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# Biomonitoring of Lead, Zinc, and Cadmium in Streams Draining Lead-Mining and Non-Mining Areas, Southeast Missouri, USA

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**Abstract** We evaluated exposure of aquatic biota to lead (Pb), zinc (Zn), and cadmium (Cd) in streams draining a Pb-mining district in southeast Missouri. Samples of plant biomass (detritus, periphyton, and filamentous algae), invertebrates (snails, crayfish, and riffle benthos), and two taxa of fish were collected from seven sites closest to mining areas (mining sites), four sites further downstream from mining (downstream sites), and eight reference sites in fall 2001. Samples of plant biomass from mining sites had highest metal concentrations, with means 10- to 60-times greater than those for reference sites. Mean metal concentrations in over 90% of samples of plant biomass from mining sites were significantly greater than those from reference sites. Mean concentrations of Pb, Zn, and Cd in most invertebrate samples from mining sites, and mean Pb concentrations in most fish samples from mining sites, were also significantly greater than those from reference sites. Concentrations of all three metals were lower in samples from downstream sites, but several samples of plant biomass from downstream sites had metal concentrations significantly greater than those from reference sites. Analysis of supplemental samples collected in the fall of 2002, a year of above-average stream discharge, had lower Pb concentrations and higher Cd concentrations than samples collected in 2001, near the end of a multi-year drought. Concentrations of Pb measured in fish and invertebrates collected from mining sites during 2001 and 2002 were similar to those measured at nearby sites in the 1970s, during the early years of mining in the Viburnum Trend. Results of this study demonstrate that long-term Pb mining activity in southeast Missouri has resulted in significantly elevated concentrations of Pb, Cd, and Zn in biota of receiving streams, compared to biota of similar streams without direct influence of mining. Our results also demonstrate that metal exposure in the study area differed significantly among sample types, habitats, and years, and that these factors should be carefully considered in the design of biomonitoring studies.

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## 1 Introduction

Southeast Missouri has been a major producer of lead (Pb) since the 1700s. In the Old Lead Belt, where mining ceased in 1972, erosion of large quantities of mine tailings into area streams has led to Pb contamination of fish and other aquatic biota, alteration of fish and invertebrate communities, and advisories

that warn against human consumption of Pb-contaminated fish (Brumbaugh, Schmitt, & May, 2005; Czarneski, 1985; Humphrey & Lister, 2004; Schmitt et al., 1993). Since the 1960s, Pb mining activity in Missouri has shifted to the Viburnum Trend or New Lead Belt (Figure 1), which remains a major producer of Pb and other metals. Mining in the Viburnum Trend has developed concurrently with increased environmental regulation and improved technology for metal recovery and pollution control. Studies conducted in the 1970s reported amelioration of adverse biological effects on stream biota and attributed these changes to improved environmental controls, such as recycling of wastewater from milling

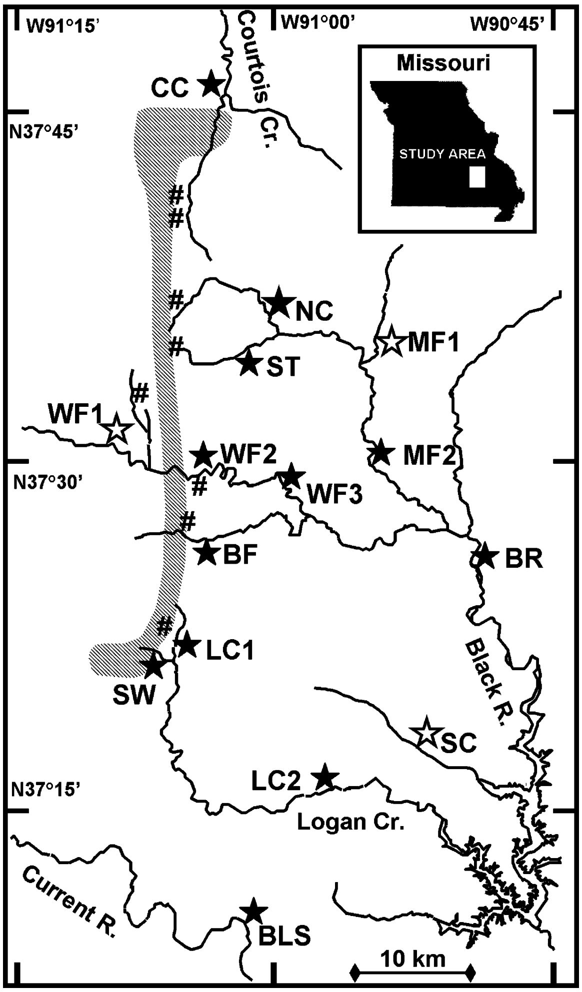


**Figure 1** Map of study sites near the Viburnum Trend mining area. *Stars* indicate study sites, with *hollow stars* indicating stream reference sites. *Hatched area* indicates approximate extent of ore deposits and *symbols* (#) indicate locations of tailings deposits.

operations and development of passive wastewater treatment systems (Ryck & Whitley, 1974; Wixson, 1978). However, mining in the Viburnum Trend has been associated with environmental problems. Discharge of large quantities of metal-contaminated mine tailings into Logan Creek, a tributary of the Black River, during a heavy rainfall event in 1977 resulted in short-term reductions in invertebrate density and taxa richness (Duchrow, 1983). Other studies have reported longer-term environmental problems in streams draining the Viburnum Trend, including elevated concentrations of Pb and other metals in fish tissues (Schmitt et al., 1993) and reduced taxa richness of benthic invertebrate communities (Humphrey & Lister, 2004).

Mining in the Viburnum Trend has progressed from north to south over time as ore deposits have been depleted and new mines have been developed. Most of these deep-shaft mines and ore-processing facilities have been developed on and under lands of the Mark Twain National Forest, which are managed for multiple uses, including mining, forestry, and recreation (USDA Forest Service, 2005). In recent years, mining companies have prospected for ore further south in the National Forest, raising concerns about possible adverse environmental effects on water quality and aquatic biota of high-quality streams. Streams potentially affected by mining in this area include the Current River and Jacks Fork River, managed as the Ozark National Scenic Riverways by the National Park Service, and the Eleven Point River, managed as a National Scenic River by the US Forest Service.

Streams draining the Viburnum Trend and prospecting areas are incised into limestone and dolomite bedrock typical of the Ozark Plateau. Besides mining, land use in the region is dominated by forestry (in the uplands) and cattle grazing (in the limited bottomland areas). Ozark headwater streams are typically low-nutrient systems with substrates dominated by coarse gravels (Petersen et al., 1998). Metals from mining areas may enter streams by seepage from tailings or mine-water impoundments, discharge from passive treatment systems, or erosion of tailings deposits during runoff events (Duchrow, 1983; Wixson, 1978). In the karst landscape of the Ozarks, metals may also enter underground waterways that discharge as springs. For example, Blue Spring, a tributary of the Current River, receives subsurface flow from “losing”

reaches of Logan Creek (Feder & Barks, 1972; Kleeschulte, 2000). Similarly, both Greer Spring, a tributary of the Eleven Point River, and Big Spring, a tributary of the Current River, receive subsurface flow that originates in an area where recent prospecting for Pb ore has occurred (Kleeschulte, 2000). Concerns about possible effects of current and future mining activities on water quality of streams in the Missouri Ozarks have prompted a series of multidisciplinary studies by the US Geological Survey (USGS; Imes, 2002).

