissues (μg g^{-1} d^{-1}), k_u = metal uptake rate constant from the dissolved phase (l g^{-1} d^{-1}), C_w = dissolved metal concentration (μg l^{-1}), AE = assimilation efficiency of ingested metal (%), IR = ingestion rate of mussels (mg g^{-1} d^{-1}), Kd = partition coefficient of metal for suspended particles (l kg^{-1}), k_{ew} = efflux rate constant after uptake from the dissolved phase (d^{-1}), k_{ef} = efflux rate constant after uptake from ingested particles (d^{-1}), and g = growth rate constant (d^{-1}).

The model predicts concentrations of Ag, Cd, Se and Zn in mussels that are nearly identical to tissue concentrations measured independently in South San Francisco Bay and Long Island Sound. Sensitivity analysis shows that the metal concentrations in bivalve tissue is directly proportional to total ambient metal concentrations and to AE and that total suspended solids load significantly influences metal bioaccumulation primarily for particle-reactive elements such as Ag and Am. The model predicts that under conditions typical of coastal waters, > 96% of Se in mussels is obtained from ingested food. For Ag, Am, Cd, Co and Zn, the relative contribution from the dissolved phase decreases significantly with AE and with increasing trace element partition coefficients for suspended particles; values range between 33% and 67% for Ag, 5% and 17% for Am, 47% and 82% for Cd, 4% and 30% for Co, and 17% and 51% for Zn.

Reference

Wang, W.-X., N.S. Fisher and S.N. Luoma. 1996. Kinetic determinations of trace element bioaccumulation in the mussel *Mytilus edulis*. Mar. Ecol. Prog. Ser. 140:91-113.

Table 8. *Mytilus edulis*. Model-predicted trace element concentration in mussel tissues (C_{ss}) and its comparison with field-measured concentration ($C_{measured}$) in mussels collected from Dumbarton Bridge, South San Francisco Bay (data from NS&T Program, T. P. O'Connor pers. comm.) and Long Island Sound (data from Goldberg et al. 1983). Seston concentrations at these stations were >7 mg l^{-1}, thus a maximum ingestion rate of 270 mg g^{-1} d^{-1} was assumed for mussels (calculated from Eq. 10). Both the lowest and highest dissolved concentrations were included in modeling (Ag, Cd and Zn dissolved concentration in SFB were from Flegal et al. 1991; Se data was from Cutter 1989). Also shown are the proportion of total body burden of each metal predicted to come from the dissolved phase. See text for detailed discussion of parameters. Sample weights are on dry wt basis

Element	C_w (µg l^{-1})	*Kd*	AE	C_{ss} (µg g^{-1})	$C_{measured}$ (µg g^{-1})	% dissolved
San Francisco Bay						
Ag	0.0026	150000	0.04	0.29	0.35–0.77	57
	0.0097	150000	0.04	1.15		59
	0.0026	150000	0.12	0.54		31
	0.0097	150000	0.12	2.09		33
Cd	0.07	5000	0.1	2.7	4.4–9.4	76
	0.17	5000	0.1	6.8		76
	0.07	5000	0.3	4.1		51
	0.17	5000	0.3	10.1		52
Se	0.025	10000	0.3	1.0	2.5–6.7	3.6
(selenite)	0.065	10000	0.3	2.5		3.6
	0.025	10000	0.7	2.2		1.6
	0.065	10000	0.7	5.6		1.6
Zn	0.5	20000	0.15	54	54–130	52
	1.7	20000	0.15	176		50
	0.5	20000	0.3	80		35
	1.7	20000	0.3	265		33
Long Island Sound						
Ag	0.0038	150000	0.04	0.43	0.04–0.44	58
	0.0044	150000	0.04	0.51		58
	0.0038	150000	0.12	0.80		31
	0.0044	150000	0.12	0.93		31
Cd	0.074	5000	0.1	2.9	1.5–6.2	76
	0.120	5000	0.1	4.8		76
	0.074	5000	0.3	4.3		51
	0.120	5000	0.3	7.0		51
Zn	0.315	20000	0.15	34	52–142	52
	1.000	20000	0.15	105		51
	0.315	20000	0.3	51		36
	1.000	20000	0.3	157		34

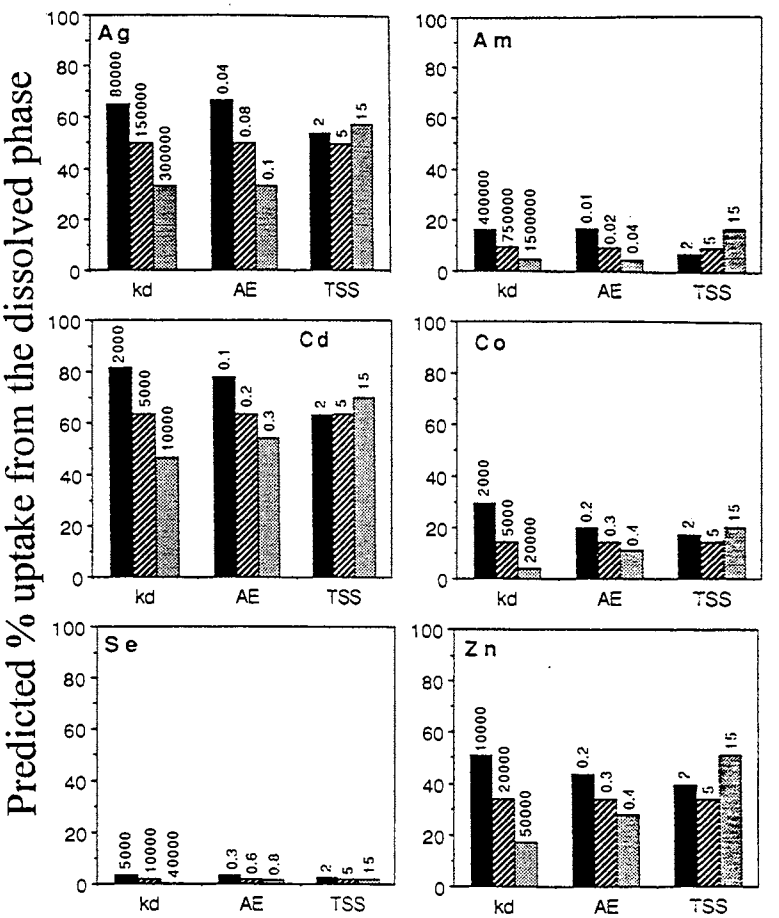

Fig. 10. *Mytilus edulis*. The calculated proportion (%) of the total trace element concentration in mussels from the dissolved phase over a range of different partition coefficients (*Kd*), assimilation efficiencies (AE), and seston (TSS) loads (2 to 15 mg dry wt l^{-1}). The range of *Kd* and AE values used are specific to each element and are based on observed values (see also Tables 6 & 7). Calculations for each parameter (e.g. *Kd*) assume intermediate values of the other 2 parameters (e.g. AE and TSS load) shown in the figure. See text for further explanation

Questions & Answers: Application of Radiotracers for Studying Trace Element Accumulation in Mussels

Q. DAVID ARMSTRONG (University of Wisconsin): I think you said that DOC had very little effect on the influx rate of the dissolved metal. I was wondering what your interpretation of that was.

A. That came as a bit of a surprise. We took some Long Island Sound water, 28 ppt salinity, and we divided it in half. This was filtered through a 0.2-micron polycarbonate membrane. Then half of the water was not amended, and half of it was UV-irradiated. We removed the DOC to concentrations of about 0.2 mg/L. Prior to the UV oxidation, I think the DOC was about 3 or 4 mg/L. We then exposed the mussels in either UV-irradiated or non-UV-irradiated water, and we only saw a small effect, but it was very small. It was on the order of 10 percent difference, in the direction that you would expect (that is, the UV-irradiated water produced slightly higher influx rate constants), but I'm not even sure they're statistically significant. So when I'm talking about DOC here, I'm talking about that which can be photo-oxidized with the Armstrong method.

Q. JIM KRAMER (McMaster University): Given the discussion this morning about colloidal phases, how should we interpret "dissolved" in your talk?

A. My definition of dissolved is less than 0.2 microns. I have some data which I could share at another time; we have specifically looked for evidence of colloidal association of silver in different parcels of seawater from around Long Island (estuarine water and full strength seawater), and, unlike many other metals, we see no evidence of colloidal silver (by "no" I mean less than one percent). So that which passes through a 0.2 micron filter, which we have traditionally called dissolved, and that which passes through a 1 kDa ultrafilter, using an Amicon stirred cell, you get virtually the same response, and we size-fractionated the colloids at 1, 50 and 300 kDa. For silver we saw no evidence, but for other elements there was pronounced association with colloids.

Reassessment of Silver Concentration Trends in the Coastal U.S.

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Previous work suggested low and variable silver recoveries in marine mussel and oyster tissue. For this reason, previously determined trends of silver concentrations in the marine environment are suspect. NOAA Mussel Watch samples from 1986-93 were originally analyzed during the collection year after HNO_3 or HNO_3-$HClO_4$ digestion. Archived samples were reanalyzed in 1995 using HNO_3-HCl and ICPMS. Results suggest that less than half of the reanalyzed samples have Ag concentrations withing 20 percent of the original values. A large number of samples had significantly higher concentrations after reanalysis. For trend detection, the Spearman correlation coefficients of all sites with at least six data points have been calculated. It was found that, on the average, there are more sites with decreasing than increasing Ag concentrations. These trends are particularly strong for the Northeastern U.S.

Questions & Answers: Reassessment of Silver Concentration Trends in the Coastal U.S.

Q. CHRISTIAN KRAHFORST (University of Massachusetts): I was wondering what the response time is for accumulation in shellfish to environmental changes. Do you have any idea what the time scales are on that?

A. Yes. Nick [Fisher] has done quite a bit of work on that. For oysters it seems that they accumulate silver and it never comes out; it just stays there. Mussels seem to depurate silver a lot faster. Am I right? [Nicholas Fisher answers in the affirmative] So we have a problem: we cannot collect mussels everywhere along the U.S. coasts. South of Delaware Bay we have to go to oysters, all the way to the Gulf of Mexico. For that part of the world we have to depend on oysters, and silver doesn't seem to cooperate very well.

Sediment Quality Criteria for Metals: Implications for Silver Regulation

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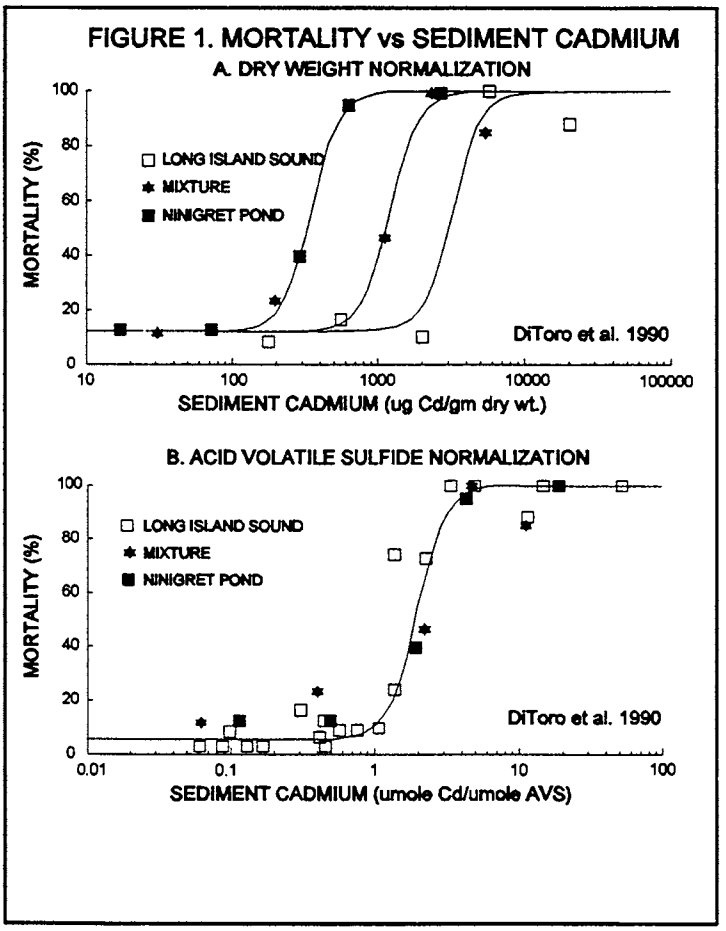
Bioavailability must be a primary consideration in the development of sediment quality criteria (SQC) for metals. Various studies have shown that the relationship of toxicity to total metal concentrations can vary greatly among different sediments (e.g., Figure 1A). However, the relationships among different sediments are often very similar when toxicity is expressed relative to interstitial (pore) water concentrations. A key factor controlling metal concentration in pore water is sulfide, which forms low solubility precipitates with a number of the cationic metals of environmental concern, including silver. Short-term (10 d) laboratory studies with a variety of marine and freshwater benthic organisms have demonstrated that when AVS concentrations in spiked or field-collected sediments exceed those of metals simultaneously extracted (SEM) with the AVS, interstitial water metal concentrations remain below those predicted to cause effects, and toxicity does not occur (e.g., Figure 1B). Similar observations have been made in life-cycle laboratory toxicity tests with amphipods and chironomids in metal-spiked marine and freshwater sediments. In addition, field colonization experiments with metal-spiked freshwater and marine sediments, varying in length from several months to more than one year, have demonstrated a lack of biological effects when there is sufficient AVS to limit metal concentrations in interstitial water. It should be noted, however, that the relationship of AVS to metal toxicity is rendered somewhat uncertain and variable by the association of



metals with other solid phases (such as organic carbon and metal oxides), by temporal and spatial heterogeneity of AVS concentrations, by the role of heavy metals in the regulation of AVS concentrations, and by the control organisms have over their own exposures due to their location in the sediment and effects on their microenvironment.

Information from several recent studies on metal bioavailability and toxicity in sediments is being used as the basis for formulating SQC for cadmium, copper, nickel, lead, and zinc. Four approaches for deriving criteria have been proposed (Ankley et al. 1996). First, if interstitial water dissolved metal concentrations ($[Me_d]$) are measured, "interstitial water criteria toxicity units" (IWCTU) can be computed by dividing the concentration of each metal by its water quality criteria chronic value (FCV_d). Concentrations are considered lower than those like to produce unacceptable effects if the sum of IWCTUs is less than one:

$$\sum_i IWCTU_i = \sum_i \frac{[Me_{i,d}]}{FCV_{i,d}} \leq 1$$

Second, if SEM concentrations are measured, SQC can be based on the expected effects of AVS on bioavailability. Because of the low solubility of metal sulfides, interstitial metal concentrations should be below toxic levels if the molar AVS concentration exceeds the sum of the molar SEM concentrations of the toxic cationic metals present:

$$\frac{\sum_i [SEM_i]}{[AVS]} \leq 1$$

Third, toxicity can be absent even when AVS < $\Sigma[SEM_i]$ if adsorption to organic matter limits the dissolved metal concentration. The amount of each metal <u>not</u> incorporated into sulfides ($\Delta[SEM_i]$) can be estimated based on the relative amounts of AVS and SEM and the solubilities of different metal sulfides. The interstitial metal concentrations (and thus the IWCTUs) then can be estimated based on the sediment organic content (f_{oc}) and metal partition coefficients for organic matter (K_{oc}):

$$\sum_i \frac{\Delta[SEM_i]}{K_{oc,i} f_{oc} FCV_{i,d}} \leq 1$$

Fourth, for sediments with low AVS and organic carbon, the interstitial metal concentrations (and thus the IWCTUs) can be similarly estimated based on a minimal value expected for the partition coefficient between water and sediment solids:

$$\sum_i \frac{[SEM_i]}{K_{min,i} FCV_{i,d}} \leq 1$$

For a number of reasons, SQC derived using these approaches generally should be considered to be "no effect" values; i.e., with these techniques, it is possible to predict when sediment metals should not be toxic, but not necessarily when metal toxicity will be manifested.

Although not included in current proposed SQC, the theoretical underpinnings for deriving sediment criteria for silver are similar. The solubility of silver sulfide is actually lower than the metals mentioned above; in fact, it is low enough that silver sulfide is generally not measured under AVS procedures. Thus, silver should be least likely among these metals to directly contribute to toxicity. However, to the extent that silver will reduce the amount of AVS available to form other metal sulfides, it could potentially contribute to the toxicity of sediments by increasing the available concentrations of other metals. Investigations of the chemistry and toxicity of silver in sediments are needed to determine how sediment quality criteria should be set.

References:

DiToro, D.M., J.D. Mahony, D.J. Hansen, K.J. Scott, M.B. Hicks, S.M. Mayr, and M.S. Redmond. 1990. Toxicity of cadmium in sediments: the role of acid volatile sulfide. Environ. Toxicol. Chem. 9:1487-1502.

Ankley, G.T., D.M. DiToro, D.J. Hansen, and W.J. Berry. 1996. Technical basis and proposal for deriving sediment quality criteria for metals. Environ. Toxicol. Chem. (In Press).

Questions & Answers: Sediment Quality Criteria for Metals: Implications for Silver Regulation

Q. NICHOLAS FISHER (SUNY-Stony Brook): It seems to me that it's implicit in what you've been talking about that the dominant source of the silver, or the metal, is the dissolved phase. That's probably true for the amphipods, where you're expressing toxicity in the toxicity studies. However, if you have deposit-feeding animals, or even some suspension-feeders that might filter and ingest particles that are resuspended by tidal action, or what have you, the ingested particulate matter may well be a source of metals for those organisms which could then pass them on to diving ducks or fish or something like that. In fact, we've been finding that at least both deposit-feeding and suspension-feeding marine bivalves are able to assimilate silver and other metals from contaminated sediment.

A. I agree fully. That's why I threw that editorial comment in earlier when talking about this, because it is a concern of mine. Except for providing some assistance to the sediment toxicity group at the lab, my work has mainly been involved in water-column toxicity, and we've run tests with both copper and lead which indicate that there is, for certain filter feeding organisms and even some embryonic fish, that potential effect from the solid phase. That's exactly why I threw that in; the scope of organisms and conditions, especially for chronic toxicity, that have been looked at are in fact rather limited. It's an impressive scope, when you look at the mass of the information. A variety of organisms have been tested, and a lot of these tests are fairly lengthy. I think there's an impressive set out there which shows a good correlation to the interstitial water concentration. And it's not just the correlation of the interstitial water concentration, because if the food is correlated to the interstitial water concentration, you get the same correlation. But it's correlated to water-only exposures with those organisms, and so I think it's an area of uncertainty which I would identify as a research area that should be better resolved.

Q. KOSTAS DASKALAKIS (NOAA/NOS/ORCA): You said earlier that as long as there is a little acid volatile sulfide (AVS), we shouldn't expect any toxicity. Now, if there is a ligand there that binds to the metals — and we know of ligands that are stronger than plain sulfide — then we will have a lot of metal in solution, so that statement would be a little simplistic. Would you like to elaborate on that?

A. Yes. First of all, when I said a little AVS, that was pertaining to silver since it appears that the measurement for AVS doesn't measure silver sulfide. So if there's any AVS at all, that's the same as it being in excess of the silver. In fact, if there's a ligand that binds silver that can create an appreciable dissolved concentration, you would have an appreciable dissolved concentration, but you would also have to have a ligand there, as per Dr. Campbell's talk earlier, that also contributes to the toxicity, because if you increase the dissolved concentration just by having a ligand that's not available, then the AVS is still maintaining the bioavailable fraction or metal activity or whatever we want to relate it to, to nontoxic levels. So it would have to be a complex that is itself available and toxic, and present in appreciable amounts.

Q. KOSTAS DASKALAKIS: And you saw that this is the case?

A. Well, partly from some of the work I've done, we still have some concerns along those lines. So yes, as with the particulate case, there are cases that I don't think can be dismissed that raise issues. It's just that on the data set that's been developed so far on a variety of organisms, we have not found that case yet where you have toxicity when you have metal less than the AVS.

Q. JIM KRAMER (McMaster University): Nick Fisher brought up the point of a nonsoluble phase, and we can argue what that is. However, in terms of the simulation, isn't it also going to be important to know the quality

of this nonsoluble material, whether it's readily available when it gets into this filter feeding system, or what? I guess the question is, do we know anything about this?

A. I wouldn't be able to answer that. The nature of the particulate phase should have some importance, but I don't know of any hard data for the organisms that I deal with [Nick Fisher comments off-microphone, and Russell Erickson repeats his answer] I guess the answer was to the effect that the organic coating on particles is a critical factor in the assimilation efficiency. I would add that some of the organisms that we deal with in fresh water, like the filter-feeding cladocerans, are quite selective in what they will take, as far as what will actually be ingested in terms of suspended particles.

Effect of Chloride, Hardness and Dissolved Organic Carbon on Silver Solubility in Aquatic Toxicity Tests

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Abstract

Silver solubility was measured over a four day period during toxicity tests. In these systems, silver, water hardness, chloride, and dissolved organic carbon were controlled. Total silver and silver that passed through a 0.45 μm filter were measured by graphite furnace atomic absorption spectrometry. Hardness had no observable effect on silver solubility. DOC had pronounced effects on silver solubility. Apparently DOC bound the silver, keeping it suspended in solution, but did not allow passage through 0.45 μm filters. The DOC was most noticeable factor affecting soluble silver concentrations when added silver was 2-10 ng/ml. When 20 ng/ml silver was added to test systems, chloride and DOC caused reductions in silver solubility which were similar in magnitude. Chloride had a larger effect on silver solubility than did DOC in solutions receiving 40 ng/ml of silver.

Introduction

Silver availability is a critical component when developing an understanding of the toxicological responses manifested by organisms exposed to effluents containing silver in the environment. The physical state of silver is a key to understanding availability. Evaluating total silver in aqueous solutions and silver that passes through a 0.45 μm filter should provide an indication of differing silver availability to aquatic organisms. If concentrations other constituents in the water are varied, the fraction of the total silver that will pass through a 0.45 μm filter should change.

During toxicity tests, the effects of silver and other constituents of the water were evaluated for their effect on mortality of fat head minnows *Pimephales Promelas* and *Daphnia magna*. Silver presence in size fractions less than 0.45 μm and greater than 0.45 μm was evaluated to determine dependence on other constituents in the water and to evaluate the to overall effect of silver fractionation on the toxicity of silver to aquatic organisms.

Methods

Aqueous solutions were prepared beginning with Milli-Q water to produce varying concentrations of chloride, hardness, alkalinity and dissolved organic carbon (DOC). These solutions were used in toxicity tests to determine the effect that 6 silver concentrations had on fat head minnows and Daphnia magna in aqueous solutions of different composition (Table 1).

Table 1. Nominal chemical concentrations used to determine effects of solution chemistry on silver toxicity.				
Species	Chloride mg/l	Hardness mg/l	DOC mg/l	Silver µg/l
Pimephales Promelas	0, 3, 20, 40, 60	50, 100, 200	0, 5, 10	0, 2, 5, 10, 20, 40
Daphnia magna	0, 3, 10, 40, 60	100, 200	0, 1, 2, 5	0, 0.5, 1, 2, 3.5, 5

Samples were analyzed for chloride, hardness, DOC, and silver at Day 0 (D0) and Day 4 (D4) of the toxicity test. Chloride was determined by ion chromatography, and hardness was determined by titration. Water samples for DOC and for silver analyses were acidified to pH=2 upon collection and stored in precleaned plastic jars until analyzed. DOC analyses were conducted by spectroscopic determination of carbon dioxide evolution following chemical oxidation of dissolved organic matter in each solution. All silver analyses were all completed within 72 hours of samples collection and most were completed within 48 hours. Soluble silver was determined in aliquots filtered through a 0.45 µm membrane and total aqueous silver was determined in unfiltered aliquots. Aqueous samples were introduced by an autosampler into a graphite furnace atomic absorption (GFAA) spectrophotometer. Each injection contained 35 µl of sample and 15 µl sodium phosphate modifier. Five point calibrations were performed prior to sample analysis each day and after every 50 sample analyses. A blank samples was analyzed after every tenth sample.

Results and Discussion

Filtered and unfiltered samples contained highly correlated silver concentrations as did samples collected on D0 and D4 (Figures 1-4). Multiple regression techniques showed that other components in the test solutions contributed to observed silver concentrations (Equations 1-5). These equations use bold type to denote parameters that are significant in predicting the dependent silver concentration variable. The soluble silver concentration in D0 samples was dependant on initial silver concentration. The amount of total silver remaining in solutions after four days was dependant on initial silver concentrations chloride and DOC. The soluble silver in D4 samples was dependant on initial silver, chloride, hardness and DOC. Soluble silver concentrations in solution on D4 were also explained by soluble silver concentrations on D0, chloride, and DOC. DOC did not seem to effect the correlation of soluble silver in D4 samples to total silver in D4 samples. These correlations may be somewhat misleading as they were determined data from all silver concentrations. When evaluating silver behaviors within a more limited silver concentration range, possible differences are evident although the number of samples

in a given treatment is too small to provide adequate statistical power for significance testing (Figures 5-10). These figures show large differences in soluble and total silver concentration dependencies at the two nominal concentrations (10 an 40 μg/l) depicted.

$$[Ag]_{0F} = \mathbf{0.31} + \mathbf{0.87}[Ag]_0 - 0.01[Cl] + 0.005\,[Ca] - 0.05[DOC] \quad \text{[Eq 1]}$$

$$[Ag]_4 = \mathbf{-1.47} + \mathbf{0.85}[Ag]_0 - \mathbf{0.29}[Cl] - 0.003\,[Ca] + \mathbf{0.39}[DOC] \quad \text{[Eq 2]}$$

$$[Ag]_{4F} = \mathbf{-0.54} + \mathbf{0.61}[Ag]_0 + \mathbf{0.06}[Cl] - \mathbf{0.01}\,[Ca] + \mathbf{0.18}[DOC] \quad \text{[Eq 3]}$$

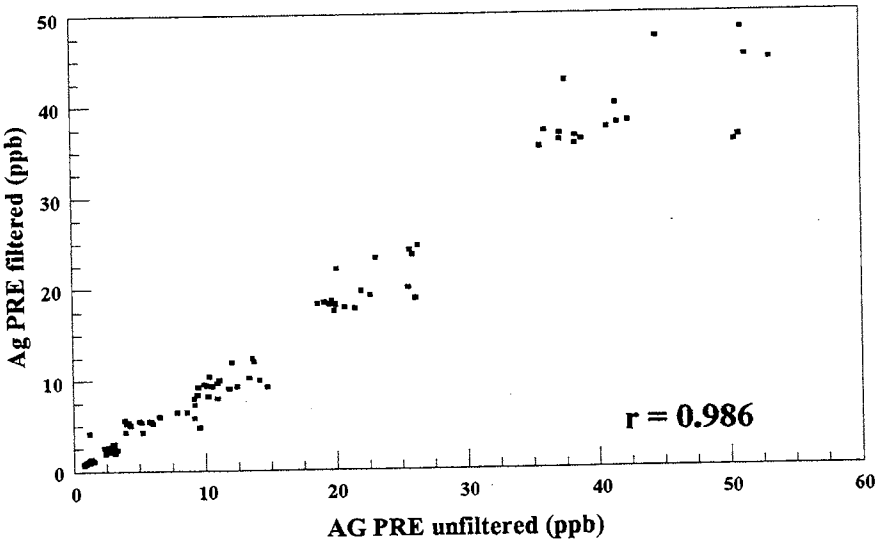
$$[Ag]_{4F} = -0.33 + \mathbf{0.66}[Ag]_{0F} + \mathbf{0.06}[Cl] - 0.01\,[Ca] + \mathbf{0.22}[DOC] \quad \text{[Eq 4]}$$

$$[Ag]_{4F} = \mathbf{-0.61} + \mathbf{0.71}[Ag]_4 + \mathbf{0.04}[Cl] - \mathbf{0.007}\,[Ca] + 0.09[DOC] \quad \text{[Eq 5]}$$

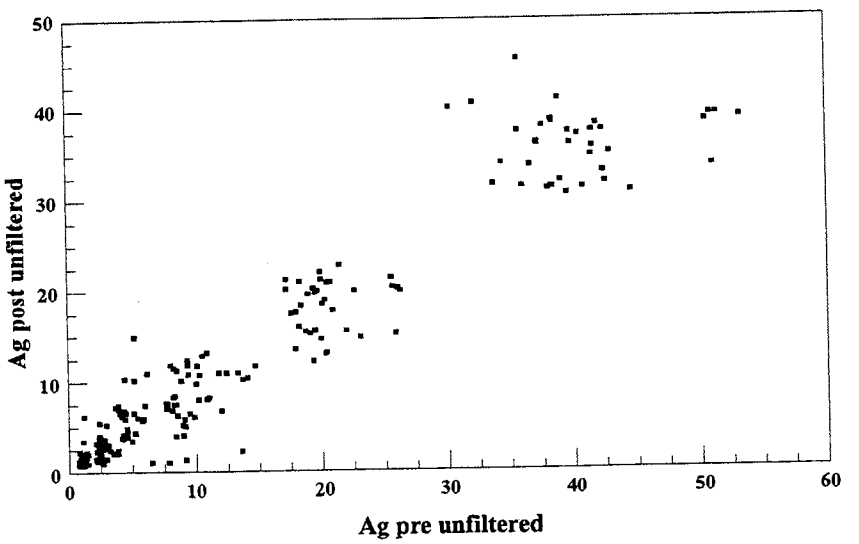
Soluble silver concentrations appear to be controlled primarily by initial silver concentration. DOC is the factor with the next highest weighting, with chloride explaining a minor amount of the dependence. Hardness was inconsistent as a factor explaining soluble silver. Both chloride and hardness are factors with weightings that are equal to or less than the error in the regressions. By comparing total silver on D0 to total and soluble silver on D4, it is obvious that quite a bit of suspended silver chloride was present in the D4 solutions as was silver bound to organic carbon. This can be seen from the large change in weightings for chloride and DOC in Eqs 2 and 3.

The DOC was most noticeable factor affecting soluble silver concentrations in solutions measured four days after test initiation and containing nominal silver concentrations of 2-10 ng/ml. When 20 ng/ml silver was added to test systems, chloride and DOC caused reductions in silver solubility which were similar in magnitude. Chloride had a larger effect on soluble silver four days after test initiation than did DOC in solutions receiving 2-10 ng/ml of silver. The trend was reversed for solutions receiving 40 ng/ml silver.

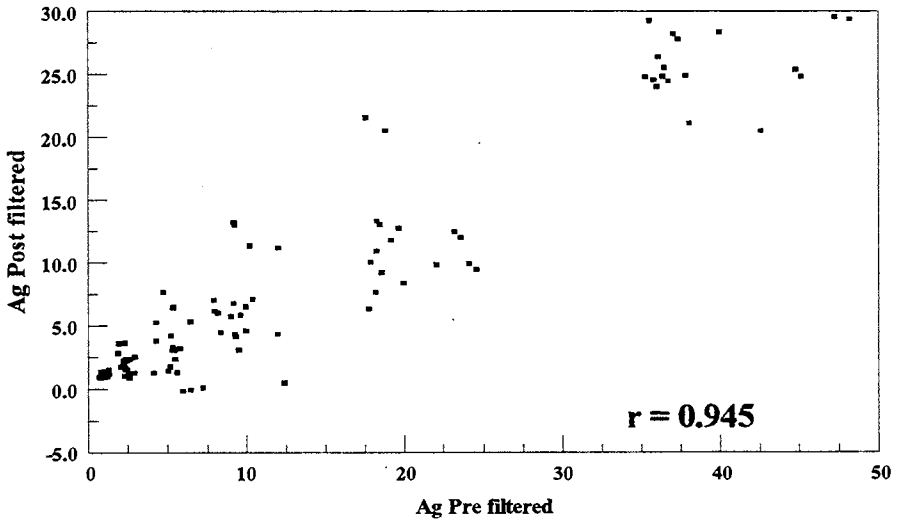
Initial Total and Dissolved Silver Concentrations



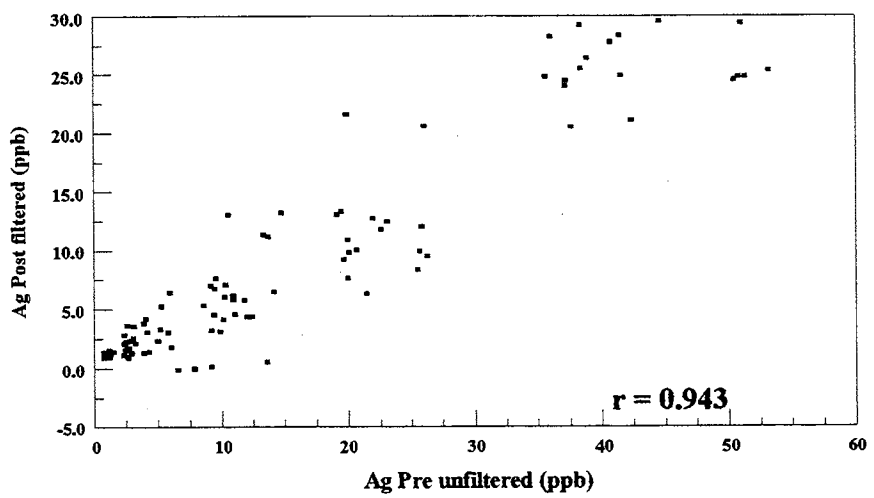
Relationship Between Total Silver Collected at 4 Day Intervals



Relationship Between Soluble Silver Concentrations



Soluble Silver Remaining on Day 4 versus Initial Total Silver



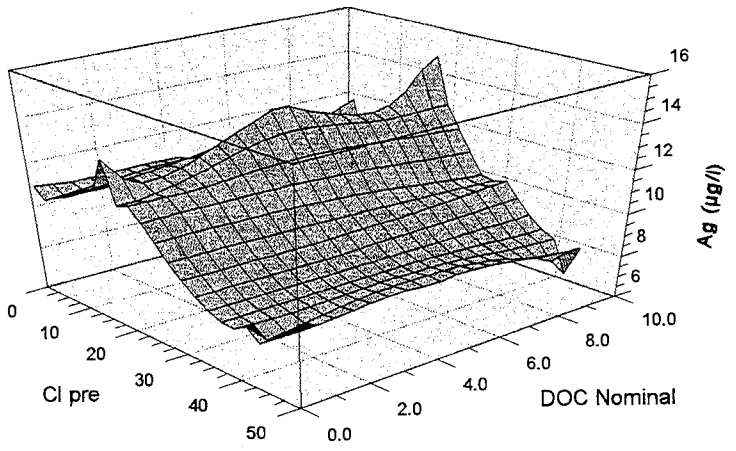


Figure 5. Effect of DOC and chloride on total silver in solutions prior to toxicity testing. Nominal silver concentrations were 10 µg/l.

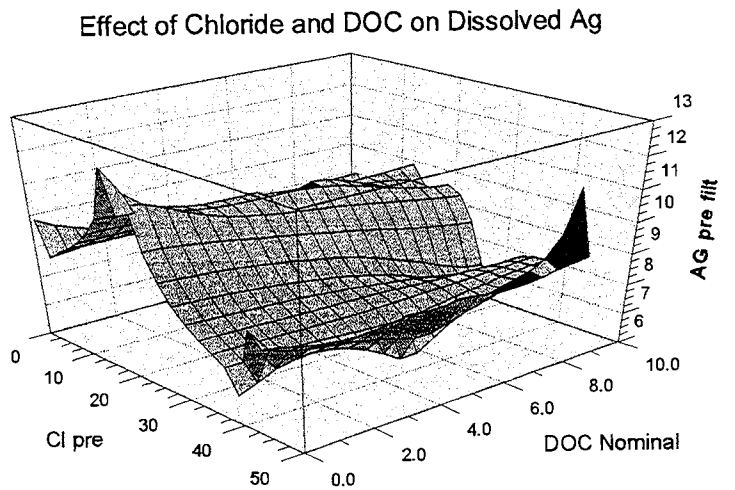


Figure 6. Effect of DOC and chloride on soluble silver at initiation of testing. Nominal silver concentrations were 10 µg/l.

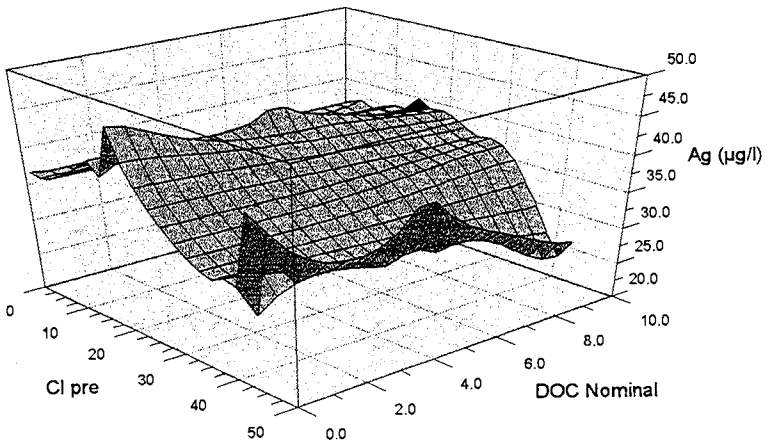


Figure 7. Effect of DOC and chloride on soluble silver at the beginning of testing. Nominal silver concentrations were 40 µg/l.

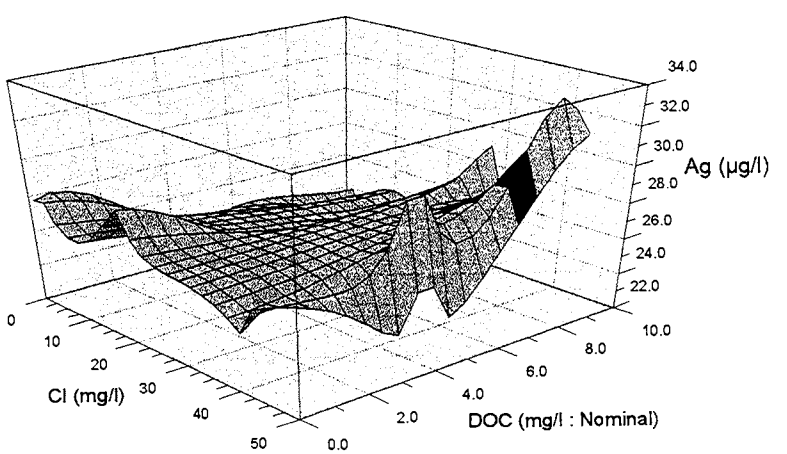


Figure 8. Effect of DOC and chloride on dissolved silver 4 days after test initiation. Nominal silver concentrations on Day 0 were 40 µg/l.

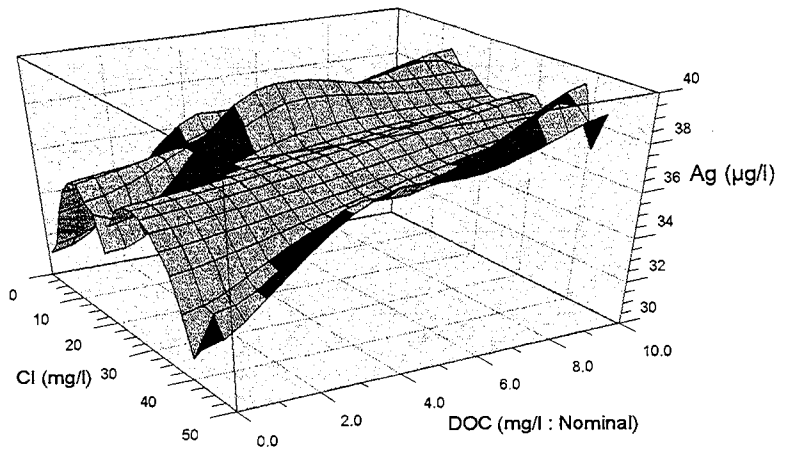


Figure 9. Effect of DOC and chloride on total silver 4 days after test initiation. Nominal silver concentrations were 40 µg/l.

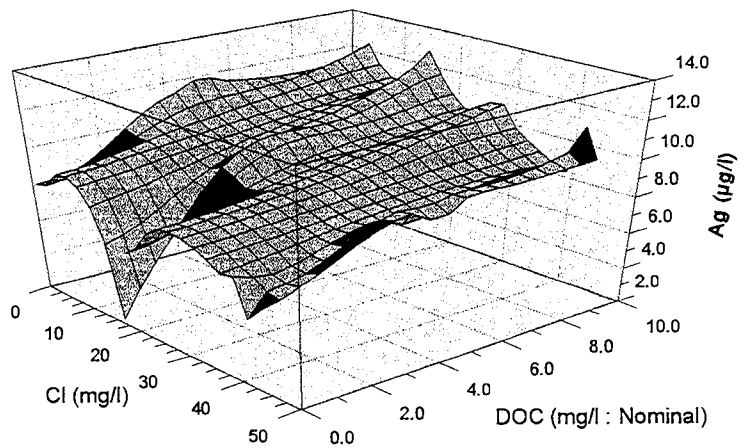


Figure 10. Effect of DOC and chloride on total silver concentrations 4 days after test initiation. Nominal silver concentrations were 10 µg/l.

Questions & Answers: Effect of Chloride, Hardness and Dissolved Organic Carbon on Silver Solubility in Aquatic Toxicity Tests

Q. ANDERS ANDREN (University of Wisconsin): Did you at any time try to understand these things mechanistically? In other words, did you try to do some modeling on complexation, and try to tease out some complexation constants to see whether things indeed made sense, chemically speaking?

A. Yes, we did. We're using some of the EPA models. The ones that we had at the time did not handle DOC well at all, so I wasn't confident in the information that was coming out. We just recently got a better version of the MINTEQ, or MINEQL model, and I hope to be running that soon, but I have not yet gotten those models that I feel confident will handle the DOC component adequately.

Q. ANDERS ANDREN: Presumably, though, you could make some guesses at stability constants and include them in there just to see whether it would work.

A. I did not do that with the DOC. I tried to run things ignoring the DOC, and things did not make sense at that point, and I did not add those back in.

Q. ANDERS ANDREN: The other question I have is that your silver concentrations probably are about 2 to 4 orders of magnitude higher than we see in most natural waters, and I wonder whether you expect to see the same surfaces and chemistry at 2 to 4 orders of magnitude lower concentrations?

A. It's hard to speculate. As you saw, from low concentration to high concentration in the experiments that we ran, the surfaces changed dramatically. So I would not expect the same type of surfaces at those very low concentrations. The rationale for conducting the experiments at the silver concentrations we were using were to evaluate the water quality criteria, if you will, and see if they were protective of aquatic species in freshwater systems in the continental U.S., and, hopefully, Canada as well.

Q. TOM BOBER (Eastman Kodak): What is the source of your DOC, and would you expect different results if you got your DOC from a different source?

A. Our DOC was a humic acid from Aldrich, and I would expect a different result with almost any type of humic acid used, based on my evaluation of the literature. Different natural sources will give you different results, and different vendors of humic acid or fulvic acid will give you quite varied results, and I believe we heard a paper earlier this morning addressing that issue.

Fate of Silver in Surface Waters, Sediments and Plant Material in an Old Mining Camp, Cobalt, Ontario

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Introduction

The Cobalt Mining Camp represents an interesting field site to study the mobility and fate of silver. In the immediate area surrounding the town of Cobalt are numerous 30-60 year old mine tailings, containing some of the highest levels of Ag found in the environment (see map). The present study is a follow-up to an earlier study (see Argentum II) that showed nondetectable concentrations (<10 ng/L) in most waters and rapid uptake in roots of plants. In this study, we used ultra-clean protocols to resample and analyze for Ag with a detection limit of 1.5 ng/L. As well, we have examined the effect of colloids by comparison of 0.2 μm and 10 kDalton filtered samples.

Cattails and indigenous grasses, along with garden plants grown on tailings, were sectioned in detail in order to gain further knowledge of the uptake and storage of Ag from contaminated soils.

Methods

Low-level trace metals techniques were used to avoid contamination and to provide sensitive and reliable analyses. Procedures for the preparation of sampling apparatus, processing of water samples, and analysis by ICP-MS were adapted from protocols used by the Lake Michigan Tributary Monitoring Project (LMTMP) (Hurley et al. Environ. Sci. Technol. (1996), 30, 2093-2098). Water samples were syringe filtered through 5mm diameter Millipore 5μm Durapore and 0.2μm polysulfone syringe filters. A third sample was syringe filtered through the 0.2μm filter into a Millipore 10kDalton Ultrafree®-15 centrifugal filter, which was filtered at the end of each sampling day (maximum 8 hour delay). Filtered samples were acidified in the field with ultrapure HNO_3 containing two rare metals (Y, Th) used to determine recovery on each sample. Ultratrace metals analyses were performed on a PE-Sciex Elan-500 ICP-MS. Table 1 summarizes the performance data for the sampling and analyses.

Table 1 Performance Data

field blanks and replicates on 10% of samples

detection limit in Laboratory: 1.5 ng/L
detection limit for field samples: 5 ng/L

relative standard deviation:

15% for samples below 25 ng/L
10% for samples 25-100 ng/L
5% for samples >500 ng/L

Mean difference for replicate field samples: 12 ±9%
Yttrium field spike (~3 µg/L) recovery: 104 ±10%

Cattails, grasses, and garden plants were thoroughly washed with milli-Q water, sectioned, and dried at 60°C. 5g samples were digested in ultrapure HNO_3 and analyzed directly by ICPMS. Sediments and soils were acid leached with ultrapure 6N HNO_3.

Results

Surface Waters

Figure 1 summarizes results for Cart Lake. Sample 2A was taken from the South End where surface water flows onto the tailings. 2B was collected from a stream flowing north along the north west edge of the tailings pond. The 2C sample was taken from the North End of Cart Lake which is covered ~1m of water.

Figure 2 shows results for the Sasaginaga-Mill-Farr Creek system. Samples 1A-1D were taken at increasing distances from the sewage (untreated) outfall for the town of Cobalt. Location 1E is at the confluent of Sasaginaga Creek and the sewage water (approximately equal flows). Sample 1F was taken along Mill Creek. 1G was taken at the confluent of the outflows from Crosswise Lake and Peterson Lake (Farr Creek), and 1H was taken where Mill Creek joins Farr Creek. Sampling locations 1A through 1F are on relatively young tailings (<30 years), while 1G and 1H sit on some the oldest tailings in the Cobalt area.

Vegetation

Figures 3a and 3b show the upper and lower values along with the median value (n=6) for cattail and grass segments. Figure 4 shows results for garden plants grown on Cart Lake tailings enriched with red loam and manure.

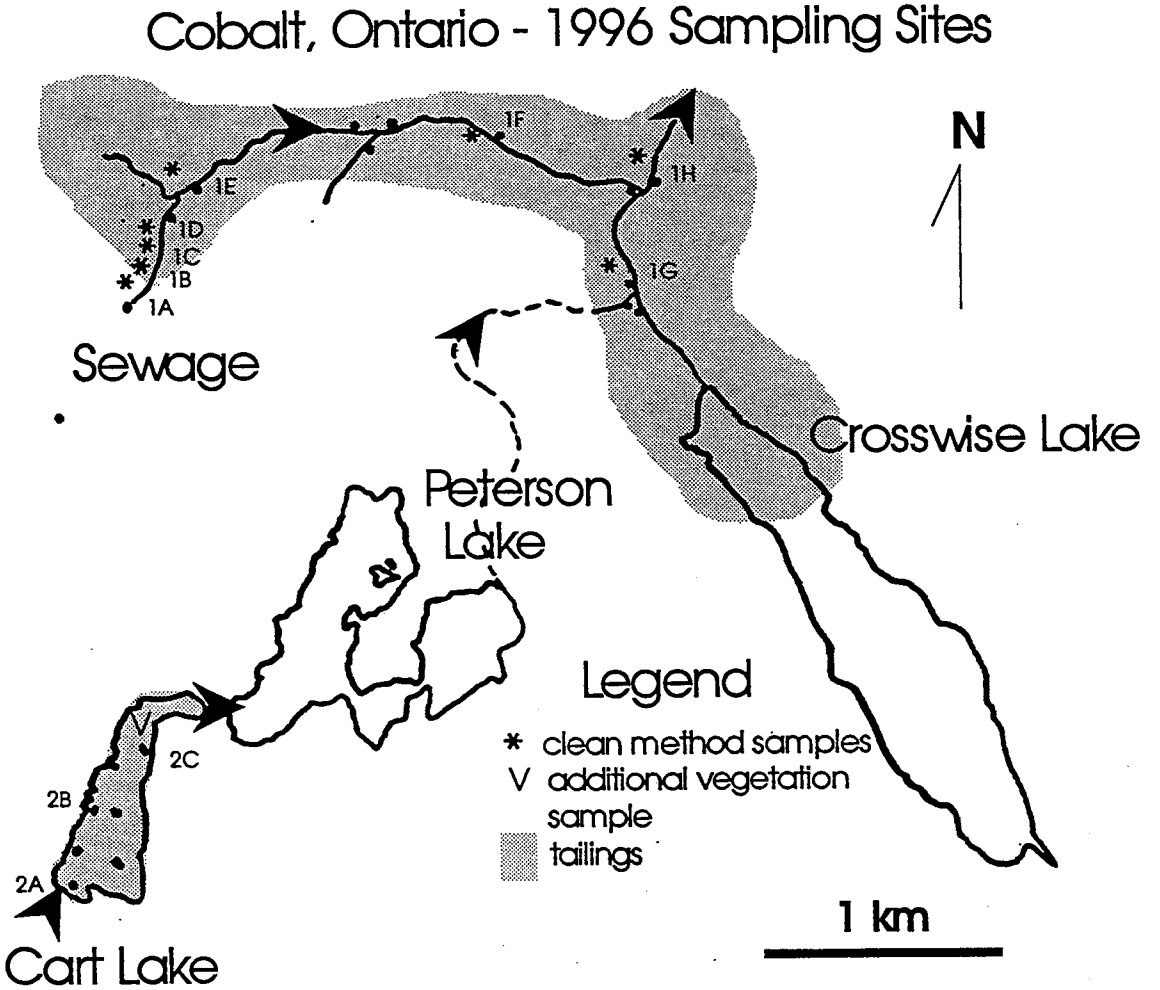
Discussion

Results from samples taken at Cart Lake (Figure 1) and samples taken at locations 1A through 1F (Figure 2) suggest that oxic surface waters flowing over tailings elevate Ag levels by at least an order of magnitude in the 5 and 0.2 μm filter fractions. However, no change from background is observed in the 10kDalton size fraction, indicating that Ag associated with colloidal material or humic matter is probably responsible for the elevated Ag levels at these sample locations.

The low levels of Ag observed at location 1G may be due to the age of the Crosswise Lake tailings, but may also be due to the lower energy flow at this site. Levels at 1H are possibly explained by assuming a mixing of Mill and Farr Creeks (see 1F and 1G).

Generally, plants growing on the tailings show an accumulation of Ag in the roots. Cattails (see Figure 3a) exhibit significant bioaccumulation in the roots, minimal accumulation in the bottom and mid-section of the shoots with more accumulation in the tips (upper 10cm) which are the oldest section of the shoots. Garden plants collected from the North End of Cart Lake (see Figure 4) display a variety of accumulation responses. Again roots seem to accumulate more Ag than other parts of the plants. Beet roots were found to have the highest levels of Ag of all the garden plants; however, levels found in the root tissue were approximately half of the leachable concentrations found in the sediment amended with red loam and manure.

In summary, the 10kDalton "soluble" Ag is at or below the level of detection (5 ng/L). Vegetation exhibits some accumulation of Ag; magnification of Ag appears to be most pronounced in the roots of cattails and local grasses.


Cobalt, Ontario - 1996 Sampling Sites
N
1A
1B
1C
1D
1E
1F
1G
1H
Sewage
Peterson Lake
Crosswise Lake
Legend
* clean method samples
V additional vegetation sample
tailings
1 km
2A
2B
2C
V
Cart Lake

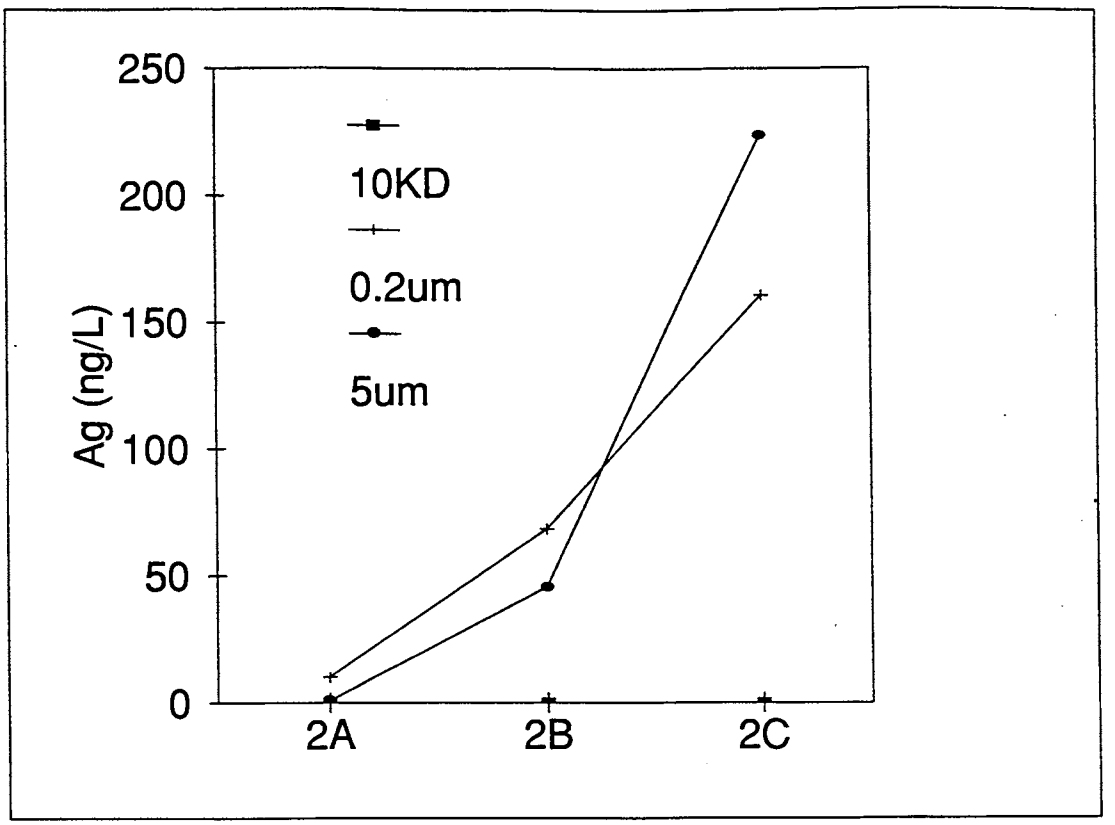


Figure 1. Ag levels in various filter fractions for surface waters flowing over Cart Lake tailings.

