

DEVELOPMENT AND APPLICATION OF FIELD METHODS FOR DETERMINATION OF
THE EXTENT OF ACID MINE DRAINAGE CONTAMINATION, AND GEOCHEMICAL
CHARACTERISTICS OF STREAM SEDIMENT RECOVERY

By

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ABSTRACT

Metal-mining processes can negatively affect surface and ground waters through the generation of acidic mine drainage (AMD). Introduced metals typically are deposited from the water column onto the stream bed downstream of the AMD input(s). The drivers of metal deposition onto stream sediments are through either the formation of insoluble colloids, adsorption to particles, or complexation with sediment surfaces. Metals can be removed from stream sediments through physical (scouring) or chemical processes (dissolution and desorption). These processes have been examined in published literature but those studies largely used *ex-situ* analysis techniques because experimental conditions are easier to control in the laboratory. In the studies presented herein, I developed a novel method to examine AMD metal deposition *in-situ*.

The study site was the North Fork of Clear Creek (NFCC), located in Gilpin County in central Colorado, which receives 2 point sources of AMD as it flows through the city of Black Hawk, Colorado. I modified an existing field method to monitor benthic invertebrate populations *in-situ*, to examine the flux of metals on stream sediment in both deposition and recovery scenarios. Also using my developed methods, I was able to examine the effects of scouring (physical removal), stream flow, and sediment surfaces on metal sediment deposition. I found that significant metal loss can occur on sediments after 8 weeks of reference water exposure, and that biofilm growth on sediment surfaces can accumulate significant concentrations of metals from the water column.

A combination of in-situ and ex-situ analysis can allow for greater realism and applicability in the data while not sacrificing rigor, replicates and reproducibility. Examination of the processes of metal deposition and removal from sediment can lead to a better understanding of the fate of AMD-associated metals and better prediction of the time course and extent of post-remediation recovery from AMD contamination.

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LIST OF ABBREVIATIONS

AAS.....	Atomic absorption spectroscopy
Al.....	Aluminum
AMD.....	Acid mine drainage
ANOVA.....	Analysis of variance
°C.....	Degrees Celsius
Ca.....	Calcium
CaCO ₃	Calcium carbonate
CCV.....	Continuing calibration verification
Cd.....	Cadmium
CI.....	Confidence interval
cm.....	Centimeter
Cu.....	Copper
CV.....	Coefficient of variation
d.....	Day
DOC.....	Dissolved organic carbon
DOM.....	Dissolved organic matter
DO.....	Dissolved oxygen
EDTA.....	Ethylenediaminetetraacetic acid
ESP.....	Extracellular polymeric substances
°F.....	Degrees Fahrenheit
Fe.....	Iron
Fe ²⁺	Ferrous iron

Fe ³⁺	Ferric iron
Fe(OH) ₃	Ferric hydroxide
FeS ₂	Iron sulfide, pyrite
g.....	Gram
GI	Gregory Incline
h.....	Hour
HFO.....	Hydrous ferric oxide
HNO ₃	Nitric acid
H ₃ PO ₄	Phosphoric acid
H ₂ SO ₄	Sulfuric acid
IDL.....	Instrument detection limit
ICP-MS	Inductively coupled plasma-mass spectrometer
ICP-OES	Inductively coupled plasma-optical emission spectroscopy
K.....	Potassium
kg.....	Kilogram
km	Kilometer
L	Liter
LC50	Median lethal concentration (concentration causing 50% lethality)
M.....	Metal (generic)
Mg.....	Magnesium
m	Meter
mg	Milligram
ml	Milliliter

mm	Millimeter
Mn	Manganese
Mohm	Megohm
NFCC	North Fork of Clear Creek
Ni	Nickel
NIEHS	National Institute of Environmental Health Sciences
NT	National Tunnel
OES	Optical emission spectroscopy
O ₂	Oxygen (molecular)
POM	Particulate organic matter
PVC	Polyvinyl chloride
QA/QC	Quality assurance/Quality control
S	Sulfur
Sc	Scandium
SD	Standard deviation
SEM	Scanning electron microscopy
SHM	Suwanee Humic Model
SO ₄	Sulfate
TEM	Transmission electron microscopy
TOC	Total organic carbon
μg	Microgram
μL	Microliter
μm	Micrometer

USA..... United States of America
USEPA..... United States Environmental Protection Agency
USGS United States Geological Survey
V..... Vanadium
XRD X-ray diffraction
WWTP Waste water treatment plant
Zn Zinc

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CHAPTER ONE

INTRODUCTION

The goal of this thesis research is to provide unique methodologies for the examination of metallic contaminant fate and transport in a natural surface-water system.

1.1 Environmental implications of metal mining

Geological excavation, extraction, and refinement processes have led to legacy environmental damage throughout the world. Although mining activities can scar the landscape, produce large quantities of solid waste, and result in ground and surface water contamination, mining activities are needed to excavate geological material for either energy or materials production. Metal mining and extracting activities have been occurring for thousands of years to facilitate production of tools, while mining for coal greatly increased during the industrial revolution as a source of thermal energy for steam generation.

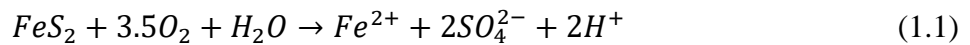
Many past mining activities have led to legacy environmental damage that has persisted for many years after the mining activities have ceased. Large quantities of waste are generated during the process of mining, because typically the metal of interest is found in low concentrations in the excavated ore. Mining and mineral processing in the USA produce ~1.8 billion tons of solid waste every year [1], which is more than 10x the amount of solid municipal waste generated [2]. The subsequent smelting and refining processes of metals can generate air pollution, solid waste, and large quantities of liquid chemical waste, which can also negatively impact ground and surface waters [3]. Mismanagement of the large quantities of solid waste, and mine shafts, and tunnels that were not properly managed or contained, can cause for the formation of acidic drainage that may escape the waste pile and enter surface and ground waters [4].

Solid mine waste has two different forms; waste rock or tailings, both of which may contain high concentrations of sulfide-containing minerals that are susceptible to oxidation [5]. The typical particle size of waste rock is ≥ 20 cm, while the typical particle size of tailings is ≤ 0.2 mm. Waste rock piles allow for increased permeation of oxygen and water into the waste pile because of the larger particle size, which results in increased reactivity. Tailings are composed of fine-grained particles and are thus more uniform in composition and lower in sulfide concentration; however, oxidation is limited because of the ability of oxygen and water to penetrate deeper beyond the surface of the waste pile [6].

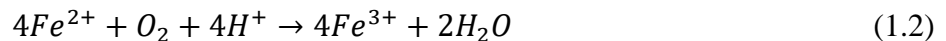
1.2 Sulfide oxidation and acid generation

Depending on the specific mineralogy of the region, waste rock and tailings can contain high concentrations of minerals that have acid-generating potential. Sulfidic minerals (the most common being pyrite – FeS_2) have the potential to oxidize when exposed to O_2 and water, generating acid, sulfate and metal cations [7,8,9].

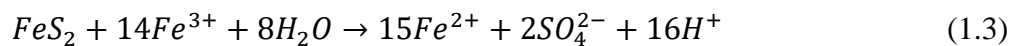
The reaction that starts this process is the oxidation of pyrite with O_2 and water. This reaction results in generated acid, sulfate and ferrous iron (Equation 1.1).



Ferrous iron is soluble in water and thus highly mobile in the environment, and it has the potential to oxidize as well. This oxidation is primarily pH-driven (Equation 1.2).



After ferrous iron oxidizes to form ferric iron, it can aid in the oxidation of pyrite by acting as the oxidant in place of O_2 (Equation 1.3).



Many metals are very soluble in acidic environments and are released by mineral dissolution of surrounding waste rock, which can contribute waterborne metals in addition to Fe. Silicate- and carbonate-containing minerals can mediate this process through neutralization of the generated acid [10,11]. AMD generation in a waste-rock pile may be highly variable because of differences in the concentrations of acid-generating and acid-neutralizing minerals as well as the unique physical and chemical features of the mine and/or waste piles. Temperature, oxygen, water and presence of sulfide-oxidizing bacteria in the waste pile affect the rate of acid generation [12,13]. Some species of bacteria can survive the acidic environment in a waste-rock pile containing sulfides, which they use as electron donors in their metabolic processes [14,15]. This can greatly increase the rate of sulfide oxidation and thus acid generation. To counter this important acid-generating process, modern, regulated mining practices include the use of bactericides to limit the growth of sulfide-oxidizing bacteria in the mine waste [16,17].

1.3 Aqueous metals

AMD affects the water and sediment chemistry of receiving aquatic systems [18,19]. Aqueous carbonate and bicarbonate species are consumed by the introduced acid, resulting in decreased alkalinity.

Fe and Mn released from minerals through chemical weathering are typically in a reduced oxidation state (Fe^{2+} and Mn^{2+}) [20]. The rates of oxidation of Fe and Mn are very slow in acidic environments [13]. Typically the majority of oxidation will occur after the AMD has been introduced into a circumneutral receiving aquatic system, thus increasing the pH [13]. The oxidation of Mn is very slow at $\text{pH} \leq 8$ and does not significantly occur in a circumneutral pH environment unless the oxidation of Mn is catalyzed on a surface [21,22,23,24]. This catalysis is through electron-accepting surface groups or by microbial biofilms on the sediment surfaces. The

results of aqueous Mn^{2+} oxidation are Mn-oxides (i.e., hausmannite and birnessite), which form a hard black substance typically found plated on stream-bed surfaces [25].

Dissolved metals can undergo hydrolysis reactions, depending on the pH of the water. As the pH increases, more hydrolysis reactions may occur, ultimately leading to the formation of metal-oxyhydroxide particles [26]. This reaction also generates acid, due to the formation of hydroxide complexes with OH^- ions in the water [26]. This may continue to affect the pH of the stream downstream of AMD inputs.

Water chemistry conditions control the speciation of aquatic metals [27] and thus the environmental mobility, toxicity and solubility of those metals [28,29,30]. Metals can form inorganic complexes with anions such as hydroxide, carbonate and chloride; however, chloride complexes need high ionic strength and low concentrations of other more strongly-binding anions.

Metals can also complex with dissolved organic matter (DOM) [31,32], which is operationally defined as organic carbon molecules that can pass through a 0.45 μm filter. If the organic matter is larger than 0.45 μm , it is defined as particulate organic matter (POM). DOM is primarily composed of humic substances, which are a mixture of humic and fulvic acids. Fulvic acids are composed of unidentified, low-molecular-weight, polyelectrolyte, organic-acids in the water column, while humic acids are of larger molecular weight. DOM also contains a minor component of identifiable organic molecules, mainly low-molecular weight acids such as acetate. DOM is also typically characterized by its origin, which is either through the decomposition of land-based plant material (allocthonous) or decomposition of aquatic plants (autocthonous) [33]. These two types of DOM vary in their chemical properties due to their origin. The metal-binding sites on DOM are primarily deprotonated carboxyl or phenolic groups, which results in a pH

dependency on the metal-binding capacity of DOM [34]. As the pH increases, the ability of DOM to complex metals also increases.

1.4 Metals on sediment

Metals have very minimal solubility at alkaline pH values, due to the previously discussed formation of insoluble hydroxide complexes. The increased binding capacity of surfaces in both the water column (e.g., onto suspended particles) or on the stream-bed are due to deprotonation of weakly acidic surface sites. Metals can leave the water column either through precipitation to form an insoluble phase, or by complexation with the surface of a sediment particle. If metals form or are bound to an insoluble phase, the metal may settle out of the water column and accumulate as loose metal oxy-hydroxide floc on/in the stream bed [35]. Depending on water composition, especially pH, poorly crystalline iron-oxides include ferrihydrite, schwertmannite, and jarosite [36,37].

These settled metal flocs and/or surface coatings on stream-bed sediment can crystallize to form minerals. In the case of iron, these minerals could be goethite or hematite [36,37]. Formation of these minerals is kinetically limited and depends on ambient pH and concentrations of other anions such as sulfate [38].

In addition to metals being deposited onto sediments, metals can also be removed from stream sediments [39,40,41]. The three primary routes of metal removal from sediment are dissolution, desorption or mechanical scouring. Dissolution can occur if the chemical conditions allow. For the majority of metals to dissolve, an acidic environment is needed. If the metals were originally deposited due to their insolubility in the receiving water, re-dissolution is unlikely unless there is a change in the aquatic geochemistry. Metal oxyhydroxide solubility is also favored at high pH, but the basic conditions needed (> 9) rarely occur in surface waters.

Desorption can remove metals from sediments, if either the chemical conditions (metal concentration, pH, ionic strength) change and surface-complexed metals are released, or if an aqueous ligand with stronger binding affinity for metals is present in the water column. Scouring is a physical removal process that is typically associated with high discharge, which may physically separate metal floc or coatings from the bed sediments. Also, because scouring is a physical removal process, the released metals may not re-dissolve or desorb, leaving the metals to be deposited downstream in a region of lower stream velocity.

Environmental effects will continue after mining operations have ceased, if the sources of AMD are not eliminated and/or the stream sediments retain previously accumulated metals and metal oxides [42]. After an incoming source of AMD is removed or remediated, the stream might not recover for an extended period of time. The time-scale for recovery in part depends on the recovery of the bed sediments. As previously discussed, metal-containing sediment coatings and particles can remain in the streambed or be transferred into the water column through chemical and physical process [43,44]. Metals in the surficial sediment can be covered by further sediment deposition or washed further downstream due to scouring. Metals released to the water column can either be in the dissolved phase or adsorbed to suspended particles. All of these processes can influence the rates of recovery of the water and sediment quality.

1.5 Environmental effects

Mine-drainage waters are complex mixtures of metals and major ions that can alter the chemistry of receiving waters in a difficult to predict manner. AMD is a reactive solution; and after it enters receiving water, the speciation of metals and ions can change depending on conditions such as temperature, pH, and O₂ exposure [45,46]. These reactions are kinetically limited, thus changing the concentrations of chemical constituents over time as the mixture of

AMD and receiving water flows downstream. Therefore, the aquatic toxicity of the mixture can vary greatly and depends on the water chemistry at a specified point in the stream.

There are two main strategies for treating water contaminated with AMD [47,48]. The first is passive treatment, which typically involves the use of specially constructed wetlands. If the treatment is successful, the wetlands will continue to treat the contaminated water into the future. The drawbacks to this method are mostly due to the possibility of the wetlands failing. The second type of contaminated water treatment is active treatment, in which a water treatment facility is constructed to process the water before releasing it back into the stream. Most AMD treatment plants use lime to increase the pH to a point where metal solubility is minimal. These precipitated metals can then be settled and/or filtered out of the water before lowering the pH to close to neutral. An active treatment plant is scheduled to come on line in early 2017 in Black Hawk, CO, the site of the dissertation research.

1.6 Thesis objectives

The research reported here has focused on the development and validation of in-situ methods for determining the extent of aquatic sediment contamination as well as examination of the time-scale of AMD-contaminated sediment recovery, in a post-remediation scenario. In this thesis, I present the developed methodology and the results of in-situ experiments to investigate the deposition and removal of relevant metals (e.g., Cu, Fe, Mn, Zn) from AMD-contaminated sediment. To examine the behavior of the modified in-situ sampler, I measured metal deposition and removal at a contaminated and a background site. The background site was used to simulate the conditions that may exist following remediation of the AMD inputs.

In order to fully understand the environmental impact of AMD, several geochemical characteristics of the stream must be taken into consideration. These include water chemistry

factors such as pH, hardness, alkalinity, temperature, dissolved oxygen, etc. There are also elemental concentrations (primarily trace metals and sulfur) introduced by AMD contamination, which are suspended or dissolved in the water, as well as elements present in the sediment. The physical and chemical speciation of these elements must also be taken into account, because this affects their geochemical behavior and bioavailability. In my research, I attempted to develop and implement a method for determining the extent of contamination resulting from AMD in stream sediment as well as to gain insights into the time-scales of AMD-contaminated sediment recovery post-remediation.

The research presented in Chapter 2 is the initial development and testing of my rock-tray method. Methods for monitoring *in-situ* recovery of stream-sediment geochemistry are not well developed. A previously published method that utilized substrate-containing trays, had been developed by Clements et al. [49] for the examination of benthic invertebrate communities *in-situ*. Methods for monitoring recovery of benthic macroinvertebrate communities from ecological impacts are relatively well-established, and I will demonstrate that these methods are transferable to monitoring geochemical recovery of contaminated sediments. However I needed to adapt this rock-tray method for the goal of allowing examination of the physicochemical processes by which stream sediments retain or release metal contamination.

Trays similar to those used by Clements et al. were deployed in the early spring of 2012. Because I observed variation in local stream velocity at each sampling site, I examined how this would affect flocculated sediment deposition by placing trays in different flow regimes at each site. The trays were removed from the stream after 4 weeks of deployment and redeployed with controls at an upstream reference site for 4 weeks for an examination of sediment recovery. During this field experiment, I noted loss of some loose metal floc during the collection and

redeployment of the contaminated rock trays. The original design of the trays had side-holes to facilitate benthic invertebrate colonization. To examine the effects of the side-holes, I deployed another set of trays in early spring 2013 at the same deposition site for 4 weeks. When the trays were redeployed at the reference site for 10 d, half of the trays were deployed with side-holes and half of the trays were deployed without side-holes for comparison of recovery. In Chapter 2 I discuss the influence of tray design on sediment deposition and loss.

The research presented in Chapter 3 was a continued development of the rock-tray method, but with a focus on only the deposition phase. After two wash-outs of deployed samplers in 2014, I modified the deployment method to be more resilient to high discharge in the stream by placing the trays in a large holding bin, which was a more compact design than the linear deployment used by Clements et al. [49]. I deployed two set of trays in the holding bins (one anchored to the stream bed and one anchored on the side of the stream) with an irrigation tube to route stream water into the bins that were placed beside the stream. The stream-side deployment was used because it is more resilient to high discharge compared to the in-stream deployment, but I was concerned that it may affect the quantity of sediment deposition. In 2015, I deployed trays again for 4 weeks using the stream-side deployment method. A high-discharge event occurred in the stream after the 2-week sampling date, which allowed for 3 comparisons of metal deposition. Trays were removed before and after the high-discharge event occurred, thus allowing for an examination of scouring effects on the deposited metals. In the second 2 weeks of deployment, I compared deposition onto clean substrate and the scoured substrate. Finally, I was able to compare temporal variability in deposition between the first 2-week period and the second 2-week period.

Chapter 4 contains results from another application of the rock-tray method, with a focus on sediment recovery and data processing in the 2015 experiment. My initial contaminated trays had been previously deployed at the deposition site for 4 weeks to accumulate a considerable amount of AMD metals on the substrate surfaces. They were then redeployed at the reference site for 8 weeks. The extraction results were inconclusive with respect to the temporal changes in metal concentration, with high variability, no statistically-significant recovery (i.e., decrease in metal concentration), and a statistically-significant increase in metal concentration on the control rocks after 8 weeks. For this latter observation I assumed that biofilms were growing on the substrate and accumulating metals, which would mask the sediment recovery. In order to aid the data interpretation, I developed a 3-component model to describe the metals extracted from the samples. Metals are extracted from the substrate, a sediment biofilm, and the AMD coatings. I calculated contributions to total extracted metals from both the substrate and biofilms for all sample time points, providing a means to calculate metals associated with coatings. These data were then normalized to the total extracted metal concentrations to provide a percentage of extracted metal that was acid extracted from the coatings. This normalization reduced variability and allowed for statistical examination of temporal trends in the metal-oxide coatings. Using this approach I was able to demonstrate significant removal of the deposited metal coatings over the 8 weeks of deployment at the reference site. An attempt to normalize data to surface area, through use of an element extracted only from the rock substrate was not successful.

In Appendix A the results of an aquatic toxicity study conducted in 2012 are presented. In this study, I and my co-authors compared the results of laboratory metal toxicity tests to *in-situ* toxicity tests performed in the NFCC. The *ex-situ* and *in-situ* toxicity tests both utilized *Daphnia magna*, a freshwater cladoceran, as the test organism. We found that aquatic metal mixtures can

result in non-additive toxicity, and that toxic effects of metals can cause a decrease in the electrolyte (Na, K, Ca, Mg) concentrations contained in *Daphnia magna*. Because this study was a part of the research I conducted in 2012 but was tangential to my primary research goals, it has been included in the appendix of this thesis.

In summary, the methods presented here provide a novel means to examine the fate and transport of aquatic metal contamination *in-situ*. Information about time-scales of removal of each metal can be used to design more-effective remediation strategies and to lead to more-realistic predictions of the time-scale for stream sediment recovery post-remediation. This information could also be used to understand the contribution to aquatic toxicity from sediment-deposited metals.

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CHAPTER TWO

DEVELOPMENT OF AN IN-SITU METHOD FOR SIMULATING POTENTIAL POST-REMEDICATION SEDIMENT RECOVERY IN A STREAM AFFECTED BY ACID MINE DRAINAGE

Modified from a paper submitted to *Environmental Science: Processes & Impacts*

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2.1 Abstract

Mine-waste drainage can greatly impact aquatic ecosystems due to the precipitation of metal oxides and subsequent formation of surface coatings on stream sediments. Toxicity resulting from elevated concentrations of dissolved and particulate metals in the water column and sediment can lead to decreased population abundance and species diversity of aquatic biota. The North Fork of Clear Creek (NFCC) in central Colorado is currently receiving acid mine drainage (AMD) from legacy mine wastes, but planned future remediation of two mine-drainage tunnels will significantly decrease metal loading to the stream. Several factors including water chemistry and stream flow will control the recovery of the streambed and overlying water through their influence on scouring and on dissolution or desorption kinetics. To examine potential recovery rates of stream-sediment chemistry in NFCC, we modified a previously existing method for examining *in-situ* benthic community recovery. Standardized trays filled with cleaned gravel and cobble, were anchored in a contaminated reach downstream of the AMD inputs in spring of 2012 and 2013. The trays were allowed to accumulate metal coatings and particles for one

month. To simulate probable post-restoration conditions, the trays were subsequently transported upstream of the AMD inputs to an unpolluted reference site and sampled at 1 week and 1 month of recovery in 2012 and at 10 d of recovery in 2013. At 1 month in the upstream reference water in 2012, the average Cu concentration in the samples decreased 34% and the average Fe concentration decreased 30%. At 10 d in the reference water in 2013, the average Fe concentration in the samples only decreased ~10% and the average Cu concentration decreased ~28%. The lower overall percent removal in 2013 likely reflects methodological improvements that decreased the loss of particulate metal from the trays during their removal from the downstream contaminated site. Placement of the trays in different flow regimes at the contaminated site did not significantly affect metal deposition on the rocks. Eliminating holes in the sides of the trays, which were included in the original design to assist in benthic macroinvertebrate colonization, may have allowed greater retention of loose sediment. This method balances the realism of field data and the rigor of laboratory analysis, to provide a way to predict biological and chemical recovery of contaminated stream sediments *in situ*.

2.2 Introduction

Historical metal-mining activities have resulted in legacy environmental damage throughout the world, with the majority of this damage occurring before implementation of responsible mining practices [1,2,3]. Oxidation of mine waste can generate acidic mine drainage (AMD)[4,5], which can increase the environmental mobility of trace metals and can degrade the quality of a receiving stream's water and sediment. Although much is known about potential damage caused by AMD, relatively little is known about recovery processes and potential hysteresis during recovery (i.e., a slower rate and/or different physical-chemical-biological path of recovery relative to the rate and physical-chemical-biological path of damage). This lack of

knowledge about recovery from AMD contamination is in part a result of the limited availability of methods to study post-remediation recovery of sediments in realistic settings.

Depending on the specific mineralogy of the region, waste rock and tailings can contain high concentrations of sulfide minerals, in particular pyrite (FeS_2). The oxidation of sulfides generates acidity, sulfate, and dissolved metal cations [6,7]. The acid generated can also dissolve surrounding minerals, resulting in additional release of acid-soluble metals.

Some metals are released into AMD in a reduced state (e.g., ferrous iron (II)) during either the oxidation of sulfide or the dissolution of metal-containing ore minerals by the generated acid. Exposure to O_2 and sulfide-oxidizing bacteria results in oxidation of iron (II) and manganese (II), and the resulting hydrolysis reactions lead to formation of metal-hydroxides and/or metal-oxy-hydroxides [8,9,10]. Additionally, mixing with receiving water typically increases the pH of the acidic water, thus fostering the formation of insoluble oxy-hydroxide precipitates of Fe(III), Al(III), and Mn(IV) [11]. Metal oxy-hydroxide particles and colloids adhere to sediment surfaces or settle out of the water column, leading to layers of flocculent precipitates on the stream bed [1]. These amorphous coatings have varying metal concentrations and mineralogy, giving them diverse chemical properties that affect environmental mobility and bioavailability of associated metals [12,13,14].

Water chemistry parameters (e.g., pH, hardness, alkalinity) control the speciation of metals in the water column and the sediments. Potentially toxic trace metals can adsorb to sediment or suspended particles in the stream [15]. Hydrated metal-oxides [16,17,18], and particulate organic matter [19,20,21] are especially important for sorbing aqueous metal ions. The importance of metal complexation to particle surfaces typically increases as AMD waters are neutralized, in part due to the increased amount of deprotonated cation-binding sites.

Adverse environmental effects can continue after mining operations have ceased if the sources of AMD are not eliminated and/or the stream retains previously accumulated metals and metal oxides. After an incoming source of AMD is removed or remediated, the stream might not recover for an extended period of time. The time-scale for recovery in part depends on the recovery of the bed sediments. Metal-containing sediment coatings and particles may remain in the stream bed or be transferred into the water column through chemical and physical processes. Metals released to the water column may either be in the dissolved phase or adsorbed to suspended particles. In particular, metals in the surficial sediment may be washed farther downstream in high flow events that promote scouring. Furthermore, metal-contaminated surface sediments may be covered by deposition of clean sediments originating from upstream. All of these processes can influence the rates of recovery of the water and sediment quality.

Methods for monitoring *in-situ* recovery of stream-sediment chemistry are not well developed. Tools for sampling substrate have been limited to fine sediments that core samplers [22] and ponar dredges [23] can penetrate, or to suspended sediments [24]. Cobble, gravel and boulders common in high-gradient mountain streams limit the ability to monitor chemical changes in substrate. However, methods for monitoring recovery of benthic macroinvertebrate communities from ecological impacts are relatively well-established and might be transferable to monitoring chemical recovery of sediments.

Benthic macroinvertebrates are excellent *in-situ* biomonitoring tools, due to their ease of collection and sensitivity to contamination. This has allowed field approaches to determine the ecological status of a freshwater system and to quantify the toxic effects of contamination on aquatic biota. For example, substrate-containing trays used by Clements et al. were designed to house a benthic invertebrate community *in situ* [25,26,27]. Each tray contains a mixture of gravel

and small cobble and is placed in a reference or contaminated stream reach for a specified amount of time to determine the effects of exposure to contaminants. We speculated that this method can also be used to examine ecosystem recovery post-remediation, because the trays can be initially deployed in a contaminated stream reach and then be re-deployed in an uncontaminated reach. If the metals are sufficiently removed by natural physical-chemical processes, the benthic invertebrate population should also recover. In our research, we are adapting this rock-tray method to examine the physical-chemical processes by which stream sediments retain or release metal contamination. If allowed to equilibrate to conditions representative of a cobble-dominated river system, *in-situ* substrate tray samplers can be an alternative method to monitor recovery of substrate.

The research reported here has focused on the development and application of *in-situ* methods for determining the extent of aquatic sediment contamination as well as examination of the time-scale of recovery of AMD-contaminated sediment in a post-remediation scenario. In this paper, we present the methodology we have developed and the results of an *in-situ* experiment to investigate the deposition and removal of relevant metals (e.g., Cu, Fe, Mn, Zn) from AMD-contaminated sediment. To examine the behavior of the modified *in-situ* sampler, we measured metal deposition at a contaminated site and subsequent removal at a reference site. The background site was used to approximate the conditions that may exist immediately after remediation of the AMD inputs. Although the focus of this study was to develop and test the methodology, time scales of metal removal were also examined by sampling over the course of 4 weeks (2012) and 10 d (2013). Information about time scales of removal of each metal can be used to design more-effective remediation strategies and to lead to more-realistic predictions of

the time scale for post-remediation stream sediment recovery. This information could also be used to help to understand the contribution to aquatic toxicity from sediment-deposited metals.

