

# **Status of Stream Biotic Communities in Relation to Metal Exposure**

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Chapter E18 of

**Integrated Investigations of Environmental Effects of Historical  
Mining in the Animas River Watershed, San Juan County, Colorado**

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# Chapter E18

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### Abstract

The fish community of the Animas River watershed study area is dominated by the introduced brook trout (*Salvelinus fontinalis*), which has replaced native trout because it can better tolerate acidity and metals contamination and because it is well adapted to small stream habitats. The distribution of brook trout in the watershed is discontinuous. Fish are absent from the Cement Creek and upper Mineral Creek drainages, which are heavily affected by acidic drainage from both natural and mining-related sources. The upper Animas River has reaches without fish near the headwaters, due to acidic conditions, and near Silverton, due to elevated concentrations of dissolved metals and precipitated iron and aluminum oxyhydroxides downstream of the mixing zone with Cement Creek. Self-sustaining trout populations occur in other reaches of the Animas River upstream of Silverton, and trout populations recover gradually in the Animas River canyon downstream of Silverton. The status of benthic invertebrate communities generally parallels that of trout populations: reductions in abundance and number of invertebrate taxa, and shifts from metal-intolerant to metal-tolerant taxa are evident in stream reaches affected by acidity and metals.

Metal concentrations in components of stream food webs—periphyton, benthic invertebrates, and brook trout—reflect differences in metal bioavailability and indicate differing risks of chronic metal toxicity to stream biota. Concentrations of the toxic metals cadmium, copper, lead, and zinc in periphyton, benthic invertebrates, and liver tissue of trout from one or more sites in the Animas River in the study area were significantly greater than those in biota from reference sites. Periphyton from sites in acidic streams and in mixing zones contained high concentrations of aluminum and iron and reduced algal biomass. Metal concentrations in benthic invertebrates reflected differences in feeding habits and body size as well as gradients of metal bioavailability. Concentrations of copper and cadmium remained stable or increased in biota across several trophic levels, suggesting that these metals were more efficiently transferred via dietary exposure.

Copper concentrations in invertebrate diets and liver tissue of brook trout from the Animas River were closely associated with differences in resident fish and invertebrate populations and approached levels associated with adverse effects on trout populations in field studies and with toxic effects in laboratory studies. These results suggest that chronic copper toxicity is an important factor limiting the distribution and abundance of brook trout in the study area.

### Introduction

The water quality, habitats, and biota of streams in the Animas River watershed study area are affected by metal contamination associated with acidic drainage from inactive mines, deposits of mine and mill wastes, and naturally acidic soil and altered rock. Biota of streams receiving acidic drainage can be adversely affected by exposure to metals via multiple exposure routes. These adverse effects can result from short-term exposure to stream water (Henry and others, 1999), suspended colloids (Smith and Sykora, 1976), and bed sediment (Kemble and others, 1994) containing toxic levels of acid or metals. Toxicity tests conducted on-site and in the laboratory have demonstrated that stream water and, to a lesser extent, fine streambed sediment from sites with impaired biotic communities in the Animas River watershed are directly toxic to fish and benthic invertebrates (Nimmo and others, 1998; Besser, Allert, and others, 2001; Besser and Leib, this volume, Chapter E19). Metal-contaminated diets can be a significant source of metal bioaccumulation and chronic toxicity to trout in stream habitats where aqueous exposure alone does not cause toxicity (Miller and others, 1993; Woodward and others, 1994, 1995; Farag and others, 1999).

Metal bioavailability to higher order consumers such as trout can be substantially modified by the processing of metals in stream food webs. Periphyton, the community of attached algae, bacteria, and fungi that develops on stream substrata, can accumulate high concentrations of metals and may be an important source of metal exposure to benthic

macroinvertebrates in streams affected by mining (Kiffney and Clements, 1993; Farag and others, 1998; Beltman and others, 1999). Differential accumulation of metals among different invertebrate taxa and differences in taxonomic composition of invertebrate communities among locations can lead to substantial variation in metal concentrations in the diets available to stream-dwelling trout (Moore and others, 1991; Clements and Kiffney, 1994; Farag and others, 1998).

## Purpose and Scope

This chapter summarizes available information from recent surveys of stream biological communities of the Animas River watershed study area and from a study of metal bioavailability in stream ecosystems, based on metal concentrations in tissues of biota. Data from these sources are used to identify associations between metal exposure and biological effects and to identify factors that may limit recovery of stream communities of the Animas River watershed.

## Acknowledgments

Tom May, Mike Walther, and Ray Weidmeyer conducted metal analyses. Win Wright, Ken Leib, and Paul von Guerard provided water chemistry data and logistical assistance in the field. Briant Kimball and Stan Church provided advice, assistance, and useful data. Barry Poulton provided taxonomic verifications of invertebrate samples. Brad Mueller, Chester Anderson (Animas River Stakeholders Group), and Bob Brantlinger (USDA Forest Service) assisted with field collections. Mike Japhet (Colorado Division of Wildlife), Robert Gallegos (Colorado Dept. of Public Health and Environment), and David Gerhardt (USDA Forest Service) provided fish and invertebrate community data. Durango & Silverton Narrow Gauge Railroad provided transportation to sites in the Animas River canyon.

