Toxicity of nickel-spiked freshwater sediments to benthic invertebrates: Spiking methodology, species sensitivity, and nickel bioavailability

By

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Abstract

This report summarizes data from of studies of the toxicity and bioavailability of nickel in nickel-spiked freshwater sediments. The goal of these studies was to generate toxicity and chemistry data for development of broadly-applicable sediment quality guidelines for nickel. The studies were conducted as three tasks, which are presented in three chapters: Task 1, Development of methods for preparation and toxicity testing of nickel-spiked sediments; Task 2, Comparison of the sensitivity of benthic invertebrates to toxicity of nickel-spiked sediments; and Task 3, Evaluation of the influence of sediment characteristics on nickel bioavailability. Additional details about the methods for the three Tasks and compilations of raw chemistry and toxicity data are available online at [insert persistent URL].

Task 1 compared three spiking methods: Direct (direct addition of aqueous Nickel solution to sediment at target Nickel concentrations); Indirect (direct spiking of high-Ni 'super-spike' sediments, followed by dilution with un-spiked sediment to target Nickel concentrations); and Indirect+Iron (indirect spiking of nickel plus equi-molar concentrations of ferric chloride or ferrous sulfide -- to oxidized or reduced sediments, respectively). All sediments were pH-adjusted after spiking and were equilibrated under anaerobic conditions. Studies in Task 1 also varied the length of the equilibration period and the rate of replacement of overlying water in sediment toxicity tests. Results were evaluated on the basis of the stability of sediment characteristics (for example, acid-volatile sulfide or AVS); distribution of nickel among sediment, pore-water (PW) and overlying water; and toxicity of spiked sediments. The methods selected for subsequent studies were: indirect spiking; minimum 10-week anaerobic equilibration followed by one week of equilibration with aerobic overlying water in toxicity test chambers;

and a high rate of replacement of overlying water (8 volume-additions/day) during the pre-test and toxicity testing periods.

Task 2 evaluated the relative sensitivity of invertebrate taxa to toxic effects of two nickel-spiked sediments: sediment from the Spring River, Missouri, which had low concentrations of the important metal-binding components, total organic carbon (TOC) and acid-volatile sulfide (AVS), and sediment from West Bearskin Lake, Minnesota, which had high TOC and high AVS. Eight taxa were tested in flow-through sediment exposure systems with automated replacement of overlying water: the amphipods, *Hyalella azteca* and *Gammarus pseudolimnaeus*; the midges, *Chironomus dilutus* and *C. riparius*; the oligochaetes, *Lumbriculus variegatus* and *Tubifex tubifex*; a mayfly, *Hexagenia* sp.; and a freshwater mussel, *Lampsilis siliquoidea*. These tests lasted at least 28 days and included multiple chronic toxicity endpoints (including survival, growth, and biomass for all eight taxa; adult emergence and egg production for *Chironomus* spp.; and number of offspring for *Hyalella* and *Tubifex*) to determine the most sensitive responses of each species. The nematode, *Caenorhabditis elegans*, was tested in small test chambers without water replacement, with endpoints of survival and production of larvae. Water-only nickel toxicity tests with these species were also conducted to aid in interpreting results of sediment tests.

Results of sediment toxicity tests were used to estimate chronic toxicity values (10% and 20% effect concentrations; EC10s and EC20s) for sediment nickel (total-recoverable Nickel concentrations or TR-Ni). Reliable toxicity values were generated for four species in the Spring River sediment and for seven species in West Bearskin sediments. Toxicity values from one flow-through test (*Gammarus* in Spring River sediment) were flagged due to low control survival and several other tests did not produce significant toxic effects. Static tests with

Caenorhabditis also did not allow reliable comparisons with other taxa, due to low control survival in some sediments and high Nickel concentrations in overlying water. The taxa most sensitive to toxicity of nickel-spiked sediments were *Hyalella*, *Gammarus,* and *Hexagenia*. Toxicity values for TR-Ni were consistently lower for Spring River sediment than for West Bearskin sediments, with lowest EC20s (for *Hyalella* biomass) of 202 µg/g in Spring River sediment and 1177µg/g in West Bearskin sediment. Lowest TR-Ni EC10s (for the same endpoint) were 131 µg/g and 855 µg/g, respectively.

In Task 3, the three most sensitive taxa (plus *Tubifex*) were tested with six additional sediments that made up a gradient of physicochemical characteristics, including AVS, TOC, and particle size distribution. Nickel distribution coefficients (Kd = concentration in sediment/concentration in pore-water) differed by more than a factor of ten among the sediments tested, suggesting a similar wide range of nickel-binding capacity. The endpoints, *Hyalella* survival, *Gammarus* survival, and *Hexagenia* growth, were selected to evaluate differences in Nickel bioavailability among the eight sediments tested in Tasks 2 and 3, based on their sensitivity and low variability. For all three taxa, toxicity values based on TR-Ni differed greatly among sediments. Toxicity values for TR-Ni had significant positive correlations with AVS for *Hyalella* and *Gammarus*, but not for *Hexagenia*. Toxicity values based on sediment nickel concentrations normalized to AVS (or AVS and TOC) did not have substantially lower variation among sediments, but toxicity values based on PW-Ni had lowest among-sediment variation, especially for the two amphipods. Toxicity of nickel-spiked sediments to the amphipods, *Hyalella* and *Gammarus,* was consistent with the hypothesis that AVS is a primary control on PW-Ni concentrations and on toxicity of nickel in sediments. For these taxa, nickel-spiked sediments were not toxic if nickel concentrations were less than AVS concentrations on a molar

basis. In contrast, toxic effects on the burrowing mayfly *Hexagenia* occurred in several sediments with Nickel concentrations that were less than the theoretical AVS binding capacity. These divergent results could indicate that AVS does not strongly control nickel bioavailability to *Hexagenia*, perhaps because ingestion of sediment particles was an important route of nickel exposure for this species. Alternatively, it is possible that our sampling methods did not adequately measure localized concentrations of AVS and/or PW-Ni in the burrows inhabited by *Hexagenia*.

Chapter 1: Development of methods for preparation and toxicity testing of nickel-spiked freshwater sediment

1.1 Introduction

Recent studies have identified technical problems associated with preparation and testing of sediments spiked with nickel, which are related to the low binding affinity and slow equilibration kinetics of Nickel with sediment, compared to other toxic metals (Simpson and others, 2004). For example, a recent study of the toxicity of nickel-spiked sediment to oligochaetes (Vandeguchte and others, 2006) was unable to estimate realistic toxicity thresholds for Nickel in sediment because toxic concentrations of nickel accumulated in overlying water during whole-sediment toxicity tests. This problem apparently resulted from a combination of high concentrations of nickel in pore-water (due to incomplete equilibration with sediment or spiking levels that exceeded sediment binding capacity) and low rates of replacement of overlying water. Accumulation of high nickel concentrations in overlying water would not be expected in either lotic or lentic ecosystems, due to rapid dispersal and/or dilution of aqueous nickel by large volumes of overlying water.

These findings indicate that care is required to achieve stable and environmentally realistic partitioning of nickel in spiked sediments. Simpson and others (2004) demonstrated that Ni spikes required a relatively long time for equilibration with sediment: up to 70 d, compared to 15 d for copper, 40 d for zinc, and 45 d for cadmium. This time required for equilibration reflects the natural rates of incorporation of metals into various solid phases, and these rates may be affected by several aspects of spiking methodology, notably control of pH and redox. Addition of

aqueous metals to sediments typically results in decreases in pH due to hydrolysis reactions of metal ions, and acid conditions inhibit sorption of nickel and other metals to sediment particles. Additional acidity may also be generated during spiking procedures by increased rates of oxidation of ferrous iron. Thus, equilibration of nickel to sediment particles may be enhanced by controlling pH and maintaining anaerobic conditions in spiked sediments (Simpson and others, 2004). A two-step ('indirect') spiking methodology, with metal-spiked sediments diluted with unspiked sediment to achieve targeted sediment nickel concentrations, has been suggested to produce more realistic nickel partitioning by providing additional binding sites for spiked nickel while reducing disruption of pH (Hutchins and others, 2008). Hutchins and others (2007) also recommended that metal spiking strategies should consider the prevailing redox conditions of the sediments and the resulting differences in the geochemistry of iron (Fe). For oxidized or partially reduced (sub-oxic) sediments, particulate organic matter and oxides of ferric iron and manganese are assumed to be the primary metal-binding components, whereas in highly reduced sediments, organic matter and amorphous sulfides (primarily ferrous sulfide, the primary constituent of acid volatile sulfide or AVS) are assumed to most affect metal binding (USEPA 2005). Carbonaro and others (2005) attributed large initial fluxes of soluble nickel from spiked sediments into overlying water to high pore-water Ni concentrations due to because insufficient ferrous sulfide or other metal-binding constituents were available to effectively bind the added nickel. This explanation suggests that addition of appropriate Fe solutions along with nickel spikes may generate fresh metal-binding phases of either hydrous Fe iron oxides or Fe iron sulfides to enhance binding of spiked nickel to sediment particles.

In addition to appropriate spiking methods, care must also be taken to ensure environmentally realistic partitioning of nickel among sediment, pore-water, and overlying water

in laboratory sediment toxicity tests. The transition of nickel-spiked sediment from anaerobic equilibration containers to toxicity test chambers with aerobic overlying water necessarily involves development establishment of a redox gradient, typically including an oxidized layer on the sediment surface. During this transition, rapid fluxes (diffusive losses) of nickel from pore-water to the overlying water is likely to occur whenever there is strong nickel concentration gradient between the pore-water and overlying water. Toxicity test systems for nickel-spiked sediments should be designed to prevent accumulation of unrealistically high nickel concentrations in overlying water, either by dilution in a large volume of overlying water (e.g., Borgmann et al 2001) or by frequent replacement of overlying water.

The goal of Task 1 was to develop methods for spiking freshwater sediments with nickel and for conducting whole-sediment toxicity tests with benthic invertebrates. Specific objectives of Task-1 studies were:

1. Evaluate spiking and equilibration methods to establish stable and environmentally-realistic partitioning of nickel between sediment and pore-water (PW).
2. Evaluate rates of replacement of overlying water (OW) needed to avoid development of high concentrations of nickel in the overlying water that could influence results of sediment toxicity tests.
3. Evaluate the effects of spiking treatments and water-replacement rates on toxicity of nickel-spiked sediments to the amphipod *Hyalella azteca*.

Task 1 evaluated three spiking methods (Direct, Indirect, and Indirect+Iron) during a 16-week equilibration period by characterizing of the distribution of nickel between pore-water and sediment, quantifying the fluxes of nickel from sediments to overlying water during toxicity

testing, and evaluating the toxicity of spiked sediments to *H. azteca*. We evaluated the spiking methodologies based on the following criteria:

- Water-sediment partitioning of nickel during equilibration: How much time was needed for equilibration? How much of the spiked nickel was retained by the sediment? Did the spiking method alter the native sediment characteristics? Did nickel partitioning in spiked sediments resemble that observed in field-collected sediments?
- Nickel partitioning during toxicity testing: Was nickel released into overlying water at concentrations that could influence the outcome of the sediment toxicity tests? Were pore-water nickel concentrations consistent for the duration of tests?
- Practical Considerations: Was the method technically straightforward and reproducible? Was the method successful over wide range of sediment types and nickel exposure concentrations?

1.2 Methods

Sediment selection

Sediment spiking studies were performed with two base sediments with very different physicochemical characteristics (Appendix 1-1). These sediments were known to have low background concentrations of nickel and other chemicals of concern. The Spring River (SR) sediment (SR: mean TR-Ni=7.2 µg/g) was collected from the upper Spring River in southwest Missouri, USA (Ingersoll and others, 2008). This sediment was chosen because it had low concentrations of AVS (<1.0 µmol/g) and organic matter (TOC < 1%) and was expected to have a low binding capacity for nickel. The West Bearskin sediment (WB TR-Ni=52 µg/g) sediment

was collected from West Bearskin Lake in northeast Minnesota, USA (Ingersoll and others, 1998). The WB sediment was chosen because it had high concentrations of both AVS (>40 µmol/g) and TOC (10%), and was expected to have a high binding capacity for nickel. Sediments were collected in fall 2008 and stored in the dark at 4 °C in sealed 21-L polyethylene buckets. Portions of each sediment from multiple containers were combined and homogenized with a stainless steel auger before Task-1 spiking studies were conducted in early 2010.

Spiking methodologies

Experimental treatments for evaluating sediment spiking methods are summarized in Table 1. All reagents were deoxygenated with nitrogen just before spiking. The SR and WB sediments were each spiked with two levels of nickel to produce high and low nickel concentration for evaluating each of the three different spiking methods. Pre-cleaned glass jars (3.8-L) with tetrafluoroethylene (TFE) lined lids were used to prepare and equilibrate all spiked sediments.

Direct spiking. Aqueous nickel was added directly (as $NiCl_2$) to 3-L portions of each wet sediment in glass jars at high and low concentrations. At the same time, a 10 N NaOH solution was added to maintain target pH (7.3 ±0.2), based on results of pilot studies. Contents of each jar were homogenized with a stainless steel paint-mixing blade, the headspace was purged with nitrogen, and jars were capped and placed in a darkened water bath at about 20 °C. During the first four weeks of equilibration, the pH of each spiked sediment was measured with a mini-electrode and adjusted by additions of NaOH or HCl as needed. After each pH adjustment, sediments were homogenized, purged with nitrogen, sealed and returned to the water bath. After four weeks, jars remained sealed and sediments were held in the dark under anaerobic conditions

for 12 weeks, with biweekly mixing (1 hr @ 20 rpm) on a rolling mill. Although direct spiking was the most straightforward approach tested, this method had several drawbacks, including the likelihood that several pH adjustments would be needed for each nickel spike concentrations to avoid unrealistically high PW-Ni and OW-Ni concentrations.

Indirect spiking. The Indirect spiking treatment involved two steps. Initially, aqueous nickel was added at a high concentration to 3-L portions of each sediment (termed 'super-spikes'), which were treated the same as the Direct spike sediments for the first four weeks. After four weeks, super-spikes were diluted with larger volumes of un-spiked sediment (with no pH adjustment) to produce targeted nominal high and low nickel concentrations, then equilibrated for 12 weeks as described above. This method was similar to the approach described by Hutchins and others (2008). Indirect spiking was intended to produce more environmentally realistic pore pore-water metal concentrations and this method also had the practical advantage that pH adjustment was required only for one superspike for each base sediment. A possible disadvantage of indirect spiking is that the high Ni concentration required for the super-spike might exceed the adsorption capacity of the sediment.

Indirect spiking plus iron. This treatment was the same as the Indirect treatment, except that the superspikes were spiked simultaneously with equimolar quantities of nickel and iron. Iron was added to the low-AVS SR sediment as ferric chloride, which was expected to precipitate as hydrous ferric oxides. Iron was added to the high-AVS WB sediment as equimolar mixtures of ferrous chloride and sodium sulfide, which was expected to precipitate as ferrous sulfide. Equilibration jars for the Indirect+Iron treatments with the SR sediment were opened to the atmosphere after day 96 to allow precipitation of ferric hydrous oxides before the third toxicity test. The equimolar ratio of nickel and iron in the Indirect+Iron treatment was intended

to ensure the presence of substantial amounts of labile iron hydrous oxide or iron monosulfide for binding nickel, while avoiding potential effects or larger amounts of iron on pH and toxicity. Maximum iron amendments represented only about 2% (WB sediment) to 6% (SR sediment) of the iron present in the base sediments, but the maximum sulfide amendment for WB was about 41% of the native AVS concentration in that sediment. The combination of the equimolar FeS addition plus the native AVS would be predicted to completely bind all added nickel (USEPA 2005). However, the efficacy of AVS for binding nickel may be lower than for other metals, as suggested by the higher solubility of nickel sulfide relative to other metal sulfides and by results of a previous nickel-spiking study (Carbonaro and others, 2005).

Four control sediments were prepared without nickel spikes. Portions of each control sediment were carried through the Direct spiking procedure and the Indirect+Iron procedure (at the highest Fe iron level for each sediment). In addition, a sediment presumed to be contaminated with nickel was collected from Lake Petit Pas (LPP) in Ontario, Canada and included in the study. This sediment was not spiked and was treated the same as the control sediments because it was intended to serve as an example of nickel partitioning in an unspiked natural sediment. However, the LPP sediment was non-toxic and had relatively low nickel concentrations. Data from the LPP samples are presented in the Appendices, but these results were generally not relevant to the spiking studies and are only minimally presented and discussed in the text.

Chemical Analyses.

Samples of sediment and water were collected for chemical analysis during equilibration and during toxicity tests according to the sampling plan summarized in Appendix 1-2). During

the equilibration period, sediments were sampled at four-week intervals that corresponded to starting dates for three sets of 21-d toxicity tests. Sediment nickel concentrations were analyzed in three fractions: total recoverable (TR-Ni; USEPA 2007a; Brumbaugh and May 2008), simultaneously-extracted (SEM-Ni; USEPA 1996) and pore-water (PW-Ni), which was sampled with 'peeper' diffusion samplers (Brumbaugh and others, 2007). The digestion procedure used for TR-Ni determinations in sediments is similar to USEPA method 3051A; it includes addition of equal volumes of concentrated nitric and hydrochloric acids followed by, and microwave heating. The method has been termed "total-recoverable" because it is a relatively aggressive oxidative dissolution procedure, but it does not yield a complete solubilization of all elements, especially of iron and aluminum, as well as any fractions of other elements that are tightly bound within lattices of silicates and other refractory minerals (USEPA, 2007). Based on information in USEPA method 3051A, as well as results for various certified reference soils and sediments obtained by our laboratory, recovery for this type of method typically is greater than 80% for most trace metals, including Cd, Co, Cu, Pb, Ni, and Zn. Bettiol and others (2008) conducted comparative digestion studies of sediments and who concluded that microwave-assisted digestion using nitric acid alone provided good estimates of most total metal concentrations.

Samples of pore-water from bulk sediment (extracted by centrifugation for 15 minutes at 7400 g) were analyzed for Ni, dissolved organic carbon, (DOC), major cations, and major anions. During toxicity tests, nickel concentrations in overlying water (OW-Ni) were analyzed weekly or bi-weekly and pore-water samples were collected from test beakers on days 7 and/or 21 for analysis of PW-Ni using "peepers" (in-situ dialysis chambers) equilibrated in sediment for about 7 days). Peepers were fabricated from acid-cleaned, 2.9-mL polyethylene vials, each filled with de-oxygenated, de-ionized water and fitted with a 0.45 µm pore-size polyethersulfone

membrane. Sediments from selected test beakers were analyzed for TR-Ni. In addition (in high-nickel treatments only), vertical gradients of aqueous nickel in overlying water and in pore-water (at three depth strata: surface to -0.5 cm; -0.5 to -1.0 cm; and -1.0 to -2.0 cm) were characterized using "diffusive gradient in thin film" (DGT) sediment-probe samplers (Zhang and others, 1995).

Measurements of nickel concentrations in all water, sediment, and DGT samplers, were conducted by inductively-coupled plasma mass spectrometry (ICP-MS) in accordance with USEPA method 6020A (USEPA 2007). Sediments were characterized for particle-size distribution (as percent by mass of sand-, silt-, and clay-sized particles), TOC, cation exchange capacity (CEC), oxidation-reduction potential (ORP), and pH. Water analyses included pH, major ions, conductivity, alkalinity, hardness, and DOC. All water and sediment analyses were performed using standard methods (e.g., APHA 2005) with rigorous quality assurance/quality control procedures according to USEPA guidelines (USEPA 2004). Results of selected quality control (QC) measurements for nickel analyses are presented in Appendix 1-3.

Toxicity Testing

Three sets of whole-sediment tests (Tests 1, 2, and 3) were conducted with all 16 treatments (12 spike treatments and four controls). Sediments for toxicity testing in Tests 1, 2, and 3 were removed from the equilibration jars 8, 12, and 16 weeks after the start of the spiking process, respectively. Toxicity tests with *H. azteca* were conducted for 21 days, based on a modification of USEPA (2000) and ASTM (2010) methods (Table 2). Test water was diluted well water (100 mg/L hardness as $CaCO_3$). The pH of test water was automatically adjusted to 7.3 by addition of dilute hydrochloric acid (Wang and others, 2008). Overlying water in test beakers was replaced automatically at two different rates during each toxicity test: 2X (two

volume-additions added per day) and 4X in Test 1; and 2X and 8X in Tests 2 and 3. After sediments were added to test beakers, they were held in the exposure system for six days (with water additions) before each test to facilitate diffusion of 'excess' unbound nickel from sediments and flushing of nickel from overlying water to avoid the accumulation of toxic concentrations of nickel in the overlying water. The endpoint for these tests was survival after the 21-d exposure period.

1.3 Results and Discussion

Equilibration of nickel-spiked sediments.

Sediment TR-Ni concentrations measured during the 112-day equilibration period were close to nominal spike levels for SR sediments, but were about 25% above targets for WB sediments, due to a miscalculation of the solids content of WB sediment (Figure 1a). Nickel concentrations measured in AVS extracts (SEM-Ni) were typically 80% to 90% of TR-Ni in all spike treatments with the SR sediment, but were lower in WB spike treatment (Figure 1b). The smaller SEM-Ni fraction observed for the spiked WB sediment is consistent with greater formation of NiS, from which nickel is only fractionally recovered by the SEM-AVS extraction procedure. For example, Carbonaro and others (2005) reported only 20% recovery as SEM-Ni from NiS, and unpublished experiments at our laboratory with freshly precipitated NiS produced 40% recovery of SEM-Ni and no recovery of AVS. Consistent with these findings, the formation of NiS was apparently enhanced in the Indirect+Iron (ferrous sulfide) treatment, which had the smallest SEM-Ni fraction (40-50%).

Indirect spiking treatments generally resulted in AVS close to the pre-spike levels for each base sediment (Figure 2). Differences among treatments were small for the low-AVS SR

sediment. After a consistent initial decrease evident on Day 56, AVS concentrations were stable or increased inmost treatments. The exception to this trend was the Indirect+Iron (ferric chloride) treatment for the SR sediment, which had lower AVS by day 112, because incubation jars were opened to atmospheric oxygen after day 96 to allow added iron to precipitate as hydrous ferric oxides. Differences in AVS among treatments were more pronounced for the WB sediments, with decreases of about 50% in the Direct treatments, compared to concentrations initially measured in base sediments. Physical and chemical manipulations of sediments (i.e., pH adjustments and homogenization) during the first four weeks after spiking probably affected the Direct treatments more than the Indirect and Indirect+Iron treatments, where the superspikes were mixed with unspiked base sediments on Day 28. Sulfide added as FeS to control sediments was fully recovered as AVS, resulting in AVS concentrations that were greater than the base sediment, but sulfide added as FeS with equimolar nickel (Indirect+Iron treatments) was minimally recovered as AVS. These results suggest that much of the nickel spiked into sediments containing "natural" AVS (rather than freshly precipitated FeS) probably did not react to form pure NiS. If pure NiS had formed, greater decreases in AVS would be expected with increased additions of nickel plus FeS.

As expected, most spiked sediments had initial pH higher than target (baseline) pH levels (Figure 3). The addition of excess NaOH along with nickel spikes was planned with the expectation that pH of spiked sediments would drift lower over time, as was reported by Simpson and others (2004). However, downward drift of pH in our spiked sediments was minimal, necessitating adjustments with HCl in some cases. This pH 'overshoot' was greatest (0.5-2.0 units above target pH) in the super-spikes in the Indirect treatments. Only minor pH adjustments were required in the Direct treatment and in the two Indirect treatments after initial

corrections. No pH adjustments were made after day 22 and subsequent changes in pH were minimal.

Pore-water nickel concentrations stabilized more rapidly in the SR sediments than in the WB sediments (Figure 4). In SR sediments, PW-Ni concentrations remained nearly constant throughout the equilibration period in all treatments, with clear differences between low- and high-nickel treatments. Different spiking methods produced a wide range of PW-Ni concentrations in the High (500 µg/g) nickel spike treatments, with highest concentrations in the Indirect+Iron treatment and lowest concentrations in the Direct treatment. The low PW-Ni concentrations in the Direct/High-Ni treatment may reflect lower nickel solubility at the higher initial pH (about 0.5 units higher) in this treatment. Greater PW-Ni concentrations in the Indirect+Iron treatment, contrary to our expectations, may indicate that added ferric iron (Fe^{3+})was reduced to ferrous iron (Fe^{2+}) under the anaerobic conditions in the equilibration jar, which would have competed with dissolved nickel (Ni^{2+})for sediment binding sites. Pore-water nickel concentrations in spiked WB sediments were much lower than those for comparable treatments of the SR sediment. Only the WB/Direct/High treatment (nominal nickel spike 3000 µg/g) had PW-Ni concentrations that approached those in the spiked SR sediments. The high PW-Ni concentrations in this treatment remained stable after about 42 d, but PW-Ni decreased slowly in other WB spike treatments until about day 84.

