CENTER FOR THE STUDY OF METALS IN THE ENVIRONMENT
Annual Report

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Table of Contents

The Center for the Study of Metals in the Environment is a multi-institutional consortium of scientists and engineers working to further the understanding of processes affecting the fate and effects of metals in aquatic and terrestrial ecosystems. Significant gaps in the ability to predict the fate and effects of metals in both aquatic and terrestrial systems continue to hamper appropriate risk assessments and cost-effective risk management. In these situations, decisions include many assumptions and the application of safety factors. The focus of the Center is to develop appropriate information so that regulatory decisions will be based on sound scientific principles. Much of the existing methodology for hazard identification and for risk assessment is based on experience with persistent organic pollutants such as DDT and PCBs. The large differences in environmental behavior and potential for toxicity between organic compounds and metals are not incorporated into these methods. Assessment methodology is currently focused on the extent to which chemicals exhibit PBT (persistent, bioaccumulative, and toxic) characteristics. All three characteristics are important aspects of the assessment of risk, but their applicability to metals and the evaluation of metals data for these criteria differ from organic compounds.

As a replacement for the current methods for evaluating the effect of metals in the environment, the Center is developing a model for the behavior of metal compounds that can be used as a tool in the hazard assessment of metals and metal compounds. This model will include the physical and chemical mechanisms that control the fate and resulting bioavailability of metals discharged to natural waters. In particular the transformations that affect metal fate and toxicity will be included. It is anticipated that it would be similar to the Unit World models, for example EUSES that are used for evaluating PBT organic chemicals. Metal behavior in watersheds, streams, lakes and reservoirs will be considered. The focus of the research efforts is to provide the information necessary to formulate and parameterize the model.

Unit World Model for Metals in Aquatic Environments

Introduction

There is a clear need for methods that can be used for evaluating the environmental hazard associated with the release of metals and metal compounds to the environment (Adams et al., 2000). The purpose of the Unit World Model (UWM) is to provide such a framework. The idea for a UWM comes from the fugacity and regional models developed for organic chemicals (Mackay 1979, 1991, Mackay et al. 1992). Models of this sort have previously been applied in various forms to pesticides (USEPA, 1986) and industrial organic chemicals (European Commission, 1996). The UWM for metals is structured such that it can be used to estimate both the exposure and effects of metal and metal compounds. It will incorporate the necessary metal specific processes that differentiate the behavior of metals from organic chemicals.

Approach

The Unit World Model is designed to represent the major processes that determine the fate and transport of metals in the aquatic environment. In the water column these processes include solubilization for particulate metal compounds, speciation among inorganic and organic dissolved ligands, and partitioning to suspended particles. The WHAM series of aqueous speciation models (Tipping, 1994) have been extensively calibrated and are well suited for incorporation in the UWM.

A metal particle sorption model (SCAMP) has also recently become available (Lofts and Tipping 1998). Although not as well calibrated, it provides a useful starting point.

The sorption of metals to water column particulate matter leads to the transfer of the metal to the bottom sediments. Hence, sediments are the ultimate repositories of metals in aquatic settings. A number of sediment models have been developed that successfully predict levels of sulfide (AVS−Acid Volatile Sulfide) and partitioned metal (SEM) in sediments and resulting fluxes of dissolved metal from the sediments to the overlying water column (Di Toro, 1996; Carbanaro, 1999; Di Toro, 2001). Therefore the frameworks exist for at least most of the processes, in various stages of development, for the water column and sediment compartments.

Effects Concentration and Bioavailability

In addition to the exposure concentrations in the water column and sediment, it is necessary to predict the effects to be expected. The traditional method is to use an effects concentration for the water column and the sediment. For the water column the EPA Water Quality Criteria (Stephen et al., 1985, USEPA 1986, 1996) or the PNEC (Predicted No Effect Concentration) derived following the EU Technical Guidelines (EU, 1996) are possibilities. However, these criteria make only limited bioavailability corrections (for water hardness only). This is a much more critical issue for metals than for organic chemicals. To remedy this situation, the Biotic Ligand Model (BLM) has recently been developed (USEPA, 1999, 2000; Di Toro, et al., 2000; Paquin et al., 2002). It incorporates the WHAM speciation model and in addition models the competitive metal binding at the toxic site of action (the Biotic Ligand). BLMs are currently available for copper and silver (Di Toro, et al., 2000; Santore et al., 2000), zinc (Santore et al., 2002) and are under development for cadmium, nickel and lead.

The situation in the sediment is similar. There are guideline values that do not take bioavailability into account−the sediment PNEC−or which are empirical and therefore are not predictive of individual metal toxicity (e.g. Long and Morgan, 1991; Field et al., 2002).

For metals, EPA has developed sediment quality guidelines that are causally related to metal effects and do take bioavailability into account. They are based on the relative magnitudes of acid volatile sulfide AVS and simultaneously extractable metal SEM, and organic carbon (Di Toro et al., 1990, 1992; Ankley et al., 1993, 1996; USEPA, 2000).

Unit World Model Design and Testing

The design of the UWM is based on the processes required for calculating both the exposure concentration and the variables required for making the bioavailability corrections for the effects concentration. Since the model is expected to be used for site-specific evaluations as well as in regulatory settings, it needs to be predictive to the extent possible. This forces a more complex model structure than would be necessary for a strictly evaluative model with fixed physical, chemical and biological variables. The current plan is to build the UWM using water column/sediment eutrophication model as a basis (Di Toro, 2001). Modern eutrophication models compute most of the required auxiliary variables required for metal modeling−carbon and sulfur cycles–and water column/sediment models for manganese and iron are also available, which are required for metal partitioning.

The steps in producing the model are to synthesize the available components into a unified modeling framework, and then to test the model with laboratory and field data for a variety of metals. The testing will begin with experiments in which a suite of metals were added to freshwater (Diamond, 1990) and marine (Santschi, 1987) mesocosms, and a long term dosing experiments to a Canadian lake (Hesslein, 1980). There is one data set (O'Connor 1988) that can be used for calibrating the model to freshwater streams. However, it is somewhat limited and it was not collected using modern clean techniques. It is anticipated that most of the data for streams will need to be generated.

Year 1 projects were completed using money appropriated for FY2001. Funding was received from EPA in April 2002 for these projects, many of which were designed to provide data for use in the initial development of the model. The projects for the first year of the Center are

Investigator	Institution	Project Title
Ross, P.	Colorado School of Mines	Role of Dietary Exposure for Bioaccumulation and Toxicity of Metals in Aquatic Ecosystems Affected by Mining
Allen, H. E.	University of Delaware	The Role of Organic Matter and Metal Oxides in the Retention of Trace Metals by Soil and Suspended Particles
Di Toro, D. M.	Manhattan College	Developing a Model to Predict the Persistence of Metals in Aquatic Environments
McDonald, G.	McMaster University	Effects of Dietary Metal Exposure on Fish and Aquatic Invertebrates
Adams, C D.	University of Missouri-Rolla	Aquatic Toxicity and Exposure Assessment
Lanno, R.	Oklahoma State University	Development of a Model to Predict the Bioavailability of Metals to Soil Invertebrates
Meyer, J. S.	University of Wyoming	Bioaccumulation and Toxicity of Dietborne Particulate Metals to Benthic Invertebrates

Reports of the results of activities for each of these projects are contained in Appendix I. A brief summary of each project is presented here:

Ross, P. Colorado School of Mines "Role of Dietary Exposure for Bioaccumulation and Toxicity of Metals in Aquatic Ecosystems Affected by Mining"

This project has investigated copper, iron, manganese and zinc in the dissolved and suspended phases in the North Fork of Clear Creek (NFCC) in Colorado. Acid mine drainage (AMD) enters the creek and results in high concentrations of heavy metals, including iron, zinc, copper, and manganese in the water and the associated sediments. The goal of this research was to examine the spatial and temporal trends in both dissolved and suspended metal concentrations. These results will be used in the development of the Unit World Model.

Iron is present in the NFCC system nearly entirely in the particulate phase and iron oxyhydroxides are observed to dominate the suspended sediments present in NFCC. *In-situ* precipitation of iron oxyhydroxides appears to occur during low flows and is especially prevalent in locations close to

AMD inputs. Copper is also seen to be present to a significant extent in the suspended sediments. In contrast, Mn is essentially only present as a dissolved phase. Zinc is present in both the dissolved and particulate phases with no clear trend. These results are consistent with the known behavior of metals in AMD. Copper is known to be more readily adsorbed than Zn. Mn is problematic, as it has been shown to be difficult to remove from AMD.

During high-flow the suspended sediments are likely principally derived from the flocculated bed sediments. The amount of suspended sediments may represent an equilibrium between re-suspension and flocculation/settling, which is controlled by the hydrologic conditions. Elevated concentrations of metals associated with suspended sediments are transported during high flows, especially during rain events. Colloids dominate the suspended sediment during lower flow; these may be formed by precipitation in the water column. Colloids have been seen to be present during low flow conditions at various areas in the creek. These colloids are likely what is contributing to the increased mass % of suspended sediment in the < 1 um fraction during low-flow sampling dates

Allen, H. E. University of Delaware "The Role of Organic Matter and Metal Oxides in the Retention of Trace Metals by Soil and Suspended Particles"

The objective of this project is to develop models to predict Cd, Cu, Ni, and Zn uptake and release from soil particles into the soil solutions. The models focus on kinetics aspects and thus will essentially improve the existing approaches based on equilibrium assumptions. The effect of soil properties (organic matter, oxides, and metal loading, etc.) and solution composition (pH, dissolved organic matter, and Ca content) on Cd, Cu, Ni and Zn release will be studied to find the necessary relationships and to evaluate the parameters of the model. Key soil and solution properties will be selected to use the minimal number of input parameters providing reasonable accuracy of predictions. The developed models will form an important component part of the "Unit World" model of metal behavior in the environment.

Initial studies have been carried out for the desorption of copper and zinc added to 2 soils. A stirred-flow method, which minimizes diffusion effects and readsorption phenomena by effective mixing in the reaction chamber and continuously removing desorbed products from solution, was used to study the Cu and Zn release from the soil particles. A first order equation described the kinetics of Cu and Zn desorption from soils: $q(t)=q_1*(1-\exp(-k_d*t)+ q_2$. The total desorbable metal is $q_1 + q_2$ and k_d is the desorption rate coefficient.

Lowering pH increased the total desorbable amount of both copper and zinc. A linear relationship was found for the total desorbable amount of metal as a function of the pH. The fraction of the copper released is generally less than 10%, while for zinc it is greater than 50%. The addition of dissolved organic matter enhanced the release of copper, but not zinc. The rate constant for the release of copper was linearly correlated to the concentration of dissolved organic matter.

Di Toro, D. M. Manhattan College "Developing a Model to Predict the Persistence of Metals in Aquatic Environments"

The objective of this project is the development of the Unit World Model for Metals as described above in detail. The initial focus is on the development of a metals model for lakes. For this purpose, a coupled water column/sediment flux model in is being developed in VBA for EXCEL. The code to track the cycling of organic carbon, nutrients, oxygen, and redox-sensitive species (S, Fe) between the water column and sediments has been developed and the computer code is being tested. The next step will be to add the metals overlay to the model. The model will then be used to perform a more detailed evaluation of available data for lakes.

McDonald, G. McMaster University "Effects of Dietary Metal Exposure on Fish and Aquatic Invertebrates"

The focus of this study is the dietary uptake of metals via metal-contaminated sediment particles in invertebrates. The initial phase of this study has used *Daphnia magna* to investigate dietary metal uptake from sediment particles. Because *Daphnia* are filter feeders, they sieve large quantities of water to collect suspended particles. Although their preferred food is algae, any suspended particles over 0.45µm will be retained on the filtering appendages and may be ingested.

Sediments used in this study are collected from Clear Creek, Colorado. Clear Creek is a high-gradient stream, which receives metal-rich effluent from a number of mining sites, and has elevated metal levels in both the water and sediments.

Due to the high concentration of easily mobilized metals associated with the sediments of Clear Creek, washing of particles is necessary to determine whether the dissolved fraction or the sediment was the main source of bioavailable metal. Work is ongoing to clearly define the role of particle-bound metal on the receptor organisms.

Adams, C D. University of Missouri-Rolla "Aquatic Toxicity and Exposure Assessment"

The University of Missouri - Rolla is conducting a metals fate and transport study on two adjacent river systems in of southeastern Missouri. These systems include the watersheds of the Big River and the West Fork of the Black River. Each river transects a world-class sized lead-zinc district that is hosted in carbonate bedrock. It is the intent of this project to develop the necessary data for calibration and validation of comprehensive fate and transport models for heavy metals to be developed within the Center for the Study of Metals in the Environment (CSME). The working hypotheses of this proposed research is that the transport and bioavailability of heavy metals in streams impacted by mining activity are a function of particle size distribution, mineralogy, density, acid leachability, specific surface area, and other key parameters. The heavy metals to be the focus of this work include lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), and nickel (Ni).

The first year samples were collected from the Big River and West Fork of the Black River in southeastern Missouri. Although our results are preliminary, some interesting observations have been made in the river systems.

- Metal concentrations in waters being emitted from the tailings piles are very high with a significant contribution from soluble and colloidal sized materials. The high metal concentrations occur despite the moderately alkaline nature of the water. Generation of an acidic microenvironment on the surfaces of sulfide minerals located within the tailings piles is believed to be the cause of the high metal contents.

- Metal concentrations decrease from the tributary creeks adjacent to the tailings piles to the Big River system. Decreases may result from dilution, adsorption onto sediment particles, or both.
- Storm surges in the river result in an increase in the concentration of most metals in the water column and an increase in the proportion of metals contained on particulate material. In some cases a disproportionally high amount of metals occur on the particles, suggesting that storm surges may result in the removal of metals from the water column by particle scavenging and sedimentation as the fiver flow subsides. A similar process was observed in the West Fork of the Black River following a construction related disturbance of the river gravels.
- Metals in the water column and bed sediments correlation with manganese, suggesting that manganese oxides are acting as adsorbents for metals in the river systems.
- Improved environmental mining practices in the New Lead Belt have resulted in substantially lowered metal release to the Black River system when compared to the Old Lead Belt. A disturbance of sedimentary material above all known mining operations has released enough metals from the river to swamp the release of metals from mining operations.

Lanno, R. Oklahoma State University "Development of a Model to Predict the Bioavailability of Metals to Soil Invertebrates"

This project will examine the relationship between physical and chemical characteristics of soils (e.g., organic matter, pH, cation exchange capacity) and the bioavailability, uptake, metabolism, and toxicity of zinc (Zn) and cadmium (Cd) by soil invertebrates. Metal speciation in pore water, and in the invertebrate gastrointestinal system and tissues will also be determined in order to develop a Biotic Ligand Model for soil invertebrates which would enhance our ability to predict the toxicity of metals in soil systems. Research during this past year has focused on the development of a method for the fractionation of earthworms to provide a tool for examining the partitioning of metals within a soil invertebrate species. This is the first step in understanding metal metabolism in various organisms in order to determine which fraction of metal in the organism best represents a biotic ligand associated with a toxic response.

Preliminary findings of this study and continuing collaborative studies with Willie Peijnenburgh (RIVM, The Netherlands) and Martina Vijver (Vrije Universiteit, Amsterdam) show that metal distribution within the earthworm body is different for essential and non-essential metals. Whereas Cd is predominantly stored in the metal fraction associated with the protein fraction and induction of this fraction was shown to occur upon exposure to Cd, Zn is more evenly distributed over the various metal fractions and induction of the protein fraction was not observed following exposure to Zn. Significantly increased Cd levels were found in the metal fraction associated with the cell tissue and the cell membranes following feeding of the litter feeding worm *L. rubellus* with Cd-contaminated leaf material in soils that contained high metal levels (total concentration) and low Cd concentrations in the pore water. Exposure of the non-litter feeding *A. caliginosa* did not lead to an increase in Cd levels whereas Zn levels did not increase in either species. This is probably due to regulation of internal Zn levels in the worms. These findings are in full agreement with metal levels found in field-collected worms and internal Cd and Zn distribution also agrees with laboratory observations. Following exposure of worms to soils spiked with metal salts, a marked increase in levels of metals associated with the protein fraction was observed. These observations allow for distinguishing the kinetics of elimination of the metal fractions associated with the protein fraction and the cell membranes/cell tissue. These experiments are currently in progress.

Meyer, J. S. University of Wyoming "Bioaccumulation and Toxicity of Dietborne Particulate Metals to Benthic Invertebrates"

The goal of this project is to investigate the ability of the BLM to predict the acute toxicity of Cu and Zn to fish when water quality parameters vary temporally. To this end, we have begun to (1) identify water quality parameters that vary diurnally in receiving waters and (2) conduct preliminary experiments needed to prepare for toxicity tests under time-varying exposure conditions.

We conducted acute toxicity tests with larval fathead minnows (FHM; *Pimephales promelas*) exposed to Zn in the presence of elevated concentrations of $Ca(NO_3)_2$, $CaCl_2$, $Mg(NO_3)_2$, $MgCl_2$ and $NaNO_3$. These tests demonstrate that the paired anion with which a major cation is added to an exposure water can affect the toxicity of Zn to larval FHM. This has important implications for testing the ability of the BLM to predict acute toxicity of metals to fish.

The results of these flow-through Zn toxicity tests suggested that either (1) Ca^{2+} is not as protective as traditionally thought, and Mg^{2+} and Na^+ interact synergistically with Zn, (2) NO_3^- interacts synergistically with Zn, or (3) NO_3^- interferes with the protective effects of Ca^{2+}, Mg^{2+} and Na^+. However, we could not exclude the possibility that the ACS Grade of these nitrate salts contained an unidentified contaminant, despite analyses of the Ag, Cd, Cu, Ni, Pb and Zn contents of those salts in our laboratory that showed no concentrations of trace metals high enough to be of toxicological concern. Therefore, we conducted two separate sets of four side-by-side static-renewal Zn toxicity tests to test if the purity of the added nitrate salt and/or the anion paired with the cation affected the Zn toxicity. The first set of four side-by-side tests was conducted in basewater, basewater containing elevated $Ca(NO_3)_2$ (ACS Grade), basewater containing elevated $Ca(NO_3)_2$ (ReagentPlus Grade -- a higher purity than ACS Grade), and basewater containing elevated $CaCl_2$ (ACS Grade). The second set of four side-by-side tests was conducted in basewater, basewater containing elevated $Mg(NO_3)_2$ (ACS Grade), basewater containing elevated $Mg(NO_3)_2$ (ReagentPlus Grade), and basewater containing elevated $MgCl_2$ (ACS Grade).

The purity of the nitrate salts did not affect Zn toxicity, for either $Ca(NO_3)_2$ or $Mg(NO_3)_2$. In fact, the 96-h LC50s for the two purities of $Ca(NO_3)_2$ differed by only 2%; and the 96-h LC50s for the two purities of $Mg(NO_3)_2$ differed by only 4%.

However, Ca^{2+} and Mg^{2+} differed in their protective effects against Zn toxicity. When Ca^{2+} was added as 2.2 mM $Ca(NO_3)_2$, the 96-h Zn LC50 was 2.4-fold greater than in the basewater; whereas when Mg^{2+} was added as 2.0 or 2.3 mM $Mg(NO_3)_2$, the 96-h Zn LC50 did not differ from the LC50 in the basewater. And when Ca^{2+} was added as 2.2 mM $CaCl_2$, the 96-h Zn LC50 was 4.3-fold greater than in the basewater; whereas when Mg^{2+} was added as 2.3 mM $MgCl_2$, the 96-h Zn LC50 was only 2.0-fold greater than in the basewater.

Moreover, the anion paired with the cation affected Zn toxicity. For Ca^{2+} and Mg^{2+}, the 96-h Zn LC50 almost doubled when the cation was paired with Cl^- compared to NO_3^-. Unfortunately, based on these results alone, we cannot determine whether (1) Cl^- protects against Zn toxicity and NO_3^- has no effect, (2) NO_3^- interacts synergistically with Zn to increase the Zn toxicity and Cl^- has no effect, or (3) some combination of the previous possibilities occurs. Direct toxicity of NO_3^- probably is not a contributor, because we observed no mortalities or outwardly apparent signs of stress in FHM larvae at 10 mM $NaNO_3$ during a $NaNO_3$ toxicity test we conducted in November 2002. To test for indirect interactions of NO_3^- with Zn, we plan to conduct an analogous set of side-by-side static-renewal Zn toxicity tests in basewater, basewater containing elevated KNO_3, and basewater containing elevated KCl -- assuming that K^+ neither protects against Zn toxicity nor interacts synergistically with Zn.

Year 2 Program Development

Additional funding was appropriated for FY2002 that has allowed expansion of the research program. The Science Advisory Committee of the Center reviewed a series of proposals for research projects. Nine projects were approved for funding with FY2002 appropriations. Planning has been carried out with the Science Advisory Committee (SAC) of the Center to allow the development of the Unit World Model to be carried out in a 3-year program. The investigators met with the SAC in May 2002 to review project plans. The following are the projects that were approved

Investigator	Institution	Project Title
Adams, C. D.	University of Missouri-Rolla	Metals Speciation and Transport in the Black River of Missouri's New Lead Belt
Allen, H. E.	University of Delaware	Release of Metals from Particulate Matter
Capitani, J. F.	Manhattan College	Quantitative Structure Activity Relationships for Toxicity and Fate Parameters of Metal and Metal Compounds
Church, T. M.	University of Delaware	Metal Speciation in Watersheds
Di Toro, D. M.	University of Delaware	Developing a Unit World Model for Metals in Aquatic Environments
Imhoff, P. T.	University of Delaware	Evaluation of Automobile Sources for Metals in Urban Areas
Meyer, J. S.	University of Wyoming	A Test of the Biotic Ligand Model: Fish Exposed to Time-variable Concentrations of Cu and Zn
Ross, P.	Colorado School of Mines	Ecotoxicology of Mining-Related Metal Oxides in a High-Gradient Mountain Stream
Sparks, D. L.	University of Delaware	The Impact of Surface Precipitation on Sequestration and Bioavailability of Metals in Soils

Most of the projects for Year 2 are focused on a central theme related to the development of a "Unit World Metal Model." This new modeling framework that will allow prediction of the distribution and, more importantly, the toxicity of metals in aquatic systems is shown in Figure 1. The lack of a model that accurately predicts metal distribution and toxicity significantly hampers the implementation of scientifically credible TMDLs and other important EPA programs. The importance of the technical factors being incorporated in the Unit World Model for Metals have been pointed out by the EPA Science Advisory Board (SAB) in its review of the plans for the Metals Action Plan.

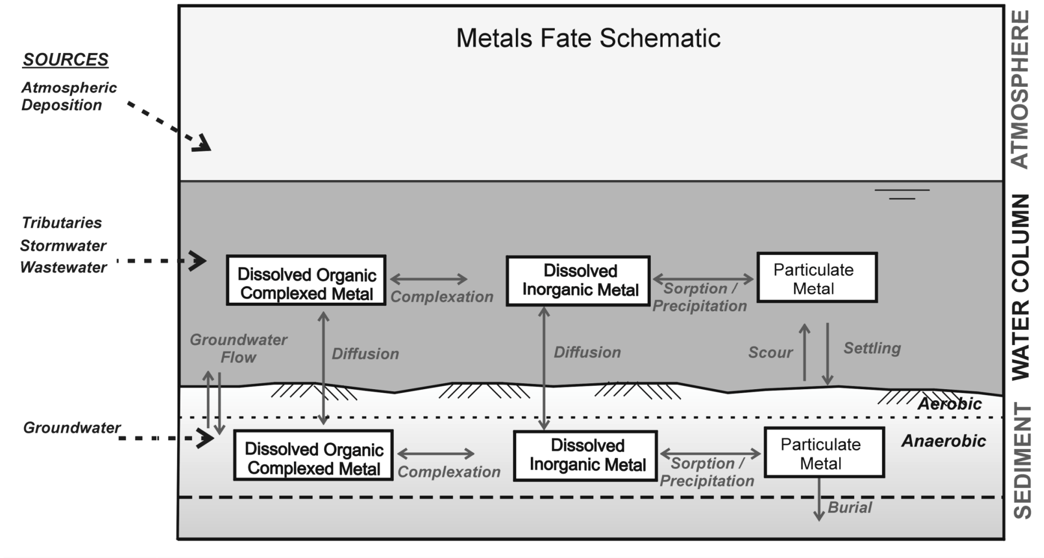

Figure 1. Fate of Metals in the Aquatic Environment

In February 2003 the Center held an international workshop on the Unit World Model Workshop at the University of Delaware. Attendees included University researchers, EPA scientists and program managers, representatives from the metal industry, and many of the Center's scientists. They considered technical and regulatory issues.

Following the recommendation of the Science Advisory Committee, the Center Director and Associate Director convened two meetings with the investigators conducting field work. A conference telephone call in January 2003 was followed by a workshop on field sampling and laboratory analytical issues. The workshop was held immediately preceding the Unit World Workshop.

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APPENDIX I

Role of Dietary Exposure for Bioaccumulation and Toxicity of Metals in Aquatic Ecosystems Affected by Mining

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Annual Report prepared for:
Center for the Study of the Bioavailability of Metals in the Environment

Introduction

Acid-mine drainage (AMD) is an important source of metal contamination in regions of current or past mining of sulfide ores. AMD impacted streams pose toxicity risks to a variety of aquatic and sediment-dwelling organisms. In the Blackhawk-Central City area of the Colorado Mineral Belt, gold, silver, copper, lead, and zinc were extensively mined in the late 1800's (Wildeman et al., 1974; Cunningham et al., 1994; U.S. EPA, 1999). Effects from these past mining activities are still seen today in the Clear Creek Watershed (Ficklin and Smith, 1994; Morris et al., 1999). For example, the North Fork of Clear Creek (NFCC), a major tributary of Clear Creek, does not support fish in locations below AMD inputs (U.S. EPA and CDPHE, 1997).

AMD enters NFCC, near the towns of Blackhawk and Central City, and results in high concentrations of heavy metals, including iron, zinc, copper, and manganese in the water and the associated sediments. Once the metal-laden water mixes with the upstream NFCC water, there is a transformation from ferrous to ferric iron. This results in the visible precipitation of colloidal ferric oxyhydroxides. These colloids can scavenge other metals present in the water column, and can either be transported downstream or aggregate and then settle to the bed sediment (Stumm, 1992; Schemel et al., 2000).

Sediments accumulated on the streambed can be resuspended and transported. Deposition and scouring of metal-laden bottom sediments is mostly controlled by flow conditions in the stream. NFCC is an example of a high-gradient mountain stream, where flows are quite variable over the year and depend on seasonal (snow-melt, fall/winter low-flow) and short-term (localized rain storms) weather conditions. In addition, drought during 2002 resulted in the lowest recorded flows for Clear Creek and likely affected metal transport.

Both the dissolved phase and suspended sediments were analyzed for a variety of metals (iron, copper, manganese, and zinc data are presented) over May-October, 2002. Water column samples were collected from both NFCC and the main-stem of Clear Creek (CC), Colorado. Bed sediment samples were collected from NFCC. The suspended and bed sediments were examined for relative particle-size distributions. Coatings on rocks, which were dissolved with acid, were examined for relative metal concentrations, based on estimated surface area. The results of the chemical analyses were used to determine the relative importance of suspended versus dissolved transport of metals over the study period.

The goal of this research was to examine the spatial and temporal trends in both dissolved and suspended metal concentrations. A spatial study of NFCC was conducted in May 2002 with the Colorado Department of Public Health & Environment (CDPHE). Collection of temporal data at several sites in NFCC and the main stem of Clear Creek (CC) are ongoing. Both the dissolved phase and suspended sediments were analyzed for a variety of metals. This report presents and discusses spatial (May 2002) and temporal (from April 2002 to January 2003) data for Fe, Cu, and Zn. More complete water chemistry data are contained in an Appendix to this report. The results of the chemical analyses were used to determine the relative importance of suspended versus dissolved transport of metals over the study period.

Methods

Spatial Study:

Water samples were collected with the assistance of the CDPHE in May 2002; sampling sites are indicated on Figure 1.

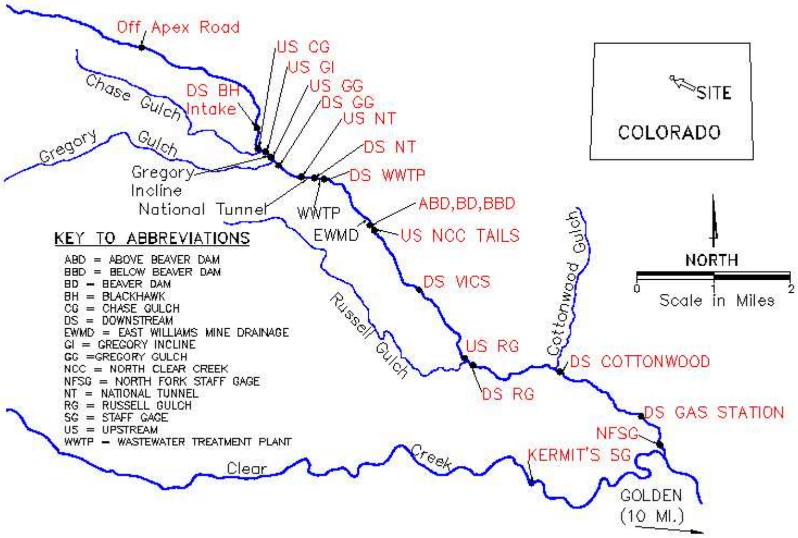

Figure 1. Spatial and Temporal Study Sites, North Fork Clear Creek (NFCC), Colorado

Temporal Study:

Water samples were collected once per month for April-May 2002, bi-weekly for the months of May 2002-November 2002, once per month for December 2002-March 2003 at the NFSG site indicated on Figure 1. Samples were collected and analyzed as described for the ‘Spatial Study’, with the exception that all analyses were performed by CSM. This report presents the results from eleven sampling events from May 21 to October 1, 2002 in NFCC. Full results from the May 21, 2002 spatial sampling are available from CDPHE.

