Field Deployed Ni-Amended Sediments Shows Varying Effects In Two Central Michigan Streams

By

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A thesis submitted
in partial fulfillment of requirements
for the degree of Master of Science
(Natural Resources and Environment)
at the University of Michigan

August 2013

Thesis Committee:
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ABSTRACT

Most research on Ni contaminated sediments has occurred under laboratory conditions, which can lead to exposure to formulated water that is not representative of environmentally relevant conditions. For example, laboratory conditions don't accurately represent natural fluctuations in temperature, light, or flow. The purpose of our study was to 1) assess the bioavailability and toxicity of Ni contaminated sediments to stream macroinvertebrate communities under *in situ* conditions and 2) compare these effects across two similar streams in close proximity of each other. Sediments were removed from two, second order streams in central Michigan (Black Creek (BC) and Little Molasses (LM)) that were similar in hardness, pH, dissolved oxygen (DO), dissolved organic carbon (DOC), and alkalinity. The sediments were directly spiked with Ni at two treatment levels, low nickel (210 mg/kg dry weight) and high nickel (1889 mg/kg dry weight), equilibrated for 10 days, and re-deployed in macroinvertebrate colonization baskets within the respective streams. Sediment geochemistry (e.g. AVS, SEM_{Ni}, total Ni, total Fe, and total Mn) and water quality measurements (e.g. hardness, alkalinity, pH, and dissolved organic carbon (DOC)) were determined at deployment and three successive sampling periods (14, 28, and 56 days post-deployment). *In situ* chambers with *Hyallela azteca* were used to conduct 96-h acute toxicity tests at day 0 and 14. In addition, we deployed a microbial decomposition test from day 0 to 14 to assess Ni effect on the microbial community. We found varying responses to Ni treatments in each stream. The 96-h acute toxicity tests only showed significant toxicity in the sediment-water interface exposure at day 0 for BC. Both Ni treatments adversely impacted the microbial community in BC, but not in LM. The benthic community recolonization response was limited with only the family Gammaridae showing a response to Ni treatment in both streams on day 14. Stepwise stream-dependent regression of the measured

variables showed that the SEM_{Ni}/AVS model of potential toxicity significantly predicted Gammaridae abundance in BC, but not LM. BC showed a trend of toxicity from Ni-amended sediments with increased mortality to *H. azteca* (acute exposure), the microbial community, and Gammaridae.

ACKNOWLEDGEMENTS

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INTRODUCTION AND LITERATURE REVIEW

Metals occur naturally in the environment, but may become problematic when anthropogenic introduction causes concentrations above background levels. They do not biodegrade and have intricate environmental chemistry. Numerous cases exist that illustrate the negative impacts metal contamination can have on aquatic ecosystems. For example, Allert et al. (2009) showed that lead contamination from mining operations in the Ozark Mountains caused reductions in the woodland crayfish (*Orconectes hylas*) in the waters downstream of the mining operation. Clements et al. (2000) found that streams moderately contaminated with Al, Cu, and Zn had heptageniid mayfly communities that were reduced by >75% as compared to reference streams.

Anthropogenic contributions of Ni into the environment (and aquatic systems) include waste water treatment plants (WWTP), smelters, mining, galvanized products, and manufacturers of stainless steel and nickel alloys (Cempel and Nikel 2006, Chakraborty and Chakrabarti 2006). According to Pane et al. (2003), uncontaminated freshwater systems have dissolved Ni concentrations that range from 1-10 μg/L while contaminated areas can have concentrations up to 1000 μg/L. Naturally occurring background levels of Ni in soils and sediments range from <5 to 700 ppm (Shacklette 1984). Bioaccumulation studies have shown that aquatic organisms have varying tolerances to whole body Ni concentration burdens. Lebrun et al. (2011) showed that *Gammarus pulex* is very tolerant to Ni bioaccumulations with 48-h LC_{50} = 477 mg/L of Ni and body burden concentrations exceeding 12 μg/g. Borgmann et al. (2001) used *Hyallela azteca* to investigate the utility of determining body burden Ni concentration vs. sediment Ni concentrations. Because Ni

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bioavailability varies based on sediment properties, Borgmann et al. (2001) assessed bioaccumulation using three different sediments and concluded that determining body concentrations is a reliable predictor of Ni toxicity. Bervoets et al. (2004) conducted *in situ* caged bioaccumulation tests with *Chironomus riparius* in several different types of sediments and found that Ni body burden concentrations varied from 1 to 120 μg/g depending on sediment characteristics.

Excess divalent Ni^{2+} may cause aquatic toxicity, but the exact physiological mechanisms of uptake, storage, and clearance have been poorly described in aquatic organisms. Chowdhury et al. (2008) investigated the effects of waterborne Ni on rainbow trout (*Oncorhynchus mykiss*) and found that during exposure to excess Ni the gut, kidney, and scales all played a role in maintaining Ni homeostasis within the tissues. This suggests that Ni is an essential micronutrient. Franzellitti et al. (2011) performed 96-h toxicity tests on a mussel species to assess the synergistic effects of Ni and the pesticide chlorpyrifos which are often detected together in aquatic ecosystems. They found that within the four day test gene expression levels and their protein products of the *MgPgp* and *MgMvp* genes were up-regulated to induce a physiological response that increases extracellular transport mechanisms that are capable of removing harmful xenobiotics (Franzellitti et al. 2011). However, research linking effects to specific physiological or molecular mechanisms is still lacking with sparse amounts of research done on fish, mussels, and crustaceans (Pane et al. 2003, Franzellitti et al. 2011, Leonard et al. 2011).

Different Modes of Metal Partitioning

The chemical properties of divalent metals (e.g. Ni^{2+}) allow them to be easily dissolved in water, but quickly adsorb to particles (Heimann et al. 2002, Doig and

Liber 2006a). Therefore, groundwater, overlying water, and sediments are all ecologically relevant when assessing metal contamination risk. Once in any of these compartments a multitude of factors can influence metal partitioning between dissolved and particulate phases and ultimately bioavailability: acid volatile sulfides (AVS), dissolved/particulate organic matter (DOC/POC), particle size, pH, hardness/alkalinity, Fe/Mn oxyhydroxides, and physical processes such as – groundwater flow, stream gradient, and resuspension events from storm-flow and dredging (Heimann et al. 2002, Simpson et al. 2004, Carbonaro et al. 2005, Doig and Liber 2006b, Fairbrother et al. 2007).

AVS

Acid-volatile sulfide (AVS) refers to a heterogeneous complex of sulfides in sediment that are comprised of a variety of reduced sulfur components. Specifically, AVS is defined by the fraction that is released when sediments are treated with acid. Although a complex mixture, the sulfides are often dominated by Fe and Mn mono-sulfides (e.g. FeS) (Rickard and Morse 2005, Hammerschmidt and Burton 2010). AVS is typically higher in anoxic sediments with high organic carbon content (Hammerschmidt and Burton 2010). It has been well established that AVS is very important to metal bioavailability in sediments because of the affinity of reduced sulfur for divalent metals and the limited bioavailability of those metal monosulfides. Essentially, metal bioavailability can be estimated by the molar relationship between the concentrations of the simultaneously extractable metal (SEM) to the concentration of AVS. The concept is derived from the fact that dissolved metals in the porewater (i.e. the bioavailable fraction) are a function of the amount of AVS and the amount of SEM which can form insoluble metal sulfide complexes that render the metal un-available (USEPA 1991, Ditoro et al. 1992, Hammerschmidt and Burton

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2010, Nguyen et al. 2011). At equilibrium, the AVS acts as a permanent sink for metals, but the metal sulfides are also a potential source of toxicity because they are not completely un-reactive. Upon oxidation the metals can become mobilized and flux into the porewater (Carbonaro et al. 2005).

AVS has become a widely accepted measure in assessing the potential toxicity of metal contaminated sediments. The U.S. Environmental Protection Agency (USEPA) promotes using the relationship between AVS and SEM in assessing metal toxicity, and many investigations have applied the logic to assessing metals, including Ni (USEPA 2005). For example, DiToro et al. (1992) used amphipods, oligochaetes, and snails to test the toxicity of Cd and Ni spiked sediments. With the SEM/AVS model they correctly predicted non-toxic sediments 51% of the time and toxic sediments 42% of the time. Liber et al. (1996) showed that spiked sediments placed into a pond had increased AVS over time. The increased AVS was correlated with increased SEM_{Zn} which resulted in protection of benthic re-colonizers. Therefore, AVS protected the benthic community from adverse effects from the Zn.

Because AVS is such an important measure for estimating bioavailability, Billon et al. (2001) and Hammerschmidt and Burton (2010) investigated the methods of AVS determination and found that methodological artifacts can occur at multiple stages including preparation, sample storage, homogenization of the sample, and variation from non-standardized methods. Hammerschmidt and Burton (2010) sent sub-samples of four stream sediments to seven different laboratories for AVS and SEM determination. They got back results where AVS concentrations varied 70 to 3500x and SEM concentrations varied by 17 to 60x (Hammerschmidt and Burton 2010). Such variation greatly alters the outcome of the SEM and AVS models.

Takematsu (1979) determined the sorption kinetics of several transition metals (i.e., Co, Cu, Ni, and Zn) on Fe and Mn oxide surfaces and found that these metals are readily scavenged when dissolved in water. Since, many researchers have documented the importance of highly-sorptive Fe and Mn oxides on binding metals and reducing bioavailability, especially in oxic sediments (Sundelin and Eriksson 2001, Yu et al. 2001, Tebo et al. 2004). Burton et al. (2007) documented a weak, but significant correlation between total Fe and Mn levels to $\sum$SEM while surveying wadeable streams in Europe. More recently, Costello et al. (2011) documented in Ni-spiked sediments deployed under field conditions that Ni partitioned to OC in the beginning of the experiment then shifted to binding to Fe and Mn oxides by the end of the experiment. Thus, indicating that Fe and Mn oxides be incorporated into the SEM and AVS models to help improve the assessment of potential toxicity from Ni contaminated sediments.

DOC & TOC

To have a better understanding of the effects of organic carbon on metal toxicity, a background on the definitions and types of organic carbon are described first. Dissolved organic carbon (DOC) in freshwater systems can come from external sources (i.e., allochthonous carbon) and internal sources (i.e., autochthonous carbon). Sources include terrestrial vegetation, organic matter in the upper soil layer, wetlands/bogs, and the organisms that live within (Prusha and Clements 2004). However, the majority of freshwater DOC is derived from allochthonous sources which can lead to spatial and temporal variability in DOC concentrations associated with physical occurrences such as runoff and snowmelt (Canavan et al. 2007). The chemical nature of DOC is very complex, but most investigators examine the two

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major components, humic and fulvic acids, which are produced through cellulose and lignin biodegradation (Prusha and Clements 2004).

It is generally accepted that DOC plays a role in binding free metals, which influences toxicity and bioavailability (Boucher and Watzin 1999, Christensen and Christensen 2000, Di Toro et al. 2001, Chakraborty and Chakrabarti 2006, Doig and Liber 2006a, Butler 2009). Prusha and Clements (2004) used a GIS approach to establish a correlation between the amount of forested habitat in 16 watersheds and their respective DOC stream values. They then compared metal toxicity to several stream invertebrates to see if there was a relationship to DOC. They concluded that percent forested area within a watershed was an accurate predictor of stream DOC content, and that DOC was a likely factor to the reduced toxicity seen in one of the invertebrates. However, their definition of what constitutes DOC was different than the majority of the literature; defining it as the portion of organic matter that passes through a 0.7 μm filter.

The fate of DOC and its influence on metal toxicity is of particular importance when considering urbanization and the discharge of waste waters (from WWTPs) into receiving waters. According to Katsoyiannis and Samara (2007) the portion of non-biodegradable DOC that goes through a WWTP may bind persistent organic pollutants (POPs) and heavy metals that may not be removed during the treatment process. The multiple sources of heavy metals to WWTPs include: household effluents, car washes, dental offices, and automotive contributions (exhaust, tires, brakes, gas/oil leakage) through storm water runoff (Katsoyiannis and Samara 2007). Therefore, DOC and the associated pollutants may be directly discharged into receiving waters. The authors found that DOC was not the source of the pollutants, but that DOC influenced the partitioning between dissolved and sorbed phases. They

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also calculated that approximately 69% of DOC was removed during the WWT process. To conclude, POPs and heavy metals that aren't sorbed to particulate carbon may become sorbed to DOC and stay in the dissolved phase. WWT effluent discharge would then deposit these contaminants into receiving waters. Evans et al. (2005) looked at surface water DOC concentrations from the last 15 years and noticed that concentrations are increasing around the world. They suggest that a combination of factors related to global climate change is responsible, most notably a decrease in acid deposition and increasing temperatures. Suggested environmental effects of increased DOC ranged from increased metal mobility to complications with drinking water treatment (excess DOC reduces the effectiveness of disinfection and the reaction of chlorine with humic substances can form harmful byproducts) and changes in energy, nutrient, and light regimes of lakes and estuaries. Continued monitoring of these changes may provide useful information for climate change related research.