The goal of this study was to characterize metal exposure in stream ecosystems draining the Viburnum Trend mining district and the prospecting area, relative to regional background exposure levels. Our approach was to conduct a comprehensive survey of metal concentrations in stream food webs, including plant biomass, aquatic invertebrates, and fish. Food-web biomonitoring has proven to be a valuable component of research on the ecological effects of mining, as it provides information on spatial and temporal variation of metal levels, trophic pathways of metal exposure, and hazards of toxicity to fish and invertebrates (Besser et al., 2001; Farag et al., 1999; Farag et al., 2003). We determined concentrations of Pb, Zn, and Cd in samples collected in fall 2001 and fall 2002 from streams in the vicinity of the Viburnum Trend mining district and in the prospecting area. Our objectives were: (1) to characterize metal concentrations in biota of reference sites within the Ozark region that have no direct influence of mining; (2) to document differences in metal exposure of stream biota between reference sites and sites downstream from mining areas; and (3) to evaluate annual and longer-term temporal variation of metal exposure in study streams.

## 2 Materials and Methods

### 2.1 Study area

Samples of eight components of stream food webs were collected from 19 sites during 2001 (Table I). Eleven sites were located in perennial streams downstream of mining areas in the Viburnum Trend, in the Black River and Meramec River watersheds (Figure 1). Seven of these sites (mining sites) were located close to known mining sites, subject to accessibility and landowner permission, and four additional sites (downstream sites) were located

**Table I** Location of study sites in Missouri USA

| Site ID | Location | Type | County | Latitude/Longitude (°) |
|---|---|---|---|---|
| CC | Courtois Creek | Mining | Washington | 37.7678 N/91.0711 W |
| NC | Neals Creek | Mining | Iron | 37.6081 N/91.0174 W |
| ST | Strother Creek | Mining | Iron | 37.5980 N/91.0384 W |
| MF1 | Middle Fork Black River (upper) | Reference | Iron | 37.6270 N/90.9664 W |
| MF2 | Middle Fork Black River (lower) | Downstream | Reynolds | 37.5247 N/90.9352 W |
| WF1 | West Fork Black River (upper) | Reference | Reynolds | 37.5071 N/91.1612 W |
| WF2 | West Fork Black River (middle) | Mining | Reynolds | 37.4973 N/91.0873 W |
| WF3 | West Fork Black River (lower) | Downstream | Reynolds | 37.4775 N/91.0078 W |
| BF | Bee Fork | Mining | Reynolds | 37.4427 N/91.0894 W |
| BR | Black River | Downstream | Reynolds | 37.4169 N/90.8253 W |
| SW | Sweetwater Creek | Mining | Reynolds | 37.3306 N/91.1369 W |
| LC1 | Logan Creek (upper) | Mining | Reynolds | 37.3408 N/91.1194 W |
| LC2 | Logan Creek (lower) | Downstream | Reynolds | 37.2469 N/90.9665 W |
| SC | Sinking Creek | Reference | Reynolds | 37.3089 N/90.8772 W |
| BLS | Blue Spring | Spring | Shannon | 37.1662 N/91.1632 W |
| BGS | Big Spring | Spring | Carter | 36.9482 N/90.9904 W |
| EP | Eleven Point River | Reference | Oregon | 36.7963 N/91.4054 W |
| HC | Hurricane Creek | Reference | Oregon | 36.7813 N/91.2772 W |
| GRS | Greer Spring | Spring | Oregon | 36.7913 N/91.3439 W |

further downstream of mining areas. Five stream sites (reference sites) were located in streams with no known upstream mining activity (Table I). Three reference sites (MF1, WF1, SC) were in the Black River watershed, near the Viburnum Trend, and two sites (EP and HC) were in the Eleven Point River watersheds, near the prospecting area. Samples were also collected from Blue Spring (BLS; Figure 1), a tributary to the Current River that receives subsurface flow from Logan Creek, and from Big Spring (BGS) and Greer Spring (GRS), which receive subsurface flow originating from the prospecting area. Locations of sample sites were documented by hand-held GPS units based on the WGS84 geodetic datum.

### 2.2 Food web samples

Samples of eight different food web components, including plant biomass (periphyton, filamentous algae, and detritus), invertebrates (crayfish, snails, and riffle benthos), and two taxa of fish were collected from stream sites during September 2001 and from springs during November 2001, with the exception that most of the September samples (detritus, periphyton, algae, snails, and benthos) from Strother Creek (ST) were lost and a second set of samples was collected in December 2001. Additional samples of snails and crayfish were collected (at four sites each) during September 2002. Individual fish were placed in polyethylene bags and each fish was analyzed separately to allow matching metal analyses with measurements of biochemical responses (Schmitt et al., in press). Other samples were composites of multiple individuals, to assure enough biomass for metal analyses and to reduce the influence of individual variation in metal concentrations. Samples of crayfish (three or more individuals per sample) were held in glass jars with Teflon-lined lids. Samples of detritus, periphyton, algae, snails, and benthos (10- to 25-ml of biomass per sample) were held in 30-ml plastic vials. All sample containers were acid-washed before use. Three replicate samples of each component were collected from each study site whenever possible. Each component was collected from at least 11 sites and at least five components were collected from all sites. Samples were stored on ice in the field, then frozen until analysis.

Samples of plant biomass were collected to represent the predominant sources of plant biomass in the study streams. Periphyton, consisting of attached algae and associated organic and inorganic particles, was scraped from rocks along stream margins with an acid-cleaned plastic spatula. Aquatic mosses were collected from the three spring sites where algal periphyton was not available. Samples of filamentous algae were collected from shallow areas in pool habitats. Organic detritus was collected from leaf packs in pool habitats with dip nets. Fine detritus particles were flushed from the dip net with site water into a 300-μm mesh stainless steel sieve, rinsed to remove fine sediments, and then decanted to eliminate heavier sand particles.

Invertebrate samples represented the dominant invertebrate groups of the study streams. Small crayfish (*Orconectes* spp.; <25 mm carapace length) were collected by kick-net in riffle habitats. Crayfish samples were not sorted to species, but dominant species were *O. hylas* (all sites in the Black River watershed), *O. luteus* (sites CC, BLS, and BGS), *O. ozarkae* (sites EP and HC) and *O. eupunctus* (GRS). Mixed-species composite samples of snails were collected from shallow margins of pool and riffle habitats. Mixed-species composite samples of benthic macroinvertebrates (benthos) were collected from riffle habitats. The biomass of riffle benthos samples was dominated by the large, predatory larvae of dobsonflies (Megaloptera: Corydalidae).

Two taxa of fish representing different trophic positions were collected at each site by netting, electrofishing, or hook-and-line. Target fish taxa were large-scale stonerollers (*Campostoma oligolepis*), which feed on periphyton, and longear sunfish (*Lepomis megalotis*), which feed predominantly on small invertebrates (Pflieger, 1997). Neither fish species was collected from site HC, stonerollers were not collected at site SW and sunfish from sites LC1 and CC were collected 2–4 km downstream from the primary collection sites. Alternate fish taxa were collected from the three spring sites, where target taxa were not present: bleeding shiners (*Luxilus zonatus*) and Ozark minnow (*Notropis nubilus*) were substituted for stonerollers; Ozark sculpin (*Cottus hypselurus*) and banded sculpins (*C. carolinae*) were substituted for sunfish. Carcasses of stonerollers and sunfish were analyzed after small amounts of blood and liver tissues were removed for characterization of biochemical responses to metal exposure (Schmitt et al., in press).

