

# Bioavailability of metals in stream food webs and hazards to brook trout (*Salvelinus fontinalis*) in the upper Animas River watershed, Colorado

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## Abstract

The water quality, habitats, and biota of streams in the upper Animas River watershed of Colorado USA are affected by metal contamination associated with acid drainage. We determined metal concentrations in components of the food web of the Animas River and its tributaries -- periphyton (*aufwuchs*), benthic invertebrates, and livers brook trout (*Salvelinus fontinalis*) -- and evaluated pathways of metal exposure and hazards of metal toxicity to stream biota. Concentrations of the toxic metals cadmium (Cd), copper (Cu), lead (Pb), and zinc (Zn) in periphyton, benthic invertebrates, and trout livers from one or more sites in the upper Animas River were significantly greater than those from reference sites. Periphyton from sites downstream from mixing zones of acid and neutral waters had elevated concentrations of aluminum (Al) and iron (Fe) in periphyton, reflecting deposition of colloidal Fe and Al oxides, and reduced algal biomass. Metal concentrations in benthic invertebrates reflected differences in feeding habits and body size among taxa, with greatest concentrations of Zn, Cu and Cd in the small mayfly *Rhithrogena*, which feeds on periphyton, and greatest concentrations of Pb in the small stonefly *Zapada*, a detritivore. Concentrations of Zn and Pb decreased across each trophic linkage, whereas concentrations of Cu and Cd were similar across several trophic levels, suggesting that Cu and Cd were more efficiently transferred via dietary exposure. Concentrations of Cu in invertebrates and trout livers were more closely associated with impacts on trout populations and invertebrate communities than were concentrations of Zn, Cd, or Pb. Copper concentrations in livers of brook trout from the upper Animas River were substantially greater than background concentrations and approached levels associated with reduced brook trout populations in field studies and toxic effects on other salmonids in laboratory studies. These results indicate that bioaccumulation and transfer of metals in stream food webs is a significant component of metal exposure for stream biota of the upper Animas River watershed, and suggest that chronic toxicity of Cu is an important factor limiting the distribution and abundance of brook trout populations in the watershed.

## Introduction

The water quality, habitats, and biota of streams in the upper Animas River watershed of southwest Colorado USA are affected by metal contamination associated with acid drainage from abandoned mines, deposits of mine and mill wastes, and naturally acidic soils and rocks. The upper Animas River and its two major tributaries, Cement and Mineral Creeks (Fig. 1), drain a geologic structure known as the Silverton caldera that has been altered by hydrothermal activity, resulting in extensive deposits of economically valuable metal ores. More than 1500 mining claims have been staked in the watershed. The first claims were staked in 1873, mining activity peaked in the 1920's, and the last mine closed in 1991. Weathering of sulfide minerals in mine shafts, deposits of mine and mill tailings, and exposed rocks and soil generates acid drainage that enters streams of the upper Animas watershed. This acid drainage contains high concentrations of iron (Fe), aluminum (Al), and manganese (Mn), along with lesser concentrations of more toxic metals such as zinc (Zn), copper (Cu), lead (Pb), and cadmium (Cd; Church *et al.* 1997).

Biota of streams receiving acid drainage can be adversely affected by exposure to metals via multiple exposure routes. Toxic effects in habitats affected by acid drainage can result from short-term exposure to metal-contaminated stream water (Henry *et al.* 1999), suspended colloids (Smith and Sykora 1976), and sediments (Kemble *et al.* 1994). Toxicity tests conducted on-site and in the laboratory have demonstrated that stream water and, to a lesser extent, fine streambed sediments from the most highly-impacted sites in the upper Animas River watershed are directly toxic to fish and benthic invertebrates (Besser *et al.* 2000). 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 *et al.* 1993; Woodward *et al.* 1994, 1995; Farag *et al.* 1999). Metal bioavailability to higher order consumers such as trout can be substantially modified by processing of metals in stream food webs. Periphyton, or *aufwuchs*, 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 mining-impacted streams (Kiffney and Clements 1993, Farag *et al.* 1998; Beltman *et al.* 1999). Differential accumulation of metals among invertebrate taxa and differences in taxonomic composition among locations can lead to substantial variation in metal concentrations in the diets available to stream-dwelling trout (Moore *et al.* 1991, Clements and Kiffney 1994, Farag *et al.* 1998).

This study sought to characterize metal bioavailability and hazards of chronic toxicity in stream habitats throughout the upper Animas River watershed, based on metal concentrations in tissues of stream biota. The objectives of this study were to: (1) assess pathways by which metals enter and move through stream food webs of the upper Animas watershed, and (2) assess potential hazards of chronic metal toxicity to resident brook trout.

## Study Area

Twelve study sites in the upper Animas River and its tributaries were selected for sampling of biota for metals analyses (Fig. 1, Table 1). These sites were classified, based on the status of resident brook trout (*Salvelinus fontinalis*) populations, as high-impact, intermediate-impact, or reference sites. High-impact sites, which did not support permanent fish populations during community surveys in 1992 and 1998, included acid headwater streams (Cement Creek, CEM; and upper Mineral Creek, UMC) and neutral-pH sites downstream of mixing zones of acid

tributaries and neutral waters (lower Mineral Creek, LMC; Animas River above Eureka, AR1; and Animas River below Silverton, AR4). Intermediate-impact sites on the Animas River (Howardsville, AR2; above Silverton, AR3; Elk Park, AR5; and Needleton, AR6) had circumneutral pH and supported varying densities of brook trout and benthic invertebrates. Reference sites supported relatively diverse and abundant benthic invertebrate communities and reproducing populations of brook trout (personal communication; M. Japhet, Colorado Division of Wildlife, Durango, Colorado) and relatively diverse and abundant benthic invertebrate communities. One reference site, Cascade Creek (CAS) is located in a watershed with little or no history of mining. Two other reference sites, Cunningham Creek (CUN) and South Fork of Mineral Creek (SMC) are located in drainages which have experienced significant historic mining activity. However, the presence of apparently healthy biological communities at these sites suggests that the bioavailability of metals in these streams is reduced by the lesser degree of alteration of the ore-bearing formations in these drainages and by the greater abundance of rocks which contribute buffering capacity to stream water (Church *et al.* 1997).

Fish and invertebrate communities of the study sites reflected the range of natural and mining-related impacts on stream biota of the upper Animas watershed (Table 1). Brook trout was the predominant fish species throughout the study area. Native Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*), rainbow trout (*O. mykiss*) from earlier stocking efforts, and rainbow-cutthroat hybrids occurred in very low numbers (1-3% of fish sampled) except at the most downstream site in the Animas River canyon (AR6), where these species made up about 10% of the population. Cunningham Creek (CUN), which was not sampled during recent surveys, supports both brook trout and cutthroat trout. Taxonomic diversity of benthic invertebrates was generally low in recent surveys, even at reference sites, reflecting low levels of taxonomic resolution (family or genus) and naturally low taxa richness of high-altitude streams as well as effects of mining on sensitive taxa. The status of the fish and invertebrate communities at high-impact sites in the study area changed little between 1992 and 1997-98, despite the initiation of remediation efforts including sealing mine openings, diversion and/or treatment of runoff from deposits of mine waste, and removal of mine waste from stream channels (Table 1). Some recovery of fish and invertebrate populations was evident at some intermediate-impact sites in the Animas River, notably in the near Howardsville (AR2) and the lower Animas canyon (AR5, AR6).