Sample Collection

All water samples were grab samples collected in high-density polyethylene (HDPE) bottles. Bottles were rinsed three times with the stream water before sample collection. The bottles were placed below the surface water and then filled by moving the bottle through the water column. It was assumed that the water was well mixed due to the turbulent nature of the stream and thus, samples were not depth integrated. Water was filtered in the field using 25 mm filter holders (Gelman) with disposable 0.45-micron (Supor-450) filters. Filtered and raw water samples were acidified with metal-free hydrochloric acid to a $pH < 2$. Unfiltered samples were also collected for total organic carbon, alkalinity, and particle-size analysis (organic carbon and alkalinity results are not presented).

Bed sediments were collected at the NFSG, DS Gas Station, and BD sites on May 21, 2002. Based on visual observations, samples of varied sediment types were collected in order to assess the range of sediment types present in the stream. Loose sediments were collected using plastic beakers. The sediments were transferred to polyethylene bags with overlying stream water. Cobbles and rocks of approximately 5-25 cm diameter were collected twice at NFSG (10 on May 21, 2002 and 8 on August 20, 2002) and at two sites below the beaver dam (BBD) (6 rocks for each) in August.

NFSG flow data were obtained from the U.S. Geological Survey (USGS) Lakewood, Colorado office.

Analytical Methods

Temperature and pH were measured in the field; all other analyses were performed in the laboratory. All alkalinity measurements were conducted at the Colorado School of Mines (CSM) using a HACH kit and titrating to the Bromocresol-Green endpoint. For the spatial study, the USEPA Region VIII Laboratory, using Inductively Coupled Plasma Spectroscopy (ICP-Mass Spectroscopy for Cd, Cu, Pb, and Ag and ICP-Atomic Emission Spectroscopy for all other elements), assessed the majority of samples for metal concentrations. Metals from sites ABD, BD, and BBD were measured by CSM using a Perkin-Elmer Optima 3000 Inductively Coupled Plasma Atomic Emission Spectrometer (ICP-AES). All metal analyses for the temporal study were performed at CSM. Samples were analyzed for total and dissolved metals on a Perkin-Elmer Optima-3000 ICP-AES using EPA Method 200.7. TOC measurements were performed at CSM by high-temperature combustion using a Shimadzu TOC-500 instrument.

Particle number and size distribution measurements were performed on unfiltered water samples using a Nicomp-780 Single Particle Optical Sizer (SPOS). SPOS uses a combination of light obscuration and scattering to determine the size distribution of a sample over the range of 0.4-250 micron. The upper limit of particles per ml for injection into the Nicomp-780 SPOS is $\sim 10^4$ particles/ml. Thus, samples were diluted in deionized water before analyses and the results were adjusted with the dilution factor to determine particles per ml in each original sample.

Bed sediments were wet sieved into > 2 mm, 2 mm to 63 μm, and <63 μm fractions. The fraction > 2 mm was discarded and the two remaining fractions were dried. The 2 mm to 63 μm fraction was air-dried and the <63 μm fraction was oven-dried at 50 ˚C. The dry mass in each size fraction was determined.

Sediment coatings on the rocks were removed by soaking the rocks in 0.5 M HCl for 3-10 days with occasional agitation. Loose, flocculated material was rubbed off the rocks (by hand, using nitrile gloves) before the rocks were submersed in the acid solution. The acidic solution was filtered and diluted before analysis for metals by ICP-AES. The dimensions of the rocks were determined and an approximate geometric surface area was computed and used to normalize the metals data.

Results and Discussion

Spatial Study:

AMD inputs to NFCC include Gregory Gulch, Gregory Incline, National Tunnel, and, to a lesser degree, East Williams Mine Adit. Each of these locations is indicated on Figure 1. Concentrations of iron, copper, and zinc, from above Blackhawk to just above the confluence with CC, are shown in Figure 2.

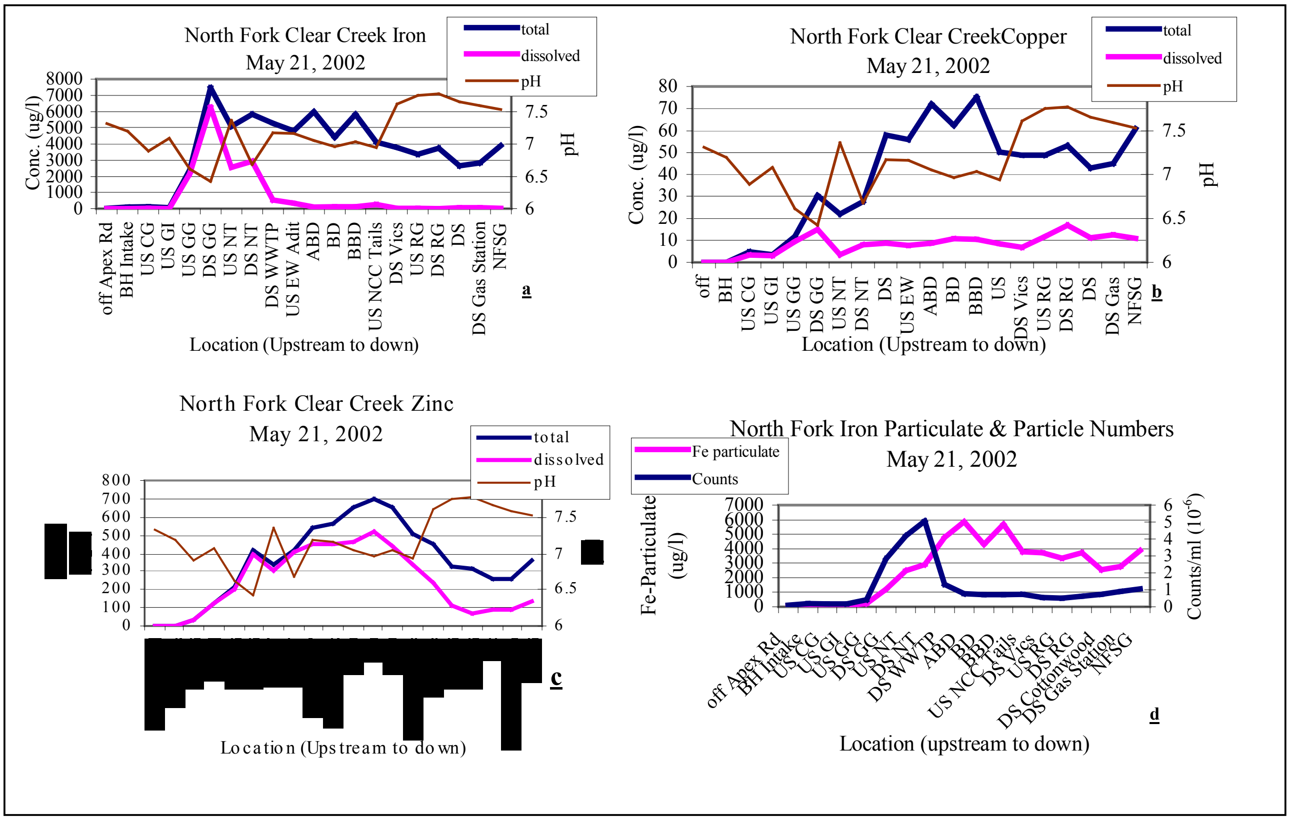

Figure 2. a) Fe Total and Dissolved and pH; b) Cu Total and Dissolved and pH; c) Zn Total and Dissolved and pH; d) Particulate Fe and Suspended Particle Numbers

Iron (Figure 2a) increases rapidly in concentration from the AMD inputs and then begins to oxidize and precipitate as iron oxyhydroxides (also called hydrous ferric oxides, HFO) after the Gregory Gulch site, as indicated by the difference between total and dissolved iron concentrations. This precipitation is observed in the creek: water is relatively clear, then becomes cloudy, and then becomes laden with visible particles of HFO as one travels downstream. Copper (Figure 2b) follows the same trend as the iron, also increasing in the particulate fraction below Gregory Gulch. This is likely due to adsorption of the Cu onto the HFO. Although there is some particulate zinc below Gregory Gulch, the amount is negligible. Significant Zn particulate is not observed until the DS WWTP site and then it gradually increases from ~16 to 65% going downstream, with the exception of the DS RG site, which has 80% particulate Zn.

Particle numbers begin to increase in the same region of the creek where the iron begins to precipitate, and numbers rapidly increase as precipitation increases. This is a result of Fe oxidation forming many small HFO particles. Then, below the WWTP, the particulate iron concentration changes very little, but the particle numbers dramatically decrease. This is because the large numbers of smaller particles are aggregating into larger particles, having the same concentration of iron. At the DS GG site, the maximum particle size was <8 μm; at US NT it was < 6 μm; at DS NT it was 6 µm; and at the DS WWTP site it was 16 µm. For all sites downstream of the WWTP, the maximum particle size was always greater than 15 µm.

Temporal Study:

Flow in NFCC was very low in 2002, with a range from 0.4 to 25 cfs (Figure 3).

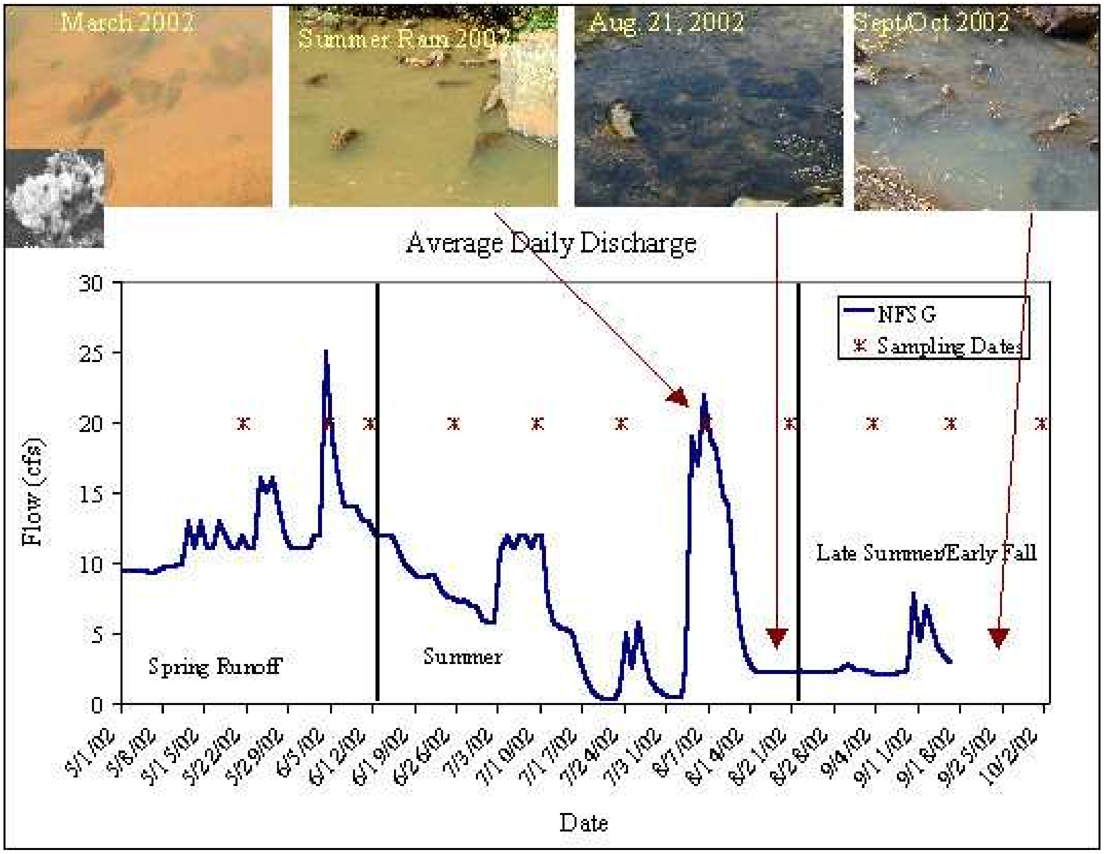

Figure 3. Flow, sampling dates, and photos showing sediment changes and transport during varied hydrologic events.

Flow dynamics in the creek were seen to control particulate-metal transport, as illustrated by the photographs in Figure 3. During the winter and early spring, very fine iron oxyhydroxides had accumulated and appeared to dominate the bed sediments (left-most photograph in Figure 3). These iron oxyhydroxides typically form initially as submicron colloids, as shown in the SEM photomicrograph (Figure 3 insert). These colloids aggregate and form a loose floc that deposits in the stream. As the flow increased during spring runoff, these iron oxyhydroxides were re-suspended and carried downstream. As the summer progressed, and flow decreased, iron oxyhydroxides re-accumulated on the creek bed. A summer rain event on August 5th increased the flow dramatically over a short period. This increased flow resuspended and transported the iron oxyhydroxides downstream (2nd photograph in Figure 2). Once these particles were scoured away by the increased flow, a black coating, suspected to be manganese oxide, was seen on the rocks (3rd photograph in Figure 3). Over several weeks of low flow, iron oxyhydroxides once again began to accumulate on the creek bed. In addition, increased turbidity in the stream was observed.

The particle-size distribution of the suspended sediments (0.4 to 250 μm) was determined using SPOS. The resultant data were simplified by summing the number of particles in each of three size ranges (< 1, 1-10, > 10 μm). The results for each of the sampling dates are presented in Figure 4.

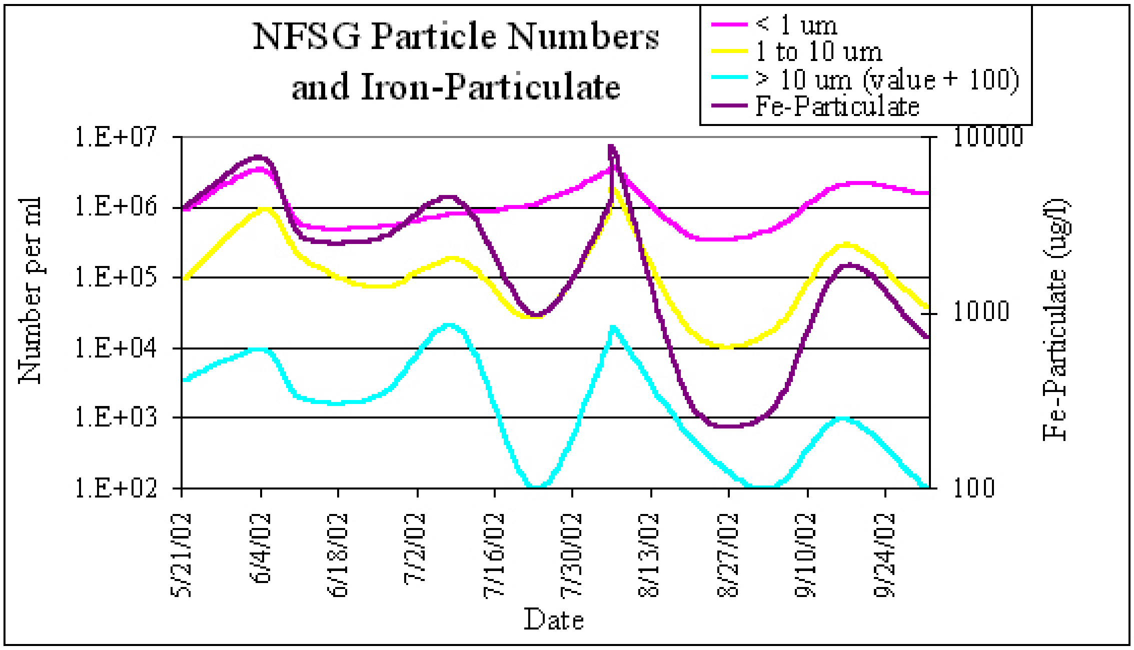

Figure 4. Concentration of suspended particles and iron-particulate.

These results show that the majority of suspended sediment particles are colloidal (less than 1 micron in diameter). The number of particles greater than 10 micron is very small (for ease of comparison to other data presented in Figure 4, 100 particles per ml was added to the particle numbers in this size fraction). Iron particulate in Figure 4 was computed as the difference between iron concentration in the raw (total) and filtered (dissolved) samples. Particle numbers in all size classes are correlated to particulate iron concentrations for all dates. This result is consistent with the fact that iron oxyhydroxide precipitates dominate the suspended sediments.

Particle number-based size distributions can be mathematically converted to volume-based size distributions assuming spherical particles. Particle-volume size distributions are directly related, assuming a relatively uniform density, to the size distribution of particle mass, and is used as a surrogate for mass-based size distributions.

As shown in Figure 5, during low flow conditions (e.g. July 23rd and October 1st), particles in the < 1 um fraction significantly contribute to volume % (~ mass %). This is consistent, especially for the latter date, with our observation of a greenish-white turbidity in the stream (see Sept/Oct photo in Figure 3).

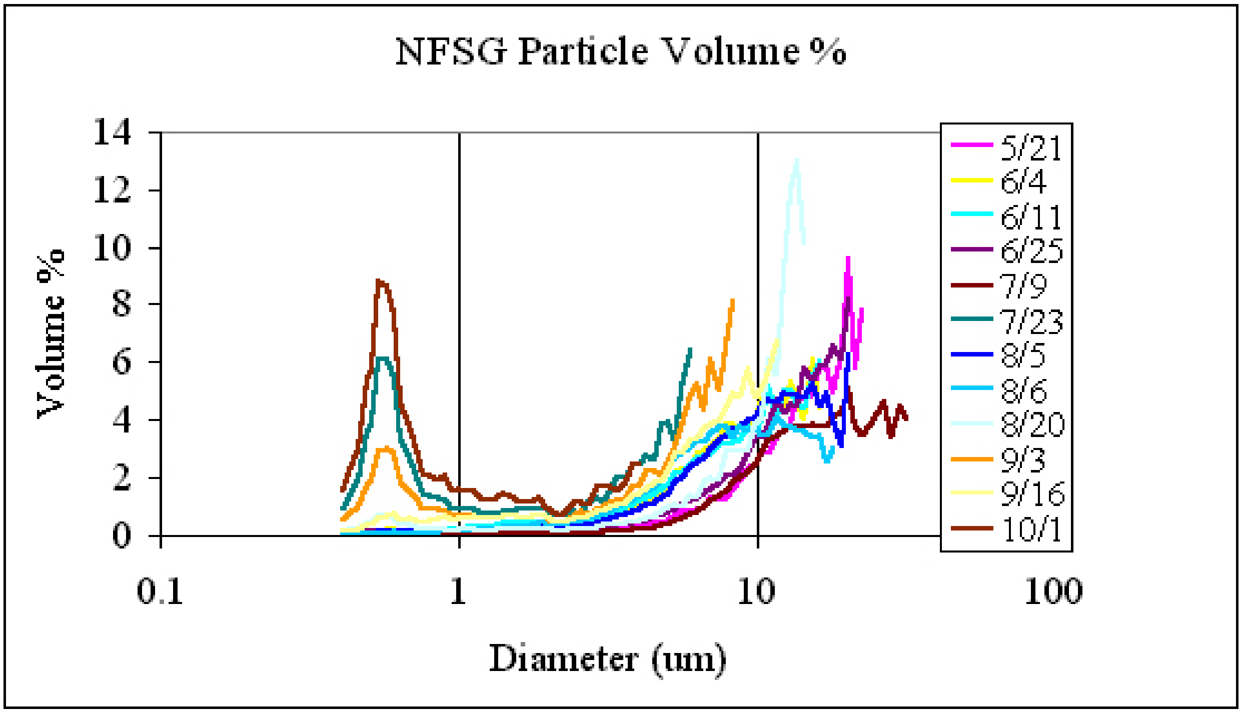

Figure 5. Volume-based size distribution over study period.

Filtration of samples left a reddish-orange colored material on the filter. This observation and the ICP-AES results for iron indicate the suspended sediments are primarily iron oxyhydroxides. The color in the water column may be a result of wavelength-dependent scattering by sub-micron colloids. The absence of larger particles is due to the low flow being unable to suspend these larger sediments. Thus, colloidal transport of metals may be important during low flows in NFCC. Larger particles (> 5 micron) are dominant at higher flows, during both spring runoff and the rainstorm event. During higher flows, the suspended sediments represent particles that are scoured from the streambed and re-suspended in the water column.

Results from ICP-AES analyses of four metals in the water column are shown in Figure 6. Although pH remains relatively constant over the study period (6.9 to 7.6, shown in Figure 6), it was observed that for the spring and summer rain events (June 4th and August 5th), the pH was lower. This has been seen in other AMD impacted stream systems (Ortiz et al., 1995; Rupert, 2001) and is likely due to flushing of more AMD into the creek during higher flow events. Figure 5a shows that iron is present only in the particulate phase at NFSG during the study period. Elevated levels of particulate iron coincide with the high flows that occur during spring runoff and the summer rainstorm.

Copper is predominantly in the particulate phase (66-96%) during higher flows and in the dissolved phase (0-26% particulate) during lower flows (Figure 5b). For most of the sampling dates, copper transport by suspended sediments was dominant.

Manganese (Figure 6c) is present only in the dissolved phase during all dates, with the exception of a spring rain event (June 4th), where 25% of the total manganese was in the particulate phase.

Manganese concentrations are also seen to increase over the course of the year and dissolved phase transport is always dominant.

Zinc in the particulate phase varies widely (0- 66%) and does not appear to have a clear temporal trend (Figure 6d). These results suggest that both dissolved and particulate zinc transport is important in NFCC.

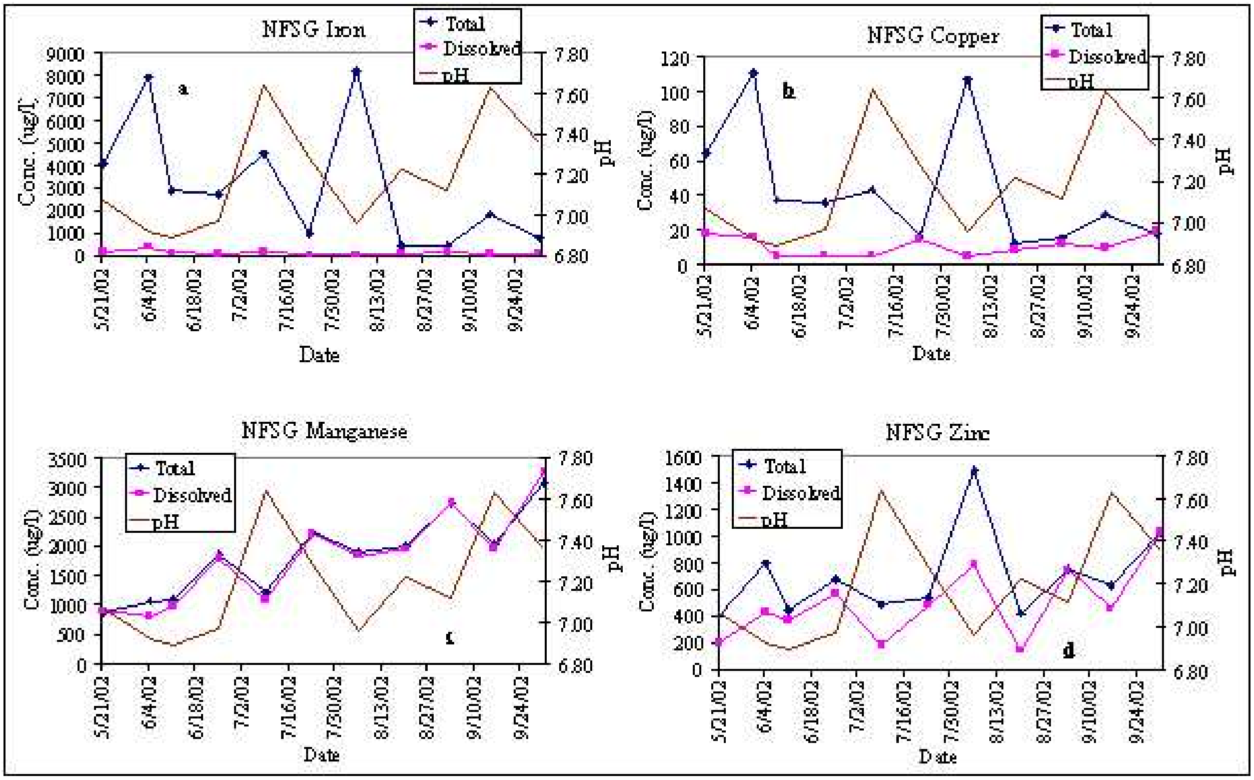

Figure 6. pH, total and dissolved iron (a), copper (b), manganese (c), and zinc (d) over time.

Bed Sediment

Sediment collected on May 21, 2002 from three different sites in NFCC varied greatly in size (Table 1) and sediment material.

Table 1. Bed sediment size distribution.

Sampling Site	Size Fraction	
	2 mm to 63 μm	< 63 μm
DS Gas Station (a)	98%	2%
DS Gas Station (b)	29%	71%
DS Gas Station (c)	82%	18%
Beaver Dam (BD)	4%	96%
NFSG	89%	11%

Three areas within a 15-foot reach of the creek were sampled at the ‘downstream Gas Station’ site. The sediment in this area was very diverse, including coarse sand, organic-rich sediments mixed with iron oxyhydroxides, and fine sand (Table 1 ‘DS Gas Station’ a, b, and c, respectively). Very fine iron oxyhydroxide floc dominates the sediments in the beaver dam. NFSG sediments were a mix of iron oxyhydroxides and coarse sand. Acid digestion and metals analysis of these sediments is not complete.

Rock Coatings

The surface area of the rocks was estimated from measurements of the major faces performed after removal of coatings. Based on this computation, an estimate of metal concentrations on a surface area basis was made (Table 2). The 0.5 M HCl easily removed the coatings present on the NFSG and BBD rocks collected in May. The black coating on the NFSG rocks collected in August was removed more slowly (about 10 days of submersion in the acid with occasional agitation), and included a black/brown colored 0.5 M HCl-insoluble fraction. This material was dissolved in concentrated HCl. From Table 2, it is seen that the dominant component of the coatings for all May samples is iron, which is consistent with the observed reddish color of the coatings.

Table 2. Major components of rock coatings.

Analyte	Site Name and Concentration (mg/cm^2 rock surface)				
	BBD-01	BBD-02	NFSG May 2002	NFSG August 2002	
Soluble in:	0.5 M HCl	0.5 M HCl	0.5 M HCl	0.5 M HCl	Conc. HCl
Iron	1114	819	551	309	324
Calcium	438	469	274	145	143
Aluminum	168	142	238	169	151
Manganese	90	47	91	259	360
Phosphorus	97	149	71	24	27
Zinc	76	38	56	96	106
Lead	36	7	1	1.2	1.6
Copper	30	10	12	6	6

A comparison of the manganese and iron concentrations in the August versus May rock coatings shows that manganese was higher in August and iron was lower. This is consistent with the observation of the observed black coating (expected to have been manganese oxides) and the lack of an orange coating (see Figure 3). In addition, it was found that the insoluble fraction of the rock coating in August was predominantly manganese (~100 mg/cm^2 rock surface more Mn was present in the concentrated HCl versus the 0.5 M HCl) with an iron component. These results indicate that the amount and composition of the rock coatings is not consistent throughout the study period, and may indicate a seasonal trend.

Summary

Iron is present in the NFCC system nearly entirely in the particulate phase and iron oxyhydroxides are observed to dominate the suspended sediments present in NFCC. *In-situ* precipitation of iron oxyhydroxides appears to occur during low flows and is especially prevalent in locations close to AMD inputs. Copper is also seen to be present to a significant extent in the suspended sediments. In contrast, Mn is essentially only present as a dissolved phase. Zinc is present in both the dissolved and particulate phases with no clear trend. These results are consistent with the known behavior of metals in AMD. Copper is known to be more readily adsorbed than Zn. Mn is problematic, as it has been shown to be difficult to remove from AMD.

During high-flow the suspended sediments are likely principally derived from the flocculated bed sediments. The amount of suspended sediments may represent an equilibrium between re-suspension and flocculation/settling, which is controlled by the hydrologic conditions. Elevated concentrations of metals associated with suspended sediments are transported during high flows, especially during rain events. Colloids dominate the suspended sediment during lower flow; these may be formed by precipitation in the water column. Colloids have been seen to be present during low flow conditions at various areas in the creek. These colloids are likely what is contributing to the increased mass % of suspended sediment in the < 1 um fraction during low-flow sampling dates.

There are a wide variety of bed-sediment types throughout the creek, as well as at a given site, using the ‘DS Gas Station’ site as an example. Bed-sediment composition and particle-size distribution changes during the year. As seen with the NFSG site, larger particles dominated in May, but over the sampling period, accumulation and loss of more fine-grained material (iron oxyhydroxides) was observed. Very fine iron oxyhydroxides dominate the May beaver dam sample and have been seen to dominate throughout the sampling period.

Fe, Ca, Al, and Mn (in decreasing order) comprise the majority of elements present in coatings on rocks at NFSG in May 2002. Mn, Fe, Al, Ca, and Zn (in decreasing order) comprise the majority of elements present in the black coatings (considering both the 0.5M HCl soluble and insoluble fractions) on rocks at NFSG in August 2002. Loosely associated rock coatings are likely to change over time in the creek due to scouring at higher flows and deposition at lower flows. It is currently uncertain as to the formation and fate of the harder, black (manganese-dominated) coatings on the rocks. The observed differences in both the rock coatings and the bed sediments may affect metal attenuation in NFCC.

Future studies will include further examination of particle formation, the relationship of particle formation to water column metal concentrations, particle transport, and the effect of the Waste-Water Treatment Plant (WWTP) on particulates and metal concentrations. Additionally, the relationship between suspended sediment and coatings (loose and armor) on rocks will be examined.

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Poster: Harvey, B.B., Ranville, J.F., Ross, P.E., Pearson, J., Walski, K., Clements, W. Transport and Metal Chemistry of Sediments in a High-Gradient Mountain Stream Impacted by Mining, Presented at the 2002 Annual Meeting of the Geological Society of America, Oct. 27-30, 2002, Denver, CO. Abstract published in GSA Abstracts with Programs Volume 34, No. 6, September 2002.

