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Toxicity of Nickel-Spiked Freshwater Sediments to Benthic Invertebrates—Spiking Methodology, Species Sensitivity, and Nickel Bioavailability




Scientific Investigations Report 2011–5225

U.S. Department of the Interior
U.S. Geological Survey

Cover. Laboratory photographs (clockwise from upper left): Mixing composite sample of field-collected sediment; Adding nickel-spike solution to sediment in equilibration jars; Stocking invertebrates into test chambers with spiked sediment; Test chambers and water-delivery system during sediment toxicity test.

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Contents

Figures

Tables

Appendix Tables

Chapter 1

Note: These appendixes are available online (in Excel format) at [*http://pubs.usgs.gov/sir/2011/5225/downloads/appendix-1.xls*].

Chapter 2

Note: These appendixes are available online (in Excel format) at [*http://pubs.usgs.gov/sir/2011/5225/downloads/appendix-2.xls*].

Chapter 3

Note: These appendixes are available online (in Excel format) at [*http://pubs.usgs.gov/sir/2011/5225/downloads/appendix-3.xls*].

Conversion Factors

SI to Inch/Pound

Multiply	By	To obtain
	Length	
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
meter (m)	1.094	yard (yd)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	2.113	pint (pt)
liter (L)	1.057	quart (qt)
liter (L)	0.2642	gallon (gal)
liter (L)	61.02	cubic inch (in^3)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound avoirdupois (lb)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8×°C)+32$$

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Concentrations of chemical constituents in sediment are given either in micrograms per gram (µg/g) or micromoles per gram (µg/g).

Abbreviations

Chemical constituents	
Ni	nickel
TR-Ni	total-recoverable nickel (in sediment)
SEM-Ni	simultaneously extracted metal-nickel (in sediment)
OW-Ni	nickel in overlying water (of sediment toxicity tests)
PW-Ni	nickel in pore water
NiS	nickel sulfide
Fe	iron
FeS	iron sulfide
AVS	acid-volatile sulfide
TOC	total organic carbon
DOC	dissolved organic carbon
NaOH	sodium hydroxide
HCl	hydrochloric acid

	Toxicity values
EC10	10-percent effect concentration
EC20	20-percent effect concentration
LC50	Median (or 50%) lethal concentration
LOEC	lowest-observed effect concentration
NOEC	no-observed effect concentration
	Sediments
DOW	Dow Creek (Michigan)
P30	Pond 30 (Missouri)
RR2	Raisin River site 2 (Michigan)
RR3	Raisin River site 3 (Michigan)
SR	Spring River (Missouri)
STJ	St. Joseph River (Michigan)
STM	south tributary of Mill Creek (Michigan)
WB	West Bearskin Lake (Minnesota)
	Species
CD	*Chironomus dilutus* (midge)
CE	*Caenorhabditis elegans* (nematode)
CR	*Chironomus riparius* (midge)
GP	*Gammarus pseudolimnaeus* (amphipod)
HA	*Hyalella azteca* (amphipod)
HS	*Hexagenia* species (mayfly)
LS	*Lampsilis siliquoidea* (mussel)
LV	*Lumbriculus variegatus* (oligochaete)
TT	*Tubifex tubifex* (oligochaete)

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Toxicity of Nickel-Spiked Freshwater Sediments to Benthic Invertebrates—Spiking Methodology, Species Sensitivity, and Nickel Bioavailability

By John M. Besser[1], William G. Brumbaugh[1], Nile E. Kemble[1], Chris D. Ivey[1], James L. Kunz[1], Christopher G. Ingersoll[1], and David Rudel[2]

Abstract

This report summarizes data from 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 to support development of broadly applicable sediment quality guidelines for nickel. The studies were conducted as three tasks, which are presented here as three chapters: Task 1, Development of methods for preparation and toxicity testing of nickel-spiked freshwater sediments; Task 2, Sensitivity of benthic invertebrates to toxicity of nickel-spiked freshwater sediments; and Task 3, Effect of sediment characteristics on nickel bioavailability. Appendixes with additional methodological details and raw chemistry and toxicity data for the three tasks are available online at [*http://pubs.usgs.gov/sir/2011/5225/downloads/*].

Task 1 compared three spiking methods: Direct (direct addition of aqueous nickel solution to sediment at target nickel concentrations); Indirect (direct spiking of high-nickel 'super-spike' sediments, followed by dilution with unspiked sediment to target nickel concentrations); and Indirect+Iron (indirect spiking of nickel plus equimolar concentrations of ferric chloride or ferrous sulfide—to oxidized or reduced sediments, respectively). All sediments were pH-adjusted after spiking and were equilibrated in anaerobic conditions. Studies in Task 1 also evaluated the effects of the duration of the equilibration period for spiked sediments and the rate of replacement of overlying water in sediment toxicity tests. Results were evaluated based on the stability of sediment characteristics (for example, acid-volatile sulfide or AVS); distribution of nickel among sediment, pore water and overlying water; and toxicity of spiked sediments to the amphipod, *Hyalella azteca*. The methods selected for subsequent studies were indirect spiking; minimum 10-week anaerobic equilibration followed by 1 week of equilibration with aerobic overlying water in toxicity test chambers; and a high rate of replacement of overlying water (eight 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 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: two amphipods, *Hyalella azteca* and *Gammarus pseudolimnaeus*; two midges, *Chironomus dilutus* and *Chironomus riparius*; two 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 (survival, growth, and biomass for all eight taxa; adult emergence and egg production for *Chironomus* spp.; and number of offspring for *Hyalella azteca* and *Tubifex tubifex*) to determine the most sensitive responses of each species. The nematode, *Caenorhabditis elegans*, was tested in small test chambers without

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water replacement, with endpoints of survival and production of larvae. Water-only nickel toxicity tests with all nine species also were conducted to aid in interpreting results of sediment tests.

Results of sediment toxicity tests were used to estimate the chronic ten-percent and twenty-percent effect concentrations (EC10 and EC20, respectively) for sediment nickel, expressed as total-recoverable nickel concentrations (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 because of low control survival, and several other tests did not produce statistically significant toxic effects. Static tests with nematodes also did not allow reliable comparisons with other taxa, because of 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 micrograms per gram (µg/g) in Spring River sediment and 1,177µg/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 represented 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 10 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 statistically positive correlations with AVS for *Hyalella* and *Gammarus*, but not for *Hexagenia*. Toxicity values based on sediment nickel concentrations normalized to AVS (or to AVS and TOC) did not have substantially less variation among sediments, but toxicity values based on pore-water nickel concentrations 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 pore-water nickel 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 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 the sampling methods used in this study did not adequately measure localized concentrations of AVS or pore-water nickel (or both) 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 of sediments spiked with nickel (Ni) for toxicity testing. For example, a study of the toxicity of nickel-spiked sediment to oligochaetes (Vandegehuchte and others, 2007) 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 (because of incomplete equilibration with sediment or spiking levels that exceeded sediment binding capacity) and low rates of replacement of overlying water. These problems may be related to the low binding affinity and slow equilibration kinetics of nickel with sediment, compared to other toxic metals (Simpson and others, 2004). Accumulation of high nickel concentrations in overlying water would not be expected in either lotic or lentic ecosystems, because of rapid dispersal 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: as much as 70 days (d), compared to 15 d for copper, 40 d for zinc, and 45 d for cadmium. The 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 oxidation-reduction potential. Addition of aqueous metals to sediments typically results in decreases in pH because of hydrolysis reactions of metal ions, and acidic conditions inhibit sorption of nickel and other metals to sediment particles. Additional acidity also may 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 oxidation-reduction potential of the sediments and the resulting differences in the geochemistry of iron. For oxidized or partially reduced (sub-oxic) sediments, particulate organic matter and hydrous oxides of 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 (U.S. Environmental Protection Agency, 2005). Carbonaro and others (2005) attributed large initial fluxes of soluble nickel from spiked sediments into overlying water to high pore-water Ni concentrations because insufficient ferrous sulfide or other metal-binding constituents were available to effectively bind the added nickel. This explanation suggests that addition of appropriate iron solutions along with nickel spikes may generate fresh metal-binding phases of either hydrous iron oxides or iron sulfides to enhance binding of spiked nickel to sediment particles.

In addition to appropriate spiking methods, care also must 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 establishment of an oxidation-reduction 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 (for example, Borgmann and others, 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 affect 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 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 *Hyalella azteca*. Spiking methodologies were evaluated 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 affect 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 for a wide range of sediment types and nickel exposure concentrations?

1.2 Methods

Sediment Selection

Sediment spiking studies were conducted with two base sediments with different physicochemical characteristics (*appendix 1–1*). These sediments had low background concentrations of nickel and other chemicals of concern. The Spring River (SR) sediment, which had a sediment Ni concentration of 7.2 micrograms per gram (µg/g), was collected from the upper Spring River in Jasper County Missouri, USA (not shown; Ingersoll and others, 2008). The SR sediment was chosen because it had concentrations of acid-volatile sulfide (AVS) less than 1.0 micromoles per gram (µmol/g) and organic content (expressed as total organic carbon or TOC) less than 1 percent and was expected to have a low binding capacity for nickel. The West Bearskin sediment (WB; sediment Ni concentration = 52 µg/g) was collected from West Bearskin Lake in Cook County, Minnesota, USA (not shown; Ingersoll and others, 1998). The WB sediment was chosen because it had high concentrations of AVS (about 40 µmol/g) and TOC (about 10 percent), 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 (degrees Celsius) in sealed 21-liter (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 2009.

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 concentrations for evaluating each of the three different spiking methods. Pre-cleaned glass jars (3.8–L) with tetrafluoroethylene-lined lids were used to prepare and equilibrate all spiked sediments.

Direct spiking.—Aqueous nickel was added directly (as nickel chloride) to 3–L portions of each wet sediment in glass jars at high and low concentrations. At the same time, a solution of sodium hydroxide (NaOH; 10 normal) was added to maintain target pH (7.3 plus or minus 0.2 units), 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 20°C. During the first 4 weeks of equilibration, the pH of each spiked sediment was measured with a mini-electrode and adjusted by additions of NaOH or hydrochloric acid (HCl) as needed. After each pH adjustment, sediments were homogenized, purged with nitrogen, sealed and returned to the water bath. After 4 weeks, jars remained sealed and sediments were held in the dark in anaerobic conditions for 12 weeks, with biweekly mixing on a rolling mill for 1 hour at 20 revolutions per minute (rpm). 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 concentration to avoid unrealistically high concentrations of nickel in pore water and overlying water.

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 4 weeks. After 4 weeks, super-spikes were diluted with larger volumes of unspiked sediment (with no pH adjustment) to produce high and low nickel concentrations, then equilibrated for 12 weeks as described above under "Direct spiking". This method was similar to the approach described by Hutchins and others (2008). Indirect spiking was intended to produce more environmentally realistic pore-water metal concentrations and this method also had the practical advantage that pH adjustment was required only for one super-spike 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 super-spikes were spiked simultaneously with equimolar quantities of nickel

Table 1. Summary of spike treatments, nominal additions of nickel and iron, and estimated simultaneously extracted metal-nickel minus acid-volatile sulfide concentrations in nickel-spiked sediments.

[mg/kg, milligram per kilogram; SEM-Ni; simultaneously extracted metal-nickel; AVS, acid-volatile sulfide; µmol/g, micromole per gram; OC, organic carbon; SR, Spring River; WB, West Bearskin Lake]

Sediment	Spike treatment	Nickel treatment	Nickel spike (mg/kg)	Iron spike (mg/kg)	SEM-Ni minus AVS (µmol/g OC)
Control treatment					
SR	Direct, Indirect	Control	0	0	-100
SR	Indirect+iron	Control	0	1,893	-100
WB	Direct, Indirect	Control	0	0	-476
WB	Indirect+iron sulfide	Control	0	1,893	-588
Nickel-spike treatment					
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+iron	Low	167	316	256
SR	Indirect+iron sulfide	High	500	947	965
WB	Direct	Low	1,000	0	-250
WB	Direct	High	3,000	0	91
WB	Indirect	Low	333	0	-363
WB	Indirect	High	1,000	0	-250
WB	Indirect+iron	Low	333	630	-476
WB	Indirect+iron sulfide	High	1,000	1,893	-588

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 percent (WB sediment) to 6 percent (SR sediment) of the iron present in the base sediments, but the maximum sulfide amendment for WB was about 41 percent of the native AVS concentration in that sediment. The combination of the equimolar ferrous sulfide (FeS) addition plus the native AVS would be expected to completely bind all added nickel (U.S. Environmental Protection Agency, 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).

For this study 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 iron level for each sediment). In addition, sediment presumed to be contaminated with nickel was collected from Lake Petit Pas (LPP), near Havre-Saint-Pierre, Quebec, Canada (not shown). The LPP 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 nontoxic and had relatively low nickel concentrations. Data from the LPP samples are presented in the appendixes, but these results generally were 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 4-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 nickel (TR-Ni; U.S. Environmental Protection Agency, 2007a; Brumbaugh and May, 2008), nickel in the simultaneously extracted metals fraction (SEM-Ni), defined as the fraction solubilized along with AVS (U.S. Environmental Protection Agency, 1991); and pore-water nickel (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 U.S. Environmental Protection Agency (2007b) method 3051A; it includes addition of equal volumes of concentrated nitric and hydrochloric acids followed by 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. According to U.S. Environmental Protection Agency (2007b) and analyses of certified reference soils and sediments at the Columbia Environmental Research Center (CERC) of the U.S. Geological Survey (USGS) (W. Brumbaugh, USGS; unpub. data, 2007), recovery for this method typically is greater than 80 percent for most trace metals, including cadmium, cobalt, copper, lead, nickel and zinc. Bettiol and others (2008) conducted comparative digestion studies of sediments and determined 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 7,400 times standard gravity) were analyzed for PW-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 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-milliliter (mL) polyethylene vials, each filled with de-oxygenated, de-ionized water and fitted with a 0.45 micrometer (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 below the sediment surface (surface to 0.5 centimeter (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 in accordance with U.S. Environmental Protection Agency (2007a) method 6020A. Sediments were characterized for particle-size distribution (as percent by mass of sand-, silt-, and clay-sized particles), TOC, cation exchange capacity, oxidation-reduction potential, and pH. Water analyses included pH, major ions, conductivity, alkalinity, hardness, and DOC. All water and sediment analyses were performed using standard methods (for example, American Public Health Association and others, 2005; U.S. Environmental Protection Agency, 1994) with rigorous quality assurance/quality control procedures according to U.S. Environmental Protection Agency (2004) guidelines. Results of selected quality control 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 4 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 the amphipod, *Hyalella azteca,* were conducted for 21 days, based on a modification of methods from U.S. Environmental Protection Agency (2000) and American Society for Testing and Materials (2010a) (table 2). Test water was diluted well water (100 mg/L hardness as calcium carbonate). The pH of test water was automatically adjusted to 7.3 by addition of dilute hydrochloric acid (Wang and others, 2007). Overlying water in test beakers was replaced automatically at two different rates during each toxicity test: 2X (two volumes 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 6 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 percent greater than targets for WB sediments, because of a miscalculation of the solids content of WB sediment (fig. 1*A*). Nickel concentrations measured in AVS extracts (SEM-Ni) were typically 80 percent to 90 percent of TR-Ni in all spike treatments with the SR sediment, but were lower in WB spike treatment (fig. 1*B*). The smaller SEM-Ni fraction observed for the spiked WB sediment is consistent with greater formation of nickel sulfide (NiS,) from which nickel is only fractionally recovered by the extraction procedure for SEM and AVS (U.S. Environmental Protection Agency, 1991). For example, Carbonaro and others (2005) reported only 20 percent recovery as SEM-Ni from NiS, and experiments at CERC with freshly precipitated NiS produced 40 percent recovery of SEM-Ni and no recovery of AVS (W. Brumbaugh, USGS; unpub. data, 2008). Consistent with

Table 2. Test conditions for Task-1 whole-sediment toxicity tests with the amphipod *Hyalella azteca*, based on U.S. Environmental Protection Agency (2000) and American Society for Testing and Materials (2010a).

[±; plus or minus; °C, degrees Celsius; $CaCO_3$, calcium carbonate; DGT, diffusion-gradient thin-film]

Test condition	Description
Test type	Spiked whole-sediment toxicity tests with water replacement
Temperature	23 ± 1°C
Lighting	Ambient laboratory light; 16 hour light/8 hour dark
Test chamber	300-milliliter beakers
Sediment volume	100 milliliters, with 175 milliliters of overlying water
Test water	Well water diluted with de-ionized water to hardness of 100 milligrams per liter 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 per day (all tests); high treatment, 4 volumes per day (Test 2) or 8 volumes per day (Test 3)
Age of organisms	About 7 days
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 (U.S. Environmental Protection Agency, 2000), 1 milliliter per day (1.8 milligrams per day)
Aeration	None
Test duration	6-day pre-stocking period and 21-day amphipod exposure
Endpoints	Survival
Test acceptability	Survival greater than 80 percent survival in control sediment

these findings, the formation of NiS apparently was enhanced in the Indirect+Iron (ferrous sulfide) treatment, which had the smallest SEM-Ni fraction (40–50 percent).