## Status of Stream Biotic Communities

### Sources of Data

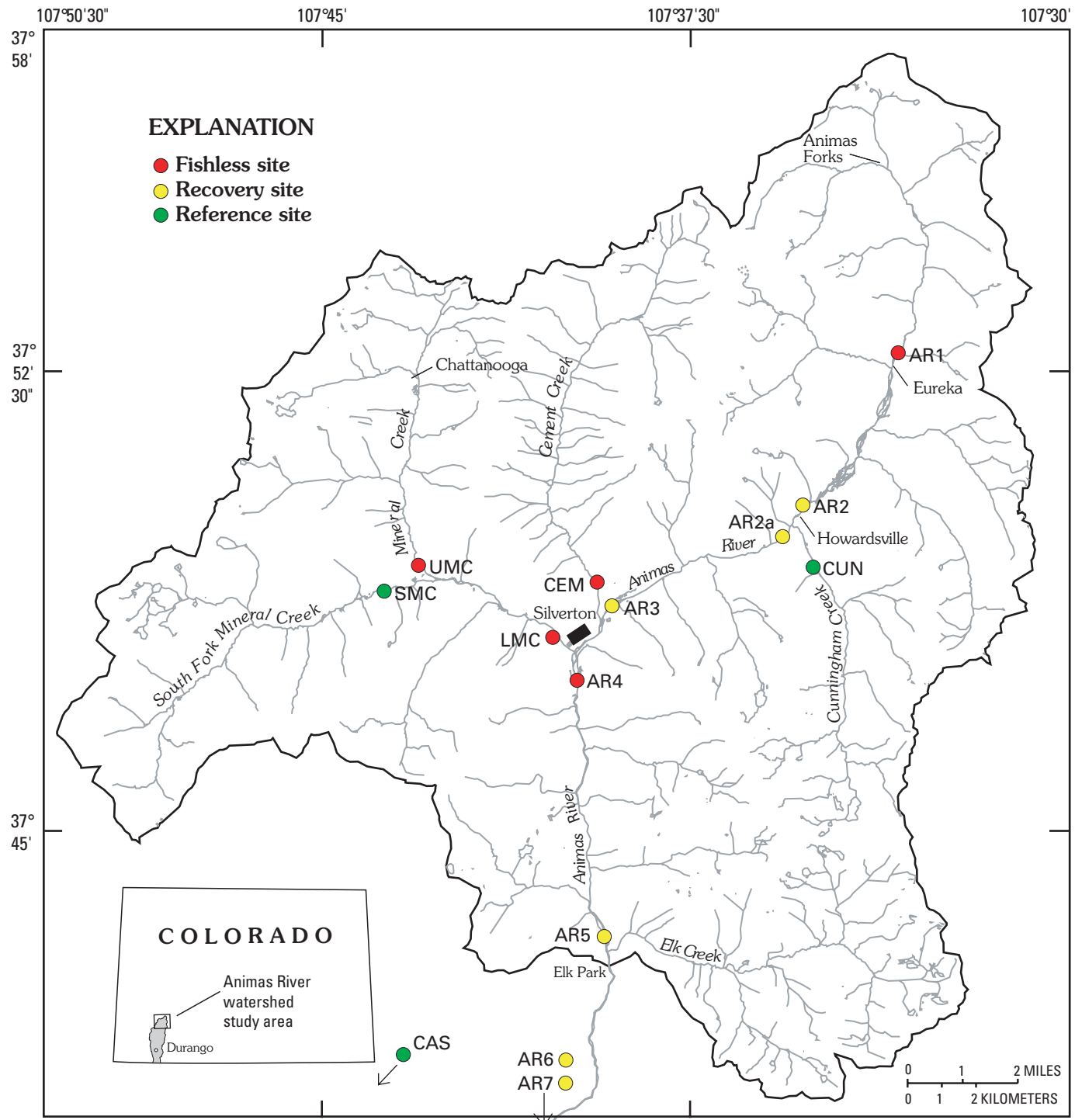
The fish and benthic invertebrate communities of the Animas River watershed have been surveyed repeatedly since the 1970s, with recent fish sampling conducted primarily by the State of Colorado, Division of Wildlife (Robert Gallegos, written commun., 1996), and sampling of benthic invertebrate communities conducted by the State of Colorado, Department of Public Health and Environment, and the Animas River Stakeholders group (Peter Butler, Robert Owen, and William Simon, Unpublished report to

Colorado Water Quality Control Commission, Animas River Stakeholders Group, 2001). Sample sites reported in this chapter are shown in figure 1 and described in table 1.

