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Laboratory Toxicity and Benthic Invertebrate Field Colonization of Upper Columbia River Sediments: Finding Adverse Effects Using Multiple Lines of Evidence

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Abstract From 1930 to 1995, the Upper Columbia River (UCR) of northeast Washington State received approximately 12 million metric tons of smelter slag and associated effluents from a large smelter facility located in Trail, British Columbia, approximately 10 km north of the United States–Canadian border. Studies conducted during the past two decades have demonstrated the presence of toxic concentrations of heavy metals in slag-based sandy sediments, including cadmium, copper, zinc, and lead in the UCR area as well as the downstream reservoir portion of Lake Roosevelt. We conducted standardized whole-sediment toxicity tests with the amphipod *Hyalella azteca* (28-day) and the midge *Chironomus dilutus* (10-day) on 11 samples, including both UCR and study-specific reference sediments. Metal concentrations in sediments were modeled for potential toxicity using three approaches: (1) probable effects quotients (PEQs) based on total recoverable metals (TRMs) and simultaneously extracted metals (SEMs); (2) SEMs corrected for acid-volatile sulfides (AVS; i.e., $\sum$SEM − AVS); and (3) $\sum$SEM − AVS normalized to the fractional organic carbon (f_{oc}) (i.e., $\sum$SEM − AVS/f_{oc}). The most highly metal-contaminated sample ($\sum PEQ_{TRM} = 132$; $\sum PEQ_{SEM} = 54$; $\sum$SEM − AVS = 323; and $\sum$SEM − AVS/$_{foc}$ = 64,600 umol/g) from the UCR was dominated by weathered slag sediment particles and resulted in 80% mortality and 94% decrease in biomass of amphipods; in addition, this sample significantly decreased growth of midge by 10%. The traditional $\sum$AVS – SEM, uncorrected for organic carbon, was the most accurate approach for estimating the effects of metals in the UCR. Treatment of the toxic slag sediment with 20% Resinex SIR-300 metal-chelating resin significantly decreased the toxicity of the sample. Samples $\sum$SEM − AVS > 244 was not toxic to amphipods or midge in laboratory testing, indicating that this value may be an approximate threshold for effects in the UCR. In situ benthic invertebrate colonization studies in an experimental pond (8-week duration) indicated that two of the most metal-contaminated UCR sediments (dominated by high levels of sand-sized slag particles) exhibited decreased invertebrate colonization compared with sand-based reference sediments. Field-exposed SIR-300 resin samples also exhibited decreased invertebrate colonization numbers compared with reference materials, which may indicate behavioral avoidance of this material under field conditions. Multiple lines of evidence (analytical chemistry, laboratory toxicity, and field colonization results), along with findings from previous studies, indicate that high metal concentrations associated with slag-enriched sediments in the UCR are likely to adversely impact the growth and survival of native benthic invertebrate communities. Additional laboratory toxicity testing, refinement of the applications of sediment benchmarks for metal toxicity, and in situ benthic invertebrate studies will assist in better defining the spatial extent, temporal variations, and ecological impacts of metal-contaminated sediments in the UCR system.

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The Upper Columbia River (UCR) is part of a major river system that drains a large area of southern British Columbia, Canada, and northeastern Washington, United States. Grand Coulee Dam, which was completed in 1942, lies approximately 240 km (150 miles) downstream from the Canadian border to form Franklin D. Roosevelt Lake, commonly referred to as "Lake Roosevelt." This large reservoir and its riverine section extend approximately 240 km north of the dam at full pool; riverine conditions prevail in the upper 16 to 25 km below the Canadian border. Annually, reservoir level fluctuations of ≤25 m can occur in response to dam operations and river-management requirements.

Approximately 12 million metric tons of smelter slag, along with effluents, reportedly were discharged to the UCR between approximately 1930 and 1995 from a large smelter facility located in Trail, British Columbia, approximately 16 km north of the United States–Canadian border (Cox et al. 2005; Teck American Incorporated [TAI] 2008). A large percentage of these materials are now found in the UCR as weathered slag dominated by sand-sized and smaller particles that are enriched with several heavy metals, including cadmium (Cd), copper (Cu), zinc (Zn), and lead (Pb) (Johnson et al. 1988, 1990; Johnson 1991; Bortleson et al. 1994).

In 1998, Lake Roosevelt was listed as impaired under Section 303 (d) of the Clean Water Act for several stressors, including metals. In 1999, the Colville Confederate Tribes petitioned the United States Environmental Protection Agency (USEPA) to conduct environmental assessments of Lake Roosevelt and the UCR due to concerns regarding environmental degradation of the river and reservoir. TAI is currently conducting a Remedial Investigation Feasibility Study (RI/FS) in cooperation with the USEPA to assess the environmental and human health risks associated with contaminated water and sediments in Lake Roosevelt and the UCR. Several previous UCR studies demonstrated that metal concentrations in UCR sediments are increased higher than concentrations of ecological concern (e.g., Johnson 1991; Bortleson et al. 2001; USEPA 2006a, b; Paulson and Cox 2007). For example, Besser et al. (2008) demonstrated that slag-enriched UCR sediments decreased survival of amphipods (*Hyalella azteca*) and growth of midge (*Chironomus dilutus*) in laboratory sediment-toxicity tests. These studies imply that metal-contaminated sediments may have adverse effects on benthic invertebrate communities in the UCR.

The goal of the current study was to further evaluate the potential effects of metal-contaminated sediments on benthic invertebrate communities from five sampling stations within Lake Roosevelt and the UCR. The four study objectives were to determine if (1) UCR sediments elicited a toxic response to invertebrates under standardized laboratory test conditions; (2) the application of a metal-chelating resin as a toxicity identification evaluation (TIE) procedure would sequester divalent metal cations and subsequently decrease toxicity under laboratory conditions; (3) benthic invertebrate communities would colonize UCR sediments at equivalent rates compared with reference sediments, manipulated sediments, and artificial substrates; and (4) observed differences in laboratory toxicity studies and in-field sediment colonization rates are due to metal contamination, physical sediment characteristics, or other factors, such as behavioral avoidance.

Methods

Sediment Sampling, Preparation, and Physical Characterization

Relative distances in the UCR in this study are discussed as river miles as opposed to metric units to facilitate comparison with sample locations evaluated in previous studies. Sediments were collected from a 33-mile stretch of the UCR between river miles 705 and 738 (Fig. 1). Eleven different sediments or sample matrices were studied. Samples, locations, and treatment categories are listed in Table 1. The two upstream samples (Dead Man's Eddy 1 [DME1] and Dead Man's Eddy 2 [DME2]) were collected from a perennially riverine portion of the UCR system. The China Bend (CHNB) sample was collected from a portion of the UCR that is transitional between riverine and reservoir conditions depending on seasonal fluctuations in the level of Lake Roosevelt. The two downstream samples (Marcus Flats East [MFE] and Marcus Flats North [MFN]) were collected from a predominantly reservoir-influenced section of the UCR. Sediments were sampled May 28 and June 11, 2008, using a stainless-steel spoon. Semiwet sediment samples were obtained from shallow pits (0- to 30-cm depth) on exposed point bars immediately adjacent to the shore water interface just before inundation due to rapidly increasing river levels fed by snow melt.

Sediments were placed in acid-cleaned 20-L polyethylene buckets and shipped overnight to the United States Geological Survey's (USGS) Columbia Environmental Research Center (CERC), located in Columbia, MO. On arrival at the CERC, the samples were refrigerated at 4°C for 6 weeks before the start of laboratory and field testing. In addition, several laboratory control and reference sediments, low in metals but varying in particle size and organic matter content, were obtained and stored in the dark at 4°C along with the UCR sediments.