Indirect spiking (with or without iron) generally produced greater PW-Fe concentrations than Direct spiking (Figure 5), presumably indicating that more consistent reducing conditions in these treatments favored formation of the soluble ferrous iron species – whereas ferric iron is highly insoluble (precipitates as $FeOH_3$) at either neutral or basic pH. For the SR sediment, PW-Fe roughly tracked PW-Ni, including decreases in the Indirect+Iron treatments on day 112, after

the jars were opened. In spiked WB sediments, PW-Fe increased slowly as PW-Ni decreased, consistent with PW-Ni slowly displacing ferrous iron in AVS:

$$Ni^{2+}\,(aq) + FeS\,(s) \rightarrow NiS\,(s) + Fe^{2+}\,(aq)$$

Notably, PW-Fe was considerably lower in the WB/Direct/High (3000 µg/g) treatment compared with all other WB treatments. One explanation for this behavior is that concentrations of PW-Ni in the WB/Direct/High treatment might have been high enough to be toxic to iron-reducing bacteria, effectively limiting the overall soluble PW-Fe concentrations in that treatment. Hutchins and others (2007) suggested this mechanism to explain similar PW-Fe behavior observed for a series of copper–spiked sediments.

Nickel concentrations during toxicity testing

Concentrations of PW-Ni in test beakers decreased during the six-day pre-test equilibration period and during the toxicity tests (Figure 6). Rapid decreases in PW-Ni in spiked SR sediment between sampling of bulk spiked sediments and sampling in test beakers (Day 7 of tests) indicate that a considerable fraction of dissolved or weakly-bound nickel was present in spiked SR sediments, perhaps indicating spiking amounts that exceeded nickel-binding capacity in that sediment. Changes in PW-Ni were more gradual between Days 7 and 21 of toxicity tests. In contrast, PW-Ni in several WB spike treatments did not change substantially over this time period. The Direct/High (3000 µg/g) and Indirect+Iron treatments showed some initial loss of PW-Ni, but only the Indirect+Iron/Low treatment showed continuing losses like those seen in the SR sediments. Differences in PW-Ni between 2X and 8X water-replacement treatments (measured on Days 7 and 21 of tests) were minimal, indicating that replacement of overlying

water had little effect on PW-Ni concentrations, at least at the sediment depths sampled by the peepers (about 1 to 2 cm below the surface).

Water replacement strongly influenced nickel concentrations in overlying water during toxicity tests (Figure 7). At the lowest water-replacement rate (2X), mean OW-Ni in several spike treatments exceeded the USEPA (2004) chronic water quality criteria for nickel at 100-mg/L hardness (52 µg/L), with means as high as 120 µg/L, indicating a substantial risk of toxicity from nickel in overlying water. The 4X and 8X treatments reduced OW-Ni proportionately across all treatments, with most treatments averaging OW-Ni less than 20 µg/L. The 8X treatment reduced mean ratios of OW-Ni to PW-Ni ratio to less than 0.2 for all treatments except the WB/Indirect+Iron treatments, which had ratios as high as 0.33. Lower OW-Ni/PW-Ni ratios presumably indicate a lesser contribution of OW-Ni to toxicity observed during sediment toxicity tests.

Vertical diffusion gradients for aqueous nickel (measured in high-nickel spikes only) showed different trends for the two sediments (Figure 8). In the SR sediment, PW-Ni increased with depth, consistent with diffusive losses to overlying water and depletion of PW-Ni in upper sediment layers. In contrast, PW-Ni decreased with depth in WB sediments, suggesting control by AVS in subsurface sediments and mobilization of nickel by oxidation of AVS in the surface layer. The same trends were evident in both low and high water-replacement treatments, suggesting that increasing water replacement rate did not substantially alter Ni fluxes or redox gradients during the toxicity tests.

Toxicity of nickel-spiked sediments

Amphipod survival was consistently high in control sediments and was sensitive to effects of nickel-spiking treatments (Fig. 1-9; Appendix 1-11). Mean control survival in all control groups in the three tests met test acceptability requirements (ASTM 2010, USEPA 2000), with survival in control groups ranging from 83% to 100% (overall control mean=93.5%). Amphipod survival varied widely among nickel-spike treatments, with means ranging from 0% to 100% (Table 3). Of the 36 spiking treatments (combinations of 3 spike methods, 2 sediments, 2 nickel levels, and 3 tests), 24 had at least one mean that was significantly less than controls (rank ANOVA with Dunnett's test; Table 3). For most combinations of sediment type and spiking method, amphipod survival was lower in high-nickel treatments than in low-nickel treatments, as expected. However, these differences were relatively small for tests with the SR/Direct spike treatment, apparently due to the low binding capacity of the SR sediment.

Toxicity of nickel-spiked sediments showed little change with increasing equilibration time from Test 1 (started on Day 56 of the equilibration period) through Test 3 (started on Day 112; Table 3). For the low (2X) water-replacement treatment, which was included in all three tests, survival in most treatment groups (10 of 12) did not differ significantly among tests indicating high test repeatability (Table 3). The two treatment groups with significant differences showed opposing trends: decreasing survival in SR/Direct/High vs. increasing survival in WB/Indirect+Iron/High. Increased survival in the WB/Indirect+Iron/High treatment after Test 1 was consistent with gradual decreases in PW-Ni (Figure 4).

Water-replacement treatments had no significant effects on amphipod survival for either sediment in any of the three tests (Table 3). However, 15 of 24 comparisons between 2X and 8X

treatments (in Tests 2 and 3) showed greater mean survival at the higher water-replacement rate, consistent with reduced nickel exposure via OW-Ni (Table 3).

Variation in amphipod survival in treatment groups with similar sediment nickel concentrations was related to spike treatments, but not water-replacement treatments (Figure 9). In both sediments, variation in survival among spike treatments was greatest at TR-Ni levels that caused intermediate levels toxicity: the three low-nickel treatments (nominal nickel spikes=167 µg/g) in the SR sediment and the three 'intermediate' nickel treatments in the WB sediment (nominal nickel spikes=1000 µg/g: Direct/Low, Indirect/High, and Indirect+Iron/High), which had nominal nickel spikes of 1000 µg/g. Differences among spike treatments were greater for the SR sediment, with survival in the low-nickel treatments ranging from about 80% in the Indirect treatment to about 30% in the Direct treatment. This variation among treatments is consistent with stronger binding of nickel to sediment particles (i.e., lower bioavailability) in the Indirect treatment. In the WB sediment, survival in the 1000 µg/g spike treatments was generally higher for the Direct treatment than the Indirect+Iron treatment, but there was considerable overlap among all three spike treatments. Variation in survival was not consistently related to water replacement treatments in either sediment.

Concentration-response relationships suggest that toxicity of nickel-spiked sediments across different spiking treatments corresponded closely to nickel concentrations in pore-water. Median lethal concentrations (LC50s) calculated from PW-Ni were consistent across the three tests and across three water-replacement treatments, with LC50s ranging from 81 to 117 µg/L (Table 4). In contrast, LC50s for OW-Ni were consistent across tests but differed among water-replacement treatments, with mean LC50s for OW-Ni ranging from 33 µg/L in the 2X treatment to 13 µg/L in the 8X treatment. These trends suggest that toxicity of nickel-spiked sediments was

primarily driven by exposure to nickel in pore-water and was little affected by differences in OW-Ni.

1.4 Conclusions

Methods selected for spiking and toxicity testing in Tasks 2 and 3

- Spiking Method: Indirect. The Indirect method required less pH manipulation than the Direct method (only in the superspike, not in individual treatments), yet it produced consistent sediment pH across nickel levels and resulted in less change in AVS concentrations, presumably due to the stabilizing effects of the dilution with unspiked sediments. The Indirect method also produced stable pore-water nickel concentrations and consistent toxicity for all three tests. This method has the practical advantage of greater flexibility in preparing multiple spike levels from a single superspike.

- Equilibration Period: 10-weeks (anaerobic) plus 1 week (aerobic). An equilibration period of eight to 12 weeks (4-8 weeks after sediment dilutions) was adequate for the Indirect spike method. We observed no change in PW-Ni in spiked SR sediments after the first four weeks, and only minor decreases in PW-Ni in spiked WB sediments after eight weeks. A one-week pre-test equilibration period in toxicity beakers (with addition of overlying water) facilitated the removal of unbound or weakly-bound nickel from the SR sediment and allowed the development of an oxidized boundary layer at the surface of the WB sediment.

- Water Replacement Rate: High (8 volume-additions/d) . The highest rate of water addition (8 volumes/d) was necessary to maintain low nickel concentrations in overlying water. Overlying water of Indirect spike treatments that received 8X water additions had nickel concentrations that were 10% or less, compared to nickel concentrations in pore-water.

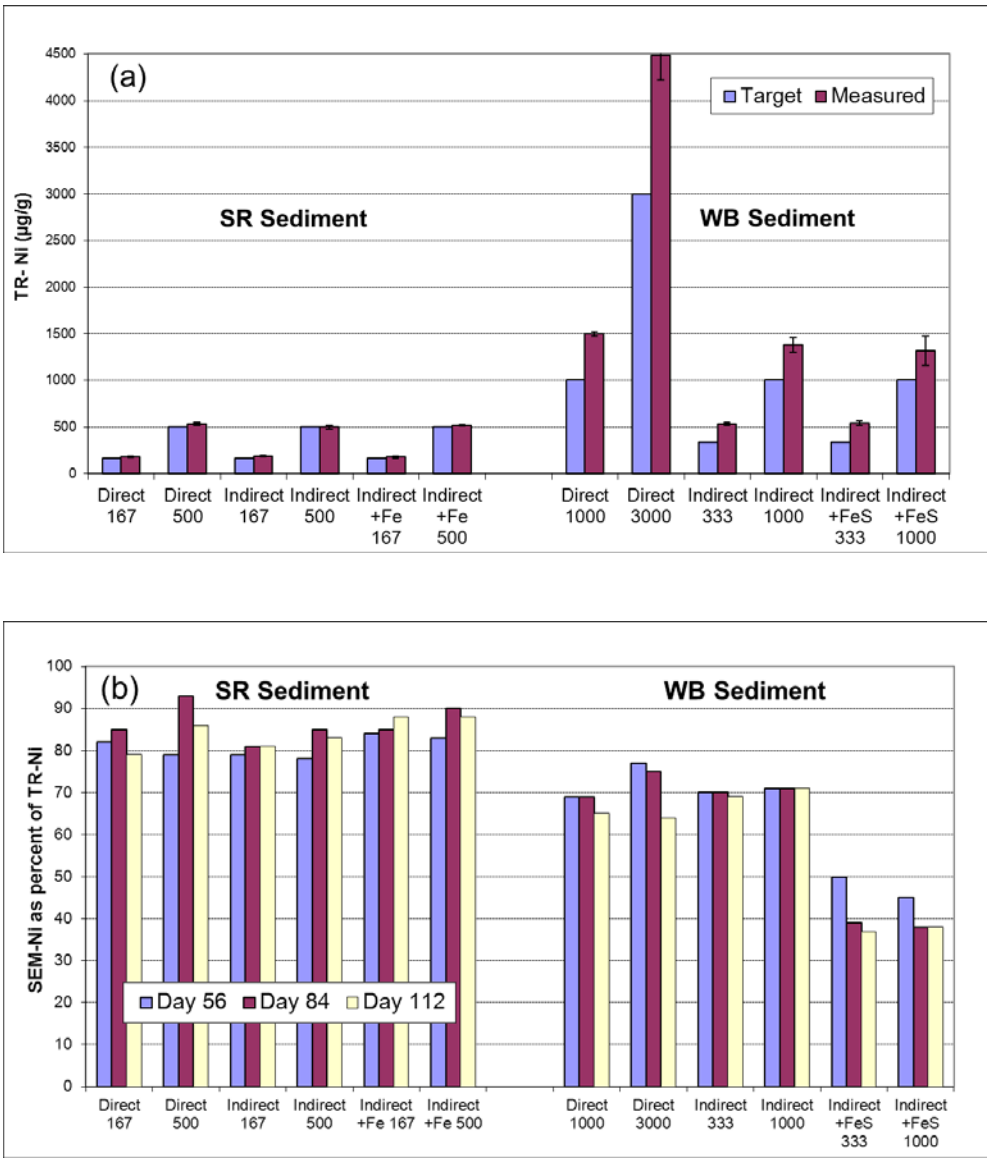


Figure 1. Target and measured sediment nickel concentrations in spiked sediments(a) target vs. measured total recoverable nickel (TR-Ni); (b) measured SEM-Ni expressed as a percent of TR-Ni.

[X-axis labels indicate spiking treatment and target nickel concentration (µg/g; Table 1). Measured TR-Ni concentrations are means (with standard error) for samples from separate containers on days 56, 84, and 112).]

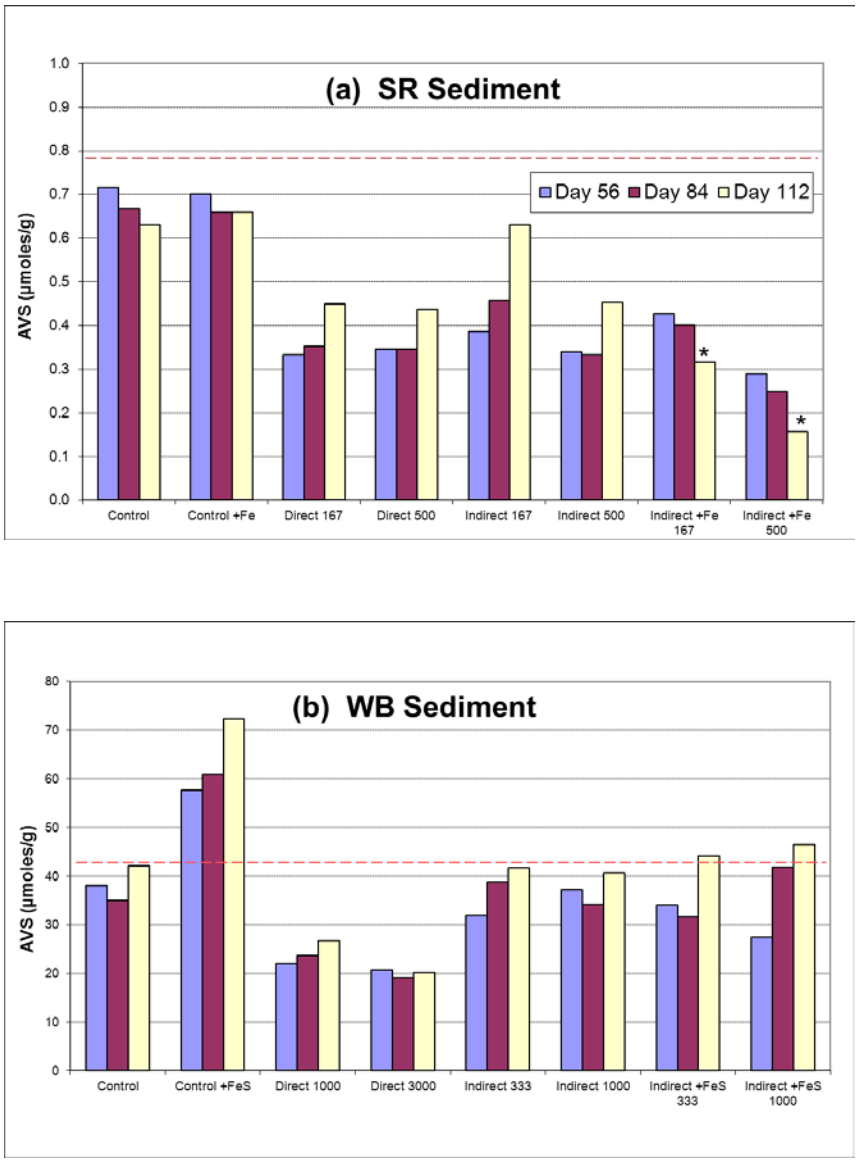
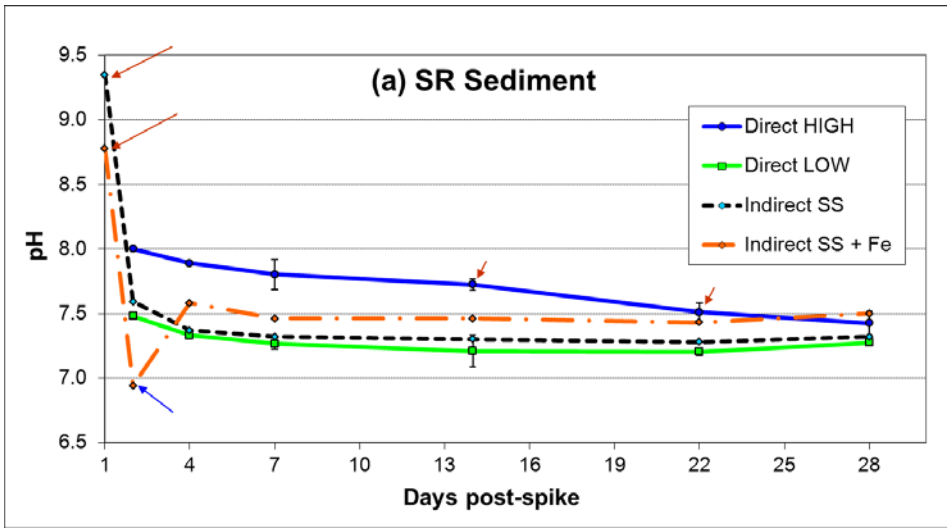


Figure 2. Acid-volatile sulfide (AVS) concentrations in nickel-spiked sediments: (a) SR sediment; (b) WB sediment.

[X-axis labels indicate spiking treatment and target nickel concentration (µg/g; Table 1). Red dashed line=Initial AVS (October 2008). Asterisks indicate jars (SR sediment; Indirect+Iron treatment) that were exposed to atmospheric oxygen on day 96.]



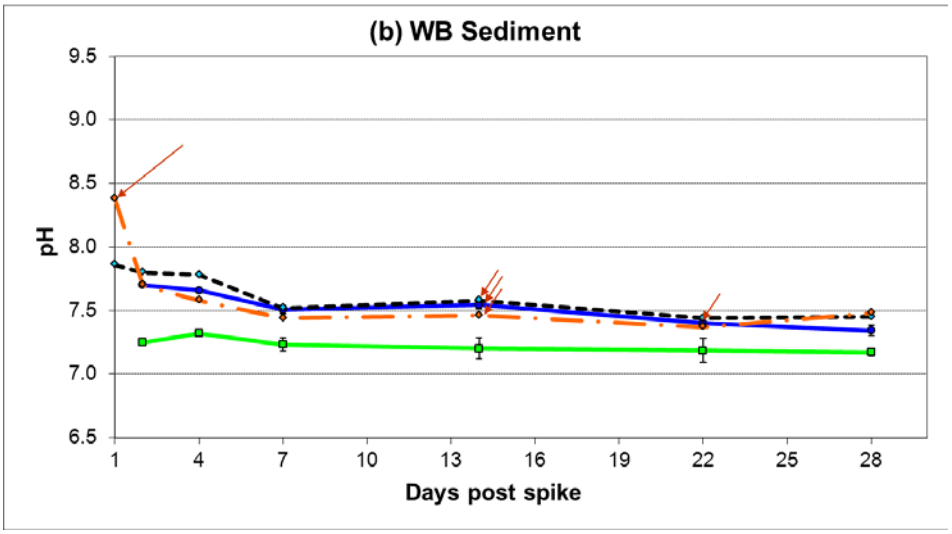


Figure 3. Adjustment of pH in nickel-spiked sediments during first four weeks of equilibration: (a) SR sediment; (b) WB sediment.

[Target pH=7.35 (SR), 7.15 (WB). Arrows indicate pH adjustment with NaOH (blue) or HCl (red). Error bars = 2 standard deviations for Direct treatment (n=3). SS=superspike]

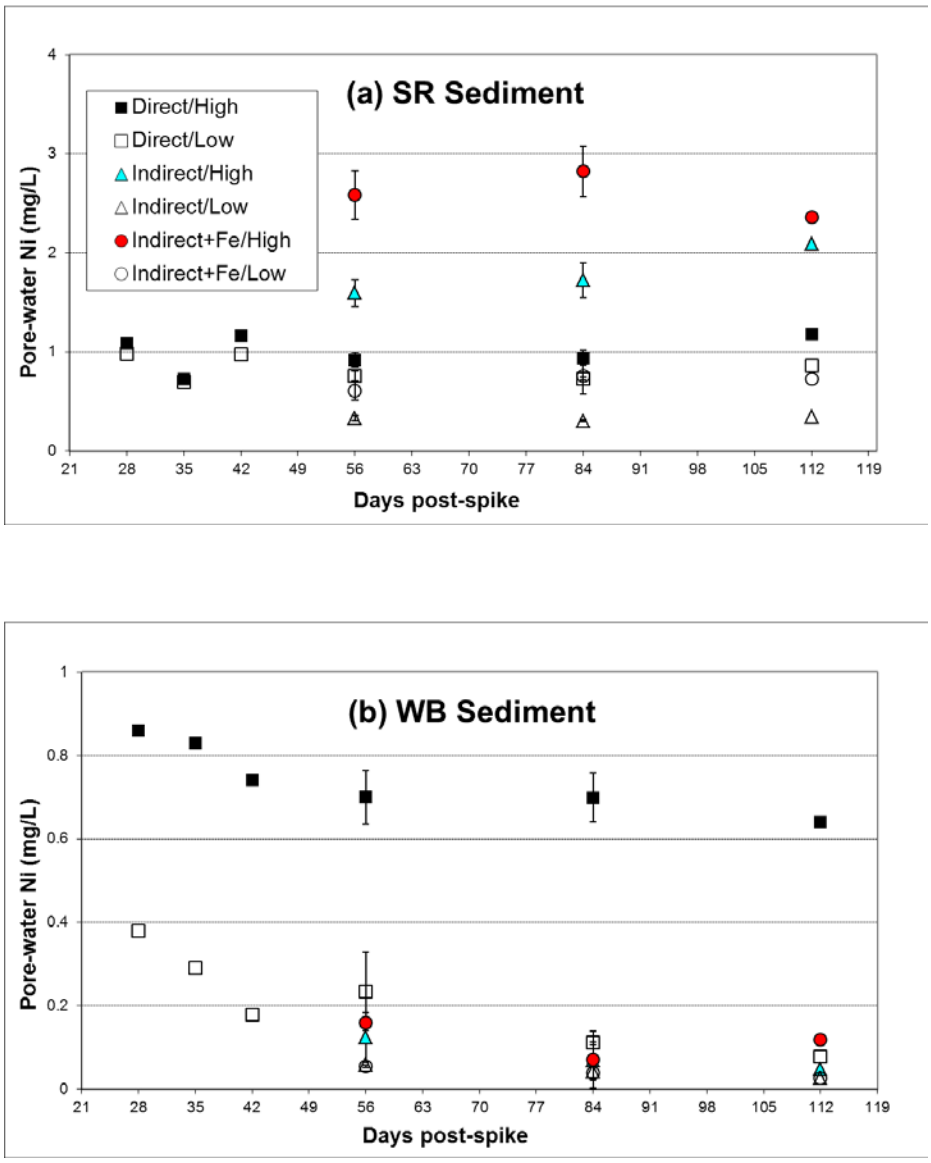


Figure 4. Pore-water nickel in bulk spiked sediment during equilibration period: (a) SR sediment; (b) WB sediment.

