2009

Ecological impacts of lead mining on Ozark streams: Toxicity of sediment and pore water

John M. Besser
US Geological Survey, Columbia Environmental Research Center

William G. Brumbaugh
US Geological Survey, Columbia Environmental Research Center

Ann L. Allert
US Geological Survey, Columbia Environmental Research Center

Barry C. Poulton
US Geological Survey, Columbia Environmental Research Center

Christopher J. Schmitt
US Geological Survey, Columbia Environmental Research Center, cjschmitt@usgs.gov

See next page for additional authors

Authors
John M. Besser, William G. Brumbaugh, Ann L. Allert, Barry C. Poulton, Christopher J. Schmitt, and Christopher G. Ingersoll

Ecotoxicology and Environmental Safety 72 (2009) 516–526

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Ecological impacts of lead mining on Ozark streams: Toxicity of sediment and pore water

John M. Besser *, William G. Brumbaugh, Ann L. Allert, Barry C. Poulton, Christopher J. Schmitt, Christopher G. Ingersoll

US Geological Survey, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, MO 65201, USA

ARTICLE INFO

Article history:
Received 7 April 2008
Received in revised form
19 May 2008
Accepted 23 May 2008
Available online 7 July 2008

Keywords:
Sediment toxicity
Metals
Mining
Amphipod
Pore water
Sediment quality guidelines
Lead
Nickel
Zinc
Cadmium

ABSTRACT

We studied the toxicity of sediments downstream of lead–zinc mining areas in southeast Missouri, using chronic sediment toxicity tests with the amphipod, *Hyalella azteca*, and pore-water toxicity tests with the daphnid, *Ceriodaphnia dubia*. Tests conducted in 2002 documented reduced survival of amphipods in stream sediments collected near mining areas and reduced survival and reproduction of daphnids in most pore waters tested. Additional amphipod tests conducted in 2004 documented significant toxic effects of sediments from three streams downstream of mining areas: Strother Creek, West Fork Black River, and Bee Fork. Greatest toxicity occurred in sediments from a 6-km reach of upper Strother Creek, but significant toxic effects occurred in sediments collected at least 14 km downstream of mining in all three watersheds. Toxic effects were significantly correlated with metal concentrations (nickel, zinc, cadmium, and lead) in sediments and pore waters and were generally consistent with predictions of metal toxicity risks based on sediment quality guidelines, although ammonia and manganese may also have contributed to toxicity at a few sites. Responses of amphipods in sediment toxicity tests were significantly correlated with characteristics of benthic invertebrate communities in study streams. These results indicate that toxicity of metals associated with sediments contributes to adverse ecological effects in streams draining the Viburnum Trend mining district.

Published by Elsevier Inc.

1. Introduction

Lead (Pb) and other metals have been mined in Missouri since the early 18th century (Proctor, 1984). Since the 1960s, active mining has occurred in the Viburnum Trend (VT) mining district in the Ozark Plateau region (Wixson, 1978; Fig. 1). Over time, active mining has gradually shifted from north to south within the VT, and recent prospecting activity has focused on areas further south, in the Mark Twain National Forest, that are drained by high-quality streams that have federal protection as a National Wild and Scenic River (Eleven Point River) or as a National Park (Current and Jacks Fork Rivers). Concerns raised about possible expansion of mining into these environmentally sensitive areas have focused on the need for a better understanding of the ecological impacts of mining on stream ecosystems of the Missouri Ozarks.

A variety of ecological changes have been reported from streams downstream of mining areas in Missouri. Early studies reported growth of algal mats downstream of mining areas (Gale et al., 1973; Wixson, 1977) and several subsequent studies documented changes in benthic macroinvertebrate communities of streams downstream of inactive mines in the Old Lead Belt and active mines in the VT during the 1960s and 1970s (Duchrow, 1983; Ryck, 1974; Ryck and Whitley, 1974) and in recent years (Humphrey and Lister, 2004; Lister and Humphrey, 2005). Other recent studies have documented increased concentrations of Pb, cadmium (Cd), and zinc (Zn) in plant biomass, aquatic invertebrates, and fish in streams downstream of mining areas (Besser et al., 2007; Schmitt et al., 2007a). Fish from habitats affected by mining activities also exhibit biochemical effects of metal exposure (Schmitt et al., 1984, 2007b; Dwyer et al., 1988).

The mechanisms responsible for observed ecological impacts in streams draining Missouri lead-mining areas are poorly documented. In some areas, notably in the Big River downstream of the Old Lead Belt, erosion of mine tailings into stream channels has resulted in degradation of physical habitats in addition to contamination with toxic metals (Schmitt and Finger, 1982). Waste-management practices at VT mining sites keep tailings and ore-processing wastes in large impoundments, except for rare discharges into streams during storm events (e.g., Duchrow, 1983). Water discharged into tailings impoundments may also contain organic chemicals used in the milling process, notably the large

* Corresponding author. Fax: +1 573 876 1896.
E-mail address: jbesser@usgs.gov (J.M. Besser).

0147-6513/$ - see front matter Published by Elsevier Inc.
doi:10.1016/j.ecoenv.2008.05.013

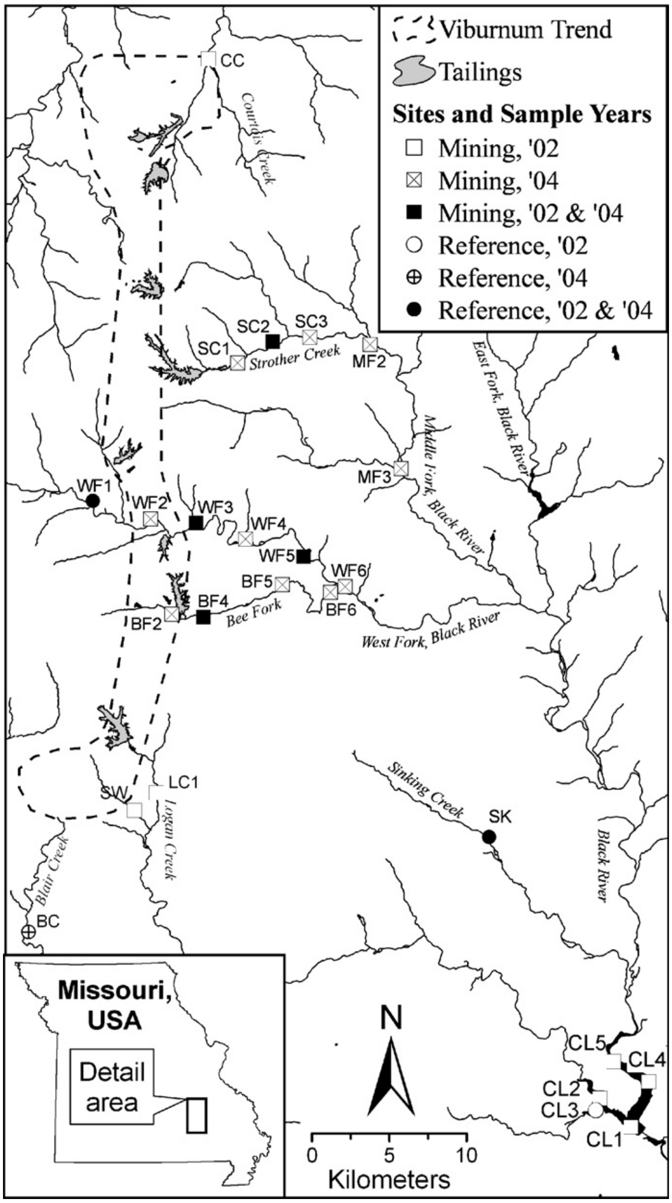

Fig. 1. Map of sediment sampling sites in Viburnum Trend study area in 2002 and 2004.

quantities of xanthates used to separate ore minerals by flotation (Wixson, 1977). Xanthates may be toxic to aquatic biota at concentrations less than 1 mg/L, but they degrade rapidly in aquatic ecosystems (Xu et al., 1988). Recent analyses of water samples from mill discharges and tailings reservoirs did not detect xanthates and detected only trace amounts of degradation products (Rostad et al., 2007), suggesting that toxicity risks from these compounds in receiving streams are minimal. In contrast, substantial loadings of metals enter receiving streams by a variety of routes, including transport of fugitive particles by wind and/or storm-water runoff and release of metals in surface discharge or subsurface seepage from tailings impoundments (Proctor, 1984; Wixson, 1978; Kleeschulte et al., in press). Metal concentrations in surface waters of receiving streams are typically less than water quality criteria and are often below routine analytical detection limits, but fine-grained sediments at sites downstream of VT mines contain high concentrations of Pb and other potentially toxic metals, including Cd, Zn, nickel (Ni), and cobalt (Co) (Brumbaugh et al., 2007; Petersen et al., 1998, Kleeschulte et al., in press). Large quantities of metal-rich sediments have been transported downstream by the Black River and its tributaries and deposited in Clearwater Lake, an impoundment of the Black River located downstream of most VT mining areas (Kleeschulte et al., in press; Brumbaugh et al., 2007; Petersen et al., 1998; Schmitt and Finger, 1982; Wixson, 1978).

Geo-environmental models developed by Leach et al. (1995), based on geochemical characteristics of ore bodies and host rocks of the VT mining district, predict that Pb and Zn should pose the greatest ecological risks to aquatic ecosystems, with other metals including Cd, Ni, and Co posing lesser risks. However, the bioavailability and toxicity of metals in tailings and sediments from VT mining areas have been little studied. We studied the bioavailability and toxicity of metals associated with sediments from several streams draining the VT and from Clearwater Lake. The objectives of these studies were: (1) to evaluate spatial and temporal patterns of metal concentrations and toxicity of sediments and pore waters, relative to mining activities; (2) to evaluate relationships between metal concentrations and toxic effects in laboratory tests; and (3) to evaluate relationships between responses in laboratory toxicity tests and characteristics of stream invertebrate and fish communities.