Poster: Harvey, B.B., Ranville, J.F., Ross, P.E., Pearson, J., Walski, K., Clements, W. Transport and Metal Chemistry of Sediments in a High-Gradient Mountain Stream Impacted by Mining, Presented at the 23rd Annual North American Conference of the Society of Environmental Toxicology and Chemistry, Nov. 10-14, 2002, Salt Lake City, UT. Abstract published in SETAC Abstracts, 23rd Annual Meeting.

Poster: Harvey, B.B., Ranville, J.F., Ross, P.E., Pearson, J., Walski, K., Clements, W. Transport and Metal Chemistry of Sediments in a High-Gradient Mountain Stream Impacted by Mining, Presented at the 2003 Annual Meeting of the Rocky Mountain Regional Chapter of the Society of Toxicology and Environmental Chemistry, April 16, 2003, Denver, Colorado. *Best Student Presentation Award.*

Paper/Oral Presentation: Harvey, B.B., Ranville, J.F., Ross, P.E. Seasonal Changes in the Characteristics of Suspended Sediment Metal Transport in a Mining-Impacted Stream, Presented at the 2003 Joint Conference of the 9th Billings Land Reclamation Symposium and the 20th Annual Meeting of the American Society of Mining and Reclamation, Billings MT, June 3-6, 2003. Published by ASMR, 3134 Montavesta Rd., Lexington, KY 40502

The Role of Organic Matter and Metal Oxides in the Retention of Trace Metals by Soil and Suspended Particles

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Center for the Study of the Bioavailability of Metals in the Environment

Introduction

Contamination of surface waters with trace metals has become a serious problem in many regions of the USA and all over the world. To assess the risk of water contamination in any area it is necessary to predict the impact of different kinds of management practices on water quality based on general data on soil and water properties available from the soil surveys and monitoring of surface water composition. Soils provide an important source of trace metals to rivers, both in the dissolved form through leaching and in the particulate form through erosion (McColl & Pohlman, 1986; Rasmussen, 1986; Bergkvist, Folkeson & Berggren, 1989). Furthermore, the bioavailability, and thus potential toxicity or deficiency, of trace metal ions in soils and sediments depends largely on their concentrations in the pore waters and the ability of the soil matrix to uptake or release them. The Center is developing a "Unit World Model" for fate and exposure of metals. Interaction of dissolved metals with natural particles is a key component of this model.

It is generally accepted that both natural organic matter and metal oxides, both of which are ubiquitous in natural particles, are capable of sorbing large amounts of trace metal ions (Bunzl, Schmidt & Sansoni 1976; Kuo & Mikkelsen 1980; Barrow 1986 a&b; Ainsworth et al. 1994; Backes et al. 1995). Metal uptake and release may strongly depend on metals content, soil organic matter (SOM), iron and manganese oxides-hydroxides and clay fraction (Kabata-Pendias, 2001; Sauvé, 2002). Solution pH, dissolved organic matter (DOM) content and ionic strength (especially the calcium concentration) may be the principal solution properties affecting the metals' uptake and release. These properties may influence both equilibrium relationships and desorption kinetics. Moreover, the release of trace metals from the soil particles appears to be a slow process and equilibrium between solid and solution hardly can be attained in soils (Sparks, 1988). However, the mechanisms underlying the sorption and desorption processes have not been established. Nonetheless, there have been some consistent observations that are discussed in the following paragraphs.

Two contiguous phases have been invariably observed in trace metal sorption and desorption by soil particles and their components (Bunzl, Schmidt & Sansoni 1976; Kuo & Mikkelsen 1980; Barrow 1986 a&b; Ainsworth et al. 1994). The initial rapid phase is followed by the second phase, which is very slow and prolonged. There is clear and growing evidence that the sorption process takes days to months and even longer to reach equilibrium (Bruemmer, Gerth & Tiller 1988). Ecotoxicological effects of trace metals in spiked soils and sediments are based on the solution phase concentrations. As equilibrium is approached, the continued sorption during the slow phase will have a dramatic effect on the solution phase concentration. At pH 6.41, the initial Zn^{2+} adsorption by goethite accounted for 80% of the initial loading within the first 2 hours. The subsequent slow phase was observed to continue for 42 days and beyond (Barrow, Gerth & Bruemmer 1989). So, depending on one's choice of the period (beyond 2 hours) for equilibrium, the ratio between the solution phase and the solid phase could vary tremendously. Therefore, the kinetics of the slow process should be considered to assess the environmental risk of trace metals in soils and sediments.

Another phenomenon that has been observed is the desorption hysteresis; the desorption isotherm does not coincide with corresponding sorption isotherm (McKenzie 1967; Padmanabham 1983; Ainsworth et al. 1994; McLaren et al. 1983, 1986 & 1998; Strawn &

Sparks 2000). What this suggests is that the sorption and desorption behaviors are different. For example, the partition coefficient will depend on whether a sorption or a desorption process is taking place. Thus models using partition coefficients based only on sorption may lead to unrealistic prediction for desorption situations.

Yet another phenomenon that relates sorption with desorption is that of the “aging effect.” It has been shown that the percentage of metals desorbable decreases as sorption time increases (Nelson & Melsted 1955; Kuo & Mikelsen 1980; McLaren, Lawson & Swift 1986; Ainsworth et al. 1994; Backes et al. 1995; McLaren et al. 1998; Eick et al. 1999; Ford et al. 1999). This phenomenon suggests a natural sequestration of metals in the solid phase and subsequent decrease in bioavailability of metals over time. However, a consensus on the extent of the aging effect and the underlying mechanisms is lacking. Thus we have initiated investigation of metal uptake and release by natural particles with particular focus on the mechanisms involved in the aging effect and hysteresis.

Most of the studies on trace metal-NOM interaction are predicated on chemical kinetics although there is clear and growing evidence for the possibility of physical processes like intra-particle diffusion. NOM has been suggested to consist of two phases: glassy and rubbery (Weber & Huang. 1996; Weber et al., 2001). The crystal-like glassy phase is suggested to contain micro-pores into which organic pollutants could diffuse. Metal oxides, goethite in particular, have also been used to study sorption and desorption of trace metals. Solid-state diffusion within oxide particles (Bruemmer et al. 1988), diffusion into micropores and intraparticle spaces (Backes et al. 1995) is very likely involved.

Based on the existing studies, the following hypothesis is proposed for the sorption and desorption of trace metals by natural particles. First, metals sorb on the external organic matter, then proceed to diffuse into interior oxides. The adsorption in the external layer is relatively rapid and dominates the initial stage. The subsequent diffusion of adsorbed metals into the oxide is characteristically slow and is thus the rate-limiting process. In this case, hysteresis occurs because oxides have greater affinity towards metals than does the organic matter. The aging effect is observed because with increasing sorption time, the fraction of sorbed metals that have reached the interior will increase and thus the subsequent desorption is increasingly difficult.

Objective

The objective of this project is to develop models to predict Cd, Cu, Ni, and Zn uptake and release from soil particles into the soil solutions. The models will focus on kinetics aspects and thus will essentially improve the existing approaches based on equilibrium assumptions. The effect of soil properties (organic matter, oxides, and metal loading, etc.) and solution composition (pH, dissolved organic matter, and Ca content) on Cd, Cu, Ni and Zn release will be studied to find the necessary relationships and to evaluate the parameters of the model. Key soil and solution properties will be selected to use the minimal number of input parameters providing reasonable accuracy of predictions. The developed models will form an important component part of the “Unit World” model of metal behavior in the environment being designed in the Center for the Study of Metals in the Environment.

We have adopted two complimentary approaches in our studies. In the first approach we are preparing particles with controlled physical and chemical properties. Sorption and desorption of metals onto these particles provides a direct check of the models. In the second approach, we are using natural soil particles.

Materials and Methods

Synthesis of particles

Goethite (α-FeOOH) was synthesized by the procedure recommended by Schwertmann & Cornell (1991). Commercial sand (60/80-grade Accusand by Unimin Corp., Le Suer, MN) was coated with goethite by the method of Scheidegger, Borkovec and Sticher (1993). The $NaNO_3$ concentration and the pH of the suspension were varied to achieve different amounts of coating. The amount of goethite coated on the particles was determined by microwave-assisted digestion and analysis of the iron that was released. Pure goethite was also digested and the recovery of the procedure was determined to be 93.7 ± 6.4 %. The BET analyses involving N_2-adsorption and the 5 Point BET method was performed using High Speed Gas Sorption Analyzer (Quantachrome Corp., NOVA 2000). For the standard reference material, aluminum oxide, of specific surface area (5-point BET) 0.576 m^2/g with 95% confidence interval for reproducibility of ±0.046, the measured value was 0.569± 0.008 m^2/g. The densities of the particles were measured by the standard method.

Soil humic acid (HA) was extracted from natural soil in accordance with the method (Swift 1993) recommended by International Humic Substances Society. Total organic carbon (TOC) analyses showed that 2.84mg of HA corresponded to 1mg of TOC. For preliminary investigation, 3g of particles was suspended in 5 ml 0.01 M $Ca(NO_3)_2$ solution and HCl or NaOH are added to achieve a desired electrolyte concentration and $pH \approx 4$. After a few hours of stirring, desired amount of HA was added from stock solution (prepared from dissolving Aldrich HA in 0.01 M NaOH solution). The pH was adjusted to $pH \approx 4$ and the suspension was then shaken continuously for 10 hours at 22°C. The final TOC of the supernatant was analyzed. The particles were washed and analyzed for TOC to determine the coating. The resulting HA-coated particles were washed and equilibrated for 5d in 0.01 M $Ca(NO_3)_2$ to investigate the desorption of the HA coating. A 13 ± 4% desorption of the coated HA was observed.

Soil samples

The study was carried out using a Matapeake silt loam soil from Delaware and a Codorus clay loam soil from Maryland (Table 1). Both were obtained from the 0-10 cm layer. Portions of each soil were spiked with Cu (48 mg/kg) and Zn (103 mg/kg) using metal salts. The moist soil was mixed, then air-dried for 4 days.

Dissolved organic matter was separated from the water of the Edisto River in South Carolina by reverse osmosis. The trace metals in organic matter were removed by acid washing of the precipitated humic acid and cation exchange of the fulvic acid. The humic and fulvic acid fractions were recombined prior to their use in our experiments. DOM stock solution was added into 3 mM $Ca(NO_3)_2$ solution to reach the desired DOM concentrations.

Table 1. Soil parameters

Soil type	pH (H_2O, 1:1)	Sand (%)	Silt (%)	Clay (%)	WB-OM (%)	Cu (mg/kg)	Zn (mg/kg)
Matapeake silt loam	6.38	13	63	24	2.46	21.4	73.5
Codorus clay loam	6.05	27	45	28	2.68	14.2	58.5

A stirred-flow method (Yin et al. 1997), which minimizes diffusion effects and readsorption phenomena by effective mixing in the reaction chamber and continuously removing desorbed products from solution, was used to study the Cu and Zn release from the soil particles. Soil was ground to pass a 2-mm sieve and 0.3 g samples were placed in a 6.4 mL stirred flow chamber and then leached at 22 ± 2 ^{o}C with a solution containing $Ca(NO_3)_2$ as a background electrolyte. The solution was buffered at constant pH with MES buffer.

A first order equation described the kinetics of Cu and Zn desorption from soils: $q(t)=q_1*(1-exp(-k_d*t)+ q_2$. The total desorbable metal is $q_1 + q_2$ and k_d is the desorption rate coefficient.

Results

Sorption experiments

Sorption experiments with Cd, Cu, Ni and Zn have been initiated on goethite-coated particles. All of the experiments have been conducted at pH $\approx$ 5.85, buffered using 1 mM MES, and with $Ca(NO_3)_2$ concentration of 10 mM. The samples were equilibrated for 12h on a shaker before introducing Cu. The samples were then shaken for appropriate period of time and filtered. Then the metal concentrations of the resulting supernatant solutions were determined by ICP-EOP. The following results are for Cu sorption.

Figure 1 shows the kinetics of Cu uptake by three different goethite-coated particles. The sorption of Cu by goethite-coated particles does not reach equilibrium even after 45h. This shows that kinetics could play a major role in the uptake of trace metals by natural particles. Second, the larger the amount of coating the greater the extent of sorption as expected due to greater sorbent availability. Figure 2 shows the corresponding solid phase concentrations based on mass balance on Cu. The sorption process is hypothesized to be a two-phase process: initial adsorption on the external surface and subsequent diffusion into the interior of the coated goethite layer. If this is the case, then the initial sorption will be dependent on the total surface area and the total sorption on the amount of goethite coating.

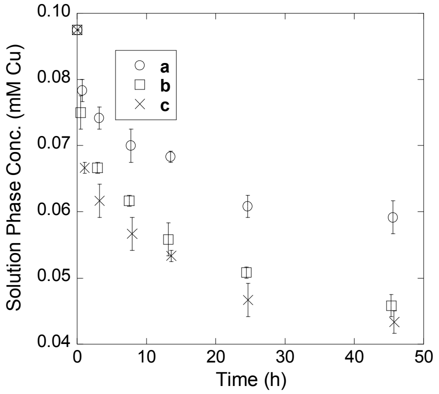

Figure 1. The kinetics of Cu sorption (solution concentration vs. time) by goethite coated sand particles a, b and c with 0.29 (± 0.04), 0.57 ((± 0.03) and 1.34 (± 0.04) mg goethite/g sand, respectively.

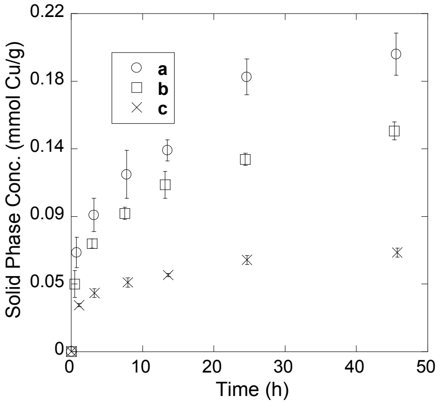

Figure 2. The kinetics of Cu sorption (solid phase concentration vs. time) by goethite coated sand particles a, b and c.

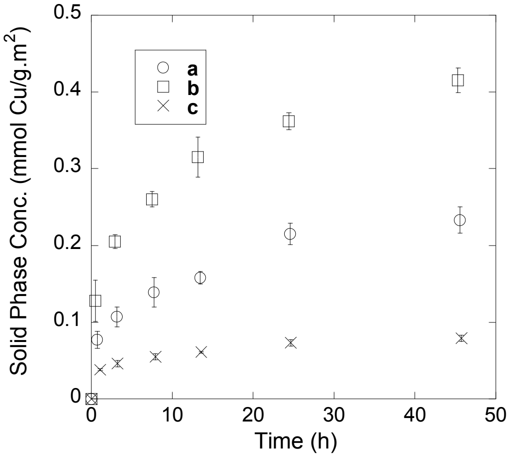

Figure 3. Solid phase concentrations adjusted for the amount of coating and the total surface area of the coated particles a, b and c.

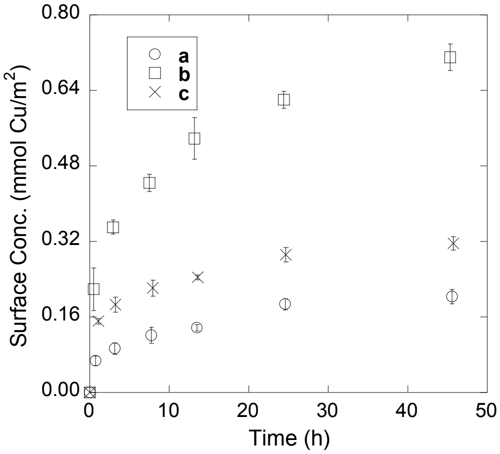

Figure 4. Surface concentrations based on the specific surface area of the particles, 0.283 (±0.002), 0.118 (±0.002), 0.278 (±0.004) m^2/g for a, b and c, respectively.

In particles with similar specific surface area but different amount of coating, the sorption process will be similar initially but more prolonged for the particle with greater amount of coating (compare particles a and c). Where as for particles with similar amount of coating but different specific surface area, the initial rate will be different. Figures 3 and 4 shows the surface area normalized solid phase concentration of Cu and surface density of the sorbed Cu, respectively.

The above data was modeled using two different models; One treats the adsorption and desorption as chemical processes and the other treats sorption as a diffusion process. The following is the formulation of the chemical model. For this model, the kinetic parameters- adsorption and desorption constants- are determined by minimizing the root mean squared value between the observed and the modeled values.

$$C_T = C + C_p; \quad r = \frac{C_p}{m}$$

$$C + m \quad \overset{k_{ads}}{\underset{k_{des}}{\leftrightarrow}} \quad C_p$$

$$\frac{dC}{dt} = -k_{ads}Cm + k_{des}C_p$$

$$= -k_{ads}Cm + k_{des}\left(C_T - C\right)$$

$$\Rightarrow \frac{dC}{dt} + \left(k_{ads}m + k_{des}\right)C = k_{des}C_T$$

$$\textit{With Boundary Condition at } t = 0, C = C_T = C_0$$

$$C = \left(\frac{k_{des}}{k_{ads}m + k_{des}}\right)C_0 + \left(\frac{k_{ads}m}{k_{ads}m + k_{des}}\right)C_0 \exp\left(-\left(k_{ads}m + k_{des}\right)t\right)$$

The diffusion (or penetration model) follows and it is illustrated in Figure 3. In this model, the concentration of the metal at the particle surface-solution interface is constant and this surface density or the concentration drives the diffusion of the metals into the interior of the particle. The assumption that initial surface density is a non-zero constant is obviously not true for the Cu data as presented. However, given the apparently fast the initial adsorption on the surface, it could be realistic. This is a simple model and will have to be modified to account for the initial adsorption to match our hypothesis of initial adsorption followed by diffusion.

$$\frac{\partial r}{\partial t} = D\frac{\partial^2 r}{\partial x^2}$$

$$\textit{Boundary Conditions}:$$

$$\textit{at } x = 0, r = r_0,$$

$$\textit{at } x = \infty, r = 0 \textit{ and}$$

$$\textit{at } t = 0, r = 0$$

$$r = r_0 erfc\frac{x}{2\sqrt{Dt}}$$

$$C = C_0 - mr_0 erfc\frac{x}{2\sqrt{Dt}}$$

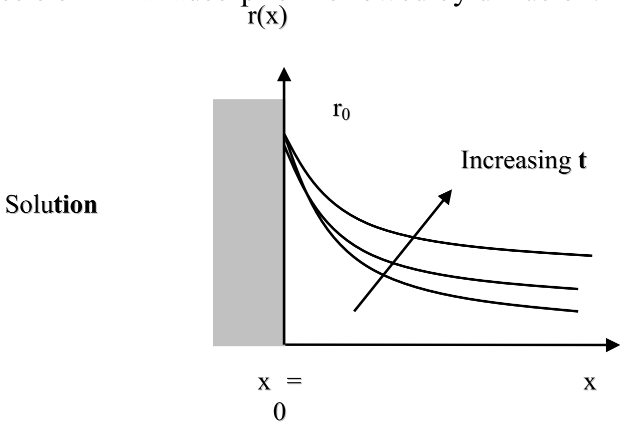

Figure 5. Schematic of diffusion results.

Figure 6 and 7 shows the predictions based on the chemical and diffusion models, respectively. The corresponding parameters as determined from the model optimization are shown in Table 2 and 3, respectively.

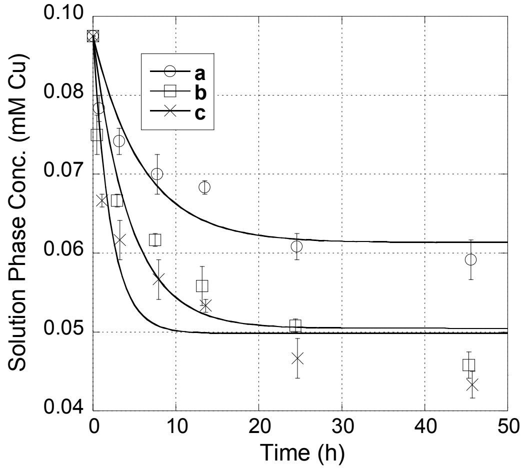

Figure 6. Fitting the chemical model to the sorption kinetics of Cu by goethite coated particles a, b and c.

Particle	a	b	c
m (g/L)	0.1742	0.3418	0.8040
k_{ads}(L/gh)	0.2874	0.2472	0.2036
k_{des}(1/h)	0.0932	0.0831	0.1536
$K_p=k_{ads}/k_{des}$(L/g)	3.084	2.975	1.326

Table 2. Parameters as determined by model fitting in Figure 6.

Comparing the model predictions and the observed data points, the models are under-predictive for shorter time periods (< 20h) and over-predictive for longer time periods (>20h). However, the diffusion model is the better of the two. From the estimated parameters for the chemical model, the partition coefficient of Cu on goethite is similar for particles a and b, but that for particle c is significantly lower. Similar observation is also made for the second parameter involving the penetration depth (x) and the diffusion coefficient (D) in the diffusion model estimates (Table 3). Whether or not such a discrepancy is due to the difference in particle properties is to be validated with the use of particles with different coatings in the future.

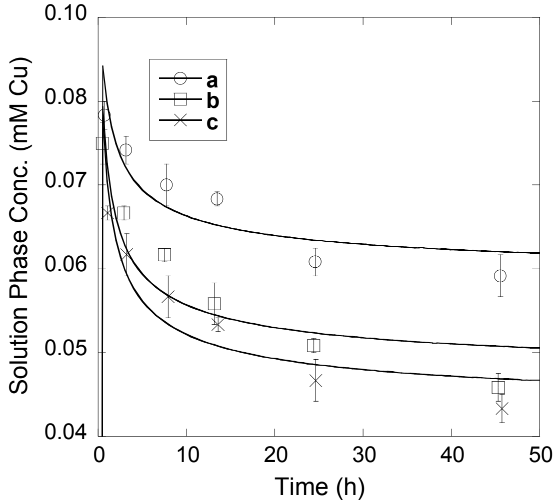

Figure 7. Fitting the diffusion model to the sorption kinetics of Cu by goethite coated particles a, b and c.

Particle	a	b	c
r_0 (mmol/mg)	0.214	0.1588	0.0707
$x/(2\sqrt{D})$ ($h^{1/2}$)	0.0896	0.0829	0.697
rms error	0.0442	0.0667	0.0591

Table 3. Parameters as determined by model fitting in Figure 7.

Desorption from soils

The effect of flow rate was studied by varying flow rate at the following values: 0.5 mL/min, 1 mL/min, 2 mL/min, and 4 mL/min (Figure 8 and 9). Spiked Matapeake soil was used in this study. Experiments were carried out at fixed Ca concentration (3 mM) and constant pH (5.5). Higher flow rates increased Cu and Zn release from spiked soils.

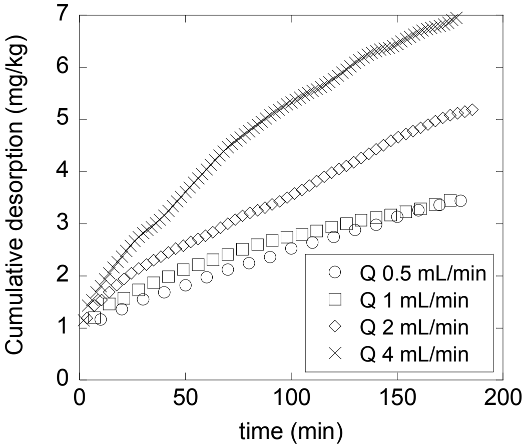

Figure 8. Effect of flow rate on Cu desorption from spiked Matapeake soil (pH=5.5, [Ca]=3mM).

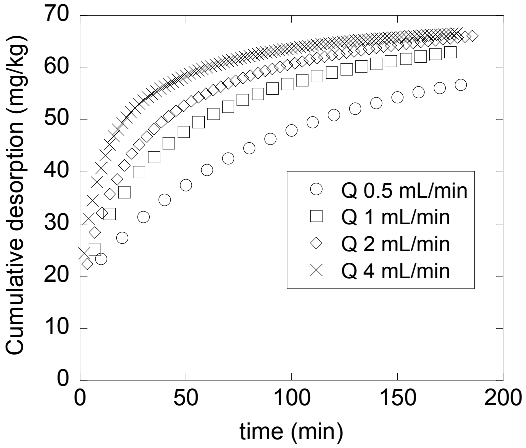

Figure 9. Effect of flow rate on Zn desorption from spiked Matapeake soil (pH=5.5, [Ca]=3mM).

The effect of pH was studied by varying the pH in leaching solutions containing MES buffer. The flow rate was 1 mL/min. Solid lines in Figures 10, 11, 12 and 13 are 1st-order model fittings. Decrease of pH enhances Cu and Zn release from spiked soils. Total desorbable Cu and Zn are negatively correlated with solution pH as is shown in Figures 14 and 15, respectively.

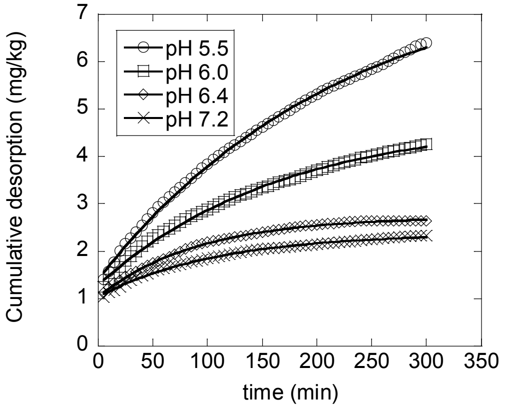

Figure 10. Desorption of Cu from spiked Matapeake soil at different pH ([Ca] = 3mM).

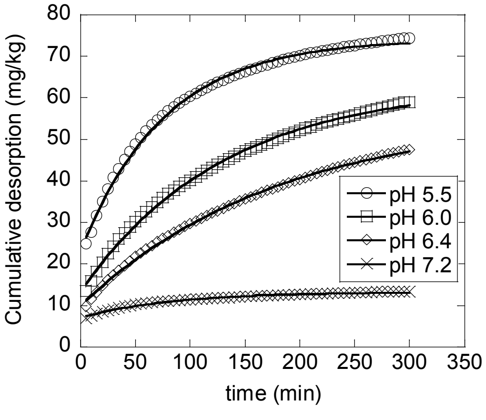

Figure 11. Desorption of Zn from spiked Matapeake soil at different pH ([Ca] = 3mM).

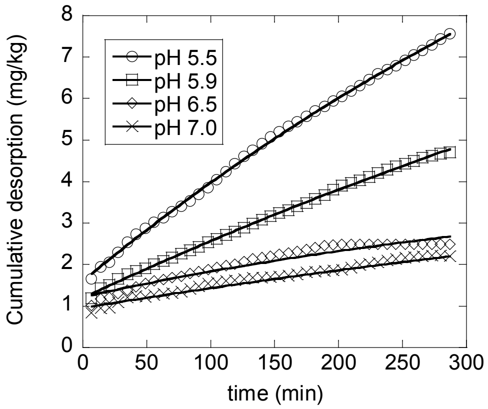

Figure 12. Desorption of Cu from spiked Codorus soil at different pH ([Ca]=3mM).

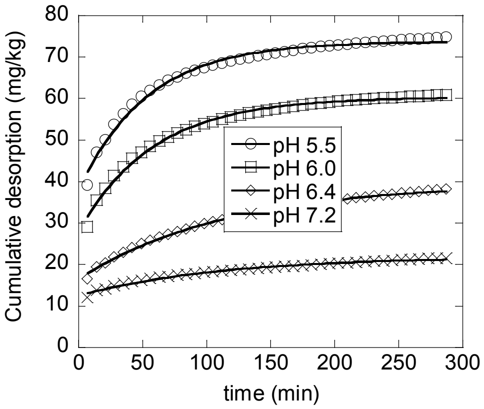

Figure 13. Desorption of Zn from spiked Codorus soil at different pH ([Ca]=3mM).

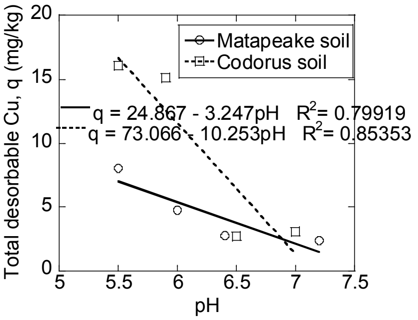

Figure 14. Correlation of total desorbable Cu with pH.

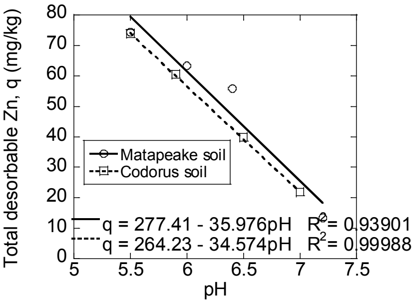

Figure 15. Correlation of total desorbable Zn with pH.

The effect of DOM was studied by varying DOM concentrations in leaching solutions. The flow rate was set to 1 mL/min. Solid lines in figures are 1st-order model fittings (Figure 16 and 17). DOM greatly enhances Cu release from spiked soils, but it has little effect on Zn release (data not shown). Cu desorption rate coefficients are positively correlated with DOM concentrations (Figure 18).

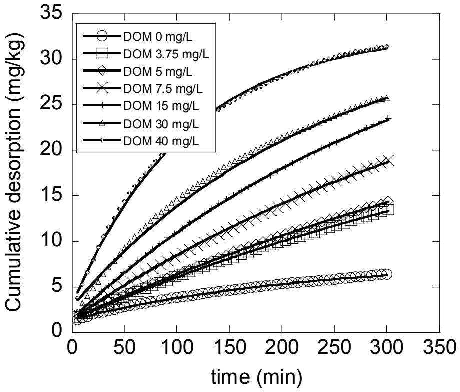

Figure 16. Desorption of Cu from spiked Matapeake soil at different DOM concentrations (pH=5.5, [Ca] = 3mM).