2.3 Experimental

2.3.1 Study area

The headwaters of the North Fork of Clear Creek (NFCC) originate on the east side of the Continental Divide in central Colorado, USA. The NFCC is a high-gradient mountain stream fed by snowmelt and groundwater. Stream discharge greatly increases due to snow melt in spring, then declines to a minimum in fall and winter. Discharges from local abandoned metal mines and drainage tunnels also contribute to the NFCC flows. The NFCC and its tributaries drain high alpine forests before flowing through the cities of Black Hawk and Central City in Gilpin County, Colorado (Figure 2.1). Metal-mining operations began there in 1859 following the discovery of gold and silver deposits. The NFCC watershed, which was placed on the National Priority List (Superfund) by the United States Environmental Protection Agency (USEPA) in 1983 comprises 200 km² and is contained in the 1000 km² Central City/Clear Creek watershed [28].

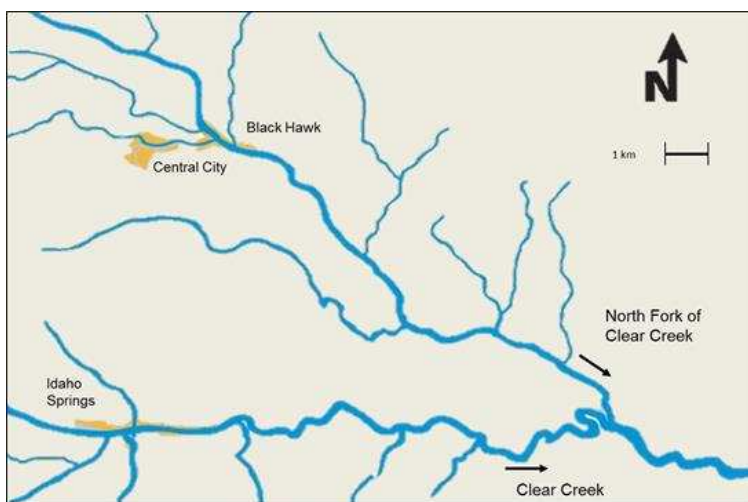


Figure 2.1: AMD-contaminated reach of North Fork of Clear Creek in central Colorado.

Currently, two major legacy mine drainages (Gregory Incline [GI] and National Tunnel [NT]; Figure 2.2) enter the stream reach in Black Hawk. The AMD-affected reach of NFCC cannot sustain fish populations [28] and has a depauperate population of aquatic macroinvertebrates [29]. Additionally, the AMD discharge decreases the pH and alkalinity of NFCC and elevates water hardness and concentrations of dissolved and particulate metals (e.g., Cu, Fe, Mn, Zn) in comparison to upstream waters. The two major AMD discharges are scheduled to be diverted to a lime-treatment plant in 2017.

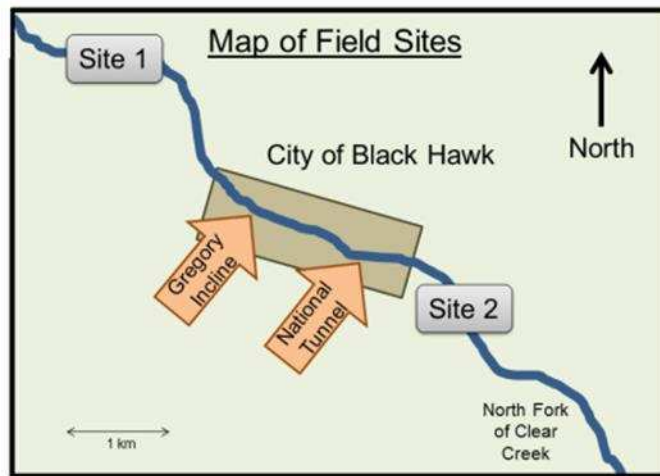


Figure 2.2: Locations of the field sites on the North Fork of Clear Creek.

2.3.2 Sampling sites

Upstream of the two primary AMD point sources, NFCC water contains low dissolved and particulate metal concentrations and has species diversity and population density of benthic macroinvertebrates that are typical of uncontaminated Rocky Mountain streams. A relatively pristine reach of NFCC was chosen as the reference site (Site 1), ~2 km upstream from GI (the first major AMD point source). Site 2 was chosen for its potential to deposit AMD metals/precipitates into the experimental rock trays (see below), because it is located ~100 m downstream of NT (the second major AMD point source). This choice of location assured

complete mixing of NT water with NFCC water (i.e., ~60 stream widths downstream of NT, with a stream width of approximately 1.6 m) while still being close to the highest metal loadings. At this site, the water chemistry and macroinvertebrate communities are most severely affected by the AMD inputs (i.e., elevated dissolved and particulate metal concentrations, and decreased pH and alkalinity). During the 2012 and 2013 experiments, the average stream flow was measured at USGS gage station #06718550 ~12 km downstream of Site 2. Continuous discharge from the Black Hawk Wastewater Treatment Plant (~10 km downstream) and a minor seasonal contribution from Russel Gulch (~6 km downstream) results in a higher discharge at the gage station than at the upstream sites, but these inputs are minor. Experiments were conducted during winter low-flow conditions when metal contamination was at or near its maximum, because these AMD sources have a relatively constant discharge throughout the year while the upstream NFCC flow decreases to its lowest in winter.

2.3.3 Rock trays

The trays used in this study (Figure 2.3) were originally developed for analysing benthic invertebrate communities under a variety of stream conditions. Construction of the rock trays is described in detail in Clements et al. [26]. Briefly, 10-cm x 10-cm x 6-cm polypropylene containers (Stor-Keeper® Arrow Plastic Manufacturing Company, Elk Grove, Illinois, USA) were filled with small cobble (6-10 cm diameter) and coarse gravel (2-6 cm diameter) to represent a typical range of bed sediments present in high-gradient mountain streams. Sample uncontaminated sediments, obtained from a local river bed, were washed in 10% nitric acid (JT Baker concentrated reagent-grade) for 20 min, rinsed in DI water, and dried before placing them in the trays. The trays had three 2-cm-diameter holes drilled into each of the four sides, originally intended to facilitate aquatic invertebrate colonization and to promote water flow through the

substrate. For in-stream deployment, the trays were tightly strapped onto a 1.5-m-long wooden rack anchored to the stream bed. All of the racks of trays were positioned perpendicular to the direction of stream flow. Rock trays were first deployed in-stream at Site 2 to allow for AMD metal coatings and particles to accumulate in/on the tray substrate (Figure 2.4). The trays were later removed from Site 2 and redeployed upstream at Site 1 to simulate post-remediation conditions.



Figure 2.3: Rock trays strapped to a wooden rack before deployment in stream.

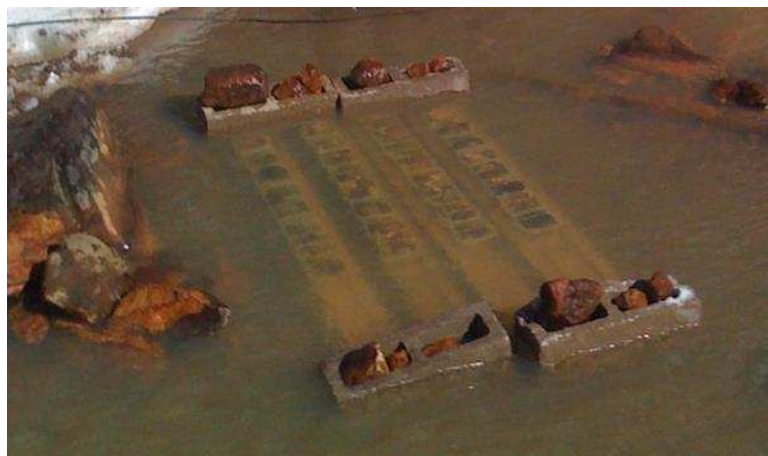


Figure 2.4: Rock trays deployed at metal-contaminated Site 2 on North Fork of Clear Creek.

2.3.4 Experimental field procedures

Twenty-four trays were deployed in the stream at Site 2 on February 14, 2012. To examine the possible impacts of localized stream flow on metal deposition onto the stream bed, one-half of the trays were anchored in a reach of stream that appeared to have fast, unimpeded stream flow and one-half were anchored in areas of the stream that appeared to have slower, more-circulating backflow downstream of large boulders. On March 20, 2012, the trays were collected by placing a clean linen cloth on top, unstrapping them from the rack, and removing them from the stream. Some of the trays, designated as “deposition” samples, were removed for chemical analysis. The remaining trays were transported upstream to Site 1 to examine sediment recovery. Eight control trays, containing clean acid-washed rocks were co-deployed at Site 1 with the AMD-contaminated trays. When the trays were redeployed in the stream, control trays were alternated with trays from Site 2 to minimize potential positional bias. On March 27, 2012, some of the trays (4 controls and 4 metal-contaminated trays from Site 2) were collected as 1-week upstream “recovery” samples. The remaining trays (4 controls and 4 metal-contaminated trays from Site 2) were sampled on April 20, 2012, to represent 1-month upstream “recovery” samples.

In 2013, a variation of the 2012 experiment was conducted in which identical and modified versions of the rock trays were deployed on March 29, 2013, at the same AMD-contaminated stream site (Site 2) as in the 2012 experiment. During the 2012 experiment, some loose sediment had been lost through the side holes when the trays were removed from the stream and redeployed at Site 1. In 2013, eight trays were deployed with half of the trays having the same design as in 2012 (i.e., with side holes) and half of the trays having a modified design without side holes. To maintain equivalent conditions for both types of trays during the deposition period, the trays with side holes were nested inside empty trays that had no holes

(Figure 2.5). Therefore, during deposition in the 2013 experiment, all of the trays did not have water flowing in laterally through the sides but instead only had water flowing over the sediment surface. After a 28-d AMD exposure at Site 2, the trays were transported upstream to Site 1 on April 26, 2013. Before trays were collected from the stream, plastic container lids (which replaced the linen cloths used in 2012) were attached to the trays to minimize substrate loss from the top of the tray during removal. The trays were then carefully removed from the board and placed in large re-sealable plastic bags for transport upstream. Before redeployment at Site 1, the outer trays were removed from 4 trays with holes (Figure 2.5). Clean control rock trays (4 with holes and 4 without holes) were also deployed at Site 1. All of the trays deployed at Site 1 were collected on May 6, 2013, after 10 d of upstream recovery. The 2013 “simulated recovery” period was shorter than in 2012 because an early snowmelt increased discharge enough that sampling would have been unsafe at later dates.

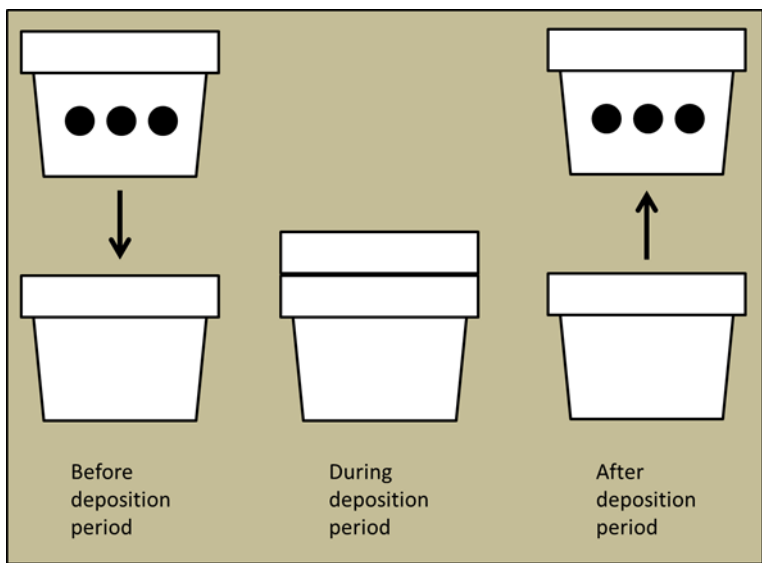


Figure 2.5: Schematic of enclosure of rock trays that contained side holes inside a plastic tray that contained no side holes, before, during and after deployment at metal-contaminated Site 2 on North Fork of Clear Creek.

2.3.5 Extraction procedures

For the 2012 experiment, at the time of collection, each rock tray was placed in a new 4-L resealable Ziploc® polyethylene bag (Ziploc®, S. C. Johnson & Son, Inc., Racine, WI, USA) and stored in a laboratory freezer (~ -40°C) until it was removed for the extraction procedure and subsequent chemical analysis. The samples were allowed to thaw overnight at room temperature before extraction.

All rocks and associated floc from each tray were transferred into a new, labelled 8-L resealable polyethylene bag, and the wet mass was recorded. Then 150 ml of a 10% nitric acid solution, prepared by diluting concentrated trace-element-grade nitric acid (Macron) with Milli-Q water (>18 Mohm), were added to the bag. The bags allowed the use of a minimal volume of acid solution while still allowing complete contact with the sediment. An initial set of experiments was performed using this method to determine if the plastic bag would leach metals into the acid solution, thereby biasing the measured concentrations. Only Zn concentrations were elevated above the control (5-10 µg/L), and those concentrations were several orders of magnitude less than the Zn concentrations in the rock-tray extracts and were also close to the analytical detection limit for ICP-OES. Therefore, background contamination was deemed negligible. No other metal was above its detection limit in the method-blank leachate.

The extraction was performed for 96 h in a fume hood at room temperature with the bag sealed. The acid concentration and extraction time used were based on preliminary method-development experiments (data not shown). Forty ml of the acidic solution in the bag was then sampled with a disposable polyethylene pipette, and transferred into a conical Falcon tube (50-ml polypropylene). A 20x dilution was prepared with Milli-Q water in another conical Falcon tube (10-ml polypropylene), and later analysed for metal concentrations.

Filtration of the highly acidic rock-tray extracts was problematic because, in a preliminary experiment, metals were leached out of the filters by the acid, resulting in biased metal concentrations. Thus, the extracts were not filtered and instead were allowed to settle overnight and then were decanted to remove the majority of particles before dilution for analysis by ICP-OES (Perkin Elmer 5300;). Consequently, the analysed metal concentrations might include some colloidal/particulate matter.

Several of the procedures were modified for the 2013 experiment. During processing of the 2012 samples, some of the plastic bags leaked. Thus, extractions in 2013 were conducted in hard-walled 2-L polypropylene containers (Kartell® Polypropylene Beakers, Kartell S.P.A., Noviglio, Milan, Italy). Additionally, the extraction procedure was changed to a modification of USEPA Method 3050B [30]. Modifications to the USEPA method were made to accommodate the mass and type of sediment collected from the rock trays. The USEPA method was developed for extraction of “environmentally available metals” from approximately 1 g of solid sediment sample, which is generally fine grained or ground material. Given the larger sample mass and the nature of the rock materials used in this experiment (i.e., cobble and coarse gravel), only a small fraction of the sample mass was dissolved during the extractions.

All sediment and associated floc in each rock-tray were transferred into a tared 2-L container, and the wet mass was recorded. One hundred ml of a 50% nitric acid solution (Macron concentrated trace-element-grade HNO₃ diluted with Milli-Q water) was added to the container. A Teflon watch glass was placed over the opening before placing the container in a hot-water bath (~90°C) for 15 min. The container was then removed from the bath and allowed to cool, after which 50 ml of concentrated nitric acid (Macron trace-metal-grade) was added. After 30 min at

room temperature, the container was returned to the hot-water bath for 2 h. After the container was removed from the bath, a solution of 50 ml of Milli-Q water and 75 ml of hydrogen peroxide (Macron, 30% reagent grade) was added to the container. It was then returned to the hot-water bath for 15 min, after which the container was removed from the bath and allowed to cool for 30 min. This also provided time for most undigested sediments, if any, to settle. The final combined mass of the sediment and extraction fluid was recorded. Forty ml of the acidic solution was sampled with a disposable polyethylene pipette, transferred into a conical Falcon tube (50-ml polypropylene), diluted 20x with Milli-Q water as in 2012, and later analysed for metal concentrations.

2.3.6 Water sampling and chemical analysis

Grab samples of stream water were collected in polypropylene containers on March 29, April 26, and May 6, 2013 for analysis of water quality parameters and metal concentrations. Samples were filtered (Thermo Scientific Target2 Nylon + Glass Syringe Filter, 0.45- μ m pore size) in the field for dissolved metals. Unfiltered samples were collected for total metals and total organic carbon (TOC) analyses. Water samples were acidified with nitric acid (Macron concentrated trace-element-grade) before being analysed for major elements and metals, or they were acidified with reagent-grade phosphoric acid (EM Science) before being analysed for TOC.

Field pH (VWR SP80PC meter/VWR 14002-860 combination gel electrode), alkalinity (HACH Model AL-DT Alkalinity Test Kit), and ferrous iron (HACH DR/890 Colorimeter/1-10 phenanthroline AccuVac ampules) were measured immediately after water collection at each site. Water hardness was calculated from the Ca and Mg concentrations measured by ICP-OES. Sulfate concentrations were calculated from the sulfur concentrations, obtained by ICP-OES, assuming that the only sulfur species was sulfate. Because sulfate and bicarbonate are the

dominant aqueous anions in this freshwater AMD-affected system [31], additional anions were not analysed.

All ICP-OES analysis used a continuously introduced Sc internal-calibration standard. Deionized water blanks (Barnstead Nanopure system, Thermo Fisher Scientific) and certified continuing calibration verification (CCV) standards were analysed after every 20 samples for quality assurance and quality control. The ranges of instrument detection limits for the metals, and hardness ions (Ca and Mg) during 2012 and 2013 were (in $\mu\text{g/L}$): 1.4-8.0 Ca, 0.3-0.9 Cu, 0.1-2.5 Fe, 0.1-0.3 Mg, 0.02-0.1 Mn, and 0.2-0.3 Zn.

2.3.7 Data analysis

Analysed metal concentrations in the rock-tray extracts were multiplied by the measured mass of the extraction solution to calculate the mass of extracted metals. These values were then divided by the total measured mass of the sediment sample to calculate the mass concentration in units of mg metal/kg wet sediment. The 2012 and 2013 populations of wet-sediment-normalized metal concentrations were analysed separately by year and metal for homogeneity of variances using Levene's Test at an alpha value of 0.01. Because the variances of both populations were not significantly non-homogenous for any of the four metals of interest (Cu, Fe, Mn, and Zn), the data were not transformed before subsequent analyses.

Statistical analysis of the 2012 extraction data for the metals of interest began by testing for significant differences among the 3 control groups (initial, 1 week, and 1 month). We tested for significant differences in metal concentrations between "fast" and "slow" samples at each recovery time point (i.e., 1 weeks and 1 month) and for significant differences between AMD-exposed trays and controls. All of these tests were 1-way analysis of variance (ANOVA) followed by a Tukey HSD Test ($\alpha = 0.1$). We chose an alpha value (the probability of a Type I

false-positive error) of 0.1 because the variability in field studies can be higher than in laboratory studies, for which the more traditional alpha value of 0.05 is often considered appropriate. Increasing the alpha value decreased the probability of a Type II false-negative error, thus avoiding overly-conservative inferences in this type of field study. Analogous statistical analysis of the 2013 results began with comparison of the two control groups (with and without holes). We tested for significant differences in metal concentrations between the initial deposition samples and the recovery samples (with and without holes), and for significant differences in metal concentrations between the recovery samples (with and without holes) and the controls.

2.4 Results and discussion

To illustrate the utility of this exposure-and-extraction method, results are presented herein for Cu, Fe, Mn and Zn. These metals were chosen because Cu and Zn can be toxic to aquatic organisms [32,33], and Mn and Fe comprise the main oxy-hydroxide phases in this system. We did not include results for Al because the extracted Al concentrations in the treated rock-tray sets did not differ significantly from the controls (i.e., the calculated amounts of AMD-deposited Al were small compared to the amounts of Al extracted from the control-substrate rocks). The stream's discharge was very similar for both the 2012 and 2013 field experiments ($\sim 0.1 \text{ m}^3/\text{s}$ to $\sim 0.3 \text{ m}^3/\text{s}$).

2.4.1 2012 Experiment

Metal concentrations in the 2012 rock-tray extracts, normalized to total mass of wet substrate, are shown in Figure 2.6 and Table 2.1. Metal concentrations did not significantly differ among the 3 sets of control rock trays (pre-deposition, 1 week in recovery water, and 1 month in recovery water). Additionally, no statistically significant differences occurred between the “fast”

and “slow” treatments at any of the three time points (i.e., at the end of the deposition period, after 1 week of recovery, and at the end of the 1-month recovery period; Table 2.1).

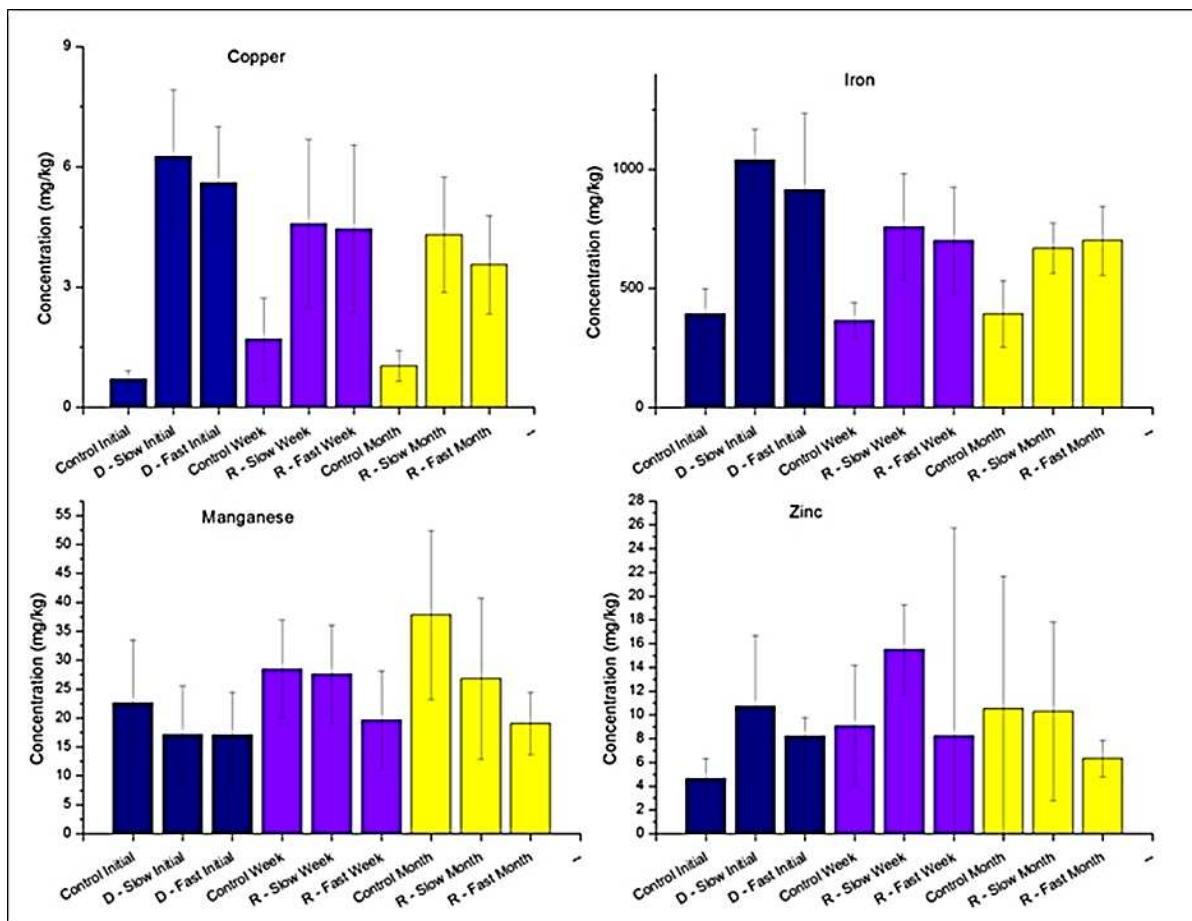


Figure 2.6: Concentrations (mg/kg wet sediment) of Cu, Fe, Mn, and Zn extracted from sediment in rock trays deployed in North Fork of Clear Creek in 2012 (mean of 4 replicates; error bars are 90% confidence intervals). D = deposition (metals-contaminated Site 2), and R = recovery (upstream Site 1). Flow conditions for the depositional phase consisted of a fast and slow flow rate. The two time points for recovery were 1 week and 1 month. Control Initial = clean rocks never placed in stream water; Control Week and Control Month = clean rocks placed only in upstream Site 1 water for 1 week and 1 month, respectively.

After 1 month of sediment deposition at Site 2, Cu, Fe, and Zn concentrations in both the “slow” and “fast” trays were significantly greater than in the initial controls (Figure 2.6, Table 2.1). The average Cu, Fe, and Zn concentrations in the “slow” contaminated rock trays significantly increased by 800%, 160%, and 130% respectively, compared to the initial controls.

Similar patterns occurred in the “fast” contaminated rock trays, in which average Cu, Fe, and Zn concentrations significantly increased by 700%, 130%, and 80%, respectively. However, the average Mn concentrations after 1 month of deposition did not differ significantly from the initial controls in either the “slow” or “fast” deposition treatments.

Table 2.1: Concentrations (mg/kg wet sediment) of Cu, Fe, Mn, and Zn extracted from sediment in rock trays deployed in North Forth of Clear Creek in 2012 (mean of 4 replicates), 90% confidence intervals in parentheses; C = control, D = deposition (metals-contaminated Site 2), and R = recovery (upstream Site 1). Flow conditions for the Site 2 depositional phase consisted of a fast and slow flow rate. The two time points for recovery were 1 week and 1 month. C - Initial = clean rocks never placed in stream water; C - Week and C - Month = clean rocks placed only in upstream Site 1 water for 1 week and 1 month, respectively.

Treatment	Cu	Fe	Mn	Zn
C - Initial	0.70 (± 0.22)	394 (± 105)	22.6 (± 10.9)	4.66 (± 1.68)
D - Slow Initial	6.26 (± 1.66)	1040 (± 130)	17.1 (± 8.40)	10.7 (± 5.96)
D - Fast Initial	5.60 (± 1.39)	914 (± 321)	17.1 (± 7.42)	8.21 (± 1.56)
C - Week	1.70 (± 1.02)	365 (± 73.9)	28.5 (± 8.45)	9.08 (± 5.09)
R - Slow Week	4.58 (± 2.10)	759 (± 222)	27.6 (± 8.51)	8.15 (± 3.78)
R - Fast Week	4.45 (± 1.00)	702 (± 300)	19.7 (± 9.84)	8.26 (± 1.80)
C - Month	1.03 (± 0.38)	393 (± 140)	37.8 (± 14.6)	10.5 (± 11.2)
R - Slow Month	4.30 (± 1.44)	668 (± 105)	26.8 (± 13.9)	10.3 (± 7.53)
R - Fast Month	3.57 (± 1.23)	701 (± 144)	19.1 (± 5.38)	6.33 (± 1.54)

During the recovery period, the average Cu and Fe concentrations in the rock trays previously deployed at Site 2 decreased temporally following upstream deployment. After 1 month, the Cu and Fe concentrations in the “slow” deposition rock trays significantly decreased from the “initial” contaminated concentration by averages of 31% and 36%, respectively. After 1 month, Cu concentrations in the “fast” deposition rock trays significantly decreased by an average of 36%, and Fe concentrations in the “fast” deposition rock trays decreased (but not significantly) by an average of 23%. However, Fe and Cu concentrations in the AMD-coated rock trays were still significantly higher than the control concentrations after 1 month at the upstream reference site.

Also during the recovery period, the average Zn concentration decreased (but not significantly) in the “slow” and “fast” deposition treatments from their “initial” contaminated concentrations by averages of 4% and 23%, respectively. The Zn concentrations were not significantly different from the control concentrations after 1 month in the upstream reference water due to high among-replicate variability. Surprisingly, the average Mn concentrations on the AMD-contaminated rocks increased by 57% and 12%, respectively, in the “slow” and “fast” samples during the 1-month recovery period. However, none of the increases in average Mn concentration was statistically significant due to high among-replicate variability. Even more surprising, the Mn concentrations in the “fast” samples were significantly lower than the control concentrations after 1 month in the upstream reference water, but the Mn concentrations in the “slow” samples were not significantly lower than the 1-month controls.