In the process of conducting this literature review it has become evident that the complexity of DOC chemistry has resulted in great experimental variation. To begin, there doesn't seem to be a standard definition for DOC that everyone can reference. Most widely used is a physically-based classification meaning that the dissolved vs. particulate fraction is distinguished by what passes through a certain pore-sized filter. The problem with this definition is that there is not an accepted standard as to what pore-size to use. The majority of investigators separate DOC with either 0.22 μm or 0.45 μm filters. According to Filella (2009) this is not necessarily an accurate method because filtration artifacts include fractionation of some of the natural organic matter (NOM) compounds while filtering, and some adherence of the compounds to the filter. Because NOM has different sources and is extremely

chemically complex, various methods have been developed to look at particular

fractions (Filella 2009). Unfortunately, this complexity has led to many inconsistent

methods that make comparing data across studies difficult, and with end users that

often doesn't understand the correct methods to use for the appropriate type of DOC

(Filella 2009). To illustrate variability, Hoang et al. (2004) obtained water from the

Black River (Andrews, S.C.) and used a 0.45 μm Gelman nylon mesh filter while

Doig and Liber (2006a) obtained humic and fulvic acid components from three

natural water sources and filtered them using 0.45 μm polysulfone membranes.

Jackson et al. (2005) obtained DOC for their experiments by obtaining sediments

from an annual pond and isolated DOC using 0.22 μm cellulose acetate filters. In

disagreement with the accepted pore sizes in the majority of the literature, Prusha and

Clements (2004) defined DOC as any portion of organic matter passing through a 0.7

μm filter.

An additional problem associated with physical size separation is that dissolved and

colloidal DOM fractions may pass through a 0.22 μm filter (Jackson et al. 2005).

Colloidal species may be suspended within the aqueous phase and according to

Jackson et al. (2005) they may bind metals and affect bioavailability, but may also

increase mobility. Cantwell and Burgess (2001) used an ultrafiltration method to

separate dissolved and colloidal fractions of DOC from marine sediment interstitial

waters. They confirmed that colloidal organic carbon influenced metal speciation

and was a major complexing agent for five metals, including Ni.

The variability of the metal binding capacity of DOC is due to the heterogeneity of

natural waters. DOC is derived from abundant sources and contains many

components which results in complex chemistry (Evans et al. 2005). Regardless,

there are two main components of DOC that are usually the target of metal-DOC

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investigation, humic acid and fulvic acid. The low molecular weight compounds in

DOC (e.g. amino acids and carbohydrates) are generally ignored due to their

insignificant proportion (Evans et al. 2005). Jackson et al. (2005) mentions that most

field studies don't properly characterize DOM, and most of the time DOC is just

assumed to be either humic or fulvic compounds. In regards to proportion,

Chakraborty et al. (2009) makes the statement that DOC is predominantly comprised

of humic substances which are now thought to be a collection of low molecular mass

molecules with diverse hydrophobic-hydrogen bond-forming properties that can bind

metals under different binding energies. No other information was found to

corroborate the claim, but Evans et al. (2005) defined humic substances as medium to

high molecular weight molecules with functional groups (e.g. ketone, amide,

carboxyl) attached to varying aromatic and aliphatic hydrocarbons. Another

definition of DOC by Winch et al. (2002) was thorough, but again, illustrates the

inconsistency of the working definition. They use Curtis' 1998 definition describing

DOC as an assortment of compounds (organic polymers) ranging in molecular weight

from approximately 1,000 to 10,000 Daltons, which pass through a 0.45 μm filter.

The authors also state that DOC metal complexation is driven by aromatic and

aliphatic carbon structures that are bound to carboxyl and phenolic functional groups

(Winch et al. 2002). Nadella et al. (2009) conducted metal toxicity experiments

using water-only exposures of Cu, Zn, Ni, and Cd to the blue mussel, and found that

Cu toxicity was greatly reduce by DOC. However, analysis of the DOC waters with

fluorescent spectroscopy showed a difference in the make-up. DOC with higher

fulvic acid content was more protective than DOC predominated by humic acids.

Cloran et al. (2010) found that under laboratory Ni toxicity testing, the addition of

humate reduced Ni toxicity in exposures to *D. magna*.

To conclude, inconsistencies in defining DOC may make comparing data between experiments difficult or even erroneous. Some investigators test metal-DOC interactions using humic substances while others use fulvic components, and some generalize DOC as the portion passing through either 0.22 μm or 0.45 μm filters.

Nickel and DOC Effects

Spatial and Temporal Variation

Canavan et al. (2007) used two methods to assess the depth profiles of metals in lake contaminated sediments. Porewater and sediment concentrations of Ni were measured and they determined what Ni was binding to and at what depths. Then, using a reactive transport model (RTM), they predicted the availability of Ni throughout a 20 cm profile. At the sediment-water interface, metals were bound to organic matter, clay, and Fe/Mn-oxides, but deeper in the sediments (anoxic conditions) there was a shift to metals binding to the insoluble sulfides. These sulfides originated from the decomposition of organic matter (Canavan et al. 2007). Therefore, the oxidation of the deeper sediments (via resuspension or bioturbation) could result in the release of metals to the water column or porewater. They found that pore-water DOC and Ni concentrations varied spatially (depth) and temporally. Sediment profiles for Ni were performed based on total Ni, AVS-SEM, Fe-oxide, and reducible Fe (III) phases. Extractions indicated a shift from oxide- to sulfide-bound Ni with a stronger association to FeS_2 with increasing depth. The modeling simulated Ni as being more mobile due to unstable Ni-sulfide compounds. Temporally, DOC was variable in the spring, but tended to increase to a maximum of approximately 1.5 mM at 5 cm and then decreasing to ~ 0.75 mM at 20 cm. Summer measurements indicated a gradual increase from ~ 0.75 mM at 1 cm to ~1.5 mM at 20 cm.

Rember and Trefry (2004) sampled several Alaskan rivers to document total suspended solids (TSS), dissolved metal, and DOC concentrations during spring floods. As rivers reached peak discharge, DOC concentrations increased from 167 to 742 μmol/L. Correspondingly, Rember and Trefry (2004) documented temporal changes in dissolved metals which increased 3 to 25 fold over off peak discharge. They attribute the flux to low pH soils seeing increased weathering (i.e. snowmelt) which releases dissolved metals and DOC.

Competition with Ions

Chakraborty and Chakrabarti (2006) used mining effluent to see the interactions between DOC-metal complexes and ions (Ca^{2+} and Mg^{2+}) as competing ligands. They proposed a kinetic model, the competing ligand exchange model (CLEM), which suggested that when a competing ligand (in this case Ca^{2+} or Mg^{2+}) is in concentrations much higher than the metal of concern, the competing ligand in highest concentration dominates the first order reaction. To simulate what happens when mining effluent enters a freshwater system, they used two scenarios: a diluted and undiluted effluent. Humic substance complexation was created and all ions measured. Dimethylglyoxime (DMG) was used as the competing ligand in the Ni experiment. DMG was added to the effluents and then percent Ni released was calculated over a 60 second interval. The diluted effluent showed a drastic decrease in release of Ni over the undiluted. Next, a Ni (II)-DOC complex solution was created to test the effects of adding Ca^{2+}. Ca^{2+} concentrations ranged from 0.1 mM to 10 mM and resulted in a gradual increase of Ni from 6% to 22%, respectively. The authors explain the decreased release of Ni from the diluted effluent as an effect from having less Ca^{2+}. To conclude, they suggest this model needs to be interpreted with caution because of the complex nature of DOC binding affinities, and the complexity

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of natural waters. However, this was evidence that further investigation needs to occur to understand metal-DOC complexes and the possibility of competing ligands releasing metals into freshwater systems (Chakraborty and Chakrabarti 2006).

Because natural waters are heterogeneous with complex mixtures of ions and cationic divalent metals that compete for binding sites on DOC, Rey-Castro et al. (2009) developed a conditional affinity spectrum model (CAS) to help explain metal ionic binding affinities to humic substances. A sample of natural river water was used to identify all of its ions and then CAS was used to describe the binding affinities to the fulvic acid component. The method established three distinguished groups of cations that preferentially bind to specific sites on the fulvic ligand: 1) cations that bind to the phenolic sites Al, H, Pb, Hg, and Cr 2) cations which have high affinity for carboxylic sites Ca, Mg, Cd, Fe (II) and 3) an overlap between phenolic and carboxylic sites Fe (III), Cu, Zn, and Ni. Application of this model may help to characterize metal binding and thus mobility and bioavailability. Because DOC is so chemically complex refinement of this model is necessary for field application. However, it is a step in the right direction to help further the understanding of metal dynamics in aquatic systems (Rey-Castro et al. 2009).

Increased water hardness has been shown to be protective against metal toxicity. This effect results from the competitive binding of Ca^{2+}, Mg^{2+}, and metal ions (M^{2+}) for sites on biota called the biotic ligand (discussed below) (Keithly et al. 2004). For example, Keithly et al. (2004) used *C. dubia* and *H. azteca* to determine acute and chronic toxicity values for Ni. The goal was to use the data to construct a Ni-BLM. Hardness values of 50, 113, 161, and 253 mg/L $CaCO_3$ were tested with *C. dubia* in 48-h toxicity tests. LC_{50} values showed an increase as water hardness increased 81, 148, 261, and 400 mg/L, respectively. Chronic EC_{20s} for *C. dubia* were 3.8, 4.7, 4.0,

and 6.9 mg/L at the listed hardnesses, respectively. In comparison, *H. azteca* 96-h
LC_{50} was 3,045 mg/L and the EC_{20} (14-d) was 61mg/L in a hardness of 98mg/L.
Another study by Deleebeeck et al. (2007) used ten different species of cladocerans
to investigate whether or not species collected from soft water (<10mg/L $CaCO_3$) and
hard water (>25mg/L $CaCO_3$) environments were effected differently by Ni toxicity.
Organisms from soft water environments were tested in waters of hardness 6.25 and
16.3 mg/L CaCO3 while hard water organisms were tested using hardnesses of 16.3
and 43.4 mg/L CaCO3, respectively. Acute and chronic tests revealed that soft
water-collected cladocerans were not significantly more sensitive to Ni toxicity than
the hard water organisms. However, both groups exhibited protective effects as
water hardness increased (Deleebeeck et al. 2006). Lastly, Pyle et al. (2002) used
larval *P. promelas* to illustrate a five-fold increase in 4-d Ni LC_{50} with increases from
0.45 to 2.27 mg/L in water hardness of 20 to 140 mg/L CaCO3, respectively.

The last major competitive ion model to discuss is the biotic ligand model (BLM).
The BLM is an extension of two previously developed aqueous models of toxicity:
the gill surface interaction model and the free ion activity model (Di Toro et al.
2001). It was developed to help provide a better understanding of the complex nature
of aqueous metal-toxicity interactions. Essentially, the BLM is an equilibrium-based
model that quantifies the competition of ions for a site of action on an organism
called the biotic ligand (Di Toro et al. 2001, Paquin et al. 2002). The utility of the
model stems from the concept that it can be used to estimate the degree of metal
binding at the biotic ligand, and thus, ultimately predict a potential toxicological
response (Di Toro et al. 2001, Santore et al. 2001, Paquin et al. 2002). The BLM is
an extensive topic, and is beyond the scope of this paper. Therefore, I only make
mention of a few experiments related to Ni toxicity. Deleebeeck et al. (2008)

performed laboratory experiments to test the toxicity of aqueous Ni against various

concentrations of the competing ions Ca^{2+}, Mg^{2+}. They found that as the

concentration of Ca^{2+} and Mg^{2+} increased 21-d reproductive effects on *D. magna*

were decreased. To further improve the accuracy of the BLM for predicting chronic

effects of Ni to *D. magna*, they modified the model to include interactions with DOC

and the effects of pH (Deleebeeck et al. 2008). Kozlova et al. (2009) completed a

study to refine a BLM for waterborne Ni to *D. pulex*. In order to increase the

efficacy of the model they tested the effects of Ca, Mg, Na, K, Cl, pH, and NOM on

Ni toxicity. Ca and Mg had the strongest effect and thus reduced toxicity, but Na, K,

and Cl did not change Ni toxicity to *D. pulex*.

pH Dependence

pH values in sediments, porewater, and overlying water can affect Ni toxicity. Low

pH environments increase metal mobility because of the competition between

hydrogen and free metal ions. Therefore, hydrogen ions bind to sediment particles

increasing metal bioavailability (Nowierski et al. 2005). To the contrary, higher pH

values can deprotonate OC carboxylic and phenolic functional groups resulting in

increased metal binding sites and the partitioning of metals to the sediments (Doig

and Liber 2006b). Doig and Liber (2006b) demonstrated that change in pore-water

pH from 6 and 8 influences Ni sediment bioavailability by increasing complexation

and changing speciation. Pyle et al. (2002) illustrated that Ni LC_{50} values were

correlative to pH. *Pimephales promelas* LC_{50} values at pH 5.5 and 8.5 increased

from 0.69 to 2.21 mg/L, respectively. Jackson et al. (2005) used Ni contaminated

sediments from a pond and sub-sampled by adding DI water in a 1:1 –

sediment:water ratio. Next, the subsample was re-suspended, shaken for 16 h, and

the supernatant filtered through a 0.22 μm filter. Leachate contained high levels of

DOC ranging from 44-200 mg/L. These levels did not correlate to sediment organic carbon content, but these high levels suggested that any metals would be complexed to the DOC. However, the strength of binding has been shown to be influenced by pH. Christensen and Christensen (2000) showed that at pH 5-6, Ni would be present mostly as its free cation. Four different methods were implemented to study the binding of metal to DOM in the extracts. Flow field-flow fractionation (FI FFF), size exclusion chromatography (SEC), UV-absorbance (UVA), and inductively coupled plasma mass spectrometry (ICP-MS) were used because these combined techniques do a good job of discerning whether metals were bound to DOM or as free-labile forms . Using these techniques, the authors concluded that Ni was present as the free-cation or labile complex suggesting potential bioavailability. These findings were explained by the fact that all samples ranged in pH from 4.5 to 5.9 (Jackson et al. 2005).