Indirect spiking treatments generally resulted in AVS close to the pre-spike levels for each base sediment (fig. 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 in most 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 percent in the Direct treatments, compared to concentrations initially measured in base sediments. Physical and chemical manipulations of sediments (that is, pH adjustments and homogenization) during the first 4 weeks after spiking probably affected the Direct treatments more than the Indirect treatments and Indirect+Iron treatments, where the super-spikes were mixed with unspiked base sediments on day 28. Sulfide added to control sediments as FeS 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 (fig. 3). The addition of excess NaOH along with nickel spikes was planned with the expectation that pH of spiked sediments would drift lower with time, as was reported by Simpson and others (2004). However, downward drift of pH in spiked sediments was minimal, necessitating adjustments with HCl in some cases. This pH "overshoot" was greatest (0.5–2.0 units greater than 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 (fig. 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 expectations, may

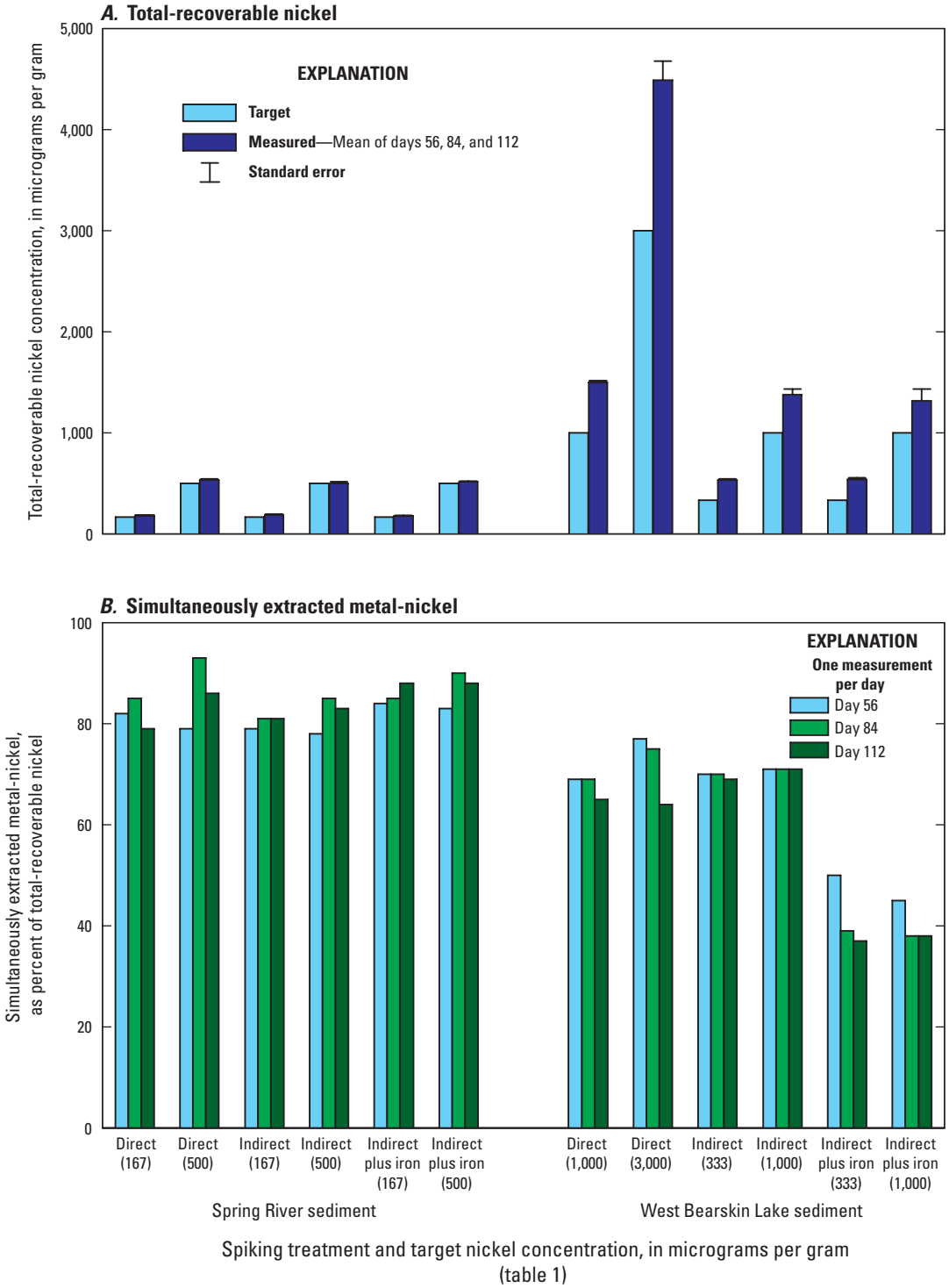

Figure 1. Target and measured nickel concentrations in nickel-spiked sediments (Task 1).

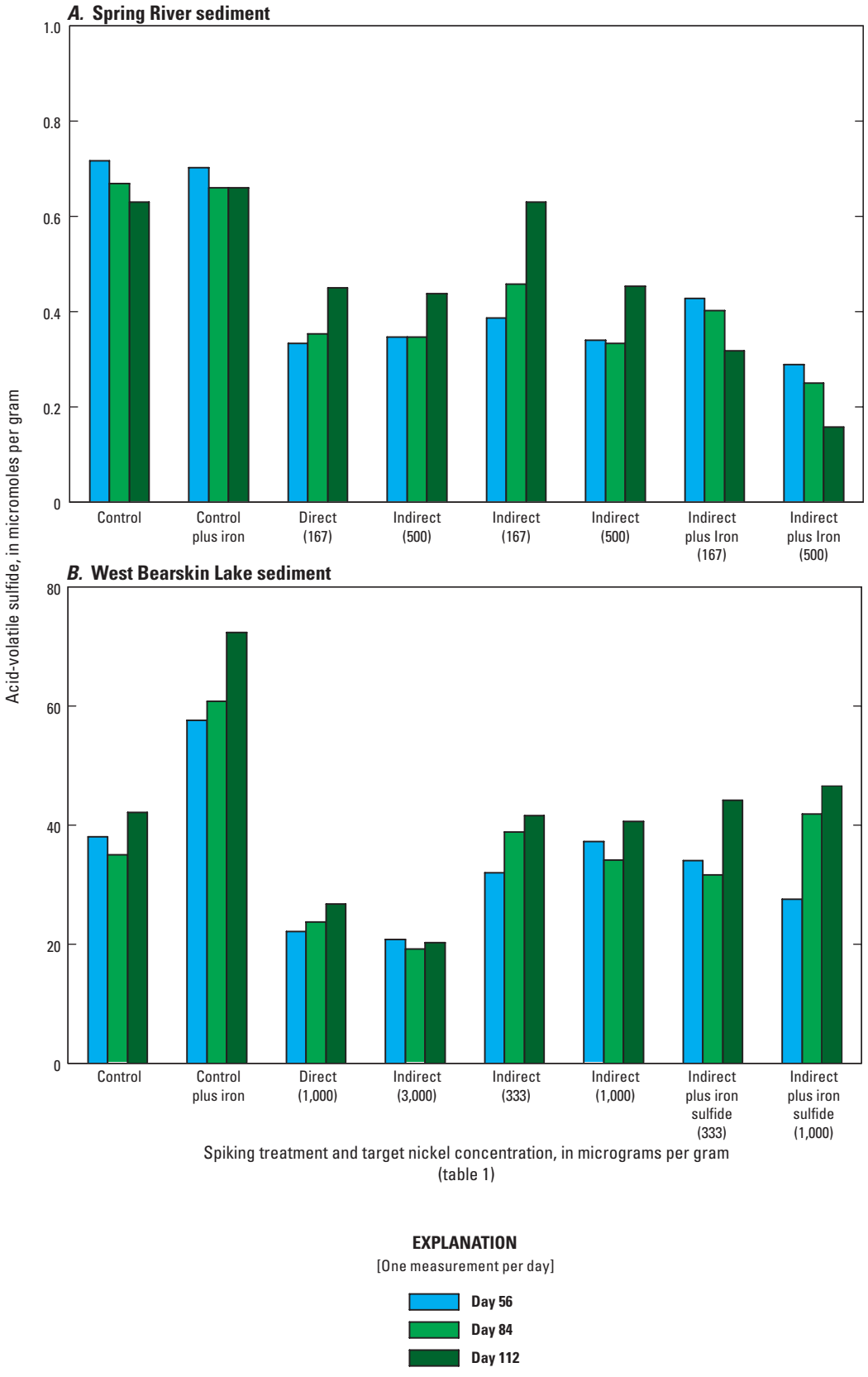

Figure 2. Acid-volatile sulfide concentrations in nickel-spiked sediments during equilibration (Task 1).

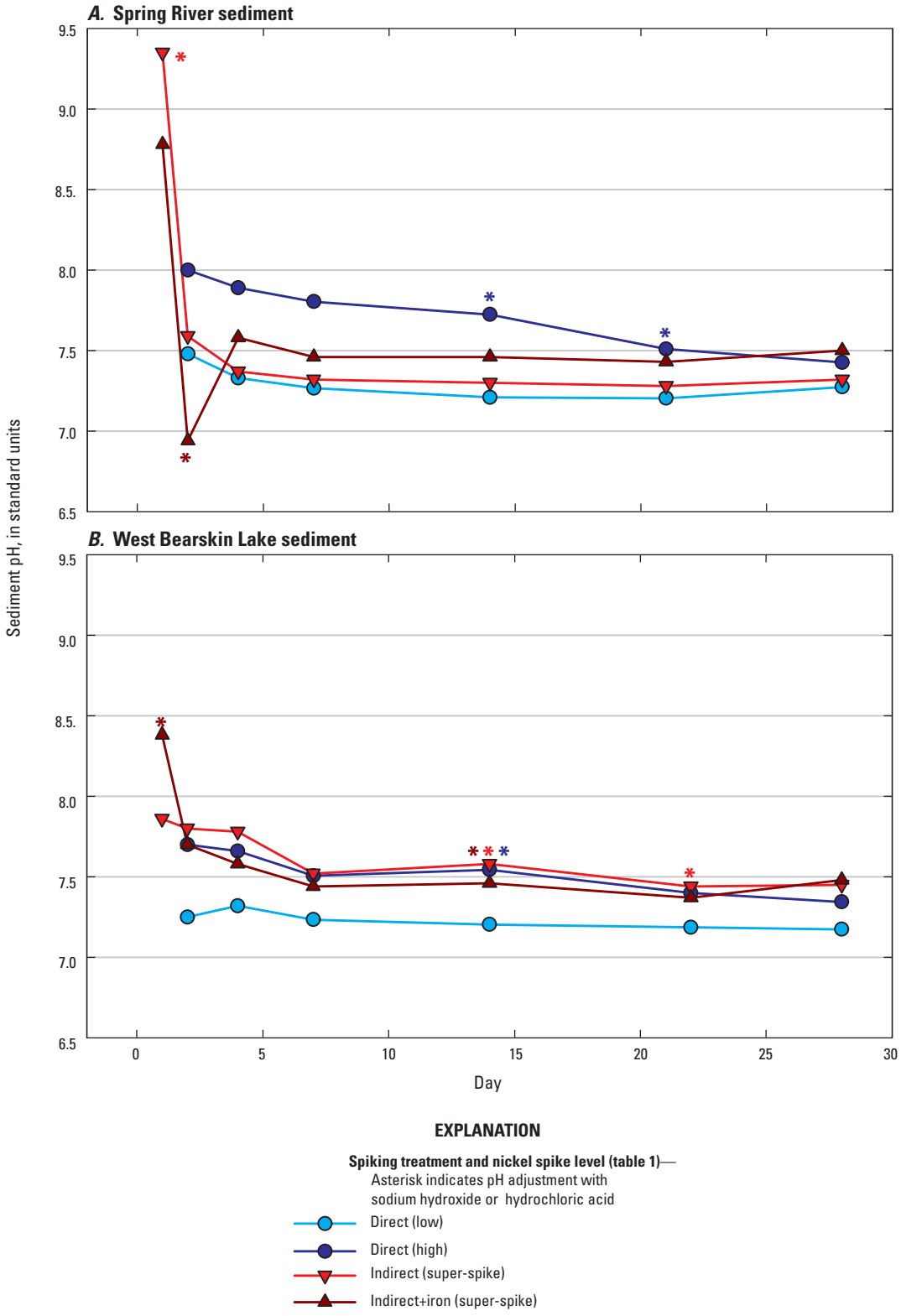

Figure 3. Adjustment of pH in nickel-spiked sediments (Task 1).

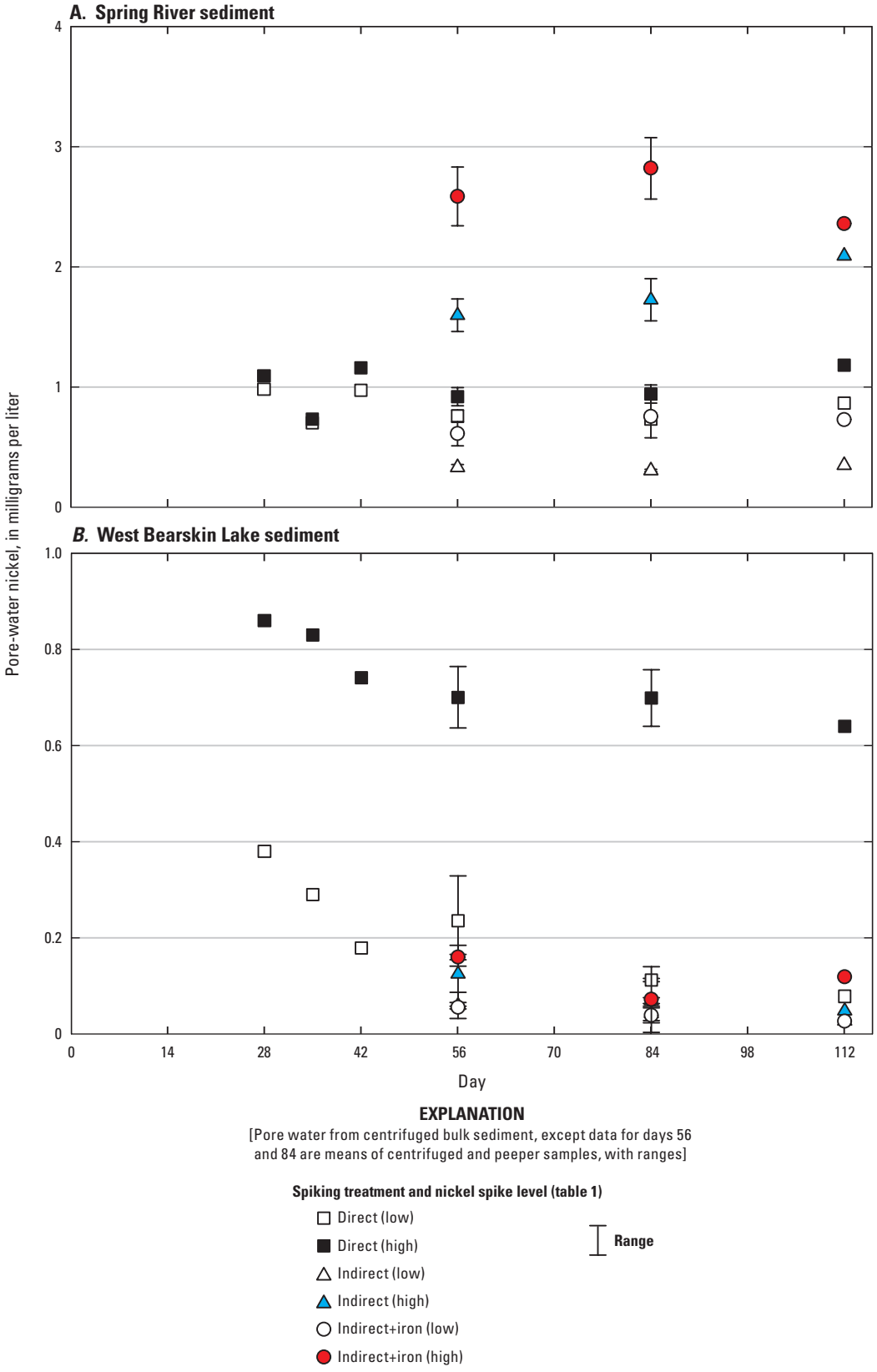

Figure 4. Pore-water nickel concentrations in nickel-spiked sediments during equilibration (Task 1).

