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Response of benthic invertebrate assemblages to metal exposure and bioaccumulation associated with hard-rock mining in northwestern streams, USA

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Abstract. Benthic macroinvertebrate assemblages, environmental variables, and associated mine density were evaluated during the summer of 2000 at 18 reference and test sites in the Coeur d'Alene and St. Regis River basins, northwestern USA as part of the US Geological Survey's National Water-Quality Assessment Program. Concentrations of Cd, Pb, and Zn in water and (or) streambed sediment at test sites in basins where production mine density was ≥0.2 mines/km^2 (in a 500-m stream buffer) were significantly higher than concentrations at reference sites. Zn and Pb were identified as the primary contaminants in water and streambed sediment, respectively. These metal concentrations often exceeded acute Ambient Water Quality Criteria for aquatic life and the National Oceanic and Atmospheric Administration Probable Effect Level for streambed sediment. Regression analysis identified significant correlations between production mine density in each basin and Zn concentrations in water and Pb in streambed sediment (r^2 = 0.69 and 0.65, $p < 0.01$). Metal concentrations in caddisfly tissue, used to verify site-specific exposures of benthos, also were highest at sites downstream from intensive mining. Benthic invertebrate taxa richness and densities were lower at sites downstream than upstream of areas of intensive hard-rock mining and associated metal enrichment. Benthic invertebrate metrics that were most effective in discriminating changes in assemblage structure between reference and mining sites were total number of taxa, number of Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa, and densities of total individuals, EPT individuals, and metal-sensitive Ephemeroptera individuals.

Key words: mining, metal exposure, macroinvertebrates, bioaccumulation, structural response.

Mining activities over the last century have profoundly altered water-quality, aquatic-biological, and hydrologic conditions in the Coeur d'Alene (CDA) River basin (Ellis 1940, Hoiland et al. 1994, Horowitz et al. 1995, Woods and Beckwith 1997, Maret and Dutton 1999). From the late 1800s to early 1980s, the CDA Mining District, located in the CDA valley of northern Idaho, was among the USA's leading producers of Pb, Ag, and Zn. These past mining activities have resulted in US Environmental Protection Agency (USEPA) Superfund investigations and remediations in the South Fork CDA River. The second-largest USEPA Superfund site is located in the study area—the Bunker Hill Smelter near Kellogg, Idaho (Doppelt et al. 1993). In addition, the US Department of the Interior is currently conducting Natural Resource Damage Assessments and other remediation activities in the CDA River basin. Despite treatment and clean-up of mine wastes, Cd, Pb, and Zn concentrations continue to exceed the USEPA Ambient Water Quality Criteria (AWQC) and streambed sediment guidelines for the protection of aquatic life (Farag et al. 1998, Brennan et al. 1999).

Metals can affect aquatic organisms as toxic substances in water and sediment, or as a toxicant in the food chain (Sorensen 1991, Rainbow 1996). Metals associated with mining activities near CDA River basin streams have reduced native fish species richness and abundance (Maret and MacCoy 2002), reduced survival and growth of fish (Farag et al. 1999), and reduced diversity and densities of benthic invertebrate species (Hoiland and Rabe 1992, Hoiland et al. 1994).

Some insect taxa accumulate metals in proportion to metal concentrations in their environment and, therefore, are effective as biomonitors (Cain et al. 1992, Hare 1992). However, interpretation of bioaccumulation data is complicated by the presence of both absorbed and adsorbed forms of metals, with the latter form not incorporated into tissue but contributing to the over-

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all body concentration. Metals associated with intracellular tissues or biochemical fractions are unambiguous indicators of exposure to biologically available forms of metals. Analysis of metals in cytosol of resident invertebrates is a simple approach to assess site-specific metal bioavailability (Cain and Luoma 1998).

Metals in invertebrate tissues also represent a concentrated source that may be toxic in the diet of fish (Woodward et al. 1994). Farag et al. (1999) concluded that ingestion of metal-contaminated invertebrates may be the principal route of metal exposure to fish in streams of the CDA River basin.

Responses of benthic invertebrate assemblages have been used to assess ecological impacts of metal contamination in streams (Kiffney and Clements 1994, Poulton et al. 1995, Beltman et al. 1999, Deacon et al. 2001, Mebane 2001). Reduction in the abundance, diversity, and composition of functional-feeding groups of macroinvertebrates are related to elevated metal concentrations associated with mining (Clements et al. 2000, Mebane 2001). Taxa richness and abundances of metal-sensitive taxa are among the most reliable measures of metal effects on invertebrate assemblages (Carlisle and Clements 1999). Metal-sensitive taxa have been identified in studies of Rocky Mountain streams of Colorado (Clements 1994, Clements et al. 2000) and Montana (McGuire 2001). These studies showed that specific taxa of Ephemeroptera are highly sensitive to metal contamination, and that their abundances in mining-affected streams can be a reliable measure of metal impacts on benthic invertebrate assemblages.

Studies based on spatially extensive, synoptic sampling of multiple reference and contaminated sites are one way to characterize metal impacts on stream biota (Clements et al. 2000). The most effective assessments of anthropogenic stressors account for the influence of natural stressors (Hughes et al. 1986). Information from these types of studies provides a basis for identifying attainable conditions that can be used to set remediation goals for stream habitats affected by past mining activities.

Benthic invertebrate assemblages, selected environmental variables, and associated basin mine density were evaluated at 18 stream sites in the CDA and St. Regis River basins during the summer of 2000 as part of the US Geological Survey (USGS) National Water-Quality Assessment (NAWQA) Program. The objectives of our paper are to 1) describe the extent and severity of hard-rock mining impacts on benthic invertebrates; 2) evaluate correlations among metal concentrations in surface water and streambed sediment, metal concentrations in benthic invertebrates, and changes in assemblage structure; and 3) characterize the biotic and abiotic conditions of reference streams in the study area to help resource managers formulate recovery goals and implement effective remediation actions. We test the hypothesis that streams affected by mining and associated metals support fewer invertebrate taxa and lower abundances because invertebrates are exposed to elevated concentrations of metals.

Study Area

The study area in northern Idaho and western Montana (Fig. 1A) is entirely in the Northern Rockies ecoregion (Omernik and Gallant 1986). Land use for all study basins ranges from 85 to 99% forested land, and the remainder consists primarily of rangeland. Agricultural and urban land uses constitute $<1\%$ of all study basins. The study area has a complex geologic history of sedimentation, compressional deformation, igneous activity, and, most recently, extensional block faulting (Kendy and Tresch 1996). Rock types in the study area are almost entirely metasedimentary. Because of the extensively mineralized rocks, metal concentrations can be elevated in streambed sediment in undisturbed streams (Maret and Skinner 2000).

Streams in the study area are exclusively coldwater. The main source of surface and ground water is snowmelt runoff from April to July. Streamflow conditions during the study period were similar to or below long-term average conditions. The mean August streamflow for 2000 was $\sim$84% of the long-term average streamflow for the North Fork CDA River near Enaville and the South Fork CDA River near Pinehurst (Fig. 1A, sites 10 and 17). Streams in the study area typically have high water clarity, coarse-grained substrates (cobble and boulders), high gradients ($>0.5\%$), well-defined riffles and pools, and very sparse macrophyte growth. Mining and logging practices, channelization, and roads have affected riparian areas, reduced instream habitat, and degraded stream and