2.4.2 2013 Experiment

In 2013, total and dissolved metal concentrations in the water column were considerably higher at Site 2 than at Site 1 (Table 2.2), due to the AMD inputs. The pH of Site 2 stream water was just under 7, while Site 1 stream water had a pH of just over 7; and the alkalinity of the stream decreased from Site 1 to Site 2, indicating neutralization of the incoming AMD. In contrast, the hardness of the stream water increased from Site 1 to Site 2, indicating AMD input that resulted from acidic dissolution of Ca- and Mg-containing minerals underground. Temperature and dissolved oxygen were comparable between the 2 sites, but the TOC concentration was slightly higher at Site 2. Results obtained in 2013 are likely also representative of 2012, because the hydrologic conditions and deployment season were similar in both years. Butler et al. [34] reported a more-detailed NFCC water chemistry.

Table 2.2: Ranges of measured water chemistry parameters and metal concentrations during the 2013 field experiment in North Fork of Clear Creek (3/29/2013 – 5/6/2013). Site 1 = upstream reference location; Site 2 = downstream metal-contaminated depositional location; D = dissolved; T = total.

	Site 1	Site 2
pH	7.4 - 7.9	6.7 - 6.7
Alkalinity (mg CaCO ₃ /L)	21 - 28	1.0 - 6.4
Temperature (°C)	3.3 - 5.6	4.1 - 5.0
Dissolved Oxygen (mg/L)	9.4 - 11	9.5 - 9.6
Total Organic Carbon (mg/L)	1.7 - 3.1	2.8 - 5.1
Hardness (mg CaCO ₃ /L)	56 - 68	190 - 240
Copper - T	0.001 - 0.002	0.08 - 0.16
Copper - D	0.001 - 0.002	0.02 - 0.05
Iron - T	0.1 - 0.6	10 - 18
Iron - D	0.04 - 0.60	6.7 - 12
Manganese - T	0.01 - 0.11	3.2 - 4.2
Manganese - D	0.007 - 0.100	3.1 - 4.1
Zinc - T	0.01 - 0.03	1.2 - 1.3
Zinc - D	0.03 - 0.14	1.2 - 1.4

Metal concentrations in the 2013 rock-tray extracts, normalized to mass of substrate, are shown in Figure 2.7 and Table 2.3. None of the concentrations of the 4 metals differed significantly between the two types of controls (without holes and with holes) deployed at upstream Site 1 during the recovery period.

Clean rock trays exposed to the metals-contaminated Site 2 water for 1 month resulted in average concentrations of Cu, Fe, Mn, and Zn being 23 mg/kg, 3090 mg/kg, 28 mg/kg, and 29 mg/kg, respectively in the “D – initial” trays (Figure 2.7). After 10 d of recovery at Site 1, Cu, and Zn concentrations in the rock trays without holes decreased (but not significantly) from their initial contaminated concentrations by 28% and 14%, respectively. After 10 d of recovery at Site 1, Fe concentrations in the rock trays without holes decreased significantly from their initial contaminated concentrations by 17%. However, the Cu, Fe, and Zn concentrations in rock trays without holes remained significantly higher than the recovery controls without holes. In contrast,

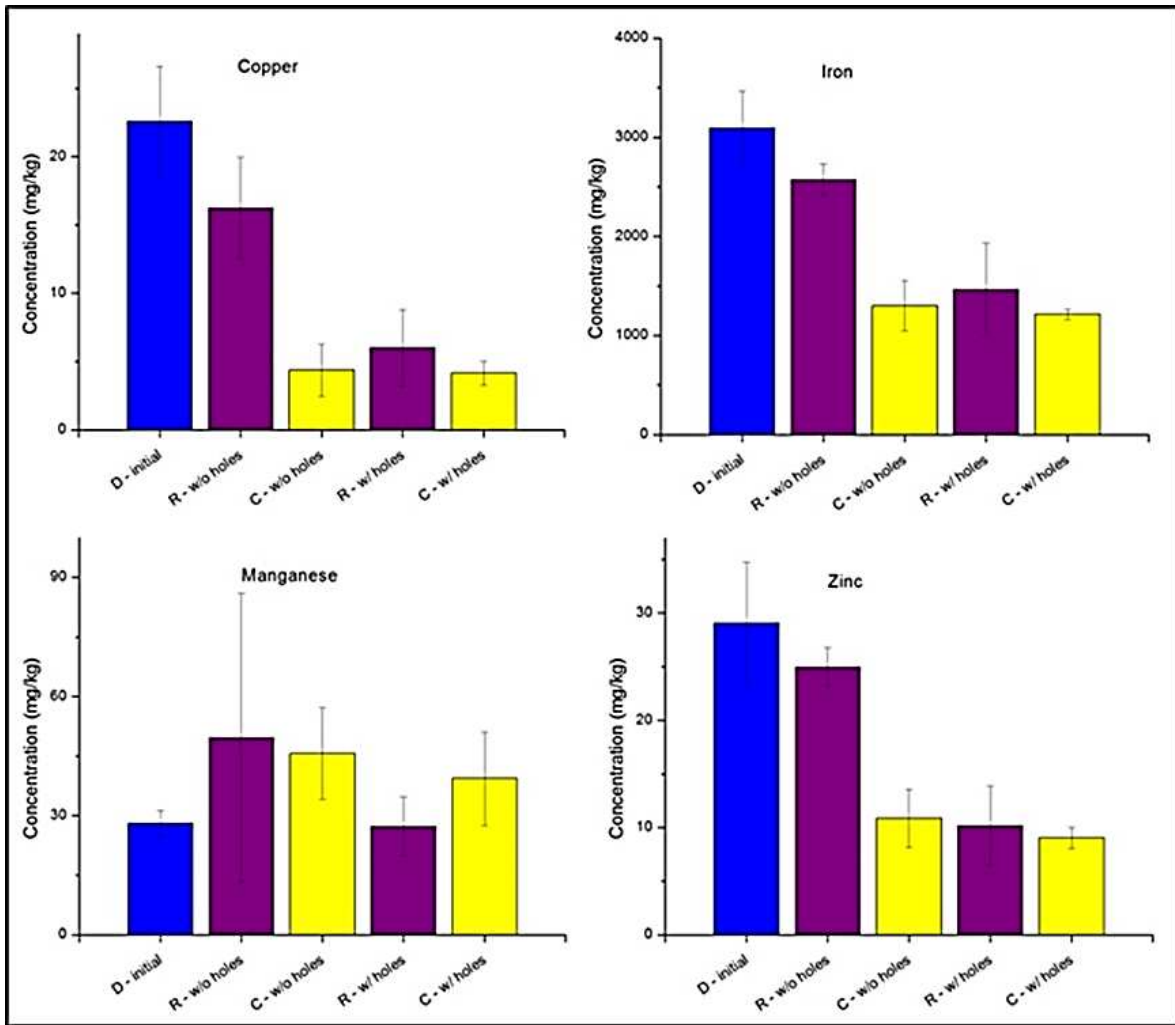


Figure 2.7: Concentrations (mg/kg wet sediment) of Cu, Fe, Mn, and Zn extracted from sediment in rock trays deployed in North Fork of Clear Creek in 2013 (mean of 4 replicates; error bars are 90% confidence intervals). C = control, D = depositional, and R = recovery. After 28 d of accumulation of metals at metals-contaminated Site 2 (D – initial), half of the trays were deployed with holes (R – w/ holes) and half without holes (R – w/o holes) for 10 d in recovery water at upstream Site 1. Control rock trays with holes (C – w/ holes) and without holes (C – w/o holes) were concurrently deployed for 10 d in recovery water at Site 1. These control rock trays had not been in stream water before deployment at Site 1.

Table 2.3: Concentrations (mg/kg wet sediment) of Cu, Fe, Mn, and Zn extracted from sediment in rock trays deployed in North Fork of Clear Creek in 2013 (mean of 4 replicates; 90% confidence intervals in parentheses). C = control, D = deposition, and R = recovery. After 28 d of accumulation of metals at metals-contaminated Site 2 (D – initial), half of the trays were deployed with holes (R – w/ holes) and half without holes (R – w/o holes) for 10 d in recovery water at upstream Site 1. Control rock trays with holes (C – w/ holes) and without holes (C – w/o holes) were concurrently deployed for 10 d in recovery water at Site 1. These control rock trays had not been in stream water before deployment at Site 1.

	Copper	Iron	Manganese	Zinc
D - Initial	22.6 (±5.80)	3090 (±529)	28.0 (±4.63)	29.1 (±8.09)
R - w/o holes	16.2 (±5.30)	2570 (±226)	49.6 (±52.1)	25.0 (±2.58)
Control w/o holes	4.37 (±3.99)	1300 (±664)	45.6 (±10.6)	10.9 (±5.32)
R - holes	6.00 (±2.73)	1460 (±361)	27.2 (±16.4)	10.1 (±3.90)
Control holes	4.14 (±1.24)	1220 (±71.7)	39.3 (±16.8)	9.02 (±1.41)

significant amounts of Cu, Fe, and Zn (73%, 53%, and 65%, respectively) were released from the rock trays with holes during the 10-d recovery period at Site 1. At the end of that recovery period, the Cu, Fe, and Zn concentrations in rock trays with holes were not significantly different from the recovery controls with holes. The Mn concentrations did not differ significantly among the metal-coated rock trays at the end of the 10-d recovery period and their corresponding controls due to high among-replicate variability.

Visually, during the recovery period, the rock trays without side holes retained fine-grained sediments that had been deposited in the trays during the deposition period, but the rock trays with side holes lost most of the fine-grained sediments. This observation was supported by statistically significant differences in Cu, Fe, and Zn concentrations between the metals-contaminated rock trays with and without holes at the end of the recovery period.

2.4.4 Synthesis

Metal contamination resulting from mining activities is a world-wide water quality concern due to non-potability of the water, decreased or extirpated aquatic vertebrate and invertebrate populations, and increased environmental mobility of potentially toxic metals.

Depending on the chemistry of the receiving water body, the majority of introduced metals may eventually be deposited onto stream or lake sediments. Long-term sediment deposition of metals can result in high concentrations of accumulated metals. Even if the overlying waters are remediated, an extended period of time may be needed for the bed sediments to recover enough to sustain healthy populations of aquatic organisms. The fate and behavior of AMD-related metals is not well understood due to the difficulty of representative sampling and analysis of unique and dynamic environmental systems, covering large areas of land and watersheds. However, the results in this study could aid in restoration, treatment, and remediation of contaminated systems.

The goals of our “field-mesocosm” methodology were to examine relative rates of metal deposition and removal from sediments *in situ*. Use of these “field-manipulated” sediments also allows for *in-situ* examination of contaminated sediments under a variety of stream conditions. This approach has not been previously described in published literature; instead, analysis of metal desorption from natural aquatic sediments is primarily limited to *ex-situ* experiments using field-sampled sediments. Laboratory analysis of aquatic metal deposition and release from sediments is easier to control and manipulate, but at the cost of realistic representation of the geochemical processes occurring in a natural aquatic system.

Although comparison of our results to other studies is illustrative, it is difficult to directly relate results from *ex-situ* experiments to our *in-situ* approach. For example, Davis et al. examined metal desorption *ex-situ* from resuspended NFCC bed sediments in a simulated storm event in order to obtain information for metal-transport modelling [35]. This is analogous to our examination of NFCC sediment recovery after simulated remediation of the overlying water. The sediments used by Davis et al. were collected from the NFCC in a reach close to our Site 2. The sediments were wet-sieved, dried and analysed before use in the laboratory resuspension

experiment. The researchers attempted to mimic resuspension of bed sediments by matching total suspended solids (TSS) concentrations in the laboratory to TSS data measured in NFCC after a storm event. They found that metal desorption from suspended sediments depended on pH, which was tested over a range of values (pH 4.4 – 6.5). Using the <2.0 mm fraction of collected bed sediments, and water from a nearby, uncontaminated lake aided in controlling experimental conditions, but the results are limited in their representation of the actual geochemical processes occurring in the NFCC.

Gao et al. examined the *ex-situ* release of Cd and Pb from harbor sediments under different overlying water conditions in order to predict aquatic bioavailability of the metals [36]. After field collection, the sediments were dried, homogenized, sieved, and analysed before being placed in ultra-filtered water that contained dissolved Cd and Pb, to allow adsorption to the sediments. Water chemistry conditions (ionic strength, pH, and chelator concentration) were then varied to examine Cd and Pb release from the sediments. Use of those laboratory-manipulated sediments and waters allowed a general examination of desorption processes, but the results are not directly applicable to a specific natural aquatic system.

Lietz et al. examined the *ex-situ* desorption of Zn, Cd, and Pb from river sediments to determine desorption characteristics in water devoid of possible complexing agents [37]. They used dialysis chambers that contained a semi-permeable membrane to isolate the sediment from the overlying water and then analysed the concentrations of dissolved metals that had diffused through the membrane under varying conditions (chelating agents, incubation, and addition of algae). This study utilized field sediments but under controlled laboratory conditions, in contrast to our *in-situ* variation of water conditions after metals had accumulated on sediment.

Carbonaro et al. examined the *ex-situ* release of Cd, Ni, Pb, Zn and Ag from spiked sediments to examine metal transport and partitioning [38]. The sediments were collected from a pond, sieved, homogenized, and analysed before being spiked with metal-nitrate salts. Metal fate and transport following release from sulfidic sediment was then examined in O₂-saturated water. Analogously, Calmano et al. examined remobilization of Cd and Cu from sediments to determine the kinetics of metal-sediment exchange and which sediment components were acting preferentially as metal sorbants [39]. The experimental sediments were artificial model sediment and mixtures of model sediment, sea water, and dredged harbor sediments. Again, both of these studies allowed a general analysis of processes, but the results are not directly applicable to a specific natural aquatic system.

Ex-situ laboratory studies are ideal for examining a broad range of geochemical conditions, but metal deposition onto bed sediments is the result of several competing geochemical processes, as well as real-time environmental kinetics. Although experimental examination of geochemical processes *in-situ* is difficult and results in greater variability than under controlled laboratory conditions, this approach adds a level of real-world applicability and prediction of metal fate and transport under changing environmental conditions. This type of *in-situ* environmental data can aid in the development and implementation of more-effective remediation of contaminated waters and sediments, because data on metal deposition and release, specific to the site of interest and in the actual geochemical conditions, can be obtained. We are unaware of other studies that utilized trays filled with artificial sediments specifically for the purpose of chemical analysis of accumulated sediment contamination, because the tray method was originally developed to examine benthic invertebrate populations [25,26,27].

We attempted to develop our methodology to incorporate some aspects of laboratory experimental rigor, while using field-collected information and field-deployed sediment samplers. This approach allows for more versatility and reproducibility in field deployments without the added difficulty of accurately and representatively collecting undisturbed bed sediments from a stream. We found no significant differences in metal deposition based on placement of the trays in two stream microhabitats that had different flow regimes. The low velocity and generally turbulent flow, combined with the relatively narrow stream width, may have contributed to the uniformity of inorganic contaminant deposition.

After the initial experiment in 2012, we modified the method in three important ways in 2013. The presence or absence of side holes in the rock trays affected the accumulation and retention of fine-grained sediments. Using both tray designs (with holes and without holes) may be useful, because they could be representative of different stream-bed habitats. The rock trays without holes are more representative of pool reaches that have more fine-grained sediments through which lateral flow of water is limited. The rock trays with holes are more representative of riffle reaches with cobbles and gravel, in which greater lateral flow through the stream bed might be expected. Also starting in 2013, lids covered the rock trays during upstream deployment and collection. We believe this resulted in less loss of sediment during retrieval and transport of the rock trays. Finally, the control metal concentrations in 2013 were an order of magnitude greater than in the 2012 controls. Although inter-annual variability is a possible cause, it is also important that the 2013 extraction procedure used a more-concentrated acid and elevated temperature. Based on visual observation, this increased extraction rigor produced a more complete extraction of the surface coatings; however, it also increased the contribution of metals

from the rock substrate on which the AMD coating formed, thus decreasing the ability to detect statistically significant differences between treatment and control rock trays.

In 2012, Cu and Fe that accumulated during 1 month of deposition at the contaminated site did not decrease to control concentrations after 1 month in uncontaminated reference water. The results for Mn and Zn were inconclusive due to higher among-replicate variability. Of the Cu and Fe lost from the metals-contaminated rock trays during the recovery period, most was removed during the first week; during the following 3 weeks, the rates of Cu and Fe removal decreased. This could have been the result of (1) the lower total metal concentrations after 1 week of recovery, which would result in lower subsequent removal of metals per unit time even though the percentage-removal rates might have remained constant, or (2) scouring of the loosely-bound metal-oxide flocculent on the rocks, leaving more-armored metal-oxide surface coatings that are more slowly removed by the reference water. Also in 2012, the lack of a significant difference in metal accumulation between the “slow” and “fast” treatments during the deposition period suggests that the accumulation of metals in the rock trays was not affected by small-scale differences in the flow conditions at the depositional site. Furthermore, the loss of metals during the recovery period was not affected by the conditions under which the metals were deposited on the rocks.

Based on the 2013 results, the majority of metal deposited on the sediment appeared to reside in loosely-settled metal-oxide flocculent. This conclusion is based on visual observation of deposited AMD materials in the trays and the significant differences between the metals concentrations in trays with and without holes. Rock trays with side holes lost metals faster than trays without side holes. We predict that after remediation, the loose metal oxides will be scoured away quickly, especially during periods of high flow when stream discharge can be up to two

orders of magnitude greater than during our experiments. However, the plated metal coatings that form on sediment surfaces may take much longer to be released because they are not as susceptible to physical removal through scouring.

There were no significant trends for Mn concentrations in either the deposition or recovery periods in 2012 and 2013. This might be due to the slow oxidation kinetics of aqueous Mn(II) if uncatalyzed [40], thus accounting for a lack of accumulation during the deposition period. Consequently, if no Mn accumulated during the deposition period, there would have been no Mn lost during the recovery period.

2.5 Conclusions

We have developed an *in-situ* method to simulate post-remediation recovery of metal-contaminated sediments, and laboratory procedures for analysis of the samples. This methodology was used to demonstrate trends in metal concentrations in sediment under simulated stream-recovery conditions. This approach allowed us to determine that Cu and Fe are removed from contaminated sediment at a slower rate during the recovery phase than they are gained during the depositional phase. This hysteresis of metal uptake and loss could have important implications for successful remediation of AMD-contaminated sediments. The method we have developed could be used to examine temporal trends in inorganic contaminant partitioning between the water column and sediment phases, in order to maximize the effectiveness of remediation and to predict the time necessary for stream sediment to recover. This type of information could improve the current understanding and prediction of the fate, transport, and toxicity of aquatic inorganic contaminants in streams.

2.6 Acknowledgements

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CHAPTER THREE

EXAMINATION OF METAL DEPOSITION FROM ACID MINE DRAINAGE ON SEDIMENT IN THE NORTH FORK OF CLEAR CREEK, COLORADO, USA

To be submitted to *Environmental Science & Technology*

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3.1 Abstract

Mine waste, when not properly contained and managed, may result in the release of acidic waters containing high concentrations of metals. Acid mine drainage (AMD) can impair the ecological health of receiving aquatic systems due to metal toxicity and benthic habitat destruction. One such system is the North Fork of Clear Creek (NFCC) in Gilpin County in Colorado, USA, which is currently receiving AMD discharge from 2 drainage tunnels. Additional AMD originates from uncontained mine-waste from mid-to-late 1800s gold- and silver-mining activities that pre-dated modern mine-waste management practices. We developed and tested a methodology to examine the time-scales of sediment contamination by accumulated metal-rich surface coatings and settled flocculent. A key feature of the deployment methodology is that it is resilient to short-term high-discharge events occurring in the stream, which allows for longer periods of deployment of samplers. In 2014 and 2015, we deployed samplers filled with clean rock substrate at a site downstream of the AMD inputs. We performed several consecutive sets of 2-week deposition experiments to determine how water chemistry and flow conditions affect metal deposition. The collected trays underwent an acidic partial-extraction procedure to examine the concentration of deposited metals in the trays. In 2015, the extracted Cu and Fe

concentrations from the trays collected from the first 2-week deployment significantly increased relative to the initial controls by 15 mg/kg wet sediment and 490 mg/kg wet sediment, respectively. The extracted Cu and Fe concentrations from the trays collected from the second 2-week deployment increased relative to the initial controls by 7.3 mg/kg wet sediment and 230 mg/kg wet sediment, respectively. The cause of the variability in the rate of deposition likely was a combination of water composition, flow rate, and the nature of the substrate surface. Using our rock-tray method combined with water chemistry data and geochemical modeling, we successfully observed trends in NFCC metal deposition *in situ*, which allows for more realism than *ex-situ* laboratory studies. This method can be widely used to examine aquatic contaminant deposition onto stream sediment to gain a better understanding of contaminant fate and transport.

3.2 Introduction

Metal-mining activities that occurred before environmentally responsible mining practices were developed have led to unintended contamination of many aquatic systems around the world [1,2,3,4]. The drivers of acid-mine drainage (AMD) generation are the concentrations of acid-generating and acid-consuming minerals in the mine waste, climate (precipitation and temperature), sulfide-oxidizing bacteria and mineral surface area [5,6,7]. AMD is generated through oxidation of sulfide-containing minerals, primarily iron disulfide (pyrite; FeS_2), which are commonly found in waste rock and tailings [8]. The acid generated from sulfide oxidation can accelerate the release of metals from other non-sulfide minerals [9], because most metals are soluble in acidic environments, thus leading to high environmental mobility of metals.

After entering a stream, AMD typically increases the dissolved and particulate metal loads in the receiving water column, as well as decreasing the pH and alkalinity. The pH of the AMD typically increases after mixing with receiving waters, resulting in lower metal solubility and thus

leading to the formation of insoluble metal oxy-hydroxide colloids in the water column [10,11,12] and directly on the stream substrate. Metal-oxide sediment coatings and settled oxy-hydroxide particles covering the stream bed can adversely affect the aquatic ecosystem due to metal toxicity, increased acidity, and destruction of benthic macroinvertebrate habitats [13,14]. The metal oxy-hydroxide particles formed may adsorb other dissolved metals through surface complexation, and may facilitate transfer of metals from the water column to the bed sediments. The metals deposited onto the sediment may vary greatly in composition, speciation and bioavailability because the chemical properties (such as pH) of the stream water affect the formation of metal oxide particles [15] and their subsequent sorptive properties [16,17]. Commonly observed mineralogy of iron deposition in AMD-affected systems includes ferrihydrite, goethite, schwertmannite, and jarosite. Jarosite typically forms at low pH values (≤ 2), and schwertmannite typically forms at intermediate acidic pH values ($\sim 2 - 5$). At pH values > 5 , the formation of ferrihydrite is kinetically favored, and over time the deposited ferrihydrite may form thermodynamically favored goethite minerals [15,18,19].

A majority of dissolved metals originating from AMD eventually leave the water column by either settling onto the stream bed in the form of precipitated particles; adsorbed to iron, manganese, or aluminum hydroxides [16]; or through direct adsorption to the sediment surface. Metal-oxide coatings and oxy-hydroxide particles continue to accumulate on the stream bed if the incoming source of AMD is not abated, this processes being heavily-influenced by discharge. Even after AMD inputs are remediated and dissolved metals in the overlying water are decreased, the stream sediments may require a long period of recovery to release the accumulated AMD metals. Metals can be released from the bed sediment through chemical removal, which includes dissolution, desorption, and adsorption to suspended particles, or by physical removal (scouring)

[20,21,22,23,24]. Metal removal from the sediment must occur for the aquatic ecosystem to recover, and the chemical and physical properties of the stream control the rate and extent of this recovery. Deposited loose metal oxide-floc is scoured away more easily than the plated metal-oxide coatings armoring the sediment bed, which may take an extended period of time to be released or may require more extreme scouring events. In addition to the period in which stream discharge is at its annual maximum (spring runoff from snowmelt), short-term summer rainstorms can also generate significant scouring. Periods of high discharge would be interesting to examine; but due to high discharge and fast flows, it would be unsafe to work in most streams during this period.

There have been many studies on the effects of AMD on waters, soils, sediments and aquatic organisms, but to the best of our knowledge no previous studies chemically examined AMD metal accumulation on clean sediment over a period of time. Several studies have characterized field-collected metal-rich sediment from AMD affected aquatic systems. Common analytical techniques included x-ray diffraction (XRD), scanning electron microscopy (SEM), and transmission electron microscopy (TEM) [25,26,27,28]. This type of direct examination allows for analysis of precipitated mineral phases from AMD to determine speciation and adsorption properties.

Many of these studies were designed to determine the fate and behavior of metal contamination in stream water or river-bed sediments used AMD characterization at sampling points located downstream from the incoming point source of contamination. Characterization of water and sediment composition is useful in understanding the specific geochemical processes leading to metal deposition, because the specific speciation of particulates and sediment coatings can be determined. However, when using field-collected sediment samples, it can be difficult to

ensure that the resulting data are representative of the study system because of sampling biases or disturbance to the sample during collection. Also, this type of analysis provides limited information about the time scales of metal deposition.

Previous studies have focused on elemental analysis of acid-extracted AMD-deposited metals using field-collected sediments [29,30,31,32,33]. The most common analytical methods used to examine the elemental composition of AMD deposition are atomic absorption spectroscopy (AAS) and optical emission spectroscopy (OES). These analytical techniques allow for a quantitative examination of deposited metal concentrations in different reaches of stream or river. Extraction methods vary between studies ranging from a 1-step single-acid extraction to multiple steps involving multiple acids. Also, the method of bed sediment collection often varies among the studies. Mining commonly, although not exclusively, occurs in mountainous terrain. Stream sediments in this environment are particularly difficult to representatively sample due to their extremely heterogeneous grain size, from fine sand to boulders.

The research presented here is an examination of the geochemical processes that control aquatic AMD-metal deposition onto streambed sediment. Our rock-tray method (publication in review), described in Chapter 2, uses trays filled with clean sediment substrate that can be deployed in reaches of stream affected by AMD. The use of gravel and cobble as the substrate mimics the characteristics of bed sediments in high-gradient streams that are typical of many AMD-impacted sites. The trays are then analyzed for deposited metal concentrations extracted from the rock surfaces.

Presented below are the results from 2014 and 2015 of our continued methodological development of the rock-tray approach. The effects of a short-term scouring event on metal deposition were captured in the 2015 experiment. Geochemical modeling was used to examine

the processes of metal deposition in the water column. A combination of *in-situ* field data, *ex-situ* laboratory analysis and the results of geochemical modeling of the stream-water chemical properties allows for a more detailed examination of AMD-metal deposition in stream sediment and provides tools for possible prediction of metal deposition and removal in contaminated aquatic systems. Our study differs from previous AMD studies because we examine the AMD metals deposited in our samplers, which allows for an indication of the time required for significant concentrations of metal to deposit onto the sediment. Elemental analysis can also be used in conjunction with stream ecological and toxicological data to determine how the AMD-sourced metal concentrations in the water and sediment might affect aquatic biota [34].