Samples of minnows and sculpins were analyzed without removal of blood or liver samples.

### 2.3 Determination of metal concentrations

Samples were analyzed for Pb, Zn, and Cd at the USGS Columbia Environmental Research Center (CERC) by inductively coupled plasma-mass spectroscopy (ICPMS; PE/SCIEX Elan 6000 with CETAC ADX-500 autosampler/autodiluter; May et al., 1997). Samples were prepared for analysis by lyophilization, followed by homogenization to a coarse powder with a cryogenic mill (for snails and crayfish) or standard grinding equipment. Neither shells nor gut contents were removed before metal analyses. Portions of dried and homogenized samples (0.20 – 0.25 g) were digested and analyzed according to the methods described by Brumbaugh et al. (2005).

Quality control measures for digestion and ICPMS analysis included digestion blanks, reference materials, replicates, and spikes. Instrumental quality control included calibration checks, laboratory control solutions, duplicate analyses and digestate spikes. Method recoveries were evaluated with several tissue reference materials: striped bass, a CERC in-house reference material; dogfish (DORM-2) and lobster (TORT-1) from National Research Council of Canada; aquatic moss (CRM 61) and plankton (CRM 414) from the Institute for Reference Materials and Measurements; and oyster (NIST 1566a and 1566b) from National Institute of Standards and Technology. Recoveries for 96 individual analyses of reference materials ranged from 87% to 108%, except for single high recoveries of Cd (253%) and Pb (276%), apparently due to contamination during digestion. Recoveries from 144 method spikes (spiked into food web matrices) averaged 97% and ranged from 75% to 124%, except for one high recovery for Pb (146%). Method precision, based on triplicate digestion and analysis of 24 food web samples, averaged 6.2% relative standard deviation (RSD; standard deviation as percent of mean) and was less than 17% RSD for all elements in all samples except for one RSD of 32% for Pb in a fish carcass. Method detection limits for food web samples were 0.18 to 1.6 μg/g (dry wt.) for Zn, 0.005 to 0.03 μg/g for Cd, and 0.003 to 0.02 μg/g for Pb. Most blank-equivalent concentrations (38 of 51) were at or below method detection limits and none were remarkably high relative to sample concentrations.

### 2.4 Statistical analysis

Statistical analysis was performed using Statistical Analysis System software (Version 10; SAS Institute, Cary, NC). Differences among sites were evaluated by analysis of variance (ANOVA) after metal concentrations were log-transformed to improve normality and homogeneity of variance. Comparisons of means between stream sites and spring sites were made by linear contrasts. Comparisons among means for individual sites were made by Fisher's protected least significant difference (LSD) test. Comparisons of means between 2001 and 2002 samples were made with *t*-tests. Associations among metal concentrations in different sample types were evaluated by Pearson product-moment correlation. Statements of statistical significance refer to 5% Type I error rate ($P \leq 0.05$).

## 3 Results and Discussion

### 3.1 Metals in biota from non-mining areas

Metal concentrations in food chain components from sites with no known upstream mining activity often differed between warm-water streams and cold-water springs (Figure 2). For four of eight components, Pb concentrations in samples from streams were significantly greater than in samples from springs. Concentrations of Zn and Cd followed an opposite trend, with greater concentrations in samples from springs. These differences were most pronounced for Cd concentrations, which were significantly greater in samples from springs for all eight sample types. The different taxa collected in spring and stream habitats (aquatic mosses, shiners, and sculpins in springs; periphyton, stonerollers, and sunfish in streams) could explain some of the observed differences in measured metal concentrations between habitats. However, there is no reason to expect that substitution of taxa would consistently produce the same trends of lesser concentrations of Pb and greater concentrations of Zn and Cd in springs relative to streams. Greater Pb concentrations at stream sites may reflect elevated 'background' Pb exposure near the Viburnum Trend mining district, from naturally occurring mineral deposits or

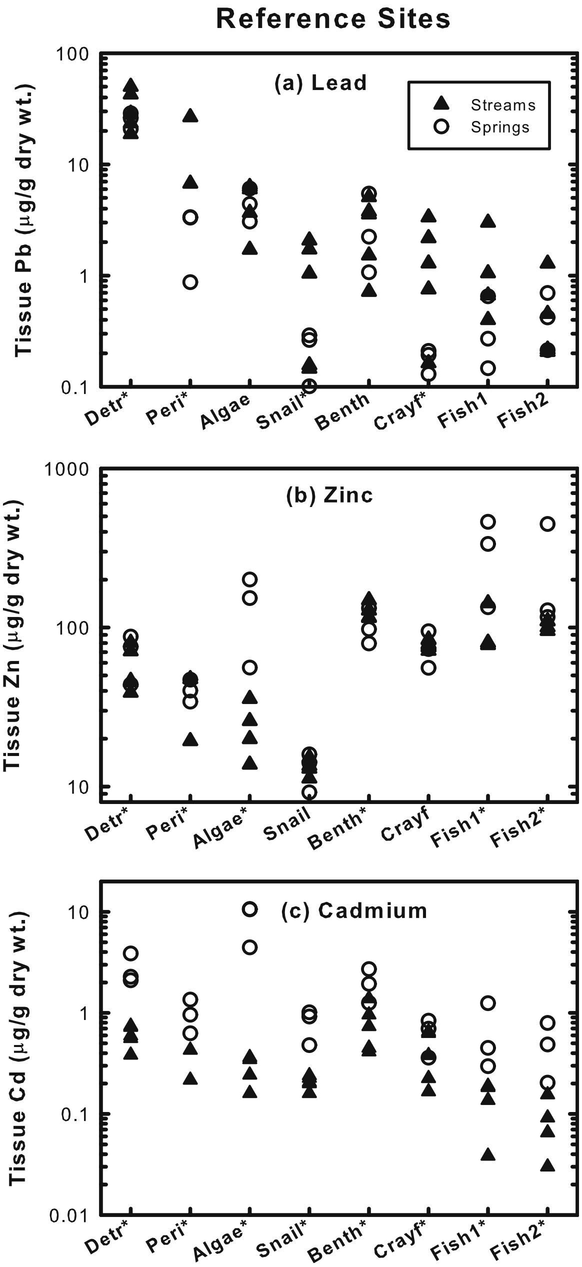


**Figure 2** Metal concentrations in food-web components in streams and springs without direct influence of mining. Sample types: *Detr* = detritus; *Peri* = periphyton; *Algae* = filamentous algae; *Snail* = snails; *Benth* = riffle benthos; *Crayf* = crayfish; *Fish1* = stonerollers (*in streams*) or other Cyprinidae (*in springs*); *Fish2* = sunfish (*streams*) or sculpins (*springs*). For each sample type, *asterisks* indicate significant differences between streams and springs.

from dispersed anthropogenic sources. Femmer (2004) reported that Pb concentrations in sediments from reference sites in the Viburnum Trend were greater than those in sediments from in the Eleven Point River watershed, outside the Viburnum Trend. In our study, food-web samples from reference streams near mining areas (MF1 and WF1) had greatest mean Pb concentrations for five of eight sample types, but greatest mean Pb concentrations in other sample types occurred at stream or spring sites outside the mining area. The consistent differences between spring and stream sites across multiple food-web components, even when the same or similar sample types were collected in both habitat types (e.g., detritus), suggests that differences between springs and streams represent underlying differences in processes controlling metal bioavailability. Characteristics of the spring habitats (e.g., low suspended sediment concentrations, low nutrient levels, and cold temperatures) may favor bioaccumulation of Cd and Zn, which are relatively soluble in water, compared to Pb, which is more strongly associated with sediment and organic matter. Regardless of the underlying cause(s), these apparent differences between spring and stream habitats should be considered in studies intended to evaluate metal bioavailability in aquatic ecosystems.