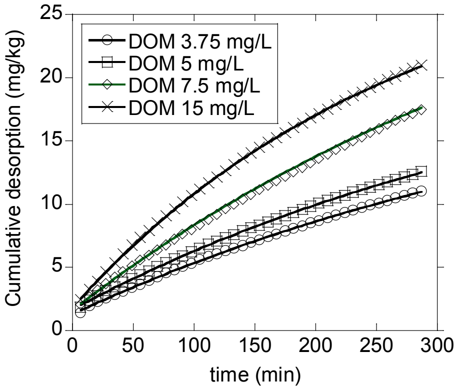

Figure 17. Desorption of Cu from spiked Codorus soil at different DOM concentration (pH=5.5, [Ca] = 3mM).

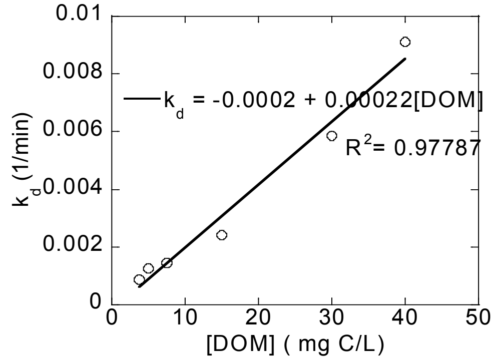

Figure 18. Correlation of Cu desorption rate coefficient with DOM concentration.

The effect of Ca was studied by varying the Ca concentrations in leaching solutions ranging from 1 mM to 10 mM (Figures 19 and 20). The flow rate was set to 1 mL/min. Ca concentration has little effect on either Cu or Zn release from spiked soils.

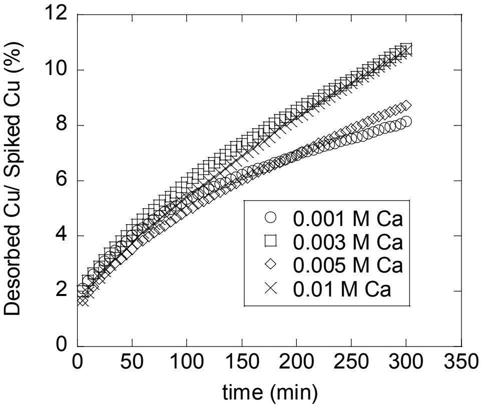

Figure 19. Effect of Ca on Cu desorption from spiked Matapeake soil (pH=5.5).

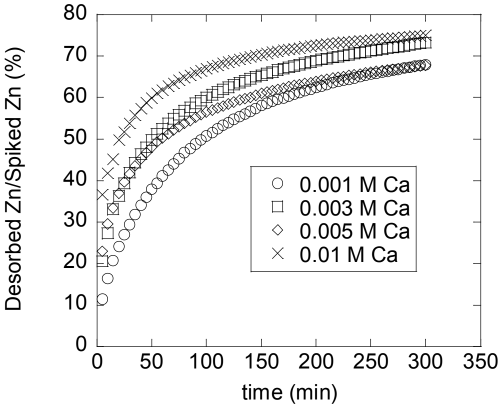

Figure 20. Effect of Ca on Zn desorption from spiked Matapeake soil (pH=5.5).

Future Investigations

The sorption of Cd, Ni and Zn, in addition to Cu, on goethite-coated particles has been initiated and the desorption will follow. This change has allowed us to obtain higher concentrations of organic matter on the particles which better simulates the percentage found in field soils. Analysis of literature data that was not shown in this report indicates that the organic matter coating of the soils does indeed govern the sorption kinetics and that the rate depends on the concentration of the soil organic matter. A model of the process is under current development and the experiments using organic-coated goethite will be an important part of the validation.

We have initiated development of a kinetic model for both the adsorption and the desorption of metal from soils. This is being used both to design experiments and for the evaluation of experimental results. We are expanding to include Cd and Ni in the studies and we will determine the effect of solution chemistry on Cd and Ni desorption from soils. We will then begin the study of the effect of soil properties on trace metals desorption from soils. This will allow us to design and validate models to predict the effect of pH, dissolved organic matter, Ca, and soil properties on trace metals desorption from soils.

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Developing a Model to Predict the Persistence of Metals in Aquatic Environments

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Center for the Study of the Bioavailability of Metals in the Environment

Introduction

The overall scope of the project is directed at the development of methods to evaluate environmental hazards associated with the release of metals and metal compounds to the environment. This is largely being addressed through the development of computer models to assess the transport, fate, and bioavailability of metals in aquatic systems. Processes to be incorporated into the model include:

- hydrodynamics and sediment transport as determined by the type of water body, flow, density structure of the water column, settling, resuspension, and burial rates
- water and sediment chemistry, including pH, alkalinity, hardness, and concentrations of DOC, POC, carbonate, iron, manganese, and sulfide, and
- equilibrium and kinetic reactions for chemical speciation of metals in the water column and sediments

The model will be applied to mesocosm studies and selected field sites for model calibration and validation. Based on these results, a screening level model for the transport and fate of metals in aquatic systems will be developed for an intact aquatic environment; i.e., a "unit world." Toxicological effects of metals in the water column and sediment compartments will be included by the addition of suitable representations of metal bioavailability.

During the first year of the project, we have:

- conducted preliminary model evaluations using available metals data (e.g., Perch Lake, the Canadian Experimental Lakes)
- reviewed computer models that could in part be used in developing a more detailed modeling approach
- developed a more detailed modeling approach for assessing the transport, fate and bioavailability of metals in aquatic systems
- developed a coupled water column/sediment flux model for organic carbon and nutrients to serve as the basis of our lake metals model
- laid out plans for developing a "unit world" model(s) that can ultimately be used in regulatory decision making.

Further information on each topic is given below.

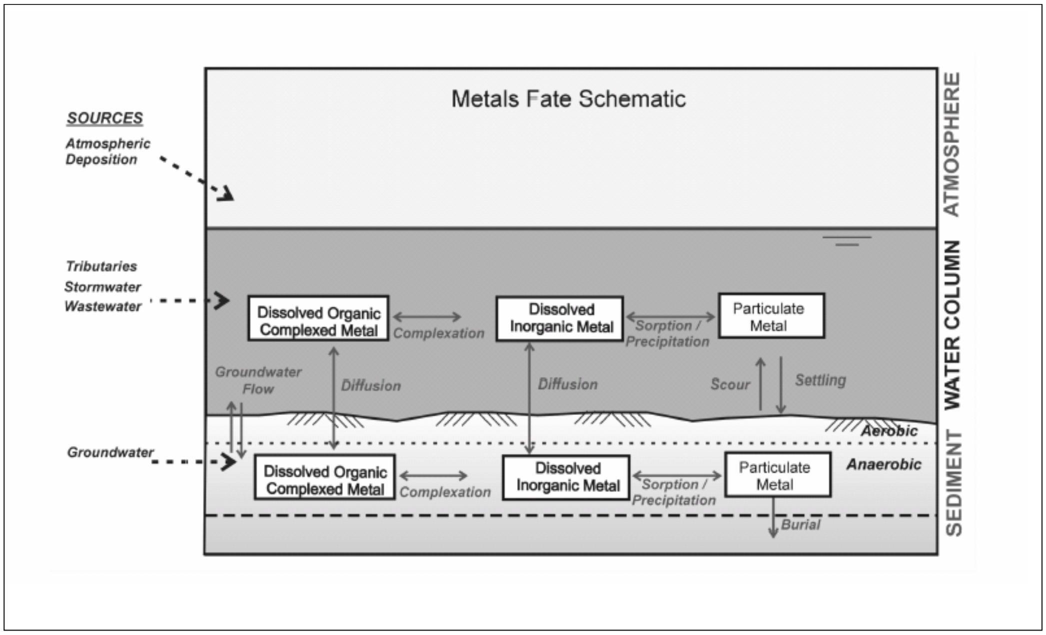

Figure 1. Scematic diagram for the fate of metals in aquatic environments

Preliminary Model Evaluations

A conceptual model framework for the transport and fate of metals in aquatic systems is given in Figure 1. In this diagram, metals enter the aquatic environment via surface runoff, storm water flow, groundwater inflow, atmospheric deposition, and direct discharges of wastewater. In the water column and sediments, the metals are distributed among the dissolved inorganic phase (which can include both the ionic form and inorganic complexes of the metal), the dissolved organically complexed phase, and the particulate phase (which can include sorbed metal, newly precipitated metal, and stable mineral phases). The specific distribution of metals will depend on chemistry in the water column and the sediments. For example, the pH, hardness, salinity, total dissolved solids, and dissolved organic carbon impact the distribution of metals in the water column, and particulate concentration of metal in anaerobic sediments typically include significant levels of metal sulfides. The transfer of metal between the water column and the sediment is dependent on the physics of the system (which controls the movement of water and particles), and on the chemistry (which controls the distribution of metal among dissolved and particulate phases).

For preliminary model evaluations, the distribution of metal between the dissolved and particulate phases is assumed to be in a dynamic equilibrium and described by equilibrium partition coefficients following previous approaches that have been developed for hydrophobic organic contaminants (e.g., see DiToro et al., 1991). A time-variable version of the model was constructed in Visual Basic Applications (VBA) for EXCEL to examine field data from enclosures in Perch Lake (Diamond et al., 1990). In the field study, fifteen radioisotopes (^{22}Na, ^{57}Co, ^{59}Fe, ^{63}Ni, ^{65}Zn, ^{74}As, ^{85}Sr, ^{99}Tc, ^{113}Sn, ^{125}I, ^{131}I, ^{133}Ba, ^{134}Cs, ^{203}Hg, and ^{241}Am) were added to the enclosures and monitored in time as the radioisotopes were lost from the water column via transfer to the sediments and radioactive decay. After twenty one days, the overlying waters in some enclosures were flushed for three days, a new enclosure was placed above the contaminated sediments, and the overlying waters were monitored for an additional fifty days to examine the release of the radioisotopes from contaminated sediments.

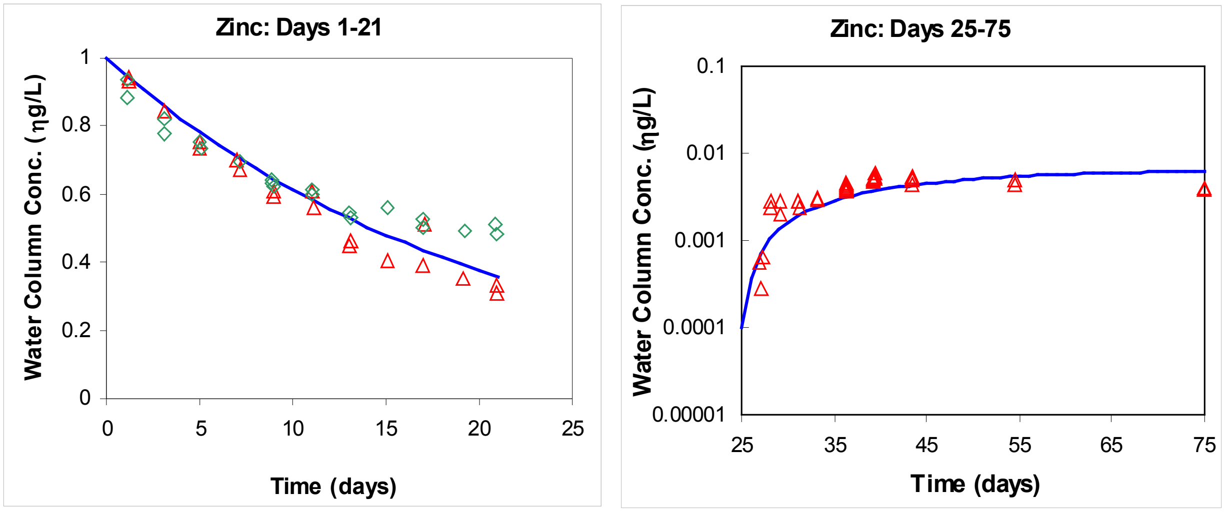

Figure 2. Comparison of model results and field data for ^{65}Zn concentrations in the water column. Data from Diamond et al. (1990).

For our model evaluations, apparent equilibrium partition coefficients for metal distributions between the particulate and dissolved (organic + inorganic) phases were adjusted separately for the water column and surficial sediment layer to obtain a close correspondence between model results and field data. All other model parameters and coefficients were taken directly from Diamond et al. (1990). Results for zinc (^{65}Zn) concentrations in the overlying water are shown in Figure 2 for both the initial contamination period and subsequent release of zinc from sediments. For the metals analyzed to date (^{59}Fe, ^{65}Zn, ^{74}As, ^{113}Sn, ^{203}Hg), partition coefficients for the overlying water varied by only a factor of 3 (from $10^{4.9}$ to $10^{5.4}$ L/kg) suggesting that partition between particulate organic matter and dissolved organic matter may be playing an important role in Perch Lake. Partition coefficients for ^{59}Fe, ^{65}Zn, ^{113}Sn, and ^{203}Hg in the sediments were typically about a factor of two lower than partition coefficients for the water column. Similar behavior has been observed for hydrophobic organic contaminants and is oftentimes related to a lower fraction of organic carbon on sediments. Finally, the partition coefficient for ^{74}As in sediments was approximately an order of magnitude lower than the water column partition coefficient and may be associated with arsenic redox chemistry.

Additional studies for metals is continuing for Perch Lake and for other experimental systems; e.g., MERL (Adler et al., 1980), MELIMEX (Gachter, 1979), and the Canadian Experimental Lakes (Hesslein and Broecker, 1980).

Review of Current Models for Metal Speciation and Organic Carbon Cycling

Although simple partitioning models are useful for preliminary evaluations of field data, these models do not provide a predictive method for determining distributions among the various metal phases, or ultimately, the transport, fate and bioavailability of metals in aquatic environments. In addition, simple partitioning models do not provide any information on how water chemistry may change in time or space (e.g., seasonal variations in organic carbon cycling and redox chemistry that are associated with phytoplankton growth and organic matter decomposition). A number of models have previously been developed to address issues of metal speciation and bioavailability, and organic carbon cycling and redox chemistry in aquatic systems. A brief review of available models that may be considered as part of our development of the "unit world" model for metals is given in Table 1.

Table 1. Modeling frameworks that will be considered in the development of the "unit world" model for metals in aquatic environments

Model	Description
MINEQL+ (Schecher and McAvoy, 1998)	A generalized chemical equilibrium speciation program. Pieces of the program may be used in developing a database of chemical equilibrium constants for complexation and precipitation reactions.
WHAM, Version 6 (Centre for Ecology & Hydrology, 2002)	A generalized chemical equilibrium speciation program. Pieces of the program may be used in developing model descriptions for metal complexation to dissolved organic carbon (DOC) and to particles.
RCA (HydroQual, 1992)	A generalized water quality model that has been applied to the cycling of nutrients, organic carbon, and dissolved oxygen in a number of eutrophication studies. Pieces of the RCA model may be used in describing vertical transport in lakes, and in defining organic carbon distributions and redox conditions in the water column.
Sediment Flux Model (DiToro, 2001)	A generalized model for describing diagenesis, redox chemistry, precipitation, and partitioning of nutrients and metals to sediments. The model may be used in describing redox conditions, sulfide and iron distributions, and metal precipitation and sorption to sediments.
AQUATOX (USEPA, 2000)	A generalized ecosystem model that represents the combined environmental fate of nutrients, sediments, and toxic chemicals in aquatic ecosystems. The program structure may be used in developing an overall framework for the "unit world" model for metals.
Biotic Ligand Model (DiToro et al, 2001)	A generalized model for describing metal toxicity as a function of water chemistry. The program may be used to describe metal toxicity effects.

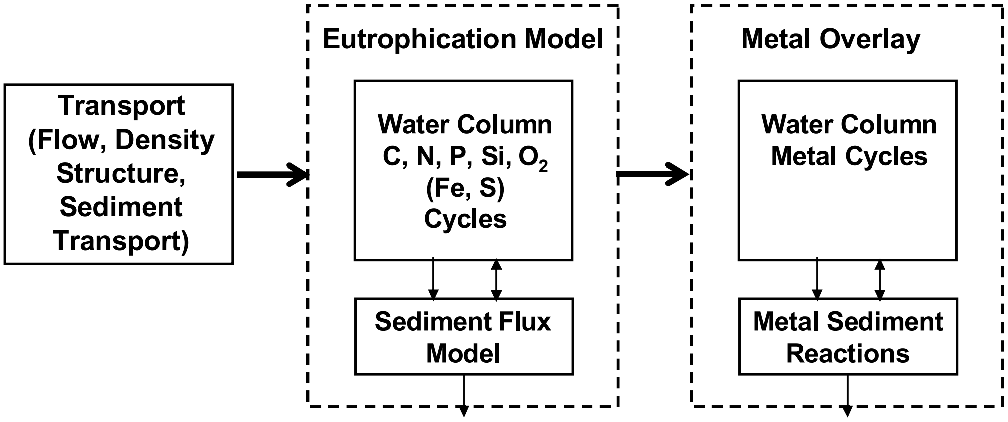

Figure 3. Detailed framework for modeling metals in lakes.

Development of a More Detailed Modeling Framework

A more detailed framework for modeling metals in lakes is given in Figure 3. In this approach, hydrodynamic transport is determined by tributary inflows, wind mixing, and thermal stratification, and can be adequately described using a number of available models. Sediment transport is a function of particle properties such as particle size and density and hydrodynamic characteristics such as bottom shear and can also be described by models. For lakes, however, direct evaluation of field data (e.g., from sediment traps and radionuclide dating) is often used in specifying settling, resuspension, and burial parameters. Water and sediment chemistry can be defined explicitly using current eutrophication models. In several cases, eutrophication models have been linked to sediment models (e.g., the Sediment Flux Model (DiToro, 2001)) to track the cycling of organic carbon, nutrients, oxygen, and redox-sensitive species (S, Fe, Mn) between the water column and sediments.

This information on hydrodynamics, sediment transport, and water/sediment chemistry will serve as a basis for a water column/sediment flux model for metals in lakes. Descriptions for metal complexation, precipitation, sorption, and redox reactions, along with species-dependent transport of metals by hydrodynamic and sediment transport will be explicitly considered in model development. The model would most likely include equilibrium descriptions for metal complexation to dissolved inorganic ligands and DOC; for metal precipitation as oxide, carbonates, and sulfides; and for metal sorption to solid phases, including POC, and iron and manganese oxides. Redox reactions may require kinetic descriptions.

Extension of the "lake" model to streams and rivers will likely be performed by considering a series on "lake" compartments to describe variations in the longitudinal direction. Simple hydrologic and more complex hydrodynamic models will be used in specifying steady-state or time-variable transport in rivers and streams. Sediment transport models (such as SEDZL, HEC-6, ECOM-SED) will be examined to determine their usefulness. These models however typically require a large amount of site-specific information for proper application. For larger, low-gradient rivers, phytoplankton-based eutrophication models similar to those developed for lakes will likely be used to describe the cycling of organic carbon, nutrients, oxygen, and redox -sensitive species (S, Fe, Mn) between the water column and sediments. Extension of the model to metals will also follow the approach discussed above for lakes. For shallow, high-gradient streams, periphyton and submerged aquatic vegetation typically play a more dominant role. Models such as AQUATOX which explicitly include periphyton will be reviewed for this purpose. In addition, the effects of chemical weathering reactions on pH may also need to be considered in these streams. The development of a metals model for high-gradient streams will more likely require a greater emphasis of aerobic reactions (e.g.,metal precipitation as oxides and carbonates, coprecipitation, and sorption) and on the role of periphyton on metals cycling.

The level of detail to be included in all of these efforts however will be based on the ultimate use of the models.

Development of a Coupled Water Column/Sediment Flux Model for Lakes

Our initial focus is on the development of a metals model for lakes. For this purpose, we are constructing a coupled water column/sediment flux model in VBA for EXCEL. To date, we have completed the code to track the cycling of organic carbon, nutrients, oxygen, and redox-sensitive species (S, Fe) between the water column and sediments and are testing the computer code. The next step will be to add the metals overlay to the model. The model will then be used to perform a more detailed evaluation of available data for lakes.

Development of a "Unit World" Model for Regulatory Use

A workshop was held at the University of Delaware in February 2003 in part to discuss the potential use of a "unit world" model for metals in regulatory programs. The overall consensus was that, although the modeling frameworks described above are doable, they could easily become overly complex for regulatory programs. Simple models that are currently in use however are too limited to be useful for management decisions. As a middle ground, it was recommended that the "unit world" model for metals should follow a tiered approach as outlined below:

- Tier 1 (Ranking Model): For this purpose, a "unit world" model for metals would be developed and applied to several idealized environments including a stratified lake, a shallow lake, a high-gradient stream, and a low-gradient stream. For each application, the physical and chemical parameters that would be required for the metals calculations would be generated from models (e.g., using results from a validated eutrophication model for a lake) and/or from summaries of field data. From these analyses, default values would be developed for each idealized environment (i.e., stratified lakes, shallow lakes, etc.) with additional sub-classifications based on low, moderate, and high ranges for productivity, alkalinity, and hardness. Model simulations for each environment would be run with default values to determine a relative ranking of hazards associated with the release of metals. This assessment would initially be conducted on a subset of metals (cadmium, copper, nickel, lead, and zinc) and would subsequently be expanded to a larger list of metals.

- Tier 2 (Preliminary Site Assessment): Application of a "unit world" model for preliminary site assessments of metals would follow the modeling approach given Tier 1. For these applications, prescribed default values would be used along with specific information for the site. As in Tier 1, prescribed default values would again be specified according to classifications for aquatic environment (i.e., stratified lake, shallow lake, etc.), and additional sub-classifications for levels of productivity, alkalinity, and hardness. Site-specific information could include external loads (e..g., from direct discharges, watershed runoff, atmospheric inputs, and groundwater inflows), sediment contamination levels, stream flow, and other easily obtainable parameters such as pH, alkalinity, and hardness. Model application could be performed for steady state (e.g., summer low flow and/or wet weather conditions), time-variable simulations (e.g., to evaluate seasonal variations in vertical mixing and pH, or daily variations in stream flow and event-driven loads), or probabilistic simulations (e.g., to evaluate exceedance frequencies based on a distributions for external loads, stream flows, or ranges in certain default parameters). In all cases, current metal concentrations in sediments should be specified and used in evaluating their effects of water column and possibly pore water responses. One difficulty in applying this modeling approach from site to site will likely be the assignment of sediment transport parameters.

- Tier 3 (Site-Specific Applications): For sites where Tier 2 evaluations are not sufficient for regulatory decisions, a more detailed evaluation would be required. This would include a more intense field data collection effort and the development of a site-specific model. In addition to information on external loads and current concentrations of metals in sediments, flows, and other easily obtainable parameters such as pH, alkalinity, and hardness, Tier 3 would likely include a more complete sampling of ambient concentrations and processes. This could include dissolved organic carbon, major ions, iron, manganese, and metal concentrations in the overlying water and possibly pore water; grain size, density, and organic carbon, iron, manganese, sulfide and metal content of suspended and sediment particles; planktonic and benthic algae concentrations and growth rates; benthic organism densities; and measurements of settling rates, sediment erosivity, burial, sediment mixing, and pore water diffusion rates. The field data would be used in specifying model parameters and in conducting model calibration and validation studies.

A critical component for the Tier 1 and Tier 2 model applications will be the development of default values for the aquatic environment classifications (i.e., stratified lake, shallow lake, etc.), and the additional sub-classifications for levels of productivity, alkalinity, and hardness. These values should initially be developed from an examination of field data, and where appropriate, should be tested for consistency using available models. The level of effort required for this evaluation is significant and is probably best handled through a separate grant. Peer review of the default values and classifications should be an integral part of the process.

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Effect of Dietary Metal Exposure on Fish and Aquatic Invertebrates

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Annual Report prepared for:
Center for the Study of the Bioavailability of Metals in the Environment

Introduction

The results presented in this report are based on five months work. The McMaster portion of year one funding commenced December 2002. Once the funding was in place, Patricia Gillis, a post-doctoral research associate, was hired on to the project.

One of the goals of the original proposal (McDonald, 2001) was to investigate the dietary uptake of metals by *Daphnia* using algae that had been cultured in the presence of metals, but since that time a number of studies (Barata *et al*. 2002a and b, Yu and Wang 2002a and b) have documented the importance of algae-borne metal as a route of metal exposure. Barata *et al*. (2002a) found that *Daphnia* accumulated twice as much Cd from water than from food (algae), but Cd from algae was assimilated much more (30 times) efficiently than the Cd acquired from water. These studies agree that although metal uptake from the overlying water is the major source of metal for a filter-feeding organism like *Daphnia*, dietary exposure is also an important route for metal uptake. The area that remained relatively unexplored was the uptake of metals from the ingestion of metal-contaminated particles, in particular, inorganic particles. The focus of this study is the dietary uptake of metals via metal-contaminated sediment particles in invertebrates.

Test Organisms

Due to its sensitivity to environmental pollutants and ease in laboratory culture, the freshwater planktonic Cladoceran, *Daphnia magna,* is often used as a model organism for investigating environmental exposure. The initial phase of this study will use *Daphnia magna* to investigate dietary metal uptake from sediment particles. Because *Daphnia* are filter-feeders, they sieve large quantities of water to collect suspended particles. Although their preferred food is algae, any suspended particles over 0.45µm will be retained on the filtering appendages and may be ingested. *Daphnia* may also ingest resuspended sediment particles when browsing at the sediment-water interface. *Daphnia* will periodically browse on detritus and sediment particles if suspended food (i.e. algae) fall below a threshold (Horton, 1979). *Daphnia* stir up sediments and detritus by scraping the bottom of shallow waters with their thoracic appendages and then produce water currents to remove particles from the water (Lampert, 1987). This switch in feeding behavior is important in overcoming periods of food shortage (Lampert, 1987). Although *Daphnia* are planktonic organisms, feeding mainly on algae, they may ingest sediment and any associated contaminants including metals through both inadvertent sieving or through intentional resuspension of sediment.

Sediments

Sediments used in this study are collected from Clear Creek, Colorado. Clear Creek is a high-gradient stream which receives metal-rich effluent from a number of mining sites, and has elevated metal levels in both the water and sediments. Sediments were collected from a site referred to as Beaver Dam on Clear Creek by Jim Ranville (Colorado School of Mines) and shipped to McMaster University. The first shipment of sediments was received Jan 22nd 2003.

An advantage of using these sediments in exposures is that they are 'naturally' contaminated as opposed to artificially contaminated (spiked) with metals. Walski (2002) used Clear Creek sediments to investigate the influence of particle size on metal bioavailability in chironomids. Jim Ranville and Phil Ross (Colorado School of Mines, (CSM)) are also using sediments from this stream in their research program. By collaborating with the CSM group, we combine a wide range of expertise to investigate metal uptake and toxicity associated with these sediments. J. Ranville will be conducting size fractionation on the sediments and measuring the chemical composition using Inductively Coupled Plasma Mass Spectrometry as well as providing guidance on any geochemical aspects of the project.

Walski *et al*. (2002) and Ranville *et al*. (2002) reported that Zn, Cu, Fe and Mn were the main metals of concern in the Clear Creek sediments. In our initial investigations we chose to focus on Cu and Zn in exposure water and in tissues of the exposed *Daphnia*.

Progress to Date

Cultures

Daphnia Cultures

Daphnia clone (lot #090600 DM) purchased from Aquatic Research Organisms (Hampton, NH, USA) in November 2002 was held in continuous culture according to USEPA protocol 16.60049002F (1993). *Daphnia* fed on a combined diet of yeast, cerophyll and trout chow (YCT) and cultured unicellular algae produced more neonates compared to animals fed on either YCT or algae alone (Table 1). Since neonate production in dechlorinated Hamilton city tap water (HCTW; which comes from Lake Ontario) was comparable with that of synthetic Lake Ontario water (SLOW) for simplicity, we chose to use HCTW as the culture media. HCTW is dechlorinated on site and routinely monitored for chlorine, metals and major ions. Animals were fed a mixture of *Pseudokircheriella subcapita* and YCT daily. Culture media was changed three times per week. Neonates were used to initiate new cultures once a week.

Algae Cultures

A pure culture of *Pseudokircheriella subcapita* purchased from University of Toronto Culture Collection (Toronto, ON) in November 2002, was cultured using Bristol's medium according to USEPA protocol 16.60049002F (1993). Algae were cultured in 3-L volumetric flasks and held under 24 h light in climate-controlled chambers at 22°C.

Hyalella Cultures

A culture of *Hyalella azteca* (source Environment Canada, Burlington, ON) is currently being established for use in future sediment exposures. Procedures for culturing *Hyalella* follow the methods of Borgmann *et al*. (1989). Briefly, *Hyalella* are held at 23°C and fed crushed Nutrafin fish flakes. Young are removed once a week to initiate new cultures.

Daphnia Sediment Exposures

Unless otherwise stated, *Daphnia* exposures were conducted in glass beakers (250 mL glass) with a water to sediment ratio of 4:1 at room temperature. Dechorinated HCTW was used as overlying water except in treatments where site water was required. Test vessels were held at ambient room temperature (22±3°C) without aeration and covered with plastic Petri dishes to prevent evaporation. *Daphnia* were not fed during exposures so that hunger (from lack of food) would motivate them to browse at the sediment-water interface. We also wanted to avoid any possible binding of dissolved metals with supplemental food (algae) which could potentially affect metal uptake and toxicity. Samples of the sediments used in the exposures have been sent to J. Ranville at the CSM to determine size distribution of the particles and sediment metal concentrations.

Preliminary Exposure

Preliminary trials (no replication) using field collected sediment and either site water or HCTW were conducted to determine if the sediment and/or the overlying water was acutely toxic to *Daphnia*. Five adult *Daphnia* were added to each test beaker and monitored for survival over 5 days (Table 2). Adult *Daphnia* were used in the preliminary exposure because they were much easier to visually detect without disturbing the sediments compared to the smaller juveniles when assessing survival in the test vessels. The low survival (20%) after 96 h of exposure indicated that either the *Daphnia* were starving during the exposure and/or that there was toxicity associated with the sediment. The lower number (2) of neonates surviving in the treatments with sediments compared to the 'site water only treatment' (8) indicates that the presence of sediment contributed to the mortality of the neonates. Although, mortality in the control water treatment suggests that the adults may also be affected by starvation. In order to ensure there was sufficient tissue for metal analysis, an exposure time of 48 h was chosen for subsequent exposures.

