Missouri Department of Natural Resources

Assessment of metal-contaminated sediments from the Southeast Missouri (SEMO) mining district using sediment toxicity tests with amphipods and freshwater mussels

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Executive Summary

We conducted an assessment of sediment quality of the Big River, which drains inactive lead mining areas in southeast Missouri (SEMO; St. Francois and Washington counties). This study was conducted to support a natural resource damage assessment and restoration (NRDAR) project for the SEMO lead mining district. Sediments were collected in September 2008 from 16 sites in the Big River (15 sites downstream of the St. Francois County mining area), one site in Mineral Fork (a Big River tributary that drains the Washington County mining area), two sites in the Meramec River (upstream and downstream of the mouth of the Big River) and one site in the Bourbeuse River (unaffected by mining activity). Sediments were processed by wet sieving to produce two size fractions for toxicity testing and chemical analyses: bulk sediments (<2 mm particle diameter) and fine sediments (<0.25 mm).

Sediments were analyzed for metal concentrations (total-recoverable metals and simultaneously-extracted metals or SEM), acid-volatile sulfides (AVS), total organic carbon (TOC) and particle-size distribution. Pore waters were prepared from bulk sediments by two methods (centrifugation and peeper samplers) and were analyzed for dissolved metals, dissolved organic carbon, major ions, and routine water quality parameters.

Metal toxicity hazards were evaluated using several sediment toxicity indices: (1) probable effects quotients (PEQ=total recoverable metal concentration/probable effect concentration; MacDonald et. 2000); (2) equilibrium-partitioning sediment benchmark index (ESB Index=ΣSEM-AVS/f_{OC}; USEPA 2005); and pore-water toxic units (pore-water metal concentration/water quality criteria; USEPA 2005).

Sediment toxicity tests were conducted with juvenile amphipods (*Hyalella azteca*) and juvenile fatmucket mussels (*Lampsilis siliquoidea*) according to standard methods (USEPA 2000, ASTM 2008a,b) with endpoints of survival, growth (length), and biomass determined after a 28-d exposure period. Amphipod tests were conducted with bulk sediments and mussel tests were conducted with fine sediments, to facilitate recovery of small mussels at the end of the exposure. Results of sediment toxicity tests were judged to be acceptable based on performance of test organisms in the control sediment, a wetted soil from Florissant MO.

Toxic effects of sediments were evaluated using a reference envelope approach. Four sites were classified as reference sites, based on low values of the metal toxicity indices,

including one site on the Big River upstream of mining areas (Site 1), two sites on the Meramec River (Sites 19 and 20) and one site on the Bourbeuse River (Site 21). Mean test responses for these reference sites were assumed to represent the normal range of responses of organisms to sediments from the study area in the absence of metal contamination. Statistical differences in toxicity endpoints among sediments were evaluated by analysis of variance (ANOVA). If the ANOVA for a particular endpoint was significant ($p<0.05$), the toxicity of individual sediments was evaluated by comparison to the reference envelope. Specifically, a response of organisms in individual sediments was designated as 'toxic' if the mean response was less than the lowest mean response in the reference sediments.

Sediments from the Big River showed strong longitudinal gradients of physical characteristics and metal contamination. Sediments from the upstream reach were dominated by sand-sized particles, but the proportion of fine (silt- and clay-sized) particles increased with distance downstream. Metal concentrations in Big River sediments (both bulk and fine fractions) increased dramatically in the reach close to the St. Francois County mining areas (Sites 2-4) and decreased gradually downstream. Metals were highly enriched in fine sediments in the upstream reach but were more evenly distributed across size fractions in the lower reach of the Big River.

Metal concentrations in sediment and in pore water indicated high risks of sediment toxicity in the Big River. Lead (Pb) concentrations in sediments and centrifuged pore water exceeded toxicity thresholds (sediment PECs and water quality criteria for pore water) throughout the entire reach of the Big River downstream of mining areas. Concentrations of zinc (Zn) and cadmium (Cd) in bulk and fine sediment exceeded PECs in the upstream reach downstream of mining areas (Sites 2-7). Other metal toxicity indices (sediment ESB index and water quality criteria for peeper pore waters) showed a different spatial pattern, with elevated values at some sites near the mining areas and some sites further downstream.

Big River sediments were more toxic to mussels than to amphipods. Mussel survival, growth, or biomass were reduced, compared to reference sites, in sediments from five sites in the reach near mining areas (Sites 2-6). In contrast, amphipod growth and biomass were reduced in sediments from three sites in the downstream reach (Sites 12, 13, and 15). Toxic effects on mussels in laboratory tests corresponded closely to reduced mussel taxa richness in field surveys (Appendix A-7; Roberts et al. in prep.), although fewer sites had toxic effects in laboratory tests (5 of 15 sites evaluated in both studies) than had reduced taxa richness in field surveys (9 of 15

sites). When results of both toxicity tests are considered (i.e., classify as toxic all sites with toxic effects on any endpoint for either amphipods or mussels), classification of sites based on laboratory results agreed with classifications based on mussel field survey results for 80% (12 of 15) sites. These results suggest that toxic effects on mussels in 28-d laboratory sediment toxicity tests are an accurate, but somewhat conservative, predictor of adverse effects in wild mussel populations.

Mussel toxicity endpoints were strongly associated with metal concentrations in Big River sediments. Mussel survival, growth, and biomass had significant negative correlations with concentrations of Pb, Cd, and Zn in both bulk and fine sediment size fractions, with most consistent correlations with Zn and Cd in fine sediments. Mussel toxicity endpoints also had significant negative correlations with percent sand and concentrations of TOC and AVS, reflecting the higher levels of these constituents occurring in metal-contaminated sediments. Amphipod toxicity endpoints did not have significant negative correlations with Zn, Cd, or Pb concentrations in sediments, but did have significant correlations with metal concentrations in pore water.

Sediment toxicity thresholds based on sediment Zn and Cd concentrations reliably predicted toxicity to mussels in laboratory tests and impacts on mussel communities in field surveys. Sediment toxicity thresholds based on PEQs for Zn and Cd (separately and in combination) in both bulk and fine sediments reliably predicted both toxic effects on mussels in the laboratory (85-100% of sites classified accurately) and reductions in mussel taxa richness in the field (93% accurate). Thresholds based on PEQs for Pb (or mixtures containing Pb) reliably predicted mussel taxa richness but were less reliable for predicting mussel toxicity. Impacts on mussel taxa richness were better predicted by lower thresholds (e.g., Zn or Cd PEQ >0.5) than those that predicted mussel toxicity (e.g., Zn or Cd PEQ >1.0).

Introduction

This study was conducted to support a natural resource damage assessment and restoration (NRDAR) project in streams associated with the southeast Missouri (SEMO) lead mining district. The SEMO lead mining district contains large piles and impoundments of mine tailings and other waste that cover thousands of acres of land. Movement of tailings and associated metals from these sites has led to extensive contamination of aquatic sediments and biota in streams that drain these areas, especially the Big River and its tributaries, which drain the St. Francois County and Washington County sites (Schmitt and Finger 1982; Schmitt et al. 1984, 1987). Concentrations of lead (Pb), cadmium (Cd), and zinc (Zn) in sediments from Big River exceed probable effects concentrations (PECs; MacDonald et al. 2000) have been reported at sites in a reach extending over 48 kilometers downstream from the St. Francois County mining area (MDNR 2003, Madden et al. 2006). Sediments that exceed PECs for one or more contaminants are associated with increased frequency of toxic effects on benthic invertebrates (Ingersoll et al. 2001, Ingersoll 2007) and MDNR (2003) documented reductions in benthic invertebrate density and diversity below the St. Francois County mining area.

Freshwater mussel populations in the Big River have also decreased in recent years in a reach extending nearly to the confluence of the Meramec River, over 150 km downstream from the St. Francois County mining areas (Roberts et al. in prep.). Buchanon (1979) and Oesch (1995) reported low mussel abundance and low mussel taxa richness in the Big River, presumably due to release of metal-contaminated mine tailings. Similar impacts have been reported for mussel communities of the Spring River system, which drains zinc-lead mining areas of the Tri-State (Missouri, Kansas, and Oklahoma) mining district (Angelo et al. 2007). Schmitt et al. (1987) reported elevated metal concentrations in soft tissues of freshwater mussels in the Big River below lead mining sites. Roberts and Bruenderman (2000) surveyed some of the same locations as Buchanan (1979) in the Big River and noted additional declines in mussel populations. The Meramec River downstream of the Big River supports some of the largest remaining populations of the federally endangered pink mucket (*Lampsilis abrupta*) and scaleshell (*Leptodea leptodon*), which may be affected by contaminated sediment from the St. Francois County and Washington County sites (Roberts and Bruenderman 2000).

Until recently, there was uncertainty associated with the reliability of data from

laboratory toxicity tests with freshwater mussels, because of their unique life history, because of limited expertise in laboratory culture of the sensitive early life stages, and because of a lack of standardization of test methods (Ingersoll et al. 2006). However, ASTM International (ASTM 2008a) has recently published a standard guide for conducting water-only laboratory toxic tests with glochidia and juvenile stages of freshwater mussels. Laboratory water-only toxicity tests have documented the high sensitivity of early life stages of mussels to several contaminants, including ammonia and some metals (e.g., Augspurger et al. 2007; Wang et al. 2007a,b,c). Intra- and inter-laboratory toxicity studies have demonstrated relatively uniform sensitivity of different mussel taxa to aquatic contaminants, indicating that testing conducted with surrogate mussel species (e.g., fatmucket, *Lampsilis siliquoidea*) may adequately define acute or chronic responses of listed mussel species (Wang et al. 2007a,b,c). Recent studies have adapted the ASTM (2008a,b) methods to include laboratory tests with contaminated sediments (Ingersoll et al. 2008).

This study reports results of chronic (28-d) toxicity tests with juvenile freshwater mussels (fatmucket, *Lampsilis siliquoidea*) and amphipods (*Hyalella azteca*) exposed to metal-contaminated sediments collected downstream of the St. Francois County and Washington County sites in the SEMO Mining District. The objective of these toxicity tests was to evaluate potential injury to mussel communities exposed to metal-contaminated sediment in the Big River drainage. Standardized sediment toxicity tests with amphipods (USEPA 2000; ASTM 2008b) were included to allow comparison of sediment toxicity tests conducted with mussels to previous studies of metal-contaminated sediments from other mining areas. Results of these toxicity tests with amphipods and mussels were evaluated relative to results of physical and chemical characterization of test sediments and relative to results of concurrent surveys of mussel abundance and taxa richness at most of the same study sites (Roberts et al. in prep.).

Methods

Site selection

Sediments were collected in September 2008 from 21 study sites in southeast Missouri (St. Francois, Washington, Jefferson, and Franklin Counties; Table 1, Figure 1). Seventeen sites

were located on the Big River, which drains most of the mining areas in St. Francois County. Additional sites were located on Mineral Fork, a tributary of the Big River that drains much of the Washington County mining area; on the Meramec River, upstream and downstream of the mouth of Big River; and on the Bourbeuse River, a tributary to the Meramec River that has no known mining areas in its watershed. Many of these sites were selected to correspond to locations sampled in mussel population studies (Roberts et al. in prep.), fish population studies (unpublished data; Mike McKee, Missouri Department of Conservation, Columbia MO) and population studies and in-situ toxicity studies with crayfish (Allert et al. in prep.).

Several sites were selected as possible reference sites for evaluation of toxicity test results. Sediments from these candidate reference sites were expected to have sediments low metal concentrations and to have physical-chemical characteristics (e.g., particle size distribution, organic carbon content) similar to sediments from sites on the Big River downstream of mining areas. Candidate reference sites included Site 1 on the Big River (upstream of mining) and sites on the Bourbeuse and Meramec Rivers (Sites 19, 20, and 21).

Collection of bulk and fine sediment samples

Composite samples of bed sediments were collected from depositional areas at each site. Sediments were collected from the top 10 cm of the sediment profile using PVC scoops (Ingersoll et al. 2008). These sediments were wet-sieved with a minimum quantity of site water using plastic wash buckets equipped with stainless steel mesh (2 mm pore diameter; Wildlife Supply Co, Buffalo NY), to remove coarse sediments and detritus (Ingersoll et al. 2008). Sieved sediment (henceforth called bulk sediment) and rinse water were collected in acid-washed 20-L polyethylene buckets, as needed to ensure collection of 20 L of settled bulk sediment from each site. The resulting composite samples were stored in the dark at 4 ºC in a refrigerated truck (in the field) and in a walk-in cooler. After each site, scoops and wash buckets were scrubbed with nylon brushes and site water to remove sediment particles and stored in clean plastic bags between sites.

After 11 to 14 days of cold storage, bulk sediments from each site were processed in preparation for toxicity testing and chemical analyses. Bulk sediments from each site were homogenized using an electric drill and stainless steel auger, incorporating enough of the

overlying site water to allow easy mixing. A portion of the homogenized bulk sediment from each site was then wet-sieved through a stainless steel #60 sieve using a minimum quantity of site water to obtain about 500 ml of sediments smaller than 0.25 mm particle diameter (henceforth called fine sediments) for use in mussel toxicity tests and associated analyses. The fine sediment fraction was used for the mussel toxicity tests to allow recovery of the small juvenile mussels (typical shell length 1-2 mm) from sediments at the end of the tests (Ingersoll et al. 2008).

Analysis of whole sediment and pore water

Sediments and pore waters were analyzed to characterize metal concentrations and other characteristics that may affect toxicity of metals. Analyses were conducted at CERC unless otherwise indicated. Analytical methods and performing laboratories are summarized in Appendix A-1. Bulk sediments from each site were analyzed to determine percent water (by weight), particle size distribution (hydrometer method) and total organic carbon (LECO carbon analyzer). Samples of both bulk and fine sediments samples from each site were analyzed for total recoverable metals and other elements by inductively-coupled plasma-mass spectrometry (ICPMS) in semi-quantitative mode (Brumbaugh and May 2008) using a Perkin-Elmer/Sciex ELAN DRC-e. Pore waters extracted from bulk sediment samples by centrifugation (20 minutes at 7,000 g at 15 °C) were filtered (polypropylene cartridge filter, nominal 0.45-µm pore diameter) and analyzed for dissolved metals by semi-quantitative ICPMS, dissolved organic carbon (OI Model 700 TOC analyzer), major cations (by ICP-atomic emission spectroscopy) and anions (ion chromatography), and routine water quality parameters (Appendix A-1).