## Trout Populations

The fish community of the Animas River watershed study area originally consisted of native Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) and possibly mottled sculpins (*Cottus bairdi*). Streams and tributaries in drainages with extensive areas of alteration (Bove and others, this volume, Chapter E3) and (or) extensive past mining (Jones, this volume, Chapter C), such as Cement Creek, Mineral Creek (except the South Fork) and the headwaters of the upper Animas River, probably never supported trout populations. The fish community is now dominated by the introduced species, brook trout (*Salvelinus fontinalis*), with cutthroat trout occurring only in high-altitude tributaries with good water quality, such as Cunningham Creek and Minnie and Maggie Gulches (Wright, Simon, and others, this volume, Chapter E10). Two other non-native salmonids, rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*), have been stocked in the study area in the past. Although healthy populations of rainbow trout and brown trout occur in downstream reaches of the Animas River canyon, and a few rainbow trout may still occur in the study area, neither species has maintained self-sustaining populations in the study area. The current distribution of brook trout in the study area is discontinuous (table 1), with reaches supporting fish separated by reaches that are fishless due to the influence of acidic drainage and metal contamination (Unpub. report to Colorado Water Quality Control Commission, ARSG, 2001).

The replacement of cutthroat trout by brook trout reflects some combination of the ability of brook trout to compete for habitat in small streams, and the greater tolerance of brook trout for acidity and metal contamination. The principal current populations of brook trout in the study area occur in South Fork Mineral Creek and in two segments of the Animas River: from Minnie Gulch downstream to near Silverton, and from approximately Molas Creek downstream (fig. 1). Greatest densities of brook trout occur in the Animas River downstream from Minnie and Maggie Gulches and in South Fork Mineral Creek (table 1). Current brook trout populations in the Animas River upstream of Silverton are substantially greater than those reported in the 1970s (N.F. Smith, unpub. report, Colorado Division of Wildlife, 1976), and populations in the Animas River canyon, downstream from Silverton, have increased since 1992 (table 1). These changes suggest that anthropogenic degradation of water quality and (or) habitat changes have been partially reversed by reduced mining and milling activity and by reclamation activities in the watershed.



**Figure 1.** Location of sampling sites for aquatic biological community surveys and food-web metals study. Not shown on map: AR6, Animas River above Needle Creek (lat 37°37.845' N., long 107°41.702' W.); AR7, Animas River above Cascade Creek (lat 37°35.909' N., long 107°46.331' W.); and CAS, Cascade Creek (lat 37°39.573' N., long 107°48.714' W.).

**Table 1.** Sites for aquatic community sampling and food-web metals study.

[Brook trout population data from Colorado Department of Public Health and Environment (1992; Rob Gallegos, Denver, Colo., written commun., 1992) and Colorado Division of Wildlife (Mike Japhet, Durango, Colo., written commun., 1998). No fish sampling occurred at sites indicated by --]

Site ID (fig. 1)	Site	Elevation (m)	Site category (see text)	Brook trout (number/300 m)	
				1992	1997–98
Animas River					
AR1	Animas at Eureka	3,006	Fishless	--	--
AR2	Animas at Howardsville	2,940	Recovery	110	270
AR2a	Animas below Cunningham Creek	2,910	Recovery	24	--
AR3	Animas above Silverton	2,854	Recovery	57	2 <sup>a</sup>
AR4	Animas below Silverton	2,805	Fishless	0	0
AR5	Animas at Elk Park	2,707	Recovery	13	23
AR6	Animas at Needleton	2,500	Recovery	--	--
AR7	Animas above Cascade Creek	2,353	Recovery	36	102
Tributaries					
CEM	Lower Cement Creek	2,835	Fishless	0	--
UMC	Upper Mineral Creek	2,903	Fishless	0	--
LMC	Lower Mineral Creek	2,817	Fishless	0	--
CUN	Cunningham Creek	3,008	Reference	--	--
SMC	South Fork Mineral Creek	2,975	Reference	66	--
CAS	Cascade Creek	2,664	Reference	--	--

<sup>a</sup>1997 fish sampling upstream of Arrastra Creek.

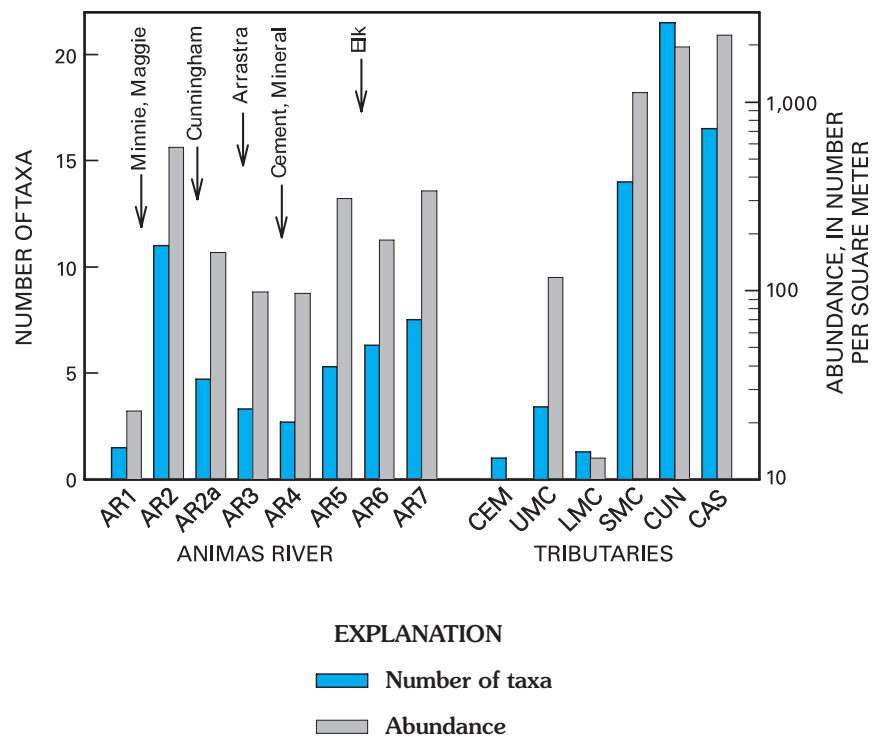
For the purposes of this chapter, sites were classified into three categories based on their populations of brook trout and degree of influence of upstream mining or acid rock drainage: fishless sites (no fish present; high levels of acidity and metals); recovery sites (fish present; downstream of fishless sites); and reference sites (fish present; relatively little influence of upstream acidity or metals) (table 1; fig. 1). Fishless sites included acidic headwater streams (Cement Creek, CEM; and upper Mineral Creek, UMC) and neutral-pH sites downstream of mixing zones of acidic tributaries (lower Mineral Creek, LMC; Animas River upstream of Eureka, AR1; and Animas River downstream of Silverton, AR4). Recovery sites on the Animas River (Howardsville, AR2; upstream of Silverton, AR3; Elk Park, AR5; and Needleton, AR6) had circumneutral pH and supported varying densities of brook trout and benthic invertebrates. Reference sites (South Fork Mineral Creek, SMC; Cunningham Creek, CUN; and Cascade Creek, CAS) supported reproducing populations of brook trout and relatively diverse and abundant benthic invertebrate communities. Cascade Creek flows into the Animas River in northern La Plata County, south of the Animas River watershed study area.