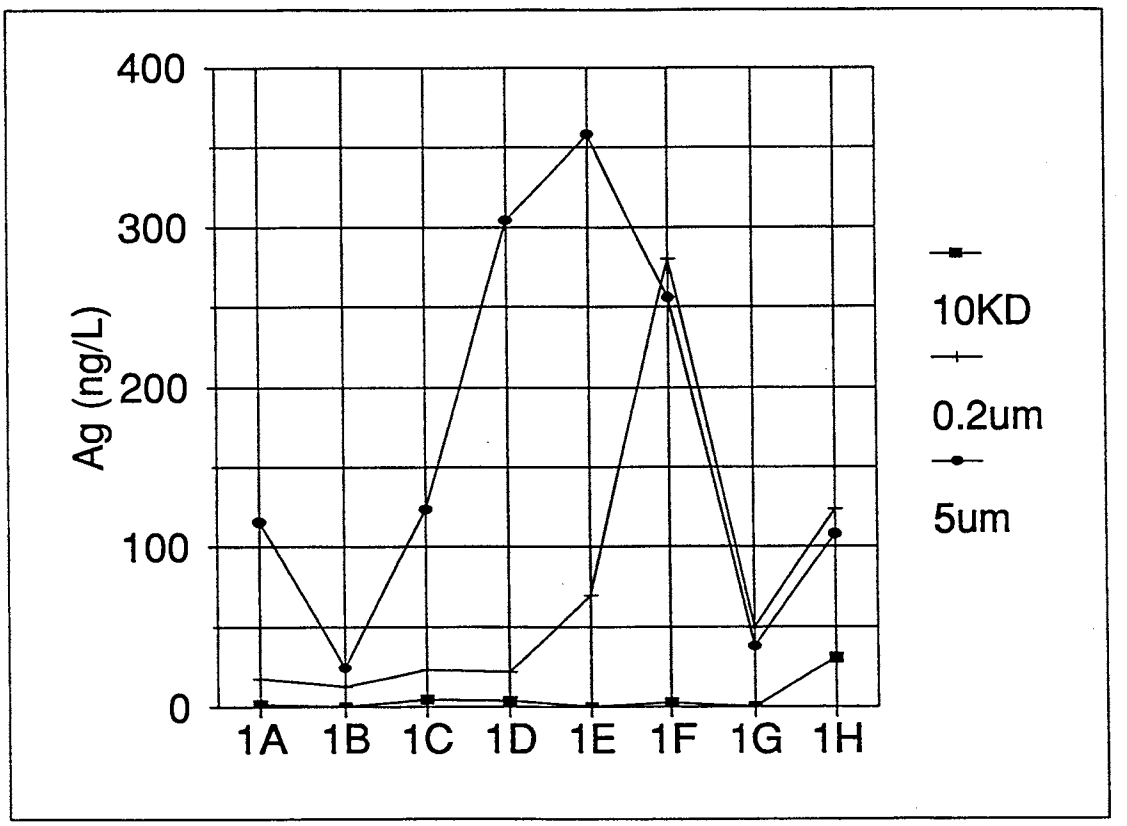


Figure 2. Ag levels in filter fractions from the Sasaginaga-Mill-Farr Creek system.

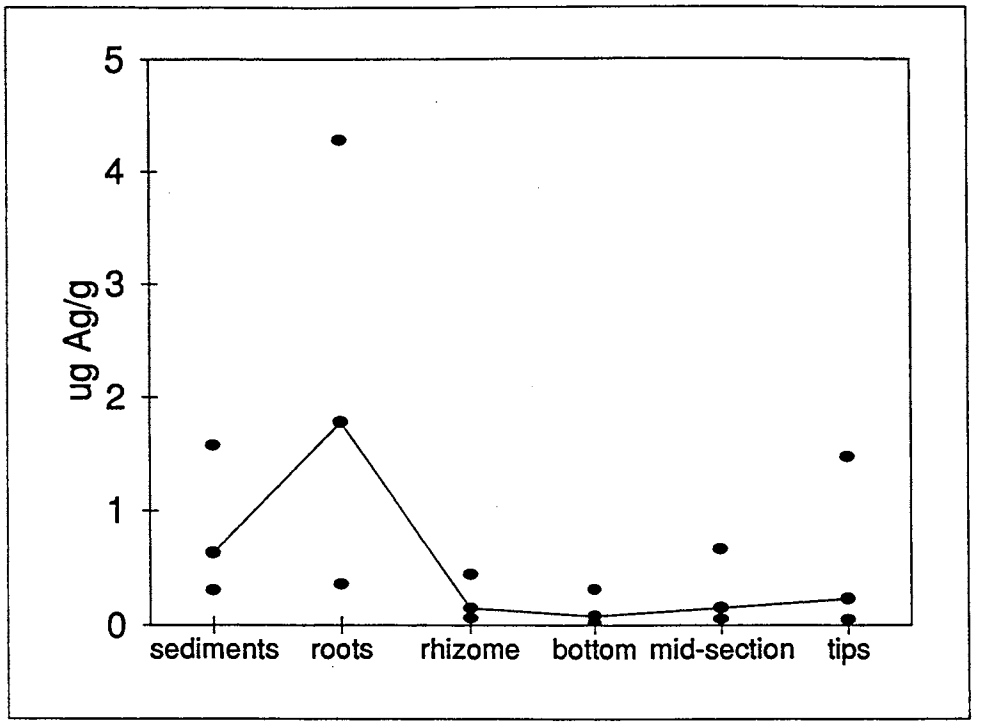


Figure 3a. Ag in cattails growing on tailings in the Cobalt area. Median, high, and low values are reported for n=6.

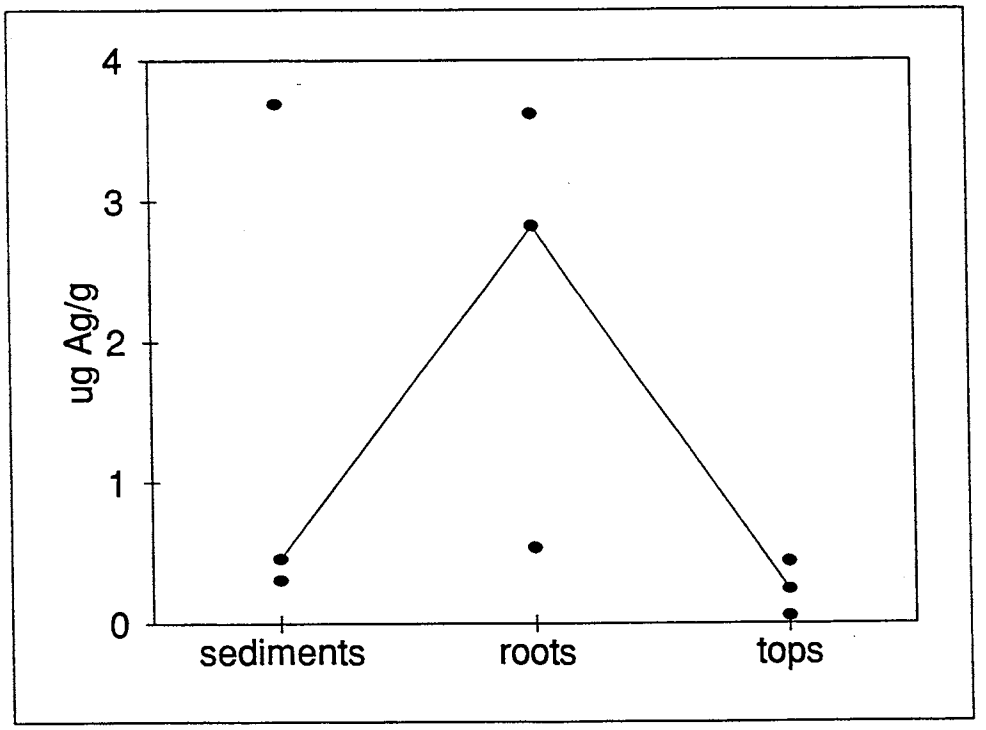


Figure 3b. Ag in grasses growing on tailings. Median, high, and low values are reported for n=5.

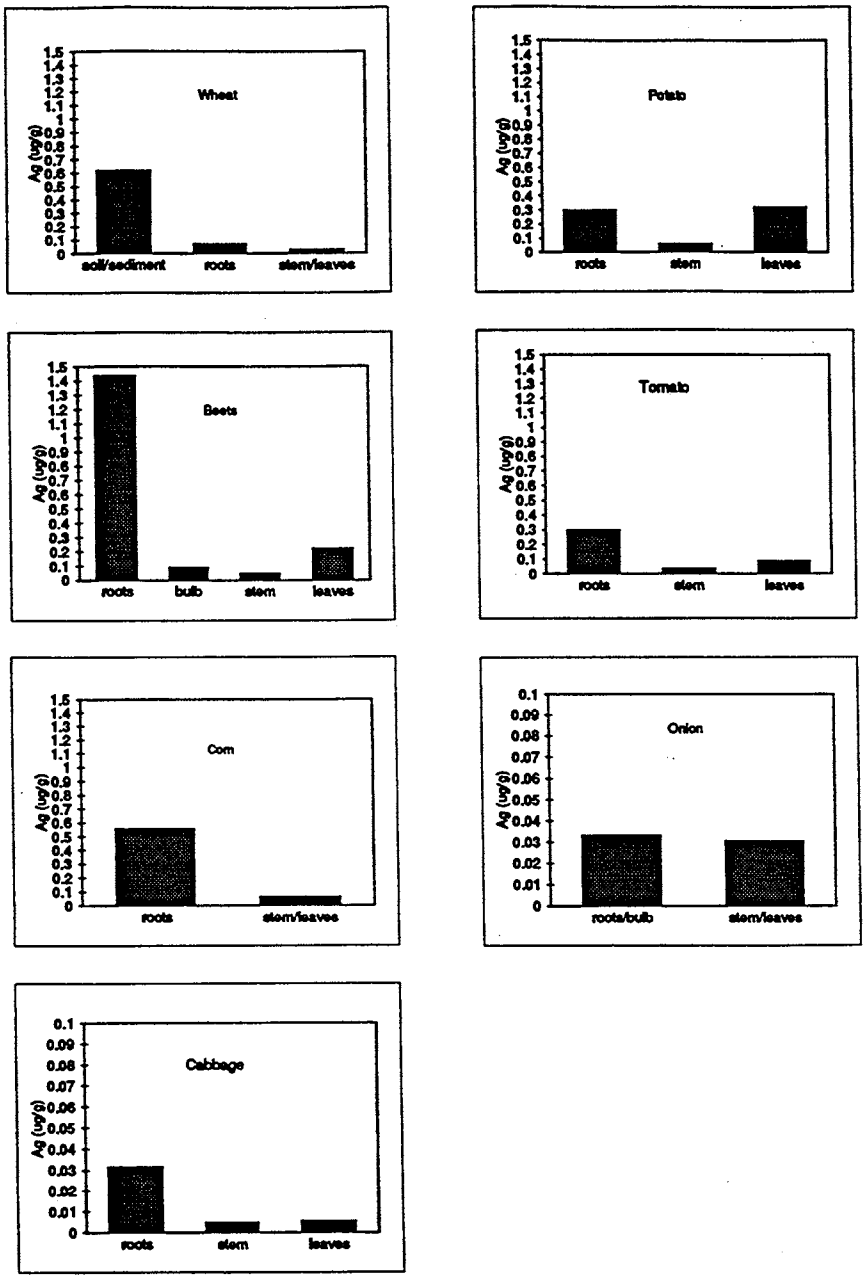


Figure 4. Ag levels in garden plants grown in tailings amended with red loam and manure.

Questions & Answers: Fate of Silver in Surface Waters, Sediments and Plant Material in an Old Mining Camp, Cobalt, Ontario

Q. PETER SANTSCHI (Texas A&M): You showed that silver is not bioaccumulated in grass and roots and vegetables and so on; how about organisms in the lake? Did you look at that? There were low silver concentrations but still, there could have been some food chain accumulation and transfer.

A. In terms of the lake, there are some small fishes that we measured in the '94 study. The lake, because of the arsenic and other things, in terms of, let's say clams and mollusks, is particularly barren. So no, we have not had the opportunity. One of the other, deeper lakes does have some clams in it. The cattails do show some bioaccumulation in the tips. It may be a temporal effect; the cattails have been around for ten years or longer, the grasses for a shorter time. The big problem is an area where it goes from desiccation to wetting very quickly, depending upon the ambient climate. It's a very porous system. So I can't give you an answer.

Performance of Activated Sludge Reactors Fed with a Silver-Bearing Photoprocessing Wastewater

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Introduction

Photofinishing laboratories generate wastewaters as a result of photosolution replenishment and wash water used between the various production steps for both negatives and prints. These wastewaters vary significantly in quantity, strength and composition depending on the size, process type and schedule of the photoprocessing facility. In most cases, discharge of photofinishing wastewaters into municipal sewers has been permitted. Past and current research on the biological treatment of photofinishing wastewaters have demonstrated that these wastewaters are amenable to both aerobic and anaerobic treatment[1-8].

During photoprocessing, in particular the fixing step, silver is removed from the film or paper and becomes part of the photoprocessing wastes, typically in the form of a silver thiosulfate complex. However, very low silver concentrations (less than 5 mg/L) remain in the photoprocessing effluents, because of commonly practiced silver recovery[1,8].

The objective of the work presented here was to assess the performance of laboratory-scale activated sludge reactors fed with photoprocessing wastewaters at a silver level expected after desilverization had taken place as well as to determine the leachability of silver from the resulting waste activated sludge.

Materials and Methods

Photoprocessing wastewater: Wastewaters simulating the fixing step of a film photoprocess -- KODAK FLEXICOLOR Process C-41 -- were prepared from a concentrated fixer solution provided by Kodak. To arrive at the simulated photoprocessing wastewaters, actual replenisher rates and dilution commonly attained with wash water in photoprocessing facilities were considered. It was assumed that the simulated fixer overflow was desilvered to a silver concentration of 200 mg/L.

Activated sludge reactors: Three laboratory-scale (150-L liquid volume) fill-and-draw reactors were set up with activated sludge mixed liquor taken from the R. M. Clayton Wastewater Treatment Plant, Atlanta, GA. The reactors were fed with either a synthetic mixture (control reactor) simulating municipal wastewaters, synthetic mixture plus C-41 fixer without silver, and synthetic mixture plus C-41 fixer with silver. The composition of the synthetic mixture was as follows (in mg/L): dextrin, 1,500; peptone, 500; sodium acetate ($C_2H_3O_2Na$), 500; sodium citrate ($C_6H_5O_7Na_3 \cdot 2H_2O$), 500; glycine ($C_2H_5O_2N$), 250; $MgSO_4$, 25; $MnSO_4 \cdot H_2O$, 5; $FeSO_4 \cdot 7H_2O$, 5; KCl, 7; $CaCl_2 \cdot 2H_2O$, 7; $K_2HPO_4 \cdot 3H_2O$, 260. Ammonium chloride (400 mg/L) was added to the influent of the control reactor in order to achieve the same ammonia level with that of the fixer-amended reactors. pH controllers with acid- and base-delivering pumps (1 N HCl and 1 N $NaHCO_3$) were used to maintain a narrow pH range between 7.0 and 7.2 in all three reactors. The feed fixer level was equivalent to a 40% volumetric contribution of photoeffluents based on the concentrations of the fixer constituents attained in the influent to the reactors. The silver concentration in the influent of the third reactor was 2 mg Ag/L, based on a fixer overflow silver concentration between 2.5 and 3 g/L and assuming that the fixer was desilvered electrolytically to a silver level equal to 200 mg/L. The reactors were operated with a 2-day hydraulic retention time (HRT) and a 10-day solids retention time (SRT). All three reactors were seeded and operated similarly to the control reactor (all reactors fed with only the synthetic mixture) for more than two SRT (24 days).

Analyses: The following parameters were measured according to procedures outlined in *Standard Methods*[9]: pH, alkalinity (pH = 4.0), acidity (pH = 8.3), dissolved oxygen (DO), ammonia, total and volatile suspended solids (TSS and VSS), sludge volume index (SVI), and dissolved organic carbon (DOC). Chloride, nitrite, nitrate, phosphate, sulfate and thiosulfate were determined by ion chromatography/conductivity using a Dionex AS4a-SC column and the eluent was 1.7 mM $NaHCO_3$/1.8mM Na_2CO_3. Silver was determined by acid digestion/atomic absorption and the toxicity characteristic leaching procedure (TCLP) was used following standard procedures[10].

Results and Discussion

Characteristics of photoprocessing wastewaters: Analyses of simulated C-41 fixer photoprocessing wastewaters (i.e., fixer overflow diluted by wash water at levels encountered in photoprocessing facilities) resulted in the following composition: pH, 7.1; alkalinity, 143 mg/L as $CaCO_3$; acidity, 255 mg/L as $CaCO_3$; DOC, 34 mg/L; ammonia-N, 674 mg/L; sulfate-S, 22 mg/L; and thiosulfate-S, 1,610 mg/L.

Reactor performance: The control reactor was operated for 138 days. C-41 fixer without silver was added to the influent of the second and third reactor after being operated similarly to the control reactor for 24 days. The second reactor fed with the fixer-amended influent was operated for another 74 days. After approximately six SRT had elapsed since the introduction of the fixer, fixer-containing silver was added to the influent of the third reactor and its operation continued for another 116 days. The influent characteristics of the three reactors are shown in Table 1. The steady-state (i.e., after three SRT had elapsed since the last change in each reactor's feed) data for the three reactors are shown in Table 2.

Removal of the organic mixture (quantified as DOC) was more than 98% in all three reactors. The rate of DOC removal over the feeding cycle was very similar in all three reactors (Figure 1). The DO level in all three reactors was above 7 mg/L 20 h after feeding (Figure 2). The fixer amended reactors lagged behind the control reactor in achieving the DO saturation level as a result of additional oxygen demand due to the thiosulfate oxidation. Sludge settling was excellent and when compared to the control reactor, the fixer and fixer-plus-silver amended reactors had better sludge settling (i.e., lower SVI values; see Table 2, Figure 3). Nitrification occurred, but it took a very long time (over 60 days of operation) to achieve complete ammonia removal. The following ammonia removals were achieved by the control, fixer, and fixer-plus-silver amended reactors (at the corresponding days of operation in parenthesis), respectively: 90 (61 d), 92 (68 d), and 100 (122 d). With prolonged operation, all three reactors achieved 100% ammonia removal. Compared to the control reactor, the two fixer-amended reactors had elevated nitrite concentrations and relatively low nitrate concentrations, indicating partial nitrification. Complete removal and oxidation of thiosulfate to sulfate was observed in both fixer-amended reactors. After 97 days of operation since the addition of silver to the influent of the third reactor, the sludge silver concentration reached a value of 1.96±0.02 mg Ag/g mixed liquor suspended solids (dry weight basis). The unfiltered effluent silver concentration was equal to 0.08±0.01 mg/L. The silver concentration in filtered (GF/C) effluent samples was below the method detection limit (0.01 mg Ag/L). By developing a silver mass balance over the operation time of the third reactor and taking into account solids wastage (and therefore silver lost) intentionally and inadvertently via the effluent, the predicted steady-state silver concentration was found to be 1.86 mg Ag/g MLSS which compares favorably with the measured value of 1.84±0.16 mg Ag/g MLSS (Figure 4).

TCLP analyses: Sludge from the silver-amended reactor at steady-state when subjected to the TCLP analysis resulted in a silver concentration of the extract equal to 0.08±0.02 mg Ag/L which is two orders of magnitude lower than the regulatory level of 5 mg Ag/L. When silver-bearing activated sludge was aerobically digested for 40 d and subjected to the TCLP analysis, the silver concentration in the sludge extract was equal to 0.11±0.02 mg Ag/L.

Conclusions

Based on the results of this study, we conclude that biological treatment of silver-bearing photoprocessing wastewaters, at the levels used in this study which assumes photoeffluent desilverization, is feasible by the activated sludge process without any operational problems. The resulting silver-bearing activated sludge passed the TCLP test.

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Table 1. Influent characteristics

Parameter	Control Reactor	Fixer-Amended Reactor	Fixer/Silver-Amended Reactor
pH	8.1	8.2	8.0
Alkalinity, mg/L as $CaCO_3$	1,940±24[a]	2,390±17	2,385±6
DOC, mg/L	1,630	1,610	1,650
Ammonia-N, mg/L	250±25	261±8	258±15
Chloride, mg/L	23	29	29
Phosphate-P, mg/L	79	68	79
Sulfate-S, mg/L	3.7	12.8	2.5
Thiosulfate-S, mg/L	--	752	703
Silver, mg/L	--	--	1.85±0.05

[a] Mean ± standard deviation (n ≥ 3);

Table 2. Reactor Performance Data

Parameter	Control Reactor	Fixer-Amended Reactor	Fixer/Silver-Amended Reactor
Mixed Liquor:			
pH	7.2	7.0	7.0
Alkalinity, mg/L as $CaCO_3$			
Before feeding	159 ± 54[a]	213 ± 15	201 ± 36
After feeding	411 ± 62	509 ± 91	713 ± 11
TSS, mg/L	4,616 ± 83	5,108 ± 199	4,336 ± 406
VSS, mg/L	3,903 ± 156	4,231 ± 188	3,677 ± 79
SVI, mL/g	65 ± 1	42 ± 6	37 ± 4
Effluent[b]:			
TSS, mg/L	130 ±20	120 ± 30	150 ± 30
DOC, mg/L	26 ± 5	25 ± 6	25 ± 3
Ammonia-N, mg/L	26 ± 5 (61)[c]	20 ± 4 (68)[c]	ND[d] (122)[c]
Nitrite-N, mg/L	72 (138)[c]	195 (68)[c]	222 (159)[c]
Nitrate-N, mg/L	151 (138)[c]	7 (68)[c]	12 (159)[c]
Sulfate-S, mg/L	6.5 ± 0.6	745 ± 11	681 ± 11
Thiosulfate-S, mg/L	ND	ND	ND
Silver, mg/L	ND	ND	0.08 ± 0.01

[a] Mean ± standard deviation (n ≥ 3); [b] Supernatant 24 h after feeding and after 1 h settling; [c] Number in parenthesis indicates reactor operation time in days; [d] ND, not detected

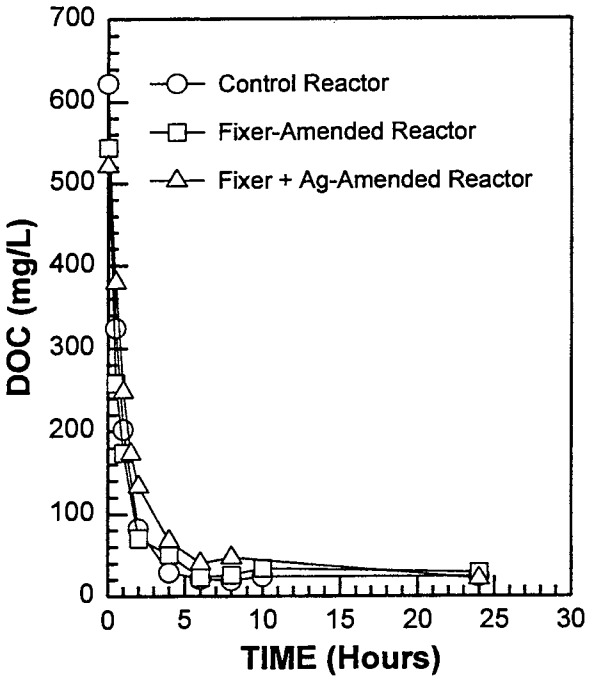

Figure 1. Dissolved organic carbon (DOC) profiles over the 24-h feeding cycle of the three reactors.

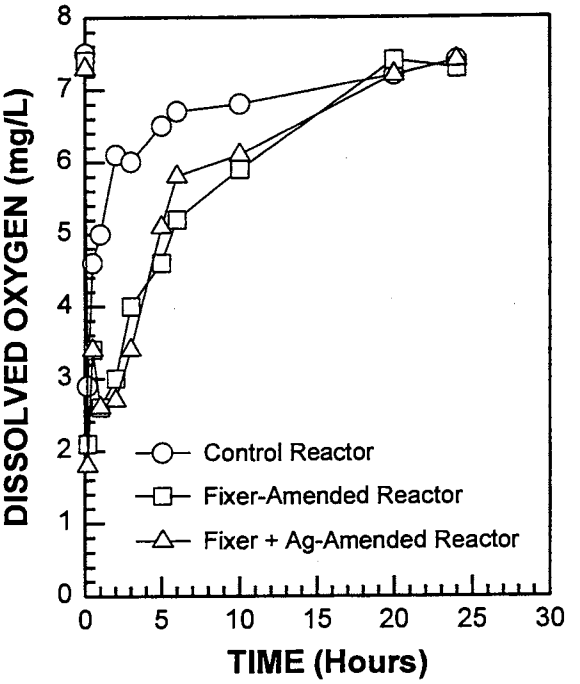

Figure 2. Dissolved oxygen (DO) profiles over the 24-h feeding cycle of the three reactors.

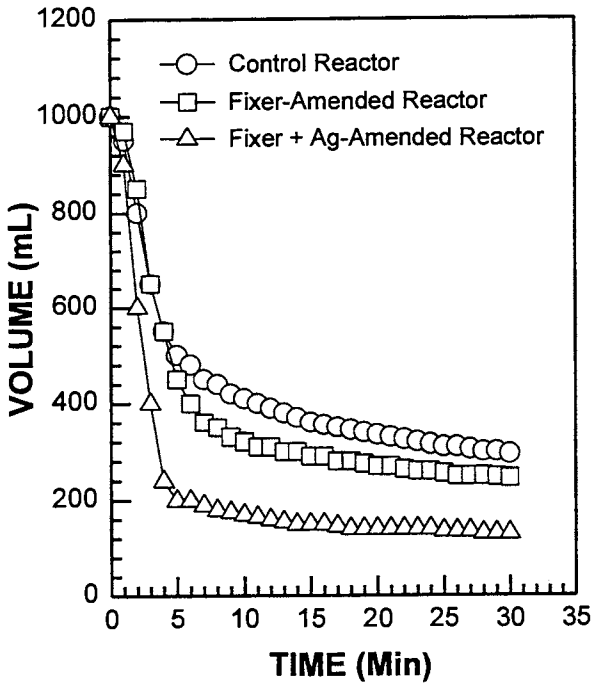

Figure 3. Settling characteristics of the mixed liquor activated sludge.

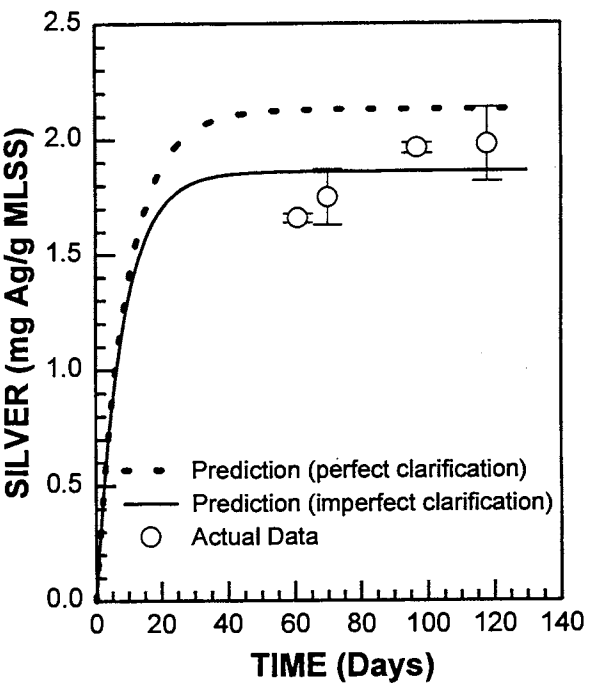

Figure 4. Development of the steady-state mixed liquor activated sludge silver concentration.

Questions & Answers: Performance of Activated Sludge Reactors Fed With a Silver-Bearing Photoprocessing Wastewater

Q. MARIANNE HIRSCH (Eastman Kodak): Did you measure the removal efficiency of silver from the photoprocessing effluent?

A. I caution you in terms of analytical capabilities; we're working at milligrams per liter, not nanograms per liter. But for the scale at which we're working and the analytical capability we had (our detection limit was 0.01 mg/L for digestions followed by atomic absorption), we could not measure any silver in the filtered effluent. So the treatment efficiency is 100 percent as far as the effluent. However, when you take into account the solids, it would probably drop to about 95 percent, maybe 98 percent, if you account for the solids that inadvertently go out and which carry some silver. The good news is that the silver concentration of those solids is much lower than the silver concentration of the overall solids. Obviously, coprecipitation has taken place, and basically the silver is embedded in the solids that have very good settlability. That is what differentiates that silver concentration in the retained solids from the concentration of silver in the effluent solids.

Environmental Aspects of Silver Research in Russia: A Review

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General Information: Ores, Provinces, and Available Resources

Silver is a precious metal with an average crustal content equal to 7-11 $*10^{-6}$ (20 times higher than that of gold). There are 60-80 minerals containing silver. Those characterized by Ag content over 50%, are considered to be Ag ores; others (with Ag content of 10-15%), are polymetallic ores of nonferrous and heavy metals. Eighty percent of Ag is produced along with other metals from polymetallic ores and ores of Au and Cu. The most important silver mines in Russia are located in the Okhotsko-Chukotsky volcanic ribbon, the Sikhote-Alinsky volcanic ribbon and in the Norilsk region.

Production and Use of Silver

Presently there are no reliable figures reflecting the production of silver in the USSR. Most Russian publications contain evaluations of Ag production in developed and developing countries. These evaluations are based on silver prices. At the end of the 1980s, the world production (without the USSR) was estimated as 10-15 thousand tons per year. In 1912, Russian silver (3240 kilograms) formed only 2.92% of all silver produced in the world.

At the beginning of the 1990s, the photographic industry was the largest consumer of silver in Russia and used 30-40% of Ag. Secondary silver (mainly recovered from photographic industry wastes), formed about 60-70% of the total consumption of this metal. Silver alloys with palladium, gold, copper, zinc (20% of consumption) were used in the electronic industry. Alloys of silver with gold and copper, as well as with mercury, tin and zinc were used in stomatology. Ag-Zn and Ag-Cd accumulators (storage batteries) were consumed by the space and military industry (20-25%). Other areas of silver consumption were jewelry, vacuum devices, silverware, mirror production, chemical catalysis, medicine, food industry (special layer inside some reactors), etc.

Silver in the Environment

Anthropogenic Sources.

According to the assessment published in Russia at the beginning of the 1980s, about half of the silver contained in coal is emitted to the atmosphere by coal burning power stations. It is predicted that in the year 2000, over 6,500 tons of Ag will be emitted in the world due to the coal burning.

By the beginning of the 1990s, Russia was producing from 410 to 440 tons of coal per year. This figure decreased in 1993 to 305 million tons. From 75% to 85% of coal was used by thermoelectric power plants. If the silver content was ~ 0.5 $mg\text{-}kg^{-1}$, and half of it was emitted to the atmosphere, the Soviet annual share by the end of the 1980s was close to 200 tons, while that from Russian amounted to about 110 tons. The world coal production at the same time was estimated as 4 billion tons; the Russian share in annual world silver emissions was then about 10% (or the Soviet share was over 18%).

On the other hand, 10% of the world emission in 1985 means over 400 tons of silver. In principle, these figures are not that contradictory; they are of the same order of magnitude. Continuing our estimations, we can assume that at the end of the 1980s, industries (including metallurgy, chemical and pharmaceutical industry) and transport in Russia could annually emit from 175 up to 700 tons of silver.

Without presentations of exact figures, most Russian publications devoted to the topic of silver removal from photographic wastes agree that, due to economic restrictions, professional photo and film studios do not form the most of silver discharge to the aquatic environment. The only official data on silver discharge to fresh water bodies was published in 1993 in a report of the Perm region (located in the Urals). The region is responsible for 94% of the overall silver discharged to the surface water bodies in the Russian Federation, namely for 900 kilograms.

The Urals (including the Perm area) are known as one of the most import centers of metallurgy, chemical and coal industries in Russia. The Perm area itself contributes substantially toward discharges of various metals. Still, it is difficult to make a conclusion concerning the predominant source of silver discharges. On the other hand, it seems reasonable that such cities and areas as Norilsk, Nickel or Monchegorsk play an important role in Ag discharges to water bodies.

Distribution of Silver in Environmental Media

Soils. Most of the studies described in the literature are industry oriented. Such a study was undertaken in one of the largest Russian polymetallic production areas located in the north of the country (Monchegorsk). Copper-nickel ore has been mined and processed in this region since the 1930s. Atmospheric emissions of the plant contain heavy metals and acid gasses. It is found that along with copper, nickel, cobalt, lead and chromium, upper soil contains enlarged amounts of silver (10-20 higher than baseline concentrations).

Another region of polymetallic (copper-molybdenum) production was studied in the Balkhash arid area of the former USSR. Maximum silver content in neighboring lake terrace soils was close to 20 $mg\text{-}kg^{-1}$ (120 times higher than in baseline soils of the same genesis, or 200 times higher than the average crustal content). Copper, molybdenum, zinc, and lead are found in concentrations in 50-100 times higher than those in baseline soils of the same genesis. Saline soils (enriched in sulfates) intensively accumulate heavy metals in the upper A_0 horizon; silver has the highest coefficient of accumulation (300) comparatively to copper (166.7), lead (100), zinc (6).

The maximum registered concentration of silver in Moscow area soils is 30 $mg\text{-}kg^{-1}$. Higher silver contents are located nearby Kashira and Shatura thermoelectric plants, Balashikha military and electronic industry center, Schelkovo chemical plant, Podolsk city (the most polluted city in the Moscow area, center of electroplating, machinery, building, chemical industry).

In the Vladimir area, silver is found in enriched concentrations at several sites, some of which are located in the area of influence of the large thermoelectric power plant. However, there is no information on any increased levels of Ag nearby the historical mirror production.

Marine Water. According to several well-known Soviet handbooks, the average concentration of silver in marine water varies between 0.019 $\mu g\text{-}l^{-1}$ and 120 $\mu g\text{-}l^{-1}$; most typical levels are close to 0.3 $\mu g\text{-}l^{-1}$ or 0.1 $\mu g\text{-}l^{-1}$. Due to anthropogenic factors, the silver content in the upper 200m layer of ocean can annually increase by 4%. Silver bioaccumulation by marine fauna and flora is characterized by factor $\sim 10^3$; marine vegetation contains about 0.025 mg of silver per 100 kg of dry mass.

Silver in sea water is thought to exist predominantly as $AgCl_2^-$ and $AgCl_3^{2-}$ complexes; the total mass of dissolved silver in the ocean is computed to be $1.37 \cdot 10^8$ tons. Suspended solids contain Ag as well; Suspended silver

concentrations have been assessed as 0.003 mg-l^{-1} (total mass is $4.11 \cdot 10^6$ tons, respectively). Rivers can carry up to 65% of silver in particulate form; the average dissolved Ag concentration in river water is about 0.2 µg-l^{-1}.

Zinc, cobalt, mercury, silver and chromium concentrations were investigated in **The Azov Sea**. Increased concentrations of dissolved silver (0.46-2.1µg-l^{-1}) were often registered in littoral areas. It is hypothesized that silver entered the sea from the atmosphere and from rivers in particulate form. The silver then dissolved in the sea water. Thus, in October 1979, the upper layer of water contained 62% of suspended silver (1.47 µg-l^{-1}); by April 1989, silver was concentrated in the benthic water layer. In July 1980, the average concentration of suspended silver in all the mass of warm water was 0.3 µg-l^{-1} (27%).

The Mediterranean Sea was studied in 1983. The surface microlayer (300 µm), surface layer (0-0.5m) and benthic layer were investigated. Average concentrations of dissolved silver were 0.3-0.4 µg-l^{-1}. In most samples (29),suspended silver was either not detected or detected at concentrations lower than 0.01 µg-l^{-1}; in 10 samples, suspended silver concentrations varied between 0.01 and 0.09 µg-l^{-1}; only one surface microlayer sample contained detectable silver at 0.01 µg-l^{-1} of silver. Atmospheric depositions seem to play a certain role in the silver balance at this station (**Ag** concentrations in the air above the sea varied between 0.001 and 0.009 µg-m^{-3}). Special experiments strongly indicated that oil spills could contribute toward the increase of silver concentrations.

The equatorial zone of the **Pacific Ocean** was studied in 1988. The main conclusion of the authors is that concentrations of all microelements varied significantly; surface layers in many cases showed much higher levels of iron, cobalt, etc. As far as silver is concerned, the highest concentration registered for the surface layer was 0.103 µg-l^{-1}; the deeper layer (47 m) value was 0.134 µg-l^{-1}. All concentrations measured were in the interval from 0.004 µg-l^{-1} to 0.134 µg-l^{-1}.

Silver Extraction From Marine Water. The total amount of dissolved and suspended silver in the world's oceans is evaluated as $141*10^6$ tons. Iron concretions in sediments of the Pacific Ocean contain about 0.003% silver. Modified silica gels can be used for silver sorption; concentration factors vary from 10^2 (Fe form) to 10^4 (Ca and Na forms). Activated carbon in Ca, Mg, Fe, Na forms can be used to extract silver from sea water. Concentration factors varies from 10^3 to 10^4. Anion exchange sorbents in the chloride form are suitable for Ag removal from sea water, due to the formation of silver complexes with the resin. The overall conclusion from this study was that sorption of silver, along with other elements (U, Mo, V, etc.) can be economically sound.

Air. Silver is sometimes discussed along with other metals as quite a typical component of aerosols, in particular in urban areas. The highest concentration of silver registered in cities is $5.3*10^{-2}$ µg-m^{-3}. Incineration plants, coal burning thermoelectric stations, cement and paint productions are considered to be the principal industrial sources of atmospheric silver.

Analytical Methods. Silver is a typical trace metal and occurs in the environment at very low concentrations (see above). Analytical methods applied to detect silver are similar to those used for other heavy metals. Recent articles and reviews suggest that atomic absorption, inductively coupled plasma and neutron activation analysis are methods of choice for silver analysis in environmental media. Sample pretreatment in aqueous samples, by sorption and extraction, is often necessary because of very low concentrations.

Organic sulfides have been applied to extract silver from aqueous solutions and natural waters; the final extract can be 50-100 times enriched in Ag[I] compared to the initial aqueous solution. Both flame atomization and graphite furnace cells can be used. The method is applicable for the determination of silver in the range of 0.003 to 10 µg-l^{-1}. Preliminary sorption of Ag on a polymer thioester, allows the determination of dissolved concentrations from 0.01 µg-l^{-1}. For the analysis of silver in the suspended sediment fraction, chemists use membrane filtration followed by HNO_3 treatment of the precipitate.

Toxicity and Environmental Standards

In the former Soviet Republics, toxicological and environmental standards called maximum allowable toxicant (MATC) have been adopted. MATC for silver have been introduced for air (in working zones); these vary from 1 to 0.01 mg-m^{-3}. MATC in water (for sanitary water bodies) is 0.05 mg-l^{-1}. Some fragmentary information indicates that free silver in water can be harmful for plants (9.8 µg-l^{-1} for corn crop; 4.9 µg-l^{-1} for lupine; 10 mg-l^{-1} for kidney bean). But it is believed that silver in the concentration range of about 0.25 mg-l^{-1} does not affect waste water treatment in aeration tanks.

'Silver Water'. Silver is applied for sanitation purposes. The antibacterial effects of silver have been compared to that of penicillin and other antibiotics. Viruses are more tolerant. Drinking water containing 50 µg-l^{-1} of silver, is believed to be harmless for humans. A level of 100 µg-l^{-1} guarantees bacterial safety of water. It is estimated that drinking 1 liter of such 'silver water' per day, a human will get 2.5 grams of Ag by the age of 70. Silver is considered to be a compulsory metal for man; its average daily doze should not be less than 88 µg.

Economic Aspects of Environmental Standards.
Officially it is presently impossible to induce an enterprise to pay fees for silver emissions/discharges. This seems to be reasonable, since professional photographic laboratories or electronic plants are considered – silver is just too expensive. Roughly estimated pollution rates for silver are on the order of $600 per ton (in the air); $1,000 per ton (in water).

Research Needs

There are several reasons why Russian research is undeniably needed to evaluate global trends, sources, distribution and fate of silver in the environment. These include: a *huge territory, numerous polymetallic ore deposits, non-registered industrial and municipal sources of silver, etc.*

The available data are too fragmentary and sporadic to allow appropriate assessments. Due to the historical approach to "the strategic resources," there are no figures on the production and use of silver in Russia. At the same time, all precious metals are registered in each institution and enterprise. Thus, *a series of regional environmental audits* should not be very complicated, provided data kept at various levels are open for scientists.

Silver determination in the air *should be included in environmental monitoring programs, especially* for cities (first of all where industries emitting silver into the atmosphere are located) and for Biosphere Reserves (base line levels). Regions with natural anomalies also deserve special attention. Silver concentrations in fresh water bodies has not been reported in the Russian scientific literature; it seems like this should become a new research branch; marine investigations are to be systematized.

In order *to evaluate the silver balance in the Russian Federation, it would,* in principle, *be necessary to bring together governmental bodies* (such as Ministry for Environmental Protection, Committee for Hydrometeorology and Monitoring), *technological and academic institutions* (Institute for Geochemistry and Analytical Chemistry, Institute for Oceanology, Institute for Human Ecology, St-Petersbourg Observatory, etc.). A comprehensive review of scientific, technical and economic data (both published and unpublished) could become the first step of such a collaborative study.

It would also be very valuable if Russian Scientists studied *environmental toxicity of silver and further developed new methods suitable for trace analysis of various species of silver in the collaboration with their foreign colleagues.*

Questions & Answers: Environmental Aspects of Silver Research in Russia: A Review

No questions.

Session 3

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

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

New Proposals for Regulating Metals in the Aquatic Environment: Geochemical, Toxicological and Physiological Bases

Harold L. Bergman
University of Wyoming
Laramie, Wyoming, USA

There is a growing consensus in the scientific and regulatory communities that a departure is needed from past empirical approaches for evaluating the toxicity of metals to aquatic organisms. New approaches show promise for linking organism responses to fundamental changes in the target tissue (e.g., gills of fish) and to the details of metal chemistry at the target tissue. Mathematical models of both the biological and chemical processes can be used to estimate the bioavailable metal concentration at the target tissue. The next steps required include further validation of these models in additional species, additional metals, and a range of water quality conditions, along with the incorporation of receiving water exposure models. These models would explicitly take into account what has been termed "bioavailability" and have the potential to be predictive across a wide range of environmental conditions. This approach marks a turning point in developing a mechanistically based, workable method for assessing the effects of metal exposure on aquatic organisms, and the method could lead to a more fundamental approach to regulatory decisions about metals in the aquatic environment. The proposed approach, including the geochemical, toxicological and physiological bases, will be summarized in this presentation.

Questions & Answers: New Proposals for Regulating Metals in the Aquatic Environment: Geochemical, Toxicological and Physiological Bases

Q. NICHOLAS FISHER (SUNY-Stony Brook): Harold, I enjoyed your talk. You have focused, in your toxicity discussion, primarily on fish, and in particular on gill as the target tissue. But as we know, and as you alluded to in your concluding statements, many of the most sensitive organisms in aquatic ecosystems — important components of those aquatic ecosystems — are not fish, but invertebrates, phytoplankton, etc. They may be sensitive at concentrations that are far below the sensitivity shown in fish tissue. I know you were focusing on acute toxicity studies, where the toxicity caused by silver or other elements requires metal concentrations five or six or more orders of magnitude higher than what naturally exist in waters, even the most contaminated natural waters. I would argue that — given that there are other components of these ecosystems that are much more sensitive, and looking at your tripartite diagram of toxicity, geochemistry and physiology — we need to focus on the physiological mechanism of toxicity to the most sensitive components of those ecosystems, because this may be most telling with respect to regulation.

A. I agree. As a matter of fact though, most of the discussion at the SETAC meeting was about invertebrates, in particular cladocerans but also other invertebrates including benthic macroinvertebrates. There is an opportunity to explore physiological consequences and mechanisms in those types of organisms as well as algae and bacteria. A lot more work, though, needs to be done. What we have now is correlation, some of it very good: Bill Sunda's work, your own, and others. Unfortunately, I didn't hear Peter Campbell's talk yesterday, but it was reviewed for me; there's a lot of good correlation work that has been done. You don't understand physiological mechanisms in those cases, but we have some opportunity to move to invertebrates, which are very sensitive organisms. I think those of us that are interested in the application of geochemical modeling to estimate bioavailability and effect on aquatic organisms have to move away from fish, at least, in part, and to work with algae, *Daphnia*, and other critters that are, in some cases, driving the water quality criteria because they're more sensitive.

Q. CHRISTER HOGSTRAND (University of Kentucky): My concern is somewhat similar to Nick's. In the environment, most of the concerns that you will get from metals and most other contaminants, I'd say, is chronic toxicity. As you pointed out, what you are modeling here is acute toxicity. As Nick said, these concentrations we see here, we're probably not going to see out in the wild. But we are going to see toxicity. Take a metal like cadmium, for example; we know that cadmium screws up the calcium metabolism, and acute toxicity does it at the gills. But during long-term exposure you'll get uptake of cadmium which is not over the gills, but more likely over the gut and accumulation in the kidney. We'll still get calcium disturbances, not because the gills are affected, but because the kidney gets disrupted. So I just want to point out that I think it's very important that we think about that other issue as well, because I think that's more environmentally realistic.

A. Absolutely. A very difficult problem; we have to keep working on it. But, the acute water quality criteria still are a very important issue, and very important regulatory conflict in certain parts of the country and in certain types of environments; in old mining waste areas we still have fish kills. We still have acute lethality occurring in some environments, though much less than some number of years ago. Acute effects and acutely lethal concentrations of metals still occur, sometimes in mixing zones, sometimes because of accidental releases. It's still a problem, and it still needs to be regulated. But you're right, the chronic effect is going to be the tougher nut to crack, and we've got to continue to work on that.

Q. FERNANDO GALVEZ (McMaster University): Just a few comments, actually. You were mentioning the Reid and McDonald data — the gill dip. Actually, I did a gill dip in the fourth year, looking at zinc, and one of the

things about those data that gave such low values for K was the fact that it was done at really high concentrations. So, instead of looking at specific transport sites, what you're likely going to have is nonspecific binding to the gill. Nathan did some subsequent work on zinc and found that if you use much lower concentrations like Rick is using, you're starting to attack the actual transport sites. It's really important in determining the binding affinity to the gill that we're actually isolating the transport sites. In addition, the binding affinities are based on looking at saturable kinetics. So we're saying that we have a metal in the water, and if we increase the concentration we'll eventually saturate that site. It might not necessarily be applicable to metals which might form neutral complexes, which might actually enter into the gill without having to go through certain protein channels [BERGMAN interjects: Including, possibly, low molecular weight natural organic matter.] Because I know there's been some talk about using the gill model, I think some of these things have to be taken into consideration.

The Toxicity of Silver to Marine Fish

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McMaster University, Hamilton, Ontario, Canada

Salinity has a remarkable effect on Ag toxicity to aquatic organisms. The free Ag^+ ion, which is only encountered in freshwater systems in traceable quantities, is one of the most toxic commonly occurring metals with a typical 96-h LC50 of 0.1 μM to fish (Hogstrand *et al.*, 1996). In contrast, Ag is virtually non-toxic in brackish water, due to precipitation of Ag as cerargyrite, but in seawater where dissolved $AgCl_n$ complexes prevail Ag is moderately toxic. The 96-h LC50 for Ag the tidepool sculpin (*Oligocottus maculosus*) and seawater acclimated rainbow trout (*Oncorhynchus mykiss*) in seawater ranges from 4-6 μM, depending on salinity (Ferguson *et al.*, 1995; Shaw *et al.*, 1996). Thus, Ag, added as $AgNO_3$, is 50 times less toxic in seawater than in freshwater.

The mechanism of acute Ag toxicity to freshwater fish is now relatively well delineated. Ag acts at the level of the gills where it seems to non-competitively block the Na^+/K^+-ATPase at the basolateral membrane (Ferguson *et al.*, 1996; Morgan *et al.*, 1996). This blockage drastically reduces the branchial Na^+ and Cl^- uptake, resulting in a large net loss of Na^+ and Cl^- from the blood (Wood *et al.*, 1996). The subsequent sequence of events during acute silver toxicity to freshwater fish can be traced back to the progressive net loss of Na^+ and Cl^- across the gills. In contrast to the well described etiology of acute silver toxicity in freshwater fish, the mechanisms behind toxicity of Ag to fish in seawater is unknown. Thus, the aim of the present study was to characterize the mechanisms that lead to acute toxicity of Ag in marine fish.

The experimental approach was similar to that of Wood *et al.* (1996). Starry flounders (*Platichtys stellatus*) were collected by bottom trawl in Barkley Sound off the west coast of Vancouver Island. The fish were fitted with chronically indwelling catheters in the caudal portion of the dorsal aorta to allow repetitive blood sampling. After surgery, the fish were placed in individual 10-l plastic tubs, supplied with a continuous flow (350 ml/min) of well aerated seawater with a salinity of 32 ppt and a temperature of 12 ± 1°C. The tubes were covered by a plastic mesh to avoid visual stress. The exposure system consisted of a header tank which delivered a constant flow (3.0 l/min) of aerated sea water to a 20-l vigorously aerated mixing chamber. Silver nitrate, dissolved in distilled water and acidified with 0.05% HNO_3, was dispensed from a stock solution into the mixing chamber by a peristaltic pump at a rate of 1.0 ml/min. The silver stock solutions were kept in a darkened bottle and renewed every 48 h. The concentrations of the stock solutions were set so that the concentrations of Ag in the tubs were 2.3 μM for one group of eight fish and 9.3 μM for a second group. A third group of eight fish served as sham treated control group. Blood samples were withdrawn from experimental fish and controls before the start of the exposure and then 12, 24, 48, 96, and 144 h after onset of exposure. The blood samples were analyzed for plasma Cl^-, plasma glucose, plasma ammonia, plasma protein, blood gasses (P_{O2} and P_{CO2}), pH, hematocrit, hemoglobin, and lactate.

While there were few physiological disturbances in starry flounders exposed to 2.3 μM Ag, the higher concentration of Ag, 9.3 μM, was in the lethal range. Three out of eight fish exposed to 9.3 μM Ag died between the 96-h and 144-h sampling points. The increased stress in fish within this group was reflected in a rapidly increasing plasma glucose concentrations (Fig. 1). At the 1- and 2-day sampling points there were slightly elevated plasma glucose concentrations also in the group exposed to 2.3 μM of Ag, but this effect was not found later in the experiment (Fig. 1).

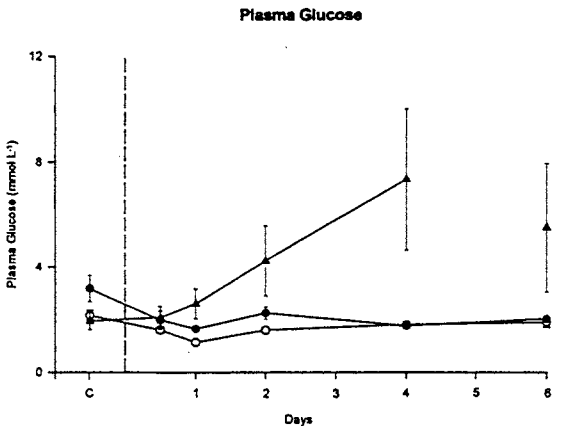

Figure 1. Plasma glucose concentrations in starry flounder during exposure to waterborne Ag added as $AgNO_3$. The start of the exposure is indicated by the dashed vertical line. Open circles=sham-treated controls; filled circles=2.3 μM Ag; filled triangles=9.3 μM Ag. Values are presented as mean±1 SEM. N is 8 for all points exept for the sampling point on Day 6 of the 9.3 μM group, which included the 4 surviving fish. Because of the mortality, this point is not representative of the group and, therefore, no line connects the point with the other points in the time series.