Sediments were passed through a 2-mm sieve to separate fine gravel from the sand/silt/clay fraction. The >2-mm size

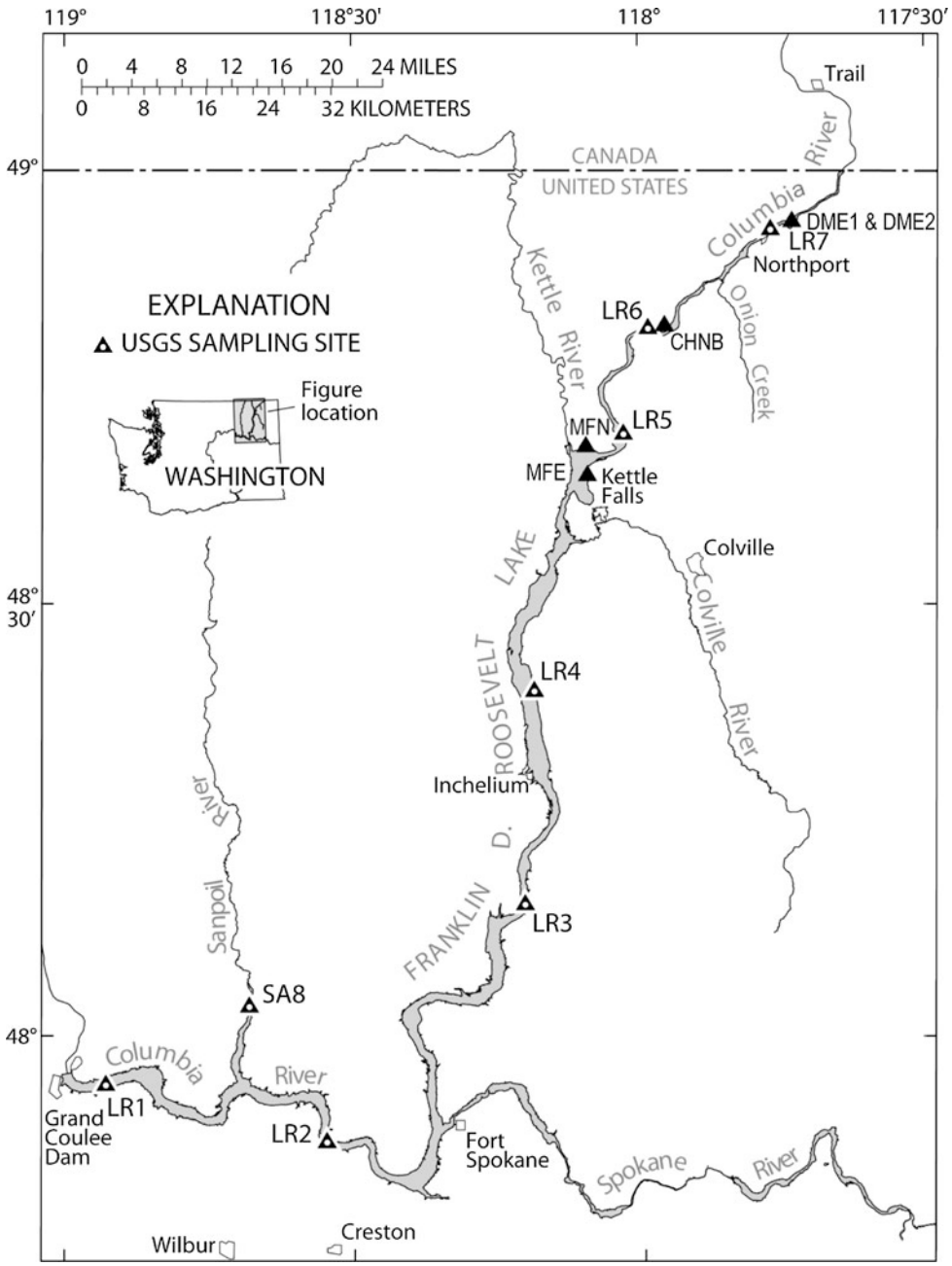

Fig. 1 Location of sampling sites from this study (*solid triangles*) compared with sites studied by Paulson and Cox (2007) and Besser et al. (2008) (*open triangles*)

fraction was dried at 105°C and weighed to determine fine gravels. Particle size of the sand/silt/clay fraction (i.e., sand 0.0625 to 2 mm; silt = 0.004 to 0.0625 mm; and clay <0.004 mm) was determined using the Bouycous hydrometer method (Bouycous 1962). The coefficients of variation (COVs) within size classes were <15% among triplicate samples. Total organic carbon (TOC) was determined using a Coulometrics Model 5020 Carbon Analyzer (Joliet, IL). Accuracy of the TOC analysis, which was defined as the average recovery of 10 sucrose standards, was 97%. Precision, which was defined as the coefficient of variance of duplicate analyses of sediments containing approximately 0.5% organic carbon, was 5%.

A Niton x-ray fluorescence device (Thermo Scientific, Boston, MA) was used to initially classify sediments in terms of their relative metal concentration and potential toxicity compared with literature-based probable effect concentrations (PECs) and other sediment-quality guidelines (MacDonald et al. 2000). DME1 and DME2 sediment samples contained a high percentage of metal-contaminated slag material and were classified as "high" in metals; the CHNB sediment sample was classified as "intermediate" in metal

Table 1 Description of sediment sources (type), sediment codes, sampling locations, and assigned treatment category

Sediment source	Sediment code	River mile	GPS coordinates[a]	Treatment category
Dead Man's Eddy 1 (slag)	DME1	737	446928.53, 5421152.64	High
Dead Man's Eddy 2 (slag)	DME2	737	447046.15, 5421127.52	High
Dead Man's Eddy 2 (slag) with 20% SIR-300 resin)	DME2 + RESIN	737	447046.15, 5421127.52	High manipulated
100% SIR-300 resin	RESIN	NA	NA	Manipulated
China Bend	CHNB	724	431501.25, 540784.24	Intermediate
Marcus Flats East	MFE	706	419976.61, 5389284.88	Low
Marcus Flats North	MFN	708	420347.86, 5392800.24	Low
Commercial River Sand	CSAND	NA	NA	Physical reference
CERC pond sediment	CERC	NA	NA	Reference
Florissant control	FLSNT	NA	NA	Control
Spawntex	SPTX	NA	NA	Artificial substrate

NA not applicable

[a] GPS Coordinate System NAD 83 UTM Zone 11 N (m)

content, with only a trace of visually observable slag; and the MFE and MFN sediment samples were classified as "low" in metals according to published sediment-quality guidelines (MacDonald et al. 2000). Two additional sediment samples were classified as "manipulated": (1) DME2 treated with 20% Resinex SIR-300 resin by volume (DME2 + RESIN) and (2) 100% Resinex SIR-300 resin (RESIN) (Resintech, West Berlin, NJ). Resinex SIR-300 is a cation exchange resin that preferentially sorbs divalent metals in the order of Cu^{+2}>Pb^{+2}>Ni^{+2}>Zn^{+2}>Cd^{+2}>Fe^{+2}>Mn^{+2}>Mg^{+2}>Ca^{+2} and has been proposed for use in toxicity identification procedures (TIEs) for metals in marine and freshwater sediment samples (Burgess et al. 2000; Ho et al. 2007). The resin was prepared by rinsing in deionized water three times (minimal rinse duration 1 minute; 4:1 water-to-resin ratio) followed by refrigeration (≤4°C) for 24 hours in a solution of 33 g/L NaCl in deionized water (4:1 water-to-resin ratio) as recommended by others (Burgess et al. 2000; Ho et al. 2007). After the 24-hour equilibration time, the Resinex SIR-300 resin was rinsed 10 times in deionized water to remove remaining NaCl before mixing and use. Florissant (FLSNT), a silty/clay sediment routinely used in other toxicity tests at CERC (e.g., Kemble et al. 1994; Ingersoll et al. 1998), was used as the toxicity control. Two additional sediments were classified as "reference" sediments (commercial river sand [CSAND] and CERC pond sediment [CERC]). CSAND was used as a clean, quartz-based particle-size reference material for comparison with the DME samples. CERC sediment was used as a second clean silt/clay reference material. In addition, Spawntex (SPTX; Aquatic Ecosystems, Apopka, FL), an aquaculture material (2.5-cm thickness) consisting of a three-dimensional woven coconut fiber, was tested as an "idealized artificial substrate material" (Cairns 1982) in the colonization experiments described later in the text.