2. Methods

2.1. Collection of sediment and pore water

Sediments were collected during July 2002 and August 2004. In 2002, sediments were collected from 10 stream sites and 5 sites in Clearwater Lake, which were selected to represent a wide range of possible mining influences (Fig. 1). Seven sites were located on streams draining VT mines: Courtois Creek (CC) in the Meramec River watershed, and Neals Creek (NC), Strother Creek (SC2), West Fork Black River (WF3, WF5), Bee Fork (BF4), Logan Creek (LC) and Sweetwater Creek (SW) in the Black River watershed. Stream reference sites sampled in 2002 were WF1, located upstream of mining in the West Fork Black River watershed and SK, located on Sinking Creek, which has no mining activity in its watershed. Five additional sites were located in Clearwater Lake: four sites (CL1, CL2, CL4, and CL5) in arms of the reservoir fed by watersheds containing mining activity, and one reference site (CL3) in the Webb Creek arm, which has no upstream mining inputs. In 2004, sediments were collected from 14 sites in 3 stream segments extending downstream of mining activities in the Black River watershed (Fig. 1): Strother Creek-Middle Fork Black River (SC1, SC2, SC3, MF2, MF3); West Fork Black River (WF2–WF6); and Bee Fork (BF2, BF4, BF5, and BF6). Reference sites for the 2004 study included WF1 and a site in Blair Creek (BC), a tributary of the Current River. Detailed descriptions of study sites are provided in the on-line Supplemental Information and by Brumbaugh et al. (2007).

Sediments were extracted from the gravel-dominated substrates of study streams using a gasoline-powered diaphragm pump equipped with an intake manifold covered with stainless steel screen to exclude particles larger than 2 mm (Brumbaugh et al., 2007; Schmitt and Finger, 1982). This apparatus generates a slurry of surficial sediments extracted from depths up to about 10 cm, depending on substrate characteristics. Sediment slurries were pumped into conical 50-L polyethylene settling tanks and allowed to settle for 30 min, after which clear overlying water was decanted and sediments were transferred to 20-L polyethylene storage containers. Sediment samples from Clearwater Lake were composites of multiple ponar grabs. Sediments samples were stored in the dark at 4 °C for 5–14 d (2002) or 13–20 d (2004) and then homogenized before testing and analysis.

Pore waters were extracted from composite sediment samples for chemical analysis and toxicity testing. Pore waters for chemical analysis were prepared on days 0 and 27 of sediment toxicity tests by centrifugation (30 min at 3500g). Day-0 samples were extracted from the composite sample and day-27 samples were extracted from extra test beakers carried through the amphipod test. Samples of pore water for metal analysis were filtered through polypropylene syringe filters (pore diameter 0.45 um) and preserved with ultrapure nitric acid to 1% (v/v). Samples were also analyzed immediately for routine water quality parameters or refrigerated for analysis of dissolved organic carbon (DOC). Larger volumes of pore-water (2 L) required for pore-water toxicity testing were extracted with a pressurized-nitrogen squeezing apparatus (squeezed pore waters; Carr and Chapman, 1995), then centrifuged for 30 min at 3500g and stored at 4 °C until use (up to 8 d). Filtered samples of squeezed pore waters were collected for metals determinations on days 0, 4, and 7 of the pore-water toxicity test.

2.2. Toxicity testing

Whole-sediment toxicity tests with the amphipod, *Hyalella azteca,* were conducted in 2002 and 2004, and pore-water toxicity tests with the daphnid, *Ceriodaphnia dubia*, were conducted in 2002, using standard test methods (ASTM, 2006; USEPA, 2000, 2002). Details of the toxicity test methods are reported in the Supplemental Information. Endpoints in the amphipod test included survival and growth (measurement of dorsal carapace length with a digital image analysis system; Ingersoll et al., 1998) after a 28-d sediment exposure and reproduction (neonates per surviving female) in clean water during a 14-d period following the sediment exposure. In 2002, all 14 sediments were tested simultaneously. In 2004, 16 sediments were tested in two batches, started 1 week apart, to reduce differences in storage time. A control sediment (wetted terrestrial soil collected in Florissant, Missouri) was tested with each batch of field-collected sediments to document test acceptability (ASTM, 2006; USEPA, 2000). For the daphnid test, endpoints of survival and reproduction (neonates per surviving female) were determined after an 8-d exposure. Treatments included 100% (undiluted) pore water from each sediment plus a control water (CERC well water).

2.3. Characterization of sediment and pore water

Metal concentrations in sediment and pore water were determined by inductively coupled plasma mass spectroscopy (ICP-MS) (Brumbaugh et al., 2007). Sediments were analyzed for total-recoverable (TR) Ni, Zn, Cd, Pb, iron (Fe), and manganese (Mn) in both years. In 2002, sediments were also analyzed for acid-volatile sulfide (AVS) and simultaneously extracted metals (SEM; Ni, Zn, Cd, Pb, and copper) on days 0 and 27 of the whole-sediment toxicity test. All pore-water samples were analyzed for Fe, Mn, Zn, Cd, and Pb. In addition, Ni concentrations were analyzed in squeezed pore waters in 2002 and Ni and Co were analyzed in all pore waters in 2004. Metal concentrations in sediments and pore waters are summarized in the Supplemental Information and raw data, detailed methods, and quality assurance data for extraction and analysis of metals are presented by Brumbaugh et al. (2007). Sediments were also analyzed for moisture content, particle-size distribution (hydrometer method), and total organic carbon (TOC; by combustion and coulometric titration). Pore-water samples were analyzed for routine water quality parameters (pH, conductivity, alkalinity, hardness, ammonia, and DOC; APHA et al., 2005).

Risks of toxicity from individual metals (Pb, Zn, Cd, and Ni) and metal mixtures in sediment in pore water were estimated by three methods. Total-recoverable metal concentrations were converted to probable effect quotients (PEQs) by dividing TR metal concentrations by the probable effect concentration (PEC; MacDonald et al., 2000) for each metal, with PEQs of 1.0 or greater associated with an increased probability of toxic effects in laboratory tests. Individual PEQs for the four metals were summed ($\sum$PEQ) to estimate risks from the metal mixtures (Ingersoll et al., 2001). A second index of toxicity risk from sediment metals was derived by subtracting the concentration of AVS from the molar sum of SEM metal concentrations and dividing this value by the organic carbon fraction content of the sediment: $\sum$SEM$-$AVS/f_{OC} (USEPA, 2005). Equilibrium partitioning sediment benchmarks (ESBs; USEPA, 2005) based on this index predict that sediments with less than 130 μmol/g OC (including negative values) will be non-toxic and sediments with greater than 3000 μmol/g OC will be toxic, with intermediate values indicating 'uncertain toxicity'. A third index of toxicity risks, pore metal toxic units (TU, also known as criteria units; USEPA, 2005), were derived by dividing concentrations of each metal (Cd, Pb, Ni, and Zn) by its chronic water quality criterion (adjusted for pore-water hardness; USEPA, 2004). Toxic units normalize differences in toxicity among different cationic metals so they can be summed ($\sum$TU) to estimate cumulative risks from metal mixtures, with sums less than 1.0 predicted to be non-toxic.

2.4. Statistical analysis

Data were analyzed using SAS/STAT software (version 9.1; SAS Institute, Cary, NC). Statements of statistical significance refer to a probability of Type I error of less than 5% ($p<0.05$). Survival data from the *Ceriodaphnia* test were analyzed using Fisher's exact test to determine significant reductions, relative to the control. Reproduction data from the *Ceriodaphnia* test and all toxicity data from amphipod tests were rank-transformed and analyzed by one-way analysis of variance (ANOVA). Differences from the control (for the daphnid test) or from reference samples (for amphipod tests) were determined by one-way Dunnett's test. Separate ANOVAs and Dunnett's tests were conducted for amphipod toxicity tests with stream and reservoir sediments in 2002 to allow comparisons with separate stream and reservoir reference sediments. Differences in amphipod toxicity endpoints between years were evaluated with two-tailed Wilcoxon tests. Pearson correlation analysis was used to examine associations of toxicity endpoints with constituents of sediment and pore water, toxicity risk indices, and characteristics of stream biotic communities.

3. Results

3.1. Characteristics of sediments and pore waters

Physical and chemical characteristics of sediments collected in 2002 and 2004 were reported by Brumbaugh et al. (2007) and are summarized in the Supplemental Information. Sediments from Clearwater Lake were dominated by silt- and clay-sized particles (83–95% by weight) and stream sediments were dominated by sand-sized particles (69–90%), except the SC1 stream sediment was dominated by silt- and clay-sized particles (53%). Sediment from SC1 also had highest concentrations of Fe (1.2%), and Mn (0.43%). Both stream and reservoir sediments had relatively low organic content (TOC 0.2–2.1%), with lowest TOC in several stream sediments (WF1, SK, LC, and SW). AVS concentrations (measured only in 2002) varied both among locations and during the course of tests. AVS concentrations on day 0 of the amphipod test were generally lower in stream sediments (range: 0.01–3.4 μmol/g) than in reservoir sediments (1.4–9.1 μmol/g). AVS concentrations increased between days 0 and 28 in all but one of the VT stream sediments and decreased during the tests in three of five reservoir sediments. Physical characteristics of stream and reservoir reference sediments were generally similar to stream and reservoir sediments from sites downstream of mining areas.