Metal bioavailability was further characterized in samples of bulk sediments that were carried through the amphipod toxicity test in extra test beakers (chemistry beakers). Chemistry beakers were stocked with amphipods and treated identically to other test beakers, except that passive pore-water samplers (‘peepers’; Brumbaugh et al. 2007) were deployed in chemistry beakers. Peepers consisted of polyethylene vials containing a small volume (2.9 mL) of deoxygenated deionized water, which was separated from sediment particles by polyethersulfone filters with 0.45 µm pore diameter). Peepers were deployed by inserting them surficial into 100 mL of sediments for 7 d (test days 0-7) to allow the water in peepers to equilibrate by diffusion

with dissolved constituents in the pore water. Sediments from chemistry beakers were collected at the time of peeper retrieval for analysis of acid-volatile sulfide (AVS) and simultaneously-extracted metals (SEM) (Brumbaugh and Arms 1997). Peeper samples and SEM extracts were analyzed for Pb, Zn, Cd, copper (Cu) and nickel (Ni) by ICPMS in quantitative mode (Appendix A-1).

Quality assurance/quality control

Results of quality control (QC) samples indicated satisfactory accuracy and precision of analyses for the five primary metals of interest (Pb, Cd, Zn, Cu, and Ni). Results for spiked samples, replicate samples, and reference samples are summarized in Appendix A-2. All of those results were within targeted ranges (i.e., 80-120% recovery from spikes and reference samples; <20% variation for replicated samples). Recoveries of the five metals of interest from a reference sediment using the AVS/SEM extraction method ranged from 34% to 71% of certified total concentrations, within the typical ranges historically obtained at CERC using this method. Complete recovery of sediment metals is not expected when using this weak acid extraction, so results of SEM analyses were not adjusted for differences in recovery.

Additional QC samples analyzed included continuing blank and calibration verification solutions, a laboratory control sample, a five-fold dilution check for selected samples, an interference check solution, blanks, and method detection limit determinations (Appendix B). Among these additional QC measures, the only result of concern was the finding of elevated Zn in the peeper blanks. However, these elevated Zn concentrations were later determined to be associated with leaching from the container used to store the blank peepers after the remainder had been deployed in the sediment samples. That finding, combined with the fact that most of the sample peepers had much lower concentrations than the blank peepers, indicate that peeper blank concentrations did not accurately reflect blank contributions for peepers actually deployed in sediment samples.

Analysis of duplicate samples of sediment and pore water for other constituents of sediment and pore water indicated high reproducibility of analytical methods. Relative percent difference (difference/mean) for duplicate samples ranged from 0% to 13% (Tables 2 and 3).

Metal toxicity indices

Because metal toxicity in environmental samples represents cumulative toxicity of multiple metals with similar modes of toxic action (USEPA 2005), metal toxicity risks were estimated for each site using three different indices of metal-mixture toxicity based on metal concentrations measured in sediments and pore waters:

1. Probable effect quotients (or PEC-based hazard quotients; PEQ=total-recoverable metal concentration/PEC; Ingersoll et al. 2001, Besser et al. 2008). PEQs were calculated for individual metals and mean PEQs were calculated for various combinations of metals in each sediment (Table 4 and Appendix A-3) to estimate the toxicity risks of metals mixtures (MacDonald et al. 2000; USEPA 2000b; Ingersoll et al. 2002, 2009).

2. Equilibrium-partitioning sediment benchmark index (ESB index; USEPA 2005). The ESB index is calculated as the molar sum of SEM metal concentrations (Cd, Cu, Pb, Ni, Zn) minus the molar concentration of AVS, normalized to the TOC fraction of sediment (ΣSEM-AVS/*f*oc; Table 5). USEPA (2005) predicted that sediment metal mixtures would not be toxic in sediments with values of the ESB index less than 130 µmol/g organic carbon (OC), that toxicity would be uncertain between 130 and 3,000 µmol/g OC, and that toxicity would occur in sediment with values of 3,000 µmol/g OC or greater.

3. Toxic units (or criteria units; USEPA 2005) based on metal concentrations in sediment pore waters. Toxic units for individual metals (Cd, Cu, Pb, Ni, and Zn) were calculated by dividing dissolved metal concentrations by the hardness-based chronic water quality criterion for each metal (USEPA 2006) and toxic units for were summed for each sample (Table 6). Sediments with less than 1.0 pore-water toxic units are predicted to be non-toxic (USEPA 2005).

These metal toxicity indices were used to verify that candidate reference sediments had low metal concentrations associated with minimal risks of metal toxicity. Specifically, candidate reference sediments were considered to be acceptable if metal toxicity indices were: (1) mean PEQ (for Cd, Cu, Ni, Pb, and Zn) less than 0.2, (2) ESB index less than 130 µmol/g OC, and (3)

sum of pore-water toxic units (for Cd, Cu, Ni, Pb, and Zn) less than 1.0 (Table 7; Ingersoll et al. 2009, MacDonald et al. 2009).

Sediment toxicity testing

Sediment toxicity tests were conducted using juvenile (about 2 months old) mussels and juvenile (about 7 days old) amphipods (Appendix A-4). Amphipods were obtained from cultures maintained at CERC (Ingersoll et al. 2002) and the juvenile mussels were propagated following methods outlined by Wang et al. (2007c). Amphipod toxicity tests were conducted with bulk sediments according to published test methods (ASTM 2008b; USEPA 2000). Mussel toxicity tests were conducted with fine sediments using methods adapted from ASTM (2008a,b) and USEPA (2000) as described by Ingersoll et al. (2008). Both tests lasted 28 days, with endpoints of survival, growth, and biomass. Growth of individual animals was assessed by digital measurement of body length (amphipods) or shell length (mussels). Total biomass for each replicate was determined using length-weight relationships for amphipods or direct measurements of dry weight for mussels (Ingersoll et al. 2008). A negative control sediment (wetted soil from Florissant MO; Ingersoll et al. 1998) was be tested concurrently with field-collected sediments to characterize performance of test organisms relative to control test acceptability criteria established by ASTM (2008a,b) and USEPA (2000). The cohort of mussels used for sediment toxicity tests was tested concurrently in a water-only reference toxicant test with sodium chloride (NaCl). The median lethal concentration (LC50) in a 96-h exposure was 3.0 g/L of NaCl (95% confidence limits, 2.5 to 3.6 g/L). This LC50 is consistent with results of previous reference toxicant tests conducted with fatmucket mussels at our laboratory in ASTM hard water (e.g., LC50s of 3.1 and 3.3 g/L; Ingersoll et al. 2008). Similarly, the LC50 for amphipods in a 48-h NaCl exposure was 5.3 g/L (95% confidence limits, 4.8-5.9 g/L), similar to results of recent NaCl reference toxicant tests conducted with amphipods at our laboratory in ASTM hard water (e.g., LC50s of 5.7 to 6.1 g/L; Ingersoll et al. 2008).

Toxicity tests were conducted in temperature-controlled water baths (23 ^{o}C) with automated replacement of overlying water (Ingersoll et al. 1998; Appendix A-4). The overlying water used in the tests was CERC well water diluted with de-ionized water to a hardness of 200 mg/L as $CaCO_3$, typical of water quality at sediment collection sites in the Big River watershed

(unpublished data from USGS National Water Information System web site http://waterdata.usgs.gov/nwis/sw). Sediment and overlying water were placed in exposure chambers with test water (under static conditions) one week before the start of the toxicity tests to allow re-equilibration of sediment and pore water (Ingersoll et al. 2008). Automated replacement of overlying water started on Day -1 (the day before test organisms were added to the sediments). Table 3 summarizes pore-water chemistry data and Appendix A-5 summarizes overlying water quality measured during the sediment exposures.

Data analyses

Statistical analyses were performed using SAS statistical software (SAS/STAT version 9.2; SAS Institute, Cary NC). Differences in toxicity endpoints among sites were determined by analysis of variance (ANOVA). Toxicity data were transformed before ANOVA to improve normality, as indicated by the Shapiro-Wilks test, in accordance with guidance from USEPA (2000) and ASTM (2008a,b). If transformations (arcsine square root for survival; square root or log for growth) did not improve normality, data were rank-transformed before analysis (Conover and Iman 1981). Pearson correlation coefficients were used to evaluate relationships between responses in the toxicity tests and physical or chemical characteristics of sediments.

Toxicity endpoints were also evaluated using a reference envelope approach. This approach compares responses of test organisms in test sediments to responses in reference sediments (Hunt et al. 2001, Ingersoll and MacDonald 2002, Ingersoll et al. 2009). The reference envelope, or the range of mean responses observed in reference sediments with minimal levels of metal contamination (as described above) was assumed to represent the normal range of responses of test organisms in uncontaminated sediments in the study area. Test sediments were classified as toxic if they mean responses of one or more toxicity endpoint (survival, growth, or biomass) were less than the lowest mean for reference sites (Table 7).

Reliability of sediment toxicity thresholds

We evaluated the reliability of sediment toxicity thresholds (STTs) based on metal toxicity indices for assessment of injury to benthic invertebrates and other aquatic receptors in the SEMO study area. STTs were used to classify sediment samples as contaminated or not contaminated, and the reliability of STTs were evaluated using procedures established by MacDonald et al. (2003; 2005a,b). An STT was considered to be reliable if the incidence of toxicity was less than 20% below the STT and greater than 50% above the STT, and if the overall correct classification rate was greater than 80%. Thresholds that met these criteria were considered to provide a reliable basis for classifying sediment samples as toxic or not toxic, with an overall error rate of less than 20%. Reliable STTs would also minimize the potential for false-negative errors (i.e., the frequency of toxicity below the STT would be less than 20%) and would have a low probability of false-positive results (i.e., frequency of non-toxic samples above the STT would be less than 50%).

Results and Discussion

Characteristics of whole sediments and pore waters

Bulk sediments (sieved to <2 mm particle diameter) from Big River sites generally followed a gradient from a predominance of sand-sized particles in the upstream reach (i.e., lower numbered sites; Figure 1) to greater proportions of silt- and clay-sized particles in the downstream reach (Table 2). Big River sediments had low to moderate concentrations of TOC and AVS, constituents that are important controls on metal bioavailability, and these parameters did not follow clear upstream-downstream trends (Table 2).

Sediment pore waters prepared by centrifugation were slightly alkaline (pH range, 7.41-8.02) and were strongly buffered with carbonates (Table 3). Pore waters from all Big River sites downstream of mining areas had high hardness (224-350 mg/L as $CaCO_3$) and most had high DOC concentrations (>10 mg/L). Both of these characteristics tend to reduce the bioavailability and toxicity of dissolved metals.

Concentrations of total-recoverable of Pb, Zn, and Cd were elevated in both bulk sediments (Table 4a) and fine sediments (Table 4b) from sites downstream of mining areas. Lead

concentrations exceeded PECs in 14 of 15 samples of bulk sediment and all 15 samples of fine sediment from sites between the Leadwood tailings pile and the confluence with the Meramec River. Bulk sediments in the reach from Site 2 to Site 6 (Hwy EE) and fine sediments in the reach from Site 2 to Site 7 (Hwy CC) also exceeded PECs for Zn and Cd. Overall levels of metal contamination, as indicated by mean PEQs for five metals (Table 4, Appendix A-3) peaked further upstream (Site 2) for fine sediments than for bulk sediments (Site 4). High levels of sediment metal contamination persisted throughout the reach from Site 2 to Site 10 (excluding Site 8 on Mineral Fork). Mean PEQs in this reach exceeded 1.0 for bulk sediments and 3.0 for fine sediments.

Metal concentrations in Big River sediments showed strong longitudinal gradients downstream of mining areas. Metal concentrations in the fine sediment fraction decreased by a nearly a factor of five between the upstream reach of the Big River (Sites 2-7) and the downstream reach (Sites 9-18), compared to a two-fold decrease in metal concentrations for bulk sediments. In the upstream reach, PEQs for the fine sediment fraction averaged 2.7 times greater than PEQs in bulk sediments, compared to a factor of 1.3 for sediments from the downstream reach. Concentrations of SEM in bulk sediment samples exceeded the binding capacity of AVS at 13 of 15 Big River sites downstream of mining areas, indicating that some fraction of these metals was potentially bioavailable at these sites (Table 5). Eight Big River sites downstream of mining areas had values of the ESB index in the range that indicates an increased risk for metal toxicity (>130 μmol/g OC; USEPA 2005).

Pore-water samples obtained by centrifugation (Table 6a) and from peeper samplers (Table 6b) indicated high concentrations of Pb, and to a lesser extent Cd, in many Big River samples. Pore-water toxic units calculated for filtered centrifuged pore waters indicated elevated risks of Pb toxicity (i.e., toxic units >1.0) at all Big River sites downstream of mining areas, and elevated risks of Cd toxicity at six sites downstream of mining areas (Table 6a). Metal concentrations and toxic units were consistently lower for pore-water samples collected with peepers, especially for Pb (Table 6b). This difference between sample types suggests that some of the Pb measured in centrifuged samples may have consisted of solid-phase (colloidal) particles that may be less toxic than dissolved metals (USEPA 2005). The nominal pore diameter of the filters used for both types of pore water sample was 0.45 microns, but the more variable pore sizes in PP-fiber filters may have allowed more particles to pass through into pore

water samples, compared to the discrete pores of PES filters. Consequently, analyses of centrifuged pore water may have included greater amounts of colloidal metals. This difference could have been exacerbated by the greater pressure generated by the syringe filter apparatus, compared to passive diffusion through the peeper membrane. Toxic units for peeper samples indicated toxicity risks for Pb in seven samples and for Cd in one sample (Table 6b).

Sediments from Site 8 in Mineral Fork, which drains much of the Washington County mining area, had relatively low levels of metal contamination. Sediment Pb concentrations exceeded the PEC for fine sediments, but not bulk sediments (Table 4a,b) and all other metals were below PECs in both sediment fractions. Neither the ESB Index (Table 5) nor pore-water toxic units (Table 6) indicated elevated risks of toxicity from this sediment. However, samples from Site 8 may not be representative of levels of metal contamination at sites closer to mining areas.