Metal Analysis and PECs

Total recoverable metals (TRMs; concentrated nitric acid digestion) were extracted according to Brumbaugh et al. (1994). Simultaneously extracted metals (SEMs; 1-N HCl digestion) were analyzed according to Brumbaugh and Arms (1996) using inductively coupled–plasma mass spectrometry. Acid-volatile sulfides (AVS) were determined using 1-N HCl extraction and a sulfide-specific probe as also described by Brumbaugh and Arms (1996). All units were converted to dry-weight based on moisture determinations in subsamples. SEM and AVS analysis of a National Institute of Standards (NIST) river sediment no. 145 using these procedures resulted in the following recoveries: Cu 55%, Zn 83%, cadmium (Cd) 72%, lead Pb 74%, and AVS 100%. Pre-extraction matrix spike recoveries were as follows: Cu 105%, Zn 110%, Cd 104%, Pb 103%, and AVS 105%. Method detection limits for the ICP-MS analysis were as follows: Cu 0.05 μg/g, Zn 0.5 μg/g, Cd 0.008 μg/g, Pb 0.2 μg/g, and AVS 0.01 μmol/g. Samples were not corrected for recovery. All concentrations are reported as dry-weight concentrations.

Metal concentrations (TRMs and SEMs) were converted to probable-effects quotients (PEQs) by dividing metal concentrations by the PEC (MacDonald et al. 2000; Ingersoll et al. 2001) for each metal. Individual mean PEQs for the five metals were summed ($\sum$PEQ) to estimate potential risks from the metal mixtures. For the purposes of this article, we interpolated a benchmark of $\sum$PEQ = 10 from Fig. 1 of Ingersoll et al. (2001) as the level that would exhibit 100% toxicity to aquatic invertebrates. Equilibrium partitioning sediment benchmarks for toxicity were calculated as $\sum$(SEM − AVS) or $\sum$(SEM − AVS)/f_{oc}) (Di Toro et al. 2005; Hansen et al. 2005).

Laboratory Invertebrate Culture and Toxicity Tests

Amphipods (*H. azteca*) were cultured at 23°C with a luminance of approximately 800 lux using 80-L glass aquaria containing 50 L of CERC well water (hardness 283 mg/L as $CaCO_3$; alkalinity 255 mg/L as $CaCO_3$ [pH 7.8]). Artificial substrates were placed in the amphipod culture aquaria (6 20-cm^2 sections/aquarium of "coiled-web material" [3 M, Saint Paul, MN]). Amphipods used to start the tests (approximately 7 days old) were isolated from laboratory cultures using a sieving sequence that allowed organisms to pass through a no. 35 United States–standard size (500-um opening) and collect on a #40 (425-um opening) sieve placed under water (ASTM 2011). Amphipods were held in 3 L of water with gentle aeration and a small amount of Tetramin and maple leaves for 24 hours before the start of the test. This sieving method resulted in mean amphipod lengths at the start of the exposures of 1.95 ± 0.05 mm (mean ± 1 standard error of the mean, SEM).

Midge (*C. dilutus*) were mass cultured under static conditions in 5.7-L polyethylene cylindrical chambers containing approximately 3 L of water and 25 mL of silica sand as substrate (USEPA 2000; ASTM 2011). Cultures were maintained at a temperature of 25°C and a light intensity of approximately 870 lux. Midge in each culture chamber were fed 5 mL/d of a 100 g/L suspension of fish food flakes (i.e., Tetrafin) or a 5-mL Chlorella suspension (deactivated Algae-Feast Chlorella [Earthrise Farms, Calipatria, CA]) on alternating days. Second-instar midge were obtained by isolating <24-hour-old midge larvae 10 days before starting the test. Ash-free dry weights of midge at the beginning of the study were 0.12 mg/individual based on an average ash-free dry weight of 10 individuals.

Whole-sediment toxicity tests with *H. azteca* were conducted for 28 days, and whole-sediment toxicity tests with *C. dilutus* were conducted for 10 days in well water diluted by 50% with deionized water (hardness 150 mg/L as $CaCO_3$; alkalinity 110 mg/L as $CaCO_3$, and pH 8.1) according to methods described in USEPA (2000) and ASTM (2011). End points measured at the end of the amphipod exposures included 28-day survival, length, and total biomass. End points measured at the end of the midge exposures included 10-day survival, ash-free dry weight/individual, and total biomass.

Test sediments were homogenized in a stainless-steel bowl using a plastic spoon and added to exposure beakers 1 day before test organisms were added (day 1). Amphipods and midge were exposed to 100 ml of sediment with 175 ml of overlying water in 300-ml beakers with a total of four replicates per species/treatment combination. The photoperiod was 16 hours of light to 8 hours of dark at an intensity of approximately 200 lux at the surface of the exposure beakers. Exposure temperatures averaged 23° C. Each beaker received two volume additions/day of overlying water starting on day –1. Diluters cycled every 4 hours (±15 min) with each diluter cycle delivering 50 ml of water to each beaker. Tests were started on day 0 by placing 10 amphipods or 10 midge into each beaker using an eye dropper. Amphipods in each beaker were fed 1.0 ml of yeast-Cerophyll-trout chow (YCT [1.8 g/L]) in a water suspension daily. Midge in each of the beakers were fed 1.5 ml of Tetrafin fish food (6.0 mg dry solids) in a water suspension daily (USEPA 2000; ASTM 2011). Midge and amphipods were isolated at the end of the exposures from each beaker by pouring off most of the overlying water, gently swirling the remaining overlying water and upper layer of sediment, and washing the sediment through a no. 50 United States–standard stainless-steel sieve (300-µm opening). The materials that were retained on the sieve were washed into a glass pan, and the surviving midge and amphipods were removed. Amphipods from each sediment treatment were counted and preserved in 8% sugar formalin for subsequent length measurements (Kemble et al. 1994). Length of amphipods was measured along the dorsal surface from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface. Amphipod length measurements were made using an EPIX imaging system (PIXCI SV4 imaging board and XCAP software; EPIX, Buffalo Grove, IL) connected to a computer and a microscope (Ingersoll et al. 2001). Total biomass of surviving amphipods from each replicate was estimated as the sum of individual amphipod weights calculated from the empirical relationship of Ingersoll et al. (2008) as follows: weight (mg) = $((0.177*\text{length (mm)}) - 0.0292)^3$. Ash-free dry weights of midge were obtained by first drying the surviving animals at 100°C in a drying oven and then recording the weights. The midge were then ashed at 500°C for 24 hours. Ash-free dry weights were determined by subtracting the ashed weight from the total dry weight and dividing by the total number of organisms.