Pore waters isolated by centrifugation had neutral to slightly alkaline pH (7.05–8.35). Hardness and concentrations of Mn, DOC and total ammonia in pore waters were greater and more variable for stream sediments than for reservoir sediments (Table 1). Pore water from most stream sediments had very high concentrations of DOC (>100 mg/L) and several had high concentrations of ammonia (>10 mg N/L) and Mn (>20 mg/L), especially in 2002. Pore-water quality did not differ markedly between mining sites and reference or downstream sites in either year, or among the three stream segments studied in 2004. The strongest signature associated with mining occurred in pore waters from upper Strother Creek (SC2 and SC3) in 2004, which had sulfate concentrations greater than 300 mg/L, compared to range of 2.6–60 mg/L in the other two study watersheds (Brumbaugh et al., 2007). Concentrations of pore-water constituents were similar for squeezed samples (used in pore-water toxicity tests) and centrifuged samples in 2002, except for higher pH (8.04–8.65) in squeezed pore waters, apparently due to equilibration with the atmosphere during storage (Table 1; Brumbaugh et al., 2007). Dissolved oxygen of pore waters was adequate (4.5 mg/L or greater) throughout the daphnid test.

3.2. Metal exposure and sediment quality guidelines

Total-recoverable metal concentrations in sediments in 2002 toxicity tests (Brumbaugh et al., 2007) indicated elevated risks of metal toxicity to benthic invertebrates. The $\sum$PEQ index (Fig. 2a) indicated highest risks of toxicity for three VT stream sediments (CC, SC2, and WF3), which had $\sum$PEQ values greater than 3.0. The LC sediment and the four non-reference sediments from Clearwater Lake also had $\sum$PEQ values slightly greater than 1.0, suggesting lower risks of metal toxicity, although concentrations of individual metals did not exceed PECs in these sediments.

The ESB index for 2002 sediments, based on averages of day-0 and day-27 values for SEM and AVS, did not characterize any of the sediments tested as 'likely to be toxic' but five VT stream sediments had index values in the 'uncertain toxicity' range. High values of the ESB index for sediments from CC, SC2, and WF3 were predominantly due to high SEM metal concentrations, whereas high values for sediments from LC and SW reflected lower SEM concentrations combined with low concentrations of AVS and

Table 1
Water quality of pore waters from 2002 (C: centrifuged, S: squeezed) and 2004 (centrifuged)

ID	Hardness (mg/L as $CaCO_3$)			Manganese (mg/L)			DOC (mg/L)			Ammonia (mg/L as N)		
	2002-C	2002-S	2004	2002-C	2002-S	2004	2002-C	2002-S	2004	2002-C	2002-S	2004
Stream sites												
CC	300	510	–[a]	20	23	–	220	220	–	18	19	–
SC1	–	–	640	–	–	8.2	–	–	48	–	–	5.3
SC2	280	370	624	40	17	12	180	170	63	17	18	4.3
SC3	–	–	560	–	–	7.8	–	–	66	–	–	4.7
MF2	–	–	600	–	–	13	–	–	160	–	–	7.3
MF3	–	–	588	–	–	13	–	–	146	–	–	9.3
WF1 (R)[b]	500	630	446	32	35	11	370	330	109	18	18	6.5
WF2	–	–	300	–	–	13	–	–	159	–	–	4.0
WF3	580	690	340	42	37	11	320	310	90	33	35	8.9
WF4	–	–	373	–	–	9.7	–	–	43	–	–	3.3
WF5	1160	1210	576	49	61	4.6	570	540	178	23	24	6.5
WF6	–	–	1000	–	–	21	–	–	477	–	–	15
BF2	–	–	240	–	–	8.8	–	–	26	–	–	2.4
BF4	850	880	610	57	67	17	450	470	206	41	43	13
BF5	–	–	240	–	–	3.5	–	–	8	–	–	1.5
BF6	–	–	290	–	–	13	–	–	140	–	–	12
SK (R)	180	200	–	11	11	–	20	26	–	3.9	3.8	–
LC	200	510	–	5.6	4.9	–	8	8	–	5.2	4.9	–
SW	330	390	–	8.4	20	–	200	200	–	4.4	4.6	–
BC (R)	–	–	600	–	–	12	–	–	145	–	–	2.2
Reservoir sites												
CL1	200	240	–	17	13	–	10	7	–	4.7	5.1	–
CL2	160	180	–	12	8.0	–	11	5	–	2.5	2.6	–
CL3 (R)	170	190	–	14	13	–	12	4	–	5.0	5.0	–
CL4	150	150	–	7.8	4.6	–	33	3	–	1.9	1.7	–
CL5	200	230	–	16	14	–	7	7	–	5.9	5.7	–

Values represent single analyses from day 0 of toxicity tests, except hardness ($n = 2$) and ammonia ($n = 4$) in squeezed samples.
[a] No sample or missing data.
[b] 'R' indicates reference site.

TOC. The ESB index predicted no toxicity for four VT stream sediments (reference sites plus WF5 and BF4) and all five of the reservoir sediments (Fig. 2b).

Pore-water toxic units also indicated substantial risks of metal toxicity for most stream sediments from sites downstream of mining areas in 2002. Average toxic unit scores ($\sum$TU) for samples derived by centrifugation were generally 10-fold greater than those for samples derived by squeezing, but toxicity risks generally followed similar trends among sites for pore waters prepared by both methods (Fig. 2c). Toxic units for centrifuged samples indicated highest risks of metal toxicity (>10 toxic units) for sediments from four VT stream sites closest to mining (CC, SC2, BF4, and LC). Toxic units for both centrifuged and squeezed pore-water samples indicated high toxicity risks for the BF4 sediment, which was not predicted to be toxic by either index based on whole-sediment metal concentrations. Toxic units for both centrifuged and squeezed samples indicated negligible toxicity risks for pore waters from VT stream reference sites, but centrifuged samples indicated some risks of toxicity (two to five toxic units) for four of the five sites in Clearwater Lake, including the CL3 reference sediment. Toxic units for all centrifuged samples were dominated by Pb, and lower toxic units for squeezed samples reflect lower Pb concentrations in these squeezed samples (except BF4 and LC). Lower toxicity risks estimated for the squeezed pore waters reflect lower initial (day-0) metal concentrations, compared to day-0 centrifuged samples, plus further decreases occurring during storage and testing. For the seven VT sites closest to mining, Pb concentrations in squeezed pore waters averaged 65% lower on day 0 of the daphnid test and decreased an additional 52% by day 7 (Brumbaugh et al., 2007).

Total-recoverable metal concentrations indicated substantial differences in risks of metal toxicity among the three stream segments sampled in 2004 (Fig. 3a). Probable effect quotients indicated greatest toxicity risks for sediments from upper Strother Creek-Middle Fork Black River, intermediate risks for Bee Fork, and lowest risks for West Fork Black River. Toxicity risks from Strother Creek sediments reflected contributions from several metals, with PEQs for Ni, Pb, and Zn greater than 1.0 at one or more sites. The PEQ for Pb was also greater than 1.0 in Bee Fork sediments. Concentrations of individual metals did not exceed PECs in sediments from West Fork Black River, although WF3 sediments had a $\sum$PEQ greater than 1.0. Toxicity risks showed clear upstream–downstream gradients in sediments from Strother Creek-Middle Fork and Bee Fork, with risks decreasing by 97% between SC1 and MF2 and by 95% between BF2 and BF6. In contrast, $\sum$PEQ values for sediments from the two upstream sites in the West Fork Black River were only about twice those for the three downstream sites.

Pore-water toxic units also indicated substantial longitudinal variation of toxicity risks in all three watersheds in 2004 (Fig. 3b). Greatest toxicity risks were predicted for pore waters from the three sites immediately downstream of tailings ponds (SC1, WF3, and BF4), with $\sum$TU for these samples ranging from 9.1 to 32. Toxicity risks were lower by a factor of 10 in samples collected furthest downstream in each stream segment. Contributions of individual metals to toxic unit scores were similar to patterns observed for whole sediment toxicity indices, with Pb, Cd, and Ni exceeding chronic criteria values for Strother Creek pore waters, and Pb dominating for Bee Fork and West Fork pore waters. Toxicity risks estimated by toxic units were similar to those estimated from $\sum$PEQ, except toxic units indicated proportionally greater toxicity risks for pore waters from WF3.

Data for the sites downstream of mining that were sampled in both 2002 and 2004 indicated differences in metal exposure