[Symbols indicate spiking treatments and nickel level (Table 1). Data for days 28-56, 84, 112 are from separate replicate jars. Data for days 56 and 84 are means of peeper and centrifuged samples. Error bars indicate ranges]

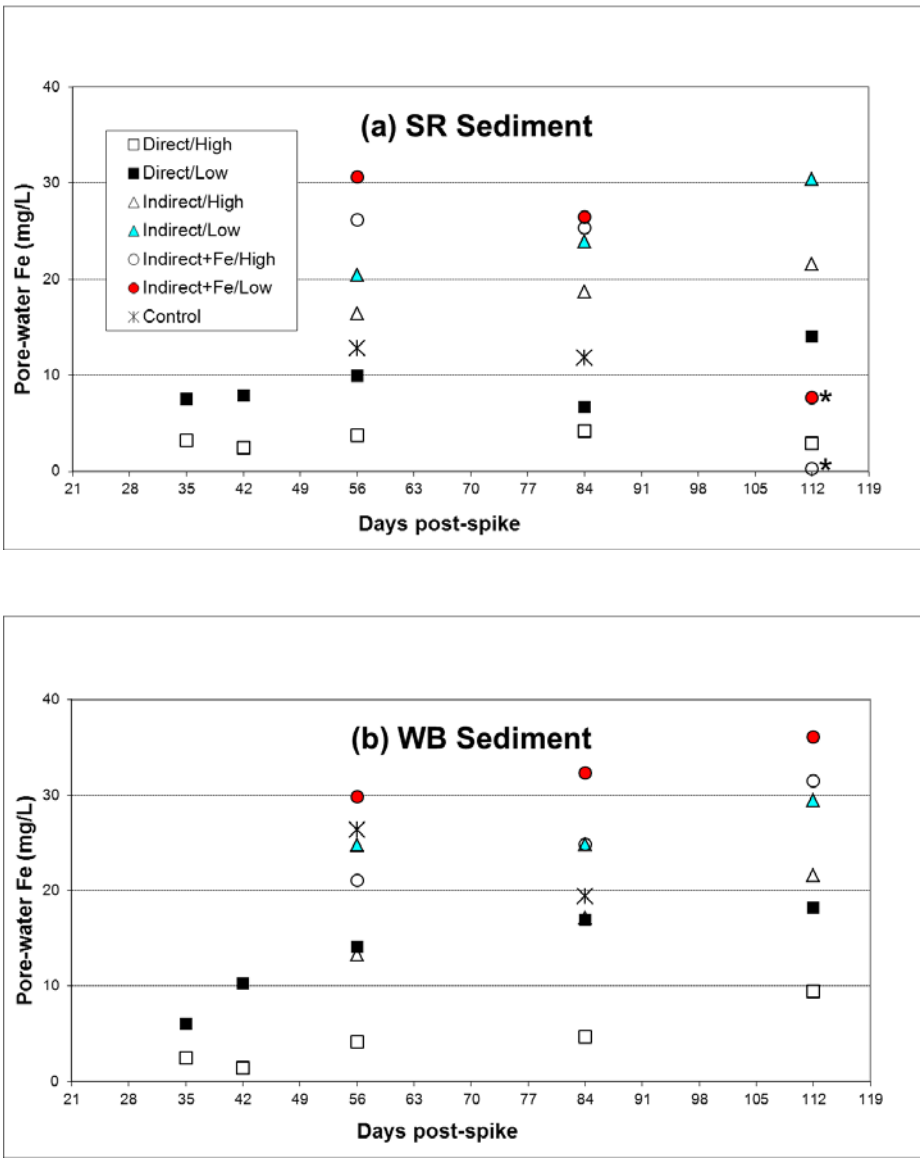


Figure 5. Pore-water iron in spiked sediment during equilibration period: (a) SR sediment; (b) WB sediment.

Symbols indicate spiking treatments and nickel levels (Table 1). Data for days 28-56, 84, 112 are from separate replicate jars. Asterisks indicate jars (SR sediment; Indirect+Iron treatment) that were exposed to atmospheric oxygen on day 96.]

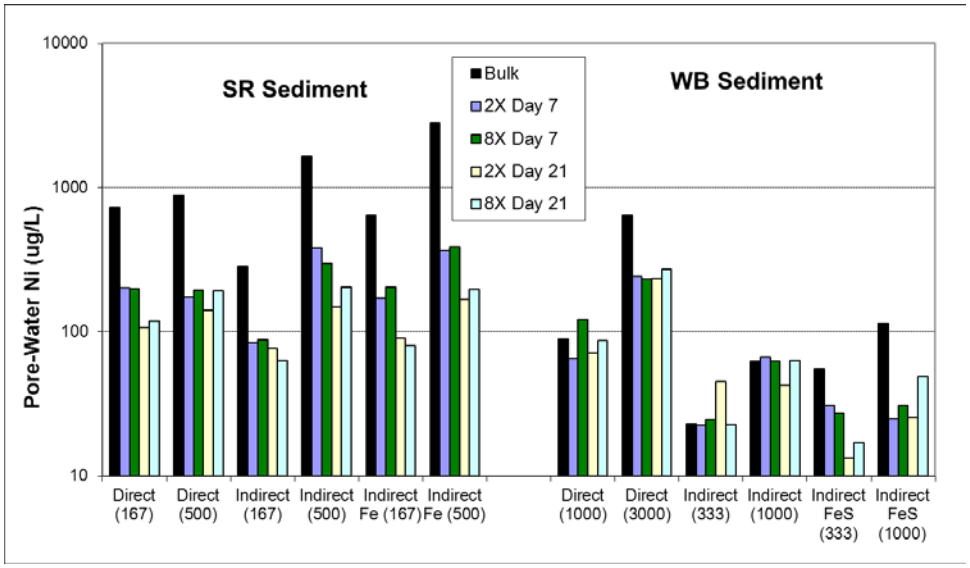


Figure 6. Pore-water nickel concentrations in spiked sediments before and during toxicity tests.

[Bulk pore-water sampled by centrifugation on day 84 of equilibration study. X-axis labels indicate spiking treatment and target nickel concentration (µg/g; Table 1). Bulk samples=pre-test samples. Peeper samplers removed from test chambers on day 7 or day 21 during Test 2. 2X, 4X, and 8X refer to water volumes added per day during toxicity testing.].

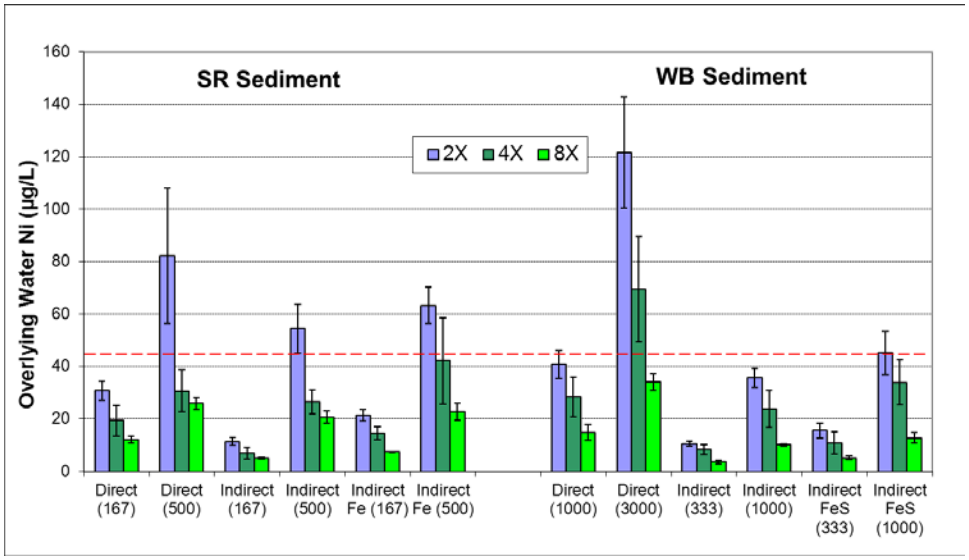
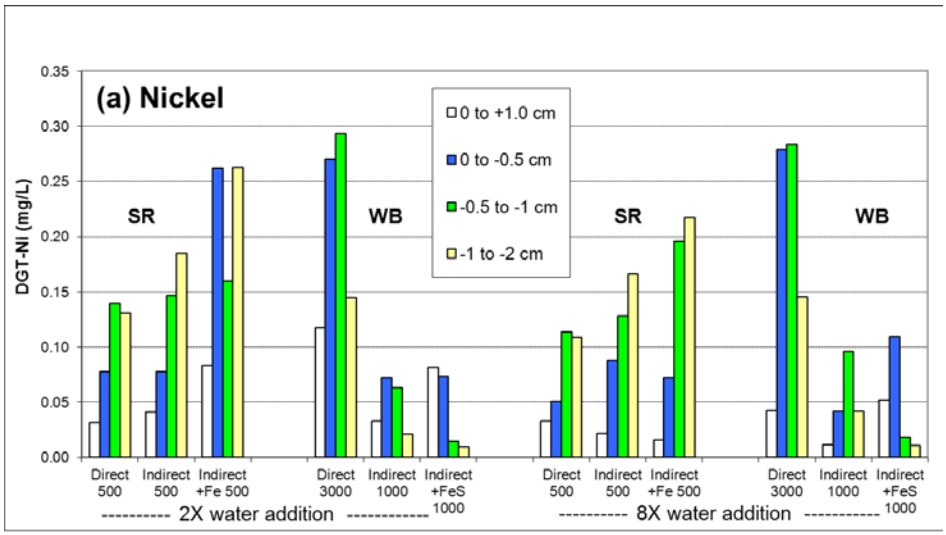


Figure 7. Nickel concentrations in overlying water of spiked-sediment toxicity tests.

[X-axis labels indicate spiking treatment and target nickel concentration (µg/g; Table 1). Data are means and standard error of samples collected on days 1, 8, and 14 of Test 2. Red dashed line= USEPA water quality criterion at 100 mg/L hardness.]



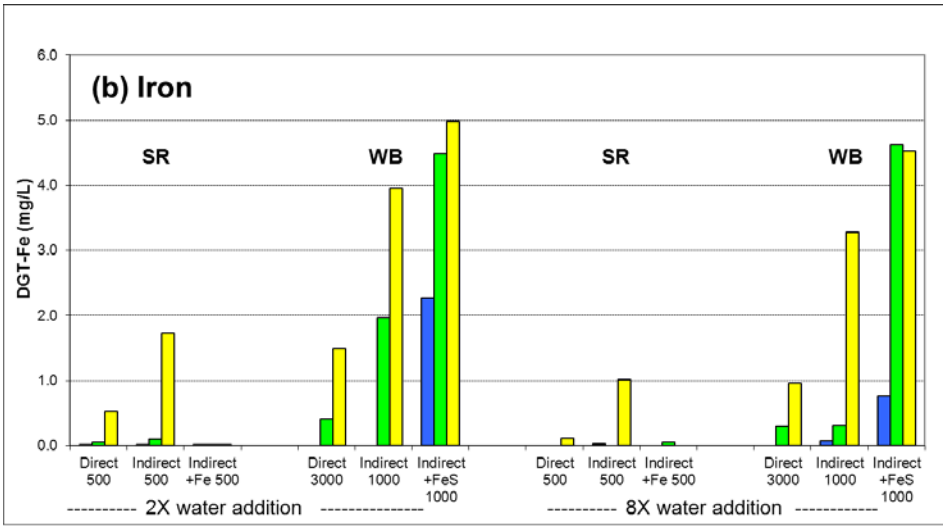


Figure 8. Depth gradients of dissolved metal concentrations estimated by DGT samplers during sediment toxicity tests: (a) Nickel; (b) Iron.

[X-axis labels indicate target nickel concentration (µg/g; Table 1) and rate of water addition. Positive depths=overlying water; negative depths=pore-water. Data from days 1-2 of Test 3 (high spike treatments).]

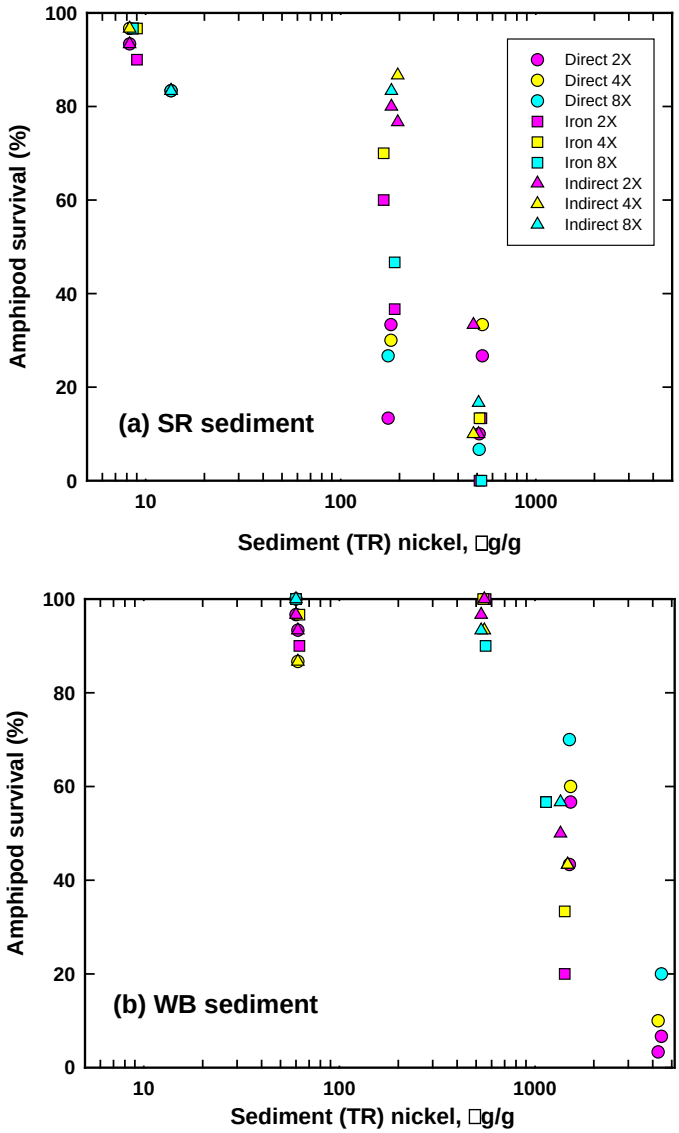


Figure 9. Effects of spiking treatments and water replacement on amphipod survival in nickel-spiked sediments: (a) SR sediment; (b) WB sediment.

[Symbols and colors indicate spiking treatment (Iron = Indirect+Iron) and rate of water addition.]

Table 1. Summary of spike treatments, nominal additions of nickel (Ni) and iron (Fe), and estimated [SEM(Ni)-AVS] concentrations in nickel-spiked sediments

[Base sediments: SR=Spring River (Missouri), WB=West Bearskin (Minnesota).]

Sediment	Spike treatment	Nickel treatment	Ni spike (mg/kg)	Fe spike (mg/kg)	SEM(Ni)-AVS (μmol/g OC)
Control treatments:					
SR	Direct, Indirect	Control	0	0	-100
SR	Indirect+Fe	Control	0	1893	-100
WB	Direct, Indirect	Control	0	0	-476
WB	Indirect+FeS	Control	0	1893	-588
Spike treatments;					
SR	Direct	Low	167	0	256
SR	Direct	High	500	0	965
SR	Indirect	Low	167	0	256
SR	Indirect	High	500	0	965
SR	Indirect+Fe	Low	167	316	256
SR	Indirect+FeS	High	500	947	965
WB	Direct	Low	1000	0	-250
WB	Direct	High	3000	0	91
WB	Indirect	Low	333	0	-363
WB	Indirect	High	1000	0	-250
WB	Indirect+Fe	Low	333	630	-476
WB	Indirect+FeS	High	1000	1893	-588

Table 2. Test conditions for Task-1 whole-sediment toxicity tests with the amphipod *Hyalella azteca*, based on USEPA (2000) and ASTM (2010a).

Test condition	Description
Test type:	Spiked whole-sediment exposures with water replacement
Temperature:	23 ± 1 °C
Lighting	Ambient laboratory light; 16 hr light/8 hr dark
Test chamber:	300-ml beakers
Sediment volume:	100 mL, with 175 ml mL of overlying water
Test water:	Well water diluted with de-ionized water to hardness of 100 mg/L as $CaCO_3$. pH of incoming test water was adjusted to 7.3 ± 0.2 for water entering test chambers..
Water Additions:	Low treatments: 2 volumes/d (all tests); high treatment, 4 volumes/d (Test 2) or 8 volumes/d (Test 3)
Age of organisms:	About 7-d old
Organisms/beaker:	10
Number of replicates:	4 replicates per treatment for toxicity endpoints, plus additional replicates for peeper sampling and DGT samplers.
Feeding:	Yeast-cereal leaf-trout chow suspension (USEPA 2000), 1 mL/d (1.8 mg/d).
Aeration:	None
Test Duration:	6-d pre-stocking period and 21-d amphipod exposure
Endpoints:	Survival
Test acceptability:	Survival >80% survival in control sediment.

Table 3. Effects of experimental treatments and equilibration time on survival of *Hyalella azteca* in toxicity tests with nickel-spiked sediments.

[Mean percent survival by treatment group (n=3), with results of ranks analysis of variance. Asterisks indicate means significantly less than controls (Tukey's test). P-values indicate significance of ANOVA for differences among water-replacement treatments and among repeated tests.

Treatments			Mean Survival (percent)						Difference among tests (p-value)	
			SR Sediment			WB Sediment				
Spike	Nickel	Water	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3	SR sediment	WB sediment
Direct	Low	2X	33*	13*	30*	57	43*	60	0.223	0.456
		4X	30*	--	--	60	--	--		
		8X	--	27*	30*	--	70*	83		
	High	2X	27*	10*	3*	3*	7*	7*	0.003	0.729
		4X	33*	--	--	10*	--	--		
		8X	--	7*	27*	--	20*	17*		
Indirect	Low	2X	77	80	97	97	97	97	0.154	1.000
		4X	87	--	--	93	--	--		
		8X	--	83	57	--	93	97		
	High	2X	33*	10*	23*	43*	50*	63	0.232	0.222
		4X	10*	--	--	43*	--	--		
		8X	--	17*	37*	--	57*	70		
Indirect Plus Iron	Low	2X	60	37	57	100	97	80	0.164	0.212
		4X	70*	--	--	97	--	--		
		8X	--	47*	53	--	90	93		
	High	2X	0*	13*	10*	20*	57*	40*	0.254	0.009
		4X	13*	--	--	33*	--	--		
		8X	--	0*	23*	--	57*	57		
Difference among water treatments (p-values):			0.909	0.871	0.355	0.805	0.502	0.210		

Table 4. Median lethal concentrations (21-d LC50s) for *Hyalella azteca* based on nickel concentrations in pore-water and overlying water of sediment toxicity tests.

[Nickel LC50s with 95% confidence intervals, expressed as µg/L. LC50s were calculated from data for all spike treatments for each test and water-replacement treatment.]

Test	Water-replacement treatment		
	2X	4X	8X
	Pore-water		
Test 1	100 (89-113)	102 (90-116)	NT*
Test 2	81 (68-96)	NT	103 (87-123)
Test 3	81 (63-104)	NT	117 (90-152)
	Overlying Water		
Test 1	30 (26-34)	23 (20-26)	NT
Test 2	33 (28-38)	NT	12 (10-13)
Test 3	37 (33-42)	NT	13 (11-15)

*NT=not tested.

Chapter 2: Sensitivity of benthic invertebrates to toxicity of nickel-spiked freshwater sediments

2.1 Introduction

In Task 2, two sediments were spiked using in direct spiking methods (developed in Task 1) to produce a wide range of sediment nickel concentrations for toxicity testing. Chronic toxicity tests with nickel-spiked freshwater sediments were conducted with nine taxa benthic invertebrate taxa, representing both taxonomic diversity (3 insects, 2 crustaceans, 2 oligochaetes, 1 mollusk, and 1 nematode) and diverse ecological and behavioral traits. Toxicity test methods for these taxa were based on standard toxicity test methods or other published methods. Eight of nine taxa were tested in exposure systems with automated replacement of overlying water, consistent with the findings of Task 1, and nematodes were tested under static conditions.

The primary objective of Task 2 was to characterize the relative sensitivity of nine freshwater benthic invertebrates to nickel-spiked sediments. Multiple chronic toxicity endpoints were evaluated for each species, including survival and sublethal endpoints such as growth, biomass, and reproduction. Responses of these endpoints were characterized with concentration-response models based on measured nickel concentrations and the most sensitive endpoints for each species were selected for comparisons. Analysis of concentration-response curves focused on estimation of nickel concentrations that caused ten percent and twenty percent reductions of an endpoint relative to the response in the absence of nickel exposure -- EC10s and EC20s, respectively. USEPA uses EC20s to develop chronic water quality criteria for protection of aquatic life (for example, USEPA 2007b) and the European Union uses EC10s for establishing predicted no-effect concentrations (PNECs)

under REACH (ECHA, 2008). Toxicity tests with aqueous nickel (without sediment) were also conducted with each of the nine species. Results of these water-only tests provided a separate line of evidence to characterize differences in nickel sensitivity among species.

2.2 Methods

Sediment Spiking Procedures

Task-2 studies were conducted with the same two base sediments used in Task 1: Spring River, Missouri, USA (SR); and West Bearskin Lake, Minnesota, USA (WB). These sediments had very different physico-chemical characteristics, notably differences concentrations of metal-binding phases total organic carbon (TOC) and acid-volatile sulfide (AVS; Table 1). The SR sediment had low TOC and low AVS and was expected to have a low-binding affinity for nickel (i.e., high nickel bioavailability) and the WB sediment had high TOC and high AVS and was expected to have a high binding affinity for nickel (i.e., low Ni bioavailability).

Sediments were spiked with nickel using an indirect spiking method based on results of Task 1 (Table 5; Appendix 2-1). Sediments were spiked and equilibrated with nickel over a 10-week period that consisted of two phases: (1) prepare pH-adjusted, high-nickel 'superspike' sediments and equilibrate for four weeks; and (2) dilute equilibrated superspikes with unspiked base sediment to produce target nickel concentrations and equilibrate for additional six weeks. In the first phase, three separate superspikes (3.0-3.3 L each) for each sediment type were prepared in 3.8-L glass jars by addition of nickel chloride stock solutions, sodium hydroxide (NaOH) solutions to maintain target pH of 7.25 (±0.20), and deoxygenated water to facilitate mixing. Superspikes were homogenized with a stainless steel auger; subsequently, the headspace was purged with nitrogen, and jars were sealed (with Teflon-lined lids). Once sealed, the jars were rolled for two hours on a rolling mill at 20 rpm and

then placed in a 20 °C water bath in the dark for x h. During the first two weeks after spiking, the pH of the superspikes was monitored regularly and pH was adjusted as needed to maintain conditions within 0.1 unit of the target pH by addition of dilute NaOH or dilute hydrochloric acid (HCl). After each pH check or pH adjustment, jars were homogenized (if pH adjustment was performed); purged, sealed, and rolled; and returned to the water bath, as described above. Superspike jars remained sealed for two more weeks, with weekly mixing (1 hr @ 20 rpm) on the rolling mill.

Four weeks after initial nickel spiking, portions of each superspike were diluted with base sediments in varying proportions to produce a series of five target nickel concentrations (for example, SR-1 through SR-5, with SR-5 being the highest nickel spike level) plus an unspiked control for each base sediment (Appendix 2-1). Control sediments were prepared in 3.8-L glass jars as described for the superspikes but without nickel spikes or pH adjustment. Three replicate jars were prepared for each spike treatment and controls. These jars were purged with nitrogen, sealed, and equilibrated in the 20 °C water bath for at least six weeks, with each jar mixed on the rolling mill (1 hr @ 20 rpm) every two weeks. The first set of toxicity tests were started with sediments from the first set of the replicates ten weeks after preparation of superspikes.

Sediment Toxicity Tests

The chronic toxicity of nickel-spiked sediments to eight species of benthic invertebrates was tested in flow-through test systems. These species (and species IDs) were:

1. Amphipod, *Hyalella azteca* (HA)
2. Amphipod. *Gammarus pseudolimnaeus* (GP)
3. Midge, *Chironomus dilutus* (CD)
4. Midge, *Chironomus riparius* (CR)

5. Oligochaete, *Lumbriculus variegatus* (LV)
6. Oligochaete, *Tubifex tubifex* (TT)
7. Mussel, *Lampsilis siliquoidea* (LS)
8. Mayfly, *Hexagenia* sp. (HS)

Test organisms were obtained from ongoing cultures maintained at the USGS Columbia Environmental Research Center (CERC) in Columbia, Missouri, except cohorts of mussels and mayflies were obtained from outside sources and reared at CERC to appropriate age/size for testing. Juvenile mussels were obtained from Dr. Chris Barnhart of Missouri State University, Springfield, Missouri. Fertilized mayfly egg masses were obtained from Dr. Jan Ciborowski of University of Windsor, Ontario, Canada.