Metal concentrations in stream water of the upper Animas River, during the late summer/fall sampling period, show longitudinal trends of increased metal concentrations associated with tributary metal loadings followed by downstream attenuation (Table 2). Aqueous metal concentrations throughout the entire study reach of the Animas were an order of magnitude greater than in two reference tributaries, Cunningham Creek (CUN) and South Mineral Creek (SMC). High concentrations of dissolved and total metals at the most upstream site on the Animas River site (AR1) reflected loadings of metals from acid drainage in the headwaters. Concentrations of Fe, Pb, and Cu decreased in the reach between AR1 and Silverton (AR3), but Zn remained stable or increased, due to inputs from floodplain deposits of mine tailings and milling wastes (Leib and Wright, 2000). Tributaries entering the Animas near Silverton, Cement Creek (CEM) and lower Mineral Creek (LMC), add substantial loadings of dissolved and colloidal metals (Church *et al.* 1997, Lieb and Wright 2000), resulting in increased concentrations of total Cu and Pb and total and dissolved Fe downstream at AR4. Although data is limited, elevated aqueous concentrations of some metals apparently persisted further downstream in the Animas Canyon (AR5 and AR6), despite minimal metal loadings from

downstream tributaries. Aqueous metal concentrations also vary seasonally, with concentrations of dissolved Zn and Cu typically near annual minima during summer and reaching annual maxima in late winter, when metal concentrations may be greater by a factor of three or more (Leib and Wright 2000). Seasonal or year-round concentrations of aqueous Zn and Cu at stations in the vicinity of Silverton (AR3, AR4, LMC) exceed water quality criteria for protection of aquatic life and/or exceed levels shown to be toxic to sensitive taxa of invertebrates and fish in site-specific tests (Besser *et al.* 2000).

Concentrations of metals in fine streambed sediments of the upper Animas River and tributaries reflect the occurrence of metal-rich fine particles derived from historic mining and milling operations, deposition of hydrous metal oxides generated by acid drainage (Kimball *et al.* 1995), and variable retention of these particles in the stream bed (Church *et al.* 1997). Concentrations of extractable Fe in bed sediments were greatest in acid tributaries (CEM and UMC) and at sites immediately downstream (LMC and AR4), which experience large loadings of colloidal Fe (Table 3). Sediment Al concentrations were greatest at upstream sites in the Animas River (AR1-AR3). Greater concentrations of extractable Fe in sediments from most of the Animas River sites, relative to reference sites, reflected the widespread degradation of benthic habitats by embedded deposits of Fe oxide precipitates. Greatest concentrations of extractable Cd, Cu, Pb, and Zn occurred at Animas River sites upstream of Silverton, where the relatively broad valley floor led to development of milling facilities and where lesser stream gradients have allowed greater retention of fine sediments in alluvial deposits. Concentrations of these metals in sediments of acid tributaries were generally no greater than those in the reference tributaries, apparently reflecting reduced sorption to Fe- and Al-rich sediments under acidic conditions (Church *et al.* 1997). Sediments from Cascade Creek (CAS), located outside the most mineralized portion of the upper Animas watershed, had low concentrations of all metals studied.

## Methods

Samples of periphyton (considered here to be synonymous with *aufwuchs* or biofilm) were collected by scraping the surface of rocks in shallow near-shore areas with a polyethylene spatula. Periphyton samples were collected from all twelve study sites: eleven sites were sampled in October 1996, when access to the final site (AR5) was blocked by adverse weather conditions; and samples from AR5 and a second set of samples from AR4 were collected in September 1997. Six composite samples from each site were placed in acid-washed polyethylene vials and frozen on dry ice in the field. Four samples were analyzed for metals and the remaining two samples were split for determination of moisture content, chlorophyll-a, and total organic carbon (TOC). Samples for chlorophyll analysis were rinsed onto glass-fiber filters and extracted overnight in buffered ethanol. Chlorophyll concentrations in the extracts were determined by fluorometry (Turner Designs Model 10-AU) and corrected for pheophytin (APHA *et al.* 1989). Carbon analyses were conducted by coulometric titration of CO<sub>2</sub> (Coulometrics Model 5020), with total organic carbon (TOC) determined as the difference between total carbon (CO<sub>2</sub> released on combustion) and total inorganic carbon (CO<sub>2</sub> released on acidification).

Benthic invertebrates were collected with kick-nets (800 X 900  $\Phi$ m mesh) at eight of the twelve study sites. No invertebrate samples were collected at AR1, CEM, UMC, or LMC, where invertebrate populations were sparse or nonexistent. Samples from six of the eight sites were collected in October 1996. Samples from two sites in the Animas Canyon (AR4 and AR5),

which were inaccessible in 1996, were obtained from these sites in September 1997. Dominant taxa at each site were sorted into acid-washed polyethylene vials. Two to four composite samples per taxon, depending on the mass available, were immediately frozen on dry ice for metals analysis. Sample splits for each taxon were preserved with 70% ethanol for taxonomic identification. No attempt was made to eliminate the gut contents of invertebrates before analysis. The mayfly, *Rhithrogena* sp. (Heptageniidae), and the caddisfly, *Arctopsyche* sp. (Hydropsychidae), were collected from six sites; the stonefly, *Megarcys* sp. (Perlodidae) was collected from five sites; and another stonefly, *Zapada* sp. (Nemouridae) was collected from one site (AR2). Predominant species in these genera reported in recent surveys were *R. robusta*, *A. grandis*, *M. signata*, and *Z. oregonensis* (unpublished data; Robert Gallegos, Colorado Department of Public Health and Environment, Denver, Colorado, and David Gerhardt, U.S. Forest Service, Durango, Colorado). The accuracy of field identifications at the genus level, as confirmed by laboratory examinations of split samples, was 100 % for eight of nine samples (3 *Rhithrogena*, 3 *Megarcys*, 1 *Arctopsyche*, and 1 *Zapada*), and 90% accuracy for one sample of *Rhithrogena*, which contained individuals of another small mayfly, *Drunella doddsi* (Ephemereillidae).

Fish were collected in October 1996 with a backpack electrofishing unit (Smith-Root Inc., Vancouver, WA). Three brook trout of uniform size (200-250 mm total length), were selected from fish obtained at six sites (AR2, AR3, AR6, CUN, SMC, and CAS.). These fish were held on ice and taken to a field laboratory, where livers were dissected (within 8 hr of collection) and frozen prior to metals analyses.

Biological samples were prepared for analysis of metals by lyophilization, homogenization, and digestion by microwave with nitric acid and hydrogen peroxide. Metal determinations were performed by inductively-coupled plasma-mass spectroscopy (Perkin-Elmer/SCIEX Elan 6000; May *et al.* 1997). Fish and invertebrate samples were analyzed for arsenic (As), Cd, Cu, Pb, nickel (Ni), silver (Ag), and Zn. Periphyton samples were analyzed for these metals plus Al, Fe, and manganese (Mn). Concentrations of As, Ni, and Ag from biotic samples were typically below detection limits and are not reported here.

Quality assurance measures for metal analyses included blanks, reference tissues samples, matrix spikes, and replicate analyses. Recoveries of Cd, Cu, Mn, Pb, and Zn from standard reference materials (fish, invertebrate, and plant tissues) were within 10% of nominal for 90% of analyses. Recoveries of Al and Fe from reference tissues were consistently low (18-53%), due to incomplete solubilization of refractory forms of these metals and interferences in the mass spectra. However, recoveries of all metals from matrix spikes ranged from 82% to 120%. Repeated analyses for Al, Fe, Mn, Cd, Cu, and Zn resulted in relative percent differences less than 20% for 93% of pairs, whereas 67% of repeated analyses for Pb met this standard. Analysis of procedural (reagent) blanks indicated that blank contamination contributed very low levels of metals to tissue samples (blank-equivalent concentrations: Cd, Cu, Mn, and Pb, <0.1  $\Phi$ g/g; Al, <1.0  $\Phi$ g/g; Zn and Fe, <15  $\Phi$ g/g).

Data analyses were performed using the SAS statistical software program (SAS Institute 1990). Metal concentrations in periphyton, benthic invertebrates, and fish tissues were log-transformed before analysis to improve normality and homogeneity of variance. 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). T-tests indicated no significant differences between in metal concentrations between periphyton samples collected in 1996 and 1997 (station AR4), so samples of periphyton and

invertebrates collected in 1996 and 1997 were pooled for analysis of differences among locations. Associations among metal concentrations and other characteristics of sediment and periphyton were evaluated by Pearson correlation analysis. Statements of statistical significance indicate a probability of Type I error of 5% or less ( $p \leq 0.05$ ).