The four candidate reference sediments were screened using the three metal toxicity indices and found to be substantially free of metal contamination (Table 7). All four sites had mean PEQs less than 0.2 (for five metals) in both bulk sediments used in the amphipod test and fine sediments used in the mussel toxicity test. Mean PEQs or 0.2 for the five metals is assumed to represent a risk of toxicity comparable to a single metal occurring at its PEC (Ingersoll et al. 2002, 2009; MacDonald et al. 2004, 2005]). All four reference sediments also had values of the sediment ESB index less than 130 µmol/g OC and pore water toxic units index less than 1.0, with both values predictive of no metal toxicity (USEPA 2005).

Sediment toxicity

Control performance for both toxicity test organisms met test acceptability requirements of greater than 80% survival of test organisms in control sediment (Tables 8 and 9; Appendix A-4). Water quality of overlying water (Appendix A-5) was consistent among treatments for both tests and concentrations of ammonia and dissolved oxygen were maintained within acceptable ranges (ASTM 2008a,b; USEPA 2000). Survival, growth and biomass of controls in amphipod and mussel tests were similar to or greater than the mean for the four reference sites (Table 7). Variation among mean responses for reference sites was greatest for biomass (both species) and lowest for amphipod survival and mussel length.

Amphipods showed relatively little toxicity in tests with bulk sediments (Table 8). Five sites had mean survival less than the lowest mean for reference sites, but the range of mean survival among sites was narrow (minimum survival=83% for Site 4), and the ANOVA indicated that amphipod survival did not differ significantly among sites. Amphipod growth and biomass varied more widely than survival among sediments and ANOVAs indicated significant differences among sites for both endpoints (Table 8). The reference envelope approach indicated toxic effects on amphipod growth and biomass in sediments from three sites in the middle reach of Big River (Sites 12, 13, and 15).

Mussels showed greater responses in toxicity tests with fine sediments, with ANOVAs indicating that all three mussel endpoints differed significantly among sites (Table 9). The three endpoints followed similar trends among sites, with toxic effects indicated by the reference envelope method for the five sites closest to the St. Francois County mining areas (Site 2 through Site 6). Fine sediments from all five sites in this reach caused reduced mussel growth (mean shell length), with four sites having reduced biomass and three sites having reduced survival. Lowest mussel survival (35%) occurred in sediment from Site 3, lowest growth occurred in sediment from Site 6, and lowest biomass occurred in sediment from Site 5. There were no toxic effects on mussels in toxicity tests with sediments from the Mineral Fork (Site 8) or sediments from sites in the reach of the Big River from Site 7 (Hwy CC) to Site 18 (near the confluence with the Meramec River).

The responses of mussels and amphipods in toxicity tests with SEMO sediments followed very different geographic patterns. All fine sediments that were toxic to mussels (reduced survival, growth, or biomass) were collected from sites in the upstream reach, near the large tailings deposits of the St. Francois mining area. In contrast, bulk sediments that were toxic to amphipods (reduced growth or biomass) were collected from sites in the downstream reach. No sites were classified as toxic to both species. As a result, the amphipod tests and the mussel tests agreed on the classifications of sediments as toxic or non-toxic for only 60% of sites (Table 10).

Relationships between laboratory toxicity tests and field surveys

Results of the laboratory sediment toxicity tests with mussels corresponded closely to results of concurrent field surveys of mussel communities reported Roberts et al. (in prep).

Qualitative surveys (timed searches) of mussel taxa richness and abundance were conducted at 15 sites located close to sites of sediment collection for toxicity testing (Appendix A-7) and quantitative surveys (quadrat counts) of mussel density were conducted at seven sites. Results of both qualitative and quantitative field surveys (Appendix A-7) were in good agreement with results of sediment toxicity tests conducted with mussels, but not with results of sediment toxicity tests with amphipods (Table 10). Overall, classification of sites based on laboratory toxicity tests with amphipods (i.e., toxicity to any amphipod endpoint) agreed with classification based on field mussel surveys (i.e., reduced mussel taxa richness) for only six of the 15 common study sites (40%). In contrast, classification of sediments as toxic based on mussel toxicity tests agreed with classification based on mussel taxa richness in the field survey for 11 sites (73%). All five sites with sediments that were toxic to mussels in laboratory tests had reduced mussel taxa richness in the field survey. When results of both toxicity tests are considered (i.e., sites with toxic effects on any endpoint for either amphipods or mussels), laboratory results agreed with mussel field survey results for 12 sites (80%) (Table 4d).

Relationships between laboratory and field responses of mussels are illustrated in Figure 2. Sites with reduced mussel growth in sediment toxicity tests consistently had low mussel taxa richness (Figure 2a) and low mussel abundance (catch per unit effort; Figure 2b). Both qualitative and quantitative mussel sampling identified impacts on mussel communities at several sites (4 of 13 non-reference sites in the qualitative survey and 3 of 5 non-reference sites in the qualitative study; Appendix A-7) that did not show toxicity in mussel toxicity tests (Table 10). These results suggest that toxic effects on mussels in 28-d laboratory sediment toxicity tests are conservative predictors of adverse effects in wild mussel populations.

Relationships between toxicity endpoints and sediment characteristics

The responses of the two test organisms in laboratory toxicity tests followed very different patterns among sites, resulting in significant negative correlations between mussel endpoints (survival, growth, and biomass) and amphipod endpoints (growth and biomass; Table 11). Mussel and amphipod toxicity endpoints also followed different trends with respect to mussel taxa richness or CPUE from the field survey, although none of the correlations of toxicity endpoints with field survey metrics were statistically significant. Mussel toxicity endpoints had

positive (non-significant) correlations with taxa richness and CPUE, whereas amphipod endpoints had negative (non-significant) correlations with these metrics.

These differences between responses in the amphipod and mussel toxicity tests was reflected in different patterns of correlation with metal concentrations and other characteristics of SEMO sediments and pore water (Table 11). Toxic effects on mussels was only observed in sediments from sites near the St. Francois County mining area, which had highest levels of metal contamination, and all three mussel toxicity endpoints had significant negative correlations with PEQs for Zn, Cd, and Pb and with mean PEQs for metal mixtures (in fine sediments). In contrast, toxic effects on amphipod growth and biomass occurred in sediments from sites further downstream from mining areas and no amphipod endpoints had significant negative correlations with metal PEQs in bulk sediments. Mussel and amphipod endpoints also had very different patterns of association with pore-water metal concentrations and metal toxic units. Amphipod endpoints had significant negative correlations with pore-water Pb (in centrifuged samples), Zn (in peeper samples), and summed pore-water toxic units (in both sample types), whereas mussel growth and biomass had significant positive correlations with pore-water Pb and toxic units (in centrifuged samples). Amphipod and mussel endpoints also had opposite patterns of association with percent sand, TOC, and AVS: significant positive correlations for amphipod endpoints, significant negative correlations for mussel endpoints. These contrasts reflect differences in characteristics of sediments from the upstream reach (Site 2 through Site 6) that were most toxic to mussels, compared to sediments from the downstream reach (Site 12 through Site 15) that were toxic to amphipods. Sediments from the upstream reach had high sediment metal concentrations (especially in fine sediments), lower pore-water metal concentrations, and higher levels of sand, TOC, and AVS.

The responses of mussels and amphipods to sediments and pore waters from the SEMO study area were somewhat different from those observed in a 2007 toxicity study with these same species exposed to metal-contaminated sediments (fine sediments for mussels, bulk sediments for amphipods) from the Tri-State Mining District of Missouri, Kansas, and Oklahoma (Ingersoll et al. 2008). In the Tri-State study, toxic effects on the two species followed similar trends with respect to metal concentrations in sediment and pore water, although amphipods were more sensitive than mussels (MacDonald et al. 2009). The primary metals of concern (Pb, Zn, and Cd) were the same in both Tri-State and SEMO study areas, but the relative abundance

of these metals in sediment and pore water differs markedly between the two areas. Most notably, concentrations of Zn and Cd in both sediment (based on PEQs) and pore water (based on toxic units) were substantially greater in toxic sediments from the Tri-State study area than in sediments from either the upstream or downstream reach of the Big River in the SEMO study area (Table 12). Toxic sediment samples from the two study areas had similar Pb concentrations in sediments (bulk and fine fractions) and in pore water (peepers), but Pb concentrations in centrifuged pore waters were substantially greater for SEMO sediments, especially those from the downstream reach. The lack of significant amphipod toxicity in the contaminated SEMO sediments from the upstream reach of the Big River is consistent with the lower concentrations of Zn and Cd in bulk sediments and pore waters, compared to toxic sediments from the Tri-State study area (Table 12). The reduced amphipod growth in SEMO sediments from the downstream reach, despite lower sediment metal concentrations, reflects greater Pb concentrations in pore waters. The range of pore-water (peeper) Pb concentrations in sediments from the downstream reach of the Big River that were toxic to amphipods is consistent with concentrations associated with significant toxic effects on amphipods in water-only toxicity tests (Besser et al. 2005).

Toxic effects on mussels in SEMO sediments from the upstream reach were consistent with high concentrations of metals (especially Pb, Zn, and Cd) in fine sediments, but not with the relatively low pore-water metal concentrations in these sediments. This discrepancy suggests that toxic effects on mussels exposed to SEMO sediments were primarily caused by exposure to metal-contaminated sediment particles rather than exposure to aqueous metals in pore water. The greater sensitivity of mussels to metal-contaminated sediments from the SEMO study area, compared to sediments from the Tri-State area, may be related to the use of younger juvenile mussels (2 months old) in tests with SEMO sediments, compared to somewhat older juveniles (3-4 months old) used in the Tri-State study. Although sensitivity to toxic effects of aqueous metals may not change substantially with age for juvenile mussels (unpublished data; Chris Ingersoll, USGS, Columbia MO), juvenile mussels undergo a transition in feeding habits from primarily benthic feeding (pedal feeding on benthic particles and filter feeding of particles from pore water) to filter feeding from overlying water (Yeager et al. 1994, Gatenby et al. 1996). Hence, the younger juvenile mussels tested with SEMO sediments may have experienced greater metal exposure via ingestion of metal-contaminated sediment particles.

Metal concentrations in sediments from the SEMO study area were strongly associated

with adverse effects on mussels in both laboratory toxicity tests and field surveys. Mussel growth (shell length) in toxicity tests was reduced in fine sediments with elevated metal concentrations. The five fine sediments that caused toxic effects on mussel growth also had highest PEQ or mean PEQ values For Zn (Figure 3a), Cd (Figure 3b), and the Zn-Cd mixture (not shown). This relationship was weaker for Pb (Figure 3c) and for metal mixtures that included Pb, because several fine sediments with high Pb concentrations did not cause toxicity in the mussel toxicity test. The sediment ESB index (based on concentrations of five metals, AVS and TOC in bulk sediments) also had a weak association with mussel growth. Mussel taxa richness in the field survey followed similar overall trends with respect to metal PEQs, but severe reductions on mussel taxa richness occurred at lower concentrations of Pb, Zn, and Cd than those associated with reduced mussel growth in the laboratory tests (Figure 4a and 4b). In contrast to the results of mussel toxicity tests, field surveys showed consistent decreases in mussel taxa richness with increases in association with Pb PEQs (Figure 4c). This contrast was especially evident in the reach of the Big River downstream of the Mineral Fork. Sites in this reach had lower concentrations of Zn and Cd in fine sediments (less than PECs) and were not toxic to mussels in laboratory tests, but several of these sites (Sites 9, 10, and 12) had reduced mussel taxa richness. This difference may be related to elevated levels of Pb in fine sediment, which remained above the Pb PEC throughout the lower reach of the Big River.

The greater impacts on mussel populations in the field, compared to toxic effects in sediment toxicity tests, probably reflect differences in both the duration and the nature of the metal exposure. Juvenile mussels used in laboratory toxicity tests have already survived the glochidia stage and the difficult transition to the juvenile stage. The short (28-d) duration of the laboratory test with juvenile mussels represents a small portion of the first growing season for juvenile mussels, during which time they must have robust growth to decrease their vulnerability to predators and to accumulate energy stores to survive their first winter, and a much smaller portion of the multi-year period before reproductive maturity (Bauer 2001). In addition, mussels in the field probably experience greater exposure via consumption of metal-contaminated particles (both bed sediments and suspended particulates), compared to the uncontaminated diets provided during the toxicity test. Alternatively, mussel populations in the field may be responding to other factors, such as water quality or habitat quality, independent of or in combination with metal contamination. Roberts et al. (in prep.) reported significant negative

correlations of mussel taxa richness and CPUE with concentrations of Pb, Zn, and Cd in both bulk and fine sediments from mussel survey sites, along with significant correlations with several habitat variables (embeddedness, sediment deposition, and channel stability). They concluded that metal contamination of sediments downstream of the St. Francois County mining area was the primary driver for reductions in mussel diversity and abundance in the Big River.

Evaluation of toxicity thresholds

Sediment toxicity thresholds (STTs) based on metal concentrations in sediment and pore water had varying success in predicting the incidence of toxicity in laboratory toxicity tests (Table 13 and Appendix A-8). These STTs were selected based on findings of recent studies (MacDonald et al. 2008) and on sediment quality benchmarks recommended by USEPA (2005). Thresholds were judged to be highly reliable if they met the following criteria: (1) less than 20% incidence of toxicity below the STT, (2) greater than 50% incidence of toxicity above the SST, and (3) greater than 80% overall correct classification of the sediment samples (toxic or not toxic) based on the STT (MacDonald et al. 2002; 2003; 2005a,b; 2008). None of the STTs were reliable predictors of amphipod toxicity endpoints, but several STTs reliably predicted mussel toxicity endpoints. All three mussel toxicity endpoints were reliably predicted by both high and low PEQ-based thresholds for Zn and Cd, separately and in combination (high threshold; PEQ or mean PEQ=1.0). These STTs were successfully applied to data for both fine and bulk sediments, producing correct predictions for 85% to 90% of sites. In contrast, only the high STTs for Pb or mixtures containing Pb (PEC or mean PEC=5.0) met the reliability criteria. Predictions based on Pb or mixtures containing Pb were consistently reliable for the mussel biomass endpoint. Thresholds based on the Pb- Zn-Cd mixture were more reliable than other STTs based on Pb, but less reliable than STTs based on Zn and/or Cd.