Conditions for conducting flow-through sediment toxicity tests are summarized in Table 6. These tests were conducted in temperature-controlled water baths at 23 ^{o}C (except 15 ^{o}C for GP tests) with automated replacement of overlying water. Test water consisted of well water diluted with de-ionized water to a hardness of about 100 mg/L (as $CaCO_3$). The pH of test water was adjusted to about 7.3 using an automated pH controller that added dilute HCl as needed. A volume of test water equal to eight times the volume of overlying water was added to each test chamber daily to maintain low concentrations of nickel in overlying water, based on results presented in Chapter 1.

For most of these test organisms, standard procedures for conducting sediment toxicity tests have been published by ASTM (2010a,c); USEPA (2000), and/or OECD (2004, 2007). Species-specific test conditions (described below and in Table 6) were selected to facilitate efficient, concurrent testing of multiple species in our laboratory, while remaining consistent with existing test methods, published scientific literature, and preliminary studies in our laboratory.

Amphipods (HA and GP). Test methods for both amphipods generally followed standard methods for HA (ASTM 2010a, USEPA 2000). Tests with GP were started with juveniles 3 to 5 mm long and were conducted at 15 ^{o}C (Nebeker and others, 1984) for 28 d. Endpoints for GP were survival, growth (length), and biomass (based on ash-free dry weight; Oseid and Smith, 1974).

Midges (CD and CR). Methods for midge life-cycle tests closely followed standard methods (USEPA 2000, ASTM 2010a) except that tests were started with 7-d old CD larvae rather than <24-h-old larvae, in order to improve control performance (Ingersoll and others, 2008), and were stocked with 10 animals (CD) or 12 animals (CR) per chamber. Dates for measurement of survival, growth, and biomass were adjusted to day 10 (from day 14) for CR and day 13 (from day 20) for CD. This approach has been found to produce more consistent emergence in the controls (Ingersoll and others, 2009).

Oligochaetes (TT and LV). Adult TT were isolated from the culture by sieving organisms (<500 µm) and each replicate was stocked with 4 adult TT (Reynoldson and others, 1991; ASTM 2010a). Both species were tested under a 16:8 photoperiod with flow-through conditions and were fed a suspension of Tetrafin® fish food at 16 mg/d (4 mL of 4 g/L stock). Preliminary testing with TT was conducted to ensure adequate performance of TT and LV tests under the specified test conditions (water replacement, feeding, and lighting; (Ingersoll and others, 2009). Endpoints in 28-d oligochaete tests were total abundance (for LV), biomass (for both species) and reproductive endpoints (for TT). At the end of the exposure, TT were isolated by sieving sediments to >250 µm to obtain adults, juveniles, and cocoons and sieved samples were preserved and stained to facilitate counting of juveniles and cocoons (Reynoldsen and others, 1991; Maestre and others, 2009). After counting, ash-free biomass was determined separately for adults and offspring (unhatched cocoons plus juveniles).

Mayflies (HS). Tests were started with small mayfly nymphs about 6-8 weeks post-hatch (about 5-10 mg wet weight). Four replicate groups of 10 HS were stocked into 200 mL of sediment in 1-L beakers (Nebeker and others, 1984, Day and others, 1998, ASTM 2010a). Each replicate was fed daily with YCT food suspension (7.2 mg per beaker in Task 2 and 3.6 mg per beaker in Task 3), based on the weekly ration used by Day and others (1998). Preliminary testing documented adequate performance of HS under the test conditions (water replacement, feeding, and chamber size) described in Table 6 ((Ingersoll and others, 2009). Tests were conducted for 28 days, with endpoints of survival, growth (mean dry weight), and biomass.

Mussels (LS). Methods for whole-sediment tests with LS were based on methods for chronic water-only toxicity tests with juvenile mussels (ASTM 2010b, Wang et al 2007) and were similar to standard methods for the amphipod, HA (USEPA 2000, ASTM 2010a). Endpoints of the 28- tests were survival, growth (length) and biomass. The reliability of this method has been demonstrated in tests with metal-contaminated sediments from Missouri mining areas (Ingersoll and others, 2008; Besser and others, 2009). Unlike these previous tests with juveniles LS, nickel-spiked SR and WB sediments were not sieved before testing.

A sample of animals from each batch of test organisms was collected at the start of the study to document starting size (length and/or weight; Table 7). Starting size and final growth and biomass were determined as body length (determined digitally) and/or ash-free dry weight. The status of cultures of test organisms used in toxicity tests was evaluated by conducting acute toxicity tests with a reference toxicant (sodium chloride) following standard test methods (USEPA 2000, ASTM 2010a,b).

Water-only Toxicity Tests

Toxicity tests with aqueous nickel were conducted with the same eight invertebrate species using methods similar to those used for spiked-sediment tests (Appendix 2-2). Aqueous nickel solutions were delivered by proportional diluters, with a control and five nickel concentrations in a 50% dilution series. Test solutions were delivered at a rate of four volume-additions per day. The highest nominal nickel concentrations for each species, based on results of range-finding tests, ranged from 80 µg/L (for HA, GP, LS) to 1000 µg/L (for HS). A substrate of 5 mL clean sand was provided for most species. Because the burrowing mayfly HS did not perform well with a sand substrate, the water-only test with this species was conducted with a substrate of 200 mL of unspiked SR sediment – the same sediment volume used in sediment toxicity tests.

Nematode Toxicity Tests

Toxicity tests with the nematode, *Caenorhabditis elegans* (CE) were conducted using static test methods modified from ISO (2010) methods (Appendix 2-3). One week before the start of the sediment tests, 10-mL portions of each sediment were placed in clean vials and 10 mL of test water (diluted well water: hardness 100 mg/L, adjusted to target pH for each sediment) was added. Overlying water in each vial was removed with pipets and replaced with clean test water twice daily for seven days. The last portion of overlying water from each vial was filtered and analyzed to estimate nickel concentrations in overlying water during subsequent toxicity tests. At the start of the tests, sediment from each vial was mixed and 1-mL portions of sediment were added to each well of a six-well culture plates. Tests were started with addition of a suspension of antibiotic-killed *E. coli* in test water (0.5 mL) and 10 synchronized L1 larvae per well, and plates were held at 20 degrees C. After four days, nematodes were fixed by the addition of Bengal Red, harvested, and placed on slides

for counts of surviving adults and larvae. Water-only toxicity tests with CE were conducted using similar methods, except 1.0 mL of nickel chloride solution in test water was added to each cell, instead of sediment.

Characterization of Sediment and Water

Physical and chemical characteristics of sediment, pore-water, and overlying test water were determined before and during flow-through sediment toxicity tests according to the sampling schedule in Table 8. Characterization of spiked sediments included measurements of TR-Ni, SEM-Ni, AVS, particle size distribution, cation exchange capacity (CEC), andtotal organic carbon (TOC). Centrifuged pore-waters were analyzed for pH; dissolved Ni, Fe, and Mn; major cations and anions; and dissolved organic carbon (DOC), and routine water-quality parameters. Samples of sediment and pore-water were collected from jars of spiked sediments (bulk sediments) before the spiked sediments were placed in the exposure chamber (7 days before the start of tests). Additional chemical analyses were conducted on samples of sediment (SEM-Ni and AVS), peeper pore-water (Ni, Fe, and Mn), and overlying waters (Ni and water-quality) from test beakers during toxicity tests. Separate test beakers designated for all chemistry sampling were stocked with test organisms and maintained in the same manner as those used for assessing toxicity. For all flow-through tests, peepers were deployed in chemistry beakers (between 1 and 2 cm below the sediment surface) on day 7 and collected on day 14. Samples of whole sediment from treatment 3 for each sediment type were collected on day 14 for all tests. Overlying water samples for nickel analyses were collected near the sediment/water interface on day 1 and day 28 for all chronic tests. For the static CE tests, aqueous nickel concentrations in overlying water were estimated by sampling overlying water before tests, after one week of daily water replacements and 24 hours after the previous water replacement.

Sediment Toxicity Testing Schedule

The schedule for Task- 2 sediment toxicity testing is presented in Table 9. Due to the limited capacity of flow-through exposure systems, tests with all species except CE were conducted at CERC in three groups (2-4 four species per group) over a four month period, with sediment for all tests in a group coming from the same replicate spiking jar. Spiked-sediment equilibration times for the three groups ranged from 10 weeks (group 1) to 22 weeks (group 3). Tests with HA were conducted with both the first and last test groups to document any long-term changes in nickel toxicity. Before each group of sediment tests, jars were homogenized on a rolling mill (2 hr at 20 rpm), sediment was removed for chemical analyses and for distribution into test chambers, and automated additions of overlying water to test chambers were started. Water additions continued for one week before the chambers were stocked with test organisms, to flush unbound nickel, sodium, and chloride from the spiked sediments and to allow sediments to develop an oxidized surface layer in contact with overlying water. Tests with CE were conducted with sediments remaining after Group-3 tests. These sediments were stored at 4 °C until they were prepared for testing as described above.

Data Analysis and Interpretation

Results of toxicity tests and chemical analyses were used to evaluate the relative sensitivity of the nine test species to toxicity of nickel-contaminated sediments. Data from sediment tests were analyzed using two statistical approaches. Analysis of variance (ANOVA) with Dunnett's test was conducted with rank-transformed data to estimate lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC), using Statistical Analysis System software (SAS/STAT, version 9.2; SAS Institute, Cary NC). Concentration-response relationships were modeled using Toxicity Relationship Analysis

Program (TRAP, version 1.20; provided by Russell Erickson, U.S. Environmental Protection Agency, Duluth Minnesota) to estimate EC10s and EC20s and associated 95% confidence intervals. The primary focus of concentration-response models was on estimation of toxicity values for total nickel concentrations in sediment (TR-Ni), but toxicity values were also estimated for sediment nickel concentrations normalized to AVS (i.e., [SEM(Ni)-AVS]); or to AVS and the organic carbon fraction of sediment, [(SEM(Ni)-AVS)/fOC] (USEPA 2005); and for nickel concentrations in pore-water and overlying water. Similar ANOVAs and concentration-response modeling were conducted with data from water-only toxicity tests.

Concentration-response models were evaluated based on both quantitative performance (i.e., convergence of model estimates, significance of regression, and width of confidence intervals) and qualitative inspection of model fit (especially in the low-effect range). Models were considered to have a good fit if they met all these criteria. Models that met some criteria but not others (for example, limited range of toxic response, high variation or poor fit in background or low-effect ranges, and/or failure to generate confidence intervals) were considered to have a marginal fit. For each species and sediment, toxicity values were obtained from the most sensitive model with good fit or, if necessary, the most sensitive model with marginal fit. If no acceptable model could be generated, toxicity values were estimated as greater than the maximum exposure concentration ('unbounded NOEC').

2.3 Results and Discussion

Sediment and Pore-water Characteristics

Physico-chemical sediment characteristics differed substantially between the two Task-2 sediments (Appendix 2-4). Spiked WB sediments had greater concentrations of AVS and TOC, greater cation exchange capacity, and a larger fraction of fine particles, compared to spiked SR sediments. All these characteristics are consistent with the WB sediment having

greater binding capacity and stronger binding affinity for nickel and other cationic metals. Most of these characteristics were unaffected by the spiking treatments, but AVS concentrations in bulk sediments decreased with increased nickel additions in both sediments. Some of these decreases may reflect oxidation of AVS during spiking, but the trend for lower AVS concentrations with increasing nickel spikes probably also reflects greater amounts of spiked nickel reacting with AVS to form NiS, which is poorly recovered by the AVS method (Carbonaro and others, 2005; W. Brumbaugh, USGS, unpublished data). Some decreases of AVS apparently also occurred due to oxidation during toxicity tests. AVS concentrations in test beakers (day 14 of tests) were consistently 10% to 20% lower than concentrations in bulk samples (7 days before the start of tests). This oxidative loss of AVS presumably occurred at the surface of the sediment and resulted in the release of some AVS-bound nickel, which could either bind to other sediment components or increase nickel concentrations in surficial pore-water or overlying water.

Nickel distribution coefficients for spiked sediments, expressed as log Kd (log Kd=log [concentration in sediment/concentration in pore-water]), averaged 3.5 in the SR sediment and 4.6 in the WB sediment (Appendix 2-4). These values were consistent with the log Kd of 3.7 estimated from the field-collected LPP sediment (Appendix 1-10) and with a median log Kd of 4.0 for nickel previously reported for field-collected sediment samples (Allison and others, 2005; cited in USEPA 2005). The difference in log Kd indicates that a given TR-Ni concentration in the SR sediment would be associated with ten-fold higher PW-Ni concentration than the same TR-Ni concentration in the WB sediment, suggesting that SR sediments had roughly a ten-fold lower binding affinity for nickel, and presumably greater nickel bioavailability. Nickel Kd values were constant across controls and spike treatments for SR sediment, but Kd decreased with increasing nickel spikes for the WB sediment. This

trend may reflect progressive ‘saturation’ of high-affinity binding sites in the WB sediment at higher nickel-spiking levels.

Several constituents of bulk pore-waters differed among spike treatments (Appendix 2-5). Sodium and chloride concentrations in bulk pore-waters increased with greater additions of nickel chloride (from spike solutions) and sodium hydroxide (from pH adjustment), with maximum concentrations of sodium plus chloride in bulk pore-waters of 3.4 g/L in SR-5 and 3.1 g/L in WB-5. These maxima approached levels that could be acutely toxic to some of the invertebrates tested, based on in reference toxicity tests conducted in our laboratory. Acute toxicity tests with sodium chloride were conducted at CERC with all eight test species (except nematodes) between 2008 and 2010, producing toxicity values that ranged from 4.0 g/L for LS to 11 g/L for TT (unpublished data; John Besser, USGS). However, the sodium chloride exposure of organisms during sediment toxicity tests was probably much lower than concentrations in bulk pore-waters, due to diffusion of these ions from pore-water to overlying water, where they would be rapidly diluted and flushed from test chambers. During Task-3 sediment tests, concentrations of sodium in pore-water of test beakers from the highest nickel-spike treatments averaged about 10% of sodium concentrations in bulk pore-waters (Appendices 3-5, 3-6).

Concentrations of iron, calcium, and to a lesser extent other cations in bulk pore-water also increased with increasing nickel spikes (Appendix 2-5), presumably reflecting displacement from binding sites by added nickel. DOC concentrations in bulk pore-waters followed opposite trends in SR sediment (increasing with added nickel) and WB sediment (decreasing with added nickel), particularly for the highest treatment of each sediment. These trends may indicate that that elevated ionic constituents in the pore-water (for example, Ni, Na, Fe, Cl) affected distributions of organic matter between soluble and insoluble forms differently between the two sediments. The DOC of pore-waters from different sediments

may contain different proportions of fulvic and humic acids, which are known to precipitate differently in response to changes in pH and ionic composition (Lawrence, 1989).

Water-quality characteristics of overlying water during flow-through sediment toxicity tests are summarized in Appendix 2-6. Most parameter remained close to expected ranges across tests with different species, sediment types, and nickel spike treatments. Some treatments in tests with two burrowing species, HS and TT, had elevated conductivity in overlying water, apparently reflecting effects of bioturbation on release of pore-water ions (e.g., sodium and chloride). This phenomenon was most evident in the second-highest nickel spike levels, which had highest conductivity values (2,290-4,040 µS/cm) for tests with both HS and TT in both SR and WB sediments. Lower conductivity values measured in the highest nickel treatments (1,151-1,517 µS/cm), despite higher concentrations of pore-water ions, suggesting that toxic nickel concentrations inhibited burrowing activity in these treatments. For the WB sediment, some of the same HS and LV treatments with high conductivity also had reduced pH (as low as 6.50), suggesting that bioturbation enhanced oxidation of reduced iron associated with AVS, leading to release of H^+ during formation of hydrous ferric oxides.

Nickel Concentrations

Sediment nickel concentrations (TR-Ni) in spiked sediments were within 20% of target concentrations (Figure 10; Appendix 2-7). The lower nickel spikes added to the SR sediment resulted in a lower range of TR-Ni concentrations (maximum=762 µg/g) than in spiked WB sediments (maximum=7990 µg/g). As was observed in Task-1, a higher percentage of sediment nickel was recovered in the SEM fraction in spiked SR sediments (77-87%) than in spiked WB sediments (62-78%). This lower recovery of SEM-Ni from the WB sediments may reflect greater formation of insoluble NiS by reaction of spiked nickel

reacting with AVS to form NiS, because SEM-Ni is only partially recovered from NiS (Carbonaro 2005; W. Brumbaugh, USGS, unpublished data). Accordingly, lower recovery of Ni when measured as SEM-Ni was most evident for WB sediments that were spiked with both Ni and FeS (for example, Task 1; Figure 1b). Concentrations of TR-Ni and SEM-Ni in treatments selected for intensive sampling (SR-3 and WB-3) were consistent between bulk samples and samples from toxicity test beakers on day 14 (Figure 10).

The difference between concentrations of SEM-Ni and AVS ([SEM-AVS]) in spiked sediments, an estimate of the potentially bioavailable fraction of sediment nickel (USEPA 2005), was stable across testing groups over the three-month toxicity testing period (Figure 11). Due to lower AVS concentrations, four of five nickel spike treatments in the SR sediment had positive levels of [SEM-AVS], compared to two of five treatments with WB sediments. In the SR-3 treatment, [SEM-AVS] in test beakers was slightly less than in bulk samples, apparently as a result of loss of nickel to overlying water during tests. Conversely, [SEM-AVS] in WB-3 sediments in test beakers was slightly greater (more positive) than in bulk sediments, presumably as a result of loss of AVS by oxidation.

Spiking levels produced the expected gradients of bulk PW-Ni concentrations in spiked sediments, and these gradients remained stable across the three groups of toxicity tests in both sediments (Figure 12; Appendices 2-8 and 2-9). Decreases in PW-Ni between samples from bulk sediments and test beakers varied among spike treatments, but marked decreases occurred only in the three highest spike levels of the SR sediments and the highest spike level of the WB sediment (Figure 12). These large decreases represent diffusive losses of “excess” unbound nickel to overlying water, which were most rapid during the 7-d pre-test equilibration period and early in the test (see Chapter 1; Figure 6). Presumably, this equilibration period also resulted in decreased concentrations of other cations and anions (as was documented in Chapter 3.) These decreases in PW-Ni also point out the importance of

the pre-test equilibration of sediments in test beaker for producing environmentally realistic nickel partitioning. Although results of Task 1 indicate that the indirect spiking approach is superior to direct spiking methods, the occurrence of large pools of unbound nickel in some spike treatments in Task 2 suggests that spiking cannot be expected to produce environmentally realistic nickel partitioning when spiking levels approach limits of sediment binding capacities. In both sediments, greatest losses of PW-Ni occurred in treatments with greatest [SEM-AVS]. Measured PW-Ni concentrations in test beakers were consistent across three test groups and four test start dates (Figure 12).

Despite the loss of large amounts of aqueous nickel from spiked sediments in several treatments, nickel concentrations generally remained low in overlying water of toxicity tests (Figure 13; Appendix 2-10). Mean OW-Ni concentrations differed among tests with different species, but remained well below chronic water quality criterion for nickel (52 µg/L at a hardness of 100 mg/L; USEPA 2009) except for treatments WB-5 (all species) and WB-4 (LV only). For both WB-4 and WB-5 treatments, OW-Ni means for LV tests were substantially greater than means for other species (maximum = 200 µg/L in WB-5), suggesting that bioturbation by these oligochaetes increased the release of aqueous nickel from sediments into the overlying water. In contrast, mean PW-Ni concentrations in test beakers consistently exceeded the chronic water-quality criterion in the three highest spike treatments for both sediments, with a maximum of nearly 1,000 µg/L in the WB-5 treatment (Figure 12).

Attempts to maintain acceptable OW-Ni concentrations during the static CE tests with nickel-spiked sediments were not successful. Despite daily replacements of overlying water in the week preceding CE tests, aqueous nickel concentrations at the end of the week (before the final water replacement) were substantially greater than those in tests with water replacement (Appendix 2-10). Nickel concentrations in samples of overlying water collected

at the end of the pre-test period for the nematode tests (24 hr after water replacement) were 10- to 100-fold greater than mean OW-Ni concentrations in other tests, and OW-Ni concentrations in the four highest WB spike treatments exceeded the water quality criterion.

Sediment Toxicity Tests

Seventeen of 18 flow-through sediment toxicity tests conducted in Task 2 met test acceptability criteria (Appendix 2-11). The exception was the 28-day test with GP (the amphipod, *Gammarus pseudolimnaeus*) in the SR sediment, which had unacceptably low control survival (mean = 55%). This low control survival was apparently caused by a short-term (<24 hr) malfunction of a pH-controller which resulted in a period of low pH in test chambers. This malfunction apparently affected amphipods across all six nickel treatments. Except for lower survival in control and low-nickel treatments, GP endpoints (survival, growth in length, and biomass) followed trends similar to those observed in the GP test with WB sediment. The results of this test were flagged but are included in the following discussions for comparative purposes.

Responses of invertebrates to nickel-spiked sediments differed between SR and WB sediments and among species and endpoints (Appendix 2-11). In spiked SR sediments, five of eight species (CD, CR, LS, LV, and TT) had no significant reductions of any endpoint, relative to controls. In contrast, seven of nine species (all except LS and LV) had significant toxic effects in tests with spiked WB sediment.

Amphipods (HA and GP) and mayflies (HS) showed the most consistent toxic responses to nickel-spiked sediments. Effects on HA were very similar in duplicate tests started 12 weeks apart, suggesting that nickel bioavailability remained stable throughout the Task-2 testing period. Reduced HA survival and biomass were the most consistent responses in both sediments (Figures 2-5a and 2-5b), but small reductions in growth and large (but

variable) effects on reproduction were evident in most tests. Tests with GP and HS showed consistent decreases in survival and biomass and lesser reductions in growth in both sediments (Figures 2-5c and 2-5d). For GP in spiked SR sediments, these responses followed consistent decreasing trends with increasing nickel spikes in both sediments, despite the low control survival. For HS, effects in both sediments were more restricted to the highest spike treatments.

Midges (CD and CR), oligochaetes (TT and LV) and mussels (LS) were less sensitive to nickel-spiked sediments. For CD (Figure 14e), adult emergence was the only endpoint that showed a significant dose-related reduction relative to controls, and this response was only evident in the WB-5 treatment. The CR test (Figure 14f) had small but significant reductions in growth and biomass in several WB spike treatments, but the most consistent dose-related response was reduced fecundity (eggs per egg mass) in WB-5. Hatching success of CR eggs could not be evaluated because few eggs were fertilized by males in the 300-mL egg-deposition chambers. The TT test showed significant reductions in biomass and reproduction in the WB sediment (Figure 14g). Neither LV (Figure 14h) nor LS (Figure 14i) showed significant toxic effects in either sediment.

Water-only Toxicity Tests

Test conditions during chronic water-only toxicity tests remained close to nominal. Minor deviations from nominal nickel concentrations occurred in the HS test (30% greater than nominal) and in the tests with CD and CR (14% less than nominal) (Appendix 2-12). Water-quality of test waters was within normal ranges throughout all tests, except for a small increase in alkalinity in the HS test, which apparently reflects the influence of the (unspiked) SR sediment added to provide a substrate for burrowing (Appendix 2-13).

Chronic water-only toxicity tests with eight species met test acceptability criteria (Appendix 2-14). Significant toxic effects of nickel occurred in six of eight tests, with LOECs ranging from 17 µg/L for HA (survival, growth, and biomass endpoints) to 1715 µg/L (emergence endpoint) for CR (and EC20s ranging from 8.5-1201 µg/L; Table 10). The EC20 for HA survival (12 µg/L) was substantially lower than the survival EC20 of 61µg/L previously reported for HA in 14-day tests with a comparable test water (hardness = 98 mg/L; Keithly and others, 2004). The relative sensitivity of species and endpoints in water-only tests were generally consistent with results of spiked-sediment tests, except that the mussel LS, which had significant reductions of growth and biomass at a waterborne nickel concentration of 71 µg/L, did not show any significant toxic effects in tests with nickel-spiked sediments. Tests with the oligochaetes LV and TT did not have significant toxic effects at the highest aqueous nickel concentration tested (494 µg/L), although this level was less than the LOECs for the two midge species. The rankings of water-only toxicity values were consistent with rankings from a previous comparison of nickel toxicity to several of these species: HA (most sensitive) > LV > CD (Phipps and others, 1995).