3.3 Experimental

3.3.1 Study area

The North Fork of Clear Creek (NFCC) is an alpine stream, fed primarily by snow melt, in Gilpin County in the Rocky Mountains west of Denver, Colorado, USA. Its headwaters start at high elevation (>3000 m) on the east side of the continental divide. The NFCC then flows southeast through the city of Black Hawk, Colorado. Two point sources of ground water currently discharge AMD into the NFCC in Black Hawk. Gregory Incline (GI) is the furthest upstream of the two tunnels that discharge into the NFCC, followed by National Tunnel (NT) ~1 km downstream of GI. The pH of the incoming AMD typically is ~5 – 6, and it contains high concentrations of dissolved metals (Al, Cu, Fe, Mn, and Zn), including ferrous iron (Fe^{2+}) and very low concentrations of particulate-associated metals [35,36]. Metal oxy-hydroxides begin to form in the water column shortly after the discharged AMD mixes with the higher-pH stream water, resulting in increased turbidity. Downstream of the AMD inputs, the water column and the stream bed contain high concentrations of metals and the stream bed is coated with orange metal-

oxyhydroxides. The discharge from GI and NT remains relatively constant throughout the year, while the NFCC's discharge is highly variable and typically reaches the annual maximum in mid-spring when mountain streams and rivers experience elevated discharge due to snow melt. In mid-summer, thunderstorms commonly occur nearly every afternoon, which often can result in short-term flash-flood events with instantaneous discharges approaching spring-runoff discharges. The majority of the creek freezes in the winter, when discharge typically is at its annual minimum, leading to high metal concentrations in the stream water due to greater percentage contributions by the more-seasonally consistent discharge of the AMD inputs. The NFCC flows for ~11km southeast from Black Hawk before converging with the main stem of Clear Creek. The NFCC represents only a small northern part of the Clear Creek watershed, which encompasses 1000-square km. Below the confluence with Clear Creek, the water conditions improve due to dilution, resulting in healthier, more diverse aquatic biological communities than in the AMD-affected reach of the NFCC.

3.3.2 Study site

In our previous method-development study (Williamson et al., submitted), rock trays were deployed in a reach of stream ~100 m downstream of NT. Due to construction at that site starting in 2014 that impeded access to the stream, we moved our primary sampling/deployment site to a site ~1 km downstream of NT (Figure 3.1).

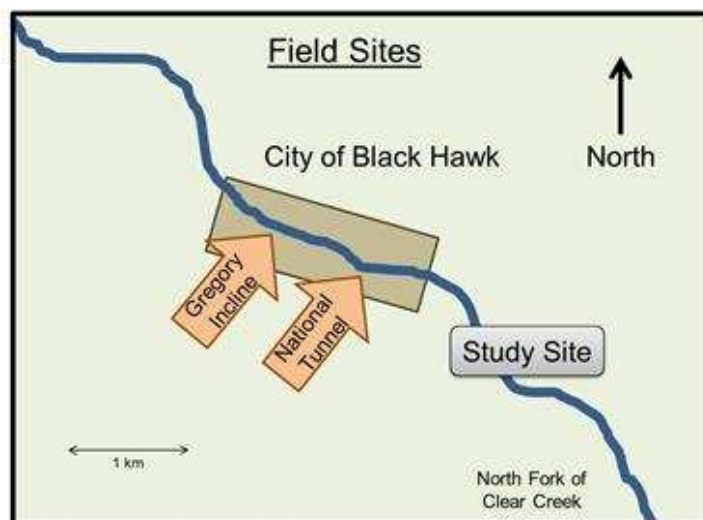


Figure 3.1: Locations of the field sites on the North Fork of Clear Creek.

Discharge measurements were taken from a United States Geological Survey (USGS) stream-flow gauge station located on the NFCC ~50 m upstream of the confluence with the main stem of Clear Creek (USGS 06718550). There are no significant surface-water inputs between the sample site and the USGS gauging station. Thus, the measured discharges approximate those at the sampling site.

3.3.3 Rock trays

The rock trays were constructed from 10 x 10 x 6cm polypropylene food-storage containers (Stor-Keeper® Arrow Plastic Manufacturing Company, Elk Grove, Illinois, USA). In 2014, they were filled with clean gravel and cobble collected from a stony sand bar in the main stem of Clear Creek ~15 km downstream from the NFCC confluence, in the city of Golden. The stream rocks were rinsed with de-ionized water and then allowed to sit for 24 h in a 10% trace-metal-grade nitric acid solution (Macron HNO₃, diluted with deionized water). Although the streambed sediment at this location is not noticeably influenced by upstream metal sources, we wished to remove any possible metal-oxyhydroxide coatings and biofilms. The rocks were then

removed from the acid bath, rinsed with de-ionized water, allowed to dry, and placed in the trays. In 2015, the trays were filled with small cobble (6-10 cm diameter) and coarse gravel (2-6 cm diameter) obtained from a local landscaping company and were not pre-cleaned with acid, because they had not experienced recent exposure to stream water. The 2014 and 2015 average total mass of substrate contained in each of the filled trays was ~1 kg. Even though the substrate used in trays differed between the 2014 and 2015 field experiments, the interpretation of the final extraction results is unaffected because the changes in metal concentrations are relative values which are compared to the initial rock substrate (also used as control samples) that underwent the extraction procedure and analysis.

3.3.4 Experimental field procedures

We attempted a rock-tray deployment in a reach of stream ~1 km downstream of NT on April 9, 2014, using the in-stream deployment rack method of Williamson et al. (in review). However, due to high discharge resulting from intense precipitation, the deployed racks of rock trays were washed away after <2 weeks of exposure. Immediately deploying another set of trays would have been impossible due to increasing stream discharge resulting from the spring snow melt. On August 8, 2014, we attempted another rack deployment again at the same site, and ~2 weeks later a flash flood again washed away the deployed racks of rock trays.

To prevent further loss of deployed rock trays, we modified the deployment method, which was originally employed by Clements et al. [37,38,39], in two ways. The linear wooden racks that hold 10 trays in-stream were replaced by deployment chambers that can hold 18 trays. This deployment method was designed to be more resilient to high stream discharge due to the more compact and heavier design. The deployment chambers were constructed from 70 x 42 x 18cm polypropylene containers (Rubbermaid®, Atlanta, Georgia, USA). After placement of the

trays in the deployment chambers, plastic netting was stretched over the top and attached to the sides of each chamber, to contain the trays and to keep coarse debris from settling in the trays. Rock trays were sampled by detaching the mesh from the top of the deployment chamber; attaching lids to the trays, while still submerged; and then carefully removing the trays from the stream. The attached lids minimize loss of loose sediment and disturbance to the rock trays during retrieval. After sampling, the mesh was reattached to the top of the deployment chamber.

In a second modification to further improve the resiliency of our deployed samplers, we developed a flow-through field mesocosm by anchoring the deployment chambers on the stream bank instead of the previous method of in-stream deployment (Figure 3.2). The deployment location was adjacent to the stream on a flat sand bar just downstream of a small waterfall, where the stream flowed over some large boulders and descended ~1 m in elevation. The opening of a polypropylene irrigation tube (~0.15 m diameter and ~6 m long) was anchored to the top of the waterfall to route stream water to the large deployment chambers using gravity flow. The downstream end of the tube was attached to the first stream deployment chamber, and water was able to flow freely over all of the rock trays. The streamside deployment chambers were positioned so the overflowing water from the first deployment chamber flowed into the second deployment chamber.



Figure 3.2: Pictures of the side-of-the-stream deployment method at Site 2. (a) The field-mesocosm bin holding 18 rock trays, with stream rocks placed in between the trays for additional weight; (b&c) the stream-side deployment showing the placement of the irrigation tube and holding bins.

In 2014, to determine if the new flow-through field mesocosm deployment method significantly affected metal deposition in the trays, we deployed another set of trays for comparison. This additional deployment was located in the stream ~3 m lateral from the mesocosm deployment, to examine if deposition varied between in-stream and side-of-the-stream deployments in the same water chemistry conditions. The trays for this in-stream deployment were placed in the same type of chamber used in the stream-side deployment, and the chamber was completely submerged in the water and secured to the stream bed. In 2015, we continued to only use the stream-side deployment approach at the same site, and did not include any additional in-stream deployments.

On September 9, 2014, 8 rock trays were deployed in the flow-through stream-side mesocosm and 8 trays were deployed in-stream. At 2 weeks of AMD exposure, 3 trays were collected from both sets of deployments (stream-side, and in-stream). At 4 weeks of exposure, 5 trays were collected from both sets of deployments. I refer to the 2014 samples by their deposition time (D-2w, D-4w) and their location (Stream-side, In-stream).

In 2015, 12 rock trays were deployed in the flow-through mesocosm on July 7, 2015. On August 8, 2015, 5 trays were collected from the deployment chambers for analysis at the 2-week exposure time (sample D-2w). On August 8, 2015, I returned to check the field deployment apparatus and found that a flash flood resulting from an evening thunderstorm on August 8, 2015, had dislodged the stream-side drainage tube, so that the stream-side deployment chambers were not receiving flowing water and the holding bins had been displaced from their original positions. Two trays (sample D-2w*) were collected to examine the effects of the flash-flood scouring on metal deposition. The deployment chambers were placed back into position and the drainage tube was re-anchored to the top of the small waterfall. Upon return to the deployment site on August 8, 2015, I added 5 clean rock trays (sample D-2w**) to the deployment chambers for comparison to the metal accumulation during the first 2 weeks of AMD exposure. On August 8, 2015, 5 of the trays deployed at the start of the experiment were collected for analysis (sample D-4w), as well as the 5 trays deployed on August 8, 2015 (sample D-2w**). I refer to the samples by their deposition period and their relationship to the scouring event (i.e., D-2w, D-2w*, D-2w**, and D-4w). When trays were collected from the deployment chambers, they were randomly selected from different chambers and different areas of the chamber to ensure unbiased exposure for each sampled set of trays.

The high-discharge event allowed for an unintended interesting comparison of metal deposition during two different 2-week periods onto clean and scoured substrates. The rocks visually appeared to have been partially scoured of their coatings by the high-discharge event. The 2-week samples collected on August 8, 2015 and the sample trays (deployed on August 8, 2015) that had been exposed to AMD water for the following 2 weeks both represented deposition onto clean substrate. These first 2 sets of 2-week deposition data represent the accumulation of metals over consecutive 2-week periods. The third set of 2-week deposition data for the substrate deployed on July 7, 2015 represented deposition onto substrate that already had some surface coatings that were resistant to scouring.

3.3.5 Extraction procedures

To analyze the concentrations of AMD metals deposited into the rock trays, a surface-extraction procedure was performed as described in Chapter 2, which was modified from USEPA method 3050b. At the time of collection, trays were placed inside 2-gallon re-sealable polyethylene bags (Ziploc®, S. C. Johnson & Son, Inc., Racine, WI. USA), and stored in a laboratory freezer (-40°C) until they were thawed overnight before performing the extraction. The extraction method used concentrated trace-metal-grade nitric acid (Macron), concentrated reagent-grade hydrogen peroxide (Macron) and a hot water bath (90°F). The total time required to perform the extraction procedure was 5 hours. Overnight settling and decanting of the solution removed the majority of particles before dilution for analysis. I found from preliminary extractions that filtration of the highly-acidic extraction solution was problematic because metals were leached out of the syringe filters, resulting in biased metal concentrations. Forty mL of the resulting extracted solution was sampled using a disposable polyethylene pipette and transferred to a conical Falcon tube (50-mL polypropylene). The solution was diluted with Milli-Q water (>

18Mohm) to approximately 2% nitric acid and analyzed using ICP-OES (Perkin Elmer Optima 5300) for metal and major element concentrations.

3.3.6 Water sampling and chemical analysis

During every deployment, collection, or water-sampling event during the field experiment, stream-water samples were collected for chemistry analysis. Grab samples of unfiltered and filtered (Thermo Scientific Target2 Nylon + Glass Syringe Filter, 0.45- μ m pore size) stream water were collected for analysis of total and dissolved metal and major cation concentrations using ICP-OES (Perkin Elmer Optima 5300). Table 3.1 lists the laboratory and field instrumentation. Hardness was calculated using the measured concentrations of Ca and Mg, and sulfate concentration was calculated using the concentration of S from the ICP-OES analysis. Unfiltered and filtered (Thermo Scientific Target2 Nylon + Glass Syringe Filter, 0.45- μ m pore size) stream-water samples were also analyzed for total organic carbon (TOC) and dissolved organic carbon (DOC) concentrations, respectively.

All ICP-OES analysis used a continuously introduced Sc internal-calibration standard. Deionized water blanks (Barnstead Nanopure system, Thermo Fisher Scientific) and certified continuing calibration verification (CCV) standards were analysed after every 20 samples for quality assurance and quality control. The instrument detection limits for the metals, and hardness ions (Ca and Mg) were (in μ g/L): 0.1 Ca, 0.3 Cu, 0.4 Fe, 0.6 Mg, 1.3 Mn, and 0.4 Zn.

Table 3.1: Instrumentation used in the analysis of collected NFCC water from the field experiment.

pH	VWR SP80PC meter/VWR 14002-860 combination gel electrode
Alkalinity (unfiltered)	HACH Model AL-DT Alkalinity Test Kit
Ferrous Iron	HACH DR/890 Colorimeter/1-10 phenanthroline AccuVac ampules
Elemental Concentrations (Metals and Major Cations)	ICP-OES; Perkin Elmer Optima 5300 DV
Organic Carbon	Sievers 5310C TOC analyzer

3.3.7 Data analysis

All of the data analyses to test for significant differences employed a 1-way analysis of variance (ANOVA) followed by Tukey HSD post-hoc pairwise comparisons ($\alpha = 0.1$) (calculated using Microsoft Excel). I chose an alpha value (the probability of a Type I false-positive error) of 0.1 because the variability in field studies can be higher than in laboratory studies, for which the more traditional alpha value of 0.05 is often considered appropriate. Increasing the alpha value decreased the probability of a Type II false-negative error, thus avoiding overly-conservative inferences in this type of field study.

Statistical analysis of the 2014 extraction data for the metals of interest (Cu, Fe, Mn, Zn) began by testing for significant differences between the initial controls and the D-2w samples, between the initial controls and the D-4w samples, and between the D-2w samples and the D-4w samples. This was to determine if concentrations of metal had increased significantly during either 2 weeks or 4 weeks of deposition, and to determine if the deposition during the first 2-week period differed significantly from the second 2-week period. I then tested for significant differences in metal concentrations between the stream-side and in-stream samples at the 4-week time point, to determine if the deployment method significantly affected metal deposition.

Analogous statistical analyses of the 2015 results began with comparison of the initial controls to the other deposition sample groups (i.e., D-2w, D-2w*, D-2w**, and D-4w). These comparisons allowed me to determine if concentrations of metals increased significantly in the trays at a given time point; and for the D-2w* trays, comparison to the controls allowed me to determine if the scouring event removed enough metals to cause the metal concentrations to not differ significantly from the controls (i.e., complete scouring). I then tested for significant

differences in metal concentrations between the 3 different 2-week depositions (i.e., D-2w, D-2w**, and (D-4w – D-2w**)).

3.3.8 Geochemical modeling

I input water chemistry data collected during the field experiment into Visual MINTEQ, a speciation-solubility geochemical modeling program [40]. I used the model to predict the aqueous speciation of metals and mineral saturation in the water column. The sources of the water chemistry inputs are shown in Table 3.2, and all other options in the model not listed in Table 3.2 were left at the program default. The concentration of Fe^{3+} used in the modeling was calculated as the difference between the filtered Fe concentration in a given water sample and the field measurement of Fe^{2+} concentration in the waters that were sampled for later laboratory analysis. I also allowed Fe redox chemistry to occur in the model. I assumed that the primary particulate form of Fe in this system is ferrihydrite because the pH of the stream is typically near neutral. Because I also assumed that the primary sorbent in this system is ferrihydrite, precipitation of ferrihydrite was allowed, as well as surface complexation to form ferrihydrite from oxidation of Fe^{2+} . A small initial concentration of ferrihydrite is required for the model to run, and the lowest concentration of ferrihydrite allowed by Visual MINTEQ (1×10^{-6} g/L) was input.

The modeling results were a prediction of the water composition after equilibrium, including dissolved, precipitated and adsorbed forms of metal. As a consequence, the model predictions may not be fully representative of a non-equilibrated, flowing reactive system such as the NFCC. However, modeling goals were to predict the quantity and composition of precipitates, because this should be indicative of the metals deposited on the sediment in the stream. A “modified” water chemistry model was also run that did not allow Fe redox chemistry

to occur and did not allow ferrihydrite to form. Instead, the measured Fe^{3+} concentration was divided into dissolved and particulate (as ferrihydrite) concentrations, which were entered into the model separately. This allowed me to examine the situation when only the measured particulate iron concentration was used in the sorption calculation, possibly more representative of the non-equilibrium flowing system.

Table 3.2: Visual MINTEQ inputs.

	“Normal” Water Chemistry	“Modified” Water Chemistry
pH	Field measurement	Field measurement
Temperature	Field measurement	Field measurement
Alkalinity	Field measurement	Field measurement
Solid Phases Allowed to Precipitate	Ferrihydrite	None
Surface Complexation Isotherm	Ferrihydrite-CDM	Ferrihydrite-CDM
Connect Solid Conc. to Possible Solids	Ferrihydrite	n/a
Redox Equilibrium	$\text{Fe}^{+2}/\text{Fe}^{+3}$	n/a
Dissolved Sulfate	Calculated from measured S concentration	Calculated from measured S concentration
Dissolved Al^{3+} , Ca^{2+} , Cu^{2+} , K^+ , Mg^{2+} , Mn^{2+} , Na^+ , Ni^{2+} , and Zn^{2+}	Measured concentrations	Measured concentrations
Dissolved $\text{O}_2(\text{g})$	Field Measurement	Field Measurement
Dissolved Fe^{2+}	Field Measurement	Field Measurement
Dissolved Fe^{3+}	Measured Fe concentration	Measured Fe concentration
Ferrihydrite	n/a	Measured particulate Fe concentration
Dissolved Organic Carbon (DOC)	Measured concentration (Suwanee Humic Model - SHM)	Measured concentration (Suwanee Humic Model - SHM)

3.4 Results and discussion

In 2014, on the date of the first deployment (September 9, 2014), the stream’s discharge was $0.2 \text{ m}^3/\text{s}$; and on the date of the final collection (November 7, 2014), the stream’s discharge was $0.1 \text{ m}^3/\text{s}$. In 2015, on the date of the first deployment (July 31, 2015), the stream’s discharge was $0.3 \text{ m}^3/\text{s}$; and on the date of the final collection (October 23, 2014) the stream’s discharge was $0.2 \text{ m}^3/\text{s}$.

3.4.1 Water chemistry

The 2014 and 2015 ranges of water chemistry properties measured at the study site are shown in Table 3.3. The total and dissolved concentrations of Cu, Fe, Mn, and Zn are also presented in Table 3.3. These elements are reported because Fe and Mn represent the main oxide phases in this system, and Cu and Zn are the most abundant trace elements in the NFCC water column that are considered toxic [41,42]. We did not include results for Al because the extracted Al concentrations in the treated rock-tray sets did not differ significantly from the controls (i.e., the calculated amounts of AMD-deposited Al were small compared to the amounts of Al extracted from the substrate rocks).

Table 3.3: Range of NFCC water chemistry data collected during the 2014 and 2015 field deployments.

	Study Site 2014 (9/3/14-10/1/14)	Study Site 2015 (7/31/15-8/28/15)
pH	6.3 - 6.9	6.6 - 7.3
Temperature (°C)	5.6 - 11.5	12.2 - 18.1
TOC (mg/L)	2.5 - 3.3	2.5 - 3.4
DOC (mg/L)	1.9 - 2.4	2.0 - 2.4
Dissolved Oxygen (mg/L)	8.0 - 8.9	8.0 - 8.9
Sulfate (mg/L)	150 - 200	120 - 210
Hardness (mg CaCO ₃ /L)	150 - 180	120 - 210
Alkalinity (mg CaCO ₃ /L)	10 - 15	2.4 - 9.9
Total Cu (mg/L)	0.07 - 0.15	0.19 - 0.27
Dissolved Cu (mg/L)	0.003 - 0.006	0.012 - 0.082
Total Fe (mg/L)	11 - 27	8.6 - 14
Dissolved Fe (mg/L)	5.0 - 9.3	2.2 - 8.2
Ferrous(II) Fe (mg/L)	1.5 - 6.9	0.75 - 3.0
Total Mn (mg/L)	2.9 - 3.8	2.5 - 4.5
Dissolved Mn (mg/L)	2.7 - 3.8	2.5 - 4.4
Total Zn (mg/L)	1.1 - 1.3	1.4 - 1.8
Dissolved Zn (mg/L)	0.9 - 1.2	1.3 - 1.7

3.4.2 Sediment chemistry

The 2014 extraction results for the stream-side and in-stream deployments are presented in Figure 3.3 and in Table 3.4.

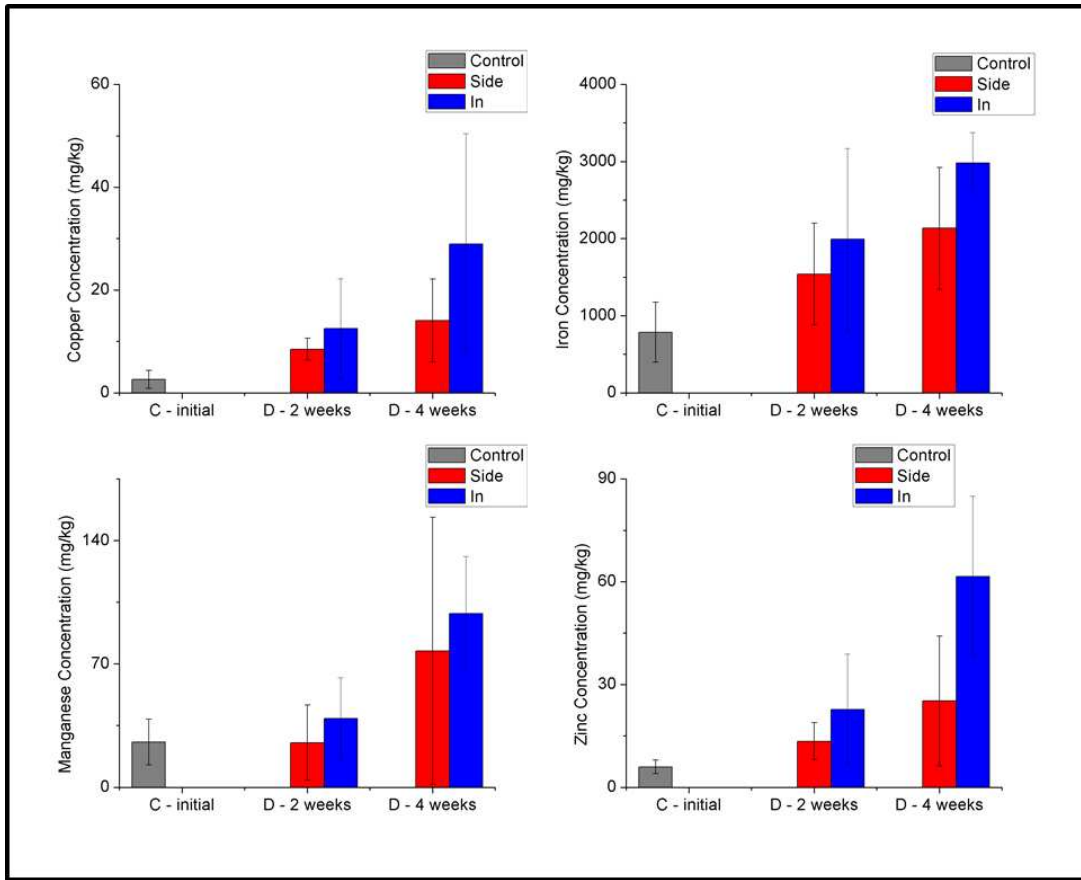


Figure 3.3: Concentrations (mg/kg wet sediment) of Cu, Fe, Mn, and Zn extracted from sediment in rock trays deployed in North Fork of Clear Creek in 2014 (mean of 5 replicates, except for D – 2 weeks, which is the mean of 3 replicates; error bars are 90% confidence intervals). “Side” = rock trays deployed in the stream-side mesocosm, and “In” = rock trays deployed in-stream at the study site. The two time points for deposition were 2 weeks and 4 weeks. C - Initial = clean rocks never placed in stream water; D – 2 weeks = clean rocks exposed to contaminated water for 2 weeks; D – 4 weeks = clean rocks exposed to contaminated water for 4 weeks.

Table 3.4: 2014 average concentrations of Cu, Fe, Mn, and Zn (mg/kg wet sediment; 90% CI in parenthesis). “Initial Controls” = clean rocks never placed in stream water, “Stream-side” = rock trays deployed in the stream-side mesocosm for 4 weeks, and “In-stream” = rock trays deployed in-stream at the study site for 4 weeks.

	Initial Controls	Stream-side	In-stream
Cu	2.59 (± 1.73)	14.1 (± 8.06)	29.0 (± 21.5)
Fe	786 (± 388)	2130 (± 790)	2980 (± 393)
Mn	25.7 (± 13.0)	77.4 (± 75.8)	98.5 (± 32.5)
Zn	5.96 (± 1.95)	25.2 (± 18.9)	61.5 (± 23.4)

Deposition in Stream-side Mesocosm

The extracted metal concentrations from the “C - initial” rock trays represent metals extracted from control rocks that were never placed in stream water. The extracted Cu, Fe, and Zn mean concentrations from the “D – 2 weeks” rock trays significantly increased relative to the controls by 5.9, 750, and 7.5 mg/kg, respectively, and the mean Mn concentrations decreased (but not significantly) by 0.33 mg/kg relative to the controls. The extracted mean Cu, Fe, and Zn concentrations in the “D – 4 weeks” rock trays significantly increased by 11, 1300, and 19 mg/kg, respectively, compared to the controls, and the mean Mn concentrations in the “D – 4 weeks” rock trays increased (but not significantly) by 52 mg/kg compared to the controls. The mean Cu, Fe, Mn, and Zn concentrations extracted from the “D – 4 weeks” rock trays increased (but not significantly) by 5.6, 590, 52, and 12 mg/kg, respectively, compared to the “D – 2 weeks” rock trays.

Deposition in In-Stream Mesocosm

The extracted Cu, Fe, and Zn mean concentrations from the “D – 2 weeks” rock trays significantly increased by 9.9, 750, and 17 mg/kg wet sediment, respectively, compared to the controls, and the mean Mn concentrations increased (but not significantly) by 13 mg/kg compared to the controls. The extracted mean Cu, Fe, Mn, and Zn concentrations in the “D – 4 weeks” rock trays significantly increased by 26, 2200, 73, and 56 mg/kg wet sediment, respectively, compared to the controls. The mean Cu, Fe, and Zn concentrations extracted from the “D – 4 weeks” rock trays were 16, 990, and 39 mg/kg wet sediment significantly greater, respectively, than for the “D – 2 weeks” rock trays, and the mean Mn concentration extracted from the “D – 4 weeks” trays was 59 mg/kg greater (but not statistically significant).

Method Comparison

For Cu, Fe, Mn, and Zn, the extracted concentrations in the “D – 2 weeks” samples did not differ significantly between the stream-side-deployed trays and the in-stream-deployed trays. At 4 weeks, the Cu, Fe, Mn, and Zn concentrations in the in-stream trays were greater than in the stream-side trays by 14.9, 849, 21.1, and 36.3 mg/kg wet sediment, respectively. For Cu and Mn, the extracted concentrations in the “D – 4 weeks” samples for the stream-side and in-stream deployments did not differ significantly. For Fe and Zn, the extracted concentrations in the “D – 4 weeks” samples for the stream-side were significantly lower than in the in-stream deployments.

The 2015 extraction results are presented in Figure 3.4 and Table 3.5.

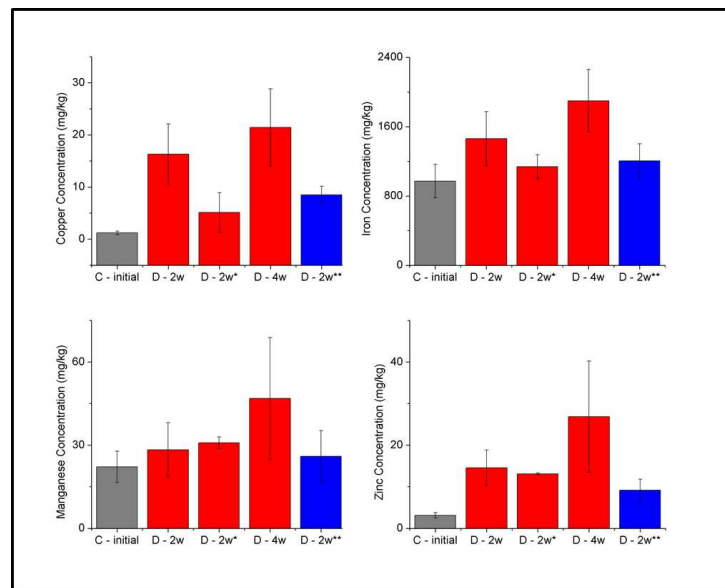


Figure 3.4: Concentrations (mg/kg wet sediment) of Cu, Fe, Mn, and Zn extracted from sediment in rock trays deployed in North Fork of Clear Creek in 2015 (mean of 5 replicates, except for D – 2w*, which is the mean of 2 replicates; error bars are 90% confidence intervals). C - Initial = clean rocks never placed in stream water; D – 2w = clean rocks exposed to Site 1 water for 2 weeks; D – 2w* = clean rocks exposed to Site 1 water for 16 days; D – 4w = clean rocks exposed to Site 1 water for 4 weeks; D – 2w** = clean rocks exposed to Site 1 water for the final 2 weeks of deployment.