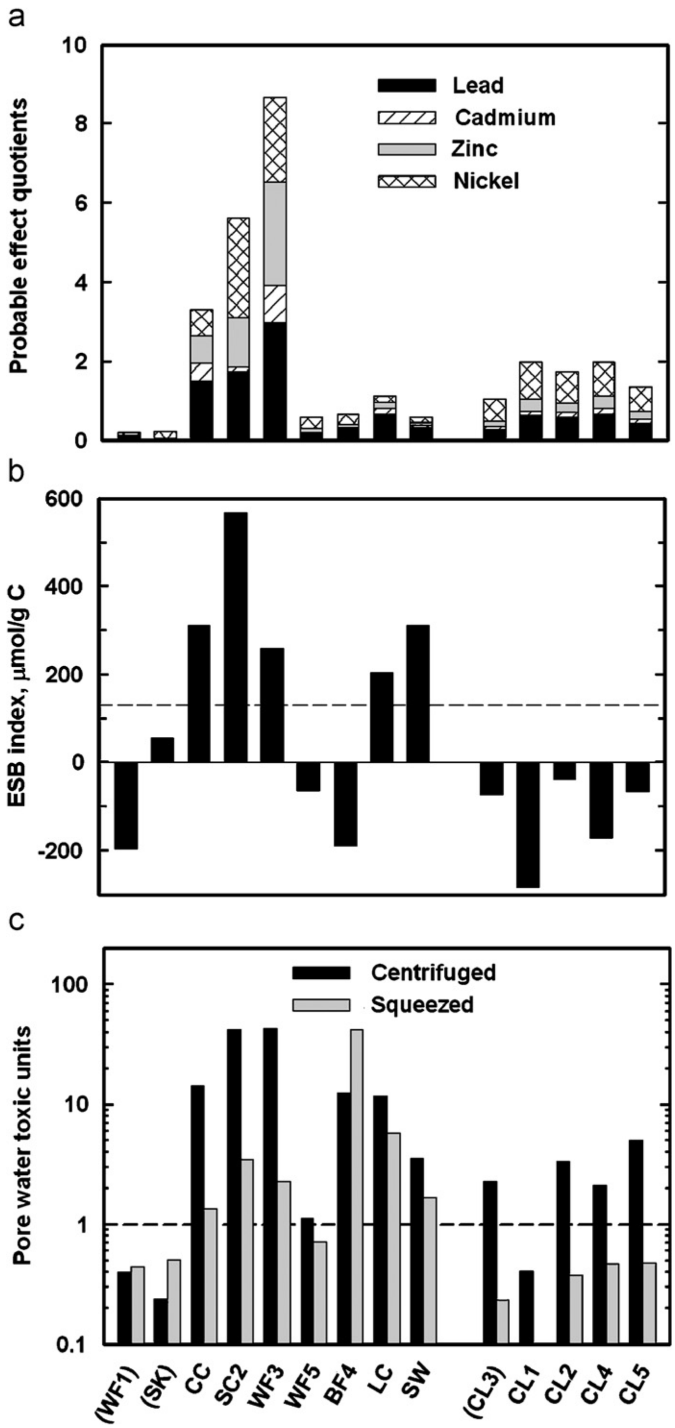

Fig. 2. Risks of metal toxicity for 2002 sediments: (a) sum of sediment probable effect quotients (concentration/probable effect concentration; MacDonald et al., 2000); (b) equilibrium sediment benchmark (ESB) index ($\sum$SEM$-$AVS/f_{OC}; USEPA, 2005); (c) sum of toxic units (concentration/water quality criterion; USEPA, 2004) for centrifuged and squeezed pore waters. Parentheses indicate reference sediments. Dashed lines indicate 'non-toxic' ranges.

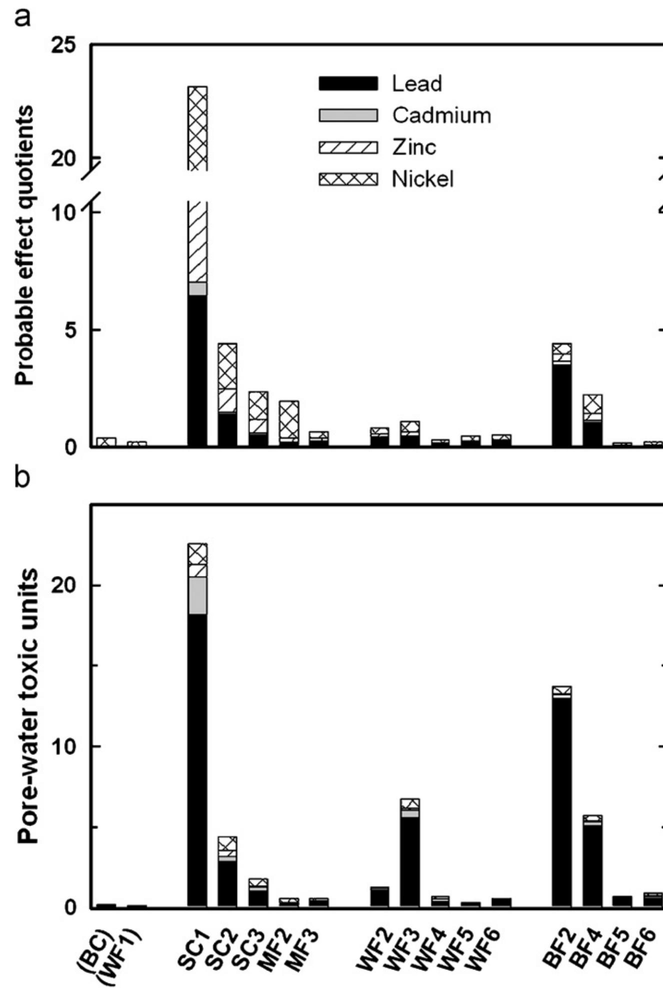

Fig. 3. Risks of metal toxicity in sediments collected in 2004: (a) sum of sediment probable-effect quotients (concentration/probable effect concentration; MacDonald et al., 2000) and (b) sum of pore-water toxic units (concentration/water quality criterion; USEPA, 2005). For either index, samples with values <1.0 are predicted to be non-toxic.

between years (Figs. 2 and 3). Toxicity risks estimated by $\sum$PEQ were lower in 2004 for sediments from WF3 and SC2, but toxicity risks for BF4 sediments were greater in 2004. Toxicity risks estimated by pore-water toxic units were lower for all three sites in 2004, with greatest decreases occurring at SC2 (95% reduction in $\sum$TU) and lesser decreases occurring at WF4 (70%) and BF4 (50%).

3.3. Toxicity of sediment and pore water

Sediments collected from several stream sites downstream of VT mining areas in 2002 were toxic to amphipods. Survival, growth, and reproduction of amphipods differed significantly among the nine VT stream sediments (Table 2). Survival ranged from over 90% in six sediments (including the two stream reference sites) to 17% in the SC2 sediment. Mean survival was significantly lower in the SC2, BF4, and WF3 sediments than in either reference sediment. Amphipods from the SC2 sediment also had lowest growth and reproduction of all sediments tested, but none of the site means for growth or reproduction were significantly less than both reference means. Differences in toxicity endpoints among sites were less pronounced for reservoir sediments (Table 2). Survival did not differ significantly among the five reservoir sediments. Growth differed significantly among the reservoir sediments, but mean growth in sediments from reservoir sites downstream of mining areas was not reduced significantly relative to the reference sediment (CL3: Webb Creek arm). Reproduction did not differ significantly among reservoir sites, although reproduction in sediments from CL4 (lower Black River arm) and CL1 (main reservoir near dam) averaged only about one-half of that in the reference sediment.

Table 2
Toxicity of stream and reservoir sediments to the amphipod, *Hyalella azteca*, in 2002

Sample ID	Survival, % ($n = 16$)	(SE)	Length, mm ($n = 4$)	(SE)	Young per female ($n = 4$)	(SE)
Control	82	(4)	4.31	(0.18)	8.8	(1.1)
Stream sediments						
WF1 (R)[a]	93	(3)	5.33	(0.08)	20	(5.0)
SK (R)	97	(2)	4.61	(0.17)	7.8	(0.7)
CC	96	(2)	5.58	(0.14)	17	(3.3)
SC2	17*	(4)	3.44	(0.27)	0.6	(0.6)
WF3	69*	(5)	5.11	(0.03)	12	(1.1)
WF5	94	(2)	5.38	(0.13)	20	(3.3)
BF4	59*	(6)	4.60	(0.08)	8.2	(2.8)
LC	98	(1)	4.55	(0.06)	8.4	(2.0)
SW	97	(2)	5.30	(0.06)	17	(1.6)
ANOVA (p)	<0.0001		<0.0001		<0.0001	
Reservoir sediments						
CL3 (R)	99	(1)	4.21	(0.06)	7.0	(0.8)
CL1	98	(1)	4.11	(0.16)	3.2	(0.9)
CL2	92	(3)	4.30	(0.04)	5.8	(0.4)
CL4	97	(2)	4.50	(0.05)	3.8	(0.6)
CL5	91	(5)	4.61	(0.04)	8.3	(2.9)
ANOVA (p)	0.0557		0.0030		0.0981	

Means (with standard errors) and p-values for one-way ANOVAs for stream and reservoir sediments. Asterisks indicate means that are significantly lower than reference mean (lower of WF1 or SK for stream sediments; CL3 for reservoir sediments), determined by Dunnett's test.

[a] (R) indicates reference site.

Table 3
Toxicity of pore waters to *Ceriodaphnia dubia* in 2002

Sample ID	Survival (of 10)	Reproduction (neonates/survivor)	(SE)
Control	10	20.1	(1.5)
WF1 (R)[a]	2*	0.0*	(0.0)
SK (R)	8	11	(2.0)
CC	2*	0.0*	(0.0)
SC2	0*	0.0*	(0.0)
WF3	0*	0.0*	(0.0)
WF5	6*	0.0*	(0.0)
BF4	0*	0.0*	(0.0)
LC	10	32	(1.9)
SW	3*	0.0*	(0.0)
CL3 (R)	7	5.6*	(1.5)
CL1	10	14	(3.2)
CL2	7	11*	(3.4)
CL4	7	21	(6.3)
CL5	7	6.8*	(2.6)
ANOVA (p)	–	<0.0001	

Number of survivors, mean reproduction per survivor (with standard error), and p-value for differences among means (for reproduction; one-way rank ANOVA). Asterisks indicate values that are significantly less than controls (Fisher's exact test for survival; Dunnett's test for reproduction).

[a] (R) indicates reference site.

Pore waters from 2002 sediments were highly toxic to daphnids (Table 3). Daphnids performed well in the control water, but results in pore waters from the three reference sediments varied widely, with survival ranging from 20% to 80% and reproduction ranging from 0 to 11 young per female. Pore waters from six of the seven VT stream sites had survival significantly less than the control, with no reproduction. Pore waters from all VT sediments except those from LC had survival and reproduction significantly less than controls. Survival was not significantly lower than controls in pore waters from any reservoir sediments. Reproduction was significantly lower, relative to controls, in pore waters from CL2 (Logan Creek Arm) and CL5 (upper Black River arm), but mean reproduction in both of these pore waters was greater than that in the reference pore water (CL3).