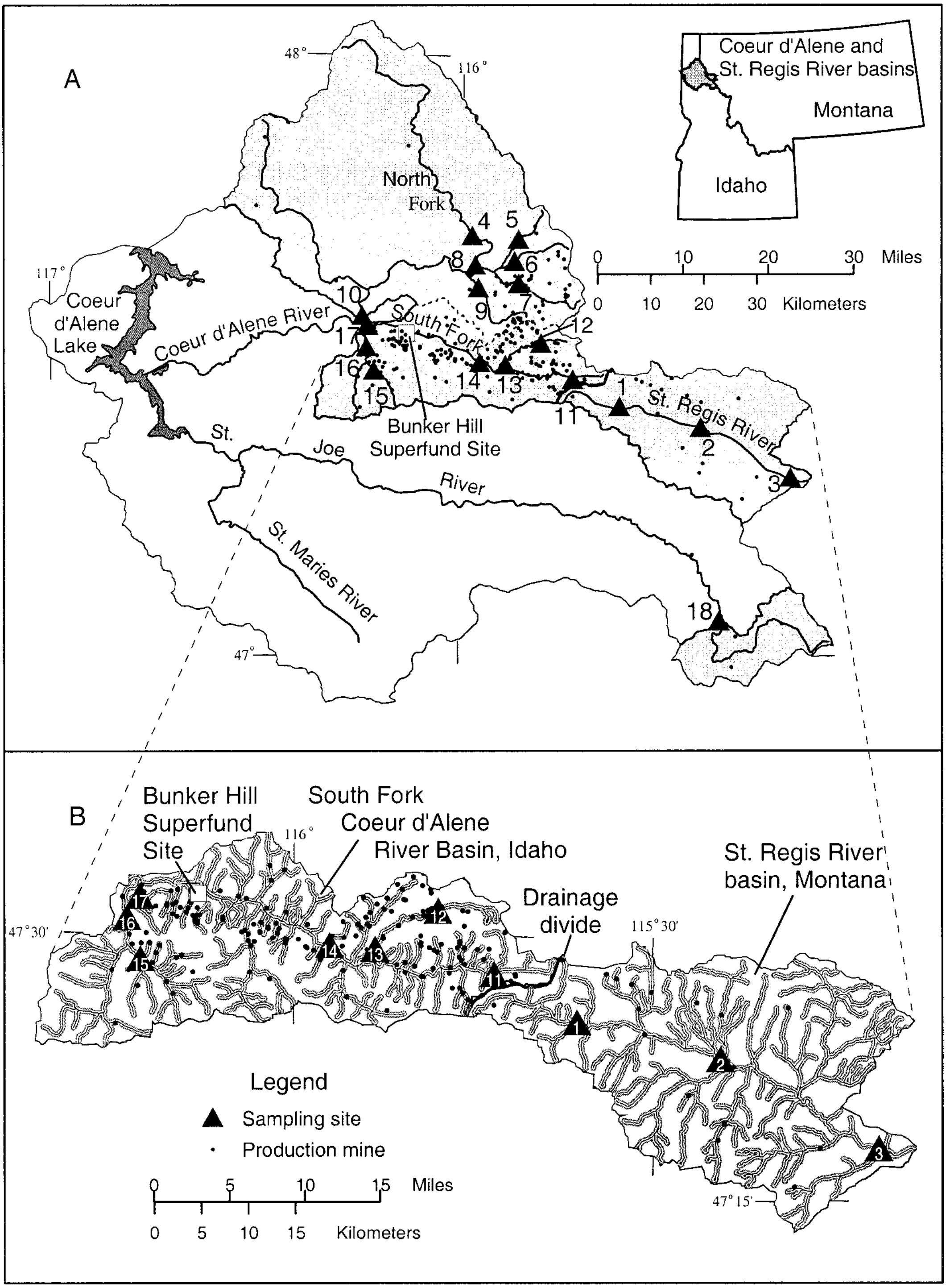

FIG. 1. A. Study area (shaded), sampling sites, and production mines (active and inactive) in the Coeur d'Alene and St. Regis River basins. B.—South Fork Coeur d'Alene and St. Regis River basins showing the 500-m stream buffer used to quantify production mine density in the study basins. See Table 1 for site names.

TABLE 1. Sampling sites, production mine density in the basin and in a 500-m buffer (250 m from each bank) upstream from each site, and site type, Coeur d'Alene and St. Regis River basins. R = reference site, T = test site. Site numbers are shown in Fig. 1.

Site number	Site name	Production mine density/km[2 a]		Site type
		Basin	Buffer (500 m)	
1	St. Regis River above Rainy Creek, MT	0.040	0.000	R
2	St. Regis River near Haugan, MT	0.039	0.075	R
3	St. Regis River near St. Regis, MT	0.025	0.058	R
4	North Fork Coeur d'Alene River near Prichard, ID	0.001	0.003	R
5	West Fork Eagle Creek below Settlers Grove, ID	0.068	0.162	R
6	East Fork Eagle Creek near Murray, ID	0.076	0.200	T
7	Upper Prichard Creek near Murray, ID	0.218	0.354	T
8	Prichard Creek at Prichard, ID	0.154	0.315	T
9	Beaver Creek near Murray, ID	0.180	0.200	T
10	North Fork Coeur d'Alene River near Enaville, ID	0.027	0.034	R
11	South Fork Coeur d'Alene R above Mullan, ID	0.176	0.000	R
12	Canyon Creek near Burke, ID	0.145	0.197	R
13	Canyon Creek at Woodland Park, ID	0.420	1.038	T
14	South Fork Coeur d'Alene River at Silverton, ID	0.314	0.498	T
15	East Fork Pine Creek above Nabob Creek near Pinehurst, ID	0.162	0.396	T
16	Pine Creek below Amy Gulch near Pinehurst, ID	0.218	0.483	T
17	South Fork Coeur d'Alene River near Pinehurst, ID	0.233	0.412	T
18	St. Joe River at Red Ives Ranger Station, ID	0.011	0.032	R

[a] Production mines (active and inactive) taken from US Bureau of Mines (1995)

groundwater quality (Woods and Beckwith 1997, Beckwith 1998).

More than 250 active and inactive hard-rock mines of various sizes are located in the study area (Fig. 1A), and many of these mines are located near streams. Production mine density ranges from 0.001 to 0.42/km^2 (Table 1). Historically, metal extraction and processing were relatively inefficient, yielding large volumes of metal-rich tailings that were deposited in and around nearby streams. Mine tailings in the study area typically contain elevated concentrations of trace metals such as As, Cd, Cu, Pb, Hg, and Zn (Woods and Beckwith 1997). These tailings and mines continue to provide a source of trace metals to streams, lakes, and reservoirs as streams meander through and erode tailings deposits and transport them downstream. Mine tailings entering the South Fork CDA River have been transported and deposited along the river channel and floodplain into CDA Lake and downstream into the Spokane River (Woods 2000, Grosbois et al. 2001). There is concern these elevated metals from upstream mining sources may be affecting aquatic life and bioaccumulating in fish tissue at levels exceeding human consumption guidelines for the Spokane River.

Methods

Site selection

Sampling sites in the CDA and adjacent St. Regis River basins were selected to represent streams affected by a range of mining activities, from minimally disturbed reference sites (little or no prior mining activity in the basin) to highly disturbed test sites (affected by cumulative mining impacts in the basin). Criteria suggested by Hughes et al. (1986) were used to select reference sites: 1) examination of existing data, 2) consultation with local land management agencies familiar with streams in the area, and 3) reconnaissance of candidate sites. Reference sites represented regional conditions (site 18), conditions upstream from major mining activities (sites 4, 5, 11, and 12), and conditions in similar (paired) basins (reference sites 1, 2, and 3 in the St. Regis River basin, reference site 11, and test sites 14 and 17 in the South Fork CDA River basin). To reduce the effects of stream size, each pair of sites in the South Fork CDA and St. Regis River basins were approximately the same distance (river km) from the mouth of the St. Regis or the South Fork CDA River. Both basins are similar in size, and site pairs are similar in elevation and stream geomorphology (see Reiser 1999). The location of test sites was based on the location of production mines identified in the US Bureau of Mines (1995) Minerals Availability System.

Eighteen sites were selected for sampling (Table 1). A site consisted of a representative reach generally containing repeating geomorphic channel units (riffles, runs, and pools). Because the length of stream sampled was a function of stream width, reach lengths ranged from 150 to 500 m (Fitzpatrick et al. 1998). The stream sites represented 2nd- through 5th-order streams (Strahler 1957). All stream sites were wadeable except sites 10 and 17.

Environmental variables

Environmental variables were evaluated for each site (Tables 1, 2) and consisted of basin and reach characteristics, physical habitat, and water and streambed sediment physicochemistry.

Geographic characterization.—Basin area and production mine density were determined using Arc/Info, a geographic information system (GIS). Several sources were used to construct the geographic data layers. Basin boundaries were constructed using hydrography and hydrologic unit boundary data layers (USGS 1975). Because most of the mining activities in this area occurred near streams, a proximity analysis also was conducted on mine locations relative to streams in each basin. A 500-m buffer width (250 m from each bank) was selected for the entire drainage network (at 1:100,000 scale) upstream from each site (Fig. 1B) after extensive analysis and comparison of different buffer widths (including none). This buffer width best characterized mining activities directly affecting local instream conditions. The buffer included only ~35% of the total study basin area but included 59% (158 of 269) of all production mines. Site elevations were derived from USGS 1:24,000-scale Digital Raster Graphic maps. Information on production estimates of individual mines was unavailable.

Physical habitat.—Instream habitat variables were measured following procedures outlined by Fitzpatrick et al. (1998). Stream gradient was determined onsite for each reach by using a rod and level. Discharge at the time of sampling was measured onsite using a Marsh–McBirney flow meter or was determined from streamflow-gauging records. Stream width was measured with a laser rangefinder at 6 locations throughout the reach. At each riffle location where invertebrates were sampled, estimates of depth, % open canopy, velocity, and % embeddedness were measured or estimated. Velocities were measured at 0.6 of the stream depth. Percent open canopy was determined by measuring left and right canopy angle using a clinometer. Percent embeddedness was visually estimated to the nearest 10%. Dominant substrate size was visually categorized into size classes (i.e., silt, sand, gravel, cobble, and boulder). For data analysis, a mean value was calculated for each habitat variable at each site.

Water and sediment chemistry.—Water-quality and streambed sediment samples were collected at each site during low-flow conditions in August and September 2000 using methods described by Shelton and Capel (1994). Water-quality samples were collected for analysis of selected nutrients and trace metals. A portion of each sample was filtered through a 0.45-μm filter. Analytes in the filtered aliquot are hereafter referred to as dissolved. Samples were shipped on ice to the USGS National Water Quality Laboratory (NWQL) in Arvada, Colorado, for analysis (Fishman 1993). Water temperature, dissolved oxygen, pH, and specific conductance also were measured onsite (Wilde and Radtke 1998).