## Benthic Invertebrate Communities

The status of benthic invertebrate communities provides additional resolution of differences in water quality and habitat conditions throughout the Animas River watershed study area. A study of benthic invertebrate communities conducted

over three sampling periods in 1996–98 (Unpub. report to Colorado Water Quality Control Commission, ARSG, 2001) demonstrated both dramatic differences in the status of benthic communities of different portions of the watershed and more subtle longitudinal shifts in communities resulting from gradual changes in water quality and stream habitats (fig. 2). Average taxonomic richness in these samples was relatively low, even at reference sites, reflecting both the expectation for low taxonomic richness in high-altitude streams and the small area sampled by individual Surber samplers (0.093 m<sup>2</sup>). Benthic communities at sites with persistent acidic conditions and high metal concentrations averaged fewer than five taxa per sample and fewer than 100 organisms per square meter, whereas communities of reference sites consistently had 15 or more taxa and more than 1,000 organisms per square meter. Communities also differed qualitatively between sites: fishless sites were dominated by tolerant taxa such as fly larvae (Diptera; for example, Chironomid midges); whereas recovery and reference sites had greater representation of more metal-sensitive taxa such as mayflies (Ephemeroptera), stoneflies (Plecoptera), and caddis flies (Trichoptera).

Longitudinal changes in benthic invertebrate communities of the Animas River were generally consistent with the patterns in the distribution of brook trout. Communities exhibited moderately high diversity and abundance in the reach between Eureka Gulch and Arrastra Creek, but they showed evidence of increasing adverse effects in the reach that includes the confluences of Cement and Mineral Creeks near Silverton, followed by a gradual recovery in the downstream



**Figure 2.** Characteristics of benthic invertebrate communities of Animas River watershed study area. Mean number of taxa and mean abundance from three sample dates in 1996–97. See table 1 and figure 1 for site identifications and locations. Data from Peter Butler, Robert Owen, and William Simon, Unpublished report to Colorado Water Quality Control Commission, Animas River Stakeholders Group (2001).

reach between Molas and Cascade Creeks. Comparison of these data to the 1992 invertebrate survey conducted by the Colorado Department of Public Health and Environment shows relatively little change in benthic communities at most sites during this period, but it suggests that some recovery of benthic invertebrate communities is occurring in certain reaches of the Animas River, both upstream and downstream of Silverton.

## Metal Bioaccumulation in Stream Food Webs

### Methods

Samples of stream biota of the Animas River watershed study area were collected for analysis of metals during fall low-flow periods in 1997 and 1998 (Besser, Brumbaugh, and others, 2001). Samples of periphyton, benthic invertebrates, and fish tissue were collected at some or all of the study sites. Periphyton samples were collected at all 12 study sites and

analyzed for metals, chlorophyll-*a* (a measure of algal biomass), and total organic carbon (TOC). Benthic invertebrates were collected at eight sites, and dominant taxa at each site were sorted into separate samples for metal analysis. Samples of the mayfly, *Rhithrogena* sp. (Heptageniidae), and the caddis fly, *Arctopsyche* sp. (Hydropsychidae), were collected from six sites. Samples of the stonefly, *Megarcys* sp. (Perlodidae), were collected from five sites, and samples of a second stonefly, *Zapada* sp. (Nemouridae), were collected from one site. Adult brook trout were collected with a backpack electrofishing unit at six sites (AR2, AR3, AR6, CUN, SMC, and CAS). Livers were dissected for metal analysis as an indication of chronic metal exposure (Crawford and Luoma, 1993). Biological samples were analyzed by inductively coupled plasma–mass spectroscopy after microwave digestion with concentrated nitric acid (May and others, 1997; Besser, Brumbaugh, and others, 2001).