Nematode Toxicity Tests

Static sediment tests with nematodes (CE) gave highly variable results (Appendix 2-15). The test with nickel-spiked SR sediment failed completely, with no live organisms recovered from either controls or spike treatments, but a test with nickel-spiked WB sediments had good control survival and strong concentration-response trends for survival and larvae production (Figure 15a). Significant reductions in these endpoints produced a LOEC of 353 µg/g as TR-Ni, suggesting that CE was among the most sensitive species tested in the WB sediment. In contrast, the results of water-only toxicity tests indicated that nematodes were not highly sensitive to toxicity of waterborne nickel (Appendix 2-15).

Survival of CE adults was low (80%) in controls and was not significantly reduced by any of the nickel concentrations in the water-only test, but production of larvae differed significantly among treatments, producing a LOEC of 800 µg/L.

The very different results of the nematode test with SR and WB sediments raised the question whether the nematode test method could produce meaningful results in sediment tests across a wide range of physicochemical characteristics. This question was addressed in a supplemental test conducted with eight unspiked base sediments from Tasks 2 and 3. This study showed a wide range of nematode survival across different sediment types (Figure 15b). Nematode survival was generally greater in sediment with higher organic content, with low survival (0-33%) in sediments with 0.8% to 1.9% TOC and higher survival (55-81%) in sediments with 4.1% to 10 % TOC. However, even the high-TOC sediments did not meet the ISO (2010) test-acceptability criterion for control survival (>90%).

It was also unclear whether the toxicity observed in the nematode test with nickel-spiked WB sediment could be attributed to a 'natural' partitioning of nickel between sediment and pore-water. Pre-test samples of overlying water from sample cups with spiked WB sediment (collected <24 hr after water replacement) had high nickel concentrations, as was reported in previous static toxicity tests with nickel-spiked sediments (Vandeguchte and others 2006). Treatments that were toxic to nematodes had OW-Ni concentrations (68-5700 µg/L) that exceeded chronic water quality criteria for nickel (for example, 52 µg/L at hardness of 100 mg/L). In the most toxic treatments, OW-Ni also exceeded the nickel LOEC from the nematode water-only test. Nickel concentrations in overlying water presumably increased over time during the 4-day tests with no water replacement. In contrast, OW-Ni concentrations in flow-through tests exceeded 50 µg/L in only one treatment (WB-5), and generally decreased during tests (Appendix 2-10). These compparisons suggest that exposure

to aqueous nickel was a greater contributor to observed toxicity in the static CE sediment tests than in flow-through tests with the other eight taxa.

Concentration-Response Relationships

Concentration-response models based on TR-Ni were evaluated separately for tests with spiked SR and WB sediment (Appendix 2-16) to identify the endpoints that would generate the most sensitive and reliable toxicity values for each species. Results of each successful model are reported as both EC10s and EC20s, with corresponding 95 % confidence intervals. Trends among species and endpoints were similar for EC10s and EC20s. This discussion will focus primarily on EC20s, but will note any substantive differences between the two metrics.

Several species had endpoints that were sensitive for both sediments, including GP biomass (flagged for low survival in SR control), HA biomass, and HS biomass, and LV abundance, plus other endpoints (survival and/or growth) that produced acceptable models but were less sensitive. For HA, models selected for each sediment were derived using merged data from duplicate tests. The oligochaetes LV and TT each had one acceptable model for each sediment, despite low levels of effects. For TT, the most sensitive endpoint differed between the SR sediment (adult biomass) and the WB sediment (number of juveniles). Both midges showed effects only in WB sediments and for only one endpoint: emergence for CD and egg production for CR. The CE test produced models for adult survival and larvae production in the WB sediment, but no toxicity data for the SR sediment.

The sensitivity of invertebrates to nickel-spiked sediments, expressed as either EC20s (Figure 16a) or EC10s for TR-Ni, differed widely among species and between sediments. Based on responses in both sediments, the three most sensitive species were HA, GP, and HS. Previous toxicity tests with nickel-spiked sediments reported a similar ranking of sensitivity

for four of the species we tested: HA (most sensitive) > HS > CR > TT (Milani and others, 2003). The sensitivity of GP to nickel-spiked sediments was consistent with the responses of *Gammarus* to nickel-spiked sediments in field colonization studies (Costello and others, 2011). The relative sensitivity of the other species is less certain because of the lack of defined toxicity values for four species in tests with the SR sediment. For the four species that had toxicity models for both sediments, EC20s for TR-Ni were consistently lower (i.e., nickel toxicity was greater) in SR sediments. EC20s averaged 5.7 times greater for WB sediments than for SR sediments (range: 4.0 for HS to 8.5 for GP), and these differences were more pronounced for EC10s (7.3 times greater for WB sediments). The apparent differences in nickel bioavailability between the sediments are consistent with the differences in Kd values, which averaged 5.2 times greater for WB sediment.

Concentration-response models based on the 'potentially-bioavailable' nickel fractions, [SEM-AVS] or PW-Ni, greatly reduced differences in toxicity values between sediments. For the four species that had defined TR-Ni toxicity values for both sediments, pore-water EC20s for two species (GP and HA) were greater for the WB sediment and pore-water EC20s for the other two species (HS and TT) were greater for the SR sediment. The similarity of concentration-response data for the two sediments allowed estimation of toxicity values for a broader range of species (five species for [SEM-AVS] and seven species for PW-Ni) using merged data from tests with both sediments (Appendix 2-17; Figures 2-7b,c). For the three most sensitive species (GP, HA, HS), the widths of confidence intervals (expressed as a percentage of the EC20) were similar for EC20s calculated for individual sediments based on TR-Ni (means = 166% for SR, 300% for WB) and for EC20s calculated from merged data based on [SEM-AVS] (mean = 291%) or PW-Ni (mean = 180%). The convergence of toxicity values based on [SEM-AVS] and PW-Ni, despite the widely differing nickel-binding behavior of the WB and SR sediments, is consistent with the

development of models to predict nickel toxicity based on factors controlling nickel partitioning and bioavailability in sediments (for example, Ankley and others, 1996, USEPA, 2005).

The importance of nickel exposure via pore-water was supported by the close agreement between EC20s from water-only exposures and EC20s for PW-Ni for most species (Figure 16c). Water-only EC20s fell within or close to the confidence intervals for PW-Ni EC20s for all species except LS and HA, both of which had water-only EC20s that were much lower than PW-Ni EC20s. These apparent differences in sensitivity to aqueous nickel between tests may reflect differences in age/size of these species at testing. For both species, average starting size (shell length of juvenile mussels; dry weight of mayfly nymphs) was substantially larger for the Task-2 sediment tests than for the water-only tests (Table 7). The smaller starting size of these two species in the water-only test could have contributed to their greater sensitivity to aqueous nickel. The discrepancy in EC20s for PW-Ni in sediment tests and for nickel in the water-only test may also indicate that: (1) peeper samples overestimated actual PW-Ni exposures during sediment tests (for example, due to microhabitat differences); and/or (2) water-only toxicity did not accurately represent the sensitivity of these species to nickel in a sediment environment (for example, due to inadequate substrate). Another exception to the convergence of water-based toxicity values is the contrast between results of the nematode sediment and water-only tests. The nematode EC20 for reduced survival based on OW-Ni concentrations (pre-test) was 105 µg/L in WB sediment, but survival in the water-only test was not significantly reduced at a nominal concentration of 800 µg/L, the highest concentration tested. This discrepancy may indicate that pre-test OW-Ni measurements underestimated PW-Ni and/or OW-Ni that occurred during the four-day static test.

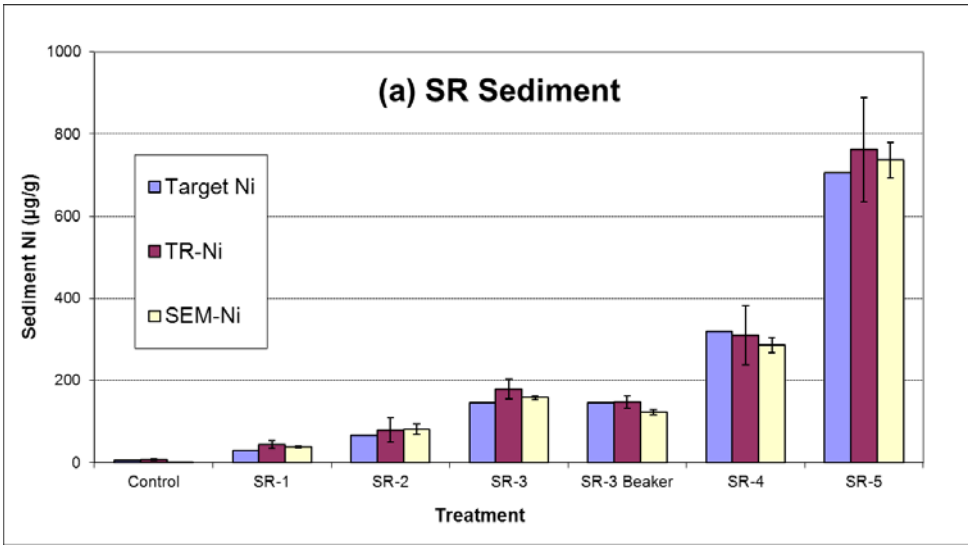
2.4 Conclusions

- The sediment spiking protocol (10-week indirect spiking plus 1-week pre-test equilibration) produced consistent concentrations of nickel and AVS across a wide range of spike levels in two sediments over a four-month toxicity testing period. The pre-test equilibration of sediment in test chambers allowed formation of an oxidized surface sediment layer and allowed diffusive loss of unbound "excess" nickel from pore-waters of the highest nickel-spike treatments. Nickel concentrations in overlying waters remained below levels of concern during tests, except in treatment WB-5.

- Flow-through sediment toxicity tests generated chronic toxicity values for seven species (of eight species tested) in spiked WB sediment and for four species in spiked SR sediment. Other tests produced no toxic effects at the highest nickel spike levels. Static sediment toxicity tests with the nematode *Caenorhabditis* did not produce toxicity values that could be reliably compared to toxicity values from flow-through tests. The nematode tests were problematic because of the wide variation in nematode survival among different sediments and because of greater OW-Ni concentrations in the static nematode tests, compared to flow-through tests.

- Toxicity values for sediment nickel (TR-Ni) differed between sediments by a factor of six, with toxicity occurring at lower nickel concentrations in the SR sediment. These differences are consistent with the greater nickel binding capacity of the WB sediment, as indicated by nickel distribution coefficient or Kd. In contrast, toxicity values estimated from [SEM-AVS] or PW-Ni did not differ substantially between sediments, suggesting that these measurements were better estimators of the bioavailable nickel fraction.

- The amphipods *Hyalella* and *Gammarus* and the mayfly *Hexagenia* were the three most sensitive species in tests with both sediments. The lowest valid EC20 value for TR-Ni in SR sediment was 202 µg/g (for *Hyalella* biomass), compared to the lowest EC20 of 1177 µg/g (for *Hyalella* biomass) in WB sediments. The lowest EC20s estimated from merged data for both sediments were 6.8 µmol/g as [SEM-AVS] (for *Gammarus* biomass) and 63 µg/L as PW-Ni (for *Hexagenia* biomass). The corresponding lowest EC10 values were: 131 µg/g (SR sediment) and 855 µg/g (WB sediment) for TR-Ni; 2.9 µmol/g for [SEM-AVS]; and 45 µg/L for PW-Ni.



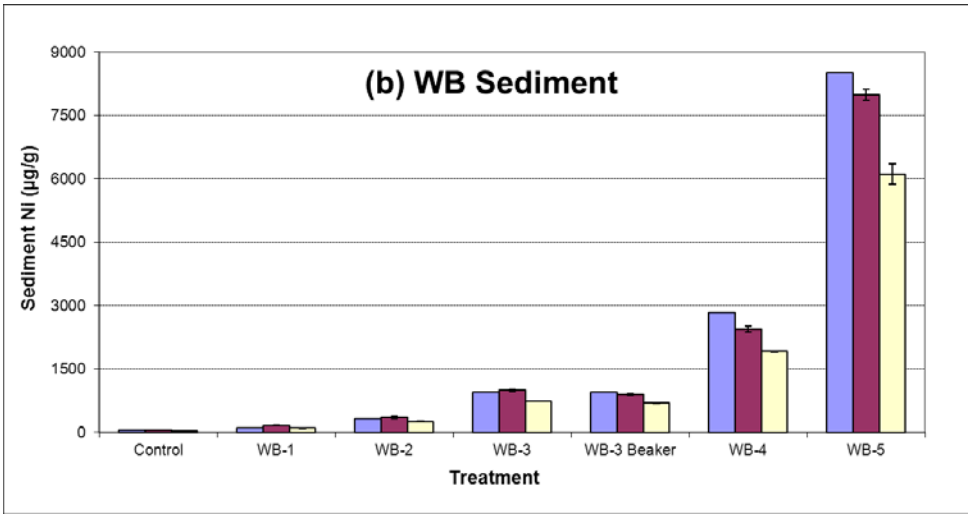
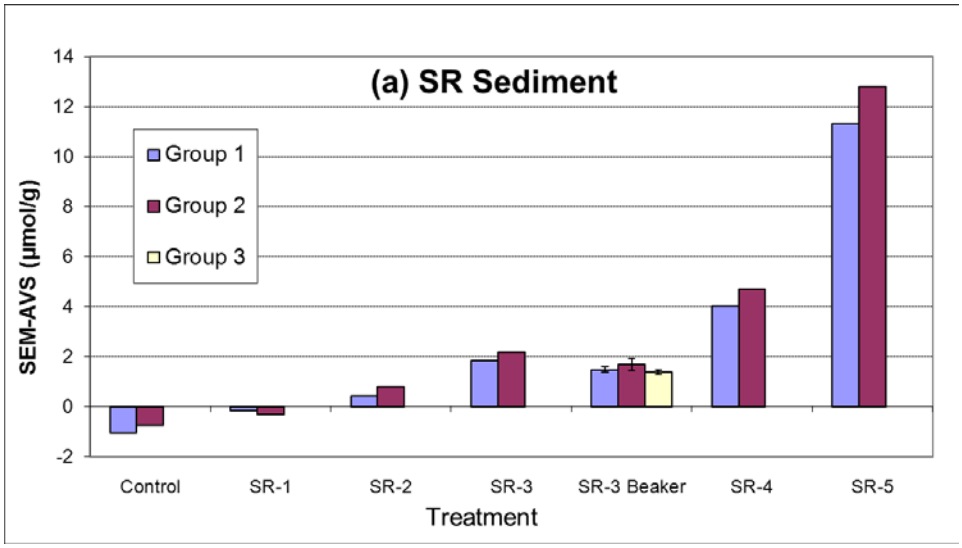


Figure 10. Target and measured nickel concentrations in spiked sediments: (a) SR sediment; (b) WB sediment.

[TR-Ni = total-recoverable nickel; SEM-Ni = simultaneously-extractable nickel; means and standard deviations of analyses of bulk sediments used for test groups 1 and 2 (n=2), and sediments from Day 14 test beaker treatment 3 of all test groups (n=9).]



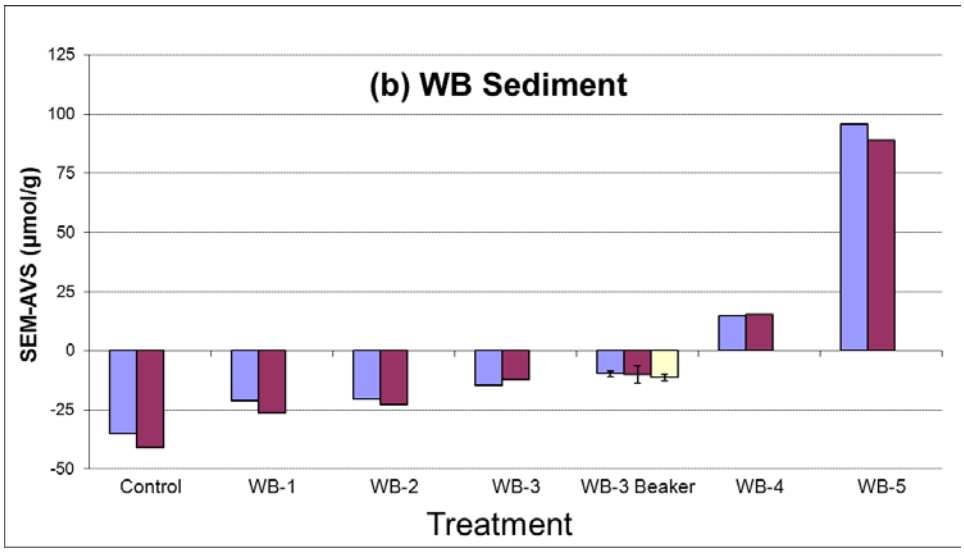


Figure 11. Difference between simultaneously-extracted nickel (SEM-Ni) and AVS in spiked sediments: (a) SR sediment; (b) WB sediment.

[Single analyses of bulk sediments for test groups 1 and 2: means and standard deviation of analyses of beaker sediments for all three groups (treatment 3 only; n=2-5).]

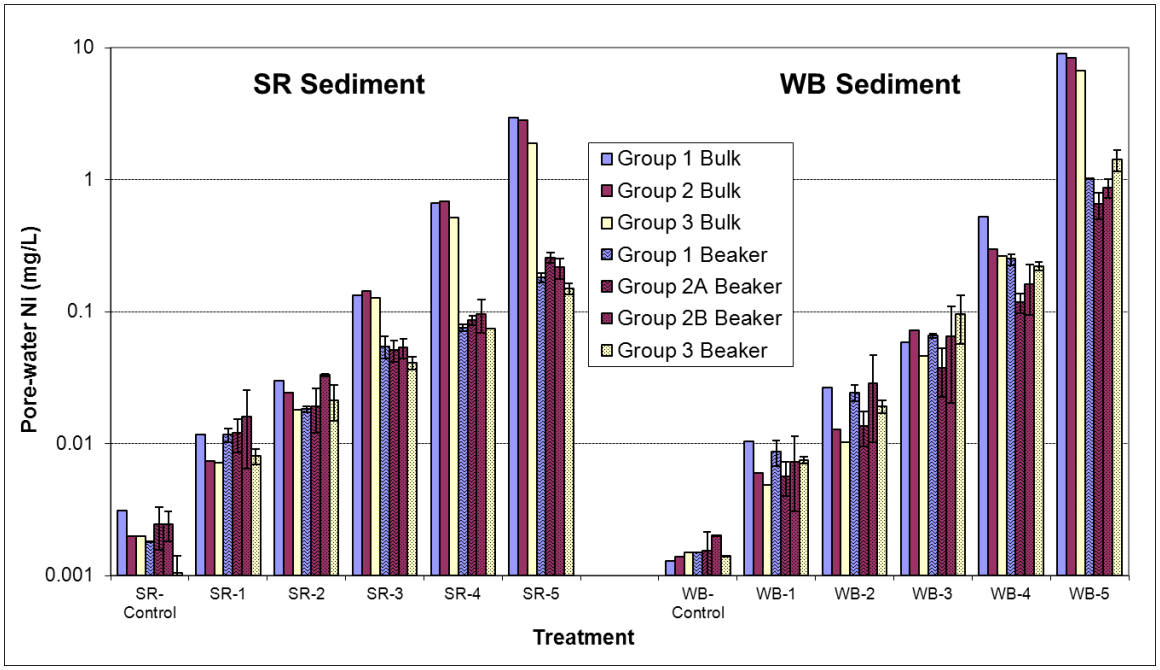


Figure 12. Pore-water nickel concentrations in nickel-spiked sediments: (a) SR sediment; (b) WB sediment.

[Single analyses of centrifuged samples from each of the three bulk sediment containers (day-7) and means and standard deviations of multiple analyses of peeper samples from test beakers (day 14).]

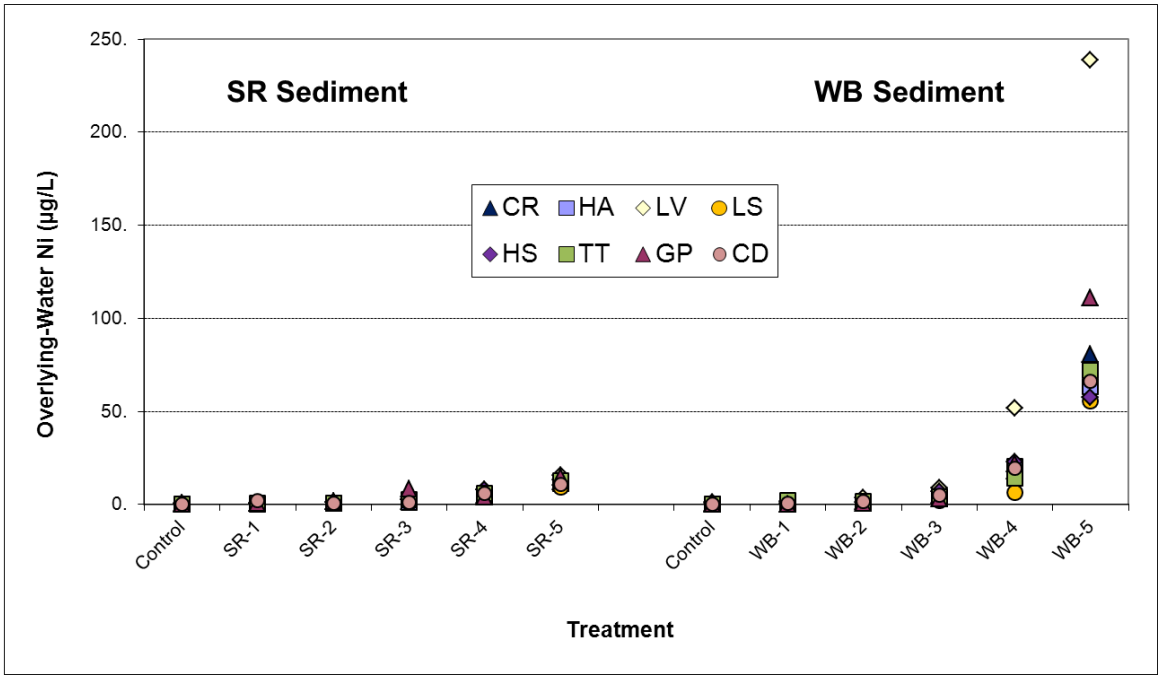


Figure 13. Measured nickel concentrations in overlying water of sediment toxicity tests.

[Means, (n = 2 or 3). Species: HA=*Hyalella*; GP=*Gammarus*; CD=*C. dilutus*; CR=*C. riparius*; LV=*Lumbriculus*; LS=*Lampsilis*; HS=*Hexagenia*; TT=*Tubifex*.]