Streambed sediment was sampled at 5 to 10 locations within a reach. The upper 2 cm of bed sediment was collected from undisturbed, continuously wetted, depositional zones using a plastic scoop. The subsamples were composited and wet-sieved through a 63 μm nylon mesh. Sediment $<$63 μm was retained to normalize the size fraction among sites. This sieved sample was placed on ice and submitted to the NWQL for analysis. Metal analysis of the sediment focused on the partially extractable fraction (5% hydrochloric acid extraction) (Hornberger et al. 1999). This method extracts metals from the sediment surface and represents a metal fraction that has high potential for exposure

TABLE 2. Environmental variables from the Coeur d'Alene and St. Regis River basins. Variables in bold are significantly different ($p < 0.05$) between site types, based on a Mann–Whitney t-test. All measurements of water and streambed sediment physicochemistry were made during low flow. SD = standard deviation.

Variable	Reference (n = 9) Mean (SD)	Reference Median	Reference Range	Test (n = 9) Mean (SD)	Test Median	Test Range
Environmental						
Basin area (km^2)	517 (756)	233	17–2325	194 (220)	100	52–738
Site elevation (m)	954 (187)	1039	664–1155	783 (77)	798	668–908
Gradient (%)	0.89 (0.75)	0.52	0.06–2.30	1.00 (0.49)	1.20	1.08–1.34
Discharge (m^3/s)	2.05 (2.54)	1.30	0.13–8.02	0.82 (1.06)	0.45	0.04–3.34
Width (m)	18.6 (16.7)	10.3	4.8–53.6	10.3 (4.4)	8.1	6.1–19.2
Depth (m)	0.21 (0.06)	0.20	0.14–0.32	0.22 (0.09)	0.19	0.12–0.39
Open canopy (%)	51 (20)	43	24–80	57 (5)	57	51–68
Velocity (m/s)	0.63 (0.17)	0.63	0.27–0.83	0.60 (0.15)	0.59	0.36–0.80
Embeddedness (%)	7 (7)	8	0–20	13 (14)	5	0–42
Dominant substrate (mm)	78 (36)	96	8–96	96 (0)	96	96
Specific conductance (µS/cm)	51 (22)	52	15–93	89 (80)	50	30–272
Dissolved oxygen (mg/L)	9.7 (0.5)	9.8	8.9–10.3	9.2 (0.5)	9.0	8.5–10.0
Water temperature (°C)	11.5 (2.6)	11.3	8.0–15.0	15.1 (2.2)	15.0	12.6–18.9
pH	7.4 (0.3)	7.4	6.8–7.7	7.1 (0.4)	7.0	6.6–7.8
Hardness (mg/L)	21 (9)	22	6–33	38 (37)	19	10–120
Total NO_3 (mg/L)	0.015 (0.007)	0.009	0–0.021	0.043 (0.067)	0.013	0–0.208
Total P (mg/L)	<0.008	<0.008	<0.008	<0.008 (0.024)	<0.008	<0.008–0.071
NH_3, dissolved (mg/L)	0.003 (0.001)	0.002	0.002–0.004	0.030 (0.084)	0.002	0.002–0.254
Cd						
Dissolved, water (µg/L)	<1	<1	<1	3 (5)	<1	<1–13
Streambed sediment (µg/g)[a]	1 (0.5)	0.9	0.3–1.9	24 (41)	3	2–124
Pb						
Dissolved, water (µg/L)	<1	<1	<1	5 (10)	<1	0.5–30
Streambed sediment (µg/g)[a]	52 (49)	41	3–125	1851 (2394)	358	132–6252
Zn						
Dissolved, water (µg/L)	4 (4)	3	0–12	503 (630)	110	16–1510
Streambed sediment (µg/g)[a]	69 (71)	66	2–199	2022 (2724)	322	112–7346

[a] Partially recoverable analysis (weak acid extraction) of <63-µm size fraction

to and ingestion by resident biota (Luoma 1989, Cain et al. 1992, Luoma et al. 1995). Metals were determined by inductively coupled plasma optical emission spectrophotometry (Hornberger et al. 1999).

Quality assurance.—Field quality assurance (QA) consisted of blank water samples and duplicate sediment samples. Laboratory QA consisted of routine blank and replicate samples for water analyses (Fishman 1993) and analyses of reference material for sediment samples (Arbogast 1990). Concentrations of metals analyzed in all blank water samples were below the minimum reporting limit. Concentrations of Cd, Pb, and Zn in duplicate sediment samples had relative differences of 0 to 15%, and concentrations in reference material were within QA guidelines of ± 3 SD. All other analytical results for QA laboratory samples were within acceptable USGS method standards.

Benthic invertebrate collection and analysis

Invertebrate sampling.—Benthic invertebrates were collected at all sites during low streamflow conditions in August and September 2000. Samples from 5 separate riffles were collected and composited throughout each reach using a Slack rectangular kicknet sampler (total area of 1.25 m^2) equipped with a 425-μm-mesh net (Cuffney et al. 1993). Large gravel and cobbles within the sample collection area were brushed to dislodge organisms, and the entire area was disturbed by kicking for 30 s. Onsite processing consisted of bucket elutriation of each sample by repeated washing through a 425-μm-mesh sieve. Samples were placed in 1-L plastic jars and fixed with 10% buffered formalin. Invertebrate taxonomic and abundance data were provided by the Biological Group at the NWQL (Moulton et al. 2000). Organisms were identified to the lowest practical taxonomic level (genus or species for most taxa). Voucher collections of invertebrates are deposited at the NWQL.

Metrics.—Benthic invertebrate assemblages were analyzed using relative abundance data and metrics useful in evaluating structural changes resulting from elevated concentrations of metals in Rocky Mountain streams: 1) number of total taxa; 2) number and % Ephemeroptera, Plecoptera, and Trichoptera (EPT) taxa; 3) number and % of Ephemeroptera taxa; 4) number of metal-sensitive Ephemeroptera taxa; 5) density (number/m^2) of all taxa; 6) density of EPT taxa; 7) density of metal-sensitive Ephemeroptera (Hoiland et al. 1994, Kiffney and Clements 1994, Carlisle and Clements 1999, Clements et al. 2000). We used metal-tolerance rankings from 0 (sensitive) to 10 (tolerant) developed by McGuire (2001) for Ephemeroptera in the Clark Fork basin, Montana. In our study, metal-sensitive Ephemeroptera (tolerance values ≤ 2) included *Ameletus* spp., *Caudatella* spp., *Drunella* spp., *Serratella* spp., *Timpanoga hecuba, Cinygmula* spp., *Epeorus* spp., *Rhithrogena* spp., and Leptophlebiidae.

Taxa were assigned to functional-feeding guilds (i.e., filterers, gatherers, predators, scrapers, and shredders) following guidelines of Merritt and Cummins (1996). Relative abundances of each feeding guild were used in the analyses.

Tissue analysis.—The net-spinning caddisflies *Ceratopsyche* spp., *Hydropsyche* spp., and *Arctopsyche grandis* were collected with kicknets and by hand from riffle habitats throughout each reach for tissue analysis. Caddisflies initially were placed in plastic containers (previously acid rinsed), cleaned with stream water, and sorted onsite. Sorted samples were transferred to sealable plastic bags with a small volume of river water, placed on dry ice, and stored at −70°C in the laboratory.

Caddisfly larvae were partially thawed in batches, rinsed with cold, deionized water to remove adhering particles, and then transferred to a chilled sorting dish for identification (Merritt and Cummins 1996). Larvae were then immediately transferred to an ice cooler, and combined randomly into subsamples, each weighing between 0.4 and 1.6 g wet mass. One to 6 subsamples from each site were processed for metal analysis.

Larvae were homogenized with a stainless-steel, high-speed, tissue homogenizer in cold, 0.05-M Tris-hydrochloric buffer (pH 7.4, previously degassed and bubbled with N_2) under an N_2 atmosphere for 1 min. An aliquot of the homogenate was collected to obtain a whole-body concentration, weighed, and then frozen at −40°C. The cytosol then was isolated from a 2nd aliquot of the homogenate by differential centrifugation following procedures modified from Wallace et al. (1997) and Cain and Luoma (1998).

All tissue samples were prepared for elemental analysis using procedures described by Cain and Luoma (1998). Samples were analyzed by inductively coupled plasma optical emission spectrophotometry. The median CV for replicate analysis of whole-body and cytosol samples for the reported metals ranged from 5 to 15% and 6 to 19%, respectively. QA consisted of analyses of procedural blanks, National Reference Council of Canada Tort-2 reference material, and metal-spiked (predigestion) blanks and samples. Sample concentrations were not corrected for blanks because procedural blanks for Cd and Pb were below the method detection limits (0.3 and 2.0 μg/L, respectively), and measurable Zn blanks (>0.3 μg/L) were <5% of the sample concentrations. Measured concentrations in spiked blanks and samples were within 7% of nominal concentrations. Relative SD for measured concentrations of Cd and Zn in Tort-2 reference material were 2 and 4%, respectively, and the mean concentrations ±3 SD were within the certified concentrations. Lead concentrations in prepared Tort-2 reference materials were at or near the method detection limits and, thus, were not quantifiable.