Metal concentrations in tissues of stream biota were log-transformed before statistical analysis to improve normality and homogeneity of variance. Associations among metal concentrations and other characteristics of sediment and periphyton were evaluated by Pearson correlation analysis. Differences in metal concentrations among locations were

evaluated by analysis of variance (ANOVA), with differences among means compared by Duncan's multiple range test (Snedecor and Cochran, 1980). Statements of statistical significance indicate a probability of Type I error of 5 percent or less ( $p \leq 0.05$ ).

## Periphyton

Concentrations of metals in periphyton differed significantly among sites. Greatest concentrations of cadmium, copper, manganese, lead, and zinc occurred in the Animas River upstream of Silverton (table 2). Periphyton samples from this reach contained zinc concentrations approaching 2 percent on a dry-weight basis. Concentrations of copper in periphyton from the Animas River decreased gradually from upstream to downstream, whereas concentrations of zinc, cadmium, and lead were consistently high at sites upstream of Silverton and dropped off sharply downstream. As a result, concentrations of copper, but not other metals, in periphyton from downstream recovery sites in the Animas River remained greater than those at reference sites.

Metal concentrations in periphyton samples can reflect both trapping of metal-rich colloids and uptake of dissolved metals, by bioaccumulation or sorption (Newman and others, 1985). Concentrations of most metals in periphyton were significantly correlated with concentrations in fine stream-bed sediment, although these associations usually explained less than half of the overall variation (Besser, Brumbaugh, and others, 2001). Periphyton from the upstream reach of the Animas River (AR1-AR3) contained both high concentrations

of several metals and concentrations of chlorophyll and TOC that were similar to those of reference sites (fig. 3). Concentrations of chlorophyll and TOC were positively correlated with concentrations of zinc and copper in periphyton, suggesting that the presence of algae and other organic constituents resulted in increased accumulation of metals. In contrast, chlorophyll and TOC were negatively correlated with concentrations of iron and aluminum. Samples of periphyton from two acidic sites, with pH <4.5 at low flow (CEM and UMC; Wright, Simon, and others, this volume), and two neutral-pH sites downstream from acid tributaries (AR1, LMC) contained little algal biomass and were dominated by precipitated iron and aluminum oxyhydroxides (fig. 3). These minerals made up approximately 50–90 percent of periphyton dry mass at the sites most affected by precipitates, compared to 3–10 percent at the reference sites. This finding is consistent with experimental studies that found copper and cadmium to be preferentially sorbed by algal biomass, relative to Fe, Al, or Mn oxyhydroxides (Calmano and others, 1988). Overall, our data suggest that periphyton in stream reaches with circumneutral pH and low levels of iron or aluminum precipitates can provide a substantial source of metal-contaminated food for stream food webs.

## Benthic Invertebrates

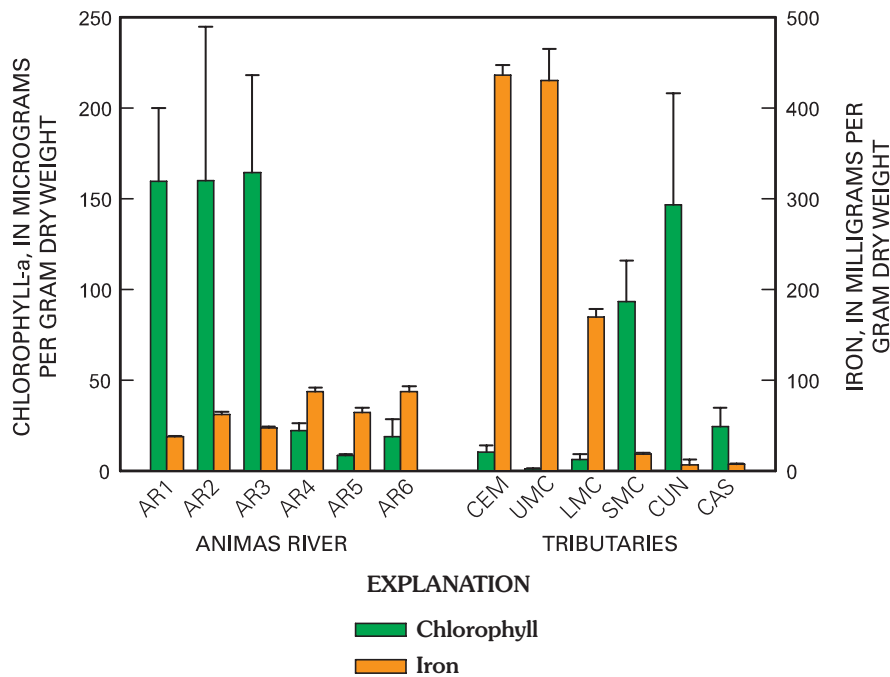
Concentrations of metals in benthic invertebrates differed significantly among taxa and among sites. Concentrations of cadmium, copper, lead, and zinc differed significantly among invertebrate taxa (Besser, Brumbaugh, and others,