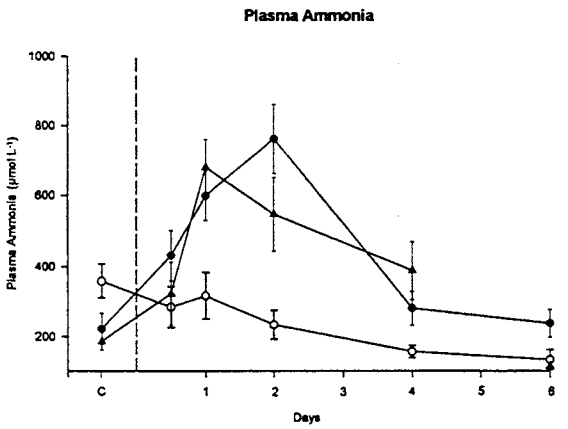

Figure 2. Concentrations of total plasma ammonia in starry flounders during exposure to waterborne Ag added as $AgNO_3$.. Other details are as in Figure 1.

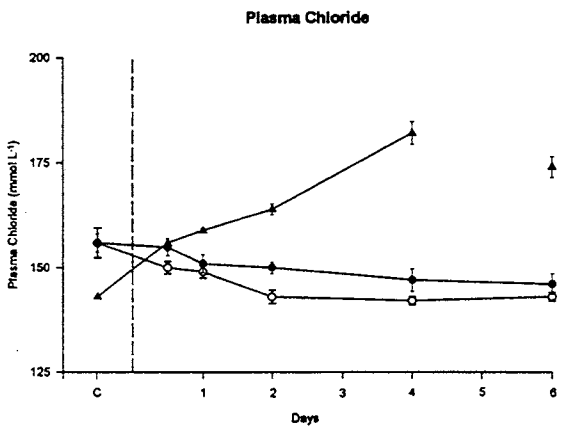

Figure 3. Plasma chloride concentrations in starry flounder during exposure to waterborne Ag added as $AgNO_3$. Other details are as in Figure 1.

Plasma ammonia was the only variable that markedly changed in fish exposed to the lower concentration, 2.3 μM, of Ag (Fig. 2). The plasma ammonia level peaked within the first two days of exposure, followed by a partial recovery. A similar increase and subsequent recovery was present in the group exposed to Ag at 9.3 μM. Thus, the plasma ammonia concentration did not increase dose-dependent fashion with increasing level of Ag exposure and it was partially restored in moribound fish (Fig. 2). Recently, Webb *et al.* (1996) found that Ag stimulates an increase in plasma ammonia levels also in freshwater fish, which establishes elevated plasma ammonia levels as a general effect of Ag exposure in fish. However, the lack of dose-dependency of the effect, together with the fact that the plasma ammonia level was on the decline later in the experiment when fish from the 9.3 μM Ag group started to die, strongly suggest that ammonia toxicity was not the primary cause of death in Ag exposed starry flounders. However, it remains possible that ammonia could contribute to the toxicity of silver in other fish species and during conditions that impair ammonia excretion (Shaw *et al.*, 1996).

The pattern of plasma Cl^- concentration changes in Ag exposed fish was characteristic of a key toxic mechanism for a lethal effect. Whereas plasma Cl^- in starry flounders exposed to 2.3 μM of Ag was only slightly elevated in comparison to the sham-treated control, fish exposed to 9.3 μM of Ag showed dramatically escalating plasma Cl^- concentrations (Fig. 3). Furthermore, the individuals that died between the 96 and 144-h sampling point exhibited critically high plasma Cl^- concentrations. Thus, just as in freshwater fish, the mechanism of Ag toxicity to starry flounder in seawater seems to be a disturbance in the ability to regulate Cl^- (and presumably Na^+). In seawater this disturbance is manifested by a net accumulation of Cl^- (rather than a net loss of Cl^- which is the case in freshwater fish) because of the higher concentrations of Cl^- in seawater than in the blood. To this point, the effect of Ag resembles that of Cu; in freshwater fish exposure to waterborne Cu results in reduced plasma Cl^- and Na^+ levels and in seawater fish the plasma Cl^- and Na^+ levels increase during Cu exposure (Stagg and Shuttleworth, 1982; Wilson and Tyler, 1993a,b).

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Questions & Answers: The Toxicity of Silver to Marine Fish

Q. DOMINIC DI TORO (Hydroqual, Inc.): At the high concentrations (mg/L) of silver you must be getting silver chloride precipitation.

A. No, we don't. This is 32 parts per thousand seawater.

Q. DI TORO: At the concentrations we've operated at, you would be seeing it.

A. We actually measured the silver concentration in 0.45-micron filtered water in the tubs where the fish are located. We don't see any silver chloride precipitation. As a matter of fact, we modeled it — not at 1 milligram per liter, but at 250 micrograms per liter — and it shouldn't precipitate.

Q. NICHOLAS FISHER (SUNY-Stony Brook): Have studies been done that have examined the toxicity of dietary silver to either marine or freshwater fish? I ask in light of the implication of the intestine in the fish. I'm not aware of any studies.

A. There will actually be a presentation by Fernando Galvez about that dietary tomorrow. I should add we have also conducted work now in freshwater at close to environmentally realistic concentrations of silver to compare how the effects that we see at these industrial concentrations work at the lower concentrations. This is the obvious question that you ask yourself, and part of it has been done. We think that we probably want to do more of that.

Persistence, Bioaccumulation and Toxicity of Silver in Freshwater Systems

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Columbus State University, Columbus, Georgia, USA

A bioassessment study was conducted on the transport, fate and effects of silver and other metals in Big Bayou Creek in Western Kentucky. Results of environmental chemistry, effluent and ambient toxicity studies and ecological surveys were integrated to assess the effects of metal pollution. The stream system was a second to third order tributary of the Ohio river; was of moderate gradient; and was characterized by good habitat quality and frequent riffles, runs and pools. Sediment characteristics generally were comparable throughout the study area. The creek received four continuous and four intermittent effluent outfalls from a uranium enrichment plant. The discharge volume of the continuous effluent totaled 3.96 million gallons per day (MGD) and, in downstream order, included approximately 0.44 MGD for 009, 1.18 for 008, 0.71 for 006, and 1.63 for 001. The study area encompassed 10.7 stream km and included nine stream monitoring stations (BB1-BB9) and eight effluent monitoring points. Water column residence time for metals calculated from the effluent 009 outfall were 0.65 hr, 1.2 hr, and 2.31 hr for downstream stations BB7, BB8 and BB9, respectively. The principal reference station (BB1) was 1.4 km upstream of the first effluent outfall (009). A nearby secondary reference station (BB2) also was located just upstream of effluent 009 and was used primarily for the collection of fish and macroinvertebrate taxa used for "sentinel monitoring" to characterize metal bioavailability.

Ecological surveys included the collection of resident biota from all stream stations, including sites located downstream of each effluent. Ecological perturbation primarily affected macroinvertebrate populations. The latter were sampled in spring, summer, and fall of the first year of the study and annually thereafter for five years. Average results from the first two-years for total macroinvertebrate density and number of taxa are illustrated in Figure 1. Macroinvertebrate species richness and density were reduced perceptibly in the effluent receiving zone, amounting to losses of 33 and 77 %, respectively. Abundance of mayfly taxa was reduced by more than 50 % immediately below the 009 outfall. Multimetrics based on U.S. EPA Protocol III (Plafkin *et al.*, 1989), were used to calculate bioassessment (BA) scores, which indicated moderate to substantial impact for stations BB3-BB7.

Toxicological characterization of effluents and receiving waters was performed using chronic biomonitoring procedures with *Ceriodaphnia dubia* and the fathead minnow, *Pimephales promelas* (Weber *et al.*, 1989). Of approximately 300 on-site tests, 92 were conducted on the continuously flowing effluents that discharged into Big Bayou Creek (Fig. 2). Significant toxicity was observed for effluent 008 50 % of the time. Effluent 004, which tested positive in 100% of tests conducted, originated from a secondary waste treatment plant and entered 008 just prior to its entry into Big Bayou Creek upstream of station BB4. As discussed below, metal uptake in resident biota was highest at this station.

The six metals considered most likely to impact aquatic life were cadmium (Cd), copper (Cu), chromium (Cr), nickel (Ni), lead (Pb), and silver (Ag). Though each was elevated in the effluent receiving zone, ecological impact could not be attributed to individual metals. Therefore, an additivity model, based on Cumulative Criterion Units (CCUs), was used to assess combined metal impact (Birge *et al.*, 1992; NAS/NAE, 1972). Criterion units for each of six metals were calculated as the ratio of the mean analyzed concentration in water to the U.S. EPA chronic water quality criterion concentration (U.S. EPA, 1986). Values for each metal were then added to give a cumulative criterion unit (CCU).

This additive model is summarized by the following equation:

$$CCU = \frac{Cd_A}{Cd_B} + \frac{Cu_A}{Cu_B} + \frac{Cr_A}{Cr_B} + \frac{Ni_A}{Ni_B} + \frac{Pb_A}{Pb_B} + \frac{Ag_A}{Ag_B}$$

where A is the mean measured water concentration, and B is the freshwater chronic criterion value. As there was no U.S. EPA chronic criterion for Ag (U.S. EPA, 1980; Birge *et al.*, 1996), a value of 0.2 µg/L was used in the calculations summarized in Figure 3. A CCU index of "1" or less was considered protective of aquatic life. However, the CCU values were above "1" for stations BB4-BB7 where ecological impact was measurable and where effluent and ambient toxicity occurred at significant frequencies (Figures 2, 3, 4).

Though CCUs based upon total recoverable metal concentrations provided a reasonable fit with ecological conditions in the effluent receiving zone, they clearly overestimated impact at downstream stations (*e.g.* BB8, BB9). As shown for Figure 5, total recoverable silver peaked at station BB6, due principally to loading from effluent 001, and remained relatively constant downstream where ecological impact was not discernable. Stream loading rates for silver of 0.32, 0.39, 1.48 and 4.63 g per day were calculated for effluents 009, 008, 006 and 001, respectively. Attempts to determine filterable (*i.e.* dissolved) metal concentrations proved inconclusive. Therefore, "animal sentinels" were used to assess instream bioavailabilty of metals. Body burden determinations for the specified metals were performed on three taxa, including the stoneroller minnow (*Campostomum anomalum*, gut removed), mayfly (*i.e. Stenonema sp.*), and a caddisfly (*i.e. Cheumatopsyche sp.*). On a comparative basis, body burden increased in the order of *Cheumatopsyche* < stoneroller minnow < *Stenonema*. Depending on the specific metal, body burden in the mayfly was up to fifty times greater than for the other species and consistently lower for *Cheumatopsyche*, known to be a metal-tolerant, opportunistic species (Plafkin *et al.*, 1989).

The stoneroller minnow proved to be the best indicator of bioavailable metal and body burden values for such metals as Cu, Cd, and Ag provided close correlations with ecological impact (Fig. 4). Although most silver loading to Big Bayou Creek came from effluent 001, bioavailable silver originated principally from the upstream effluents, especially 008 that affected stream station BB4 (Fig. 5, 6).

Proportional differences in body burden were used to calculate the bioavailable fraction of total recoverable silver, using the following formula:

$$M_{BF} = \frac{M_{BB}}{M_{RBB}} * M_{TR}$$

where:

M_{BB} = Body Burden, Sentinel Organisms
M_{RBB} = Reference Body Burden
M_{TR} = Instream Total Recoverable Metal

Using a conversion factor 0.85 for silver at BB4-BB5 (Davies, 1996) there was a decrease in calculated bioavailable silver to ≈ 10% at BB9. This overall decrease in silver bioavailability occurred within about two hours of instream residence time. It was also clear that the conversion factor for silver would greatly overestimate potential impact of the 001 effluent (Fig. 5). Sentinal monitoring with the stoneroller minnow was useful in characterizing metal exposure conditions throughout the study area.

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Figure 1. Density and Number of Taxa for Macroinvertebrates in Big Bayou Creek

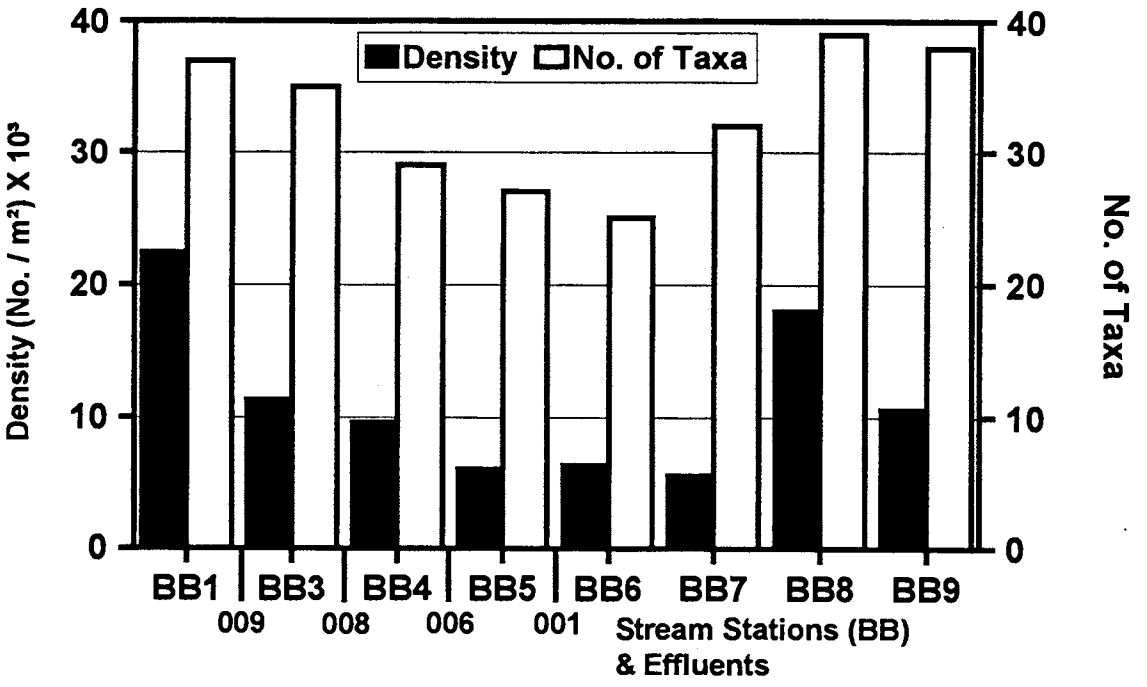


Figure 2. Frequency of Significant Effluent Toxicity, Big Bayou Creek

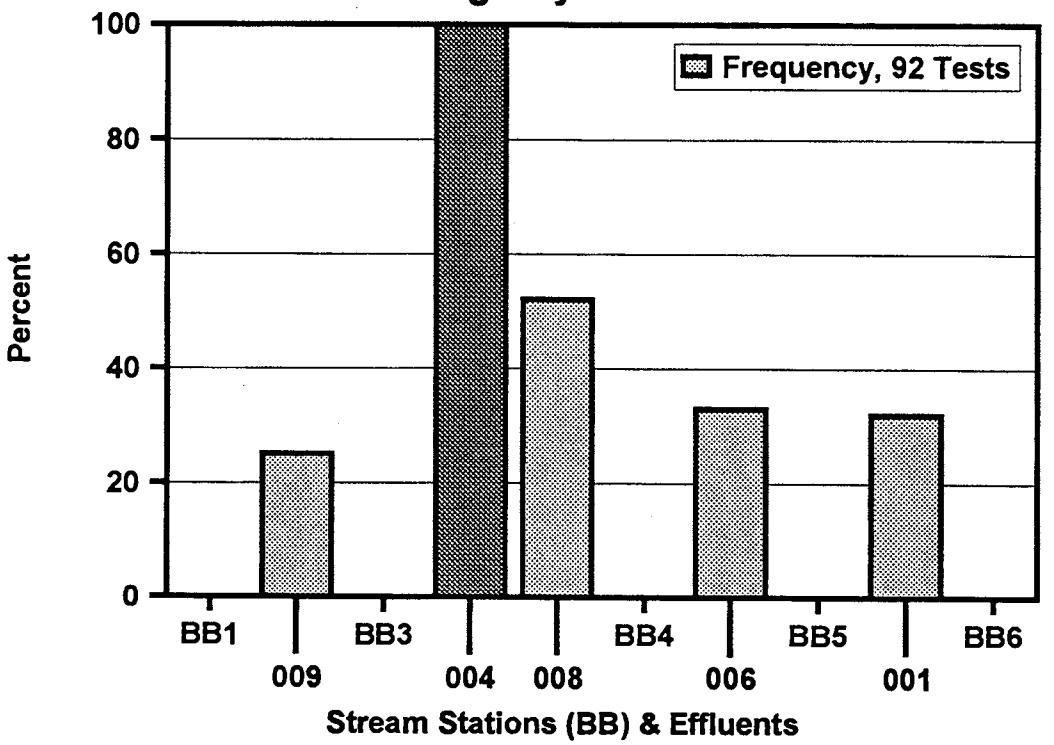


Figure 3. Metal Cumulative Criterion Units (CCUs) for Big Bayou Creek

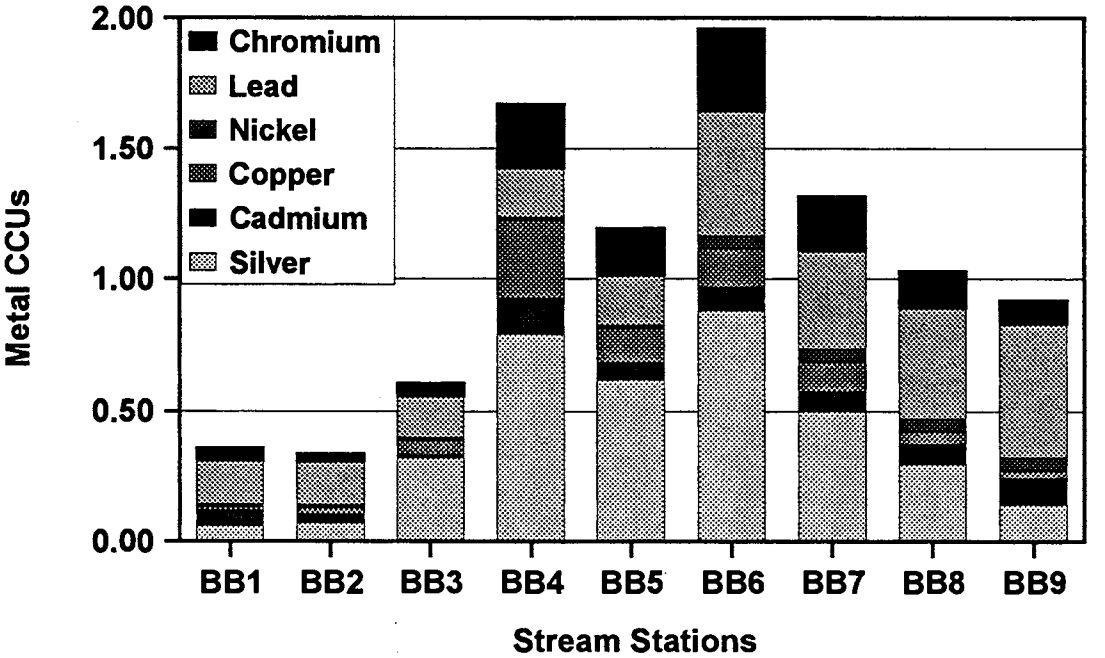


Figure 4. Relationship of Cumulative Criterion Units (CCUs) to Bioassessment (BA) Score and Number of Macroinvertebrate Taxa

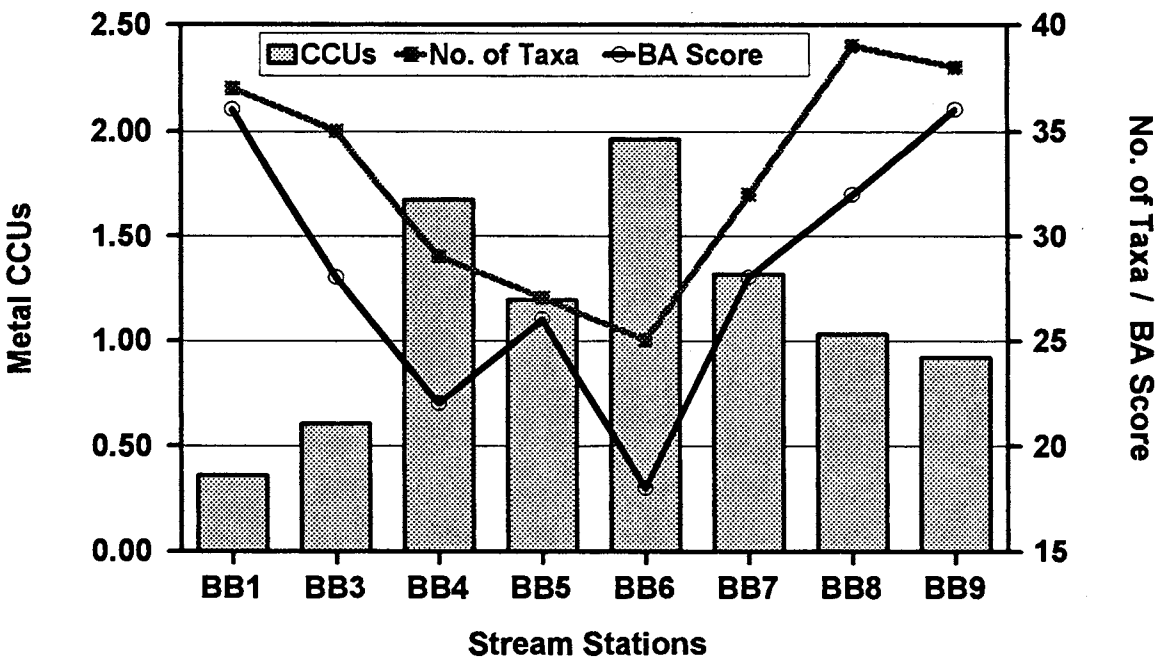


Figure 5. Silver (Ag) Loading into Big Bayou Creek and Uptake in Stoneroller Minnows

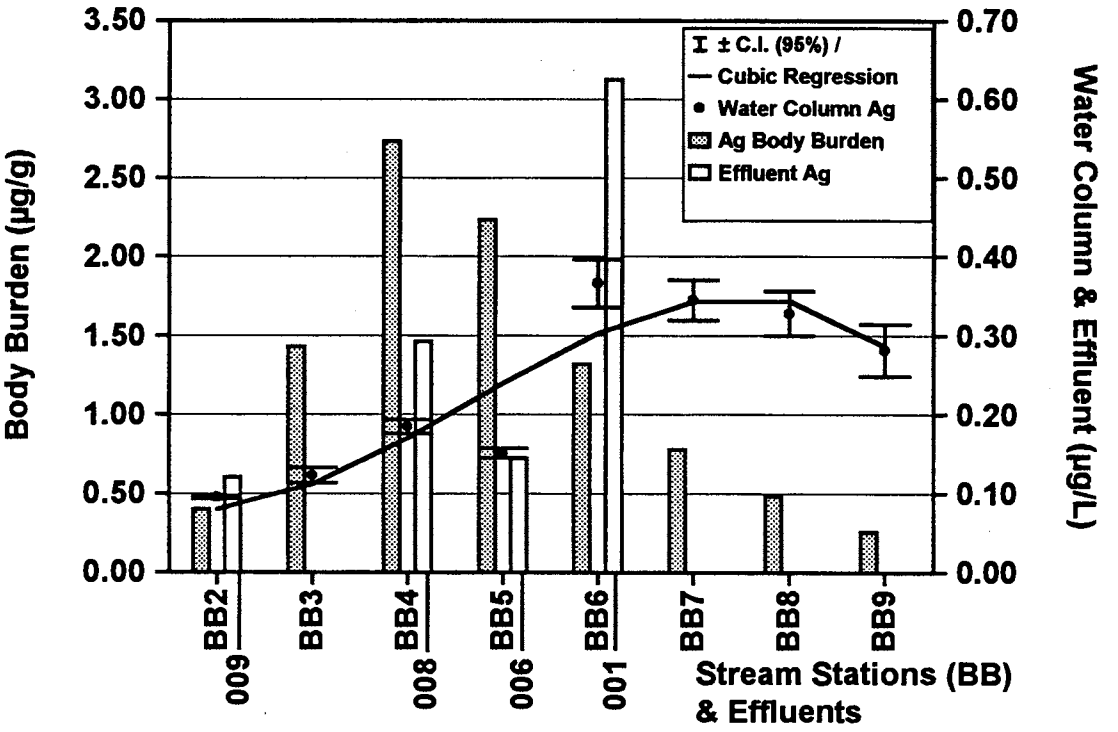
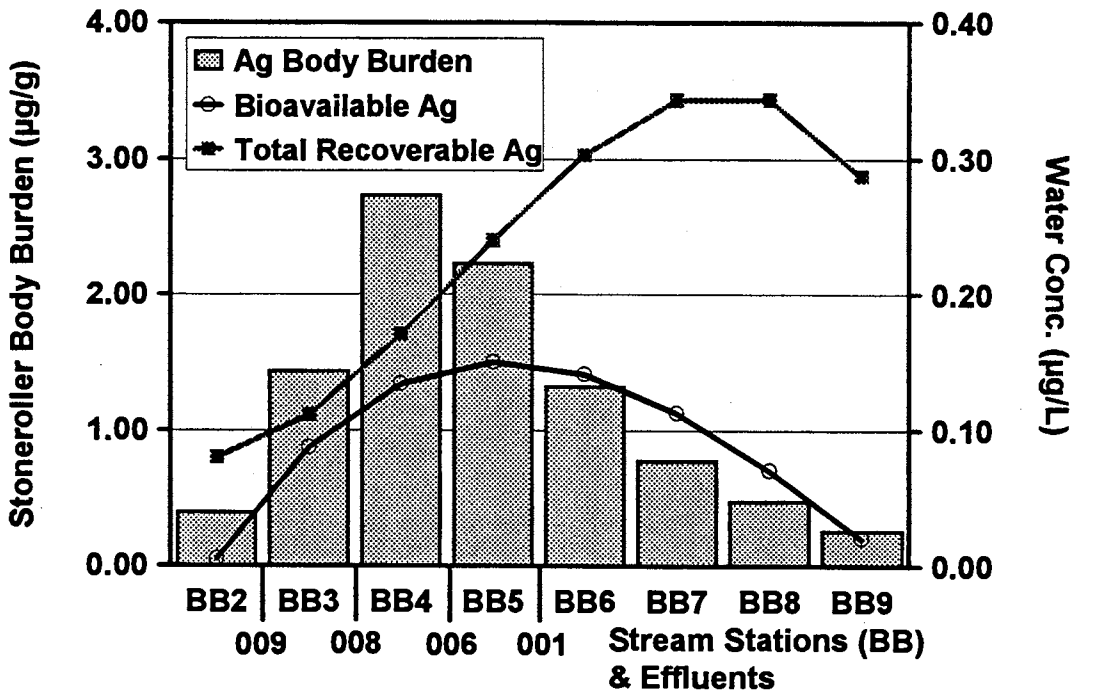


Figure 6. Bioavailability of Silver (Ag) in Big Bayou Creek



Q. DOMINIC DI TORO (Hydroqual, Inc.): As I understand your idea, you take the total recoverable metal and then you correct for bioavailability by looking at the bioaccumulated version in tissue. I would agree with you that a good measure of bioavailability is tissue body burden. But then you compare the calculated distribution of bioavailable metals to the tissue body burden from which you calculated the bioavailable metals, so it's bound to lay one on top of another. My problem is, how would you make a prediction of bioavailable metals if you had to change the situation? Isn't that the problem we face?

A. I think, first of all, we're not saying that this is a fully confirmed approach; but then, acid-volatile sulfides aren't all that certain either. I think we have to look at all the possibilities. We work with lotic systems. A good part of the sediment is washed in the water column, transported, settles out, is resuspended, moves, and settles out again. I see a lot of things in terms of the sediment approach that really don't take into account the kinds of things we see in the field. I'm saying that this is an idea, and I don't think that this is just a fit of convenience; I think that there is too much coincidence here in terms of this correlating with ecological trends. We have a ton of ecological data. I've only shown you a mere fraction.

Q. DI TORO: I think that I would conclude that the body burden data appears to correlate with ecological responses. Is that a fair statement? Because, if you look at the shape of the body burden stuff, and then you look at the way your ecological . . .

A. It does for some species, but not for others. I think a good part of this is knowing what sentinels are going to give you the most typical, representative kind of readout. If we were to show you this based on *Cheumatopsyche sp.*, the body burden data wouldn't mean much, and you would get the idea that there should be no ecological impact.

Q. DI TORO: One other question. Have you done toxicity tests where you look at the body burden as a function of the exposure?

A. Sure, we probably did some of the longest chronics on record: 5-year studies with cadmium and mercury back in the old days. And we did look at reproductive readout relative to body burden and relative to gonadal burden. There are times when we can find good correlations. That's really demanding work; it's time consuming, and I don't know how we accumulate enough of a data base for that to be usable in a routine monitoring situation. This other thing with the stone roller is something that can be done, but I believe that body burden is very important, and we've looked at it for a long time. We're attaching more relevance to it now, particularly when we see the mayfly-*Cheumatopsyche sp.* relationship. I think we need more work on body burden, and we need to have better answers to the questions you're raising.

Q. JAMES LEAGAN (Eastman Kodak): That outfall that was number 008, the effluent where they were accepting photoprocessing wastes: what form of treatment was it receiving prior to discharge?

A. We don't have that information. We have some, but I don't think I'm at liberty to release it yet. This is one of those federal installations that is careful about such things. It's something that is high on our list, and I think we have avenues now of looking into that to a greater extent. We would really love to understand the relationship between the 008 and 001 effluents. We get more silver from one, and none of it appears to have any impact on body burden of any of the sentinel monitors. Then we get about half as much coming from this one effluent,

and we get a substantial uptake. If you were to use those data, you'd get a pretty good-sized bioaccumulation factor for silver. We don't want to try to take on too many things at one time, but part of this is to get back and look at bioaccumulation factors for silver, and we're going to be using some of these organisms with which to do it.

Q. RUSSELL ERICKSON (USEPA): Lacking the ability to comment on the effluent properties, what about the in-stream chemistry? Do you have any information on how that is changing downstream of these effluents that might relate to the bioavailability question?

A. We have some information on that. There is an upswing, for example, in total suspended solids at that 011 effluent site, and that remains relatively stable on downstream, so that seems to be unrelated to the continual, progressive decrease in body burden. I'm sure it's complexing metal, but that seems to be happening there at that one point, and we don't see a downstream correlation on that. We've looked at other kinds of things; we don't have as much general stream chemistry data as we'd like. We've looked for other kinds of outfalls and other complicating factors, but it's a relatively simple stream, moderately clear water without a lot of suspended solids, and a lot of this just seems to be the normal dynamics that stem from what's occurring in the effluent outfall area. But those are good points, and those are some of the things that we have on our list of questions.

Water Quality Components Affecting Silver Toxicity in *Daphnia magna* and *Pimephales promelas*

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We characterized silver toxicity to *Daphnia magna* (DM) and *Pimephales promelas* (FHM) for a variety of water quality conditions. The need for such characterization results from a limited understanding of the interactions between silver toxicity and Ca-hardness, chloride, and organic carbon. 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 $CaCO_3$, and up to 5 or 10 mg/L dissolved organic carbon (DOC) for DM or FHM, respectively. For FHM, mortality was 100% at silver concentrations of 20 or 40 ug/L, regardless of water quality parameters. For DM, mortality was generally complete at silver concentrations $\geq$ 3.5 ug/L. For either species, there was little protection afforded by increased $CaCO_3$, alone, whereas DOC has a major ameliorating influence on measured silver toxicity. Lower concentrations of chloride ($\leq$ 20) had little effect on reducing silver toxicity. We noted an appreciable reduction in free silver during the course of the exposures. The individual toxicity responses of FHM and DM are described elsewhere in this Volume. The objective of this paper is to describe the intercorrelations among the independent water quality parameters and the dependent toxicity responses of FHM and DM..

Specifically, we determined the simultaneous influence of water quality parameters, alkalinity, hardness, chloride and organic carbon, on acute silver toxicity to DM and FHM. The method of choice was partial correlation analysis. The existence of a correlation between two or more variables does not necessarily imply a direct causal link between them. Variable 1 might "control" 2 (or vice versa). In fact, a third variable may be responsible for the mutual correlation between variables 1 and 2. Hence, when several variables are quantified in a study, it is appropriate to use correlation coefficients to discern potential underlying causal mechanisms. Although partial correlation analysis has been described in numerous basic multivariate statistics texts, including Bailey (1981) Johnson & Wichern (1982) and Sokal & Rohlf (1995), it is a technique not widely used. One of us (TLP) has used partial correlation to describe the independent linear effects of pH, calcium, and aluminum on fish survival, weight and hatching success (Ingersoll, *et al.* 1990).

The formula for determining the partial correlation (in this example, for three variables) is:

$$r_{12,3} = \frac{r_{12} - (r_{13}\, r_{23})}{((1 - r_{13})^2\,(1 - r_{23})^2)^{1/2}}$$

where $r_{12,3}$ implies the first-order partial correlation of variables 1 and 2, holding 3 constant and r_{12} is the zero-order bivariate correlation of variables 1 and 2. In this study of silver, hardness, alkalinity, pH, DOC, ionic strength, and organism mortality, we desire to know, for example, how silver is related to mortality, when the effect of hardness or DOC has been excluded ("held constant"). Sokal & Rohlf (1995) elaborate on the analysis of partial correlation coefficients by using path analysis. A path coefficient is the standard partial regression coefficient and measures the strength of a relationship (between two or among threee or more variables) as the proportion of the total standard deviation. Such partial correlation coefficients can be tested for significance in much the same way as total correlation coefficients, with adjustments made for the varying degrees of freedom for the number of variables held constant.

The results of the partial correlation analysis indicate that at all silver concentrations tested for DM and FHM, there was a statistically negative correlation between silver and survivorship when dissolved organic carbon, chloride concentration and hardness were held constant (Table 1; -0.64 and -0.73, respectively; $\alpha = 0.05$). The stronger effect for FHM is viewed in Figure 1. In Figures 1 and 2, choride concentrations of 40 and 60 mg/L do not appear to be as protective against silver toxicity for either DM or FHM. This apparent trend is not supported by the partial correlation analyses and needs further clarification.

Table 1. Partial correlation of water quality parameters with survivorship in *P. promelas* and *D. magna*. The asterisk (*) implies statistical significance at 0.05.

	SILVER	DOC	CHLORIDE	HARDNESS
Fathead Minnows	-0.73*	0.19	0.03	-0.001
Daphnia magna	-0.64*	0.28*	0.04	0.01

Our conclusions concerning the effects of water quality parameters on silver toxicity to daphnia are as follows: Alkalinity, hardness and chloride had no-to-minimal influence on silver toxicity; and chloride, at concentrations of 40 to 60 mg/L, as used in these experiments, tended to enhance silver toxicity. As for silver toxicity to fathead minnows, although DOC did lessen the effect of silver, the response was of borderline statistical significance. Overall, exposure to silver, and resultant effects on behavior or survivorship, is best viewed with consideration of complexing factors in the environment.

Figure 1. *Daphnia magna* survivorship as a function of silver and chloride.

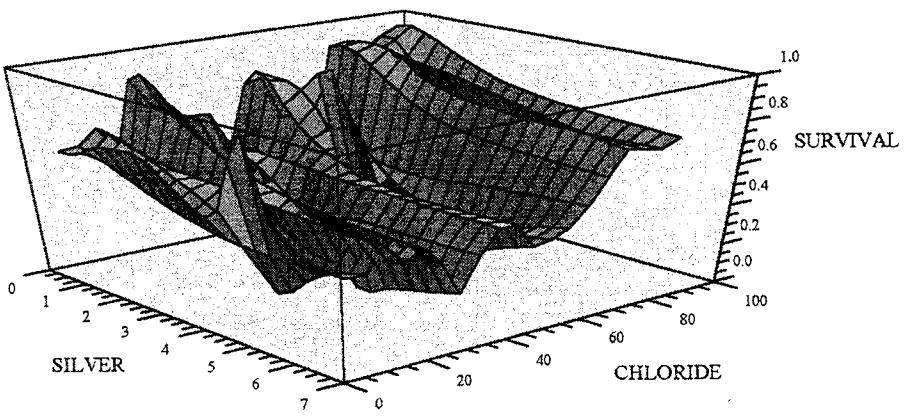
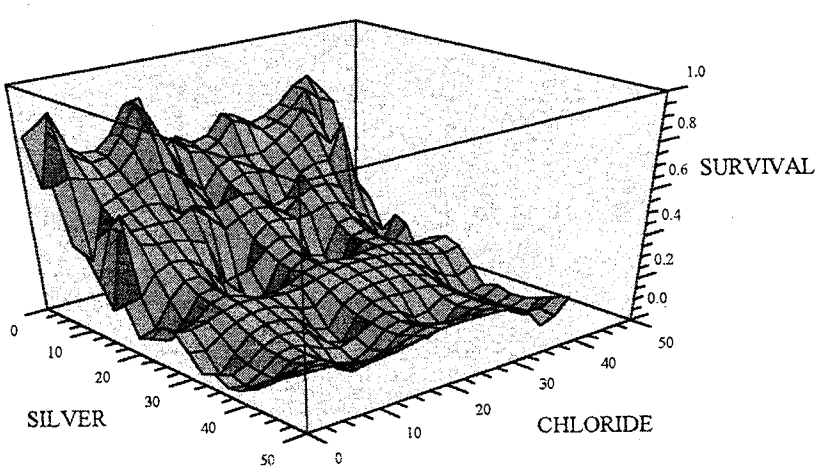


Figure 2. Fathead minnow survivorship as a function of silver and chloride.



Questions & Answers: Water Quality Components Affecting Silver Toxicity in *Daphnia magna* and *Pimephales promelas*

Q. CHRISTER HOGSTRAND (University of Kentucky): What was the lowest chloride concentration that you used in the tests? And do you get a decrease in toxicity when you increase it?

A. We used three milligrams per liter as the lowest concentration. And the toxicity increased at 40 [milligrams per liter] chloride. It decreased at really low chloride concentrations. It served a protective function at the lower concentrations.

Q. HOGSTRAND: Do you have any speculations as to what that could be?

A. No, unfortunately I don't. [George Cobb adds: If you remember the discussion yesterday, as we added chloride, up to about 20 or 30 milligrams per liter, the silver concentration in solution decreased, and then when we got to 40, it began to increase.]

Q. HOGSTRAND: So the values that you showed for silver, were they nominal or measured?

A. The values shown on the screen are nominal, but not on the 3-dimensional surface. We used measured.

Q. ANDERS ANDREN (University of Wisconsin): I'm interested in the *Daphnia* measurements. For instance, you had chloride going from a few milligrams per liter to 40, which is a freshwater range for, say, Wisconsin rivers not impacted by road salt. What was your mechanistic understanding? Did you think that by changing the chloride you would change the speciation of silver, or did you think you would change something in the osmoregulatory aspect of your *Daphnia*, or both?

A. I think it's the latter; it would have been the osmoregulatory capacity.

Q. ANDREN: Because you can compute the effect of that chloride range on the speciation of silver even though you don't know the complexation constants for your DOC. You can make a reasonable stab, at least.

A. I agree, and I think your comment and question yesterday were perfect. It's something we need to do with these data, and we've got the information and the model structure to do that.

Influence of Age Sensitivity on the Acute Toxicity of Silver to Fathead Minnows at Various Water Quality Parameters

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The data to adequately characterize silver toxicity to freshwater fish for a variety of water quality conditions are lacking or poorly 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; these silver limits may be underprotective or overprotective. Work described earlier provided a response for 4-day old fathead minnows at varying water qualities. These LC50 values for 4-d old fish were found to be significantly lower than those reported in the literature for older fish (Davies *et al.*, 1978; Goettl and Davies, 1978; Lemke, 1980; Nebeker *et al.*, 1983). Thus, it is the objective of this study to generate a silver ($AgNO_3$) toxicity data set for 28-day old fathead minnows, *Pimephales promelas*, that accounts for variations in water quality parameters such as chloride, hardness, and dissolved organic carbon.

Fathead minnow larvae, *Pimephales promelas*, (24-days old) were purchased and inspected for viability upon receipt, from Charles River-ARO (Hampton, NH). An acute (96-hour), static non-renewal test was conducted with juvenile fathead minnows (28-d old) at 22°C ± 1°C with a 16-hr. light (10-20 $\mu E/m^2/s$):8-hr. dark cycle. Hardness as $CaCO_3$ (50 mg/L) and alkalinity (100 mg/L) were the same for all treatments. Mean alkalinity, hardness, and pH was 107 mg/L, 52 mg/L, and 8.26 respectively. Dissolved oxygen averaged 8.0 mg/L and never fell below 4.7 mg/L. The effects of two different water quality parameters on acute silver toxicity were examined. Chloride (3 , 20 , 40 , and 60 mg/L) and humic acid (0 , 5 , and 10 mg/L) were used in conjunction with the six concentrations of silver (0 , 2 , 5 , 10 , 30 , and 60 μg/L) in a complete factorial design. This results in a total of 72 treatments. Each treatment had three replicates containing 10 fish each.

Chloride was found to have no significant effect on the 96-hr LC50 values. Without any dissolved organic carbon added, the LC50 values ranged from 19.6 to 24.3 μg Ag/L over the tested chloride concentrations. The mitigating effect of DOC was significant. However, the difference between 5 and 10 mg/L DOC was not always significant. If 5 mg/L DOC was present the LC50 values ranged from 21.8 to 26.2 μg Ag/L over the tested chloride concentrations. An additional 5 mg/L dissolved organic carbon produced a range of 26.0 to 33.8 μg Ag/L. All NOEC values corresponded to the nominal silver treatment of 10 μg Ag/L.

All LC50 values were significantly greater than those produced with 4-d old fathead minnows. At 0 mg/L DOC, the LC50 values for 28-d old fish averaged 7.6 times higher than those produced with 4-d old fathead minnow larvae. This difference decreased to 3.8 times at 5 mg/L DOC. An additional 5 mg/L DOC did not significantly affect this difference (4.0). A chloride concentration of 3 mg/L exhibited similar LC50 differences between 4-d and 28-d old fish among the dissolved organic carbon treatments (i.e., 8.1, 3.9, and 2.7 times)(Figure 1). The 4-d old fish were unusually sensitive to silver at 40 mg/L chloride for unexplained reasons. This phenomenon was not seen with 28-d old fathead minnows. At 60 mg/L chloride and 0 mg/L DOC the difference in LC50 values was 4.4 times greater for 28-d old fish; appreciably lower than the difference exhibited at 3 or 20 mg/L chloride (Figure 2). The differences were similar however at 5 and 10 mg/L DOC. Even though LC50 values increased, the same significant increase in NOEC values was absent.

Lemke (1980) reported the lack of effect produced by chloride with concentrations ranging from 1 to 32 mg/L. I found that chloride concentrations of 20 mg/L or greater test waters were cloudy. However, this precipitation did not correspond with significantly decreased toxicity at any level except 60 mg/L chloride and 0 mg/L DOC. This effect was less evident with dissolved organic carbon present. Chloride concentrations above 60 mg/L may indeed reduce silver toxicity. The increased toxicity exhibited by 4-d old fish at 40 mg/L chloride was absent in this study. This may indicate that the response may have been physiologically based. Gill development may play a role in the age sensitivity of the fathead minnow.

Results of this study indicate that an organic carbon coefficient should be incorporated into the criteria for silver. Acute silver toxicity was diminished significantly with 5 or 10 mg/L DOC. The difference in response between these concentrations of DOC was not as pronounced as that found with 4-d old fathead minnows.

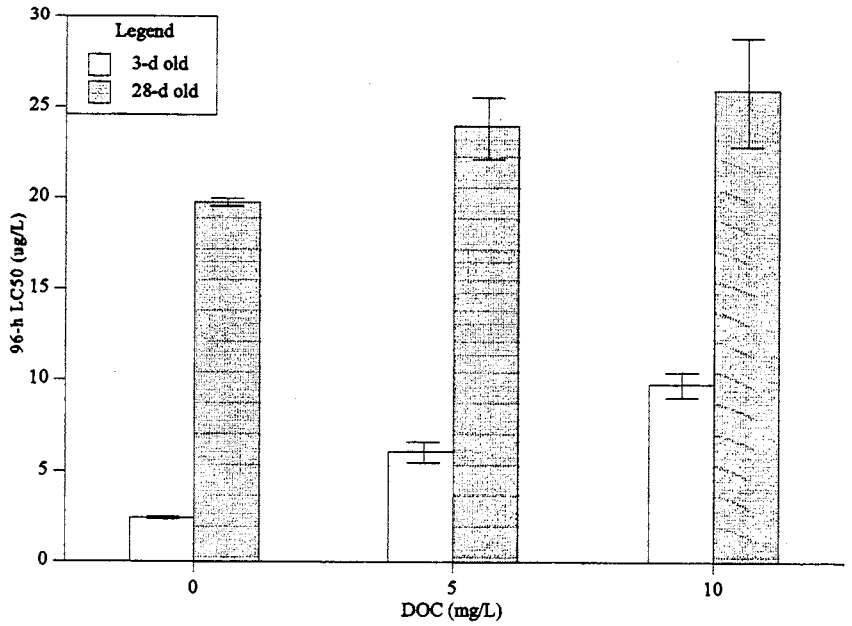

Figure 1. Age comparison of fathead minnows for acute silver toxicity at hardness of 50 mg/L and chloride 3 mg/L.

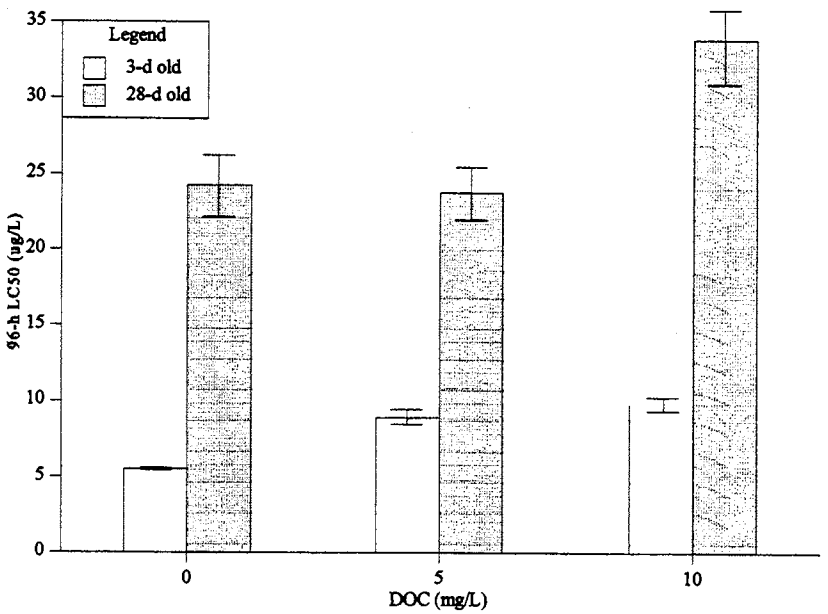

Figure 2. Age comparison of fathead minnows for acute silver toxicity at hardness of 50 mg/L and chloride 60 mg/L.

Questions & Answers: Influence of Age Sensitivity on the Acute Toxicity of Silver to Fathead Minnows at Various Water Quality Parameters

Q. FERNANDO GALVEZ (McMaster University): More of a comment. We didn't do DOC; we looked at chloride and hardness. Previously we maintained our chloride at 50 micromolar and 250 micromolar, which is low. We saw that there wasn't much of an effect on the LC50, but we also maintained the silver levels at 100 micrograms per liter and varied the chloride concentration. We saw the same effect. So we went to MINEQL, and we modeled this. We saw that, in order for chloride to have a major effect on speciation, the silver concentrations have to be elevated. You don't start to see a major effect on speciation until you get above 10 micrograms per liter, or around 40 to 50 micrograms per liter of total silver. I am hypothesizing that something such as DOC may modify toxicity enough that you have to have a higher concentration of silver to reach the LC50. When that condition is being met, then you have a high total silver concentration, so as you increase the chloride, then you'll start to see the effect of chloride coming in. The effect of chloride, I think, is more of a secondary effect; you need something else to modify the LC50 enough to bring it up to that concentration, and then chloride will have an additional effect. But if the DOC in natural water is low so that your LC50 is low, you'll never get to those conditions. Your silver concentrations are going to be so low that the AgCl that forms is going to be aqueous and potentially bioavailable to the fish.

A. Did you use Aldrich humic acid, or did you use some natural DOC?

Q. GALVEZ: We didn't do DOC. We saw that, when we had our silver concentrations high, we saw an effect on chloride. In my discussion I mentioned the fact that this might be what was going on. Your data seem to support that hypothesis.

A. Did MINEQL come out with similar types of responses to what George showed yesterday? Because we haven't run that yet.

Q. GALVEZ: There was so much data there that I'd have to look at it a little more closely.

Q. CHRISTER HOGSTRAND (University of Kentucky): It is also really a range phenomenon. Fernando did some work on this some years ago. If you would increase the chloride concentration more to get up to, say, 50 or more, you wouldn't be able to kill the fish with silver.

Q. JOSEPH GORSUCH (Eastman Kodak): Yesterday George Cobb reported that he did not see any dissolved silver in the presence of the humic acid. Was that the case with the 28-day-old exposure?

A. I think we saw the same trends with silver concentrations between dissolved and total with the 28-day-old as we saw with the four.

Q. GORSUCH: Is that true with all variations?

A. I don't remember. I think so. I don't think there was anything peculiar in the difference between experiments in terms of chemistry.

Q. JAMES KRAMER (McMaster University): With regard to the time change, I'm wondering: how were the fish conditioned before you started counting? Were they in the same solution without silver, or . . .

A. They were held in a culture chamber, and then we transferred them in within 30 minutes.

Q. KRAMER: But is that medium different from the experimental medium, except maybe for one parameter?

A. It is a big difference. There's no DOC, and obviously they're not all the same hardness.

Q. KRAMER: How do you account for that change versus the experimental change?

A. Certainly that is an important factor, but when you vary this many water quality parameters, what you have to do is control for that with your control organisms. If we saw high control mortality in any particular treatment, then we had to redo the test.

The Effect of Stabilized Silver-Laden Waste-Activated Sludge on the Growth of Terrestrial Plants

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An important consideration in assessing the environmental impact of photoprocessing solutions is the consideration of the effects on plants grown in soils amended with sewage sludge that has been used in the treatment of photoprocessing waste. A greenhouse study was performed to evaluate the effect of the test article, an artificial silver-laden waste-activated sludge generated exclusively from the treatment of photographic solutions, on the emergence, growth, and yield of lettuce, oats, turnip, and soybean seeds planted in soil mixtures amended with the sludge. Two soil mixtures were amended with the sludge at the levels of 0.125% and 1.0% (w/w). A control soil mixture was also prepared. The control, 0.125% and 1.0% sludge-amended soil mixtures contained silver at analyzed concentrations of 0.25, 5.2, and 120 mg Ag/kg, respectively. Twelve plants of each species (turnip, soybean, and oats) and 20 lettuce plants were grown from seed in each of the three soil mixtures. The growing pots were observed daily for emergence. The general health of the emerged plants within the growing pots was also observed daily. Each species was harvested at maturity and the yield determined. The uptake of silver into the mature plants was also investigated. Conclusions regarding "significance" refer to statistical comparisons of the data that were performed using an alpha level of 0.05.

There was no effect of the sludge on the mean emergence time or the mean percent emergence of any of the seeds sown in the sludge-amended soil mixtures used in this study. Refer to Figure 1.

The mean wet weights of leaves from lettuce plants grown from seed in both soil mixtures amended with the sludge were significantly greater than that of the control group. However, the mean dry weights of lettuce plants grown from seed in either sludge-amended soil mixtures were not significantly different from that of the control group. The mean wet and dry weights of soybean plants (stalk and leaves) and turnip leaves grown from seed in the soil mixture amended with 1.0% sludge were significantly heavier than those of the corresponding control plants. The mean dry weights of oat plants (stalk and leaves), and wet and dry weights of oat seeds grown in the soil mixture amended with 1% test article were significantly heavier from those of the control plants. Although the oat plants grown in soil amended with the sludge at a concentration of 1% (w/w) were significantly heavier than the controls, the mean height of the same oat plants was significantly *shorter* than the controls. Therefore, the overall growth of oat plants was not adversely affected by the presence of the sludge in the soil at concentrations up to 1% (w/w). Refer to Figures 2 and 3.

Lettuce leaves, oat seeds, and oat stalks from plants grown in soil mixtures amended with both 0.125% and 1% sludge contained significantly more silver than the corresponding controls. In addition, soybean stalks from plants grown in soil mixtures amended with sludge at a rate of 1.0% (equivalent to a land application rate of approximately 10 tons/acre) contained significantly more silver than the corresponding controls. Refer to Figure 4.

The plant growth effects and tissue silver accumulation seen in this study may be observed after growing plants in soils amended with sludges arising from the treatment of only photoprocessing solutions. It is likely that the growth effects and tissue silver accumulation reported in this study would not be observed in practice, as the effluent arising from photographic activities would contribute only a small amount to the total waste entering a publicly-owned-treatment-works (POTW).

Figure 1. Mean (± SD) time to emergence of seedlings from control and sludge-amended soils.