In 2004, sediments from all three VT stream segments showed evidence of toxicity to *H. azteca*. Amphipod survival and growth in control and reference sediments met test acceptability criteria (ASTM, 2006) and all three endpoints differed significantly among sites during both Tests 1 and 2 (Table 4). In both tests, sediments from one or more sites caused significant reductions of one or more endpoint relative to the reference sediments (BC in Test 1; WF1 in Test 2). Sediments from Strother Creek-Middle Fork produced greatest toxic effects and had the clearest upstream–downstream toxicity gradient of the three watersheds. Sediments from SC1 caused nearly complete amphipod mortality, lowest growth, and no reproduction, and these endpoints were also significantly reduced, to lesser extents, in sediments from SC2. No significant effects were evident in the next two sites downstream (SC3, MF1), but significant reductions in survival and growth also occurred at MF2, the study site furthest downstream from mining (22 km downstream of Buick tailings). Sediments from all four Bee Fork sites showed some evidence of toxicity. There were no significant reductions in survival in Bee Fork sediments, but amphipod growth was significantly reduced, relative to reference sediments, at all sites except BF6, the site furthest downstream (14 km below Fletcher tailings), and reproduction was significantly reduced at all four Bee Fork sites. West Fork sediments showed the least evidence of toxicity and the least evidence of an upstream–downstream gradient in toxic effects. There were no significant reductions of any endpoint in sediments from the upstream sites WF2 and WF3 (closest to Brushy Creek tailings and West Fork tailings, respectively), but there were significant reductions in survival and reproduction in sediments from WF4 and significant reductions in growth and reproduction in sediments from the downstream site, WF6 (17 km below West Fork tailings).

Amphipod toxicity tests indicated reduced toxicity at sites affected by mining in 2004, compared to 2002. Amphipod survival was significantly greater in 2004 for the three sites close to

Table 4
Toxicity of stream sediments to *H. azteca* in 2004

Sample ID	Test	Survival, % ($n = 8$)	(SE)	Length, mm ($n = 4$)	(SE)	Young per female ($n = 4$)	(SE)
Control	1	96	(1.8)	4.46	(0.11)	4.6	(1.2)
Control	2	93	(2.5)	4.31	(0.14)	0.9	(0.3)
BC (R)[a]	1	98	(1.6)	5.28	(0.20)	15	(0.4)
WF1 (R)	2	95	(3.8)	5.44	(0.06)	17	(4.3)
SC1	2	7.5*	(3.1)	2.86*	–[b]	0.0*	(0.0)
SC2	2	81*	(3.0)	4.17*	(0.30)	0.9*	(0.9)
SC3	2	98	(1.6)	5.23	(0.13)	9.2	(1.2)
MF2	1	99	(1.3)	5.57	(0.06)	13	(1.1)
MF3	2	81*	(5.2)	5.25	(0.17)	7.6*	(1.3)
WF2	1	95	(3.3)	5.25	(0.07)	14	(3.0)
WF3	1	98	(1.6)	5.54	(0.14)	16	(2.6)
WF4	1	90	(2.7)	5.24	(0.13)	10*	(1.8)
WF5	2	98	(1.6)	5.45	(0.08)	13	(1.2)
WF6	2	95	(2.7)	4.79*	(0.17)	3.5*	(1.3)
BF2	1	100	(0.0)	4.70*	(0.02)	8.2*	(1.8)
BF4	2	91	(3.0)	4.83*	(0.08)	5.1*	(1.0)
BF5	1	99	(1.3)	4.51*	(0.03)	2.6*	(0.2)
BF6	1	84	(12)	5.25	(0.24)[c]	3.9*	(1.5)
ANOVA (p)	1	0.0178		<0.0001		<0.0001	
	2	<0.0001		<0.0001		<0.0001	

Site means (with standard errors in parentheses; $n = 4$ unless noted) and p-values for one-way ANOVAs for differences among means. Separate ANOVAs were conducted for Tests 1 and 2. Asterisks indicate means significantly less than reference means (BC for Test 1, WF1 for Test 2; by Dunnett's test).
[a] (R) indicates reference site.
[b] $n = 1$.
[c] $n = 3$.

mining activities (SC2, WF3, BF4; Wilcoxon tests). Mean growth was greater in 2004 for all three sediments, but differences between years in growth and reproduction were not significant. In contrast to results from sites close to mining activities, there were no significant differences between years for sediments from a reference site (WF1) or from a site located further downstream from mining activities (WF5).

4. Discussion

4.1. *Associations of toxicity with sediment and pore-water constituents*

Results of amphipod toxicity tests were significantly correlated with several characteristics of sediments and centrifuged pore waters (Table 5). Growth and reproduction were significantly correlated with percent sand (and negatively correlated with percent silt and percent clay; data not shown) and sediment Fe concentration, and all three endpoints were significantly correlated with sediment Mn concentration. Correlations with particle size and Fe reflected differences in these constituents between stream sediments and Clearwater Lake sediments, combined with slightly lower growth and reproduction in the reservoir sediments. When these analyses were repeated with only stream sediments, only sediment Mn was significantly correlated with all three endpoints (results not shown), reflecting high Mn levels in the toxic Strother Creek sediments. Correlations of amphipod endpoints with pore-water quality were generally weak. The only significant correlation was a positive correlation of reproduction with pore-water DOC, perhaps reflecting organic enrichment or an amelioration of metal toxicity. Amphipod survival had a non-significant negative correlation with total ammonia, reflecting

Table 5
Pearson correlation coefficients(r) for associations of toxicity endpoints with sediment and pore water constituents and metal toxicity indices

	Amphipod survival	Amphipod growth	Amphipod reproduction	Daphnid survival	Daphnid reproduction
Sediment characteristics					
Sand	−0.02	0.41*	0.44*	–	–
TOC	−0.20	−0.40*	−0.47	–	–
AVS	0.07	0.02	−0.25	–	–
Manganese	−0.60*	−0.65*	−0.47*	–	–
Nickel	−0.60*	−0.57*	−0.43*	–	–
Zinc	−0.56*	−0.51*	−0.36	–	–
Cadmium	−0.38*	−0.39*	−0.23	–	–
Lead	−0.47*	−0.49*	−0.35	–	–
∑PEQ[a]	−0.54*	−0.53*	−0.37*	–	–
ESG index ($n = 14$)[b]	−0.50	0.04	0.003	–	–
Pore-water characteristics					
Hardness	−0.17	0.20	0.21	−0.43	−0.41
Ammonia	−0.34	0.11	0.19	−0.76*	−0.58*
DOC	−0.11	0.33	0.40*	−0.66*	−0.66*
Manganese	0.01	0.25	−0.03	−0.68*	−0.83*
Nickel	−0.52*	−0.01	0.07	−0.79*	−0.48
Zinc	−0.64*	−0.36*	−0.21	−0.76*	−0.45
Cadmium	−0.62*	−0.30	−0.06	−0.46	0.06
Lead	−0.59*	−0.34	−0.16	−0.66*	−0.28
∑TU[c]	−0.55*	−0.43*	−0.25	−0.58*	−0.13

Sample size is 30 for amphipods, 14 for daphnids, except as noted. Asterisks indicate correlations that are statistically significant. Metal concentrations were log-transformed before analysis.
[a] ∑PEQ = sum of probable effect quotients (PEQ = metal concentration/PEC; MacDonald et al., 2000).
[b] ESB index = [∑SEM−AVS]/f_{OC} (USEPA, 2005). Data for 2002 samples only.
[c] ∑TU = sum of toxic units (TU = metal concentration/chronic water quality criterion; EPA, 2005).

reduced amphipod survival in the two samples with greatest ammonia concentrations: 2002 samples from WF3 (33 mg N/L) and BF4 (41 mg N/L). Available LC50s for ammonia toxicity to *H. azteca* (9.2–18 mg N/L; Whiteman et al., 1996; Borgmann, 1994, Nile Kemble, USGS, unpublished data) suggest that ammonia could have contributed to reduced survival in WF3 and BF4 other sediments, although amphipod survival was unaffected in several sediments with pore-water ammonia concentrations greater than these LC50s.

Amphipod toxicity endpoints had consistent negative associations with metal concentrations and metal toxicity indices in sediment and pore water. Concentrations of TR Ni, Zn, and Pb in sediments had significant negative correlations with both amphipod survival and growth, with Ni having the strongest correlations (Table 5). A plot of amphipod survival vs. sediment (TR) Ni (Fig. 4a) suggests a strong sigmoidal concentration–response relationship (logistic regression, $r^2 = 0.87$), with sharp decreases in survival occurring at Ni concentrations about twice the PEC. The only exception to this relationship was the low survival (59%) in the BF4 sediment (2002), which may reflect ammonia toxicity. Amphipod growth had a similar relationship with sediment Ni (Fig. 4b), although growth varied more widely among low-Ni sediments. Reproduction also had a significant negative association with sediment Ni, despite the wide range of reproduction in low-Ni sediments (Fig. 4c). All three amphipod endpoints had a significant negative correlation with the sum of PEQs for these four metals and a similar, but non-significant negative association with the ESB index ($\sum$SEM$-$AVSf_{OC}) (Table 5). The lack of significant correlations between toxicity endpoints and the ESB index reflects the smaller sample size for these correlations, because SEM and AVS were measured only in 2002 samples. Concentrations of Ni, Zn, Cd, and Pb in pore water, and sums of toxic units for these metals, all had significant negative correlations with amphipod survival, with sharp reductions in survival occurring in sediments with greatest pore-water metal concentrations (e.g., Fig. 5a).