**Table 2.** Concentrations of metals in periphyton from the Animas River and tributaries.

[Means, with standard errors in parentheses ( $n=3-7$ ). For each metal, means followed by the same letter are not significantly different]

Site	Metal concentration ( $\mu\text{g/g}$ dry wt.)			
	Cadmium	Copper	Lead	Zinc
Animas River				
AR1	33.3 (0.5) <sup>a</sup>	6,587 (371) <sup>a</sup>	1,051 (40) <sup>abc</sup>	18,950 (743) <sup>a</sup>
AR2	25.3 (1.3) <sup>a</sup>	1,031 (64) <sup>bc</sup>	2,023 (33) <sup>a</sup>	8,520 (465) <sup>ab</sup>
AR3	27.8 (1.8) <sup>a</sup>	952 (45) <sup>bc</sup>	1,597 (24) <sup>ab</sup>	11,903 (314) <sup>a</sup>
AR4	5.6 (0.4) <sup>b</sup>	764 (54) <sup>bc</sup>	680 (80) <sup>abc</sup>	2,179 (124) <sup>cd</sup>
AR5	6.1 (0.3) <sup>b</sup>	362 (36) <sup>c</sup>	465 (26) <sup>abc</sup>	2,656 (272) <sup>cd</sup>
AR6	9.5 (0.6) <sup>b</sup>	709 (72) <sup>bc</sup>	480 (9) <sup>abc</sup>	3,673 (243) <sup>bc</sup>
Fishless sites				
CEM	0.2 (0.04) <sup>c</sup>	31 (1) <sup>e</sup>	207 (9) <sup>c</sup>	44 (8) <sup>c</sup>
UMC	0.2 (0.1) <sup>c</sup>	105 (7) <sup>d</sup>	753 (54) <sup>abc</sup>	40 (13) <sup>c</sup>
LMC	5.2 (0.9) <sup>b</sup>	1,571 (296) <sup>b</sup>	605 (150) <sup>abc</sup>	1,135 (3,830) <sup>c</sup>
Reference sites				
CUN	2.7 (1.4) <sup>c</sup>	40 (36) <sup>f</sup>	266 (265) <sup>d</sup>	338 (300) <sup>e</sup>
SMC	6.7 (0.3) <sup>b</sup>	89 (5) <sup>d</sup>	276 (5) <sup>bc</sup>	1,783 (87) <sup>cd</sup>
CAS	0.4 (0.01) <sup>d</sup>	9 (1)	9 (0.5) <sup>abc</sup>	84 (6) <sup>cd</sup>





**Figure 3.** Algal biomass (represented by chlorophyll-*a*) and iron in periphyton. Mean and range for chlorophyll ( $n=2$ ); mean and standard error for iron ( $n=4$ ).

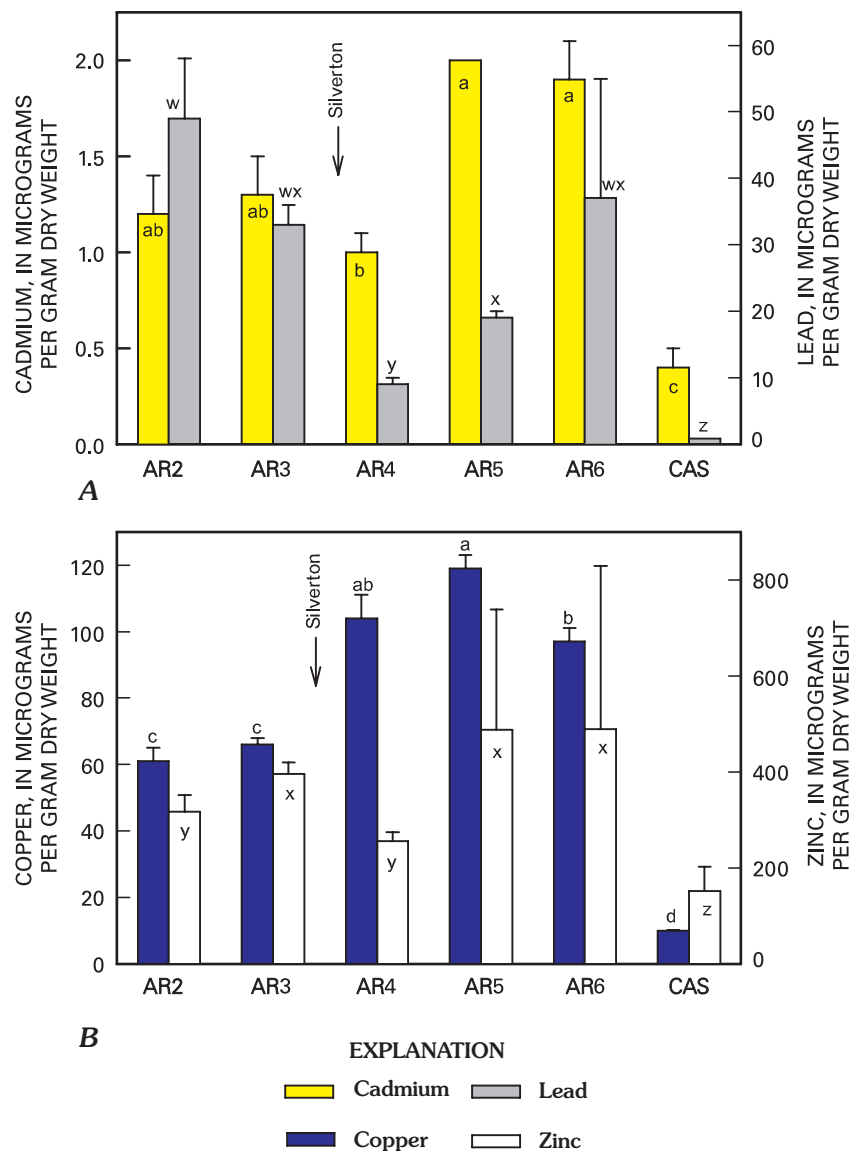
2001). Concentrations of all four metals were significantly greater in two smaller taxa (*Rhithrogena* and *Zapada*), consistent with previous reports that metal accumulation by invertebrates decreases with increasing body size (Smock, 1980). However, differences in metal concentrations among taxa also reflected differences in feeding ecology. Of the two small taxa, the periphyton grazer, *Rhithrogena*, accumulated greater concentrations of zinc and cadmium, and the detritivore, *Zapada*, accumulated greater concentrations of lead. Two larger taxa, *Megarcys* and *Arctopsyche*, with diets that include substantial components of invertebrate tissue, accumulated proportionally greater concentrations of copper. Metal concentrations in invertebrates also differed significantly among sites, reflecting variation in metal bioavailability in the watershed. Lowest concentrations of all metals occurred in invertebrates at Cascade Creek (CAS), the reference site outside the highly mineralized portion of the watershed (Yager and Bove, this volume, Chapter E1), but metal concentrations at the other reference sites (SMC and CUN) were not consistently less than those from recovery sites in the Animas River. Of the metals studied, only copper occurred at consistently greater concentrations in invertebrates from fishless and recovery sites in the Animas River, relative to reference sites.