Alkalinity, hardness, pH, specific conductivity, dissolved oxygen, and total ammonia were measured in overlying test water on day 0 (the day the animals were stocked) and at the end of the tests. Conductivity and dissolved oxygen in overlying water were also measured weekly. Temperatures in the water baths holding the exposure beakers were monitored daily and averaged 23°C ± 0°C. Methods used to characterize overlying water quality in the whole-sediment tests are described in Kemble et al. (1994).

In Situ Invertebrate Colonization Tests

In situ sediment-colonization tests were conducted in an outdoor experimental pond (earthen bottom; 0.1 ha, 1.5-m maximum depth) located at the CERC (Boyle et al. 1985, 1996; Fairchild et al. 1992; Ingersoll et al. 2005). A total of

11 benthic treatments (sediment sources/types) were evaluated (Table 1).

Sediments were wetted with 50% well water (CERC well water diluted 50% with deionized water) and then homogenized in glass jars placed on mechanized rollers (20 revolutions/min for 30 minutes). Sediments were then transferred to individual polyethylene trays (0.36-L volume; 15-cm length; 12-cm width; 3-cm depth) and frozen (lids attached) for 48 hours at –20°C to kill indigenous invertebrates. A total of 10 replicate trays were tested for each treatment. Before the start of the field study, the water level in the pond was drawn down to expose the pond sediments (i.e., the same CERC sediment used in toxicity testing and colonization studies) and allow the in situ placement of sediment-colonization trays. Macrophytes were removed using a rake to minimize any physical habitat bias. Sediments were thawed and placed by hand on the pond bottom level with the sediment surface in a completely randomized 10-block experimental design (Electronic supplementary material Fig. S1). The pond was then reflooded using well water to a depth of approximately 1 m. The colonization area was protected from intruding wildlife (e.g., turtles, muskrats, and geese) using plastic construction fencing (1.2-m height, 4-cm mesh) extending from the sediment surface to 20 cm above the water level. Subsequent plant growth was minimized using overhead shade material (90% light decrease) mounted on a wooden frame placed 2 m above the pond surface. Additional CERC well water was added to the pond on September 19, 2008, to replace water lost due to evaporation. Dissolved oxygen, temperature, pH, and conductivity were sampled weekly at the surface and bottom of the pond using a YSI hand-held unit (YSI, Yellow Springs, OH). In addition, weekly 1-L grab samples were transported to the laboratory and analyzed for alkalinity and hardness using the same methods as for toxicity tests.

After the 8-week colonization period, the water level in the pond was drawn down to a depth of approximately 0.5 m during a 48-hour period to facilitate retrieval of colonization trays using a small rubber raft. An individual wearing a mask and snorkel retrieved individual colonization trays by hand while leaning over the edge of the raft to minimize sediment disturbance. On retrieval, the colonized trays and associated invertebrates were chilled on ice and then preserved in a 0.5-L jar filled with 90% ethanol. Samples were rinsed 24 hours later and refilled with 90% ethanol and stored at room temperature until enumeration and identification.

Invertebrates in each sample were stained using Rose-Bengal solution; isolated using tweezers; identified to major taxonomic group (class, order, family, or subfamily) according to Pennak (1978); and counted using a dissecting microscope. All individual invertebrates were counted from each sample tray (i.e., no subsampling).

Statistical Analysis

All statistical analyses were conducted using SAS version 9.2 (SAS 2008). Data were tested for normality using the Shapiro-Wilk's statistic. Neither laboratory nor benthic-colonization data were normally distributed. Therefore, statistical tests among treatments were conducted using ranked data (Snedecor and Cochran 1982). Laboratory data (invertebrate survival and growth data) and benthic community–colonization data (total numbers and numbers within major groups) were tested using one-way analysis of variance (ANOVA) followed by means discrimination using Dunnett's test (laboratory toxicity data) and the Duncan's multiple range test (benthic community data). Principal component analysis (PCA), using the untransformed raw data, was used to examine differences among sediment treatments based on physical and chemical characteristics (SAS 2005). Cluster analysis, using untransformed (raw) benthic invertebrate data, was used to compare the relative distribution of major taxonomic groups among treatments (SAS 2005). All significance levels were tested at $p \leq 0.05$.

Results

Metal Chemistry

Total recoverable metal concentrations of sediments, along with PEC and PEQ guidelines published by MacDonald et al. (2000) and Ingersoll et al. (2001), are listed in Table 2. TRMs were operationally defined by the extraction technique as a general reflection of metal content but do not represent total extractable metals used in mineral characterization. Sediments from DME2 contained the highest concentrations of TRMs with a $\sum PEQ_{Total} = 132$ due to increased concentrations of Cu (2870 μg/g), Pb (266 μg/g), and Zn (20,100 μg/g); however, concentrations of Cd were low (1.00 μg/g). DME1 sediment contained lower concentrations of TRMs ($\sum PEQ_{TRM} = 67$), primarily due to 39% lower levels of copper (1750 μg/g) compared with DME2. However, DME1 contained similar concentrations of TRMs for Cd (1.00 μg/g), Pb (313 μg/g), and Zn (19,900 μg/g) compared with DME2. CHNB sediment was moderately contaminated with TRMs (Cd 4.09, Cu 269, Pb 275, and Zn 3100 μg/g) and contained a $\sum PEQ_{TRM} = 11$. Calculated $\sum PEQ_{TRM}$ for DME2, DME1, and CHNB exceeded our threshold for toxicity (10) by factors of 13×, 7×, and 1×, respectively. The remaining sediment samples from MFE, MFN, FLSNT, and CERC were low in metals and contained a $\sum PEQ_{TRM} < 1$ (Table 2).

Concentrations of the molar sum of simultaneously extracted metals ($\sum SEM$) were highest in DME2

Table 2 TRMs, PECs, and PEQs for metal-contaminated sediments

Sediment source and PECs	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	Zn (μg/g)	$\sum PEQ_{TRM}$
DME1	1 (0×)[a]	1750 (12×)	313 (2×)	19,900 (43×)	67
DME2	1 (0×)	2870 (19×)	266 (2×)	20,100 (44×)	132
CHNB	4 (1×)	269 (2×)	275 (2×)	3,100 (6×)	11
MFE	1 (0×)	17 (0×)	19 (0×)	92 (0×)	<1
MFN	1 (0×)	10 (0×)	28 (0×)	121 (0×)	<1
CSAND	ND	1 (0×)	2 (0×)	7 (0×)	<1
CERC	ND	10 (0×)	12 (0×)	40 (0×)	<1
FLSNT	ND	11 (0×)	14 (0×)	43 (0×)	<1
Guidelines (PECs)[b]	5	149	128	459	10

ND, not detected

[a] Individual metal PEQs are shown in parentheses

[b] Guidelines for PECs for freshwater invertebrates are from MacDonald et al. (2000). Note that the guideline for $\sum PEQ_{TRM}$ was interpolated from Fig. 1 of Ingersoll et al. (2001)

Table 3 SEMs, PECs, and PEQs for metal-contaminated sediments

Sediment source and PECs	Cd (μg/g)	Cu (μg/g)	Pb (μg/g)	Zn (μg/g)	$\sum PEQ_{SEM}$
DME1	1 (0×)[a]	8 (0×)	324 (2×)	18,560 (40×)	42
DME2	0 (0×)	1,160 (8×)	295 (2×)	20,160 (44×)	54
CHNB	3 (1×)	138 (1×)	301 (2×)	2480 (5×)	8
MFE	1 (0×)	9 (0×)	12 (0×)	23 (0×)	1
MFN	1 (0×)	10 (0×)	32 (0×)	58 (0×)	1
CERC	nd	4 (0×)	12 (0×)	13 (0×)	<1
FLSNT	nd	4 (0×)	7 (0×)	7 (0×)	<1
Guidelines (PECs)[b]	5	149	128	459	10

ND, not detected

[a] Individual metal PEQs are in parentheses

[b] Guidelines for PECs for freshwater invertebrates are from MacDonald et al. (2000). Note that the guideline for $\sum PEQ_{SEM}$ was interpolated from Fig. 1 of Ingersoll et al. (2001)

($\sum PEQ_{SEM} = 54$), DME1 ($\sum PEQ_{SEM} = 42$), and CHNB ($\sum PEQ_{SEM} = 8$) (Table 3). Measured SEM values exhibited similar trends as the bulk metal measurements with the exception of Cu, which was lower in DME1 (8 μg/g) compared with DME2 (1160 μg/g).