## Results and Discussion

### Metal concentrations in stream biota

Concentrations of metals in periphyton differed significantly among sites (Table 4). Greatest concentrations of Fe in periphyton occurred at acid sites (CEM and UMC), and greatest concentrations of Al occurred in periphyton from neutral-pH sites downstream of acid loadings (AR1, LMC; Fig. 2). Assuming that Fe and Al in periphyton occurred as hydrous oxides,  $\text{Fe}(\text{OH})_3$  and  $\text{Al}(\text{OH})_3$ , these minerals made up approximately 50% to 90% of periphyton dry mass at the most impacted sites, but only 3% to 10% at the reference sites. In contrast, greatest concentrations of Cd, Cu, Mn, Pb, and Zn in periphyton occurred in the reach upstream of Silverton (AR1-AR3). Periphyton samples from this reach contained Zn concentrations approaching 2% of dry mass. Copper concentrations in periphyton decreased gradually from upstream to downstream, whereas concentrations of other metals were consistently high upstream of Silverton and dropped off sharply downstream. Despite these decreases, metal concentrations in periphyton from downstream sites in the Animas River generally remained greater than concentrations in reference tributaries. Lowest metal concentrations in periphyton occurred in samples from acid tributaries (CEM and UMC). This trend, similar to that observed for sediment metals, may reflect low algal biomass in these samples and/or low metal bioavailability or under acid conditions.

Metals concentrations in periphyton samples can reflect uptake of dissolved metals, by bioaccumulation or sorption (Newman *et al* 1985), or trapping of metal-rich particles. The interpretation of influences on metal accumulation by periphyton was limited by the limited data available on metal concentrations in water and sediment in the study area, especially for the more remote study sites, and by the absence of data on metal concentrations in suspended solids (Tables 2 and 3). Metal concentrations in periphyton generally reflected metal concentrations in fine streambed sediments, as concentrations of all metals studied in periphyton, except Al, were significantly correlated with concentrations of these metals in sediments. However, these correlations generally explained little of the total variation in periphyton metal concentrations ( $r^2$  ranged from 0.36 for Cu to 0.56 for Mn). Concentrations of Fe in periphyton were more strongly correlated with total Fe in water ( $r^2=0.80$ ) than with dissolved Fe ( $r^2=0.52$ ), suggesting that Fe-rich suspended solids contributed substantially to Fe accumulation by periphyton. In contrast, concentrations of Zn and Cu in periphyton were not significantly correlated with total or dissolved concentrations of these metals in water.

Organic constituents of periphyton samples (algal biomass, estimated by chlorophyll-*a*; and total biomass, estimated by TOC) varied widely among sites (Fig. 2). Chlorophyll and TOC concentrations in periphyton from the upstream reach of the Animas River (AR1-AR3) were similar to those in reference tributaries. Periphyton biomass was lowest in samples from acid tributaries (CEM, UMC) and from sites most affected by deposition of Fe oxides (LMC, AR4-AR6). Chlorophyll-*a* and TOC had significant negative correlations with Fe concentrations in periphyton ( $r^2 = 0.40$  and  $0.35$ ) and with dissolved and total Fe concentrations in water ( $r^2$  from

0.69 to 0.80). Chlorophyll also had significant negative correlations with total and dissolved Cu ( $r^2 = 0.55$  and  $0.52$ ), reflecting the elevated concentrations of these metals in water from acid sites. These associations suggest that periphyton productivity was reduced at acid sites and in downstream mixing zones.

Variation in the abiotic components of periphyton samples, especially hydrous Fe oxides, may affect the concentrations and physicochemical forms of metals associated with these samples. Newman *et al.* (1985) suggested that sorption to hydrous metal oxides plays a dominant role in metal accumulation by periphyton. However, studies of sorption of metals by competing solid phases found that sorption of Cu and Cd by algal biomass was up to four times greater, per unit mass of sorbent, than sorption to Al, Fe, or Mn oxides (Calmano *et al.* 1988). In the current study, accumulation of Zn and Cd by periphyton was positively correlated with chlorophyll and TOC ( $r^2 = 0.36-0.46$ ), reflecting the greater metal accumulation by more productive periphyton communities at locations with neutral pH and relatively low loadings of hydrous Fe oxides (e.g., the Animas River above Silverton).

Concentrations of metals in benthic invertebrates differed significantly among taxa and among sites. Concentrations of Cd, Cu, Pb, and Zn differed significantly among invertebrate taxa at station AR2, the least impacted of the Animas River sites (Fig. 3). Concentrations of all four metals were significantly greater in the two smaller taxa (10-15 mm total length), the mayfly *Rhithrogena* and the stonefly *Zapada*, than in the larger taxa (30-50 mm TL), the stonefly *Megarcyus* and the caddisfly *Arctopsyche*. This contrast is consistent with previous reports that metal accumulation by invertebrates decreases with increasing body size (Smock 1983). However, differences in metal concentrations among taxa were not simply proportional to size. *Rhithrogena* accumulated greatest concentrations of Zn and Cd, whereas the similar-sized *Zapada* accumulated much lower concentrations of these metal, but much greater concentrations of Pb. Differences in metal concentrations between smaller and larger taxa were less pronounced for Cu, with smaller taxa accumulating Cu concentrations about three times as great as those in *Arctopsyche* and less than twice as great as those in *Megarcyus*.

The observed trends in metal concentrations among the four invertebrate taxa may be related to their different feeding habits. The grazer, *Rhithrogena*, feeds on periphyton scraped from the surfaces of rocks; the detritivore, *Zapada*, feeds on fine particulate organic matter; the omnivore *Arctopsyche* feeds on detritus and small invertebrates trapped by its coarse silk mesh; and the predator, *Megarcyus*, feeds on other invertebrates (Wiggins 1977, Merritt and Cummins 1989). Greater accumulation of Zn and Cd by *Rhithrogena* reflects the proportionally greater enrichment of these metals in periphyton relative to sediments. Studies of other streams affected by mining have also found that grazers accumulated high concentrations of these metals. In the Arkansas River, Colorado, another small, grazing mayfly (*Baetis* sp.) accumulated significantly greater concentrations of Zn and Cd than *Arctopsyche* or a predaceous stonefly, *Skwala* sp. (Kiffney and Clements 1993). In the Coeur d'Alene River, Idaho, herbivorous taxa also accumulated significantly greater concentrations of most metals, including Zn and Cd, than other invertebrates, but Cu concentrations did not differ between herbivores and predators (Farag *et al.* 1998). The relatively high concentrations of Cu in *Megarcyus* and *Arctopsyche* from the Animas River also suggest that Cu was efficiently accumulated from diets containing invertebrate tissue. The accumulation of high concentrations of Pb by *Zapada* is consistent with a previous report of preferential accumulation of Pb by invertebrate detritivores (Cain *et al.* 1992). Although sediment samples do not adequately reflect the diet of *Zapada*, Pb was more enriched in sediment samples (relative to periphyton) than other metals and Pb has a greater

affinity than Zn or Cd for sorption to soil or organic detritus (Elliott *et al.* 1986).

Metal concentrations in invertebrates differed significantly among sites, although these comparisons were limited by the absence of one or more invertebrate taxa from some study sites (Table 5). Lowest concentrations of all four metals studied in *Rhithrogena*, *Arctopsyche*, and *Megarcys* occurred at CAS, the reference site outside of the highly-mineralized portion of the watershed. However, metal concentrations in invertebrates from the other reference sites (SMC and CUN) were not consistently less than those from intermediate-impact sites in the upper Animas River. Greatest concentrations of Cd in *Rhithrogena* and *Megarcys* occurred at SMC and CUN, respectively. Of the metals studied, only Cu concentrations were consistently greater in invertebrates from intermediate- and high-impact sites in the Animas River relative to reference sites.