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 3,000 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 pore-water iron concentrations (PW-Fe) than Direct spiking (fig. 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 and precipitates as hydrous ferric oxides at 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 (3,000 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 6-day pre-test equilibration period and during the toxicity tests (fig. 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 during this period. The Direct/High (3,000 µg/g) and Indirect+Iron treatments indicated some initial loss of PW-Ni, but only the Indirect+Iron/Low treatment indicated continuing losses like those 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 treatments strongly affected nickel concentrations in overlying water during toxicity tests (fig. 7). At the lowest water-replacement rate (2X), mean OW-Ni in several spike treatments exceeded the chronic water-quality criteria for nickel (52 µg/L, at a water hardness of 100 milligrams per liter (U.S. Environmental Protection Agency 2009) 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 (fig. 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 low and high water-replacement treatments, suggesting that increasing water replacement rate did not substantially alter Ni fluxes or oxidation-reduction 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. 9; *appendix 1–11*). Mean control survival in all control groups in the three tests met test acceptability requirements (American Society for Testing and Materials 2010a; U.S. Environmental Protection Agency, 2000), with survival in control groups ranging from 83 percent to 100 percent (overall control mean = 93.5 percent). Amphipod survival varied widely among nickel-spike treatments, with means ranging from 0 percent to 100 percent (table 3). Of the 36 spiking treatments (combinations of 3 spike methods, 2 sediments, 2 nickel levels, and 3 tests), 24 had at least 1 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 because of 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

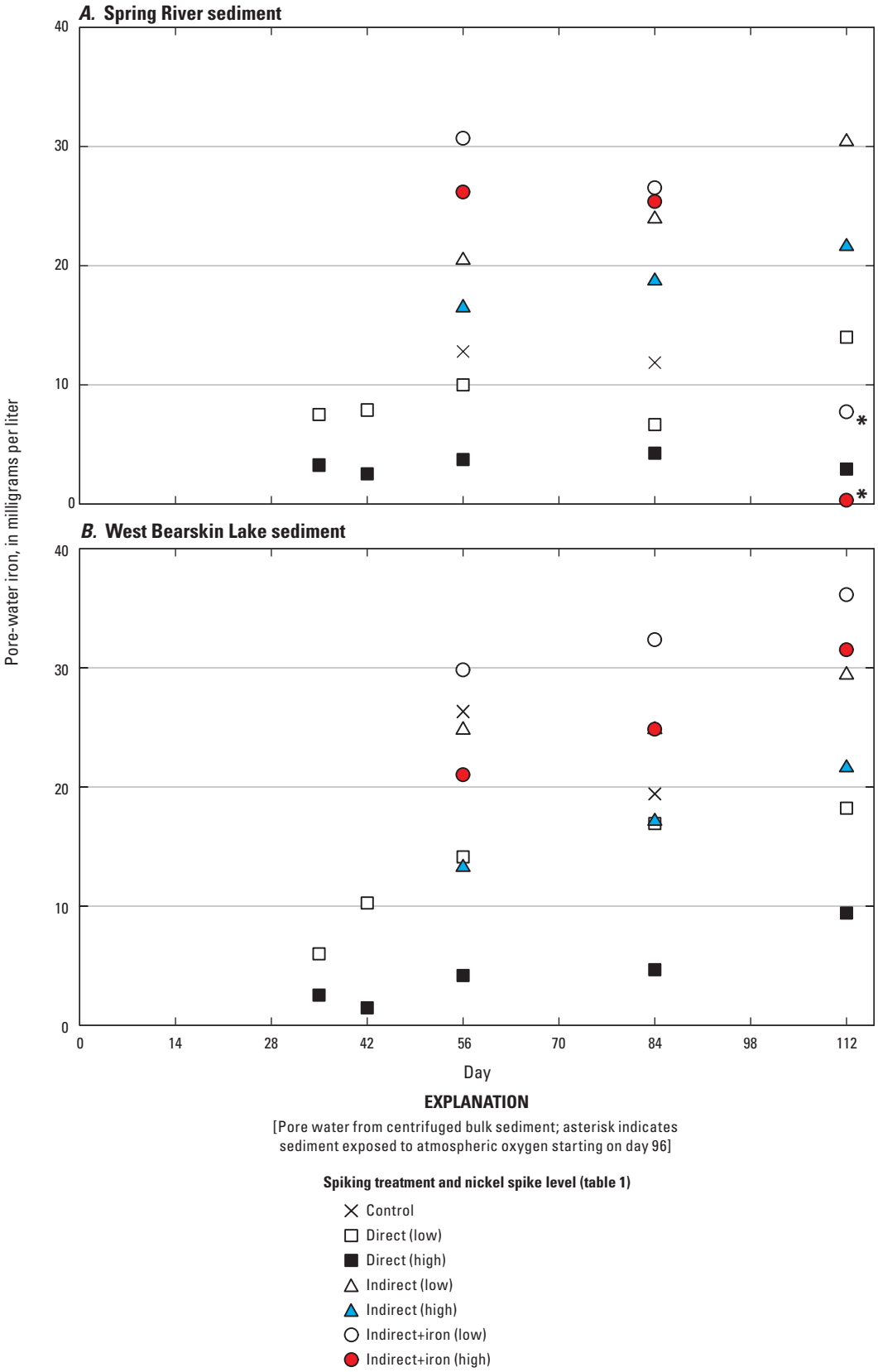

Figure 5. Pore-water iron concentrations in nickel-spiked sediments during equilibration (Task 1).

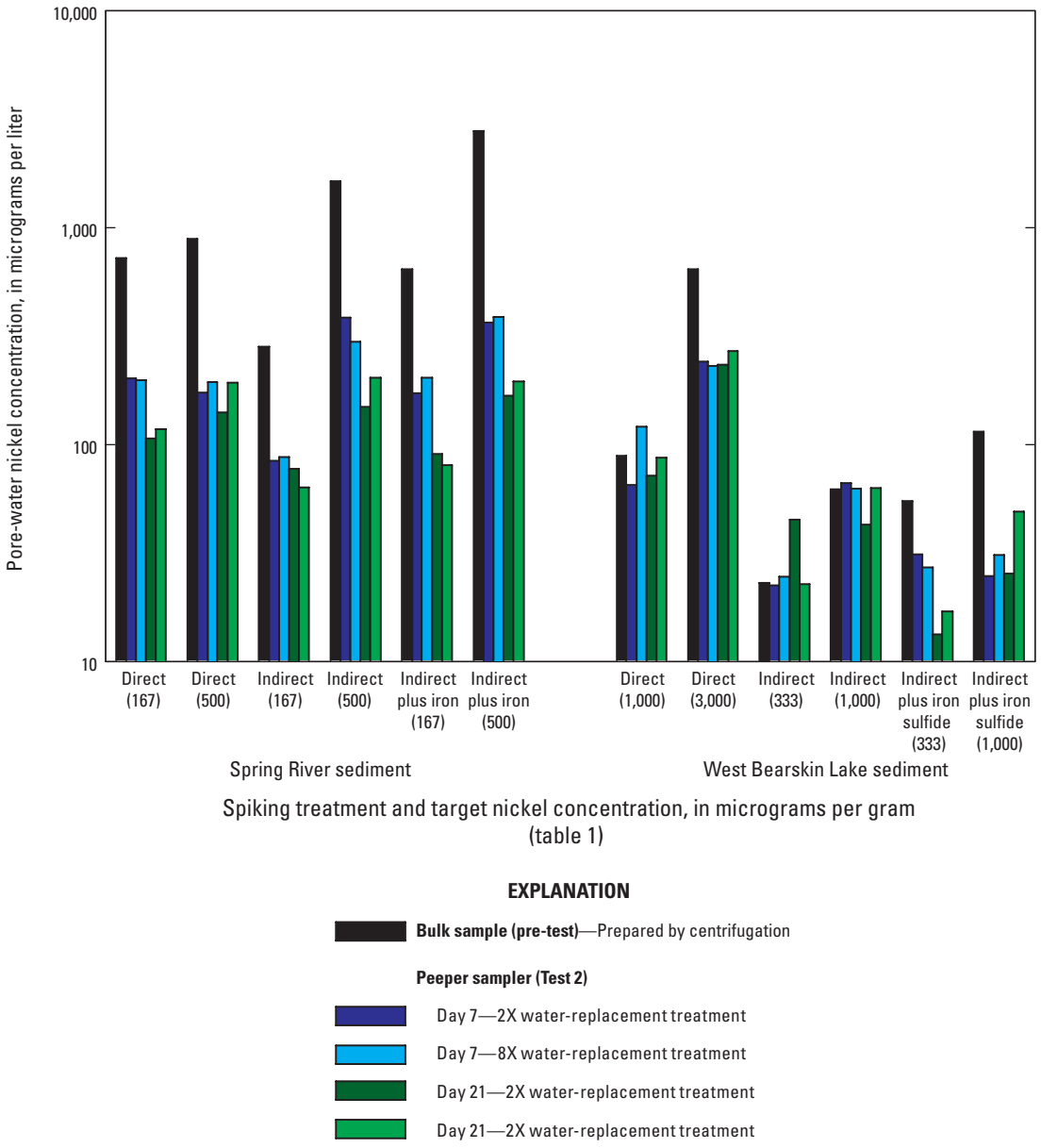

Figure 6. Pore-water nickel concentrations in nickel-spiked sediments before and during toxicity tests (Task 1).

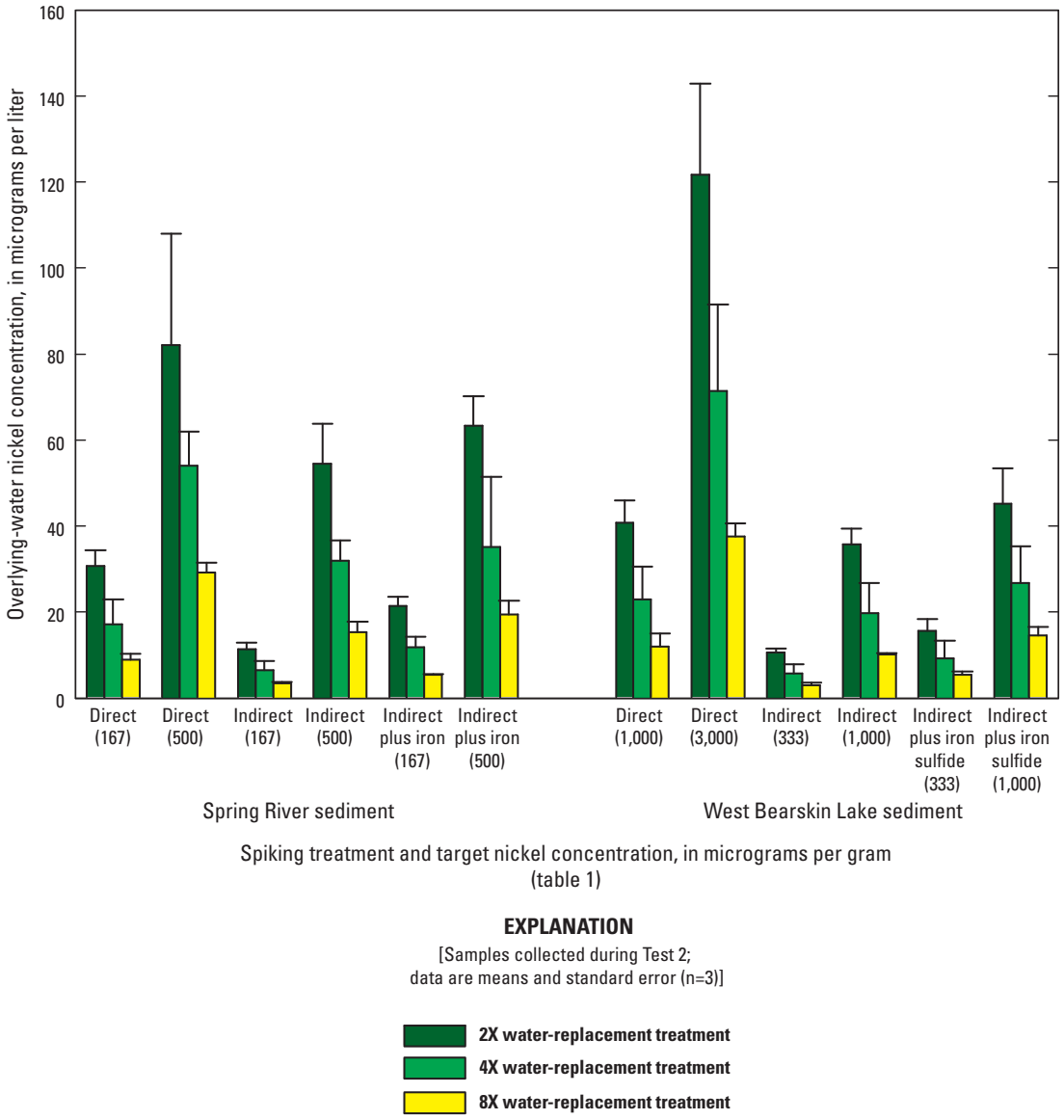

Figure 7. Overlying-water nickel concentrations in toxicity tests with nickel-spiked sediments (Task 1).

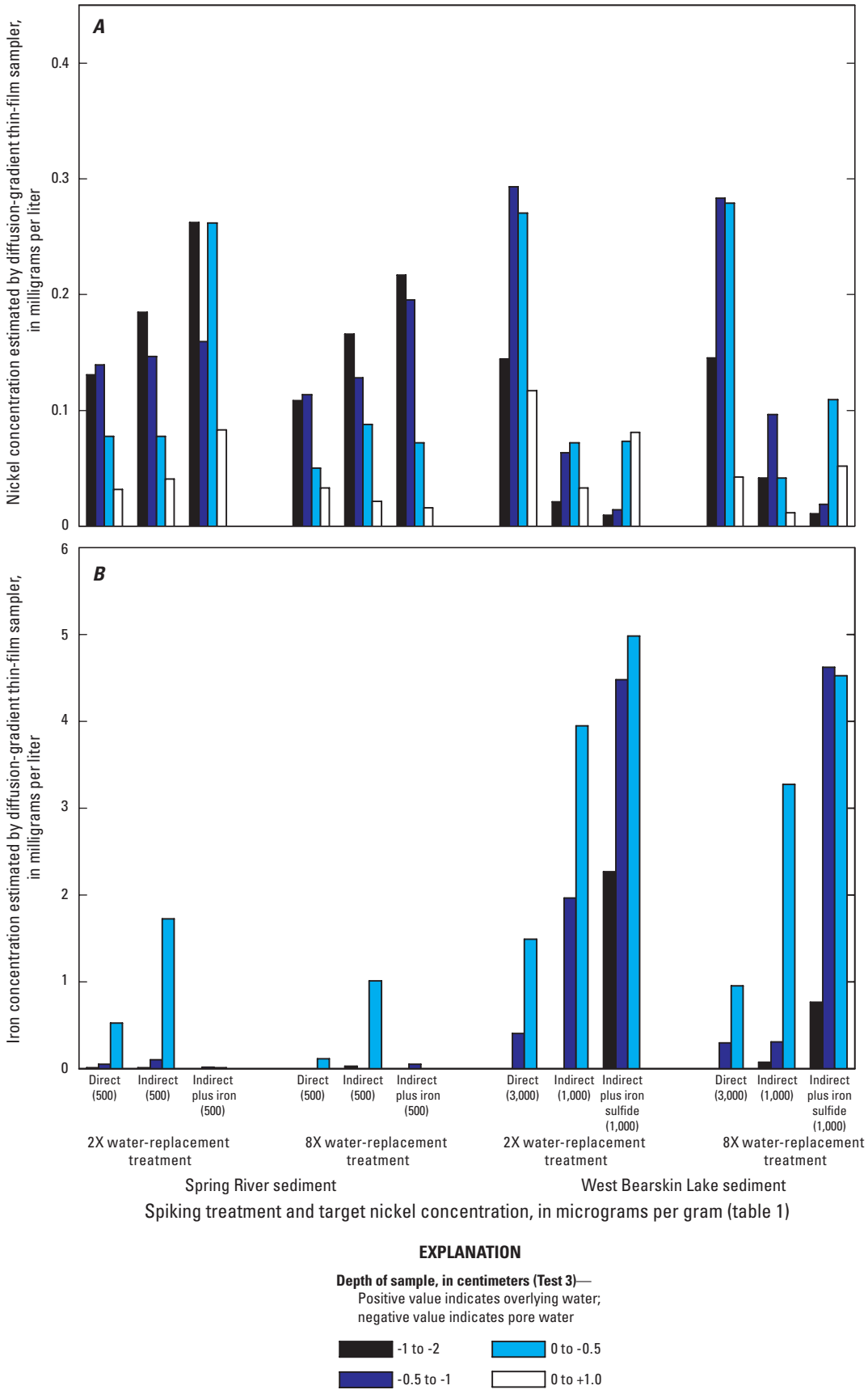

Figure 8. Depth gradients of dissolved metal concentrations estimated by diffusion-gradient thin-film samplers (Task 1).

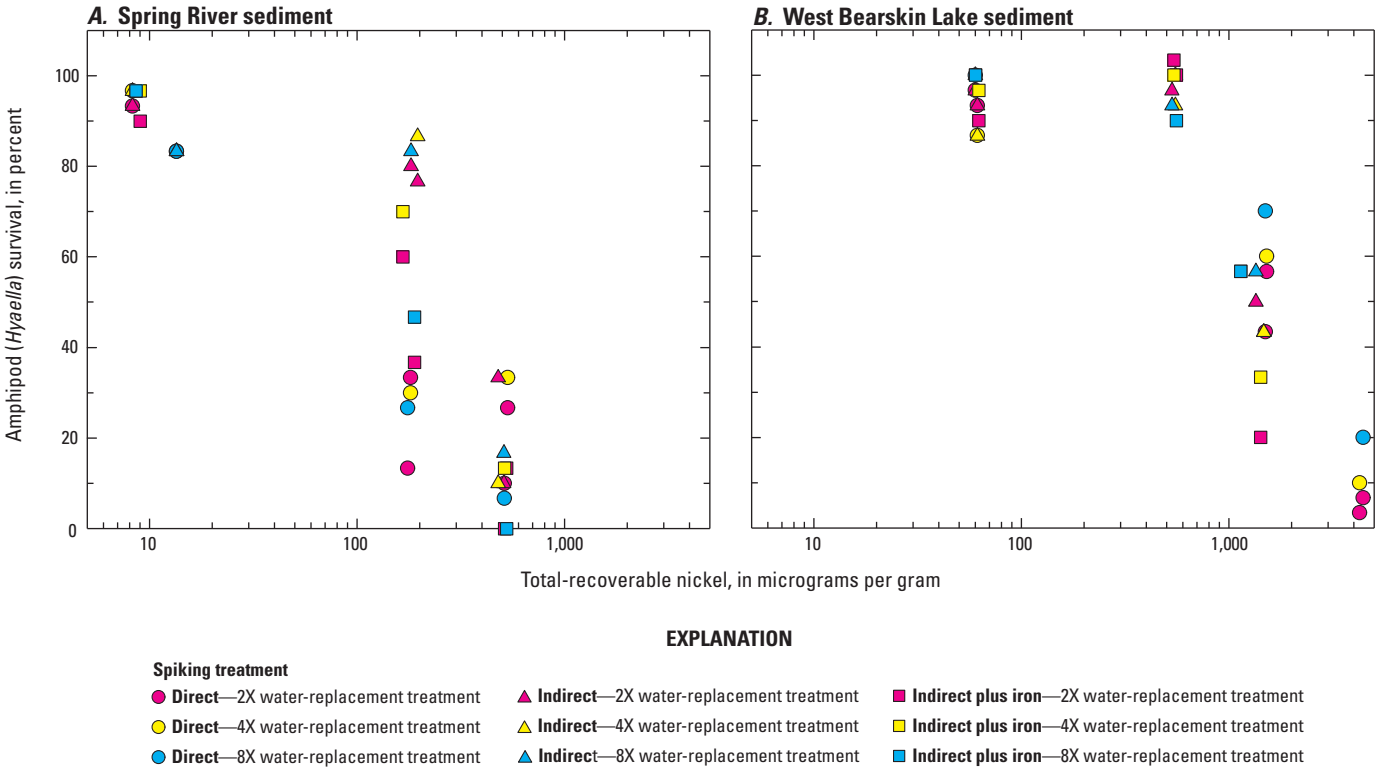

Figure 9. Effects of spiking treatments and overlying water-replacement treatments on survival of amphipods (*Hyalella azteca*) in nickel-spiked sediments (Task 1).