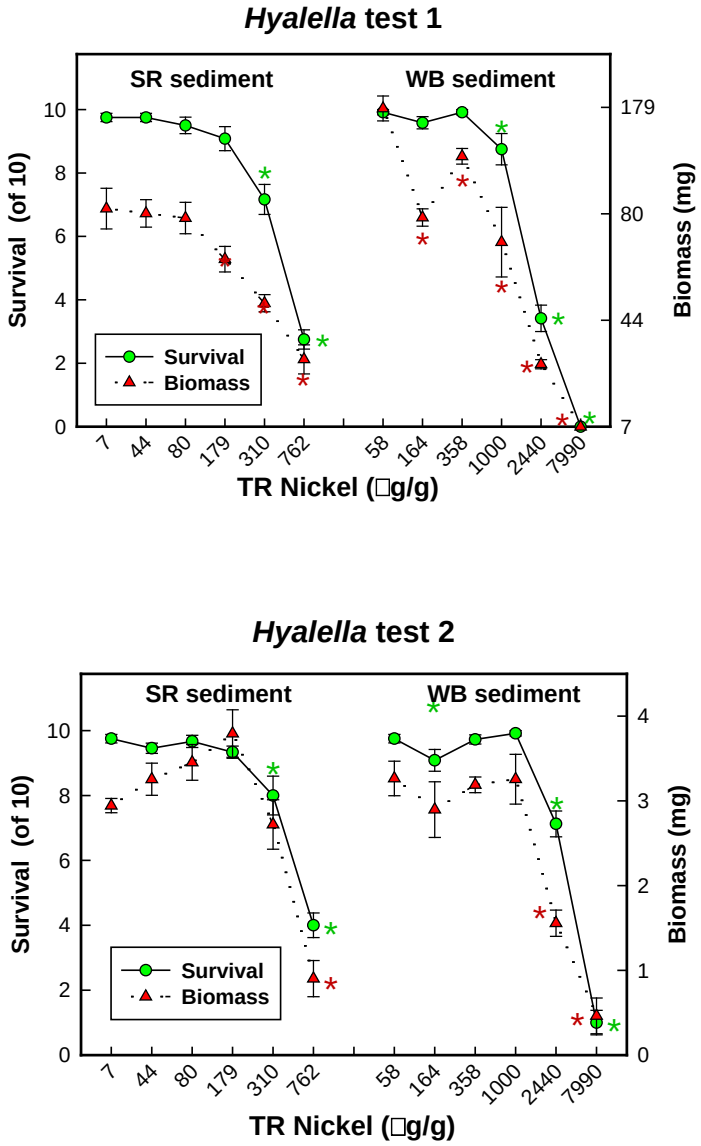
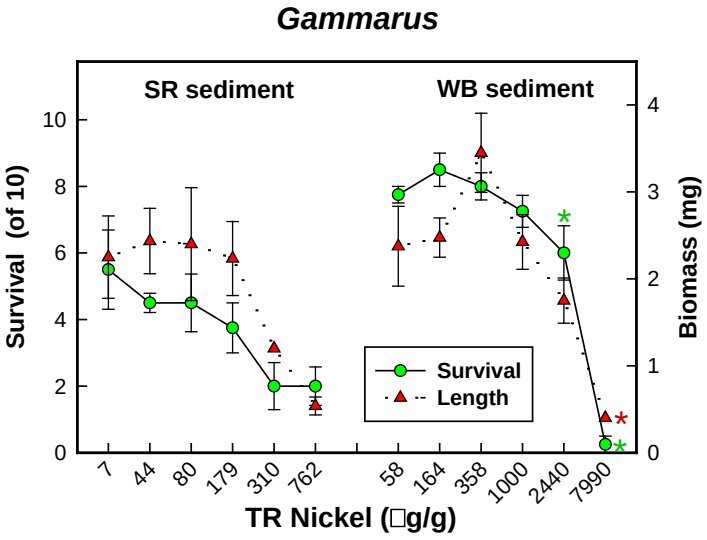


Figure 14. Responses of selected endpoints in sediment toxicity tests: (a) *Hyalella* (amphipod) test 1; (b) *Hyalella* test 2.

[Mean and standard error (n=4); asterisks indicate significant difference from control. X-axis is not to scale.]



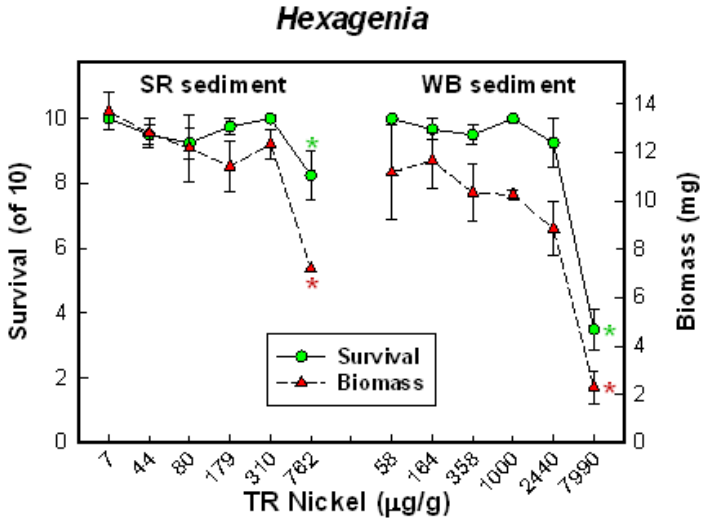


Figure 14 (continued): (c) *Gammarus* (amphipod); (d) *Hexagenia* (mayfly);

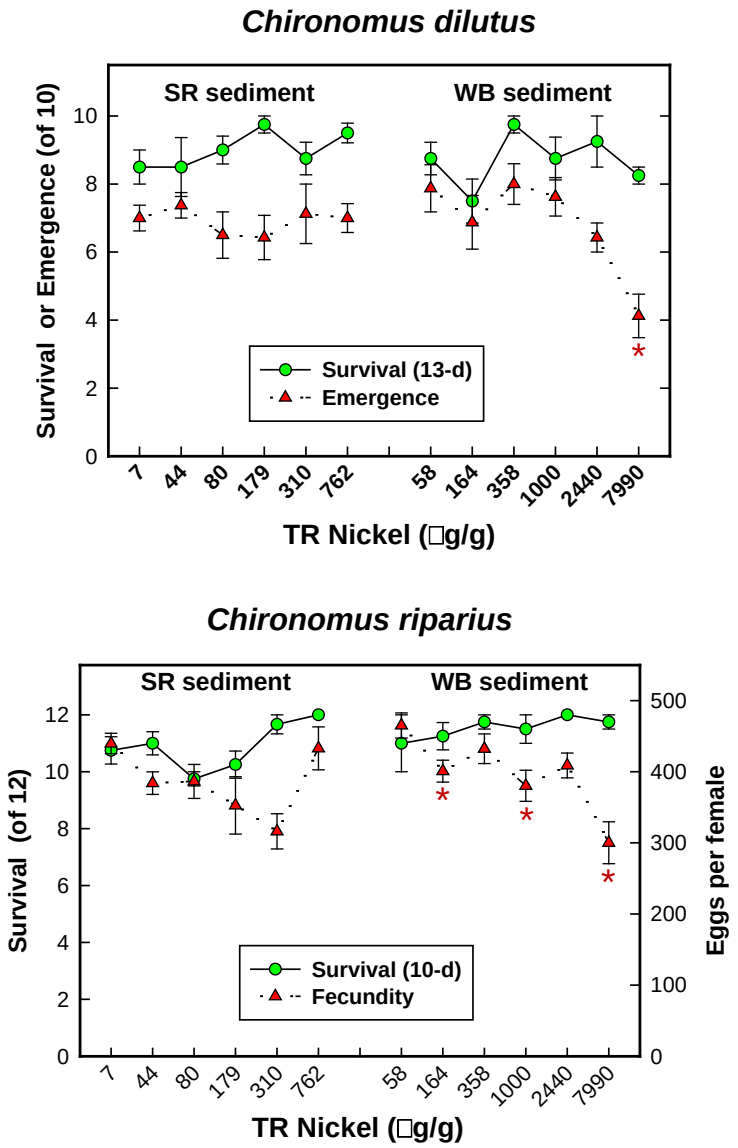
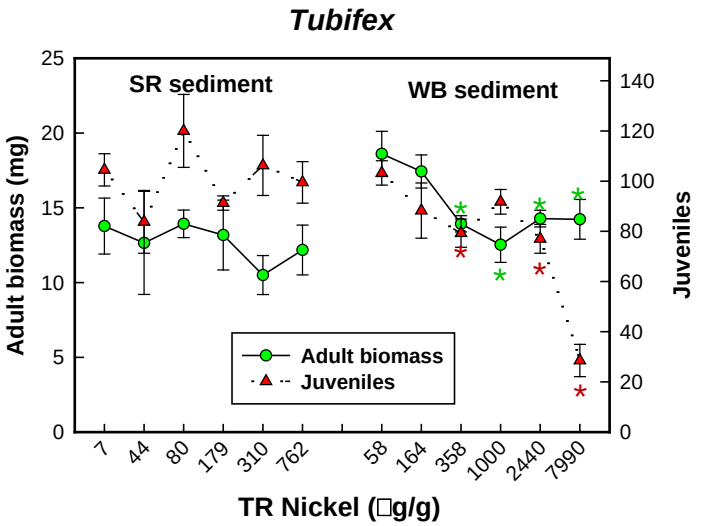


Figure 14 (continued): (e) *Chironomus dilutus* (midge); (f) *Chironomus riparius*;



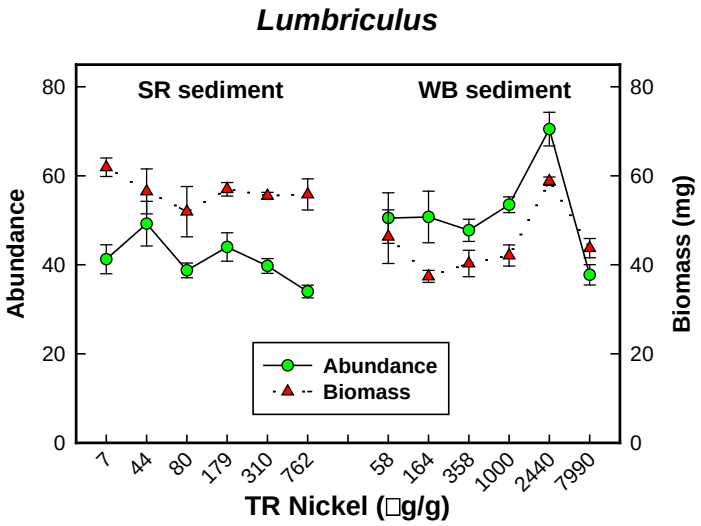


Figure 14 (continued): (g) *Tubifex* (oligochaete); (h) *Lumbriculus* (oligochaete).

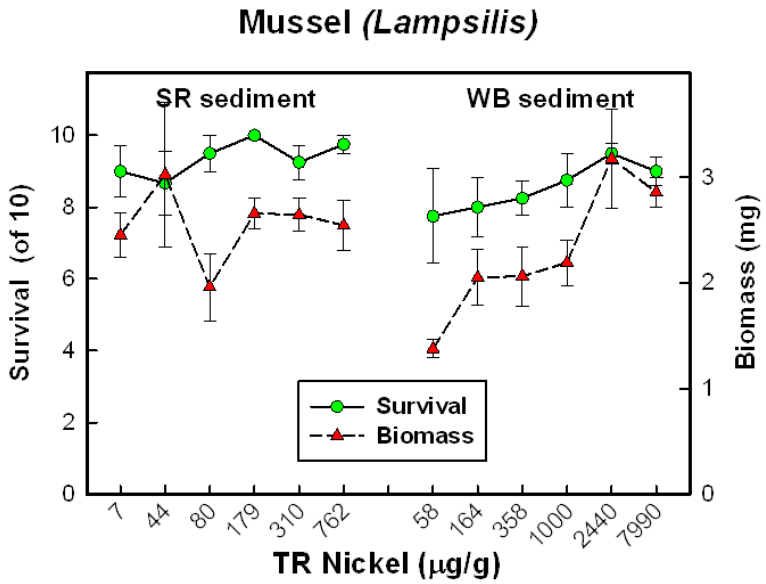


Figure 14 (continued): (i) *Lampsilis* (mussel).

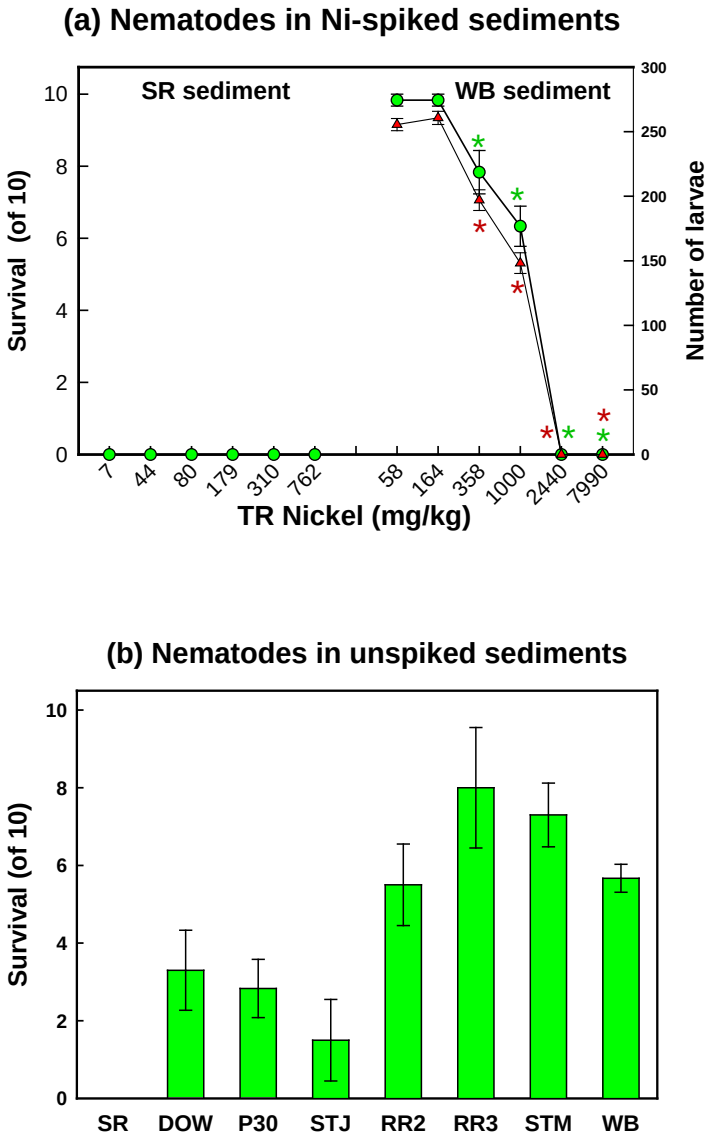


Figure 15. Survival and reproduction of nematodes (*Caenorhabditis*) in nickel-spiked and unspiked sediments: (a) nickel-spiked sediments; (b) unspiked sediments.

[Plot (a): Means (n=6) with standard error; asterisks indicate significant difference from control. X-axis not to scale. Plot (b): Means (n=6) with standard deviation. Sediments arranged from low to high TOC. Three-digit sediment IDs refer to sediments described in Chapter 3 (see Table 11 and Appendix 3-2)]

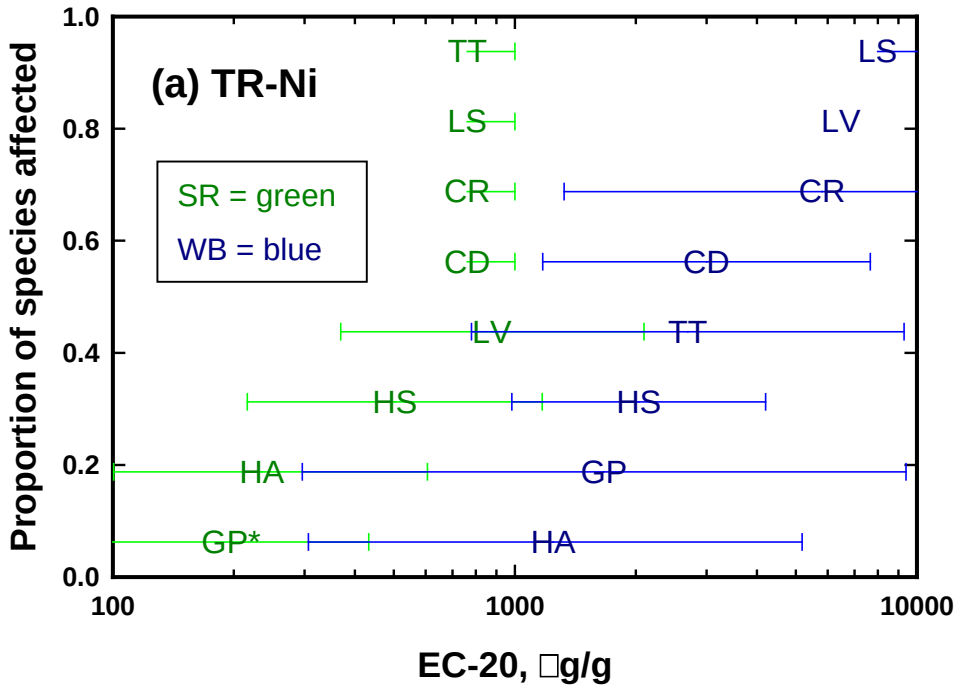
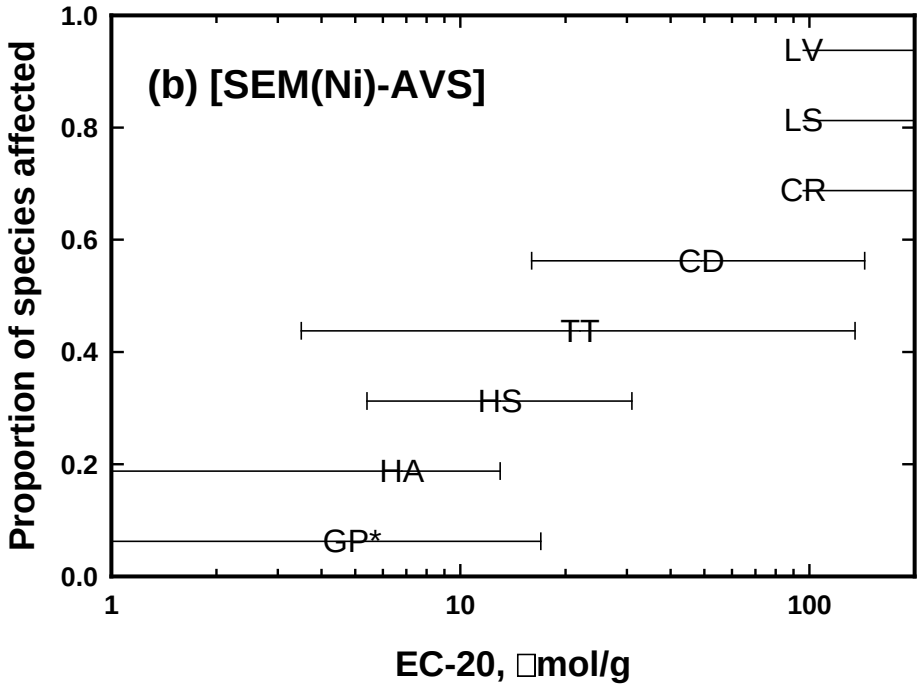


Figure 16. Sensitivity of invertebrates to nickel toxicity in spiked-sediments: (a) total-recoverable nickel (TR-Ni); (b) SEM-Ni minus AVS [SEM(Ni)-AVS]; (c) pore-water nickel (PW-NI).

[EC20s are labeled with species IDs (with 95% confidence intervals). For Plot (a), EC20s were plotted separately for each sediment: green=SR, blue=WB. For plots (b) and (c), data for both sediments were combined for EC20 determinations. In plot (c), water-only EC20s are plotted as stars. One-sided confidence intervals indicate unbounded NOECs. Asterisk indicates test flagged for low control survival.]



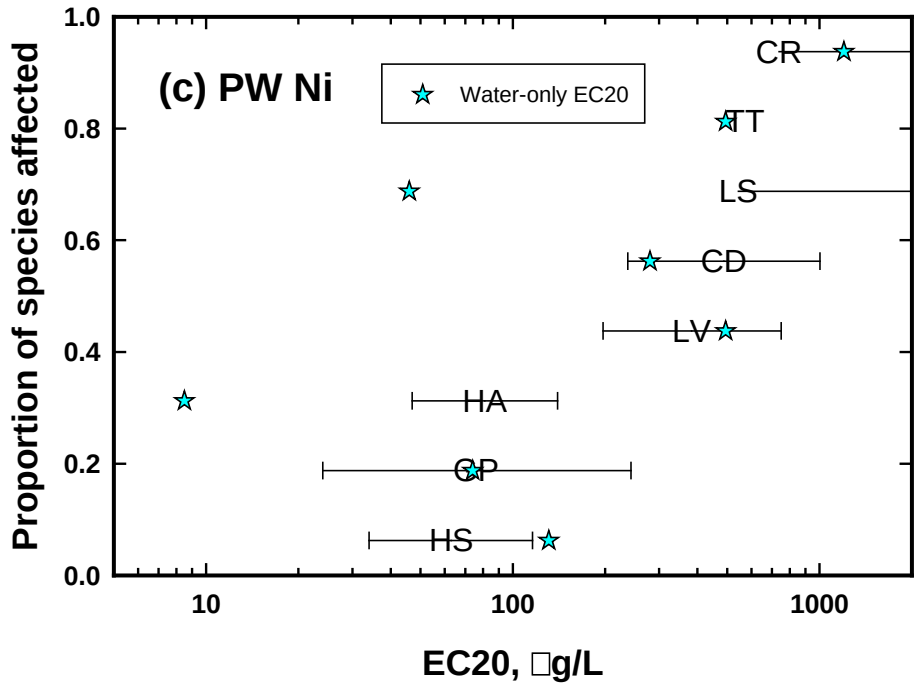


Figure 16 (continued): (b) SEM-Ni minus AVS [SEM(Ni)-AVS]; (c) pore-water nickel (PW-NI).

Table 5. Target nickel spike concentrations for Task-2 sediment toxicity tests.

Treatment	TR-Ni (μg/g)	[SEM(Ni)-AVS] (μmol/g)	(μmol/g OC)
	WB sediment:		
WB-5	8506	103	1000
WB-4	2835	31	62
WB-3	945	-5.7	-251
WB-2	315	-30	-355
WB-1	105	-39	-389
	SR sediment:		
SR-5	705	8.0	1400
SR-4	320	3.6	582
SR-3	146	1.4	210
SR-2	66	0.30	41
SR-1	30	-0.25	-36

Table 6. Test conditions for flow-through sediment toxicity tests with benthic invertebrates.

[HA=Hyalella; GP=Gammarus; CD=C. dilutus; CR=C. riparius; LV=Lumbriculus; LS=Lampsilis; HS=Hexagenia; TT=Tubifex. S=Survival, A=Abundance, G=Growth, B=Biomass, R=Reproduction, E=Emergence, F=Fecundity, H=Hatching.]

Test Condition	Description
Temperature	23 °C for all species except GS (15 °C)
Lighting	Wide-spectrum fluorescent lights (about 200 lux); 16 hr light:8 hr dark
Chamber volume	300 ml for all tests except HS (1,000 ml)
Sediment volume	100 mL except HS (200 ml)
Overlying water volume	About 175 ml except HS (about 700 ml)
Test water	Diluted CERC well water (100 mg/L hardness as CaCO3); pH adjusted to 7.3 by an automated pH controller.
Overlying water renewal	8 volume-additions s per day
Replicates per treatment	4, except HA, 12 reps; CD and CR, 16 reps
Organisms per replicate	10 per replicate except TT (4) and CR (12)
Age of organisms	CR, 4 d old; CD and HA, 7 d old;
	HS, 6-8 weeks old (5-10 mg wet weight);
	GS, juveniles (about 3-5 mm length),
	LS, juveniles (about 2 months old); LV and TT, adults.
Feeding	HA and GS: YCT diet, 1.8 mg/d (1.0 ml from 1800 mg/L stock)
	HS: YCT diet (1800 mg/L stock): 4 mL/d
	CD and CR: Tetrafin® suspension, 6 mg/d (1.5 ml/d of 4 g/L stock)
	LV and TT: Tetrafin® suspension, 16 mg/d (4 ml of 4 g/L stock)
Aeration	None
Water & sediment quality (see Table 4)	Test water pH, conductivity, major ions, DOC (day 0)
	Overlying water pH, dissolved oxygen, temperature, conductivity (weekly)
	Overlying water Ni, hardness, alkalinity, ammonia (day 0 and end of test)
	Sediment Ni, particle size, TOC, CEC, solids (day -7); SEM, AVS (day 28)
	Pore-water Ni, pH, conductivity, ions, DOC (day 0); Ni, Fe (day 14)
Duration and endpoints	GP, HS and LS: 28 d (S/G/B)
	LV: 28 d (A/B)
	TT: 28 d (S/G/B/R)
	HA: 28 d (S/G/B), 42 d (R)
	CD and CR: 10 d (S/G/B); about 42 d for CR (E); about 56 d for CD (E/F/H)
Acceptability criteria	CD and CR: ≥70% control survival (day 10); ≥50% emergence
	HA, GP, LS, HS: ≥80% control survival (day 28)
	TT: ≥90% control survival (day 28)
	LV: >60% increase in biomass (day 28)
	Additional performance criteria from ASTM (2010a,b,c), OECD (2004, 2007), USEPA (2000),and Norberg-King et al. (2006).