Data analysis

Site categorization.—Sampling sites were categorized as either reference or test sites on the basis of production mine density upstream from each site and concentrations of metals in water and sediment. The median mine density of 0.2 mines/km^2 in the 500-m buffer was initially used to divide sites into 2 equal groups of reference (low mining density) and test (high mine density) sites. Test sites were finally selected by satisfying 2 criteria: 1) production mine density of ≥0.2 mines/km^2 in the stream buffer (Table 1) and, 2) elevated metal concentrations in water and (or) sediment that exceeded one or more of the guidelines for the protection of aquatic life. Although the North Fork CDA River near Enaville (site 10) is located downstream of 4 test sites, it was categorized as a reference site because metal concentrations did not exceed Idaho's Water-Quality Standards (Woods 2000). Only Cd, Pb, and Zn concentrations are reported in our study because they have been identified as the metals most elevated in CDA River basin streams and are of greatest ecological concern (Brennan et al. 1999).

Water and sediment toxicity.—Metals in water were compared with USEPA (2000) acute AWQC for the protection of aquatic life. Dissolved, rather than total recoverable, concentration was evaluated because the dissolved fraction more accurately reflects site-specific metal bioavailability and toxicity (Prothro 1993). Because water hardness affects bioavailability and toxicity of most metals, criterion values were modified to account for variation in water hardness among streams (USEPA 2000). Chronic criteria were not evaluated because they are based on 4-d average metal concentrations, which were not collected during our study. Our study may not accurately describe Cd criteria exceedances because of the low water hardness and high laboratory detection limit (1.0 μg/L). On occasions where hardness is <30 mg/L, acute criteria of <1.0 μg/L for Cd are possible.

National Oceanic and Atmospheric Administration (NOAA) screening values were used to evaluate the severity of the metal contamination in sediment (Buchman 1999). The Probable Effect Level (PEL) for each metal, defined as the concentration above which adverse effects to aquatic life are predicted to occur frequently, was used. Because the <63-μm size fraction, rather than bulk sediment (used to develop these guidelines), was analyzed, the likelihood of exceeding a PEL may be increased.

Cumulative toxic units (CTU) for Cd, Pb, and Zn in water and sediment were calculated for our study similarly to Clements et al. (2000). Acute AWQC and the NOAA PEL values were used as toxicity reference values (TRV), similar to the approach used by Dyer et al. (2000). Each CTU value represents the sum of Cd, Pb, and Zn concentrations in water and sediment at each site divided by the appropriate TRV. A CTU value >1.0 represents potentially toxic levels of cumulative Cd, Pb, and Zn concentrations in water or sediment at a site, and provides resource managers with a measure of the severity of metal contamination in the streams sampled.

Bioaccumulation data.—Not all genera of caddisflies were collected at every site. Previous studies (Cain et al. 1992, Cain and Luoma 1998), and analysis of samples from our study (ANOVA) indicated that interspecific differences in bioaccumulation would not confound among-site comparisons of metal exposure. Therefore, *Ceratopsyche* spp. and *Hydropsyche* spp. were treated as a single taxon, *Hydropsyche* spp., and metal data for *Hydropsyche* and *A. grandis* were combined at sites where both taxa were collected.

Statistics.—All statistical analyses were performed using SYSTAT (L. Wilkinson. 1999. SYSTAT for Windows statistics, version 9.0, SYSTAT, Inc., Evanston, Illinois.). All possible combinations of responses and explanatory variables were examined using Spearman's rank correlation matrices. Regression analyses between mine densities and metal concentrations were used to predict metal concentrations and potential biological impacts. Correlations between mine density and the sum of Cd, Pb, and Zn concentrations in water and streambed sediment were described using regression analyses. The LOWESS (LOcally WEighted Scatterplot Smoothing) technique, a robust, nonparametric description of data patterns (Helsel and Hirsch 1992), was used to evaluate trends between benthic invertebrate metrics and CTUs for metals in water and sediment. Significant differences in the environmental variables and benthic invertebrate metric data between reference and test sites were determined with the nonparametric Mann–Whitney test. Nondetections of chemical constituents were set to ½ the minimum reporting limit prior to statistical analysis. Results of statistical tests were considered significant if $p < 0.05$.

Results

Environmental variables

Site elevation and water temperature were the only environmental variables (excluding Cd, Pb, and Zn concentrations) significantly different between reference and test sites (Table 2). Median elevations were significantly higher at reference sites (1039 m) than at test sites (798 m) because some reference sites necessarily were located upstream from mining areas. Median water temperatures were significantly lower in reference sites (11.3°C) than test sites (15.0°C). Moreover, unlike many Rocky Mountain streams affected by acid-mine waste, pH of the stream water at the test sites was near neutral and ranged from 6.6 to 7.8.

Cd and Zn in water and streambed sediment and Pb in streambed sediment were significantly higher at test sites than at reference sites (Table 2). More than 75% of the concentrations of dissolved Cd and Pb in water were less than the reporting limit; only at test sites 13 (Canyon Creek at Woodland Park), 15 (East Fork Pine Creek), and 14 and 17 (South Fork CDA at Silverton and near Pinehurst) were concentrations of one or both of these metals quantifiable. Metal concentrations in water and sediment often exceeded the acute AWQC and PEL, and were highest at test sites 13, 15, 14, and 17. However, metal concentrations in sediment at reference sites 1, 10, 11, and 12 also exceeded the PEL of 91.3 and 315 μg/g for Pb and Zn, respectively. Concentrations of dissolved metals in water at reference sites did not exceed acute AWQC.

Concentrations of Zn in water and Pb in sediment were significantly correlated ($r^2 = 0.69$ and 0.65, $p < 0.01$) with the number of mines in close proximity to streams upstream from sampling sites (Fig. 2A, B). Metal concentrations in both water and sediment at test sites generally exceeded concentrations at reference sites, some by as much as 1 to 2 orders of magnitude. Mean Zn concentrations in water were 503 and 4 μg/L at test and reference sites, respectively (Table 2). Similarly, mean Pb concentrations in sediment were 1851 and 52 μg/g at test and reference sites, respectively.

The relative severity of Cd, Pb, and Zn contamination in water and sediment is shown by ranking all sites using the CTU values (Fig. 3A, B). CTUs for water and sediment at all sites were significantly correlated ($r = 0.93$). Zn contributed the most CTUs for water and Pb contributed the most CTUs for sediment. CTUs varied greatly among sampling sites, ranging from 1 to 35 for water and 1 to 111 for sediment. CTUs for most reference sites did not exceed 1, indicating that cumulative effects from Cd, Pb, and Zn contamination are unlikely. CTUs for water and sediment were highest at sites 13, 14, and 17.

Metal concentrations in caddisflies

Cd, Pb, and Zn concentrations in whole-body caddisfly tissue were significantly higher at test sites than at reference sites (Table 3). Pb in the cytosolic fraction was also significantly higher at test sites than at reference sites. Caddisflies were absent at 5 of the 9 test sites. Metal concentrations in the whole body and cytosol were highest at test sites 14 and 17 (Fig. 4A–C). Concentrations in the cytosol of caddisflies at those sites were generally 1 to 2 orders of magnitude