Sediment Exposure I

In order to determine the relative contribution of sediment-associated metals and water-borne metals to metal accumulation and toxicity, *Daphnia* were exposed to various combinations of the two components (water and sediment). *Daphnia* were exposed (48 h) to the same sediment treatments as in the preliminary exposures but with four replicates (beakers) per treatment. Juvenile *Daphnia* (5-day old), were used in these experiments in accordance with ASTM (2000) protocol for whole sediment bioassays with invertebrates. Ten *Daphnia* were added to each test vessel. Water chemistry parameters (pH, dissolved oxygen, and conductivity), dissolved (filtered through an Acrodisc 0.45 µm in-line filter) and total (unfiltered) Zn and Cu concentrations in the overlying water were measured at initiation (t=0) and upon completion (t=48) of the exposure. Surviving *Daphnia* were transferred to HCTW for one hour to purge their gut and then dried at 60°C for 24 h. A composite dry weight measurement was taken for all surviving animals in each replicate. *Daphnia* tissue samples were digested with 50 µL of concentrated metals grade Nitric acid in 2 mL centrifuge tubes for 24 h at 60°C. Tissue metal concentrations were measured using either Graphite Furnace (Cu) or Flame (Zn) Atomic Absorption Spectroscopy.

Dissolved and total concentrations of Zn and Cu in the overlying water at the beginning and end of the exposure are given in Table 3. Most (78 to 100%) of the metal present in the overlying water at the end of the exposure was in the dissolved phase. The concentrations of Zn and Cu in the overlying water (dissolved and total) were significantly ($p<0.05$) higher in the 'sediment and site water' treatment than in either the 'site water only' or the 'sediment and culture water' treatments, suggesting that both the sediment and the overlying site water were sources of metals. Survival was lower (<50%) in treatments containing sediment (Table 4) compared to the site-water-only treatment (90%). Tissue concentrations of Zn and Cu were respectively about two- to eight-fold higher in all treatments compared to culture water controls, demonstrating that there is substantial bioavailable metals associated with these sediments, though the differences between treatments were small.

Sediment 'Washing'

The dissolved concentrations of Zn and Cu in the overlying water in the first exposure were as high as 1200 µg/L for Zn and 53 µg/L for Cu. These levels were within the range of reported EC50 values for water-only exposures of these metals. De Schamphelare *et al*. (2002) reported 48 h EC50 for *D. magna* for total copper that ranged from 115 to 1455 µg/L in media containing varying amounts of TOC, and De Schamphelare and Janssen (2002) found that without the addition of TOC, 48 h EC50 ranged from 4 to 52 µg/L. Muyssen and Janssen (2001) reported 48 h EC50 of 1.1 mg/L in unacclimated *D. magna*. In order to differentiate toxicity associated with the dissolved fraction from toxicity associated with the particle-bound metals, sediments were subjected to a series of 'washings' to remove any easily mobilized metals from the sediment particles. Two methods of washing were compared.

In the first method of sediment washing (Method A) the sediments used in the first exposure (Exposure I) underwent a series of resuspensions (by stirring) and settlings and finally replacement of the overlying water after three resuspensions/stirring events. For Method A, the overlying water was replaced three times over the course of a month. In a second method (Method B), fresh sediments were subjected to a series of mixing (Vortex mixer for 5 min) followed by centrifugation (3000 rpm for 10 min) and replacement of overlying water. For Method B, this process was repeated six times in the course of a day. In both methods the overlying water was sampled for dissolved metals and the remainder of the overlying water was gently decanted before the addition of new water.

In both types of washing Zn and Cu levels in the overlying water decreased with repeated washing of the sediments (Figure 1). The final concentration of Zn in the overlying water was 89 (43) µg/L after washing with Method A and 194 (16) µg/L using Method B. Final Cu concentrations were 16 (5) µg/L and 12 (9) µg/L after washing with Method A and B, respectively. After washing, Cu concentrations were reduced to 40% (Method A 38%, B 42%) and Zn concentrations reduced to 20% (Method A 20%, B 23%) of initial concentrations in the overlying water of unwashed sediments.

Sediment Exposure II

Following the removal of the easily mobilized metals from the sediment, *Daphnia* were exposed to the washed sediments in whole sediment bioassays. Experimental setup (sediment to water ratio, parameters measured etc.) was the same as in the first sediment exposure. Metal uptake and toxicity from the sediments washed with Methods A and B were compared.

Zn and Cu concentrations in the overlying water at time 0 and time 48 h are given in Table 5. The dissolved fraction accounted for 20 to 70 % of the total metals at the end of the exposure. Survival was high with a minimum of 95% (Table 6). *Daphnia* accumulated similar concentrations of Zn from sediments washed with both methods but the tissue Cu concentrations were significantly (p=0.002) higher in *Daphnia* exposed to sediments washed with Method B (Vortex and centrifuge) compared to sediments washed with Method A (stirring and settling). Note that the Zn accumulations were lower than those previously seen with unwashed sediments or site water but tissue copper accumulations were comparable (Table 5). The concentration of Cu in the control organisms appears to have increased over the course of the exposure. The mean weight of control *Daphnia* decreased by nearly 50% over the course of the exposure, presumably due to starvation, thereby having a concentrating effect of tissue Cu concentrations.

Discussion of Findings

Due to the high concentration of easily mobilized metals associated with the sediments of Clear Creek, washing of particles is necessary to determine whether the dissolved fraction or the sediment was the main source of bioavailable metal. The decrease in toxicity and tissue concentrations in Exposure II compared to Exposure I would suggest that dissolved metals significantly contributed to the observed mortality and metal tissue concentrations in the first exposure. Because the physiological condition of the digestive tract can be very different from the surrounding water (Weltens *et al*. 2000) we expected that metals adsorbed to the particles would be biologically available to *Daphnia* even though most of the easily mobilized metals had been removed. The accumulation of Cu and Zn even after washing the particles confirms this. We will continue to investigate metal uptake from 'washed' settled sediments and 'washed' suspended particles (both together and in separate exposures) in order to determine the relative importance of these sources of metal uptake.

The higher total Cu concentration in the overlying water of sediments washed using Method B may be due to the way in which the particles were washed. In Method B the particles are vigorously mixed with a 'Vortex' mixer. This process may break up large aggregates into smaller particles which may not only stay in suspension longer but may now be within the size range that can be retained by the filtering mechanism of the *Daphnia*. Both of these scenarios could increase the bioavailability of particle-bound metals to the *Daphnia*. The different methods used to settle the sediment between washings may also contribute to the higher total Cu concentration in the water of sediments washed using Method B. In Method A, sediments are allowed to settle by gravity (minimum 24 h) before the overlying water is sampled and replaced. In Method B centrifugation was used to quicken the settling process (10 min). The forced sedimentation of all particles into the pellet in Method B would prevent smaller particles from being decanted off in the successive water renewals as could have occurred in Method A. Further investigation as to the most appropriate washing method will continue. Removing easily mobilized metals using rigorous mixing and centrifugation (Method B) is a much quicker (day vs. month) than repeated stirring and resettling over time (Method A). The increased bioavailability of Cu when method B is used to wash sediments is something that will be further examined and likely utilized for future exposures.

When *Daphnia* ingest mineral-based particles, bacteria adsorbed to the particles are also consumed (G. –Toth, 1984), although a diet consisting solely of bacteria is an inadequate source of food. Most field bacteria (<0.5 µm) are close to the lower size limit (0.45 µm) and therefore are not efficiently retained (Brendelberger 1986, DeMott, 1985). Pace (1983) reported that a population of *D. parvula* experienced negative growth when offered only bacteria as food and Tezuka, (1971) found that *D. pulex* would feed and grow when offered a pure bacteria diet, but they would not reproduce. By not feeding the *Daphnia* in our exposures, we may be inflicting a stress that could interfere with metal uptake and toxicity; therefore, we may consider feeding in future experiments. However, the complication of feeding may be that particles will sorb to food, a factor which conceivably could either increase or decrease their bioavailability.

Problems Encountered

The level of Zn associated with the Clear Creek sediments is very high and this lends to substantial (mg/L) Zn in the overlying water; therefore measurement of Zn concentrations using the Flame Atomic Absorption Spectrometer (AAS) is relatively quick and simple. On the other hand, tissue levels of Zn (µg/g) in control animals at least, are significantly lower requiring substantial time and effort to fine-tune the method to accurately measure these low levels (within the lower 10% of the instrument range). This is further complicated by the small size of *Daphnia* and the small (1-2 mL) sample volume (based on the dry weight and metal concentration). Typically Graphite Furnace AAS is used to measure trace levels (µg/L) of metals, but because of background problems associated with zinc measurements and the increased time commitment involved when using the Furnace AAS (10 times longer) because of the large number of samples generated in this project, we chose use the Flame AAS mode. After repeated trials and tweaking of the method we think we have found a combination of parameters (0.5-1 mg dry tissue, 2 mL sample volume, and shortened 'reading' during analysis) that will work to measure Zn tissue concentrations in *Daphnia* using the Flame AAS.

As mentioned earlier on, one of the major drawbacks of using *Daphnia* as a test organism for accumulation studies is their small size, and working with juveniles (10% of the adult dry wt) is even more restrictive. We have found that 0.5-1 mg of dry tissue is adequate for determining Zn tissue concentrations. In the second *Daphnia* exposure (Exposure II), even though survival was over 80%, the mean dry weight of a composite sample was only 0.2 mg. Therefore, conducting an exposure with ten 5-day organisms for 48 h will not provide even half of the minimum tissue. Clearly we will need to scale up. Muyssen and Janssen (2002) found no significant difference between Zn concentrations in juveniles and adults (although no data were shown) and typically use adult *Daphnia* in their accumulation studies. Considering the 10-fold difference in dry weight between adults and young, using adults would certainly simplify Zn measurements using a Flame AAS. Only 2-3 adults would provide 1 mg of dry weight whereas more than 20 juveniles would be required. Currently, we are investigating if tissue concentration of Zn varies with age or reproductive status of *Daphnia*. If we confirm the findings of Muyssen and Janssen (2002) and find no significant difference between the concentration of Zn in the tissue of unexposed juvenile and adults, we will consider conducting future experiments with adults.

Future Directions

Benthic organisms, because of their close association with the sediment would be expected to have considerable exposure to any sediment-associated contaminants (e.g. metals) both through bodily contact, and through feeding. Warren *et al*. (1998) investigated the influence of invertebrate habitat on exposure route of Cd. They reported that for endobenthic organisms such as tubificids and chironomids, Cd accumulation was significantly and linearly correlated with sediment cadmium concentration, whereas in *Chaoborus punctipennis*, an organism that seeks refuge in the sediment during the day but migrates nightly into the water column to feed, Cd accumulation did not respond to increases in sediment Cd. In the Warren *et al*. (1998) study, *Hyalella azteca* which lives at the sediment water interface and feeds on detritus and algae, also accumulated more of its Cd from the overlying than the sediment. Walski (2002), investigated the dietary uptake of metals from Clear Creek sediment particles in *Chironomus tentans*, an endobenthic organism**.** Walski (2002), reported that *C. tentans* accumulated Zn from both the overlying water and the sediments and that smallest size fraction (<53 µm) of particles was the main source of Zn. In future studies, we plan to build on the findings of Walski (2002) and use other benthic organisms such as *Hyalella azteca* to determine the relative importance of dietary uptake of metals from sediment particles.

It is well known that pH of overlying water can have significant effects on metal bioavailability and toxicity. Yu and Wang (2002b) found that Zn uptake in *Daphnia magna* was strongly pH dependent. We plan to manipulate the pH of the overlying water to determine the effect of pH on the mobilization of metals from the sediments into the overlying water and resulting uptake and toxicity to *Daphnia*. One of the aims of the study is to increase pH in the system to mimic that in a mining impacted stream following remediation in order to determine how increased pH would affect uptake of metals through particle ingestion by *Daphnia*.

We are also interested in looking at the effect of suspended metal-contaminated particles on metal uptake and toxicity in Daphnia. In a preliminary experiment where sediment particles were suspended and allowed to settle, we found that over 90% of the particles settled out of suspension within 3 hours. We plan to conduct *Daphnia* exposures with the fine particles that remain in suspension after an initial settling period.

As mentioned at the outset, the majority of time for which we have been funded to date (6 months) has been dedicated to method development and preliminary experimentation. We have now hired an undergraduate assistant (Ms. Sonia Sharma) who will work full-time for the summer and part-time throughout the school year. Once the student is trained she will take over the time-consuming tasks of maintaining algal and animal cultures, thereby allowing the post-doctoral associate (P. Gillis) to focus on conducting experiments and analyzing data. We are currently expecting a second and larger shipment of sediment from the Colorado School of Mines and will soon begin the next, more experimentally intensive phase of the study.

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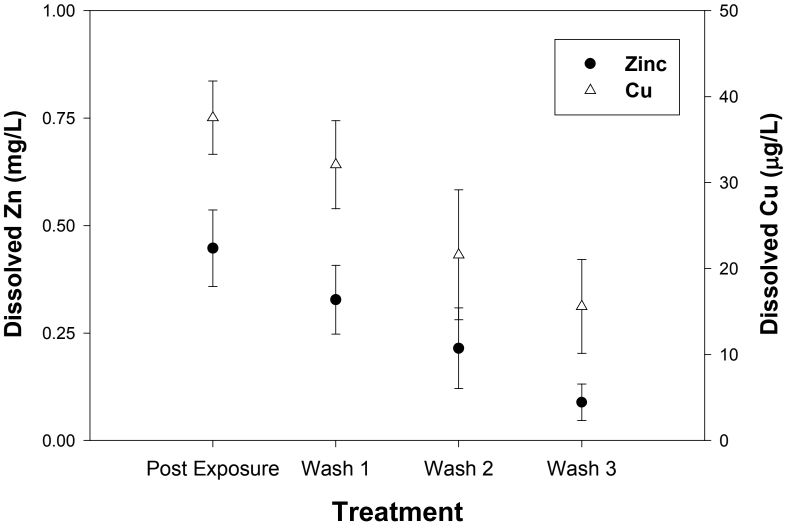

Figure 1. Dissolved Zn (mg/L) and Cu (µg/L) concentrations in overlying water following repeated 'washing' of sediment using Method A (Bench top stirring and settling). Error bars are standard deviations, n = 4. ‘Post Exposure’ indicates concentrations in overlying water after Exposure I.

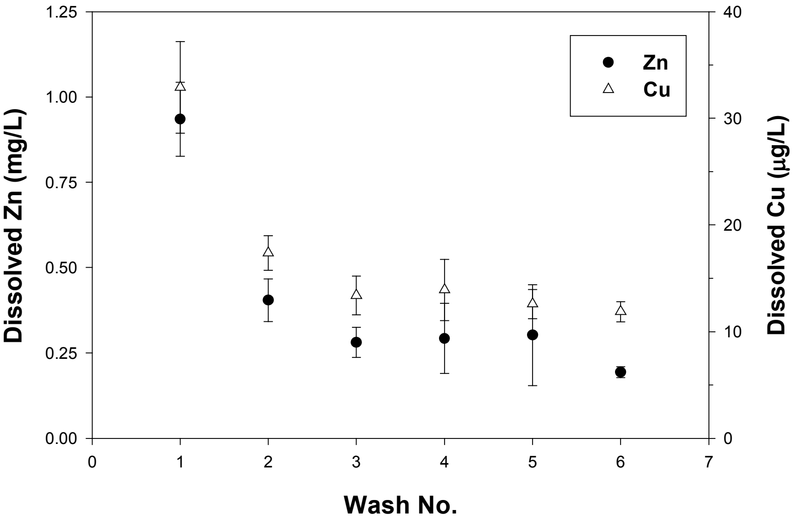

Figure 2. Dissolved Zn (mg/L) and Cu (µg/L) concentrations in overlying water following repeated 'washing' of sediment using Method B (Vortex mixing and centrifugation). Error bars are standard deviations, n = 8.

Table 1. Mean (standard deviation) number of neonates produced per brood using either dechlorinated Hamilton city tap water (HCTW) or synthetic lake Ontario water (SLOW) as culture media with either yeast cerophyll and trout chow (YCT) and/or unicellular algae (*Pseudokircheriella subcapita*) as food. Reproduction was followed for 3wk.

Media	**Food Source**	**Mean Brood Size**
HCTW	YCT	7.5 (7.6)*
	Algae	35.7 (18.8)
	Algae + YCT	43.2 (15.5)
SLOW	YCT	20.3 (9.8)
	Algae	37.3 (11.2)
	Algae + YCT	43.5 (15.7)

*Adult *Daphnia* in this treatment died and had to be replaced twice

Table 2. Survival and reproduction in preliminary exposure of adult *Daphnia* to field sediments. Five adults were initially added to each test vessel.

Treatment	**24 hr**	**96 hr**		**120 hr**	
	Adults	**Adults**	**Young**	**Adults**	**Young**
Site Water and Sediment	4	0	1	0	1
HCTW and Sediment	4	2	1	2	1
Site Water	4	1	8	0	8
HCTW	5	1	0	1	8

Table 3. Mean (standard deviation) total and dissolved concentrations (mg/L) of Zinc and Copper metal in overlying water at the beginning (time=0) and end (time=48 h) of Sediment Exposure I (unwashed particles).

Metal	**Treatment**	**Time 0**			**Time 48 h**		
		Total Metal (mg/L)	**Dissolved (mg/L)**	**Dissolved (%)**	**Total Metal (mg/L)**	**Dissolved (mg/L)**	**Dissolved (%)**
Zn	Dechlorinated Water (Control)	ND	ND	-	ND	ND	-
	Site Water Only	0.759 (0.153)	0.643 (0.139)	84.7	0.973 (0.162)	0.972 (0.223)	99.9
	Sediment + Site Water	1.300 (0.029)	1.143 (0.045)	87.9	1.222 (0.146)	1.205 (0.096)	98.6
	Sediment + Dechlorinated Water	0.482 (0.107)	0.423 (0.100)	87.8	0.454 (0.090)	0.443 (0.094)	97.6
Cu	Dechlorinated Water (Control)	0.014 (0.010)	0.006 (0.005)	42.9	0.013 (0.012)	0.013 (0.011)	100.0
	Site Water Only	0.050 (0.011)	0.030 (0.002)	60.0	0.046 (0.009)	0.041 (0.002)	89.1
	Sediment + Site Water	0.078 (0.005)	0.050 (0.002)	64.1	0.068 (0.004)	0.053 (0.004)	77.9
	Sediment + Dechlorinated Water	0.046 (0.008)	0.028 (0.003)	60.9	0.045 (0.007)	0.037 (0.006)	82.2

Note: ND=not detected

Table 4. Mean (standard deviation, n=4) survival (percent) and tissue concentrations (µg/g) of Zn and Cu in *Daphnia* after 48 h of exposure (Sediment Exposure I).

Treatment	Survival (%)	Zn (µg/g)	Cu (µg/g)
Dechlorinated Water (Control t=0)	-	246 (130)	24.57 (10.18)
Dechlorinated Water (Control t=48)	77 (25)	191 (61)	93.63 (40.91)
Site Water Only	90 (8)	1192 (375)	174.18 (20.42)
Sediment and Site Water	12 (12)	1586 (209)	243.23 (201.56)
Sediment and Dechlorinated water	42 (21)	1390 (400)	221.78 (31.26)

Table 5. Mean (standard deviation) total and dissolved concentrations (mg/L) of Zinc and Copper metal in overlying water at the beginning (time=0) and end (time=48 h) of Sediment Exposure II (washed particles). Dechlorinated Hamilton City tap water was used a control.

Metal	Treatment	Time 0			Time 48 h		
		Total Metal (mg/L)	Dissolved (mg/L)	Dissolved (%)	Total Metal (mg/L)	Dissolved (mg/L)	Dissolved (%)
Zn	Control	ND	ND	-	ND	ND	-
	Method A	0.159 (0.040)	0.063 (0.025)	39.6	0.069 (0.046)	0.049 (0.035)	71.0
	Method B	0.345 (0.048)	0.080(0.004)	23.2	0.175 (0.032)	0.036 (0.004)	20.6
Cu	Control	0.033 (0.026)	0.004 (0.002)	12.1	0.004 (0.001)	0.004 (0.001)	100.0
	Method A	0.032 (0.004)	0.010 (0.002)	31.2	0.018 (0.007)	0.012 (0.003)	66.7
	Method B	0.092 (0.016)	0.014 (0.001)	15.2	0.057 (0.003)	0.016 (0.001)	28.1

Table 6. Mean (standard deviation, n=4) survival (percent) and tissue concentrations (µg/g) of Zn and Cu after 48 h of exposure to washed sediments (Sediment Exposure II).

Treatment	Survival (%)	Zn (µg/g)	Cu (µg/g)
Control (t=0)	-	162.38 (63.42)	17.11 (2.12)
Control (t=48)	100	189.91 (85.67)	77.13 (69.63)
Method A	96 (0.7)	599.11 (127.00)	226.77 (33.50)
Method B	95 (0.6)	517.82 (95.19)	394.20 (57.02)

Aquatic Toxicity and Exposure Assessment

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Annual Report prepared for:
Center for the Study of the Bioavailability of Metals in the Environment

Introduction

Objectives

The University of Missouri - Rolla is conducting a metals fate and transport study on two adjacent river systems in of southeastern Missouri. These systems include the watersheds of the Big River and the West Fork of the Black River. Both watersheds are located in the St. Francois Mountains Region, approximately 50 to 80 miles (80 to 130 km.) south-southwest of Saint Louis. Each river transects a world-class sized lead-zinc district that is hosted in carbonate bedrock. This study is being jointly conducted by faculty researchers from the Departments of 1) Geology and Geophysics (D.J. Wronkiewicz) and 2) Environmental Engineering (C.D. Adams and C. Mendosa). Student researchers include Gary Krizanich (Ph.D. G&G), Anne Faeth (M.S. EE), Josh McBurnett (M.S. G&G), Sara Struttman (G&G undergraduate), and Robert Hemmann (G&G undergraduate).

Risk assessment and risk management evaluations require accurate fate and effects models for heavy metals in the aqueous environment. While effective models exist for many organic contaminants, there currently is a significant deficiency in the models available for heavy metals in aqueous systems particularly river systems. Thus, it is the intent of this project to develop the necessary data for calibration and validation of comprehensive fate and transport models for heavy metals to be developed within the Center for the Study of Metals in the Environment (CSME). The working hypotheses of this proposed research is that the transport and bioavailability of heavy metals in streams impacted by mining activity are a function of particle size distribution, mineralogy, density, acid leachability, specific surface area, and other key parameters. The heavy metals to be the focus of this work include lead (Pb), zinc (Zn), copper (Cu), cadmium (Cd), and nickel (Ni). The specific objectives of the proposal are to:

- Characterize a portion of the Black River in the New Lead Belt of Missouri with respect to hydraulics, metal inputs, and sinks
- Develop hydrographs for the study reach
- Measure the bed load heavy metals concentrations and their temporal and spatial variability within the study system
- Measure the water column heavy metals concentrations (including both "dissolved" and "particulate" fractions) and their temporal and spatial variability in relation to hydraulic flows within the study system during both types of hydraulic events
- Assess the bioavailability of heavy metals within the study system through determination of acid extractability and speciation
- Develop, calibrate and validate a transport model for heavy metals within the Black River study system to provide guidance for implementation of sampling regimes
- Coordinate the sample collection, chemical analysis, and other methods with collaborators within the CSME to provide comparable data for varied aquatic systems, and to provide appropriate data for calibration of a Unit World Model
- Characterize metal transport for two distinct hydraulic events: 1) a slow rise and fall (i.e., "spring melt"), and 2) a steep rise and fall (i.e., "summer storm event"). It is hypothesized that these two events may be significantly different with respect metal transport

properties and hydraulics, with both of these factors significantly impacting the complexation and transport of metals through the system.

The Old Lead Belt

Over three centuries of mining in the "Old Lead Belt" of Missouri has resulted in the accumulation of over 250 million tons of waste tailings that serve as a potential source for metal contaminants in the Big River Watershed. The Big River drainage basin also includes within its confines the Washington County barite ($BaSO_4$) district and numerous smaller historic mining and smelting operations. The Old Lead Belt ores are composed primarily of the lead sulfide mineral galena, with economically recoverable concentrations of zinc sulfides, copper-iron sulfides, and silver. Silver occurs as a trace constituent in the zinc and lead ores. Non-economic concentrations of Co, Ni, Cd, and As also occur. These elements are released into tailings pile drainage waters in notable concentrations. All mineralization is hosted in carbonate rich bedrock. The ores of the Old Lead Belt were exposed at the surface by natural weathering processes and were easily exploitable by Native American Peoples and early European explorers to the region. These early operations were relatively modest in scale. Industrial scale mining in the district began around 1860 and continued nearly uninterrupted until the last mines were closed in 1972.

The Big River watershed encompasses a region of approximately 955 square miles (2473 km^2). The river flows approximately 138 nautical miles northward from its headwaters in the St. Francois Mountains until it merges with the Meramec River near St. Louis (Fig. 1a). Over this course it has an average stream gradient fall of 6.6 feet per mile. Gradients for the tributary creeks in the vicinity of the Old Lead Belt mines range from approximately 40 to 15 feet per nautical mile. The term "nautical mile" will be used throughout this report to denote a statute mile (5280 feet), which has been traced along the course of a river. Stream gradients of the Big River are much lower, ranging from a drop of approximately four feet per mile near the Old Lead Belt tailings piles to approximately two feet per mile in the lower stretches of the Big River near our Brynesville sampling location. Major tributaries of the Big River include the Flat River Creek, Mineral Fork, Cedar Creek, and Terre Bleue Creek systems. The sediments of the Big River reflect their low stream gradient, being dominated by sand- and silt-sized particles.

Land usage and geological features in the Big River Watershed change over the course of the river with the upper portions being primarily forested wilderness on a bedrock geology of middle-Precambrian aged high alkali-silica granite and rhyolite. The lower reaches (where our samples were collected) are characterized by increasing agricultural usage including pasture and row cropping. Small urban centers are located within the Old Lead Belt Mining District including the adjoining communities of Park Hills, Bonne Terre, Leadville, Desloge, and Leadington. These cities have a combined population of approximately 18,000 (2000 census figures). Paleozoic-aged dolomites and calcareous shales dominate the bedrock geology in the lower watershed, except in the extreme lower reaches where sandstones become more prevalent. These rock units exert a considerable influence on the chemistry of the river waters maintaining a slightly alkaline pH and high bicarbonate content of the river system. Southeast Missouri is also noted for its karst terrane (caves, springs, and sinkholes), although these features are not predominant in the Big River watershed.

The New Lead Belt (Viburnum Trend)

The "New Lead Belt" or "Viburnum Trend" ore district rivals the size and productivity of the "Old Lead Belt" district. The deposits, mineralogy, and host rock lithology of the New Lead Belt ores are similar to those of the Old Lead Belt, although local differences in trace metal compositions are noted both within and between mining districts. Improved metallurgical ore processing has resulted in greater metal recoveries in the New relative to the Old Lead Belt. Thus metal contents in the tailings piles are likely to be lower in the New Lead Belt Region, although we do not have any analytical data support this observation. All of the major ore horizons of the New Lead Belt are confined to strata located below the surface although Doe Run Company mining personnel have indicated that small historic prospect pits had exploited surface shows of mineralization within the West Fork drainage basin. Initial discoveries of the present-day ore horizons were made in 1955. The first ore production occurred in 1960 and continues today. Ore processing in the New Lead Belt has resulted in the accumulation of over 235 million tons of waste tailings.

The West Fork of the Black River is located in Reynolds County Missouri (Fig. 1b). The river emerges from its headwaters near the Reynolds-Dent County line and flows approximately 45 miles in an easterly direction until it merges with both the Center and East Forks of the Black River near Lesterville, Missouri. A stretch approximately 18 miles of the center portion of the West Fork is being characterized in the present study. Five of the six sampling sites from this study lie within a 4.6 nautical mile zone of the river that transects the north-south trending Viburnum Zone ore trend at a right angle. The sixth sample location is located an additional 13.2 miles downstream. The West Fork watershed receives run off drainage from three large mining operations in the region, the Brushy Creek, the West Fork, and Fletcher mines. Over the course of the sampling region, the river drops a total elevation height of approximately 200 feet, for an average fall of 11.2 feet per nautical mile. Major tributary creeks that join the West Fork over this segment include Toms Creek, Bills Creek, and Bee Fork, the latter two include active mines within their drainage basins. The sediments of the West Fork reflect their high stream gradient, being dominated by cobble, pebble, and sand-sized particles.