Metal concentrations in invertebrates were used to indicate differences in overall metal bioavailability (aqueous plus dietary) among the Animas River sites. Concentrations of Cu, Pb, and Zn in *Rhithrogena* and *Megarcys* were generally greatest at upstream sites in the Animas River (AR2, AR3), consistent with trends in concentrations of these metals in periphyton. However, each of these taxa occurred at only one of the three downstream sites. *Arctopsyche*, which occurred at all Animas River sites except the headwater site (AR1), allowed the best comparison of longitudinal trends in metal bioavailability. Concentrations of Cu in *Arctopsyche* were significantly greater at sites AR4 and AR5, downstream of Cement and Mineral Creek, than at upstream sites. In contrast, concentrations of Zn, Pb, and Cd in *Arctopsyche* were generally lowest at AR4, and otherwise showed few consistent differences between sites upstream and downstream of the two major tributaries. These results suggest that bioavailability of Cu in the Animas River was greatest at AR4, which is located in the mixing zone downstream of the greatest tributary metal loadings and which exhibits the greatest impacts on stream biota. Due to its wide distribution and tolerance of elevated metal burdens, *Arctopsyche grandis* has been widely used to monitor metal bioavailability in streams of the western U.S. (Cain *et al.* 1992, Kiffney and Clements 1993, Farag *et al.* 1998). Conclusions about metal bioaccumulation by *Arctopsyche* at sites AR4 and AR5 must consider the possibility that metal bioavailability differed between 1997, when these samples were collected, and 1996, when this taxon was collected at other sites. However, metal concentrations in periphyton from AR4 did not differ significantly between these two years, and water samples collected monthly at the USGS gaging station at AR4 also did not indicate substantial annual differences in dissolved metal concentrations (K. Leib, USGS, Durango, CO; pers. commun.).

Concentrations several metals in liver tissue of brook trout differed widely among three sites on the upper Animas River and three reference sites. Concentrations of Pb, Zn, and Cu in liver tissue differed significantly among sites (Fig. 4), although Pb concentrations did not differ significantly between intermediate-impact sites in the Animas River and the reference tributaries (except CAS). Concentrations of Zn and Cu in livers of brook trout from the Animas River sites corresponded to general longitudinal trends in impacts on stream biota: lowest concentrations at AR2, the site with the greatest density of trout and invertebrates; greatest concentrations at Silverton (AR3), reflecting increased metal loadings from deposits of mine wastes; and decreased concentrations in the zone of recovery in the lower Animas Canyon (AR6). Differences among sites were more pronounced for Cu, as trout from two Animas River sites (AR3 and AR6) contained significantly greater liver Cu concentrations than fish from the reference sites, whereas only trout from AR3 contained greater liver Zn concentrations than the reference sites. Concentrations of Cd in trout livers were relatively high throughout the



watershed, but did not differ significantly among sites. Previous studies have reported similar elevated concentrations of Cd, Cu, and Zn in livers of trout from metal-contaminated sites (Wilson *et al.* 1980, Moore *et al.* 1991, Farag *et al.* 1995).

### Food-web transfer of metals

Bioaccumulation factors (BAFs), defined here as ratios of metal concentrations between consumers and diets, may provide useful information about differences in trophic transfer of metals among locations, among metals, and among trophic links. BAFs for clearly defined trophic links (e.g., from periphyton to invertebrate grazers or from benthic invertebrates to brook trout) reflect variation in the contribution of dietary metals, but not aqueous metals, to total metal bioaccumulation. However, comparison of BAFs for different trophic links across the same set of sites normalizes the contribution of aqueous metal exposure, which can be assumed to be similar for different taxa at the same location. Interpretation of BAFs must consider sources of variation in metal concentrations in both consumers and their diet. BAFs for the same trophic link may vary among sites due to variation in the bioavailability of dietary metals. For example, BAFs for uptake of Cd, Cu, Pb, and Zn from periphyton by the grazer *Rhithrogena* were lower for sites in the Animas River than for tributary sites (data not shown), reflecting significant negative correlations between BAFs and metal concentrations in periphyton. These trends suggest lesser bioavailability of metals from diets of highly-contaminated periphyton, perhaps due to influences of other periphyton characteristics such as greater proportions of Fe and Al oxides. BAFs for transfer between invertebrate taxa (*Rhithrogena* to *Megarcys*) and between invertebrates and fish were more consistent across sites.

BAFs for the four metals studied followed similar trends across different trophic links (Table 6). High BAFs (>1.0) for uptake of Cd by *Rhithrogena* from periphyton and for Cd uptake by brook trout from invertebrates both suggest that Cd is highly bioavailable in the stream food web. BAFs suggest that periphyton Cu was less readily accumulated by *Rhithrogena*, but that Cu accumulated by *Rhithrogena* was available to the invertebrate predator, *Megarcys*, and to brook trout. In contrast, BAFs suggest that Zn associated with periphyton was accumulated efficiently by *Rhithrogena*, but was transferred less efficiently to invertebrate predators and brook trout. BAFs for Pb were lower than those for other metals for three of the four trophic links, suggesting that Pb was the least biologically available of the metals studied. These trends suggest that exposure pathways for stream biota differed among different metals. Despite low concentrations in sediment and water, Cd became relatively bioavailable by accumulation in periphyton and efficient trophic transfer to invertebrates and fish. The occurrence of elevated Cd concentrations in biota at two of the three reference sites and at the 'recovery' site downstream in the Animas canyon, far from sources of Cd loading, is consistent with the previous finding that Cd contamination of stream food chains persisted further downstream than other metals (Moore *et al.* 1991). In contrast, Pb concentrations in periphyton did not reflect high Pb concentrations in sediment, and Pb in tissues of stream biota was not efficiently transferred to higher order consumers. High concentrations of Zn occurred in sediment, water, and stream biota of the upper Animas, but Zn concentrations in biota were consistently lower at higher trophic levels. Concentrations of Cu were generally lower than concentrations of Pb or Zn in sediment and periphyton, but Cu concentrations were greater than Pb concentrations in invertebrates and greater than concentrations of both Pb and Zn in trout livers.

The shift in the relative concentrations of metals in livers of brook trout (Cu > Zn > Cd >

Pb), compared to benthic invertebrates and periphyton ( $Zn > Cu > Pb > Cd$ ), could reflect differential accumulation of metals among trout tissues as well as differential trophic transfer. Trout from metal-contaminated habitats were found to accumulate greater concentrations of Zn, Cd, and especially Cu in liver tissues than in skeletal muscle or whole-body samples (Wilson *et al.* 1980, Farag *et al.* 1995). Concentrations of Cu in liver tissues of rainbow trout were not regulated by rainbow trout during laboratory exposures to Cu in water or diets (Miller *et al.* 1993, Lanno *et al.* 1985), whereas internal Zn concentrations were partially regulated by rainbow trout during exposures to waterborne and dietary Zn (Spry *et al.* 1988). BAFs calculated based on metal concentrations in trout livers probably overestimate the efficiency of trophic transfer for Cu, and possibly Cd, although this bias would not affect BAFs for transfer between lower trophic levels.

### **Metal hazards to brook trout**

The uneven distribution and abundance of brook trout in the upper Animas watershed and the near absence of other trout species suggest that trout populations at sites heavily impacted by acid drainage are limited by toxic effects of metals and associated habitat degradation. Cutthroat trout are largely restricted to high-elevation streams that are least affected by mining or natural acid drainage, and rainbow trout occur in significant numbers only at the furthest downstream study site (pers. commun.; M. Japhet, Colorado Division of Wildlife, Durango Colo). This pattern suggests that these species cannot tolerate the levels of metal exposure that are typical of much of the upper Animas River watershed, although the distribution of these species may also be influenced by differences in habitat preferences among species and by competition with brook trout. Although brook trout are widely distributed in the watershed, this distribution is discontinuous and population densities vary (Table 1). The absence of brook trout at high-impact sites such as AR4 and LMC may reflect direct toxicity of aqueous Cu, as dissolved Cu concentrations at these sites exceed chronic toxicity thresholds for brook trout during winter low-flow periods (Besser *et al.* 2000). At sites with lower aqueous metal concentrations, brook trout may experience levels of metal exposure associated with chronic toxicity via combined aqueous and dietary exposure. These hazards can be estimated by evaluation of metal exposure via the available invertebrate diets and by metal bioaccumulation in trout tissues.