AVS were low and ranged from minimally detectable to 42.3 μmol/g in DME1 (Table 4). Organic carbon concentrations, also known to affect the bioavailability of metals, ranged from 0.01% (DME1 and DME2) to 2.31% (MFE). The $\sum SEM - AVS$ data indicated that sites DME2 and DME 1 exceeded a threshold level of probable effects (120 umol/g) by a factor of 3× (323 umol/g) and 2× (244 umol/g), respectively. The $\sum SEM - AVS/_{foc}$ data indicated that DME2 and DME1 exceeded a threshold level of probable effects (3000 $\mu mol/g_{oc}$) by a factor of 22×(64,600 $\mu mol/g_{oc}$) and 16×(48,800 $\mu mol/g_{oc}$), respectively.

Physical and Chemical Characteristics of Sediments

Physical properties of the sediments are listed in Table 5. DME1, DME2, and CSAND (particle size reference material) consisted of 100% sand and were low in organic carbon (<0.09%). The remaining sediments (CHNB, MFE, MFN, CERC, and FLSNT) averaged 63% sand, 24% silt, and 8% clay and ranged from 0.88% to 2.31% organic carbon. PCA was used to analyze and graphically compare the physical properties of sediments based on Euclidean distances among sites (Fig. 2). PCA axis 1 (PCA1) explained 74% of the total variance among physical properties of samples due to relative levels of sand, clay, and silt content. PCA axis 2 (PCA2) explained an additional 25% of the variance (i.e., 98% total cumulative variance) due to particle size >2 mm and organic carbon. The CSAND particle-size reference treatment loaded directly over DME1and DME2 on PCA1,

Table 4 Concentrations of simultaneously extracted metals (∑SEM), acid-volatile sulfides (AVS), total organic carbon (TOC), and ∑SEM − AVS/$_{foc}$) for sediments

Sediment source	Cd	Cu	Pb	Zn	∑SEM	AVS	SEM − AVS	TOC (%)	∑SEM − AVS/$^{a}_{foc}$
DME1	ND	0	2	284	286	42	244	0.01[a]	48,800
DME2	ND	18	2	308	329	6	323	0.02[a]	64,600
CHNB	ND	2	2	38	42	7	35	1.20	2917
MFE	ND	ND	ND	ND	1	<1	0	2.31	43
MFN	ND	ND	ND	1	1	<1	0	0.88	114
CERC	ND	ND	ND	ND	<1	13	–13	1.82	<1
FLSNT	ND	ND	ND	ND	<1	<1	0	0.95	<1
Guideline[b]	NA	NA	NA	NA	10	NA	120	NA	3000

All units except TOC are expressed as umol/g

ND not detected, *NA* not applicable

[a] ∑(SEM − AVS)/$_{foc}$) is expressed as the fractional concentration of TOC in the sediment. A minimum default value of 0.50% for TOC used for DME samples as suggested by Robert Santore (HydroQual Environmental Engineers and Scientists, East Syracuse, New York, personal communication, February 2011)

[b] Guidelines for upper 90% values of observed toxicity interpolated from Fig. 1 in Ingersoll et al. (2001) or published by DiToro et al. (2005) and Hansen et al. (2005)

Table 5 Physical characteristics of benthic colonization and toxicity study sediments

Sediment source	TOC (%)	Particle size distributions			
		>2 mm (%)	Sand (%)	Silt (%)	Clay (%)
DME1	0.01	0.1	100	0	0
DME2	0.02	0.0	100	0	0
CHNB	1.20	6.9	72	18	4
MFE	2.31	7.7	52	28	11
MFN	0.88	0.9	66	26	8
CSAND	0.09	0.1	100	0	0
CERC	1.82	4.3	9	62	25
FLSNT	0.95	0.1	9	71	20

which indicates that it would serve as a good physical reference material for invertebrate colonization experiments. The CHNB and MFE samples loaded on the upper portion of PCA2 due to particle size >2 mm and higher TOC. The other reference and control sediments loaded at separate points due to relative differences in TOC, silt, and clay.

When SEM metals (Cd, Cu, Pb, and Zn) were added to the PCA analysis in addition to TOC and sediment particle size fractions, PCA1 explained 63% of cumulative variance to the model, with Pb, Zn, and sand contributing the most discrimination among sites (Fig. 3). PCA2 explained an additional 16% of variance (i.e., 79% total cumulative variance) among sites, with sediment >2 mm and total organic carbon being the two primary discriminating variables. The two slag-based sediments (DME1 and DME2) grouped together on the right of PCA1 due to high levels of metals and sand but low TOC. The CSAND reference treatment plotted left of the DME treatments due to low concentrations of metals and TOC; this again indicated that CSAND should serve as a useful colonization reference material for comparison with the DME1 and DME2 treatments containing high metals. The CHNB and MFE treatments loaded high on PCA2 and separate from the other sites due to moderate levels of metals, sand, and TOC. The remaining sites (MFN, CERC, and FLSNT) loaded lower on PCA1 and PCA2 due to variations in texture and TOC similar to that observed in Fig. 2.

Laboratory Toxicity Testing

Survival and growth of amphipods and midge in the control achieved test acceptability requirements outlined in USEPA (2000) and ASTM (2011). Water quality during the laboratory toxicity tests was within acceptable limits as described by ASTM (2011) and USEPA (2000) (Electronic supplementary material Tables S1, S2).

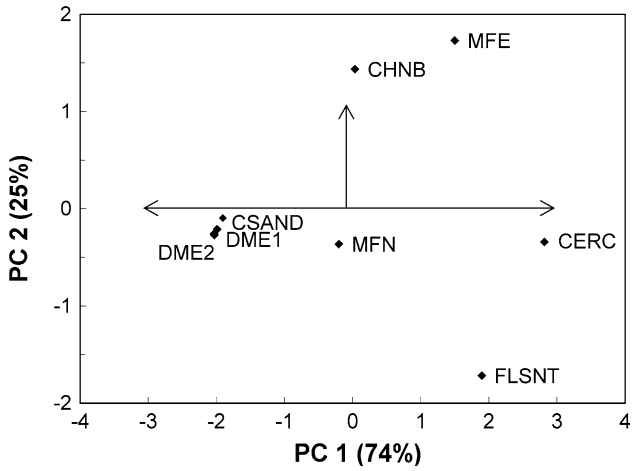

Fig. 2 PCA of sediments tested based on particle size (>2 mm, sand, silt, and clay) measurements and TOC