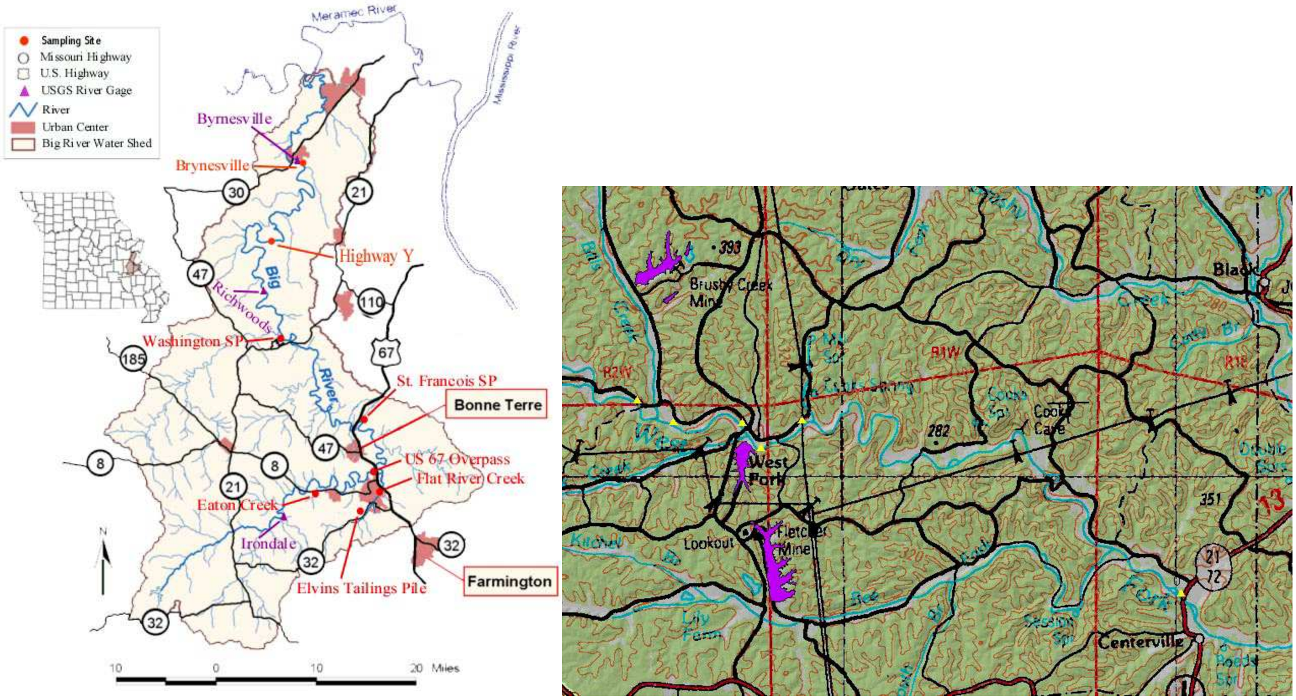

Figure 1. a) (to left) showing location of the Big River and associated tributaries, sampling sites, USGS gage stations, and other anthropogenic features. The inset map of Missouri shows the Big River drainage basin. b) (to right) showing the location of sample collection sites on the West Fork of the Black River, topographic features, and anthropogenic features. The West Fork of the Black River is located just to the southwest of the Big River watershed. The base of the Figure 1b is approximately 14 miles in length.

Land usage and geological features in the West Fork of the Black River are limited to minor grazing. Most of the watershed above the study area is forested wilderness. Reynolds County has a total population of approximately 6700 and there are no major urbanized centers that affect the drainage within our study segment of the river. The surface bedrock geology is characterized by Paleozoic-aged dolomites, with lesser calcareous shales and sandstones. As with the Big River system, these rock units maintain a slightly alkaline pH and high bicarbonate content of the river waters. The Black River watershed is known for its development of caves and springs, these features may complicate local water travel pathways.

Experimental Procedures

A significant portion of the research effort in the first year of study has been dedicated to the selection of sampling sites, development of appropriate sampling protocol, procuring equipment, and training personnel. Eight sampling sites were selected on the Big River system and six on the West Fork of the Black River. These sites were selected to fulfill several criteria including ease of accessibility, proximity to known mining operations, and correspondence to sampling locations from previous historical studies.

Sampling Dates & Locations –Big River

Water column and bed load sediment samples were collected from the Big River and associated tributaries during four successive sampling events spaced approximately three months apart. Sampling dates (all in the year of 2002) were on March 9th, June 9th, September 8th, and December 19th. These sampling events allowed for a preliminary evaluation of metal transport parameters as a function of differing river flow conditions and seasonal variability. All water column samples collected have been analyzed whereas only the March 9th bed load samples have been analyzed to date.

Samples were collected from six primary sites during each sampling trip to the Old Lead Belt (Fig. 1a). These sites include three tributaries to the Big River (Elvins, Eaton, and Flat River Creeks) that are located in close proximity to tailings piles. Three additional sites on the Big River were also sampled on a quarterly basis. These included the US Highway 67 overpass for the Big River, St. Francois State Park which is located approximately 10 nautical miles downstream from the Highway 67 bridge, and Washington State Park which is located an additional 25 miles downstream from the St. Francois site. Two additional sampling sites were included during the September 9th sampling period. These sites were located at Highway Y overpass on the Big River and the Brynesville location. The highway Y site is located 25 nautical miles downstream from Washington State Park, while the Brynesville location is another 23.5 miles downstream from Highway Y.

During each sampling trip, the Big River was sampled from north to south, moving progressively upstream to each successive site. This protocol allowed us to collect samples first from sites that were located furthest from the mine tailings piles, thus reducing the potential for particle contamination from metal rich sediments. Both sediment and water column samples were collected upstream of all bridges and at distances sufficiently far enough downstream from any tributaries to allow for homogenization of river waters.

Because of rapid flow conditions, samples were collected using extension poles from the shoreline of the Big River March 9th and the Flat River Creek on December 19th. All other samples from the Big River and Flat River Creek were collected by wading into the main river channel. Samples were always collected upstream of the sampling personnel to avoid incorporating any bottom sediment material that had been suspended during the sampling processes. The Elvins and Eaton Creek samples were also collected from their respective shorelines, with the relatively small sizes of the creeks allowing complete access to the waterways using an extension pole.

Big River Conditions

General river and weather conditions for each of the sampling trips are indicated as follows:

- March 9th – Samples were collected following an overnight rainstorm that resulted in a moderate flood rise on the Big River and some of its tributaries. The rain had tapered off and ended as the initial sample was being collected at the Washington Park site, however, water levels in the Big River were on the rise during the morning hours when the samples were collected. Water currents were faster than normal but the river and tributary creeks remained within their high-water channels. Maximum discharge and gauge height readings for three US Geological Survey stations maintained on the Big River are shown in Table 1while continuous flow readings for the Richwoods station during the entire twelve-month collection period of collection are shown in Figure 2a. Waters were turbid for all sites on the Big River and Flat River Creek with an abundance of fine-grained suspended material plus organic debris. Waters in the Elvins and Eaton Creeks were very clear and devoid of all particulate material.

- June 9th – Very light rain occurred the night before sampling. The weather system cleared through the region by morning with clear skies prevailing during the day of sampling. Water levels remained stable for all waterways during the day of sampling with all systems remaining within their normal channels. All waters were relatively clear and devoid of particulate material.
- September 8th – The weather was clear during the sampling day with no precipitation occurring during the prior two weeks before sampling. Water levels in the drainage systems were low and remained at a constant level during the day of sampling. Waters in all waterways were relatively clear and devoid of any particulate material.
- December 19th – Moderate rains occurred the night before and during the first half of the sampling date. The Flat River Creek was in moderate flood stage during the time of sampling but remained within its primary channel boundary. Flat River Creek waters were highly turbid with abundant suspended particulate material. Waters within the Big River were less affected by the rains, however, downstream of the confluence with the Flat River Creek the waters were moderately turbid and were rising during the day of sampling. Waters in the Elvins and Eaton Creeks were clear and devoid of any particulate material.

Sampling Dates & Locations – West Fork of the Black River

Water column and bed load sediment samples were collected from the West Fork Black River and associated tributaries during four successive sampling intervals spaced approximately three months apart. Sampling dates were on May 28th, 2002, August 23rd, 2002, November 23rd, 2002, and February 21st 2003. The water column was generally devoid of particulate material on all four sampling dates, however, an abundance of low density, organic-rich, particulate material was noted on the river bottom in the shallow water zones on the February 21st date. This material had presumably been suspended during high flow conditions initiated from rains two days prior to our sampling trip and was stranded during the drop in water level that occurred just prior to our visit (Table 2, Figure 2b). Water column samples collected from the May 28th and August 23rd sampling trips has been analyzed, while the remaining samples have been submitted for chemical analysis. All sediment samples will be analyzed during the upcoming summer months. Additional sampling events are planned for West Fork of the Black River in the future, including the sampling of a summer rainstorm event.

Table 1. Flow conditions of the Big River during sampling events.

Date	Conditions	USGS Gage Richwoods	River Water Temp. °C
3/09/02	-Moderate flood stage -High turbidity water	4040 ft^3/sec 8.77′	10.4 – 11.5
6/09/02	-Water slightly above normal seasonal flow	440 ft^3/sec 3.57′	22.9 – 24.5
9/08/02	-Water slightly above normal seasonal flow	188 ft^3/sec 2.77′	24.8 – 26.2
12/19/02	-Minor flooding of Flat River Creek	1050 ft3/sec	8.4 – 10.6

Table 2. Flow conditions of the West Fork of the Black River during sampling events.

Date	Conditions	USGS Gage Annapolis	River Water Temp °C
5/28/02	low flow	669 ft^3/s 4.80 ft	17.3-18.6
8/23/02	low flow	289 ft^3/s 3.87 ft	24.1-26.1
11/23/02	low flow, extreme clarity	169 ft^3/s 3.43 ft	6.9-11.3
2/21/03	water high from previous rain light rain throughout the day	1520 ft^3/s 6.05 ft	5.1-6.7

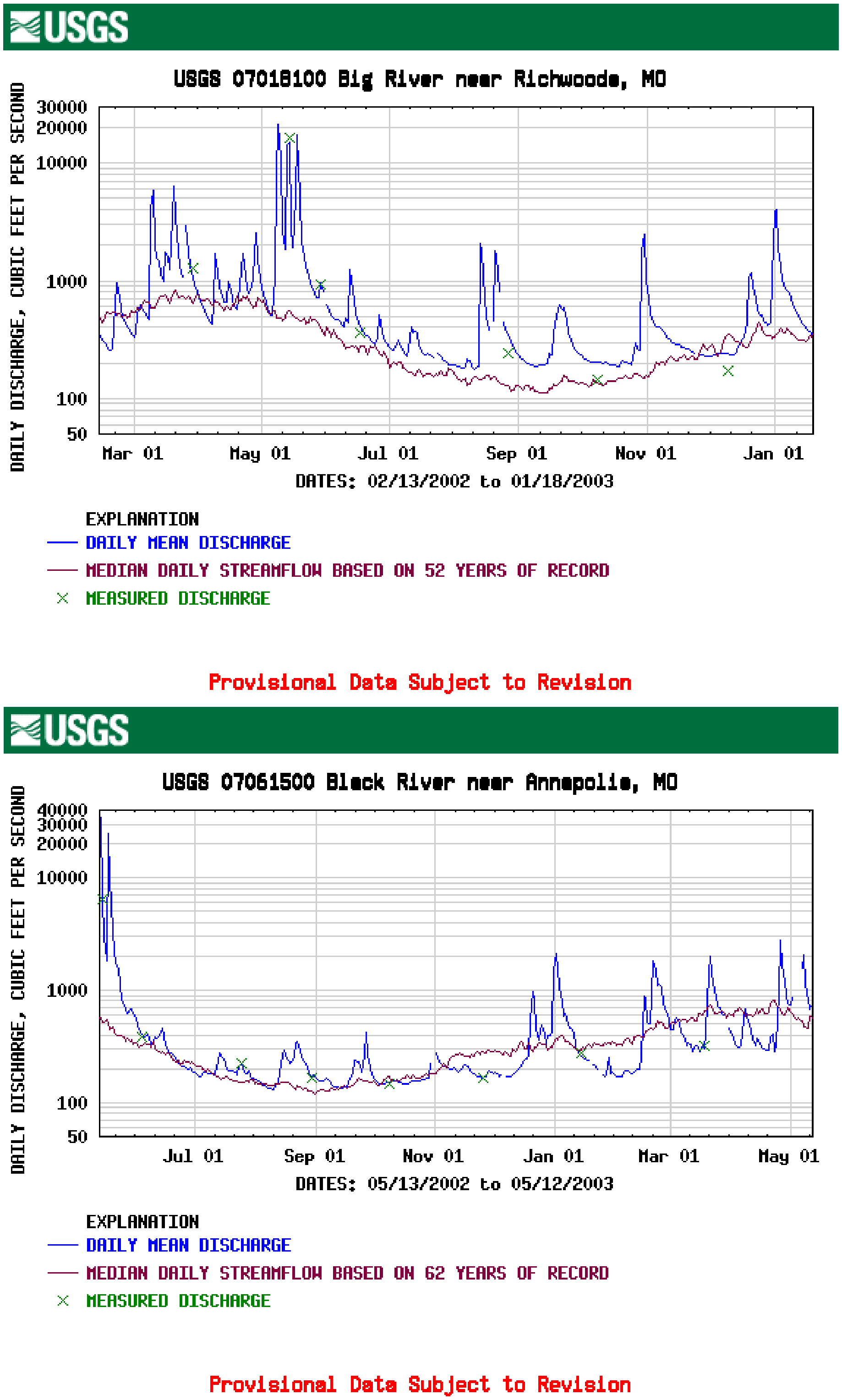

Figure 2. Continuous trace of river discharge from the USGS gauge stations located at a) Richwoods on the Big River (top figure), and b) Annapolis on the main branch of the Black River (bottom image).

Samples were collected from six primary sites on the West Fork during each sampling trip (Fig 1b). Site A lies just above Bills Creek and was chosen to represent the control site as it is located upstream of all known mining activities. Site B lies further downstream, below the confluence with Bills Creek, which drains the area where the Brushy Creek Mine is located. Site C lies just to the west of the Highway KK Bridge and is located above both Toms Creek and the West Fork mine. Site D and E are located further downstream, below the drainage of the West Fork mine. Sites A through E span a collective distance of 4.6 nautical miles. Site F lies a further 13.2 miles downstream from site E at the Centerville Public Fishing Access. This last site receives distal drainage from the Brushy Creek, West Fork, and Fletcher Mines.

For each collection trip, the Black River was sampled first at site A, above all known mining activities. Remaining sites were progressively collected downstream and to the east from the control site. Both sediment and water column samples were collected upstream of all bridges and at distances sufficiently far enough downstream from any tributaries to allow for homogenization of river waters. All of the samples were collected by wading into the main river channel. Samples were always collected upstream of the sampling personnel to avoid collecting bottom sediment that had been suspended during the sampling processes.

Experimental Methodology

Bottle Cleaning Procedure

Each of the polyethylene gallon jugs, 1000 ml, 125 ml, and small bottles used for Ion Chromatography were first washed with tap water and a mild dishwashing soap solution. The bottles were subsequently rinsed with tap water and filled with a 5% HCl solution and allowed to soak for 30 minutes. After soaking in HCl, the bottles were rinsed 3 times with ultrapure DIW, filled with the DIW, and then placed in a 90^{o}C oven overnight. The next day, the bottles were again rinsed 3 times with ultrapure DIW and dried at 90^{o}C for 30 min before they were weighed and labeled. All bottles, syringes, filters, and sample holders were placed into clean zip-lock bags for storage. One zip lock bag was prepared in such a manner for each of the sampling sites.

Water Column Sample Collection

Water samples were collected by submerging a presterilized 1000 ml polyethylene bottle just below the water surface of the rivers. Samples were collected in such a manner to avoid the inclusion of any visible suspended organic material and sedimentary particles that may be moving by a hopping process near the bottom of the river channel. Four different aliquots of the water column samples were segregated for cation analysis. These samples included: #1) an unfiltered water sample that was poured from the one liter bottle into a 150 ml polyethylene after waiting a period of 20 seconds. This aliquot was collected as the unfiltered river water sample. The remaining three samples were collected by pouring an aliquot of water into a 60 ml Becton Dickson brand polypropylene syringe with various sized syringe filters attached (described below). The first several milliliters of water passed through each filter were discarded, with the remaining filtrate solution collected into a presterilized 150 ml polyethylene bottle. All filters and syringes were discarded after use.

The filtrate samples collected included the following sizes: #2) a sample passed through a 5.0 μm Cameo nylon membrane syringe filter housed in a polypropylene mounting, #3) a sample passed through a 0.45 μm Nalgene surfactant-free cellulose acetate syringe filter housed in an acrylic mounting, and #4) a sample passed through 0.02 μm Whatman inorganic alumina matrix syringe filter housed in a polypropylene mounting. The syringes were typically refilled a second time so that a total sample filtrate volume between 100 and 120 ml could be collected. The 0.02 μm filters often exhibited increasing resistance to fluid passage during filtering indicating that the filter pores were becoming plugged with particulate matter. The remaining filters typically did not exhibit this phenomenon, except during flood stage when a large amount of particulate material was suspended in the water column (e.g., the March 9th, 2002 sampling on the Big River). Upon return to the laboratory, all sample aliquots destined for cation analysis were acidified with high purity Ultrex nitric acid to achieve a pH of between one-to-two.

Sediment Sample Collection

Sediment samples were collected for chemical analysis using two different sampling devices. A scoop constructed from PVC pipe and an end-fitting cap was outfitted and attached to a stainless steel sampling pole with an external clamp. The metallic parts of the sampling pole were attached to the external portion of the PVC scoop so as to avoid any contact with the sediment sample that was being collected. This sampling device proved effective in sampling sediment from the Big River where sand and silt sized particles generally dominated the sediment load. Sediments in the West Fork of the Black River contain a higher proportion of pebble and cobble size material. The larger sized particles prevented the effective use of the PVC scoop, so a syringe type device was constructed by cutting the tip off a "turkey baster" to enlarge the opening to four or five millimeters. The syringe device allowed us to collect the finer grained material located between the larger sized sediment particles. All sediments were wet sieved in the field using native river water and stainless steel sampling pans to collect a –10 to +80 mesh (coarse to medium sand), –80 to +230 mesh (medium to fine sand), and a –230 mesh fraction (silt + clay). The >10 mesh size fraction was discarded. Sediment samples were dried in a clean mechanical convection oven at 40°C prior to analysis.

Additional sediment samples were collected in the field for heavy particle analysis. Two techniques were used to collect the heavy metal particles. The first technique used a high-density lithium metatungstate solution ($G \approx 3.0$) to which several grams of sediment were added. The mixture was agitated in an ultrasonic cleaner (with frequent changing of the bath water to prevent heating of the lithium metatungstate solution and associated changes in specific gravity). The heavy mineral fraction that collected in the bottom of the vessel was withdrawn with a pipette, transferred to a clean petri dish, and rinsed several times with deionized water. A second technique utilized a plastic gold pan to concentrate the heavy mineral fraction directly in the field. After processing of initial samples it was noted that residual tungsten from the lithium metatungstate solution spectrally interfered with the detection of silicon during Scanning Electron Microscopy analysis. At this point, the heavy liquid separation technique for collecting high-density particles was abandoned in favor of the gold pan technique.

Hardness and Alkalinity Test Procedure

The hardness and alkalinity tests were performed using HACH digital titration portable test kits. The alkalinity test was done on-site at each of the sampling locations using the HACH method 8230. Total and Ca hardness tests were performed in the geochemistry lab the day following the sampling trip. The procedures used for these tests were HACH methods 8213 and 8204.

Conductivity, Temperature, pH and Eh readings

The conductivity, pH and temperature readings were measured *in situ* at each of the sampling locations. The conductivity meter was calibrated in the field using 97, 252, and 510 µS conductivity standards while the pH meter was calibrated using 4.00, 7.00 and 10.00 pH standards. A single standard for conductivity and pH was subsequently run at each sampling site as a check standard. Instruments were recalibrated if significant instrumental drift was detected. Water and air temperature readings were made from the combination pH meter and a separate temperature probe. Eh measurements were also made *in situ* as standardless analysis in the field for a limited number of sample locations.

Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS)

All solutions were analyzed by Inductively Coupled Plasma – Mass Spectroscopy (ICP-MS) at Indiana State University – Terra Haute. The values reported have been corrected for the diluting effect of acid addition. Estimated accuracy values are better than +/- 10%.

Optical and Scanning Electron Microscopy-Energy Dispersive Spectroscopy (SEM-EDS)

Optical microscopy and SEM-EDS analyses were conducted on the density separated (gold panned) sedimentary particles. These particles were first inspected and photographed using an optical microscope and then specific particles of interest were mounted using probes and tweezers on carbon tape attached to polished resin disks. Pictures were taken of the entire density separated population and of the selected mounted particles before SEM analysis. The SEM analysis was done on a JOUL JSM T33M-A scanning electron microscope. Pictures were taken and quantitative results were obtained in a graphical form.

X-ray diffraction analysis (XRD)

X-ray diffraction analysis was conducted both on bulk powdered samples (of sieved sediments) and river particle samples that were prepared by gravity separation in a water column and deposited on glass slides. The latter sample preparation technique is designed to maximize the recovery of clay-sized particles. Samples were analyzed in a Rigaku Miniflex x-ray diffractometer.

Results and Discussion

Big River Results

Tailings Pile Waters

Metal release to fluvial systems from the tailings piles occurs in a complex and dynamic fashion. In the Old Lead Belt area, many of the metals are being released from the tailings piles in a soluble or colloidal form. Zinc contents are highest in tributary streams near tailings piles (ranging up to

20,825 ppb) with up to 82 and 88% of the zinc from this sample passing through a 0.02 and 0.45 μm filter, respectively. Lead is also released in a soluble or colloidal form, though not to the same extent that zinc is. Maximum lead concentrations are 623 ppb, with 13 and 23% passing through a 0.02 μm and 0.45 μm filter, respectively. The remaining maximum metal (and As) concentrations and percent passing through a 0.45 μm filter from the tributary creeks are as follows: Ba-63 ppb-100%; Cd-53 ppb-83%; Cu-26 ppb-45%; Ni-711 ppb-99%; Co-853ppb-98%; As-13 ppb-100%; Ag-0.53 ppb-<10%.

While it is difficult to provide a statistically meaningful set of data results with only a limited number of samples, especially when those samples have been collected under variable meteorological conditions, some interesting trends are still apparent in the data set. Metal concentrations are quite high in the solutions being released from the tailings piles with a large proportion of the metals being released in a soluble and/or colloidal form (passing through the 0.45 μm filter). Release of Zn, Ba, Cd, Ni, Co, and As is dominated by the fine size fractions. The proportion of soluble and/or colloidal Pb and Cu is lower that that of other metals, but still represents a significant fraction of the total. These high metal concentrations may be typical for acid mine drainage systems, but the pH of the waters emanating from the tailings piles in the Old Lead Belt are approximately 7.8. The equilibrium concentration for zinc in the presence of smithsonite ($ZnCO_3$) is calculated to be 654 ppb at a pH of seven; the 20,825 ppm value measured from tailings water exceeded this equilibrium concentration by more than 30 fold. The aqueous solutions emanating from the tailings piles are clearly out of equilibrium, an observation further substantiated by our inability to obtain a stable conductivity reading from these waters and the observed precipitation of a Zn-Mg-carbonate phase upon evaporation of the tailings pile waters in the laboratory. It is proposed that these high metal concentrations are produced when microfilms of acidified water develop on the corroding surfaces of sulfide minerals such as pyrite (FeS_2) and sphalerite (ZnS) within the tailings piles. The acidified waters are quickly neutralized through reactions with the surrounding carbonate tailings sands, but not before the metals have been released to solution. Waters emanating from the tailings piles are supersaturated with respect to many of the metals present, with concentrations of the metals decreasing with time and distance from the sulfide mineral surfaces.

Metal concentrations in solution tended to be diminished slightly following meteoric precipitation events. While localized variations in precipitation and a lag-time before a hydrologic flux is transmitted through a tailings pile may play a yet undetermined role in these patterns it does appear that metal concentrations in tailings waters may be diluted slightly following rain events.

Big River Water Column

Zinc concentrations in unfiltered water column samples collected from the three Big River sites during low-water stages ranged from <1 to 219 ppb, with values progressively decreasing with distance downstream. The samples collected during the March 2002 flood event displayed higher zinc concentrations (between 144-227 ppb) and also progressively decreasing downstream. Lead concentrations from the Big River determined in the same manner indicate that low flow waters exhibited a concentration range of between 16 to 82 ppb, while lead levels during the March 9th flood event were between 197 to 286 ppb. Unlike zinc, however, lead levels often increased with distance downstream, possibly reflecting additive contributions from multiple point sources. The maximum metal (and As) concentrations and percent passing through a 0.45 μm filter from the Big River waters during low water stages are: Ba-260 ppb-97%; Cd-3.9 ppb-77%; Cu-41 ppb-97%; Ni-5 ppb-80%; Ag-3.45 ppb-100%; with Co and As occurring at concentrations below the detection limit of one ppb. Although the proportion of metals in a dissolved or colloidal state (<0.45 μm) was lower in the Big River when compared to the tributary systems, a significant fraction was still maintained in the finer-sized fraction.

The proportion of particulate material increased dramatically during flooding, indicating that metal-bearing particles can easily be resuspended from the bed load sediments during high flow stage and thus contribute to the overall metal transport process. The maximum total metal concentration and percent passing through a 0.45 μm filter from the Big River waters during the March 9th flood event are: Ba-317 ppb-33%; Cd-4.1 ppb-10%; Cu-17.7 ppb-15%; Ni-11.4 ppb-15%; Co-11.0 ppb-6%; As-5 ppb-100%; with silver at 0.22 ppb with the filtered fraction being below the detection limit of 0.22 ppb. The proportion of metals contained on the particulate material thus increased dramatically during flooding. Some metals such as Ba and Cd had total concentrations that increased only slightly, yet the particulate fraction displayed a disproportionally large increase relative to the total metal variation. Overall nickel concentrations decreased yet the proportion of nickel contained on particulate material still displayed a dramatic increase during flooding. Such trends suggest that the suspension of particulate material during flooding may actually remove metal species from the water column by particle scavenging and settling as the particles move through the water column.

The distribution patterns for trace metal elements from each water aliquot sample were plotted against their respective Mn, Fe, and Al concentrations to see if any correlations may exist between metal contaminants and any potential adsorptive phases. Only weak correlations were noted with Fe and Al suggesting that iron-bearing phases iron and aluminum-clays play only a limited role in influencing the transport properties of metals in the Big River system. By contrast, Mn tends to correlate fairly well with zinc and lead, suggesting that it may be strongly coupled to the transport of metal species as particulate manganese oxide particles (Figure 3). Particulate organic materials may also represent a potential adsorptive phase for metals, however, no analyses of the organic fraction have been conducted to date.

Lead vs Manganese in Water Samples

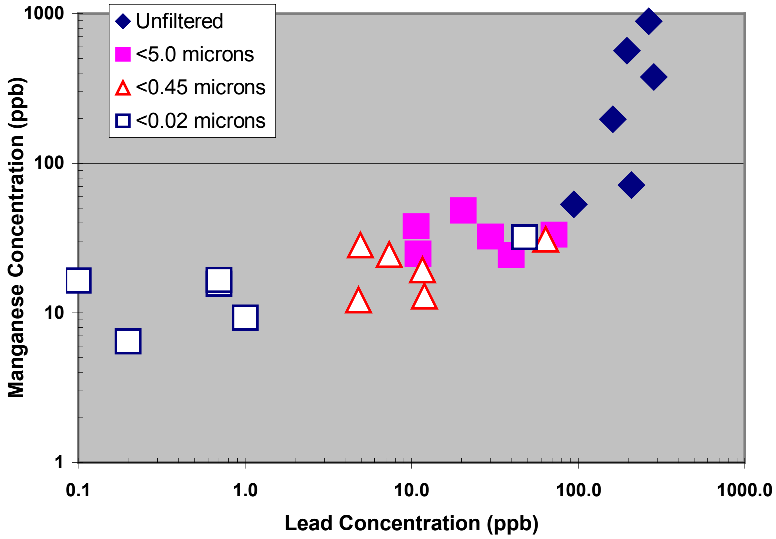

Figure 3. Log concentration of manganese versus log concentration of lead in waters from the Big River. Samples are segregated into four size fractions by filtration.

Bed-Load Sediments of the Big River and Associated Tailings Piles

Concentrations of Zn were greatest in the silt- to clay-sized sediments, ranging up to 1600 and 26,400 ppm for Big River and tailings pile sediments, respectively. Distributions of Cd closely paralleled those of Zn, although overall concentrations were considerably lower. Lead concentrations in sediments were also highest in the finer fractions with concentrations ranging up to 2800 and 24,800 ppm for Big River and tailings pile sediments, respectively.

X-ray diffraction analysis of the various sieve-sized sediment fractions from the tributary creeks and the Big River indicate that the sediments are dominated by dolomite and quartz. No other minerals were detected, including an absence of any clay minerals. Detection limits for XRD may vary from instrument to instrument, and with the types of minerals being analyzed, but generally minerals cannot be detected when they are present in concentrations that are lower than 5%. Sand to silt sized galena (PbS), pyrite-marcasite (FeS_2 polymorphs), iron oxides-oxyhydroxide, and manganese oxides were detected in the sediments by SEM/EDS analysis of the heavy mineral fraction (Figure 4). The sulfide grains generally decreased in size and increased in degree of roundness with transport distance down river. Sulfides have yet to be detected at any locations downstream of the St. Francois State Park site. Iron oxides appeared to be dominant over manganese oxides but no quantitative determinations have been made on the relative proportions of the two phases.