Concentrations of Cu and other metals in invertebrate diets from the upper Animas River may approach levels associated with dietary toxicity in trout. Average Cu concentrations in available invertebrate diets, estimated by means of all taxa collected at each site, were substantially greater at Animas River sites with reduced densities of brook trout (104-157  $\mu\text{g/g}$  at AR3-AR5) than at reference sites (22-45  $\mu\text{g/g}$  at CUN, SMC, and CAS). In contrast, mean concentrations of Zn and Pb in invertebrates from these Animas River sites overlapped broadly with those in reference sites. There are no published studies of dietary toxicity thresholds for metals in brook trout, although toxic effects of metal-contaminated diets has been reported for other salmonid species. Woodward *et al.* (1994, 1995) and Farag *et al.* (1999) found that diets of metal-contaminated invertebrates collected from the Clark Fork River, Montana, and the Coeur d'Alene River, Idaho, caused reduced survival and/or growth of brown, rainbow, and cutthroat trout. Woodward *et al.* (1995) found reduced growth of brown trout and rainbow trout fed diets of invertebrates from the Clark Fork River containing Cu concentrations as low as 176  $\mu\text{g Cu/g}$ , similar to the highest mean concentrations at sites in the upper Animas River. However, diets in these studies contained different proportions of toxic metals than those occurring in Animas invertebrates: greater Cu and lower Zn, Cd, and Pb in the Clark Fork diets; and greater Pb and

lower Cu and Cd in the Coeur d'Alene diet. Studies with diets spiked with metals in the laboratory suggest lesser hazards of dietary metal toxicity. Reduced growth of rainbow trout fed a Cu-spiked formulated diet (Lanno et al 1985) occurred at dietary Cu concentrations (664-730  $\mu\text{g/g}$ ) greater than the most contaminated Clark Fork diet and almost four times greater than the greatest average Cu concentrations in invertebrates from the Animas River. Another study found no toxic effects in rainbow trout a live diet dosed with a mixture of metals based on metal concentrations in invertebrates from the Clark Fork River (250  $\mu\text{g Cu/g}$ ), or with greater concentrations of individual metals (up to 1000  $\mu\text{g Cu/g}$ ; Mount *et al.* 1994). These contradictory results, which may be related to differences in nutritional characteristics or metal bioavailability (Cain *et al.* 2000) between field-collected and formulated diets, limit the assessment of hazards of metal toxicity in the upper Animas watershed based solely on metal concentrations in available invertebrate diets.

Concentrations of Cu in liver tissues of brook trout, which reflect chronic metal exposure via both diet and water, provide more direct evidence of hazards of chronic Cu toxicity (Table 7). Brook trout from impacted sites in the upper Animas (AR3, AR6) had liver Cu concentrations greater than those reported from a life-cycle study conducted at 'no-observed-effect' concentrations of 9.4  $\mu\text{g/L}$  in water (McKim and Benoit 1974). Unfortunately, no liver concentrations were reported from brook trout exposed at 17  $\mu\text{g Cu/L}$ , the lowest aqueous concentration that caused reduced survival and growth of early life stages (McKim and Benoit 1971). Concentrations of Cu in livers of brook trout from Animas sites were within the range reported for moderately impacted (approx. 100 fish/km) to 'depauperate' brook trout populations in the upper Blackfoot River, Montana (Moore *et al.* 1991) and were similar to or greater than liver Cu concentrations associated with reduced growth and survival of rainbow trout in laboratory dietary exposures (Lanno *et al.* 1985). Studies of brown trout from the Clark Fork and Blackfoot Rivers suggest that this species can tolerate liver Cu concentrations similar to those measured in brook trout from the Animas and Blackfoot Rivers (Moore *et al.* 1991, Farag *et al.* 1995). These comparisons suggest that brook trout are less sensitive to chronic Cu toxicity than rainbow trout (and, presumably, the closely-related cutthroat trout) and about equally sensitive to brown trout. The reduced abundance of brook trout at sites where with liver Cu concentrations approach levels associated with toxicity (AR3, AR6), and the absence or greatly reduced populations of brook trout at sites where water and invertebrate diets contained greatest Cu concentrations (AR4 and AR5), support the hypothesis that chronic Cu toxicity is an important factor controlling the distribution and abundance of brook trout in the upper Animas River watershed.

## Conclusions

Metals derived from acid drainage from mines, mine wastes, and naturally acidic rocks and soils in the upper Animas River watershed accumulated to high concentrations in tissues of stream biota upper level consumers. Periphyton or *aufwuchs* from streams in the upper Animas watershed contained large, but variable, percentages of Fe and Al oxides and high concentrations of Zn, Cu, Pb, and Cd. High concentrations of Fe and Al in periphyton were associated with reduced algal biomass, suggesting that degradation of benthic habitats by deposition of Fe and Al oxides could cause reduced primary production and reduced food base for benthic invertebrates and fish. Metals associated with periphyton were biologically available, as evidenced by

accumulation of high concentrations of metals by grazing invertebrates. Zinc occurred at greater concentrations than other toxic metals in water, sediment, periphyton, and invertebrates, but Cu and Cd were more efficiently transferred via the food web, resulting in proportionately greater exposure of brook trout to these metals via contaminated diets. Greatest concentrations of Cu, but not other metals, in benthic invertebrates and in livers of brook trout occurred at sites with greatest impacts on benthic invertebrate communities and brook trout populations.

Concentrations of Cu in liver tissue of brook trout from the upper Animas River approached concentrations associated with adverse effects on populations of wild brook trout and with toxicity to other salmonids in laboratory studies. These results suggest that chronic toxicity of Cu is an important factor limiting the distribution and abundance of brook trout in the upper Animas River watershed. The effects of Cu 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 upper Animas watershed. Based on these findings, management efforts focused on expanding the range and increasing the population density of trout in the upper Animas River watershed should select techniques and locations remediation that will result in greatest reductions in Cu loadings.

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Table 1. Characteristics of benthic invertebrate communities and brook trout populations at sites sampled for tissue metals analysis, upper Animas River and tributaries, Colorado. Sources: unpublished data from Colorado Department of Public Health and Environment, Denver, Colo. (1992 data); U.S. Forest Service, Durango Colo. (1997 invertebrate data); and Colorado Division of Wildlife, Durango Colo (1997-98 trout data).

Location (Site ID)	Elev. (M)	Benthic Invertebrates				Brook Trout	
		(taxa)		(no./m <sup>2</sup> )		(no./300M)	
		1992	1997	1992	1997	1992	'97-98
<u>Upper Animas River</u>							
Above Eureka (AR1) <sup>a</sup>	3006	6	1.5	12	23	-- <sup>b</sup>	--
Above Howardsville (AR2)	2945	12	11	163	575	134	327
Above Silverton (AR3) <sup>c</sup>	2854	14	3.3	171	98	21	1
Below Silverton (AR4) <sup>a</sup>	2805	9	2.7	20	97	0	0
Elk Park (AR5)	2707	12	5.3	84	309	13	23
Needleton (AR6) <sup>c</sup>	2500	--	6.3	--	185	73	102
<u>'Reference' tributaries</u>							
Cunningham Cr. (CUN)	3012	16	16.5	1305	2255	--	--
South Mineral Cr. (SMC)	2988	20	15	1090	2769	80	--
Cascade Cr.	2670	--	--	--	--	--	--
<u>Impacted tributaries</u>							
Cement Cr. (CEM)	2835	1	0	3	0	0	--
Upper Mineral Cr. (UMC)	2903	--	--	--	--	--	--
Lower Mineral Cr. (LMC)	2817	3	1.8	4	24	0	--

<sup>a</sup> High-impact sites

<sup>b</sup> Dashed line indicates no data available.