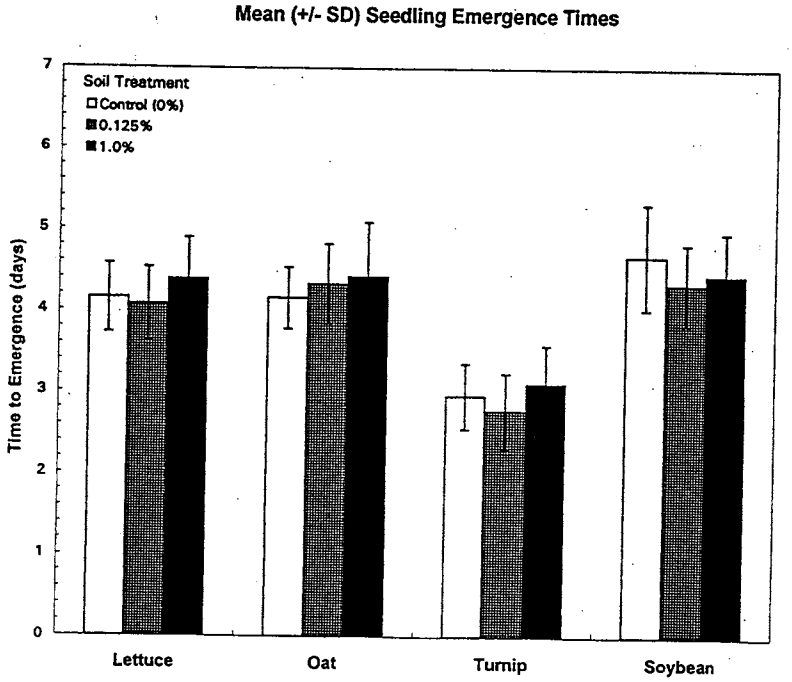
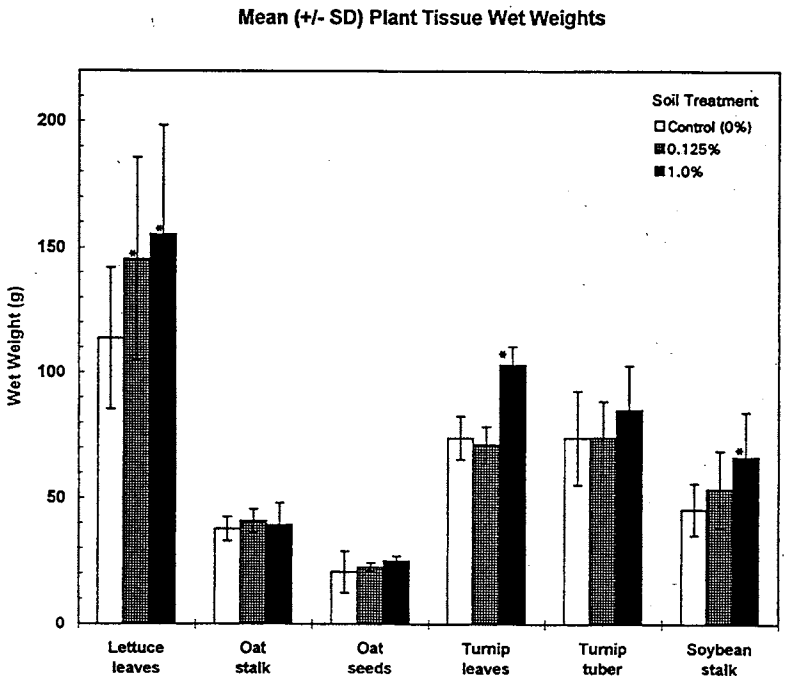


Figure 2. Mean (± SD) wet weights of mature plants grown from seed in control and sludge-amended soils.



*Significantly greater than control value ($p<0.05$)

Figure 3. Mean (± SD) dry weights of mature plants grown from seed in control and sludge-amended soils.

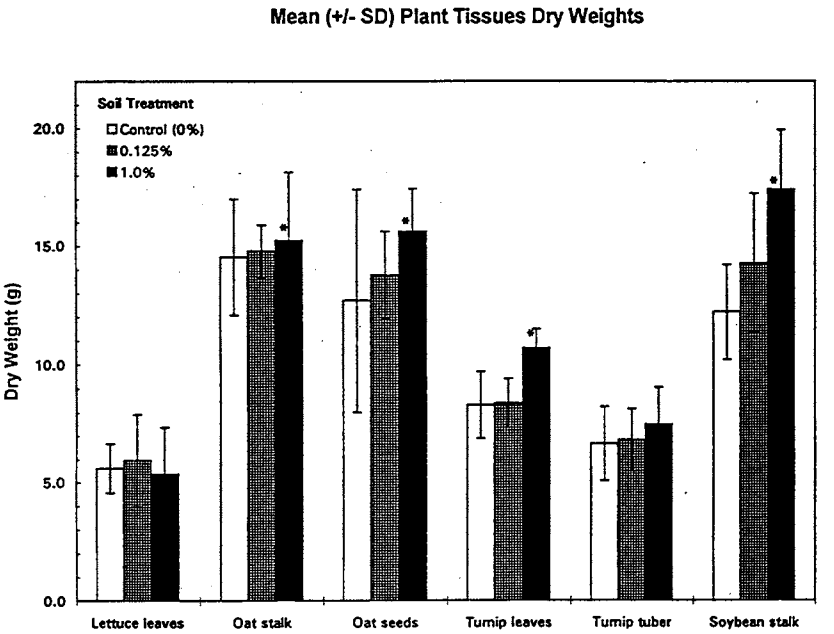
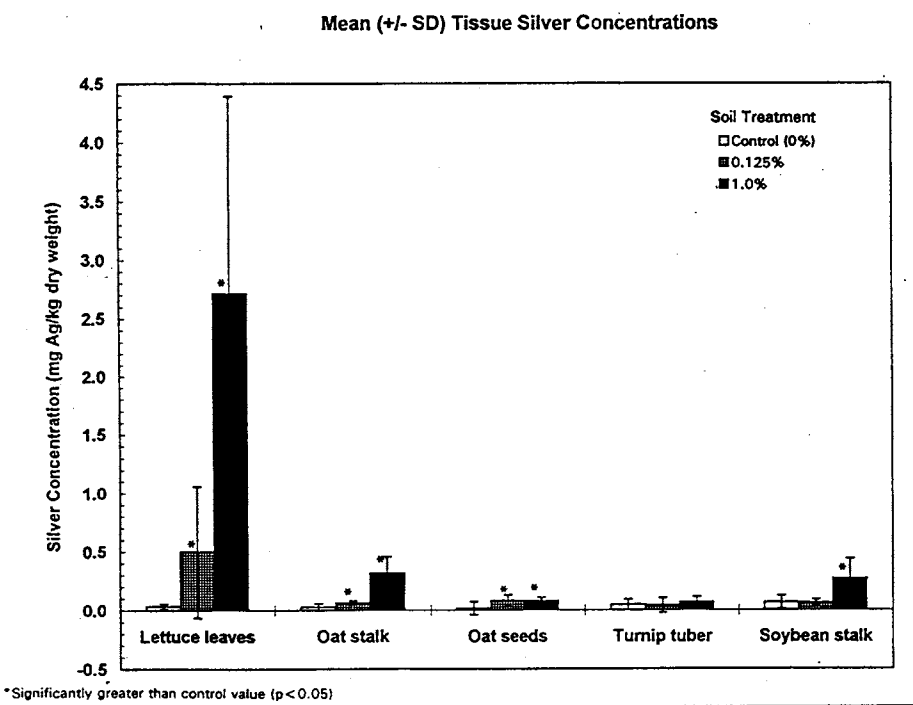


Figure 4. Mean (± SD) concentrations of silver in tissues of mature plants grown in control and sludge-amended soils. Control, 0.125% sludge-amended, and 1.0% sludge-amended soils contained 0.25, 5.2, and 120 mg Ag/kg, respectively.



Questions & Answers: The Effect of Stabilized Silver-Laden Waste-Activated Sludge on the Growth of Terrestrial Plants

Q. ERIC CRECELIUS (Battelle Marine Sciences Lab): There seems to be no conceivable problem with silver as far as bioaccumulation or effects on plants using existing sludges that come from POTW's.

A. As best we can tell. I collected, for the first two studies, actual sludge from POTW's, but I had to spike it in because the levels of silver were so low. But even using those as controls, the silver was not accumulated into the plants in those studies.

Q. JAMES KRAMER (McMaster University): We talked a little bit last time about why lettuce may preferentially take up silver. Do you have any further comments?

A. I'm not sure why it seems to take up silver. I know there are some studies that show that it takes up organics. Some plants are hyperaccumulators. There has been some work to suggest that plants tend to accumulate those metals that are necessary, but as far as we know there's no requirement for silver in the plant. So I don't have a guess yet for that. In fact, I was going to ask you about your cattails.

Q. KRAMER: There is a known phytochelatin in the cattails. I've forgotten what it is right now. We found that in the literature. I wondered if there was an equivalent in lettuce.

A. I have seen phytochelatins, and I could just assume that that's the case.

Q. DOMINIC DI TORO (Hydroqual, Inc.): When we were reviewing the sludge regulations that EPA promulgated, when we reviewed the procedure that they went through in order to avoid the same fate that befell them when we were doing sediment quality criteria, one of the things that we noticed was that there was a real divergence of opinion on whether pot studies (greenhouse studies) bore any resemblance to what happens out in the field. This was an issue that was raised by the reviewers of all of the work that was done, so if you need another thing to do . . .

A. In fact, I've asked for my own farm, but Kodak is telling me no. I have seen some articles that suggest that, within a greenhouse situation, the metals are taken up to a greater extent than in the field.

Q. DI TORO: The other thing you might want to look at is biota samples from sludge-amended lands that are already out there.

Q. DALAND JUBERG (Kodak): Do you have any thoughts on relative localization within the plant? The reason I ask this is, you showed — toward one of the last slides — that oftentimes in human health and risk assessment and multipath risk assessment, indirect exposure is one of the critical pathways that people look at. In particular, with respect to ingestion of plants, localization of silver and other metals may be important in terms of human health dose. Do you have any thoughts on localization: tubers, roots, leafy structures, things like that? Beyond just silver.

A. All I can tell you is that in the first two studies, we did analyze roots, and roots are incredibly difficult to clean. There was quite a bit higher silver concentrations in the roots, and part of it could have just been associated with the roots, or it could have been stuck at the roots. There's been some work to suggest that the roots do

act as a very nice barrier to some of these metals. As far as localization of silver goes within the plant, I'm not sure if it localizes to certain areas. I know that corn plants can localize certain metals along the leaf veins. We didn't do that; in fact there was only one study where I used corn. It gets incredibly large in a greenhouse and you have to hand-pollinate it, so I chose not to use corn in this last study and instead jack up the number of plants in this last study. I guess my answer is, no, I don't know about localization, other than I do know that it accumulates in the roots, and the roots may act as a barrier. It was interesting when we did the turnips, because that is a root crop, and that was one of the plants that just showed no silver uptake. There was obviously silver in the peelings, but not massive amounts, either.

Q. FERNANDO GALVEZ (McMaster University): Do you have any idea why the plants are growing better in the silver-contaminated sludge?

A. The sludge is applied to the land as a soil conditioner and as a fertilizer as it's usually high in nitrogen and phosphorus. Probably the reason is that it's acting the way we'd expect it to act if we were to apply it to the land. From what I've read about regulations on sludge application to soils, basically you have to show that it is going to be beneficial to the crops that will be grown there. So it's nice to see that we went into this thinking sludge is a fertilizer, and it appears to be acting as a fertilizer. Whether it's the nitrogen, the phosphorus, or something else as a result of photoprocessing, I don't know.

Q. ANDERS ANDREN (University of Wisconsin): In keeping with the plea of Harold Bergman about a more mechanistic understanding vis à vis aquatic organisms; do you have any idea how silver travels through the soil — perhaps through interstitial water — into the plant? Whether it's passive diffusion or whether you have active pumps working across the membrane? Do you have a conceptual mechanism as to how these things get into the plants?

A. From what I've read, it depends on the metal. There appears to be some difference in how it's handled, based on whether it's a required element for the plant or whether it is something that's not required. For required elements, there seems to be a mechanism for uptake, whereas for the metals that aren't required it seems to be more of a diffusion at the root zone. I don't know if this is the case for silver; I'm only speaking for other metals. That might be something that's worth looking at, as far as silver goes.

Q. GEORGE COBB (Clemson University): We did some research with Kennecott Corporation using some mine tailings-amended soil. We found results that were very similar to yours. One thing I'd like to add to the comment Dominic made is that part of the reason that some of the laboratory studies are not mimicking field studies is that, sometimes in the laboratory studies you have extremely heterogeneous soils. And when you're dealing with nominal concentrations in those heterogeneous plots, you often get skewed results.

A. Thank you.

Q. NICHOLAS ADAMS (McMaster University): With the cattails, we found some correlations with nickel, zinc and some other metals. So that's a possibility; to look at correlations and relate it to what's known about those other metals.

A. Thank you.

Q. JAMES KRAMER (McMaster University): Relative to Anders' comment about mechanisms, and whether it's a diffusion or maybe a ligand-activated phenomenon: A long time ago we did a study on aluminum transport in white pine, and we found all the action is right during germination. One of the studies you might want to consider is to take your reference plant and grow it in the noncontaminated topsoil; then right after germination, replant it into the contaminated topsoil. Then, of course, do it in all the combinations and see if there is a

difference. Now, I know white pine seedlings are not lettuce and so on, but we found that, going into the literature, it looked like right when the tap root starts going that all of the really good ligands are out there to grab everything and scoop them up in there. Maybe you've already done that.

A. No, we haven't done that yet.

Influence of Complexing Agents on Uptake of Silver by Rainbow Trout and by *Daphnia*

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Previously, we quantified Ag-gill binding strength in comparison to Ag-thiosulphate and Ag-dissolved organic carbon (DOC) binding. These binding affinities were log $K_{Ag\text{-}gill} = 10.0$, log $K_{Ag\text{-}S203} = 8.80$, and log $K_{Ag\text{-}DOC} = 9.0$ (Janes and Playle 1995). These values were entered into an aquatic chemistry program, MINEQL$^+$ (Schecher and McAvoy 1992) to predict Ag accumulation by trout gills. From our work it is clear that complexing agents such as thiosulphate and DOC can keep Ag from binding to gills of freshwater fish.

We have been expanding this approach by studying adsorption of Ag to gills of rainbow trout and the subsequent active uptake of Ag into the fish, as modified by the complexing agent thiosulphate. Adult rainbow trout (*Oncorhynchus mykiss*, ~200 g) were fitted with dorsal aortic cannulae for repetitive blood sampling during 5 d exposures to ~0.11 μM Ag (~12 $\mu g \cdot L^{-1}$) in synthetic soft water with 0 or 5 μM thiosulphate added.

In the absence of thiosulphate, Ag accumulates on trout gills and in the plasma: accumulation of Ag in the plasma is against the electrochemical gradient for Ag, so is an active uptake process. With thiosulphate in the water, Ag accumulation on trout gills and in the plasma is reduced or eliminated. Impaired gas transfer at the gills caused by Ag, as indicated by low arterial PO_2, and ionoregulatory effects of Ag (e.g. decreased plasma Na) are eliminated when thiosulphate is present. However, thiosulphate alone is toxic to the fish.

We have been studying the protective effects of DOC against metal uptake by fish using Cu and Cd, plus terrestrial (Aldrich) humic acid and natural, aquatic DOC. Rainbow trout were exposed to a mixture of ~0.15 μM Cd (~17 $\mu g \cdot L^{-1}$) and 0.75 μM Cu (~48 $\mu g \cdot L^{-1}$) in synthetic soft water, in the presence and absence of 40 mg $C \cdot L^{-1}$ Aldrich humic acid or 5 mg $C \cdot L^{-1}$ DOC isolated from a marsh. The DOC was concentrated from Luther Marsh, near Grand Valley, Ontario, by reverse osmosis, and was passed through a cation exchnage resin to remove Cu contamination of the DOC (Hollis et al. 1996). Repetitive blood samples from the fish were taken throughout the exposures *via* dorsal aortic cannulae.

Forty mg $C \cdot L^{-1}$ humic acid protected fully against active uptake of Cd and Cu into the fish, and protected fully against respiratory (low arterial PO_2, high arterial PCO_2) and ionoregulatory (e.g. decreased plasma Cl concentrations) effects of the Cd and Cu mixture. Forty mg $C \cdot L^{-1}$ humic acid itself had no effect on any of the measured blood parameters. That is, high concentrations of humic acid have entirely positive effects against waterborne metals, with no adverse effects.

Natural DOC at concentrations of about 5 mg $C \cdot L^{-1}$ also fully protected against active Cd and Cu uptake by the fish and against the respiratory and ionoregulatory effects of the metals. When comparing natural DOC and terrestrial humic acid at equal concentrations, there was no difference in the protection provided against Cu binding to trout gills. However, the terrestrial humic acid did protect better than the natural DOC against Cd binding at the gills. Aquatic DOC from different sources protects against Cu accumulation on fish gills to an equal degree (Playle et al. 1992), but further research is needed with Cd and different aquatic DOC sources to determine whether aquatic DOC sources vary in their ability to bind Cd and thus prevent Cd binding by fish gills.

Although our previous work has demonstrated the protective effects of DOC against Ag binding by trout gills (Janes and Playle 1995), work still needs to be done with adult rainbow trout and natural DOC to determine if Ag uptake by trout is affected by DOC in the same way as is uptake of Cu and Cd. In addition, it is important to determine whether the protective effect of DOC against Ag binding to gills and subsequent uptake by the fish is independent of the source of aquatic DOC. If the source of DOC is not a factor in determining protection against Ag (or Cd) uptake by fish - that is, if DOC *concentration* is the master variable - then prediction of the physiological and toxicological effects of these metals becomes much easier. We have recently shown that the age of the metal-DOC complex is not important in keeping Cu and Cd off fish gills (Hollis et al. 1996), which again indicates that DOC concentration is the master variable.

The applicability of the metal-gill modelling approach (Janes and Playle 1995; Playle et al. 1993a,b) to aquatic invertebrates is not known. We have started examining Ag binding to Daphnia (Cladocera) in the presence and absence of thiosulphate, as an initial step in extending the Ag-gill modelling approach to other aquatic organisms. Silver binding to *Daphnia* was relatively fast, reaching ~8 $nmol \cdot g^{-1}$ (wet tissue) in about 6 to 8 h, and stabilizing after that to 96 h, in exposures to 0.012 μM Ag (1.3 $\mu g \cdot L^{-1}$) in synthetic soft water.

Accumulation of Ag by whole *Daphnia* was similar to Ag accumulation by trout gills, even though the Ag concentration to which the *Daphnia* were exposed was about one-tenth that used in the trout exposures. The very low exposure concentration was necessary because of the extreme sensitivity of *Daphnia* to Ag in our soft water system. Background Ag concentrations were about ten times

higher in *Daphnia* ($\sim$2 nmol·g^{-1} wet tissue) than in trout gills. The high sensitivity of *Daphnia* to Ag, their small size (about 3 mm long, $<$0.5 mg each), and their high background Ag concentrations, all make measurement of Ag accumulation by these invertebrates difficult. However, there is no indication so far that a Ag-*Daphnia* binding constant cannot be determined then entered into a computer model for predictive purposes.

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Questions & Answers: Influence of Complexing Agents on Uptake of Silver by Rainbow Trout and by *Daphnia*

Q. PETER CAMPBELL (Universite du Quebec, INRS-Eau): In your experiments with adult fish, exposing them to cadmium and copper in the presence of DOC, I think I saw you show results where the DOC was protecting in terms of uptake into the plasma, yet you were getting metal onto the gills. Can you explain that discrepancy?

A. For copper there was a little bit, but it's not significant. But yes, it does look like there's some, and with cadmium there certainly was. The discrepancy would be that we don't have a fish that died. We don't know how much metal they would've accumulated on the gills. The assumption is that even though there are significant amounts there, it's much less than if the fish had not been in the presence of organic carbon. In that figure we don't have what we'd like to compare it to.

Q. PETER CAMPBELL: In your talk, and in Harold's yesterday, there was considerable emphasis on speciation of metals in the exposure solution. I would suggest that the concept of speciation be extended to the gill surface itself. You're talking about tissue level in the gill after, I presume, short-term exposure. In such short-term exposure you have metal in the mucous, on the gill surface and on the gill tissue. You've got at least three different "species" of metal on the gill, and do you not think that the biological response is going to vary as the distribution among those different forms varies?

A. That's a good point. When we prepare the gill samples we rinse them off. Presumably it does get rid of loosely-bound metal and probably mucous-bound metal. But whether the metal is on the surface of the gills or inside we can't tell. You could use an EDTA rinse to remove surface-bound metal and do it by difference, but the way we're looking at it, it really doesn't matter whether it's surface bound or inside, because what we're dealing with is presence or absence. If it's not on the gill it's not toxic, is the theory. If it is on the gill, then it might be toxic. So we're talking presence or absence. In this simple model, we're not worried about what compartment it's in.

Q. JIM KRAMER (McMaster University): Rick, as other people have pointed out before, I think you should be congratulated for making this linkage; some might say it's obvious, but you did it and did it very well. Having said that, there is a very important assumption that has not been brought out, and I think it's extremely important that it be brought out and you think about it. That is, in your basic experimental setup and calculation, you are assuming — and this is true in all ligand competition work which is done in sediments and everywhere else — that the metal alone adsorbs on the gill (or say, on the gill ligand), and certainly in sediments this is not the case. This assumption is where the weakness of an otherwise elegant ligand competition model is. In other words, the ligand can adsorb on there, and the metal-ligand can adsorb on there. This will not qualitatively change your results, necessarily, unless the metal-ligand has a specific toxicological effect. But it will certainly change your conditional stability constants. You have to bring this in. I would suggest that the one ligand you're dealing with, and I can tell you a case with fish where this happens, is DOC. DOC, as a fulvic acid, does adsorb on the gill. This was shown in some aluminum work we did. So you have to consider not just metal partitioning between solution and gill ligand, but you have to consider that ligand adsorbed on the gill and the binding constant of that at the gill, which is not necessarily the same as that in solution. We have these problems in sediments. We'd like to do this in sediments, but the ligand adsorbs on the sediments, and you have to consider that. There's two different ways that can occur.

A. I see the point, and possibly we're talking about disjunctive and adjunctive reactions. I think you're talking about an adjunctive reaction, where you get the two sets of ligands joining together and then there's a reaction, as opposed to the free metal sort of being in an equilibrium between them.

Q. JIM KRAMER: No, that's another complication, where you can get bidentate relationships. But you have a charge distribution on the gill, and if you have a ligand on the gill, and you put a metal on that, the binding constant is not the same as in solution. It's different, and it can be markedly different. If you go into the work on other surfaces, this comes up. In some cases this can be negligible, but this is a fundamental assumption in your work.

A. I guess the assumption behind that is that dissolved organic carbon is a complex amorphous molecule. Assume that that's negatively charged; even if there are metals on it, it's going to be negatively charged. Mucous is negatively charged. I would argue that there's more repulsion and there probably isn't a large degree of metal-DOC binding on the gill. Certainly, in the model it's assuming that it's the free metal that interacts.

Q. JIM KRAMER: When you say a gill or any surface is net negatively charged, you're saying the overall average charge. There are domains of positive and negative charge. I think it's important that this assumption be made up front. If you look at the sediment literature, people do address this, and the molecules are no different.

A Review of Silver Hazards to Plants and Animals

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Abstract

A review of available literature on ecological and toxicological data of silver (Ag) and silver salts in the environment are briefly summarized, with an emphasis on natural resources. Subtopics include sources and uses of silver, chemistry and metabolism, concentrations in field collections, lethal and sublethal effects, and recommendations for the protection of natural resources (Eisler, in press).

Elevated silver concentrations in biota occur in the vicinities of sewage outfalls, electroplating plants, mine wastes, and silver-iodide seeded areas; in the United States, the photography industry is the major source of anthropogenic silver discharges into the biosphere. Maximum silver concentrations found in field collections, in mg total Ag/kg dry weight (tissue), are 1.5 in mammals (liver), 6 in fish (bone), 14 in plants (whole), 30 in annelid worms (whole), 44 in birds (liver), 110 in mushrooms (whole), 185 in bivalve molluscs (soft parts), and 320 in gastropods (whole). Humans with silver poisoning (argyria) contain as much as 72 mg total Ag/kg dry weight skin and 1,300 mg total Ag/kg fresh weight whole body. Silver and its compounds are not known to be mutagenic, teratogenic, or carcinogenic.

Under normal routes of exposure, silver does not pose serious environmental health problems to humans at less than 100 ug total Ag/L drinking water or less than 10 ug total Ag/m^3 air. Free silver ion (Ag^+), however, is lethal to representative species of sensitive aquatic plants, invertebrates, and teleosts at nominal water concentrations of 1.2 to 4.9 ug/L; at sublethal concentrations, significant adverse effects occur between 0.17 and 0.6 ug/L. No data are available on effects of silver on avian or mammalian wildlife; all studied effects are on poultry, small laboratory animals, and livestock. Silver is harmful to poultry at concentrations as low as 1.8 mg total Ag/kg whole egg fresh weight by way of injection, 100 mg total Ag/L in drinking water, or 200 mg total Ag/kg in diets; sensitive mammals are adversely affected at total silver concentrations as low as 250 ug/L in drinking water, 6 mg/kg in diets, or 13.9 mg/kg whole body.

Proposed criteria for the protection of sensitive resources are discussed in terms of silver speciation in natural waters, the significance of silver residues in tissues, silver interactions with other metals, the environmental fate of silver, and silver absorption and retention by animals.

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Questions & Answers: A Review of Silver Hazards to Plants and Animals

Q. TOM BOBER (Eastman Kodak): Ron, you acknowledged the difference in toxicity between ionic silver and total recoverable silver, and yet your proposed criteria were in terms of total recoverable silver. Does this not ignore chemical realities in the environment as well as biological ones?

A. There's an imperfect correlation between total recoverable silver and acid-soluble silver. For example, the freshwater criterion of 2.3 micrograms per liter; in the '87 draft document to EPA, this is 0.92, but they have lots of catch-22's in there. It could go as high as 7.3 for an hour, which is like saying that, on average, you can put your head underwater for five seconds a year, but over a 30-year period, if you do it all at once . . . you know. A chain is as strong as its weakest link. So this is still a problem: how to speciate the silver. I had a lot of help on this; Professor Kirschenbaum from the University of Rhode Island has spent his whole life on hypervalent silver species, so I had to give something on that, and this is now in the marketplace. But what is particularly distressing to me, and this is my goal, is to see some work on terrestrial and avian wildlife. It probably will not be profound, but it's a gap, along with the other 30 gaps. And I have a galley proof of the entire manuscript if anyone wants to look at it; it has a lot of numbers in it. I just went for concepts in this talk.

The Bioavailability of Silver in Sediments

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Introduction

The toxicity of certain cationic metals in sediments is strongly influenced by the quantities of the amorphous metal sulfides that are present [1]. It has been demonstrated that if the molar concentration of acid volatile sulfide (AVS) that is extracted from a sediment exceeds the molar sum of the simultaneously extracted metals (ΣSEM) that form more insoluble sulfides than iron sulfide, e.g., NiS, ZnS, CdS, PbS, and CuS, then those metals will not be toxic to sediment dwelling organisms [2, 3, 4]. The chemistry and the initial toxicity results, presented below, support the hypothesis that for Ag/AVS < 2 (the 2 accounts for the stoichiometry of Ag_2S) the pore water concentrations of silver are low and there is no toxicity. The results also demonstrate that there is additional binding of silver in sediments beyond that provided by AVS. This is presumably due to silver partitioning to organic carbon. Organic carbon is suspected because silver binds strongly to organic carbon and organic carbon is the source of the additional binding observed for Cd, Cu, and Pb [5].

If the conclusion—that for Ag/AVS < 2 no toxicity is found—is sustained through further testing, then it is highly unlikely that the level of silver found in most sediments will cause toxicity to benthic animals. The reason is that the typical AVS concentrations found in sediments ($> 1\mu$mol/g) are much larger than typical silver concentrations found in sediments ($\sim 1\mu$g/g $\simeq 0.01$ μmol/g) so that the molar ratio will almost always be less than 2. For sediments with no AVS, the sediment organic carbon concentration can provide significant binding and work is ongoing to evaluate its magnitude and significance.

FeS and AVS Binding Capacity

The fundamental displacement reaction which converts silver to silver sulfide is:

$$2Ag^+ + FeS(s) \rightarrow Ag_2S(s) + Fe^{2+} \tag{1}$$

We have tested whether this reaction occurs in a reasonable length of time. Two anaerobic titration experiments (Fig.1A) have been performed in which Ag was added as $AgNO_3$ to a suspension of freshly precipitated FeS(s) following the procedures used for previous determinations [1, 5]. Concentrations of dissolved silver ranged in the low μg/L range— the limit of detection for the methods employed—until the molar ratio of 2 is exceed, after which the silver concentration increases dramatically. This demonstrates that freshly precipitated FeS(s) can rapidly complex silver to form silver sulfide. The results of analogous titrations using natural sediments with varying AVS are shown in Fig.1B. There is a considerable difference between the results using a reaction time of 2 hrs, which was employed for the previous experiments [1, 5] and the results for which the reaction eq.(1) is allowed to take place for 24 hrs before the dissolved silver concentrations are measured. The results of a kinetics experiment (Fig.1C) demonstrated that the reaction eq.(1) takes longer than expected to complete when sediments are used instead of synthetic FeS. The results of two titrations using a 24 hr. equilibration period (Fig.1B – stars) result in low dissolved concentrations for Ag/AVS < 2 and an increase thereafter. This is what is predicted by the AVS binding model. Therefore we expect no toxicity for sediments where Ag/AVS < 2.

The effects of additional binding capacity in sediments can be seen by comparing the results of using only FeS (Fig.1A) and sediments (Fig.1B). The vertical lines denote a molar ratio of two. Note that for FeS, the increase in dissolved silver is abrupt to the right of a molar ratio of 2. By contrast, the dissolved silver concentrations for the sediments exhibit a slower rise. This is due, presumably, to the binding of silver to the particulate organic carbon in the sediment.

Sediment Toxicity Results

We have been working in collaboration with our colleagues at the EPA Narragansett Laboratory who are conducting sediment toxicity tests to examine the validity of the SEM/AVS procedure for silver [6]. The results of these tests are shown in Fig.2. The interstitial water data for silver are plotted against the nominal SEM/AVS in Fig.2 (top) using the $\times\times$ convention where SEM is divided by 2 to account for the stoichiometry in eq.(1). The data are identified as T = 0 (C) which is time = 0 data for centrifugation as the separation technique. Peepers were also employed and that is denoted by (P). Note that the peeper data (filled diamonds) are consistent with expectations based on the titration data. For SEM/AVS $< 1\times\times$, no pore water silver should be detected.

The mortality and peeper data are presented in Fig.2 (bottom). The mortality date is consistent with expectations except that there is an inversion in response between the $0.8\times\times$ and $1.2\times\times$ treatment. What makes this results suspicious is that the peeper chemistry data in inconsistent with the mortality data. The $0.8\times\times$ pore water silver concentration is low and mortality is high. The $1.2\times\times$ pore water silver concentration is high and the mortality is low. The causes for this anomaly appears to be toxicity due to ammonia in the pore waters [6].

Silver Sulfide Oxidation

We have performed an initial experiment to examine whether silver sulfide oxidizes in sediment. The experimental design is similar to that used for previous experiments used in the cadmium sediment model [4, 7]. Replicate sediment cores spiked with silver with an Ag/AVS = 1.0 ($0.5\times\times$ treatment) were set up in the laboratory. A parallel set of cores were spiked with cadmium at Cd/AVS = 0.5. The buffered overlying water, which was aerated with filtered air to maintain aerobic conditions, was sampled periodically. The time course data is presented in Fig3A,B. The cadmium concentration increased relatively quickly reaching its maximum at approximately 25 days (Fig.3A). Since the initial Cd/AVS ratio was 0.5, the release to the overlying water occurred due to CdS oxidation. At the termination of the experiment after 185 days of oxidation, the SEM_{Cd} and AVS concentrations were measured in 0.5 cm sections of the sediment. The results are shown in Fig.3C. Initially the profiles of AVS and SEM_{Cd} were constant in depth at the concentrations found at depth. The AVS concentration decreased to a depth of 4 cm. The SEM_{Cd} also decreased but only near the surface 1 cm. As a consequence the SEM/AVS ratio increased dramatically due to the oxidation of CdS. This is also reflected in the increase of the Cd concentration in the overlying water (Fig.3A).

By contrast, the silver concentrations in the overlying water (Fig.3B) stayed at low concentration until about 100 days, when the concentration increased. Thereafter it decreased to low levels. Both replicates exhibited this unexpected behavior. The SEM_{Ag} and AVS are shown in Fig.3D with both plotted to the same scale. Since silver sulfide is not extracted using nitric acid, the AVS found in these sediments measures only FeS. Note that the FeS is completely oxidized to 2 cm of depth. The SEM_{Ag} concentration is quite small, although it is greater than zero.

A presentation of the AVS and SEM_{Ag} concentrations for each replicate are shown in Fig.4. The plotting scale for the SEM is chosen so that the results are more clearly seen. A decrease in surface layer AVS and in the bottom layer occurred (Fig.4A,B). We attribute the lower layer oxidation to oxygen that diffused into the bottoms that sealed the sediment cores. The experiment lasted almost 200 days and some air apparently diffused into the reactor. Future experiments will employ better seals. The SEM_{Ag} (Fig.4C,D) showed an increase in the surface layers corresponding to the decrease in AVS. The same increase occurs in the bottom layer. We suspect that this increase in SEM_{Ag} is due to oxidation of Ag_2S.

Note that the concentrations of SEM_{Ag} are small and that the exposure time is long. Nevertheless this is evidence that silver can be released in the oxic layer. Therefore, it is possible that there was a silver flux to the overlying water which caused the observed increase (Fig.3B). The subsequent decrease in overlying water silver may be due to the formation of iron oxyhydroxide (FeOOH) and the re-sorption of the silver from the overlying water onto this phase.

Conclusions

It appears to us that the question of the ultimate fate of silver in sediments is still to be answered. For sediments with any appreciable AVS, the silver is present as silver sulfide. If silver sulfide were a *permanent* sink for silver, then that would imply that once silver forms silver sulfide, it has been permanently rendered environmentally unreactive. This line of reasoning applies to the other sulfide forming metals as well. Since toxicity depends on the relative magnitude of AVS and SEM, processes which create or destroy either the iron or other metal sulfide components of AVS and SEM are important in determining the toxicity and fate of metals in sediments. A number of laboratory and field data sets demonstrate that changes in SEM and AVS can occur for cadmium and zinc [4, 8, 9, 10]. These results indicate that metal sulfides in sediments are both formed and degraded affecting the SEM and AVS as time progresses.

There are two consequences of these observations. From the point of view of the application of sediment quality criteria, sediments that are judged to be non-toxic from measurements of SEM and AVS at one time in the year (e.g. the summer), may become toxic at a later time (e.g. the winter), due to the oxidation of the iron and the other metal sulfides in the intervening time. Therefore, in order to be sure that sediments remain non-toxic throughout the year, it is necessary to understand the seasonal cycles of the components of AVS and SEM. From the point of view of the fate and transport of metals in natural waters, the oxidation of metal sulfides liberates metal that can then escape from the sediment to the overlying water. Hence although the silver is not toxic in the sediment as silver sulfide, dissolved silver may be released to the overlying water by diffusive transport.

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Silver Titration

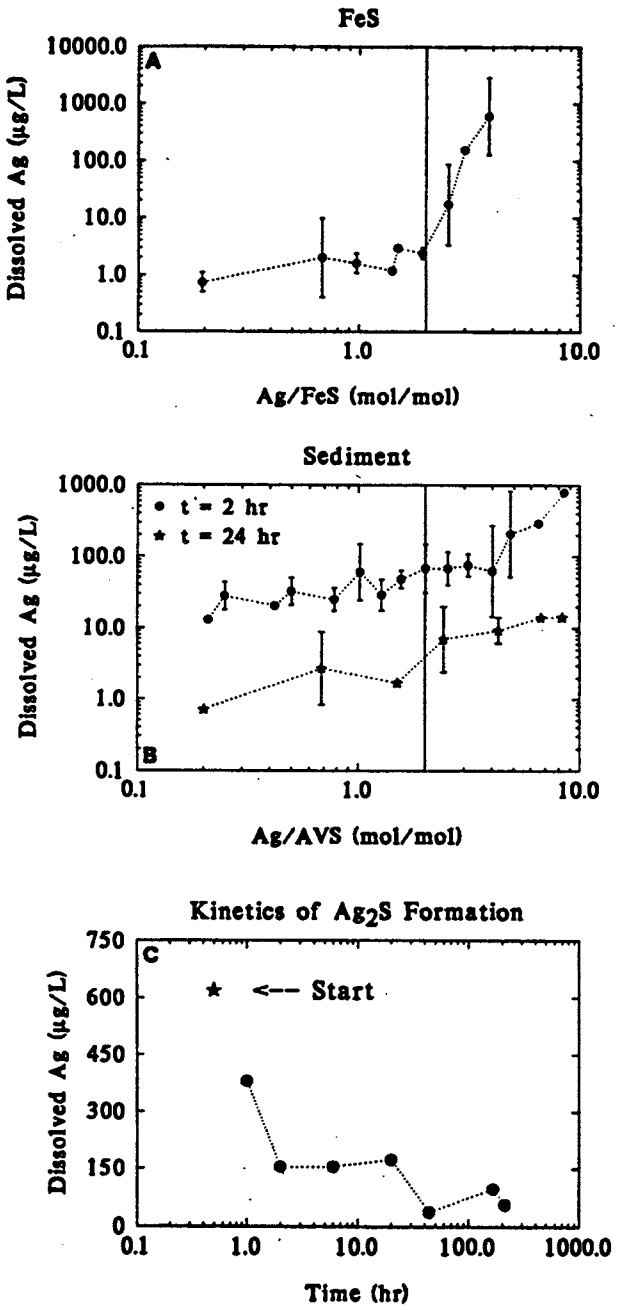
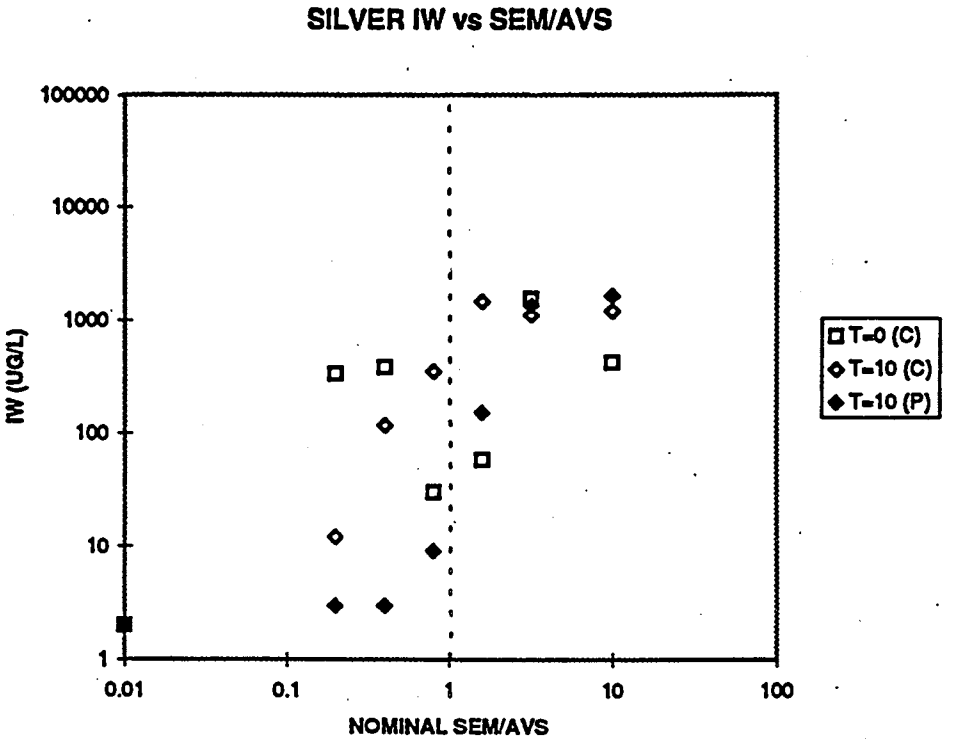


Figure 1


SILVER IW vs SEM/AVS
IW (UG/L)
100000
10000
1000
100
10
1
0.01
0.1
1
10
100
NOMINAL SEM/AVS
T=0 (C)
T=10 (C)
T=10 (P)

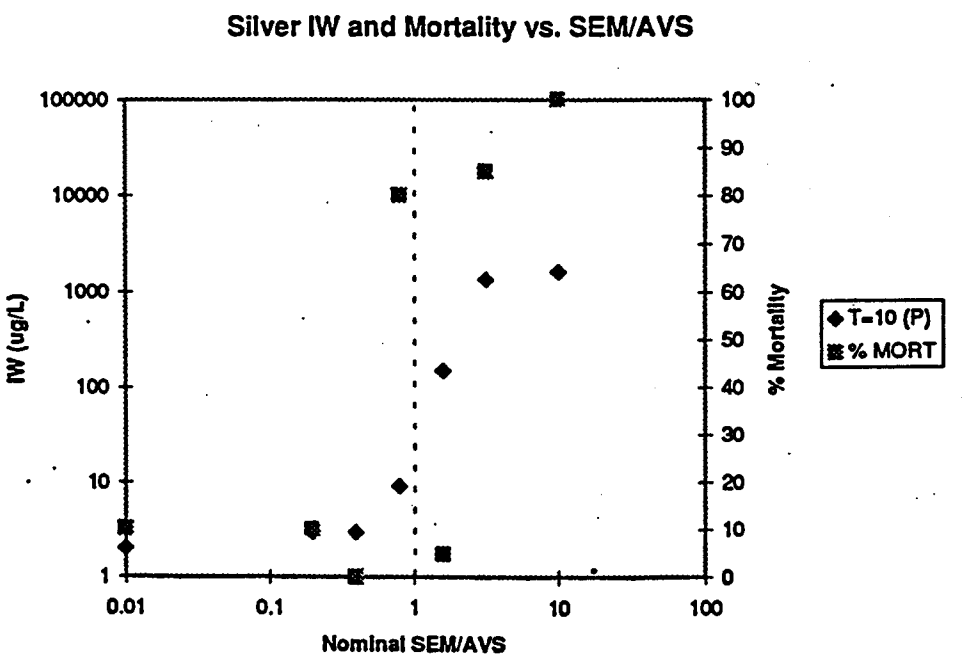
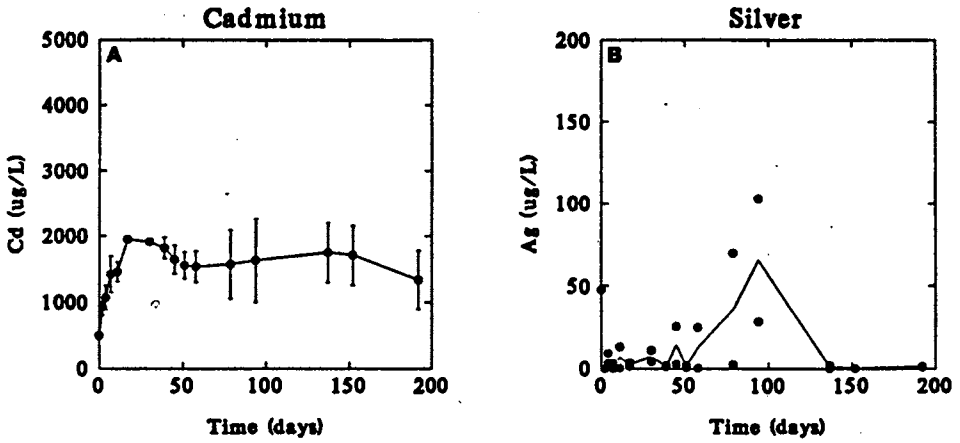

Silver IW and Mortality vs. SEM/AVS
IW (ug/L)
100000
10000
1000
100
10
1
0.01
0.1
1
10
100
Nominal SEM/AVS
% Mortality
100
90
80
70
60
50
40
30
20
10
0
T=10 (P)
% MORT

Figure 2

Sediment Oxidation Experiment

Overlying Water Concentrations



Final Sediment Concentrations

★ SEM
● AVS

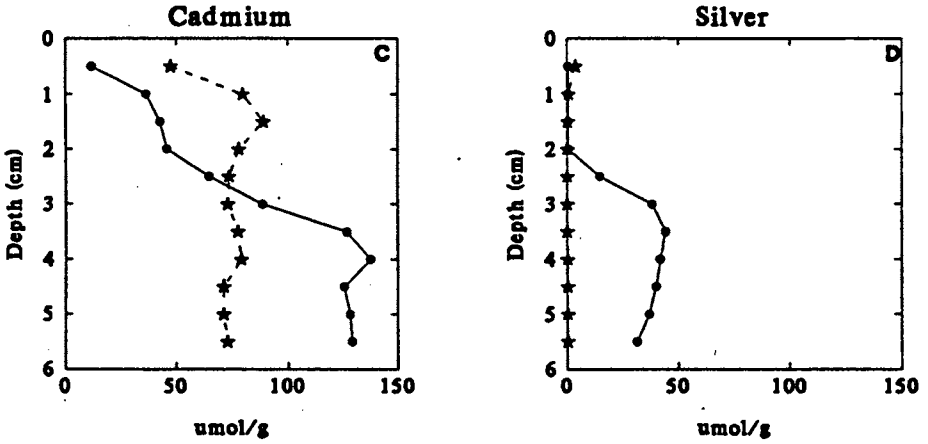


Figure 3

Silver Oxidation Experiment

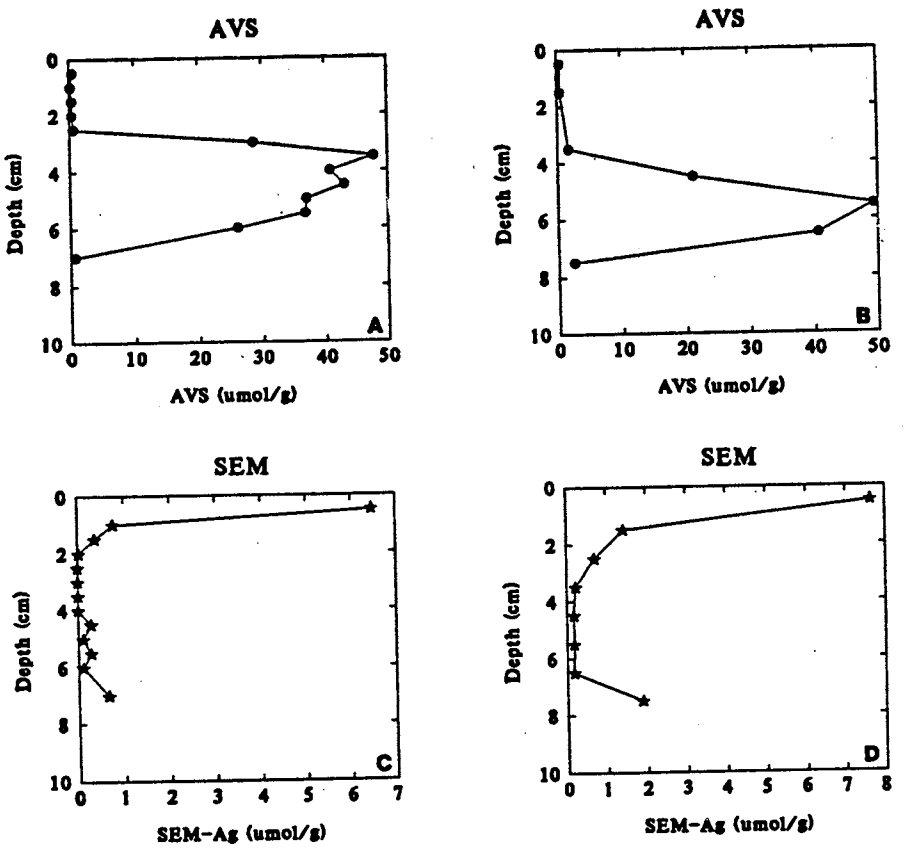


Figure 4

Questions & Answers: The Bioavailability of Silver in Sediments

Q. NICHOLAS ADAMS (McMaster University): Two questions. First, do you really know whether you have silver sulfide, or do you have a silver that's adsorbing onto your iron sulfide?

A. If it was an adsorption onto the iron sulfide, I would expect that the cold nitric acid extraction would extract it. We don't actually know, because we don't know the strength of the binding of silver to iron sulfide, but we run this thing in 0.3 molar nitric acid; that's a lot of protons competing with the silver. So my guess is that it's silver sulfide. Do you mean that we form silver sulfide absolutely? (Adams: Yes.) It's hard to explain this stoichiometric result if it was just sorption. The break occurs at exactly the molar ratio you'd predict from the formation of that. It would be damned convenient if the sorption was a two-to-one molar ratio. I think that's the first evidence that it's not sorption.

Q. NICHOLAS ADAMS: The second question I had is, on the last overhead, where you're saying that you don't think that it's some kind of solubility. What is your detection limit?

A. About a part per billion. Way above the solubility limit. (Adams: So you wouldn't see that . . .) If we were looking in porewater, absolutely. But what we're looking at here is the extraction of the whole sediment. This concentration is measured by taking a slice of the sediment and extracting it in nitric acid, and then measuring, with a detection limit of a part per billion, in the extract. So it's not a part per billion in that sense. But you're right; if we were looking in porewater, we'd have no idea what we were seeing.

Q. ANDERS ANDREN (University of Wisconsin): You have maximized the oxidation. In the environment you will have sedimentation with material that perhaps would consume oxygen, and we might not see oxygen diffusing that far down. Could you just comment on how you think that would affect a general interpretation of these experiments?

A. We designed this specifically to get as much oxidation as we could to make the thing happen. It was a proof of principle experiment, essentially. If there were any particle mixing at all, this material would be mixed up into the layer that's devoid of oxygen, and you'd see all of this reacting with that to make silver sulfide again. So as long as the molar ratio of sulfide — of SEM sulfur — to AVS stays less than one . . . this experiment was much more realistic. This experiment had animals in it, so you're getting constant mixing. You have to be pretty close to a molar ratio of one before you can actually manage to kick it over the edge. I think in the environment, the oxidation of silver is going to be a relatively slow thing. We know that because we don't see a lot of silver in the ambient waters. The people that have measured silver fluxes get tiny numbers: nanograms per meter squared per day. It's a very small flux. But the point is that it has to be looked at, just like it has to be looked at with any other metal sulfide. That's why one of the things we talked about at Pellston was trying to build these sorts of models into fate models, because it clearly is a phenomenon that we have to worry about.

Q. ANDERS ANDREN: Do you postulate that you have an inorganically-driven oxidation from oxygen, or do you think it's bacterially mediated?

A. I don't know. We're going to do some poisoning experiments to see what happens. In what looks like inorganic systems — just suspensions — it oxidizes. We haven't done silver yet, but cadmium sulfide oxidizes pretty well. So it's likely oxygen. Whether it's oxygen itself or radicals, I have no idea. We're thinking about whether it's free radical oxidation. But anyway, oxygen is the terminal electron acceptor.

Q. WESLEY BIRGE (University of Kentucky): As you know, we're concerned about the aerobic, biological region of the sediment and riverine systems. And these sediments are subject to suspension, resuspension, and downstream transport, which means further aeration, further washing, and so on. Then, depending on porosity and texture there can actually be substratum flow of the water column in the sediments, which starts to gnaw away at the integrity of the porewater compartment. With these kinds of concerns in mind, do you think that the acid-volatile sulfide-SEM ratio would still hold in these kinds of systems?

A. If you've got a purely aerobic sediment, you should see no AVS, in which case, it's of no use to you in deciding whether it's toxic or not. In gravels, for example, there is no iron sulfide, I would think. You can't have a ratio less than zero, so in gravel situations and in situations where there's no anaerobic sublayer, it doesn't work.

Q. WESLEY BIRGE: I'm not talking about just gravel substrates. Where you have 50 percent fine-grained soils and some sand and fine gravel, you can still get substratum flow. Irrespective of the texture analysis, you can still get suspension and resuspension into the water column.

A. What we know is that if you have an excess of AVS, acute toxicity is ameliorated. That's all we know. If the situation you're describing is a situation where there isn't any excess AVS, then you're on your own. We can't make any predictions.

Q. WESLEY BIRGE: But then what do the state regulators do in Kentucky, where most of the stream systems are of this sort?

A. What I would do in that situation is look in the porewaters and see what the porewater concentration of metals looks like. That has been the most useful diagnostic tool that we have, once the other methods fall apart, for example in systems where there's no AVS. And I say that to anyone who has a field situation. Whenever we're in trouble, whenever we see something we don't understand, we start looking at the porewater concentrations of everything we can measure. That's the best correlation.

Q. WESLEY BIRGE: The bottom line here, then, is AVS-SEM ratio is not universal.

A. It's a sufficient condition to prevent toxicity. It is not a necessary condition. So in that sense it's not universal. Correct.

Q. JIM KRAMER (McMaster University): Before you came, there was quite a discussion by a number of us on colloidal materials. I would suggest that many of the things you're showing us, as important as they are, have a colloidal effect. But before I do that, I have to ask you two questions. Aside from the marine situation, were these all low-concentration and low-ionic strength waters? The second question is, what was your separation size?

A. We have three sets of experiments, two of which I've shown you pieces of. One was a marine system. But the best porewater data comes from Landes, Hare and company. They used peepers with, I think, 0.1 micron, or . . . (Kramer: I'm talking about your lab experiments.) Oh, the lab experiments. The lab experiments are filtered with a 0.2 micron in-line syringe filter.