Table 3.5: 2015 average concentrations given in mg/kg (90% CI in parenthesis). Initial Controls = clean rocks never placed in stream water; D – 2w = clean rocks exposed to Site 1 water for 2 weeks; D – 2w* = clean rocks exposed to Site 1 water for 16 days; D – 4w = clean rocks exposed to Site 1 water for 4 weeks; D – 2w** = clean rocks exposed to Site 1 water for the final 2 weeks of deployment.

	Initial Controls	D – 2w	D – 2w*	D – 4w	D – 2w**
Cu	1.20 (±0.320)	16.3 (±5.76)	5.13 (±3.79)	21.4 (±7.42)	8.49 (±1.66)
Fe	973 (±194)	1460 (±313)	1140 (±138)	1900 (±359)	1210 (±197)
Mn	22.2 (±5.65)	28.3 (±9.74)	30.9 (±2.10)	46.9 (±22.0)	26.0 (±9.27)
Zn	3.12 (±0.653)	14.6 (±4.24)	13.1 (±0.223)	26.9 (±13.4)	9.16 (±2.65)

After 2 weeks (August 1, 2015 – August 14, 2015) of AMD deposition onto clean substrate (D-2w), the average Cu, Fe, and Zn concentrations in the trays significantly increased relative to the controls by 15, 490, and 11 mg/kg wet sediment, respectively, and Mn increased (but not significantly) relative to the controls by 6.1 mg/kg. After the following 2 weeks (August 14, 2015 – August 28, 2015) of deposition onto clean substrate (D-2w**), the average Cu and Zn concentrations significantly increased relative to the controls by 7.3, and 6.0 mg/kg, respectively, and Fe and Mn increased (but not significantly) relative to the controls by 230, and 3.8 mg/kg, respectively. The Cu and Zn concentrations in the D-2w and D-2w** trays differed significantly from each other, but the Fe and Mn concentrations in the D-2w and D-2w** trays did not differ significantly from each other.

After the scouring event, the average Cu and Zn concentrations significantly decreased relative to the D-2w concentrations by 69, and 1.5 mg/kg wet sediment, respectively. The average Fe concentration decreased (but not significantly) relative to the D-2w concentrations by 320 mg/kg, and the average Mn concentration increased (but not significantly) relative to the D-2w concentration by 2.5 mg/kg. For the first 2 weeks after the scouring event, the average Cu and Fe concentrations increased relative to the average D-2w* concentrations by 16 and 760 mg/kg,

respectively, and the average Mn and Zn concentrations increased (but not significantly) relative to the average D-2w* concentrations by 16 and 14 mg/kg, respectively.

The average stream discharge for the first 2-week deposition period was 0.24 m³/s, and the average stream discharge for the second 2-week deposition period was 0.19 m³/s. The measured total, dissolved, and by difference, particulate metal concentrations in the water column are shown in Figure 3.5. The concentrations of particulate Cu and Zn in the water column were lower during the last 2 weeks.

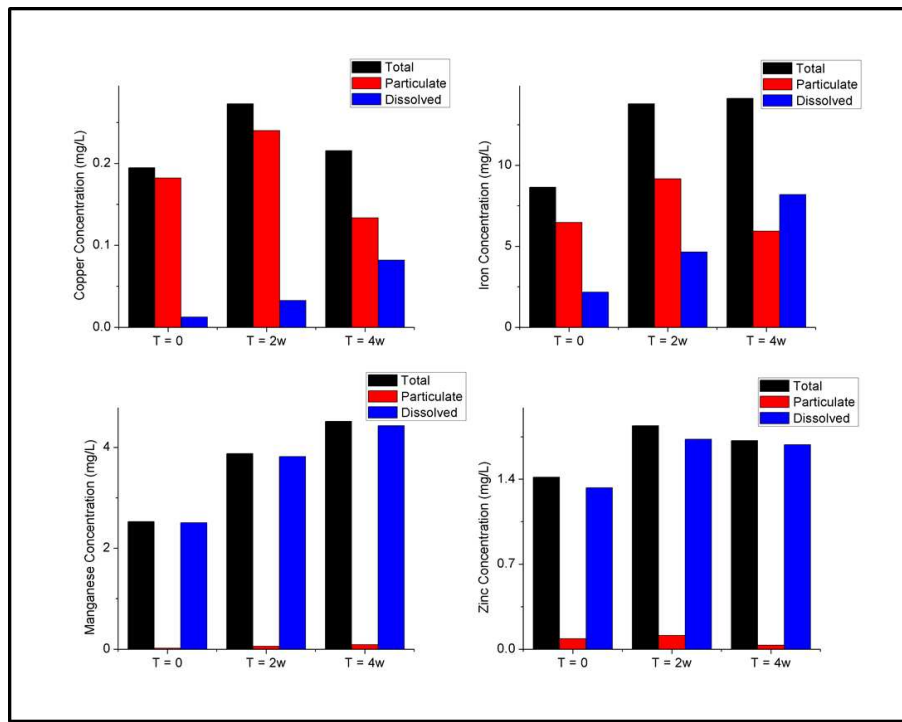


Figure 3.5: The Cu, Fe, Mn, and Zn concentrations measured in the water column at Site 1 during the 2015 field experiment. “T = 0” = the metal concentrations measured on August 2, 2015. “T = 2w” = the metal concentrations measured on August 14, 2015. “T = 4w” = the metal concentrations measured on August 28, 2015. “Total” = the metal concentration in an unfiltered stream water sample; “Particulate” = the difference between the “Total” and “Dissolved” metal concentrations; “Dissolved” = the metal concentration in a filtered (0.45µm) stream water sample.

The total and dissolved Fe, Cu, and Mn concentrations varied by up to 2.5x during the 4-week deployment period; however, the Zn concentrations were less variable.

3.4.3 Geochemical modeling results

The Visual MINTEQ-predicted percentages of metal sorbed and metal precipitated in the water column are shown in Table 3.6. The percentage of metals predicted to adsorb to ferrihydrite at equilibrium was similar between the two modeled sets of NFCC water chemistry. The difference between the two models was the amount of ferrihydrite present. The “normal” model allowed ferrihydrite to form from all of the total Fe concentration that was measured in the water. The “modified” model had ferrihydrite equal to the measured particulate fraction of Fe in the water. The modeling results from both water chemistry data sets were very similar.

Table 3.6: The predicted percentage of total metal concentration, measured in the water column, adsorbed to ferrihydrite using Visual MINTEQ.

Water Chemistry Inputs			
Date	8-2-15	8-14-15	8-28-15
Cu (mg/L)	0.19	0.27	0.22
Mn (mg/L)	2.5	3.9	4.5
Zn (mg/L)	1.4	1.8	1.7
pH	6.61	7.04	7.33

Normal Water Chemistry Modeling Results			
Date	8-2-15	8-14-15	8-28-15
Cu (% sorbed to ferrihydrite)	95	96	96
Mn (% sorbed to ferrihydrite)	0	0	0
Zn (% sorbed to ferrihydrite)	12	34	48
Ferrihydrite (mg/L)	13.8	21.8	22.4

Modified Water Chemistry Modeling Results			
Date	8-2-15	8-14-15	8-28-15
Cu (% sorbed to ferrihydrite)	95	97	99
Mn (% sorbed to ferrihydrite)	0	0	0
Zn (% sorbed to ferrihydrite)	9.3	14	53
Ferrihydrite (mg/L)	10.3	14.6	9.46

3.5 Synthesis

Laboratory analysis and examination of field-collected sediments may allow for broad geochemical data interpretation, but if information on temporal trends in specific geochemical aquatic contamination in a natural system is desired, my field-deployment methodology can be more useful. The combination of water sampling, geochemical modeling and rock-tray sampling have allowed us to examine the fate and transport of introduced AMD metal contamination in-situ, and this method can be used to examine these process in other contaminated aquatic systems. Specifically, in this study I examined the deposition of AMD contamination onto streambed sediments over short time periods in 2014 and 2015. I was able to examine removal by scouring of deposited metal in 2015. An unforeseen high-discharge event that occurred in the NFCC provided comparisons of metal deposition between different sample sets. Because of this event, we were able to compare deposition during two consecutive 2-week time periods, to allow an estimate of short-term variability, and also to compare deposition on clean substrate and substrate that had resisted scouring.

The use of my method provides a means for relevant contaminant fate and transport analysis of a natural study system, although the data reported here are specific to the NFCC and the stream conditions during the field experiment. The overall chemistry of NFCC and the AMD inputs (GI and NT) that enter the NFCC, the specific NFCC conditions that occurred during our field deployment, and the substrate used in our trays may influence the observed sediment composition. In this study, I wanted to focus on the formation of AMD metal coatings on sediment and to not analyze the existing bed sediments, which has been described previously in literature. To observe metal concentration increases on sediment, I needed an approach that is not dependent upon field sediment sampling. Temporal trends in metal concentrations may be difficult to determine because of potential high variability resulting from field sampling. Uniform

field collection of sediments may not be possible in aquatic systems containing high amounts of coarse gravel, stones and boulders on the stream bed, which are typical in mountainous regions. My approach is not dependent on the stream-bed composition and allows for comparison to a negative control (substrate not exposed to NFCC water), which is not the case when utilizing a field sampling approach. The controls and replicates of our relatively uniform samplers allow for the use of statistical analysis on the deposition results, which broadens the applicability of my methodology for use in other aquatic systems.

In the NFCC, our field experiments are limited to periods in the early Spring or the Fall, in which the stream is not experiencing high discharge (i.e., Spring runoff and summer thunderstorms) or has not frozen over in the Winter. Seasonal changes in stream discharge can result in significant differences in sediment metal concentrations, and this limits the total experimental time scale when attempting to avoid discharge related variability in a sediment deposition experiment. Field-collection of contaminated stream sediment during these periods may not provide results with significant temporal differences in metal concentration. My approach of utilizing samplers with clean substrate allows for a more controllable examination of metal deposition onto sediment over time, compared to analysis of field sediments.

In 2014, deposition of Cu, Fe, and Zn in the field mesocosm was sufficient at 2 weeks to see a difference from controls. Average concentrations continued to increase to 4 weeks but could not be described as significantly different from the samples that experienced 2 weeks of deposition. I believe that deposition was still occurring in the second 2 weeks of deployment because the average metal concentrations increased, but high variability in the results and slow deposition in the NFCC during our field experiment did not allow for significant differences to be determined. An increased deposition period and/or increased amount of replicates may result in

the observation of significant increases. The high variability suggests that either the substrate needs to be more uniform in its physical composition or placement in the chamber affects the results. Further refinement in the method may allow a reduction in the observed variability.

Due to its different geochemical behavior Mn concentrations did not increase. This is consistent with the observation that black Mn oxide coatings are generally absent from this site. Although deposition of Mn appears to be a slow process, the average Mn concentration increased (but not significantly) after 4 weeks of deployment. Additional deployment time may have allowed the Mn concentrations to increase enough to result in a significant difference despite the large variability. Results are consistent with the fact that manganese typically remains dissolved at circumneutral pH [43] and Mn removal from the water column requires a surface catalyst [44,45,46] for Mn oxidation and the subsequent precipitation and/or sorption to the sediment. Biofilms have been shown to accumulate Mn due to alteration of the small-scale geochemical environment on a sediment surface, typically through increased pH [47,48]. My samplers initially contained clean substrate before deployment and thus did not have the biofilms typically found on sediment surfaces. The lack of significant Mn deposition in the 4 weeks of our field deployment may have been due to the lack of sufficient growth of biofilm needed to accumulate Mn.

Similar observations were made for the in-stream deployment, but in this case the increases of Cu, Fe, and Zn during the second two weeks were statistically significant. Although the variability in metal concentration was similar in both deployment approaches, the Cu, Fe and Zn concentrations were higher in the in-stream deployment.

The 2014 experiment demonstrated that different deployment methods (stream-side and in-stream) can result in different amounts of extractable metals deposited on the rock-tray substrate. Both deployment methods resulted in significant increases in extractable metal

concentrations after 4 weeks of deployment, but the stream-side mesocosm resulted in lower average extractable metal concentrations compared to the in-stream mesocosms at both the 2-week and 4-week time points. This could be explained by the accumulation of some sandy bed sediments in the in-stream rock trays, while the stream-side mesocosm trays were primarily exposed to suspended or dissolved metals present in the water column. Visually, the trays deployed in-stream had accumulated more sandy sediment material after 4 weeks of deployment, compared to the trays deployed in the stream-side mesocosm. For all four metals, the concentrations (mg metal/kg wet sediment) did not differ significantly between both the two deployment methods at 2 weeks. This was also observed for the Fe and Zn concentrations at 4 weeks but not for Cu and Mn. This may have been due to different flow conditions between the stream-side mesocosm and in-stream deployments that would affect delivery of dissolved metals and suspended sediments to the trays. Alternatively, it may have been due to fine bed sediments accumulating in the in-stream deployed trays that were not captured by the irrigation tube due to the open being positioned near the water surface.

Regardless of the explanation, this effect required more than 2 weeks of deposition to result in a significant difference. Deposited fine sediment material in the trays will likely also have extractable AMD metal surface coatings, but due to its higher surface area to mass ratio than the rock and gravel in the control substrate, the extraction procedure may result in increased substrate dissolution compared to extraction of the tray rock substrate. The extraction of the in-stream trays lacks a true control for comparison, because the initial control trays contained only gravel and cobbles and not fine material. Future refinements of the method should include analysis of upstream fine-grained sediments. The field mesocosm deployment method does show lower Fe and Zn deposition after 4 weeks compared to the in-stream deployment but it is more

resilient against temporary high-discharge events in the stream that may result in loss of the deployed rock tray samplers. Furthermore, the flocculated materials are likely of greater concern than the fine-grained sediments washed into the in-stream sampler, because the flocculent material is composed of primarily AMD sourced metal oxyhydroxides in contrast to the fine grained sediments which translocated from upstream. However, these sediments may have sorbed AMD metals on the surface of stream-bed particles and thus could contribute to the metal load of the bed sediments.

In 2015, the extracted Cu and Zn concentrations from the D-2w trays and the D-2w** trays were significantly different. The D-2w trays and the D-2w** trays had the same deployment method (stream-side), deployment duration (2-weeks), stream site, and rock substrate, but the 2-week deposition periods were not identical; instead, they were consecutive.

The different amount of deposited Cu and Zn between the D-2w trays and the D-2w** trays may have been due to differences in stream properties such as temperature, which affects the kinetics of deposition. The pH, which affects aquatic-complex formation, may have also contributed to the significant differences in deposition. Finally, water column total metal concentration and distribution between dissolved and suspended particles could play a role. Less particulate metal was observed in the water column during the second two weeks, perhaps providing an explanation of the lower amount of sediment deposition.

An alternative possibility is that differences in stream flow, and thus variation in flow through the stream-side mesocosm between the first 2-week deployment and the second 2-week deployment may have also affected metal deposition. The average stream discharge was slightly lower in the second 2-week deployment period compared to the first 2-week deployment period. Less water flowing through the stream-side mesocosm may have decreased the exposure to AMD,

which resulted in less metal deposition onto the trays. Also the irrigation tube required repositioning after the flood event, and this may have led to less flow through the holding bins, resulting in less metal deposition in the trays. Measuring the actual flow through the irrigation tube to the mesocosms would improve future application of this approach.

If differences in accumulated metals in the trays are not attributable to variable flow, it may have been due to differences in water chemistry, which could affect metal particulate formation, adsorption, and deposition onto sediment. This was investigated by using Visual MINTEQ to predict mineral saturation and metal sorption to both existing ferrihydrite particles and those predicted to form if ferrous iron is allowed to react. Over the 3 sampling dates, the total Fe concentration increased, but the particulate Fe concentration did not show an overall trend. The model predicted that the concentration of precipitated ferrihydrite would increase over the 3 sampling dates when ferrous iron oxidation was allowed, which is indicative of the increasing total Fe concentration.

The Fe deposition did not differ significantly between the first 2-week and second 2-week period, in contrast to Cu and Zn. This may have been due to variability in the high Fe concentration present in the water column and a difference in deposition that was not statistically significant, or that the Fe deposition processes were similar between the 2 periods but changes in water chemistry affected Cu and Zn adsorption and thus sediment deposition. The Cu deposition was significantly greater and Zn deposition was significantly less in the second 2-week depositional period compared to the first 2-week depositional period. The geochemical model predicts that Zn adsorption to ferrihydrite should continue to increase over the entire 4-week depositional period, but this does not explain the decrease in deposited Zn, because increased adsorption to ferrihydrite should result in increased sediment deposition. The increasing

adsorption of Zn may be explained by the increasing pH measured over the entire 4-week depositional period. The Zn concentration in the water did not vary by more than 10% during the entire deployment. The lower amount of Zn deposition in the second 2-week period may have been due to a lower concentration of ferrihydrite in the water column, even though the percentage of Zn sorbed was greater. Lower stream water flow through the stream-side mesocosms appears to be a more likely explanation for the decreased deposition of Zn. The predicted percentage adsorption of Cu to ferrihydrite slightly varied for our three modeled water chemistry conditions but the Cu deposition was greater in the second 2-week period. It appears that the hydrology of the study system and/or the hydrology in the stream-side mesocosm must have affected Cu and Zn deposition.

Visual MINTEQ predicted that 0.000 percent of Mn present in the water column would adsorb to insoluble suspended ferrihydrite. The precision of the modeled prediction was limited to 3 decimal places, which includes the possibility of < 0.0005 percent Mn sorption ferrihydrite, but this is negligible. A low concentration ($\sim 1.5\%$ of the total Mn concentration in the water column) was observed to be particulate ($> 0.45 \mu\text{m}$) in the results of the water analysis. This may have been due to Mn adsorption to DOC or another suspended insoluble particle. Also Mn^{2+} oxidation and thus deposition is a very slow process at circumneutral pH and often requires a surface catalyst [48,49]. The low particulate Mn concentration in the water column may have been the result of this surface-catalyzed oxidation and thus adsorption to the surface of a suspended particle, and this chemistry may not have been accounted for in the model. The microbial biofilms needed for Mn catalyzed oxidation have not have grown sufficiently in 4 weeks on our initially clean tray substrate to affect Mn deposition.

In 2015, I had the opportunity to examine an unplanned deposition situation by the high-discharge scouring event, which occurred 2 weeks into the planned 4-week deployment period. This allowed for an examination of scouring effects on the concentrations of deposited AMD metals. There were significant losses of Cu and Zn from the trays after the scouring event. The model predicted that almost all of the Cu but less than half of the Zn in the water should have adsorbed to ferrihydrite particles, so unless the water chemistry conditions were significantly different during the high discharge event, chemical removal (i.e., dissolution, and desorption) is not likely to be a contributing factor to the metal removal during the scouring event. There was a decrease in the average Fe sediment concentration after the scouring event, but it was not significant. If the route of metal removal was completely physical, I should have also observed a significant loss of Fe. The scouring event did not affect deposited Mn in the trays, because the concentration did not change significantly after the scouring event and the average Mn concentration in fact increased slightly. This is most likely due to Mn having a chemical bond with the sediment surface and a more crystalline oxide form [45,50,51], in contrast to settled particulate matter consisting of amorphous metal oxy-hydroxides with metals sorbed to the surface of those particles.

In the second 2-week depositional period I were able to compare 2 sets of samples; one with clean substrate trays, and one with apparently scour-resistant metal oxyhydroxide coatings on the substrate. The Cu and Fe concentrations from the 2 different tray deployments differed significantly between the 2 sample sets. The deployment time, length, and exposure were identical between the 2 sets of trays, but significantly more Cu and Fe was extracted from the trays that had started the 2-week depositional period with the metal coatings that resisted scouring (“D-4w” minus “D-2w*”), compared to trays that began the 2-week depositional period with

clean substrate (control-adjusted “D-2w**”). Significant differences in Mn or Zn concentrations between the 2 sets of trays in the second 2-week depositional period were not observed. Our measured sediment data suggests that the substrate surface may affect the deposition of Cu and Fe. This would only affect possible surface complexation and not the chemical processes controlling metal-oxide particle formation and subsequent metal adsorption in the water column. Sediment surface chemistry appears to have a significant effect on the accumulation of AMD metals on the bed sediments, because the trays with coatings that had resisted scouring accumulated significantly more Cu and Fe than the initially clean trays, in the same time period.

3.6 Conclusions

I determined that stream-side mesocosm-deployment method is preferable to in-stream deployments because it reduces fine bed sediment deposition in the trays and is more resilient to high discharge wash-outs. Thus the rates we observed are more indicative of the chemically-driven transfer of metals from the water column to the sediment than the physical transport of sediment from upstream sources. While the use of the flow-through mesocosm may underestimate the rate of metal accumulation in the sediments, this procedure was actually developed to obtain material that could be transported upstream to simulate recovery. These experiments are described in Chapter 4. Thus, it was less important to exactly match the stream deposition conditions as it was to have a robust method to obtain coated media.

As expected, loose flocculent appears to be easily scoured off the sediment bed surfaces, while harder metal-oxide plating on the substrate is very resilient to physical removal. This could mean that sediment recovery post-remediation of the overlying water may require much more time than the loss of the loose floc. The surface chemistry of the sediment substrate was found to have a significant effect on metal deposition. When the substrate in the trays had scouring-resistant metal-oxide coatings instead of clean substrate, metal accumulation significantly

increased. This effect appears to have been due to increased adsorption to the scour-resistant coatings on the substrate, because the exposure conditions were identical.

This field method can also be combined with water chemistry analysis and geochemical modeling to examine trends in metal concentration in both the sediment and the water column. The specific conditions of an aquatic system are difficult to predict and replicate in the laboratory; however, combining field methods with laboratory methods allows for increased rigor and realism in the results.

3.7 Acknowledgements

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CHAPTER FOUR

***IN-SITU* EXAMINATION OF GEOCHEMICAL TRENDS IN RECOVERY OF ACID-MINE-DRAINAGE-CONTAMINATED STREAM SEDIMENTS**

To be submitted to *Applied Geochemistry*

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4.1 Abstract

Acidic mine drainage (AMD) can negatively impact receiving aquatic systems due to increased concentrations of potentially toxic elements, lowered pH and alkalinity, and impairment of aquatic benthic macroinvertebrate habitats resulting from the metal-hydroxide surface coatings accumulated on the stream bed. This latter process is a result of dissolved Fe(II) and Mn(II) introduced by AMD that can oxidize to insoluble Fe(III) and Mn(IV) after entering neutralizing oxic surface waters. The drivers of the deposition of dissolved metals (Al, Cd, Cu, Fe, Ni, Mn, Zn) onto the stream-bed sediments are the formation of and pH-dependent adsorption to insoluble particles in the water column, as well as surface complexation directly to bed-sediment surfaces. The North Fork of Clear Creek (NFCC) in central Colorado currently receives 2 point sources of legacy AMD that enter the stream in the city of Black Hawk. Our primary interest is to use a field mesocosm method to examine the rates and processes that remove these coatings under background water chemistry conditions; leading to a better estimate of the potential for recovery after the AMD remediation has occurred. During 4 weeks in late summer of 2015, I employed the field method at a site downstream of the AMD inputs in order to accumulate AMD metal particles and coatings on rock substrate surfaces. The contaminated and uncontaminated control substrates were then redeployed at a reference site located upstream from the AMD inputs. Conditions

(water chemistry, stream discharge, temperature, etc.) at this site approximated those likely to be present at the downstream site post-remediation of the AMD inputs. The compositions of acid extractions of the substrate were determined at a number of time points during a 2-month period. The resulting sediment metal composition data was interpreted by developing a mathematical approach that used a 3-component model to describe the total extracted metals. From this I calculated the mass concentrations of metal that the substrate, metal coatings and biofilms each contributed to the extraction solution, which allowed for examination of the temporal changes in the metal coatings. I then normalized the data to the total extracted metal concentrations to calculate a percent metal contribution from the coating. This normalization approach reduced the variability in the raw data and allowed for statistical analysis of recovery trends (loss of metal-oxide coatings) when concurrent accumulation of metals, likely due to biofilm growth, is occurring. Copper and Fe in the deposited coatings decreased by 0.080 and 7.4 mg metal/kg wet-substrate/day, respectively, during 8 weeks of deployment at the recovery site. The percentage of Cu and Fe extracted from the coating decreased by 0.20 and 0.39 %, respectively, during 8 weeks of deployment at the recovery site. Using a combination of field techniques, laboratory analysis, data transformations and statistical analysis, I was able to observe significant trends in metal release from AMD-generated coatings and accumulation in biofilms within stream sediments with this novel *in-situ* approach.

4.2 Introduction

Before implementation of environmentally responsible regulation of mining practices, legacy environmental damage occurred in aqueous systems, sediments and soils throughout the world [1]. A majority of the water quality problems caused by mining is due to the lack of proper containment of the mining wastes and drainages. Mining processes generate extremely large

quantities of solid waste because the metals of interest typically occur in low concentrations in the excavated ore. This leads to large quantities of waste rock and/or tailings, which may contain high concentrations of sulfide minerals that are reactive upon exposure to water and oxygen [1,2]. This reaction produces sulfate through the oxidation of sulfide, and releases metal cations from the sulfide mineral into a dissolved form [3]. A product of sulfide oxidation is sulfuric acid, which may result in additional metal release through dissolution of metal-containing minerals present in the waste rock pile [4,5]. Many metals are highly soluble in acidic environments, leading to their elevated environmental mobility in the form of acidic mine drainage (AMD) [6,7,8]. Although the detrimental effects of AMD on waters, soils and sediments are generally well known, much less is known about the geochemical processes of recovery from AMD contamination, demonstrating the need for further research.

AMD typically affects the aquatic chemistry of a receiving stream by lowering pH and alkalinity, and increasing the concentration of dissolved and particulate metals. Some metals are initially released in a reduced oxidation state (e.g., ferrous iron (II) and manganese (II)) [9,10,11]. After entering an oxic receiving water, these metals oxidize and undergo hydrolysis reactions leading to the formation of insoluble metal oxyhydroxide particles [12,13,14]. The geochemical conditions of the receiving stream affect the fate, behavior and bioavailability of the AMD metals.

Water chemistry conditions control the speciation of aqueous metals; they may remain in the dissolved phase as free ions and various aqueous complexes, form oxyhydroxide particles in the water column (Fe and Mn) [15], adsorb to a suspended particle or colloid (inorganic or organic) [16,17,18], or form a metal-oxide coating directly on sediment surfaces [19]. The amount of surface complexation of metal cations, both in the water column and in the bed sediments, increases with increasing pH, due to the deprotonation of hydroxyl-groups on possible

adsorbent surfaces [20]. Metal oxyhydroxide particle formation also generates acid as a byproduct [5], which can affect the pH of the stream farther downstream from the point source.

In AMD-affected stream systems, the fate of the majority of the introduced metals is accumulation on stream-bed sediments through the aforementioned processes of precipitation, settling, and sorption. After mining activities have ceased, AMD may continue to be generated if the source of sulfides is not contained or removed. Restoration of mining areas includes the removal of waste materials and the treatment of the metal-rich acidic effluents from tunnels and waste piles. Numerous approaches for treatment exist, with most relying on methods that increase the pH of the AMD and cause metal loss through precipitation before discharging to local surface waters [1]. Even after remediation of the metal sources, the time scale of stream recovery depends on the processes that control removal of metal accumulated in the bed sediments.