The five stream sites without upstream mining activity (MF1, WF1, SC, HC, and EP) were used as reference sites for comparisons with stream sites downstream of mining. Lead concentrations at reference sites were more variable, both among sites and among sample types, than were concentrations of Zn or Cd. Among-site variation of metal concentrations, expressed as RSDs for the eight sample types, ranged from 40% to 94% for Pb, 9% to 62% for Cd and 5% to 60% for Zn. Lead concentrations also differed more widely among the eight sample types, with median Pb concentrations ranging from 0.34 μg/g (for sunfish) to 28 μg/g (for detritus). Greatest concentrations of Zn and Cd occurred in riffle benthos. The two fish species had lowest median concentrations of Pb (0.86 μg/g for stonerollers, 0.34 μg/g for sunfish) and Cd (0.08 μg/g for stonerollers, 0.16 μg/g for sunfish) but comparatively high levels of Zn (80 μg/g for stonerollers, 105 μg/g for sunfish), presumably due to regulation of internal concentrations of this essential element (Sorensen, 1991). Median concentrations of all three metals in both fish species from reference streams were greater than 85th percentiles of metal concentrations from the 1985 National Contaminant Biomonitoring Program (NCBP), which analyzed 315 composite fish samples from 109 stations on major

rivers in the US (Schmitt & Brumbaugh, 1990). The apparent elevated 'background' metal concentrations in fish from these remote streams are unlikely to reflect anthropogenic metal inputs, but they may reflect an elevated regional enrichment of these metals in the Ozark region, due to dispersed mineral deposits (Lee, 2000). However, they may also reflect differences in habitats and fish species sampled between the two studies.

## 3.2 Metals in stream food webs downstream from mining areas

### *3.2.1 Invertebrates and plant biomass*

Concentrations of Pb, Zn and Cd in plant biomass and invertebrates were greater at mining and downstream sites than at reference sites (Tables II, III, and IV). For all six of these sample types, mean concentrations of Pb, Zn, and Cd from one or more mining site were significantly greater than those from all five reference sites. Concentrations of all three metals were high in samples of plant biomass from mining sites and over 90% of these samples were significantly greater than samples from reference sites. Metal bioaccumulation in invertebrates was more variable among metals and among sample types. Benthos samples generally contained greater concentrations of all three metals than samples of snails or crayfish. Significant increases in metal concentrations in benthos from mining sites (relative to reference sites) were more frequent for Pb and Zn (6 of 11 sites), than for Cd (3 of 11 sites). Snails generally had lower metal concentrations than other invertebrates, but concentrations of all three metals were significantly greater in snails from most mining sites than in those from reference sites. Crayfish from mining and downstream sites had fewer significant differences from reference sites for all three metals: 4 of 10 sites for Pb and Cd and only one site (CC) for Zn.

We assessed the degree of metal contamination associated with mining activities by calculating 'enrichment factors' (EFs), defined as ratios of mean metal concentrations in samples from mining or downstream sites to corresponding means for samples from reference sites. Average EFs for Pb in plant biomass from mining sites ranged from nine for periphyton to 61 for filamentous algae. Enrichment factors for other metals were nearly as large: from 10 (periphyton) to 20 (algae) for Zn, and from 7 (periphy-

**Table II** Lead concentrations (μg/g dry wt.) in plant biomass and invertebrates from sites downstream of mining areas, compared to reference sites

| Site | Detritus | Periphyton | Algae | Snail | Benthos | Crayfish | Rank |
|---|---|---|---|---|---|---|---|
| *Mining sites* | | | | | | | |
| CC | **522 (55)** | **165 (4)** | **326 (48)** | **11 (1)** | **68 (3)** | **16 (1)** | 3 |
| NC | **142 (5)** | 9.5 (1.6) | **13 (3)** | **14 (2)** | 5.9 (0.9) | 1.5 (0.2) | 8 |
| ST | **779 (33)** | **149 (10)** | **413 (110)** | –[a] | **106 (20)** | – | 1 |
| WF2 | **204 (39)** | **63 (11)** | **58 (9)** | **3.9 (0.8)** | 8.0 (0.2) | 2.7 (0.4) | 6 |
| BF | – | **309 (11)** | **547 (42)** | **7.1 (0.9)** | **35 (6)**[b] | **16 (2)** | 2 |
| SW | **496 (60)** | **96 (9)** | – | **17**[c] | **22 (3)**[b] | **16 (5)** | 4 |
| LC1 | **282 (15)** | **253 (28)** | – | **9.1 (1.7)**[b] | **16 (2)** | **4.7 (0.4)** | 5 |
| *Downstream sites* | | | | | | | |
| MF2 | **168 (21)** | **78 (9)** | 9.1 (0.9) | 1.9 (0.2) | **9.9 (1.1)** | 2.5 (0.3) | 7 |
| WF3 | **117 (26)** | – | 9.0 (2.5) | 1.5 (0.4) | 5.3 (1.8) | 1.3 (0.2) | 9 |
| LC2 | **70 (3)** | – | 6.4 (0.0) | 0.9 (0.1) | 3.8 (1.2) | 0.8 (0.2) | 13 |
| BR | **66 (3)** | 22 (1) | **28 (4)** | – | 2.8 (0.6) | 0.5 (0.1) | 12 |
| *Reference sites* | | | | | | | |
| (range) | 19–50 | 6.7–27 | 1.7–6.3 | 0.1–2.1 | 0.7–3.4 | 0.2–1.3 | 10–16 |

Means (standard error in parentheses; $n$ = 3 unless noted), with ranges of site means for reference sites. Site means in bold text are significantly greater than means from all reference sites. Rank = site rank based on average rankings for all sample types.

[a] '–' indicates no sample.

[b] $n$ = 2.

[c] $n$ = 1.