Endpoints from pore-water toxicity tests with *C. dubia* had strong correlations with both water quality parameters and metal concentrations of pore waters (Table 5). Daphnid survival and reproduction had significant negative correlations with total ammonia, DOC, and Mn in squeezed pore waters. Both ammonia and Mn are plausible contributors to observed toxic effects on daphnids at the concentration measured in the VT pore waters. All seven pore waters that caused reduced daphnid survival and complete reproductive failure had Mn concentrations of 17 mg/L or greater and six had ammonia concentrations of 18 mg/L or greater, consistent with published chronic values for toxicity of Mn (Lasier et al., 2000) and ammonia to *C. dudia* (Johnson, 1995, USEPA, 1999). Daphnid survival and reproduction also had strong negative correlations with metal concentrations in pore-water, with significant negative correlations of daphnid survival with pore-water Ni, Zn, and Pb. Plots of daphnid survival and reproduction vs. pore-water Ni (Fig. 5b and c) suggest that daphnids were more sensitive to metal toxicity than amphipods, with reduced survival below 0.5 toxic units and total reproductive failure occurring at less than 0.1 toxic units. These results are consistent with available toxicity data, which indicates that *C. dubia* is the most sensitive aquatic taxon tested for Ni toxicity (USEPA, 1996). Chronic (7-d) LC20s of 12 μg/L or less and EC20s for reproduction of 7 μg/L or less were reported for *C. dubia* in tests with water hardness up to 253 mg/L (Keithly et al., 2004; USEPA, 1996). However, our data cannot distinguish possible toxic effects of Ni (or other metals associated with mining) from possible toxicity due to elevated concentrations of ammonia or Mn, which occurred at high concentrations in sediments with and without direct influence of mining.

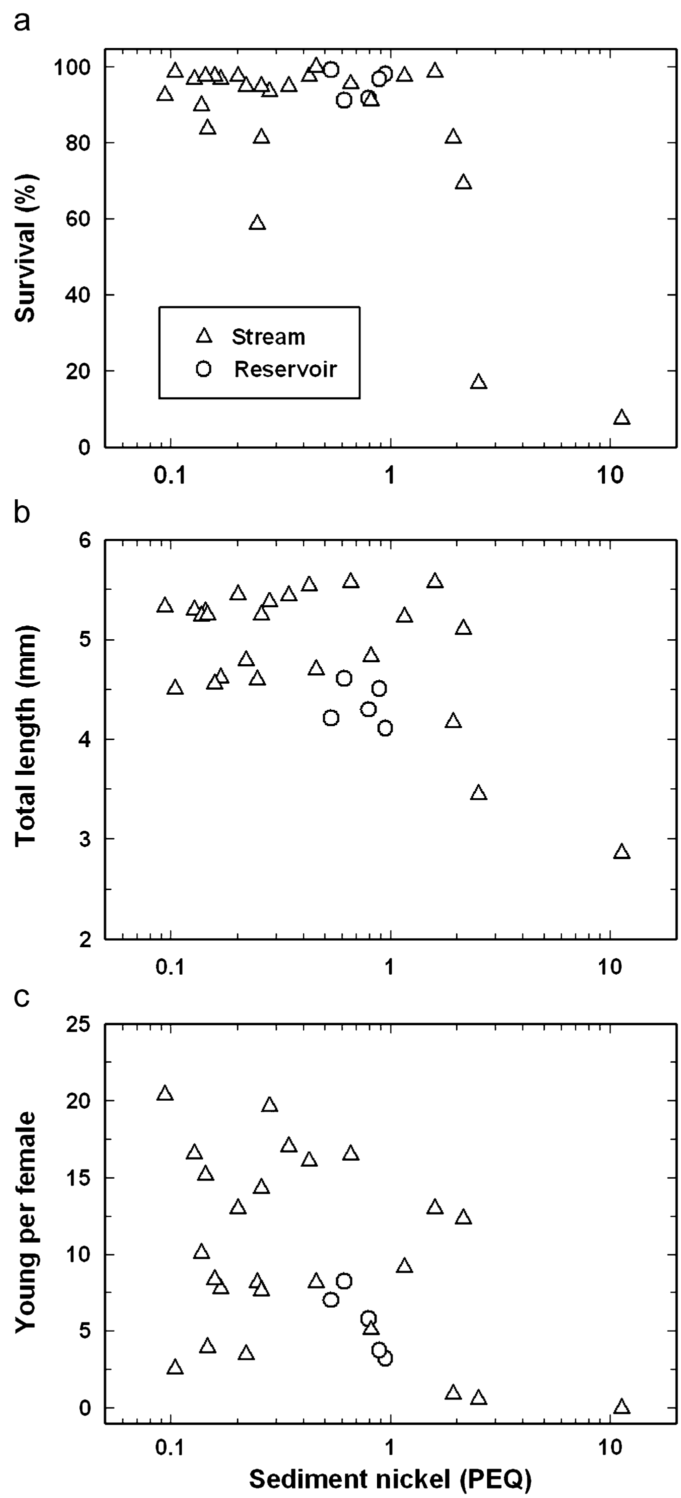

Fig. 4. Relationships between amphipod toxicity endpoints and nickel concentrations in sediment: (a) survival; (b) growth; (c) reproduction. Total-recoverable nickel concentrations are expressed as probable effect quotients (PEQ = nickel concentration/probable effect concentration; MacDonald et al., 2000).

Observed toxic effects on amphipods probably reflect cumulative action of a mixture of metals. The metals we studied (Ni, Zn, Cd, Pb) all exceeded concentrations associated with increased toxicity risks in sediments (PECs) and pore waters (water quality criteria). Risks estimated from sediment metals were dominated about equally by Pb and Ni, with lesser contributions from Zn and Cd (Figs. 2 and 3). Although toxicity risks for pore water metals

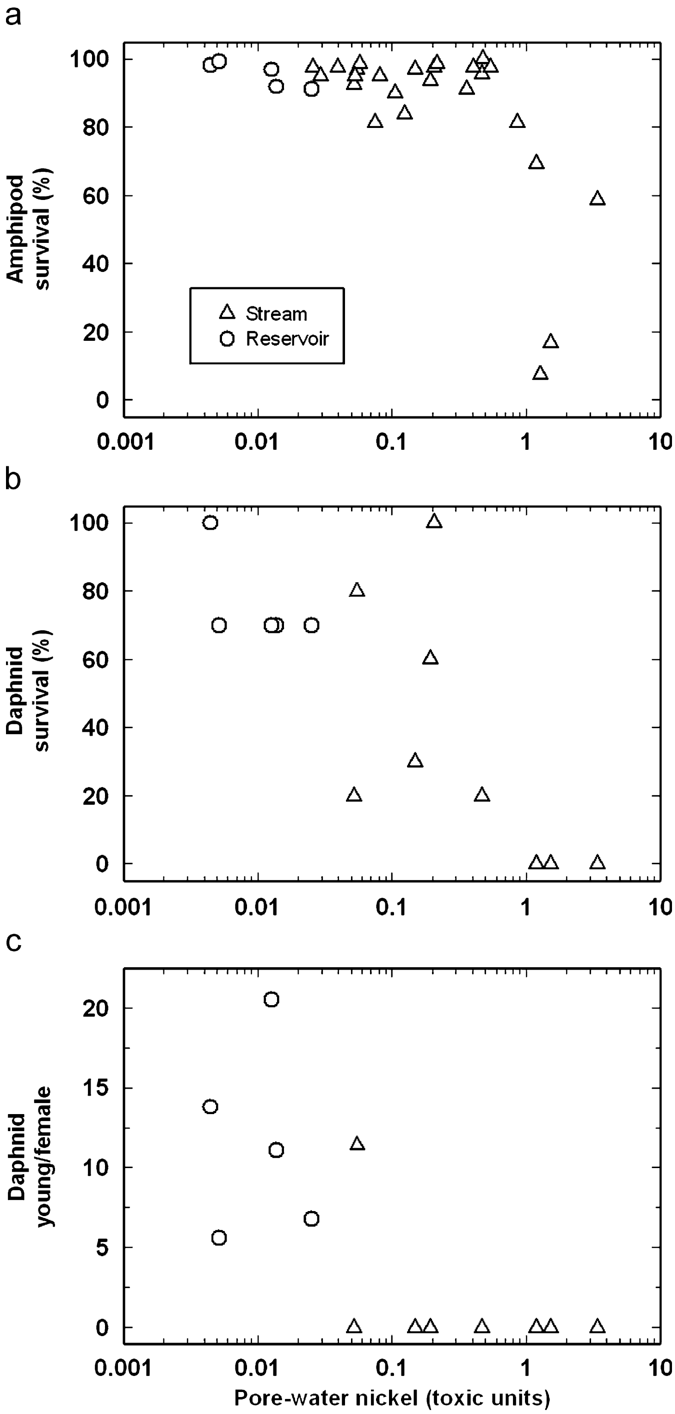

Fig. 5. Relationships between toxicity endpoints and nickel concentrations in pore water: (a) amphipod survival; (b) daphnid survival; (c) daphnid reproduction. Pore-water nickel concentrations are expressed as toxic units (nickel concentration/chronic water quality criterion; USEPA, 2004, 2005).

were dominated by Pb, with several samples exceeding 10 toxic units (Fig. 3b), toxic effects on amphipods corresponded most closely with Ni concentrations in sediment and pore water (e.g., Figs. 5a and 6a). The strong association of toxicity with Ni concentrations reflects the occurrence of high Ni concentrations in the highly toxic sediment (and pore water) from Strother Creek. Reduced survival in VT sediments is consistent with water-only chronic values for Ni toxicity to *H. azteca* (LC20 = 61 μg/L at hardness of 91–91 mg/L). In VT sediments, amphipod survival was significantly reduced in five of six samples with PW Ni concentrations over 150 μg/L.

The strong associations of toxicity in VT sediments with Ni concentrations, and the weaker associations with Pb concentrations, may indicate relatively high bioavailability of Ni in VT pore waters. Free Ni ions (Ni^{2+}) may predominate over free Pb ions (Pb^{2+}) in VT pore waters due to greater complexation of Pb^{2+} by carbonates (Lee et al., 2005) and due to competitive displacement of Ni^{2+} from strong binding sites on organic ligands by high concentrations of calcium and magnesium (Mandal et al., 2000). Mandal et al. (2002) reported that free Ni^{2+} predominated in waters draining another mining area (Sudbury, Canada) that has similar calcium- and magnesium-rich geology. This hypothesis of greater concentrations of free Ni^{2+}, compared to free Pb^{2+}, was supported by experimental characterization of pore water metal speciation in selected VT sediments (SC2, WF3, BF4) during the 2004 sediment toxicity tests (Brumbaugh et al., 2007). This study used metals sequestered into 'diffusion gradient thin-film' (DGT) samplers (Zhang et al., 1995) to indicate the relative availability of free metal ions in pore waters. DGT samplers accumulated more Ni than Pb from all three sediments, despite consistently greater concentrations of Pb measured in filtered pore waters. The relative sampling efficiency of pore water metals by the DGT samplers (mass of metal in DGT sampler divided by concentration in pore water) was greater for Ni than for Pb by factors from 25 to 970 (Brumbaugh et al., 2007).