Relationships between mussel growth (shell length) and STTs are shown in Figure 3. Figures 3a and 3b show consistent reductions in growth of mussels in sediments with elevated Zn or Cd concentrations. Five of six sites that had PEQs of 1.0 or greater for Zn or Cd in fine sediments were toxic to mussels. These plots indicate that STTs of 1.7 PEQ for Zn and 2.4 PEQ for Cd would accurately categorize 100% of sites. In contrast, Figures 3c and 3d show that sites exceeding the high STT for Pb-PEQ or the low threshold for the ESB Index included roughly

equal number of toxic and non-toxic sediments.

Mussel taxa richness determined during field surveys (Roberts et al. in prep.) was also reliably predicted by STTs based on PEQs for sediments used in laboratory toxicity tests (Table 13). For PEQ-based STTs, predictions of impacts on taxa richness were generally more accurate for the low STT values applied to data from fine sediments. The six PEQ-based STTs for fine sediments met all criteria for reliability and correctly classified 80 to 93% of sites (Table 13b). In contrast, none of the STTs for bulk sediment based on the SEM mixture met reliability criteria, and these STTs only classified 40 to 73% of sites correctly (Table 13a). The greater reliability and accuracy of the low STTs for predicting reduced mussel taxa richness reflects the fact that adverse effects on mussel communities in the field occurred at lower levels of metal contamination than those affecting growth in the laboratory. Mussel taxa richness in field surveys decreased sharply with increasing concentrations of sediment metals. All eight sites with Zn or Cd PEQs greater than the low STTs (PEQ=0.5) had reduced mussel taxa richness compared to historic data (Figures 4a and 4b; Roberts et al. in prep.). Taxa richness at sites exceeding the low STTs ranged from zero to three, compared to a range from six to 26 taxa at reference sites. Similarly, eight of nine sites that exceeded the high STT for sediment Pb (Pb-PEQ=5.0) had reduced taxa richness. In contrast, the ESB Index (Figure 4d) had a weak predictive relationship with mussel taxa richness, with several impacted sites having values of this index in the range predicted by USEPA (2005) to have no toxicity (<130 μmol/g OC). The poor predictive ability of this index, which is based on predicting the presence of dissolved metals in pore water (Ankley et al YEAR, USEPA 2005), is consistent with our hypothesis that adverse effects on juvenile mussels may reflect exposure to metal-contaminated particulates.

Conclusions

1. Sediments from the Big River showed strong longitudinal gradients of physical characteristics and metal contamination. Sediments from the upstream reach were dominated by sand-sized particles, but the proportion of finer (silt- and clay-sized) particles increased with distance downstream. Metal concentrations in Big River sediments increased dramatically in the upstream reach close to the St. Francois County mining area (Sites 2-4) and decreased gradually downstream. Metals were highly enriched in fine sediments in the

upstream reach but were more evenly distributed across size fractions in the downstream reach.

2. Metal concentrations in sediment and pore water indicated high risks of sediment toxicity in the Big River. Lead concentrations in bulk and fine sediments and in pore water exceeded toxicity thresholds (PECs for sediment and water quality criteria for centrifuged pore water) throughout the entire reach of the Big River downstream of mining areas. Concentrations of Zn and Cd in sediment exceeded PECs in the upstream reach near mining areas (Sites 2-7). Other metal toxicity indices (sediment ESB index and water quality criteria for peeper pore water) showed a different spatial pattern, with elevated values at some sites near the mining areas and some sites in the downstream reach.

3. Big River sediments were more toxic to juvenile fatmucket mussels (*Lampsilis siliquoidea*) than to juvenile amphipods (*Hyalella azteca*). One or more mussel toxicity endpoints (survival, growth, and/or biomass) were reduced, compared to reference sites, by fine sediments from five sites in the reach near mining areas (Sites 2-6). In contrast, amphipod growth and biomass was reduced by bulk sediments from three sites in the downstream reach (Sites 12, 13, and 15). Toxic effects on mussels in laboratory tests corresponded closely to reduced mussel taxa richness in field surveys reported by Roberts et al. (in prep.), although fewer sites had toxic effects on mussels (5 of 15 sites evaluated in both studies) than had reduced mussel taxa richness (9 of 15 sites). When results of both toxicity tests are considered (i.e., sites with toxic effects on any endpoint for either amphipods or mussels), laboratory results agreed with mussel field survey results for 80% of the sites. These results suggest that toxic effects on mussels in 28-d laboratory sediment toxicity tests are conservative predictors of adverse effects in wild mussel populations.

4. Sediment from a site near the mouth of Mineral Fork (Site 8), which drains the Washington County mining district, had lower metal concentrations in bulk sediments, fine sediments, and pore waters compared to sediments from the reach of the Big River downstream of the St. Francois County mining district. Lead concentrations in fine sediments from this site exceeded the PEC, but metal concentrations at this site did not exceed sediment quality

guidelines for metal mixtures in sediments or pore waters (mean PEQ, ESB index, or pore-water toxic units). Sediments from this site were not toxic to either mussels or amphipods.

5. Mussel toxicity was strongly associated with metal concentrations. Mussel toxicity endpoints had significant negative correlations with concentrations of Pb, Cd, or Zn in both bulk and fine sediment size fractions, with consistently strong correlations with Zn and Cd in fine sediments. Mussel toxicity also had significant negative correlations with percent sand and concentrations of TOC and AVS in bulk sediments, reflecting the higher levels of these constituents in metal-contaminated sediments. Amphipod toxicity did not have significant negative correlations with Zn, Cd, or Pb concentrations in bulk or fine sediments, but did have significant correlations with metal concentrations in centrifuged and peeper pore water.

6. Sediment toxicity thresholds based on sediment Zn and Cd concentrations were the most reliable predictors of mussel toxicity and impacts on mussel communities. Sediment toxicity thresholds based on PEQs for Zn and Cd (separately or in combination) in bulk and fine sediments reliably predicted both mussel toxicity (85-100% of sites classified accurately) and reductions in mussel taxa richness (93% accurate). Thresholds based on PEQs for Pb (or mixtures containing Pb) reliably predicted mussel taxa richness but were less reliable for predicting mussel toxicity. Impacts on mussels taxa richness were better predicted by lower thresholds (e.g., Zn or Cd PEQ >0.5) than those that predicted mussel toxicity (e.g., Zn or Cd PEQ >1.0).

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Yeager MM, Cherry DS, Neves RJ. 1994. Feeding and burrowing behaviors of juvenile rainbow mussels, Villosa iris (Bivalvia: Unionidae). Journal of the North American Benthological Society:217-222.

Figure 1. Map of study area, showing locations of sediment collection sites (numbered circles), paired mussel survey sites (squares), and major mine waste sites (triangles).

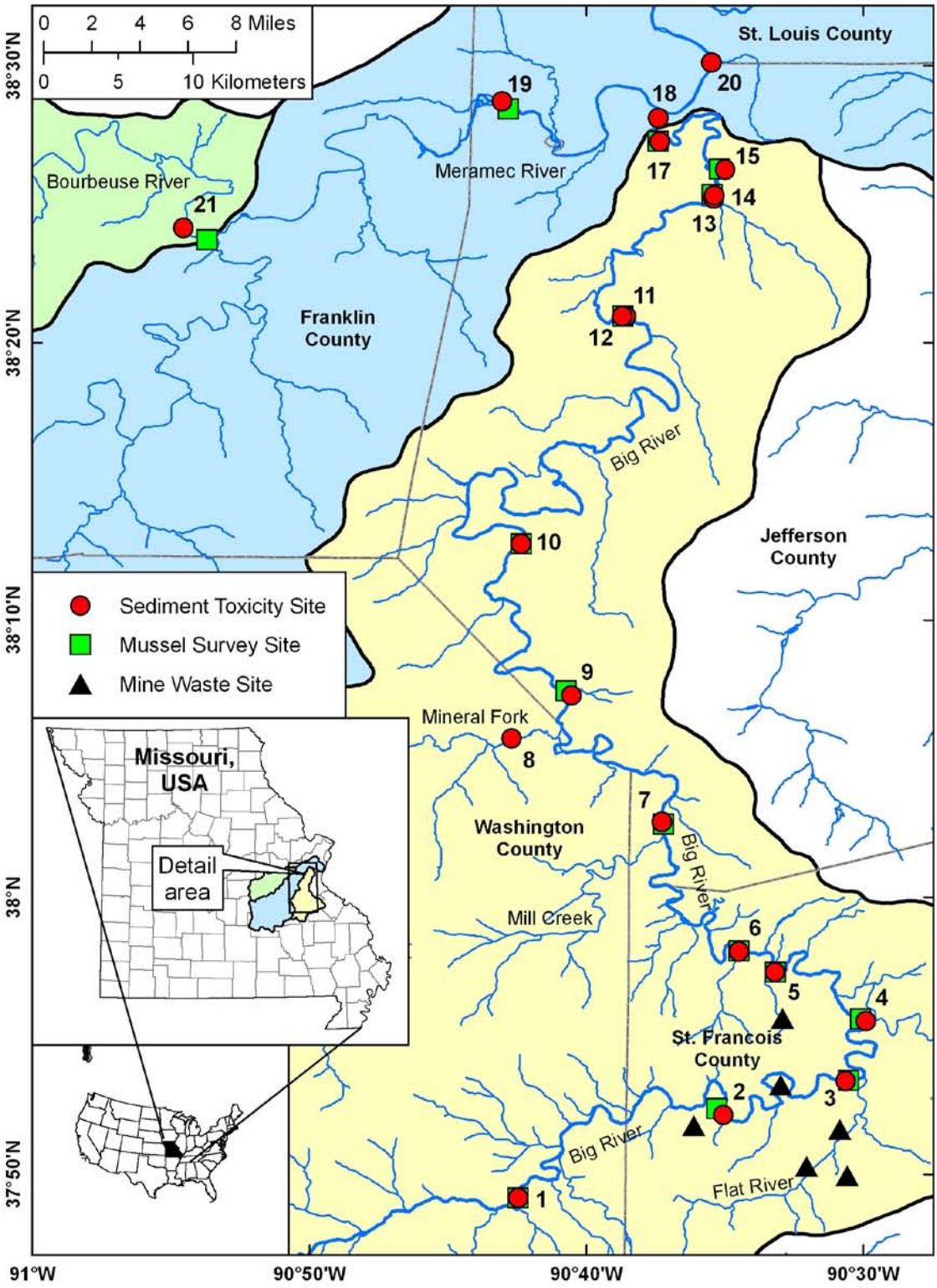
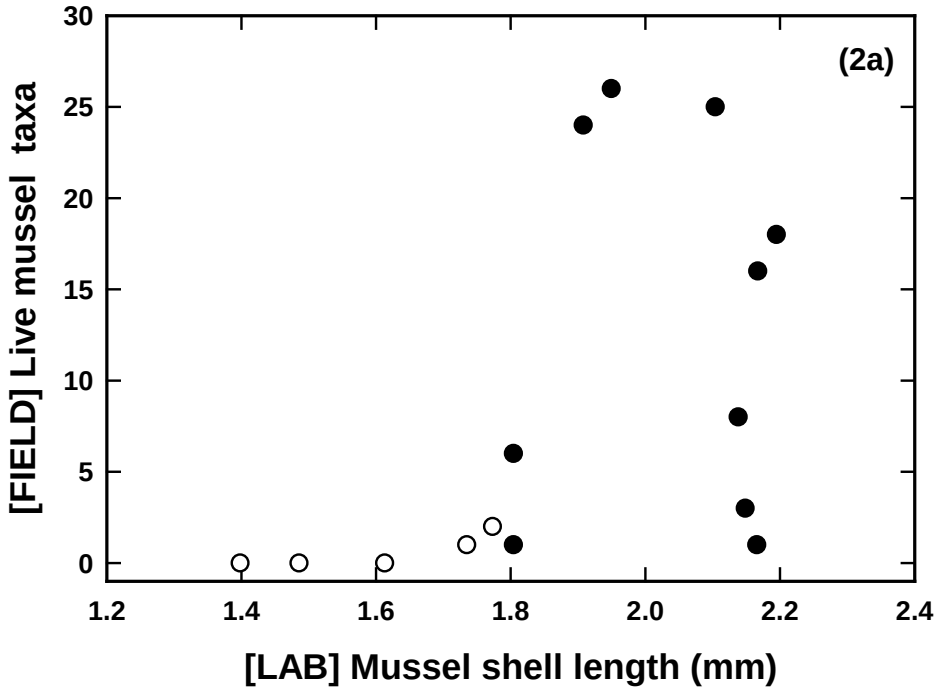


Figure 2. Relationships of mussel growth in laboratory toxicity tests with results of field mussel surveys: (a) number of live mussel taxa; (b) mussel catch per unit effort (CPUE). [Hollow symbols indicate sites that were toxic in laboratory tests (Table 9)]