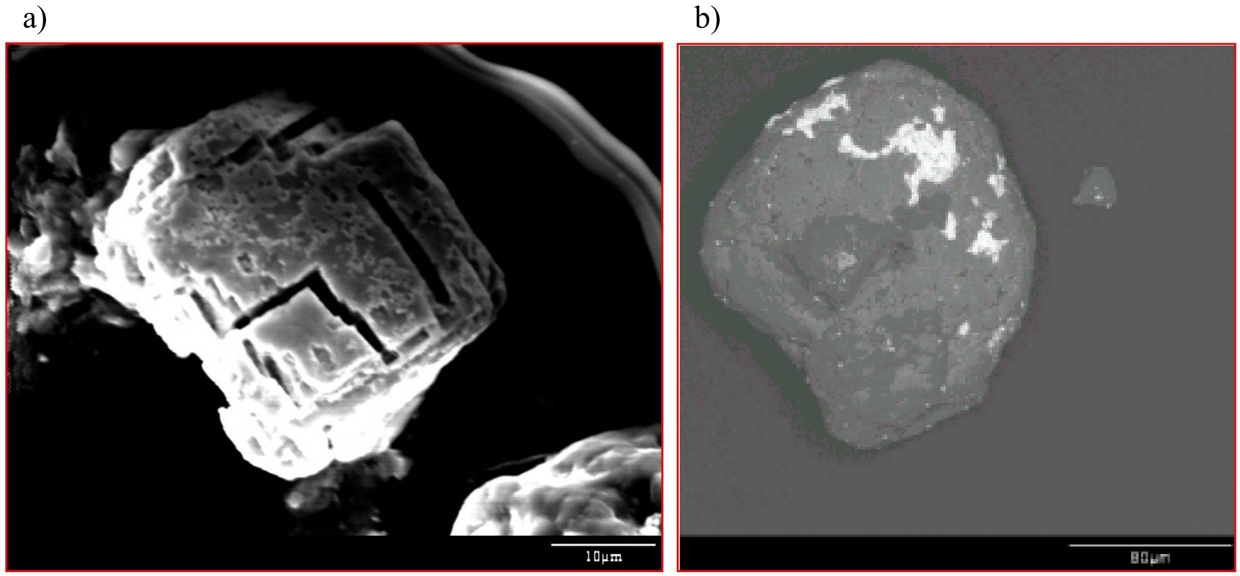

Figure 4. Scanning Electron Microscopy images of river sediment material collected from the Big River at the Highway 67 site. a) Secondary electron image of galena (PbS) grain displaying cubic morphology and corroded surface features, b) Backscattered electron image of rounded stream particle displaying galena (bright white), sphalerite (ZnS; small light gray triangular grain to left center of particle), iron oxide (gray matrix comprising the majority of the sample), and dolomite ($(Ca,Mg(CO_3)_2$ dark gray).

Black River Results

West Fork - Black River Water Column

Metal concentrations were generally low for most water column samples collected during the May 2002 sampling trip. A comparison of total metal concentrations between the West Fork of the Black River versus the relatively high concentrations noted previously for the Big River water samples attests to the improved environmental management practices employed by the mining companies in the New Lead Belt area. Values of lead Pb ranging from <1.0 (detection limit) to 6.7 ppb across the entire sampling area (Figure 5a). Lead concentrations were lowest at sites A, C, and F, all three of which are also located either upstream or the furthest downstream from known mining operations. Lead concentrations increased at site B, which receives effluent from the Brushy Creek Mine *via* Bills Creek. Lead concentrations were highest at sites D and E, which are located in close proximity downstream of the West Fork Mine. The higher lead concentrations at sites D and E, relative to B, probably reflect this close proximity of the West Fork mining operation rather than any notable difference in metal release. Lead distributions in the water column appear to be dominated by particulate material > 0.45 µm in size.

Zinc concentrations in water column samples from the May 2002 trip ranged from <1 to 163 ppb (Figure 5b). Metal distribution patterns roughly parallel those of lead with the highest concentrations occurring at sites D and E and the lowest concentrations occurring at sites A, C, and F.

Samples collected during the August 2002 sampling period displayed an anomalous distribution of lead in the water column (Figure 5c). There is minimal variability in the lead concentrations across the entire stretch of the sampling 17.8 mile long sampling region and most of the lead appears to be associated with the <0.02 µm size fraction. Lead values were relatively constant, between 3 and 5 ppb. The higher concentration of lead also extended to the control site A, an entirely unexpected result. It was later discovered that at approximately the same date of our sampling event there was a county construction crew working approximately 1.3 miles upstream from our site A location. The county operation involved the excavation and possible removal of some gravel from a 1500 foot or longer segment of the river in order to facilitate the construction of a low water bridge.

Zn values were also affected by the county construction operations during the August sampling period, but in a manner that varied somewhat from that of lead. Overall zinc concentrations were lowered following the county operation with the maximum concentration at 21 ppb (Figure 5d).

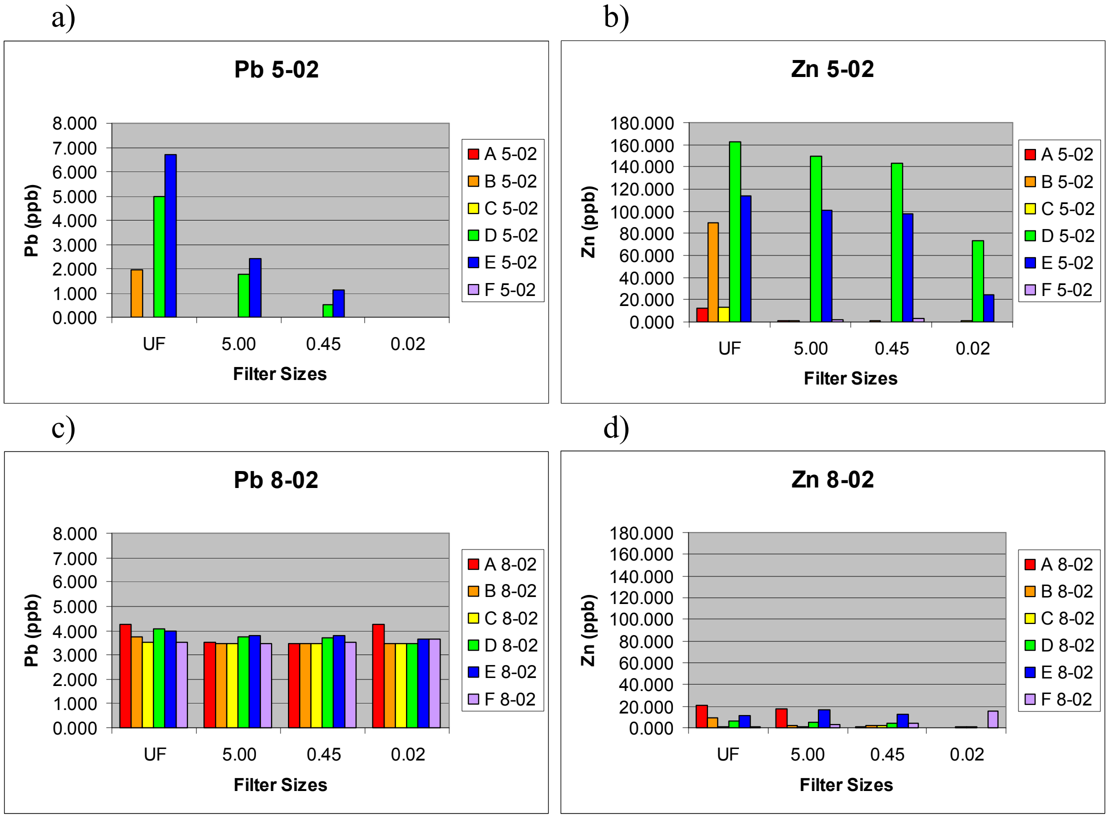

Figure 5. Metal concentrations from water column samples collected from the West Fork of the Black River. a) Zinc concentrations from May 2002, b) Lead concentrations from May 2002, b) lead concentrations from August 2002 reflecting the disturbance event caused by gravel mining , d) zinc concentrations from the August 2002 sampling event.

Since the highest zinc contents were noted at site A, and the zinc distribution at this location is dominated by the 5 µm and larger size fractions, the pattern suggests that the resuspension and later sedimentation of particulate grains may result in the adsorption and scavenging of some metals from the water column. A similar pattern was noted during the flood stage of the Big River. Additional sampling would be required to validate the potential for such a process. Although the zinc pattern produced by the county operation appears to dominate the metal distribution patterns for sites A, B, and C, the effect is diminished for sites D and E where a subdued increase in zinc concentration is noted. These sites are located just downstream from the West Fork Mine.

The identification of the "disturbance event" resulting from the county road crew construction activity has several important ramifications to our program of study. First of all, the detection of the disturbance event attests to the sensitivity of our sampling program to detect changes in the river system dynamics that influence the distribution of metals. Secondly, the disturbance of the river sediment at a location ***upstream*** of all known mining activity completely dominated the lead distribution patterns, effectively swamping any potential input from the three mines located in the watershed area. Such an event suggests that the release of metals directly from the tailings piles has a minimal impact on lead levels in the river. Finally, the disturbance event may mimic potential transport pathways for metals during flood events, except that the sediment was suspended by manmade rather than natural causes.

Black River Conductivity Results

Conductivity readings were taken as part of the regular site measurements. The readings display a nearly consistent increase from site A (the control site) to site E, a pattern that likely reflects increasing contributions from waters being released from the mine tailings sites (Figure 6). Site D consistently displayed lower readings than site E, even though D was located closer to the West Fork Operation than E. Conductivity readings at site F fall back down to levels that are just above those of control site A. Site F is located more than 13 miles downstream from site E. The higher overall conductivity patterns during each sampling date are inversely correlated with the level of stream flow. The lowest stream flow recordings were recorded for the 8/02 and 11/02 sampling events, which also display the highest conductivity readings. The highest stream flow event of 2/03 also correlates to the lowest overall conductivity readings, although there is some cross over between sites from the 5/02 and 2/03 sampling periods. The 8/02 disturbance event does not appear to have influenced the conductivity readings.

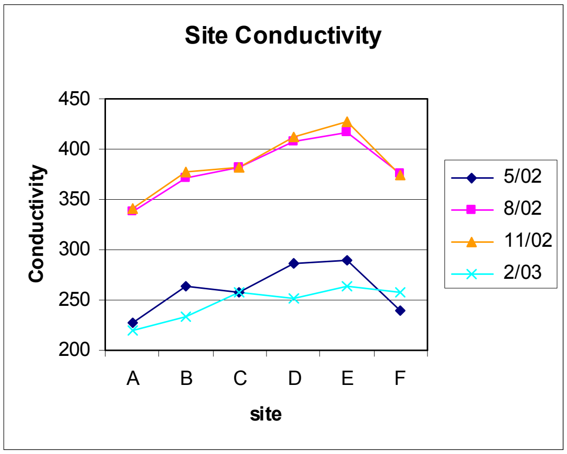

Figure 6. Conductivity readings from sampling locations on the West Fork of the Black River. All measurements are in µS.

Black River Optical Microscopy and SEM Results for Sediments

Optical microscopy and SEM-EDS analyses were conducted on the density-separated particles (gold panned) of sedimentary material from sites A, D, E, and F. Many of the density separated particles were composed mostly of Al, Mn, Fe, and Si, along with frequent Ca, Mg, K, S, O, and C. This is consistent with the presence of many clear grains that are coated with orange, red, and black minerals, which is expected when grains are covered with iron and/or manganese oxide coatings. A few particles displayed a Zn peak during EDS analysis, but no lead was found. Minor Ni and Cu peaks were also detected. One particle from site F displayed growths of small oval sphere of biologic material (possibly algae or blue-green algae). Overall, the most consistent trend was the Mn and Fe peaks and the orange color on most grains, either in patches or as a complete surface coating. Most of the coatings appeared to be composed of hematite (Fe_2O_3), based on their red color and iron-rich composition.

Conclusions

The first year samples were collected from the Big River and West Fork of the Black River in southeastern Missouri. Although our results are preliminary, some interesting observations have been made in the river systems.

- Metal concentrations in waters being emitted from the tailings piles are very high with a significant contribution from soluble and colloidal sized materials. The high metal concentrations occur despite the moderately alkaline nature of the water. Generation of an acidic microenvironment on the surfaces of sulfide minerals located within the tailings piles is believed to be the cause of the high metal contents.
- Metal concentrations decrease from the tributary creeks adjacent to the tailings piles to the Big River system. Decreases may result from dilution, adsorption onto sediment particles, or both.
- Storm surges in the river result in an increase in the concentration of most metals in the water column and an increase in the proportion of metals contained on particulate material. In some cases a disproportionally high amount of metals occur on the particles, suggesting that storm surges may result in the removal of metals from the water column by particle scavenging and sedimentation as the river flow subsides. A similar process was observed in the West Fork of the Black River following a construction related disturbance of the river gravels.
- Metals in the water column and bed sediments correlation with manganese, suggesting that manganese oxides are acting as adsorbents for metals in the river systems.
- Improved environmental mining practices in the New Lead Belt have resulted in substantially lowered metal release to the Black River system when compared to the Old Lead Belt. A disturbance of sedimentary material above all known mining operations has released enough metals from the river to swamp the release of metals from mining operations.

Development of a Model to Predict the Bioavailability of Metals to Soil Invertebrates

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Annual Report prepared for:
Center for the Study of the Bioavailability of Metals in the Environment

Project overview

Soil invertebrates, such as earthworms, springtails, and isopods, are important in nutrient cycling and maintaining the fertility and structure of soils and as food sources for terrestrial vertebrates. Each of these groups of animals have evolved different mechanisms for the uptake, metabolism, and excretion of metals. However, these mechanisms in this important group of animals are poorly understood, especially as they relate to metal toxicity. In soil systems, the fraction of metals available for interaction with soil organisms is determined by the physical and chemical characteristics of soil such as pH, organic matter content, and cation exchange capacity. These modifying factors interact with metals to determine the amount of metal that is present in solution in soil pore water, which is thought to represent the fraction of metal available for dermal uptake. However, dermal uptake is not the only route of metal exposure in soil invertebrates. Although it may be important in soil invertebrates lacking a cuticle (e.g., annelids), dietary exposure may also be important, especially in soil arthropods. The Biotic Ligand Model (BLM) not only relates dissolved metal concentrations to toxicity during aqueous exposures, but also considers the interaction of metals with organic and inorganic ligands. If the dissolved metals are indeed the toxic fraction in soils, the BLM should be applicable to soil systems as well. If it is not predictive of metal bioaccumulation and toxicity, that would suggest that other routes of exposure (diet) may be involved in metal uptake. The model would then need to be modified to include metal speciation in the intestine and within the organism.

The proposed research will examine the relationship between physical and chemical characteristics of soils (e.g., organic matter, pH, cation exchange capacity) and the bioavailability, uptake, metabolism, and toxicity of zinc (Zn) and cadmium (Cd) by soil invertebrates. Metal speciation in pore water, and in the invertebrate gastrointestinal system and tissues will also be determined in order to develop a Biotic Ligand Model for soil invertebrates which would enhance our ability to predict the toxicity of metals in soil systems.

Objectives

1) Examine the relationship between Zn and Cd levels in soil and toxicity and bioaccumulation by earthworms.
2) Develop methods for determine physiological conditions and metal speciation in the intestine of these soil invertebrates
3) Examine the partitioning of metals within the tissues of each of these soil organisms
4) Incorporate these parameters into the BLM

Research during this past year has focused on the development of a method for the fractionation of earthworms to provide a tool for examining the partitioning of metals within a soil invertebrate species. This is the first step in understanding metal metabolism in various organisms in order to determine which fraction of metal in the organism best represents a biotic ligand associated with a toxic response. This research was conducted in conjunction with visiting scientists Willie Peijnenburg (RIVM, The Netherlands), Martina Vijver (Vrije Universiteit, Amsterdam), and Post-doctoral associate Bintoro Gunadi (Ohio State University).

Introduction

Development of a fractionation procedure for examining the partitioning of metals in earthworms

The expression of metal exposure for earthworms for the purposes of ecological risk assessment is confounded by a number of factors. The environmental availability of metals in soils is determined by the metal interaction with components of the soil matrix such as organic carbon, pH, and cation-exchange sites (Lee et al. 1996; Janssen et al. 1997; Peijnenburg et al. 2001). The subsequent bioavailability of metals to earthworms is determined by factors that affect metal uptake and depuration by the worm, such as metal desorption kinetics from soil, routes of exposure (dermal vs intestinal tract), and metabolism, sequestration, and excretion within the earthworm. Another issue in expressing metal exposure in soil systems is the adherence of regulatory agencies to total metal levels as a measure of exposure, even though abundant literature suggests that total metals are not predictive of metal bioavailability or toxicity (Lock & Janssen 2001; Van Wensem et al. 1994). Critical body residues (CBRs) are concentrations of chemicals in organisms that are associated with toxicological effects and have been successfully developed and applied as measures of exposure to organic chemicals in aquatic systems (McCarty 1991). The development of CBRs for metals is more complex. Although total metal residues can be used as an absolute measure of metal bioavailability, this measure may not be useful as a CBR. Metal detoxification/defense mechanisms in earthworms involve the binding of metals to proteins such as metallothioneins or sequestration in granules (Melancon et al. 1992; Dallinger 1993; Reinecke et al. 1999; Spurgeon & Hopkin 1999). Once these detoxification mechanisms are exceeded, metal toxicity may be observed. In order to develop a CBR for metals in earthworms it is necessary to determine metal levels in organismal compartments that are not associated with detoxification. For instance, the amount of Cd that is bound to metallothionein and other ligands and is present as granules needs to be accounted for during the development of metal CBRs in earthworms. Simple tissue fractionation methods exist for the isolation of the protein-bound Cd fraction (primarily metallothionein-bound Cd) in invertebrates (Honeycutt et al. 1995; Jenkins & Mason 1988). Other researchers have applied fractionation schemes to examining Cd toxicokinetics in earthworms, using more of a mass balance approach and quantifying Cd in two fractions (Conder et al. 2002). The objective of this research was to adapt an empirical approach developed for examining the partitioning of metals in benthic invertebrates (Cain et al. 2000) and fish liver (Giguere 2002) to earthworms (*Eisenia andrei*) exposed to cadmium (Cd) in various types of soil. A data matrix of Cd and Zn levels in the various earthworm fractions was obtained. Trends in observed levels are discussed in terms of the exposure regime, and future research needs to substantiate the concept are discussed.

Materials and Methods

Earthworm bioassays

Twenty-eight day bioassays using *Eisenia andrei* were conducted with field soils individually spiked with 300 mg Cd kg^{-1}, dry weight, in triplicate using mature (clitellate) manure worms according to a standard protocol (ASTM 1997). The 200-g soil samples were moistened and maintained between –0.33 bar and saturation, placed in glass jars with 3 small air holes in the lid, and acclimated in an environmental chamber maintained at 20±1°C for 24 h prior to the addition of ten earthworms per replicate. Twenty-four hours prior to the addition of earthworms to test soils, mature (clitellate) earthworms weighing approximately 200-400 mg were removed from synchronized in-house cultures, rinsed with reagent grade water, and placed on moist filter paper for 24 hours to depurate most of the bedding material from their intestinal tracts. Randomly chosen earthworms were removed from the filter paper, rinsed, separated into replicates of ten earthworms, blotted dry, weighed, and transferred to test jars. Testing was conducted in an environmental chamber maintained at 20±1°C with constant light. Dead earthworms were removed on a daily basis, rinsed thoroughly with reagent grade water, individually wrapped in aluminum foil, and frozen at -20°C for subsequent analysis. At day 28 of each study, live earthworms were depurated for 24 hours on moist filter paper, rinsed, weighed, and stored as described above until metals analysis.

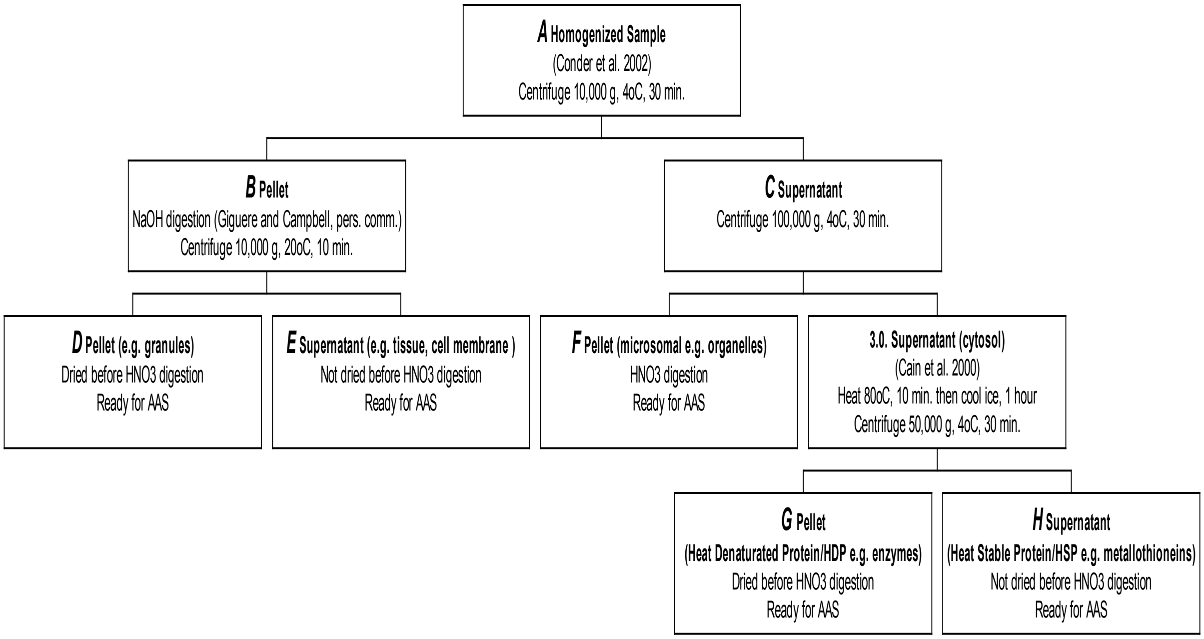

Figure 1. Fractionation scheme for the determination of metals in *Eisenia fetida*. Tissue fractionation procedure.

A - Homogenized sample

Two to four earthworms (400-1600 mg wet weight) were briefly thawed, weighed, placed in a 15-ml polyethylene centrifuge tube, and homogenized (OmniTH 30311 tissue homogenizer, 7-mm saw-tooth blade, Omni International Inc., Warrenton, VA) in 5 ml ice-cold 0.01M Tris HCl buffer (pH 7.0, Fisher Scientific, Houston, TX) for 3 minutes (1 min. full speed, 2 min. 1/2 - 3/4 speed). Homogenizer blades were rinsed twice with 1 ml of 0.01M Tris HCl buffer into the centrifuge tube. Homogenates were then centrifuged at 10,000 *g*, 4°C, for 30 minutes to obtain a pellet fraction enriched in cellular debris, nuclei, and mitochondria-lysosomes (Nash et al. 1981). The supernatant from this centrifugation process was decanted into a 10-ml polycarbonate ultracentrifuge tube in preparation for ultracentrifugation.

B - Pellet

The pellet remaining from the homogenization procedure was resuspended in 4 ml ultra pure (grade 1/millipore) water, poured into a 15-ml Pyrex test tube and heated at 100°C for 2 minutes. This fraction was then subjected to NaOH digestion (Giguere 2002) by adding 4 ml NaOH (1N) and heating at 60-70°C for 1 hour. The sample was then centrifuged at 10,000 *g*, 20°C, for 10 minutes.

The resuspension, digestion, and centrifugation of fraction B yielded the two fractions (D and E) discussed below.

C - Supernatant

The supernatant from the initial homogenization (A) was decanted to an ultracentrifuge tube and centrifuged (Beckman L7) at 100,000 *g*, 4°C, for 30 minutes to yield fraction F (pellet) and a supernatant.

D - Pellet (e.g. granules)

The remaining pellet fraction, representing granules, was resuspended in 8 ml 0.01M Tris HCl buffer and poured into a preweighed, 15-ml glass test tube and dried at 105°C overnight (12-15 hours), weighed to determine pellet dry weight, and digested in 2 ml concentrated HNO_3 at 105°C overnight, and resolubilized in 3 ml 0.5N trace metal grade HNO_3 prior to metal analysis by flame atomic absorption spectroscopy (FAAS; Perkin-Elmer Analyst 700).

E - Supernatant (e.g. tissue, cell membranes)

The supernatant from the NaOH digestion procedure, containing intact cells, tissues, and cell membranes was poured into a 15-ml glass test tube and dried at 105°C overnight, weighed to determine pellet dry weight, and digested in 2 ml concentrated HNO_3 at 105°C overnight prior to metal analysis by FAAS.

F - Pellet (e.g. microsomal)

The pellet fraction from the centrifugation of fraction C contains cell membrane microsomes (Nash et al. 1981) and was resuspended in 8 ml 0.01M Tris HCl buffer and poured into a preweighed, 15-ml glass test tube and dried at 105°C overnight, weighed to determine pellet dry weight, and digested in 2 ml concentrated HNO_3 at 105°C overnight prior to metal analysis by FAAS.

The supernatant from the centrifugation of fraction C contains ribosomes and other cytosolic constituents and was decanted into an ultracentrifuge tube, heated at 80°C for

10 minutes, and then cooled on ice for 1 hour. The sample was then centrifuged at 50,000 *g*, 4°C, for 30 minutes to yield fractions G and H.

G - Pellet (Heat Denaturated Protein/HDP e.g. enzymes)

The remaining pellet fraction was resuspended in 8 ml 0.01M Tris HCl buffer, poured into a preweighed, 15-ml glass test tube and dried at 105°C overnight, weighed to determine pellet dry weight, and digested in 2 ml concentrated HNO_3 at 105°C overnight prior to metal analysis by FAAS. Fraction G is thought to contain proteins such as enzymes that are denatured by heating to 80°C.

H - Supernatant (Heat Stable Protein/HSP e.g. metallothioneins)

The supernatant from the digestion and centrifugation of the supernatant of fraction C was decanted into a preweighed, 15-ml glass test tube and dried at 105°C overnight, weighed to determine pellet dry weight, and digested in 2 ml concentrated HNO_3 at 105°C overnight prior to metal analysis by FAAS.

Method detection limits for Cd and Zn in earthworm digests were estimated to be approximately 0.05 ug/g and 1.5 ug/g, respectively. Quality assurance/quality control (QA/QC) procedures included Cd analysis of procedural blanks, spikes, and certified reference material (lobster hepatopancreas, TORT-2, National Research Council, Canada). Analysis of the certified reference material (lobster hepatopancreas) by the metal analysis methods used in this research resulted in recovery of Cd and Zn within 95% confidence limits. All other Cd and Zn QA/QC measurements (e.g., spikes) were within 95-105% of expected values.

Soil analyses

0.1M $Ca(NO_3)_2$-extractable Cd concentrations in tests soils were determined following procedures detailed in Conder & Lanno (2000). QA/QC measures included duplicate analyses, metal spikes, blanks, and analyses of standard reference soil “Sandy Soil B” (CRM-SA-B, Environmental Express, Mt. Pleasant, SC). Measured Cd concentrations of the standard reference soil were within performance acceptance limits.

Data analysis

The fractionation procedure was used to generate a data matrix of Cd and Zn levels in the various earthworm fractions. Neither total body levels (fraction A) nor fraction D (granules, Cd/Zn levels below detection limit of flame atomic absorption spectroscopy) were determined in this study. Data analysis was by inspection or simple t-test comparisons between means (α=0.05).

Results

Cd and Zn were above detection limits in all of the fractions except fraction D (Table 1). Several trends in Cd and Zn concentration were observed:

1) Cd levels in the H fraction, thought to contain metallothionein, increased after exposure to Cd, especially in live worms or worms that did not perish rapidly (e.g., within 2 days)

2) Cd levels in fractions B and C of control worms were above detection limits but significantly lower than in Cd-exposed worms
3) Absolute levels of Cd in *E. andrei* appear to be higher than Zn levels, even in control worms. This was an unexpected result and may be due to food sources used in the culture of earthworms and requires further investigation.
4) Zn appears to be relatively evenly distributed over various fractions in both half procedures (B and C fractions) or full procedures (E,F,G,H).

Table 1. Cd and Zn concentrations (mg/kg) in tissues of earthworms exposed to Cd in field soils amended with a total nominal Cd level of 300 mg/kg (dry weight). Measured $Ca(NO_3)_2$ -extractable Cd levels (mg/kg) are also presented. (nd – not determined).

Fraction	Cd analysis	Bernow B - dead	Canisted A	Canisted control	Dennis A	Dennis A control
B		nd	30.5	1.9	17.6	1.1
C		91.7	62.0	41.5	88.0	43.0
D		9.7	nd	nd	nd	nd
E		13.7	14.9		13.3	
F		7.5	4.1		4.7	
G		217.4	4.1		6.1	
H			51.0		89.1	
$Ca(NO_3)_2$			43.6		235.1	
Fraction	Dennis B	Dennis B control	Dougherty A	Dougherty control	Haskel	Perkins - dead
B	18.2	1.4	40.5	1.9	16.0	196.1
C	126.5	1.9	131.2	35.5	68.1	97.0
D	nd	nd	nd	nd	nd	nd
E	11.7		16.5			73.3
F	4.1		5.4			10.3
G	3.5		7.3			10.0
H	75.3		115.0			27.6
$Ca(NO_3)_2$	197.8		253.1		242.1	230.2
Fraction	Pond Creek B	Pond Creek B - control	Pratt B - dead	Zn analysis	Bernow B – dead	Canisted A
B	10.2	0.8	129.7			10.6
C	91.0	2.5	129.7			7.6
D	nd	nd	nd			nd
E			41.8		nd	30.1
F			9.3		18.4	21.7
G			15.0		4.5	29.7
H			113.6		2.1	12.5
$Ca(NO_3)_2$	230.2		255.9		16.5	

Table 1. (cont) Cd and Zn concentrations (mg/kg) in tissues of earthworms exposed to Cd in field soils amended with a total nominal Cd level of 300 mg/kg (dry weight). Measured $Ca(NO_3)_2$-extractable Cd levels (mg/kg) are also presented. (nd – not determined).

	Canisted control	Dennis A	Dennis A control	Dennis B	Dennis B control	Dougherty A
B		14.0		16.5		18.7
C	15.2	32.1	20.6	18.0	13.5	9.6
D	nd	nd	nd	nd	nd	nd
E		24.2		22.8		13.6
F		10.3		26.3		85.5
G		25.9		21.8		31.0
H		15.2		28.6		66.0
$Ca(NO_3)_2$						
Fraction	Dougherty control	Haskel	Perkins - dead	Pond Creek B	Pond Creek B - control	Pratt B - dead
B		23.9		27.8		
C	13.6	15.6	16.0	35.8	30.4	10.1
D	nd	nd	nd	nd	nd	nd
E			16.3			17.9
F			6.0			10.6
G			5.7			11.4
H			12.8			24.3
$Ca(NO_3)_2$						

Discussion

The results of this study suggest that the fractionation procedure described in this paper can be used to separate different pools of metals within the earthworm and metal levels in specific fractions (e.g., H) will vary with exposure treatment and effect. In order to gain greater understanding of the potential of this method for determining fraction-specific relationships with metal levels in soil, it is necessary to:

1) Verify the constituents of each fraction using histological or biochemical techniques.
2) Increase the sample size and range of metal exposure concentrations in the soil.
3) Expand the database of metal levels in the fractions identified here.
4) Link the fractionation patterns observed to toxicity characteristics.
5) Generate data on the kinetics of uptake and elimination and link kinetics to available metal pools in soil and in metal fractions in the worms.