Q. JIM KRAMER: And these were low ionic strength? (DiToro: No, actually it's about a millimole of buffer.) That's low ionic strength. I think that we have to consider that you were looking at colloidal effects.

A. Yes, when we're looking at dissolved things. But I would like you to focus on the extraction. I think this is the strongest evidence. That's extracted silver from the sediments. Colloids don't really play a role.

Parameters that Influence Silver Toxicity: Ammonia and Salinity

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University of Kentucky
Lexington, Kentucky, USA

The toxicity and physiological effects of silver differ dramatically for freshwater and marine fish (Wood, 1995; Ferguson *et al.*, 1995,1996; Hogstrand *et al.* 1995, 1996). While most investigations have focused on freshwater systems, recent studies have started to pinpoint mechanisms and define silver toxicity for a few marine fish (*e.g.* rainbow trout, starry flounder, tidepool sculpin). Also, these investigations have illuminated several factors that influence the effects of silver. This study parallels these efforts and more closely investigates two such parameters, ammonia and salinity.

Ammonia

Silver exposure has opposing effects on nitrogen metabolism and excretion in fish. It causes an immediate and dramatic increase in plasma ammonia concentrations and leads to a time-delayed increase in the rate of ammonia excretion. These effects have been observed in freshwater and marine fish exposed to a wide range of silver concentrations (Hogstrand *et al.*, 1995; Hogstrand and Wood, 1996; Webb *et al.*, 1996). However, altered nitrogen metabolism is probably not the proximate cause of silver toxicity, since there was no dose-response associated with these increases (Hogstrand and Wood, 1996). While increased plasma ammonia concentrations do not appear to be the primary mechanisms of silver toxicity for the species studied, they do appear to make silver exposed fish more vulnerable to any factor that increases ammonia retention. For example, the majority of ammonia is excreted by diffusion across the fish gill. As water column concentrations of ammonia are increased, transbranchial diffusion gradients are reduced or even reversed. For marine fish, this applies not only to un-ionized ammonia but also to charged NH_4^+ (Wilson and Taylor, 1992). Thus, increased water column ammonia inhibits nitrogen excretion and results in a rapid increase in plasma ammonia, which may exacerbate silver toxicity. In the first set of experiments, a series of toxicity tests was performed on the tidepool sculpin, *Oligocottus maculosus,* to investigate this possibility.

Initially, 96-h static renewal toxicity tests were conducted with silver and ammonia according to recommendations given in EPA/600/4-90/027F. Sculpins were housed in 600 mL test chambers, ten fish per chamber, and three replicate chambers per test concentration. Test water (32 ppT salinity) was changed daily and maintained at 10±1 °C. After LC50s and LT50s were established for the individual toxicants, four concentrations of silver (0.431, 0.539, 0.647, 0.809 mg Ag/L) were tested in combination with three concentrations of total ammonia (8.6±6.1, 87.26±7.3, 224.47±2.5 mg Tamm/L). In addition, ammonia excretion rates were determined over a range of silver concentrations (0.108, 0.323, 0.421 mg Ag/L).

The 96-h LC50 values for silver and ammonia were 0.636 and 107.6 mg/L, respectively. When tested in combination, ammonia enhanced silver toxicity (Figure 1). The observed mortality for the combined toxicants, in all but one instance, was significantly greater than the predicted value for silver alone. This was true even for concentrations of ammonia that produced no effect (*i.e.* mortality) when tested alone.

In addition to concentration effects, time-course to mortality was investigated (Figure 2). In agreement with Ferguson *et al.* (1995), a time lag in the onset of silver toxicity was observed. Also, the slopes of the LT50 *vs.* concentration curves for silver and ammonia were different, which is indicative of separate mechanisms of toxicity. In the combined study, the two lowest concentrations of ammonia (8.6±6.1, 87.26±7.3 mg Tamm/L), when plotted

as LT50 *vs.* their respective silver concentrations, approached but fell below the silver curve. These curves indicate a larger silver and smaller ammonia contribution to the total toxicity. In contrast, LT50 *vs.* silver concentrations plotted for the highest concentration of ammonia (224.47±2.5 mg Tamm/L), flattened out and approached their respective time points on the curve representing ammonia. Thus, at the highest ammonia concentration, the majority of fish died from ammonia. Ammonia toxicity resulted from the dual actions of external ammonia and silver on plasma ammonia levels. Nevertheless, at all ammonia concentrations tested, the onset of silver toxicity was accelerated (*i.e.* below the LT50 *vs.* concentration curve for silver) again suggesting a dual mode of toxicity.

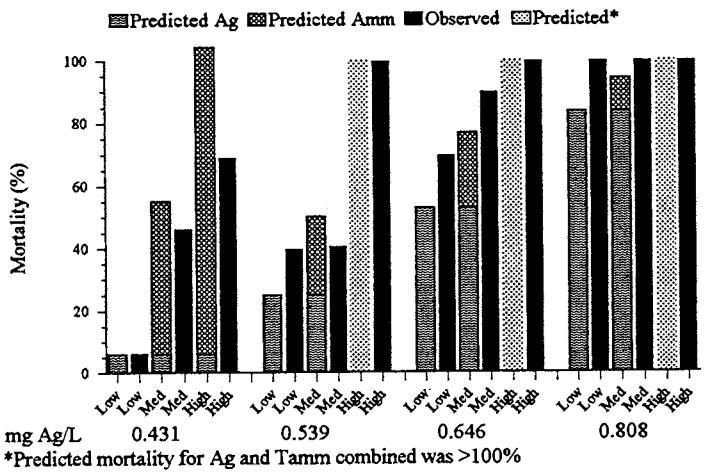

Figure 1. Predicted vs. observed mortality from combined toxicity tests with silver (Ag) and total ammonia (Tamm). *Low, medium, and high refer to Tamm concentrations of 8.6±6.1, 87.26±7.3, and 224.47±2.5 mg Tamm/L, respectively. Standard deviation for observed mortality overlapped predicted values for every combination except 0.431 mg Ag/L and high ammonia.*

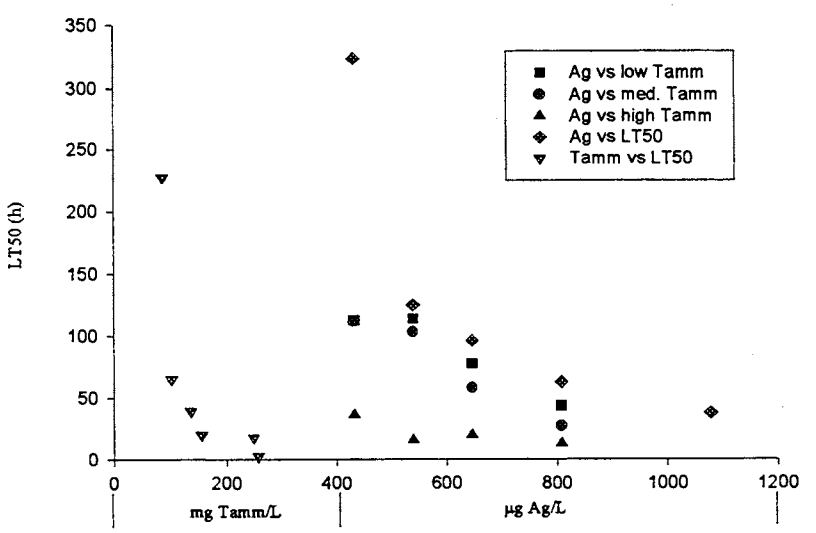

Figure 2. Concentration vs. time. *Low, medium, and high Tamm refer to 8.6±6.1, 87.26±7.3, and 224.47±2.5, mg Tamm/L. Ag concentrations are given in µg Ag/L. Tests conducted with combinations of Ag and Tamm are plotted as Ag.*

Finally, ammonia excretion was monitored over a range of silver concentrations. The rate of ammonia excretion increased compared with control fish following two days of silver exposure for all silver concentrations tested. However, there were no observable differences between silver treatments. The absence of a dose-dependent increase in the ammonia excretion rate suggests, but does not exclude, different primary mechanisms of silver and ammonia toxicity.

Salinity

The effects of salinity on silver toxicity are varied. Silver toxicity is decreased as salinity increases from freshwater to brackish systems, 0.6-50mM Cl^- (Galvez and Wood, 1994; Hogstrand *et al.*, 1996). This decrease is due to cerargyrite precipitation. If salinity is further increased dissolved $AgCl_n$ species form and moderate toxicity is observed. However, as full strength seawater is approached toxicity is once again reduced (Ferguson, 1995). In addition to the silver toxicity data for 32 ppT salinity calculated for the ammonia study, a second set of experiments was conducted to investigate the effects of salinity on silver accumulation.

Tests were conducted with the tidepool sculpin at 25 and 32 ppT salinity. Sculpins were individually housed in 30 mL scintillation vials, with ten replicate vials per silver treatment. Treatments lasted seven days and test water was renewed daily and maintained at 10±1 °C. After seven days, organisms were anesthetized, rinsed, and prepared for silver analysis. Silver accretion increased with increased water column concentrations of silver at the lower salinity(Figure 3). However, at 32 ppT salinity silver did not accumulate above basal levels for all water column concentrations tested. Reduced accumulation of silver at the higher salinity likely is due to differences in silver speciation and/or physiology. Interestingly, while very little silver was accumulated up at the higher salinity, toxicity was still observed. There was no correlation between whole body silver burden and toxicity.

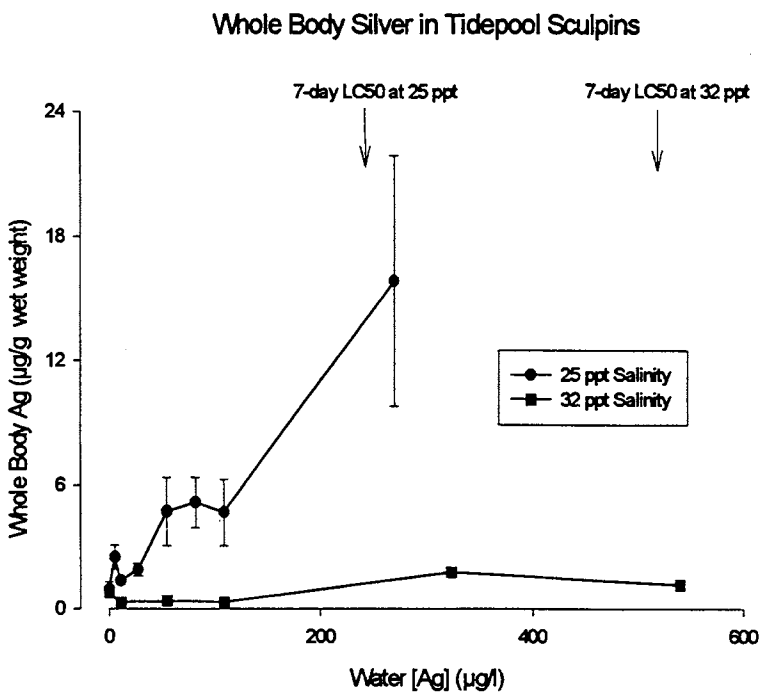

Figure 3. The effects of salinity on silver accumulation. *Whole body silver concentrations were measured following 7 d exposure to silver. The presented values are means of 5-10 fish ± SE. Arrows mark the water column Ag concentrations that correspond to the 7 d LC50 values.*

Ammonia and salinity are natural components of aquatic systems. However, their concentrations are variable and can be altered by anthropogenic intervention. These studies suggest that both parameters influence silver toxicity and thus have potential implications on criterion development and regulatory strategies. Silver toxicity to marine fish is enhanced and accelerated in the presence of ammonia. Moreover, the chloride ion is generally protective. However, increased silver toxicity is observed in the transition from esturines to seawater. Criteria values that do not account for these modulators have the potential to incorrectly predict silver toxicity.

Acknowledgments

This project was supported by a grant from the Silver Coalition/National Association of Photographic Manufacturers. JS was supported by NIEH Training Grant #ES07266. The authors wish to thank the staff at the Bamfield Marine Station, Bamfield, B.C., Canada.

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Questions & Answers: Parameters That Influence Silver Toxicity: Ammonia and Salinity

Q. NANCY NEELY (Fuji Film): The silver that you're talking about; what form is it in? What was your source of silver?

A. Silver nitrate.

Q. NANCY NEELY: What source of ammonia did you use?

A. Ammonium chloride.

Q. RUSSELL BELL (McMaster University): Can I make a plea to all to try and use moles rather than these strange milligrams and micrograms and things. My question, though, is this: did you control the pH when you were doing these ammonia experiments? Because the concentration of ammonia is going to vary with pH appreciably, and that may, therefore, change its rate of transport into the gills.

A. If my primary focus would have been on ammonia toxicity, I probably would have been a little more careful with that. It was repeated by the same methods for both studies. Also, it's been shown that the ammonium ion can diffuse into and out of the fish gill for marine fish.

Q. RUSSELL ERICKSON (USEPA): One comment and one question. I'd be a little more careful how you use the term "additive." There's quite a literature on additivity, and it's used in different ways; there's concentration additivity versus response additivity.

A. I was just saying it appears to be additive on a gross level.

Q. RUSSELL ERICKSON: By some definition of additivity your data would actually be argued to be nonadditive. So some care is needed there.

A. I think the major point there was that it was just actually enhancing toxicity.

Q. RUSSELL ERICKSON: My question was, you showed an interesting response across a range of salinities. It seems that it potentially correlates with the changing inorganic speciation of silver as you go through that series. Have you attempted to look at the dominant calculated silver chloride species as far as how it correlates?

A. Yes, and it does correlate with silver chloride speciation. In other words, you get [insoluble silver species] formation up to the brackish water system that has a protective effect, and as you push farther, you start getting some dissolved silver chloride species, and that's where you see your moderate toxicity. Then, as you go to more highly chlorinated silver chloride species, that's where you see your reduction in toxicity.

Q. ANDERS ANDREN (University of Wisconsin): In going from 32 parts per thousand down to 25 parts per thousand, you saw an increase in sensitivity to silver. I don't have the speciation calculations in my head, but I can't believe there is much of a change in the silver-chloro distribution of species going from 32 to 25, and I wonder whether it's some other regulation — some physiological regulation, for instance — that has changed the sensitivity of the fish.

A. I think that there is a large shift to the -3 and -4 silver chlorides from 25 to 32 ppt. The data that I would point to would be the body burden data, where it's just not being taken up at 32 ppt seawater.

Physiological Analysis of the Stress Response Associated with Acute Silver Exposure in Rainbow Trout (*Oncorhynchus mykiss*)

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Exposure to $AgNO_3$ causes severe ionoregulatory disturbances that appear to put fish under a great deal of stress. Wood *et al.* (1996) found that plasma Na^+ and Cl^- levels dropped dramatically during a six day exposure of rainbow trout to 10 μg/L Ag (as $AgNO_3$) in moderately hard freshwater. They also saw the plasma become acidic with a drop of P_{CO_2} and HCO_3^- levels. A comparable study by Hogstrand, Ferguson and Wood (unpubl. results) exposed starry flounder to 250 μg/l Ag (as $AgNO_3$) in seawater; plasma ammonia levels increased dramatically after the start of exposure, recovered somewhat by the end, but remained significantly higher than control values.

These results suggest that silver affects both ion exchange and ammonia excretion at the gills of fish. The current study replicated the exposure of Wood *et al.* (1996) in order to measure plasma ammonia levels and excretion rates, as well as to determine the cause of decreased plasma ions.

In adult rainbow trout exposed to 10 μg/l Ag (as $AgNO_3$) in moderately hard freshwater, blood pH fell significantly by the 6th day with P_{CO_2} values dropping to 50% of those found in control fish. Plasma glucose levels of exposed fish increased, and after 6 days were 4x control values. Plasma cortisol levels also quadrupled by the 6th day.

Na^+ and Cl^- flux rates at the gills were immediately affected after introduction of Ag. Unidirectional influx rates for both ions dropped by 50% and by 8 hours were almost completely blocked. Unidirectional efflux rates were unaffected and thus a net loss of these ions occurred throughout the exposure.

Activity levels of two gill transport enzymes were also inhibited by 10 μg/l Ag. Na^+/K^+ ATPase activity was decreased by 85%, while that of carbonic anhydrase was decreased by only 20%.

Plasma ammonia levels began to increase immediately following exposure and by the end were 2x control values. However there was no inhibition of ammonia output. To the contrary, the ammonia excretion rate doubled after only 24 hours of exposure.

We hypothesize that silver stopped Na^+ and Cl^- uptake by blocking the Na^+/K^+ ATPase which is the driving force behind ionic uptake at the gills. This inhibition caused Na^+ to build up in the gill transport cells, stopping H^+ excretion resulting in blood acidosis. The disturbance of ion regulation also elicited a stress response, evidenced by the build up of glucose and cortisol in the blood. Cortisol has be shown to mobilize glucose into the blood and to increase proteolysis which in turn elevates ammonia production in the tissues of the fish. Ammonia (as NH_3) ties up H^+ ions to form NH_4^+ and thus helps the fish to address the acidity of the blood. Hyperventilation was also evident as the Pa_{CO_2} fell. This also helps to counteract the low blood pH. However, since neither mechanism addresses the loss of plasma ions, the fish eventually dies from the consequences of ion imbalance perhaps in combination with toxic levels of plasma ammonia.

Wood C.M., Hogstrand C., Galvez G. and Munger R.S. (1996) The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) : 1. The effects of ionic Ag^+. *Aquat. Toxicol.* (in press).

Research is funded by a grant to C.M. Wood from Kodac Canada and NSERC

Figure 1. Ammonia parameters of freshwater rainbow trout exposed to 10 µg/l Ag (as $AgNO_3$) for 6 days in moderately hard freshwater. The vertical denotes the start of exposure with the first data point being taken 4 hours later. Plasma ammonia levels (top) of Ag exposed fish (solid circles and line) increase immediately reaching twice the control levels (open circles, dashed line) after only 48 hrs of exposure. The excretion rates of ammonia (bottom) in exposed fish (hatched bars) also increase immediately and remain elevated throughout the exposure. The open bars represent the excretion rates of control fish. Data points are means ± SE (N = 8).

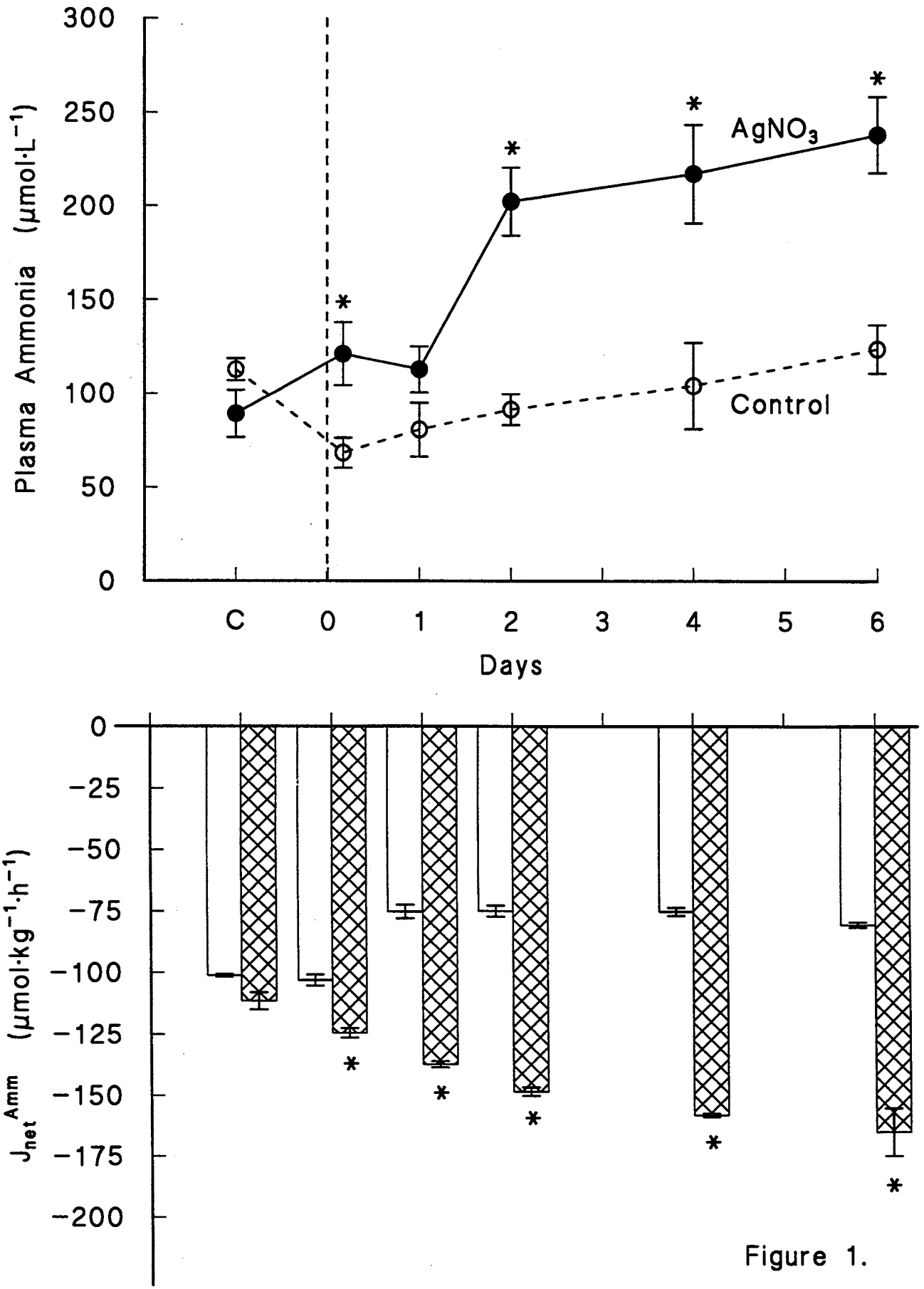
300
250
200
150
100
50
0
Plasma Ammonia (μmol·L⁻¹)
AgNO3
Control
C
0
1
2
3
4
5
6
Days
0
−25
−50
−75
−100
−125
−150
−175
−200
J net Amm (μmol·kg⁻¹·h⁻¹)

Figure 1.

Questions & Answers: Physiological Analysis of the Stress Response Associated With Acute Silver Exposure in Rainbow Trout *(Oncorhynchus mykiss)*

Q. DALAND JUBERG (Eastman Kodak): Presumably, some of the channels you mentioned are voltage-gated; have you or others ever looked at what's going on in terms of membrane depolarization and stabilization electrochemically, in terms of silver or other metals? Do you think that's playing a role? (N.A. Webb: You're asking if sodium helps depolarize the membrane?) Calcium channels, I know, can be voltage-gated, and open and close in response to differences. Do you have any thoughts on that?

A. The sodium channel isn't thought to be voltage-gated. It's thought to be linked to the hydrogen ATPase which causes the electrochemical gradient. Namely, it pumps hydrogen ions out, causing the cell inside to be negative, which allows the sodium ions to enter the cell. It's not thought that this is actually an open-close channel. Most people assume it's always open and always ready to take up sodium. Of course, you could say this might not keep sodium in the cell, but that's where the sodium-potassium ATPase comes in. It pumps three sodium ions across into the blood in exchange for two potassium ions, which causes the cell to become less negative than the blood itself. So whether silver affects voltage-gated channels, we're not exactly sure. As you saw by that little increase of loss of sodium, it may have an effect on the type of junctions in between cells, which could cause an increased leakage of ions out, but that hasn't been shown yet, or studied, as far as I know.

Q. RUSSELL ERICKSON (USEPA): One concern that's existed at this meeting is pushing our understanding to more sensitive endpoints, either organisms or chronic endpoints. You did show a slide there which showed a 50 percent reduction in sodium-potassium ATPase activity down as low as 2 micrograms per liter. I'm wondering whether you have any more information? I presume that's below the lethal levels for your fish under your test conditions. Do you have any other information about responses at lower concentration levels? Secondly, if you could speculate on whether, in fact, these same mechanisms might play a role in chronic or sublethal toxicity.

A. The point of the study with the sodium-potassium ATPase at 2 micrograms per liter and 10 micrograms per liter was to get a value between zero and 10, which we were running most of our tests at. The problem with the 2 micrograms per liter is that the inhibition wasn't significantly different from control levels. It did show a decreasing trend, but in all statistics you have to show this with little stars on top. And doing all the tests, it wasn't significantly different. As for chronic exposure, we suspect it does play a role in chronic exposure, although we have to do some tests. We did some tests this summer on marine fish, and freshwater chronic exposure is something we're thinking of getting into. We hope to get into it this fall. Whether or not it has an 80 percent inhibition, I'm not exactly sure, simply because we do these exposures at such high levels to find out the mechanism and see what the real cause of death is. Then we can go back to chronic levels and see what the effect is and relate that to the toxicity of those levels. So we think it may have an effect at chronic levels, but we're not exactly sure.

The Physiological Effects of Dietary Silver Exposure in Rainbow Trout *(Oncorhynchus mykiss)*

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University of Kentucky, Lexington, Kentucky, USA
University of Guelph, Guelph, Ontario, Canada

Introduction

Previous studies performed on adult rainbow trout have suggested that the toxic mechanism of acute waterborne Ag exposure (as $AgNO_3$ in moderately hard freshwater) involves a severe branchial ionoregulatory disturbance (Morgan et al, 1996; Wood et al, 1996a) resulting in a sharp decline of both plasma $[Na^+]$ and $[Cl^-]$. It is further proposed that death may ensue due to circulatory collapse, as a result of ionoregulatory-induced hemoconcentration. However, probably of more environmental importance (at least in freshwater fish) is that Ag levels were found to be significantly elevated in tissues such as the liver, following acute exposure (Hogstrand et al, 1996; Wood et al, 1996b). At Argentum III (Washington, D.C., August 5-9, 1995) concern was raised of the implications of Ag transfer along the food chain. Despite our current knowledge of the toxic mechanisms of acute exposure of waterborne Ag, little is known concerning the effects of dietary silver exposure in aquatic animals. The primary objective of this study was to investigate the physiological effects of food chain transfer of biologically incorporated silver.

Materials and Methods

Diet Preparation. A silver enriched diet (~3.5 μg Ag/g) was prepared from the carcasses of rainbow trout previously exposed to elevated concentrations of waterborne silver (as silver thiosulphate; 371 mmol Ag/L) for a period of one week (referred to as Ag diet). Several control diets (0.05 μg Ag/g) were also formulated from trout exposed to either Hamilton dechlorinated tapwater (control diet) or to the same concentration of thiosulphate (sodium thiosulphate diet) for the same period. A third control produced from commercially manufactured herring meal (herring diet) was used to test the palatability of these diets containing the "home-made" fish meal. In each case, the carcasses were dried, ground into a fine powder, and mixed with other ingredients to yield a nutritionally-balanced diet. Diet formulations were then steam-pelleted producing a commercial-grade pellet.

Experimental Design. Juvenile rainbow trout (~5 g at start) maintained under ambient conditions (5°C to 11°C) were fed with one of the four diets over a four month period. Two groups were fed either the control or Ag diet to satiation twice daily. An additional three groups (control, sodium thiosulphate or herring diets) were pair-fed to the Ag treatment group twice daily. This means that these groups were being fed the same amount of food on a per fish basis as the Ag treatment. Consequently, if the Ag diet were to result in a decreased food intake, it would be possible to determine whether any observed physiological effects were caused by either the toxicant or from a modified food intake (due to decreased palatibility of the food).

Over the course of the experiment, food consumption and growth were monitored. In addition, oxygen consumption, and ammmonia and urea excretion rates were measured. Blood samples were withdrawn for analysis of whole blood hemoglobin and hematocrit. Plasma was also obtained for analysis of cortisol,

glucose, protein, and a variety of plasma electrolytes (Na^+, Cl^-, Ag, and Ca^+) Liver, gills and intestine were dissected out for measurements of total Ag, Zn, Cu and metallothionein content. Finally, branchial sodium uptake rates were measured over four hour periods at different times throughout the feeding study.

Preliminary results and Discussion

a) Feeding and Growth. Food consumption rates between the control satiation-fed group and the Ag diet fed group remained relatively constant. The growth rate of fish fed the Ag diet was slightly increased between days 15 and 35 (Fig. 1). However, after the four month period, no growth differences between any of the groups (except for the herring group) were observed. Fish fed the herring diet showed slightly elevated growth rates.

b) Hematology. Elevated Ag levels in the diet produced no significant differences in plasma protein, hematocrit and whole blood hemoglobin concentrations, after approximately three months when compared with controls. This is in contrast with waterborne exposure to $AgNO_3$ which resulted in significant increases in each of these hematological parameters (Wood et al, 1996a).

c) Sodium Flux Experiments. Branchial Na^+ uptake measured after one month of Ag diet exposure was not significantly different than control values. In vitro studies by Morgan et al (1996) showed a decrease in Na^+/K^+-ATPase activity following waterborne $AgNO_3$ exposure and acute inhibition of Na^+ uptake. In contrast, preliminary results from the present study indicate that biologically incorporated Ag has no discernable effects on intestinal Na^+/K^+ ATPase activity.

d) Oxygen Consumption, Ammonia and Urea Excretion. There were no differences in oxygen consumption and urea excretion among any of the treatments. Ammonia excretion rates measured on day 36 were slightly reduced in fish fed the Ag diet, suggesting a decrease in protein catabolism during this period. This could explain the increased growth rate in these fish at this time.

e) Metal Metabolism. The Ag contents in the 16,000-g supernatants of liver homogenates from fish reared on the Ag diet were significantly elevated compared to the simultaneous controls after only 16 days. By day 60, the Ag content was approximately 12-fold greater than control values (Fig. 2). In contrast, copper concentrations in this fraction were significantly reduced by day 60 (Fig. 3), whereas Zn levels were unaffected by dietary Ag exposure. Overall, a small decrease in the Cu to Zn ratios were observed in the Ag diet-fed group. One possibility is that elevated Ag levels result in a displacement of Cu from either metallothionein or other cytosolic proteins. It is not known whether this Cu is simply being stored in other subcellular compartments, or transported out into the circulation.

Conclusion

Preliminary results suggest that dietary Ag exposure does not elicite the same physiological effects as seen during waterborne $AgNO_3$ exposure. Nevertheless, Ag is significantly accumulated in the livers of fish fed an Ag-enriched diet. In addition, hepatic copper levels are significantly reduced in these fish. It remains unclear whether accumulated silver is likely to effect metal homeostasis to the point that toxicity is produced.

Acknowledgements: This work was supported by grants from the National Association of Photographic Manufacturers/Silver Coalition, Kodak Canada, and the NSERC Industrially Oriented Research Program.

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Wood, C.M., Hogstrand, C., Galvez, F., and R.S. Munger. (1996a). The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*): 1. The effects of ionic Ag^+. Aquatic Toxicology. In press.

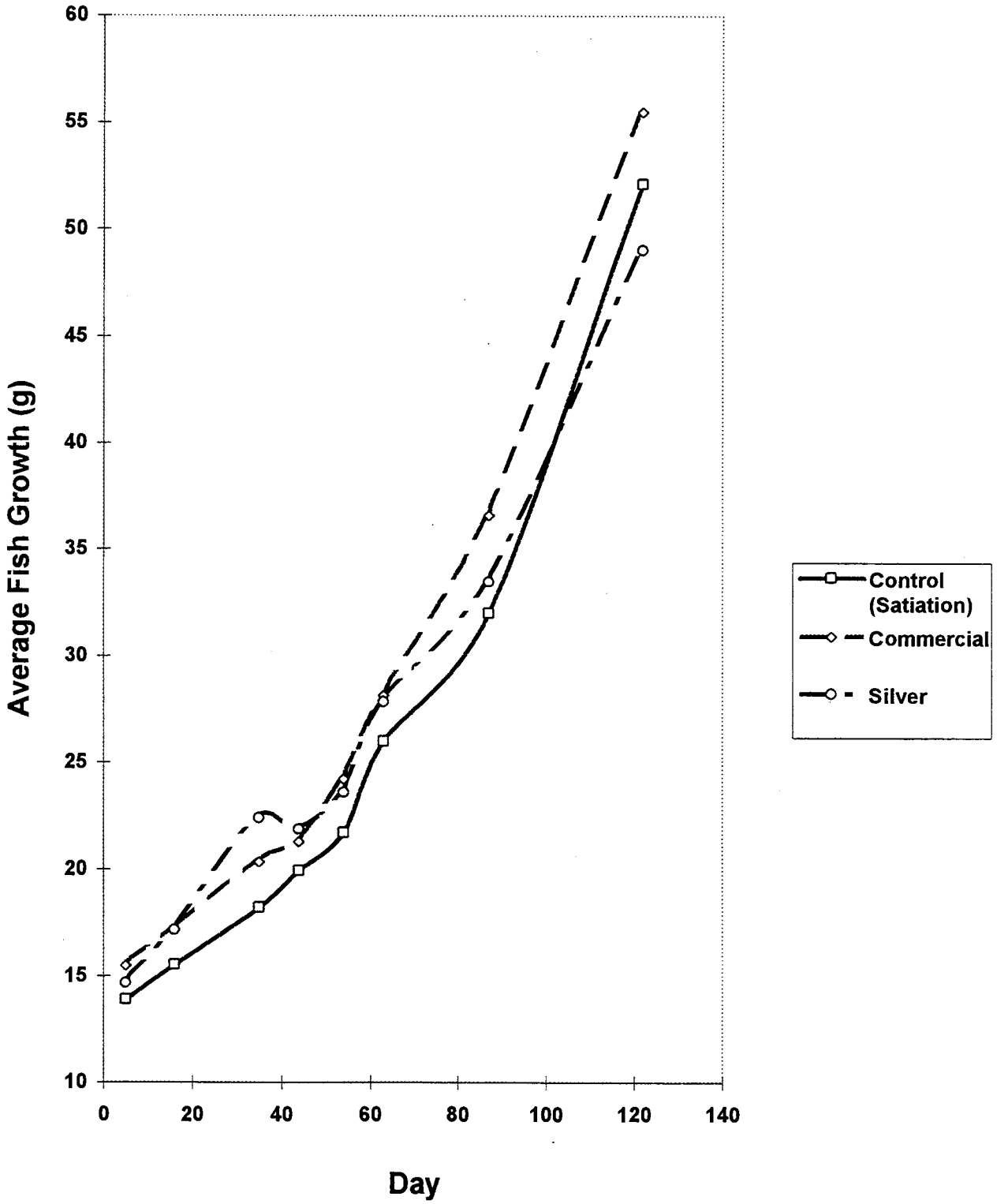
Wood, C.M., Hogstrand, C., Galvez, F., and R.S. Munger. (1996b). The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*): 2. The effects of silver thiosulphate. Aquatic Toxicology. In press.

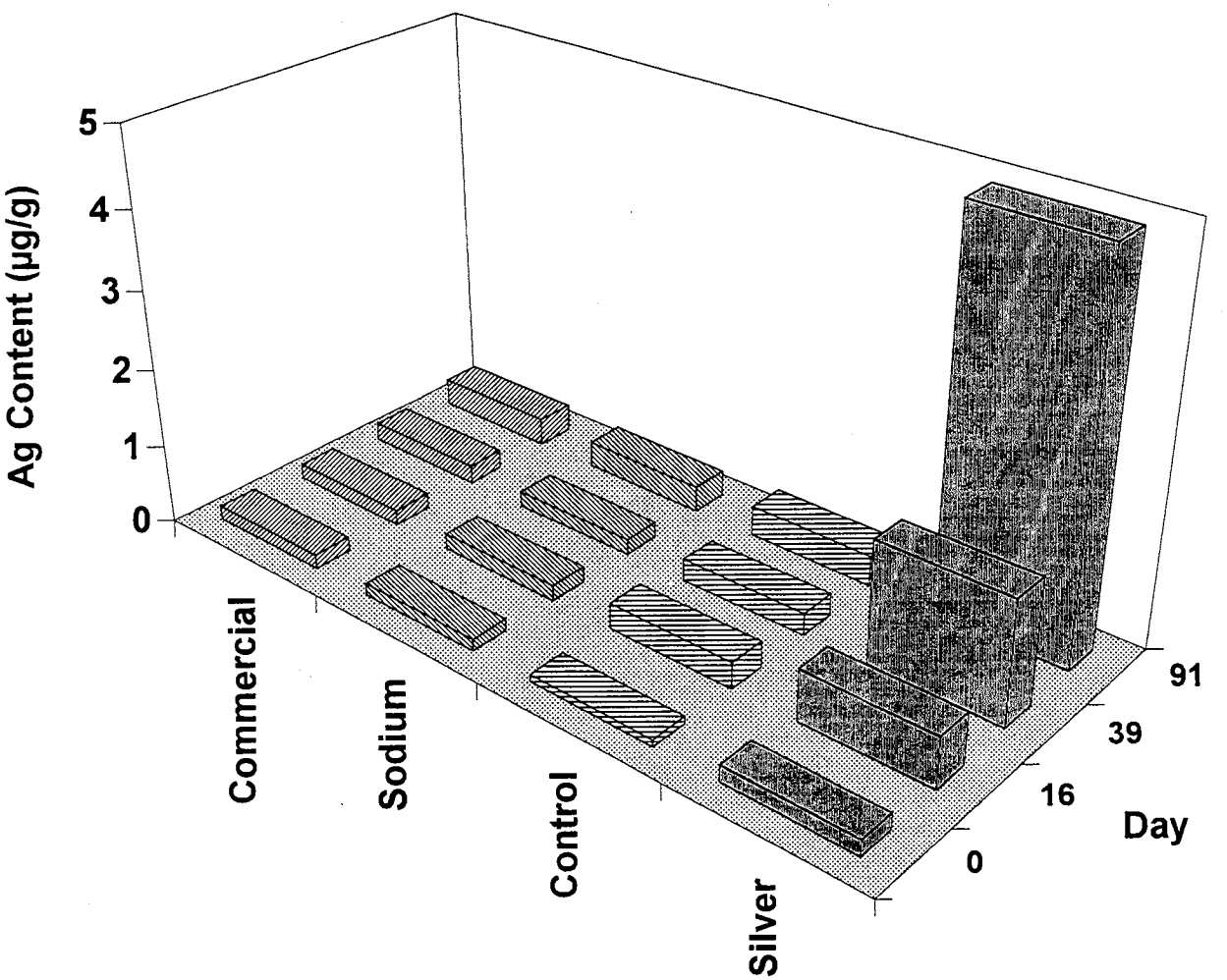
Figure Legends.

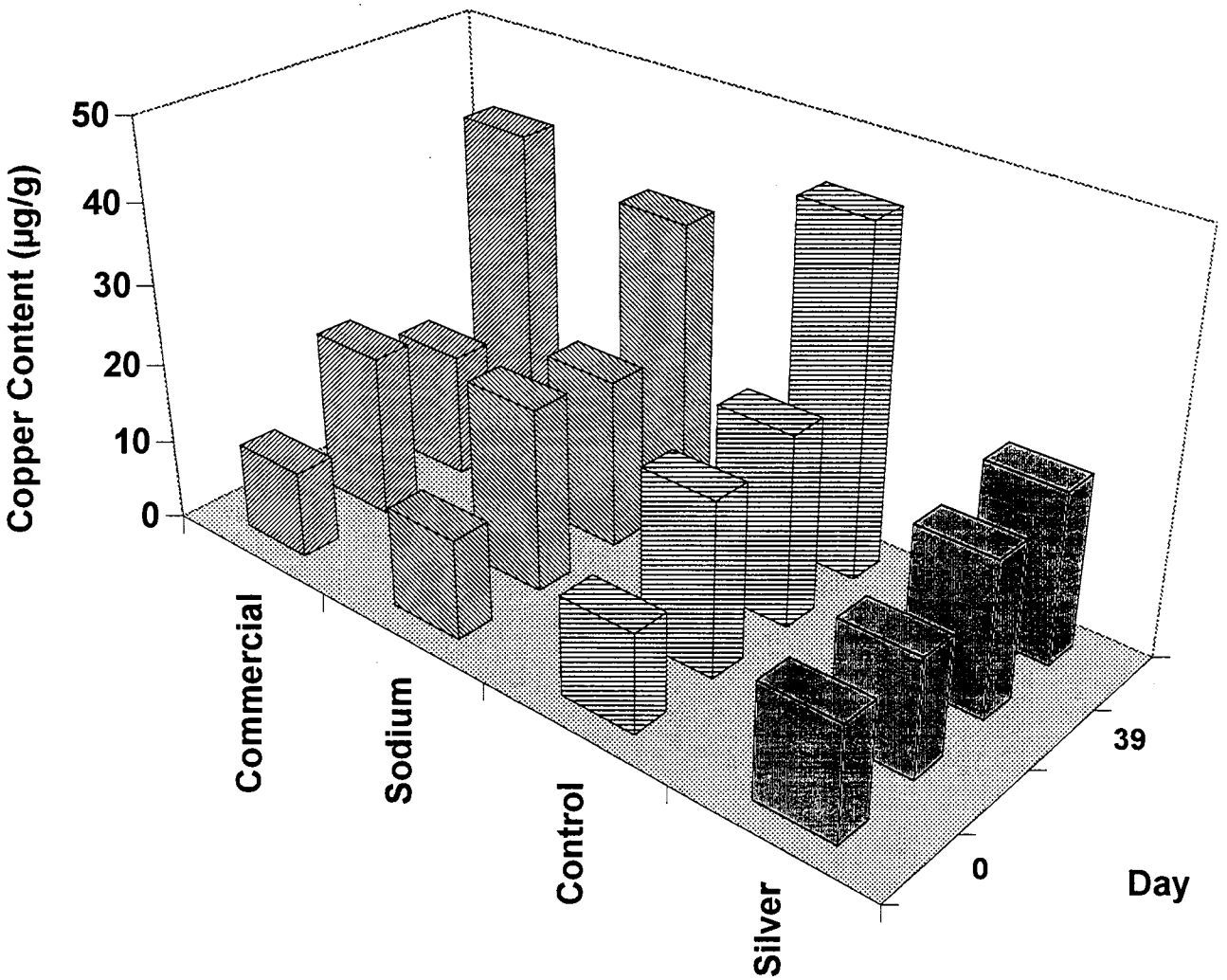
Figure 1. Average weight of rainbow trout during four month feeding study. For clarity only growth rates of groups fed either the commercial, control (satiation) and silver diets are shown.

Figure 2. Silver concentrations (µg/g wet weight) in the cytosolic fraction of liver samples from rainbow trout. Values are shown as means (N=10) for fish sampled on days 0, 16, 39 and 91.

Figure 3. Copper concentrations (µg/g wet weight) in the cytosolic fraction of liver samples from rainbow trout. Values are shown as means (N=10) for fish sampled on days 0, 16, 39 and 91.


Average Fish Growth (g)
60
55
50
45
40
35
30
25
20
15
10
0
20
40
60
80
100
120
140
Day
Control (Satiation)
Commercial
Silver


Ag Content (µg/g)
5
4
3
2
1
0
Commercial
Sodium
Control
Silver
0
16
39
91
Day


Copper Content (µg/g)
50
40
30
20
10
0
Commercial
Sodium
Control
Silver
0
39
Day

Questions & Answers: The Physiological Effects of Dietary Silver Exposure in Rainbow Trout *(Oncorhynchus mykiss)*

Q. PETER CAMPBELL (Universite du Quebec, INRS-Eau): A nice paper, Fernando. In your work on metallothionein and the copper and zinc in the liver, have you looked at the metal levels and distribution in the liver cytosol as opposed to the liver total tissue, which I presume is what you're reporting here?

A. I have homogenate still available. Basically, you take the liver sample and homogenize it in TRIS buffer. We will measure that homogenate for total silver. We'll be able to determine, at least, how much of the silver and the zinc and copper are in that fraction as compared to the total. In a month I'm going to go down to Kentucky, where we will be doing a silver-chase experiment where we'll look more closely at where the silver is being distributed within the cell. So I hope we'll be able to answer that next year.

Q. RUSSELL BELL (McMaster University): One comment: some years ago, Dennis Winge at the University of Utah did some experiments with metallothionein where he made the silver species. He found that the silver was displaceable by things like mercury, copper, platinum, palladium and bismuth, but zinc did not displace the silver. So your results are quite interesting, since the zinc seems to be OK, but the copper one is odd.

A. It seems to be consistent with what Christer's group is seeing with the zinc, as well as with several other studies. So that's good to see.

Q. CHRISTER HOGSTRAND (University of Kentucky): A comment on that. I think that most studies that you'll see in the literature would say that silver is the strongest binder to metallothionein of the commonly found elements. It has a fantastic binding affinity; the association constant is something like 10 to the power of 22. It's a little bit less for copper.

Release of Silver and Other Metals From Resuspended Harbor Sediment

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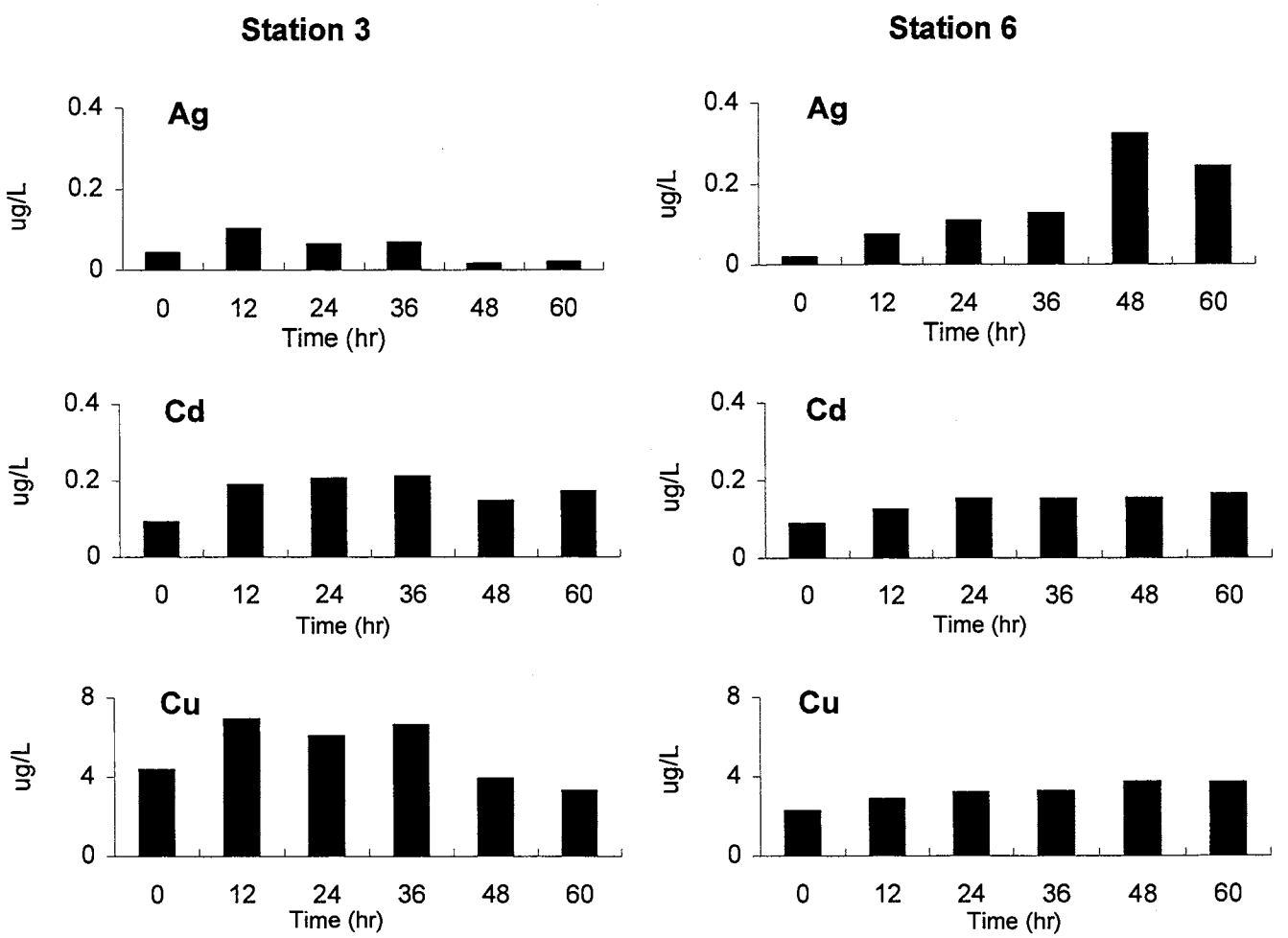
NCCOSC RDTE DIV 522, San Diego, California, USA

The release of Ag, Cd, Cu, Ni and Pb from marine sediment in San Diego Harbor, California, was estimated by using both a field-deployed benthic flux chamber and by suspending sediment in seawater in the laboratory. The concentration of metals generally increases in the flux chamber over the 2-day deployment. The dissolved oxygen concentration in the chamber was monitored and controlled to maintain oxic conditions. The sediment and sediment porewater were sampled after the flux chamber was recovered. The sediment samples contained about 1 percent to 2 percent total organic carbon (TOC) and 1 to 20 μ mole/g acid volatile sulfide (AVS). The concentrations of metals in the porewater were lower than those in the ambient bottom water in the flux chamber at the start of the deployment.

During the 2-day deployment, the concentrations of dissolved (0.4 μm filter) Ag, Cd and Pb increased by about 0.1 to 0.2 μg/L. The concentration of dissolved Cu and Ni increased by 2 to 5 μg/L, and Zn increased by 20 μg/L. Although the porewater contained relatively high concentrations of Fe and Mn, these elements did not increase markedly in the flux chamber, presumably because the oxic water in the chamber caused the precipitation of Fe and Mn in the surface sediment.

The flux of metals into the chamber may be due to the oxidation of amorphous metal sulfides that are unstable in oxic water and that release dissolved metals. To test the hypothesis that metal sulfides release metals in oxic seawater, the same sediment samples were suspended in oxic seawater in the laboratory for 24 hours. The concentrations of dissolved metals in the resuspension experiment were similar to those in the benthic flux chamber.

Concentration of Dissolved Metals versus Time in the Benthic Flux Chamber at Two Stations in San Diego Bay



Questions & Answers: Release of Silver and Other Metals From Resuspended Harbor Sediment

Q. DOMINIC DI TORO (Hydroqual, Inc.): That was very interesting, Eric. You showed the silver going up, and then going down, and you saw that in some of the other profiles. Do you think that's artifactual? Do you think that's real?

A. We've tended to see that in all six of the sites we went to. At some sites there was not as much of a silver increase, but we often saw it going down a little. I don't know if that's an artifact of the chamber. There never was a deployment of just spiking metal in the water and holding it in the chamber to see what the overall chamber effect was. Did things start to grow on the walls of it? Did metal oxides start to form? Essentially, I don't think we were fluxing iron out of the sediments being caught in the redox zone, but the manganese was fluxing out. It might eventually become a sink on the walls of the chamber, after that 60 hours.

Q. JIM KRAMER (McMaster University): It's my perception from your work, and from what we showed in Cobalt and so on, that when you take a real natural system, under aerobic conditions (oxidizing conditions) silver doesn't seem to mobilize. But then when you look at experiments like Dominic proposes, and other experiments in the laboratory, you do get mobilization. I wonder if you have any insights or suggestions of what's different in the experimental versus the real?

A. The experimental has been working with high levels of silver, up around 100 micrograms per gram. I don't know if that has something to do with it; if you're overwhelming certain phases so you're studying other processes, instead of working at one or two micrograms per gram in the sediment. I don't know if that could be part of the system.

Q. DOMINIC DI TORO: That's right. We're running our experiments at hundreds of micrograms per gram, and we're running them for 200 days, and even then we see only a little mobilization. He sees mobilization of silver, and that's consistent with the notion that there is some oxidation of the silver sulfide. Otherwise, he'd see nothing.

Transcriptional Activation of the Rainbow Trout Metallothionein-A Gene by Zn and Ag

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Umea University, Umea, Sweden

Metallothionein (MT) is a low molecular weight, cysteine rich, metal binding protein that has a high affinity for, and plays a key role in the detoxication of metals in the I-B and II-B groups of the Periodic Table (1,2). Presence of these metals (i.e. Zn, Cu, Cd, Hg, Ag), at elevated concentrations increases MT synthesis, and a high percentage of their total concentration in the cell can be found as MT complexes. Of these metals, Ag has the highest affinity for MT. The free Ag ion is also known to be extremely toxic to freshwater living animals (3).

Recent studies have suggested that disruption of branchial Na/K-ATPase activity is a primary mode of action of Ag toxicity. Ag has been shown to compete for the protein's Mg binding site, displacing the vital Mg that is needed to bind ATP which drives the catalytic activity of the enzyme. *In vitro* studies have indicated that the Ag-MT complex, however, does not have inhibitory effects on Na/K-ATPase function (4).

The promoter region of the Rainbow Trout MT-A gene contains six 12-bp Metal Responsive Elements (MRE) which act as recognition sites for sequence specific transcription factors (5). Transcription factor(s) bind(s) to these MRE in the presence of Zn, and possibly other metals, and activates transcription (6). A model for the Zn induction pathway has been illustrated as shown in Figure 1a. The mechanism of Ag induction is not known. By analogy to the Zn pathway, however, the induction pathway of Ag has two apparent options; displace Zn from preexisting Zn-MT, or directly interact with the transcription machinery to initiate MT synthesis. If Ag does directly interact with cellular transcription machinery, a direct mechanism against Ag toxicity is indicated.