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 statistically significant differences showed opposing trends: decreasing survival in SR/Direct/High in contrast to 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 (fig. 4).

Water-replacement treatments had no statistically 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 exposure to nickel in overlying water (table 3).

Variation in amphipod survival in treatment groups with similar sediment nickel concentrations was related to spike treatments, but not water-replacement treatments (fig. 9). In both sediments, variation in survival among spike treatments was greatest at TR-Ni levels that caused intermediate levels of toxicity: the three low-nickel treatments in the SR sediment, which had nominal nickel spikes of 167 µg/g; and the three "intermediate" nickel treatments in the WB sediment (Direct/Low, Indirect/High, and Indirect+Iron/High), which had nominal nickel spikes of 1,000 µg/g. Differences among spike treatments were greater for the SR sediment, with survival in the low-nickel treatments ranging from about 80 percent in the Indirect treatment to about 30 percent in the Direct treatment. This variation among treatments is consistent with stronger binding of nickel to sediment particles (that is, lower bioavailability) in the Indirect treatment. In the WB sediment, survival in the 1,000 µg/g spike treatments generally was 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 related consistently to water replacement treatments in either sediment.

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

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 rank analysis of variance (ANOVA). P-values indicate significance of ANOVA for differences among water-replacement treatments and among repeated tests. SR, Spring River; WB, West Bearskin Lake; X, water volumes added per day; *, indicates means significantly less than controls (Tukey's test); --, no data]

Treatment			Mean survival (percent)						Difference among tests (p-value)	
			SR sediment			WB sediment				
Spike	Nickel	Water replacement	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	--	--
Direct	High	2X	27*	10*	3*	3*	7*	7*	.003	.729
		4X	33*	--	--	10*	--	--	--	--
		8X	--	7*	27*	--	20*	17*	--	--
Indirect	Low	2X	77	80	97	97	97	97	.154	1.000
		4X	87	--	--	93	--	--	--	--
		8X	--	83	57	--	93	97	--	--
Indirect	High	2X	33*	10*	23*	43*	50*	63	.232	.222
		4X	10*	--	--	43*	--	--	--	--
		8X	--	17*	37*	--	57*	70	--	--
Indirect plus iron	Low	2X	60	37	57	100	97	80	.164	.212
		4X	70*	--	--	97	--	--	--	--
		8X	--	47*	53	--	90	93	--	--
Indirect plus iron	High	2X	0*	13*	10*	20*	57*	40*	.254	.009
		4X	13*	--	--	33*	--	--	--	--
		8X	--	0*	23*	--	57*	57	--	--
Difference among water-replacement treatments (p-values)			.909	.871	.355	.805	.502	.210	--	--

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

[X, water volumes added per day; NT, not tested]

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)

1.4 Conclusions

The following methods were selected for use in Tasks 2 and 3:

- *Spiking Method: Indirect.*—The Indirect spiking method required less pH manipulation than the Direct method (only in one super-spike for each sediment, not in individual treatments), yet it produced consistent sediment pH across nickel levels. The Indirect spike method also resulted in less change in AVS concentrations, presumably because of the stabilizing effects of the dilution with unspiked sediments, and produced stable PW-Ni concentrations and consistent toxicity across all three tests. This method also has the practical advantage of greater flexibility in preparing multiple spike levels from a single super-spike.
- *Equilibration Period: 10 weeks (anaerobic) plus 1 week (aerobic).*—An anaerobic equilibration period of 8 to 12 weeks (4–8 weeks after sediment dilutions) was adequate for the Indirect spike method. No change was observed in PW-Ni concentrations in spiked SR sediments after the first 4 weeks, and only minor decreases in PW-Ni concentrations occurred in spiked WB sediments after 8 weeks. A 1-week pre-test equilibration period in aerobic toxicity beakers (with replacement of overlying water) facilitated the removal of unbound or weakly-bound nickel from the SR sediment and allowed the development of an oxidized layer at the surface of the WB sediment.
- *Water Replacement Rate: High (8 volume-additions per day).*—The highest rate of water addition (8 volumes per day) 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 percent or less, compared to nickel concentrations in pore water.

Chapter 2—Sensitivity of Benthic Invertebrates to Toxicity of Nickel-Spiked Freshwater Sediments

2.1 Introduction

In Task 2, two sediments were spiked using indirect 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 9 benthic invertebrate taxa, representing taxonomic diversity (3 insects, 2 crustaceans, 2 oligochaetes, 1 mollusk, and 1 nematode), and diversity of ecological and behavioral traits. Toxicity test methods for these taxa were based on standard toxicity test methods or other published methods (discussed below under "Sediment Toxicity Tests"). Eight of nine taxa were tested in exposure systems with automated replacement of overlying water, based on 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 10-percent and 20-percent reductions of an endpoint relative to the response at low nickel concentrations—EC10s and EC20s, respectively. The U.S. Environmental Protection Agency uses EC20s to develop chronic water-quality criteria for protection of aquatic life (for example, U.S. Environmental Protection Agency, 2007c) and the European Chemicals Agency uses EC10s to establish predicted no-effect concentrations (PNECs) under the "Registration, Evaluation, Authorisation and Restriction of Chemicals" (REACH) program (European Chemicals Agency, 2008). Toxicity tests with aqueous nickel (without sediment) also were 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 spiking 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 different physicochemical characteristics, notably differences in 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 (that is, high nickel bioavailability), and the WB sediment had high TOC and high AVS and was expected to have a high binding affinity for nickel (that is, 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 for a 10-week period that consisted of two phases: (1) prepare pH-adjusted, high-nickel "super-spike" sediments and equilibrate for 4 weeks; and (2) dilute super-spikes with unspiked base sediment to produce target nickel concentrations and equilibrate for additional 6 weeks.

In the first phase, three separate super-spikes (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. Super-spikes 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 2 hours on a rolling mill at 20 rpm and then placed in a 20°C water bath in the dark. During the first 2 weeks after spiking, the pH of the super-spikes 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. Super-spike jars remained sealed for two more weeks, with weekly mixing for 1 hour on the rolling mill.

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

[TR-Ni; total recoverable nickel; µg/g, microgram per gram; SEM-Ni, simultaneously extracted metal-nickel; AVS, acid-volatile sulfide; µmol/g, micromole per gram; OC, organic carbon; WB, West Bearskin Lake; SR, Spring River]

Treatment	TR-Ni (µg/g)	SEM-Ni minus AVS	
		(µmol/g)	(µmol/g OC)
WB sediment			
WB-5	8,506	103	1,000
WB-4	2,835	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	1,400
SR-4	320	3.6	582
SR-3	146	1.4	210
SR-2	66	.30	41
SR-1	30	-.25	-36

Four weeks after initial nickel spiking, portions of each super-spike 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 (SR–C and WB–C; *appendix 2–1*). Control sediments were prepared in 3.8–L glass jars as described for the super-spikes, 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 6 weeks, with each jar mixed on the rolling mill (1 hour at 20 rpm) every 2 weeks. The first set of toxicity tests were started with sediments from the first set of jars 10 weeks after preparation of super-spikes.

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 cultures maintained at CERC, 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°C (except 15°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 milligrams per liter as calcium carbonate. 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 (about 22 additions of 63 mL) 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 American Society for Testing and Materials (2010a, 2010b); U.S. Environmental Protection Agency (2000); and Organization for Economic Cooperation and Development (2004, 2007). Species-specific test conditions (described below and in table 6) were selected to facilitate efficient, concurrent testing of multiple species while remaining consistent with existing test methods, published scientific literature, and preliminary studies at CERC (C. Ingersoll, U.S. Geological Survey; unpub. data, 2008).

Amphipods (HA and GP).—Test methods for both amphipods generally followed standard methods for HA (American Society for Testing and Materials, 2010a; U.S. Environmental Protection Agency, 2000). Tests with GP were started with juveniles 3 to 5 millimeters (mm) long and were conducted at 15°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 (U.S. Environmental Protection Agency, 2000, American Society for Testing and Materials, 2010a) except that tests were started with 7-day old CD larvae rather than less than 24-hour 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 produced more consistent emergence in the controls (Ingersoll and others, 2009).

Oligochaetes (TT and LV).—Adult TT were isolated from the culture by sieving organisms (less than 0.5 mm) and

Table 6. Test conditions for flow-through sediment toxicity tests with benthic invertebrates in Tasks 2 and 3.

[GP, *Gammarus pseudolimnaeus*; HS, *Hexagenia* species; HA, *Hyalella azteca*; CD, *Chironomus dilutus*; CR, *Chironomus riparius*; TT, *Tubifex tubifex*; LS, *Lampsilis siliquoidea*; LV, *Lumbriculus variegatus*]

Test condition	Description
Temperature	23 degrees Celsius for all species except GP (15 degrees Celsius)
Lighting	Wide-spectrum fluorescent lights (about 200 lux); 16 hour light:8 hour dark
Chamber volume	300 milliliters for all tests except HS (1,000 milliliters in Task 2)
Sediment volume	100 milliliters except HS (200 milliliters in Task 2)
Overlying water volume	About 175 milliliters except HS (about 700 milliliters in Task 2)
Test water	Diluted well water (100 milligrams per liter hardness as calcium carbonate); pH adjusted to 7.3 by an automated pH controller
Overlying water renewal	8 volume-additions per day
Replicates per treatment	4 per treatment, except HA (12), CD (16), and CR (16)
Organisms per replicate	10 per replicate except TT (4), CR (12), and HS (5 in Task 3)
Age of organisms	CR, 4 days old; CD and HA, 7 days old;
	HS, 6–8 weeks old (5–10 milligrams wet weight);
	GP, juveniles (about 3–5 millimeters length),
	LS, juveniles (about 2 months old); LV and TT, adults.
Feeding	HA and GP: Yeast-cereal leaf-trout chow diet (U.S. Environmental Protection Agency, 2000), 1.8 milligrams per day
	HS: Yeast-cereal leaf-trout chow diet, 7.2 milligrams per day (3.6 milligrams per day in Task 3)
	CD and CR: Tetrafin® suspension, 6 milligrams per day
	LV and TT: Tetrafin® suspension, 16 milligrams per day
Aeration	None
Water/sediment quality (see table 4)	Test water pH, conductivity, major ions, dissolved organic carbon (day 0)
	Overlying water pH, dissolved oxygen, temperature, conductivity (weekly)
	Overlying water nickel, hardness, alkalinity, ammonia (day 0 and end of test)
	Sediment nickel, particle size, total organic carbon, cation exchange capacity, solids (day 7); simultaneously extracted metal-nickel, acid-volatile sulfide (day 28)
	Pore-water nickel, pH, conductivity, ions, dissolved organic carbon (day 0); pore-water nickel, iron (day 14)
Duration and endpoints	GP, HS and LS: 28 days (survival, growth, biomass)
	LV: 28 days (abundance, biomass)
	TT: 28 days (survival, growth, biomass, reproduction)
	HA: 28 days (survival, growth, biomass), 42 days (reproduction)
	CD and CR: 10 days (survival, growth, biomass); about 42 days for CR (emergence); about 56 days for CD (emergence, fecundity, hatching)
Acceptability criteria[1]	CD and CR: Greater than or equal to 70-percent control survival (day 10); greater than or equal to 50-percent emergence
	HA, GP, LS, HS: Greater than or equal to 80-percent control survival (day 28)
	TT: Greater than or equal to 90-percent control survival (day 28)
	LV: Greater than 60-percent increase in biomass (day 28)

[1]Additional performance criteria from Americans Society for Testing and Materials (2010a, b, c), Organization for Economic Cooperation and Development (2004, 2007), and U.S. Environmental Protection Agency (2000).

each replicate was stocked with four animals (Reynoldson and others, 1991; American Society for Testing and Materials, 2010a). Both species were tested under a 16:8 photoperiod with flow-through conditions and were fed a suspension of Tetrafin® fish food (16 milligrams per beaker per day). 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 greater than (>) 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, 2007). 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; American Society for Testing and Materials, 2010a). Each replicate was fed daily with a yeast-cereal leaf-Tetrafin® food suspension (U.S. Environmental Protection Agency, 2000; 7.2 mg per beaker), based on the weekly ration used by Day and others (1998). Preliminary testing documented adequate performance of HS in 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 (American Society for Testing and Materials, 2010c; Wang and others, 2007) and were similar to standard sediment test methods for the amphipod, HA (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials, 2010a). Endpoints of the 28-day 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 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 (table 7), determined as body length (determined by digital imaging) or ash-free dry weight, or both. 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 (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials, 2010a, 2010b).

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

[Ash-free dry weight data from 20–80 animals weighed as 2–4 replicates. Length data from 15–20 animals, measured individually; --, no data]

Species	Dry weight (milligram)		Length (millimeter)	
	Mean	Standard deviation	Mean	Standard deviation
Task-2 sediment test				
Chironomus dilutus	0.26	0.05	--	--
Chironomus riparius	.09	.02	--	--
Gammarus pseudolimnaeus	--	--	2.84	0.49
Hexagenia species	1.12	.36	--	--
Hyalella azteca (Test 1)	--	--	1.92	.19
Hyalella azteca (Test 2)	--	--	1.59	.26
Lampsilis siliquoidea	--	--	1.85	.35
Lumbriculus variegatus	1.20	.10	--	--
Tubifex tubifex	1.52	.25	--	--
Task-2 water-only test				
Chironomus dilutus	0.13	0.06	--	--
Chironomus riparius	.02	.01	--	--
Gammarus pseudolimnaeus	--	--	2.69	0.08
Hexagenia species	.41	.05		
Hyalella azteca	--	--	2.00	.07
Lampsilis siliquoidea	--	--	1.37	.06
Lumbriculus variegatus	1.07	.03	--	--
Tubifex tubifex	.86	.03	--	--
Task-3 sediment test				
Gammarus pseudolimnaeus	--	--	2.92	0.44
Hexagenia species	0.17	0.03		
Hyalella azteca			1.83	.33
Tubifex tubifex	1.07	.01	--	--

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-percent 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 1,000 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 International Standard Organization (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 7 days. The overlying water removed from each vial on the day before the test 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 six-well culture plates. Tests were started with addition of a suspension of antibiotic-killed *Escherichia coli* in test water (0.5 mL) and 10 synchronized L1 CE larvae per well, and plates were held at 20°C. After 4 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 total-recoverable nickel (TR-Ni), simultaneously extracted metal-nickel (SEM-Ni), acid-volatile sulfide (AVS), total organic carbon (TOC), cation exchange capacity, and particle size distribution. Centrifuged pore waters were analyzed for pH; dissolved nickel (PW-Ni), iron, and manganese; major cations and anions; dissolved organic carbon (DOC), and routine water-quality characteristics. 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 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 1 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. Because of the limited capacity of flow-through exposure systems, tests with all species except CE were conducted at CERC in three groups (2–4 species per group) during a 4-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 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 1 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.

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, North Carolina). Concentration-response relations 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-percent confidence intervals. The primary focus of concentration-response models was on estimation of toxicity values for total nickel concentrations

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

[C/1/3/5, control, low, medium, and high nickel treatments]

Sample	Analyte(s)	Test day(s)	Treatments	Frequency
Bulk sediment	Particle size distribution	Day −7	Composite	First jar
	Cation exchange capacity	Day −7	Composite	First jar
	Total organic carbon	Day −7	Composite	First jar
	Total-recoverable nickel	Day −7	All	All jars
	Simultaneously extracted metal-nickel and acid-volatile sulfide	Day −7	All	All jars
Bulk pore water	Dissolved organic carbon, cations, routine water quality	Day −7	All	First jar
	Anions	Day −7	All	Second jar
	Nickel and iron	Day −7	All	All jars
Test water	Dissolved organic carbon, 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 nickel	Day 0, end	All	All tests
Beaker sediment	Simultaneously extracted metal-nickel and acid-volatile sulfide	Day 14	3 only	All tests
Beaker pore water	Nickel and iron in peeper samplers	Days 7–14	All	All tests

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

[>, greater than]

Test group (jar)	Equilibration (weeks)	Test	Start date (2009–10)	Duration (days)	Endpoints
1	10	*Hyalella azteca* (Test 1)	24-September	28 42	Survival, growth, biomass Reproduction
	10	*Chironomus riparius*	25-September	10 About 42	Survival, growth, biomass Emergence
2	14	*Lumbriculus variegatus*	20-October	28	Abundance, biomass
	14	*Lampsilis siliquoidea*	20-October	28	Survival, growth, biomass
	14	*Hexagenia* species	20-October	28	Survival, growth, biomass
	16	*Tubifex tubifex*	2-November	28	Survival, growth, biomass
	16	*Gammarus pseudolimnaeus*	2-November	28	Survival, growth, biomass
3	22	*Chironomus dilutus*	18-December	13 About 56	Survival, growth, biomass Emergence, fecundity, hatching
	22	*Hyalella azteca* (Test 2)	18-December	28 42	Survival, growth, biomass Reproduction
	>22	*Caenorhabditis elegans*	Various	4	Survival, reproduction

in sediment (TR-Ni), but toxicity values also were estimated for differences between concentrations of SEM-Ni and AVS (abbreviated as SEM-AVS); and for SEM-AVS divided bythe organic carbon fraction of sediment (abbreviated as [SEM-AVS]/*f*OC; U.S. Environmental Protection Agency, 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 quantitative performance (that is, 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, 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 assumed to be greater than the maximum exposure concentration.