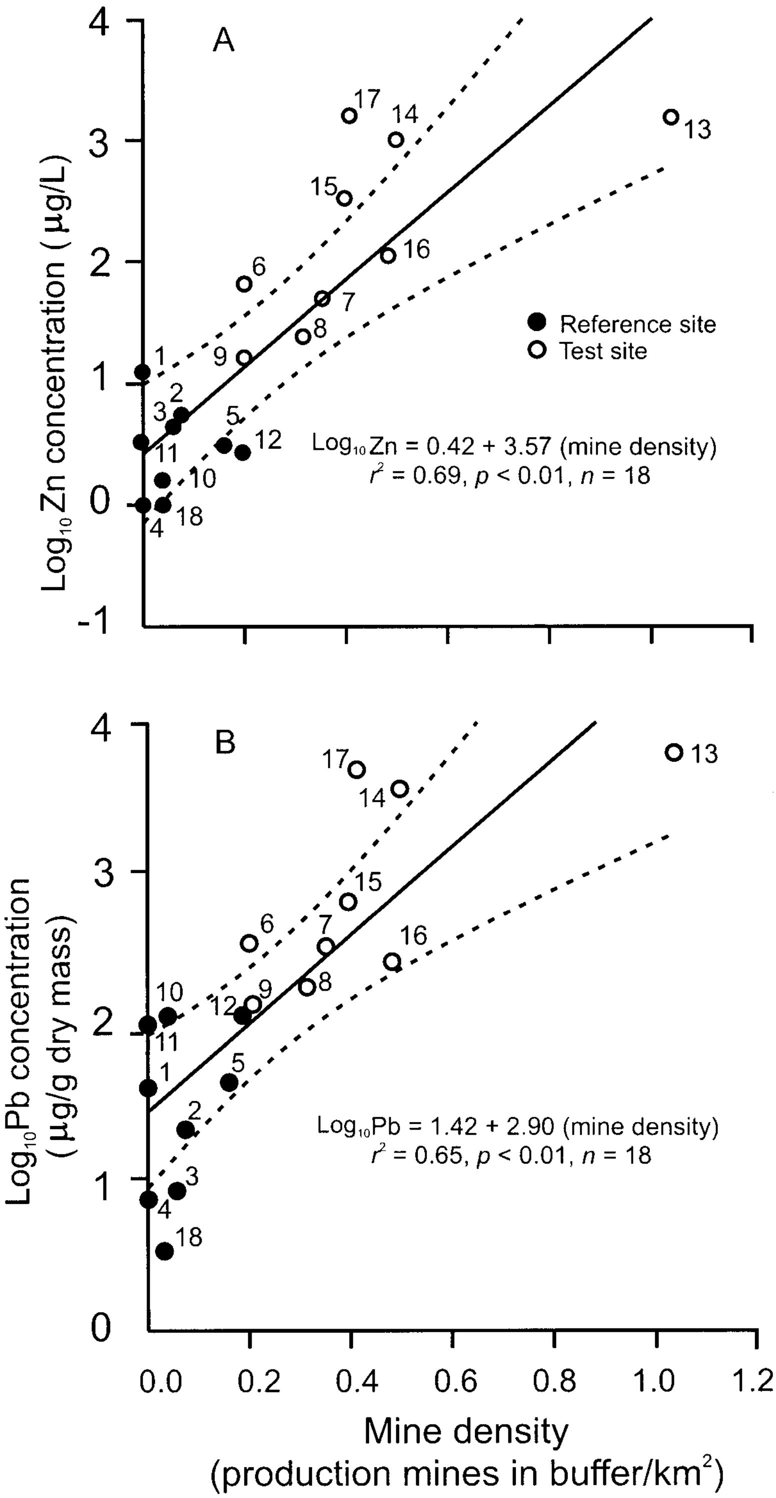

FIG. 2. Relationship between production mine density (in 500-m stream buffer) and Zn concentrations in water (A) and Pb in streambed sediment (B), Coeur d'Alene and St. Regis River basins. For details on sampling sites see Table 1 and Fig. 1. Data were fitted using a linear regression. Dashed lines are the 95% confidence intervals.

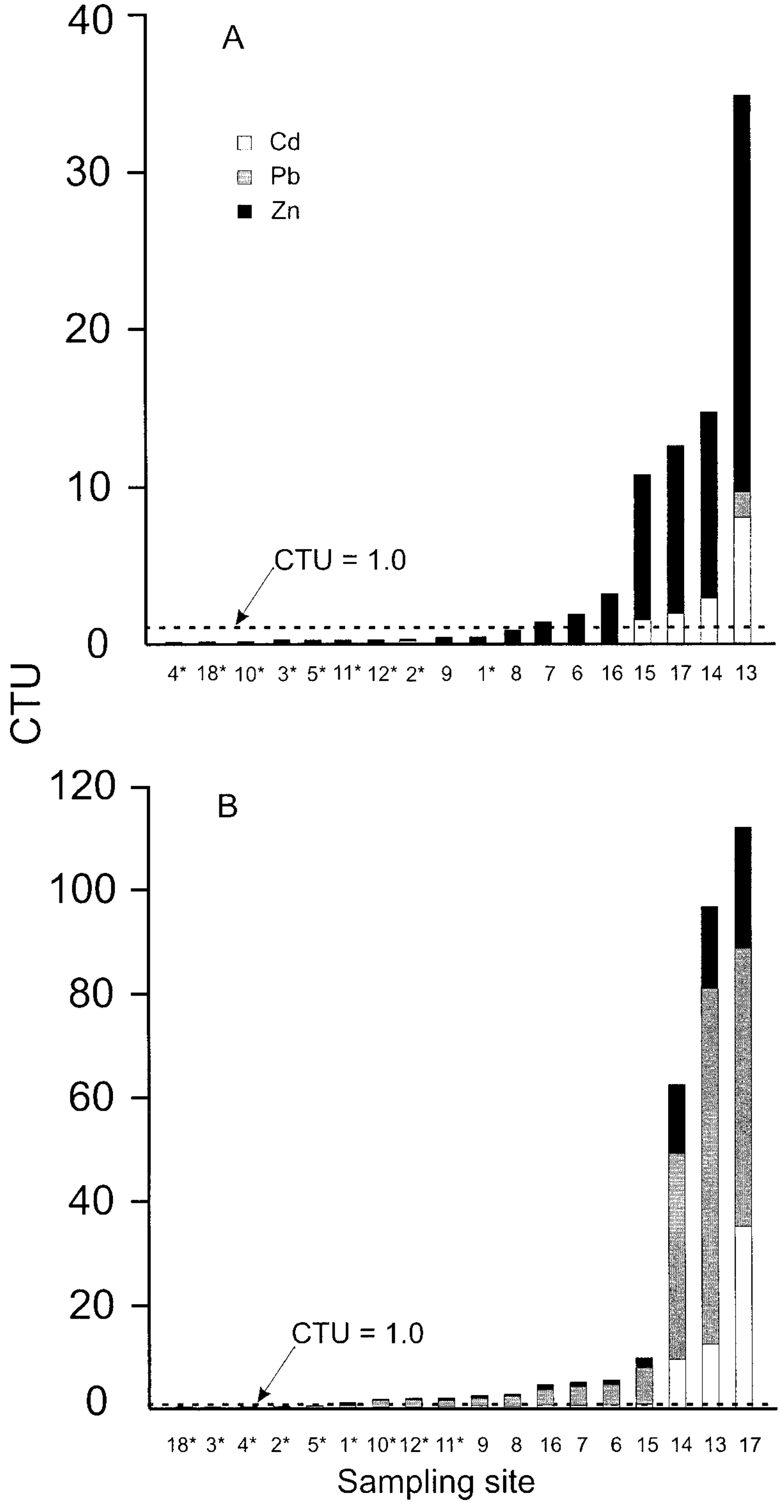

FIG. 3. Ranking of sampling sites on the basis of cumulative toxic unit (CTU) values for Cd, Pb, and Zn concentrations in water (A) and sediment (B), Coeur d'Alene and St. Regis River basins. A CTU value >1.0 represents potential cumulative toxicity of metals at a site. * = reference site.

TABLE 3. Metal concentrations in caddisfly tissue, Coeur d'Alene and St. Regis River Basins. Variables in bold are significantly different ($p < 0.05$) between site types, based on a Mann–Whitney t-test. SD = standard deviation.

	Site type					
	Reference (n = 9)			Test (n = 9)		
Caddisfly tissue[a,b]	Mean (SD)	Median	Range	Mean (SD)	Median	Range
Cd						
Whole body (µg/g)	0.58 (0.70)	0.30	0.08–2.32	6.68 (6.61)	6.80	0.42–12.7
Cytosol fraction (µg/g)	0.28 (0.36)	0.15	0.05–1.21	1.10 (1.20)	0.74	0.13–2.78
Pb						
Whole body (µg/g)	2.94 (2.83)	1.37	0.20–7.09	142 (157)	114	11–331
Cytosol fraction (µg/g)	0.61 (0.80)	0.24	0.04–2.56	7.20 (10.90)	2.48	0.42–23.4
Zn						
Whole body (µg/g)	181 (69)	172	82–326	707 (560)	642	180–1365
Cytosol fraction (µg/g)	68 (35)	58	27–148	108 (82)	84	41–221

[a] Includes test sites 8(AH), 9(A), 14(AH), 17(AH); A = *Arctopsyche grandis,* H = *Hydropsyche* spp.
[b] Includes references sites 1(A), 2(A), 3(A), 4(H), 5(A), 10(H), 11(A), 12(A), 18(A)

higher than at other sites. Cytosolic Pb concentrations at site 14 were particularly high, exceeding 20 µg/g. Metal concentrations in the whole body and cytosol were significantly correlated ($r \geq 0.85$). However, the proportional contribution of the cytosol tended to decline as whole body concentrations increased, reflecting the importance of other metal accumulation sites (Wallace et al. 1997, Cain and Luoma 1998). Cd and Zn concentrations in caddisflies at reference sites 1 (St. Regis River above Rainy Creek), 4 (North Fork CDA River near Prichard), and 12 (Canyon Creek near Burke) were higher than in samples at other reference sites. As mentioned above, metal concentrations in sediment at sites 1 and 12 also exceeded the PEL.

Metal concentrations in caddisfly tissue were significantly correlated with mine density and metal concentrations in water and sediment (Table 4). Metal concentrations in the whole bodies of caddisflies were most strongly correlated with sediment concentrations (r = 0.88, 0.90, and 0.67, for Cd, Pb, and Zn, respectively). Correlations with water were significant for all metals (r = 0.62, 0.63, and 0.47, for Cd, Pb, and Zn, respectively), while correlations with production mine density were significant for Cd and Pb (r = 0.56, and 0.64, respectively). Similarly, metal concentrations in the cytosol increased in relation to increasing concentrations of metals in sediment and water; however, corresponding changes in metal concentrations were not highly correlated ($r < 0.40$). These correlations probably were affected by site-specific differences in the partitioning of metals between the cytosol and whole-body fractions.

Benthic invertebrates and metrics

Ninety-one benthic invertebrate taxa were identified. The number of taxa collected ranged from 19 at site 13, the most contaminated site, to 38 at site 8 (Prichard Creek at Prichard). *Baetis tricaudatus* was the most common taxon (collected at all sites) and one of the most abundant organisms collected (mean density of 1173/m^2). Only *Simulium* spp. were more abundant (mean density 1254/m^2) at the 17 sites where they were collected.

Total taxa richness, EPT taxa richness, and densities of total individuals, EPT individuals, and metal-sensitive Ephemeroptera individuals were significantly higher at reference sites than at test sites (Table 5). In addition, abundances of invertebrates at reference sites were approximately twice those at test sites, for all density metrics. No significant differences in the percentages of functional feeding groups were evident between site types (data not shown).