Two fish cell lines, chosen for their differing abilities to produce MT, were used to help elucidate the role of Ag in MT induction. The rainbow trout gonadal cell line (RTG) has a normal MT gene and expresses MT normally. The Chinook salmon embryonal cell line (CHSE) has a hypermethylated MT gene and thus does not produce MT. The exploitation of this dissimilarity was essential in our experiments.

The MT-A promoter region was fused upstream of a luciferase reporter gene in a plasmid vector (pGL-6MRE). Both cell lines were transfected with this construct, exposed to various concentrations of Zn or Ag, and assayed for luciferase activity as a surrogate measure of rainbow

trout MT-A promoter activity. Cells were also transiently transfected with a construct wherein the lac Z gene was under control of the constitutive CMV promoter. Constitutive CMV-promoter driven β-galactosidase activity, therefore, was used to standardize transfection efficiency between replicates. When exposed to identical concentrations of Ag, CHSE-214 cells showed five times greater production of luciferase than did the RTG-2 cells (Figure 2a). Apropos to the hypothesis that metallothionein transcription factor inhibitor (MTI) has a lower binding affinity for metals than does MT (7), this data is consistent with the MT buffering capacity of the RTG-2 cell line. The positive results of the luciferase assay in the CHSE-214 cell line indicate that Ag can indeed induce MT transcription without displacing Zn from Zn-MT.

Many Zn reservoirs other than MT exist within the cell. To further our understanding of how Ag elicits a transcriptional response, the aforementioned cell lines were grown in a media containing the radioisotope ^{65}Zn for a period of 7 days. Subsequently, the cultures were exposed to differing concentrations of Ag, lysed, and centrifugally fractionated to yeild the following 4 subcellular fractions: Nucleus (N), Mitochondria & Lysosomes (ML), Microsomes (Mic), and Cytosol (C). Upon ascertaining the location of ^{65}Zn in the cell, no redistribution of Zn was evident. If Ag was in fact displacing bound Zn in the cell, allowing Zn to bind to the transcriptional machinery, one would expect to observe a redistribution of Zn into the N fraction. This data further supports the theory that Ag interacts directly with transcription machinery to initiate MT synthesis.

In conclusion, our results suggest that the rainbow trout MT-A gene is highly responsive to Ag and that Ag-mediated MT induction may occur by direct interaction between Ag and the transcriptional machinery. Furthermore, our data suggest that MT is an important system for Ag detoxication in fish.

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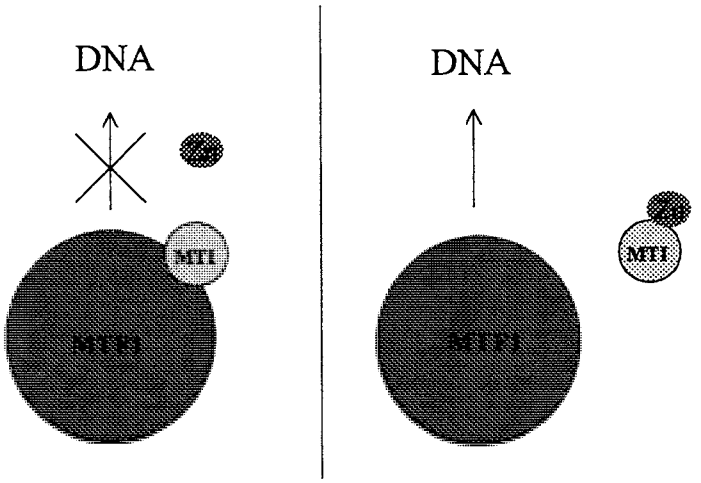

Figure 1a

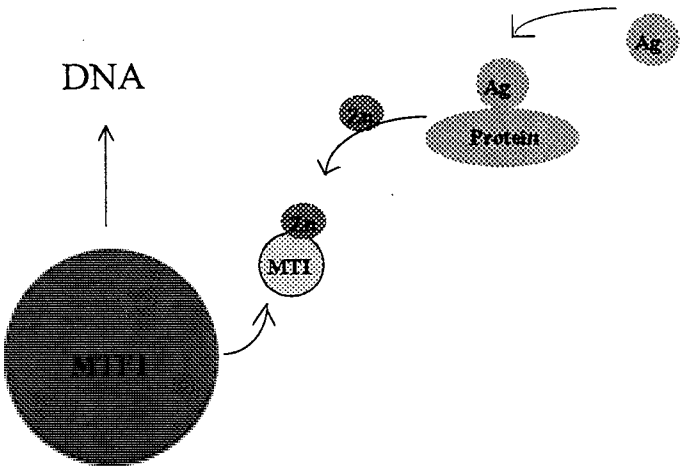

Figure 1b

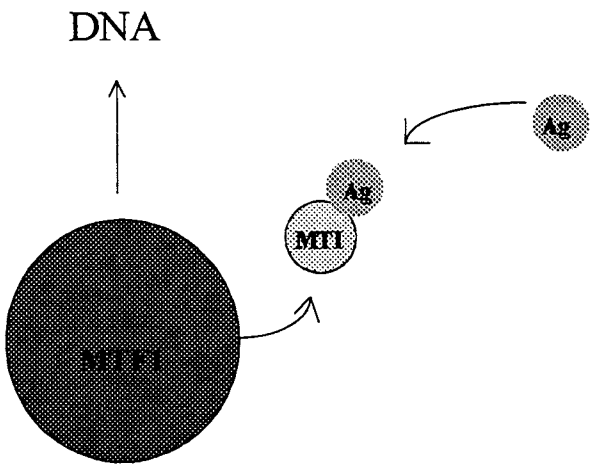

Figure 1c

A working model for the Zn induction pathway has been illustrated by Palmiter (8). (*fig 1a*)
In this model an inhibitory factor, denoted here as MTI, binds to the constitutively active transcription factor, MTF-1. In the prescence of Zn, MTI can bind Zn and be released from the transcription factor. This allows the MTF-1 to bind to the DNA binding site and initiate transcription.

Two pathways that seem obvious for the Ag induction mechanism are illustrated in figures 1a&b.

One such mechanism accounts for the displacement of bound Zn from an intracellular protein. This mobilizes free Zn to in turn bind to MTI and activate MTF-1. (*fig 1b*)

The second pathway Ag may follow in the cell is to directly bind to the MTI in turn activating MTF-1. If this is the case, Ag exhibits a direct mechanism of detoxication.

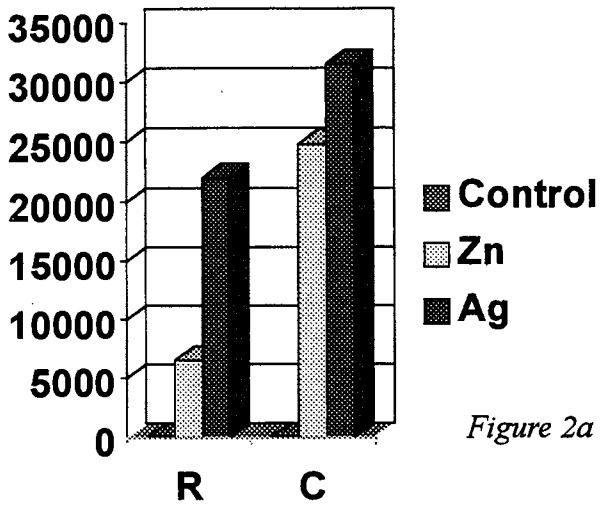

Figure 2a

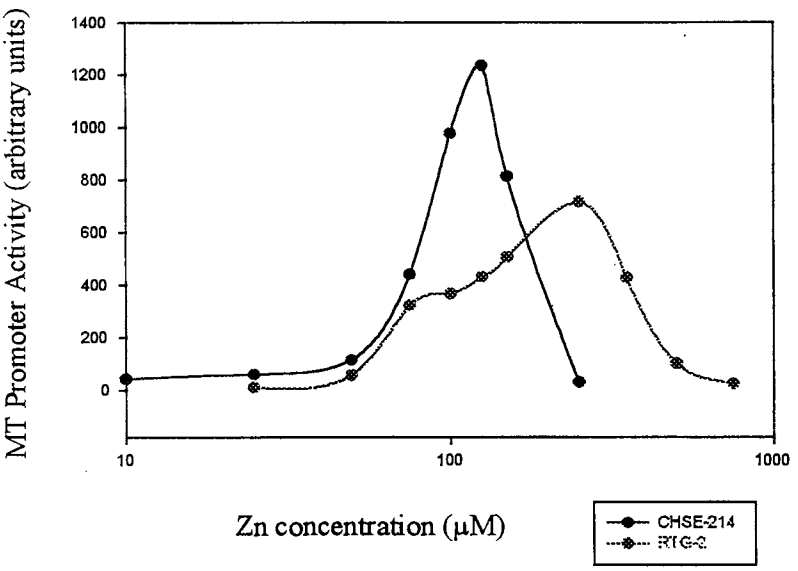

Figure 2b

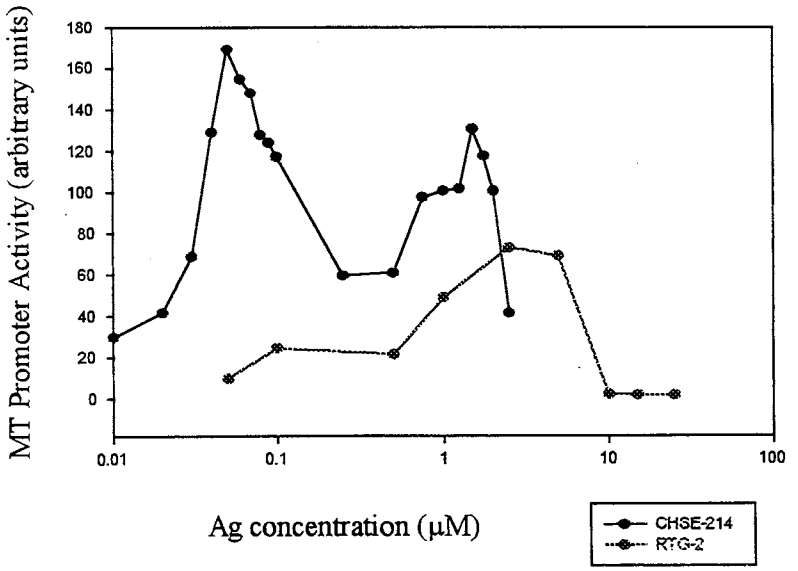

Figure 2c

When both RTG-2 and CHSE-214 cells were transfected with the pGL-6MRE and subsequently incubated with identical concentrations of either Zn or Ag, the CHSE-214 cells showed a significantly higher luciferase activity than did the RTG-2 cells. (*fig 2a*)

A dose response relationship was evident in both cell lines with respect to both of the individual metals. It is also evident that the LC_{50} was markedly decreased in the RTG-2 cell line. This is consistent with the MT buffering capacity of the RTG-2 cell line. (*figs 2b&c*)

The CHSE-214 cell line shows a significantly higher maximum luciferase activity in both of the cell lines, this could be attributed to the competition between plasmid cloned MRE's and genomic MRE's for active transcription factor(s).

Questions & Answers: Transcriptional Activation of the Rainbow Trout Metallothionein-A Gene by Zn and Ag

Q. RUSSELL BELL (McMaster University): Have you considered glutathione as a possible transcriptional-factor "beginner," if you like? All cells have a lot of glutathione in them.

A. No, I haven't. This is the first study of this type that has actually been done. We were concentrating more this time on just the redistribution of metals. When the paper came out on zinc, we thought it would be an interesting thing to do with silver. But we haven't looked at that.

Q. RUSSELL BELL: It's certainly a very nice piece of work. This is one reason that we would like to look at the structure of glutathione-silver complex, because it will form and be a very good complex, I think.

The Bioconcentration and Bioaccumulation of Silver in an Experimental Freshwater Ecosystem

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Silver is a highly toxic metal that can be found in industrial and municipal effluents and receiving waters (Bard *et al.*, 1976 and Dagon, 1973). However, little has been done to examine the possibility of foodchain effects as have been reported for other metals such as copper and selenium (Besser *et al.*, 1993 and Ogle and Knight, 1989). Terhaar *et al.* (1977) attempted to determine the extent of bioconcentration and bioaccumulation of silver, as $Ag(S_2O_3)_2^{3-}$, in a multi-trophic level system. The green alga, *Scenedesmus*, exhibited increased uptake of silver over time. *Daphnia* did bioconcentrate silver to levels higher than the water (BCF $\approx$ 7.5). It was unclear if daphnids bioaccumulated silver through ingestion of contaminated algae. Bioconcentration of silver was seen with fathead minnows, *Pimephales promelas*, but they did not accumulate silver from ingested daphnids. The objective of the studies described here was to determine the disposition of silver in a laboratory system in an effort to better understand silver's effect in receiving waters.

The green algae, *Selenastrum capricornutum*, was selected to represent the phytoplankton community for a number of reasons. It is also used as a foodsource in the culture of *Daphnia magna*, an organism representing primary consumers to be used in subsequent foodchain experiments. Following a 7-d growth period, one flask of algal suspension was fortified with 10 mg Ag/L stock (as $AgNO_3$) to a final silver concentration of 1 μg/L, while the other flask was labeled as a control. Both flasks were allowed to continue growth for 96-h at which time the algae was concentrated and washed. *Daphnia magna* were exposed to silver via two routes; the first being waterborne exposure and secondly through food consumption (*S. capricornutum*). Fifty daphnids from each replicate were sampled at the conclusion of the test period for silver analyses and the remaining 150 transferred to the following experiment with bluegill. Bluegill, *Lepomis macrochirus*, juveniles (6.4 cm $\pm$ 0.9 cm, 2.67 g $\pm$ 2 g) were purchased from Carolina Biological Supply Company (Burlington, NC) and acclimated to test conditions for one week prior to test initiation. At the conclusion of the *D. magna* experiment, bluegill (*L. macrochirus*) were exposed to silver via two routes; the first being waterborne exposure and secondly through food consumption (*D. magna*). A bioconcentration experiment was performed with duckweed, *Lemna gibba* G3. There were three treatments tested with a static non-renewal 72 hour exposure period.

S. capricornutum exhibited an ability to accumulate silver from solution. Exposure to 1 μg Ag/L resulted in the accumulation of more than 3 μg Ag/g. The bioconcentration factor (BCF) was calculated to be 4.8. Daphnids responded in a manner suggesting that direct uptake from water was the mechanism responsible for accumulation of silver (Figure 1). Significant uptake and accumulation

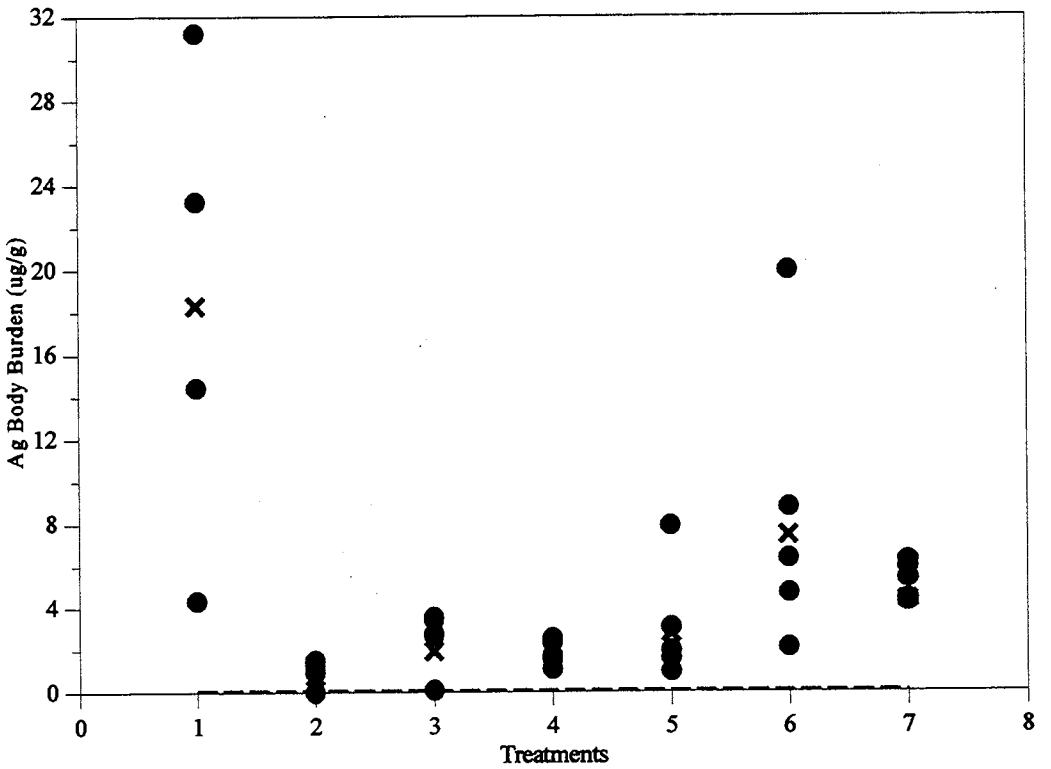

Figure 1. *Daphnia magna* body burdens of silver following aqueous or foodborne exposures to silver (Treatment 1 = 0.5 μg Ag/L, Treatment 2 = control algae, Treatment 3 = 0.5 μg Ag/L and control algae, Treatment 4 = dosed algae, Treatment 5 = 2X dosed algae, Treatment 6 = 0.5 μg Ag/L and dosed algae, Treatment 7 = 0.5 μg Ag/L and 2X dosed algae). (● replicate, ✖ mean, --- quantitation limit)

of silver was found with a 0.5 μg Ag/L aqueous exposure to silver. The calculated BCF for silver by *D. magna* was 61. The results of the first experiment involving aqueous and foodborne silver exposures revealed that mean concentrations accumulated by bluegill were below the analytical quantitation limit of 0.1 μg Ag/L. The results suggested that *L. macrochirus* did not accumulate silver via ingestion of contaminated food. The bioconcentration of silver by bluegill was also minimal. The second experiment designed to further probe the bioconcentration response of bluegill was less variable and did produce significant results. With regard to exposure time, the internal organs exhibited increased accumulation of silver (Figure 2). It was not known if equilibrium had been

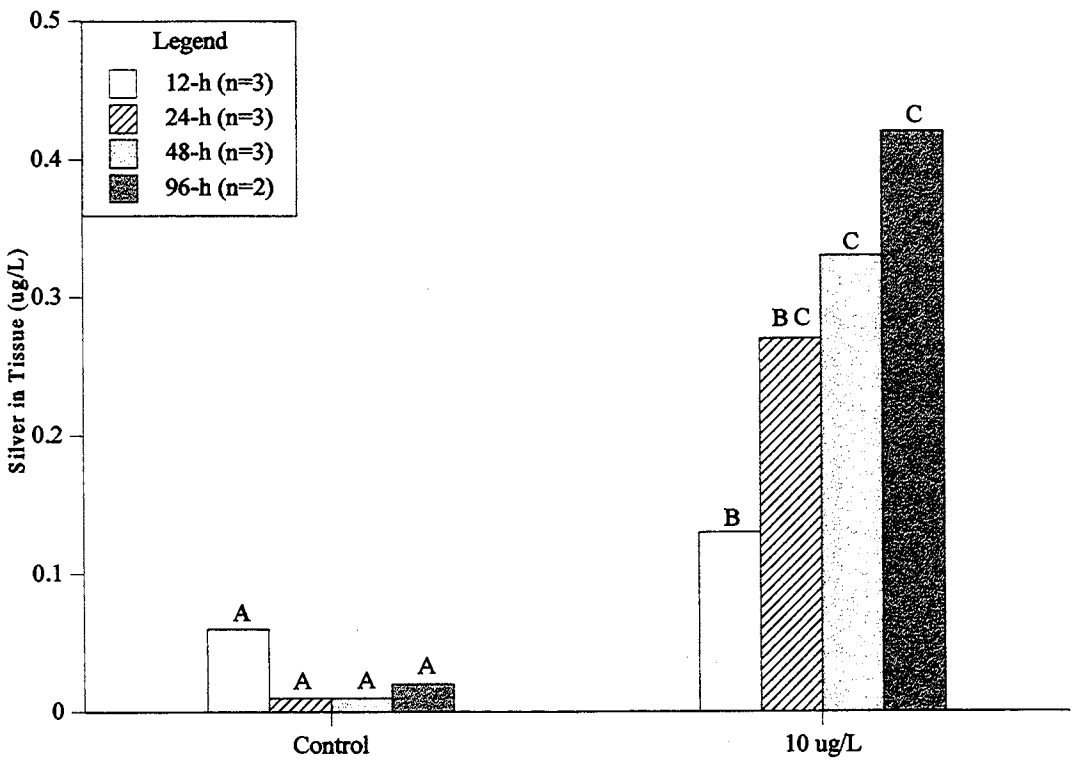

Figure 2. *Lepomis macrochirus* mean internal organ burdens of silver following a 96-h aqueous exposure to silver nitrate. Those not sharing the same letter are significantly different $p \leq 0.05$.

reached after 96-h. A bioconcentration factor of less than one (0.06) was obtained for gills of bluegill in this experiment. Concentrations of silver in gill tissue were significantly greater for exposed fish when compared to controls (Figure 3). The mean concentration did not change from 12-h to 48-h of exposure. At 96-h the concentrations were lower, but not significantly so. Duckweed, *L. gibba*, exhibited the ability to bioconcentrate silver of the aquatic organisms examined. Plants exposed to 6.9 μg Ag/L for 72 hours were able to accumulate a mean concentration of 135.3 μg Ag/g (BCF = 1.5). The mean concentration of silver found in *L. gibba* at this exposure was 3,500 μg Ag/L. A BCF of 25.4 was produced by duckweed exposed to 118.5 μg Ag/L.

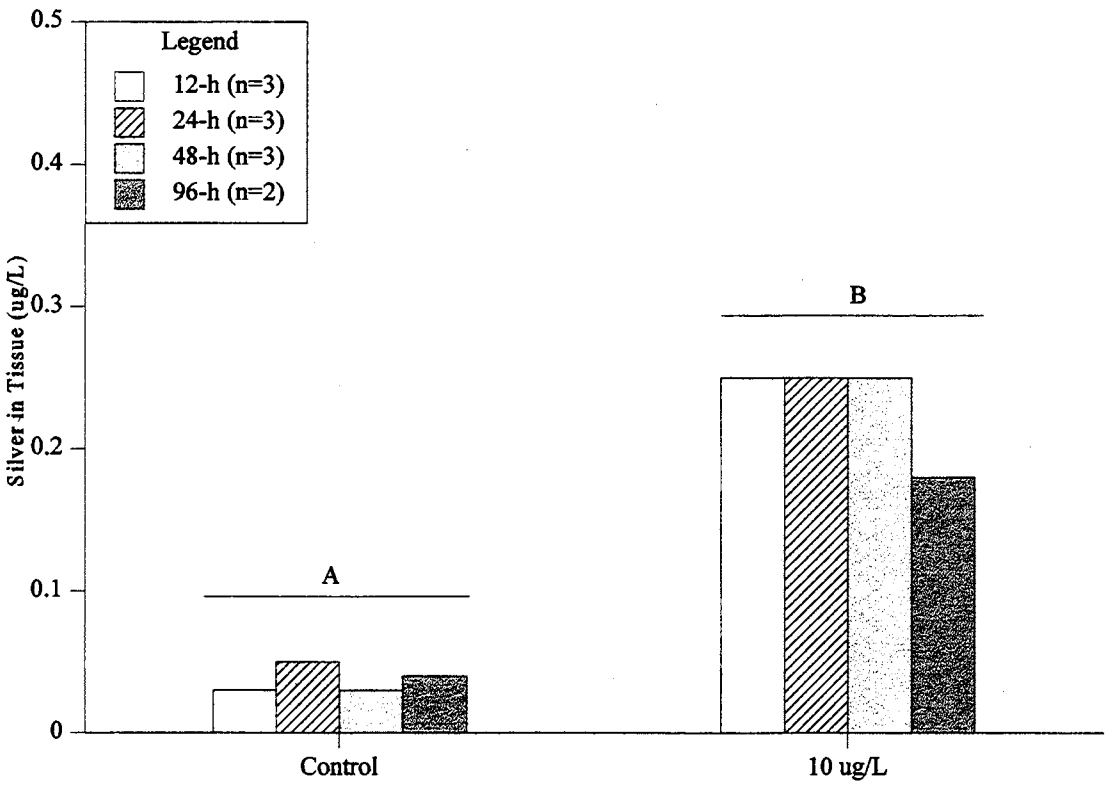

Figure 3. *Lepomis macrochirus* mean gill burdens of silver following a 96-h aqueous exposure to silver nitrate. Those not sharing the same letter are significantly different $p \leq 0.05$.

Questions & Answers: The Bioconcentration and Bioaccumulation of Silver in an Experimental Freshwater Ecosystem

Q. JOSEPH SHAW (University of Kentucky): When you delivered your dose of silver to the *Daphnia*, you did it by doubling the number of algae cells, correct?

A. That was for the food-borne exposures, yes.

Q. JOSEPH SHAW: Did you give any thought to maybe just doubling the amount of silver?

A. That's a possibility, yes. We did think about that, but we went the route of doubling the food amount, because we knew that we were getting silver into the algae.

Q. JOSEPH SHAW: Were they fed the same amount of algae? Were they brought up with control algae, or something like that?

A. Yes.

Q. JOSEPH SHAW: Do you think that you got a fair distribution of algae?

A. I think so.

Q. JOSEPH SHAW: Did you take any measure to keep it in solution?

A. The algae that we were feeding, at the rate we were feeding, was enough that we had complete clearance of algae before the next feeding.

Metallothionein Protects Against Silver Blockage of the Na^+/K^+-ATPase

Elizabeth A. Ferguson, D.A. Leach and Christer Hogstrand
University of Kentucky
Lexington, Kentucky, USA

The principle mechanism of silver toxicity to freshwater fish is likely to lie in the ability of the metal to compromise the transport of Na^+ via the sodium/potassium-adenosine triphosphotase (Na^+/K^+-ATPase) across the basolateral membrane of the gill epithelium (Wood, *et al.*, 1996: Morgan, *et al.*, 1996). The Na^+/K^+-ATPase is a membrane bound enzyme found in all animal cells. Itis primarily responsible for regulation of cell volume, maintenance of the concentration gradient of Na^+ and K^+ across the cell membrane, and is the engine that drives movement of Na^+ and Cl^- across the gill epithelium. Inhibition of this enzyme has been identified as part of the key toxic mechanism during acute exposures to other metals such as copper (Lauren and McDonald, 1987; Lorz and McPherson, 1976) and aluminum (Booth, *et al.*, 1988; Staurnes, *et al.*, 1984). Our investigation focused on localization and characterization of Na^+/K^+-ATPase inhibition by Ag^+ with respect to the Na^+, K^+, and Mg^{2+} binding sites. A second facet of the investigation looked at the possibility of protection of the enzyme by the metal sequestering protein, metallothionein, during silver exposure.

Basolateral membranes (BLM) from rainbow trout (*Onchorynchus mykiss*) gill epithelia were isolated for Na^+/K^+-ATPase activity measurements in an initial study in which the effect of Ag^+ on the Na^+ and K^+ binding sites was analyzed by Michealis-Menten kinetics. Enzyme activity was measured as the rate of dephosphoylation of ATP by the Na^+/K^+-ATPase. Na^+ concentrations were varied from 1 -160 mM, keeping the total salt concentration and free Mg^{2+} concentration constant. Na^+/K^+-ATPase activity was monitored with Ag^+ added in concentrations ranging from 0 - 1 μM. Addition of Ag^+ to the assay medium resulted in a decreased maximum velocity of the reaction (V_{max}) without significantly changing the affinity of the enzyme for Na^+ (K_M) (figure 1A & 1B). Thus the mode of inhibition can be characterized as noncompetitive with respect to the Na^+ binding sites (*i.e.* Ag^+ and Na^+ have separate binding sites).

The results on BLM were confirmed by a second study in which purified dog kidney Na^+/K^+-ATPase was incubated with Ag^+. Purified Na^+/K^+-ATPase was employed to achieve higher activity of the enzyme as well as to alleviate extraneous ligand binding of Ag^+ which may have occurred with the BLM. The same protocol was followed as above with the results of the Michealis - Menten kinetics shown in figure 2A & 2B. In this case

the enzyme showed greater sensitivity to Ag^+. The mode of inhibition was again noncompetitive suggesting that Ag^+ was not binding to the enzyme at the Na^+ binding sites. An IC_{50} for purified dog kidney Na^+/K^+-ATPase was determined to be 42 nM Ag^+. The protocol was repeated with K^+ used as the enzyme substrate. As with Na^+, the mode of inhibition could be characterized as noncompetitive thus indicating that the K^+ binding sites are not the sites of Ag^+ inhibition. Another attempt to find the location of Ag^+ binding was attempted at the Mg^{2+} binding site of the Na^+/K^+-ATPase. In a protocol similar to that described above, with Na^+ and K^+ concentrations held constant, Mg^{2+} and Ag^+ were varied to obtain Mg^{2+} concentrations ranging from 1 - 600 mM at Ag^+ concentrations of 0 nM, 1.7 nM, 8 nM, and 42 nM. The V_{max} was unaffected by these Ag^+ concentrations but the apparent K_M for the Mg^{2+} site showed a marked dose dependent increase with increasing Ag^+ concentrations (figure 3A & 3B). Thus the mode of inhibition with respect to the Mg^{2+} binding sites can be characterized as competitive which strongly suggests that the Mg^{2+} binding site as the probable location of Ag^+ binding.

In a parallel study, we determined if the metal sequestering protein, metallothionein (MT), would be able to protect the enzyme from blockage by Ag^+. Ag^+ was complexed to MT and separated from free Ag^+ by size exclusion chromatography (Sephadex G-25). The MT containing fraction from the column was analyzed by graphite furnace AAS for Ag, Zn, and Cd content. Results showed that the Ag completely displaced Cd and Zn from MT. The Ag-MT containing fraction was again passed though the same column to assure that no free Ag^+ was present in the Ag-MT fraction. The Ag-MT was then diluted to a Ag concentration of 42 nM. The enzyme activity assay was again performed with optimal concentrations of Na^+, K^+, and Mg^{2+}. The assay was run with 0 nM Ag^+, 42 nM Ag^+, and 42 nM Ag as Ag-MT. As predicted, 42 nM Ag^+ resulted in approximately 50% reduction of the control activity (figure 4). The same concentration of Ag added as Ag-MT, had no effect on the Na^+/K^+-ATPase activity. Thus, at this Ag concentration MT offered complete protection against inhibition.

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Wood, C. M., Hogstrand, C., Galvez, F., and Munger, R. S. (1996). The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*). I. The effects of ionic Ag^+. *Aquatic Toxicology,* 35:93-109.

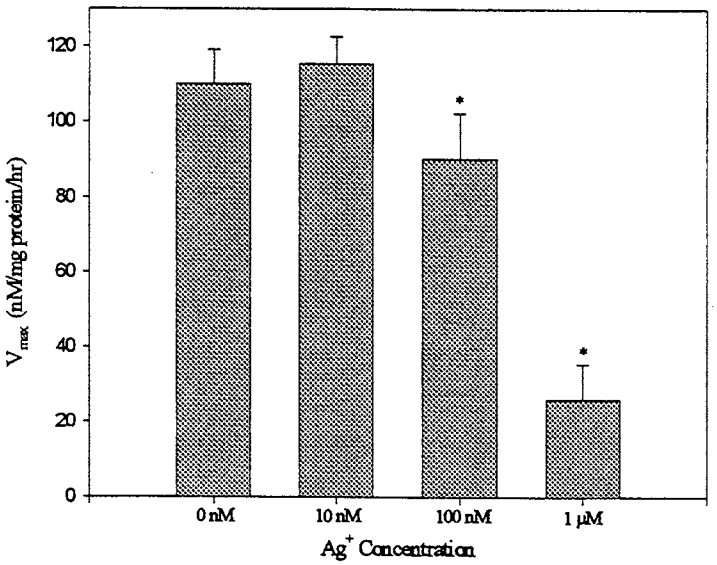

Figure 1A. *Comparison of the Na^+/K^+-ATPase V_{max} values for a range of silver concentrations with isolated basolateral membranes from rainbow trout gill epithelium. Values significantly different from the control ($p<0.05$) are indicated by an asterisk (*).*

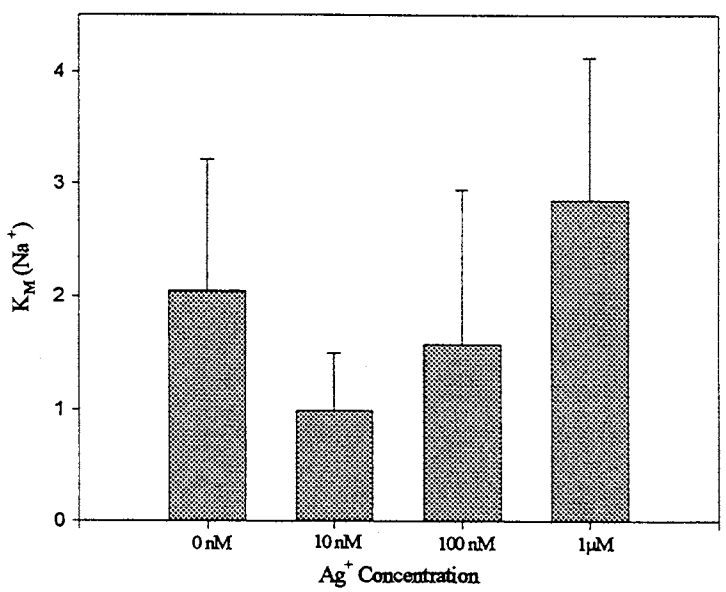

Figure 1B. *Comparison of the K_M values obtained at a range of Ag^+ concentrations. Groups were not significantly different.*

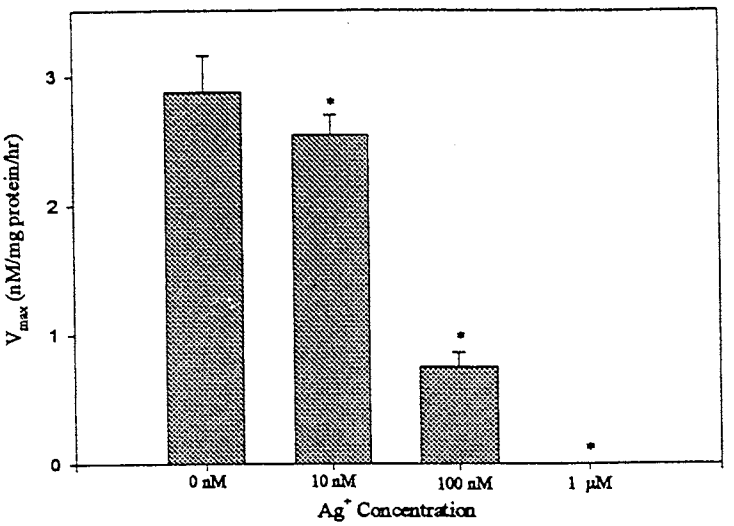

Figure 2A. *Comparison of purified dog kidney Na^+/K^+-ATPase V_{max} values for a range of silver concentrations with Na^+ as the substrate. Values significantly different from the control ($p<0.05$) are indicated by an asterisk (*).*

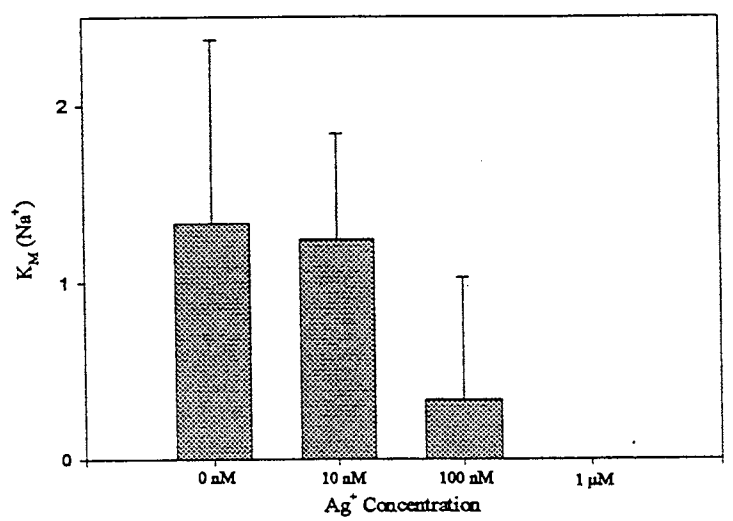

Figure 2B. *Comparison of the K_M values obtained at a range of Ag^+ concentrations.*

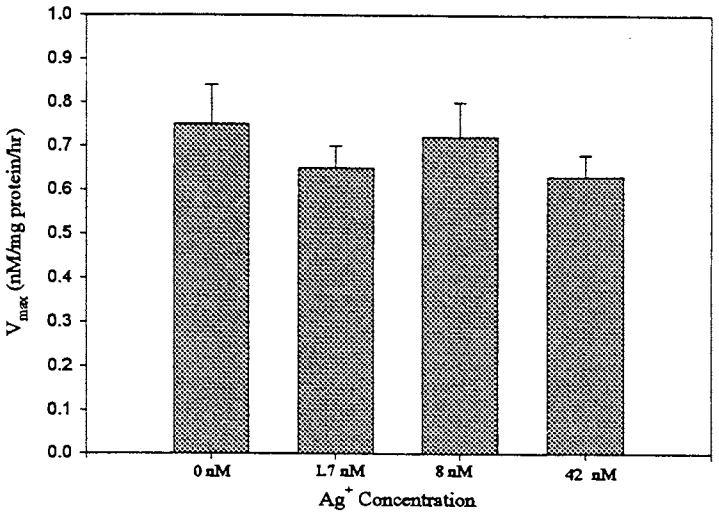

Figure 3A. *Comparison of purified dog kidney Na^+/K^+-ATPase V_{max} values for a range of silver concentrations with Mg^{2+} as the substrate. Groups were not significantly different.*

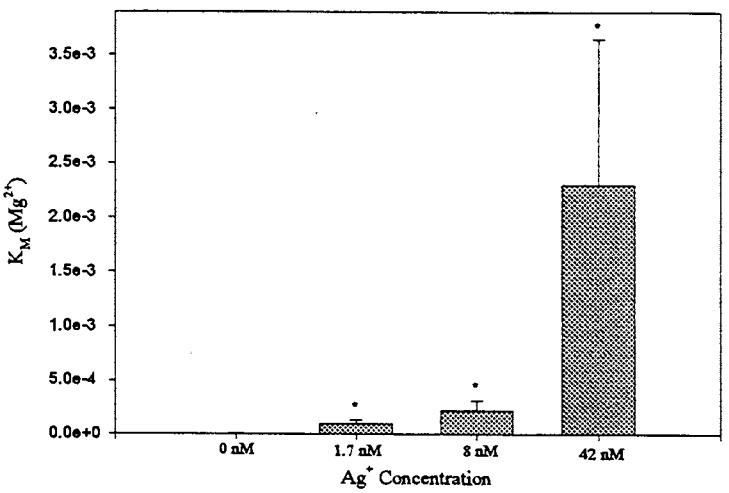

Figure 3B. *Comparison of the K_M values obtained at a range of Ag^+ concentrations. Values which are significantly different from the control ($p<0.05$) are indicated by an asterisk (*).*

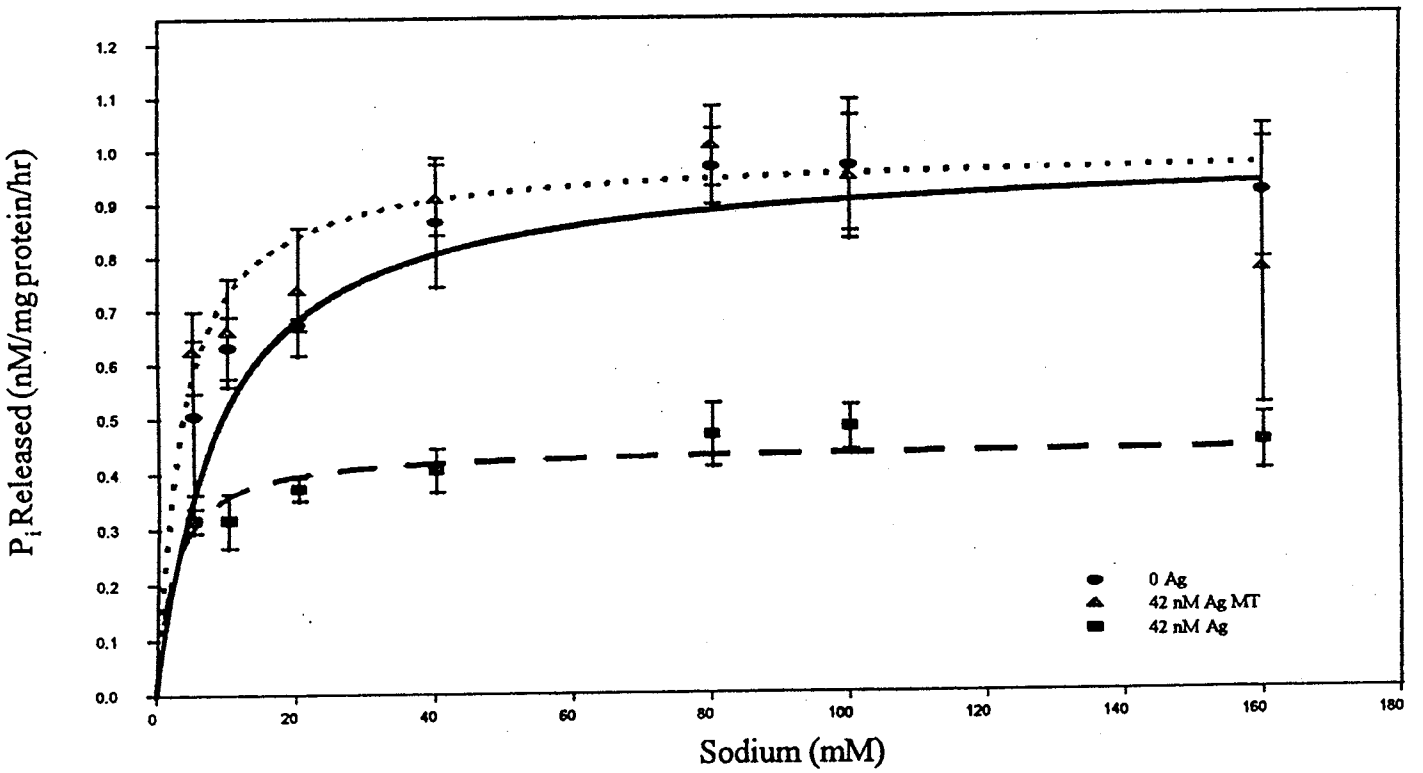

Figure 4. *Michealis - Menten kinetics curves obtained from purified dog kidney Na^+/K^+-ATPase exposed to Ag^+ and Ag-MT. Dashed line (----) represents 42 nM Ag^+, dotted line (·····) represents 42 nM Ag as Ag-MT, solid line represents control (0 nM Ag).*

Questions & Answers: Metallothionein Protects Against Silver Blockage of the Na^+/K^+-ATPase

No questions.

Panel Discussion

Regulation of Silver in Water Columns

Moderator:
D. Webb, Wis. Dept. of Natural Resources, Madison, Wisconsin

Panelists:
D. Armstrong, University of Wisconsin-Madison
H. Bergman, University of Wyoming
R. Erickson, U.S. Environmental Protection Agency, Duluth, Minnesota
N. Fisher, State University of New York-Stony Brook
C. Hogstrand, University of Kentucky
S. Klaine, Clemson University
P. Santschi, Texas A&M University

Panel Discussion: Regulation of Silver in Water Columns

Panel Chair: David Webb (Wisconsin Department of Natural Resources)

Panelists: **David Armstrong (University of Wisconsin)**
Harold Bergman (University of Wyoming)
Russell Erickson (USEPA)
Nicholas Fisher (SUNY Stony Brook)
Christer Hogstrand (University of Kentucky)
Stephen Klaine (Clemson University)
Peter Santschi (Texas A&M University)

DAVID WEBB: I'd just like to say a few things and then have a few statements from each of the panelists. After that we'll open it up to some comments and questions.

My name is David Webb. I'm an environmental toxicologist with the Wisconsin DNR, right downtown in Madison, and as Anders said, we do struggle with many a metals issue. It's great to see this kind of meeting of the minds, because I know firsthand that, while the information exchange may be slow, it's critical that folks like me get the information from meetings like this to be able to apply in permitted discharges and other monitoring-type activities. I struggle to do something that I don't think is very often successfully done, and that's to merge monitoring with water quality standards and regulatory issues. That's where accurate regulation and accurate monitoring happens. Certainly over the last couple of years, especially since the infamous 1993 Annapolis meeting that some of you may have been at. That's the meeting where folks got together at an EPA-sponsored meeting and collectively said, "we ought to regulate based on dissolved metals; it's the greatest thing since sliced bread." And then the recent Pellston Workshop in Florida earlier this year: a similar type recommendation came from a panel of experts. That leaves us in the regulatory community in a somewhat tough position because we're dealing with hundreds if not thousands of permitted discharges, and we're trying to implement this new and evolving science in a situation of hundreds of permits and limits. It's challenging to make it work accurately.

As an example, since there is a desire to regulate based on dissolved metals, and "credit" the particulate-bound fraction of a discharged metal, we as effluent-limit-calculators and toxicologists and so on have to figure out what the dissolved concentration, or filtrable concentration, is in the receiving water, equal to in total recoverable concentration in an effluent. This is true since, for a number of reasons, half of which are legal and in statutory code, effluent limits must be expressed as total recoverable metal, at least in today's world. Therein lies the problem. Reconciling dissolved in-stream with total recoverable in effluent is a huge challenge, especially on hundreds of streams with hundreds of different types of discharges. That's the kind of situation that really renders itself perfectly to information like I'm hearing today, and which I'm sure will be talked about tomorrow.

So with that, let me allow the panelists their few minutes (less than five), to air their views on the subject matter. Then we shall open it up to a stimulating discussion. As long as there is no preference, let's just go from left to right.

PETER SANTSCHI: My interests are in environmental radiochemistry, trace metal chemistry and colloid geochemistry. Based on the work we've done and what I've read, I would think that the relationship between silver and sulfur needs to be better shown. In sediments it has been done to a much greater extent than in the water column. What we found, for example — that silver is associated with macromolecular organic matter — needs to be firmed up; other people have not found that, for example in San Francisco Bay, and we've heard from Nick

Fisher today that they didn't find it in Long Island Sound. If reduced sulfur is the key element, we know it's easily oxidizable, and the importance of those associations need to be better quantified.

Now, on the other hand, from the point of view of the regulator one could say, if we regulate based on dissolved metal concentrations, does it matter if silver is associated with macromolecular organic matter? Some of our as-yet unpublished results indicate that the colloidally bound silver is bioavailable and is getting incorporated into shrimp, for example, but we don't know if it's toxic. The relationship between bioavailability and toxicity is not really established; something can be incorporated and when the pulse disappears it gets depurated and leaves no trace. That's a possibility. If it's not toxic, if it's just basically complexed, then it's the free metal ion concentration that matters, and the total dissolved metal concentration is still a conservative measure. The free silver concentration would be at least an order of magnitude lower, and that would be an OK standard, I would think.

DAVID ARMSTRONG: First of all, my area is aquatic chemistry. I guess, at least philosophically, in establishing standards for regulation what one would like to do is establish what the relative toxicity of the different silver species are and then set out to either measure or model what the levels of those species are in a particular system. Through the discussions that we've been hearing, there's been a lot of progress made toward that end; yet, as Peter has just elaborated, there are some questions that are still in front of us.

We heard a good discussion this morning about speciation, and I think that many of us would start with the free-ion model. We heard that that certainly has merit, and yet we also heard that there are some problems or questions; for example, in environmental systems the effects of DOC seem somewhat ambiguous — either enhancing or decreasing toxicity, depending on the system. Of course another factor is, what is the target organism? Whether the organism is a filter feeder or not is going to have some bearing on the forms of silver that are going to have influence. So those are some of the factors, I think, and it points to the need to have a better understanding of the interactions with colloidal material and DOC. I guess the other perspective is, what is the cycling and behavior of silver in the environment, and what is the residence time of silver? If we're talking about residence time in the water column after higher levels have been introduced, at least in some of the cases that we have looked at, where the relatively high concentrations compared to natural levels might be discharged in wastewater effluents, the rate at which the silver is removed downstream is quite rapid. This seems to be another important factor to consider in the overall problem of regulating silver.

RUSSELL ERICKSON: My work at the Duluth lab has generally dealt with looking at the effects of exposure conditions on toxicity and bioaccumulation and trying to tease out and model what processes are really regulating, again, toxicity and bioaccumulation, with the end goal of actually addressing the problem of taking laboratory toxicity data and applying it to exposure conditions in the field, because there's often quite a big mismatch between those two. As an alumnus of the Water Chemistry Program here, I should say that, for silver and other metals, I think the key to good toxicology is good chemistry, because unless we can characterize the conditions under which the toxicity tests are run, and know the conditions to which they are going to be applied, there's going to continue to be a big uncertainty.

Our chair referred to a problem in terms of just making the simple step of going to dissolved as a basis for ambient standards and criteria. Things were quite a bit simpler when the metals were based on total concentrations in the water column, and because toxicity tests in the laboratory tend to have greater toxicity and bioavailability — not always, but on average they are under more bioavailable conditions — that introduced an issue of conservatism. It was simple because you could do simple dilution models for relating effluents to in-stream concentrations, but the bottom line is that it was conservative in most cases, and sometimes extremely conservative.

We're crossing, then, the line into a risk-management decision of saying exactly what society wants; are the resources going to be put in to do a better job and get a better answer as to what the risk is, or are we going to go

with the simple, conservative assumption? We have to start with some kind of consensus or societal direction on what the risk-management goals are and how good an answer, in terms of the details of the chemistry and toxicology, we really need. From my perspective, once there needs to be a better answer than total, then there needs to be the kind of work we've seen today in chemical characterization, and then better linked into the description of toxicity with some talks we'll hear on Wednesday.

NICHOLAS FISHER: My research group focuses on the biogeochemical cycling of metals in marine and freshwater systems, mostly marine, and we look at both the effects of accumulation of metals in organisms — the effects of metals on the organisms — and as well, the effects of the organisms on the vertical flux and cycling in metals on aquatic systems. There are a few points I'd like to raise. One is, we heard, I thought, an elegant talk from Peter Campbell this morning about the difficulty of establishing a consistent relationship between metal uptake or effects in aquatic organisms and metal speciation. In a sense it's not surprising because there's a lot of different DOM out there, and it's been poorly characterized, especially in the marine environment. Some of it may act as a surfactant and enhance membrane permeability to metals, others may complex metal and make it less available. I think that, going back to first principles, we know that organisms, both from a nutritive standpoint and a toxicity standpoint, respond to metals or any substance as a function of the material that's in them or on them, not the material that's outside of them. So, however the metal may speciate in the ambient water, the organism will only respond to the metal that's already accumulated in its body. I think it's important to express toxicity as a function of the body burden of the metal, rather than as a function of the ambient metal concentration. It may turn out that you get a very nice correlation with the ambient metal concentration, but frequently you do not, as we heard from Peter.

One point I wish to emphasize is that attention ought to be given to assessing metal toxicity as a function of organism body burden and compare that with body burdens of those metals out in the environment. I would also, as you might guess, like to emphasize that attention be given to food chain transfer of metals, including silver, in helping to set water quality criteria. We know for sure that some metals are accumulated primarily from food, not from the dissolved phase. Selenium is the best example I can think of, and court cases were settled in San Francisco Bay based on the fact that selenium is accumulated primarily from food, not from the dissolved phase, and yet water quality criteria have been proposed that are primarily focusing only on the dissolved phase.

I think many toxicity tests that have been conducted and continue to be conducted, use acute toxicity exposures for usually fairly short periods of time. I would propose that experiments use more realistic environmental metal concentrations assessing sublethal or chronic toxicity tests. The parameters that could be looked at from the biological standpoint include growth, reproductive impairment — for example, hatching of eggs or what have you — and for animals, behavioral effects. Some of these are fairly easy to measure and are quantifiable.

Finally, I would like to stress that serious attention be focused on the accumulation and toxicity at the lower end of food webs, particularly phytoplankton and zooplankton. Some work has been done on phytoplankton, but very little on marine zooplankton and, as far as I know, not too much on freshwater zooplankton as well, with respect to sensitivity to silver and other metals. These organisms serve as the base of most aquatic food webs, and can serve to introduce accumulated metals to higher trophic levels, potentially leading to man. I think that gap in our knowledge of the interactions of the plankton with silver and other metals needs to be filled.

HAROLD BERGMAN: My background is basically environmental toxicology, with a physiological bent sometimes. Also my background is as an observer of government regulatory action. As our moderator pointed out, since the Annapolis meeting in 1993 and the recent SETAC meeting, it seems like things are starting to really change in regulatory practice in the EPA and, therefore, in the states. It goes back to before that; there was a meeting in December 1990 in Washington, D.C., at which Bob April of USEPA presided. A number of us were there. EPA was being beat upon rather vigorously from a number of quarters, and it was not just industry. It was also

municipal wastewater treatment plants who were having trouble meeting copper standards, I recall. From that point on, there has been a consistent move toward adjustment or changes in water quality regulation, kind of an opening of the process, and more outside science solicited and listened to by EPA than I have seen in my career, with respect to metals in the water column. True, the Prothro memo of 1993 recommending that dissolved forms of metals could be used in determining compliance with water quality criteria has been kind of an upheaval, but recall, as I think our moderator pointed out, that states and regions have the option of, based on risk-management decisions in a water body (in a lake, or a river, or a state), deciding to stay with total recoverable metals.