2.3 Results and Discussion

Sediment and Pore-Water Characteristics

Physicochemical 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 spiked nickel reacting with AVS to form nickel sulfide, which is poorly recovered by the AVS method (Carbonaro and others, 2005; W. Brumbaugh, U.S. Geological Survey; unpub. data, 2008). Some decreases of AVS apparently also occurred because of oxidation during toxicity tests. AVS concentrations in test beakers (day 14 of tests) were consistently 10 percent to 20 percent 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 the logarithm of concentration in sediment divided by concentration in pore water (log Kd), averaged 3.56 in the SR sediment and 4.12 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 Allison, 2005). The difference in log Kd indicates that a given TR-Ni concentration in the SR sediment would be associated with higher PW-Ni concentration than the same TR-Ni concentration in the WB sediment, suggesting that SR sediments had a lower binding affinity for nickel, and presumably greater nickel bioavailability. Nickel Kd values were consistent 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 reference toxicity tests. 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 grams per liter (g/L) for LS to 11 g/L for TT (John Besser, U.S. Geological Survey; unpub. data, 2010). However, the sodium chloride exposure of organisms during sediment toxicity tests was probably much lower than concentrations in bulk pore waters, because of 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 percent of sodium concentrations in bulk pore waters (*appendixes 3–5* and *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 of cations 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, sodium, chloride, and iron) 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 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 characteristic 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 (for example, sodium and chloride). This phenomenon was most evident in the second-highest nickel-spike levels, which had highest conductivity values [2,290–4,040 microsiemens per centimeter (µS/cm)] for tests with 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 hydrogen ions during formation of hydrous ferric oxides.

Nickel Concentrations

Sediment nickel concentrations (TR-Ni) in spiked sediments were within 20 percent of target concentrations (fig. 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 = 7,990 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 percent) than in spiked WB sediments (62–78 percent). 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, U.S. Geological Survey; unpub. data, 2008). Accordingly, lower recovery of Ni measured as SEM-Ni was most evident for WB sediments that were spiked with Ni and FeS (for example, fig. 1*B*). 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 (fig. 10).

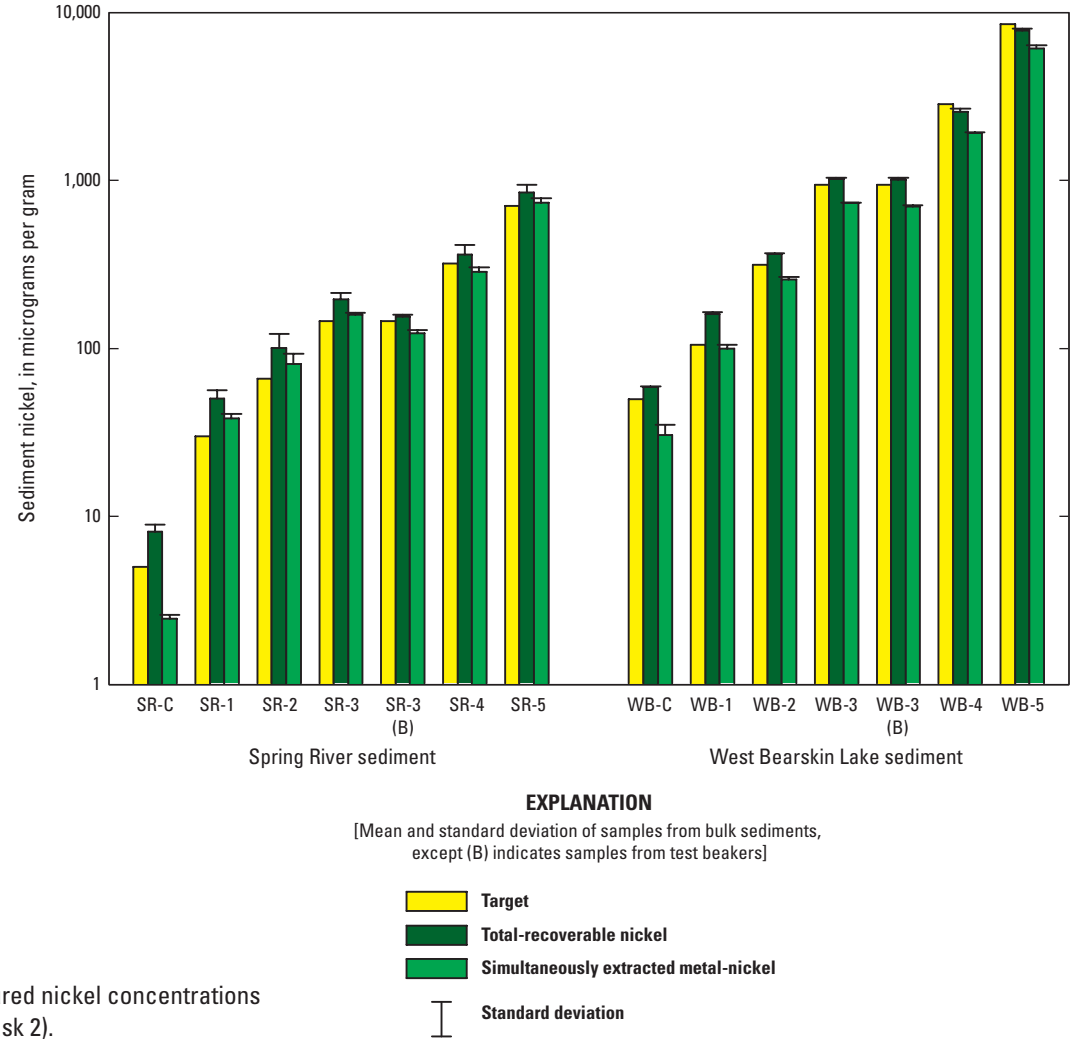

Figure 10. Target and measured nickel concentrations in nickel-spiked sediments (Task 2).

The difference between molar concentrations of SEM-Ni and AVS (SEM-AVS) in spiked sediments, an estimate of the potentially bioavailable fraction of sediment nickel (U.S. Environmental Protection Agency, 2005), was stable across testing groups during the 3-month toxicity testing period (fig. 11). Because of 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 (fig. 12; *appendixes 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 (fig. 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; fig. 6). Presumably, this equilibration period also resulted in decreased concentrations of other cations and anions (as discussed in Chapter 3, under "Sediment Characteristics and Nickel Concentrations"). These decreases in PW-Ni also point out the importance of the pre-test equilibration of sediments in test beakers 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, the greatest losses of PW-Ni occurred in treatments with the greatest SEM-AVS. Measured PW-Ni concentrations in test beakers were consistent across three test groups and four test start dates (fig. 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 (fig. 13; *appendix 2–10*). Mean concentrations of nickel in overlying water (OW-Ni) 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; U.S. Environmental Protection Agency, 2009) except for treatments WB-5 (all species) and WB-4 (LV only). For 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 (fig. 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 hours after the last 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.

Toxicity of Nickel-Spiked Sediments

Test acceptability criteria were met in 17 of 18 flow-through sediment toxicity tests conducted in Task 2 (*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 percent). This low control survival apparently was 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. Although this test was flagged as unreliable, the results 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 statistically significant reductions of any endpoint, relative to controls. In contrast, seven of nine species (all except LS and LV) had statistically 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 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 (figs. 14*A* and 14*B*), 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 (figs. 14*C* and 14*D*). 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.

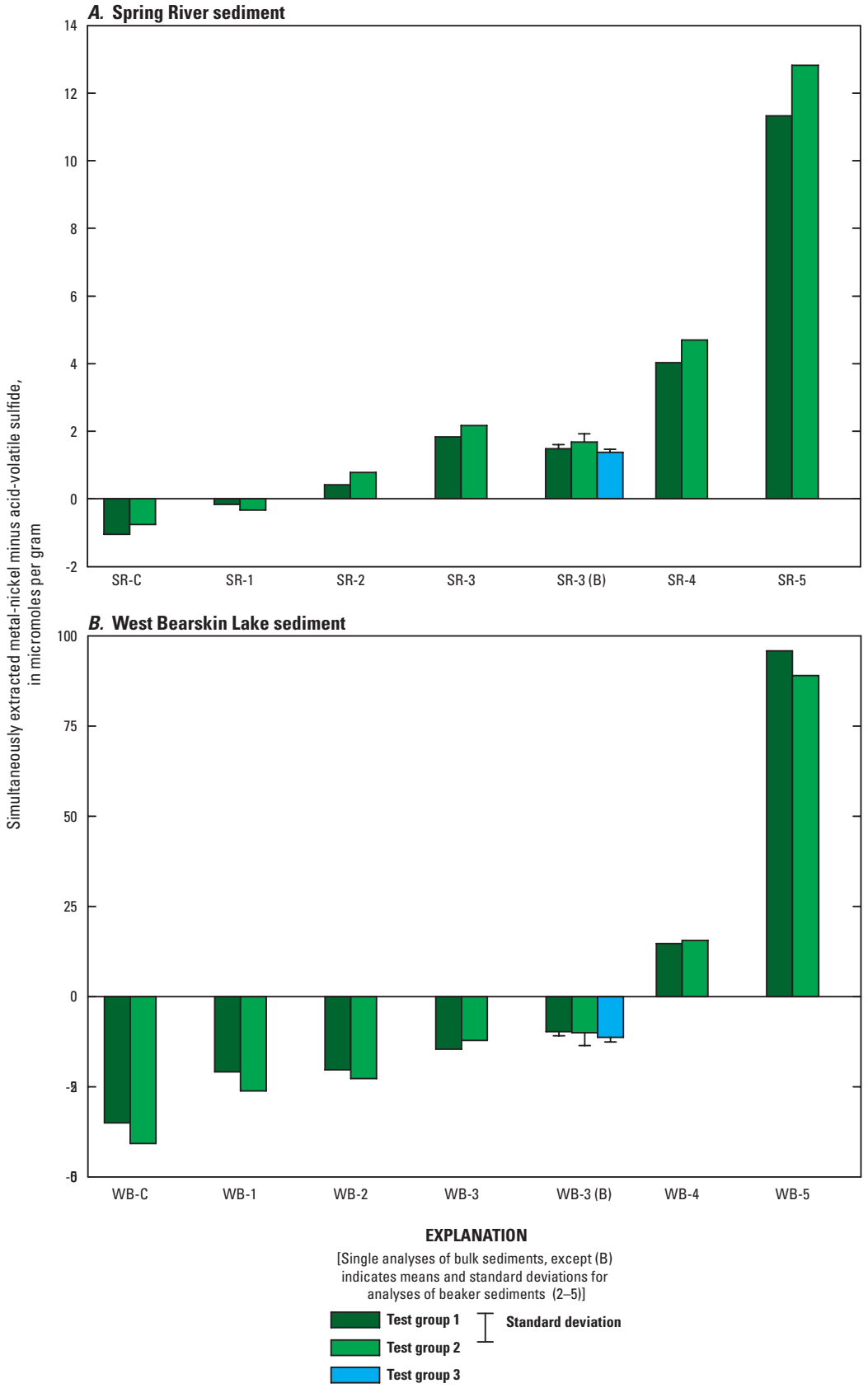

Figure 11. Difference between simultaneously extracted metal-nickel and acid-volatile sulfide in nickel-spiked sediments (Task 2).

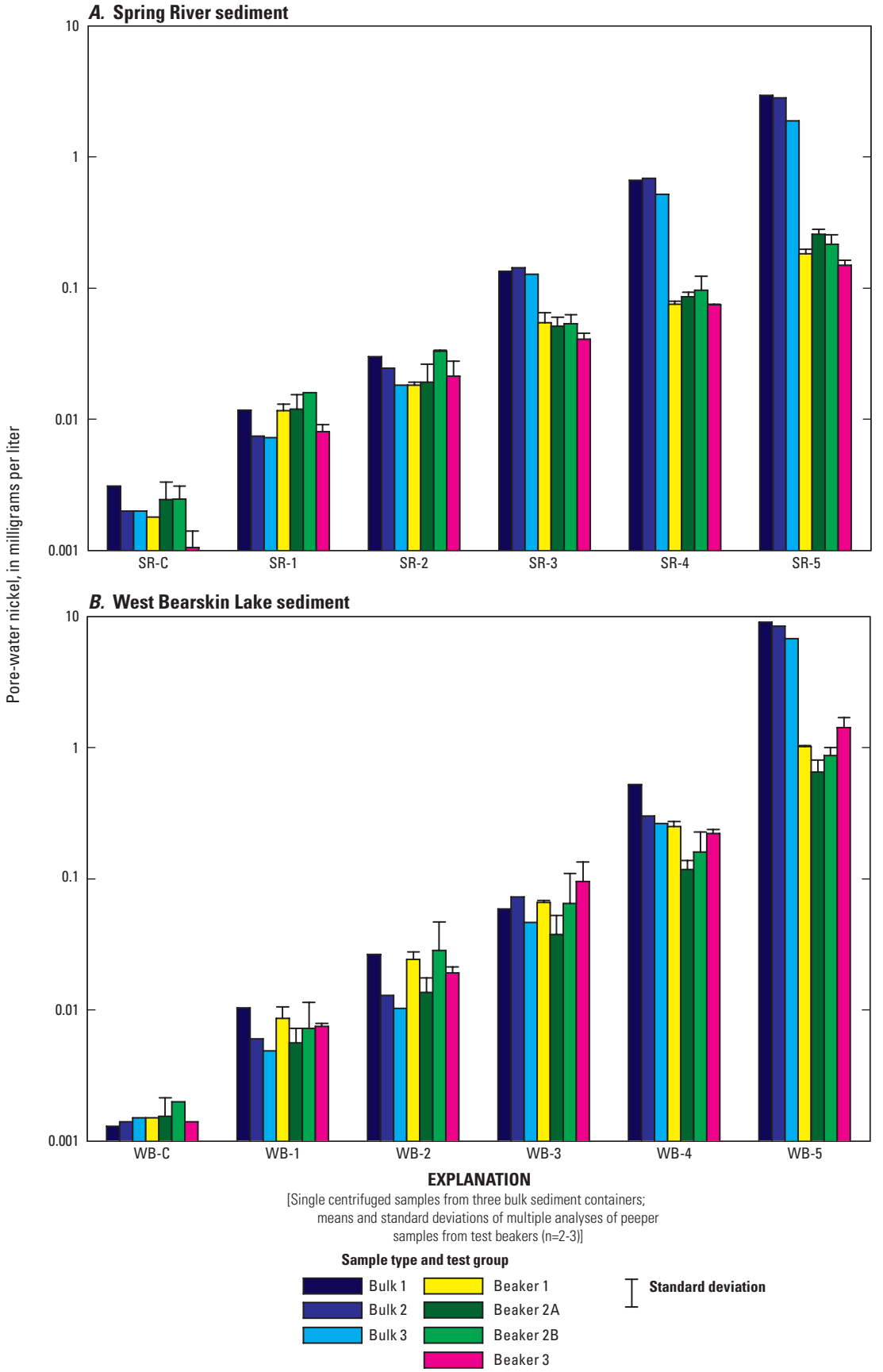

Figure 12. Pore-water nickel concentrations in nickel-spiked sediments (Task 2).