Comparisons of paired basins revealed that, for reference sites 1, 2, and 3 in the St. Regis River basin and site 11 upstream from major

TABLE 4. Spearman correlation coefficients among production mine densities; metal concentrations in water, sediment, and caddisfly tissue; and selected benthic invertebrate metrics, Coeur d'Alene and St. Regis River basins. * = $p < 0.05$.

	Production mines/km² in 500-m stream buffer	Water (μg/L)		
		Cd	Pb	Zn
Concentration (μg/g) in caddisfly tissue[a]				
Cd	0.56*	0.62*		
Pb	0.64*		0.63*	
Zn	0.35			0.47*
Metric				
Total taxa	−0.66*	−0.63*	−0.60*	−0.75*
EPT taxa[b]	−0.66*	−0.64*	−0.61*	−0.77*
Total individuals (no./m²)	−0.56*	−0.38	−0.34	−0.45
EPT individuals[b] (no./m²)	−0.47*	−0.28	−0.29	−0.38
Metal-sensitive Ephemeroptera individuals (no./m²)	−0.56*	−0.66*	−0.65*	−0.62*

[a] Whole-body concentrations at 13 sites
[b] Sum of Ephemeroptera, Plecoptera, and Trichoptera taxa

mining impacts in the South Fork CDA River basin, EPT taxa richness ranged from 10 to 19, and metal-sensitive Ephemeroptera taxa richness ranged from 3 to 7 (Fig. 5). Test sites 14 and 17 in the CDA River basin are affected by mining in Canyon and Nine Mile Creeks that flow into the South Fork CDA River upstream. These tributaries carry high concentrations of Cd, Pb, and Zn, which increase metal loads into the South Fork CDA River by an order of magnitude (Woods 2000). Fewer EPT (7 to 9) and metal-sensitive Ephemeroptera taxa (0 to 1) were collected at test sites than at reference sites; however, the % composition of EPT was higher at test sites (86–93%) than at reference sites (41–76%). *Baetis tricaudatus*, *Hydropsyche* spp., and *A. grandis*, which are relatively tolerant of metals (Clements 1994), were the predominant EPT taxa at test sites 14 and 17. Metal-sensitive Ephemeroptera taxa composed 16 to 22% of the assemblage at the reference sites but only 0 to 3% at the test sites. *Drunella doddsi* was the only metal-sensitive Ephemeroptera taxon collected at either of these test sites. This species also was collected at site 13, the most metals-contaminated site (Fig. 3A).

Benthic invertebrate measures and contaminant relations

The 5 benthic invertebrate metrics shown in Table 5 that were significantly different between reference and test sites were sometimes significantly correlated with mine density and Cd, Pb, and Zn concentrations in water, sediment, and caddisfly tissue (Table 4). Each metric was negatively correlated with ≥1 of the variables associated with mining. Some of the strongest correlations were between the total number of taxa ($r = -0.75$), EPT taxa ($r = -0.77$), and Zn concentrations in water. In addition, density of metal-sensitive Ephemeroptera was significantly, negatively correlated with production mine density and metal concentrations in caddisflies (whole-body), water, and streambed sediment ($r = -0.54$ to -0.70).

Scatterplots (including LOWESS smoothing) summarizing relations among benthic invertebrate metrics and CTUs for Cd, Pb, and Zn in water clearly showed a declining trend in biotic condition along a gradient of increasing metal toxicity (Fig. 6). Because water and sediment CTUs were strongly correlated ($r = 0.93$), there were also similar declining trends in biotic condition with increasing sediment CTUs. Correlation coefficients among CTUs for metals in water and selected benthic invertebrate metrics were all significant. Total taxa, EPT taxa, and density of metal-sensitive Ephemeroptera taxa were the most responsive metrics to site-specific changes in water and sediment toxicity. Those metrics were less variable than the abundance metrics, total number of individuals and EPT individuals.

TABLE 4. Extended.

Streambed sediment (μg/g)			Caddisfly tissue[a] (μg/g)		
Cd	Pb	Zn	Cd	Pb	Zn
0.88*					
	0.90*				
		0.67*			
−0.62*	−0.62*	−0.69*	−0.12	−0.21	−0.32
−0.56*	−0.58*	−0.68*	−0.22	−0.28	−0.50*
−0.56*	−0.64*	−0.48*	−0.43	−0.4	−0.08
−0.41	−0.50*	−0.38	−0.47*	−0.42	−0.15
−0.63*	−0.62*	−0.60*	−0.70*	−0.70*	−0.54*

The number of invertebrate taxa generally declined with increasing water and sediment CTUs, but measures of invertebrate density were highly variable among reference and test sites. For example, total and EPT densities were relatively high at site 17 in spite of the fact that CTUs for water and sediment were among the highest of any test site. Unlike habitat at other sites, the predominant habitat at site 17 consisted of dense mats of filamentous algae. This site was dominated by hydropsychid caddisflies, composing ~67% of all organisms in the sample.

Discussion

Our study showed broad agreement among the multiple lines of evidence (mine density, metal concentrations in water and sediment, bioaccumulation in caddisfly tissue, and benthic invertebrate assemblage structure) used to evaluate hard-rock mining impacts. The most pronounced differences were among reference sites and some of the most metal-contaminated test sites, including sites 13, 14 and 17. Our study demonstrated that streams downstream from areas of intensive hard-rock mining contained fewer benthic invertebrate taxa and lower densities, and that these changes in assemblage structure were associated with elevated metal contaminants in both water and sediment. There were few significant differences between reference and test sites for the instream physical habitat variables measured. Although water temperatures differed significantly between site types, temperatures at all sites were below Idaho's coldwater temperature criterion of 19°C (Grafe et al. 2002). Moreover, based on occurrence patterns of Idaho's coldwater obligate invertebrates, defined as those taxa that have <10% probability of occurring in streams where water temperatures exceeded 19°C (Grafe et al. 2002), thermal conditions at the test sites did not appear to be biologically limiting. For example, reference and test sites had at least 15 and 13 coldwater obligate invertebrate taxa, respectively. In addition, coldwater fish species are present at all sites, except in lower Canyon Creek (Maret and MacCoy 2002). Furthermore, Reiser (1999) measured continuous summer water temperatures at numerous sites in the CDA River basin and also concluded that water temperatures are likely not limiting to aquatic life.

Metal exposure

Production mine density in the upstream basin and within a 500-m buffer was a useful indicator of metal exposure. Test sites, defined as having ≥0.2 mines/km^2 in the 500-m buffer, had elevated concentrations of Cd, Pb, and Zn in ≥1 exposure indicators (water, sediment, caddisflies). Although production data for individual mines might have enhanced our analysis, this information was not available.

Concentrations of Cd, Pb, and Zn at reference

TABLE 5. Benthic invertebrate metrics for all sampling sites, Coeur d'Alene and St. Regis River basins. Variables in bold are significantly different ($p < 0.05$) between sites, based on a Mann–Whitney t-test. SD = standard deviation.

	Site type		
	Reference (n = 9)		
Metric	Mean (SD)	Median	Range
Richness			
Total taxa	34 (5.2)	35	21–37
EPT taxa[a]	17 (3.1)	18	10–21
% EPT	67 (14)	70	41–82
Ephemeroptera taxa	7 (1.7)	6	5–9
% Ephemeroptera	38 (12)	37	21–58
Metal-sensitive Ephemeroptera taxa	5 (2.1)	5	3–8
Density (no./m²)			
Total individuals	9356 (4294)	8304	3564–18,133
EPT individuals	5919 (2204)	5790	2734–10,258
Metal-sensitive Ephemeroptera individuals	2045 (553)	2005	1135–2823

[a] Sum of Ephemeroptera, Plecoptera, and Trichoptera taxa

sites in the study area were typically below AWQC. Elevated concentrations of Pb and Zn in sediment at reference sites 1, 10, 11, and 12 may be the result of limited mining upstream, natural mineral deposits, and (or) prevailing winds that historically transported airborne metal particulates from the nearby smelter operations at the Bunker Hill Superfund site near Kellogg, Idaho.

At those sites where benthic invertebrates were used for tissue analysis, metal concentrations in whole-body and cytosol samples generally corresponded with contamination levels measured in sediment and water, confirming that metals originally associated with mine waste were biologically available. The correlation between metal concentrations in whole-body and cytosol samples suggests that, in general, site-specific exposures to bioavailable metals in the CDA River basin can be inferred from the concentrations in whole-body samples.

Previous studies showed that metal contamination of streambed sediment and biota extends >70 km from major mining sources in the South Fork CDA River basin to the Spokane River downstream from Lake CDA (USGS 1999, Grosbois et al. 2001). Mean concentrations of Cd, Pb, and Zn in tissue of caddisflies ~40 km downstream from the lake were 3 μg/g, 52 μg/g, and 480 μg/g, respectively (USGS 1999). These tissue concentrations are ~3 to 18 times higher than mean concentrations in caddisflies at reference sites sampled in our study (Table 3).

Maret and Skinner (2000) noted that Cd, Pb, and Zn concentrations in livers and fillets of fish from the lower South Fork CDA River were among the highest from any sites sampled in the Spokane River basin. Farag et al. (1998) also noted that concentrations of metals in tissue of various fish and macroinvertebrates from the South Fork CDA River were higher than in macroinvertebrates at reference sites, and that metal concentrations measured in *Arctopsyche* spp. were comparable to those at sites 14 and 17 on the lower South Fork CDA River.