**Table III** Zinc concentrations (μg/g dry wt.) in plant biomass and invertebrates from sites downstream of mining areas, compared to reference sites

| Site | Detritus | Periphyton | Algae | Snails | Benthos | Crayfish | Rank |
|---|---|---|---|---|---|---|---|
| *Mining sites* | | | | | | | |
| CC | **969 (36)** | **486 (13)** | **454 (28)**[c] | **41 (8)** | **426 (20)** | **121 (8)** | 2 |
| NC | **637 (6)** | **95 (12)** | **252 (72)** | **32 (3)** | **251 (11)** | 94 (5) | 4 |
| ST | **3080 (120)** | **685 (54)** | **1110 (260)** | –[a] | **493 (83)** | – | 1 |
| WF2 | **275 (18)** | **119 (17)** | **118 (14)** | **25 (1)** | 183 (3) | 95 (4) | 7 |
| BF | – | **287 (5)** | **481 (40)** | 19 (0.3) | 177 (12)[b] | 97 (3) | 6 |
| SW | **452 (33)** | **212 (7)** | – | **27**[c] | **251 (32)**[b] | 97 (7) | 5 |
| LC1 | **620 (17)** | **425 (54)** | – | **31 (3)**[b] | **361 (42)** | 94 (2) | 3 |
| *Downstream sites* | | | | | | | |
| MF2 | **217 (16)** | **102 (4)** | **111 (2)** | 19 (2) | **205 (18)** | 90 (7) | 8 |
| WF3 | **125 (2)** | – | **78 (8)** | 14 (1) | 135 (14) | 87 (6) | 9 |
| LC2 | **989 (5)** | – | 32 (3) | 17 (1) | 128 (16) | 77 (8) | 12 |
| BR | 65 (2) | 41 (1) | 52 (13) | – | 161 (4) | 81 (6) | 11 |
| *Reference sites* | | | | | | | |
| (range) | 39–80 | 19–48 | 14–26 | 11–15 | 115–149 | 72–84 | 10–16 |

Means (standard error in parentheses; $n = 3$ unless noted), with ranges of site means for reference sites. Site means in bold text are significantly greater than means from all reference sites. Rank = site rank based on average rankings for all sample types.

[a] '–' indicates no sample.

[b] $n = 2$.

[c] $n = 1$.

**Table IV** Cadmium concentrations (μg/g dry wt.) in plant biomass and invertebrates from sites downstream of mining areas, compared to reference sites

| Site | Detritus | Periphyton | Algae | Snails | Benthos | Crayfish | Rank |
|---|---|---|---|---|---|---|---|
| *Mining sites* | | | | | | | |
| CC | **13 (0.2)** | **4.4 (0.1)** | **5.0 (0.2)**[a] | **1.5 (0.2)** | **9.5 (0.4)** | **5.1 (0.1)** | 1 |
| NC | **2.5 (0.1)** | 0.39 (0.05) | **1.4 (0.4)** | **0.37 (0.02)** | 1.8 (0.1) | 0.74 (0.11) | 4 |
| ST | **4.9 (0.2)** | **1.8 (0.1)** | **2.3 (0.6)** | –[b] | 0.56 (0.15) | – | 6 |
| WF2 | **1.3 (0.2)** | 0.48 (0.09) | 0.55 (0.06) | 0.20 (0.01) | 0.34 (0.03) | 0.32 (0.02) | 12 |
| BF | – | **2.0 (0.01)** | **2.5 (0.2)** | **0.35 (0.02)** | 0.71 (0.00)[a] | **0.96 (0.14)** | 5 |
| SW | **6.7 (0.6)** | **2.4 (0.1)** | – | **1.8**[c] | **11 (1)** | **3.9 (0.3)** | 3 |
| LC1 | **9.4 (0.5)** | **5.2 (0.3)** | – | **1.9 (0.3)**[a] | **6.4 (0.7)**[a] | **2.5 (0.2)** | 2 |
| *Downstream sites* | | | | | | | |
| MF2 | 0.91 (0.09) | **0.66 (0.04)** | 0.17 (0.02) | 0.25 (0.03) | 0.95 (0.11) | 0.46 (0.07) | 10 |
| WF3 | **1.1 (0.02)** | – | 0.36 (0.04) | 0.17 (0.01) | 1.5 (0.2) | 0.62 (0.04) | 9 |
| LC2 | **0.94 (0.02)** | – | 0.42 (0.03) | 0.12 (0.01) | 0.27 (0.11) | 0.22 (0.06) | 15 |
| BR | 0.70 (0.01) | 0.27 (0.00) | **0.96 (0.18)** | – | 1.6 (0.2) | 0.65 (0.02) | 7 |
| *Reference sites* | | | | | | | |
| (range) | 0.38–0.74 | 0.22–0.43 | 0.16–0.36 | 0.16–0.24 | 0.41–1.4 | 0.17–0.65 | 8–16 |

Means (standard error in parentheses; $n = 3$ unless noted), with ranges of site means for reference sites. Site means in bold text are significantly greater than means from all reference sites. Rank = site rank based on average rankings for all sample types.

[a] $n = 2$.

[b] '–' indicates no sample.

[c] $n = 1$.

ton) to 10 (detritus) for Cd. Average EFs for the three invertebrate groups were greatest for Pb (EFs from 6.0 to 13), intermediate for Cd (4.9–5.4), and lowest for Zn (1.3–2.4). The low average EF for Zn in crayfish from mining sites (1.3) suggests that crayfish, like fish, can regulate internal Zn concentrations despite elevated Zn concentrations in stream food webs.

Metal concentrations in plant biomass and invertebrates were lower at downstream sites than at sites closest to mining (Tables II, III, and IV). Average metal concentrations in plant and invertebrate samples decreased by factors from five to eight between mining and downstream sites. Rankings of food-web metal concentrations across sites (average site ranks across all plant and invertebrate sample types) were consistently high for mining sites, which had the three highest ranks for one or more metals: CC (all three metals); ST (Pb and Zn); BF (Pb); LC1 (Zn and Cd); and SW (Cd). Conversely, no downstream site were ranked higher than seventh for any metal. Only two mining sites (NC and WF2) had rankings lower than any downstream sites. Lower levels of metal contamination at these two sites probably reflect reduced activity at upstream mining areas. The Magmont mill (in the headwaters of Neals Creek) was closed in 1994, and ore from the West Fork mine (upstream from WF2) has been diverted to the Fletcher mill on Bee Fork in recent years (Seeger, 2005, written communication, Missouri Department of Natural Resources).

### *3.2.2 Fish tissues*

Lead concentrations in fish tissues were frequently elevated at sites close to mining activities. Stonerollers from three of six mining sites and sunfish from five of seven mining sites had Pb concentrations that were significantly greater than those at any reference site (Figures 3 and 4). Enrichment factors for Pb in tissues of fish from mining sites were similar to those for invertebrates and plant biomass, with mean EFs of 16 for stonerollers and 12 for sunfish.

Differences between mining and reference sites were less consistent for Zn and Cd. For these metals, fewer site means for fish from mining sites were significantly greater than reference sites: 3 of 13 means Zn and 2 of 13 for Cd. Most of these significant differences were for stonerollers, consistent with the greater EFs for stonerollers (average EFs: 1.6