Table 7. Average starting size of test organisms used in toxicity tests.

[Means and standard deviations (SD). Ash-free dry weight data from 20-80 animals weighed as 2-4 replicates. Length data from 15-20 animals, weighed individually.]

Species	Dry Weight (mg)		Length (mm)	
	Mean	SD	Mean	SD
Task-2 sediment tests				
C. dilutus (CD)	0.26*	0.05		
C. riparius (CR)	0.09	0.02		
Gammarus (GP)			2.84	0.49
Hexagenia (HS)	1.12	0.36		
Hyalella (HA1)			1.92	0.19
Hyalella (HA2)			1.59	0.26
Lampsilis (LS)			1.85	0.35
Lumbriculus (LV)	1.20	0.10		
Tubifex (TT)	1.52	0.25		
Task-2 water-only tests				
C. dilutus (CD)	0.13	0.06		
C. riparius (CR)	0.02	0.01		
Gammarus (GP)			2.69	0.08
Hexagenia (HS)	0.41	0.05		
Hyalella (HA)			2.00	0.07
Lampsilis (LS)			1.37	0.06
Lumbriculus (LV)	1.07	0.03		
Tubifex (TT)	0.86	0.03		
Task-3 sediment tests				
Gammarus (GP)			2.92	0.44
Hexagenia (HS)	0.17	0.03		
Hyalella (HA)			1.83	0.33
Tubifex (TT)	1.07	0.01		

Table 8. Sampling schedule for Task- 2 sediment toxicity tests

Sample	Analyte(s)	Test day(s)	Treatments	Frequency
Bulk sediment	Particle size distribution	Day -7	Composite	First jar
	Cation exchange capacity (CEC)	Day -7	Composite	First jar
	Total organic carbon (TOC)	Day -7	Composite	First jar
	Total-recoverable Ni (TR-Ni)	Day -7	All	All jars
	Acid-volatile sulfide (AVS), simultaneously-extracted Ni (SEM-Ni)	Day -7	All	All jars
Bulk pore-water	Dissolved organic carbon (DOC), cations, routine water quality	Day -7	All	First jar
	Anions	Day -7	All	Second jar
	Ni and Fe	Day -7	All	All jars
Test water	DOC, cations	Day -7	Composite	First test
	Anions	Day -7	Composite	Second test
Overlying water	pH, temperature, dissolved oxygen, conductivity	Weekly	C/1/3/5	All tests
	Hardness, alkalinity, ammonia	Day 0, End	C/1/3/5	All tests
	Filterable Ni	Day 0, End	All	All tests
Beaker sediment	AVS, SEM-Ni	Day 14	3 only	All tests
Beaker pore-water	Ni and Fe	Days 7-14	All	All tests

Table 9. Schedule for Task-2 sediment toxicity tests.

[Species: HA=Hyalella; GP=Gammarus; CD=C. dilutus; CR=C. riparius; LV=Lumbriculus; LS=Lampsilis; HS=Hexagenia; TT=Tubifex. Endpoints: S=Survival, A=Abundance, G=Growth, B=Biomass, R=Reproduction, E=Emergence, F=Fecundity, H=Hatching.]

Group (Jar)	Equilibration (Weeks)	Test	Start date (2009-10)	Duration (d)	Endpoints
1	10	HA(1)	24-Sep	28/42	SGB/R
	10	CR	25-Sep	10/~42	SGB/E
2	14	LV	20-Oct	28	AB
	14	LS	20-Oct	28	SGB
	14	HS	20-Oct	28	SGB
	16	TT	2-Nov	28	SGB
	16	GP	2-Nov	28	SGB
3	22	CD	18-Dec	13/~56	SGB/EFH
	22	HA(2)	18-Dec	28/42	SGB/R
	>22	CE	??	4	SR

Table 10. Toxicity values for Task- 2 water-only toxicity tests determined by analysis of variance (ANOVA) and concentration-response models.

[All values µg/L. NOEC=no observed effect concentration; LOEC=lowest observed effect concentration; EC10 and EC20 are 10% and 20% effect concentrations; lcl and ucl are lower and upper 95% confidence limits. Endpoints: S=survival, G=growth (length or weight), B-biomass, R=reproduction, C=coccoons, E=emergence. Dash (--) indicates no value was calculated.]

Species ID	Endpoint	ANOVA		Concentration-response models			
		NOEC	LOEC	EC10	lcl-ucl	EC20	lcl-ucl
CD	S	>1710	--	--	--	--	--
	G	689	1710	--	--	--	--
	B	689	1710	--	--	--	--
	E	363	689	208	64-684	280	117-669
CR	S	>1715	--	999		1454	
	G	>1715	--	--	--	--	--
	B	>1715	--	839	60-11,807	1201	273-5,292
	E	678	1715	1610	1,068-2,426	--	--
GP	S	47	94	56	51-62	74	69-79
	G	94	170	--	--	--	
	B	94	170	143	9.7-2,110	165	42-652
HA	S	8.3	17	8.6	6.6-11	12	9.9-15
	G	8.3	17	17	3.1-88	22	6.6-74
	B	8.3	17	6.5	0.52-82	8.5	1.1-65
	R	17	40	6.7	0.21-219	9.0	0.77-105
HS	S	>1335	--	--	--	--	--
	G	104	257	53	3.2-889	131	20-863
	B	104	257	102	29-364	204	86-485
LS	S	>71	--	--		--	--
	G	25	71	41	13-130	65	39-108
	B	25	71	32	1.4-706	46	7.6-275
LV	S	>494	--	--	--	--	--
	G	>494	--	--	--	--	--
	B	>494	--	--	--	--	--
TT	S	>494	--	--	--	--	--
	B	>494	--	--	--	--	--
	C	>494	--	--	--	--	--
	R	>494	--	--	--	--	--
CE	S	--	>800	--	--	--	--
	R	400	800	349	2-50,094	550	42-7,273

Chapter 3: Influence of sediment characteristics on nickel bioavailability

3.1 Introduction

The results of Task- 2 demonstrated substantial differences in nickel toxicity thresholds for sensitive invertebrate species between two sediments with widely differing physic-chemical characteristics. These differences in nickel bioavailability were generally consistent with general models of bioavailability of cationic metals (e.g., USEPA 2005). However, there remains uncertainty about the applicability of these models to nickel. As discussed above, nickel tends to have lower affinity for binding to sediment components, including AVS, compared to other cationic metals (Ankley and others, 1996). In Task 3, the most sensitive invertebrate species identified in Task 2 were tested with six additional nickel-spiked sediments that represented gradients in concentrations of AVS, TOC, and other sediment characteristics that may control nickel bioavailability. The primary objective of Task 3 was to characterize differences in nickel toxicity and bioavailability among the eight freshwater sediments tested in Tasks 2 and 3. These combined data provided a basis for examining relationships between nickel toxicity values and the characteristics of sediment and pore-water that control nickel bioavailability. The ultimate goal of these studies is to provide a sound basis for development of sediment sediment-quality guidelines for nickel in freshwater sediments.

3.2 Methods

Sediment Spiking

Sediments were selected for testing in Task 3 primarily to establish a gradient of concentrations of AVS and TOC between the extremes represented by the two Task-2 sediments (AVS = 0.8-42 µmol/g, TOC = 0.8-10.3 %) (Table 11). Sediments tested in Task 3 included one pond sediment from CERC, Missouri, USA (Pond 30, or P30) and five stream sediments from southern Michigan, USA: Dow Creek (DOW); Raisin River Site 2 (RR2); Raisin River Site 3 (RR3); St. Joseph River (STJ); and South Tributary of Mill Creek (STM). Several of the Michigan sediments (and the SR sediment from Task 2) were included in a companion field study of invertebrate colonization of nickel-spiked sediments (Costello and others, 2011). Task-3 sediments were collected in Fall 2009, sealed in 21-L polyethylene buckets, and stored at 4°C in the dark until spiking in Winter 2009-2010.

The six Task-3 sediments were each spiked with nickel using the two-stage spiking protocol described in Chapter 2. Nickel spike concentrations were selected to reflect the expected nickel-binding capacities of the sediments, with nominal high nickel concentrations of 1237 µg/g (for DOW sediment), 1667 µg/g (for P30, RR2, RR3, and STJ sediments), and 2400 µg/g (for STM sediment). Following the 28-d equilibration of super-spikes prepared with each sediment, sediment dilutions with unspiked sediment produced five nominal nickel concentrations in two-fold dilution series (Table 11), plus a control, for each sediment. Details of the spiking and sediment dilution procedures are presented in Appendix 3-1. Superspikes were spiked with nickel and superspikes were used to prepare sediment dilutions after 28 d. Duplicate jars were prepared for each treatment (3.6 L sediment per jar). Jars in the first duplicate set (Group 1) were opened for testing after a 10 weeks of equilibration, and the second set of jars (Group 2) was opened for Group-2 tests after 14 weeks of equilibration.

Toxicity Testing

The four species selected for tested in Task 3 included the three most sensitive species tested in Task 2: the amphipods, *Hyalella azteca* (HA) and *Gammarus pseudolimnaeus* (GP); and the burrowing mayfly, *Hexagenia* sp. (HS). A fourth, less sensitive species, the oligochaete, *Tubifex tubifex* (TT), was also tested to ensure that the results reflected broad taxonomic and behavioral diversity. Methods and endpoints for Task-3 sediment toxicity tests were the same as those described for Task 2 (Chapter 2; Table 6), except test conditions for HS were modified due to space limitations. Instead of the large (1-L) test chambers used in Task 2, Task-3 HS tests were conducted with the same smaller (300-mL) test chambers used for the other three species. Use of the smaller test chambers required reductions in the amount of sediment added (100 mL per chamber), the number of HS stocked (5 per chamber), and the feeding rate (2 mL YCT diet per day). Group-1 tests were conducted with HS and TT and Group-2 tests were conducted with GP and HA.

Sediment Characterization

Sampling schedules for characterizing nickel concentrations and other constituents of sediment and water were similar to those described in Chapter 2 (Table 8). General physico-chemical characteristics of bulk sediments and pore-waters were measured in samples from Group-1 sediments only. Concentrations of sediment nickel (TR-Ni, SEM-Ni) and AVS were measured in bulk sediments samples from both groups. Concentrations of Ni, Fe, and Mn in pore-waters (day-14 peeper samples) and Ni concentrations in overlying water (days 1 and 27) were measured during all tests.

Data Analysis and Interpretation

Routine analyses of data from Task-3 toxicity tests and chemical analyses were similar to those described in Chapter 2. Rank ANOVA and Dunnett's test were conducted using SAS/STAT software and concentration-response relationships and toxicity values (EC20s) were modeled using TRAP software. Data from Tasks 2 and 3 (8 sediments and 3 species) were merged for analysis of relationships among toxicity values and sediment characteristics. These analyses (conducted with SAS/STAT) included bivariate (Pearson's) linear correlation analysis.

3.3 Results and Discussion

Sediment Characteristics and Nickel Concentrations

Sediment characteristics are summarized in Appendix 3-2. All sediments had circumneutral pH (6.8-7.2) and suboxic to moderately reducing conditions (-160 to -198 mV). Most sediments were dominated by sand-sized particles (14-28% fine particles), except STM (47% fines) and P30 (90% fines). Cation exchange capacity ranged from 6.0 meq/100 g (DOW) to 29 meq/100 g (STM) which generally corresponded to differences in TOC and AVS. Unspiked sediments had consistently low concentrations of trace metals, but wide ranges in concentrations of the major elements Ca (0.4-8.4%), Fe (0.7-2.7%), and Al (0.6-2.6%) (Appendix 3-3).

Sediment nickel concentrations are summarized in Appendix 3-4. Measured TR-Ni concentrations in high-spike treatments were close to nominal and means differed by more than a factor of four between DOW (1341 µg/g) and STM (5080 µg/g). In unspiked sediments, SEM-Ni concentrations constituted small fractions of TR-Ni (from 18% in DOW to 33% in STM), suggesting relatively low nickel bioavailability. In spiked sediments, SEM-

Ni made up greater fractions of TR-Ni, ranging from about 65% (DOW, STJ) to over 80% (RR3, P30, STM) and these SEM-Ni fractions did not differ appreciably among spike levels. As a result, differences between SEM-Ni and AVS ([SEM-AVS]) in the highest spike treatments were lower than target values in some treatments, but [SEM-AVS] levels were still clearly separated into three target spiking ranges: low (DOW), about 15 µmol/g); medium (P30, STJ, RR2, RR3), 28-38 µmol/g); and high (STM), 61 µmol/g). However, expressing [SEM-AVS] relative to the organic carbon fraction of sediments produced a different ranking of sediments, with highest values ranging from 480 µmol/g OC in RR3 to 1780 µmol/g OC in STJ.

Characteristics of bulk pore-waters are summarized in Appendix 3-5. Pore-waters of unspiked sediments had high concentrations of calcium (182-348 mg/L), magnesium (47-70 mg/L), and DOC (21-51 mg/L). Iron concentrations (assumed to be ferrous) in bulk pore-waters in unspiked sediments ranged from 10 mg/L (STJ) to 46 mg/L (STM), consistent with anaerobic conditions during equilibration, but total dissolved sulfides were low (<20 µg/L). Some nickel-spiked sediments had increased pore-water iron concentrations, which might be expected if ferrous iron associated with FeS was displaced by nickel from spike solutions. Bulk pore-waters of spiked sediments also had high concentrations of sodium and chloride from spike solutions and pH adjustments. Increased concentrations of other cations were also evident in bulk pore-waters of nickel-spiked sediments, presumably as a result of displacement by nickel. Maximum sodium chloride concentrations in bulk pore-waters from Task-3 sediments ranged from 2.3 g/L (RR3 sediment) to 7.7 g/L (STJ and STM sediments), which overlapped with the range of acute EC50s for sodium chloride reference-toxicity tests (4.1-11 g/L; J. Besser, USGS, unpublished data). However, pore-waters from test beakers (Appendix 3-6) had lower concentrations of sodium and other cations -- by a factor of ten or more in highest spike treatments – suggesting diffusional loss of ions to overlying water as a

result of strong concentration gradients between pore-water and overlying water. Conductivity of overlying waters in high nickel-spike treatments were not substantially different from controls (Appendix 3-7), suggesting that test organisms experienced low sodium chloride levels during flow-through sediment tests. Overlying waters of nickel-spiked sediments in Task-3 HS tests did not have the increased conductivity or reduced pH reported in Task-2 HS tests (Appendix 2-6). This observation is consistent with the hypothesis of reduced bioturbation and slower oxidation of AVS in Task-3 HS tests, due to the smaller size of HS nymphs and smaller sediment surface area in the small Task-3 test chambers. Other water-quality characteristics of overlying water were also close to expected values.

Concentrations of nickel, iron, and manganese in pore-waters of bulk sediments and test beakers are summarized in Appendix 3-8. Like other cations, PW-Ni decreased substantially between bulk sediments and test beakers in the highest spike treatments. Within treatments, PW-Ni in test beakers were consistent among the four tests except in the P30 sediment, where PW-Ni in the highest spike treatment ranged from 80 µg/L in the TT test to 673 µg/L in the GP test. Spiking treatments resulted in similar ranges of mean PW-Ni across the six sediments (Figure 17a), but distribution of nickel between sediment and pore-water differed among sediments. Nickel distribution coefficients were lowest for DOW (log Kd=3.529) and highest for STM (log Kd=4.340) (Appendix 3-2; Figure 17a). The role of AVS in controlling nickel binding is illustrated by the consistently low PW-Ni in treatments with SEM-Ni less than AVS (Figure 17b). Iron and manganese concentrations also were greater in bulk pore-water (by about five- to ten-fold) than in test beakers. However, in contrast to bulk pore-water, iron and manganese concentrations in beaker pore-waters tended to decrease at increased nickel spike levels (Appendix 3-8). The reason for this trend is unclear.

Nickel concentrations in overlying water averaged less than 10% of PW-Ni (Appendix 3-9). Concentrations of OW-Ni were generally consistent within spike levels across different (for example, 20-35 µg/L in high spike treatments), but the HS test with P30 sediments generally had greater OW-Ni concentrations than tests with other species and sediments.

Toxicity Tests and Endpoints

Results of Task- 3 sediment toxicity tests are summarized in Appendix 3-10. All 24 tests met test acceptability criteria for control survival (Appendix 2-3). Three of four species tested (GP, HS, and HA) showed significant toxic effects (significant overall ANOVA and LOECs defined by significant Dunnett's tests) for one or more endpoints in all six sediments. Nickel-spiked sediments were less toxic to the oligochaete TT, with no endpoint having defined LOECs for more than two sediments. These results are similar to the responses of these four species in Task 2 tests, which produced consistent toxic effects for the three sensitive species, but only marginally significant effects on TT. These results indicated that tests with GP, HA, and HS would provide useful information for comparisons of nickel bioavailability among sediments, whereas TT would not.

The development of reliable models of nickel bioavailability in sediments required selection of toxicity endpoints that are both sensitive and have low variability. Table 12 compares the sensitivity and variability of toxicity values (EC20s) for endpoints from sediment tests with GP, HA, and HS. (The GP test with SR sediment in Task 2 was excluded from this comparison due to low control survival.) For GP, survival was less sensitive than biomass, as indicated by its higher average EC20 for TR-Ni (1516 µg/g vs. 1134 µg/g). However, survival EC20s were much less variable, with 95% confidence ranges that averaged 101% of EC20s, compared to 1025% for biomass. Toxicity endpoints for HA

followed a similar pattern, with survival being less sensitive but much less variable. For HS, growth and biomass endpoints were equally sensitive, but growth (average dry weight) was less variable. Other endpoints for these species were either less sensitive (HS survival, HA and GP growth) or were highly variable (HA reproduction) (Appendix 3-10).

Figure 18 compares responses of selected endpoints (survival of GP and HA, growth of HS) among tests with six Task-3 sediments. Survival of GP (Figure 18a) and HA (Figure 3-b) followed similar trends, with toxic effects at lowest TR-Ni concentrations in the DOW sediment and at highest TR-Ni in the STM sediment. Responses in the other four sediments were clearly separated for GP survival, suggesting a gradient of nickel bioavailability, but responses of HA survival were similar among these four sediments. The HS growth endpoint (Figure 18c) showed lesser overall differences among sediments, although the DOW and STM sediments still had highest and lowest nickel bioavailability, respectively.

Table 13 summarizes variation in EC20s for GP, HA, and HS among all eight sediments tested in Tasks 2 and 3. Toxicity values calculated based on TR-Ni and SEM-Ni followed similar trends for all three species. For HA survival, TR- Ni EC20s were lowest in SR (317 µg/g) and DOW (528 µg/g) sediments and highest in WB (1645 µg/g) and STM (3475 µg/g) sediments. The range of survival EC20s for HA among sediments was similar to the range of median lethal concentrations (LC50s) previously reported for 10-d tests with HA in four nickel-spiked sediments (150-2100 µg/g; Doig and Liber 2006a). Another study reported a lower range of 28-d survival LC50s for HA among three nickel-spiked sediments in static tests (83-543 µg/g; Borgmann and others, 2001), perhaps reflecting toxicity of nickel in overlying water (range of LC50s for OW-Ni: = 409-938 µg/L).

The variation of EC20s calculated based on different measures of nickel exposure was expressed as relative standard deviation (RSD=standard deviation as a percent of mean; Table 13). Sediment toxicity values based on TR-Ni and SEM-Ni had a similar degree of variation

among sediments. Calculating toxicity values based on [SEM-AVS] or [SEM-AVS]/$f$oc did not substantially reduce among-sediment variation in EC20s for HA or GP, and greatly increased variation in EC20s for HS. AVS-normalized EC20s for GP and HA were positive values, consistent with the hypothesis that metals will not be toxic if molar concentrations of SEM-Ni are less than AVS (USEPA 2005) -- except one EC20 for GP in the P30 sediment ([SEM-AVS]/$f$oc = -30 µmol/g oc). In contrast, several EC20s for [SEM-AVS] were negative for HS, especially those for high-AVS sediments from Task 3 (P30, RR3, STM). Toxicity values based on PW-Ni were generally less variable than those based on nickel concentrations in sediment. These results are consistent with the hypothesis that nickel bioavailability is largely determined by sediment characteristics that control distribution of nickel between sediment and pore-water (USEPA 2005).

Toxicity values for the three sensitive invertebrate taxa had varying degrees of concordance with published sediment quality guidelines. For all three species, EC20s for TR-Ni and SEM-Ni were three to 70 times greater than the empirical Probable Effect Concentration of 49 µg/g for nickel proposed by MacDonald and others (2000), suggesting that this guideline is conservative. For amphipods (HA and GP), all EC20s for [SEM-AVS] fell within the range of 'uncertain toxicity' range (1.7-120 µmol/g) of the equilibrium-partitioning sediment benchmarks developed by USEPA (2005), but several EC20s for [SEM-AVS]/$f$oc fell below the low end of the uncertain-toxicity range (130-3,000 µmol/g). All amphipod EC20s for PW-Ni fell near or above the USEPA (2009) chronic water quality criterion for dissolved nickel: 52 µg/L for overlying water at hardness of 100 mg/L as $CaCO_3$; and 86 µg/L for pore-water at a typical hardness of 180 mg/L. Water-only In contrast, most mayfly (HS) EC20s based on [SEM-AVS], [SEM-AVS]/$f$oc, and PW-Ni fell well below USEPA sediment toxicity benchmarks or water quality criteria, especially for Task-3 sediments.

Relationships of Nickel Bioavailability with Sediment Characteristics

Table 14 summarizes linear correlations among toxicity values and characteristics of sediment and pore-water. Of the three species, sediment (TR-Ni) EC20s for GP had the strongest associations with sediment characteristics, with significant positive correlations with AVS, TOC, Fe, Mn, CEC, and Kd. Correlations for HA followed similar trends but were weaker and were significant only for AVS and Kd. All these significant correlations were consistent with lower toxicity (higher EC20s) in sediments with greater nickel-binding affinity and less exposure to PW-Ni. Both amphipods also had significant positive correlations of PW-Ni EC20s with DOC, consistent with reduction in bioavailability of PW-Ni by complexation with dissolved organic ligands (Doig and Liber 2006b). In contrast, EC20s for HS did not have any significant correlations with characteristics of sediment or pore-water. Associations of nickel toxicity values with sediment characteristics are complicated by strong inter-correlations among sediment constituents (Table 14). Although sediment EC20s for both GP and HA were significantly correlated with sediment AVS concentrations, EC20s for HA were also significantly correlated with concentrations of TOC, Fe, and Mn. All of these sediment constituents are potential contributors to nickel-binding capacity, as is reflected by their significant correlations with CEC and Kd. However, correlation analysis cannot identify which of these parameters are the most important controls on nickel bioavailability.

Associations of SEM-Ni EC20s with AVS concentrations are illustrated in Figure 19. According to the equilibrium-partitioning approach for sediment guidelines presented by USEPA (2005), AVS is assumed to be the strongest binding phase for nickel and other divalent metals in sediments, and nickel-spiked sediments should not be toxic unless SEM-Ni concentrations exceed concentrations of AVS, on a molar basis. Results of our tests with HA (Figure 19a) and GP (Figure 19b) are generally consistent with this hypothesis, with

EC20s for SEM-Ni equal to or greater than AVS concentrations. Regressions for both species indicate that associations with AVS explain most of the variation in SEM-Ni EC20s. However, the equilibrium-partitioning hypothesis does not fully explain the results of the HS tests (Figure 19c). Results of HS tests in Task 2 (SR and WB sediments) produced SEM-EC20s that were similar to trends seen in HA and GP tests, but Task- 3 tests produced several SEM-EC20s that were less than corresponding AVS concentrations, especially for high-AVS sediments. Overall, there was a shift of HS toxicity values from higher nickel concentrations in Task 2 to lower nickel concentrations in Task 3. This shift may reflect the much larger starting size of HS nymphs tested in Task 2 (1.4 mg) compared to Task 3 (0.14 mg) (Table 7). Lower nickel EC20s for the Task-3 tests presumably reflect greater sensitivity of smaller nymphs to nickel toxicity. Despite these differences between tasks, which resulted in a non-significant regression for the combined data, EC20s from individual tasks showed similar trends for the association of EC20s with AVS, although the slopes were less than slopes for HA and GP. For the Task 3 data, a large proportion of the variation in HS EC20s was explained by regression with AVS (r^2=0.86).