Effect of metals on invertebrate assemblages and dietary exposure to fish

Elevated metal exposure associated with mining density appeared to be directly related to effects on benthic invertebrate assemblages. Comparisons of reference and test sites, using both upstream-versus-downstream and paired-basin approaches, provided evidence that metals associated with mining have adversely affected benthic invertebrate assemblages. Numbers of total taxa and EPT taxa at sites down-

TABLE 5. Extended.

Site type			
Test (n = 9)			Mann–Whitney
Mean (SD)	Median	Range	t-test p-value
25 (6.3)	22	19–38	0.019
13 (3.3)	14	8–18	0.014
73 (24)	84	28–93	0.145
5 (2.8)	6	1–9	0.261
48 (26)	48	15–80	0.566
4 (2.6)	4	0–8	0.326
5323 (3160)	4330	1333–10,521	0.048
3746 (2609)	3224	711–9097	0.047
1054 (814)	968	0–2199	0.015

stream from areas of mining activity were significantly lower than at reference sites. Various measures of abundance, including density of all individuals, density of EPT individuals, and density of metal-sensitive mayflies were also reduced. Other studies have documented reductions in total taxa with increasing metal contamination (Beltman et al. 1999, Carlisle and Clements 1999). Contrary to our results, Beltman et al. (1999) did not identify a decrease in total density of individuals, which Beltman et al. (1999) attributed to the highly variable numbers of chironomids in their samples.

Metal-sensitive Ephemeroptera metrics were less responsive to metal exposure. Reductions in the number and % of metal-sensitive Ephemeroptera were evident only at the most contaminated sites (13, 14, and 17) downstream from areas of intensive mining activity. Kiffney and Clements (1993) suggested that the loss of metal-sensitive Ephemeroptera taxa from metal-contaminated systems is possibly a direct result of high body burdens of metals. Our results are consistent with this notion. The exceptionally high metal exposures at sites 14 and 17, indicated by elevated concentrations in tissue of hydropsychid caddisflies, could prohibit colonization of these sites by metal-sensitive taxa. However, contaminated sites may be occupied temporarily by insects drifting downstream from

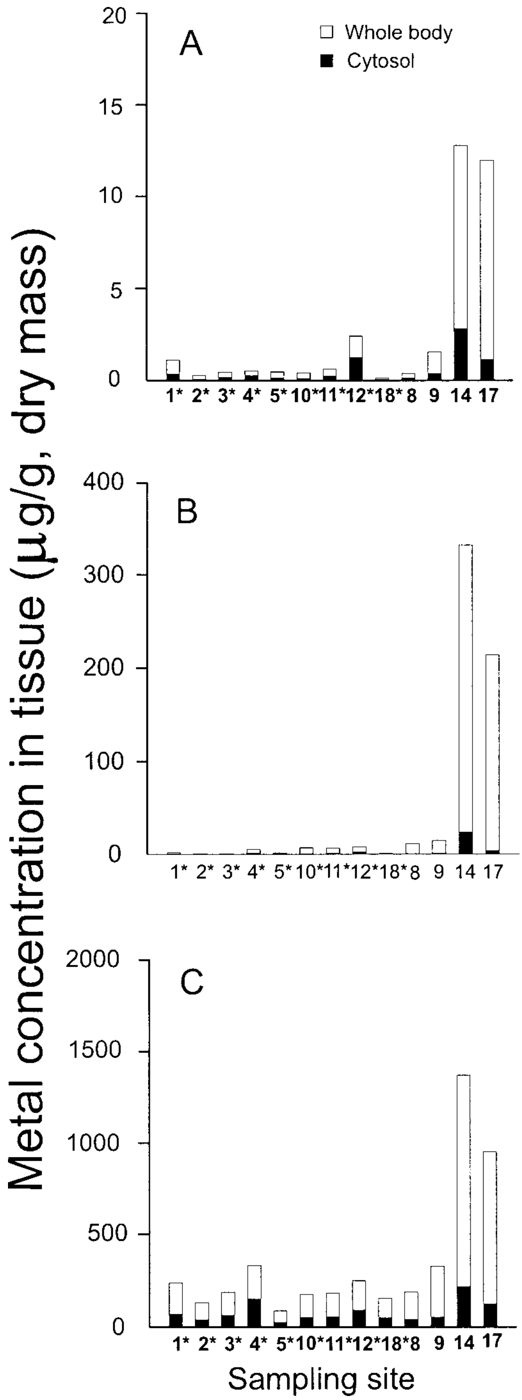

FIG. 4. Metal concentrations in whole-body caddisfly tissue and cytosol (*Arctopsyche grandis* and *Hydropsyche* spp.), Coeur d'Alene and St. Regis River basins. A.—Cd. B.—Pb. C.—Zn. Means were used to represent sites where both taxa were collected. * = reference site.

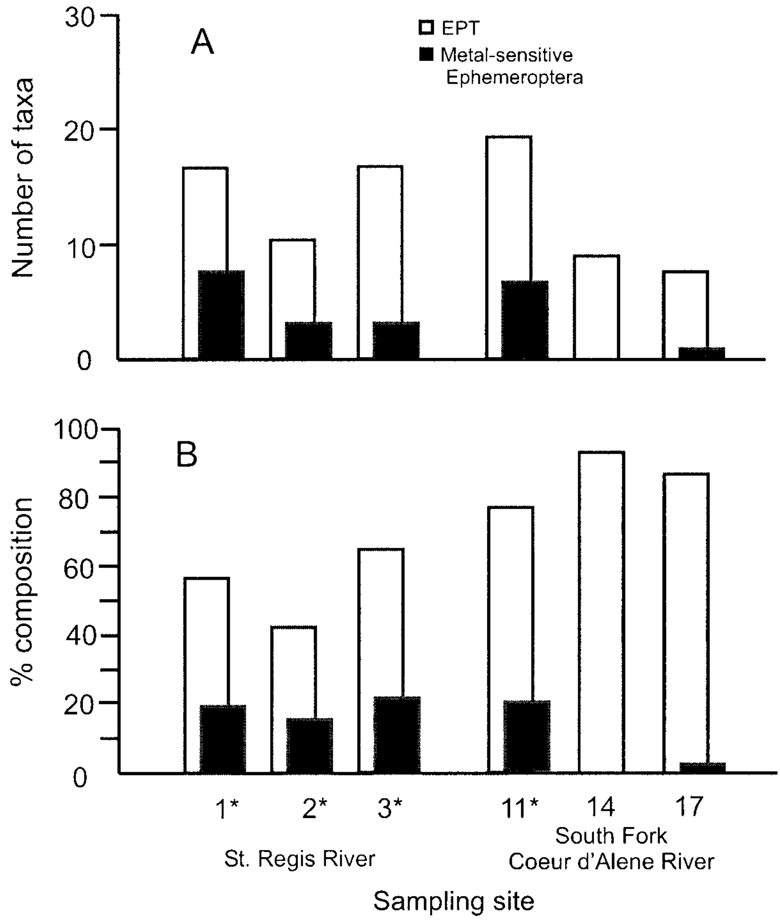

FIG. 5. Comparisons of taxa number (A) and % composition (B) of EPT and metal-sensitive Ephemeroptera among 4 reference and 2 test sites along the South Fork Coeur d'Alene River, Idaho, and St. Regis River, Montana. See Fig. 1B for site locations. * = reference site.

uncontaminated areas (Clements 1994, Beltman et al. 1999). Even though these organisms may occupy contaminated sites for only a short time, the immigration of individuals from upstream may affect sampling results. Our measures of metal-sensitive Ephemeroptera could have been affected by the presence or absence of a few rare taxa. Thus, measures of invertebrate drift may be useful where drift may affect assessment results. Also, samples collected in different seasons may help determine local survival of rare species (i.e., metal-sensitive Ephemeroptera taxa) over the year.

Percent Ephemeroptera was not an effective metric, primarily because *B. tricaudatus* was found at all sites and was often the most abundant taxon at test sites. This finding is consistent with Clements (1994), who noted the occurrence of large numbers of *Baetis* spp. during the summer downstream from areas of intensive mining activity along the Arkansas River in Colorado. Clements and Kiffney (1995) noted that the usefulness of *Baetis* spp. as an indicator of metal contamination in Rocky Mountain streams is questionable because of its potential for rapid recolonization. However, Deacon et al. (2001) noted that the abundances of *Baetis* spp. were reduced in streams in the Colorado River basin that contained elevated trace metals.