Christensen and Christensen (1999) used leachate-polluted groundwater to measure the effects of pH on DOC-metal complexation. Laboratory experiments were conducted using pH ranging from 5-8, and DOC concentrations ranging from 79-187 mg C/L. All the metals tested (including Ni) illustrated an increase in DOC complexation as pH increased. This behavior was explained by the relationship between DOC's complexing abilities and the content of its carboxylic and phenolic functional groups. Therefore, influenced by acid-base chemistry, these functional groups greatly manipulate DOC's complexation capacity (i.e. it is pH dependent). Lastly, the laboratory results were compared with two speciation/simulation models: WHAM and MINTEQA2. WHAM predicted Ni-DOC complexation at the pH range of 5-7 within a factor of 3-4 times the measured amount of free ion activity. However, at pH 8 the model overestimated values that were highly inaccurate. In

comparison, MINTEQA2 predicted almost no relationship between DOC and pH suggesting that the model should not be used to predict pH dependent, DOC-metal complexation (Christensen and Christensen 1999).

Cantwell and Burgess (2001) looked at metal speciation in marine sediments and examined the effects of salinity, pH, and colloidal organic carbon (COC). In the pH experiments, colloidal partitioning of all the metals (Cd, Cu, Ni, Pb, and Zn) increased as pH increased. The rationale was that as pH increases it removes H^+ from humic acid molecules making them more negative (-) and increasing the amount of binding sites for metals.

Doig and Liber (2006a) studied the effects of DOM (quantified as DOC) on Ni bioavailability to *H. azteca* by looking for differences in DOM source (natural, made from peat) and speciation (humic acid or fulvic acid). They found that environmentally relevant DOC concentrations (0.6-30.4 mg/L) were not enough to significantly change Ni concentrations in acute LC_{50} tests. However, at sub-lethal Ni concentrations (200 and 500μg/L) surface water DOC concentrations significantly reduce Ni bioavailability independent of the source or fraction. Free Ni concentrations were significantly reduced in comparison to the control. Therefore, they claim that under fixed conditions, pH and hardness, that the Ni:DOC ratio plays an important role in Ni bioavailability and toxicity. The concentrations used for the acute test indicate that tissue-accumulated Ni was not affected by DOC suggesting that lower Ni:DOC ratios must be present to make a difference (i.e. the DOC concentration must be much higher). Even though elevated DOC concentrations (>30 mg/L) are rarely found in surface waters, Thurman (1985), found that DOC concentrations in sediment porewater often surpasses these levels (Doig and Liber 2006a).

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Hoang et al's (2004) objectives were to assess how NOM, hardness, pH, fish age, and alkalinity influence Ni toxicity to fathead minnows. Experiments were EPA 96 h static-renewal tests, and fish age was either < 1d or 28d. It has been reported that above pH 8, alkalinity may play a significant role in Ni toxicity because the dominant species is $NiCO_3$ (Hoang et al. 2004). Hoang et al. (2004) showed that without NOM, high hardness/alkalinity, and increased pH, Ni toxicity increased. When compared to the BLM, LC_{50} values were not accurately predicted for this scenario which suggests that $NiCO_3$ may be bioavailable under certain conditions. In regards to DOC, concentrations of 5-10 mg/L illustrated reductions in Ni toxicity of up to 50% (Hoang et al. 2004).

pH and Competing Ions

Zhou et al. (2005) used two models, surface complexation model (SCM) and the diffuse-layer model (DLM), to derive the complexation constant of Ni with humic substances in the presence of high levels of Ca and Al. They then predicted the bioavailability and speciation of Ni contaminated groundwater. Because humic substances are known to effect metal bioavailability (Boucher and Watzin 1999, Christensen and Christensen 2000, Di Toro et al. 2001, Chakraborty and Chakrabarti 2006, Doig and Liber 2006a, Butler 2009), the authors used the models to investigate how pH influences competitive binding to the functional groups (carboxylic and phenolic). Contaminated groundwater from a Department of Energy research facility contained Ni, Ca, and Al in concentrations of 10, 2000, and 500 mg/L, respectively. Based on these concentrations and the humic acid content of site groundwater, the data was used to calculate complexation constants and other variables needed for the models (calculations and formulas are beyond the scope of this review). Data showed that as pH increased Ni complexation to humic acid also increased. In

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conjunction, increased humic acid concentration also increased Ni complexation due to the availability of more binding sites. The competing ion (i.e., Ca) inhibited Ni binding, but effects were nullified when either the pH increased or humic concentrations increased to amounts high enough to alleviate competitive binding (Zhou et al. 2005). In contrast, Al had almost no influence on the complexation of either Ni or Ca with the humic substances. To conclude, Zhou et al. (2005) showed that when compared to field data the SCM and DLM could be useful tools when investigating metal-humic substances interactions of contaminated groundwater.

Ultraviolet Radiation

Winch et al. (2002) investigated the potential for increased metal toxicity to phytoplankton with artificial UV-B photooxidation of DOC in natural waters. Concern for adverse UV-effects stems from the fact that DOC complexes metals rendering them non-bioavailable, and toxicity to phytoplankton could have two consequences 1) loss as an energy source for higher trophic levels and 2) the potential for bioconcentration. Algal bioassays using the green algae, *Selenastrum capricornutum,* were performed with high and low DOC waters (30.3 mg/L and 4.4 mg/L, respectively) that were irradiated for 0, 5, and 10 days. Sample water was spiked with metals (Cu^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Co^{2+}, and Pb^{2+}) to represent a range of stability constants. Due to the expected photodegradation, DOC concentrations in both waters were decreased by approximately 20%. Interestingly, UV-B exposure of the high DOC water decreased the IC_{50} concentrations (i.e. increased toxicity) of all the metals except Ni and Cd. Winch (2002) indicates that the data for Cd may be a result of its low stability constant. However, based on Ni's stability constant it was expected that Ni toxicity would also increase. Upon further examination, it was determined (through the literature) that Ni exhibits slow ligand-exchange kinetics

which results in slow speciation equilibration time (Xue et al. 2001). This suggests that Ni^{2+} may not have had sufficient time to reach equilibrium with DOC before the bioassays were performed. In conclusion, changes in DOC concentration by UV-B photodegradation can change metal bioavailability to phytoplankton (Winch et al. 2002).

Winch and Lean (2005) conducted a set of experiments similar to Winch et al. (2002), but this time they included exposure via natural solar radiation and UV-A lamps for 20 days. UV-B and UV-A exposure reduced DOC concentrations by 20% and 24%, respectively. 20 day UV-B exposure significantly reduced algal bioassay IC_{50} concentrations (toxicity was increased) for Ni by 35%. The natural exposure produced similar toxicity results. When considering UV-A only exposures, IC_{50} concentrations actually increased (toxicity decreased) suggesting different effects of irradiation wavelengths on DOM. Winch et al. (2005) suggests that UV-A targets molecules of DOM that absorb longer wavelengths which in turn affects metal binding sites. Other factors affecting irradiation-based toxicity include initial DOC concentration, the source of the DOM, and the metal (Winch and Lean 2005). The authors also point out that the effects of solar radiation on phytoplankton and DOC in a natural environment would be influenced by source and stream flow. For instance, phytoplankton and DOC of surface waters in slow moving streams would experience the majority of the solar radiation exposure and impacts, but streams with fast flowing waters would see a constant mixing of water layers and thus UV exposure may have less of an impact on DOC (Winch and Lean 2005). More recently, Brooks et al. (2007) showed that in several Rocky Mt. streams, UV light altered the complexing capacity of dissolved organic matter for copper ions which increased the availability of Cu^{2+}.

Metal Toxicity Testing – Water Only Testing

Water-only (overlying water) testing uses standard toxicity testing organisms (e,g *Daphnia magna*) and exposes them to a contaminant of concern (CoC) for a short interval (24-96 h) to assess acute toxicity by measuring survival. Chronic tests (to evaluate long term effects) assess survival and sublethal effects of exposure using organisms such as *H. azteca*, *C. dubia* or *P. promelas* (Fairbrother et al. 2007). The organisms chosen for the specific test should be based on where the exposure is occurring and which molecular/physiological receptors are likely to be affected (e.g. Is the contaminant likely to bind to oxygen exchange receptors of epithelial tissue or are effects likely to come from ingestion?) (Fairbrother et al. 2007). Results are obtained in the form of point estimates of concentrations that cause specific effects, for example: the lethal concentration that results in 50% mortality (LC_{50}), the concentration that has an effect on 50% of the individuals being tested (EC_{50}), the concentration with no observable effects level (NOEL), or the concentration with the lowest observable effects level (LOEL). These values are useful as an estimate of toxicity to a single chemical, but they don't account for realistic scenarios of exposures with multiple stressors (e.g. multiple chemicals at the site). Also, just because a contaminant is found exceeding criteria at a site doesn't prove causality (Borgmann 2003). Milani et al. (2003) performed water-only tests using four benthic invertebrates (*H. azteca, C. riparius, Hexagenia spp.,* and *T. tubifex*) to assess sensitivity to Cd, Cu, and Ni. LC_{50s} and LC_{25s} were determined and exhibited variability. For Ni, the amphipod, midge, mayfly, and tubificid LC_{50s} ranged from 0.08 to 13 mg/L, 9 to 80 mg/L, 4 to 76 mg/L, and 61 to 67 mg/L respectively. When compared to field-collected sediment tests, species sensitivity did not correlate to the 96-h water-only exposure failing to support causality. However, *H. azteca* was

found to be most sensitive in both tests, and it was suggested that more than one target species should be used to detect the adverse effects of multi-contaminant exposure (Milani et al. 2003).

Metal Toxicity Testing - Sediment Testing/Theoretical Approach

Chronic sediment toxicity tests use indicator species such as *H. azteca, C. riparius,* and/or *C. tentans,* to measure endpoints such as survival, growth, reproduction or emergence. *L. variegatus* has been used to assess bioaccumulation by measuring tissue concentration and monitoring behavior (Fairbrother et al. 2007). Assessing risk from contaminated sediments can be achieved through sediment quality guidelines. They can be categorized into two types based on the approach taken to assess the risk: theoretical or empirical/correlative. A theoretical approach, equilibrium partitioning (EqP) is used to provide guidelines for assessing potential metal toxicity by assessing the partitioning of contaminants to organic carbon and SEM and AVS interactions. EqP is based on the principles that 1) predicted toxic effects of contaminants within sediments can be made by comparing the concentration of a contaminant in the porewater to known effects data from water-only exposures and 2) porewater concentrations can be estimated based on important binding phases (e.g. organic carbon) that influence partitioning (Boothman et al. 2001). This method has been shown to be predictive of non-toxic effects. For example, Burton et al. (2005) conducted a long-term field study that documented the effects of Zn contaminated sediments to benthic invertebrates. The model correctly predicted effects at four sites 92% of the time. When Boothman et al. (2001) used Ni-spiked sediments and benthic colonization trays, the SEM-AVS model correctly predicted the no-effect seen in their treatments. In conjunction, Carbonaro et al. (2005) modeled Ni flux from spiked sediments with oxygen-saturated overlying-

water and documented change using the SEM/AVS model. In the beginning, the SEM/AVS ratio was high and large amounts of pore-water Ni fluxed into the overlying-water. However, as time proceeded Fe oxidation created additional metal adsorption sites resulting in lower SEM/AVS ratios that correlated to the diminished flux of Ni.