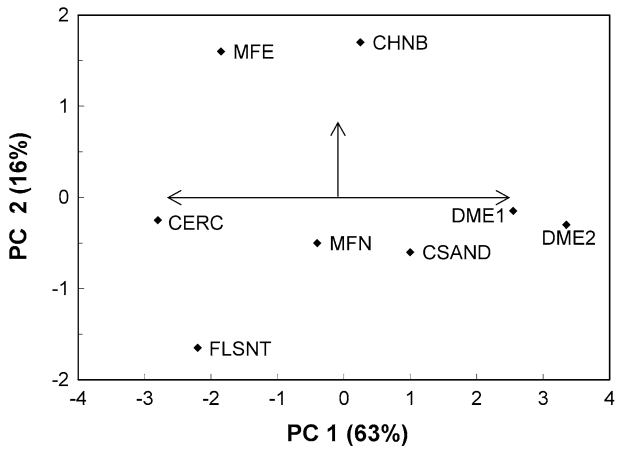

Fig. 3 PCA of sediments based on SEM metals (Cd, Cu, Zn, and Pb) in addition to TOC and particle size (>2 mm, sand, silt, and clay) measurements

H. azteca chronic toxicity testing showed that sediments from DME2 significantly decreased survival (77%), length (39%), and biomass (94%) compared with the FLSNT control sediment (Table 6). The addition of 20% SIR-300 resin to the DME2 sediment significantly increased survival, length, and biomass of amphipods compared with DME2 and control sediment. Survival of amphipods in the DME2 and DME2 + RESIN treatments were similar to that of the FLSNT control; however, length and biomass were significantly decreased. No toxicity was observed in any other sediment treatment for *H. azteca*.

No significant effects of metal-contaminated sediments were observed on survival of *C. dilutus* (Table 7). However, weight and biomass of midge were significantly decreased (24% and 30%, respectively) in DME2 sediment compared with FLSNT control sediment. Significant mortality and biomass decrease of midge occurred in the DME2 + RESIN and the RESIN treatments compared with FLSNT control sediment due to incomplete rinsing of NaCl from the resin during preparation before testing as measured by increased conductivity, alkalinity, and pH as well as decreased hardness (Electronic supplementary material Table S2).

Table 6 Mean response of the amphipod *H. azteca* in the 28-day whole-sediment laboratory toxicity test

Sediment source	Survival (%)	Length (mm/individual)	Biomass (mg)
DME1	98 (2.50)	3.74 (0.08)	2.77 (0.08)
DME2	20 (5.77)*	2.50 (0.19)*	0.18 (0.06)*
DME2 + RESIN	93 (4.79)	3.57 (0.09)*	2.15 (0.09)*
RESIN	73 (4.79)	3.17 (0.13)*	1.34 (0.27)*
CHNB	100 (0.00)	3.85 (0.07)	2.86 (0.17)
MFE	98 (2.50)	3.83 (0.05)	2.83 (0.22)
MFN	100 (0.00)	3.88 (0.06)	2.94 (0.11)
CERC	88 (12.50)	4.47 (0.09)	4.04 (0.33)
FLSNT	88 (2.50)	4.08 (0.07)	3.13 (0.11)

Means ($n = 4$ replicate beakers; + 1 SEM in parentheses)

* Significantly decreased relative to FLSNT control response ($p < 0.05$)

Invertebrate Field Colonization of Sediments

Water-quality conditions were similar to those measured in other pond studies conducted at the CERC (Boyle et al. 1985, 1996; Fairchild et al. 1992; Ingersoll et al. 2005) (Electronic supplementary material Table S3). Although the pond was originally filled with CERC well water, the water quality in general was higher in pH and lower in alkalinity and hardness compared with CERC well water due to precipitation of carbonates as carbon dioxide diffused from the water. Water-quality characteristics of the pond were similar across dates except on September 19, 2008, when CERC well water was added to the pond to replace water that had evaporated, which led to a temporary increase in alkalinity and hardness.

A total of 9316 benthic organisms were collected during the study and were divided among 14 major taxonomic groups (Electronic supplementary material Tables S4–S14). The benthic community was dominated by Ceratopogonidae (biting midges) and Chironomidae (nonbiting midges). These two groups accounted for approximately 88% of all benthic organisms identified. One-way ANOVA of ranked data showed that invertebrate community structure was significantly different among treatments for total numbers of invertebrates and numbers of Ceratopogonidae and Chironomidae (Table 8).

Total numbers of invertebrates were significantly greater in the SPTX and MFN treatments compared with all other

Table 7 Response of the midge *C. dilutus* in the 10-day laboratory sediment-toxicity test

Sediment source	Survival (%)	AFDW (mg/individual)	Biomass (mg)
DME1	98 (2.50)	0.55 (0.02)	5.39 (0.17)
DME2	95 (2.89)	0.43 (0.02)*	4.04 (0.26)*
DME2 + RESIN	60 (10.80)*	0.89 (0.13)	5.32 (0.10)*
RESIN	35 (6.45)*	0.21 (0.02)*	0.73 (0.18)*
CHNB	100 (0.00)	0.48 (0.09)	4.80 (0.26)
MFE	100 (0.00)	0.59 (0.02)	5.91 (0.24)
MFN	98 (2.50)	0.88 (0.04)	8.85 (0.41)
CERC	100 (0.00)	0.90 (0.01)	8.90 (0.13)
FLSNT	98 (2.50)	0.60 (0.02)	5.81 (0.20)

* Significantly decreased relative to the FLSNT control response ($p \leq 0.05$)

Means ($n = 4$ replicate beakers; + 1 SEM in parentheses)

sediments (Table 9). Significantly lower numbers of total invertebrates were found in the DME1, DME2, DME2 + RESIN, and RESIN treatments compared with the SPTX, MFN, and CSAND treatments. Total numbers of invertebrates were similar in the CSAND, FLSNT, MFE, CHNB, and CERC treatments.

Total numbers of Ceratopogonidae were significantly different across sediment treatments (Table 10). The total number of Ceratopogonidae colonizing sediments from MFN, CERC, and FLSNT sediments was significantly greater than the colonization counts for the DME1, DME2, SPTX, DME2 + RESIN, and RESIN treatments. The CERC, FLSNT, MFE, CSAND, and CHNB sediments contained similar numbers of total Ceratopogonidae.

Table 8 Average total numbers and major taxa of invertebrates found during the colonization study

Sediment source or treatment	Total[a]	Ceratopogonidae	Chironomidae
DME1	63 (10)	27 (7)	17 (3)
DME2	70 (12)	22 (5)	30 (6)
DME2 + RESIN	58 (17)	19 (3)	24 (3)
RESIN	80 (10)	20 (6)	42 (9)
CHNB	89 (13)	39 (11)	28 (3)
MFE	93 (14)	53 (12)	24 (3)
MFN	126 (18)	76 (13)	35 (4)
CSAND	108 (15)	53 (15)	33 (6)
CERC	83 (11)	54 (9)	20 (3)
FLSNT	111 (25)	70 (25)	25 (8)
SPTX	158 (7)	21 (5)	93 (8)

[a] Total is greater than the sum of the two groups due to low numbers of other taxa not listed in the table. For complete list of major taxa for each treatment, refer to the Electronic supplementary material. Each number represents mean (±SEM; $n = 10$ trays/sediment type)

Table 9 Results of Duncan's multiple range test on ranks of total number of invertebrates colonizing sediment treatments in CERC

Sediment source or treatment	Mean rank	Duncan's grouping		
SPTX	97	a		
MFN	75	a		
CSAND	67	b		
FLSNT	61	b	c	
MFE	55	b	c	d
CHNB	53	b	c	d
CERC	52	b	c	d
RESIN	49		c	d
DME2	37		c	d
DME1	34		c	d
DME2 + RESIN	29			d

Ranks range from 0 to 110. Means with the *same letter* are not significantly different ($p \leq 0.05$)

Total numbers of Chironomidae were significantly different among treatments ($p \leq 0.0001$) (Table 11). However, statistical significance among treatments did not correspond to measured concentrations of metals, such as that observed with total numbers of invertebrates (Table 9) and total numbers of Ceratopogonidae (Table 10).) Significantly greater numbers of total Chironomidae were found in the SPTX artificial substrate; however, no clear trends were found among reference and UCR samples.