The metals accumulated on the stream-bed sediments may require an extended period of time for removal to occur, which depends on the dominant physical and chemical processes that control metal release. Physical removal is through scouring of the deposited metals from the sediment surfaces. Scouring increases during high-discharge events because the flow rate over the surface of the sediment increases. During high-discharge events, the water column typically contains higher concentrations of fine suspended sandy material, which can also increase the amount of scouring on sediment surfaces. After the overlying water is remediated, the loose metal-oxyhydroxide floc (e.g., ferrihydrite) that previously settled on the stream-bed sediments should be removed relatively quickly by scouring, but the armored metal coatings on the stream-bed sediment may require increased scouring during even-higher-discharge events. The scouring process is primarily dependent on stream discharge and not on any of the chemical conditions.

Chemical removal of deposited metals from sediment can occur through dissolution of previously insoluble metal complexes settled on the sediment bed, or through desorption of previously adsorbed metals from a settled particle or a metal sediment coating. Both dissolution and desorption require a change in conditions to occur. Metal-oxide particles form after AMD enters the stream, due to the increased pH, because metals are much less soluble at higher pH. The pH of a natural stream is usually higher than the entering AMD, and this limits the amount of dissolution of stream sediment that can occur. Following remediation, currently-contaminated reaches of stream will likely be at higher pH as well. If the metal oxyhydroxides are near equilibrium with the stream water (i.e., at mineral saturation), post-remediation decreases of dissolved metal concentrations could drive dissolution. Sorption/desorption processes are also primarily controlled by pH, with metal sorption to oxide surfaces increasing with increasing pH. Thus, metals adsorbed to settled particles or a sediment surface are unlikely to desorb in the higher pH expected following remediation. However, metals could desorb from the sediment and adsorb to a suspended particle if the binding-site affinity and density is higher on the suspended particles.

To examine sediment recovery from AMD metal contamination, I used a field-mesocosm approach that employed rock-tray samplers (Williamson et. al., in review). These trays were originally designed by Clements et al. [21] to examine benthic invertebrate populations *in-situ*. In a previous study, I modified these rock trays to examine the processes of metal deposition onto sediments in an AMD-impacted stream. Herein I report the use of this method to study geochemical flux of metals under different stream conditions, between contaminated stream-bed sediments and pristine overlying stream water. I propose that this approach, which utilizes relocating rock trays from a contaminated site to a clean background site, adequately simulates

the changes to the stream-bed sediments after AMD inputs are remediated. The field-mesocosm approach allows for temporal examination of metal-contamination release from stream sediments *in-situ* with discrete sample replicates, which is more challenging with stream-sediment sampling. Field collection of representative contaminated stream-bed sediments can be difficult due to easily disturbed loose sediment coatings, as opposed to the field mesocosm approach which utilizes lid-attachable trays that are easily removed from the stream, which reduces loss of loose deposited metal oxide contamination. This approach also has a predictive ability because contaminated samples can be relocated upstream of the contamination input, and sediment recovery can be examined before remediation. The purpose of the research presented here is to utilize a combination of sediment extraction data and subsequent data processing techniques to examine the processes and time scales of metal release from contaminated sediments under water chemistry conditions that simulate the post-remediation environment.

4.3 Experimental

4.3.1 Study area

The study system was the North Fork of Clear Creek (NFCC), which flows through the cities of Black Hawk and Central City in Gilpin County, Colorado, USA. The headwaters of the NFCC, located on the east side of the Continental Divide in central Colorado, are primarily fed by snowmelt, groundwater, and discharge from local abandoned metal mines and drainage tunnels. The NFCC is a high-gradient mountain stream that drains high alpine forests in the Rocky Mountains and flows south-east until it converges with the main stem of Clear Creek. Legacy AMD enters the stream in Black Hawk from 2 drainage tunnels: Gregory Incline (GI) and National Tunnel (NT). In the spring, stream discharge greatly increases due to snow melt; then it declines to its lowest discharge in the fall and winter. The AMD-impacted groundwater discharge

from the tunnels is much less seasonally-variable. This creates high seasonal variation in water composition and flow velocity [22].

Currently, the AMD-affected reach of NFCC (Figure 4.1) cannot sustain an aquatic vertebrate population and has a depauperate population of aquatic benthic macroinvertebrates [24,25]. Although the pH is circumneutral (~6.5-7.5), elevated metal concentrations in the water column, both dissolved and particulate, and impaired stream-bed habitat due to the deposition of metal oxyhydroxides likely lead to the stream's poor ecological health. Additionally, the stream water downstream of the AMD discharges has lower alkalinity and increased hardness compared to upstream waters, both of which are important parameters for metal toxicity. The GI and NT waters are scheduled to be diverted to a lime treatment plant in 2017 to remove the metals before discharging the water into the NFCC.



Figure 4.1: Map showing the reach of North Fork of Clear Creek from Black Hawk to the confluence with the main stem of Clear Creek [23].

4.3.2 Sampling sites

Upstream from the GI and NT inputs to the NFCC, the stream water has a healthy population and species diversity of aquatic vertebrates and invertebrates. Because upstream water has low concentrations of dissolved and particulate metals, I chose a reach in this region as the

simulated recovery site (Site 1). After the AMD point sources are treated, the stream water composition of the NFCC downstream of Black Hawk should closely resemble the conditions at Site 1, although hardness and SO_4 concentration of the downstream water should be higher, due to both the input of these ions from the AMD sources and the added lime. The water treatment plant, which will use slaked lime to increase the pH, will discharge the treated water into the NFCC at a site ~1 km south of Black Hawk.

The AMD-affected study site used for the deposition phase of the field experiment was designated as Site 2. This site allowed for accumulation of metal-rich coatings on tray substrates for redeployment at the upstream site. Site 2 is located ~1 km south of Black Hawk, and this reach of NFCC currently receives drainage from GI and NT. The concentrations of dissolved and particulate metals are high and the alkalinity is very low at Site 2, and most of the sediment surfaces in the stream are completely coated in AMD metal contamination. Slightly upstream from Site 2, closer to the AMD inputs, the ferrous iron concentration in the water is typically in the 1 – 3 mg/L range; but by the time the water flows down to Site 2, the ferrous iron concentration in the water is typically < 1 mg/L. I knew from past water chemistry sampling along this reach of stream, that in the summer when the temperature of the stream is near its annual maximum, the ferrous iron from the AMD oxidizes quickly, but as the temperature decreases, higher concentrations of ferrous iron will flow downstream before oxidation and subsequent precipitation.

4.3.3 Rock trays

I used trays containing rock substrate that were originally developed by Clements et al. [21,26,27] for *in-situ* examination of aquatic benthic macroinvertebrate communities in various stream conditions. I modified the methods and materials to focus on geochemical analysis of the

sediments as opposed to examining biological community responses. The modified methodology is described in Chapter 2 of this thesis. Briefly, 10 cm x 10 cm x 6 cm polypropylene food-storage containers (Stor-Keeper® Arrow Plastic Manufacturing Company, Elk Grove, Illinois, USA) were filled with about 1 kg of a mixture of clean cobble (6-10 cm diameter) and coarse gravel (2-6 cm diameter) to represent a range of bed sediment substrate. The cobble and gravel was obtained from a local rock quarry and was representative of the local geology.

4.3.4 Experimental field procedures

The rock-trays were placed inside 3 large polypropylene holding bins (70 x 42 x 18 cm) and then deployed at Site 2 following the methods reported by Williamson et al. (in review), to allow for AMD metal coatings and particles to accumulate in/on the tray substrate. This deployment method is described in Williamson et al (in prep). The trays were then removed from Site 2 and redeployed upstream with clean control trays at Site 1 to simulate post-remediation conditions.

Fifty-four rock trays were deployed at Site 2 on July 31, 2015. The trays were placed inside the holding bins that were anchored adjacent to the stream bank and had stream water delivered by gravity as shown in Figure 4.2. This approach overcame the problem of washout of the sampler during short-term high-discharge events that occur during summer due to intense thunderstorms. A large (size) PVC drainage tube was used to route flowing stream water into the bins. This was accomplished by anchoring the tube opening at the top of a small waterfall in which the stream was flowing over several large boulders. The outlet of the tube was secured to one end of the first large holding bin, which was anchored below the waterfall. The 3 bins were positioned such that the stream water coming out of the tube flowed through the first bin, and then flowed through the other 2 holding bins in a cascade. Although deposition of flocculent

sediments was observed, the turbidity of the water exiting the sampler appeared similar to that entering the bins. Thus, we assumed that all three bins were exposed to similar amounts of suspended sediments. The Site 2 deployment (deposition phase) lasted for 4 weeks. While it is expected that the flow velocities in the bin were not equivalent to that in the stream, this approach was only used to provide contaminated materials for the sediment-recovery experiment, which was conducted directly in the stream at upstream reference Site 1.



Figure 4.2: Pictures of the side-of-the-stream deployment method at Site 2. (a) The field-mesocosm bin holding 18 rock trays, with stream rocks placed in between the trays for additional weight; (b&c) the stream-side deployment showing the placement of the irrigation tube and holding bins.

On August 28, 2015, five of the rock trays at Site 2 were collected for analysis to represent a maximally contaminated set of sample trays (4 weeks of AMD exposure). Before removal from the holding bins, lids were attached to the trays to contain all of the deposited material during collection and transport to the laboratory for later extraction and analysis. This appeared to

minimize loss of loose, flocculent material. Although flow through the three bins appeared relatively uniform, trays were randomly removed from each bin to avoid potential bias in the deposition conditions.

All of the remaining rock trays were removed from the holding bins, covered with lids, and transported to Site 1. There the trays were redeployed in holding bins, which were anchored to the stream bed and not on the side of the stream. The high discharge events that could disrupt the experiment were less likely at this site and time of year. Perhaps more importantly, I also wanted to expose the rock trays to the actual stream flow velocity, so as to investigate actual conditions that might contribute to metal loss. The holding bins containing the trays were lowered into the stream and anchored before removing the lids, which was done while they were under water. This approach appeared to minimize loss of loose material in the trays during redeployment. To monitor for sediment loss over time (recovery phase), 5 trays were collected out of the holding bins for analysis on each of the following dates in 2015: August 30, September 9, September 11, September 25, October 9, and October 23. This allowed for analysis of trays that had undergone simulated recovery for 2 days and 1, 2, 4, 6, and 8 weeks.

4.3.5 Extraction procedure

At the time of collection, each rock tray was placed inside a new 2-gallon re-sealable polyethylene bag (Ziploc®, S. C. Johnson & Son, Inc., Racine, WI. USA). The samples were then stored in a laboratory freezer ($\approx -40^{\circ}\text{C}$) until they were removed to perform the extraction and subsequent metals analysis. The sample trays were allowed to completely thaw overnight at room temperature before analysis.

To examine the AMD-sourced metals deposited into the rock trays, I performed a partial extraction procedure that was aimed at primarily extracting coating-associated metals as described

in Chapter 2. The extraction method was USEPA Method 3050B [28] that we modified because the original method was meant for a 1-2 g of fine-grained sediment sample, but the typical substrate samples in the rock trays were ~1 kg of coarse material. The purpose of this procedure was to extract the “environmentally available metals” from the sample. The extractions were conducted in hard-walled 2-L polypropylene containers (Kartell® Polypropylene Beakers, Kartell S.P.A., Noviglio, Milan, Italy), and used concentrated nitric acid (Macron trace-metal grade), hydrogen peroxide (Macron, 30% reagent grade) and a hot water bath (90°F).

The resulting solutions were sampled (40 mL) using a disposable polyethylene pipette and transferred to conical Falcon tubes (50-mL polypropylene), diluted with Milli-Q water (> 18 Mohm) and analyzed using inductively coupled plasma optical emission spectroscopy (ICP-OES) (Perkin Elmer Optima 5300).

Filtration of the highly acidic extraction solution was problematic because metals, primarily Zn, were leached out of the filters, resulting in biased metal concentrations. Thus, overnight settling and decanting of the extraction solution was used to remove the majority of particles before dilution for analysis by ICP-OES. The lack of observable turbidity and the high concentrations of metals dissolved by the acid limit the potential for bias being introduced by the lack of filtration. The metal concentrations were multiplied by the measured mass of the extraction solution to calculate the mass of metals extracted from the sediment substrate in the trays. These values were then divided by the total measured mass of the sediment sample to give a metal mass concentration in units of mg metal/kg wet sediment.

Normalizing to rock surface area is experimentally problematic due to the non-uniformity in the shapes of the rocks and gravel, and may not be highly relevant because not only are rock coatings generated but loose sediment is accumulated in the pore spaces and on top of the rock

substrate. This makes rock surface area less relevant than if relatively thin surface coatings were the only important components. Alternatively, we could normalize the metals content to the representative area or volume of the stream bed. While this might be more relevant than substrate mass, since these parameters were held constant, the relative temporal trends in the data would remain the same as the mass-based normalization.

4.3.6 Chemical analysis

All solution analysis by ICP-OES used a continuously introduced Sc internal-calibration standard for each sample. Continuing calibration verification (CCV) standards and deionized water blanks (Barnstead Nanopure system, Thermo Fisher Scientific) were analyzed after every 10 samples, post-calibration, for quality assurance and quality control (QA/QC). Samples were reanalyzed if acceptable QA/QC results were not obtained, and this may include $\geq 20\%$ deviations in the measurement of the internal Sc standard, or $\geq 10\%$ deviations in elemental concentrations in the CCV samples from the known concentrations. The instrument detection limits for the metals and hardness ions (Ca and Mg) were (in $\mu\text{g/L}$): 1.1 for Ca, 0.3 for Cu, 0.4 for Fe, 0.7 for Mg, 1.3 for Mn, and 0.2 for Zn.

4.3.7 Data analysis

Statistical analysis of the mass normalized extraction data for the metals of interest (Cu, Fe, Mn, and Zn) began by testing for significant differences between the various samples. All of these tests were 1-way analysis of variance (ANOVA) followed by a Tukey HSD Test ($\alpha = 0.1$). This alpha value was chosen to be consistent with previously published research on this methodology (Williamson et al). I specifically compared the initial controls (never deployed to the field) to the AMD-contaminated samples that were not redeployed upstream, in order to determine the extent to which metal deposition had occurred. I tested for significant differences

among the control groups (initial, 2 week, and 8 week) to determine if any significant metal deposition was occurring at the upstream locations during the recovery phase. I also tested for significant differences between the AMD-contaminated samples (not redeployed at Site 1) and the 8-week recovery samples. After transforming the data, I tested linear regressions for significance at 90% confidence ($\alpha = 0.1$) of corrected data (subtracted metal contributions) and transformed data (% metal from coating to total metal).

4.4 Results and discussion

The extraction results are presented for my metals of interest (Cu, Fe, Mn, and Zn). Iron, and Mn are the primary hydrous-oxide-forming elements in this system, and Cu and Zn are the likely drivers of toxicity to aquatic organisms in the NFCC [29,30]. The pH of the incoming AMD from GI or NT is typically between 5.5 and 6.0, which indicates that some neutralization (dissolution of carbonate or silicate minerals) has occurred before entry into the NFCC. Non-neutralized AMD has a pH typically in the range of 2 – 3. Although the AMD pH is not as low as might be expected, it contributes a large load of metals including ferrous iron, which subsequently deposits on the stream bed as metal-containing iron-oxyhydroxides.

4.4.1 Mass-normalized sediment metal concentration data

The metal concentrations measured in the rock-tray extract, normalized to total mass of wet substrate at the time of collection, are shown in Figure 4.3.

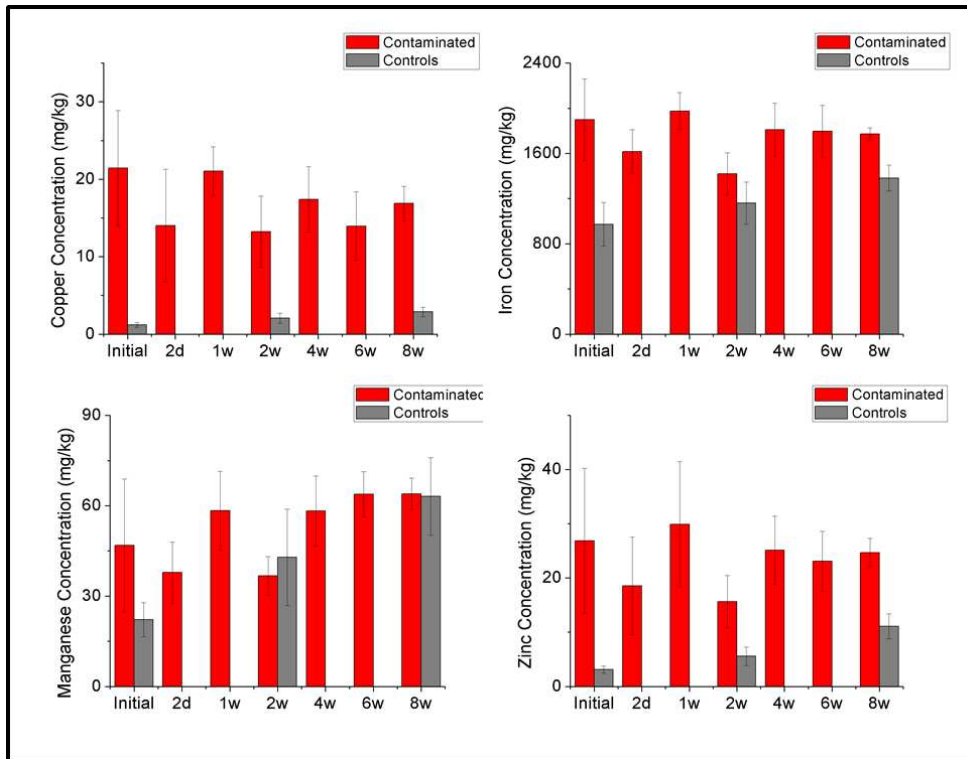


Figure 4.3: Concentrations (mg/kg wet sediment) of Cu, Fe, Mn, and Zn extracted from sediments in rock trays deployed in North Fork of Clear Creek in 2015 (mean of 5 replicates; error bars are 90% confidence intervals). Contaminated = rock trays deployed at Site 1 with previously accumulated AMD metal contamination from 1 month of deployment at Site 2; Controls = clean rock trays deployed at Site 1 that had not been exposed to AMD. Initial = recovery or control trays at the start of the recovery field experiment with no Site 1 exposure.

The initial 4 weeks of Site 2 (AMD contamination) exposure resulted in a significant increase in average metal concentrations above the average control metal concentrations. The initial contaminated samples had average Cu, Fe, Mn, and Zn concentrations greater than the initial control samples by 20 mg/kg, 930 mg/kg, 25 mg/kg, and 24 mg/kg, respectively. The contaminated trays with 8 weeks of recovery at Site 1 had less (but not significantly) average Cu, Fe, and Zn concentrations compared to the initial contaminated samples. The 8-week contaminated samples had average Cu, Fe, and Zn concentrations lower than the initial contaminated samples by 4.6 mg/kg, 130 mg/kg, and 2.2 mg/kg, respectively. In contrast, the average Mn concentration in the 8-week contaminated samples was 17 mg/kg greater than the

initial contaminated samples, but this Mn concentration increase was not statistically significant. The Cu, Fe, Mn, and Zn concentrations in the 8 week controls were significantly greater than the metal concentrations in the initial controls. The average concentrations of Cu, Fe, Mn, and Zn in the 8-week controls increased relative to the initial controls by 1.7 mg/kg, 410 mg/kg, 41 mg/kg, and 8.0 mg/kg, respectively.

4.4.2 Three-component model

It is reasonable to expect that exposing the metal-oxyhydroxide-coated substrate to a clean background site would result in loss of these metal coatings. However, no statistically-significant decreases in average extractable metal concentrations occurred after 8 weeks of recovery at Site 1 (Figure 4.3). I focused on two features in the metal extraction data to attempt to aid in data interpretation. First, the 8-week recovery control metal concentrations were significantly greater than the initial controls; and second, the extraction data from each time point had no trends of metal removal with a high amount of variability, particularly noted by the low metal concentrations at 2 days and 2 weeks (Figure 4.3). The first point had not been observed in previous field experiments that consisted of deployments of contaminated trays at Site 1 (Chapter 2).

Regarding the first point, metal accumulation during recovery at Site 1 may have also occurred on the contaminated samples, masking the metal loss due to recovery. Significant abiotic metal deposition or precipitation onto stream-bed sediments at Site 1 is unlikely, given the low concentrations of dissolved and particulate metals present in the water column. Although this phenomenon had not been observed in previous contaminated-sediment deployments at Site 1 (Chapter 2), the maximum deployment period in previous deployments at Site 1 was 4 weeks compared to the 8 weeks in the current study. I initially assumed that an 8-week recovery period

would result in increased observable metal loss from the trays, but at first analysis this appears to not be the case.

A possible hypothesis that could explain the results is that the 8 weeks of deployment at Site 1, compared to previous 4-week deployments, allowed additional time for biofilm growth, which subsequently accumulated metals on the rock-tray substrate surfaces. Aquatic microbial biofilms are very commonly found on stream-bed sediment surfaces and are primarily composed of a layer gel-like extracellular polymeric substances (EPS), which contains and is formed by colonies of microorganisms [31]. Biofilms can sorb high concentrations of particulate and dissolved metals from the water column due to high concentrations of anionic ligands (carboxyl, phosphoryl, and sulfate groups) in the EPS and their ability to alter the immediately surrounding aqueous geochemistry (e.g., elevated pH and hardness) [32,33,34,35]. Visually, the sediments in the control and recovery rock trays appeared to have accumulated organic material and gel-like surface coatings after 8 weeks of deployment at Site 1. I hypothesized that the accumulation of metals by biofilm on the contaminated substrate occurs concurrently with the metal-removal processes. This would explain the lack of significant decreases in extractable metal concentrations during the 8-week recovery period.

Regarding to the second point, variability in the extracted-metal concentrations among the five replicates was relatively consistent (similar 90% confidence intervals) for both contaminated and control samples across all time points. To reduce the variability I examined several different aspects of the experimental procedure. Collection of samples from the field mesocosms was randomized and the laboratory extraction procedure and subsequent analysis were performed as consistently as possible. The calibration and QA/QC checks for all of the ICP-OES analyses of the extraction results showed no irregularities or variability out of the accepted range. The rock

and cobble substrate contained in each tray was from the same source, and had similar mass and size variability.

The surface-extraction procedure dissolves the deposited metal coatings, as well as some minerals from the underlying substrate and any sediment biofilm, as shown in Equation 4.1 and Figure 4.4. Biofilm formation and dissolution of substrate both increase the total metal concentration in the final extracted solution, and can make it difficult to determine significant trends in sediment metals specifically resulting from AMD coatings. I could not experimentally differentiate between the metal concentrations that were extracted from the coatings, substrate or biofilm, because that would require a specialized sequential-extraction procedure (not the general surface extraction used here); and before analysis of our extraction data, I did not foresee the need for a more-specialized extraction method or for possible chlorophyll analysis to examine the microbial biofilm growth. Thus, I developed a three-component model to describe the total extractable metal from the contaminated sediment substrate.

$$[M]_{total} = [M]_{substrate} + [M]_{coating} + [M]_{biofilm} \quad (4.1)$$

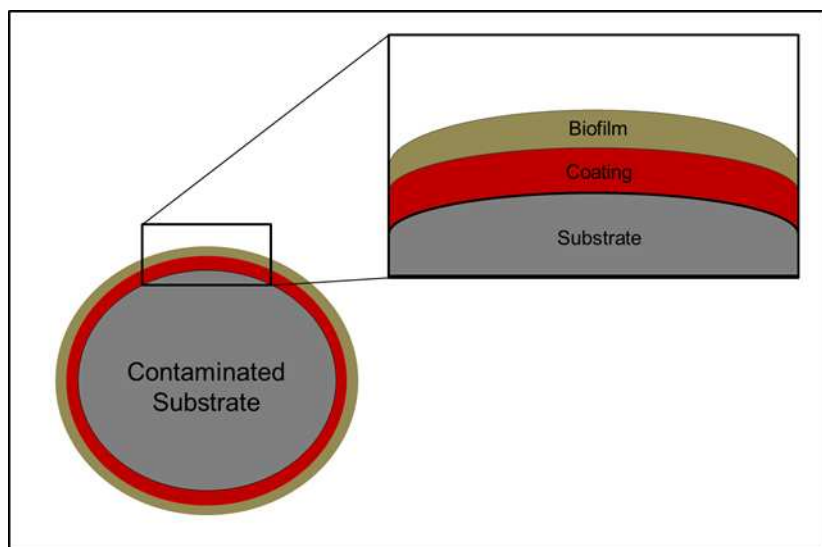


Figure 4.4: Model of the three components of extractable metals (substrate, AMD metal coatings, and Site 1 accumulated biofilms).

The contribution to the total extracted metals from the substrate was assumed to be constant for all the extractions. The metal concentration measured from the extraction of the initial controls (C-0w) corresponds to the underlying sediment substrate as shown in Equation 4.2 and Figure 4.5. These rocks and cobbles had never been exposed to field conditions and thus had no deposited metal coatings or accumulated biofilms, and any metals present in the extraction solution would have originated from dissolved substrate minerals only.

$$[M]_{\text{substrate}} = [M]_{\text{C-0w}} \quad (4.2)$$

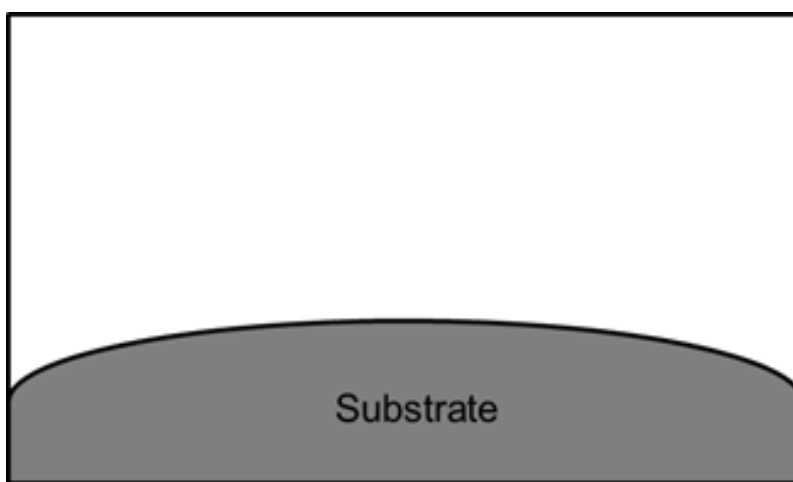


Figure 4.5: Illustration of the sediment surfaces in the initial control rock trays which are devoid of deposited AMD-metal coatings and any accumulated biofilms.

I assumed that metal accumulation at Site 1 was primarily due to biofilm growth, but contributions to this accumulation may have also resulted from additional fine bed-sediments from Site 1 collecting in the rock trays. I will use “biofilm” as a general operational term for the cumulative processes of metal accumulation at Site 1. I made an assumption that the biofilms accumulate on sediments at Site 1 at the same rate regardless of surface contamination, meaning that the biofilms accumulated on the control trays at the same rate as the contaminated trays. To determine the metal concentration contributed from biofilm accumulated at Site 1, I calculated the difference between each average control concentration at two time points (C-2w and C-8w) and

the average initial control concentration ($C-0w$) as shown in Equation 4.3 and Figure 4.6. Each average was computed from five replicates. This difference should represent metal accumulated at Site 1, because the control rock trays had never been exposed to Site 2 AMD contamination and thus had no metal coatings on the sediment surfaces in the trays.

$$[M]_{\text{biofilm}} = [M]_{(\text{Control})(t)} - [M]_{C-0w} \quad (4.3)$$

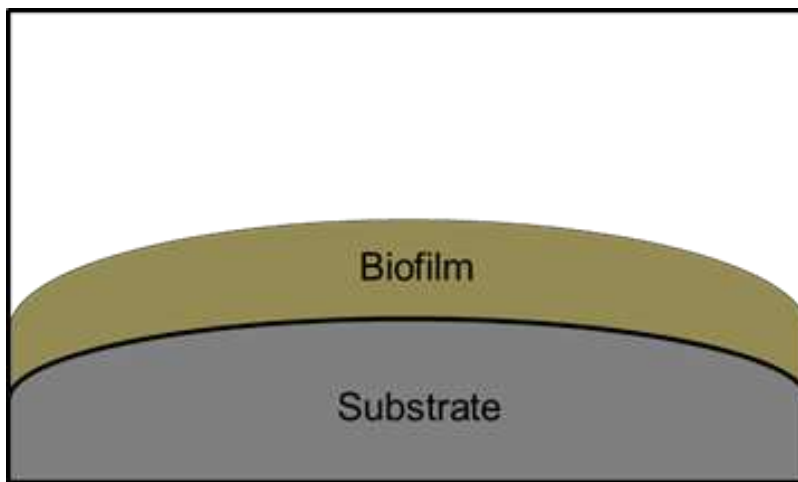


Figure 4.6: Illustration of the sediment surfaces in the control rock trays deployed at Site 1, which are devoid of deposited AMD-metal coatings.