Metal Toxicity Testing - Sediment Testing/Empirical Approaches

Empirical sediment quality guidelines compare the chemical concentration (total) of a CoC to effects seen in field or laboratory tests. These values are then referenced to criteria to develop biological risk associated with specific concentrations (Fairbrother et al. 2007, Accornero et al. 2008). Examples include threshold effect levels (TELs), probable effect levels (PELs) (MacDonald et al. 2000), and effects range median/low (ER-M, ER-L). Accorner et al. (2008) used TELs and ER-L to identify risk associated with Ni and other metals in lagoon sediments to identify which contaminants were most likely to have adverse biological affects and at which sites. To assess the toxicity of sediments below a mining operation, Allert et al. (2009) estimated risk associated with pore-water by calculating probable effect quotients (PEQs) for Ni, Pb, and Cd. PEQs were calculated by dividing total recovered metal concentrations by the probable effect concentration. Increased probability of a toxic effect was associated with PEQs ≥ 1.0. Ni had the strongest, negative correlation to *H. azteca* survival and growth. However, according to the three calculated risk indices, Pb should have been the leading contributor to toxic effects. This was explained by possible complexation of Pb by carbonates. According to Borgmann et al. (2003), TELs and PELs cannot define causality, but should be used to estimate the likelihood of toxicity. Therefore, they should be used to guide further lines of testing to establish bioavailability and the cause-effect relationship. To summarize,

empirical guidelines are quantitative standards that help predict the probability of toxic effects. Because they are based on correlations between specific contaminant concentrations and observed effects, they're often unable to define causality especially in situations with multiple stressors (MacDonald et al. 2000, Boothman et al. 2001)

Models to Predict Potential Ni Toxicity

SEM/AVS Model

The biological impact from metal contamination largely depends on which compartment the free metal resides and its partitioning. Metals can partition between sediment components (e.g. sulfide, organic matter, or Fe/Mn oxyhydroxides), porewater, and the biota. Models of simultaneously extractable metal (SEM) and acid-volatile sulfide (AVS) are used as a sediment quality guidelines to assess metal bioavailability and potential toxicity because pore-water metal is the available fraction that can cause biological effects (Boothman et al. 2001, Burton et al. 2005, Doig and Liber 2006b). The equilibrium partitioning model conveys the concept that the concentration of the metal will strive to be in equilibrium between the three compartments. In the model, SEM is the amount of metal that is removed during the cold acid extraction process to measure reactive sulfides (Burton et al. 2005). Under anoxic conditions and in the presence of a divalent metal, iron mono-sulfides in the sediment dissociate releasing Fe to the porewater allowing the more insoluble metal sulfide to form (Carbonaro et al. 2005). When the metal binds to sulfide it is no longer bioavailable and risk of toxicity is reduced. Conceptually, when the concentration of metals in the sediment is greater than the amount of sulfides available for binding/adsorption, some portion of the metals may be available and cause toxicity. In contrast, when the concentration of sulfides exceeds the

concentration of free metals, a majority of the metal will bind to the sulfides reducing

metal bioavailability and toxicity (Boothman et al. 2001, Burton et al. 2005).

Therefore, the SEM/AVS model is an important predictor of metal toxicity and

bioavailability in sediments.

∑SEM-AVS

When using the solid phase guideline it is important to consider factors that can

influence metal partitioning and availability to porewater. For example, when the

concentration of metals exceeds AVS levels it should not be automatically assumed

that toxicity will occur. It is also important to consider the magnitude of the AVS

binding capacity (Burton et al. 2005) by assessing the total amount of SEM (i.e.

∑SEM). It has been established that when ∑SEM-AVS < 0, pore-water metal

bioavailability should be decreased inferring low toxicity (Burton et al. 2005,

Vandegehuchte et al. 2007, Burton 2010). Utilizing benthic colonization trays spiked

with Cd, Cu, Pb, Ni, and Zn, Boothman et al. (2001) used the ∑SEM-AVS model to

assess toxicity of three groups of sediments with varying molar ratios of metal to

AVS. The group with the highest AVS concentration was predicted to have low

concentrations of interstitial metals (i.e. SEM-AVS<0). In contrast, groups with the

sediments having SEM-AVS >0 were predicted to have higher concentrations of

metals in the porewater resulting in biological effects. During the experiment, Ni

remained above detection limits for the first 27 days in the low AVS treatments while

all other metals were below the detection limit. By day 56 it fell below the detectable

limit. In the treatments where SEM>AVS, Ni was 2-3x above U.S.EPA marine

chronic water quality criteria (in the porewater) for the first 56 days. Thus, Ni was

following the predictions of the SEM and AVS model. However, at the end of the

119 day experiment, benthic colonization contradicted the model. No significant

differences were noted in species density or diversity across treatments. Chemical

analysis of the sediments showed that the top 1-2cm layer of each treatment had

roughly the same AVS characteristics: SEM-AVS<0. After further examination,

experimental artifacts were identified as the cause of the lack of biological effect.

ƒOC Normalized SEM/AVS Model

Metals can partition to other components of the sediment phase such as organic

carbon and Fe/Mn oxyhydroxides (Stead-Dexter and Ward 2002, Costello et al.

2011). Therefore, assessing metal availability should consider the total SEM by

evaluating the solubility of the metal of concern, and the partitioning of the metal to

non-AVS constituents like particulate organic carbon or Fe/Mn oxyhydroxides

(Boothman et al. 2001). Another model using AVS as a predictor of potential

sediment toxicity considers organic carbon (OC) as an alternative binding constituent

for metals. OC can bind free metals, reduce bioavailability, and can be used to

normalize SEM to the amount of OC (ƒOC) within sediments (Burton et al. 2005,

Doig and Liber 2006b, Vandegehuchte et al. 2007). Therefore, OC binding can

account for scenarios where SEM:AVS >1 and $\sum$SEM-AVS >0 occur without

resulting in acute metal toxicity (Doig and Liber 2006b). Burton et al. (2005) used

Zn colonization studies to measure the correlative effects of Zn toxicity and the

SEM-AVS model. 92% of the time the model correctly predicted macroinvertebrate

response. In conjunction, they used unpublished guidelines from Di Toro et al. to

predict acute and chronic toxicity using the normalized model: $\sum$SEM-AVS/ ƒOC.

According to Di Toro et al., there is a threshold that when there is an excess of

organic carbon over $\sum$SEM normalized to OC (100 to 150 mmol/g OC) metal

toxicity is reduced. Data obtained by Burton et al. (2005) supported this concept.

They found that sediment with 147.5 mmol/g OC was not chronically toxic, but

sediment showed toxic variability with concentrations between 147.5 and 154.4 mmol/g OC. Doig and Liber (2006b) used field sediments with varying levels of OC and AVS to evaluate Ni partitioning (sulfides vs. OC) over a range of pH values. In particular, sediments with high OC and low AVS were used to study Ni complexation. Toxicity tests (10-d sediment) using *H. azteca* and endpoints of survival and growth were compared to the prediction of toxicity based on ∑SEM-AVS/ fOC. Based on observed and predicted LC_{50}, LOEC, and NOEC values, mid to high AVS concentrations (27.87-44.05 μmol/g d.w.) overestimated the protective effects of OC and AVS (Doig and Liber 2006b). They concluded that because OC has an affinity to cover other inorganic coatings and a high cation exchange capacity, Ni complexation in surplus AVS could significantly affect Ni bioavailability and sediment toxicity. Lastly, Vandegehuchte et al. (2007) used the ∑SEM-AVS/ fOC model to test predictions of Ni toxicity to *L. variegates*. Biomass production was assessed with 28-d toxicity tests using sediments of varying OC and AVS concentrations, and spiked with total Ni concentrations ranging from 8-3664 mg/kg dry weight. Their results indicated that ∑SEM-AVS/ fOC were not accurate at predicting toxicity when assessing bulk sediments, most likely due to vertical variability. However, the model was accurate at predicting the magnitude of toxicity and Ni bioavailability when only surface layer sediments were evaluated. As previously mentioned, Costello et al. (2011) documented in Ni-spiked sediments deployed under field conditions that Ni partitioned to OC in the beginning of the experiment then shifted to binding to Fe and Mn oxides by the end of the experiment. Thus, indicating that OC and Fe/Mn oxides are important metal scavengers. Therefore, they suggested that Fe and Mn oxides be incorporated into the SEM and AVS models to help improve the assessment of potential toxicity from Ni contaminated sediments.

Purpose of the Study

To date, the majority of research that has been conducted on assessing Ni toxicity has been under laboratory conditions. Many of those tests occur in beakers, under unrealistic environmental conditions, and may not accurately elucidate the dynamics of Ni contamination. However, there are benefits to laboratory studies which include 1) the ability to control variables such as light, temperature, and D.O. concentrations 2) the ability to have many replicates to increase statistical power, and 3) the ability to eliminate other possible external stressors like high flow from storm events. On the other hand, performing field-based manipulative experiments provides a more realistic scenario where the organisms are subjected to not only the contaminated sediments, but also natural aquatic ecosystem variables such as diel cycling of oxygen, storm flows, pulses of increased turbidity, and daily temperature fluctuations. Therefore, the purpose of our experiment was to study the effects of Ni-spiked sediments under natural conditions for a more realistic assessment of Ni toxicity and bioavailability.

The sediments collected from two central Michigan streams had low OC content, moderate AVS, and moderate hardness. Therefore, we hypothesized that Ni-amended sediments would exhibit some toxicity. Also, waters at both sites were noticeably colored with dissolved organic matter giving the waters a dark, almost black appearance. Previous studies in the literature and one completed in the Burton laboratory by Cloran et al. (2010) indicated that under laboratory Ni toxicity testing, the addition of humate reduced Ni toxicity in exposures to *D. magna*. Consequently, we hypothesized that BC and LM would have elevated levels of dissolved organic carbon that under field conditions may be protective against Ni toxicity.

MATERIALS AND METHODS

Sample Sites

Our experiment was conducted in two streams located in central Michigan: Black Creek (BC) and Little Molasses River (LM). Both streams flow through the Gladwin State Forest in the Tittabawassee watershed with the sample sites located approximately 4.5 km apart. Black Creek is a direct tributary of the Tittabawassee River, and the sample site was located in a 2nd order reach. Little Molasses River is a tributary of the Molasses River, and the sample site was also in a 2nd order reach. The streams have similar hardness, alkalinity, pH, dissolved oxygen (DO), but sediments have varying levels of acid volatile sulfides (AVS) and dissolved organic carbon (DOC) (Table 1 and 2).

Table 1. Water chemistry (day 0) of the two streams used for Ni-spiked sediment experiments.

Stream	Latitude	Longitude	Hardness (mg/L CaCO₃)	DOC (mg/L)	Alkalinity (mg/L CaCO₃)	pH	DO (mg/L)
Black Creek (BC)	43°58'44.20"	84°19'9.02"	116.3	11.9	101.0	7.5	9.6
Little Molasses River (LM)	43°57'57.85"	84°15'58.73"	113.0	8.8	104.0	7.9	10.9

Table 2. Sediment chemistry (day 0) of the two streams used for Ni-spiked sediment experiments.

Sediment	Ni	Total Ni (mg/kg)	SEM$_{Ni}$ (μmol/g)	AVS (μmol/g)	OC (%)	SEM$_{Fe}$ (μmol/g dw)	SEM$_{Mn}$ (μmol/g dw)
BC	Ref	31.09	0.07	0.51	2.5	18.42	1.48
	Low	204.74	0.03	0.19	1.8	24.22	3.31
	High	1398.69	15.85	0.13	1.9	NA	NA
LM	Ref	32.14	0.13	0.04	1.6	11.70	1.26
	Low	216.93	2.01	0.01	1.3	7.72	0.93
	High	1333.64	25.63	0.01	1.3	17.53	1.19

NA = not applicable

Sediment Collection and Spiking

Removing the top 10 cm of sediment, we collected approximately 45 L of sediment from each stream. After returning to the lab (<2 h), the sediment containers were purged for 1 min with nitrogen gas to create an anoxic headspace prior to storage at 4 °C. Three treatments of each sediment type were used for the experiments: high nickel (~1500 mg/kg dry weight (dw)), low nickel (200 mg/kg dw) and reference (background nickel). Based on the desired final concentrations and calculated dry weights, we spiked the sediments by dissolving the appropriate amount of $NiCl_2 \cdot 6H_2O$ in 400 ml of deionized water, and gently mixing for approximately 30 seconds. The headspace of each bucket was purged with nitrogen gas and the containers were stored at 4 °C for a 10 day equilibration time. Sediment pH was measured after gently mixing the sediment every two days. After six days of equilibration, sediments were buffered with a small volume of 10M NaOH to maintain sediment pH within 0.2 units of the reference sediment. Following a 10-day equilibration period, sediments were deployed into their respective stream, and various parameters were measured at day 0, 14, 28, 56 (Figure 1).