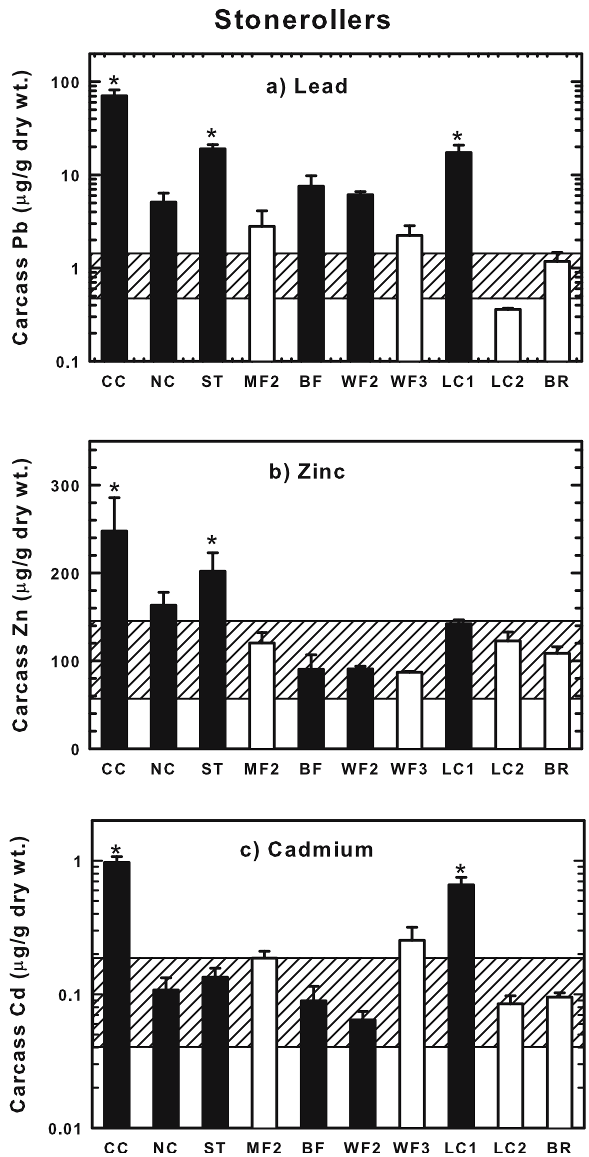


**Figure 3** Metal concentrations in large scale stonerollers (*Campostoma oligolepis*) from streams downstream of mining areas. *Bars* are site means, with standard errors ($n$ = 3). *Solid bars* indicate mining sites; *hollow bars* indicate downstream sites; and *hatching* indicates range of means for five reference sites. *Asterisks* indicate site means that are significantly greater than means for all reference sites.

for Cd and 2.5 for Zn) than for sunfish (average EFs: 1.2 for Cd and 1.0 for Zn). These apparent differences in concentrations of all three metals between the two fish species may have been biased by the fact that sunfish samples from two mining sites with high overall levels of metal contamination (LC1 and CC)

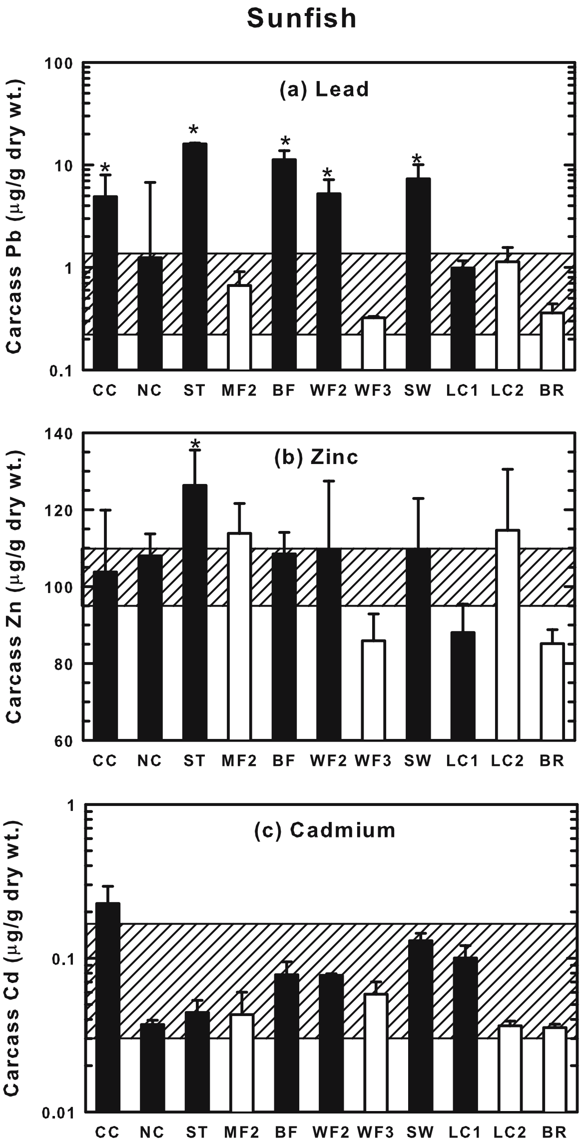


**Figure 4** Metal concentrations in longear sunfish (*Lepomis megalotis*) from streams downstream of mining areas. *Bars* are site means, with standard errors ($n$ = 3). *Solid bars* indicate mining sites; *hollow bars* indicate downstream sites; and *hatching* indicates range of means for five reference sites. *Asterisks* indicate site means that are significantly greater than means for all reference sites.

were collected 2–4 km downstream from the locations where stonerollers were collected.

Metal concentrations in fish tissues from both mining and downstream sites were substantially elevated relative to the nationwide distribution of metal concentrations in fish samples reported in the NCBP survey (Schmitt & Brumbaugh, 1990). Many mean metal concentrations in fish from several mining sites exceeded the maximum concentrations measured by the NCBP: 8 (of 13) means for Pb, 5 means for Zn and 3 means for Cd. Maximum mean concentrations of the three metals (all in stonerollers from site CC) exceeded NCBP maxima by factors of six (for Pb), four (for Cd) and two (for Zn). Although no site means for metal concentrations in fish from downstream sites were significantly greater than those for reference sites (Figures 3 and 4), nearly 90% of site means for downstream sites exceeded the 85th percentile from NCBP survey.

### 3.3 Relationships among food-web components

Associations between metal concentrations of aquatic animals and their probable diets were generally stronger for aquatic invertebrates than for fish. Metal concentrations in the three types of invertebrates were strongly correlated with those in three types of plant biomass (Table V). Almost all (43 of 45) possible bivariate correlations among plant and invertebrate sample types were statistically significant. These consistent associations indicate that metal contamination was distributed rather uniformly across the lower trophic levels of the study streams. In contrast, the strength of correlations between metal concentrations in fish carcasses and other food-web components differed among metals and between fish species. Zinc concentrations in stoneroller carcasses were significantly correlated with those in most food-web components, but stoneroller Pb concentrations were significantly correlated only with Pb concentrations in detritus, and stoneroller Cd concentrations were not significantly correlated with Cd concentrations in any food-web components. In contrast, concentrations of Pb and Cd (but not Zn) in sunfish carcasses were significantly correlated with concentrations in several food-web components.