You should be aware that, as I recall, the specific things that were listed as the kinds of risk-management decisions that would lead one to stay with "total recoverable" are food chain problems, severe sediment contamination and, obviously, adversely affected aquatic communities. Two weeks ago, as an example, the state of Montana was petitioned based on water-effect ratio and other data to issue a site-specific water quality standard for copper, specifically for the Clark Fork River. The specific request, also the petition to the state of Montana, requested that they switch from their current standing of using total-recoverable to dissolved. Montana denied the petition, on the basis of a risk-management decision that the Clark Fork River in Montana is severely affected by metals already in the sediment. There are two studies published by Woodward that show dietary effect of metals in benthic macroinvertebrates affecting survival and growth of trout, with the benthos taken from that river for these feeding studies. So, there are still ways in which this dissolved recommendation is tempered, and I don't see it taking the country by storm by any means, and I'm curious to hear what Mary Reilly or Russ has to say about it.

In the western U.S. in particular, as well as in the northern midwest, — I know, because I grew up in the Upper Peninsula of Michigan — mining-impacted rivers are a major problem. There are thousands of miles of fishless creeks and streams in Colorado, fewer in Wyoming, we think, quite a few in Montana and Idaho, caused by heavily contaminated sediments from mining practices that go back 100 years. It's true in the northern midwest, too, in several places, and it's true in certain areas of Canada. So there are a lot of complicated issues beyond this move to dissolved metals in the water column that are practical regulatory issues. There are also, as have been pointed out, many important scientific issues, food-route exposure being a very important one. So in response to some of Nick's comments, body burdens are really important, but it has to be tied, at least in fish, to target tissue, and for acute effects, that's the gill. If you try and do whole-body burden metal, and evaluate it in terms of toxicity, in past experience it doesn't work with fish, and you have to work in terms of target tissue. It's usually the gill with metals, but for chronic effects it's going to be some other tissue.

STEPHEN KLAINE: I'm an aquatic toxicologist, and in general, my interest lies in contaminants; their fate, and their effects as they move into aquatic ecosystems. I think with silver in particular I'm interested in the factors (water quality parameters in particular) that dictate and control silver bioavailability. I think that, as you've heard today and as you'll hear more tomorrow, more and more evidence is mounting that DOC is an important factor in terms of controlling silver bioavailability. I also think that the data are not all consistent, and it reflects the wide range of dissolved organic carbon that's out there in nature. In our own research, while using Aldrich humic acid makes things consistent, it in no way is predictive of what happens with natural organic carbon in different water systems. So I think one of the major issues, coming from a perspective of how to predict toxicity, is just what is it about dissolved organic carbon that actually controls bioavailability? And, Russ, I hate to agree with you, but I think that's a chemistry issue, and I think there's a tremendous amount of chemistry that has to be done on this whole issue of dissolved organic carbon and silver, everywhere from what functional groups are responsible for making the silver apparently nonbioavailable, to maybe even enhancing bioavailability, as Peter Campbell suggested earlier.

Another issue is, I'm not even sure that we're dealing in our laboratories with systems that are at equilibrium. Many people are concerned about making sure that you put your test organisms in within a half hour or an hour after you put the silver in to make sure the silver concentration is consistent. I'm not sure that things have come — in fact I'm quite sure they have not come — to equilibrium. So another major issue, again a chemistry issue, is

that we need to understand the kinetics and thermodynamics of what's going on, not only with DOC, but also with all the other water quality parameters that control silver bioavailability. With all the talk of DOC, I wonder, can we forget about chloride and hardness? I don't know, but my suspicion is, we can't. Something that I haven't seen discussed, that just came to me, is alkalinity. I'm not sure some of the disparity in the literature on hardness might not be confounded with alkalinity. George Cobb made a comment to me on that. I think that's another issue that needs to be addressed.

I think many of these issues need to be settled from a risk assessment standpoint because if you're going to do risk management, if you're going to try to change how you permit metal concentrations with water quality criteria, you need to understand what the risk is. Moving to dissolved from total represents a risk, and I don't think many states are willing to do that. They shouldn't be willing to do that until we can give them some good answers on how the metals are going to behave in the dissolved form. There probably needs to be a differentiation between truly dissolved, or free, versus apparently dissolved and partitioned into some organic matter.

CHRISTER HOGSTRAND: I'm a physiologist, and my favorite model organism is fish. Specifically, what I have been working on for years is the metabolism of essential and nonessential elements in fish. Four years ago I started to work — then together with Chris Wood, and now more independently — on silver effects on the physiology of fish. I think a lot has been said here that I wanted to say, and I can just build further on it. One important issue that's been raised is that the levels of silver as well as other metals in the environment do not need to reflect what is getting into the animal. We can take that further and say that what's getting into the animal does not necessarily need to reflect what is happening to the animal, because as Harold was saying, it also depends on where it's going, what form it's getting in, and by which route it's getting in. For example, there's a great difference between dietary silver, and other metals, compared to waterborne metals. And it also makes a difference in where these metals are getting stored in the animal. Now, in the long run we're going to get different toxicity depending on route first and then time course. If we get exposure to waterborne metals, you're going to get effects immediately at the gills. If you get a more chronic exposure you're going to get effects where the metal is stored, for example the liver, or it may be in reproduction because a lot of the stuff that is made for the eggs is actually created in the liver and transported to the ovaries. That's a big reason we have reproductive disturbances for a lot of toxicants. So I call for more physiological and biochemical variables to put into the equation that will determine what regulations we have. I think that we're moving in that direction.

I'm from Europe; I'm Swedish, and I've been involved for several years in incorporating so-called biomarkers, for example metallothionein and P-450, into long-term monitoring programs in coastal waters. It seems like it is fairly promising. There are a few of these methods that can be used to gauge not only how much contaminant is getting into the animal, but what effects it is having on the animal, because if we get effects on these systems, we know that, in the long run, we will also have effects on the health status of the individual, the population, and perhaps also the ecosystem.

DAVID WEBB: Very good. Usually, in this state, if I'm in front of the crowd, it's "blast the DNR guy", but maybe I'm immune to that. So blast 'em.

Q. RON EISLER (National Biological Service): I have a regulatory question. I've been hearing about risk management and hazard evaluation, and this is sort of based on my own ignorance. I would like to know if there has ever been a successful enforcement action that did not include a biological organism within that ecosystem that had a particular concentration of that contaminant (for which the legal action is being implemented) in a critical organ, and that concentration has been linked with adverse effects on either growth, survival or reproduction. Has there ever been such a successful prosecution?

A. HAROLD BERGMAN: Fish kills. Without even detecting it in the body, if the concentration in the water had been monitored or water samples taken. I'm dissecting your question; not being a regulator . . . Maybe DNR has some prosecution history.

A. DAVID WEBB: I can't think of anything that specific, where an action was taken due to a specific buildup in a specific organism or even a more specific environmental compartment than that. It's usually something more generic like a fish kill, or contaminated sediments leading to a biological desert in the benthos or something like that. So I don't know of anything that specific in Wisconsin.

Q. ANDERS ANDREN (University of Wisconsin): I have a question that's directed to those working in the area of analytical chemistry. There's a continuum of physical sizes in the environment with which silver is associated. I know Peter and David, among others, are working on trying to quantify where silver falls in this continuum. At the same time we need to know a little more about the dissolved part and I wonder, can you "crystal ball" for us a little bit how long it will be before we can get a much better handle on the dissolved form, as well as going up the continuum of sizes? Are we fooling the regulators and the regulated by saying that eventually we will come up with something? Are we going to be able to help everybody?

A. PETER SANTSCHI: Your question, in my mind, is related to what functional groups silver is associated with. My answer would be similar to what I was saying before: If it's related to the reduced sulfur chemistry, if that can be established, we will have made progress. At the moment, we don't know. This is just a speculation and a good guess. That question only arises because we would like to get closer analytically to ionic silver, but silver as a "B" metal can be bioavailable as an uncharged complex and could potentially do some damage there too. Certainly it's desirable from a chemical point of view to know what the truly dissolved concentration of silver is and what its ionic concentration is and to understand its speciation, but in the absence of that information for natural systems, we know from what I've heard today that silver was incorporated through food and through the dissolved form (at least in the organisms Nick looked at; he can correct me if I'm wrong). On the other hand, what I've heard is that the toxicity was still related to the ionic form of silver, so I guess the ruling is still out. But, back to what I said earlier, if we understand the functional groups, and those experiments can be done, we will be on track. You wanted to know how long that will take; my guess would be a few years.

A. DAVID ARMSTRONG: Well, I don't know that there's a lot I can add to that. I think the situation you're referring to is that our separations of dissolved silver from colloidal species or other species is operational, and what we have in what may be a dissolved phase can include a lot of different species which may differ in their behavior and toxicity. So I think what we're interested in moving toward as much as we can is defining what ligands are associated with silver in these phases that, as best we can understand, are dissolved, and then the answer to such questions as how readily transported these complexes are into organisms across membranes. I think there are some interesting things happening now that will begin to give us information about what those ligands are, but I suspect that to truly have that worked out is going to be a fairly long-term problem.

Q. ANDERS ANDREN: I don't want to occupy you for too long, but I have another question that I'd like to direct to those of you who are involved in toxicology. From a chemist's perspective it seems that it's really difficult to look at the endpoints sometimes. You look at death as an endpoint, certainly, when you are looking at phytoplankton or zooplankton, or you can look at some chronic effects in fish. Recently, however, there's been an inroad into looking at effects down at a certain part of the genome of a fish, for example. People are now looking at the base functions in the DNA that express a certain effect. Is anybody working in this particular area with metals, and is that something that EPA and the researchers should start focusing on, rather than contributions to death of an animal, which can be fairly hard to measure or at least to interpret? Rather, should we look at the part of the genetic material that expresses a sensitivity to a metal?

A. CHRISTER HOGSTRAND: There will actually be a talk on that in this conference. During the last two years, we have been starting to look at interactions of metals on gene transcription. We're also looking into exploring this further by building biosensors that can virtually measure the free silver ion concentration within cells, by measuring the donation of silver to proteins that interact with DNA to express certain proteins. Then you genetically redesign these proteins so that instead of proteins they produce light. We can do that; we can put

these into cells. For example, if we put them into an artificial gill epithelium, which we also can build, we can put water on one side and a blood substitute on the other side. From that we can determine if the silver in the water not only gets into the cell, but also if it is bioactive, that is if it interacts with the proteins in the cell. It should produce light from interaction with these DNA-binding proteins. So we're getting there.

A. NICHOLAS FISHER: Maybe I misunderstood you, Anders, but I gather that you were saying that the work done with plankton is mostly at the acute level and the work done with fish is chronic. There are well-established techniques, and other easily establishable, I think, techniques for working with sublethal concentrations with the plankton. For example, looking at depression of log-linear cell division rate in microorganisms has been well established for decades. That's all sublethal work. Moreover, looking at, let's say, development of juvenile stages to adult stages in copepods, the time necessary for that to occur as a function of particular contaminant concentration could be measured.

Q. ANDERS ANDREN: But getting down to the genetic level, what expresses the retardation of that . . .

A. NICHOLAS FISHER: That work, to my knowledge, has not been done with plankton, although I think some work has been done with freshwater bacteria.

A. RUSSELL ERICKSON: I would maybe add that with PCBs, or with some of the other endpoints that have been mentioned, there is a reason for looking, in terms of the overt endpoints on the animal, at things at a certain biochemical level. I think we have to look at some of the endpoints. Rick Playle will talk about his work, and a lot has been done with acute fish toxicity in terms of ion regulation disruption, the binding of metals to the gills and competition at the gills. It's a really good story to look forward to. But the issue is how far down the biochemical chain is it useful to go to. I'm not sure that's the case in a lot of the endpoints we deal with; you still have to make the link back to the overt effect that's going to be the basis for regulation. That's going to have the societal value.

A. CHRISTER HOGSTRAND: I'd actually argue with that. Anders was making the comment in another talk, asking if you're looking at the mechanisms. There's a reason for that; if we don't know the mechanisms involved that are causing the effects, we can't make further predictions about what the effects are going to be.

A. RUSSELL ERICKSON: I don't disagree at all. But at what level do you look at the effects to provide the benefits to the regulation? My point was that, with things like the work on gill disruption that has been done, what's to be gained from looking further down the chain? There has to be a mechanistic understanding at some level. Whether that level has to do with gene transcription or not, I think, is the issue. I don't argue with needing a better mechanistic understanding of the effects, but the direction and nature of that mechanistic understanding might differ from the example that Anders brought up.

A. CHRISTER HOGSTRAND: But this has to be judged from case to case.

Q. ERIC CRECELIUS (Battelle Marine Sciences Lab): What are the present silver water quality criteria for both marine and freshwater, and are these water quality criteria reasonable from the standpoint of the regulator? Do you feel you have a good standard to work with? Then, from industry's side, are these standards something that is causing a lot of problems? Are there examples where these criteria aren't being met, and will it become very expensive for industry and society to reduce the levels in discharges?

A. RUSSELL ERICKSON: I must admit, I won't be able to quantify this. The actual official EPA criteria for silver is quite old. There is no criteria for chronic silver toxicity. There was a draft revision of the acute toxicity (and this dates back to around 1980), and while it varied with hardness, it was on the order of several micrograms per liter. (From the audience: "2.3 in the marine environment.") I was talking freshwater. (Ron Eisler NBS: "It

varies between 1 and 1.4 depending on hardness.") That was the acute criteria. And at that time there was no chronic criteria. There was a draft silver criteria document done which updated that. It wasn't going to depend on hardness because of disagreements between some of the studies that were being cited. It did have a chronic criteria in it. That draft was never finalized, in large part because of issues we were talking about today in terms of bioavailability. It started with the whole idea of, "why not a hardness correction?" but there were other issues involved as well, and concern about actually having something that reasonably accurately represents the hazard under a particular exposure condition. If I understand things correctly, some states have implemented the old criteria, but there were some chronic studies that some states implemented as a chronic number which was submicrogram per liter. The old criteria at least cited some chronic studies, but did not specify a number. But some of those studies have been implemented as standards. I think the numbers that are included in these criteria always have to be qualified in terms of what the exposure conditions were. Under certain exposure conditions they're probably as close as is reasonable to being appropriate, but under other exposure conditions they aren't. It gets down to the issue of a better description of how the toxicity depends on the actual conditions.

A. DAVID WEBB: In Wisconsin we have, I think, only a couple of discharges that have silver limits. I'm almost positive that those discharges don't have trouble meeting them. Of all the problems we have in this state on facilities having trouble with limits, eight out of 10 of those are copper, and the other two are probably zinc. Maybe a lead or two would be thrown in. I think we have only around 50 facilities in this state with metals limits, and only a couple of those are silver. (Erickson asks, "What is your silver standard?") I think it's around one microgram per liter.

A. HAROLD BERGMAN: Just from being an observer of things that happen in the government, there have (and maybe someone from the Silver Coalition can comment on this) been concerns, of course, in the photographic industry because of pretreatment requirements that went into effect a few years ago — I don't remember what year — where, if you are discharging into a sewer that goes to a publicly-owned sewage treatment plant, there was adopted — Wisconsin and other states adopted, under the EPA's eagle eye — a limit on the concentration that could be discharged to the waste treatment plant. Hospitals, in particular, were major violators around the country. Is that right?

A. DAVID WEBB: I should've pointed that out, because we aren't really involved in those kinds of circumstances. That would be between the hospital, in that case, and the POTW. We provide guidance and so on, but we're out of that. So I don't have a handle on how many of those types of situations there are. I did forget to point out that, of all of the analytical problems and poor data histories we have with metals in general, a lot of data that we get from dischargers is either inferior or it's "no detect" at some high level of detection. So we have that sort of wild card in there, as with all the other metals. A good data set is the cornerstone of our program.

A. THOMAS PURCELL (Silver Council): I'm in the peculiar position of having been at EPA as we started working on the 1987 version of the silver criteria document, and now being with the Silver Council. I think that the issue, from an industry standpoint, with the criteria as it now stands (which is the 1980 criteria), is that the tests are done primarily using ionic silver — silver nitrate. Almost all of the toxicity data that's used to calculate the criteria are based on the silver nitrate test, so there is that issue. The second issue was already brought up, and that's the fact that, within the criteria document, a NOEL for the chronic value is stated, which is 0.13, based on, I think, a 0.17 lowest effect level that was seen in a single test. That number — when it is incorporated into a state's criteria, as it is in seven or eight states now, and then back-calculated into a pretreatment number, typically by a local pretreatment operation — is unreachable. You cannot remove silver to that extent; you have to barrel and haul all your waste. I think that's what your question was originally. I think those are basically the difficulties of the criteria as they now stand.

A. RUSSELL ERICKSON: But the basic problem with that is when it's implemented as a total silver number in the ambient stream, right?

A. THOMAS PURCELL: Yes. It's tested as ionic silver, and implemented as total silver.

A. RUSSELL ERICKSON: I would disagree that these trials actually test ionic silver; the form you add the silver in any of these tests rarely stays in that form. In principle you're right; if you compare total silver in these tests to total silver in the environment, there is an extreme mismatch as far as their bioavailability in something like a POTW. Maybe I'm quibbling a little bit to say that these tests really aren't free silver and that isn't the same as total silver. I think that's probably a bit inaccurate, but the bioavailable silver and, therefore, the toxicity is certainly greater in these tests than they would be in POTW's. We saw Martin Shafer's data from earlier as to the distribution of silver in the streamwater from Madison's POTW, and it's obviously almost entirely in a particulate form. That 0.13 is really far from the bioavailability that you would get in a stream down from a POTW.

Q. THOMAS PURCELL: As long as I'm standing here, I thought I'd bring up another point. This is a general question. In thinking about the regulation of silver or any metal (and I guess to some extent this comes from my work in the criteria program), you need to consider something that is relatively easy to do. Many of the states don't have the technical capability or the resources to measure body burden and the water column concentration and the porewater concentration, so there is this dilemma of trying to find, and I think this is something that was generally stated by the panel, a goal — something that you want to measure. Some way of defining what is the safe level; what is the safe value as the material is in the environment? That probably is the single most difficult thing that we're going to do, certainly in the short-term. I am inclined to think that you as scientists can always think of additional things to do, can always think of new projects to head off on. But the end result of that is, from a regulatory standpoint (what I'm trying to do is sort of get the thoughts of the panel back into reality), that you have to regulate now. What is the best way to go about finding something in the short-term, correcting it in the long-term if you need to, but finding something in the short-term that is usable?

A. NICHOLAS FISHER: Let me just address one small aspect of that. I think it's in many ways a lot easier to measure the concentration of metal in an organism than the dissolved concentration. There are a handful of labs in the world that can measure dissolved concentration accurately when the metal concentration is in the picomolar or low-nanomolar range, and that can do it well. But because organisms concentrate metals — sometimes by many orders of magnitude, silver on the order of ten to the fifth power, for example for phytoplankton, and ten to the third or higher for animals higher up in the food chain — the metals are that much more concentrated in the tissue, and the measurements are that much more reliable. So I would take exception to the fact that it's easy to just go out and measure the dissolved concentration. A lot of people do go out and measure dissolved concentration, but the number of values that are believable, I think, is another story entirely.

A. HAROLD BERGMAN: From a regulatory sense, of course, as you're well aware, in the last 10 to 12 years there have been three separate pieces of guidance from EPA about site-specific water quality criteria and the whole business of water-effect ratios. They all follow the Don Mount (of USEPA) maxim, "Keep it simple." I, and probably everybody in this room, have heard him say that. Russ has probably heard him say it several thousand times. As we get more scientific understanding, and we're trying to split hairs more and get one uniform policy that will apply to all waters, I'm sure glad that I'm a biologist who works in a university, and I don't have the job that this gentleman does at Wisconsin DNR or Mary Reiley's job in EPA. It's very easy to find fault with what they do, or attempt to do, but it's a tough job. And the best they've been able to do so far is site-specific standards.

A. RUSSELL ERICKSON: But Tom is right that as we go to a better understanding of the details of the response, things get more complicated and more difficult to do. I would agree with Nick's statement that residue-based criteria represent one good potential for putting in a checkpoint that might be easily measurable in terms of regulating. There are still problems in terms of backing that concentration through the water back to the effluent, in terms of getting some of the numbers you need to do that, and they end up sometimes having to do good dissolved measurements. There is some potential along that line to make things based on things that are perhaps not so easily measurable, and that has to be sort of a guiding light in trying to develop this. There's an old maxim that says you have to understand things at one or two levels of greater detail to handle them at a certain level, and research has to go to that level to get the framework, which you can perhaps turn into something simple. So the complexity we're talking about in research is not necessarily incompatible with providing reasonably simple regulation.

A. ERIC CRECELIUS: I have a comment on the analytical problems for analyzing silver in the environment. About a year ago EPA came out with some draft documents called "Clean Metals Methods" — their 1600 series. Using these methods, it's possible now to measure silver in the range of a few nanograms per liter. Texas A&M and Battelle and some other labs participated in validating these methods. It calls for a silver preconcentration step and then analysis by graphite furnace or ICP mass spectrometry. It is possible now to use those EPA methods to do clean sampling and do low-level analysis of dissolved metals. Most of this work has been done in fresh waters, doing matrix spikes and such. We did an intercomparison with the state of Florida's lab where they specially geared up to do low-level silver because they set a very restrictive level for discharges. The state of Florida was able to measure silver in water to a few nanograms per liter level. It's the only state other than Wisconsin that I know about that really has put an effort into measuring silver. They're still not always detecting silver in the environment, but it can be done. It does take the dedication in a lab to get to that.

A. DAVID WEBB: Good point. We as a state agency can't wait until methods like that get final, because it makes our jobs a lot easier for having the "authority" to require that a good method be used instead of saying "well, we can't measure compliance because we can only detect 200 nanograms per liter," of mercury for example. So anything based on good data is great.

Q. FERNANDO GALVEZ (McMaster University): I'm a fish physiologist, and I have a particular interest in zinc metabolism, similar to Christer. I guess there's been a push to get more details, and look at lower levels, subcellular and biochemical. But has there been any attempt to try to correlate this back to the population? Because it is the population of different organisms that we're trying to protect. Has there been any attempt to look at the ecology?

A. CHRISTER HOGSTRAND: This is probably the most difficult thing that there is. The evidence that you'll get is correlational evidence. But, yes, there is evidence. There have been substantial efforts, and what's been done is to go to a single spot in the field (actually Harold Bergman is probably the best one to talk about the example of the Clark Fork River) and measure whatever you can measure from the littlest molecule to the biggest animal, and try to find parity in the responses that you find at all levels. It's extremely difficult; it's only been successful in a few cases. I think the Clark Fork River might be one of those.

A. HAROLD BERGMAN: It takes, as you can imagine, a lot of time and a lot of money to do a study even at one site to make those sorts of correlations. And that's to a large extent what they are. But if the studies are designed in such a way, whether it's molecular metallothionein induction in cellular-level events linked all the way up to fish population abundance, fish growth rates in the river, fish community structure, reproductive capacity of the various species in the community, as Christer said and as Russ and anybody else in the room who's done any biology on this will attest, it's not done very often, and when it is done it's very difficult to do. In the experience of at least some of us, I know the studies have to be designed in such a way that they're

based on mechanism. What I get distressed about with my graduate students, when they come in with some hot idea, is if they're shotgunning the problem and measuring everything that they've ever read in the literature that anybody ever measured, from scale growth to RNA-DNA ratios — just shotgunning, looking for an effect. I think the only way you can even begin to do these sorts of studies is to have excellent hypotheses that are based on working mechanistic models, all the way from geochemical to toxicological to physiological mechanisms. That's one of the ways that understanding mechanisms can help us, in those situations, to be able to go in and design a study where you're actually measuring, at the mechanistic level, those things that are likely to be important in linking a metal accumulation to some cellular or organismal level effect that relates to reproduction in that organism. I don't ever want to do another one of those again. It's really important to do that, but as a society we probably can't afford to do it very often.

Q. JAMES KRAMER (McMaster University): I'd like to come back to the regulatory criteria; I have a proposal. The science, if I can capsule it briefly; let's believe what Nick Fisher said. He said pathways for metals can be both via the soluble and the food chain routes — particulate, etc. We've been told the regulations are old. They do not differentiate between total and ionic forms, but we won't get into that. We've been told by the physiologists that you've got to have good chemistry, but really, behind all this we've been told that, "well really, what happens?" You can speciate and so on, but what happens in the organism? So, what about sequential criteria? How about (and this goes back to your comment, Dave, that you have for silver maybe two problem guys in Wisconsin) a first cut where you set some concentration level? Don't worry what the silver is, total; you know, go out and analyze it. So you've got to have good analytical techniques that you can measure that at half a ppb, or wherever you want to put it. But then, once you exceed that, you say you guys have to do some kind of receptor studies. You've got to put in the right benthic organism and you've got to look at some response.

A. RUSSELL ERICKSON: That option sort of exists now, and I think, in that the EPA recognized that the criteria not just for silver but for a lot of metals basically doesn't reflect the exposure conditions at certain sites and there are procedures for, if the criteria is going to be violated at a site, to go in and do targeted tests on appropriate organisms to address that criteria. I think there has been some discussion down at the Pensacola meeting to make that even more routine and that these criteria or standards would just be a trigger level like that. Functionally right now that can be the case for any discharger that chooses to do it.

A. STEPHEN KLAINE: I think it really has to do with risk, and our level of understanding of what's going on, because the better you know the processes, the more confident you are in your risk assessment. Certainly, as we've seen, one risk assessment probably is not going to be sufficient for all of the different sites. So at some basic level of understanding, we have some conservative water quality criteria, and as you've just indicated, if you wish to go above that, you're going to have to understand more of what's going on at a particular site, and that essentially means doing more work. That's done routinely.

A. RUSSELL ERICKSON: Again, there is often a stated reluctance as far as the cost of doing those tests. Never mind that they often save a lot more money than they cost, but it's still not a trivial amount of money, and to the extent that the up-front assessment can be refined in some way by better chemistry and better toxicology to be more extrapolatable to particular sites, rather than having one low trigger value where 90 percent of the sites might have to do site tests, perhaps have a semiconservative range of values where maybe you'll only trigger the site testing on 10 percent of the sites. I think, in principle, I would agree with you that if I was a discharger I would go out and do that kind of testing to define the criteria.

Q. JOE GORSUCH (Eastman Kodak): My question is pretty much related to what you were just discussing: site-specific testing. Harold, you mentioned water-effect ratios several times, and I think you're the only one that has, but I think all three of you were alluding to it in your responses here. How valuable of a tool is water-effect ratio in your opinion?

A. DAVID WEBB: Too expensive.

A. RUSSELL ERICKSON: It is expensive, but to say it's too expensive you have to judge it against the alternatives. I don't know if it's too expensive to spend that amount of money when the alternative is to spend 10 times as much on a discharge limitation. I think the water-effect ratio, just like the toxicity tests we apply to develop the criteria, are sometimes limited. Even though I deal with toxicology, the one point I tried to make earlier was that the toxicology isn't going to mean much unless we have the representative chemistry. My opinion is that water-effect ratios can be quite useful in general, but there has to be considerable care taken as to whether the tests are run with the appropriate organisms, and the changes in the guidelines have emphasized that more. But more importantly, that there's confidence that they reflect the exposure conditions that the regulations are going to be applied to. I think in the case of silver, particularly out of POTW's, there might be some problems regarding that in terms of the chemistry of silver that goes through the treatment process relative to the chemistry of silver in a site-specific test. I think it's possible to do it appropriately, but I think there are some serious issues there.

A. HAROLD BERGMAN: I think the number one recommendation off the top of the environmental toxicology group at the Pellston metals workshop last February was that, until we can figure out how to do this better, water-effect ratios, and those sorts of tests, can be very useful for resolving many problems. Most of the recommendations dealt with using mechanistic understanding to improve things down the line, and there are some tools that are available to be used now, or in the very near future. But there was a fair amount of discussion about it, and I think, when the book finally comes out sometime before the SETAC meeting this fall, it'll still be there, and that recommendation is that in the interim, water-effect ratios and the like can be useful for resolving problems.

Q. JOE GORSUCH: Along the same lines, during the 1993 meetings both at Annapolis and Narragansett, rapid bioassessment was also suggested. Where does that fit into the scheme for the future?

A. DAVID WEBB: That's impossible to keep brief. Maybe it could be a dinner discussion.

A. HAROLD BERGMAN: Does Wisconsin use it?

A. DAVID WEBB: No. We're planning to do some things on the front of biocriteria. We have some people working on a rapid bioassessment technique, but it's in draft form right now.

Panel Discussion

Regulation of Silver in Sediments

Moderator:
M. Reiley, U.S. Environmental Protection Agency, Washington, D.C.

Panelists:
D. Call, University of Wisconsin-Superior
E. Crecelius, Battelle Northwest
K. Daskalakis, NOAA/NOS/ORCA, Silver Spring, Maryland
D. Di Toro, Hydroqual, Inc., Mahwah, New Jersey
J. Kramer, McMaster University

Panel Discussion: Regulation of Silver in Sediments

Panel Chair: Mary Reiley (US Environmental Protection Agency)
Panelists: Dan Call (Univ. of Wis.-.Superior, Lake Superior Research Institute)
Eric Crecelius (Battelle Marine Sciences Laboratory)
Kostas Daskalakis (NOAA/NOS/ORCA)
Dominic Di Toro (Hydroqual, Inc.)
James Kramer (McMaster University)

MARY REILEY: Good afternoon. I'm glad to see that some people have stuck it out to the very end here. I want to tell you a little of who I am, since I've only met a lot of you over the last couple of days. For the last four years, I've been coordinating the Sediment Quality Criteria Research Program out of the Office of Water, and I'm going to continue to do that. But I'm also picking up a few new tasks in the Aquatic Life program, and in Whole-Effluent Toxicity as well. So I'm becoming more of a "Jack of many trades."

So what's happening right now? I guess I'm going to talk on two sides here: what's happening in sediments at the agency, and a little bit about what's happening in metals at the agency. On the sediment side of the arena, we've got some short-term goals, some very immediate short-term goals, and we also have some moderate- and longer-term goals that we're trying to pursue. On the short end of things, we're trying to finish up the equilibrium partitioning methodology for nonionics, and the sediment quality criteria that go along with that. Our hope is that we will have that process of public comment and response, and Federal Register publication done this fall, just in time for the elections. We got an order in the other day from the Office of Water that had come down from the White House that says we want to look like we have the environmental agenda out there, so get to work. So we have a window of opportunity to get some stuff done that we've been trying to do for quite a while. That's a very short-term objective right now. In the longer term, metals, mixtures and bioaccumulatives are the directions that we'd like to pursue, as well as how to work with bioavailability in those areas, and how that transfers into a regulatory program for aquatic ecosystems.

As far as metals go, a lot of people have been asking how the agency is going to respond, in light of the Pellston Conference that happened this past winter. I wish I could give you a whole lot more direct information of what our ideas are and what our commitment to the conclusions of that conference are going to be. We have been asked by SETAC not to commit ourselves to any particular activity until the booklet is published, so that everybody will have an opportunity — in the academic community, the regulated community, and the regulating community — to discuss the recommendations that came out of that conference more openly rather than us jumping right in and getting started on something without having that discussion first. I can say that we're going to take the recommendations that come out of Pellston very seriously. We wouldn't have worked so hard, along with the rest of the organizing committee, to make sure that the right people were there to give their perspectives on the problems that we're having with metals in the aquatic ecosystem, if we weren't planning on taking the results seriously. I would expect that, over the next couple of years, you should look for the agency to pursue several of the recommendations that Pellston has put in front of us.

The last area that I wanted to give a heads-up on, because it's a question I get asked quite a bit, is the future direction of the criteria program in general. The program is in a moment of evolution. We're at a crossroads of having an aquatic life program that's very well established; a sediment program that's up and coming; a human health program that's well established; and whole effluent toxicity, which has been out and been used for the last 10 or 15 years. People keep saying, how are you going to bring these individual pieces together into a

comprehensive program? That is really our challenge for the next several years. How are we going to develop an integrated criteria program? As with any type of change, you have a couple of false starts before you really find the right way to do it. I think we're somewhat in that false start piece still, but starting to come out. We have formulated some ideas. I think in the long run what we'd like to be able to see is the agency come out with single criteria documents so, for example, when we issue a document "Guidance on Mercury," we issue a complete document that deals with the aquatic life aspect, the sediment aspect, the wildlife, the human health in combination, rather than having individual publications for each one of those. That way we can consider the impact of any given chemical on a watershed level rather than individual media.

That's the direction the agency would like to take; doing an integrated criterion, doing integrated exposure analysis in order to make a recommendation, and trying to figure out how we're going to deal with issues of independent application and watershed evaluation and management. I'm going to get off my soapbox there. Please feel free to ask policy questions of me.

ERIC CRECELIUS: My experience with silver has been primarily in the marine environment. I'm looking at the sources, fate and transport of silver. Most of that has been in Puget Sound. Approximately 10 or 15 years ago there was about a ton of silver per year that entered Puget Sound, mostly through wastewater treatment. Since then, the amount of silver entering the Sound has been reduced significantly, both because the price of silver went way up a few years ago and because regulation encouraged recycling of silver.

Even when the silver input was relatively large compared to the natural background, the levels in the water were a few parts per trillion, the levels in the sediment were usually less than a part per million, and the levels in tissue of the organisms that were bioaccumulating some of the silver were still down in the tenths of parts per million. There's no demonstration in that system of biological effects from silver, although there is bioaccumulation. The system had a high capacity to bind the silver and remove it. Almost all the silver went to the sediment, where it's quite tightly bound; it's released at a relatively slow rate. I would predict that the system has the capacity to bind much more silver and essentially remove it from the ecosystem, although there is potential for a small amount of it to be recycled, bioaccumulate and cause effects. But it's going to be very difficult to see these effects, and it may take some rather elaborate benthic ecology change-type studies to determine whether the silver is causing any effects in the marine environment. Most of this would probably apply as well in freshwater systems.

I think only in areas where there is a tremendous input of silver, like in mining sites, might you actually find silver causing an effect. Because the wastewater industry is now removing most anthropogenic silver that's used in industry, there's relatively small input into a system that has a relatively high capacity to immobilize silver.

JAMES KRAMER: I have five or six points here, most of which came out of the conference, at least from my perspective. I guess the first point that we have to remember with respect to silver is that it is in very low concentrations in the environment, so when we think of interactions and what this means and so on, we have to accept the proposition that anything in the system can have a pronounced effect on silver from a purely stoichiometric interaction. That makes it difficult to be completely convinced that you know what you're talking about. However, we have seen various kinds of correlations, particularly with sulfur, group B metals and so on, which suggest that we can focus down more. But I think it's important we keep that perspective.

One of the points that has come up before, but I think really was clearly developed here by different groups, is the role of the size of particulates and colloids. And this becomes important not just from academic, uptake, etc., points of view, but because we need to know more about those colloids, which have a lot more specific surface and are a lot more reactive. If we get into the areas of particulate or food uptake (if I can think of colloids as food), just how reactive are they? I know if Nick Fisher were here, he would be developing this point a lot more. Although there is information coming on, the colloids are sort of a black box, except we sort of know what form of

silver is in them. We need to know what their kinetics are, we need to know if it's sulfur, what's associated with them, so I think there's an area of effort here that needs to be carried out. I want to put on a mineralogist's hat. If you look at the mineralogical literature, in terms of silver and sulfide, you find other metals associating with silver. Now, these are crystalline materials, but I think it's important that we know — and this has come up before — if it is silver sulfide, if we, for example, add nickel and iron, whether sulfur can be accommodated in that kind of structure based on changes in the relative stability. We haven't had, except indirectly, any mixed-metal studies in a rigorous way.

The issue of oxidation is important. As Dominic said, if the stuff gets down in the sediments and stays there, the problem is over, forget about it. I think that's extremely important, and I was very happy to see Marianne Hirsch's work because, in terms of regulatory issues and whether the stuff can be incorporated, we need to see a lot more of that kind of work. I think that was a very good presentation and a good beginning on another kind of biological receptor.

Another issue that is always talked about when you can't explain your results is, throughout all of this we sort of have a pseudo-equilibrium assumption, but I think we have to look at more reversibility experiments, and we can do these now. I think that will come along. We certainly saw some pseudo-kinetic studies, but with ability to continuously measure, for example, AVS and metals, we can, perhaps, design some good experiments.

One other issue: we still need more work on answering the question, "What is AVS?" What's in there, in terms of when we do this measurement? It's not simply adding up all these metals, because, as I pointed out, silver doesn't count in the addition, it's so small. It can react with anything that's in AVS. But if this ends up just a black box, we're going to have an area of ignorance here. So I think this is an area where we need some additional development. I want to make a point on regulation. Given the ignorance, and the idea that you want something everybody can measure, it seems to me (and I tried to make this point the first day, but it got misconstrued a little bit) that in terms of measurement, from a purely regulatory point of view or pseudo-regulatory point of view, let's measure the aqueous phase if we're thinking about that uptake (and measure it however you want; filtered or total recoverable or whatever), but at the same time measure the appropriate biological receptor. It's not that difficult to measure tissues. So you have a two-way system of measurement of whether you have an uptake or not, and then whether there is a toxic effect.

The next-to-last point is, in the geochemical point of view, we need to look at geochemistry. In this game (and I'm glad Russell Bell was here), we have slipped into the traditional inorganic geochemistry. The organic sulfur species, I hope he convinced you, are very important. He didn't mention a lot of other things in the literature, and in this review they will come out. There are some tunicates that concentrate silver, I believe, to about one or two percent of silver in the marine environment. The structure of the organo-silver complex has been determined by a group in Pittsburgh. So there's another whole side to this coin in terms of silver-sulfur chemistry. Let's not be only inorganic geochemists, although the other is hard to do.

Finally, I have a cute, trite little phrase down here: molecules are not professionally labeled. We do that, and I think, to take an extreme example, listening to the many papers from the group in Kentucky on metallothionein, and their work on inhibition and silver pathways and so on, made me wonder how these things might be interpreted in terms of molecular interactions in sediments. I think we can do a lot more of this type of thing in terms of integrating what's going on. It all should make sense, from a basic molecular chemical point of view.

KOSTAS DASKALAKIS: My interest is in marine estuarine chemistry, but by association with the program where I work, I have to get involved with biology and toxicology. That messes up everything, actually. It's difficult enough to do environmental geochemistry, because of all the complexity that is out there, but this really is not enough when one talks about sediments. For sediments we have to answer specific scientific questions, and we also

have to regulate sediments. We have EPA and a few other organizations to do that. These two things don't necessarily go together. They are not in phase. Scientists want to do one thing, and can do one thing, and regulators want to do something else.

The questions that I have about sediments are basically questions that most of us have here. How can we define a contaminated sediment? How can we tell that it's contaminated? If it is contaminated, is this contaminant something that is bioavailable? And if it is bioavailable and it accumulates, then does that meant that it's toxic; does it cause any effects to the animals? For silver, we know that it bioaccumulates to a great extent in some organisms, even at the very low concentrations that are out there. What we don't know is if it causes any toxicity. I would agree with a lot of you that we don't have a lot of knowledge or a lot of indications that it causes any toxicity at all.

The association between sediments and tissue concentrations is something else that took a lot of my time recently. We have a lot of data for sediments and tissue, but we cannot associate the silver in the sediments with silver in tissue. There is variable association in mussels, but for oysters, for example, there is none. We did some similar work for cadmium, and we realized eventually that all that we could find was an association between cadmium in the tissue and freshwater input. That was for the Northeast and the Gulf of Mexico. We couldn't find any association between sediments and tissue concentrations for cadmium, and we believe that it's similar for silver. Yes, sediments are very important, and criteria should be developed, but criteria that give us total concentrations don't tell us very much. They don't tell us if this contaminant, by accumulating, creates any toxicity. I will leave it at that.

DAN CALL: I'm a biologist and an environmental toxicologist, and my experience with silver is more limited than my colleagues on the panel. I'm honored to be here with some of the pioneers in this area. We have done some work over the years looking at toxicity of various silver species in the water column. We published a paper in 1982 that described the LC50 of silver to fathead minnows and midge, which I'm happy to say was recently corroborated at Clemson and Mississippi State. Recently, we did a study on zinc where we were evaluating the SEM to AVS model in a field situation by spiking zinc at a series of concentrations, some of which had molar ratios less than one, then at one, and above one, thinking that we would see some impact of zinc upon bioassays of sediments taken back into the laboratory as well as colonization of benthic macroinvertebrates. What we found was that the zinc sulfide that was formed, formed in relationship to the amount of zinc that we had spiked into the sediment, so that it was protective. We had very few molar ratios of zinc-SEM-to-AVS greater than one. There was very little impact as well on macroinvertebrate colonization over the course of a year.

I'm interested in the connection between laboratory bioassays and what's actually happening out in the field. I think there's some progress being made in this area, with some of the benthic macroinvertebrates at present, where some of the endpoints used in laboratory bioassays may actually eventually be translated into impacts that are observed in the field in terms of the population abundance of the given species.

DOMINIC DI TORO: The operative question in our mind with regard to silver was whether it follows the SEM-AVS rules that appear to apply to the other metals. By the way, you should keep your eyes open for the December issue of Environmental Toxicology and Chemistry, which will have 13 or 14 papers, including the paper Dan Call just mentioned about the zinc colonization experiment. It is a collection of all of the data that the EPA laboratories and collaborating institutions have generated for metals over the years. Taken as a whole, I think that database allows the following statement to be made, at least for those metals: if you have a molar ratio less than one, you will not observe any acute or chronic effects for metals in sediments. We have life-cycle lab tests, and colonization experiments for both cadmium and zinc, three for cadmium that are field level tests. I don't know of any other criteria or proposed criteria for which there is that kind of backup, both in the laboratory and in the field. So from the point of view of trying to make a prediction about the lack of toxicity, I think that model seems to have stood up well over the last five or six years that it has been worked on by us and lots of other people.

The first question that comes to mind is whether that applies to silver or not. The Narragansett people have run toxicological tests on marine sediments. There are some difficulties in dealing with silver in marine sediments; it takes a long time to hit equilibrium, because the silver actually forces displacement reactions with the other metal sulfides. It'll pop copper and zinc out of copper and zinc sulfides and replace them with silver, because the solubility is so low. So that reaction takes a long time to equilibrate. It's sort of annoying, but it happens. What they've seen is that at molar ratios of around a tenth or two tenths, anything right up to the boundary, it works exactly as anticipated. But something odd has happened in at least one experiment right near the boundary, where we saw toxic response at a molar ratio of less than two, which is where you continue to form silver sulfide. So you can't say absolutely, categorically that it applied in exactly the same way that everything else has worked. But certainly at molar ratios down around less than a half or so, we see no effects.

Since silver is more insoluble than all the other metal sulfides, it'll make first grabs on the sulfide that's around, so it's hard to imagine that you could get any toxicity out of sediments for which there is a molar ratio less than one. Why then, are there silver fluxes in the environment, and why are there body burdens? If you believe what I've just told you, silver should be essentially nondetectable everywhere. It should be held at the solubility of silver sulfide plus complexing the thiols and a few other things, so the concentration should be vanishingly small. And they mostly are, once the analytical chemistry is worked out. Why, then, is there silver in organisms? Why, when you go out in San Francisco Bay (Sam Luoma's done this for years) is there silver in mussels and in oysters that the National Status and Trends program has picked up? Why should that happen? It seems to contradict the notion that sulfides and organo-sulfides are THE complexing agents, and they should win, hands down.

My explanation of that is as follows. The first observation is that, if there's no correlation between the concentration of the metals in the sediments and the concentration of the metals in the organisms, that's interesting. That suggests that there's no causation, and since what NOAA and everybody else measures is total metals and not bioavailable metals, i.e., sulfides and that kind of thing, what occurs to me is that the supply is through the water column. What the organisms must be seeing is the same source that is contaminating the sediments. So what you're looking at is a covariation. You're seeing silver being advected out by the freshwater flow, which then bioaccumulates in the organisms, and, in fact, contaminates the sediments as well. So I don't think the fact that there is silver in organisms suggests that the sediments are the source. I think the likelier explanation is that the effluents are the source. On the other hand, we've also seen that silver sulfide can oxidize slowly, so it's still an open question as to whether or not that little bit of oxidation — the fluxes that Eric Crecelius and the Navy have measured — is large enough to be the supply, in which case there would be a relationship between the silver in the sediments and the silver in the organisms. So, in this long, roundabout little story, what remains to be determined — and this is what the EPA was told by the Science Advisory Board about metal criteria — is why there appear to be body burdens in sediments where the molar ratio is less than one in our experiments and in field collected samples. Why are we seeing that? We've seen it in some experiments, we've seen it in some field-collected sediments. Maybe the story has to do with the oxidation.

With regard to toxic levels, again we've never seen, with the exception of stuff really close to the boundary, either chronic or acute toxicity for the five other metals. I don't expect to see it with silver. Oh, the other thing I should point out to you: pay attention to the experiment that Dan Call just described to you when you read about it. What happened was, the experiment was initially set up with a very large zinc-to-AVS gradient. The notion was to go from a tenth up to 10, and, following good toxicological practice, kill something. Very sensible design. What happened? They went back in six months, and all the molar ratios had settled down to about one. What happened was not that the zinc went away, but that iron sulfide was made during the summer due to the reduction of sulfate, and so all the sulfide that was generated from sulfate reduction had complexed up all the zinc, and the experiment settled down to a molar ratio of about one, and just stayed put. This is another indication that it's tough to get toxicity of metals in sediments. You've got sulfides, once they're generated, preferentially precipitating any of the metals that are available. Very interesting experiment.

MARY REILEY: Thanks to each of you. We'll open the floor up to questions and commentary.

Q. ANDERS ANDREN (University of Wisconsin): When you were talking, Dominic, something struck me about why we see concentrations in animals. I wonder, based on the work by Flegal in San Francisco Bay, and their suggestion that there must be some kind of flux back into the water column, can one hypothesize a mechanism whereby colloidal material gets back into the water column – and it might be in sulfide form – and then gets taken up by oysters? What happens to metal sulfides as they are filtered by oysters?

A. DOMINIC DI TORO: I've thought the same thought; what happens to metals associated with the thiols and the sulfhydryl groups? Can the metal come off that group and find something in the oyster that's got a stronger binding constant than what carried it up in the first instance? I don't know. It must be; the observational fact is that these things accumulate silver and other things. One has to assume that metallothionein, or whatever the protein is, has a binding constant that's larger than the sulfur groups. I don't know if anybody knows if that's true or not. Is there enough information about the binding constants to know that metallothionein is stronger than . . . [Russell Bell answers in the affirmative]. It is?

Q. ANDERS ANDREN: Perhaps a comment from Eric on that. What about the colloids?

A. ERIC CRECELIUS: I don't have data on the colloids. Most of the work was done by filtering at 0.4 microns, which is just barely getting to the range of the colloids. I think that's a very good hypothesis, that colloidal silver is cycling the silver through the water column and through the estuary, and that the organisms are competing for the silver that's on the colloids, and it sounds like they have some biochemistry to out-compete and to accumulate that in their tissues.

A. DOMINIC DI TORO: In order to make that work, you need a continual generation mechanism for colloids coming out of sediments, and I'm not sure I can see how that happens.

A. KOSTAS DASKALAKIS: Maybe they're not coming out of sediments. In most cases, you have the generation of sulfides that would precipitate the metal that is already in the water column. Some years ago when I did cadmium sulfide experiments, cadmium sulfide precipitated out and then it was supersaturated with respect to cadmium sulfide by at least two or three orders of magnitude, and it remained like that for weeks. We had colloids that were precipitated out. We filtered the whole thing and redissolved it, and then the concentrations were much lower. That's how we knew that we most likely had colloids. We could see that the solution was milky for days. Now if this happens with silver, I wouldn't know, but we are talking about much lower concentrations, so the chances are very high that it is happening.

Q. PETER SANTSCHI (Texas A&M): Maybe I can add just a bit about the colloids and silver. We are just finishing up a Master's thesis where the student equilibrated colloids which we isolated from Galveston Bay with radioactive silver and other trace metals, and then fed it to shrimp. We looked at different body parts, and the colloidal silver, which was mostly macromolecular, and in which the organic matter had one to four percent sulfur (we don't know if it was reduced) got bioaccumulated, statistically not differently from the silver which we added in ionic form. But all the accumulation curves, and also the depuration curves, looked a lot nicer. It was accumulated and depurated in a more exponential fashion, so there were a lot less irregularities. When you add trace metals in ionic form, they are absorbed in different body parts, and so your data aren't as good as with silver, which is bound to colloids. The sediments certainly have something to do with those colloids' presence, but I think they are coming from the water column. This is organic matter that is produced as exudates, and eventually they end up in the sediments and go through guts and get chewed up and all of that. Sediments, through resuspension, etc., have something to do with it, but I think the net flux is from the water column to the sediments, and not the other way.

A. DOMINIC DI TORO: Peter, can you clarify a point? You said your experiments showed that you got the same uptake whether it was ionic silver or colloid-complexed silver. Was that the same quantitative result?

Q. PETER SANTSCHI: We looked at uptake in the pancreas, the abdomen and in the gills separately. We had three replicates, and we did it over two weeks of uptake and depuration. If you go through the statistics, there was, for the whole body burden, no clear difference; it was statistically the same. But for the individual body parts, there were differences.

A. DI TORO: So a part per billion of colloid-complexed silver bioaccumulates the same, on a whole-body basis, as a part per billion of inorganic silver.

Q. SANTSCHI: Yes. We did it at ambient levels, so between one and 10 nanograms per liter.

A. DI TORO: Which means, if you think about gill speciation models or tissue complexation models, there must be a silver-DOC complex that sticks onto the body. Otherwise, that result wouldn't happen.

Q. SANTSCHI: Yes, although they are also filter feeding, so it can also get into the gut. I don't know how it's getting into the organism . . .

A. DI TORO: But these things are small, right? Your colloids are . . .

Q. SANTSCHI: The colloids were between 1,000 molecular weight and 0.4 microns. So it was that organic material that can be isolated between those values.

A. DI TORO: It isn't going to get to the liver if it just sticks to the mucous. It's got to move around in its chemical form.

Q. SANTSCHI: Yes. It has to dissociate at some point, and get into the body. The largest fraction ended up in the hepatopancreas. The other parts were a lot lower. The partition coefficients, expressed in micrograms silver per gram tissue divided by micrograms per gram water were, by an order of magnitude or more, higher in the hepatopancreas.

A. JAMES KRAMER: I'll just add a small reminder. In the first Argentum conference, when Nick Fisher gave his review paper, a very intriguing reference in there referred to the finding of silver sulfide as acanthite in the gut of one of these filter feeders. I looked the reference up, and it's equivocal; it was confirmed by electron microscopy. But if you want to accept that, you would still stay with sort of sulfur chemistry, in a way.

Q. ANDERS ANDREN: In the panel's view, when we look at criteria for metals in sediments, will we have a tougher time meeting these criteria in marine sediments, estuarine sediments, or freshwater sediments?

A. DOMINIC DI TORO: Which criteria? Which metal is one problem. The second problem is, based on what?

Q. ANDERS ANDREN: Let's talk about silver, as sediment criteria presently are discussed at proposed levels within EPA. (Mary Reiley adds: Using AVS-in-porewater approaches?) Exactly.

A. DOMINIC DI TORO: AVS levels, surprisingly enough, in freshwater sediments are not dissimilar from AVS concentrations in saltwater sediments. When we first came across that, that was a real surprise, since the sulfate concentration in seawater is two to three orders of magnitude higher than median sulfate in all of the rivers in the United States, which is 50 mg/L. The sulfate in seawater is 2.8 grams per liter. But it turns out

that the formation of iron sulfide doesn't appear to be sulfate-limited. It's limited more by the rate at which organic matter is mineralized in sediments. So the short answer, at least from my view, is that if it is based on AVS, the AVS concentrations in freshwater systems are roughly similar to saltwater systems, and there shouldn't be any difference. One of the things you have to realize is that if you believe this SEM-AVS story, there will not be a metals criteria. The notion of a single-metals criteria is incorrect. You have to just add up all of the metals, and if the sum of all of the metals are less than the total AVS there, then you'd have no impact. But one could have the following situation; suppose you had silver and nickel. Nickel is the most soluble of the metal sulfides, and silver is the least, let's say. If you have a certain amount of silver and a certain amount of nickel, and the sum of those two things exceeds the criteria, you will get an acute effect. Is it the silver's fault, or is it the nickel's fault? Nickel, by the way, will be what's in the porewater, but clearly the silver also bound up some of the sulfide. So whose fault is it? The point is that the criteria has to consider all the metals, and if the sum of the metals, on an equivalent basis, is less than the AVS, then you're OK. The current proposal, which, by the way, is also in the December journal, is to add them all up. So then the question becomes, what about the background metals? What you see a lot of in sediments are things like zinc and copper, and they use up complexing capacity. The people that have a criteria for copper, on the face of it, have to be incorrect. You can't decide whether copper is having an impact in the sediment unless you know the state of all the other metals. All of the ERM's and ERL's and all of that stuff, are just chemically incorrect. I'm glad you gave me an opportunity to make a categorical statement like that.

A. KOSTAS DASKALAKIS: If I may add; I agree with you that they are incorrect, but I would never believe that somebody would accept a criteria similar to AVS until we had toxicity when the ratio is less than one.