Figure 1. Parameters that were measured after sediment samples from Black Creek (BC) and Little Molasses (LM) were spiked to 3 Ni concentrations – reference (no Ni, only water), low Ni (200 mg/kg dry weight) or high Ni (1500 mg/kg dry weight).

Deployment

Streamside, sediments from that stream were gently mixed and placed into mesh-lined plastic trays for benthic colonization (24.5 x 7.5 x 6.5 cm) and *in situ* toxicity tests (25.5 x 35.5 x 6.5 cm) (Burton et al. 2005). Three replicates of the colonization trays were deployed for each treatment for collection after 14, 28, and 56 days (n = 27 trays per stream). The *in situ* toxicity trays were filled with sediment and covered with nylon mesh bags (5-mm opening) to reduce sediment loss. Treatment baskets and trays were placed in the center of the stream channel in the following order starting upstream and working down: reference – low – high. Steel rebar and plastic-coated wire racks were used to fasten all trays to the streambed. At each time point stream physicochemistry was monitored: pH (water and sediment porewater), DO, temperature, and pore water ammonia. Each site was also continuously monitored (hourly sampling) with a multi-parameter sonde for turbidity, pH, temperature, and DO.

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Acute Toxicity Testing

At 0 and 14 d post-deployment, *in situ* toxicity chambers containing 10 *Hyallela azteca* (7-14 d old) (Burton et al. 2005) were deployed on each sediment treatment (Figure 2). *In situ* toxicity chambers consisted of transparent cellulose acetate tubing (7 cm in diameter) cut into 12.7 cm lengths with windows (8 x 4 cm) cut out of the tube on opposite sides and covered with 80 μm mesh to allow water flow-through. After placing *H. azteca* inside, the ends were sealed with removable polyethylene caps. The chambers were attached to a plastic coated wire rack with three chambers lying directly on the sediment to measure acute toxicity at the sediment water interface and three chambers suspended in the water column to measure any toxicity due to stream conditions. At the end of the 4-d exposure, the test chambers were removed from the trays and *H. azteca* were recovered and enumerated streamside. Laboratory and travel controls were used to assess culture health and handling stress, respectively; all control survival was >95%.

Figure 2. Underwater image showing the placement of the *in situ* toxicity chambers. One set of chambers was placed on the sediment/water interface and another set suspended in the water column.

Cotton Decomposition from Microbial Activity

Tiegs et al. (2007) originally developed the test as a surrogate to study decomposition rate in aquatic environments to overcome the variation that inherently comes with using natural leaf-litter for decomposition studies. Our application was to see if the test was sensitive enough to detect adverse effects from Ni treatment on the microbial community. From days 0 to 14, rates of microbial decomposition were measured with cotton strips (Tiegs et al. 2007), which were placed 2 cm below the surface of the sediment. After two weeks the swatches were removed, rinsed, and stored in 70% ethanol. Microbial cotton degradation was determined based on loss of tensile strength as a proportion of the reference tensile strength (Tiegs et al. 2007). The level of cotton decomposition is an indirect measure of microbial activity as less tensile strength loss is expected if the rate of microbial decomposition is slower.

Benthic Sampling, Sediment, and Water Chemistry Analysis

At 14, 28, and 56 d post-deployment, three replicate colonization chambers of each treatment level were removed to streamside where 2/3 of each basket was carefully placed into individual 1-L HDPE sample bottles and then filled with 95% ethanol. The remaining 1/3 from the three replicate trays was combined and homogenized for measurement of sediment chemistry: AVS, SEM, total metals, and organic carbon (OC). All samples were transported on ice back to the lab where AVS, SEM, and total metal samples were frozen while OC samples were kept at 4°C until analysis.

Water samples were collected from each stream at each sampling period with one sample placed into certified clean, amber, 250-ml glass bottles for dissolved organic carbon analysis (DOC), and a second sample taken in 1-L plastic sample bottles for

alkalinity and hardness determination. All samples were transported on ice and then were kept at 4 °C until analysis (hardness & alkalinity processed < 10 days). Immediately back at the lab, DOC samples were filtered through a 0.2 μm GD/X nylon membrane filter, acidified to pH 2 with 2.0 N HCl. Samples were analyzed using the non-purgeable organic carbon method (NPOC) on a Shimadzu TOC-V total organic carbon analyzer. Hardness and alkalinity (as $CaCO_3$) were determined through titration (Method 2340C and 2320B, respectively, (Greenberg 1995)).

Benthic invertebrate samples preserved in ethanol were passed through a series of three sieves down to 425 μm. Samples were sorted and invertebrates identified to family (Hilsenhoff 1975). Invertebrate community composition was summarized as taxa richness and abundance (family level), Chironomidae abundance, Ephemeroptera abundance, Gammaridae abundance, Shannon and Simpson's diversity indices. Community diversity indices have been extensively used in situations ranging from assessing effects from metal contamination (Burton et al. 2005, Kovecses et al. 2005) to measuring impacts from urbanization and stormwater events (Rochfort et al. 2000, Smith and Lamp 2008).

Digestion of sediment for analysis of total nickel, iron, and manganese was conducted using a CEM MARS 5 system (USEPA 1996). For quality control each digestion run contained deionized water blanks and a National Institute of Standards and Technology (NIST) certified Standard Reference Material (SRM 2702). After digestion, contents were filtered with 0.45 μm low binding, hydrophilic, polycarbonate membrane filters and diluted to 500 ml with milli-Q water. Total nickel, iron, and manganese were determined using a Perkin Elmer Optima 4300DV inductively coupled plasma optical emissions spectrophotometer (ICP-OES).

AVS was determined colorimetrically after purging and trapping H_2S from acidified sediment (1 M HCl) (USEPA 1991). The remaining acidified sediment sample was filtered through 0.45 μm low binding, hydrophilic, polycarbonate membrane filters and diluted to 250 ml with deionized water for determination of SEM. Samples were then analyzed on a Perkin Elmer ICP-OES to determine simultaneously extractable Ni, Fe, and Mn. OC was determined using a modified loss-on-ignition protocol where sediments were dried at 100 °C for 48 h. and then ashed at 440 °C for 6 h (USEPA, 2002). To account for water trapped in clay particles, ashed samples were re-wetted with deionized water and then dried for 48 h at 100 °C prior to re-weighing. The Redfield ratio was used to estimate the amount of C in organic matter (0.36), and loss-on-ignition data were corrected accordingly to estimate OC.

Concentrations of potentially bioavailable Ni were estimated using diffusive gradients in thin films (DGTs). DGTs contain a Chelex resin that binds labile metals and can be analyzed to measure rates of flux and estimate potentially-bioavailable metal fractions (Costello et al. 2012, Davison and Zhang 2012). DGTs were deployed at each collection day (in each treatment), and were in contact with the sediment for approximately 23 h (+/- 30min). After 23 h they were pulled from the sample trays, rinsed with deionized water, individually placed in clean plastic bags, and stored at 4 °C until analysis. DGTs were cut into 1 cm sections to obtain gel samples exposed to overlying water (within a 0 - 2 cm section above sediment) and two sections from the area below the sediment-water interface (top sediment layer 0 – 2 cm below and bottom sediment layer 2 – 4 cm below). The gels were dissolved in 1M nitric acid, diluted 10x, and the samples were then analyzed on a Perkin Elmer ICP-OES.

Statistics

All statistical analyses were completed using PASW Statistics 18. *H. azteca in situ* survival toxicity testing was examined with separate one-way ANOVAs for each exposure type, Ni concentration, day, and stream combination. For these ANOVAs, the alpha value was set to 0.1 because of the low power for each test. Estimates of Fe and Mn oxides ($FeO_x + MnO_x$) were calculated by determining the amount of SEM_{Fe} and SEM_{Mn} that were in excess of AVS ($[SEM_{Fe} + SEM_{Mn}] - AVS$) (Costello et al. 2011).

Two-way ANOVAs were used to assess OC, AVS, $FeO_x + MnO_x$, total Ni, Shannon, and Simpson indices between each stream and at each time point. Three-way ANOVAs using Ni treatment, stream, and time were also used to identify any invertebrate response between treatments. Any invertebrate community metrics showing a significant response to sediment Ni concentrations were regressed against our potential measures of Ni bioavailability (total Ni, SEM_{Ni}, SEM_{Ni}/AVS, SEM_{Ni}-AVS, $(SEM_{Ni}-AVS)/fOC$). During forward stepwise regression, each variable is individually entered into the regression equation. A statistical criterion for variable entry was $p < 0.05$. Standardized residuals were saved and data was checked for normality and equal variance with Shapiro-Wilk tests and Q-Q plots of standardized residuals. To meet the assumptions of linear models, predictor variables were log transformed and response variables were either arcsine square root transformed (i.e., % survival, microbial decomposition) or log + 1 transformed (Gammarus abundance). On the last sampling date (56 d) the low Ni treatment was missing from BC, possibly from a storm event or animal tampering.

RESULTS

Water Chemistry and Sediment Characteristics

Total Ni was not significantly different between streams (p = 0.899) or over time (p = 0.984) suggesting that our Ni treatments were stable during the experiment. In contrast, OC and AVS were significantly different between the two streams (p = 0.003 and p < 0.001, respectively), but did not change significantly over time within each stream (p = 0.276 and p = 0.368, respectively) (Appendix Tables A2-A5). Fe and Mn oxides were not significantly different between streams (p = 0.953) or over time (p = 0.249) (Appendix Tables A1-A4).

Percent recovery of Ni, Fe, and Mn from the NIST material was 90%, 90%, and 98%, respectively. Deionized blanks for Ni, Fe, and Mn analysis averaged 25.62 µg/L, 11.99 µg/L, and 23.45 µg/L, respectively. Calculations of the SEM and AVS models show that in many instances our Ni treatments have the potential to be toxic and, on occasion, even some reference conditions fit the theoretical thresholds for potential toxicity (see bolded numbers in Appendix Tables A1 – A4).

In situ H. azteca **Acute Toxicity**

Survival of *H. azteca* exposed to reference and low sediments at day 0 were >90% for both sediments (appendix Table A6). Exposure to high Ni sediments in BC caused a slight reduction in survival (p = 0.09), but a non-significant reduction in survival in LM (Figure 3). At 14 d, *H. azteca* survival was no longer affected by Ni treatment (Appendix Table A6).
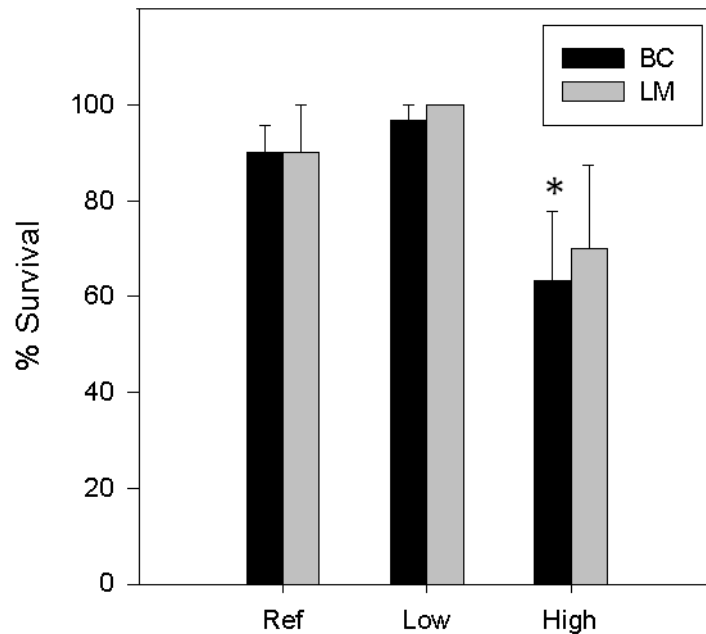
Figure 3. Mean survival (±1 SE) of *H. azteca* after 96-h exposure to contaminated sediments from Black Creek (BC) and Little Molasses River (LM) at day 0. *In situ c*hambers were placed at the sediment-water interface. Survival was measured as percent alive after 96 h. The high Ni treatment in BC was significantly lower than the reference (p = 0.089).