The lower slope of the association of HS EC20s with AVS suggests that AVS concentrations (or at least AVS concentrations measured in bulk sediments) exerted a weaker control on nickel bioavailability to this species. One hypothesis to explain this is that nickel exposure to HS occurs largely via ingestion of nickel-rich particles rather than exposure to dissolved nickel, and that gastrointestinal bioavailability of nickel is not controlled by AVS (for example, De Jong and others, 2009). This hypothesis does not explain the apparent differences in nickel bioavailability to HS between Tasks 2 and 3, which may be related to differences in test chambers and differences in the size of test organisms. In Task-2 tests, the sediment layer was shallower, the sediment surface area was larger, and larger nymphs were tested. All these factors would contribute to greater contact of sediment with overlying

water, greater physical disturbance of the sediment layer, and more rapid oxidation of sediments. In contrast, the greater sediment depth, smaller sediment surface area, and smaller nymphs in Task-3 tests would tend to reduce sediment disturbance and slow rates of sediment oxidation. By burrowing into anoxic sediment layers and circulating oxygenated overlying water into these burrows, mayflies in Task-3 presumably caused oxidation of AVS on burrow surfaces and increased fluxes of PW-Ni, which could result in toxicity at lower sediment nickel concentrations (i.e., lower sediment EC20s). Differences in chamber morphology and sediment depth may also explain differences among sediments in PW-Ni EC20s for HS growth (Table 13). In both Task-2 sediments and in the low-AVS DOW sediment in Task 3, PW-Ni EC20s were close to the water-only EC20 for HS growth (131µg/L; Table 9). However, PW-Ni EC20s for high-AVS sediments in Task 3 were much lower, as low as 11 µg/L in the STM sediment. This discrepancy suggests that low PW-Ni concentrations measured by peepers placed in AVS-rich Task-3 sediments did not accurately represent PW-Ni concentrations in the aerobic microenvironments of mayfly burrows. This sampling bias would be less evident in Task-2 sediments, where peepers were necessarily placed closer to the surface of the shallow sediment layer, or in low-AVS sediments in Task 3.

3.5 Conclusions

- The six sediments tested in Task 3 represented gradients of major metal-binding components, (AVS, 0.9-22 µmol/g; TOC, 1.5-8.2%), which ranged between the extremes of the two Task- 2 sediments. Three different spiking ranges (target nickel concentrations for high spike treatments: 1267, 2667, and 4800 µg/g) were used for low-AVS (DOW), intermediate-AVS (STJ, RR2, RR3, and P30) and high-AVS (STM) sediments, respectively. Despite differences in TR-Ni ranges among sediments, ranges of PW-Ni concentrations were similar for all sediments, reflecting differences in nickel-binding

affinity that were evident in the range of nickel distribution coefficients (range in log Kd: 3.53-4.34).

- Spiked sediments were tested successfully with four species, but only three species (the amphipods GP and HA and the mayfly HS) showed toxic effects across all six Task- 3 sediments. The endpoints, GP survival, HA survival, and HS growth, were selected for comparisons among sediments because they combined high sensitivity and low variability. Based on these endpoints, all three species showed differences in nickel toxicity values (EC20s calculated from TR-Ni) among the six sediments, with differences in toxicity being greater for HA and least for HS.

- Expressing toxicity values in terms of different nickel fractions affected the variation in EC20s among the eight sediments tested in Tasks 2 and 3. Normalizing nickel concentrations to [SEM-AVS] or [SEM-AVS/foc] did not greatly reduce variation in EC20s for the amphipods HA and GP, but greatly increased variation in EC20s for the mayfly HS. Toxicity values calculated from PW-Ni had the lowest among-sediment variation for all three species, consistent with the hypothesis that pore-water is the predominant exposure route controlling nickel toxicity.

- Sediment toxicity values (TR-Ni EC20s) for GP and HA had significant positive correlations with AVS, and GP toxicity values were also significantly correlated with other sediment components (TOC, Fe, Mn). These sediment constituents were strongly inter-correlated, and were all significantly correlated with measures of nickel-binding affinity (CEC and Kd). In contrast, sediment EC20s for HS were not significantly correlated with any sediment characteristics.

- For the amphipods, GP and HA, toxicity values for nickel-spiked sediments were generally consistent with predictions of the hypothesis that a no toxicity will occur when SEM-Ni concentrations were less than the binding capacity of AVS. In contrast, several SEM-Ni EC20s for HS in Task 3 were less than AVS concentrations, especially for high-AVS sediments. Comparison of results of HS tests between Tasks 2 and 3 was complicated by methodological differences between Tasks. Differences in the starting size of HS nymphs may have affected their sensitivity to nickel toxicity. Differences in the size and shape of test chambers may have led to differences in nickel bioavailability by affecting sediment redox and AVS concentrations.

- The divergence of HS toxicity values from those obtained from tests with GP and HA in the same sediments, and from predictions of the [SEM-AVS] hypothesis, could indicate that AVS has a lesser degree of control over nickel bioavailability to HS. For example, nickel in AVS-rich particles may be bioavailable to HS via ingestion and gastrointestinal uptake. Alternatively, HS may experience greater exposure to PW-Ni in oxygenated burrow microenvironments, due to localized oxidation of AVS, than would be predicted based on typical sampling methods for AVS or PW-Ni.

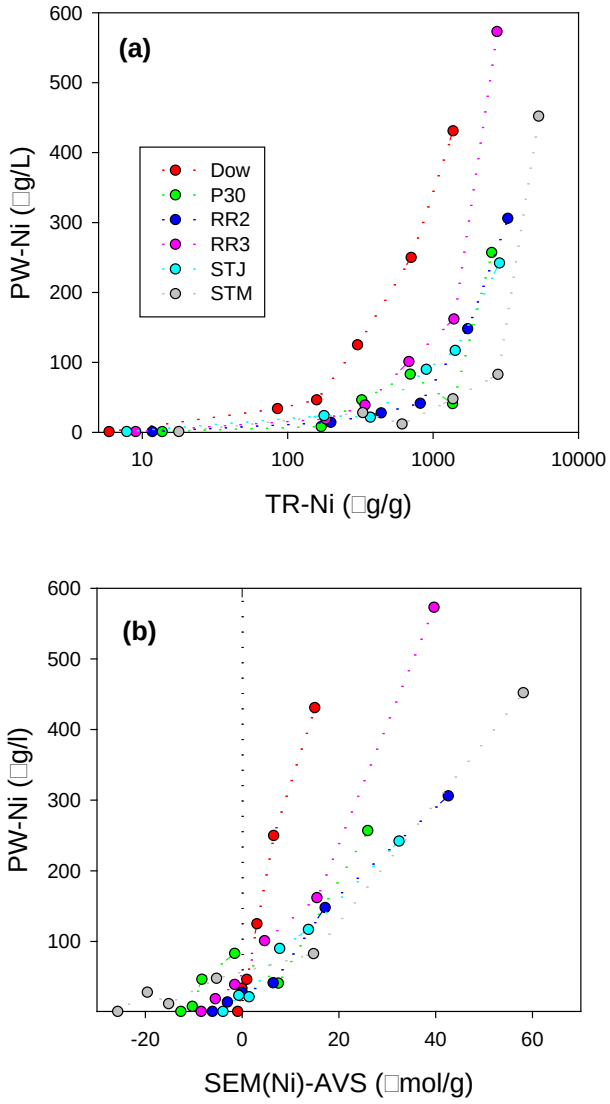


Figure 17. Pore-water nickel vs. sediment nickel fractions in nickel- spiked sediments: (a) total-recoverable nickel; (b) SEM-extractable nickel minus AVS.

[Pore-water nickel concentrations calculated from means of Day-14 peepers (n=4).]

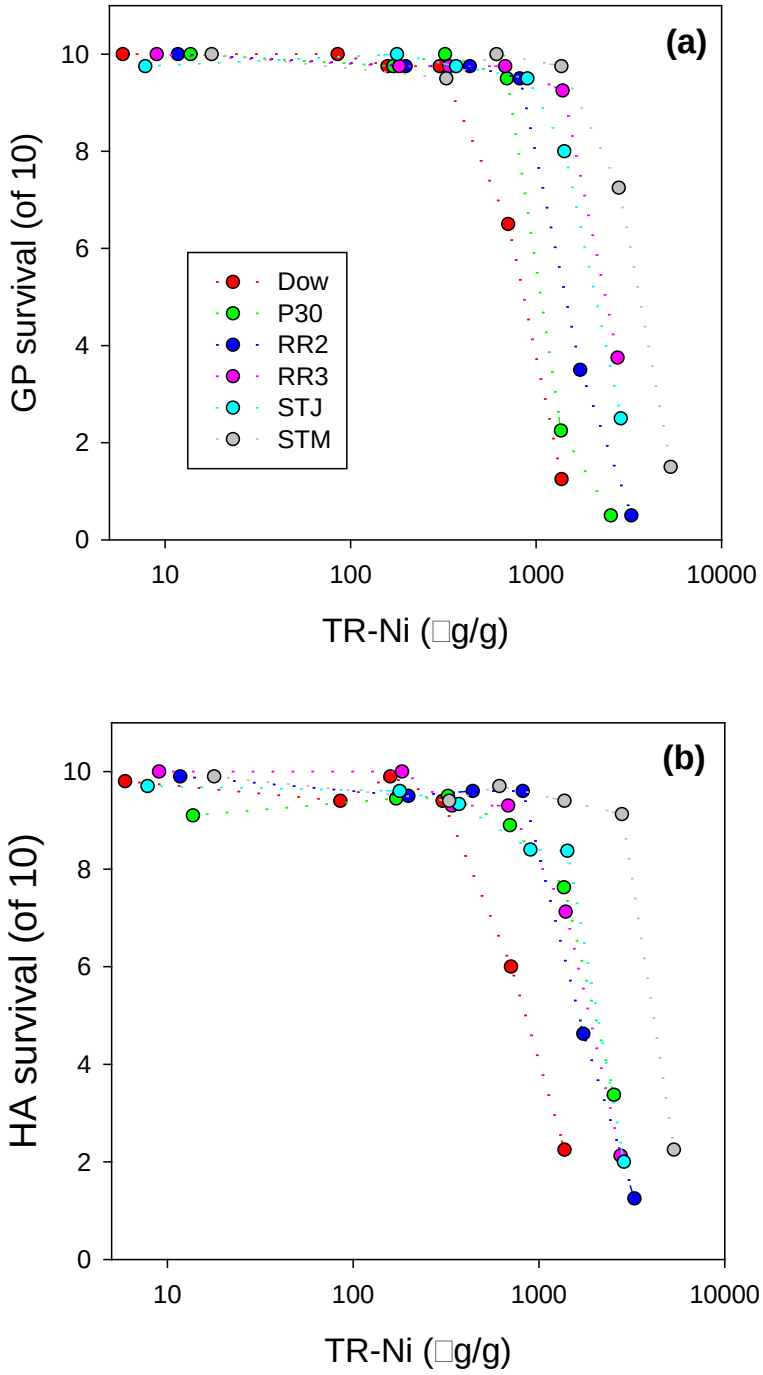


Figure 18. Concentration-response relationships for selected toxicity endpoints: (a) Gammarus (GP) survival; (b) Hyalella (HA) survival; (c) Hexagenia (HS) growth.

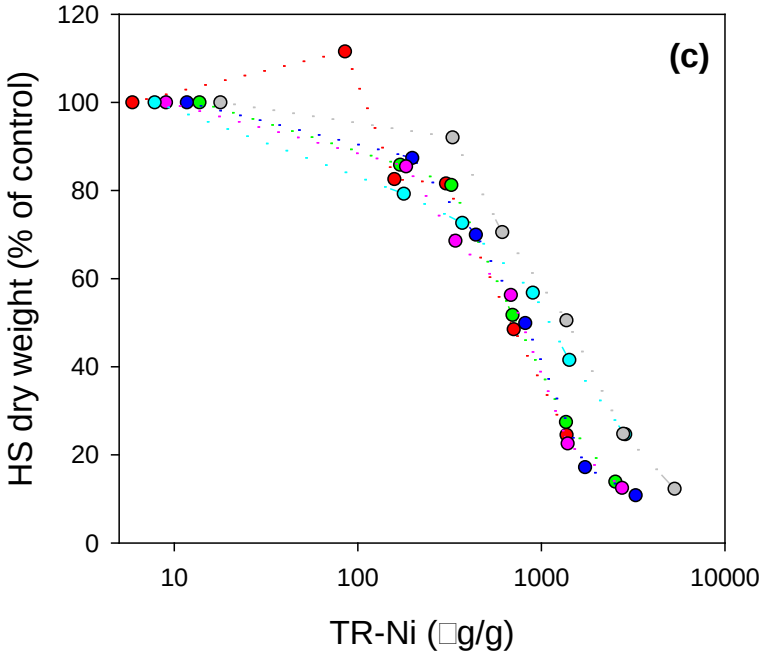


Figure 18 (continued)

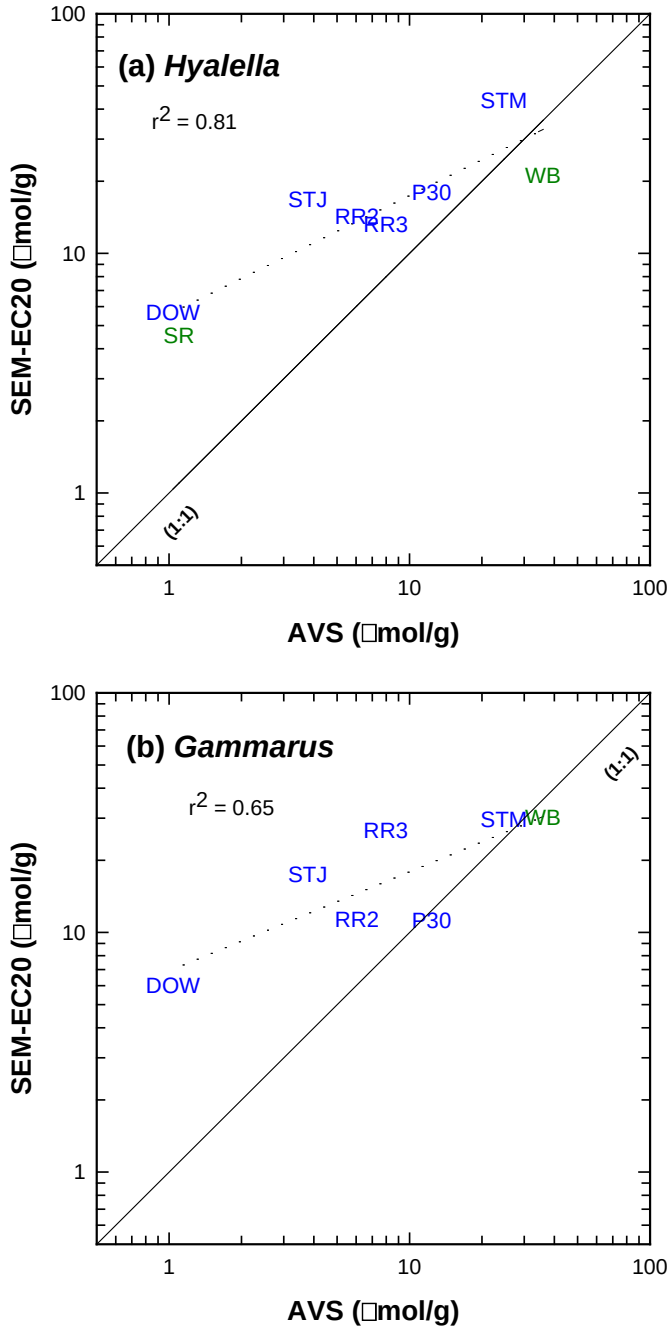


Figure 19. Relationships of sediment toxicity values (EC20s calculated from SEM-Ni concentrations) with AVS concentrations: (a) *Hyalella* survival; (b) *Gammarus* survival; (c) *Hexagenia* growth.

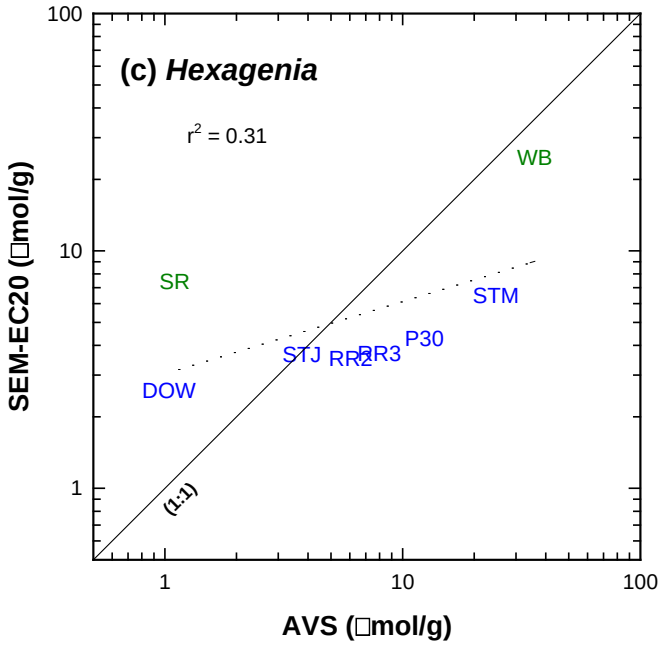


Figure 19 (continued): (c) Hexagenia growth.

Table 11. Task- 3 sediments and target nickel spike concentrations.

Characteristic	Base sediment					
	DOW	STJ	RR2	RR3	P30	STM
AVS (µmol/g)	0.9	2.7	4.8	7.2	9.5	22.0
TOC (%)	1.5	2.2	3.8	9.3	2.6	8.2

Spike level	Nickel spike (µg/g)		
1	79	167	300
2	158	333	600
3	317	667	1200
4	633	1333	2400
5	1267	2667	4800

Spike level	SEM(Ni)-AVS (µmol/g)					
1	0.4	0.1	-1.9	-4.4	-6.6	-16.6
2	1.8	3.0	1.0	-1.6	-3.8	-11.5
3	4.5	8.7	6.7	4.1	1.9	-1.3
4	9.9	20.0	18.0	15.5	13.2	19.2
5	20.7	42.7	40.7	38.2	35.9	60.1

Table 12. Sensitivity and variability of endpoints for three species of benthic invertebrates.

[EC20=20% effect concentration, expressed as TR-Ni, with lower and upper 95% confidence limits (lcl and ucl). Range = (ucl-lcl)/EC20)*100. Italics indicate less-robust concentration-response models.]

Gammarus (GP):

Sediment	Survival				Biomass			
	EC20 (mg/kg)	lcl	ucl	Range (%)	EC20 (mg/kg)	lcl	ucl	Range (%)
WB	2262	1049	4876	169	1667	296	9400	546
DOW	572	347	942	104	344	194	611	121
P30	847	584	1227	76	451	77	2656	572
RR2	1107	711	1724	92	*946*	*73*	*12240*	1286
RR3	1812	1192	2756	86	*2089*	*356*	*12258*	570
STJ	1440	948	2189	86	103	3	3944	3827
STM	2571	1656	3991	91	2338	816	6702	252
Mean:	**1516**			**101**	**1134**			**1025**

Hexagenia (HS):

Sediment	Growth (weight)				Biomass			
	EC20 (mg/kg)	lcl	ucl	Range (%)	EC20 (mg/kg)	lcl	ucl	Range (%)
SR	594	289	1217	156	503	216	1170	190
WB	1728	378	7894	435	1667	296	9400	546
DOW	221	89	548	208	239	75	763	288
P30	295	192	453	88	277	195	393	71
RR2	301	140	649	169	*283*	*149*	*538*	137
RR3	274	82	921	306	*342*	*156*	*750*	174
STJ	346	152	789	184	491	103	2337	455
STM	459	294	715	92	410	248	680	105
Mean:	**527**			**205**	**527**			**246**

Hyalella (HA):

Sediment	Survival				Biomass			
	EC20 (mg/kg)	lcl	ucl	Range (%)	EC20 (mg/kg)	lcl	ucl	Range (%)
SR	311	158	614	146	235	101	606	215
WB	1786	958	3342	133	1245	307	5182	392
DOW	528	277	1008	138	220	147	329	83
P30	1367	716	2610	139	1002	772	1299	53
RR2	1221	719	2074	111	*1391*	*96*	*20237*	1448
RR3	901	543	1495	106	*456*	*134*	*1560*	313
STJ	1482	900	2443	104	1195	1029	1388	30
STM	3475	2296	5259	85	2662	1742	4067	87
Mean:	**1384**			**120**	**1051**			**328**

Table 13. Variation of nickel EC20s for three invertebrates in nickel-spiked sediments.

[Nickel EC20s expressed as TR-Ni, SEM-Ni, [SEM(Ni)-AVS] (expressed per sediment dry mass, or per mass of organic carbon) and PW-Ni. RSD=relative standard deviation)]

Sediment	TR-Ni (mg/kg)	SEM Ni (mg/kg)	[SEM(Ni)-AVS] (µmol/g)	[SEM(Ni)-AVS] (µmol/g OC)	PW-Ni (µg/L)
Hyalella Survival:					
SR	317	267	9.0	1442	79
WB	1645	1241	8.6	10	150
DOW	528	332	4.7	378	224
P30	1367	1054	8.6	399	74
RR2	1221	834	11	293	82
RR3	901	775	13	108	99
STJ	1482	985	19	512	132
STM	3475	2557	29	332	196
RSD (%):	71	71	59	101	44
Gammarus Survival:					
WB	2262	1774	13	111	183
DOW	572	351	5.5	419	277
P30	847	656	4.2	-30	83
RR2	1107	666	10	97	80
RR3	1812	1566	25	311	247
STJ	1440	1022	15	668	150
STM	2571	1729	9.0	97	122
RSD (%):	49	52	61	101	47
Hexagenia Growth:					
SR	594	436	9.5	2074	144
WB	1728	1451	47	190	84
DOW	221	152	2.1	97	102
P30	295	252	-8.1	-236	15
RR2	301	206	-4.3	-9	14
RR3	274	216	-11	-93	19
STJ	346	214	-8.0	7	37
STM	459	382	-16	-198	11
RSD (%):	95	104	1377	331	94

Table 14. Correlations of toxicity values and characteristics of sediment and pore-water

[Pearson correlation coefficients (r), n=8. Asterisks indicate statistical significance (p<0.05). Concentrations of AVS, Fe, Mn, Ca, and Mg were log-transformed. Toxicity values (EC20s): GP=Gammarus survival; HA=Hyalella survival; HS=Hexagenia growth.]

Variable	Sediment EC20 (TR-Ni)			Sediment characteristics									
	GP	HA	HS	Fines	Clay	Silt	Sand	AVS	TOC	Fe	Mn	CEC	Kd
pH	-0.074	0.060	-0.667	**-0.752***	**-0.828***	**-0.748***	**0.785***	-0.586	-0.292	-0.322	0.165	-0.462	-0.368
Fines	0.336	0.356	0.543		**0.961***	**0.994***	**-0.996***	**0.738***	0.390	0.616	0.207	0.645	**0.723***
Clay	0.218	0.200	0.631			**0.943***	**-0.970***	0.678	0.281	0.598	0.102	0.545	0.644
Silt	0.416	0.406	0.598				**-0.995***	**0.803***	0.485	0.668	0.276	**0.721***	**0.761***
Sand	-0.367	-0.350	-0.611					**-0.777***	-0.437	-0.654	-0.228	-0.682	**-0.736***
AVS	**0.848***	**0.738***	0.507						**0.827***	**0.869***	0.516	**0.918***	**0.842***
TOC	**0.894***	0.551	0.592							**0.747***	**0.768***	**0.951***	**0.700***
Fe	**0.836***	0.656	0.688								**0.731***	**0.839***	**0.887***
Mn	**0.804***	0.510	0.291									**0.722***	0.686
CEC	**0.855***	0.565	0.667										**0.806***
Kd	**0.803***	**0.764***	0.495										

Variable	Pore-water EC20			Pore-water characteristics		
	GP	HA	HS	DOC	Ca	Mg
pH	-0.106	-0.079	-0.189	0.016	**0.715***	0.477
DOC	**0.728***	**0.906***	-0.235		0.371	0.545
Ca	0.030	0.188	-0.401			**0.883***
Mg	0.208	0.256	-0.675			

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List of Appendices

Note: These appendices are available online (in Excel format) at [persistent URL].

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