Associations between metal concentrations in fish tissues and those in other food-web components may reflect a variety of ecological and physiological mechanisms. Transfer of metals via contaminated diets may have contributed to some significant correlations, such as those between stonerollers and detritus or between sunfish and snails or crayfish. However, the use of correlation analysis to differentiate trophic pathways was limited by the strong inter-correlation of metal concentrations in many different food sources (e.g., detritus, periphyton, and filamentous

**Table V** Pearson product–moment coefficients ($r$) for correlations among metal concentrations in food-web components

| Metal | | Periphyton | Algae | Snails | Crayfish | Benthos | Stoneroller | Sunfish |
|---|---|---|---|---|---|---|---|---|
| Lead | Detritus | **0.87** | **0.91** | **0.90** | **0.92** | **0.83** | **0.68** | **0.56** |
| | Periphyton | –[a] | **0.85** | **0.79** | **0.76** | **0.80** | 0.23 | 0.51 |
| | Algae | – | – | **0.76** | **0.90** | **0.78** | 0.45 | **0.63** |
| | Snails | – | – | – | **0.81** | **0.85** | 0.41 | 0.44 |
| | Crayfish | – | – | – | – | **0.84** | 0.52 | **0.57** |
| | Benthos | – | – | – | – | – | 0.35 | 0.50 |
| Zinc | Detritus | **0.69** | **0.85** | **0.89** | **0.92** | **0.69** | **0.81** | 0.35 |
| | Periphyton | – | 0.53 | **0.73** | **0.77** | **0.71** | 0.12 | 0.06 |
| | Algae | – | – | **0.65** | **0.67** | 0.38 | **0.72** | 0.45 |
| | Snails | – | – | – | **0.62** | **0.86** | **0.79** | 0.08 |
| | Crayfish | – | – | – | – | **0.84** | **0.85** | 0.25 |
| | Benthos | – | – | – | – | – | **0.67** | 0.13 |
| Cadmium | Detritus | **0.91** | **0.80** | **0.90** | **0.75** | **0.82** | 0.52 | 0.43 |
| | Periphyton | – | **0.62** | **0.82** | **0.62** | **0.78** | 0.30 | **0.75** |
| | Algae | – | – | **0.88** | **0.60** | **0.56** | 0.48 | 0.24 |
| | Snails | – | – | – | **0.79** | **0.88** | 0.27 | **0.58** |
| | Crayfish | – | – | – | – | **0.87** | 0.35 | **0.54** |
| | Benthos | – | – | – | – | – | 0.46 | 0.52 |

Analyses performed on log-transformed site means ($n = 10-17$). Values in bold text are statistically significant ($P \leq 0.05$).

[a] '–' indicates redundant value or self-correlation.

algae). Some significant correlations (e.g., Pb in algae and sunfish, Zn in crayfish and stonerollers) that do not correspond to important trophic linkages may simply reflect similar metal uptake via different metal exposure pathways. It is less clear why some known trophic linkages (e.g., periphyton-to-stoneroller and benthos-to-sunfish) did not show significant correlations in metal concentrations. Regulation of internal Zn concentrations by sunfish (Sorensen, 1991), which is suggested by the narrow range of sunfish Zn concentrations among sites (Figure 4), may explain the lack of any significant correlation of Zn concentrations between sunfish and food-web components. However, regulation probably cannot account for weak correlations of concentrations of all three metals in stonerollers with concentrations in periphyton, their predominant diet (Pflieger, 1997). Metal concentrations in periphyton samples collected for analysis (collected from shallow, ungrazed stream margins) may not adequately represent the dietary metal exposure of stonerollers, which grazed on periphyton in deeper water. The generally weak associations of metal among food-web components observed in this study may be attributable to the sampling strategy, which was focused on comparisons among sites rather than trophic transfer. Other studies that examined clearly-defined trophic linkages have reported substantial contributions of trophic transfer to overall metal bioaccumulation in aquatic food webs (Besser et al., 2001; Croteau et al., 2005).

### 3.4 Changes in metal exposure over time

Supplemental sampling of crayfish and snails at selected sites in September 2002 indicated substantial differences in metal exposure between years for both taxa and for all three metals. Overall, concentrations of all three metals varied more between years for snails than for crayfish. The average relative percent difference (RPD = difference between means/average of means, expressed as percent) between years for all three metals averaged 50% for snails and 34% for crayfish, and there were more than twice as many significant differences between years for snails as there were for crayfish (Figure 5). Significant annual differences in concentrations of all three metals occurred at two or more sites for snails, but at only a single site for crayfish. The greater frequency of significant differences between years for snails, compared to crayfish, is consistent with the greater frequency of differences for snails in comparisons between mining and reference sites in 2001 (Tables II,

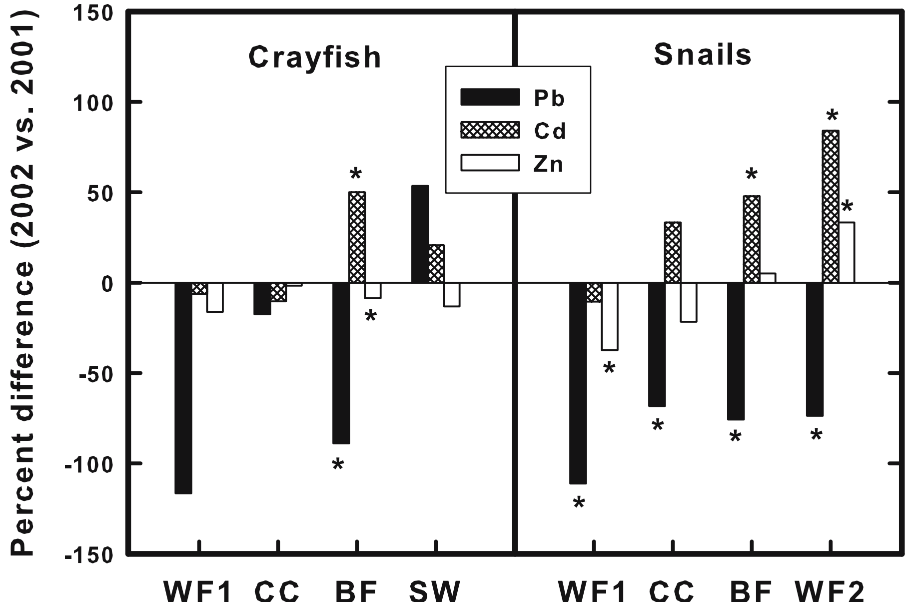


**Figure 5** Differences in metal concentrations between 2001 and 2002 in samples of crayfish and snails from selected sites. *Bars* represent difference between annual means (2002 minus 2001), expressed as a percentage of the overall mean. *Asterisks* indicate significant differences between annual means.

III, and IV). This tendency suggests that metal concentrations in snail samples are more indicative of medium-term variation in metal exposure (i.e., seasonal to annual), perhaps due to retention of metals in the snail shell compared to periodic recycling of metals associated with the crayfish exoskeleton.

Both the magnitude and the direction of changes in metal exposure between years differed for the three metals studied. For both taxa, differences between years were greatest for Pb, with an average RPD of 76%, intermediate for Cd (average RPD = 33%) and least for Zn (average RPD = 17%). Mean Pb concentrations were lower in 2002 for seven of eight sample pairs, with significant decreases for five pairs (Figure 5). In contrast, mean Cd concentrations were greater in 2002 for five of eight pairs, with significant increases for three pairs. This qualitative difference in the behavior of Pb and Cd indicates that differences between years cannot be simply interpreted as an overall increase or decrease in the influence of mining. The opposite annual trends for these metals could reflect a decrease in the influence of metals associated with fine sediments between fall 2001 and fall 2002. The 2001 samples were collected near the end of a multi-year drought in the study area. Discharge of the Black River at Poplar Bluff, Missouri (downstream of the confluence of the West, Middle, and East Forks of the Black River and Logan Creek) during calendar years 2000–2001 was the lowest for any two-year period since recordkeeping began in 1940. This drought was broken in December 2001 and was followed by a period of high stream discharge. The nine-month period preceding the supplemental sampling in September 2002 included both the peak daily discharge and highest average discharge over a comparable seasonal period (January to September) since 1985 (see http://nwis.waterdata.usgs.gov/). We hypothesize that changes in bioaccumulation of Pb and Cd between 2001 and 2002 may reflect changes in the sediment budget in study streams as metal-rich fine sediments that accumulated during the drought years were flushed from stream gravels during the high-flow period. Depletion of fine sediments could have shifted conditions in the study streams toward those observed in low-sediment spring systems, which were also characterized by relatively low bioavailability of Pb and relatively high bioavailability of Cd (Figure 2).