<sup>c</sup> Fish sampled 2 km upstream of AR3 and 5 km downstream of AR6.

Table 2. Concentrations of dissolved (<0.45  $\Phi$ m) and total metals in stream water ( $\Phi$ g/L) from the Animas River and tributaries during fall low-flow conditions, 1995-1998. Sources: Church et al . (1997); Besser *et al.* (2000); U.S. Forest Service, Durango, Colo., unpublished data.

Site	N <sup>a</sup>	Sample	Cd	Pb	Cu	Zn	Fe
AR1	3/2	<u>dissolved</u>	<u>1.3</u>	<u>0.3</u>	<u>11</u>	<u>368</u>	<u>248</u>
		total	1.2	0.9	16	232	526
AR3	3/2	<u>dissolved</u>	<u>1.1</u>	<u>&lt;0.7<sup>b</sup></u>	<u>6</u>	<u>360</u>	<u>31</u>
		total	1.2	<1.8	8	375	169
AR4	3/2	<u>dissolved</u>	<u>1.2</u>	<u>&lt;0.1</u>	<u>5</u>	<u>324</u>	<u>826</u>
		total	1.0	3.9	19	367	2081
AR5	2/2	<u>dissolved</u>	<u>0.9</u>	<u>&lt;0.3</u>	<u>8</u>	<u>302</u>	<u>512</u>
		total	1.0	5.3	22	356	1975
AR6	1/1	<u>dissolved</u>	<u>0.8</u>	<u>3.9</u>	<u>16</u>	<u>335</u>	<u>135</u>
		total	0.8	nd <sup>c</sup>	4	576	1246
-----							
CEM	2/1	<u>dissolved</u>	<u>2.5</u>	<u>6.9</u>	<u>50</u>	<u>1146</u>	<u>4425</u>
		total	2.6	8.6	54	1820	6577
LMC.	3/2	<u>dissolved</u>	<u>0.9</u>	<u>&lt;0.1</u>	<u>9</u>	<u>296</u>	<u>1747</u>
		total	0.8	6.2	47	308	3790
-----							
CUN	1/1	<u>dissolved</u>	<u>0.1</u>	<u>nd</u>	<u>1</u>	<u>nd</u>	<u>nd</u>
		total	0.1	nd	1	30	nd
SMC.	2/2	<u>dissolved</u>	<u>&lt;0.1</u>	<u>&lt;0.2</u>	<u>3</u>	<u>38</u>	<u>124</u>
		total	<0.1	<1.8	3	28	284

<sup>a</sup> First number refers to analyses for Cu, Zn, and Fe; second number refers to Cd and Pb

<sup>b</sup> '<' indicates values less than the limit of detection (LOD) were estimated as LOD/2

<sup>c</sup> 'nd' indicates no values greater than detection limits



Table 3. Concentrations of ‘weakly-bound’ metals in sediments (2M HCl-1% H<sub>2</sub>O<sub>2</sub> leach) from the upper Animas River and tributaries, 1995-97. Sources: Church *et al.* (1997); Besser *et al.* (2000).

Site	N	Metal concentration (mg/kg dry weight)						
		Al	Fe	Mn	Cd	Cu	Pb	Zn
AR1	1	8300	27000	12000	5	272	1220	1400
AR2	1	7200	29000	9800	3	183	1210	905
AR3	2	5440	19400	9700	9	273	1820	2020
AR4	2	5250	28000	4660	3	210	990	733
AR5	1	4380	21800	3880	4	174	725	830
AR6	1	3280	8870	402	0.5	18.5	68.5	166
CEM	2	4300	94800	417	<2	63	150	200
UMC	1	4900	37000	1100	<3	84	460	120
LMC	2	5990	29300	1130	<1	180	220	385
CUN	1	5900	16000	1200	<2	145	370	485
SMC	1	3900	8700	1420	2	21	246	531
CAS	1	2000	5100	460	<1	5	6	47

Table 4. Concentrations of metals in periphyton ( $\Phi$ g/g dry wt.) from the upper Animas River and tributaries, collected in fall 1996 and 1997. Means of 3-7 samples analyses (with standard errors). For each metal, means followed by the same letter are not significantly different (ANOVA/Duncan's test).

Site	Cd	Pb	Cu	Zn	Mn
<u>Animas River:</u>					
AR1	33.3 (0.5) a	1051 (40) abc	6587 (371) a	18950 (743) a	4363 (147) ab
AR2	25.3 (1.3) a	2023 (33) a	1031 (64) bc	8520 (465) ab	6970 (25) a
AR3	27.8 (1.8) a	1597 (24) ab	952 (45) bc	11903 (314) a	7053 (370) a
AR4	5.6 (0.4) b	680 (80) abc	764 (54) bc	2179 (124) cd	1683 (66) bcd
AR5	6.1 (0.3) b	465 (26) abc	362 (36) c	2656 (272) cd	1140 (85) bcd
AR6	9.5 (0.6) b	480 (9) abc	709 (72) bc	3673 (243) bc	2526 (46) abc
<u>Impacted tributaries:</u>					
CEM	0.2 (0.04) e	207 (9) c	31 (1) e	44 (8) e	123 (22) e
UMC	0.2 (0.1) e	753 (54) abc	105 (7) d	40 (13) e	56 (21) e
LMC	5.2 (0.9) b	605 (150) abc	1571 (296) b	1135 (3830) e	975 (476) cd
<u>'Reference' tributaries:</u>					
CUN	2.7 (1.4) c	266 (265) d	40 (36) f	338 (300) e	591 (555) e
SMC	6.7 (0.3) b	276 (5) bc	89 (5) d	1783 (87) cd	2517 (163) abc
CAS	0.4 (0.01) d	9 (0.5) d	9 (1) f	84 (6) e	504 (16) d

Table 5. Concentration of metals in benthic invertebrates from the Animas River and tributaries. Means (with standard error) of 2-4 replicates per site. For each species and metal, means followed by the same letter are not significantly different (ANOVA/Duncan's test).

Taxon	Site	Metal Concentration (ug/g dry wt.)											
		Cd		Cu		Pb		Zn					
<i>Rhithrogena</i> (mayfly)	AR2	18.3	(0.5)	d	214	(6)	a	129	(20)	a	3487	(98)	b
	AR3	29.0	(1.1)	b	171	(8)	b	44	(5)	b	5030	(125)	a
	AR6	23.9	(0.2)	c	181	(4)	b	28	(3)	c	2830	(113)	d
	CUN	25.3	(0.7)	c	47	(0.5)	c	60	(3)	b	2130	(320)	e
	SMC	34.4	(0.6)	a	34	(0.8)	d	10	(1.3)	d	3143	(44)	c
	CAS	6.1	(0.3)	e	19	(0.4)	e	2.0	(0.2)	e	476	(11)	f
<i>Arctopsyche</i> (caddisfly)	AR2	1.2	(0.2)	ab	61	(4)	c	49	(9)	a	317	(35)	b
	AR3	1.3	(0.2)	ab	66	(2)	c	33	(2)	ab	396	(23)	a
	AR4	1.0	(0.1)	b	104	(7)	ab	9	(1)	c	256	(18)	b
	AR5	2.0	(0.0)	a	119	(4)	a	19	(1)	b	488	(250)	a
	AR6	1.9	(0.2)	a	97	(4)	b	37	(18)	ab	489	(340)	a
	CAS	0.4	(0.1)	c	10	(0.1)	d	0.8	(0.0)	d	152	(50)	c
<i>Megarcys</i> (stonefly)	AR2	3.0	(0.1)	b	124	(8)	b	56	(6)	b	510	(20)	b
	AR3	4.1	(0.4)	b	232	(34)	a	125	(14)	a	1213	(113)	a
	AR5	1.7	(0.4)	c	140	(1)	b	41	(9)	b	404	(11)	b
	CUN	7.3	(0.4)	a	45	(1)	c	56	(9)	b	458	(34)	b
	CAS	0.8	(0.1)	d	26	(2)	c	0.8	(0.0)	c	301	(18)	c
<i>Zapada</i> (stonefly)	AR2	6.4	(0.3)		203	(11)		228	(22)		984	(69)	

Table 6. Trophic bioaccumulation factors (BAF=ratio of metal concentration in consumer to concentration in diet) for metals in aquatic organisms from the upper Animas River and tributaries. Means, with range among sites in parentheses; N = number of sites.