Functional-feeding groups did not appear to discriminate between reference and test sites. Carlisle and Clements (1999) also determined

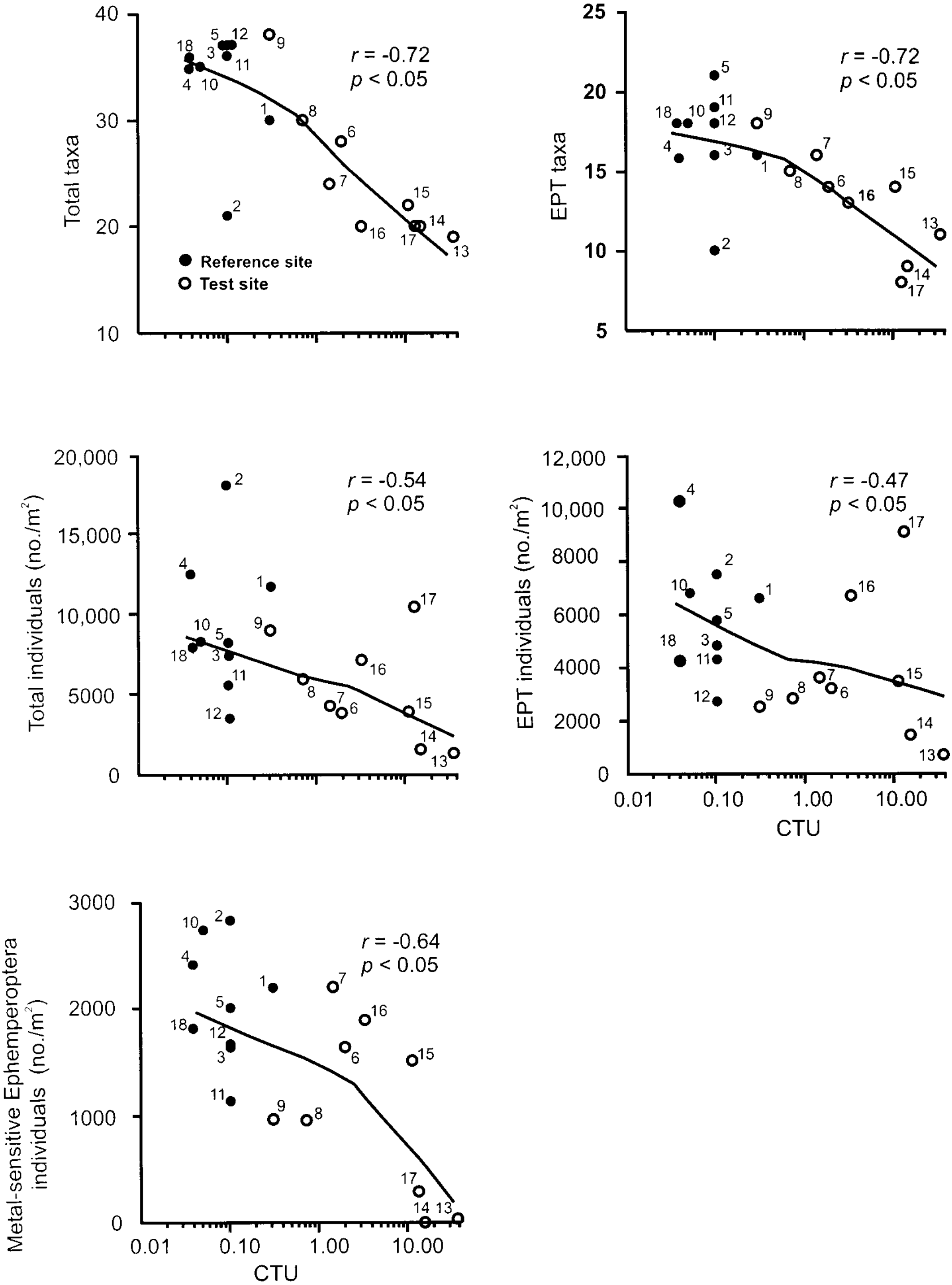

FIG. 6. Correlations between benthic invertebrate metrics and cumulative toxic units (CTU) for Cd, Pb, and Zn in water for all sampling sites, Coeur d'Alene and St. Regis River basins. Trend lines are based on the LOWESS (Locally Weighted Scatterplot Smoothing) technique (Helsel and Hirsch 1992).

that spatial assemblage patterns of functional-feeding groups were highly variable and not indicative of metal contamination.

Total and EPT taxa metrics at reference site 2 were much lower than at other reference sites; however, density metrics had some of the highest values (Fig. 6). This difference could be partly caused by the high density of *Simulium* spp. (9500 individuals/m^2) collected at this site. Reasons for this unusually high number of black flies at this location are unclear. This taxon occasionally has been found in high densities (>3000 individuals /m^2) in riffle habitats of Idaho reference streams (Maret et al. 2001).

Species groups and community metrics exhibit a variety of response patterns to increasing metal toxicity (Hickey and Golding 2002). LOWESS smoothing of the responses of taxa richness and density to increasing CTUs for water and streambed sediment suggested a threshold response for most metrics. We did not calculate effects threshold levels, but CTUs for water and sediment at 7 of 9 test sites exceeded 1.0, indicating that cumulative metal concentrations may be toxic to aquatic life, especially benthic invertebrates living on or in sediment. Invertebrate assemblages at moderately contaminated test sites 8 and 9 were not significantly different from those at reference sites. Even though sediment at these 2 test sites was enriched in Pb and Zn, concentrations in water did not exceed AWQC, and bioaccumulation of metals in caddisfly tissue was relatively low. Maret and MacCoy (2002) also reported that fish assemblages at these sites were similar to those at reference sites. Even if metals are affecting biotic assemblages at these sites, detection of these effects is probably confounded by the inherent variability in these assemblages. Likewise, demonstrating biological recovery associated with remediation efforts at these sites will be difficult.

In spite of elevated concentrations of metals in water and streambed sediment, some benthic invertebrate taxa still persisted at the most contaminated sites. For example, 19 benthic invertebrate taxa were collected at site 13, and 29 invertebrate taxa were collected at sites 14 and 17. These numbers are higher than reported in earlier studies (Savage and Rabe 1973, Hoiland and Rabe 1992, Hoiland et al. 1994), indicating that conditions at these sites have improved over time. Hoiland et al. (1994) attributed increases in taxa richness at sites 14 and 17 to mine closures and improved wastewater treatment. However, a recent study showed that fish do not inhabit site 13 (Maret and MacCoy 2002).

Abundances of benthic invertebrates were lower at test sites than at reference sites, but abundances were generally comparable to those in other Western trout streams. The lowest densities occurred at test sites site 13 and 14 (<1600 organisms/m^2). Binns and Eiserman (1979) described streams with >5000 organisms/m^2 as productive trout streams. McGuire (2001) considered densities of >5500 organisms/m^2 as typical of Montana streams containing high-quality fish habitat. However, densities alone may not be indicative of high-quality fish food, especially if the assemblage is made up of only a few abundant taxa such as chironomids. Surprisingly, site 17 contained a much higher density than expected (>10,000 organisms/m^2). This large increase in density relative to other test sites may be the result of organic material from a sewage treatment outfall upstream (G. Harvey, Idaho Department of Environmental Quality, personal communication). This sample was predominantly composed of *Hydropsyche* spp., which are tolerant of metals (Cain et al. 1992, McGuire 2001) and organic pollution (Winner et al. 1980). Dense, filamentous algal growth was observed at this site in riffle habitats where benthic invertebrate samples were collected. Moderate nutrient increases from organic inflows increase benthic invertebrate abundances (Quinn and Hickey 1993). In addition, dissolved organic matter from this source may decrease metal bioavailability and, subsequently, decrease toxicity to invertebrates (McCarthy 1989).

Temporal variability in metal toxicity can be expected as a result of seasonal influences (Nimmo et al. 1998). Clements (1994) noted that the greatest effects on invertebrates in the Arkansas River occurred during spring, when metal concentrations were highest. In contrast, dissolved Cd and Zn concentrations in streams of the CDA River basin would be highest during low-flow periods (summer, autumn, and winter) (Woods 2000). Additional studies of temporal variability in benthic invertebrate assemblages in the CDA River basin are needed to better assess mining impacts throughout the year.

Feeding on contaminated invertebrates exposes fish to high concentrations of metals that are potentially harmful. Farag et al. (1999) attributed impaired health of fish in the South Fork CDA River to elevated metal concentrations in the tissue of macroinvertebrate prey. Woodward et al. (1994) determined that metals in benthic invertebrates reduced survival and growth in hatchery rainbow trout at concentrations that were lower than Cd, Pb, and Zn in caddsiflies found in our study at sites 14 and 17. Moreover, metal concentrations of the large-sized filter feeders in our study may underestimate actual dietary exposure of fish because these concentrations can be lower than in smaller-sized taxa such as *Baetis* (Kiffney and Clements 1993, Farag et al. 1998). Thus, our results support the notion that dietary exposures to metals pose the threat of reduced growth and survival to resident fish, especially early life stages of sculpin and trout that feed almost exclusively on small-sized invertebrates. This notion is consistent with Maret and MacCoy (2002) who reported that sculpins were absent at most test sites (except 8 and 9). The most contaminated test sites, 13, 14, and 17, also had lower trout biomass than most reference sites.

Our study used a spatially extensive design, and included paired-basin comparisons and sampling along metal-contamination gradients to assess the response of benthic invertebrates to mining impacts. We conclude that metal exposure affected benthic invertebrate assemblages in the CDA River basin. Several measures of assemblage structure, including total number of taxa, number of EPT taxa, and the density of metal-sensitive mayflies, were inversely related to indicators of metal exposure and toxicity. Production mine density within a 500-m stream buffer was a significant explanatory variable of mining impacts in our study. Maret and Skinner (2000) noted significant correlations between production mine density and metal concentrations in sediment, but mine density previously has not been used to describe basin disturbance or metal concentrations that may be detrimental to benthic invertebrates. This basin descriptor can be generated from GIS, and we suggest that it may have general utility as an indicator of the severity of metal contamination and effects on aquatic life in Rocky Mountain streams.

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