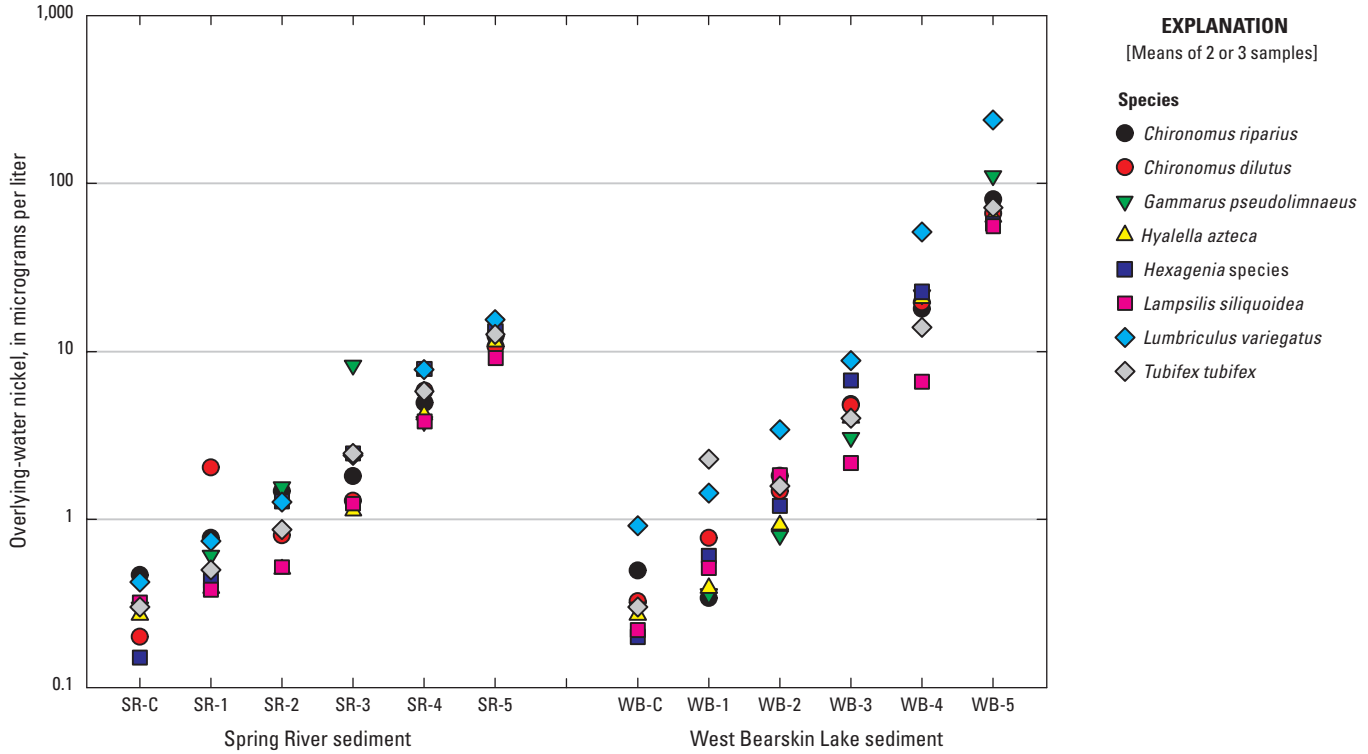

Figure 13. Overlying water nickel concentrations in toxicity tests with nickel-spiked sediments (Task 2).

Midges (CD and CR), oligochaetes (TT and LV) and mussels (LS) were less sensitive to nickel-spiked sediments. For CD (fig. 14*E*), adult emergence was the only endpoint that showed a statistically significant dose-related reduction relative to controls, and this response was only evident in the WB-5 treatment. The CR test (fig. 14*F*) had small but statistically 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 small (300-mL) egg-deposition chambers. The TT test showed statistically significant reductions in biomass and reproduction in the WB sediment (fig. 14*G*). Tests with LV (fig. 14*H*) and LS (fig. 14*I*) showed no statistically 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 percent greater than nominal) and in the tests with CD and CR (14 percent 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 an effect 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 1,715 µg/L (emergence endpoint) for CR and EC20s ranging from 8.5 to 1,201 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 tests with spiked sediments, except that the mussel had statistically significant reductions of growth and biomass at a waterborne nickel concentration of 71 µg/L, but did not have any statistically significant toxic effects in tests with nickel-spiked sediments. Tests with the oligochaetes LV and TT did not have statistically 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).

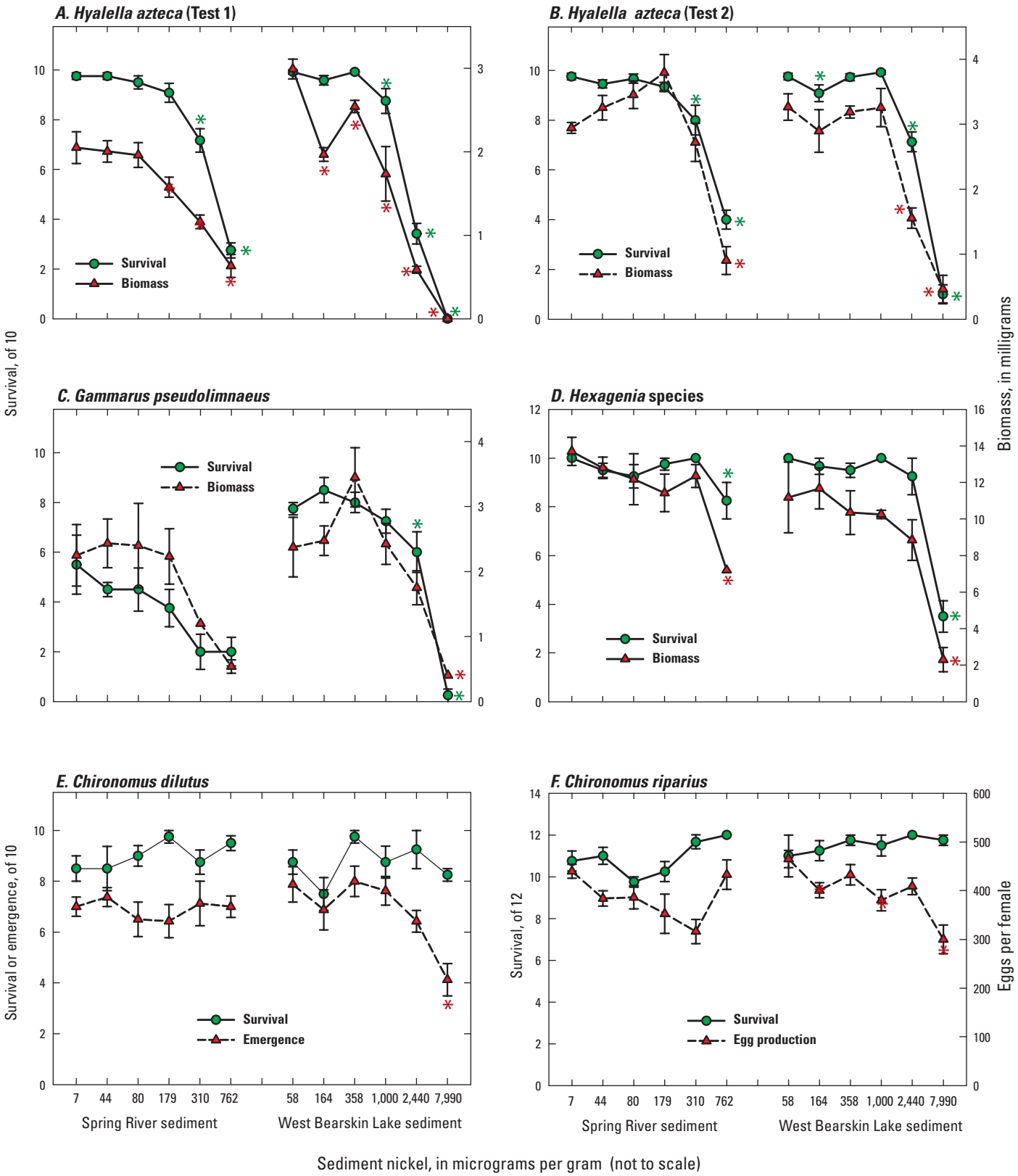

Figure 14. Responses of selected endpoints for nine invertebrate species in toxicity tests with nickel-spiked sediments.

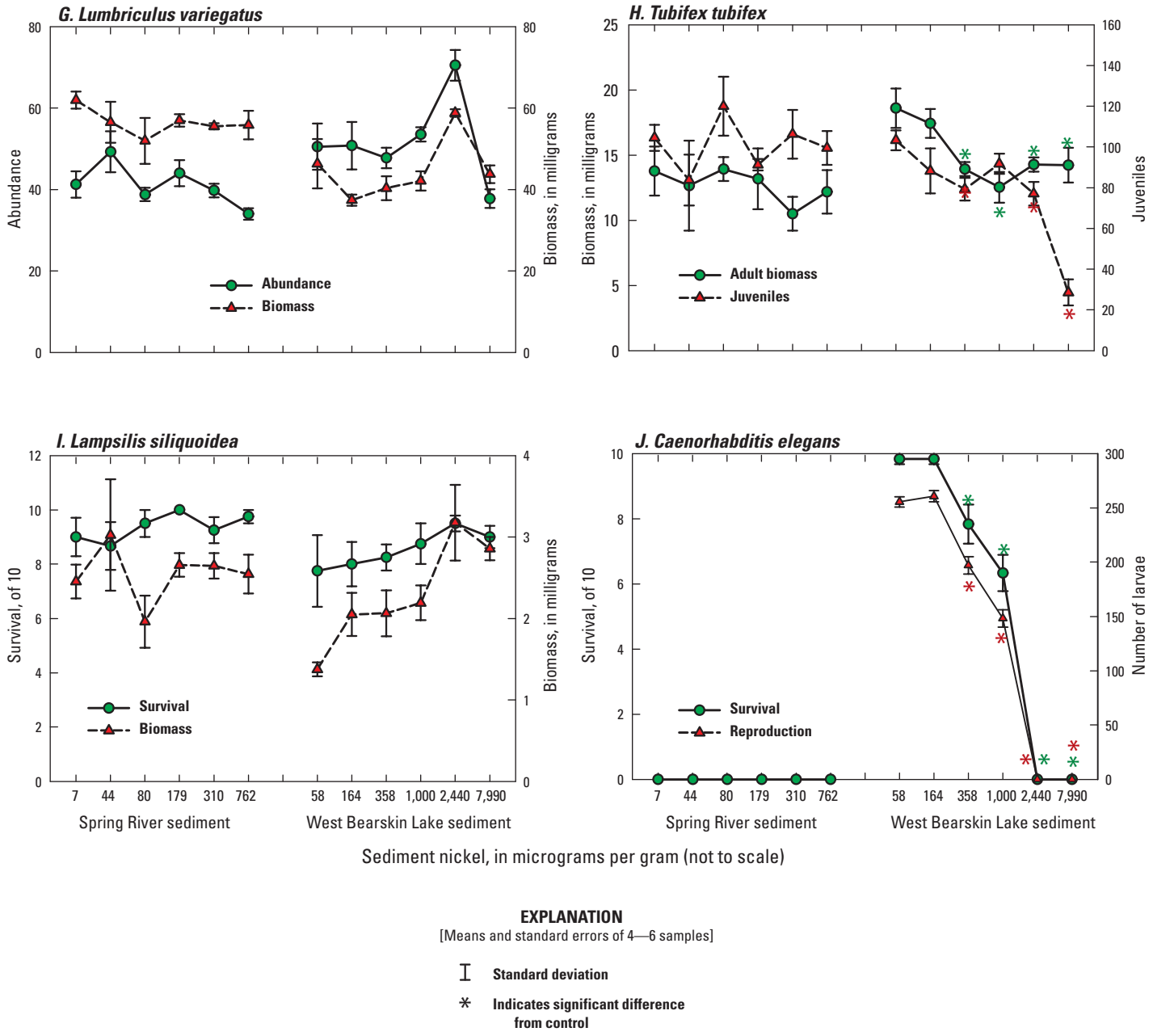

Figure 14. Responses of selected endpoints for 9 invertebrate species in toxicity tests with nickel-spiked sediments.—Continued

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. In contrast, a test with nickel-spiked WB sediments had good control survival and strong concentration-response trends for survival and larvae production (fig. 14*J*). 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 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 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

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

[NOEC, no-observed effect concentration; µg/L, microgram per liter; LOEC, lowest-observed effect concentration; EC10, 10-percent effect concentration; lcl-ucl, range from lower to upper limits of 95-percent confidence interval; EC20, 20-percent effect concentration; >, greater than; --, indicates no value was calculated]

Species	Endpoint	ANOVA		Concentration-response models			
		NOEC (µg/L)	LOEC (µg/L)	EC10 (µg/L)	lcl-ucl (µg/L)	EC20 (µg/L)	lcl-ucl (µg/L)
Chironomus dilutus	Survival	>1,710	--	--	--	--	--
	Growth	689	1,710	--	--	--	--
	Biomass	689	1,710	--	--	--	--
	Emergence	363	689	208	64–684	280	117–669
Chironomus riparius	Survival	>1,715	--	999	--	1,454	--
	Growth	>1,715	--	--	--	--	--
	Biomass	>1,715	--	839	60–11,807	1,201	273–5,292
	Emergence	678	1,715	1,610	1,068–2,426	--	--
Gammarus pseudolimnaeus	Survival	47	94	56	51–62	74	69–79
	Growth	94	170	--	--	--	--
	Biomass	94	170	143	9.7–2,110	165	42–652
Hyalella azteca	Survival	8.3	17	8.6	6.6–11	12	9.9–15
	Growth	8.3	17	17	3.1–88	22	6.6–74
	Biomass	8.3	17	6.5	.52–82	8.5	1.1–65
	Reproduction	17	40	6.7	.21–219	9.0	.77–105
Hexagenia species	Survival	>1,335	--	--	--	--	--
	Growth	104	257	53	3.2–889	131	20–863
	Biomass	104	257	102	29–364	204	86–485
Lampsilis siliquoidea	Survival	>71	--	--	--	--	--
	Growth	25	71	41	13–130	65	39-108
	Biomass	25	71	32	1.4–706	46	7.6–275
Lumbriculus variegatus	Survival	>494	--	--	--	--	--
	Growth	>494	--	--	--	--	--
	Biomass	>494	--	--	--	--	--
Tubifex tubifex	Survival	>494	--	--	--	--	--
	Biomass	>494	--	--	--	--	--
	Coccoons	>494	--	--	--	--	--
	Reproduction	>494	--	--	--	--	--
Caenorhabditis elegans	Survival	--	>800	--	--	--	--
	Reproduction	400	800	349	2–50,094	550	42–7,273

showed a wide range of nematode survival across different sediment types (fig. 15). Nematode survival generally was greater in sediment with higher organic content, with low survival (0–33 percent) in sediments with 0.8 percent to 1.9 percent TOC and higher survival (55–81 percent) in sediments with 4.1 percent to 10 percent TOC. However, even the high-TOC sediments did not meet the International Standards Organization (2010) test-acceptability criterion for control survival (>90 percent).

It also was 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 (Vandegehuchte and others, 2007). Treatments that were toxic to nematodes had OW-Ni concentrations (68–5,700 µg/L) that exceeded chronic water-quality criteria for nickel (for example, 52 µg/L at a 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 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 comparisons 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 Relations

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 EC10s and EC20s, with corresponding 95 percent 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. Other endpoints, such as survival and growth, also 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 (fig. 16*A*) or EC10s for TR-Ni, differed widely among species and between sediments. Based on responses in both sediments, the three most sensitive

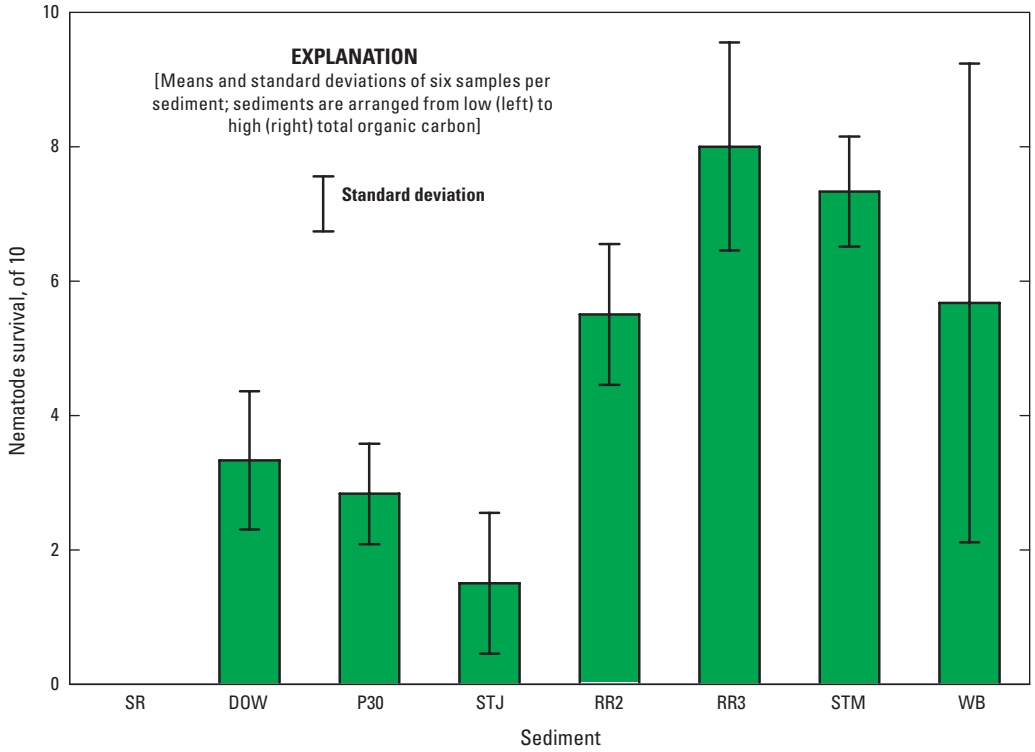

Figure 15. Survival of nematodes (*Caenorhabditis elegans*) in unspiked sediments.