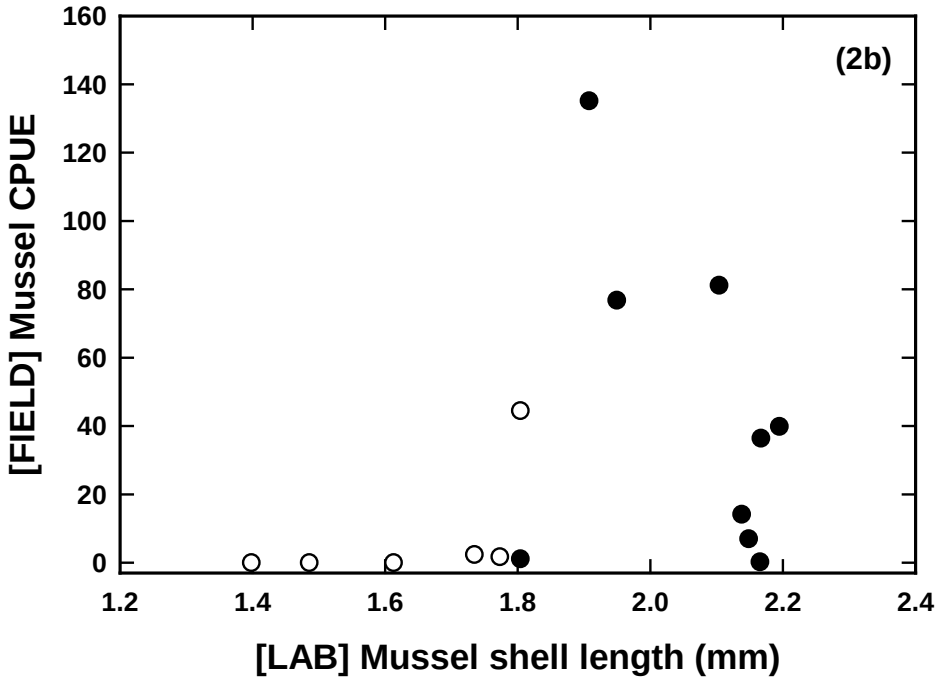
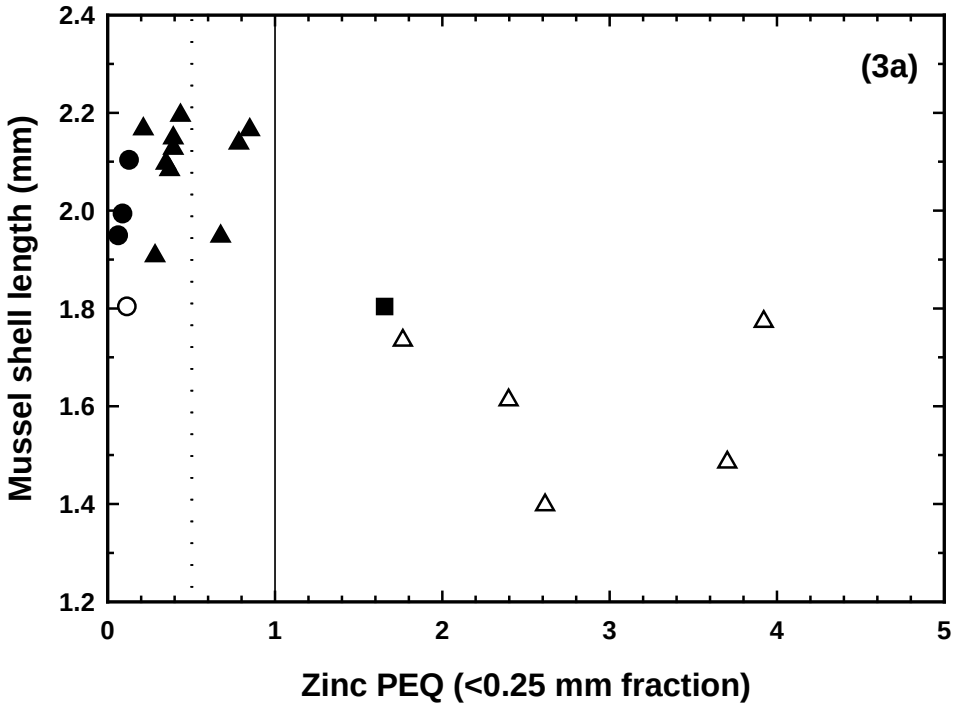


Figure 3. Relationships of mussel growth in laboratory toxicity tests with metal toxicity indices: (a) Zinc probable effect quotient (PEQ; Ingersoll et al. 2001); (b) Cadmium PEQ; (c) Lead PEQ; (d) ESB index (ΣSEM-AVS/$f$oc; USEPA 2005).[Circles indicate reference sites. Hollow symbols indicate toxic samples (reduced mussel growth; Table 9). PEQ indices were calculated for fine sediments (<0.25 mm) and ESB index were calculated for bulk sediments (<2 mm). Reference lines represent 'low' (dashed line) and 'high' (solid line) sediment toxicity thresholds for each index.]



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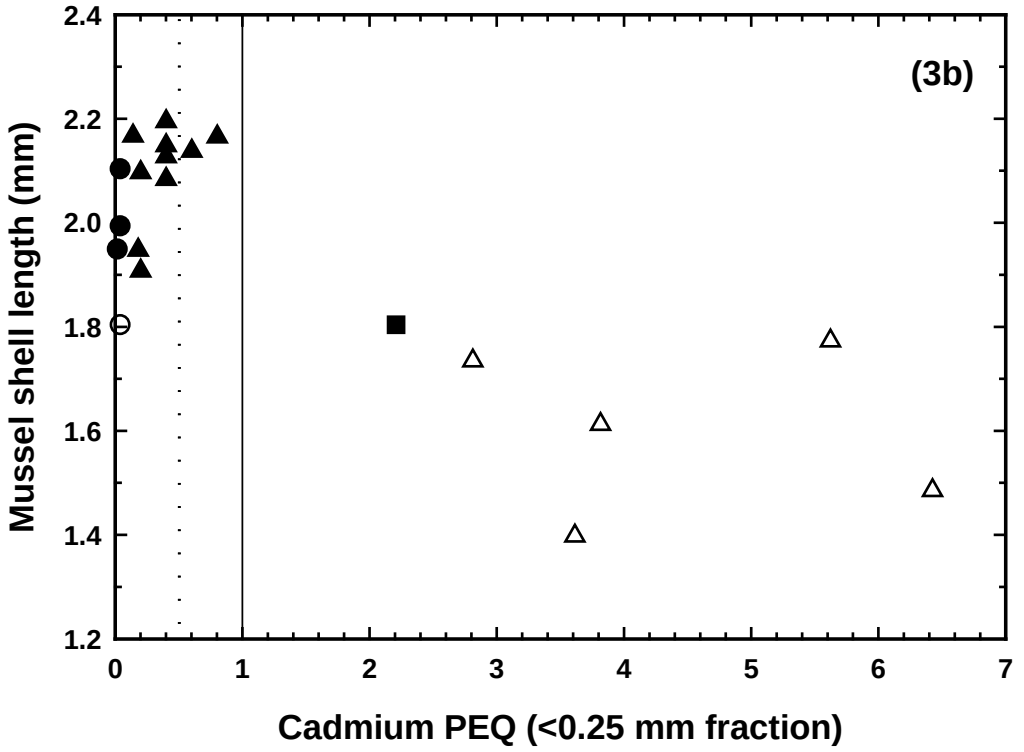

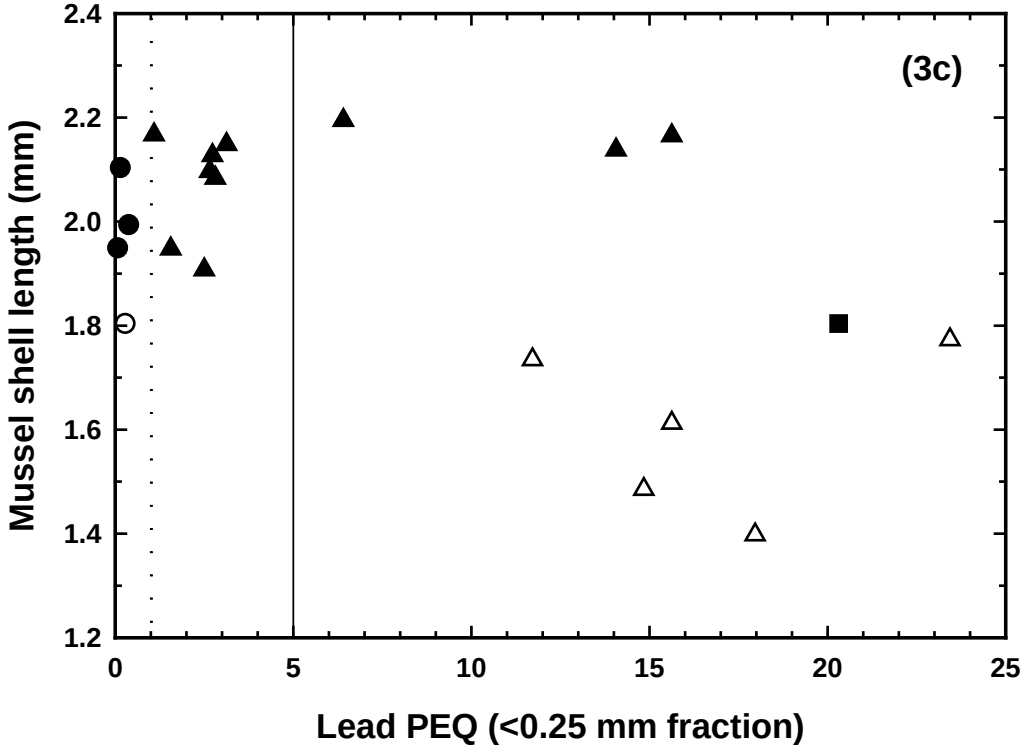

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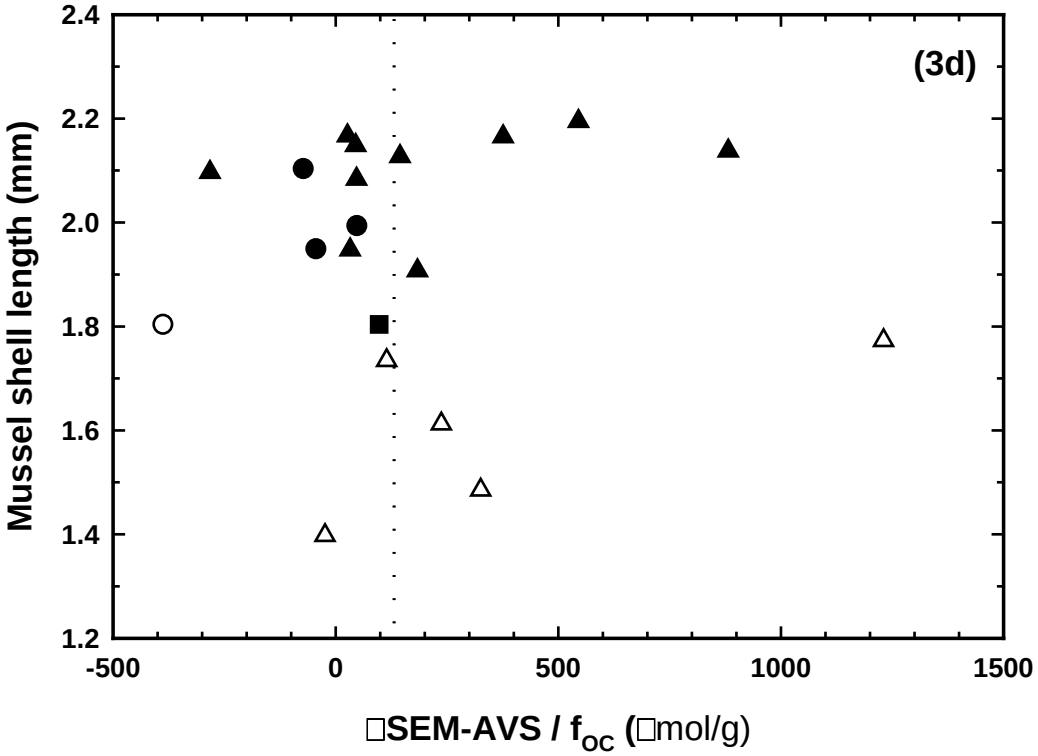
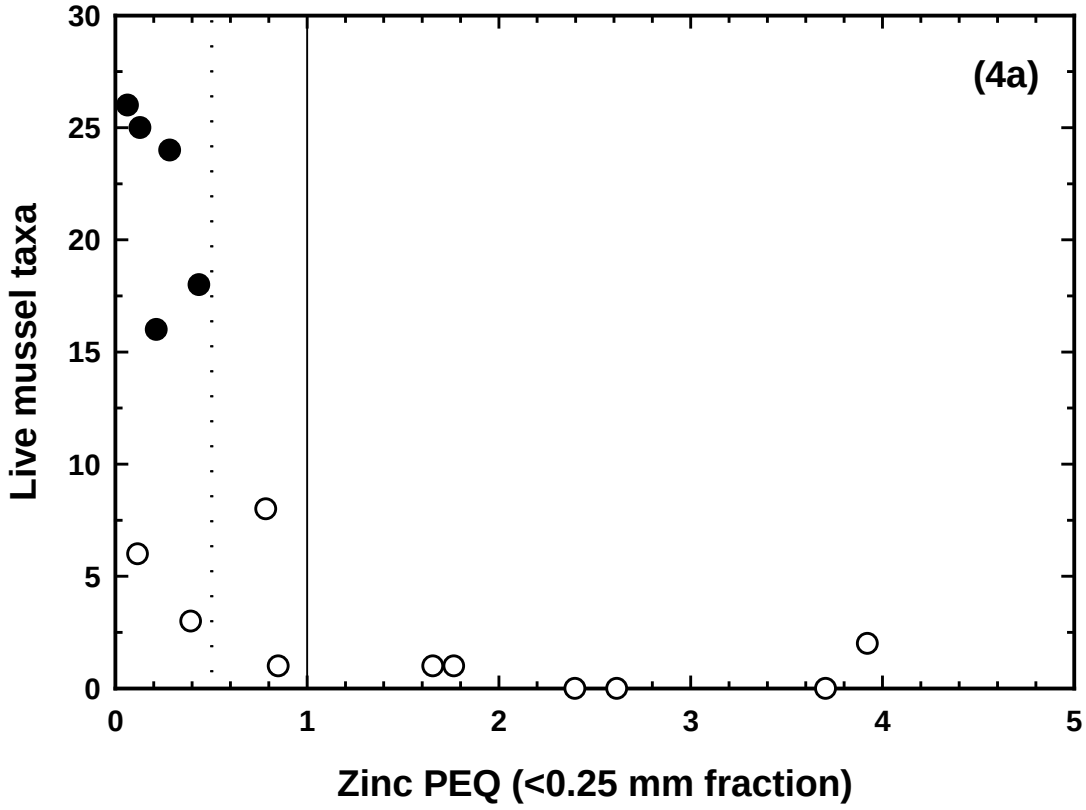


Figure 4. Relationships of mussel taxa richness in field surveys with metal toxicity indices: (a) Zinc probable effect quotient (PEQ; Ingersoll et al. 2001); (b) Cadmium PEQ; (c) Lead PEQ; (d) ESB index (ΣSEM-AVS/$f$oc; USEPA 2005). [Hollow symbols indicate sites that were determined to have reduced taxa richness relative to previous surveys (Roberts et al. in prep; Appendix A-7). PEQ indices were calculated for fine sediments (<0.25 mm) and ESB index was calculated for bulk sediments (<2 mm). Reference lines represent 'low' (dashed line) and 'high' (solid line) sediment toxicity thresholds for each index.]