Cluster analysis was used to differentiate samples in terms of relative numbers of total invertebrates and numbers of Ceratopogonidae and Chironomidae. Results indicated that there were two main clusters that separated SPTX from the other sediment treatments by a Euclidean distance of 0.80 (Fig. 4). Two secondary clusters were observed at a distance of 0.4 from each other and consisted of DME1, DME2, DME2+RESIN, and RESIN (far left) and CHNB, MFE, CERC, CSAND, MFN, and FLSNT (middle). Smaller dual clusters were observed at a distance of <0.25 for sediments from MFE/CERC, MFN/FLSNT, and DME2/DME2 + RESIN.

Discussion

The potential effects of metals in sediments are commonly evaluated using sediment-quality guidelines, such as individual PEC or ∑PEQ benchmarks derived from meta-data analysis of toxicity data of mixtures of contaminants (MacDonald et al. 2000; Ingersoll et al. 2001). These PECs were developed as concentrations above which toxicity is likely to be observed (MacDonald et al. 2000). Since these guidelines were established, additional studies using metal-spiked sediments have been conducted to determine concentrations

Table 10 Results of Duncan's multiple range test on ranks of total number of Ceratopogonidae colonizing sediment treatments in CERC

Sediment source or treatment	Mean rank	Duncan's grouping				
MFN	85	a				
CERC	75	a	b			
FLSNT	74	a	b			
MFE	68	a	b	c		
CSAND	66	a	b	c	d	
CHNB	53		b	c	d	e
DME1	44			c	d	e
DME2	40				d	e
SPTX	36					e
DME2 + RESIN	36					e
RESIN	33					e

Ranks range from 0 to 110. Means with the *same letter* are not significantly different ($p \leq 0.05$)

Table 11 Results of ANOVA and Duncan's multiple range test on ranks of total number of Chironomidae colonizing sediment treatments in CERC

Treatment	Mean rank	Duncan's grouping			
SPTX	102	a			
MFN	68		b		
RESIN	67		b		
CSAND	61		b	c	
CHNB	57		b	c	
DME2	55		b	c	d
MFE	47		b	c	d
DME2 + RESIN	46			c	d
FLSNT	38			c	d
CERC	38			c	d
DME1	29				d

Ranks range from 0 to 110. Means with the *same letter* are not significantly different ($p \leq .05$)

that are known to have effects. These known-effect levels consist of the upper 90% CIs of observed toxicity distributions, including $\sum$SEM − AVS > 120 and $\sum$SEM − AVS/$_{foc}$ > 3,000 umol/g (DiToro et al. 2005; Hansen et al. 2005). For comparable comparisons of $\sum$PEQs to the statistical criteria of DiToro et al. (2005) and Hansen et al. (2005), we applied a benchmark of 10. Our results indicate that the $\sum$SEM − AVS was reasonably accurate in predicting toxicity of UCR sediments (factor of 2 to 3); in contrast, the $\sum$PEQ and the $\sum$SEM − AVS/$_{foc}$ overpredicted toxicity by factors of 5× and 20×, respectively. The $\sum$SEM − AVS/$_{foc}$ was developed as a benchmark for toxicity for sediments containing >0.50% TOC, which is why we used this TOC level as the default value for the two DME sites. It is notable that use of the actual measured TOC levels would have increased the error of the $\sum$SEM − AVS/$_{foc}$ by another order of magnitude, which indicates that this benchmark may not be useful for evaluating sediments with low TOC values in the upper UCR.

Laboratory toxicity testing in this study showed that DME2 was the most contaminated sediment ($\sum$PEQ$_{SEM}$ = 54; $\sum$SEM − AVS = 329) and significantly decreased survival and growth of amphipods and also decreased growth of midge. Site DME1 was not toxic despite high levels of SEM-extracted metals ($\sum$PEQ$_{SEM}$ = 42; $\sum$SEM − AVS = 286). Toxicity of sediment from DME2 was significantly decreased after the addition of 20% SIR-300 resin as predicted; this indicates that metals were the primary factor causing decreased survival, length, and biomass of amphipods in the DME2 sediment. The use of the SIR-300 resin with midge in this study was not successful in "removing toxicity" from the DME2 sediment due to the residual effects of NaCl after rinsing and preparation of sediment. *Chironomus* sp. are much less euryhaline (i.e., salt tolerant) than *H. azteca* (Ingersoll et al. 1995), and the water-quality data reflected this sensitivity. Investigators using SIR-300 resin as a TIE treatment should be aware of the sensitivity of some freshwater organisms to NaCl and take additional precautions in rinsing the resins (validated by water-quality analyses) before testing (Ho et al. 2007).

Besser et al. (2008) examined the laboratory toxicity of sediments from Lake Roosevelt in relation to concentrations and bioavailability of metals. Exposure of invertebrates to samples from the riverine location of LR7 (river mile 735; near our sites DME1 and DME2) decreased survival (16%) of *H. azteca* and decreased growth (79%) of *C. dilutus* compared with their FLSNT control. Toxicity at site LR7 was attributed to high concentrations of TRMs (Cu 2800 ug/g, Zn 26,000 ug/g, Cd 4 ug/g; and Pb 1110 ug/g; $\sum$PEQ$_{TRM}$ = 85) and SEMs (Cu 99 ug/g, Zn 16,000 ug/g, Cd 1 ug/g, and Pb 590 ug/g; $\sum$PEQ$_{SEM}$ = 40) and $\sum$SEM − AVS = 260. Metal concentrations and toxicity decreased greatly downstream of site LR7 (river mile 735) as the hydrologic conditions became more lacustrine (i.e., reservoir-influenced) and sediments became more fine-grained with lower concentrations of metals.

The TRMs measured by Besser et al. (2008) were similar to those at DME1 and DME2 in our study because they came from the same sample location. However, the SEMs, considering the bioavailable fraction of metals, differed between studies in relative levels of Cu and Zn among sites DME1, DME2, and LR7. Cu appeared to be the primary metal contributing to toxicity at site DME2 because levels of Zn were similar in the two DME samples. In contrast, the toxicity observed by Besser et al. (2008) at site LR7 appeared to be driven by high Zn concentrations because Cu was low. Differences in the relative bioavailability of Cu and