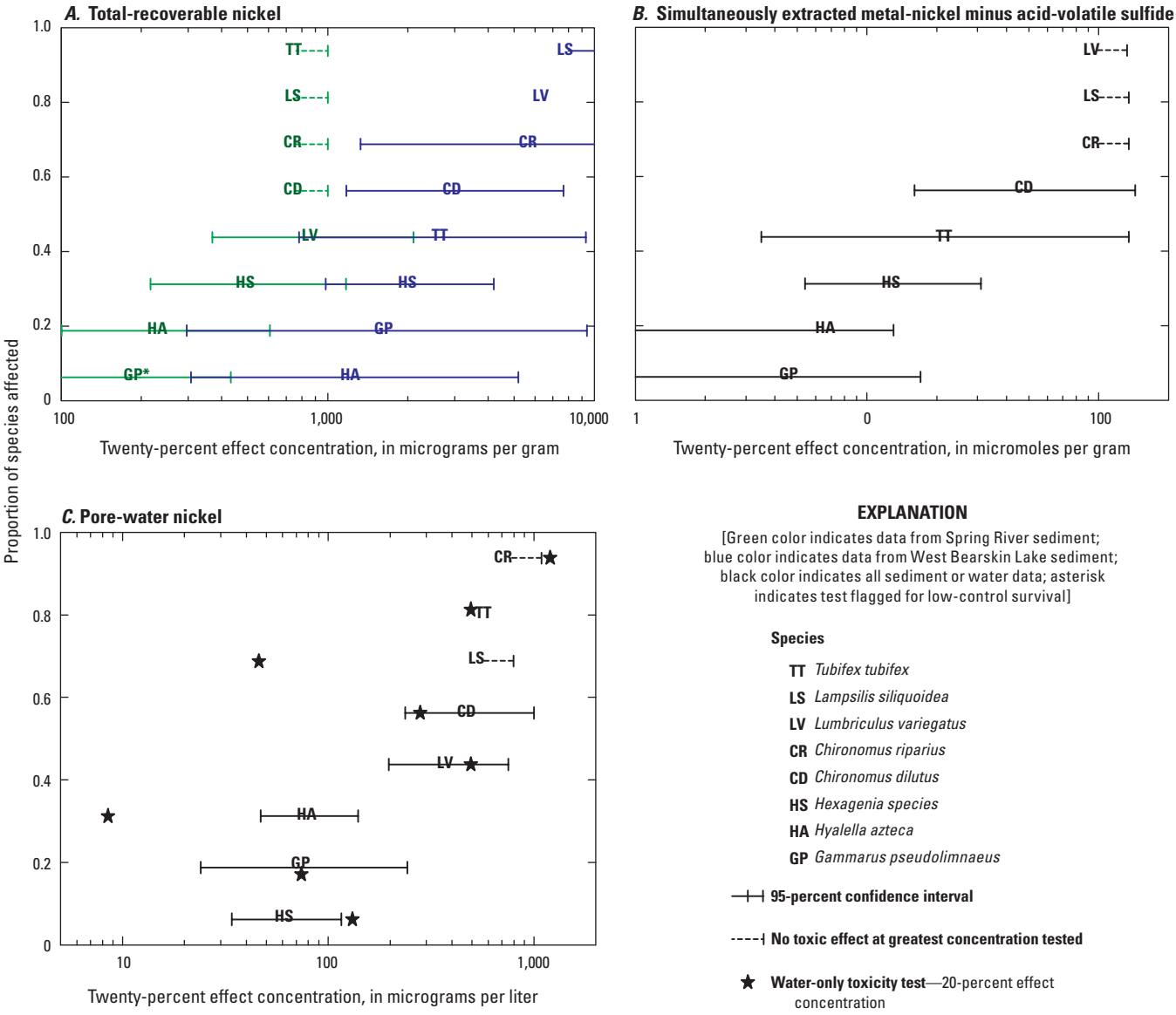

Figure 16. Species-sensitivity distribution for nickel toxicity to eight invertebrate species in nickel-spiked sediments.

species were HA, GP, and HS. Previous toxicity tests with nickel-spiked sediments reported a similar ranking of sensitivity for four of the species 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 (that is, nickel toxicity was greater) in SR sediments. The lowest EC20 value for TR-Ni in SR sediment was 202 µg/g (for *Hyalella* biomass), compared to the lowest EC20 of 1,177 µg/g (for *Hyalella* biomass) in WB sediments. EC20s averaged about six times greater for WB sediments than for SR sediments, and these differences were more pronounced for EC10s (about 7 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*; fig. 16*B*, *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 for individual sediments based on TR-Ni (means = 166 percent for SR, 300 percent for WB) and for EC20s calculated from merged data based on SEM-AVS (mean = 291 percent) or PW-Ni (mean = 180 percent). 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; U.S. Environmental Protection Agency, 2005).

The importance of nickel exposure from pore water was supported by the close agreement between EC20s from water-only exposures and EC20s for PW-Ni for most species (fig. 16*C*). 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 also may indicate that: (1) peeper samples overestimated actual PW-Ni exposures during sediment tests (for example, because of microhabitat differences); or (2) water-only toxicity did not accurately represent the sensitivity of these species to nickel in a sediment environment (for example, because of 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 or OW-Ni that occurred during the 4-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 during a 4-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 tested. 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 wide variation in nematode survival among different unspiked sediments and because of greater nickel concentrations in overlying water of the static nematode tests, compared to flow-through tests.

- Toxicity values for sediment nickel (expressed as TR-Ni) differed by a factor of six between the two sediments, with toxicity occurring at lower nickel concentrations in the SR sediment. These differences were consistent with the greater nickel-binding capacity of the WB sediment, as indicated by sediment:pore-water distribution coefficients (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 most sensitive species in tests with both sediments. The lowest EC20 value for TR-Ni in SR sediment was 202 µg/g (for *Hyalella* biomass), compared to the lowest EC20 of 1,177 µg/g (for *Hyalella* biomass) in WB sediments. The lowest EC20s derived 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.

Chapter 3—Effect 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 physicochemical characteristics. Differences in nickel bioavailability were generally consistent with proposed models of bioavailability of cationic metals (for example, U.S. Environmental Protection Agency, 2005). However, there remains uncertainty about the applicability of these models to nickel. Nickel tends to have lower affinity for binding to sediment components, including acid-volatile sulfide (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, total organic carbon (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 relations 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-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 percent) (table 11). Sediments tested in Task 3 included one pond sediment from CERC, in Boone County, Missouri, USA (pond 30, or P30) and five stream sediments from Michigan, USA: Dow Creek (DOW), in Gladwin County; Raisin River Site 2 (RR2) and Raisin River Site 3 (RR3), both in Washtenaw County; St. Joseph River (STJ), in Calhoun County; and South Tributary of Mill Creek (STM), in Cheboygan County (locations not shown). Several of the Michigan sediments (and the Spring River, Missouri, 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-liter 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 2-stage spiking protocol described in Chapter 2. Spike concentrations were selected to reflect the expected nickel-binding capacities of the sediments, with nominal high nickel concentrations of 1,237 µg/g (for DOW sediment); 1,667 µg/g (for P30, RR2, RR3, and STJ sediments); and 2,400 µg/g (for STM sediment). Following the 28-day equilibration of super-spikes prepared with each sediment, sediment dilutions with unspiked sediment produced five nominal nickel concentrations in 2-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*. Super-spikes spiked with nickel were used to prepare sediment dilutions after 28 d. Duplicate jars were prepared for each treatment, with 3.6 L of 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 testing 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), also was 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 because of space limitations. Instead of the large (1-L) test chambers used in Task 2, Task-3 HS tests were conducted with the 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 (3.6 mg per day). Group-1 tests were conducted with HS and TT and Group-2 tests were conducted with GP and HA.

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

[AVS, acid-volatile sulfide; µmol/g, micromole per gram; TOC, total organic carbon; µg/g, microgram per gram; SEM-Ni, simultaneously extracted metal-nickel]

Characteristic	Dow Creek (DOW)	St. Joseph River (STJ)	Raisin River site 2 (RR2)	Raisin River site 3 (RR3)	Pond 30 (P30)	South tributary of Mill Creek (STM)
Base sediment						
AVS (µmol/g)	0.9	2.7	4.8	7.2	9.5	22.0
TOC (percent)	1.5	2.2	3.8	9.3	2.6	8.2
Nickel spike (µg/g)						
Spike level						
1	79	167	167	167	167	300
2	158	333	333	333	333	600
3	317	667	667	667	667	1,200
4	633	1,333	1,333	1,333	1,333	2,400
5	1,267	2,667	2,667	2,667	2,667	4,800
SEM-Ni minus AVS (µmol/g)						
Spike level						
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

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 total-recoverable nickel (TR-Ni), simultaneously extracted metal-nickel (SEM-Ni), and AVS were measured in bulk sediments from both groups. Concentrations of pore-water nickel (PW-Ni), iron, and manganese (day-14 peeper samples) and overlying-water nickel (OW-Ni) (days 1 and 27) were measured during all tests.

Data Analysis and Interpretation

Methods for 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 to determine lowest-observed effect concentrations (LOECs). Concentration-response relations and toxicity values (20-percent effect concentrations or EC20s) were modeled using TRAP software. Data from Tasks 2 and 3 (tests with 8 sediments and 3 species) were merged for analysis of associations among toxicity values and sediment characteristics using bivariate (Pearson's) linear correlation analysis using SAS/STAT software.

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 millivolts). Most sediments were dominated by sand-sized particles (only 14–28 percent fine particles), except STM (47 percent fines) and P30 (90 percent fines). Cation exchange capacity ranged from 6.0 milliequivalents per 100 g (DOW) to 29 milliequivalents per 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 calcium (0.4–8.4 percent), iron (0.7–2.7 percent), and aluminum (0.6–2.6 percent) (*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 (1,341 µg/g) and STM (5,080 µg/g). In unspiked sediments, SEM-Ni concentrations constituted small fractions of TR-Ni (from 18 percent in DOW to 33 percent in STM), suggesting relatively low nickel bioavailability. In spiked sediments, SEM-Ni made up greater fractions of TR-Ni, ranging from about 65 percent

(DOW, STJ) to more than 80 percent (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 (OC) fraction of sediments produced a different ranking of sediments, with values for highest spike treatments ranging from 480 µmol/g OC in RR3 to 1,780 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 (43–70 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 sulfide 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 also were 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, U.S. Geological Survey; unpub. data, 2010). 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, because of 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 also were 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 was 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 (fig. 17*A*), 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*; fig. 17*A*). The role of AVS in controlling nickel binding is illustrated by the consistently low PW-Ni in treatments with SEM-AVS less than zero (fig. 17*B*). Iron and manganese concentrations also were greater in bulk pore water (by about 5- to 10-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 percent of PW-Ni (*appendix 3–9*). Concentrations of OW-Ni generally were consistent within spike levels across different spiked sediments (for example, 20–35 µg/L at the highest spike level), 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 statistically significant toxic effects (statistically significant overall ANOVA and LOECs defined by statistically 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 statistically 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 sensitive and have low variability. The sensitivity and variability of toxicity values (EC20s) for endpoints from sediment tests with GP, HA, and HS are compared in table 12. (The GP test with SR sediment in Task 2 was excluded from this comparison because of low control survival.) For GP, survival was less sensitive than biomass, as indicated by its higher average EC20 for TR-Ni (1,516 µg/g compared to 1,134 µg/g). However, survival EC20s were much less variable, with 95 percent confidence ranges that averaged 101 percent of EC20s, compared to 1,025 percent 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,

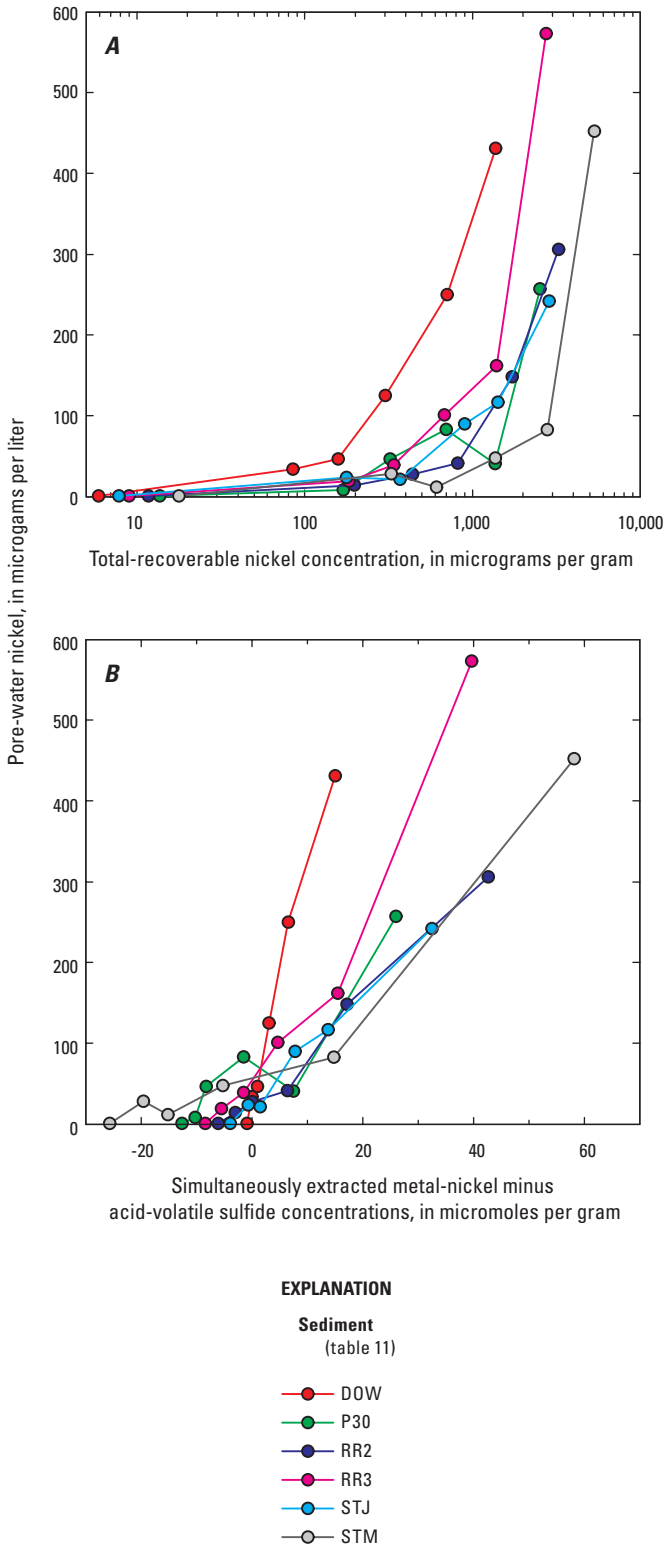

Figure 17. Pore-water nickel concentrations and sediment nickel concentrations in nickel-spiked sediments

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*).

Responses of selected endpoints (survival of GP and HA, growth of HS) among tests with six Task-3 sediments are compared in figure 18. Survival of HA (fig. 18*A*) and GP (fig. 18*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 of GP survival in the other four sediments were clearly separated, suggesting a gradient of nickel bioavailability, but responses of HA survival were similar among these four sediments. The HS growth endpoint (fig. 18*C*) showed lesser overall differences among sediments, although the DOW and STM sediments still had highest and lowest nickel bioavailability, respectively.

Variation of EC20s for GP, HA, and HS among all eight sediments tested in Tasks 2 and 3 is compared in table 13. 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 (1,645 µg/g) and STM (3,475 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 (150–2,100 µg/g; Doig and Liber, 2006a). Another study reported a lower range of 28-d 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 nickel in overlying water: 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. All AVS-normalized EC20s for GP and HA were positive values (or very close to zero), consistent with the hypothesis that metals would not be toxic if molar concentrations of SEM-Ni are less than AVS (U.S. Environmental Protection Agency, 2005). In contrast, several AVS-normalized 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 generally were 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 (U.S. Environmental Protection Agency, 2005).

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

[Green shading, Task-2 sediments; blue shading, Task-3 sediments; EC20, 20-percent effect concentration; µg/g, micrograms per gram; lcl, lower 95-percent confidence limit; ucl, upper 95-percent confidence limit; %, percent; SR, Spring River; WB, West Bearskin; DOW, Dow Creek; P30, Pond 30; RR2, Raisin River site 2; RR3, Raisin River site 3; STJ, St. Joseph River; STM, South Tributary of Mill Creek; --, no data]

Sediment	Survival				Biomass			
	EC20 (µg/g)	lcl (µg/g)	ucl (µg/g)	Range (%)	EC20 (µg/g)	lcl (µg/g)	ucl (µg/g)	Range (%)
				Hyalella azteca				
SR	311	158	614	146	235	101	606	215
WB	1,786	958	3,342	133	1,245	307	5,182	392
DOW	528	277	1,008	138	220	147	329	83
P30	1,367	716	2,610	139	1,002	772	1,299	53
RR2	1,221	719	2,074	111	1391	96	20,237	1,448
RR3	901	543	1,495	106	456	134	1,560	313
STJ	1,482	900	2,443	104	1,195	1,029	1,388	30
STM	3,475	2,296	5,259	85	2,662	1,742	4,067	87
Mean	1,384	--	--	120	1,051	--	--	328

Sediment	Survival				Biomass			
	EC20 (µg/g)	lcl (µg/g)	ucl (µg/g)	Range (%)	EC20 (µg/g)	lcl (µg/g)	ucl (µg/g)	Range (%)
				Gammarus pseudolimnaeus				
WB	2,262	1,049	4,876	169	1,667	296	9,400	546
DOW	572	347	942	104	344	194	611	121
P30	847	584	1,227	76	451	77	2,656	572
RR2	1,107	711	1,724	92	946	73	12,240	1,286
RR3	1,812	1,192	2,756	86	2,089	356	12,258	570
STJ	1,440	948	2,189	86	103	3	3,944	3,827
STM	2,571	1,656	3,991	91	2,338	816	6,702	252
Mean	1,516	--	--	101	1,134	--	--	1,025

Sediment	Growth (weight)				Biomass			
	EC20 (µg/g)	lcl (µg/g)	ucl (µg/g)	Range (%)	EC20 (µg/g)	lcl (µg/g)	ucl (µg/g)	Range (%)
				Hexagenia species				
SR	594	289	1,217	156	503	216	1,170	190
WB	1,728	378	7,894	435	1,667	296	9,400	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	2,337	455
STM	459	294	715	92	410	248	680	105
Mean	527	--	--	205	527	--	--	246