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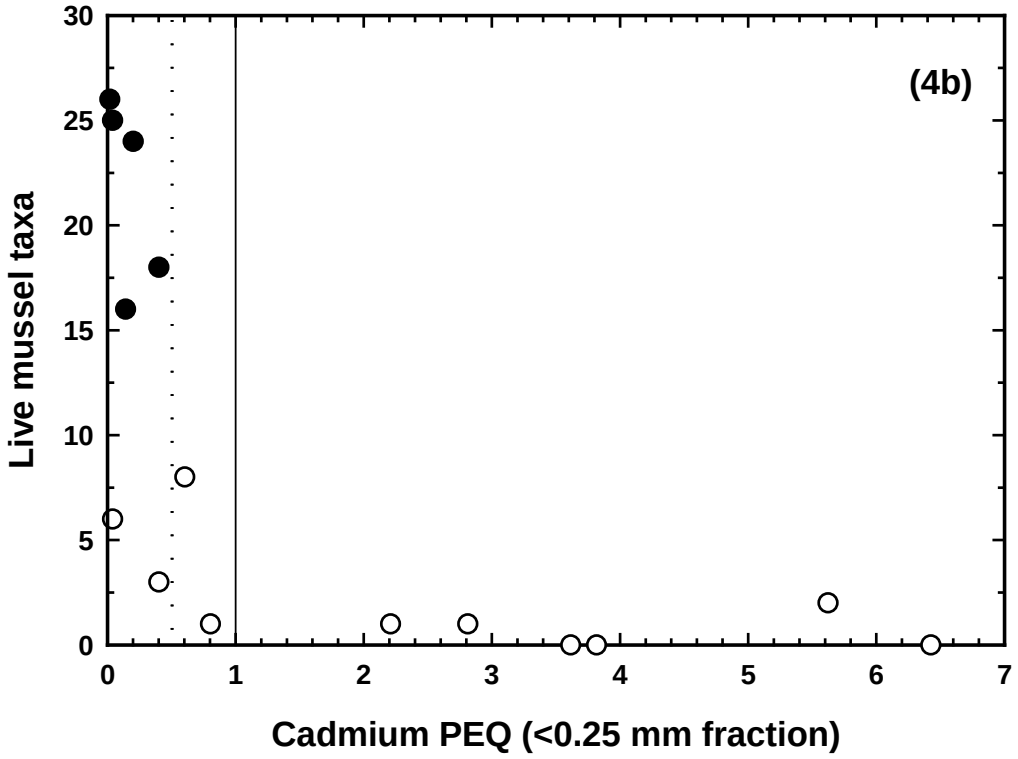

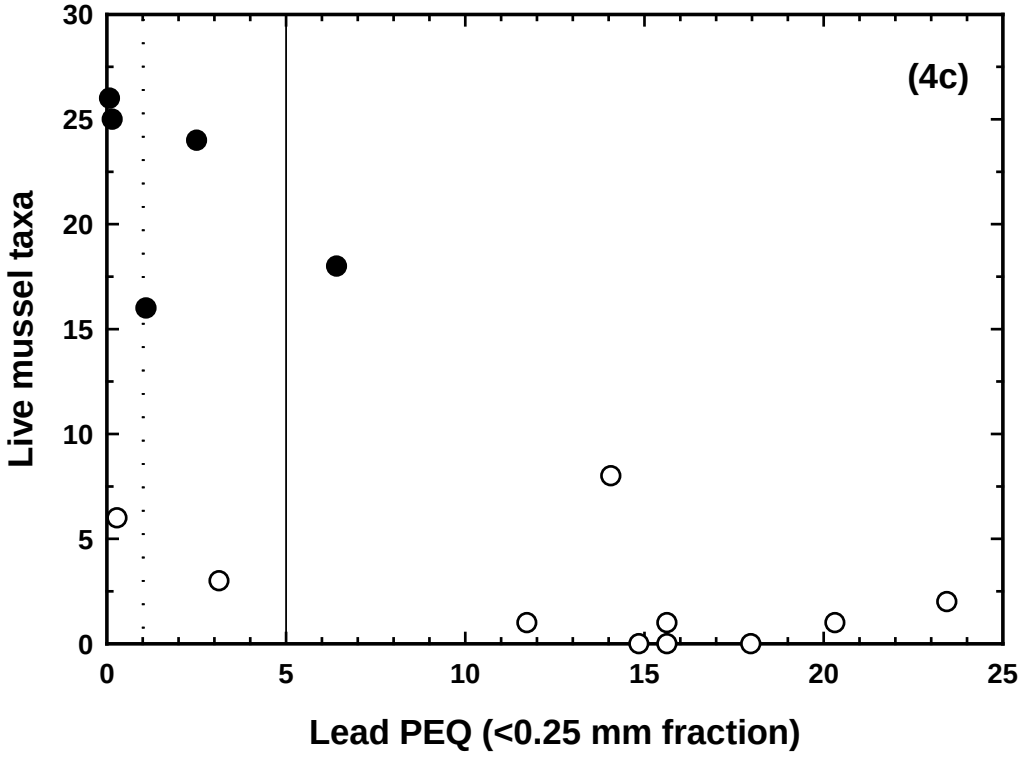

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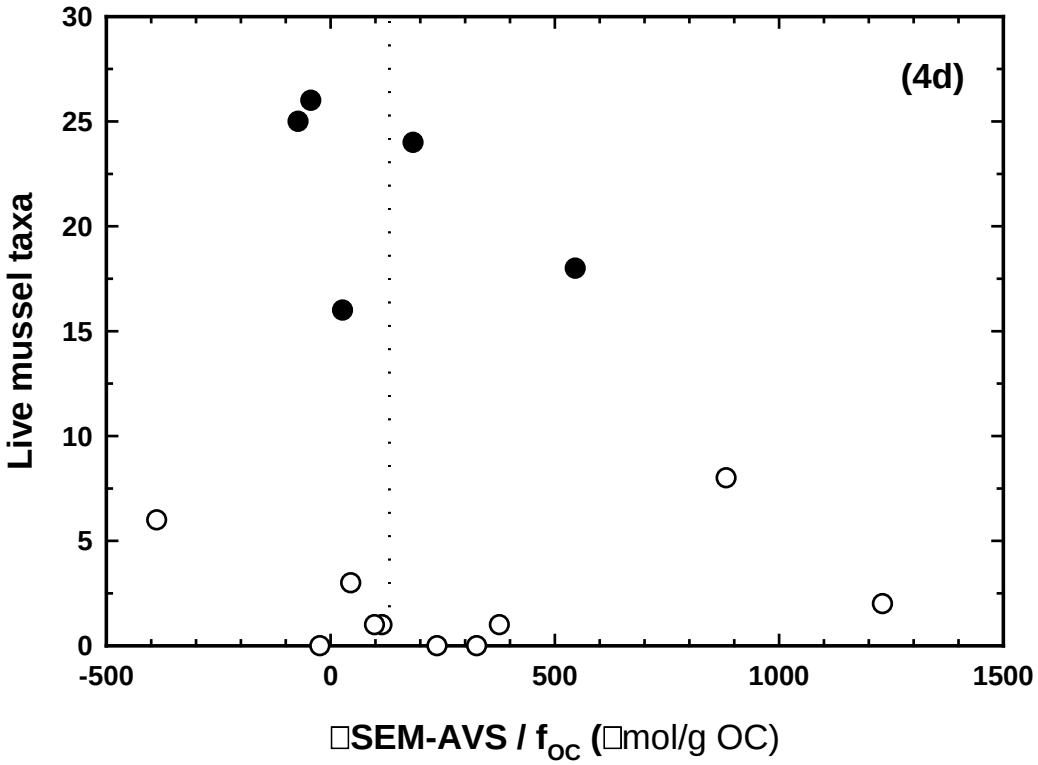

Table 1. USGS sediment collection sites in Southeast Missouri (SEMO) study area. [River miles are distance upstream from confluence of Big River and Meramece River.]

Site ID	Site Description	Short name	Date sampled	Latitude (degrees W)	Longitude (degrees N)	River mile (Big R.)
SEMO-1	Big River above Irondale	Irondale	9-Sep-08	37.8190	90.7078	129
SEMO-2	Big River at MDC Leadwood access	Leadwood	9-Sep-08	37.8690	90.5845	113
SEMO-3	Big River at Hwy 67	Highway 67	9-Sep-08	37.8898	90.5105	103
SEMO-4	Big River at Hwy K	Highway K	9-Sep-08	37.9254	90.4987	97
SEMO-5	Big River at Cherokee Landing	Cherokee	9-Sep-08	37.9550	90.5532	90
SEMO-6	Big River at Hwy EE	Highway EE	9-Sep-08	37.9676	90.5748	88
SEMO-7	Big River at Hwy CC	Highway CC	9-Sep-08	38.0453	90.6214	76
SEMO-8	Mineral Fork Creek near mouth	Mineral Fork	10-Sep-08	38.0954	90.7119	--
SEMO-9	Big River at Mammoth MDC Access	Mammoth	9-Sep-08	38.1213	90.6758	63
SEMO-10	Big River at Brown's Ford MDC Access	Browns	9-Sep-08	38.2126	90.7062	51
SEMO-11	Big River above Cedar Hill Dam	Above Cedar Hill	9-Sep-08	38.3496	90.6432	21
SEMO-12	Big River below Cedar Hill Dam	Below Cedar Hill	9-Sep-08	38.3496	90.6454	20
SEMO-13	Big River above Rockford Beach dam	Above Rockford	10-Sep-08	38.4203	90.5902	11
SEMO-14	Big River below Rockford Beach dam	Below Rockford	10-Sep-08	38.4225	90.5870	10
SEMO-15	Big River at Byrne's Mill Dam	Byrnes	10-Sep-08	38.4378	90.5832	8.2
SEMO-17	Big River at Hwy W	Highway W	10-Sep-08	38.4545	90.6228	1.3
SEMO-18	Big River above confluence w/ Meramec	Confluence	10-Sep-08	38.4688	90.6237	0.3
SEMO-19	Meramec River upstream; Pacific Palisades	Meramec upstream	10-Sep-08	38.6438	90.7154	--
SEMO-20	Meramec River downstream; Route 66 State Park	Meramec downstream	10-Sep-08	38.5021	90.5917	--
SEMO-21	Bourbeuse River near Choteau access	Bourbeuse	11-Sep-08	38.4027	90.9094	--

Table 2. Physical and chemical characteristics of bulk sediments (<2 mm particle diameter) from the SEMO study area. [RPD=relative percent difference (difference/mean) for duplicate analyses of sediments from SEMO-7 (sand/silt/clay) and SEMO-11 (AVS).]

Site ID	**Moisture (%)**	**Sand (%)**	**Silt (%)**	**Clay (%)**	**LOI (%)**	**TOC (%)**	**AVS (µmol/g)**
Control	24	15	61	24	3.2	1.0	< 0.01
SEMO-1	19	83	1	16	0.9	0.27	1.20
SEMO-2	19	88	2	10	0.7	0.12	1.78
SEMO-3	24	76	13	12	1.2	2.40	3.52
SEMO-4	21	89	0	11	2.0	4.07	1.04
SEMO-5	21	83	7	10	1.4	2.54	4.45
SEMO-6	23	84	6	10	2.0	2.49	8.74
SEMO-7	27	72	16	12	1.9	1.22	5.78
SEMO-8	22	79	11	11	1.3	0.48	0.84
SEMO-9	32	62	23	15	2.9	1.21	4.12
SEMO-10	24	62	21	18	1.8	0.58	0.85
SEMO-11	24	68	18	15	1.6	0.56	3.88
SEMO-12	28	62	23	15	2.5	0.86	1.64
SEMO-13	28	65	20	15	2.5	0.83	2.43
SEMO-14	25	69	18	13	2.3	0.67	0.62
SEMO-15	35	25	44	31	3.6	0.80	0.73
SEMO-17	24	72	15	13	1.3	0.27	1.07
SEMO-18	41	32	45	23	3.5	1.24	1.62
SEMO-19	40	27	54	18	3.1	1.07	1.24
SEMO-20	20	75	12	12	0.9	0.28	0.23
SEMO-21	32	53	34	14	1.6	0.60	0.50
RPD (%)		**2.1%**	**6.4%**	**4.8%**			**13.0%**

Table 3. Water quality of centrifuged and filtered (<0.45 µm) pore waters from SEMO sediments. [DOC=dissolved organic carbon; ND = not determined. RPD=relative percent difference (difference/mean) for duplicate analyses of pore water from SEMO-8.]

A. Routine Parameters (mg/L unless noted)

Site ID	pH	Conductivity (µS/cm)	Hardness (as $CaCO_3$)	Alkalinity (as $CaCO_3$)	Ammonia (mg N/L) Total	Ammonia (mg N/L) Unionized	DOC
FL (Control)	6.23	2340	200	68	1.70	0.001	115.0
SEMO-1	7.53	319	180	156	0.30	0.004	7.1
SEMO-2	7.90	390	230	184	0.33	0.011	6.3
SEMO-3	7.76	468	300	268	1.16	0.029	20.8
SEMO-4	7.90	474	288	208	ND	ND	9.2
SEMO-5	7.82	522	300	270	0.90	0.027	11.5
SEMO-6	7.41	556	320	324	0.50	0.006	21.6
SEMO-7	7.51	534	320	300	0.90	0.013	16.1
SEMO-8	7.68	410	250	230	0.46	0.009	5.4
SEMO-9	7.63	527	322	302	1.22	0.022	14.3
SEMO-10	7.71	421	260	222	0.41	0.009	16.2
SEMO-11	7.79	474	284	270	0.68	0.018	24.0
SEMO-12	7.88	574	350	340	0.39	0.012	20.1
SEMO-13	7.74	558	330	320	1.62	0.038	22.2
SEMO-14	7.75	542	300	290	0.66	0.016	18.2
SEMO-15	7.93	408	230	200	0.46	0.016	12.0
SEMO-17	7.77	384	250	202	0.35	0.009	7.6
SEMO-18	7.93	633	390	370	ND	0.000	26.5
SEMO-19	7.73	446	280	260	0.55	0.012	21.4
SEMO-20	8.02	443	ND	ND	0.39	0.017	15.2
SEMO-21	7.46	348	224	190	0.54	0.007	18.9
SEMO-22	7.95	347	226	183	0.26	0.010	7.9
SEMO-23	7.71	381	240	200	0.30	0.006	4.8

Table 3 (continued).