Our observation of significant inter-annual variation of metal concentrations in biota is consistent with an emerging literature describing temporal variation of metal exposure in mining-affected streams. Annual variation in metal bioaccumulation was also found to be related to variation in stream discharge in a 10-year study of the Clark Fork River (Montana, USA; Hornberger et al., 1997). Significant temporal variation in metal exposure and toxicity can also occur in response to seasonal patterns of precipitation and

**Table VI** Lead concentrations (μg/g dry wt.) in food-web samples from mining areas

| Area | Location | Plant biomass | Invertebrates | Fish |
|---|---|---|---|---|
| *Viburnum Trend (Missouri)* | | | | |
| (This study) | CC | 165–522 | 5.4–68 | 4.9–31 |
| | NC | 10–142 | 1.5–14 | 1.2–1.7 |
| | ST | 149–779 | 106–106 | 13–16 |
| | BF | 309–547 | 3.2–35 | 0.36–11 |
| | LC1 | 253–282 | 4.7–16 | 1.0 |
| (Wixson, 1977) | Near CC | –[a] | 84 (40–127) | 25 (18–42) |
| | Near NC | – | 24 (3–44) | 12 (nd[b]–22) |
| | Near ST | 140–800 | 79 (3–400) | 30 (nd–58) |
| | Near BF | – | 19 (3–40) | 16 (nd–41) |
| | Near LC1 | – | – | (nd–23) |
| *Tri-State District (Missouri/Kansas/Oklahoma)* | | | | |
| (Brumbaugh et al., 2005) | Spring and Neosho Rivers | – | – | 0.1–2.3 |
| (Allen & Wilson, 1992) | Spring River | – | 8.9–12 | nd–7.2[c] |
| *Old Lead Belt (Missouri)* | | | | |
| (Brumbaugh et al., 2005) | Big River | – | – | 8–22 |
| (Schmitt & Finger, 1982) | Big River | – | 84–140 | 48–208[c] |
| *Columbia River (Washington)* | | | | |
| (Hinck et al., 2006) | Columbia River | – | – | 0.88–37[c] |
| *Coeur d'Alene River (Idaho)* | | | | |
| (Farag et al., 1998) | Coeur d'Alene River | 450–26,000 | 46–3,900 | 74–790[c] |

Values are site means, with ranges of individual measurements in parentheses. Fish data are from carcass or whole-body samples.

[a] '–' indicates no data available.

[b] 'nd' = below detection limits.

[c] Concentrations converted from wet weight basis, assuming 75% moisture content.

snowmelt (Besser & Lieb, 2005) and daily cycles of photosynthesis and respiration (Nimick et al., 2005). Seasonal and daily variation in metal exposure can be accommodated in the design of tissue biomonitoring studies by careful selection of sampling dates and times, but characterization of longer term variation in metal bioavailability may be beyond the scope of many routine monitoring studies. Our findings demonstrate the value of multiple-year sampling efforts for studies designed to characterize spatial and temporal changes in metal contamination of stream biota.

Lead concentrations in stream biota measured during 2001 and 2002 provide a basis for an examination of long-term changes in metal exposure associated with mining activities in the Viburnum Trend. Surveys of Pb concentrations in biota downstream of Viburnum Trend mines were conducted during the period, 1972–1975, as part of a multi-year study reported by Wixson (1977, 1978). Comparison of Pb concentrations in invertebrates and fish collected from similar sample sites during these two sampling periods indicates that overall levels of metal contamination in streams downstream of active mines have not changed substantially since the 1970s (Table VI). Mean Pb concentrations in invertebrates and fish from stream reaches downstream of mining activity overlapped broadly across the two time periods. At two sites (BF and CC) where we conducted two years of sampling for crayfish and snails, mean Pb concentrations from 2002 were less than the means from the 1970s, but means from 2001 overlapped with those from the earlier survey. At only one location, Neals Creek, were Pb concentrations in both invertebrates and fish substantially less in 2001 than in 1972–1975. This finding is consistent with the cessation of milling activity in 1994 at the Magmont mill, located in the headwaters of Neals Creek (C. Seeger, 2005, written communication, Missouri Department of Natural Resources).

### 3.5 Lead contamination and effects on stream biota

Levels of Pb contamination in streams draining the Viburnum Trend, although greater than those in reference streams, are less severe than that occurring at some other Pb-mining districts. Crayfish and fish from the Big River, which drains the Old Lead Belt of Missouri, have been found to accumulate substantially greater concentrations of Pb than those in streams draining the Viburnum Trend (Schmitt & Finger, 1982; Table VI). Because the ore deposits, surficial geology, and hydrology of the Old Lead Belt and Viburnum Trend are similar, the greater Pb exposure of biota from the Big River probably reflects less-efficient recovery of ore minerals in the milling process and greater problems with containment of mine tailings in the Old Lead Belt, compared to the Viburnum Trend (Schmitt & Finger, 1982). Metal contamination and habitat disturbance in streams draining the Old Lead Belt are reflected in substantial biological effects, including alteration of benthic invertebrate communities (Lister & Humphrey, 2004) and inhibition of Pb-sensitive enzyme systems in fish (Schmitt et al., 1993). The most severe example of Pb contamination of aquatic environments in the United States is the Coeur d'Alene River watershed of Idaho, where some stream and reservoir biota contain tissue Pb concentrations that are 10- to 100-fold greater than those in the Viburnum Trend (Farag et al., 1998; Table VI). Exposure to high levels of Pb and associated metals in the Coeur d'Alene watershed has been linked to toxic effects in waterfowl and fish (Blus, Henny, Hoffman, & Grove, 1991; Farag et al., 1999).

Ecological effects associated with mining have also been reported for streams draining the Viburnum Trend. Ryck and Whitley (1974) reported decreased taxonomic richness and diversity of invertebrate communities of Indian Creek (a mining-affected tributary of Courtois Creek), Strother Creek, and Bee Fork during the early years of mining in the Viburnum Trend. A recent study of aquatic communities Indian Creek and Courtois Creek reported reduced taxa richness, reduced diversity, and increased dominance of pollution-tolerant taxa in macroinvertebrate communities compared to reference sites (Humphrey & Lister, 2004). Schmitt et al. (in press) reported significantly elevated concentrations of Pb and decreased enzymatic activity in blood of stonerollers, sunfish and northern hogsuckers (*Hypentelium nigricans*) collected downstream of Viburnum Trend mining areas during this study. These findings, together with our findings of significantly elevated exposure of stream biota to Pb and other metals, suggest that adverse biological impacts could be widespread in streams downstream of mining areas in the Viburnum Trend. Ongoing studies of fish and invertebrate communities in streams draining the Viburnum Trend, together with laboratory and field toxicity tests, are designed to characterize the extent and severity of adverse biological effects associated with mining in this region.

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