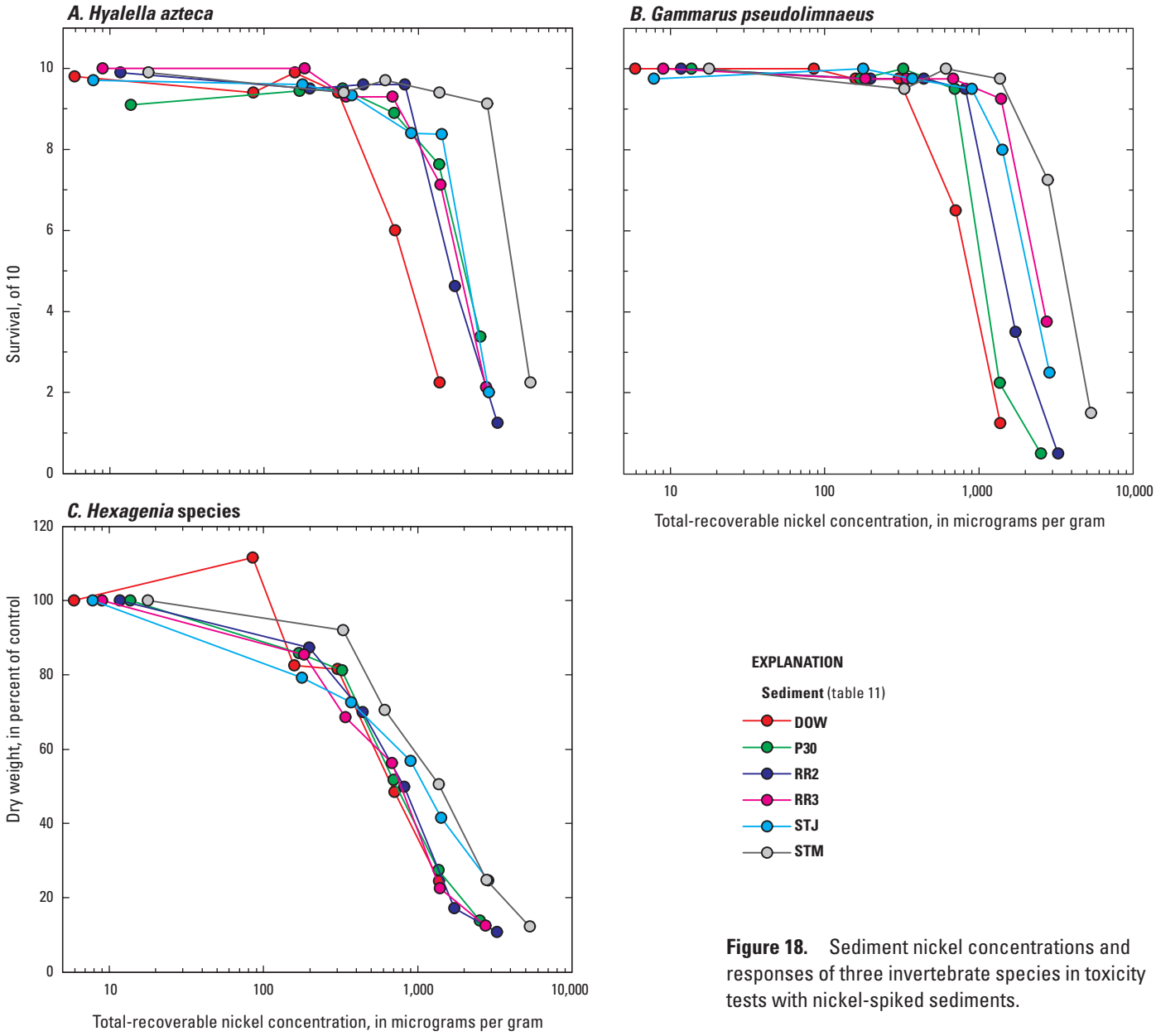

Figure 18. Sediment nickel concentrations and responses of three invertebrate species in toxicity tests with nickel-spiked sediments.

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" (1.7–120 µmol/g) of the equilibrium-partitioning sediment benchmarks developed by U.S. Environmental Protection Agency (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 were close to or greater than the U.S. Environmental Protection Agency (2009) chronic water-quality criterion for dissolved nickel: 52 µg/L for overlying water at hardness of 100 mg/L; and 86 µg/L for pore water at a typical hardness of 180 mg/L. In contrast, most mayfly (HS) EC20s based on SEM-AVS, [SEM-AVS]/$f$oc, and PW-Ni fell well below sediment toxicity benchmarks or water-quality criteria, especially for Task-3 sediments.

Relations of Nickel Bioavailability with Sediment Characteristics

Linear correlations among toxicity values and characteristics of sediment and pore water are summarized in table 14. Of the three species, sediment (TR-Ni) EC20s for GP had the strongest associations with sediment characteristics, with

Table 13. Variation of effect concentration (EC20s) for three invertebrates in nickel-spiked sediments.

[Green shading, Task-2 sediments; blue shading, Task-3 sediments; SEM-Ni, simultaneously extracted metal-nickel; AVS, acid-volatile sulfide; TR-Ni, total recoverable nickel; µg/g, micrograms per gram; µmol/g, micromoles per gram; OC, organic carbon; PW-Ni, pore-water nickel; microgram per liter; SR, Spring River; WB, West Bearskin Lake; DOW, Dow Creek; P30, Pond 30; RR2, Raisin River site 2; RR3, Raisin River site 3; STJ, St. Joseph River; STM, South Tributary of Mill Creek; RSD, relative standard deviation]

Sediment	TR-Ni (µg/g)	SEM Ni (µg/g)	SEM-Ni minus AVS		PW-Ni (µg/L)
			(µmol/g)	(µmol/g OC)	
Hyalella azteca survival					
SR	317	267	9.0	1,442	79
WB	1,645	1,241	8.6	10	150
DOW	528	332	4.7	378	224
P30	1,367	1,054	8.6	399	74
RR2	1,221	834	11	293	82
RR3	901	775	13	108	99
STJ	1,482	985	19	512	132
STM	3,475	2,557	29	332	196
RSD (percent)	71	71	59	101	44
Gammarus pseudolimnaeus survival					
WB	2,262	1,774	13	111	183
DOW	572	351	5.5	419	277
P30	847	656	4.2	-30	83
RR2	1,107	666	10	97	80
RR3	1,812	1,566	25	311	247
STJ	1,440	1,022	15	668	150
STM	2,571	1,729	9.0	97	122
RSD (percent)	49	52	61	101	47
Hexagenia species growth					
SR	594	436	9.5	2,074	144
WB	1,728	1,451	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 (percent)	95	104	1,377	331	94

statistically significant positive correlations with AVS, TOC, iron, manganese, cation-exchange capacity, and sediment:pore water distribution coefficient (Kd). Correlations for HA followed similar trends, but were weaker and were statistically significant only for AVS and Kd. All these statistically 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 statistically 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 statistically significant correlations with characteristics of sediment or pore water. Associations of nickel toxicity values with sediment characteristics are complicated by strong intercorrelations among sediment constituents (table 14). Sediment EC20s for GP and HA were significantly correlated with sediment AVS concentrations, and EC20s for HA also were significantly correlated with concentrations of TOC, iron, and manganese. All of these sediment constituents are potential contributors to nickel-binding capacity, as reflected by their statistically significant correlations with cation-exchange capacity 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 the U.S. Environmental Protection Agency (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 tests with HA (fig. 19*A*) and GP (fig. 19*B*) 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 were statistically significant and explained most of the variation in SEM-Ni EC20s. However, the equilibrium-partitioning hypothesis does not fully explain the results of the HS tests (fig. 19*C*). Results of HS tests in Task 2 (SR and WB sediments) produced SEM-Ni EC20s that were similar to trends seen in HA and GP tests, but Task-3 tests produced several SEM-Ni 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 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 may reflect greater sensitivity of smaller nymphs to nickel toxicity. These differences between tasks resulted in a nonsignificant regression for the combined data, but EC20s from each task indicated similar trends for the association of EC20s with AVS. For the Task-3 data, a large proportion of the variation in HS EC20s was explained by regression with AVS ($r^2 = 0.86$). Although the slope of this regression was less than slopes for HA and GP.

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 by ingestion of nickel-rich particles rather than exposure to dissolved nickel, and that gastrointestinal bioavailability of nickel is not

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

[Pearson correlation coefficients (r), n = 8. Concentrations of acid-volative sulfide, iron, manganese, calcium, and magnesium were log-transformed. Yellow shading indicates statistical significance ($p<0.05$). EC20, 20-percent effect concentration; TR-Ni, total recoverable nickel; Kd, concentration in sediment/concentration in pore water; AVS, acid-volatile sulfide; TOC, total organic carbon; Fe, iron; Mn, Maganese; CEC, caton exchange capacity; DOC, dissolved organic carbon; Ca, calcium; Mg, magnesium]

Variable	Sediment EC20 (TR-Ni)			Sediment characteristics										
	Gammarus pseudolimnaeus survival	*Hyalella azteca* survival	*Hexagenia* species growth	Fines	Clay	Silt	Sand	Acid-volatile sulfide	Total organic carbon	Iron	Manganese	Cation exchange capacity	Kd
pH	-0.074	0.060	-0.667	-0.752	-0.828	-0.748	0.785	-0.586	-.292	-0.322	0.165	-0.462	-0.368
Fines	.336	.356	.543		.961	.994	-.996	.738	.390	.616	.207	.645	.723
Clay	.218	.200	.631			.943	-.970	.678	.281	.598	.102	.545	.644
Silt	.416	.406	.598				-.995	.803	.485	.668	.276	.721	.761
Sand	-.367	-.350	-.611					-.777	-.437	-.654	-.228	-.682	-.736
AVS	.848	.738	.507						.827	.869	.516	.918	.842
TOC	.894	.551	.592							.747	.768	.951	.700
Fe	.836	.656	.688								.731	.839	.887
Mn	.804	.510	.291									.722	.686
CEC	.855	.565	.667										.806
Kd	.803	.764	.495										

Variable	Pore-water EC20			Pore-water characteristics		
	Gammarus pseudolimnaeus survival	*Hyalella azteca* survival	*Hexagenia* species growth	Dissolved organic carbon	Calcium	Magnesium
pH	-0.106	-0.079	-0.189	0.016	0.715	0.477
DOC	.728	.906	-.235		.371	.545
Ca	.030	.188	-.401			.883
Mg	.208	.256	-.675			

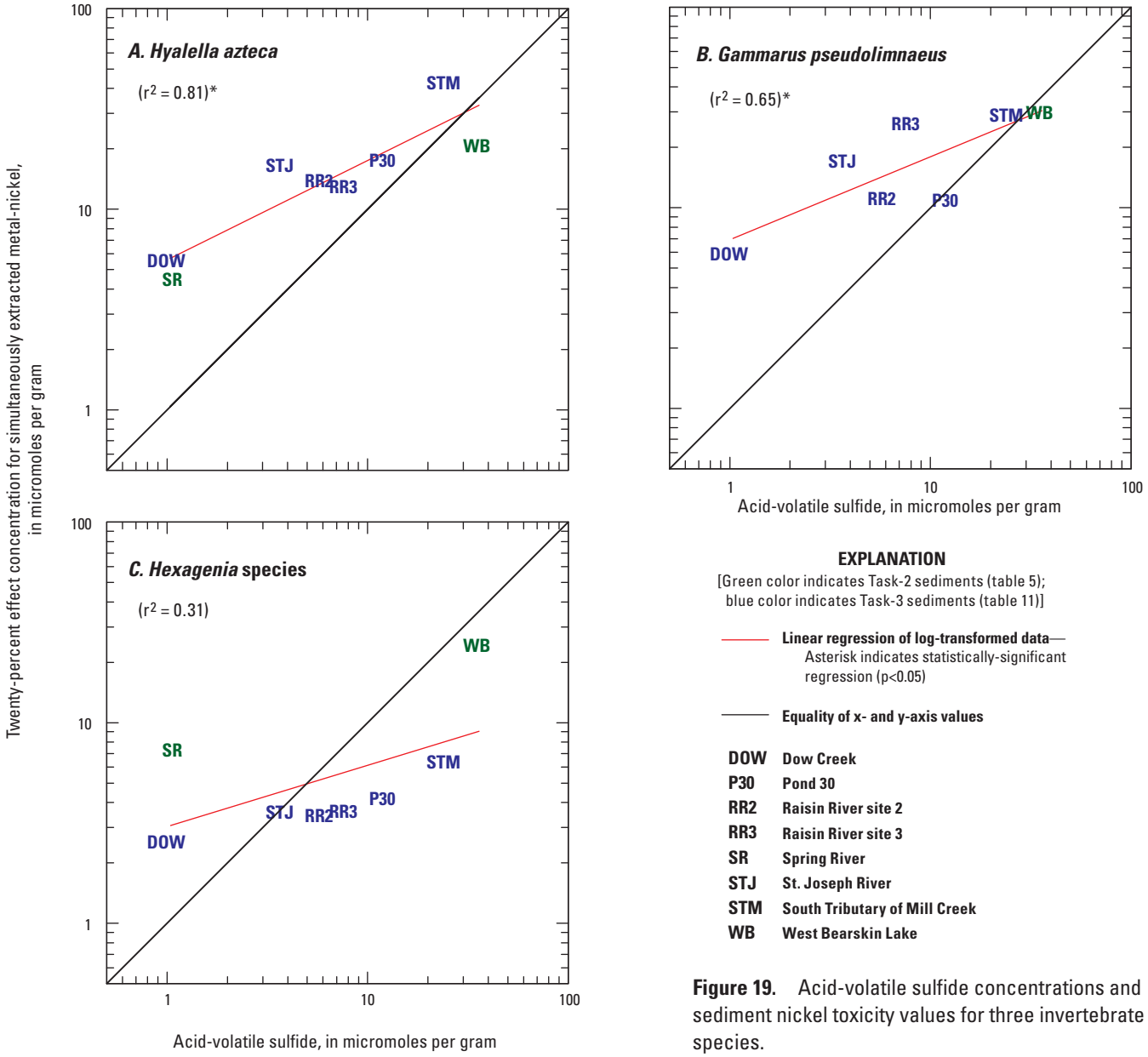

Figure 19. Acid-volatile sulfide concentrations and sediment nickel toxicity values for three invertebrate species.

controlled by AVS (for example, De Jonge 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 (that is, lower sediment EC20s). Differences in chamber morphology and sediment depth also may explain differences among sediments in PW-Ni EC20s for HS growth (table 13). In 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.

3.4 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 percent), which ranged between the extremes of the two Task-2 sediments. Three different spiking ranges (target nickel concentrations for high spike treatments: 1,267, 2,667, and 4,800 µg/g) were used for low-AVS (DOW), intermediate-AVS (STJ, RR2, RR3, and P30) and high-AVS (STM) sediments, respectively. Despite differences in ranges of sediment nickel concentrations, ranges of PW-Ni were similar for all sediments, reflecting differences in nickel-binding affinity that were evident in nickel distribution coefficients (range in log Kd: 3.53–4.34).
- Spiked sediments were tested successfully with four species in Task 3, 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 greatest 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]/$f$oc did not greatly reduce variation in EC20s for the amphipods, but greatly increased variation in EC20s for the mayfly. 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 both amphipods had statistically significant positive correlations with AVS, and GP toxicity values also were significantly correlated with other sediment components (TOC, Fe, Mn). These sediment constituents were strongly intercorrelated and all were significantly correlated with measures of nickel-binding affinity (cation-exchange capacity and Kd). In contrast, sediment EC20s for mayflies were not significantly correlated with any sediment characteristics.
- For the two amphipods, toxicity values for nickel-spiked sediments were generally consistent with the hypothesis that no toxicity would occur if SEM-Ni concentrations were less than the binding capacity of AVS. In contrast, several SEM-Ni EC20s for mayflies in Task 3 were less than AVS concentrations, especially for sediments with high AVS concentrations. Comparison of results of HS tests between Tasks 2 and 3 was complicated by methodological differences between Tasks. Differences in the starting size of mayfly nymphs may have affected their sensitivity to nickel toxicity, and differences in the size and shape of test chambers may have led to differences in nickel bioavailability by affecting sediment oxidation-reduction potential and AVS concentrations.
- The divergence of mayfly toxicity values from those obtained from tests with amphipods in the same sediments, and from predictions of the SEM-AVS hypothesis, could indicate that AVS exerts a lesser degree of control on nickel bioavailability to mayflies. For example, nickel in AVS-rich particles may be bioavailable to mayflies by way of ingestion and gastrointestinal uptake. Alternatively, mayflies may experience greater exposure to PW-Ni in oxygenated burrow microenvironments, because of localized oxidation of AVS, than would be predicted based on typical sampling methods for AVS or PW-Ni.

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Appendixes 1–3

Appendix 1. Task 1 Data

The Excel spreadsheet appendix-1.xls contains data and supplemental information from Task 1. This Excel file can be accessed at *http://pubs.usgs.gov/sir/2011/5225/downloads/appendix-1.xls*.

Appendix 2. Task 2 Data

The Excel spreadsheet appendix-2.xls contains data and supplemental information from Task 2. This Excel file can be accessed at *http://pubs.usgs.gov/sir/2011/5225/downloads/appendix-2.xls*.

Appendix 3. Task 3 Data

The Excel spreadsheet appendix-3.xls contains data and supplemental information from Task 3. This Excel file can be accessed at *http://pubs.usgs.gov/sir/2011/5225/downloads/appendix-3.xls*.

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Back cover. Photographs of invertebrate species (clockwise from top center): Amphipod (*Gammarus*); Oligochaete (*Tubifex*); Nematode (*Caenorhabditis*); Mayfly (*Hexagenia*); Mussel (*Lampsilis*); Midge (*Chironomus*).