Preliminary findings of this study and continuing collaborative studies with Willie Peijnenburgh (RIVM) and Martina Vijver (Vrije Universiteit, Amsterdam) show that metal distribution within the earthworm body is different for essential and non-essential metals. Whereas Cd is predominantly stored in the metal fraction associated with the protein fraction and induction of this fraction was shown to occur upon exposure to Cd, Zn is more evenly distributed over the various metal fractions and induction of the protein fraction was not observed following exposure to Zn. Significantly increased Cd levels were found in the metal fraction associated with the cell tissue and the cell membranes following feeding of the litter feeding worm *L. rubellus* with Cd-contaminated leaf material in soils that contained high metal levels (total concentration) and low Cd concentrations in the pore water. Exposure of the non-litter feeding *A. caliginosa* did not lead to an increase in Cd levels whereas Zn levels did not increase in either species. This is probably due to regulation of internal Zn levels in the worms. These findings are in full agreement with metal levels found in field-collected worms and internal Cd and Zn distribution also agrees with laboratory observations. Following exposure of worms to soils spiked with metal salts, a marked increase in levels of metals associated with the protein fraction were observed. These observations allow for distinguishing the kinetics of elimination of the metal fractions associated with the protein fraction and the cell membranes/cell tissue. These experiments are currently in progress.

On the basis of the findings detailed above, a comprehensive model for metal uptake can be constructed that confirms earlier literature findings that at first glance are contradictive: First of all, a distinction needs to be made between metal uptake by litter-feeding worms and worms that do not consume leaf material. A number of studies have confirmed the importance of dermal uptake. However, in these experiments worms were not fed, metal levels in the pore water were relatively high, exposure was examined for short periods of time (<1 week), the focus was on regulated metals only, or worms were used which consume solely soil material. Our findings confirm that metal accumulation is the net result of all potential uptake routes. A dynamic balance between the contribution of each route exists, and apart from external metal pools, interactions within the organism result in clearly differing contributions of each of the routes. Regulation of essential metals further impacts the dynamics of metal uptake and their adverse effects on the organisms, and in summary it may be concluded that metal accumulation and metal toxicity are the result of a well-balanced interactions of the various uptake modes. Published studies where just a narrow part of the whole uptake spectrum is considered cloud the overall picture.

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Presentations from research

Lanno, R.P., Gunadi, B., Basta, N.T., Bradham, K., Vijver, M., Peijnenburg, W. A fractionation procedure for the development of critical body residues for metals in earthworms. International Symposium on Earthworm Ecology, Sep. 1-6, Cardiff, Wales.

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Papers submitted from research

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Bioaccumulation and Toxicity of Dietborne Particulate Metals in Benthic Invertebrates

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Center for the Study of the Bioavailability of Metals in the Environment

Major Accomplishments

- We published a review of the bioavailability and toxicity of dietborne copper (Cu) and zinc (Zn) to fish (Clearwater et al. 2002). This review demonstrates that total daily ingestion of these metals appears to be a better predictor of dietborne toxicity than does concentration of the metal in the diet, because of varying feeding levels among studies. Additionally, the review demonstrates that (1) biological incorporation of Cu or Zn into a "natural" diet can either increase or decrease metal bioavailability, depending on the metal-incorporation regimen; (2) apparent contradictions related to the effect of chelation by commercial organic ligands on the bioavailability of Cu and Zn can be reconciled (i.e., dietborne Cu and Zn become more bioavailable when either metal is both organically chelated and provided in very low daily doses), and (3) the relationship between bioavailability and toxicity remains unclear. A sequel review article about the bioavailability and toxicity of dietborne silver, aluminum, cadmium and lead to fish is being prepared for publication (Clearwater et al. 2003).
- In conjunction with a study by the U.S. Geological Survey and with minor support from this project, diel cycling of water quality parameters was characterized in a Zn-contaminated headwater stream in the Rocky Mountains. The Zn concentrations in this stream vary diurnally by ~2-fold, with concomitant variation in pH but not in alkalinity and the major cations. This information will help to design exposure regimens in future laboratory toxicity studies designed to test the ability of the biotic ligand model (BLM) to predict the acute toxicity of Cu and Zn to fish when water quality parameters vary diurnally.
- We conducted acute toxicity tests with larval fathead minnows (FHM; *Pimephales promelas*) exposed to Zn in the presence of elevated concentrations of $Ca(NO_3)_2$, $CaCl_2$, $Mg(NO_3)_2$, $MgCl_2$ and $NaNO_3$. These tests demonstrate that the paired anion with which a major cation is added to an exposure water can affect the toxicity of Zn to larval FHM. This has important implications for testing the ability of the BLM to predict acute toxicity of metals to fish.

Introduction

The University of Wyoming is contributing to the research program of the Center for the Study of Metals in the Environment in two distinct areas. First, we have been preparing extensive review articles about the toxicity of dietborne metals to fish (first item under Major Accomplishments above). Second, we are investigating the ability of the BLM to predict the acute toxicity of Cu and Zn to fish when water quality parameters vary temporally. To this end, we have begun to (1) identify water quality parameters that vary diurnally in receiving waters (second item under Major Accomplishments above) and (2) conduct preliminary experiments needed to prepare for toxicity tests under time-varying exposure conditions (third item under Major Accomplishments above).

The BLM provides a useful tool for regulating aqueous discharges of metals (Di Toro et al. 2001). Although the BLM thus far has been based on data from toxicity tests conducted under constant-exposure conditions (Santore et al. 2001), concentrations of metals and routine water quality parameters often vary through time in real-world situations. In concept, the BLM is designed to account for variations in concentrations of total metal and free metal ions by accounting for variations in the amount of metal accumulated at the biotic ligand over time (Di Toro et al. 2001). However, appropriate experiments are needed to test this concept. Furthermore, to design appropriate experiments, reasonable ranges of water quality parameters that vary on short time scales (e.g., diurnally) must be identified for a variety of receiving waters, and those parameters must be varied appropriately in the experiments.

While we were preparing for the initial toxicity tests in this study, confounding results from another research project indicated the need for some preliminary toxicity tests with Zn in the presence of the salts of major cations. Specifically, in flow-through Zn toxicity tests with larval fathead minnows in that project (funded by the International Lead Zinc Research Organization -- ILZRO), it appeared that (1) Ca was not as protective against Zn toxicity as previous toxicity tests indicated (i.e., we found only a 2-fold increase in the 96-h Zn LC50 over a 10-fold increase in Ca concentration, whereas a 5-10-fold increase in the LC50 would be expected), and (2) Mg and Na were not protective against Zn toxicity, even appearing to slightly increase the toxicity of Zn at relatively low concentrations of Mg (2.7 mM) and Na (1.6 mM; see Results and Discussion below). Rather than using the more traditional chloride salts of Ca, Mg and Na, we used nitrate salts to avoid the confounding effect of adding an ionoregulated ion (Cl^-) with the cations. However, that change in the mineral form with which we added the cations to our exposure solutions appeared to alter the physiology of the organisms. If supported by additional testing, that might alter considerably how the BLM would have to be parameterized. Therefore, we conducted additional flow-through toxicity tests to repeat the initial tests with the nitrate salts and also initiated a series of static-replacement tests to conduct side-by-side comparisons of the chloride and nitrate salts of Ca, Mg and Na.

In the remainder of this report, we will discuss research related to (1) characterizing the diurnal variability in water quality of a montane stream receiving Zn from an old mine, and (2) Zn toxicity in the presence of elevated concentrations of Ca, Mg and Na.

Methods

Diurnal Variation in Water Quality in a Zn-contaminated Montane Stream

From 22 to 24 August 2002, water quality was monitored intensively in a 650-m reach of High Ore Creek, a second-order montane stream at about 2,000 m elevation in central Montana. This stream reach lies immediately downstream from a settling pond that collects drainage from the Zn-contaminated Comet Mine site. Pond water containing elevated concentrations of Zn (~2.5 mg/L) discharges into the stream reach we monitored.

Water quality was monitored periodically during a 47-h period. Stream discharge, temperature, pH, dissolved oxygen, and conductivity were measured on-site, four times daily. Samples for cation and Zn analyses were collected hourly in an ISCO autosampler, after which subsamples for dissolved Zn analyses were filtered (0.45 μm). All cation and Zn samples (filtered and unfiltered) were acidified with 0.1% trace-metal grade HNO_3, transported on ice to the University of Wyoming, and then refrigerated at 4°C until analyzed. Methods used for the physical and chemical analyses are described below.

Water quality at the downstream end of that 650-m reach of High Ore Creek was also monitored from 11 to 13 August 2002, but not as intensively (i.e., only four or five times daily). However, alkalinity and major anions were measured during that period, in addition to temperature, pH, dissolved oxygen, conductivity, dissolved organic carbon, and dissolved Zn.

Toxicity Tests

All toxicity tests were conducted according to standard procedures (ASTM 1993, Weber 1993). Initially, we used flow-through tests to study the effects of the nitrate salts of Ca, Mg and Na on Zn toxicity in a series of experiments conducted from April 2002 through January 2003. After that, we conducted static-renewal tests for side-by-side comparisons of the effects of the nitrate and chloride salts of Ca and Mg in a series of experiments conducted in April and May 2003. Although the nitrate salts we used in the flow-through tests were ACS Grade purity, we could not reject the possibility that the unusual results we obtained were due to impurities in those salts. Therefore, in the static-renewal tests, we used a purer form of those nitrate salts (ReagentPlus Grade) in addition to the same batch of the ACS Grade nitrate salts, and ACS Grade chloride salts of Ca and Mg.

The duration of all of the toxicity tests was 96 h. The basewater to which the Ca, Mg and Na salts were added was a mixture of well water and reverse-osmosis/deionized water (nominal water quality: 22°C; pH 7.5; alkalinity of 0.5 mEq/L; hardness of 1 mEq/L; dissolved organic carbon (DOC) concentration of <0.5 mg/L). The toxicity of Zn was tested in the basewater at three separate times (April, November and January) during the flow-through testing period, and once each at the same time as the three side-by-side static-renewal Ca salt tests (in April) and the three side-by-side static-renewal Mg salt tests (in May).

We used larval fathead minnows ($\leq$24 h old) produced by a stock population of adults maintained at the University of Wyoming (originating from the U.S. Environmental Protection Agency's Midcontinent Ecology Division in Duluth, Minnesota, USA) for all of the tests. The FHM adult brood stock was reared in well water (pH 8, alkalinity and hardness of 4 mEq/L, and DOC concentration of <0.3 mg/L), but each batch of FHM eggs was hatched in the water quality in which they were tested. To avoid complications associated with the sorption of metals onto food, the larvae were not fed.

Physical and Chemical Analyses

Extensive water quality analyses, including temperature (by hand-held thermometer), pH (by meter), dissolved oxygen (by meter), alkalinity (by titration), hardness (by titration), anions (by ion chromatography), cations (by flame atomic absorption spectrophotometry), dissolved organic carbon (DOC; by combustion), and dissolved (0.45-µm filtered) and total Zn (by flame atomic absorption spectrophotometry), were conducted on all exposure waters in each test using standard methods (USEPA 1979, APHA et al. 1995). Ammonia was analyzed by colorimetry (Verdouw et al. 1977). These analytical techniques are described in more detail in the UW QAPP for this research project.

Similar analyses were conducted for the characterization of diurnal water quality variation in High Ore Creek. Additionally, stream discharge was measured from velocity and cross-sectional area in-stream at the upstream end of our study reach, and in an in-stream Parshall flume at the downstream end of our study reach.

Statistical Analyses

We calculated LC50s (median lethal concentrations) for the Zn toxicity tests using the trimmed Spearman-Karber procedure in TOXSTAT Version 3.4 (WEST and Gulley 1994).

Results and Discussion

Diurnal Variation in Water Quality in a Zn-contaminated Montane Stream

From 22 to 24 August 2002, the major diurnal changes in water chemistry in High Ore Creek that are of interest for BLM modeling were ~0.5-unit changes in pH (from ≥ 7.5 to ≤ 8.3; Fig. 1b) and slightly greater than 2-fold changes in dissolved Zn concentrations (Fig. 1e). Dissolved Zn averaged ~90% of the total Zn concentration (Fig. 1f) at all three sites. Although the Zn concentration did not vary much on a daily basis at the upstream site (immediately downstream from the pond), the magnitude of the diel variations in Zn concentration increased downstream and became large by the end of the 650-m reach. Based on a NaCl tracer study and flow rates measured at the upstream and downstream sites, groundwater discharge into the stream increased the stream flow by only ~10% in the 650-m reach. The stream-water transit time was ~1 h. Discharge varied by ~20% during the 47-h sampling period (Fig. 1g), temperature varied by ~8°C (Fig. 1a), and concentrations of major cations (Ca^{2+}, Mg^{2+}, Na^+, K^+) did not vary considerably, as illustrated for example by Ca in Figure 1d. Although dissolved oxygen concentrations varied by up to 35%, they never decreased below 85% of saturation (Fig. 1c).

From 11 to 13 August 2002, the diel cycles of temperature (8.7-22.8°C), pH (7.4-8.3), dissolved oxygen (90-107% saturation) and dissolved Zn (0.7-2.3 mg/L) at the downstream site were similar to those observed at the same site from 22 to 24 August. Alkalinity (1.6-1.9 mEq/L) and concentrations of DOC (8.5-11.1 mg/L), Ca^{2+} (53-55 mg/L), Mg^{2+} (12.8-13.4 mg/L), Na^+ (5.0-5.1 mg/L), K^+ (1.9-2.1 mg/L), Cl^- (0.8-1.4 mg/L), SO_4^{2-} (126-140 mg/L), F^- (0.10-0.12 mg/L) and NO_3^- (<0.10-0.71 mg/L) varied to various extents, but with no apparent diel cycling.

Based on these results, diurnal changes in pH and dissolved Zn concentrations might be the most important components to include in a test of the BLM under time-varying water quality conditions for this type of receiving water. Because the pH was higher during daylight hours when Zn concentrations were lowest, the bioavailability of Zn might be lower during daylight hours (when most grab samples are collected) and higher at night (when pH is lower but still ≥ 7, and Zn concentrations are highest). This type of combined pH and metal cycling is not unusual in metal-contaminated streams and rivers in the Rocky Mountain region.

Toxicity Tests

In the flow-through Zn toxicity tests, elevated $Mg(NO_3)_2$ concentrations (2.2-2.7 mM) and elevated $NaNO_3$ concentrations (1.0-1.6 mM) resulted in slightly lower 96-h Zn LC50s than in the basewater; and those LC50s decreased slightly as the concentrations of $Mg(NO_3)_2$ and $NaNO_3$ increased within the ranges tested (Fig. 2). Additionally, the protective effect of $Ca(NO_3)_2$ against Zn toxicity was not as large at would be expected from traditional hardness relationships. That is, the 96-h Zn LC50 was only ~2-fold higher in the presence of a 10-fold higher Ca concentration (2.5 mM Ca in the elevated $Ca(NO_3)_2$ test vs. ~0.25 mM Ca in the basewater tests), whereas a 5-10-fold higher LC50 would be expected in this hardness range (Meyer 1999). Water quality parameters for the flow-through tests are listed in Table 1.

The results of these flow-through Zn toxicity tests suggested that either (1) Ca^{2+} is not as protective as traditionally thought, and Mg^{2+} and Na^+ interact synergistically with Zn, (2) NO_3^- interacts synergistically with Zn, or (3) NO_3^- interferes with the protective effects of Ca^{2+}, Mg^{2+} and Na^+. However, we could not exclude the possibility that the ACS Grade of these nitrate salts contained an unidentified contaminant, despite analyses of the Ag, Cd, Cu, Ni, Pb and Zn contents of those salts in our laboratory that showed no concentrations of trace metals high enough to be of toxicological concern. Therefore, we conducted two separate sets of four side-by-side static-renewal Zn toxicity tests to test if the purity of the added nitrate salt and/or the anion paired with the cation affected the Zn toxicity. The first set of four side-by-side tests was conducted in basewater, basewater containing elevated $Ca(NO_3)_2$ (ACS Grade), basewater containing elevated $Ca(NO_3)_2$ (ReagentPlus Grade -- a higher purity than ACS Grade), and basewater containing elevated $CaCl_2$ (ACS Grade). The second set of four side-by-side tests was conducted in basewater, basewater containing elevated $Mg(NO_3)_2$ (ACS Grade), basewater containing elevated $Mg(NO_3)_2$ (ReagentPlus Grade), and basewater containing elevated $MgCl_2$ (ACS Grade).

The purity of the nitrate salts did not affect Zn toxicity, for either $Ca(NO_3)_2$ or $Mg(NO_3)_2$. In fact, the 96-h LC50s for the two purities of $Ca(NO_3)_2$ differed by only 2%; and the 96-h LC50s for the two purities of $Mg(NO_3)_2$ differed by only 4% (Fig. 3).

However, Ca^{2+} and Mg^{2+} differed in their protective effects against Zn toxicity. When Ca^{2+} was added as 2.2 mM $Ca(NO_3)_2$, the 96-h Zn LC50 was 2.4-fold greater than in the basewater; whereas when Mg^{2+} was added as 2.0 or 2.3 mM $Mg(NO_3)_2$, the 96-h Zn LC50 did not differ from the LC50 in the basewater (Fig. 3). And when Ca^{2+} was added as 2.2 mM $CaCl_2$, the 96-h Zn LC50 was 4.3-fold greater than in the basewater; whereas when Mg^{2+} was added as 2.3 mM $MgCl_2$, the 96-h Zn LC50 was only 2.0-fold greater than in the basewater (Fig. 3).

Moreover, the anion paired with the cation affected Zn toxicity. For Ca^{2+} and Mg^{2+}, the 96-h Zn LC50 almost doubled when the cation was paired with Cl^- compared to NO_3^- (Fig. 3). Unfortunately, based on these results alone, we cannot determine whether (1) Cl^- protects against Zn toxicity and NO_3^- has no effect, (2) NO_3^- interacts synergistically with Zn to increase the Zn toxicity and Cl^- has no effect, or (3) some combination of the previous possibilities occurs. Direct toxicity of NO_3^- probably is not a contributor, because we observed no mortalities or outwardly apparent signs of stress in FHM larvae at 10 mM $NaNO_3$ during a $NaNO_3$ toxicity test we conducted in November 2002. To test for indirect interactions of NO_3^- with Zn, we plan to conduct an analogous set of side-by-side static-renewal Zn toxicity tests in basewater, basewater containing elevated KNO_3, and basewater containing elevated KCl -- assuming that K^+ neither protects against Zn toxicity nor interacts synergistically with Zn.

Water quality parameters for all of the static-renewal toxicity tests are listed in Table 1. Except for much higher DOC concentrations (6.5-7.3 mg/L) in the static-renewal tests, water quality was similar in the static-renewal and flow-through tests. The elevated DOC concentrations, which appears to have leached from the plastic cubitainers in which we prepared the exposure solutions, might explain why the 96-h Zn LC50s in the static-renewal tests were approximately 10 to 50% higher than in their corresponding flow-through tests. Because (1) those LC50s are based on measured dissolved Zn concentrations, (2) the 96-h dissolved Zn LC50s were 94 to 100% of the total Zn LC50s in the static-renewal tests and 91 to 95% of the total Zn LC50s in the flow-through tests, and (3) in a preliminary static-renewal test, initial and final total and dissolved Zn concentrations for the 24-h renewal periods differed by $\leq$5%, loss of Zn (via sorption to the glass beakers and/or precipitation from solution) during the static-renewal tests does not explain the increased LC50s. Fortunately, the paired LC50s in the static-renewal and flow-through tests are within the HydroQual range of acceptability for aquatic toxicity modeling (i.e., within a factor of 2; Di Toro et al. 2001).

Anticipated Year 2 Efforts

We will complete the manuscript for a review article about the bioavailability and toxicity of dietborne silver, aluminum, cadmium and lead to fish. Additionally, we will complete the series of static-renewal Zn toxicity tests in the presence of elevated concentrations of major cations by testing $NaNO_3$, NaCl, KNO_3, and KCl. After those experiments are completed, we will analyze the results and decide how best to alter major ion chemistry in the toxicity tests designed to test the ability of the BLM to predict the acute toxicity of Cu and Zn to fish. Then we will begin to conduct toxicity tests needed to calibrate the BLM and a model of time-varying toxicity to the larval fathead minnows we are using in this study.

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Table 1. Physical-chemical characteristics of the basewater used in flow-through and static-renewal toxicity tests conducted with fathead minnow (*Pimephales promelas*) exposed to Zn for 96 h. Listed are the ranges of mean values among the 9 flow-through tests conducted from April 2002 through January 2003 and the 8 static-renewal tests conducted in April and May 2003.

Parameter	Flow-through tests	Static-renewal tests
Temperature (°C)	21.2 - 23.5	21.5 - 22.7
pH	7.48 - 7.58	7.47 - 7.57
Alkalinity (mEq/L)	0.53 - 1.00	0.63 - 0.71
DOC (mg/L)	0.49 - 0.89	6.5 - 7.3
Dissolved oxygen (mg/L)	6.3 - 6.9	6.2 - 6.5
Total ammonia (mg NH_4/L)	<0.01	<0.01
Major ions (mM):		
Ca^{2+}	0.14 - 0.29	0.25 - 0.27
Mg^{2+}	0.17 - 0.27	0.29 - 0.30
Na^{+}	0.02 - 0.20	0.20 - 0.23
K^{+}	0.01 - 0.46	0.01 - 0.03
Cl^{-}	0.015 - 0.032	0.035 - 0.054
NO_3^{-}	0.018 - 0.056	0.017 - 0.028
SO_4^{2-}	0.19 - 0.37	0.32 - 0.37
F^{-}	0.001 - 0.011	0.009 - 0.011

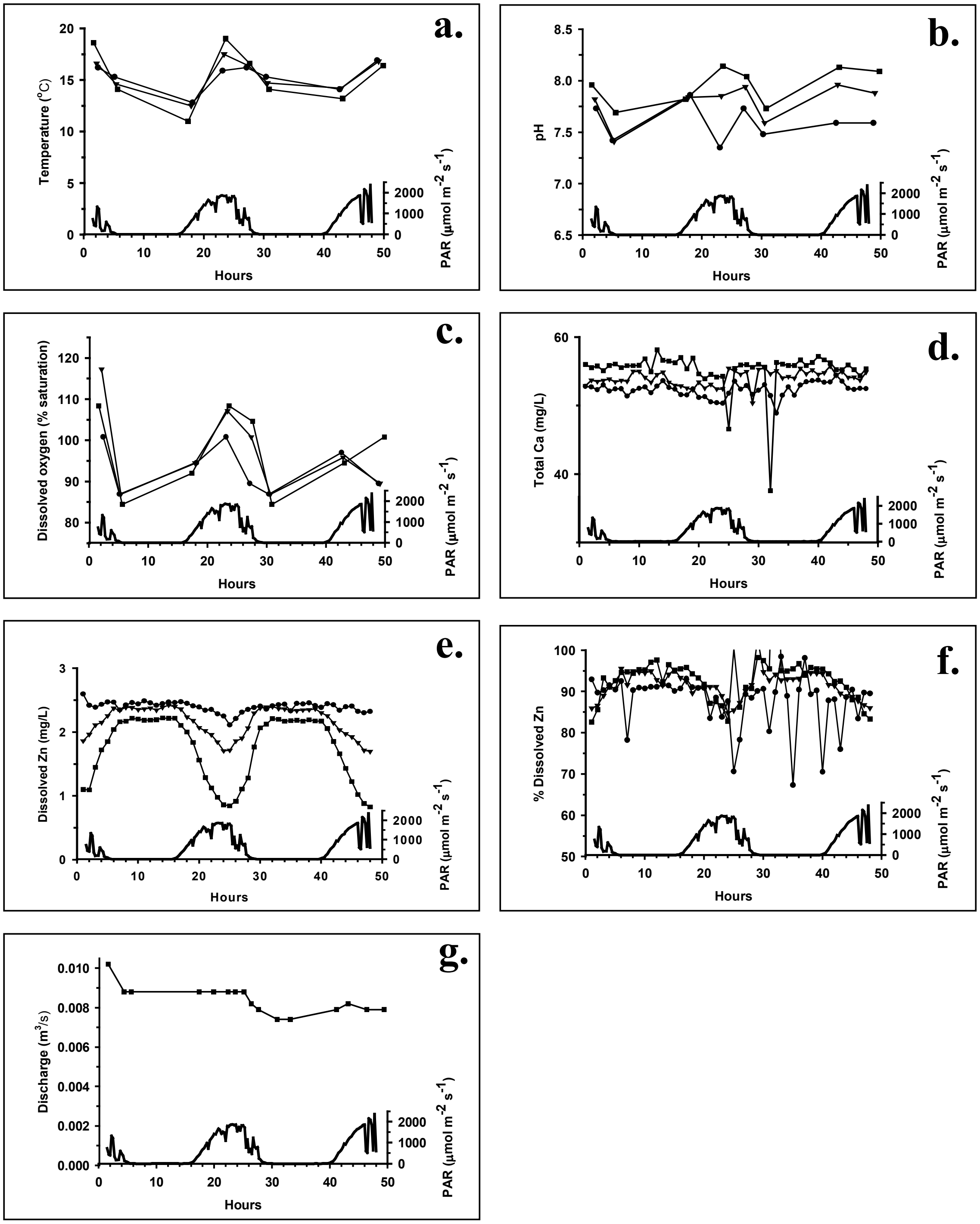

Figure 1. Water quality during 48 h of sampling along a 650-m reach of High Ore Creek, Montana, from 16:00 on 22 August to 15:00 on 24 August 2002. The upstream (•), mid (▼) and downstream (■) sampling sites were located at 0, 350 and 650 m along this reach. PAR = photosynthetically active radiation (400-700 nm).

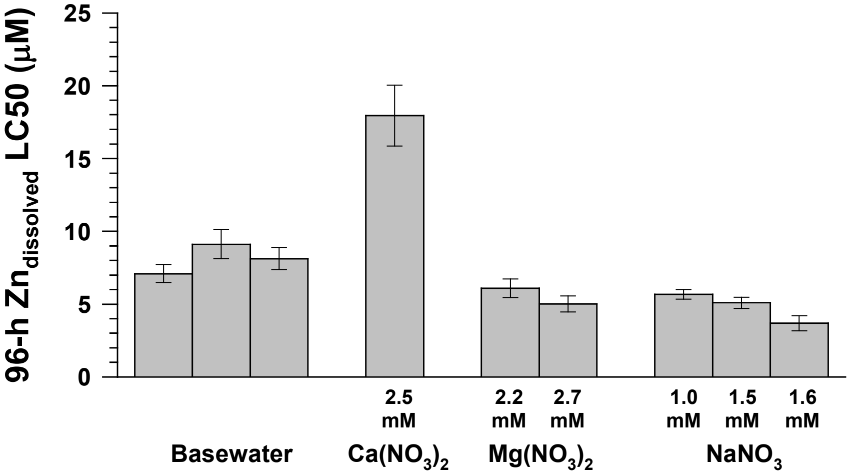

Figure 2. Toxicity of Zn to larval (≤24 h old) fathead minnow (*Pimephales promelas*) in 96-h flow-through toxicity tests conducted from April 2002 through January 2003 in a mixture of well water and reverse-osmosis/deionized water (basewater) with or without added $Ca(NO_3)_2$, $Mg(NO_3)_2$ or $NaNO_3$. Chemical characteristics of the basewater are listed in Table 1. The error bars are 95% confidence intervals.

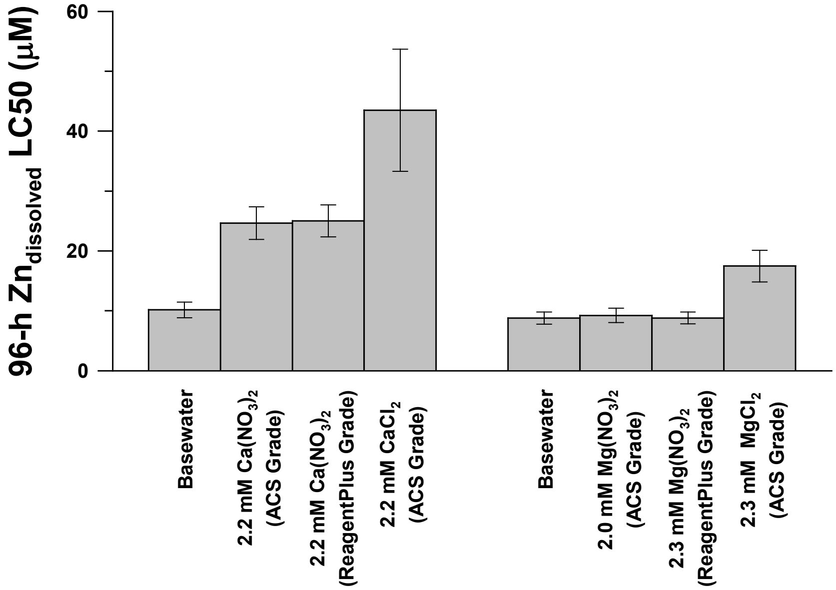

Figure 3. Toxicity of Zn to larval (≤24 h old) fathead minnow (*Pimephales promelas*) in 96-h static renewal-toxicity tests conducted in April and May 2003 in a mixture of well water and reverse-osmosis/deionized water (basewater) with or without added $Ca(NO_3)_2$, $CaCl_2$, $Mg(NO_3)_2$ or $MgCl_2$. Chemical characteristics of the basewater are listed in Table 1. The error bars are 95% confidence intervals.