I then calculated a linear regression (y-intercept forced to be 0, $n = 3$) through the calculated biofilm metal concentrations in the controls at the initial (0 day), 14-day, and 56-day sampling times. The equation of the line was used to interpolate biofilm metal concentrations for each sampling time, as shown in Figure 4.7. The calculated slopes (forced y-intercept of 0) of biofilm metal accumulation for Cu, Fe, Mn, and Zn are 0.032, 7.7, 0.77, and 0.14 mg metal/kg wet-substrate, respectively.

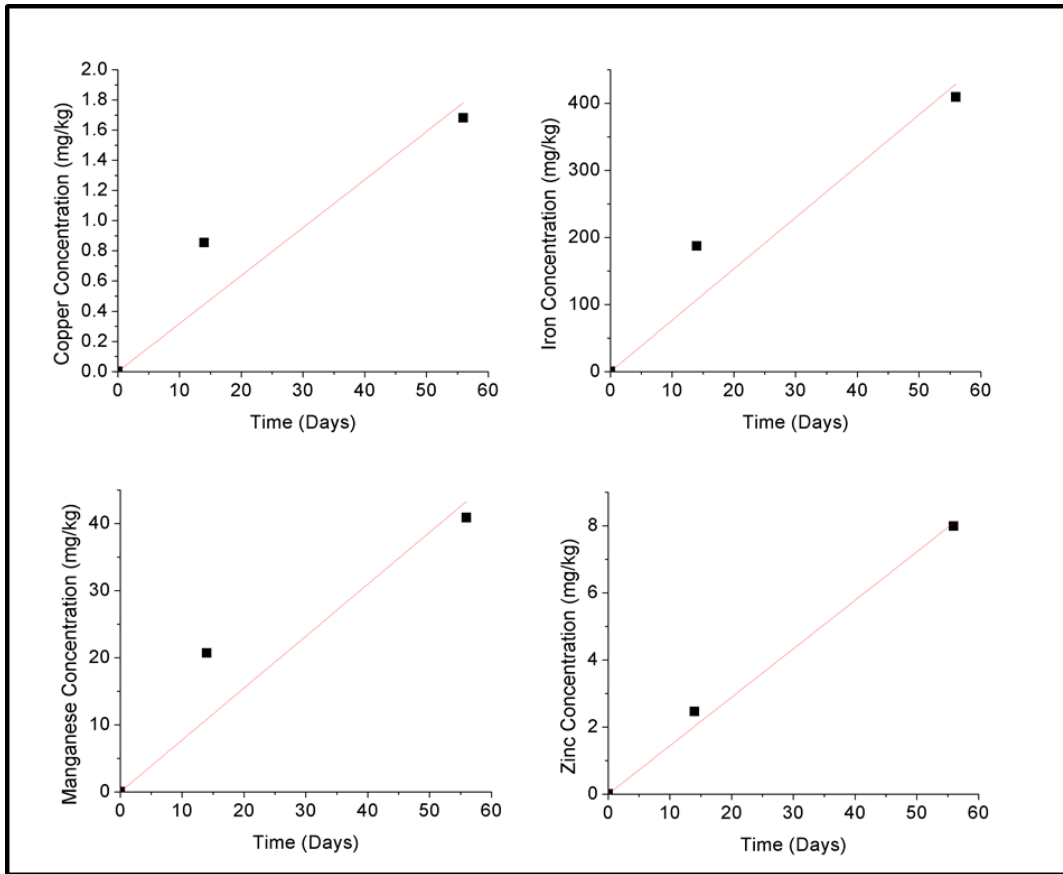


Figure 4.7: Average control-tray metal concentrations (Cu, Fe, Mn, Zn) calculated as resulting from biofilm, with a linear regression (forced y-intercept of 0).

I assumed that the extracted metal concentration resulting from dissolved substrate minerals (Equation 4.2) was constant, and subtracted that concentration from each of the contaminated samples. Next, the calculated extracted metal contribution from sediment biofilm for each time point was subtracted from each respective sample. According to my 3-component model, the remaining metal concentration is from the deposited metal coatings because the contributions from both the substrate and biofilm have been subtracted. These results are shown in Figure 4.8. The calculated slopes from the linear regressions ($n = 7$ each) for Cu, Fe, Mn, and Zn were -0.080, -7.4, -0.37, and -0.13 mg metal/kg wet-substrate/day, respectively. The calculated slopes from linear regressions for Cu, Fe, and Zn were not significantly different from zero ($p = 0.29, 0.11, 0.25$ for Cu, Fe, and Zn, respectively). The calculated slope from the linear

regression ($n = 7$) for Mn was significantly different from zero ($p = 0.07$). The R-squared values for the Cu, Fe, Mn, and Zn regressions were 0.22, 0.42, 0.50, and 0.25, respectively. Thus, although there appears to be a trend of decreasing metal concentration as recovery time increased, there were still no statistically-significant regression slopes for Cu, Fe, and Zn.

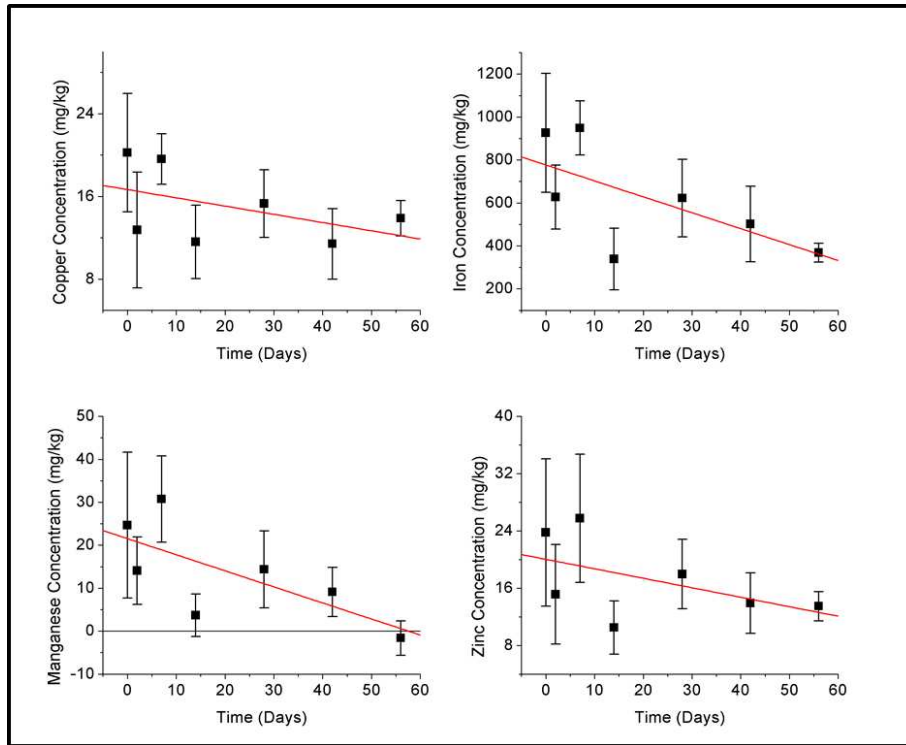


Figure 4.8: Metal concentrations (Cu, Fe, Mn, Zn) calculated as resulting from the deposited AMD coatings; linear regressions shown as red lines.

To decrease the variability in the extraction results and to possibly reveal recovery trends, we normalized the data by dividing the calculated metal concentration from the coating by the total extracted metal concentration as shown in Figure 4.9. This data-processing technique added an additional normalization to the surface area of the sample (the data is already normalized to sample wet mass), because we assumed that trays with a higher surface area to mass ratio would proportionally accumulate more metal deposition, and when only normalizing to sample mass this variability in the data will remain.

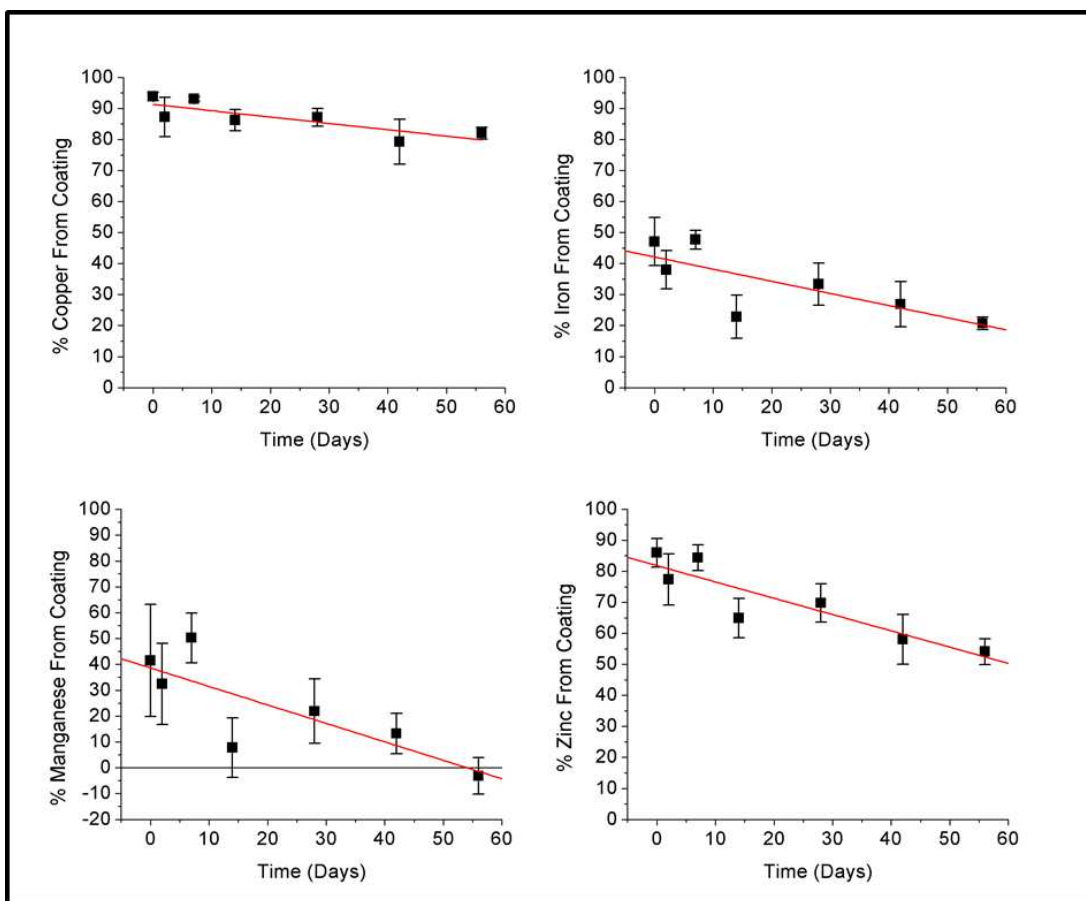


Figure 4.9: Metal concentrations (Cu, Fe, Mn, Zn) calculated as resulting from the deposited AMD coatings, normalized to total extracted metal; linear regressions shown as straight lines.

The percentage data transformation resulted in significant decreasing metal coating concentrations over the experimental time period, for all metals of interest. The calculated slopes of the linear regressions ($n = 7$) for Cu, Fe, Mn, and Zn were -0.20, -0.39, -0.72, and -0.53 % metal from coating/day, respectively. The calculated slopes from the linear regressions for all the metals of interest were significantly different from zero ($p = 0.02, 0.04, 0.03,$ and 0.01 for Cu, Fe, Mn, and Zn, respectively), and the R-squared values for Cu, Fe, Mn, and Zn are 0.68, 0.58, 0.65, and 0.82, respectively.

I also attempted to normalize the extraction data to an element that hypothetically would only result from dissolved substrate. The goal was to account for surface area differences among

the five replicate trays from each time point, which might thus decrease the variabilities shown in Figures 4.8 and 4.9. If a tracer for substrate dissolution was found, I could then normalize to an approximation of the substrate surface area contained in each sample. This tracer would theoretically be released from the substrate during the extraction procedure, and the extracted concentration of this tracer would be proportional to dissolved substrate surface. However, none of the many extracted elements examined for this purpose was suitable. A suitable normalization element would be found in similar mass concentrations (mg element/kg wet-sediment) in all extracted control samples, because a consistent concentration would imply that the extracted concentration is not dependent on deposited AMD contamination or sediment biofilm. The average concentrations of the tested elements in each of our sample sets are shown in Table 4.1. Many of the tested elements had concentration increases over time in both the control and contaminated samples. I thus concluded that no suitable tracer was available due to the temporal trends. Another untested element found almost exclusively in the substrate minerals and not in the coatings or biofilm may be suitable for surface-area normalization, but I only tested for elements that were in concentrations above the analytical detection limit of the ICP-OES. Statistical analysis was also performed on the mass concentration data for candidate normalization elements, and the sample sets differed significantly, resulting in unsuitability for my purposes. These statistical results are not shown here, but the average concentration of my possible tracer elements for each sample set is shown in Table 4.1 for illustrative purposes and general comparisons.

Table 4.1: Average concentrations of candidate tracer elements at each experimental time point. “C-0” = uncontaminated rock trays that were never deployed in the field. “C-2w” and “C-8w” = the control rock trays deployed at Site 1 for 2 weeks and 8 weeks, respectively. “0d”, “2d”, “1w”, “2w”, “4w”, “6w”, and 8w” = contaminated rock trays deployed at Site 1 for 0 days, 2 days, 1 week, 2 weeks, 4 weeks, 6 weeks, and 8 weeks, respectively.

	Ca (mg/kg)	Co (mg/kg)	Cr (mg/kg)	K (mg/kg)	Li (mg/kg)	Mg (mg/kg)	Na (mg/kg)	P (mg/kg)	Si (mg/kg)	Sr (mg/kg)	Ti (mg/kg)	V (mg/kg)
C - 0	190	0.508	0.64	96.1	0.855	212	6.25	66.9	5.73	1.23	17.3	0.797
C - 2w	256	0.676	1.24	115	0.677	238	11.0	71.4	11.5	1.83	24.3	1.15
C - 8w	353	0.912	1.75	209	0.989	325	9.44	72.7	14.5	1.90	41.7	1.65
0d	245	0.760	1.41	211	1.03	337	9.78	79.5	6.54	1.58	37.5	1.13
2d	477	0.676	1.12	162	1.03	298	13.9	120	20.2	2.21	29.1	1.04
1w	630	0.937	1.77	256	1.34	483	11.4	93.5	28.8	2.66	40.3	1.43
2w	184	0.587	1.16	136	0.673	229	7.20	50.7	24.0	1.33	25.0	0.931
4w	273	0.919	2.09	257	1.01	380	11.7	72.5	25.3	2.17	49.8	1.56
6w	336	0.993	2.25	289	1.25	395	10.6	78.3	21.3	2.42	53.0	1.76
8w	339	0.928	1.94	243	1.12	360	13.3	86.3	24.0	2.64	45.4	1.53

4.5 Synthesis

The goal of this research was to use my previously developed “field-mesocosm” methodology (Chapter 2) to advance the understanding of the processes of metal removal from aquatic sediments *in situ*. Aquatic metal deposition and release from sediments is easier to control and manipulate *ex situ*, but the results are less representative of the dynamic and non-reproducible aquatic geochemical processes occurring in a natural water system. However, environmental data can have a high amount of variability because of the lack of laboratory control of the experimental conditions. I showed that the *in-situ* sampler methodology is rigorous enough to observe significant recovery trends after processing the raw data to account for competing processes. Metal deposition and release are a combination of several geochemical processes. Use of appropriate controls and data processing can aid in isolating a specific process for statistical analysis. Data processing may be required when environmental data is being affected by several processes occurring concurrently. I expanded upon my past usage of the sediment samplers, by incorporating more robust sampling procedures, increased temporal sampling, more replicates, and some data processing techniques.

This work supports the general suggestion that processes of metal deposition are primarily chemical (precipitation and sorption), while the processes of metal removal from sediment are likely to be primarily physical. Both dissolution and desorption would not be favored by the average pH at Site 1 (~7.5). These processes would be favored, however, by the fact that metal concentrations are lower at Site 1 than Site 2. The stream would need a lower-pH environment to increase metal solubility or would need to contain dissolved metal-binding ligands in the water column for chemical removal to occur, and these types of chemical changes are not likely unless the system receives a significant discharge contribution from a differing water source. I expected equilibrium-driven dissolution to be a minor contributor to metal removal from sediment because of the low solubility of the precipitated phases in the circumneutral pH at Site 1.

It is important to note that the data gathered during this study are specific to the stream conditions in the NFCC during the course of the field experiment. The stream's discharge was relatively constant during the field experiment but slowly decreased over time from an initial ~0.2 m³/s to ~0.1 m³/s during the final sample collection. This range of discharge is representative of the annual average minimum discharge (~0.07 m³/s). The scouring of metal coatings from the trays during this period is representative of base-flow conditions and is much less than during high runoff in the spring. Also, because our field experiment was conducted in the late summer/fall, the stream water was relatively warm, which could affect the kinetics of chemical metal-removal processes.

The extraction results for this study were normalized to the final substrate mass contained in the rock trays. Normalization to surface area would be difficult to accurately measure considering our chosen tray substrate material (pebbles and cobbles from a local quarry). I

believed that filling the trays with standard sized spheres (or some other consistent geometry) would result in data that is less representative of actual stream processes, because the standardized substrate would have different surface conditions (active sites and surface area) compared to the stream-bed substrate, and this may affect the processes of metal release from sediment. Also surface area is not the only controlling factor as flocculent material is deposited in the pore spaces between the cobble and gravel. I assumed that each of our rock trays represents a 100 cm² area and 600 cm³ volume of stream bed. Since this value is constant for all trays, I normalized the extraction data to the mass contained in the trays as a general indicator of the amount surface area and volume) of streambed substrate. In this case, I chose to prioritize realism over absolute consistency between the composition of the tray substrate.

My initial assumption was that loss of deposited metal from the AMD-contaminated rock trays would be observed after 8 weeks of deployment at Site 1, but surprisingly this was not the case. It appeared that this was in part due to a high amount of variability in the sediment-surface extraction data. Additionally, the relatively consistent average metal concentrations for all of the contaminated samples, regardless of the duration of recovery at Site 1, argued against this initial assumption of expected recovery. Statistical differences in extracted metal concentrations were not observed between the initial contaminated samples and the contaminated samples that had been deployed at Site 1 for 8 weeks. The initial mass of the substrate in the trays was ~1 kg, but additional material could have accumulated during the depositional phase in the form of metal flocculent and coatings, or during the recovery phase in the form of fine sediments (sand and silt), plant debris and microbial biofilm. The raw extraction data is normalized to final substrate sample mass. This may have contributed to the observed variability in the extraction results

because any additional accumulated material at Site 1 might not have contained metal concentrations representative of the deposited metal coatings.

Visually, the contaminated trays appeared to lose the red coatings associated with deposited Fe-oxides over the 8 weeks of simulated recovery, but this was not consistent with the statistical results of the metal extraction data. Increasing the number of replicate trays may help to reduce the observed extracted metal concentration variability, but in this study the use of 5 replicate trays for each sample set did not allow for significant observable changes in extracted metal concentrations in the deployed trays during the recovery period.

The average extracted metal concentrations from the 8-week control trays were statistically significantly greater than in the initial controls. Significant metal deposition from AMD contamination in the water at Site 1 is unlikely because the measured dissolved and particulate concentrations of metals in the water column are low. Therefore, precipitation is unlikely but there may be a minor contribution from metal adsorption to the sediment surfaces. The pristine nature of the stream water at Site 1 is supportive of the growth of microbial biofilms on substrate surfaces, and visually the contaminated and control trays collected after 8 weeks of recovery at Site 1 had accumulated slippery brown coatings on substrate surfaces in the trays. Aquatic sediment microbial biofilms have been observed to significantly accumulate aquatic metals from the water column [34]. This accumulation is controlled by: the formation of biogenic manganese-oxide particles by the biofilms [36,37,38], the geochemical conditions of the stream water (e.g., toxic aquatic concentrations of inorganics and organics, temperature, sunlight) [39,40], and the physiological responses of the biofilms to these stream conditions [41,42]. I hypothesized that biofilm metal accumulation may have been masking the AMD-metal sediment losses at Site 1. The three-component extraction model (dissolved substrate metal-containing

minerals, accumulated AMD-metal coatings, and metals from biofilms accumulated at Site 1) was necessary to interpret the observed extraction results because of the lack of significant total metal removal from the contaminated trays, which was not visually confirmed or consistent with past recovery results (Chapter 2). I found that subtracting the calculated metal contributions attributable to dissolution of substrate and Site-1 biofilm accumulation from the total extracted-metal concentrations in the contaminated samples, allowed for an observation of recovery of AMD coatings. To decrease the variability in the AMD-coating data, I normalized the calculated metal concentration resulting from the coatings to the total extracted metal concentration (% recovery), which resulted in observable recovery trends in the deposited coatings.

The sediment field mesocosm method can be applied to contaminated systems other than the NFCC, because the three-component model allows for normalization to the specific tray substrate used (which affects the total extracted metals) and the specific microbial biofilm growth (metal sediment sorption) conditions of the study system. The geological characteristics of stream-bed sediments are highly variable throughout the world, but the tray methodology utilizes data normalization to dissolved metal-containing minerals from the specific substrate used for the experiment. Biofilm growth, health, and sorption properties are not constant and react to the in-stream geochemical conditions, which causes the rate of biofilm accumulation and subsequent metal sorption difficult to accurately predict in a natural aquatic system. If this prediction were possible, I could account for biofilm metal accumulation in our data; but instead, my approach uses *in-situ* empirically gathered biofilm metal accumulation on the control samples to determine the correct normalization. Even if the rate and extent of biofilm metal adsorption varies during the field experiment, as long as sufficient control samples are collected, the metal extraction data can be normalized to the observed biofilm metals for each time point in the recovery deployment.

These data-normalization methods allow for increased flexibility and applicability of the rock tray method to different contaminated aquatic systems.

To improve on this work, more replicates for each sample set may aid in reducing variability in the data set. Because many variables and geochemical processes occur concurrently in a natural aquatic system, additional replicates may be needed for statistical significance of changes in metal concentration in the trays. After I performed the field experiment and the subsequent data analysis described above, I then developed the three-component (substrate, AMD coating, and biofilms) calculation approach, which is dependent on the metal-extraction data from the controls at each given time point. In this study I collected initial controls, 2-week controls and 8-week controls. Deployment of additional controls for sampling at each of the recovery time points would allow for increased resolution of the calculated metal accumulation resulting from biofilm. Chlorophyll analysis as a tracer of biofilm accumulation would also be a useful addition to this method, but I did not anticipate the significance of biofilm metal accumulation because all of my previous recovery deployments (Chapter 2) were for a total of 4 weeks or less, which may have not have been sufficient time for biofilms to accumulate to a significant amount. A sequential extraction method for extraction of the possible AMD-metal-oxyhydroxide precipitates and adsorbed AMD-metal-oxide coatings on the sediment with minimal dissolution of the substrate minerals would also be useful in quantifying metal concentrations from a specific sediment component. Longer deployment at Site 2 may also aid in the data processing by increasing the deposited metal concentrations, thus increasing the resolution between the AMD-contamination metals and the biofilm metals in the extraction data. I attempted to begin the deployment at Site 2 as soon as possible after the end of the summer thunderstorm season to allow for a maximum possible total deployment period for this field experiment, before the stream

begins to freeze in the winter. Within the possible field deployment time window, I decided to prioritize the length of the recovery period compared to the AMD-deposition period, hoping that this would result in significant metal loss in the contaminated trays. Weather conditions at any study site can limit the applicability of this method to only a few times of the year, due to high discharge with the possibility of wash out of the trays.

4.6 Conclusions

AMD-metal coatings on stream sediment may require an extended period of time to be removed even if overlying stream water has been remediated. Physical and chemical processes in an aquatic system will remove metals from the sediment over time, but the extraction results did not show a statistically-significant decrease in total metal concentrations over the 8-week simulated recovery period. I found that biological processes resulting from biofilm can increase the concentration of metals on sediment surfaces, and this led to the development of the three-component model. Using the three-component model, I was able to support my initial hypothesis of sediment recovery at Site 1. Significant metal loss from primarily physical removal, and accumulation of metal-containing sediment biofilm, was observed using our rock-tray method and data-processing techniques. The fate and behavior of AMD-sourced metals after leaving the water column is not well understood, and robust methodologies are needed to gather information on the geochemistry of sediment recovery from metal contamination. This information will aid in facilitating effective AMD-remediation practices and in the prediction of geochemical and ecosystem recovery in AMD-affected systems.

4.7 Acknowledgements

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CHAPTER 5

CONCLUSION

5.1 Summary of results and implications

AMD can result from metal mining activities if the rock waste and tailings are not managed responsibly, which includes capping, antimicrobial agents, and proper containment. Exposure of pyritic minerals to oxygen (increased eH) and water may generate acid and sulfate through oxidation of sulfide. Pyrite oxidation releases ferrous iron and the generated acid can dissolve surrounding minerals in the waste pile leading to high concentrations of dissolved metals present in the mine drainage. This is a serious environmental concern due to the effect of AMD on natural aquatic systems which includes decreased pH and alkalinity and increased dissolved and particulate metal concentrations. Ecological consequences resulting from AMD entering an aquatic system can include loss of aquatic vertebrate and invertebrate populations, as well as a decrease in species diversity.

Metals that have been introduced into an aquatic system from a source of AMD will typically leave the water column over time and be deposited onto the stream sediment bed. This is the result of precipitation and sorption processes. Natural streams will typically have a higher pH compared to the incoming AMD, and this at least partial neutralization will lead to conditions that are not favorable to dissolved metals. Insoluble metal oxyhydroxide complexes may be formed as a result of hydrolysis at an increased pH. Depending on flow, these metal particles will eventually settle onto the stream bed. Dissolved metals may also be removed from the water column due to sorption to particles in the water column or sorption to a sediment surface.

After metals have left the water column, they are no longer an aquatic toxicity concern but can still affect the ecosystem through dietary toxicity and through the destruction of benthic macroinvertebrate habitat. This will affect the viability of organisms in higher trophic levels as well. Sediment contamination may require a long period of time for enough of the deposited metals to be removed so that the ecosystem can recover. Examination of the time scale of these processes of metal removal from sediment allow for an understanding and prediction of stream recovery that will primarily depend on metal removal from sediment. The focus of my research presented in this thesis was to develop methodologies to examine metal fate and transport post-entry of AMD into an aquatic system, which depends on the flux of metals between the water column and stream-bed sediments.

The rock tray method as developed by Clements et al., presented an opportunity to modify an existing method used to expose sediments to varying stream conditions in-situ. The original purpose of the device was to compare the stream's benthic community responses to clean and AMD-contaminated sediments. Instead of biological analysis of the tray substrate, I added an acid extraction procedure, primarily aim at dissolving surface coatings, to examine relative changes in metal concentration over time during contamination and recovery exposures. This approach provides for an understanding of the geochemistry of the accumulated AMD metal coatings through the routes of sediment deposition. It may also provide a prediction of metal removal under stream conditions that simulate that which will occur post-remediation. The methodologies presented are novel because they utilize in-situ exposure to different conditions, as opposed to ex-situ simulation of aquatic conditions.