Trophic Link	N	BAF			
		Cd	Cu	Pb	Zn
Periphyton to <i>Rhithrogena</i>	6	5.7 (1.0 - 15)	0.72 (0.2 - 2.1)	0.11 (0.03 - 0.22)	2.6 (0.4 - 6.3)
<i>Rhithrogena</i> to <i>Megarcys</i>	4	0.18 (0.13 - 0.29)	1.2 (1.0 - 1.9)	1.2 (0.4 - 2.8)	0.31 (0.15 - 0.63)
Invertebrates to trout (liver)	6	3.4 (0.5 - 13)	4.8 (2.5 - 8.8)	0.06 (0.02 - 0.12)	0.33 (0.05 - 0.7)
Periphyton to trout (liver)	6	4.8 (0.3 - 18)	4.6 (0.4 - 21)	0.005 (<0.01)	0.54 (0.03 - 2.5)

Table 7. Metal concentrations in liver tissues from affected populations of wild trout or trout exposed to metals in laboratory studies.

<u>Species</u>	Liver Cu ( $\Phi$ g/g dry wt.)	Effect type	Source	
Location or Exposure	no effect	effect		
<u>Brook Trout</u>				
Animas R., Colorado	131-350	628-788	reduced population density	This study
Blackfoot R., Montana	--	182-1113	reduced population density	Moore <i>et al.</i> 1991
Water (adults, 720-d)	208-239	--	no effect	McKim and Benoit 1974
<u>Rainbow trout</u>				
Water (adults, 360-d)	70-231	--	normal variation	Olsson <i>et al.</i> 1987
Diet (juveniles, 56-d)	102	177-637	reduced growth and survival	Lanno <i>et al.</i> 1985
Diet (juveniles, 168 d)	329	--	no effect	Lanno <i>et al.</i> 1985
<u>Brown trout</u>				
Clark Fork R., Montana	759	1079-2399	physiological impairment	Farag <i>et al.</i> 1995
Blackfoot R., Montana	494-846	--	no effect	Moore <i>et al.</i> 1991

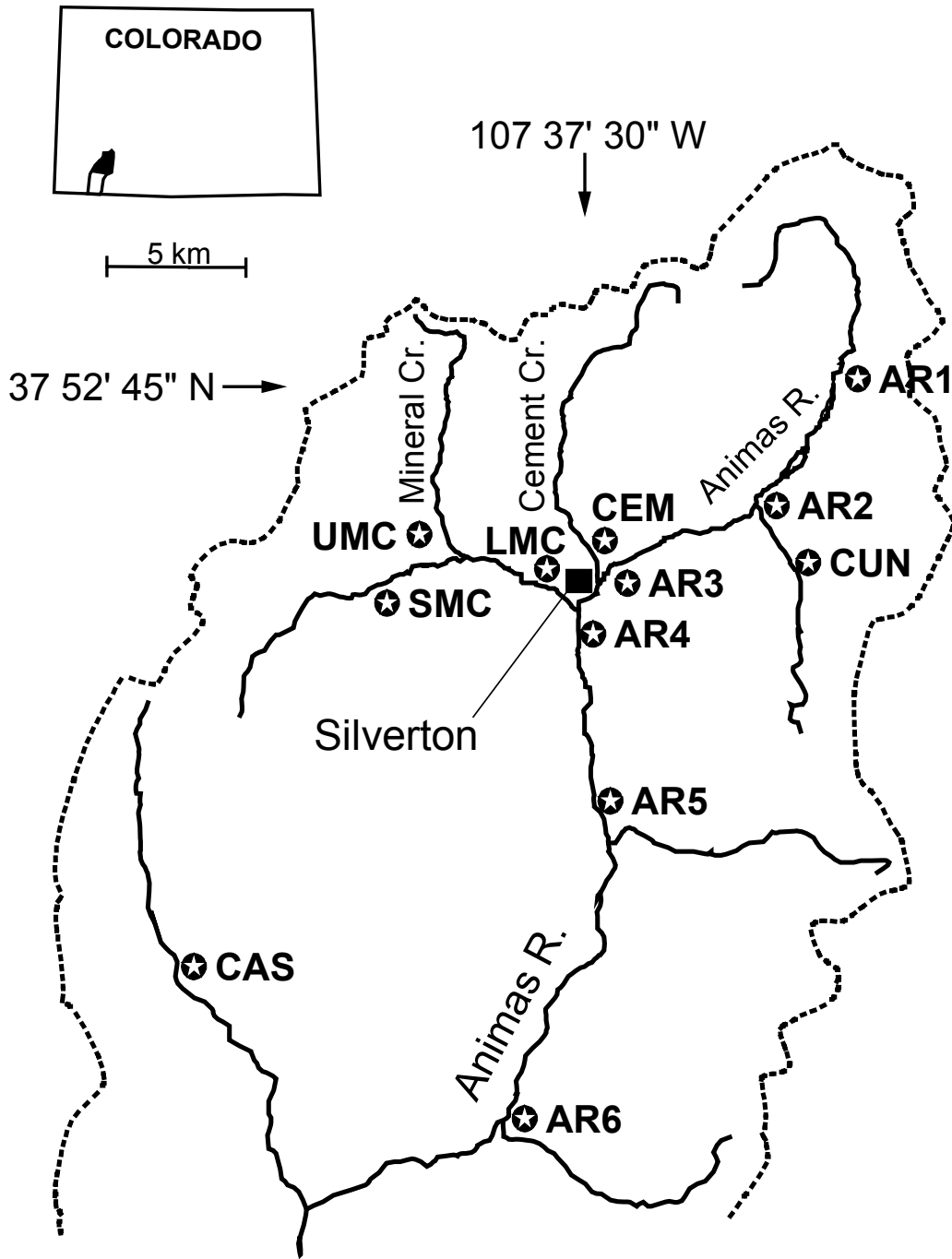


Fig. 1. Locations of sampling sites in the Upper Animas River watershed, Colorado USA.