B. Major Ions (mg/L)

Site ID	Ca	Mg	Na	K	Mn	Fe	Chloride	Sulfate
FL (Control)	348	72	26.2	8.0	11.5	1.5	35.7	103.2
SEMO-1	36	21	3.5	1.7	1.6	0.2	7.0	19.7
SEMO-2	44	26	3.9	2.4	1.0	0.1	8.1	30.5
SEMO-3	54	33	4.5	2.8	4.5	0.2	9.1	12.0
SEMO-4	53	31	7.3	3.6	0.7	0.1	16.2	51.4
SEMO-5	57	33	8.8	3.4	3.8	0.1	12.6	20.7
SEMO-6	70	32	9.4	3.6	5.2	0.5	14.0	8.8
SEMO-7	62	34	7.3	2.9	4.2	0.5	11.6	6.3
SEMO-8	49	28	3.2	1.7	1.9	0.1	6.7	10.7
SEMO-9	62	35	5.6	2.8	7.4	0.6	9.2	5.1
SEMO-10	48	26	5.0	2.3	5.3	0.5	10.6	14.2
SEMO-11	56	32	5.5	3.3	3.7	0.9	8.5	5.5
SEMO-12	72	37	5.4	3.1	7.7	0.7	9.0	7.0
SEMO-13	69	36	5.7	2.7	7.7	1.2	9.0	7.4
SEMO-14	78	20	14.3	3.2	6.7	0.3	19.7	14.7
SEMO-15	52	22	6.3	3.2	2.7	0.4	10.1	20.8
SEMO-17	45	23	5.3	2.4	4.5	0.1	9.4	19.2
SEMO-18	83	39	6.2	3.5	10.0	0.8	10.9	5.4
SEMO-19	53	27	4.9	3.2	10.1	2.9	7.0	6.8
SEMO-20	52	28	4.8	3.3	5.6	0.3	7.4	19.8
SEMO-21	36	20	4.6	3.8	9.5	6.3	7.8	4.2
SEMO-22	38	23	3.0	2.4	3.5	0.1	6.6	13.9
SEMO-23	50	21	4.0	1.6	1.0	0.1	8.5	24.8
RPD	**0.2%**	**1.1%**	**3.2%**	**0.0%**	**1.6%**	**0.0%**	**1.5%**	**0.0%**

Table 4. Concentraations of total recoverable (TR) metals and mean probable effect quotients (PEQs) in two size fractions of SEMO sediments: (a) Bulk sediments (particles <2 mm); (b) Fine sediments (particles < 0.25 mm). Gray cells indicate exceedance of probable effect concentrations (PEC; MacDonald et al. 2000). [Mean PEQ=mean of (metal concentration/PEC) for Ni, Cu, Zn, Cd, and Pb.]

A. Bulk Sediments (mg/kg unless noted)

Site ID	TR-Mn (%)	TR-Fe (%)	TR-Ni	TR-Cu	TR-Zn	TR-Cd	TR-Pb	Mean PEQ
FL (Control)	0.09	1.6	20	10	53	0.2	13	0.15
SEMO-1	0.01	0.6	5	3	20	0.04	11	0.05
SEMO-2	0.04	0.9	7	4	810	11	250	1.22
SEMO-3	0.20	1.6	10	6	980	18	840	2.51
SEMO-4	0.40	2.4	20	20	740	13	1500	3.30
SEMO-5	0.30	1.8	10	20	470	8	850	1.92
SEMO-6	0.30	2.4	20	20	530	7	950	2.10
SEMO-7	0.20	1.6	10	40	350	6	810	1.75
SEMO-8	0.03	1.1	9	10	190	0.4	110	0.32
SEMO-9	0.10	1.5	20	30	300	3	1400	2.56
SEMO-10	0.09	1.2	20	20	250	2	1200	2.17
SEMO-11	0.03	0.8	10	10	130	1	270	0.57
SEMO-12	0.07	0.9	10	10	130	1	300	0.62
SEMO-13	0.04	0.9	10	10	140	1	310	0.64
SEMO-14	0.08	0.9	10	10	65	0.5	91	0.24
SEMO-15	0.10	1.7	30	20	180	2	680	1.37
SEMO-17	0.03	0.8	9	8	110	0.6	200	0.43
SEMO-18	0.10	1.5	20	20	180	2	350	0.81
SEMO-19	0.09	1.3	10	10	56	0.2	18	0.11
SEMO-20	0.03	0.5	6	8	31	0.2	35	0.11
SEMO-21	0.04	0.7	8	5	24	0.08	8	0.07
PEC	NA	NA	49	149	459	5.0	128	--

Table 4 (continued).

B. Fine Sediments (mg/kg unless noted)

Site ID	TR-Mn (%)	TR-Fe (%)	TR-Ni	TR-Cu	TR-Zn	TR-Cd	TR-Pb	Mean PEQ
FL (Control)	0.06	1.5	10	10	43	0.2	10	0.10
SEMO-1	0.03	1.3	10	10	53	0.2	36	0.14
SEMO-2	0.06	1.4	20	10	1800	28	3000	6.69
SEMO-3	0.20	1.6	20	20	1700	32	1900	5.10
SEMO-4	0.30	2.1	30	80	1100	19	2000	4.60
SEMO-5	0.30	2.1	20	50	810	14	1500	3.41
SEMO-6	0.20	1.9	30	60	1200	18	2300	5.04
SEMO-7	0.20	2.2	30	60	760	11	2600	5.04
SEMO-8	0.08	1.6	20	20	310	0.9	200	0.59
SEMO-9	0.20	1.8	20	40	390	4	2000	3.59
SEMO-10	0.10	1.7	20	30	360	3	1800	3.21
SEMO-11	0.04	1.0	10	10	160	1	340	0.70
SEMO-12	0.10	1.3	10	20	180	2	400	0.85
SEMO-13	0.07	1.0	10	10	170	2	360	0.77
SEMO-14	0.10	1.5	20	20	98	0.7	140	0.40
SEMO-15	0.10	1.9	20	20	200	2	820	1.56
SEMO-17	0.07	1.1	10	10	130	1	320	0.65
SEMO-18	0.10	1.5	20	20	180	2	350	0.81
SEMO-19	0.10	1.3	10	10	59	0.2	19	0.12
SEMO-20	0.03	0.7	7	6	41	0.2	49	0.14
SEMO-21	0.04	0.8	9	6	29	0.09	9	0.08
PEC	NA	NA	49	149	459	5.0	128	--

Table 5. Concentrations of simultaneously-extracted metals (SEM) and values of the equilibrium-partitioning sediment benchmark (ESB) index for the bulk (<2 mm) fraction of SEMO sediments. Shaded cells indicates values of the ESB index greater than 130 µmol/g OC (USEPA 2005). [ESB index=ÓSEM-AVS/ƒoc]

Site ID	SEM-Ni (µg/g)	SEM-Cu (µg/g)	SEM-Zn (µg/g)	SEM-Cd (µg/g)	SEM-Pb (µg/g)	ESB Index (µmol/g OC)
FL (Control)	3.6	4.5	7	0.18	8	28
SEMO-1	1.0	0.7	6	0.04	8	-388
SEMO-2	1.9	0.8	133	2.64	238	1231
SEMO-3	7.1	2.1	510	11.00	680	326
SEMO-4	10.3	6.5	350	5.76	1040	237
SEMO-5	8.2	5.0	287	4.61	562	115
SEMO-6	8.6	4.8	312	4.44	646	-24
SEMO-7	6.3	7.3	234	4.10	649	98
SEMO-8	1.4	1.9	42	0.24	61	33
SEMO-9	7.2	12.9	197	3.16	1100	376
SEMO-10	6.0	11.5	152	1.96	692	882
SEMO-11	3.0	4.0	67	1.01	238	-282
SEMO-12	3.6	5.7	73	1.15	155	45
SEMO-13	3.7	5.1	85	1.27	282	47
SEMO-14	3.8	4.9	28	0.42	46	27
SEMO-15	7.9	12.5	109	1.59	637	545
SEMO-17	2.0	3.3	39	0.62	183	184
SEMO-18	5.7	9.4	102	1.71	330	145
SEMO-19	3.0	4.1	18	0.18	15	-72
SEMO-20	1.6	1.7	11	0.15	31	48
SEMO-21	2.2	2.3	8	0.08	7	-44

Table 6. Metal concentrations and toxic units in pore waters of SEMO sediments prepared by two methods: (a) Centrifuged samples (analyzed using semi-quantitative ICPMS); (b) Peeper samples (analyzed using quantitative ICPMS). Shaded values indicate concentrations exceeding chronic water quality criteria (WQC; USEPA 2002) or toxic units greater than 1.0 (USEPA 2005). [Toxic units=sum of (metal concentration/WQC)]

A. Centrifuged Samples (μg/L)

Site ID	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**	**Toxic units**
FL (Control)	4	9	20	<0.1	1	1.0
SEMO-1	3	<1	<1	<0.1	1	0.4
SEMO-2	3	<1	20	0.10	20	3.6
SEMO-3	10	2	100	1.00	100	14.7
SEMO-4	10	4	60	0.50	60	9.1
SEMO-5	8	2	20	0.20	50	6.7
SEMO-6	9	<1	8	<0.1	20	2.5
SEMO-7	4	<1	5	<0.1	20	2.4
SEMO-8	2	<1	9.5	<0.1	5	0.9
SEMO-9	6	6	20	0.20	100	12.1
SEMO-10	6	10	30	0.60	100	16.2
SEMO-11	5	10	60	1.00	400	54.8
SEMO-12	7	9	40	0.80	300	33.3
SEMO-13	8	8	40	0.80	300	35.3
SEMO-14	9	9	20	0.50	100	13.8
SEMO-15	4	10	30	1.00	200	35.5
SEMO-17	4	2	10	0.10	70	10.8
SEMO-18	10	10	40	1.00	300	30.2
SEMO-19	7	10	20	0.30	30	5.2
SEMO-20	6	3	9	0.10	30	4.4
SEMO-21	7	8	10	0.10	20	4.1

Table 6 (continued).

B. Peeper Samples (µg/L)

Site ID	Ni	Cu	Zn	Cd	Pb	Toxic units
FL (Control)	10.9	2.3	22	0.32	< 0.11	1.2
SEMO-1	2.7	0.3	9	0.05	0.6	0.4
SEMO-2	1.4	0.6	22	0.16	7.5	1.7
SEMO-3	6.8	0.2	3.79	< 0.05	2.3	0.4
SEMO-4	7.5	0.7	142	0.25	62.9	9.1
SEMO-5	4.7	0.6	3.84	< 0.05	1.9	0.4
SEMO-6	0.8	16.7	3.12	< 0.05	2.0	1.0
SEMO-7	0.8	0.3	1.47	< 0.05	1.7	0.3
SEMO-8	1.2	0.4	2.59	< 0.05	0.2	0.2
SEMO-9	2.6	0.5	9.48	< 0.05	4.4	0.7
SEMO-10	3.8	1.1	48.8	0.14	45.6	7.1
SEMO-11	2.1	0.8	13.1	0.08	16.7	2.4
SEMO-12	4.0	0.9	47	0.12	22.1	2.7
SEMO-13	2.8	0.4	0.89	< 0.05	2.3	0.4
SEMO-14	6.8	0.8	4.75	0.76	5.8	2.3
SEMO-15	4.2	12.1	16.7	0.06	33.9	6.4
SEMO-17	3.0	1.1	5.48	0.05	13.5	2.2
SEMO-18	6.8	0.6	10.3	< 0.05	7.9	0.9
SEMO-19	2.2	0.3	2.7	< 0.05	0.2	0.2
SEMO-20	1.8	0.3	6.02	< 0.05	0.7	0.2
SEMO-21	3.9	0.2	1.17	< 0.05	0.2	0.2

Table 7. Metal toxicity indices and mean toxicity endpoints for candidate reference sites. Toxicity index values less than stated guidelines were assumed to indicate minimal risk of metal toxicity. The minimum site mean for each toxicity endpoint defines the lower boundary of the reference envelope. [PEQ=mean of probable effects concentration quotients for Zn, Pb, and Cd for bulk (<2 mm) and fine (<0.25 mm) sediment size fractions (Ingersoll et al. 2001); ESB index and toxic units calculated per USEPA 2005; CV=coefficient of variation]

Site ID	Metal toxicity indices				Amphipod endpoints			Mussel endpoints		
	PEQ (bulk)	**PEQ (fine)**	**ESB Index**	**Toxic units**	**Survival (of 10)**	**Length (mm)**	**Biomass (mg)**	**Survival (of 10)**	**Diameter (mm)**	**Biomass (mg)**
Control (FL)	0.15	0.10	28	1.2	9.75	3.68	2.42	8.75	1.98	5.88
SEMO-1	0.05	0.14	-388	0.36	9.75	3.78	2.61	7.50	1.80	2.01
SEMO-19	0.11	0.12	-72	0.16	9.50	3.77	2.51	6.75	2.10	5.41
SEMO-20	0.11	0.14	48	0.24	9.50	3.49	1.99	9.25	1.99	3.06
SEMO-21	0.07	0.08	44	0.21	9.50	3.67	2.32	8.00	1.95	2.98
Guideline	0.20	0.20	130	1.00	--	--	--	--	--	--
Mean	0.09	0.12	-92.00	0.24	9.56	3.68	2.36	7.88	1.96	3.37
CV (%)					1.3%	3.6%	12%	13%	6.3%	43%
Minimum	--	--	--	--	**9.50**	**3.49**	**1.99**	**6.75**	**1.80**	**2.01**

Table 8. Results of 28-d whole-sediment toxicity tests with amphipods (*Hyalella azteca)*. Site means, with standard errors (SE) and significance levels for analysis of variance (ANOVA). Data were transformed before ANOVA as noted. Shaded cells indicate toxic effects (significant ANOVA plus means below the reference envelope; Table 7). Mean length of amphipods on day 0 was 1.95 mm (standard deviation=0.27 mm).