4.2. Associations of toxicity with responses of resident aquatic biota

Responses of amphipods in sediment toxicity tests in 2002 and 2004 were in general agreement with characteristics of invertebrate and fish communities of the VT study streams (Table 6). Poulton (unpublished data; B. Poulton, USGS, Columbia MO; see Supplemental Information) reported impairment of benthic macroinvertebrate communities at many of the VT stream sites we tested, using an index based on eight metrics of benthic invertebrate community structure (multi-metric index; see Supplemental Information). All three amphipod toxicity endpoints were significantly correlated with one or more benthic

Table 6
Pearson correlation coefficients (r) for associations of amphipod toxicity endpoints with characteristics of stream biotic communities

Community metric	Amphipod survival	Amphipod growth	Amphipod reproduction
Benthos multi-metric score	0.590*	0.700*	0.389
Benthos taxa richness	0.134	0.255	−0.039
EPT taxa[a]	0.484*	0.609*	0.324
Shannon–Wiener diversity	0.484*	0.574*	0.270
Missouri biotic index	−0.398	−0.387	−0.144
Percent Chironomid	−0.716*	−0.652*	−0.438*
Percent dominant taxon	−0.534*	−0.609*	−0.247
Percent scraper	0.542*	0.629*	0.497*
Tolerant/intolerant mayflies	−0.429*	−0.518*	−0.441*
Crayfish density	0.212	0.037	0.104
Fish CPUE[b]	0.531	0.501	0.332
Sculpin CPUE	0.403	0.562	0.486
Fish taxa	0.349	0.292	0.057
Fish IBI[c]	0.636	0.615	0.430

Benthos data from 2003 and 2004 (unpublished data; B. Poulton, USGS, Colulmbia, MO); crayfish data from 2004 (Allert et al., in press); fish data from 2003 (unpublished data; A. Allert, USGS, Columbia MO). See Supplemental Information for community data. Sample sizes (n): 23 for benthos variables; 14 for crayfish variables; 9 for fish variables. Asterisks indicate correlations that are statistically significant.

[a] EPT = Ephemeroptera, Plecoptera, and Trichoptera.
[b] CPUE = catch per unit effort (fish per minute of electrofishing).
[c] IBI = index of biotic integrity (Fausch et al., 1984).

community metrics (Table 6). Survival and growth of amphipods had similar patterns of association with benthic community metrics, including significant positive correlations with the multi-metric index and significant correlations with six of eight individual metrics. Amphipod reproduction was not significantly correlated with the multi-metric index, but was significantly correlated with two of the individual metrics. In 2004, Allert et al. (in press), documented significantly lower densities of crayfish (*Orconectes* spp.) at sites closest to mining areas, compared to reference and downstream sites, and significant negative correlations of crayfish densities with pore-water metal concentrations and pore-water toxic units from the 2004 sediment toxicity tests. Effects on crayfish were most severe in Strother Creek, which also had the greatest alteration of benthic communities and the most toxic sediments. Allert (A.L. Allert, unpublished data; USGS, Columbia, MO) also evaluated the effects of mining on fish communities in VT streams in 2004, including catch-per-unit-effort data for sculpins (Cottidae) and other fish taxa, and an index of biotic integrity (Fausch et al., 1984; see Supplemental Information). All of the crayfish and fish metrics were positively correlated with amphipod toxicity endpoints, although none of these correlations were significant (Table 6). Overall, these associations between sediment toxicity and community characteristics support the hypothesis that that toxic effects of metals from mining activities result in structural and functional changes in benthic communities in streams draining VT mining area.

5. Conclusions

The results of our studies support the hypothesis that toxicity of metals associated with sediments contributes to adverse ecological effects in streams draining the Viburnum Trend mining district. Toxicity tests found evidence of sediment toxicity in streams draining several active mining areas in 2002. Amphipod whole-sediment tests identified upper Strother Creek as the most toxic location, but amphipod survival was also significantly reduced by sediments from West Fork Black River and Bee Fork. Pore-water toxicity tests with daphnids indicated severe toxic effects at these sites, plus sites in Courtois Creek and Sweetwater. However, effects on daphnids were apparently influenced by toxic effects of elevated pore-water Mn concentrations, which occurred in sediments from both mining and reference sites. Of non-reference sites sampled in 2002, only one stream site and four sites in Clearwater Lake had no significant toxic effects in either test. The 2004 study demonstrated differences in the severity and geographic extent of sediment toxicity in three stream segments downstream of mining. The most severe toxic effects in the 2004 study were caused by sediments from upper Strother Creek, in a reach extending at least 6 km downstream from the Buick tailings pond. The longest sequence of sites with significant sediment toxicity occurred in Bee Fork, in a reach extending 16 km downstream from the Fletcher tailings pond. In all three watersheds studied in 2004, significant toxic effects also occurred at one or more sites substantial distances (14–22 km) downstream of mining activities.

Toxic effects on both amphipods and daphnids were strongly associated with exposure to metals associated with mining. Concentrations of Ni, Zn, Pb, and Cd in sediment and pore water, and sediment guidelines estimating additive toxicity risk from these metals in sediment (sums of PEC quotients and sediment ESB index) and pore water (sums of toxic units), had significant negative correlations with one or more toxicity endpoints in tests with amphipods and daphnids. Although risk indices based on measured metal concentrations predicted that Pb would be the primary contributor to observed toxic effects, Ni concentrations had the most consistent negative correlations with toxicity endpoints and DGT samplers suggested that Ni was more bioavailable than Pb in VT sediments. Elevated concentrations of ammonia and Mn, which occurred in both mining and non-mining sediments, apparently contributed to toxic effects on daphnids, but toxic effects on amphipods were not significantly associated with concentrations of ammonia or Mn in pore water.

Data from a limited number of locations also indicated differences in sediment toxicity in amphipod tests between years with reduced toxicity observed in 2004 for sediments from three sites downstream of mining. Reduced toxicity of WF3 sediments between 2002 and 2004 may represent a longer-term decrease in metal loadings at this site due to the cessation of ore processing at the West Fork mill in 2000 (Brumbaugh et al., 2007). Changes at other mining-influenced sites may reflect normal year-to-year variation in deposition and dispersal of metal-rich sediments in the study streams due to variation in stream hydrology. A long-term study of the mining-impacted Clark Fork River found strong associations between among-year variation in stream discharge and metal bioaccumulation by stream biota (Hornberger et al., 1997). Variation in toxicity among locations and sampling dates, notably the occurrence of toxic effects in sediments far downstream of known metal sources, may also reflect mobilization of metals in response to short-term or localized events such as dissolution of Mn oxides and release of associated metals under anoxic conditions. These findings suggest that long-term and short-term changes in metal concentrations and metal bioavailability in streambed sediments can affect the toxic effects of sediments and the accumulation of metals in stream food webs.

Our observations of elevated metal exposure, sediment toxicity, and altered invertebrate and fish communities in several streams draining active mines indicate that current practices for mining, milling, and disposal of metal-rich ores in the Viburnum Trend do not eliminate risks of metal toxicity in receiving streams. These observed impacts on aquatic biota were not associated with any known failure of tailings containment systems (i.e., overtopping or failure of tailings dams), suggesting that loadings of metals via seepage, surface runoff, and/or airborne dust from active mining areas were sufficient to cause the observed adverse ecological impacts in Ozark stream ecosystems.

Acknowledgments

Funding for this research was provided by the U.S. Government through the US Geological Survey. We thank Doug Hardesty, James Kunz, Chris Ivey, Eric Brunson, David Whites, Chad Vishy, and Gary Krizanich for their assistance with sediment sampling, pore-water preparation, and toxicity testing. Thomas May and Mike Walther assisted with analysis of metals and AVS. Ben Lakish and Steve Olson conducted TOC and DOC analyses. Matt Struckhoff prepared the map of the study area. We thank Jon Schumacher, Jeff Imes, Mike Kleeschulte, and Suzanne Femmer of the USGS Water Discipline and Warren Day and Rob Lee of the USGS Geology Discipline for their assistance with project planning.

Disclaimer: To ensure humane treatment of the test organisms during culture and experimentation, this research was conducted according to the CERC Animal Welfare plan, in compliance with requirements of the US Laboratory Animal Welfare Act and the Interagency Research Animal Committee.

Appendix A. Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2008.05.013.

References

Allert, A., Fairchild, J., DiStefano, R., Schmitt, C., Besser, J., Brumbaugh, W., Poulton, B., 2008. Effects of lead–zinc mining on crayfish (*Orconectes hylas*) in the Black River watershed, Missouri, USA. Freshwater Crayfish 16, in press.

APHA (American Public Health Association), American Water Works Association, Water Environment Federation, 2005. Standard methods for the examination of water and wastewater, 21st ed. American Public Health Association, Alexandria, VA.

ASTM, 2006. Test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates, E1706-05. In: Annual Book of ASTM Standards, vol. 11.05. ASTM International, West Conshohocken, PA.

Besser, J.M., Brumbaugh, W.G., May, T.W., Schmitt, C.J., 2007. Biomonitoring of lead, zinc, and cadmium in streams draining lead-mining and non-mining areas, southeast Missouri, USA. Environ. Monit. Assess. 129, 227–241.