Because it is widely distributed and tolerant of elevated metal burdens, the caddis fly *Arctopsyche grandis* has been widely used to monitor metal bioavailability in streams of the western United States (Cain and others, 1992; Kiffney

and Clements, 1993; Farag and others, 1998). *Arctopsyche* occurred at five of six sites sampled in the Animas River, and samples from these sites were used to evaluate longitudinal patterns of metal bioavailability. Concentrations of copper in *Arctopsyche* were significantly greater downstream of Cement and Mineral Creeks, at the fishless site AR4 and at recovery sites AR5 and AR6, than at sites upstream of Silverton. In contrast, concentrations of zinc, lead, and cadmium in *Arctopsyche* did not show consistent increases at sites downstream of Cement and Mineral Creeks, and were generally lower at AR4 than at other Animas River sites (fig. 4).

### Brook Trout

Concentrations of lead, zinc, and copper in liver tissue of brook trout differed significantly among sites (fig. 5). Concentrations of both zinc and copper in liver tissue corresponded to general trends in the status of stream biota, although both differences between Animas River sites and reference sites and longitudinal differences among Animas River sites were more pronounced for copper than zinc. Lead concentrations in trout livers were significantly lower at Cascade Creek than at other sites, but differences between reference and recovery sites were not evident. Previous studies have reported elevated concentrations of copper and zinc in livers of trout from metal-contaminated sites (Wilson and others, 1980; Moore and others, 1991; Farag and others, 1995).