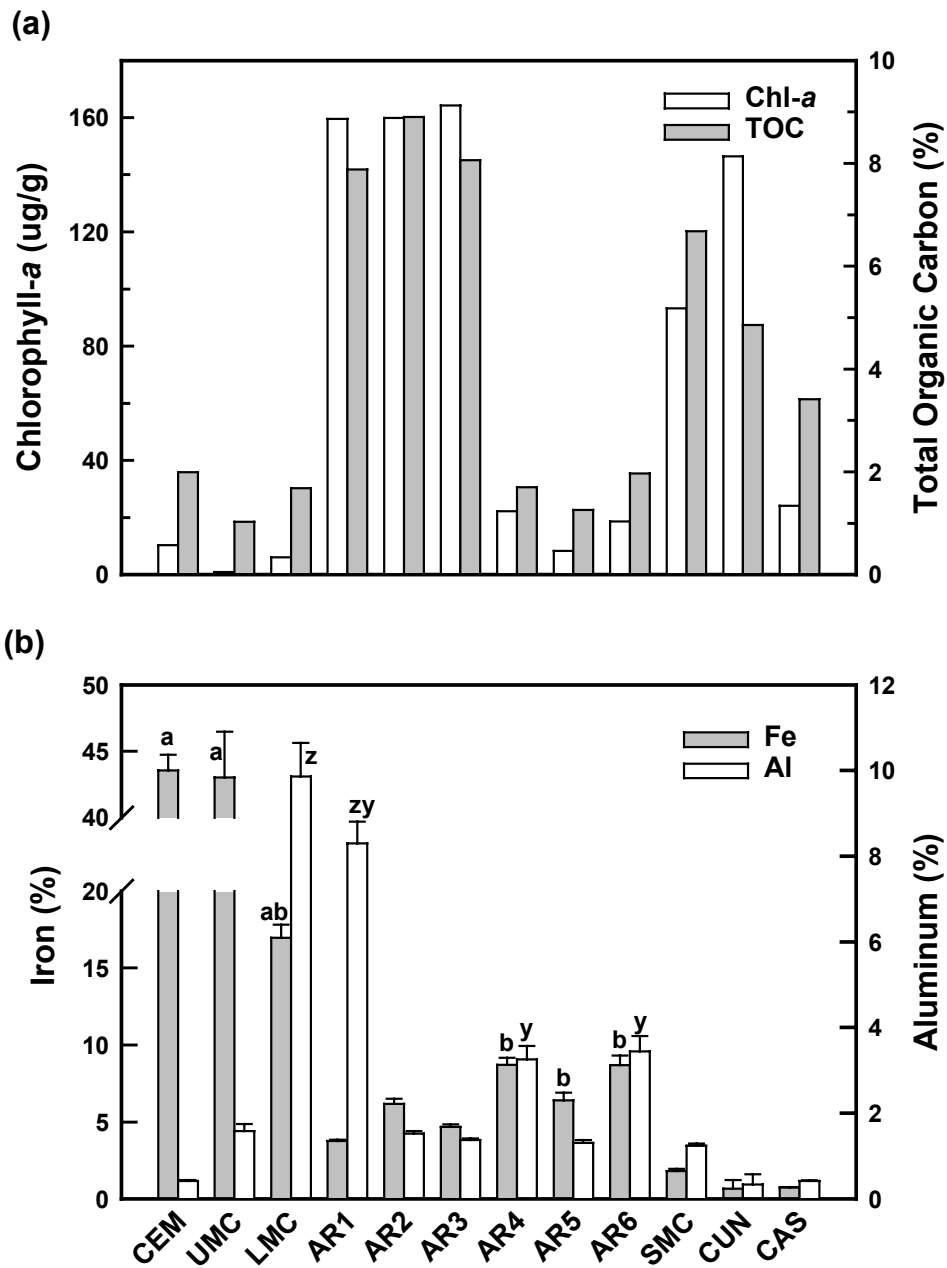


Fig. 2. Characteristics of periphyton collected from the upper Animas River and tributaries. (a) Chlorophyll-*a* and total organic carbon; means, N=2. (b) Aluminum and iron; mean and SEM, N=4. For Fe and Al, bars for each metal labeled with the same letter are not significantly different (ANOVA/Duncan's test); letters are omitted from some bars for clarity.

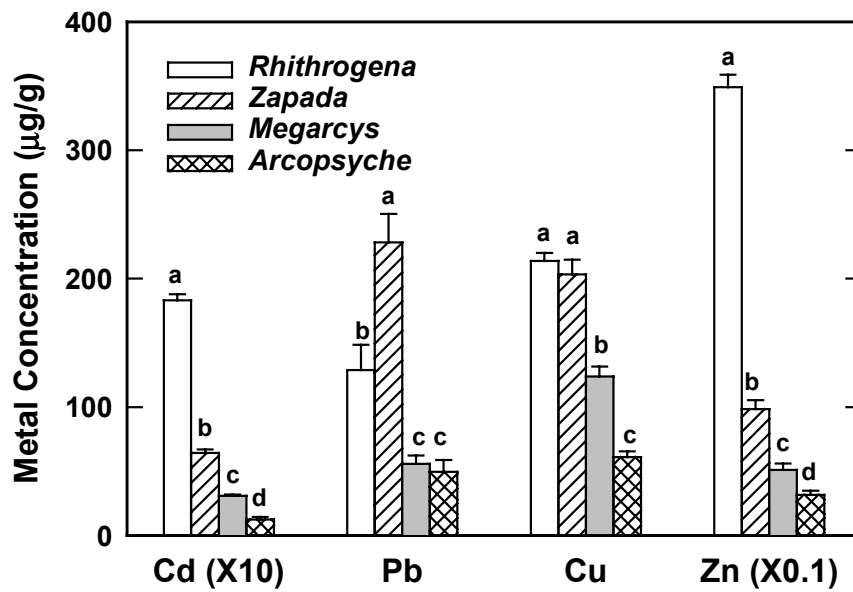


Fig. 3. Concentrations of metals in four taxa of macroinvertebrates from the upper Animas River near Howardsville (AR2). Mean and standard error of measurements in composite samples, N=3. Letters indicate significant differences among taxa. For each metal, bars with the same letter are not significantly different (ANOVA/Duncan's test).



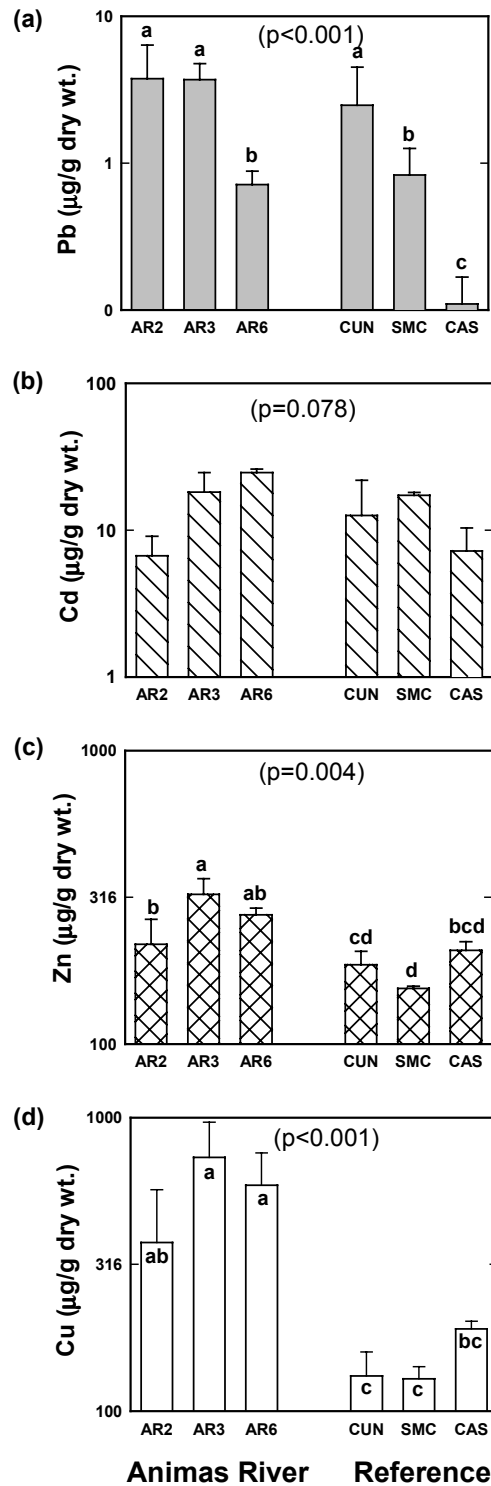


Fig. 4. Concentrations of metals (log scale) in livers of brook trout, *Salvelinus fontinalis*, from the upper Animas River and reference sites: (a) lead; (b) cadmium; (c) zinc; and (d) copper. Mean concentrations with standard errors, plotted on logarithmic axes. For each metal, means with same letters are not significantly different (ANOVA/Duncan's test).