The final version of my methodology is the result of several revisions to my initial method. The extraction procedure, deployment method, and the trays required changes to

improve the methodology. In my first attempt, the null hypothesis was that stream flow (i.e. fast or slow flowing regions of stream) would affect metal deposition onto the sediment. This was a concern because I did not want to bias my experiment in the specific placement of the trays in the stream; however, the results from that experiment did not support the null hypothesis. Thus placement at each site is not an important consideration.

The first extraction method that I attempted used plastic bags to contain the substrate that was removed from the trays, because I thought that I could use a minimal amount of extraction solution by having a flexible container which would increase the surface contact of acid and substrate surfaces. Use of the plastic bags resulted in occasional leaks in which some of the acid solution was lost, and limited the strength of acid that could be used. I then began using rigid durable containers so that I could use a modification of USEPA Method 3050b, which involved heating with a hot water bath, concentrated nitric acid and concentrated hydrogen peroxide. While efficient, this aggressive extraction did release metals from the underlying substrate, make interpretation of the results somewhat more complicated.

In 2014, two of my deployments using racks were washed out due to high discharge from heavy precipitation, so I modified the deployment method to be more resilient to high discharge with the stream-side mesocosms. I found that the stream side mesocosms will result in less extractable metals compared to trays deployed in-stream. In-stream deployed trays accumulated more fine sediment material which can possibly bias the extraction results by increased dissolution of the substrate compared to deposited metal coatings, although I did not test this. In-stream deposition is not necessary for my experimental purposes because I want to examine the AMD deposited metals and reduce extraction of substrate to a minimum. Overall, the use of the

stream-side deployments was superior due to the robustness of the method to high discharge events.

Trays with side-holes were found to increase the removal of loose deposited metal oxyhydroxides. While this may be representative of riffle-zones in the stream (increased flow through the substrate), it may not be representative of general stream conditions, especially deposition zones where loose floc might be expected to accumulate. Therefore I began using trays that did not have side-holes. The side-holes were a part of the original Clements et al. method because they facilitated transport of benthic invertebrates through the trays.

In the 3rd chapter, the results of my deposition field experiment showed that using geochemical modeling to predict speciation and sorption in the water column, combined with my sediment tray methods, can aid in the prediction of fate and behavior of metals. Geochemical modeling allowed me to determine which processes were responsible for metal deposition through prediction of metal sorption to ferrihydrite, which is the driver of metal removal from the water column. Also because I observed significantly differing deposition in the same exposure period between clean and scoured trays, the sediment surface must affect metal deposition through possible increased surface complexation.

In the 4th chapter, I demonstrated that sediment recovery from AMD contamination is a more complicated process than I initially assumed. For this study I expanded the recovery deployment period to 8 weeks compared all of my previous 4-week recovery deployments, because I assumed that significant metal loss would be observed with the additional 4 weeks of recovery exposure. Significant metal removal was not initially observed and the results had a high amount of variability. I determined that the variability can be reduced and recovery trends can be observed with additional transformations of the metal extraction data. Accumulation of

metals from biofilms was determined to contribute to the extracted metals from the recovery samples, and this realization allowed for me to formulate the three component model for extractable metals. Subtracting out the metal concentrations resulting from substrate and biofilm dissolution allowed for detection of metal coating removal from sediment. Normalization to total extracted metals resulted in significant decreasing trends in the metal concentrations resulting from the coating, indicating stream recovery, albeit more slowly than deposition.

5.2 Recommendations for future research

In the future I hope to continue developing and refining my sediment sampling methodologies to be more representative of the in-stream geochemical processes.

Although the methodology should be broadly applicable, the results from this study are: specific to the NFCC and the AMD inputs (GI and NT) that enter the NFCC, the specific NFCC conditions that occurred during our field deployment, and possibly the substrate used in our trays. The NFCC's water chemistry varies throughout the year depending on discharge and temperature. High discharge results in increased suspended sediment load, increased flow and decreased concentrations of metal contamination. Low discharge results in the inverse trends. All of these conditions will affect AMD metal deposition onto sediment. Our study represents a specific set of stream conditions and is not representative of all possible ranges of conditions that occur in the NFCC; however the data obtained with this methodology allows for a specific understanding of contaminant fate and transport in a given study system.

The deposited AMD metal coatings on the surfaces of our sample rocks may not be completely representative of NFCC stream-bed sediments because the total deposition period lasted for 4 weeks. While the materials studied may represent the loose coatings that are weakly held to the rocks, depending on water chemistry, metal oxyhydroxides deposited on stream

sediments may form crystalline metal oxides, but this process can require months. The metal coatings on the NFCC sediment surfaces likely contain crystalline metal oxide species that have not formed in the deposited metal coatings on the rock-trays. The purpose of my study however was to examine the short-term accumulation of metals on stream sediment, which likely would be removed by scouring events. Allowing for a longer deployment period could result in metal coatings that are more representative of both the loose floc and the armored coating on the contaminated NFCC sediment. Longer exposure might also integrate over the sample replicates and thus reduce sample variability.

Mineralogical analysis of field collected sediments from the NFCC would be a useful future addition to my methodology, because then I could compare mineralogical analysis of my contaminated samplers to determine the aging affects metal speciation. A sequential extraction method would also be another useful analysis of the metal phases accumulated on NFCC sediment. Both mineralogical analysis and sequential extractions can allow for a more specific analysis of metal speciation on our sample substrate. These procedures could be performed on the different temporal sample sets to determine if the sediment metal speciation changes over time. Data on the mineralogy of the metal coatings would also aid in improving the model inputs to obtain more accurate predictions of speciation and sorption.

I determined that the substrate surface can affect the accumulation of metals. I observed significant differences in metal deposition on scoured contaminated sediment compared to clean substrate. The scoured surface appears to promote metal accumulation, possibly because of increased active surface binding sites on the metal coatings that resisted scouring. Biofilms were not explicitly analyzed or measured on the sample substrate. It appeared that this biofilms did lead to metal accumulation, particularly Mn. The presence of sediment biofilms has been shown

in previous literature to greatly increase Mn deposition. Increased deployment time may allow the additional time required for microbial biofilm communities to grow on the substrate surfaces to provide sediment in our samplers that is more representative of the stream sediment.

I hope to attempt longer deployment periods using this methodology in the future. When examining deposition using my rock tray method, an increased deployment period will allow for increased resolution of metal deposition above the controls. In Chapter 3, I found that sediment surfaces affect the metal deposition, because a contaminated but scoured surface significantly increases metal deposition. The accumulated metal deposition rates on rock trays in a longer deployment period may be more representative of metal deposition onto the already contaminated stream-bed sediments. Biofilm growth on the trays at the deposition site may also increase metal accumulation on sediment as I saw at the reference site, and a longer deployment period may allow for biofilm growth on the trays. If I performed a deposition deployment for 8 weeks, similarly to my longest recovery deployment, I may observe significant increases in metal deposition due to the biofilms.

The methodologies presented in this thesis are an attempt to examine the fate and behavior of aquatic metals resulting from AMD. Field sampling of contaminated sediments is limited due to the lack of control samples for comparison and statistical analysis. The methodologies presented utilize a combination of field mesocosms, water analysis, geochemical modeling, and data transformations to represent the stream contamination

APPENDIX A

TOXICITY OF METAL MIXTURES TO *DAPHNIA MAGNA*: COMPARISON OF LABORATORY AND FIELD INVESTIGATIONS

Modified slightly from a paper published in *IMWA 2013*¹

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A.1 Abstract

In aquatic systems impacted by mine wastes, organisms are usually exposed to metal mixtures instead of individual metals. For example, legacy acid mine drainage continues to pollute the North Fork of Clear Creek (NFCC) in central Colorado. In November 2012, we collected water samples from NFCC and deployed *Daphnia magna* as in-stream biomonitoring tools. Survival and whole-body concentrations of key electrolytes in *D. magna* decreased at sites that had elevated metal concentrations. In parallel laboratory toxicity tests in which *D. magna* were exposed to binary and ternary metal mixtures, approximately additive or less-than-additive toxicity occurred.

A.2 Introduction

In the mid-to-late 1800s, mining and mineral processing operations extracted valuable metals from the Colorado Mineral Belt. These processes led to unexpected environmental consequences as a result of acid mine drainage (AMD) generation. Currently an AMD-contaminated section of the North Fork of Clear Creek (NFCC) in central Colorado cannot sustain

an aquatic vertebrate population and has a depauperate population of aquatic macroinvertebrates. Therefore, the United States Environmental Protection Agency (USEPA) has placed a high level of urgency for remediation of the stream. As a consequence of the AMD discharge into NFCC, pH and alkalinity are lowered, but water hardness and concentrations of dissolved metals (e.g., Cd, Cu, Ni, Zn) and suspended sediments are increased in comparison to upstream waters. The interplay of these water quality parameters and the dissolved organic carbon (DOC) concentration control the aquatic toxicity of individual metals [1]. However, the consequences of organism exposure to metal mixtures are less well-understood. Laboratory toxicity testing of binary and ternary metal mixtures in standard media can provide insights into potentially non-additive effects. Field studies, while less controlled than laboratory toxicity tests, offer the opportunity to examine effects under realistic conditions and compare to results of laboratory tests. Our study goal was to gain a fuller understanding of the impact of metal mixtures on aquatic life in NFCC using both approaches. Thus, we conducted *Daphnia magna* toxicity tests in the laboratory coordinated with *in situ* deployment of *D. magna* and concurrent water sampling to see if toxicity in a field setting could be predicted from laboratory toxicity tests.

A.3 Materials and methods

A.3.1 Water sampling

Six sampling sites represent changes in water chemistry as NFCC flows southeast to join Clear Creek. Two AMD point sources (Gregory Incline [GI] and National Tunnel [NT]) enter the stream in the town of Black Hawk, CO (Figure A.1). A relatively pristine reference site (Site 1) that has low metal concentrations is ~1 km upstream from GI. That upstream site contains fish and numerous species of benthic macroinvertebrates that are characteristic of uncontaminated Rocky Mountain streams. Site 2 is immediately downstream of the two point sources, and water

chemistry and stream health are severely affected by the AMD inputs. Sites 3 and 4 are situated immediately above and below the city of Black Hawk's wastewater treatment plant (WWTP), ~9 km downstream of Black Hawk. Site 4 is the last NFCC site before the confluence with the mainstem of Clear Creek. Sites 5 and 6 on the mainstem of Clear Creek are upstream and downstream of the confluence with NFCC.

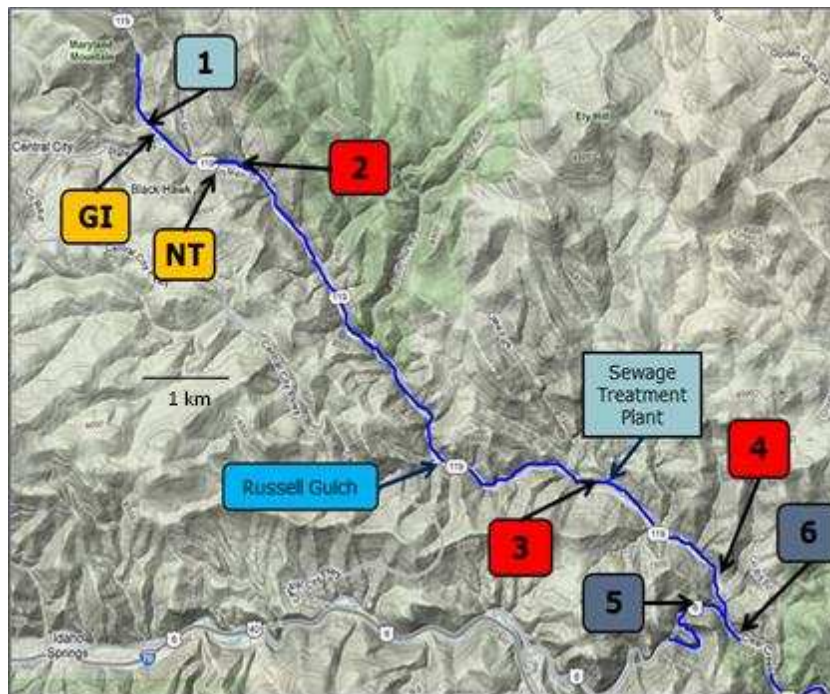


Figure A.1: Map of the sampling sites on the North Fork and mainstem of Clear Creek in central Colorado.

Grab samples of stream water, for analysis of major water quality parameters and metals, were collected using polypropylene containers on November 7, 15, and 17, 2012. Samples were filtered for dissolved metals in the field, and unfiltered samples were collected for total metals and total organic carbon (TOC) analyses. The TOC samples were collected in pre-combusted (500°C) amber glass bottles.

A.3.2 *In situ* biological monitors

Daphnia magna (a freshwater cladoceran) were deployed at the six field sampling sites on November 15, 2012, for a 48-h in-stream exposure in polypropylene test chambers that allowed free flow of water through the chamber. Surviving *Daphnia* were rinsed sequentially with Milli-Q water and 10 mL of 0.1 mM EDTA for 2 min to remove particles and weakly surface-bound metals, oven dried (60 °C, 48 h), weighed to the nearest 0.1 mg, extracted with 1 mL of trace-metal-grade nitric acid at 20°C for 24 h, and diluted to 10 mL with Milli-Q water before analysis for elemental content.

A.3.3 Laboratory toxicity tests

Daphnia magna were cultured in moderately hard reconstituted (MHR) water (USEPA 2002; nominal pH of 7.4-7.8, alkalinity of 57-64 mg/L as CaCO₃, and hardness of 80-100 mg/L as CaCO₃) and were tested in MHR water to which DOC was added as Suwannee River fulvic acid at 3 mg DOC/L. Standard 48-h lethality tests using <24-h-old neonates were conducted for all single-metal (Cd, Cu, Ni, and Zn) and metal-mixture tests (USEPA 2002). Temperature, pH, alkalinity, and concentrations of Cd, Cu, Ni, Zn, and major inorganic anions and cations were monitored during the tests; and hardness was calculated from the measured Ca and Mg concentrations. Because the concentration-response curves for individual metals can vary from test to test, we usually conducted a Cd-only, Cu-only, Ni-only, and/or Zn-only test concurrent with the binary-mixture tests. Additionally, we conducted numerous toxicity tests with each binary metal pair to determine within-day and among-week variability in results, to evaluate whether any apparently less-than-additive, additive, or more-than-additive toxicity result was due to variability alone.

A.3.4 Chemical analyses

During the field study, we measured pH (VWR SP80PC meter/VWR 14002-860 combination gel electrode), alkalinity (HACH Model AL-DT Alkalinity Test Kit), and ferrous iron (HACH DR/890 Colorimeter/1-10 phenanthroline AccuVac ampules) in the field. Later in the laboratory, unfiltered and filtered (0.45 μm) water samples from field sites were acidified with trace-metal-grade nitric acid before being analyzed for major elements and metals using inductively coupled plasma–optical emission spectrometry (ICP-OES; Perkin Elmer Optima 5300 DV). Water hardness was calculated from the measured Ca and Mg concentrations. Unfiltered water was also analyzed for total organic carbon (TOC) concentration by persulfate oxidation/UV irradiation (Sievers 5310C TOC analyzer). Acid-digested *Daphnia magna* (see above) were analyzed for elemental content using ICP-OES.

During laboratory toxicity tests, the same general analytical procedures (APHA 2012) and instruments were used. However, pH was determined using a ThermoScientific Orion2 Star Benchtop Meter/ThermoScientific Orion 815600 Ross Combination pH/conductivity probe.

A.4 Results and discussion

A.4.1 Laboratory toxicity tests

In Cu-Zn binary mixture tests, the toxicity always appeared to be either slightly more-than-additive (i.e., the observed mortality was slightly greater than the predicted mortality) or additive (i.e., the observed mortality equaled the predicted mortality) when based on dissolved-metal concentrations, whether Cu was varied while Zn was held constant (Figure A.2a), or vice versa (results not shown). Similarly, the toxicity of Cu-Ni mixtures always appeared to be more-than-additive or additive (results not shown). However, the toxicity of these Cu-Zn mixtures was less than additive when based on the concentrations of Cu and Zn bound to the biotic ligands,

when predicted using a multi-metal biotic ligand model (results not shown). These results indicate that the dominant determinant of toxicity was Cu-Zn or Cu-Ni competitive binding to DOC, thus leaving more Cu^{2+} , Ni^{2+} , or Zn^{2+} available to bind to the biotic ligand than would have been available at the same dissolved-metal concentration in a Cu-only, Ni-only, or Zn-only test. Furthermore, these results demonstrate that conclusions about additivity of metal-mixture toxicity can differ considerably when the frame of reference changes from dissolved metal to biotic-ligand-bound metal.

In contrast, in Cd-Cu, Cd-Ni, and Cd-Zn binary mixture tests, the toxicity appeared to be either less-than-additive (i.e., the observed mortality was less than the predicted mortality) or additive (i.e., the observed mortality equaled the predicted mortality) when based on dissolved metal concentrations (Figures A.2b, A.2c, and A.2d). Most notably, Ni and Zn concentrations less than 1/10 of the Ni-only or Zn-only median lethal concentration (LC50) protected against partial to 100% Cd lethality (Figures A.2c and A.2d); and Cd concentrations approximately 10x the Cd-only LC50 had to be added to the Cd-Ni and Cd-Zn mixtures before mortality began increasing above the background mortality caused by the constant Ni or Zn concentration (results not shown), again demonstrating a large protective effect of Ni and Zn against Cd toxicity. These results demonstrate a protective effect of higher-concentration metals (Cu, Ni, or Zn) against the toxicity of a more lethal metal that is present at much lower concentrations. These results also indicate that the dominant determinant of toxicity was Cd-Cu, Cd-Ni, or Cd-Zn competitive binding to the biotic ligand, whereby the relatively higher concentration of Cu^{2+} , Ni^{2+} , or Zn^{2+} allowed less Cd^{2+} to bind to the biotic ligand than would have been bound at the same dissolved-Cd concentration in a Cd-only test. An analogous protective effect against Cd toxicity also

occurred in ternary Cd-Cu-Zn mixtures, without any apparent more-than-additive interactions between Cd and Zn (results not shown).

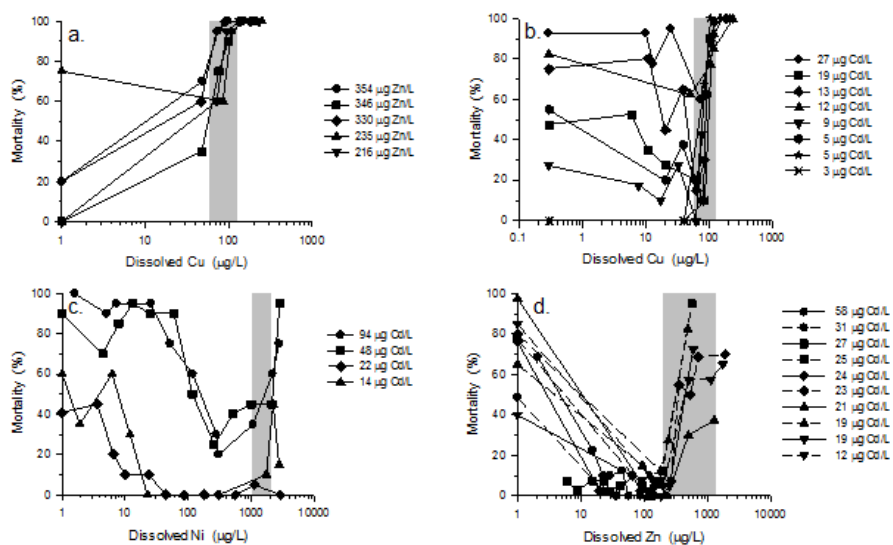


Figure A.2: Toxicity of binary mixtures of Cu and Zn and of Cd and Zn to *Daphnia magna*. (a) Zn concentration was held constant in all treatments within a given test (Zn concentrations in different tests shown in legend), while Cu was varied across a concentration series; the vertical gray bar indicates the range of concentrations at which Cu caused 50% mortality in Cu-only tests. (b) Cd concentration was held constant in all treatments within a given test (Cd concentrations in different tests shown in legend), while Cu was varied across a concentration series; the vertical gray bar indicates the range of concentrations at which Cu caused 50% mortality in Cu-only tests. (c) Cd concentration was held constant in all treatments within a given test (Cd concentrations in different tests shown in legend), while Ni was varied across a concentration series; the vertical gray bar indicates the range of concentrations at which Ni caused 50% mortality in Ni-only tests. (d) Cd concentration was held constant in all treatments within a given test (Cd concentrations in different tests shown in legend), while Zn was varied across a concentration series; the vertical gray bar indicates the range of concentrations at which Zn caused 50% mortality in Zn-only tests.

A.4.2 November 2012 field study

The pH and alkalinity of NFCC decreased and the water hardness increased at Site 2 after the two AMD point sources entered the stream, and the pH and alkalinity continued to decrease as the water flowed downstream to Site 3 (Table A.1). These continued decreases of pH and alkalinity could have been due to slow Fe^{2+} oxidation, manifested as the continuing high concentration of dissolved Fe throughout the ~9-km reach between Sites 2 and 3. Influx of the

WWTP effluent considerably increased the pH, alkalinity, and total and dissolved Fe concentrations in NFCC at Site 4, but the hardness and TOC concentration increased only slightly. Temperatures ranged from -0.5 to 3 °C at the beginning of the *D. magna* deployment.

The in-stream contributions of Cd, Cu, Fe, and Zn from the AMD point sources are manifested as large increases in dissolved and total concentrations of those metals at Site 2 (Table A.1). Trends in dissolved and particulate Cu reflect several in-stream processes, most notably pH-dependent sorption to suspended particulate iron oxides. At Site 2, Cu is largely particulate where the pH is circumneutral (6.8) and suspended iron concentration is high (15 mg/L; calculated by difference between total and dissolved Fe concentrations in Table 1). At Site 3, the particulate Cu concentration is low, consistent with the lower pH (5.7) and lower suspended particulate iron concentration (1 mg/L). Despite the higher pH (8.0) at Site 4, the particulate Cu concentration is low, likely due to the very low suspended particulate Fe concentration (<1 mg/L). In contrast, the large majority of zinc in the water is dissolved. Although the significant downstream decreases in the total and dissolved Cu concentrations might be explained by the concurrent loss of suspended particulate iron, the decreases of the total and dissolved Zn concentrations must be due to direct loss to the streambed. Decreases in metal concentrations due to dilution can be ignored based on the small decrease in sulfate concentration, which is a conservative tracer in this aerobic stream system in which sulfate is not reduced to sulfides (data not shown).

Because increasing alkalinity, hardness, and TOC concentrations tend to decrease metal toxicity (as does increasing pH above approximately 6.5-7; Meyer et al. 2007), the major longitudinal changes in water chemistry in NFCC can have major effects on the toxicity of Cd,

Table A.1: Water chemistry during 48-h field deployment of *Daphnia magna* in the North Fork and mainstem of Clear Creek in Central Colorado in November 2012.^a

Site	pH	Alkalinity	Hardness	TOC	Cd (µg/L)		Cu (µg/L)		Zn (µg/L)		Fe (µg/L)	
		(mg/L as CaCO ₃)	(mg/L as CaCO ₃)		Total	Diss.	Total	Diss.	Total	Diss.	Total	Diss.
1	8.28	24.7	43.4	1.59	<0.1	<0.1	2.6	3.2	9	27	152	15
2	6.82	12.4	247.3	1.54	4.7	3.5	70.2	3.2	1,787	1,625	38,373	23,437
3	5.67	2.5	258.0	1.41	5.8	5.4	94.0	90.8	2,004	1,963	3,580	2,520
4	8.00	52.7	268.5	1.75	1.9	1.7	21.8	14.4	740	680	629	30
5	8.20	44.9	101.1	2.28	<0.1	<0.1	10.7	5.6	219	180	168	15
6	8.19	44.0	112.6	1.50	<0.1	<0.1	11.8	6.1	267	201	290	20

^aData are averaged from the three sampling days (November 7, 11, and 15, 2012).

Cu, and Zn to aquatic organisms. Those effects were manifested in the decreased survival of *D. magna* at Sites 2 and 3, where pH and alkalinity decreased considerably even though hardness increased (Table A.2). Those toxicity results are consistent with the generalization that pH and alkalinity have more of a toxicity-modifying effect on cladocerans than does water hardness (e.g., Meyer et al. 2007), but the concurrent increases of the Cd, Cu, Fe, and Zn concentrations complicate that interpretation. The major decreases of whole-body Na and K concentrations and the smaller-percentage decreases of whole-body Ca and Mg concentrations are also consistent with expectation, because elevated concentrations of cationic metals can disrupt ionoregulation (e.g., Na for Cu exposure, and Ca for Zn exposure) and thus can be lethal to aquatic organisms (Meyer et al. 2007).

Table A.2: *Daphnia magna* survival and whole-body electrolyte concentrations during 48-h field deployment in the North Fork and mainstem of Clear Creek in Central Colorado in November 2012.

Site	<i>Daphnia</i> 48-h survival (%)	Whole-body concentration (mg/kg dry wt tissue)			
		Na	K	Ca	Mg
1	92	137	240	5,010	198
2	0	<11 ^a	<17 ^a	3,639 ^a	148 ^a
3	0	14 ^a	17 ^a	3,273 ^a	135 ^a
4	100	596	444	6,508	256
5	93	383	297	5,655	236
6	96	392	333	6,206	232

^aDead *Daphnia* analyzed for whole-body electrolytes at this site because no *Daphnia* survived.

NFCC water at Sites 2 and 3 had lower alkalinity, lower TOC concentration, and higher hardness than the MHR exposure water used in the laboratory single-metal and metal-mixture toxicity tests with Cd, Cu, and Zn (pH 7.8-8, alkalinity ~50-60 mg/L as CaCO₃, hardness ~80-90 mg/L as CaCO₃, and DOC ~3 mg/L in MHR water). At Sites 2 and 3, the Zn concentrations in NFCC were nearly double the mid-range of the median lethal concentrations (LC50 values) determined in the Zn-only laboratory toxicity tests (~600 µg Zn/L); and at Site 3, the dissolved Cu concentration in NFCC was close to the mid-range LC50 determined in the Cu-only laboratory toxicity tests (~80 µg Cu/L). Even in the relatively high hardness water, no *Daphnia* survived at Sites 2 and 3 due to the high Cu and Zn concentrations, low alkalinity, and relatively low TOC concentrations. The Cd concentrations at all sites were less than the mid-range LC50 determined in the Cd-only laboratory toxicity tests (~8 µg Cd/L); and because Cu and Zn have moderate to large protective effects against Cd toxicity (see above), the potential contribution of Cd to the observed *Daphnia* mortalities at Sites 2 and 3 was probably even less. Although the hardness at Site 4 remained approximately as high as at Sites 2 and 3, the alkalinity increased and the Cd, Cu, and Zn concentrations decreased. Therefore, despite the Zn concentration at Site 4 being near the LC50 value determined in MHR water in the laboratory, the elevated hardness in Site 4 water (compared to MHR water) probably protected against lethality. In the mainstem of Clear Creek (Sites 5 and 6), the Cd, Cu, and Zn concentrations were below the LC50 values determined for those metals in MHR water; and all the *Daphnia* survived, consistent with prediction for waters with those hardness and alkalinity concentrations.

A.5 Conclusions

Mine-drainage waters are complex mixtures of metals and major ions that can alter the chemistry of receiving waters in complex ways. The North Fork of Clear Creek is an example of

this situation. AMD is a reactive solution; and after it enters receiving water, the speciation of metals and ions can change depending on temperature, pH, oxygen exposure, etc. These reactions are kinetically limited, thus changing the concentrations of chemical constituents over time as the mixture of AMD and receiving water flows downstream. Therefore, the toxicity of the mixture can vary greatly and depends on the water chemistry at a specified point in the stream. Models of the toxicity of metal mixtures that take into account varied water chemistry (e.g., biotic ligand models) and interactions among the metals are needed to address these situations more accurately than current approaches that use water hardness as the only predictor of toxicity. Based on the laboratory study of metal-mixture toxicity that was conducted in conjunction with the field study, we conclude that metal-metal interactions among Cd, Cu, and Zn are generally either additive or less than additive based on the metal competition for binding to the biotic ligand. Failure to consider this competition can mistakenly lead to the conclusion that the mixtures are more than additive.

A.6 Acknowledgements

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