Cotton Decomposition from Microbial Activity

Cotton strips in reference sediments showed that decomposition was more rapid in BC than LM (Figure 4). BC cotton strips for both low and high treatments were significantly more decomposed than the control strip (p < 0.001) (Figure 4). In contrast, LM didn't show any significant differences between treatments (p = 0.14). This indicates that Ni-amended BC sediments were more toxic to the microbial community than Ni in LM sediments.
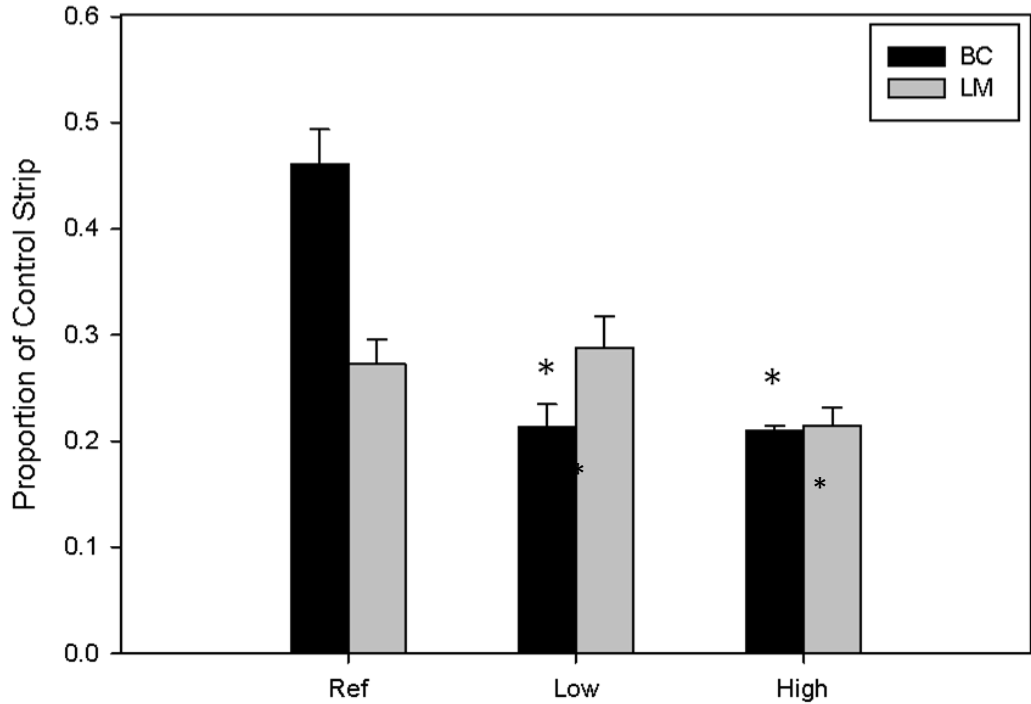
Figure 4. Proportion degradation from control strip (± 1 SE). Cotton swatches were placed 2 cm below the sediment surface and left in the field from day 0 to 14. Degradation was assessed based on loss of tensile strength relative to an unexposed control strip. Asterisks indicate significant differences between treatments and the control strip.

Benthic Indices

A total of thirty-six families were identified (Appendix Tables A6-A8). BC had a total of 27 taxa and 8 EPT taxa (Ephemeroptera, Plecoptera, and Trichoptera). LM had a total of 32 taxa and 13 EPT taxa. Chironomidae represented the most abundant taxa in both streams and comprised 54% of the total individuals. Neither the Shannon nor Simpson indices for BC or LM were significantly different between streams ($p = 0.642$, $p = 0.704$) or across Ni treatments ($p = 0.740$, $p = 0.663$)

(Appendix Table A7). Taxa richness did not significantly change over time (p = 0.220), and was not statistically different across Ni treatments (p = 0.934) (Appendix Table A7). Abundance of individuals was statistically different between streams (p = 0.001), but no differences were detected amongst the Ni treatments (p = 0.359). The total abundance of Chironomidae was different between streams (p = 0.001), but no difference was detected among treatments (p = 0.780). The mayflies (family Ephemeroptera) are often characterized as good water quality indicators, but were not significantly different between streams or Ni treatments (p = 0.90 and p = 0.447, respectively). Significant differences related to Ni treatment were only found for Gammaridae abundance. At day 14, effects from Ni treatment were seen for both BC and LM (p = 0.037, Figure 5). By day 28, effects due to Ni treatment were no longer observed.
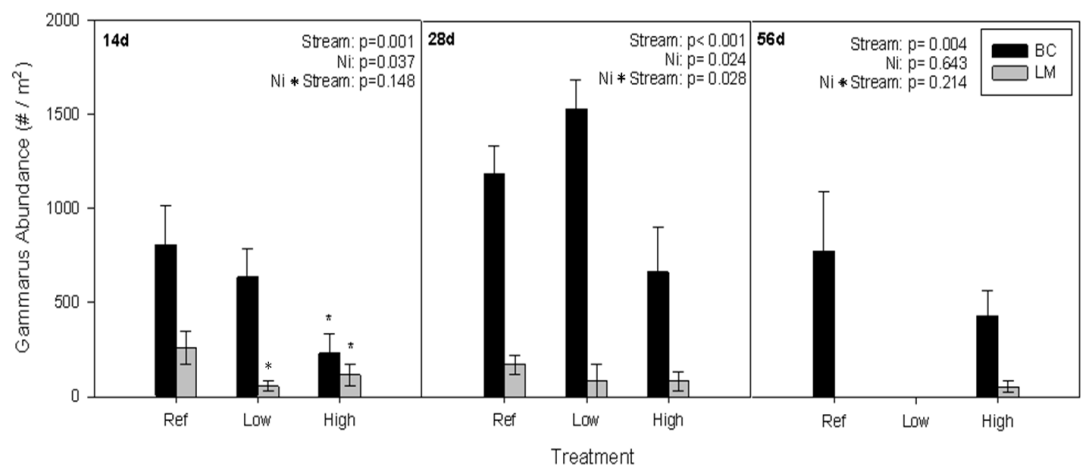
Figure 5. *Gammarus* abundance in both streams over all three time points. There were significant differences between the high and reference treatments at day 14 in both streams (*, Tukey p<0.05). However, significant effects diminished by 28 & 56 days. Note the large variation in *Gammarus* abundance between each stream irrespective of Ni treatment.

Nickel Flux

In both streams the DGT_{Ni} concentration in all three measured compartments declined through time (Figures 6-8). DGT_{Ni} concentrations in the water column and top sediment layer for BC declined faster than LM and were non-detectable by day 28 (Figure 6 and 7). The bottom sediment layer DGT_{Ni} concentrations declined less rapidly and were still detectable by day 56 for BC (Figure 8). However, many of the DGT_{Ni} samples were below detectable limits of the ICP-OES (Appendix Table A9).
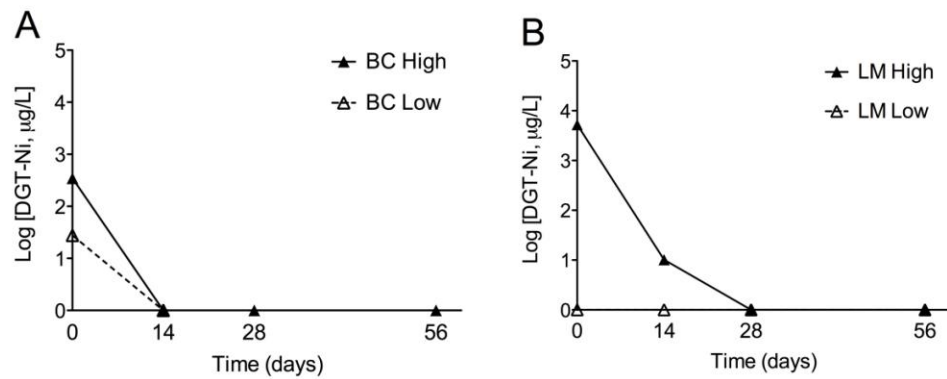
Figure 6. Water column DGT_{Ni} concentrations from day 0 to 56. Panel (A) shows Ni concentration at the DGT surface (CDGT) in BC and panel (B) shows the CDGT of Ni in LM. DGT_{Ni} was undetectable after day 14 in BC.
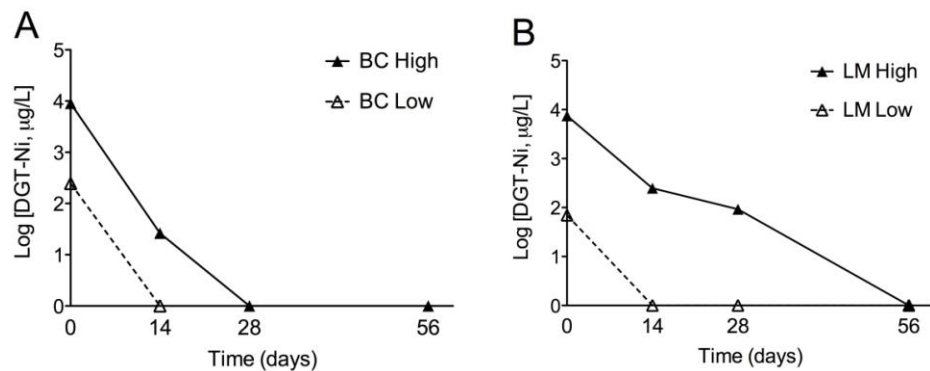
Figure 7. Surface sediment (1-2 cm) DGT_{Ni} concentrations from day 0 to 56. Panel (A) shows the CDGT of Ni in BC and panel (B) shows the CDGT of Ni in LM. DGT_{Ni} was undetectable after day 28 in BC.
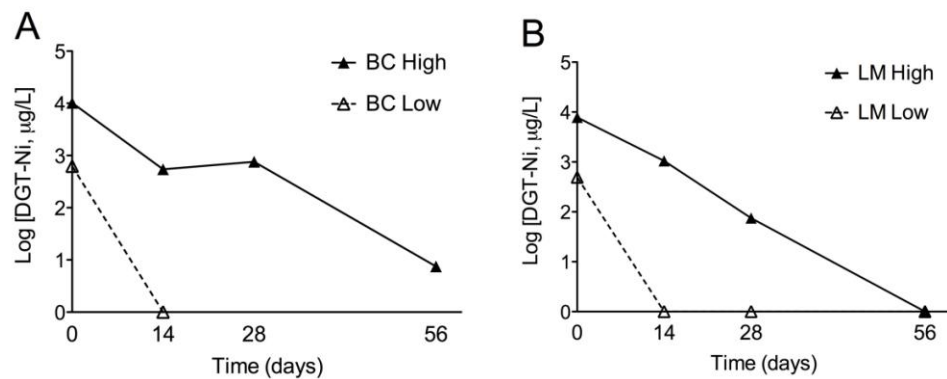
Figure 8. Deep sediment (2-4 cm) DGT_{Ni} concentrations from day 0 to 56. Panel (A) shows the CDGT of Ni in BC and panel (B) shows the CDGT of Ni in LM. DGT_{Ni} was undetectable after day 28 in BC.

Stepwise Regression and Gammaridae Abundance

Even though the streams had similar measured water chemistry, the differences in sediment chemistry (e.g. AVS, SEM_{Ni}, and OC) may influence Ni toxicity and Gammaridae abundance. Forward stepwise multiple linear regressions showed only one significant relationship from the eight metrics that were regressed. When regressing the metrics for each stream independent of the other, the SEM_{Ni}/AVS model of potential toxicity significantly predicted the relationship of Gammaridae abundance in BC ($r^2 = 0.859$, $p = 0.008$) (Figure 9). In contrast, none of the metrics were able to significantly predict Gammaridae abundance in LM (Figure 9).
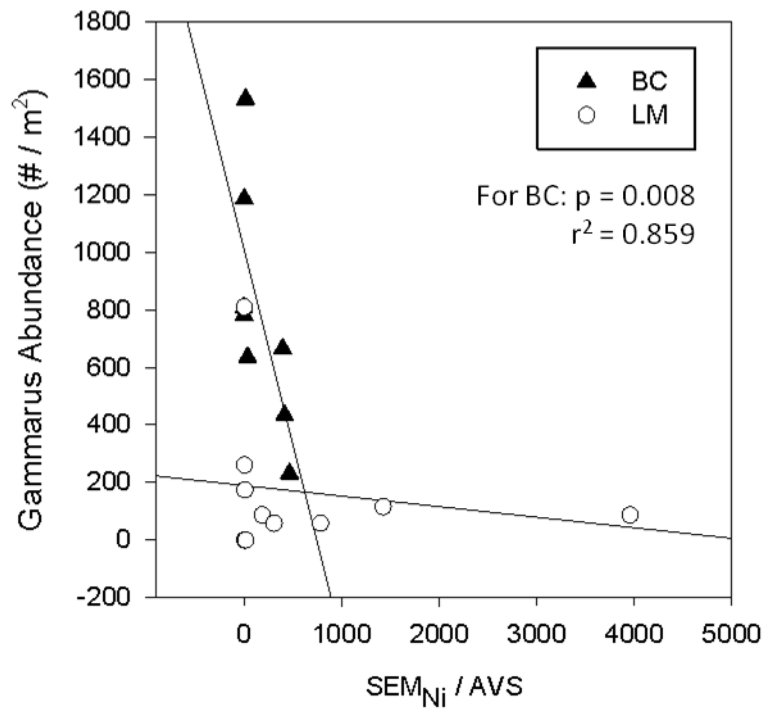
Figure 9. Stepwise multiple linear regressions showed that the SEM_{Ni}/AVS model was the best predictor of *Gammarus* abundance in BC. However, none of the variables could significantly predict *Gammarus* abundance in LM.