Site ID	**Survival (of 10)**		**Length (mm)**		**Biomass (mg)**	
	Mean	**SE**	**Mean**	**SE**	**Mean**	**SE**
FL (Control)	9.75	0.25	3.68	0.12	2.42	0.26
SEMO-1	9.75	0.25	3.78	0.04	2.61	0.13
SEMO-2	9.75	0.25	3.99	0.10	3.09	0.22
SEMO-3	9.25*	0.48	4.18	0.11	3.40	0.37
SEMO-4	8.25*	0.48	4.01	0.10	2.67	0.28
SEMO-5	9.75	0.25	3.94	0.15	3.09	0.40
SEMO-6	10.00	0.00	4.60	0.15	4.93	0.49
SEMO-7	9.75	0.25	3.87	0.05	2.80	0.09
SEMO-8	9.50	0.29	3.65	0.17	2.37	0.40
SEMO-9	9.75	0.25	3.75	0.03	2.54	0.02
SEMO-10	9.25*	0.75	3.56	0.12	2.12	0.35
SEMO-11	9.50	0.29	3.72	0.05	2.40	0.14
SEMO-12	9.50	0.29	3.34	0.07	1.73	0.14
SEMO-13	9.50	0.29	3.39	0.09	1.81	0.18
SEMO-14	9.50	0.50	3.78	0.17	2.62	0.43
SEMO-15	8.75*	0.63	3.46	0.05	1.76	0.08
SEMO-17	9.25	0.48	3.59	0.11	2.11	0.25
SEMO-18	10.00	0.00	3.56	0.04	2.21	0.07
SEMO-19	9.50	0.29	3.77	0.08	2.51	0.19
SEMO-20	9.50	0.50	3.49	0.09	1.99	0.23
SEMO-21	9.50	0.50	3.67	0.07	2.32	0.18
ANOVA	**p=0.5389 (Rank)**		**p<0.0001 (Log)**		**p<0.0001 (Log)**	

*Means are below reference envelope, but ANOVA indicates no siginficant toxicity.

Table 9. Results of 28-d whole-sediment toxicity tests with fatmucket mussels *(Lampsilis siliquoidea)*. Site means, with standard errors (SE) and results of analysis of variance (ANOVA). Data were transformed before ANOVA as noted. Shaded cells indicate toxic effects (significant ANOVA plus means below the reference envelope; Table 7). Mean shell diameter of mussels on day 0 was 0.95 mm (standard deviation=0.12 mm).

Site ID	Survival (of 10)		Shell diameter (mm)		Biomass (mg)	
	Mean	SE	Mean	SE	Mean	SE
FL (Control)	8.75	0.63	1.98	0.26	5.88	1.99
SEMO-1	7.50	0.65	1.80	0.04	2.01	0.15
SEMO-2	7.50	0.50	1.77	0.08	2.07	0.20
SEMO-3	3.50	0.65	1.49	0.13	1.58	0.90
SEMO-4	7.50	1.19	1.61	0.11	1.78	0.46
SEMO-5	6.50	0.29	1.73	0.15	1.35	0.81
SEMO-6	4.50	0.50	1.40	0.10	1.42	0.17
SEMO-7	8.50	0.65	1.80	0.11	4.05	0.92
SEMO-8	8.00	0.41	1.95	0.19	3.01	0.76
SEMO-9	8.50	0.29	2.17	0.13	4.03	0.41
SEMO-10	9.50	0.29	2.14	0.11	5.00	0.63
SEMO-11	7.50	0.50	2.10	0.11	4.36	0.47
SEMO-12	9.50	0.50	2.15	0.11	3.86	0.65
SEMO-13	7.75	0.48	2.08	0.10	4.62	1.18
SEMO-14	8.50	0.87	2.17	0.06	6.56	0.61
SEMO-15	8.75	0.48	2.19	0.09	3.93	0.61
SEMO-17	8.50	0.29	1.91	0.15	2.94	0.49
SEMO-18	7.75	0.75	2.13	0.07	5.32	0.32
SEMO-19	6.75	0.85	2.10	0.10	5.41	2.10
SEMO-20	9.25	0.48	1.99	0.05	3.06	0.08
SEMO-21	8.00	0.41	1.95	0.12	2.98	0.40
ANOVA:	**p=<0.0001(Arcsine)**		**p=<0.0001 (no trans.)**		**p=<0.0001 (Square root)**	

Table 10. Classification of impacts at SEMO sites based on lab and field studies: (a) Mussel (lab) vs. amphipod (lab); (b) Amphipod (lab) vs. mussel (field); (c) Mussel (lab) vs mussel (field); (d) Lab tests (combined) vs. mussel (field). Classifications in contingency tables are based on reference envelope method for toxicity endpoints (survival, length or biomass; Tables 8 and 9) and for mussel taxa richness in field surveys (Appendix A-8; Roberts et al. in prep.). Shaded cells indicate agreement between endpoints.

A. Mussel (lab) vs. amphipod (lab) [n=20]:

	Amphipod Toxic	Amphipod Not toxic
Mussel toxic	0%	25%
Mussel not toxic	15%	60%
	Percent agreement:	60%

B. Amphipod (lab) vs. mussel (field) [n=15]:

	Field mussel impact	Field mussel no impact
Lab amphipod toxic	7%	7%
Lab amphipod not toxic	53%	33%
	Percent agreement:	40%

C. Mussel (lab) vs. mussel (field) [n=15]:

	Field mussel impact	Field mussel no impact
Mussel toxic	33%	0%
Mussel not toxic	27%	40%
	Percent agreement:	**73%**

D. Lab tests (combined) vs. mussel (field) [n=15]:

	Field mussel impact	Field mussel no impact
Lab toxic	40%	0%
Lab not toxic	20%	40%
	Percent agreement:	**80%**

Table 11. Pearson correlation coefficients for associations of sediment toxicity endpoints with results of field mussel surveys and characteristics of whole sediment or pore water. Bold text and shaded cells indicates significant correlations ($p<0.05$; green=positive, yellow=negative). Correlations with sediment metals and PEQs use data from bulk sediments for amphipods (HA=*Hyalella azteca*) and fine sediments for mussels (LS=*Lampsilis siliquoidea*). [S=Survival; L=Length; B=biomass; Taxa=taxa richness (field); CPUE=catch per unit effort (field); PW=pore water; NH3=ammonia; TOC=total organic carbon, AVS=acid-volatile sulfide; PEQ=sediment probable effect quotient (or mean PEQ); SEM=simultaneously-extracted metals; TU=toxic units]

Variable	HA-S	HA-L	HA-B	LS-S	LS-L	LS-B
HA-L	0.156	--	--		--	--
HA-B	0.328	**0.974**	--	--	--	--
LS-S	-0.148	**-0.798**	**-0.784**	--	--	--
LS-L	0.002	**-0.848**	**-0.807**	**0.758**	--	--
LS-B	0.081	**-0.559**	**-0.524**	**0.516**	**0.830**	--
Mussel taxa	-0.156	-0.482	-0.474	0.296	0.512	0.480
Mussel CPUE	-0.100	-0.402	-0.399	0.219	0.312	0.246
Sand	0.037	**0.492**	**0.468**	-0.240	**-0.656**	**-0.639**
TOC	-0.354	**0.593**	**0.504**	**-0.551**	**-0.612**	-0.432
AVS	**0.448**	**0.684**	**0.760**	**-0.561**	**-0.535**	-0.319
PW Total NH3	0.103	0.046	0.038	-0.291	-0.027	0.115
PW Unionized NH3	-0.256	-0.066	-0.090	-0.202	-0.002	-0.034
Sediment Zn	-0.190	**0.684**	**0.598**	**-0.632**	**-0.765**	**-0.610**
Sediment Cd	-0.242	**0.676**	**0.571**	**-0.677**	**-0.784**	**-0.624**
Sediment Pb	-0.326	0.386	0.317	-0.310	**-0.543**	-0.407
Zn,Cd PEQ	-0.224	**0.682**	**0.584**	**-0.662**	**-0.779**	**-0.620**
Zn,Pb PEQ	-0.324	**0.451**	0.375	**-0.360**	**-0.583**	-0.442
Mean PEQ	-0.328	**0.516**	0.431	-0.427	**-0.637**	**-0.485**
SEM-AVS	**-0.640**	0.193	0.048	-0.174	-0.269	-0.257
SEM-AVS/TOC	-0.192	0.029	-0.006	0.132	0.009	-0.055
PW Zn (Centrifuge)	-0.400	0.101	-0.004	-0.436	-0.187	-0.113
PW Zn (Peeper)	**-0.714**	0.039	-0.100	0.164	-0.145	-0.167
PW Cd (Centrifuge)	-0.277	-0.309	-0.336	-0.062	0.303	0.319
PW Cd (Peeper)	-0.211	0.031	-0.015	0.203	0.182	0.403
PW Pb (Centrifuge)	-0.031	**-0.467**	-0.428	0.175	**0.497**	**0.448**
PW Pb (Peeper)	**-0.790**	-0.129	-0.249	0.297	0.076	0.022
PW TU (Centrifuge)	-0.159	**-0.456**	-0.439	0.178	**0.504**	0.429
PW TU (Peeper)	**-0.797**	-0.091	-0.207	0.287	0.081	0.047

Table 12. Comparison of metal toxicity indices between SEMO mining district and Tri-States mining district (TSMD). SEMO values are means for sediments within designated stream reaches; TSMD values are means for sediments that were toxic to amphipods (HA; <2 mm fraction) or mussels (LS; <0.25 mm fraction), with modeled toxicity thresholds (T-20) for 20% reductions in amphipod survival or mussel biomass relative to reference sediments (Ingersoll et al. 2008, MacDonald et al. 2009).

Sediment PEQs	**SEMO**				**TSMD**			
	Sites 2-7		Sites 9-18		Toxic-HA	Toxic-LS	T-20 for HA	T-20 for LS
	(<2 mm)	(<0.25 mm)	(<2 mm)	(<0.25 mm)	(<2 mm)	(<0.25 mm)	(<2 mm)	(<0.25 mm)
Pb	6.8	17.3	4.2	5.7	4.1	23.2	1.7	10.6
Zn	1.4	2.7	0.4	0.5	12.1	19.2	6.5	52.0
Cd	2.1	4.1	0.3	0.4	8.4	14.9	3.5	--
Pore Water Toxic Units	**SEMO**				**TSMD**			
	Sites 2-7		Sites 9-18		Toxic-HA		T-20 for HA	T-20 for LS
	(centrifuge)	(peeper)	(centrifuge)	(peeper)	(peeper)		(<2 mm)	(<0.25 mm)
Pb	5.67	1.69	25.12	2.23	1.20		0.16	0.54
Zn	0.12	0.11	0.11	0.06	3.13		0.87	3.35
Cd	0.31	0.21	1.27	0.29	3.65		0.44	6.37

Table 13. Percent correct classification of toxicity endpoints by sediment toxicity thresholds based on metal toxicity indices: (a) Bulk sediments (particles <2 mm); (b) Fine sediments (particles <0.25 mm). Highlighted cells indicates thresholds with >80% overall correct classification, <20% incidence of toxicity below the threshold and >50% toxicity above the threshold (Appendix A-6). [PEQ=probable effects quotient or mean PEQ (Ingersoll et al 2001); Taxa=mussel taxa richness (Roberts et al in prep.); SEM=simultaneously-extracted metals, AVS=acid-volatile sulfide; ESB Index=SEM-

A. Bulk sediments

Toxicity Index	Threshold		Endpoint	Amphipod		Mussel	
	Low	High		Low	High	Low	High
Zn-PEQ	0.5	1.0	Survival	--	--	75	90
			Length	45	60	85	100
			Biomass	45	60	80	95
			Taxa	--	--	93	73
Cd-PEQ	0.5	1.0	Survival	--	--	80	85
			Length	50	55	90	95
			Biomass	50	55	85	90
			Taxa	--	--	87	80
Pb-PEQ	1.0	5.0	Survival	--	--	45	75
			Length	45	55	55	75
			Biomass	45	55	50	80
			Taxa	--	--	87	80
ZnCd-PEQ	0.5	1.0	Survival	--	--	80	90
			Length	50	60	90	100
			Biomass	50	60	85	95
			Taxa	--	--	87	73
ZnPb-PEQ	0.5	1.0	Survival	--	--	50	75
			Length	50	75	60	75
			Biomass	50	75	55	80
			Taxa	--	--	93	53
ZnCdPb-PEQ	1.0	5.0	Survival	--	--	65	80
			Length	45	80	75	80
			Biomass	45	80	70	85
			Taxa	--	--	87	47
SEM-AVS	0.0	5.0	Survival	--	--	30	80
			Length	40	70	40	80
			Biomass	40	70	35	85
			Taxa	--	--	73	60
ESB Index	130	3000	Survival	--	--	55	85
			Length	55	85	65	75
			Biomass	55	85	60	80
			Taxa	--	--	60	40

Table 13 (continued).

B. Fine sediments

Toxicity Index	Threshold		Endpoint	Amphipod		Mussels	
	Low	High		Low	High	Low	High
Zn-PEQ	0.5	1.0	Survival	--	--	70	85
			Length	40	55	80	95
			Biomass	40	55	75	90
			Taxa	--	--	93	80
Cd-PEQ	0.5	1.0	Survival	--	--	75	85
			Length	45	55	85	95
			Biomass	45	55	80	90
			Taxa	--	--	93	80
Pb-PEQ	1.0	5.0	Survival	--	--	45	75
			Length	45	55	55	75
			Biomass	45	55	50	80
			Taxa	--	--	80	87
ZnCd-PEQ	0.5	1.0	Survival	--	--	75	85
			Length	45	55	85	95
			Biomass	45	55	80	90
			Taxa	--	--	93	80
ZnPb-PEQ	0.5	1.0	Survival	--	--	45	75
			Length	45	55	55	75
			Biomass	45	55	50	80
			Taxa	--	--	87	93
ZnCdPb-PEQ	1.0	5.0	Survival	--	--	50	75
			Length	50	45	60	85
			Biomass	50	45	55	80
			Taxa	--	--	93	93