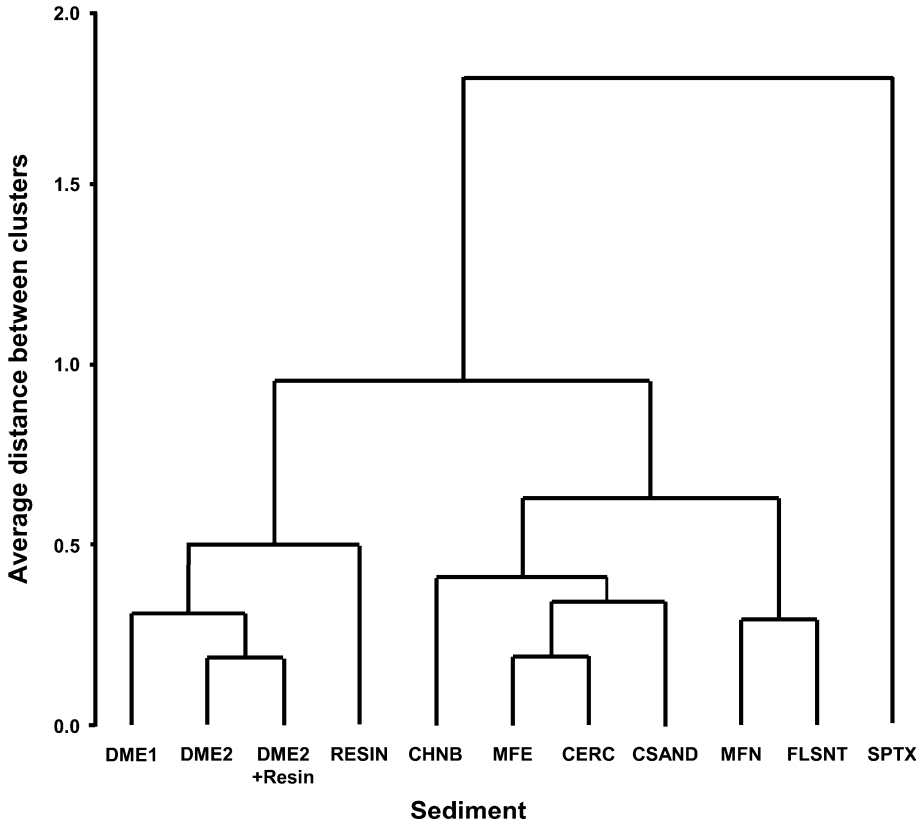

Fig. 4 Results of multivariate cluster analysis showing discrimination of major taxonomic groups among sediment treatments (Table 1). Note that there were three distinct clusters: far right = SPTX; middle = CHBND, MFE, CERC, CSAND, MFN, and FLSNT; and left = slag-based sediments DME1, DME2, and resin-based samples

Zn in these two studies was likely due to differences in sediment-sampling methods, handling, redox conditions, and subsequent chemical states of iron and manganese, which are known to control bioavailability and toxicity of metals in UCR sediments (Luoma1983; Paulson et al. 2006; Besser et al. 2008). We sampled sediments at the shore water interface to visually select sediments that were most reflective of the area of particular concern. In contrast, Besser et al. (2008) sampled sediments at minimum water depths of 12 m in areas that had been continuously flooded for 2 years to minimize the effects of reservoir dry-down on redox conditions. Thus, relative differences in the role of Cu and Zn in these two studies are understandable.

Collectively, these two studies provide strong support of the application of $\sum$SEM − AVS as a measure of the bioavailability and toxicity of metals in the UCR because these values were within a factor of 2 to 3 of benchmark toxicity values. A $\sum$SEM − AVS value of 293 could serve as a threshold for estimates of levels of concern for the upper UCR. The application of the $\sum$SEM − AVS/$_{foc}$ approach of Hansen et al. (2005) is not supported for the UCR system because it overestimates probable levels of concern by a factor of 20 due to inherent problems in normalizing $\sum$SEM − AVS to low concentrations of TOC in sediment. Therefore, additional studies of the range of oxidation conditions, TOCs, sulfide concentrations, and metal concentrations in the UCR are needed to more fully assess the degree to which these factors affect the actual bioavailability, toxicity, and ecological risks of Cu and Zn to sediment-dwelling invertebrates. Such data are forthcoming (Don MacDonald, MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia, personal communication, January 2012).

Invertebrate colonization differed significantly among UCR sediments, reference sediments, and the Spawntex artificial substrate material. In general, colonization rates were higher in the CSAND and SPTX artificial substrate compared with the slag-enriched sediments. However, results were not always similar across major taxonomic groups and treatments. Observed invertebrate colonization results were likely influenced by the experimental design (initial draw-down followed by 8 weeks of colonization), which resulted in a dominance of multivoltine insects, which reproduce by way of aerial egg deposition over water. Theoretically, aerial egg deposition should have been uniform across our experimental system due to the completely randomized block design, but certain factors, such as wind, lighting, or air temperature could have affected the results. Our taxonomic resolution, given the major groups present, appeared to be sufficient to distinguish differences in invertebrate colonization within the data set. It is unclear why colonization numbers of

Ceratopogonidae were so low in the SPTX treatments given the low concentrations of metals. Ceratopogonidae are known to prefer algal mats as habitats (Pennak 1978). The SPTX treatment may not have supported this preferred habitat compared with the other treatments. Alternatively, differential predation may have been a significant factor controlling Ceratopogonid numbers in the SPTX treatment compared with the other treatments; however, predators were not present in the actual samples when retrieved.

Although the univariate analysis of the invertebrate data was not always clear with regard to colonization patterns, cluster analysis clearly discriminated among invertebrate communities among treatments in this study. The slag-enriched DME1 and DME2 samples grouped as a separate cluster from all other groups, including the SPTX treatments and other sediments, which implies that metals were the primary factor in decreased colonization of sediments. However, the DME2 + RESIN and RESIN treatments were also colonized similarly to DME1 and DME2 treatments, suggesting that sediment color or low TOC could have been factors in the observed results. Although the CSAND treatment was similar in particle size and TOC, the sand color was much lighter than the DME1 and DME2 treatments, and it exhibited increased invertebrate colonization compared with the slag-dominated samples. Additional studies are necessary to determine if aquatic invertebrates are capable of avoiding highly contaminated slag sediments in the UCR.

The increased colonization rates in the Spawntex material compared with both reference and UCR sediments correspond to community ecology theory, which predicts that aquatic invertebrate taxa richness should be higher in benthic habitats with greater habitat complexity (i.e., expanded niche space due to increased substrate heterogeneity, surface area, and interstitial space) (Minshall 1984; Schmude et al. 1998). This may imply that Spawntex, uncontaminated sand, or other materials could be used in adaptive-management or restoration efforts in the UCR to increase taxonomic richness and biomass of aquatic invertebrates (Pratt 1994; Miller 2002). Johnson (1991) reported that the invertebrate community numbers and richness in Lake Roosevelt were highest in sand/gravel habitats compared with lacustrine silt/clay habitats; however, sampling was only conducted in habitats below river mile 728 (Little Dalles) where metal concentrations are much lower than those examined in this study. Therefore, further studies of macroinvertebrate communities are needed in the UCR to determine the relative effects of metals and physical habitat conditions on invertebrate communities because colonization of contaminated and uncontaminated substrates is known to be highly complex from an ecotoxicological perspective (Courtney and Clements 2002).

Conclusion

Results from standard laboratory toxicity tests with *H. azteca* or *C. dilutus* indicated that metal-contaminated sediment from site DME2 was toxic under laboratory conditions due to high levels of Cu compared with metal-contaminated sediments from DME1 and CHNB. Treatment of the DME2 sediments with SIR-300 resin decreased metal toxicity to amphipods under laboratory conditions. The $\sum$SEM − AVS approach was the best indicator of probable effects in the UCR whereas the $\sum$SEM − AVS/$_{foc}$ approach was inaccurate due to low levels of TOC. Benthic invertebrate colonization rates in experimental ponds were in accordance with the observed laboratory toxicity of the most heavily contaminated sample (DME2). However, invertebrate colonization was also decreased in the DME1 and resin treatments, perhaps due to avoidance. The results of this study, which considered multiple lines of evidence (analytical chemistry, laboratory toxicity, and experimental field-colonization data), indicate that metals associated with slag-enriched sediments have the potential to adversely impact benthic invertebrate communities in the UCR. Additional laboratory and field studies are needed to better define the spatial and temporal variations in metals concentrations, substrate conditions, and benthic invertebrate community dynamics in the UCR.

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