A. DOMINIC DI TORO: No toxicity when the ratio is less than one.

A. DASKALAKIS: OK, then what did we learn out of all these exercises? We learned that when it's anoxic, we have no toxicity. In most cases, I don't worry about that, because there is no oxygen, so animals will be dead anyway.

A. DI TORO: No, no. The field experiments . . .

A. DASKALAKIS: It's too simplistic, but they breathe oxygen, and we get . . .

A. DI TORO: I've tried to explain this to you and Tom O'Connor now for five years. If you look at the colonization experiments . . .

A. DASKALAKIS: It's probably the thermodynamics that mess me up. Looking at the graph, you always want to talk about the four quadrants, and you don't really talk about the two on the right side, because oxic sediments are not toxic necessarily. But on the left side, the sediment is never toxic, because it's got sulfide.

A. DI TORO: The animals are alive on all of the experiments on the left-hand side of the graph. (Daskalakis replies: "but they don't breathe the water.") Oh really? What are they breathing? (Daskalakis replies: "they are breathing the water with the oxygen.") But the point is that you don't get any toxicity from the metals associated with the sediments. That's the experimental finding.

A. DASKALAKIS: Because most likely they are not exposed to the . . .

A. DI TORO: Exactly! And why not? Because the sulfide has complexed it all up as metal sulfide. Therefore, all criteria that are based only on a single metal, that aren't properly normalized, on the face of it, can't be correct.

A. DASKALAKIS: If you have oxic sediments, you're going to have to talk about single metals.

A. DI TORO: Sediments are neither oxic nor anoxic. There's a layer of oxic on top, and then the burrows of the animals that live on top are clearly in an oxic zone . . . The way you phrased the comment suggested there aren't any organisms alive on the left-hand side of the plot.

A. DASKALAKIS: Their body may be in there, but they really don't use that water for anything else.

A. DI TORO: Well, they survive for 28-day chronic experiments. (Daskalakis replies: "They're breathing water that is outside; they've got to get oxygen.") But that's the point of it. Therefore sediments containing those metals at those concentrations don't exert toxic impacts, period.

Q. ANDERS ANDREN: Can I make a comment on that? I think the notion that no animals live in anoxic sediment is wrong. In Green Bay, which is extremely anoxic, we find worms down to 10 centimeters.

A. DASKALAKIS: Yes, but what they usually do is get oxygen from outside . . .

Q. ANDREN: Yes, but they have an opportunity to take up metals as they go down and "look for goodies" and come back up again.

A. DASKALAKIS: But, again, the concentrations there are so low that what you have to worry about mostly is the oxic sediments, not the anoxic sediments.

Q. NICHOLAS ADAMS (McMaster University): Could I just rephrase this discussion and ask what you would do under conditions of seasonal variability with AVS-type regulations?

A. DOMINIC DI TORO: It's a good question, and we've struggled with it, because it's well-known that AVS concentration fluctuates quite a lot. Measure the ratios at the time you suspect the ratios will be the lowest, which is around wintertime, we think, from northern Minnesota lakes. The stuff that the people at Duluth have sampled have seen the AVS drop to very low levels in the winter. So the answer to the question is, measure it when it's low. Or try and predict it, but certainly better than that, measure it. But the zinc experiment is very interesting in that regard. What happens is that the iron sulfide oxidizes away, and then the thing just sits there at about a molar ratio of one, because the other metal sulfides oxidize very slowly. So the experiment just poises itself at one. Now what? The short answer is, from a regulatory point of view, measure at the critical time, just like we do critical evaluations for anything else.

A. JAMES KRAMER: I think, speaking only about silver and picking up on the point that Dominic mentioned about silver replacing other sulfides, the danger I see is we don't really know what is in AVS. I mean we do in a simple, synthetic system, but, for example, seasonally we don't. We don't know whether that statement is true; the things that you measure in AVS make you think it would be, but we don't know that, for example, silver can kick off another metal which is on some fraction of AVS. We don't really have a good idea what the makeup of AVS is, and we certainly don't have an idea what the temporal changes are. Polysulfides are going to be in there; this may be one of the reasons, by the way, that you get a sub-one-to-one toxic effect. I think before we can make these statements with respect to silver – silver is a special case, because there's not much of it there – we have to know more about AVS. That's my feeling in terms of all of these things that we propose will happen.

MARY REILEY: Questions? Comments?

A. DOMINIC DI TORO: We have seen some odd behavior at very low AVS concentrations. Tenths of micromoles per gram kinds of numbers appear to be a different thing than one to 10 micromoles per gram that we usually work at. So I would echo Jim Kramer's comment. There seems to be another polymorph that oxidizes much more slowly than marcasite or whatever it is we're seeing. The reason this came up is, when we tried to analyze the Canadian data set that Landers Hare produced, oxidation rates of that stuff have to be an order of magnitude slower than the oxidation rate of the normal marcasite. You're right, Jim, there is something; I don't know what it is, but there is something in AVS that is not just run-of-the-mill amorphous FeS or marcasite.

A. JAMES KRAMER: If I could just make a quick comment to state the obvious. If we can say that any amount of AVS that we would measure is available, or reactive, with silver, then as long as you can measure AVS, it's going to cover silver. And then you can indeed say that silver is going to be with the sulfur in all cases.

MARY REILEY: The last comment that I wanted to make is that I've listened to what's going on for the three days this year, and the four days that I participated last year, and I guess I'd like to say that I've enjoyed the presentations that have been made. There's a lot of good work, a lot of good research that's going on, and I see bits and pieces of different questions being answered. I guess the challenge that I pose to this group and the organizing group is to try to start to pull this stuff together so it tells a nice story. The nice story is what the regulating community is looking for in order to answer their questions of how much is too much, in what form and how do I predict toxicity, so I can make sure that we have fishable, swimmable, and edible environments for our future?

Closing Remarks

Transport, Fate and Effects of Silver in the Environment

A.W. Andren/T.W. Bober
Conference Co-Chairs

Closing Remarks: Anders Andren and Thomas Bober

ANDERS ANDREN:

Thank you, panel members. Just a few comments before we conclude. I'd first like to thank all participants for their input. I'd like to thank the conference co-sponsors, and the scientific organizers for all of your help.

I am encouraged by the progress that we are making in pushing ahead the frontiers of our understanding of how silver behaves in the environment. I think that we are slowly catching up with other trace metals. At the same time, I also hope that some of the work that has been done on silver helps our understanding of the other trace metals, as well.

The panel members summarized some of their observations, and let me add just a couple more. While all of us have agreed now for some time that toxicologists, chemists, biologists, and biogeochemists really should work together more effectively, I still think we have a ways to go. It's my observation that all of us should do a better job of working in a cross-disciplinary fashion. When biologists design experiments on uptake, let's use chemists, and when chemists design their experiments on speciation, please talk with biologists as well as toxicologists to see what they are looking for. Related to that, just a simple example: Russell Bell made a plea for using molar units. Let's do that. It's so much easier to see relationships on equivalent or molar basis rather than using milligram or microgram or whatnot in terms of units. We can put them in parentheses if we want to, but let's all start moving in that direction.

The other comment I have is that I think our two plenary speakers made some really excellent points. Let's get more mechanistic in our approach to studying and understanding the transport and fate effects of silver. Let's get together and get better hypotheses and better conceptual models. I think that's really important. I just went to a mercury conference, and there is just too much of, "Here are the data. Here it goes up, here the levels go down. Make what you want of it."

I think that this is an opportune time to go to EPA's extramural grants division and ask them to write a fairly specific RFP that deals with the metal issues that would be of help to Mary Reiley, in terms of how she deals with metals regulation. Anybody who is interested in that, I think we should suggest this to EPA, and I know personally that they might be quite interested. The other recommendation I have is that as many of the participants as possible should please respond to Steve Klaine's challenge of writing an article. He has worked up an outline of what aspects of the silver chemistry he would like to see. We are going to send this to all of the participants, as well as others, so you'll know the categories we have proposed. I would urge you to submit papers, so more information gets out on the transport and effect of silver in the environment.

Finally, I'd like to explicitly acknowledge the contribution of those people that helped us put this conference together. Tom Bober has been very helpful, even though he is in Rochester, New York. He comes here a lot to help us out. He's an excellent detail man, and gets things done. In addition to that, I'd like to acknowledge Delphine Skinner, who many of you have probably met, and who helps out a lot. Tina Yao is the one who has made the design on our publications. She is an award-winning designer, working in the Sea Grant Institute. Gloria Gardner has done a lot of the retyping of the abstracts and put them into a nice format. Finally, Russell Herrin has assisted during the conference and will be providing the transcription services for the Proceedings. And let us also not forget the excellent services provided by the Wisconsin Center.

Thank you all for coming, and I'll ask Tom Bober to make one more set of comments about our plans for the silver conference.

THOMAS BOBER:

Initially, we had said that this was probably going to be the last annual conference and we were going to go to a biennial format. But since that time, the silver problem has been growing in importance in Europe, and we've decided that next year we would like to hold this conference outside the U.S. The photographic companies that are responsible for sponsoring this have tentatively agreed to that, provided we don't let the costs go out of hand. We're still trying to choose a location. At the moment, Krakow, Poland is probably the front-runner, and we have a few other suggestions. The lodging costs are a big factor; also, we need a place that has transportation accessibility, and does have conference facilities, but there will be a limit on the amount of funds we can make available for this. There will probably be a limited number of people coming from the United States and Canada who will be subsidized by the conference. It's probably going to be on the order of 20 people. In anticipation of that, we'd like people who propose to go to submit abstracts of what they would be presenting no later than February 1 of next year, because the conference committee is going to have to go through these abstracts and decide which are the most relevant, and which will pertain most to the problems that we're trying to resolve, particularly in Europe. If other people want to subsidize themselves, we'll be glad to entertain that; as a matter of fact, we could probably guarantee you a slot.

In a related way, if you have European colleagues who would propose doing some original research, we would welcome as many European papers as possible to really bring this home to Europe. If we could get half the papers there, that would be great for the conference. We will probably add another session to the conference next year. It might be half a day or a day long, depending on what kind of a response we get, but we've proposed adding risk assessment and life cycle analysis to the conference next year, since those are two issues that are of prime importance in Europe, and are perhaps a little more advanced there than they are here in the United States. If you have papers in those areas, they would be especially well-received, I think. We are also looking for recommendations from anybody here. If you know of a place in Europe that meets our criteria, and that is inexpensive, we're still open to suggestions. We'll have to make a decision pretty soon, though, because usually you have to make these reservations as much as a year in advance. The date we're talking about is late September 1997. Thank you.

[JAMES KRAMER asks for, and receives, a round of applause for Anders Andren and Tom Bober.]

Poster Session

Transport, Fate and Effects of Silver in the Environment

Madison, Wisconsin

Effects of Temperature and Thiosulphate on Uptake of Silver by Rainbow Trout (*Oncorhynchus mykiss*)

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We are using silver as a 'model' metal to better understand the effects of temperature and complexing agents such as thiosulphate on metal uptake by fish. Our working theory of how metals interact at the gills are as free metal ions. Two situations can occur in the water which can prevent metals from interacting and binding to the gills of fish. The first situation is the presence of competing agents in the water, such as positively charged ions (Na^+ and Ca^{++}), that can compete with free metal ions for gill binding sites (Evans, 1987; Janes and Playle, 1995). The second scenario is complexation, where negatively charged solutes (e.g. thiosulphate, dissolved organic carbon) in the water bind with free metal ions to form complexes that are unable to interact at the gills (Evans, 1987; Janes and Playle, 1995).

Theoretically, active metal uptake processes at fish gills are temperature dependant since fish metabolic rate changes directly as temperature is increased or decreased. In addition, causing an increase in fish metabolic rate results in an increase in oxygen consumption by the fish, making the fish ventilate more water to compensate for the increased oxygen demand. Greater ventilation means more water with silver passes over the gills, which could also increase silver uptake at the gills (Roch and Maly, 1979). Previous experiments of ours did not yield a difference in plasma silver concentrations in groups of fish held at 12°C and 21°C (Janes *et al.*, 1995). By decreasing the temperature to 5°C and maintaining a higher temperature of 18°C we hoped to demonstrate a metabolic rate dependant accumulation of silver in fish blood.

Adult rainbow trout (*Oncorhynchus mykiss*, ~200 g) were cannulated, using 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 per min of synthetic soft water during treatment exposures. Arterial and water PO_2 and PCO_2 were measured using a Cameron blood gas meter. Dilute (10x in E-pure water) plasma Ag concentrations were measured using graphite furnace AAS. Gill lamellae samples were removed at the completion of an experiment (~96 h) and digested in 5x their 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. Flame AAS was used to measure water and plasma Na and Ca, while plasma Cl, plasma glucose, blood lactate, and blood haemoglobin were assayed using Sigma reagents. Ventilation rates were measured visually over 30 seconds.

In the first set of experiments, trout were exposed to 0.11 μM Ag (as $AgNO_3$), or 0.11 μM Ag plus a nominal concentration of 5.0 μM $S_2O_3^{2-}$ (as $Na_2S_2O_3 \cdot 5H_2O$), or 5.0 μM $S_2O_3^{2-}$ alone. Trout used in the second set of experiments were all exposed to 0.1-0.2 μM Ag. Half of these fish were held at 5°C with the remaining fish held at 18°C.

In the Ag plus thiosulphate experiments fish were exposed to Ag only, thiosulphate only, or Ag plus thiosulphate. Fish were held at ~11°C with water pH ~6.8. Water PO_2 was ~120 torr and water PCO_2 was between 1.0 and 1.4 torr. Water Na was about 700 μM, water Cl averaged 250 μM, Ca was ~50 μM, and NH_3 averaged 35 μM. Breathing rates were around 75-80 breaths/min for the Ag only group, 60-75 breaths/min for the thiosulphate only group, and 70-75 for the Ag plus thiosulphate treatment.

In control fish in our experiments we normally observe increasing arterial PO_2 values as the experiment progresses, a partial function of repetitive blood sampling. Thus, steady or declining O_2 tensions indicate respiratory problems in the fish. Fish exposed to Ag alone and thiosulphate alone had respiratory problems compared to the Ag plus thiosulphate treatment which had significantly higher arterial PO_2 values.

Arterial PCO_2 was 2.0 torr for the Ag exposure, 2.4 torr for the thiosulphate group, and 1.8 torr for the Ag plus thiosulphate treatment. There was a slight increase in arterial PCO_2 in the Ag only group and a slight decrease in arterial PCO_2 for the Ag plus thiosulphate exposure. The fish exposed to Ag alone and thiosulphate alone appeared to be having gas transfer problems and were probably respiring anaerobically. Higher coughing rates were observed in the Ag only fish compared to the Ag plus thiosulphate group and the thiosulphate only group. This was most likely due to fish attempting to slough off mucus on the gills that had built up in response to the Ag exposure.

Blood lactate increased steadily over the course of the experiment for the Ag only treatment, while the blood lactate levels for the Ag plus thiosulphate group remained constant. This result agrees with the PCO_2 results since the Ag only fish were respiring anaerobically as blood lactate levels increased. Fish exposed to thiosulphate alone had the highest blood lactate concentrations, which again indicates anaerobic respiration.

Plasma Na levels were constant throughout the experiment for the Ag plus thiosulphate group. The Ag only group showed significant decreases in plasma Na compared to the Ag plus thiosulphate group by the second sampling time, and showed a drastic drop in plasma Na by the sixth sampling time. These results support previous work that Ag interferes with Na balance in fish (Janes and Playle, 1995; Hogstrand *et al.*, 1996). Fish exposed to thiosulphate alone showed even greater losses of plasma Na.

Fish exposed to Ag alone had significantly more Ag in the plasma, through active uptake, compared to a control treatment having no added Ag. There was also significantly more Ag in the plasma of the Ag only group compared to the Ag plus thiosulphate group. Fish exposed to Ag plus thiosulphate showed increases in plasma Ag by the first sampling time, but plasma Ag concentrations were one-half to one-third those in the Ag fish. Fish exposed to thiosulphate alone had no Ag (above background) in the plasma.

Gill Ag concentrations for both the Ag only and the Ag plus thiosulphate groups were significantly higher than the control treatment with no added Ag. However, gill Ag for the Ag alone group was significantly higher than the gill Ag of the Ag plus thiosulphate treatment. Thiosulphate was fairly effective in keeping Ag off the gills, concurring with Janes and Playle (1995).

In our temperature experiments half the fish were exposed to Ag at 5°C and half were exposed to Ag at 18°C. Water pH was ~7.5. Water PO_2 was initially ~125 torr and increased to ~150 torr in the 5°C treatment and decreased to 80-120 torr in the 18°C treatment. Water PCO_2 was ~1 torr initially and remained fairly constant throughout the experiments.

The cold group of fish exposed to Ag showed a gradual increase in arterial PO_2, while the warm group of fish exposed to Ag had a decrease in arterial PO_2 that was significantly lower than the cold group at 23 h and 72 h. The decrease in arterial PO_2 in the warm group was due to an increase in metabolic rate, therefore an increase in O_2 demand, coupled with a decrease in water PO_2. It was also likely that this respiratory problem was further induced by an increase in mucus on the gills, due to exposure to the metal toxicant, which interfered with O_2 transfer. The warm group of fish also had a slightly higher rate of coughing compared to the cold group of fish, probably due to an increase in mucus production at the gills.

With an increase in temperature and a decrease in water PO_2 that was observed in the warm group we expected to see an increase in breathing rate. The warm group of fish experienced a significant increase in breath rate over the initial value and was significantly higher compared to the cold group. The cold group breath rate decreased significantly compared to the initial rate due to a decrease in metabolic rate which decreased fish O_2 demand, combined with the increase in water PO_2.

Another problem fish face when exposed to a metal toxicant is an ionoregulatory problem. Silver is known to interfere with Na uptake (Janes and Playle, 1995; Hogstrand *et al.*, 1996) and this was seen to some extent in this experiment. Often when there is an interference with plasma Na, there is also an effect on plasma Cl. While both groups had similar initial plasma Cl concentrations, the warm group had a more significant decrease in plasma Cl compared to the initial concentration (e.g. by 30 h). The cold group also showed a decrease in plasma Cl, but this decrease was only significant at 92 h.

If metal uptake was a passive process, we would only see a plasma Ag concentration that was roughly equal to the concentration of Ag in the water (~0.1-0.2 μM). Our plasma Ag values are at least 5 times greater than the water concentration, therefore Ag was actively taken up by the fish. The warm group of fish had the greatest increase in plasma Ag, both significantly higher than the initial value and the cold group of fish exposed to Ag. This was likely due to the warm group of fish ventilating more water with Ag over the gills, increasing the 'dose' of Ag seen by the fish, and by an increased metabolic rate increasing active Ag uptake. The cold group also had a significant increase in plasma Ag, but only to one-half to one-third the extent of the warm group.

Silver concentrations on gills of fish from three different temperature experiments were determined. Gill Ag was significantly greater than the control for all groups of fish exposed to Ag (21°C Ag group consists of just 1 fish). Silver accumulation by the gills in the 12°C and 18°C groups of fish were both significantly higher than the 5°C group. These results are consistent with the theory that the warmer groups of fish, having an increased ventilation rate, would pass more water with Ag across the gills allowing for increased Ag binding to fish gills. In addition, active uptake processes may have been proceeding at a higher rate in the warm fish compared to the cold (5°C) fish.

Our thiosulphate results indicate that Ag from the Ag plus thiosulphate exposure did not accumulate in the plasma to the same extent as exposures to Ag alone. The same was also true for Ag accumulation at the gills. Therefore, thiosulphate does provide a protective effect to fish against Ag toxicity as the fish in the Ag plus thiosulphate group did not experience the respiratory or ionoregulatory problems that were evident in the Ag only exposure. It should also be noted that thiosulphate alone was toxic to the fish.

Our temperature experiments indicate that Ag accumulation in the plasma is an active process which shows temperature dependence from 5°C to 12°C. Warm fish exposed to Ag showed more respiratory and ionoregulatory problems than cold fish exposed to Ag. In future temperature experiments we intend to differentiate changes in active and passive metal uptake by isolating metabolic changes from ventilation changes. This will be done by increasing the O_2 content of the water as temperature is increased, allowing the fish to ventilate the same volume of water to accommodate its increased O_2 demand. In this case a persistently increased accumulation of Ag in the plasma of warm fish - if it occurs - would indicate that elevated active uptake of Ag due to increased fish metabolism is responsible for higher Ag accumulation in warm fish.

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X-Ray Absorption Spectroscopy Study of Silver Speciation and Distribution in Natural Aquatic Environments

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Introduction

The goal of this proposed research is to demonstrate how x-ray absorption spectroscopy (XAS) can be used to examine the physical, chemical, and biological processes that determine the distribution and fate of silver in the environment. Our poster presentation begins with a brief review of the fundamentals of XAS and the equipment used in the analysis. We conclude with a discussion of applications of XAS to environmental science and some examples related to silver.

Synchrotron Radiation and the Advanced Photon Source

Physicists began building accelerators during the 1940s to help answer questions about our physical world and the structure of subatomic particles. In the course of these experiments, scientists observed that when relativistic ($E>>m_0c^2$) charged particles moved through a magnetic field, high energy radiation was emitted. These emissions became known as synchrotron radiation.

Synchrotron radiation has grown into a valuable scientific tool; it provides a highly penetrating, broad spectrum, collimated, polarized x-ray beam that is ideally suited to a broad range of applications. For example, the development of novel catalysts, ceramics, and semiconductor materials profits from these types of studies, and the molecular structures of complex organic molecules such as metal containing proteins are becoming clearer because of x-ray work.

The Advanced Photon Source (APS) at Argonne National Laboratory, parts of which began operating in the summer of 1996, is the most intense source of x-rays for scientific research in the United States. The process of creating these x-rays begins with electron production in an electron gun. Electromagnets guide the electrons through a linear accelerator where the energy level of the electrons is increased (to 200 MeV at the APS). Leaving the accelerator, the electrons collide with a tungsten positron (anti-electron) conversion target, and electron-positron pairs are created. An electromagnetic field pulls the positrons into a positron linear accelerator, where their energy level is further increased (to 450 MeV at the APS). Bunches of these positrons collect in an accumulator ring, and then in the booster/injector synchrotron. In the booster/injector the energy level is increased one more time (to 7 GeV at the APS), and the positrons are now traveling exceedingly close to the speed of light. Finally, positrons are injected into the storage ring, where magnetic fields from bend magnets keep them circulating for hours. The energy lost from the positron beam due to synchrotron radiation is replenished by radio frequency (RF) cavities. Gradually, over many hours, positrons are lost from the beam due to collisions with residual gas atoms in the ultra high vacuum in the ring and interactions between particles in the beam, and new positrons are supplied from the injector. Typical beam currents are in the range of 100 mA.

Experiments are conducted on beamlines that extend tangentially from the storage ring. Instrumentation in the beamline is used to select particular energies (wavelengths) of x-ray photons, and to focus and filter the x-rays onto samples. Beamlines are also equipped with detectors and ancillary instrumentation. All of this equipment is kept in safety interlocked, lead shielded enclosures, and operated under computer control.

At the APS, scientists can access two different types of beamlines. All synchrotron sources use bend magnets to guide the positron beam, and broad spectrum bend magnet radiation is inevitably produced. Bend magnet lines use this radiation. The other type of beamline uses "insertion devices", which, rather than simply bending the path of the positron (or electron) beam, cause the path to wiggle back and forth. The positron (or electron) radiates strongly at each bend, producing more, and usually higher energy, x-rays than the bend magnets alone. Insertion devices allow the experimenter to manipulate and change the character of the positron beam trajectory, and the spectrum and radiation pattern of the x-rays. One type of insertion device is a wiggler magnet, which can produce intense radiation over a wide range of energies, in an angular fan of several milliradians width in the horizontal direction, and high collimation (~100 mradians) in the vertical direction. Another type insertion device is an undulator magnet, which produces light collimated (~100 mradians) in both horizontal and vertical directions, at a user-selected energy (and harmonics). Generally wigglers and undulators produce plane-polarized x-ray beams (which can be used to advantage), but special undulators can produce left and right circularly polarized x-rays, which are useful for studying magnetic materials. These specialized beams will significantly expand the types of experiments that can be conducted.

X-Ray Absorption Spectroscopy

X-ray absorption spectroscopy (XAS) is one of a few techniques that can provide *in situ* structural information about an element and its surroundings. This structural information is obtained from the analysis of modulations in the absorption of x-rays as a function of energy around x-ray absorption edges. Signals from different atomic species can be selectively excited by tuning the x-ray energy to an absorption edge of the desired element The signal starts at the absorption edges (K, L, ...edges) and extends out to energies as high as about 1 keV above the absorption edge. The two types of XAS are EXAFS (extended x-ray absorption fine structure) and XANES (x-ray absorption near edge structure). They are nowadays referred to jointly as "XAFS" – x-ray absorption fine structure.

EXAFS: Extended X-ray Absorption Fine Structure

The EXAFS regime, which is related to the wave properties of the electron, exists over a range from approximately 40 eV to 1000 eV photoelectron kinetic energies above the absorption edge. Electrons excited by photoionization interact with neighboring atoms and these neighboring atoms become secondary sources of scattering for the photoelectrons. When adjacent scattered electron waves interfere with each other, this interference influences the probability of absorption of an incident x-ray photon in an energy dependent way. Therefore, EXAFS information comes from local electron interference where the source and "detector" of the electron are the target atom. EXAFS is equally applicable to amorphous and crystalline materials. A radial structure function, centered at the x-ray absorber and usually uncorrected for phase shift, can be generated from a Fourier transform of the EXAFS spectrum from wavevector (k) space to real (r) space. Detailed analysis of the data is performed by k-space or r-space fitting or more sophisticated methods. Information typically provided by EXAFS includes average coordination number, types of neighboring atoms, bond lengths, and mean square variance in bond length.

XANES: X-ray Absorption Near Edge Structure

In XANES analysis, the x-ray absorber ejects an electron from a core level toward bound or partially delocalized empty states. These can be described in the same theoretical framework as EXAFS (multiple scattering theory), but, depending on the physical characteristics of the system, it is also sometimes useful to approach XANES from the point of view of molecular orbital and band structure theory.

Interpretation of the XANES region is more complex and less quantitatively accurate at present than is EXAFS. Nevertheless the XANES provides useful qualitative and semi-quantitative information complementary to EXAFS. For example, pre-edge transitions to 3d-derived molecular orbitals are often useful indicators of site symmetry in transition metal complexes, and characteristic multiple scattering effects in complex anions, such as MnO_4, VO_4, and CrO_4^{2-} are well understood (Bunker, 1984). Shifts in absorption edge energy provide information about the oxidation state. L2 and L3 edges probe final states of d-symmetry, which, in transition metals (e.g. Ag) participate in chemical binding. The "white lines" in the L3 edges are diagnostic of d-orbital vacancies, and are clear indications of relative oxidation state. Although not as widely used for analysis of environmental samples, relative to EXAFS, XANES is able to detect lower concentrations. For example, Pickering et al. (1995) suggest 10 ppm as a practical limit for soils analysis *with currently available equipment.* With improved detectors and sources this limit will be pushed substantially lower. In addition, time-resolved and spatially-resolved studies, which are presently "heroic" experiments, will become routine on appropriate beamlines at the APS.

XAS in Environmental Analysis

The significant advantage of XAS is that *in situ* spectroscopic studies can now be conducted at the molecular level and directly correlated with macroscopic measurements. These studies provide complementary structural and compositional information that constrain interpretations from macroscopic measurements.

Applications of the APS to environmental research were discussed in a workshop held at Argonne National Laboratory in 1990 (**ANL, 1990**). Information such as the cation environment and oxidation state in crystalline and poorly crystallized solids, and the coordination environment of sorbed surface species can be obtained. Heretofore unavailable information on process dynamics can also be obtained because complete x-ray patterns will be available within seconds. Detection limits for trace elements in soils and sediments will be pushed to lower levels because extremely bright x-rays can be used; the small beam sizes permit the use of energy resolving detectors based on multilayer optics (Zhang et al.). Finally, because environmental scientists are relatively new users of synchrotron radiation, numerous innovative and novel applications are to be expected.

Applications to Silver Speciation in the Environment

XAS can also be a valuable tool to confirm and expand on previous studies on the fate and transport of silver in aquatic environments. For example, Cutter and Radford-Knoery (1994) and Di Toro and Mahony (1994) believe that sulfide complexes can play an important role in silver distribution. Sedlak and Andren (1994) and Ilton and Veblen (1994) described how Ag^0 species could be stabilized at solid surfaces in surface waters. In their review of silver geochemistry, Kramer et al. (1994) noted that little adsorption data exist for Ag partitioning onto natural substrates. Galvez and Wood (1994) examined the roles of calcium and chloride in silver toxicity and concluded that suggestions of a "hardness correction factor" may be unfounded. Although it is clear that silver toxicity, bioavailablilty, solubility, and sorption to mineral

surfaces depend on its speciation, much of the description of that speciation remains to be quantified. XAS could be used as a complementary tool in all of these studies to help resolve remaining questions.

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Metal Assimilation in Zebra Mussels

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The zebra mussel, Dreissena polymorpha, is an efficient filter-feeder that is rapidly becoming ubiquitous in North American freshwaters. To examine the potential of using zebra mussels as bioindicators of freshwater metal contamination and to quantify the roles these bivalves can play in mediating metal cycling, we have conducted microcosm experiments to study the bioaccumulation and release of Ag, Cd, Se and Cr. Radiotracers were used to measure uptake and release rates of these elements from particulate sources typical of the freshwater portion of the Hudson River, such as diatoms, chlorophytes, cyanophytes, bacteria and suspended sediment, and from the dissolved phase. Release rates of metals from fecal pellets were measured to determine rates of metal cycling through biodeposits. Results indicate that zebra mussels have a two-phased digestion, with a gut passage time < 1.5 h and the appearance of glandular feces between 12 and 70 h. The food source does have a major influence on assimilation; assimilation efficiencies ranged from a low of 2-4% for Cr and Ag, to 72% for Cd (Table 1); biological half-lives in D. polymorpha ranged from 2-3 d for Cr and Ag, to 157 d for Cd, as determined by the slopes of depuration curves after 70 h (see Fig. 1). Leaching rates out of fecal pellets yielded mean "half-lives" of 36 d, 16 d and 10 d for Ag, Se and Cd, respectively. Absorption efficiencies (%) for dissolved Ag and other trace elements were 1.87, 1.02, 0.46, 0.27 and 0.03 for Ag, Cd, Cr(III), Cr(VI) and Se, respectively. Uptake and loss parameters from particles and the dissolved phase will be used in a quantitative model to predict metal concentrations in zebra mussel tissues and quantify the relative importance of different uptake pathways. Zebra mussels can be valuable bioindicators of freshwater metal contamination.

Figure 1. Ag Retention in the Zebra Mussel: 6 Food Types

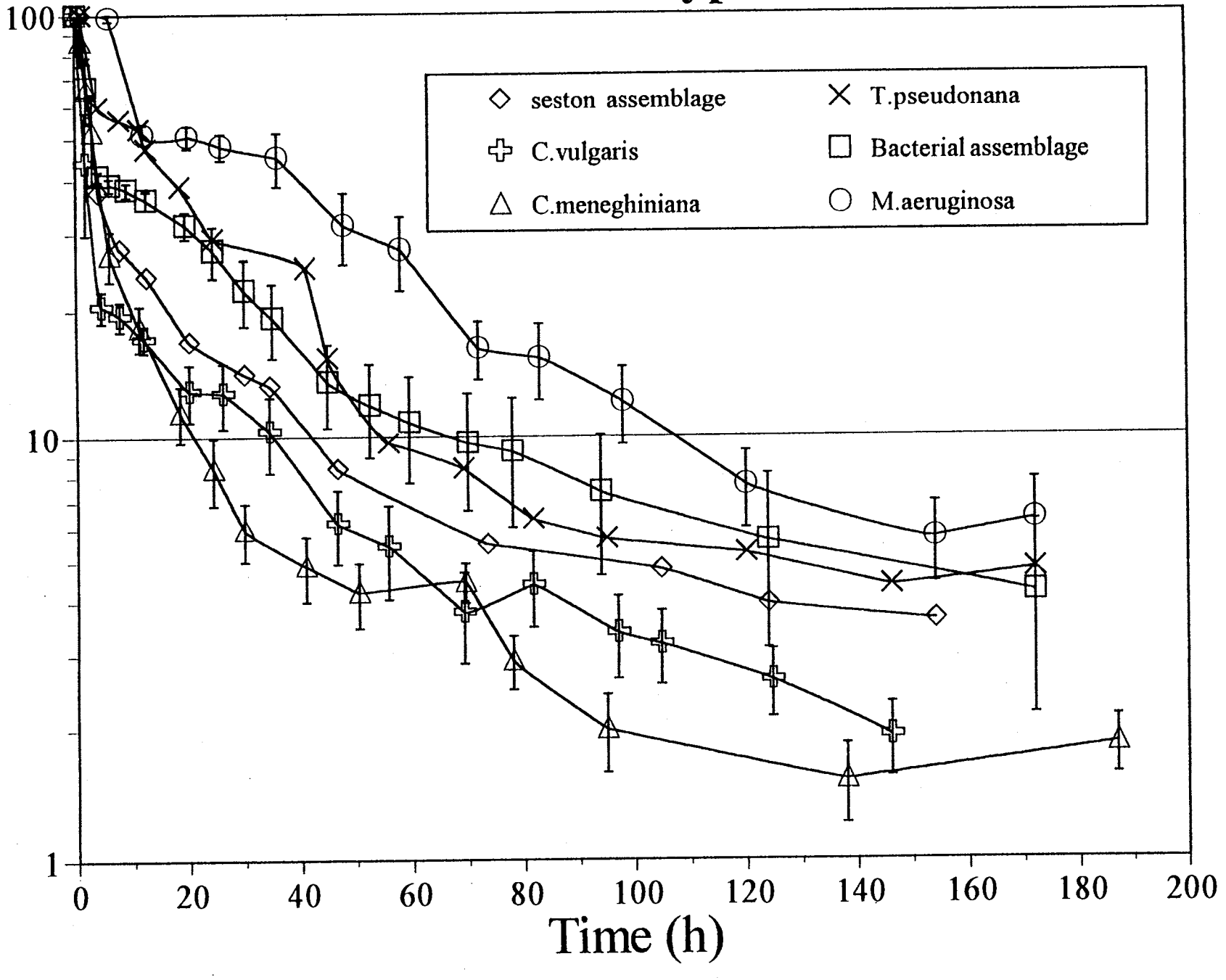

Table 1: Metal Assimilation Efficiencies (%) in the Zebra Mussel, 6 food types (mean ± SE).

Element	Seston assemblage	C. vulgaris (chlorophyte)	C. meneghiniana (large diatom)	T.pseudonana (small diatom)	Bacterial assemblage	M. aeruginosa (cyanophyte)
Ag	6±0.9	4±1	4±0.5	6±1	10±3	16±2.6
Cr	2±0.5	6±1	5±1	5±1	13±1	5±1.3
Cd	19±1	22±2	26±2	48±4	56±0.4	72±3.5
Se	8±0.4	18±1	24±3	45±2	41±1	40±3.6

The Effects of Silver on Green Algae and Prospects for Trophic Transfer

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Several investigations have documented the transport, fate, and effects of silver in freshwater fish, macroinvertebrates, and sediments. However, little attention has been given to algae. Recently, it has been demonstrated that algae play a role in determining the fate of metals in aquatic systems (Cai, 1995). Furthermore, certain metals at low concentrations increase growth of algal species (Petkov, 1995), while higher concentrations of metals such as cadmium, cobalt, copper, manganese, and nickel inhibit growth (Issa, 1995). Therefore, when investigating the transport and fate of metals in aquatic systems, it is important to consider algae. It is necessary to determine not only the effects of algae on metal transport (*e.g.* bioaccumulation), but also the effects of metals on algae *(e.g.* growth, toxicity). In addition, algae constitute a major component of the food web in most aquatic ecosystems and therefore have the potential to transfer metals to higher trophic levels. The primary objectives of this study were to investigate the: 1) effects of silver in culture medium on growth of *Selenastrum capricornutum* and the accumulation of silver in this alga; 2) effects of dietary silver, as *Selenastrum,* on reproduction and survival of *Ceriodaphnia dubia* and potential for bioaccumulation; and 3) transfer of silver along trophic levels from primary producers to primary consumers.

Ten day algal toxicity tests were conducted with silver sulfate and silver nitrate using a modification of EPA Growth Test 1000.3 (EPA 600-4-91-002). Starter cultures were grown in seven liter chambers for approximately two months with a 16:8 photoperiod. Tests were conducted in 250 mL erlenmeyer flasks, with three replicate flasks per concentration per sampling day. Growth was measured on sampling days three, seven, and ten using a hemocytometer. General water quality parameters of pH, alkalinity, hardness, salinity, and temperature also were measured at these sampling days and remained within normal ranges for all tests. Silver accumulation was calculated using algal dry weight.

The effects of dietary silver on *Ceriodaphnia dubia* reproduction and bioaccumulation were analyzed using a modification of EPA test 1002.0 (EPA 600-4-91-002). *Selenastrum* were exposed to silver in one liter test chambers, for ten days, after which water quality parameters were measured and cultures were washed in reconstituted water (100 mg $CaCO_3$/L). Then 3 mL of algae were filtered using a 0.45 μm membrane and analyzed for silver content. *Ceriodaphnia* seven day static renewal toxicity tests were conducted with dietary silver added as *Selenastrum.* Three hundred *Ceriodaphnia* were tested per dietary silver concentration. They were maintained in 30mL test chambers, with ten organisms per chamber, and 30 replicates per test concentration. Water quality parameters were measured and water changed daily. After seven days, *Ceriodaphnia* were washed in reconstituted water and pooled into groups of 100 for silver analysis. Reproductive tests were performed using ten *Ceriodaphnia* per dietary silver concentration. *C. dubia* were maintained in 30 mL test chambers, one organism per chamber with ten replicates per test concentration.

The ten day IC50 concentrations for algal growth were 85.7 and 75.8 μg/L for Ag_2SO_4 and $AgNO_3$, respectively (Figures 1, 2). Silver enhanced growth of *Selenastrum* at exposure concentrations of 20 and 40 μg/L, compared with controls. The effects of silver on growth at exposure concentrations of 30-50 μg/L met the criterion for biostimulation. Biostimulation (BS) is defined as an increase in total growth (T) above that of controls (C) according to the following :

Equation 1. $BS\ (\%)=(T - C)C^{-1}$

Growth was significantly greater than controls at 30, 40, and 50 μg/L, and significantly less than controls at 90 and 100 μg/L (Figure 3). Following 10 days in culture, silver accumulation increased in *Selenastrum* at exposure concentrations greater than 40 μg/L while growth decreased. Maximal silver accumulation was 50 times that of controls (Figure 4).

Although survival was not affected at any concentration of silver, the number of neonates per adult decreased from 23 to 0 as algal silver increased from 0.01 to 6.4 μg/g (Figure 5). Reproduction was significantly less than controls for all dietary concentrations of silver ≥ 1.1μg/g. *Ceriodaphnia dubia* silver body burdens were maximal at 1.3 to 1.33 μg/g and were over three times greater than control values. However, silver body burden decreased sharply between 1.33 and 3.7μg/g (Figure 6). This decrease may have been attributable to decreased feeding. However, further investigation is needed.

In Summary, silver enhanced the growth of *Selenastrum* at low concentrations (<50 μg/L) and transport occurred between trophic levels (*eg. Selenastrum* to *Ceriodaphnia*). Dietary silver impaired fecundity yet mortality was unaffected. Several investigations have documented the transport, fate, and effects of silver in freshwater fish, macroinvertebrates, and sediments. However, little attention has been given to algae. These studies indicate the important role of algae in silver transport

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Figure 1. Effects of Silver added as Ag_2SO_4 on *Selenastrum* Growth

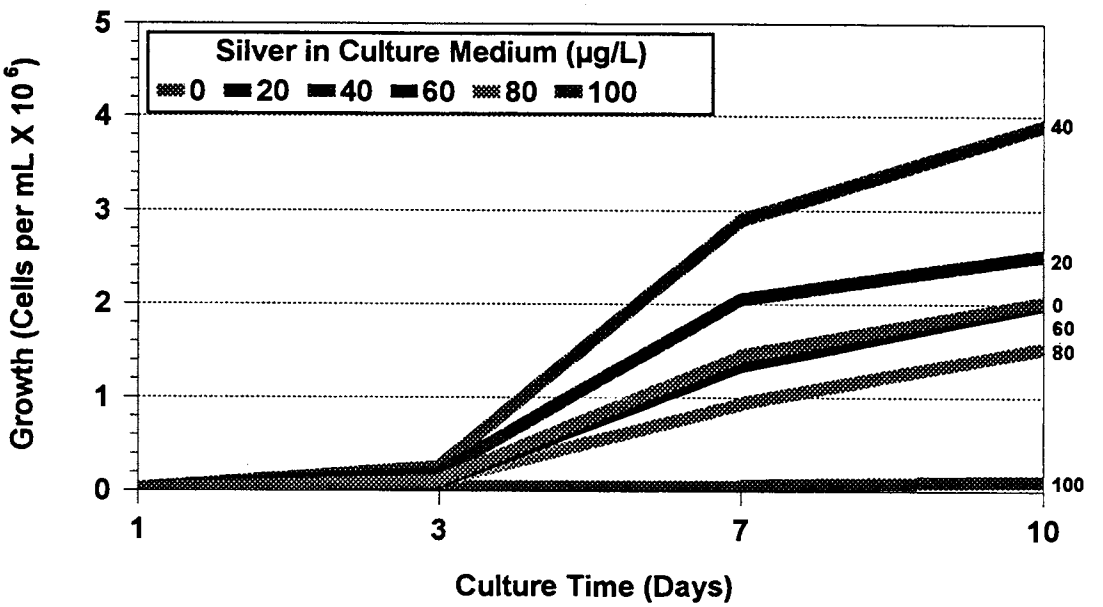


Figure 2. Effects of Silver added as $AgNO_3$ on *Selenastrum* Growth

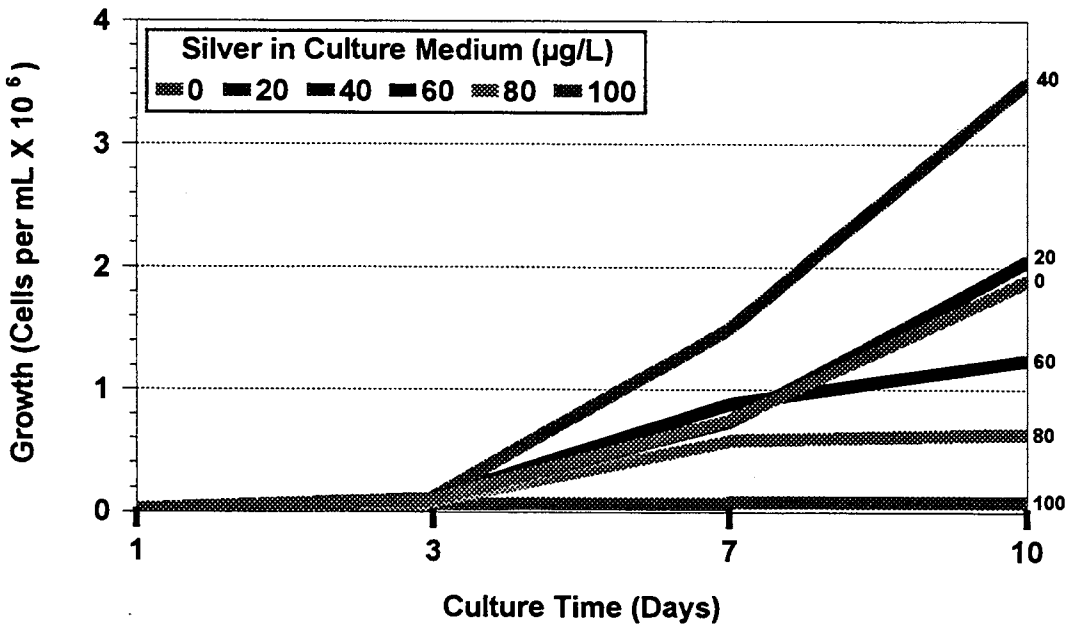


Figure 3. Effects of Silver on *Selenastrum* Growth, Ten-Day Exposure

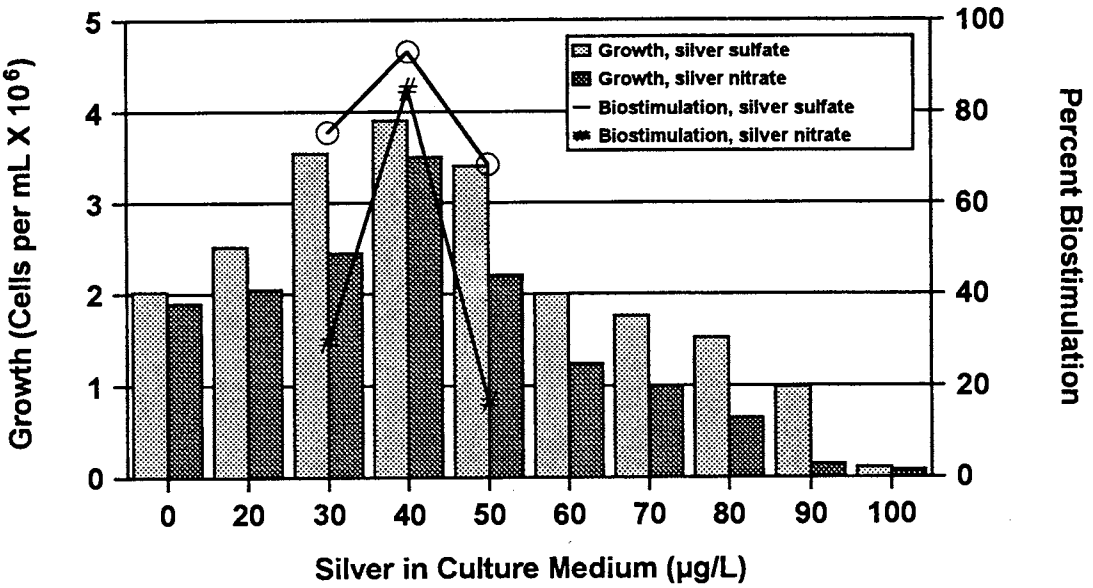


Figure 4. Silver Accumulation in *Selenastrum*, Ten-Day Exposure

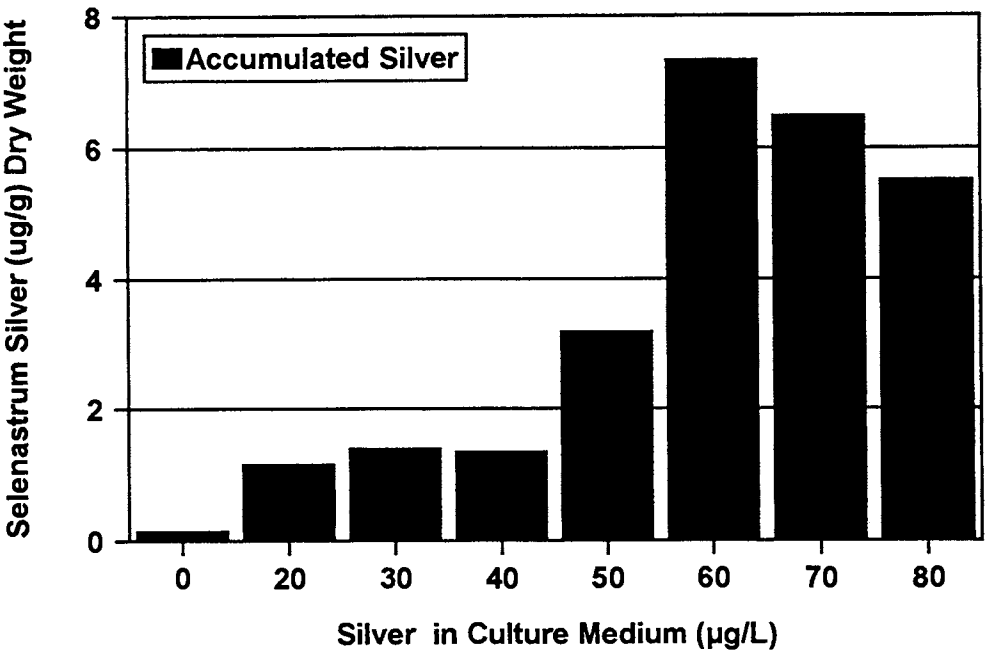


Figure 5. Dietary Silver *vs* Reproduction in *C. dubia*

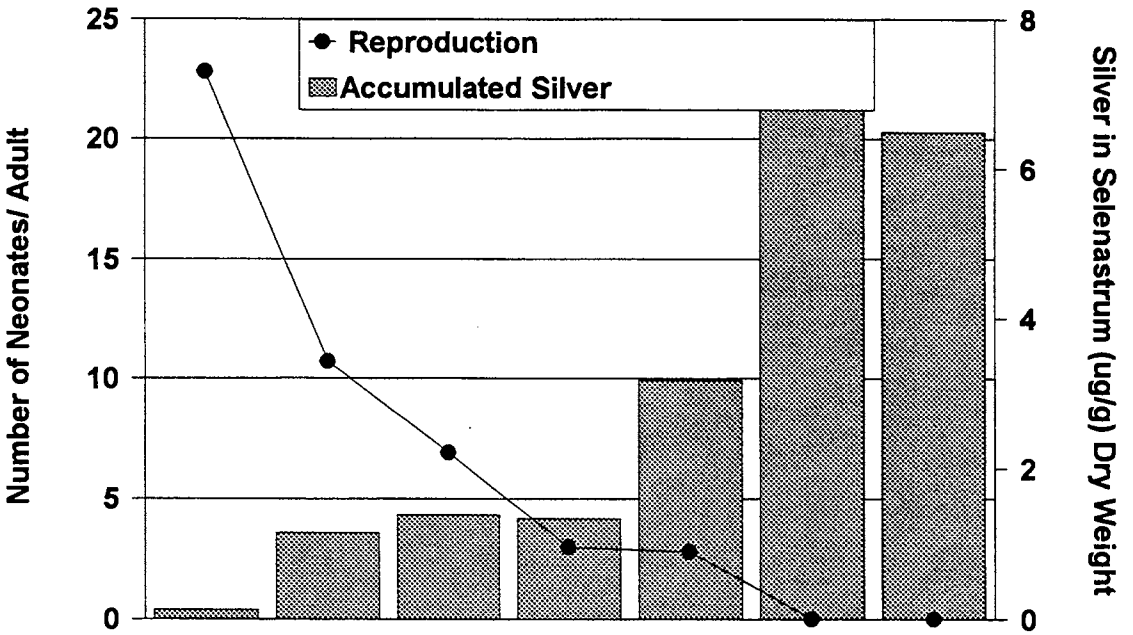
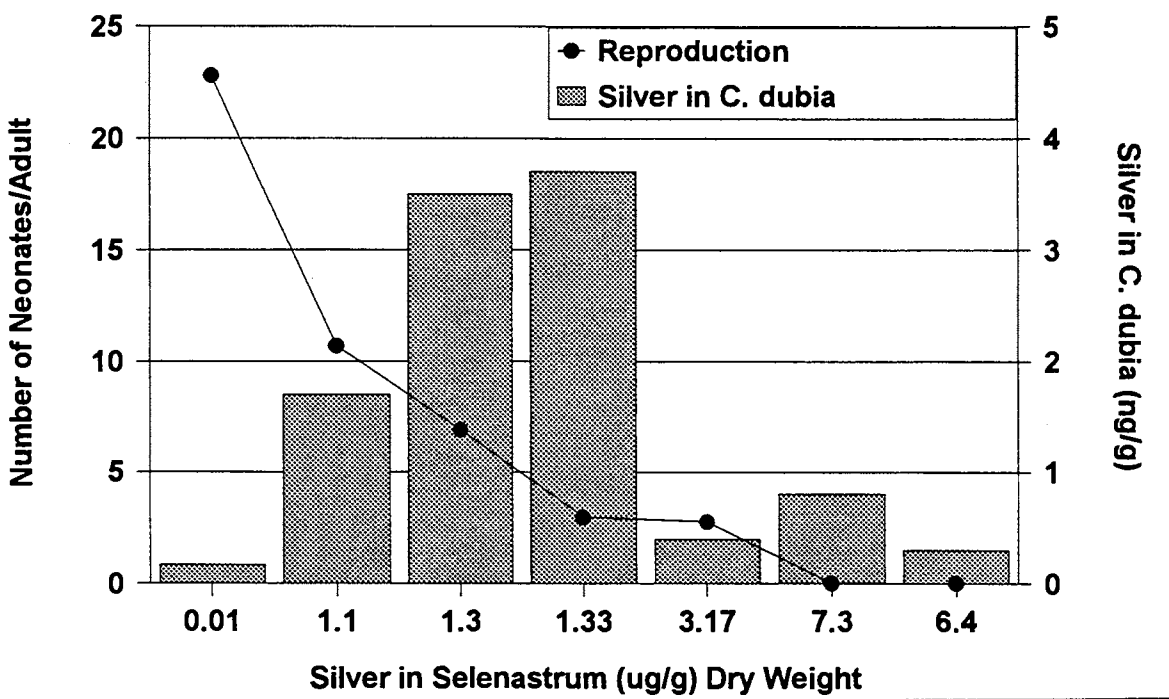


Figure 6. Effects of Dietary Silver (*Selenastrum*) on *C. dubia* Body Burden and Reproduction



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