DISCUSSION

In this study we measured long-term macroinvertebrate re-colonization responses and microbial function in addition to the acute toxicity testing to perform a weight-of-evidence approach with multiple lines of evidence to increase confidence in our assessment and overcome some limitations of acute toxicity testing.

The model test species, *H. azteca,* has been utilized in both lab (Borgmann et al. 2001, Milani et al. 2003, Liber et al. 2011) and field studies to assess metal toxicity (Burton et al. 2005, Costello et al. 2011, Nguyen et al. 2011). The 96-h acute toxicity test finds utility in assessing short term, lethal effects that may arise from metals fluxing from sediments (Burton 2010). Two recent examples using *H. azteca* to assess Ni toxicity both *in situ* and under laboratory conditions were completed by Couillard et al. (2008) and Norwood et al. (2007). Couillard et al. (2008) showed that *H. azteca* bioaccumulated metals consistent with a contamination gradient based on distance from the point source. Laboratory studies done by Norwood et al. (2007) used *H. azteca* bioaccumulation to rank sorption capacities of seven metals simultaneously. In our sediment-water interface exposures, BC and LM exhibited decreased survivorship in the high Ni treatment. Reduced survival in BC when compared to LM was surprising because BC sediment contained more AVS and OC than LM sediment, which should result in a greater proportion of insoluble unavailable Ni. Even though there are inconsistencies in the survival data between the streams, BC demonstrated a significant trend of acute toxicity to *H. azteca* under our Ni treatments. Inexplicably, Ni amended sediments in LM were not acutely toxic to *H. azteca*.

Burton (Burton 2010) points out the importance of assessing acute metal toxicity with indicator species (and particularly indigenous species), but there are also some limitations that need to be considered when making risk assessment decisions. For instance, 1) studies often don't use indigenous aquatic species 2) organismal-based tests usually only assess the effect on one species and assume that the results can be extrapolated to other individuals in that ecosystem 3) the acute tests may not be able to account for other stressors within the ecosystem that may cause synergistic effects with the contaminant (Burton and Johnston 2010), and 4) acute tests often only look at survival as an endpoint and, therefore, are likely to miss sublethal effects (Burton 2010, Burton and Johnston 2010, Franzellitti et al. 2011). Another concern of acute toxicity testing results from the common practice of only using mortality as the endpoint assessment which may miss sub-lethal effects. For instance, molecular level effects are not adequately evaluated with 96-h acute toxicity test. Franzellitti et al. (2011) performed 96-h toxicity tests on mussels to assess the synergistic effects of Ni and the pesticide chlorpyrifos which are often detected together in aquatic ecosystems. They found that within the four day test gene expression levels and their protein products of the *MgPgp* and *MgMvp* genes were up-regulated to induce a physiological response that increases extracellular transport mechanisms that are capable of removing harmful xenobiotics (Franzellitti et al. 2011). Therefore, within our weight-of-evidence approach we have two more lines of evidence that support the trend of increased Ni toxicity in BC sediments: decreased microbial decomposition and negatively impacted *Gammarus* abundance (discussed below).

The benefits of the cotton degradation test are that it's relatively simple to conduct and it may be an indicator of reduced microbial function in the presence of contaminants. Similar to the 96-h acute toxicity test, BC showed evidence of being

more sensitive to Ni than LM. Both the BC low and high Ni treatments exhibited reduced cotton decomposition, which indicates that Ni had an adverse effect on the microbial community. The cotton degradation test measures microbial function and not the composition of the microbial community. A study by Van Nostrand et al. (2007) documented that riparian sediments that were contaminated with Ni had a microbial community that shifted to Ni-tolerant species, but they never assessed whether function of the community remained the same. We showed that the degradation test is effective at measuring impairment of the microbial community in aquatic systems that are under exposure to elevated levels of Ni.

The use of benthic indices to assess metal contaminated streams has been well documented (Clements et al. 2000, Burton et al. 2005, Costello et al. 2011, Nguyen et al. 2011). In this study Gammaridae abundance differed between the streams and responded negatively to sediment Ni in BC. The Ni effects on Gammaridae were not surprising because a previous field study by Costello et al. (2011) and bioaccumulation studies by Lebrun et al. (2011) demonstrated that *Gammarus spp.* are sensitive indicators to Ni and have a high capacity for Ni bioaccumulation in fresh waters. Interestingly, Ephemeroptera, which are generally considered sensitive water quality indicators (Savic et al. 2011, Li et al. 2012), didn't show any adverse effects from Ni treatment.

In an attempt to see which measured variables might explain the effects of Ni treatment on Gammaridae abundance, we regressed eight predictor variables (see Materials and Methods) using stepwise multiple linear regressions. In BC, the SEM_{Ni}/AVS model of potential toxicity was proved to explain the most variation in Gammaridae abundance, but none of the predictor variables significantly explained Gammaridae abundance in LM. It is difficult to explain why BC was adversely

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affected by Ni treatment, but LM was not. Parameters shown to protect against metal

toxicity such as AVS and OC were both higher on average in BC than in LM. In

addition, LM had more SEM_{Ni} which would indicate more bioavailable Ni in the high

Ni treatments. However, a few possibilities may be 1) stream flow may have

effected how quickly advective transport moved any excess aqueous Ni (a result of

the short calibration time) to the overlying water, and 2) the amount of sunlight

penetration at each deployment site may have altered exposure – the BC deployment

site had little to no canopy cover, but LM had a tree canopy which limited light

penetration to the stream. Unfortunately, flow and incidence of light were not

measured during this experiment. Increased stream flow has been shown to intensify

the resuspension of metal bound colloids (Grout et al. 1999), and according to Curtis

et al. (1985) smaller watersheds may experience greater impacts from storm flows

and the subsequently altered sediment flux processes. Ni mobility and concentration

have been shown to increase during natural processes such as wetland drainage

(Rothwell et al. 2007), snowmelt (Rember and Trefry 2004), storm events (Stead-

Dexter and Ward 2004), and groundwater upwelling (Greenberg et al. 2002). During

my literature review, no articles were found that examined the effects of stream flow

alterations on Ni toxicity (only general studies on metals and storm flow).

It has been documented that sunlight penetration into lotic waters has the ability to

alter copper bioavailability. Brooks et al. (2007) showed that in several Rocky Mt.

streams, UV light altered the complexing capacity of dissolved organic matter

(DOM) for copper ions which increased the availability of Cu^{2+}. Their model

demonstrated that photooxidation of DOM could alter the bioavailability of Cu^{2+}

within four days of ambient solar radiation. With the extensive literature search for

this project, not one article was found that investigated the effects of solar radiation

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on Ni bioavailability. Even though BC and LM had similar DOC concentrations, the DOC complexation capacity of BC may have been decreased because of the open canopy and UV light exposure. Therefore, the increased toxicity of BC sediments may have resulted from the reduced ability of DOC to bind any labile Ni.

Diffusive gradients in thin films (DGTs) have been used to determine Ni speciation and thus measure the labile fraction which is generally associated with bioavailability(Chakraborty et al. 2009, Warnken et al. 2009, Costello et al. 2012, Naylor et al. 2012). By day 14 DGT_{Ni} concentrations had rapidly declined in both BC and LM, but to a lesser extent in LM. This rapid change in partitioning of DGT_{Ni} may explain the response of *H. azteca* during the first 96-h acute toxicity test in BC (day 0 to 4). The slightly toxic effects to *H. azteca* may have originated from labile Ni that was rapidly fluxing out of the surface sediments into the overlying water. Even though DGTs are often utilized to assess bioavailable fractions of metals in freshwater systems, a recent study by Costello et al. (2012) revealed that DGTs overestimated the amount of available Ni, and that DGT_{Ni} concentrations did not accurately predict macroinvertebrate community response in the experiment. The community response was more accurately predicted by either total Ni or the $(SEM_{Ni}-AVS)/fOC$ model of potential toxicity. In our study, DGT_{Ni} concentrations were similar at the beginning of the experiment for BC and LM. However, if we assumed DGT concentrations as a measure of bioavailable Ni, the DGTs would have erroneously suggested toxicity in LM.

Our short spiking equilibration time may explain the amelioration of effects to *H. azteca* by day 14 and the lability of Ni as measured by the DGTs. Recent studies have shown that different Ni-spiking methods can significantly alter sediment chemistry with decreases in pH, increased conductivity, and increased aqueous metal

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concentrations that can influence the outcome of toxicity tests (Besser 2013, Brumbaugh 2013, Vandegehuchte et al. 2013) Brumbaugh et al. (2013) conducted laboratory experiments using two sediment spiking methods for Ni in eight different types of sediments containing various combinations of AVS and OC. They found that a slow spiking method using a super-spike over at least four weeks resulted in the most consistent pH and chemical characteristics across all treatments. In addition, Brumbaugh et al. (2013) suggests that because Ni has slow reaction kinetics with AVS, environmentally realistic Ni partitioning in sediments with high AVS and OC can only be achieved with an additional four to six weeks of equilibration.

CONCLUSIONS/FUTURE RESEARCH

BC and LM showed variable responses to Ni-spiked sediments, and for the most part we found patterns that were not in agreement with the recent Ni field studies of Costello et al. (2011, 2012). However, Ni-amended sediments in BC caused an adverse response in *H. azteca*, the microbial community, and Gammaridae abundance. Our experiment shows that models of Ni bioavailability may not be as universally applied as we'd expect. In addition, relying on measures of AVS and OC to predict Ni toxicity, and using DGTs to estimate bioavailable Ni could lead to erroneous assumptions. It is likely that there are other factors that may influence Ni toxicity *in situ* such as stream flow and exposure to UV radiation. In this experiment we also showed that the cotton degradation test is an effective measure of impairment to microbial community function under conditions of elevated Ni.

Any future Ni-spiked sediment experiments should follow the super-spike method for equilibration of the sediments as indicated by Brumbaugh et al. (2013) to limit the changes to sediment chemistry and develop more realistic environmental

concentrations. In addition, recent laboratory studies by Vandegehuchte et al. (2013) and previous work by Hutchins et al. (2007 and 2009) showed that inadequate equilibration time and direct spiking methods had adverse effects by altering sediment geochemistry, influencing the partitioning of metals, and the possibility of changing toxic effects on the test species. Other factors that need to be considered: 1) the precise deployment locations within the stream to reduce variability from dynamics related to flow (e.g. oxygenation and comparable habitat between the streams for benthic recolonization – certain invertebrates colonize based on flow requirement) and to seek locations with similar canopy cover, and 2) implement a sample design that increases the replicates for acute toxicity testing and include more than one test species – preferably use at least one indigenous species. Some interesting future studies would be to investigate 1) the role of solar radiation on Ni bioavailability, and 2) a fine scale study on the effects of stream flow on Ni bioavailability in the oxic sub-layer.

APPENDIX

Table A1. Geochemistry at Day 0.

Sediment	Ni	Total Ni (mg/kg dw)	SEM_{Ni} (µmol/g dw)	AVS (µmol/g dw)	SEM_{Ni}/AVS (µmol/g dw)	SEM_{Ni}-AVS (µmol/g dw)	OC (%)	SEM_{Ni}-AVS/fOC (µmol/g dw)	Tot Fe (mg/kg dw)	Tot Mn (mg/kg dw)	SEM_{Fe} (µmol/g dw)	SEM_{Mn} (µmol/g dw)	(FeOx + MnOx)-AVS (umol/g dw)
BC	Ref	31.09	0.07	0.51	0.14	-0.44	2.5	-6	4038.39	188.26	18.42	1.48	19.4
BC	Low	204.74	0.03	0.19	0.18	-0.16	1.8	-3	3492.33	167.58	24.22	3.31	27.3
BC	High	1398.69	15.85	0.13	**125.02**	**15.72**	1.9	**298**	4010.83	170.86	-0.36	-0.03	-0.5
LM	Ref	32.14	0.13	0.04	**3.63**	**0.09**	1.6	2	4708.29	165.98	11.70	1.26	12.9
LM	Low	216.93	2.01	0.01	**161.81**	**1.99**	1.3	54	5143.82	137.66	7.72	0.93	8.6
LM	High	1333.64	25.63	0.01	**1978.26**	**25.62**	1.3	**714**	4996.03	142.84	17.53	1.19	18.7

Bold values indicate the potential for toxicity

Table A2. Geochemistry at Day 14.