Borgmann, U., 1994. Chronic toxicity of ammonia to the amphipod *Hyalella azteca*: importance of ammonium ion and water hardness. Environ. Pollut. 86, 329–335.

Brumbaugh, W.G., May, T.W., Besser, J.M., Allert, A.L., Schmitt, C.J., 2007. Assessment of elemental concentrations in streams of the New Lead Belt in southeastern Missouri, 2002–2005. US Geological Survey Scientific Investigations Report 2007–5057.

Carr, R.S., Chapman, D.C., 1995. Comparison of methods for conducting marine and estuarine sediment porewater toxicity tests—extraction, storage, and handling techniques. Arch. Environ. Contam. Toxicol. 28, 69–77.

Duchrow, R.M., 1983. Effects of lead tailings on benthos and water quality in three Ozark streams. Trans. Missouri Acad. Sci. 17, 5–17.

Dwyer, F.J., Schmitt, C.J., Finger, S.E., Mehrle, P.M., 1988. Biochemical changes in longear sunfish, *Lepomis megalotis*, associated with lead, cadmium, and zinc from mine tailings. J. Fish Biol. 33, 307–317.

Fausch, K.D., Karr, J.R., Yant, P.R., 1984. Regional application of an index of biotic integrity based on stream fish communities. Trans. Am. Fish. Soc. 113, 39–55.

Gale, N.L., Wixson, B.G., Hardie, M.G., Jennett, J.C., 1973. Aquatic organisms and heavy metals in Missouri's new lead belt. Water Resour. Bull. 9, 673–688.

Hornberger, M.I., Lambing, J.H., Luoma, S.N., Axtmann, E.V., 1997. Spatial and temporal trends of trace metals in surface water, bed sediment, and biota of the upper Clark Fork River basin, Montana. Open-File Report 97-669, US Geological Survey, 84pp.

Humphrey, S., Lister, K.B., 2004. Biological Assessment Study: Indian Creek and Courtois Creek, Washington County, 2001–2002. Missouri Department of Natural Resources, Jefferson City, MO.

Ingersoll, C.G., MacDonald, D.D., Wang, N., Crane, J.L., Field, L.J., Haverland, P.S., Kemble, N.E., Lindskoog, R.A., Severn, C., Smorong, D.E., 2001. Predictions of sediment toxicity using consensus-based freshwater sediment quality guidelines. Arch. Environ. Contam. Toxicol. 41, 8–21.

Ingersoll, C.G., Brunson, E.L., Dwyer, F.J., Hardesty, D.K., Kemble, N.E., 1998. Use of sublethal endpoints in sediment toxicity tests with the amphipod *Hyalella azteca*. Environ. Toxicol. Technol. 17, 1508–1523.

Johnson, C.G., 1995. Effects of pH and hardness on acute and chronic toxicity of un-ionized ammonia to *Ceriodaphnia dubia*. M.S. Thesis, University of Wisconsin-Stevens Point.

Keithly, J., Brooker, J.A., DeForest, D.K., Wu, B.K., Brix, K.V., 2004. Acute and chronic toxicity of nickel to a cladoceran (*Ceriodaphnia dubia*) and an amphipod (*Hyalella azteca*). Environ. Toxicol. Chem. 23, 691–696.

Kleeschulte, M.J., et al. Hydrologic Investigations Concerning Lead Mining Issues in Southeast Missouri, Science Investigations Report, US Geological Survey, Rolla, Missouri, in press.

Lasier, P.J., Winger, P.V., Bogenrieder, K.J., 2000. Toxicity of manganese to *Ceriodaphnia dubia* and *Hyalella azteca*. Arch. Environ. Contam. Toxicol. 38, 298–304.

Leach, D.L., Viets, J.B., Foley-Ayuso, N., Klein, D.P., 1995. Mississippi valley-type Pb–Zn deposits. In: du Bray, E.A. (Ed.), Preliminary Compilation of Descriptive Geoenvironmental Mineral Deposit Models. Open-File Report 95–831. US Geological Survey, Denver, CO, pp. 234–243 (Chapter 30).

Lee, L., Hageman, P.L., Besser, J., Brumbaugh, W., Deihl, S.F., 2005. Geochemical and biological impacts of modern lead mining in the mid-continental USA. Abstracts, Geological Society of America 37, 179.

Lister, K.B., Humphrey, S., 2005. Biological assessment and fine sediment study: Big River (lower): Irondale to Washington State Park, St Francois, Washington, and Jefferson Counties, Missouri. Missouri Department of Natural Resources, Jefferson City, MO.

MacDonald, D.D., Ingersoll, C.G., Berger, T.A., 2000. Development and evaluation of consensus-based sediment quality guidelines for freshwater ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31.

Mandal, R., Salam, M.S.A., Murimboh, J., Hassan, N.M., Chakrabarti, C.L., Backc, M.H., 2000. Competition of Ca(II) and Mg(II) with Ni(II) for binding by a well-characterized fulvic acid in model solutions. Environ. Sci. Technol. 34, 2201–2208.

Mandal, R., Hassan, N.M., Murimboh, J., Chakrabarti, C.L., Back, M.H., 2002. Chemical speciation and toxicity of nickel species in natural waters from the Sudbury area (Canada). Environ. Sci. Technol. 26, 1477–1484.

Petersen, J.C., Adamski, J.C., Bell, R.W., Davis, J.V., Femmer, S.R., Freiwald, D.A., Joseph, R.L., 1998. Water Quality in the Ozark Plateaus, Arkansas, Kansas, Missouri, and Oklahoma, 1992–95. US Geological Survey Circular 1158.

Proctor, P.D., 1984. Heavy metal additions to the environment near mines, mills, and smelters, southeast Missouri. In: Nriagu, J.O. (Ed.), Environmental Impacts of Smelters. Wiley-Interscience, New York, pp. 89–115.

Rostad, C., Schmitt, C., Schumacher, J., Leiker, T., 2007. Polar organic compounds in surface waters collected near lead–zinc mine and milling operations in Missouri. Poster RP132 in: Abstract Book, SETAC North America 28th Annual Meeting, Milwaukee, WI, p. 305.

Ryck, F.M., 1974. Water quality survey of the southeast Ozark mining area, 1965–1971. Aquatic Series 10. Missouri Department of Conservation, Jefferson City, MO.

Ryck, F.M., Whitley, J.R., 1974. Pollution abatement in the lead mining district of Missouri. In: Proceedings of Purdue Industrial Waste Conference, vol. 29, pp. 857–863.

Schmitt, C.J., Finger, S.E., 1982. The dynamics of metals from past and present mining activities in the Big and Black River watershed, southeastern Missouri. Final Report to Army Corps of Engineers, US Fish and Wildlife Service, Columbia, Missouri.

Schmitt, C.J., Dwyer, F.J., Finger, S.E., 1984. Bioavailability of Pb and Zn from mine tailings as indicated by erythrocyte (d)-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catostomidae). Can. J. Fish. Aquat. Sci. 41, 1030–1040.

Schmitt, C.J., Brumbaugh, W.G., May, T.W., 2007a. Accumulation of metals in fish from lead–zinc mining areas of southeastern Missouri, USA. Ecotoxicol. Environ. Saf. 67, 14–30.

Schmitt, C.J., Whyte, J.J., Roberts, A.P., Annis, M.L., May, T.W., Tillitt, D.E., 2007b. Biomarkers of metals exposure in fish from lead–zinc mining areas of southeastern Missouri, USA. Ecotoxicol. Environ. Saf. 67, 31–47.

USEPA, 1996. 1995 updates: water quality criteria for the protection of aquatic life in ambient water, EPA-820-B-96-001. U.S. Environmental Protection Agency, Duluth, Minnesota.

USEPA, 1999. 1999 update of ambient water quality criteria for ammonia, EPA-822-R-99-014. US Environmental Protection Agency, Washington, DC.

USEPA, 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Report EPA/600/R-99/064, US Environmental Protection Agency, Duluth, Minnesota.

USEPA, 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, fourth ed. EPA-821-R-02-013. US Environmental Protection Agency, Washington, DC.

USEPA, 2004. National Recommended Water Quality Criteria. US Environmental Protection Agency, Washington, DC.

USEPA, 2005. Procedures for the Derivation of Equilibrium Partitioning Sediment Benchmarks (ESBs) for the Protection of Benthic Organisms: Metal Mixtures (Cadmium Copper, Lead Nickel, Silver, and Zinc). EPA-600-R-Q2-011, US Environmental Protection Agency, Washington, DC.

Whiteman, F.W., Ankley, G.T., Kahl, M.D., Rau, D.M., Balcer, M.D., 1996. Evaluation of interstitial water as a route of exposure for ammonia in sediment tests with benthic macroinvertebrates. Environ. Toxicol. Chem. 15, 794–801.

Wixson, B.G., 1977. The Missouri Lead Study, vol. 1: an interdisciplinary investigation of environmental pollution by lead and other heavy metals from industrial development in the New Lead Belt of southeastern Missouri. Final Report to National Science Foundation. University of Missouri, Columbia and Rolla, Missouri.

Wixson, B.G., 1978. Biogeochemical cycling of lead in the New Lead Belt of Missouri. In: Nraigu, J.O. (Ed.), The biogeochemistry of lead in the environment, Part A, Ecological cycles. Elsevier/North-Holland Biomedical Press, Amsterdam/New York/Oxford, pp. 119–136.

Xu, Y., Lay, J., Korte, F., 1988. Fate and effects of xanthates in laboratory freshwater systems. Bull. Environ. Contam. Toxicol. 41, 683–689.

Zhang, H., Davison, W., Miller, S., Tych, W., 1995. In-situ high resolution fluxes of Cd, Cu, Fe, and Mn and concentrations of Zn and Cd in pore water by DGT. Geochim. Cosmochim. Acta 59, 418–419.