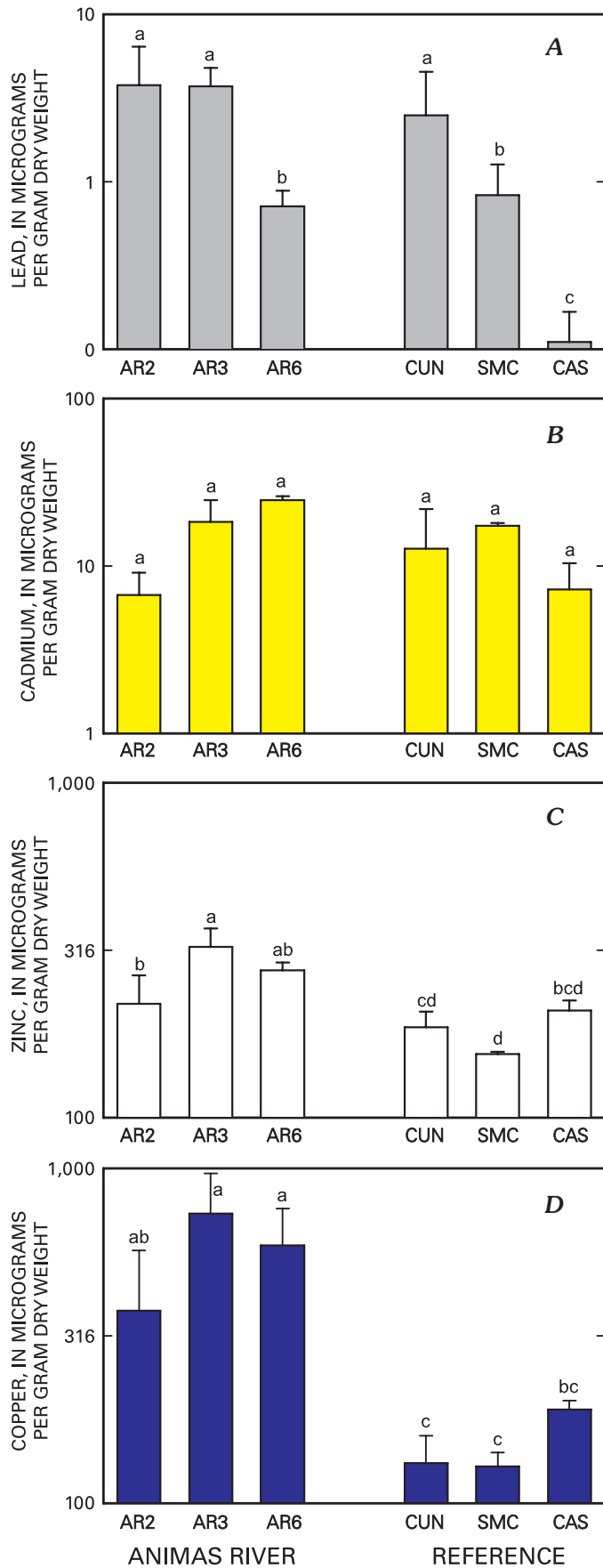


**Figure 4.** Concentrations of metals in caddis flies, *Arctopsyche* sp., from the Animas River and Cascade Creek. *A*, cadmium and lead; *B*, copper and zinc. Means with standard error ( $n=4$ ). For each metal, means with same letters are not significantly different.

## Food-Web Transfer of Metals

Comparisons of trophic transfer factors (TTFs), ratios of metal concentrations between aquatic animals and their available diets, suggested that the efficiency of transfer via the stream food web differed among the metals studied (Besser, Brumbaugh, and others, 2001). Overall TTFs for a hypothetical stream food chain (periphyton-invertebrate-trout liver) suggest that cadmium (TTF=4.8) and copper (TTF=4.6) are transferred most efficiently via diet. In contrast, zinc was apparently transferred efficiently from periphyton to invertebrates, but not to trout (overall TTF=0.54), and lead was transferred least efficiently (overall TTF=0.005). Efficient trophic

transfer of cadmium from low levels in water and sediment may explain the occurrence of elevated cadmium concentrations in biota from reference sites and the persistence of elevated cadmium concentrations at downstream recovery sites in the Animas River. This hypothesis is consistent with findings of a study of a Montana watershed, that higher levels of cadmium in stream food chains persisted farther downstream than concentrations of other metals (Moore and others, 1991). Similarly, the hypothesis of more efficient trophic transfer may partially explain the greater enrichment of copper in tissues of invertebrates and brook trout, despite low concentrations of copper (relative to zinc and lead) in sediment and periphyton. Although the presence of relatively high levels of cadmium



and copper in trout livers reflects at least in part typical patterns of enrichment of these metals in liver tissue (Wilson and others, 1980; Farag and others, 1995), internal distribution of metals cannot explain the greater concentrations of copper in liver tissue of trout from recovery sites, relative to reference sites (fig. 5).

### Association of Metal Exposure with Biological Effects

The distribution and abundance of trout in the Animas River watershed study area and adjoining area downstream are closely associated with patterns of metal bioaccumulation. The absence of brook trout at some fishless sites such as AR4 and LMC probably reflects direct toxicity of aqueous copper and (or) other metals (Besser, Allert, and others, 2001; Besser and Leib, this volume), but results of the food web study suggest that brook trout at sites with lower aqueous metal concentrations may also be subject to chronic toxicity via combined aqueous and dietary exposure. Concentrations of copper in invertebrate diets are substantially greater at Animas River sites with reduced densities of brook trout than at reference sites. However, mean concentrations of zinc and lead in invertebrates from these Animas River sites overlapped broadly with those in reference sites.

Concentrations of copper in diets and liver tissue of brook trout from the Animas River approach levels associated with chronic toxicity (Besser, Brumbaugh, and others, 2001). Dietary toxicity has been reported for other salmonid species fed diets containing elevated concentrations of copper and other metals (Woodward and others, 1994, 1995; Farag and others, 1999). Brook trout from affected sites in the Animas River had copper concentrations in liver tissue equal to or greater than those associated with toxicity to brook trout and rainbow trout in laboratory tests (McKim and Benoit, 1974; Lanno and others, 1985) or associated with reduced population density of brook trout in field studies (Moore and others, 1991). The reduced abundance of brook trout at sites where tissue copper concentrations approach levels associated with toxicity supports the hypothesis that chronic copper toxicity is an important factor controlling the distribution and abundance of brook trout in the Animas River watershed. The effects of copper toxicity on brook trout populations are probably exacerbated by toxic effects of other metals and by degradation of benthic habitats, leading to reduced biological diversity and reduced productivity in stream habitats of the Animas River watershed.

**Figure 5.** Concentrations of metals in livers of brook trout, *Salvelinus fontinalis*, from the Animas River and reference sites. A, lead; B, cadmium; C, zinc; D, copper. Means with standard errors (n=4). For each metal, means with same letters are not significantly different.

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