Sediment	Ni	Total Ni (mg/kg dw)	SEM_{Ni} (μmol/g dw)	AVS (μmol/g dw)	SEM_{Ni}/AVS (μmol/g dw)	SEM_{Ni}-AVS (μmol/g dw)	OC (%)	SEM_{Ni}-AVS/fOC (μmol/g dw)	Tot Fe (mg/kg dw)	Tot Mn (mg/kg dw)	SEM_{Fe} (μmol/g dw)	SEM_{Mn} (μmol/g dw)	(FeOx + MnOx)-AVS (umol/g dw)
BC	Ref	29.98	0.07	0.63	0.11	-0.56	1.4	-14.75	4320.36	232.76	23.85	2.62	25.8
BC	Low	198.89	2.26	0.06	**35.54**	**2.20**	2	39.03	3922.49	218.85	23.06	2.41	25.4
BC	High	1238.46	13.80	0.03	**466.26**	**13.77**	1.5	**340.95**	4282.16	174.94	21.81	1.60	23.4
LM	Ref	26.12	0.11	0.04	**2.93**	**0.07**	1.3	2.07	5314.75	179.92	17.50	2.13	19.6
LM	Low	197.29	9.77	0.01	**783.15**	**9.75**	1.1	**305.78**	5000.65	150.58	15.65	1.40	17.0
LM	High	936.13	12.25	0.01	**1423.71**	**12.24**	1.8	**241.87**	6163.03	168.14	18.56	1.53	20.1

Bold values indicate the potential for toxicity

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Table A3. Geochemistry at Day 28.

Sediment	Ni	Total Ni (mg/kg dw)	SEM$_{Ni}$ (µmol/g dw)	AVS (µmol/g dw)	SEM$_{Ni}$/AVS (µmol/g dw)	SEM$_{Ni}$-AVS (µmol/g dw)	OC (%)	SEM$_{Ni}$-AVS/fOC (µmol/g dw)	Tot Fe (mg/kg dw)	Tot Mn (mg/kg dw)	SEM$_{Fe}$ (µmol/g dw)	SEM$_{Mn}$ (µmol/g dw)	(FeOx + MnOx)-AVS (umol/g dw)
BC	Ref	26.65	0.09	0.12	0.79	-0.02	1.7	-1	4468.10	236.32	27.61	3.92	31.4
BC	Low	140.24	1.65	0.10	**16.14**	**1.55**	1.3	43	4272.90	229.97	22.77	2.94	25.6
BC	High	1160.56	16.32	0.04	**395.55**	**16.28**	1.6	**363**	4295.49	138.55	21.16	1.95	23.1
LM	Ref	26.27	0.13	0.02	**7.94**	**0.11**	1.3	3	5240.72	146.69	15.67	1.68	17.3
LM	Low	176.40	2.24	0.01	**183.06**	**2.23**	1.5	55	5751.84	147.01	17.00	2.07	19.1
LM	High	912.54	12.78	0.00	**3954.55**	**12.77**	1.0	**478**	6037.84	156.06	20.04	1.79	21.8

Bold values indicate the potential for toxicity

Table A4. Geochemistry at Day 56.

Sediment	Ni	Total Ni (mg/kg dw)	SEM_{Ni} (µmol/g dw)	AVS (µmol/g dw)	SEM_{Ni}/AVS (µmol/g dw)	SEM_{Ni}-AVS (µmol/g dw)	OC (%)	SEM_{Ni}-AVS/fOC (µmol/g dw)	Tot Fe (mg/kg dw)	Tot Mn (mg/kg dw)	SEM_{Fe} (µmol/g dw)	SEM_{Mn} (µmol/g dw)	(FeOx + MnOx)-AVS (umol/g dw)
BC*	Ref	28.02	0.08	0.38	0.20	-0.31	1.5	-7	2898.50	82.99	19.85	1.85	21.3
BC	High	867.02	13.50	0.03	**415.57**	**13.47**	2.2	**221**	4056.14	134.33	19.27	1.64	20.9
LM	Ref	44.26	0.09	0.03	**3.04**	**0.06**	1.2	2	21146.28	925.41	16.74	1.19	17.9
LM	Low	90.68	1.54	0.10	**15.50**	**1.44**	1.4	38	5312.98	130.98	13.21	1.14	14.3
LM	High	422.40	6.64	0.02	**307.42**	**6.62**	1.3	**180**	5578.24	136.42	15.05	0.98	16.0

Bold values indicate the potential for toxicity
* BC Low treatment missing from field

Table A5. *H. azteca* mean percent survival for *in situ* overlying water exposures.

		Ni Treatment		
Stream	Day	Ref	Low	High
BC	0	97	87	97
LM	0	57	97	73
BC	14	*	*	*
LM	14	63	†	67

* Only sediment/water exposures conducted because no toxicity was exhibited during the first exposure
† No low exposure because no effects were exhibited during the first exposure

Table A6. *H. azteca* mean percent survival for *in situ* sediment-water exposures.

		Ni Treatment		
Stream	**Day**	**Ref**	**Low**	**High**
BC	0	90	97	63
LM	0	90	100	70
BC	14	80	*	67
LM	14	63	*	67

* Low treatment not conducted because of lack of effects from first exposure

Table A7. Summary of benthic indices.

Sediment	Ni	Taxa richness	Abundance /m²	Shannon H	Simpson 1-D	Chironomids /m²	Gammarus /m²	Mayfly /m²
14d								
BC	Ref	8.0	5636	1.47	0.62	3295	809	867
	Low	7.7	4968	1.70	0.75	2168	636	1474
	High	7.7	5315	1.46	0.63	3093	231	1156
LM	Ref	10.0	6876	1.88	0.76	2861	260	694
	Low	9.0	3928	1.62	0.65	2226	87	376
	High	10.0	5350	1.86	0.71	2775	173	1301
28d								
BC	Ref	7.3	4596	1.48	0.67	2312	1185	87
	Low	8.0	9425	1.24	0.56	5983	1532	318
	High	8.0	5636	1.62	0.69	2890	665	462
LM	Ref	7.0	4075	1.37	0.61	2370	173	87
	Low	8.3	5922	1.43	0.61	3439	260	405
	High	7.3	1882	1.82	0.75	838	130	145
56d								
BC	Ref	9.7	9538	1.19	0.50	6619	780	260
	Low*							
	High	7.7	7656	1.11	0.49	5376	434	87
LM	Ref	4.3	4509	0.89	0.41	491	0	58
	Low	3.7	1041	1.27	0.64	231	0	29
	High	4.7	1275	1.26	0.61	202	87	0

*Missing from storm event or tampering

Table A6. Macroinvertebrate Identification at Day 14.

Family	Sediment Ni	BC Ref	BC Low	BC High	LM Ref	LM Low	LM High
Hydrachnidiae		1	0	0	4	0	3
Nematoda*		0	0	1	0	0	0
Enchytraeidae		1	2	0	0	0	0
Tubificinae		4	7	5	52	21	14
Pisidiidae		3	11	12	5	0	0
Physidae		1	0	0	0	0	0
Planorbidae		0	0	0	0	0	0
Gammaridae		28	22	8	9	2	4
Cambaridae		0	1	0	0	0	0
Baetidae		0	0	0	3	3	14
Baetiscidae		0	0	0	0	0	1
Caenidae		0	2	1	0	0	0
Ephemerellidae		0	0	0	3	0	0
Heptageniidae		0	0	0	0	2	4
Potamanthidae		0	0	0	15	3	14
Leptophlebiidae		24	27	20	2	4	2
Stonefly*		6	22	19	1	1	10
Perlodidae		0	0	0	0	2	0
Taeniopterygidae		0	0	0	0	0	0
Odonate*		0	0	0	2	0	0
Calopterygidae		2	0	1	1	1	1
Gomphidae		0	0	0	0	1	0
Belostomatidae		0	0	0	0	0	0
Limnephilidae		0	0	0	3	0	0
Psychomyiidae		0	0	0	1	0	0
Polycentropodidae		0	0	0	0	3	0
Hydroptilidae		2	0	0	0	0	1
Hydropsychidae		0	0	0	0	0	1
Elmidae		1	0	1	1	1	6
Hydrophilidae		0	0	0	0	1	0
Athericidae		0	0	0	4	0	1
Empididae		1	0	0	1	0	1
Ceratopogonidae		3	2	9	30	11	8
Chironomidae		114	75	107	99	77	96
Tipulidae		0	0	0	0	3	0
Tabanidae		4	1	0	2	0	4
Total Individuals		195	172	184	238	136	185

*Could not be identified to family

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Table A7. Macroinvertebrate identification at Day 28.

Family	Sediment Ni	BC Ref	BC Low	BC High	LM Ref	LM Low	LM High
Hydrachnidiae		0	0	1	0	3	2
Nematoda*		0	1	1	0	0	0
Enchytraeidae		3	0	0	0	0	0
Tubificinae		5	11	6	27	45	3
Pisidiidae		5	21	28	2	2	1
Physidae		0	0	1	1	1	0
Planorbidae		0	1	0	0	0	0
Gammaridae		41	53	23	6	3	3
Cambaridae		0	0	0	0	0	0
Baetidae		0	0	0	0	6	2
Baetiscidae		0	0	0	0	0	0
Caenidae		1	1	1	0	0	0
Ephemerellidae		0	0	0	0	1	0
Heptageniidae		0	0	0	0	1	1
Potamanthidae		0	0	10	0	4	0
Leptophlebiidae		2	10	3	0	2	2
Stonefly*		0	0	2	3	0	0
Perlodidae		0	0	0	0	0	0
Taeniopterygidae		0	0	0	0	0	0
Odonate*		0	0	0	0	0	0
Calopterygidae		0	0	1	0	0	0
Gomphidae		0	0	0	0	0	0
Belostomatidae		0	0	0	0	1	0
Limnephilidae		0	0	0	0	0	0
Psychomyiidae		1	0	0	0	0	0
Polycentropodidae		0	0	0	0	0	0
Hydroptilidae		0	0	0	0	0	0
Hydropsychidae		0	0	0	0	1	1
Elmidae		0	0	1	0	1	0
Hydrophilidae		0	0	0	0	0	0
Athericidae		0	0	0	0	1	0
Empididae		1	2	0	4	0	2
Ceratopogonidae		14	15	17	12	13	10
Chironomidae		80	207	100	82	119	29
Tipulidae		0	0	0	2	0	1
Tabanidae		6	4	0	2	1	8
Total Individuals		159	326	195	141	205	65

*Could not be identified to family

Table A8. Macroinvertebrate identification at day 56.

Family	Sediment Ni	BC Ref	BC Low	BC High	LM Ref	LM Low	LM High
Hydrachnidiae		0	0	0	0	0	0
Nematoda*		0	0	0	0	0	0
Enchytraeidae		1	0	1	0	0	0
Tubificinae		7	0	4	118	19	26
Pisidiidae		6	0	19	2	1	0
Physidae		0	0	0	0	0	0
Planorbidae		0	0	2	0	0	0
Gammaridae		27	0	15	0	0	2
Cambaridae		0	0	0	0	0	1
Baetidae		1	0	0	0	0	0
Baetiscidae		0	0	0	0	0	0
Caenidae		2	0	2	0	0	0
Ephemerellidae		0	0	0	0	0	0
Heptageniidae		0	0	0	0	0	0
Potamanthidae		2	0	0	1	0	0
Leptophlebiidae		3	0	0	0	1	0
Stonefly*		1	0	1	1	0	0
Perlodidae		1	0	0	0	0	0
Taeniopterygidae		0	0	1	0	0	0
Odonate*		0	0	0	0	0	0
Calopterygidae		0	0	0	0	0	0
Gomphidae		0	0	0	0	0	0
Belostomatidae		1	0	0	0	0	0
Limnephilidae		0	0	0	0	0	0
Psychomyiidae		0	0	0	0	0	0
Polycentropodidae		0	0	0	0	0	0
Hydroptilidae		0	0	0	0	0	0
Hydropsychidae		0	0	0	0	0	0
Elmidae		4	0	0	0	0	0
Hydrophilidae		1	0	0	0	0	0
Athericidae		0	0	0	0	0	0
Empididae		0	0	0	0	0	0
Ceratopogonidae		40	0	30	6	6	5
Chironomidae		229	0	186	17	8	7
Tipulidae		1	0	0	11	0	0
Tabanidae		3	0	4	0	1	3
Total Individuals		330	0	265	156	36	44

*Could not be identified to family

Table A9. DGT_{Ni} concentrations (µg/L) for BC and LM from 0 to 56 d.

Sediment/Day	Ni	DGT Section		
		Water Column	Top Sediment	Bottom Sediment
0 d				
BC	Ref	427.9	0	0
	Low	238.9	1199.7	3040.4
	High	2989.7	43648.5	49583.1
LM	Ref	0	0	0
	Low	0	335.5	2380.3
	High	44868.7	36069.7	37642.2
14 d				
BC	Ref	0	0	0
	Low	0	0	0
	High	0	112.9	2330.7
LM	Ref	0	0	0
	Low	0	0	0
	High	79.7	1098.2	4647.8
28 d				
BC	Ref	0	0	0
	Low	-	-	-
	High	0	0	2705.3
LM	Ref	0	0	0
	Low	0	0	0
	High	0	345.5	279.2
56 d				
BC	Ref	0	0	0
	Low	-	-	-
	High	0	0	25.3
LM	Ref	0	0	0
	Low	0	0	0
	High	0	0	0

Zeroes interpreted as below detection limit of ICP-OES
- Data missing

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