Evaluation of Metal Toxicity in Streams Affected by Abandoned Mine Lands, Upper Animas River Watershed, Colorado

Biological Science Report
USGS/BRD/BSR 2001-0001
May 2001

by

John M. Besser
Ann L. Allert
Douglas Hardesty
Christopher G. Ingersoll
Thomas W. May
Ning Wang[1]
Kenneth J. Leib[2]

U.S. Geological Survey
Biological Resources Division
Columbia Environmental Research Center
Columbia, Missouri

[1]University of Missouri-Columbia
Department of Fisheries and Wildlife Science
Columbia Missouri

[2]U.S. Geological Survey
Water Resources Division
Grand Junction CO

U.S. Department of the Interior
U.S. Geological Survey

science for a changing world

Table of Contents

List of Tables

List of Figures

Acknowledgments

CERC personnel. Susan Finger helped coordinate our efforts with those of researchers in other USGS divisions, through her participation in the AMLI watershed team. Bill Brumbaugh and Steve Olson provided valuable assistance with field work in 1997 and 1998, respectively. Eric Brunson, James Kunz, and David Whites provided technical assistance with both field and lab work in 1998 and 1999. Heather Willman and La Shawn Williams provided additional assistance with laboratory studies in 1999. Eugene Greer provided expert assistance with handling of brook trout embryos and fry.

USGS cooperators. Jon Evans (WRD-Durango, CO) collected samples of stream water for toxicity testing in April 1999. Win Wright (WRD-Durango) and Paul VonGuerard (WRD-Grand Junction, CO) provided logistical assistance during field sampling in 1997 and on-site toxicity testing in 1998. Del Nimmo (BRD-MESC, Ft. Collins, CO) and Janie Castle (Colorado State University, Ft. Collins, CO) provided information on preliminary toxicity testing conducted in 1997.

Other cooperators. Bill Simon (Animas River Stakeholders Group; ARSG) helped coordinate our efforts with other ongoing studies in the Upper Animas River watershed. Dave Gerhardt (U.S. Forest Service, Durango, CO) and Chester Anderson (B.U.G.S. Consulting, Choctaw, OK) provided data from 1996-97 ARSG biomonitoring study.

Disclaimer. Use of trade names does not constitute an endorsement by the USGS

Summary

Acid drainage from abandoned mines and from naturally-acidic rocks and soil in the upper Animas River watershed of Colorado generates elevated concentrations of acidity and dissolved metals in stream waters and deposition of metal-contaminated particulates in stream-bed sediments, resulting in both toxicity and habitat degradation for stream biota. High concentrations of iron (Fe), aluminum (Al), zinc (Zn), copper (Cu), cadmium (Cd), and lead (Pb) occur in acid streams draining headwaters of the upper Animas River watershed, and high concentrations of some metals, especially Zn, persist in circumneutral reaches of the Animas River and Mineral Creek, downstream of mixing zones of acid tributaries. Seasonal variation of metal concentrations is reflected in variation in toxicity of stream water. Loadings of dissolved metals to the upper Animas River and tributaries are greatest during summer, during periods of high stream discharge from snowmelt and monsoonal rains, but adverse effects on stream biota may be greater during winter low-flow periods, when stream flows are dominated by inputs of groundwater and contain greatest concentrations of dissolved metals. Fine stream-bed sediments of the upper Animas River watershed also contain elevated concentrations of potentially toxic metals. Greatest sediment metal concentrations occur in the Animas River upstream from Silverton, where there are extensive deposits of mine and mill tailings, and in mixing zones in the Animas River and lower Mineral Creek, where precipitates of Fe and Al oxides also contain high concentrations of other metals.

This report summarizes the findings of a series of toxicity studies in streams of the upper Animas River watershed, conducted on-site and in the laboratory between 1998 and 2000. The objectives of these studies were: (1) to determine the relative toxicity of stream water and fine stream-bed sediments to fish and invertebrates; (2) to determine the seasonal range of toxicity in

stream water; (3) to develop site-specific thresholds for toxicity of Zn and Cu in stream water; and (4) to develop models of the contributions of Cu and Zn to toxicity of stream water, which may be used to characterize toxicity before and after planned remediation efforts.

We evaluated the toxicity of metal-contaminated sediments by conducting sediment toxicity tests with two species of benthic invertebrates, the midge, *Chironomus tentans.* and the amphipod, *Hyalella azteca.* Laboratory toxicity tests with both taxa, exposed to fine stream-bed sediments collected in September 1997, showed some evidence of sediment toxicity, as survival of midge larvae in sediments from Cement Creek (C48) and lower Mineral Creek (M34), and growth of amphipods in sediments from these sites and three Animas River sites (A68, Animas at Silverton; A72, Animas below Silverton, and A73, Animas at Elk Park) were significantly reduced compared to a reference site, South Mineral Creek (SMC) . Amphipods were also exposed to site water and fine stream-bed sediment, separately and in combination, during the late summer low flow period (August-September) of 1998. In these studies, stream water, with no sediment present, from all five sites tested (same sites as above, except C48) caused 90% to 100% mortality of amphipods. In contrast, significant reductions in survival of amphipods occurred at two sites (A72 and SMC) in exposures with field-collected sediment plus stream water, and at only one site (A72) in exposures with sediments and clean overlying water. Concentrations of Zn, Pb, Cu, and Cd were high in both sediment and pore water (interstitial water) from most sites tested, but greatest sediment toxicity was apparently associated with greater concentrations of Fe and/or Al in sediments. These results suggest that fine stream-bed sediments of the more contaminated stream reaches of the upper Animas River watershed are toxic to benthic invertebrates, but that these impacts are less serious than toxic effects of metals in stream water.

We evaluated seasonal variation in the toxicity of stream water by repeating water-only toxicity tests with amphipods in stream water during the late winter low flow period (early April) of 1999, and by conducting toxicity tests with fathead minnows, *Pimephales promelas*, in stream water during both sampling periods. Stream water was more toxic to fathead minnows in late winter than in late summer, with only one of five sites toxic in summer 1998 and three of four sites toxic in winter 1999. Undiluted stream water from all sites was highly toxic to amphipods in both seasons, and dilutions as low as 25% from some sites caused significant toxicity to amphipods in winter 1999.

Toxic effects of stream water on amphipods and fathead minnows was associated with greater concentrations of several dissolved metals, including Zn, Cu, Fe, and Al. Complex interactions among speciation, solubility, and toxicity of Fe and Al made evaluation of the contributions of these metals to toxicity difficult. We modeled the contributions of Zn and Cu to toxicity of stream water, based on available information on dissolved Zn and Cu concentrations and on results of toxicity tests that established thresholds for toxicity of these metals at ambient water quality. We conducted toxicity tests with Zn and Cu in reconstituted waters, with ionic constituents based on stream water of the upper Animas River near Silverton (A68 and A72), to establish site-specific toxicity thresholds for toxicity of these metals. Toxicity thresholds were determined for amphipods and fathead minnows, under test conditions comparable to those used for on-site tests, and for early life stages (ELS; egg through swim-up fry) of brook trout, *Salvelinus fontinalis*, the most widespread and abundant fish species in the upper Animas River watershed. Reconstituted test waters were prepared to represent conditions in the Animas River at Silverton during the summer testing period (Animas soft water: hardness 110 mg/L as $CaCO_3$) and conditions at this location during the late-fall spawning period for brook trout (Animas hard water: hardness 180 mg/L).

The three taxa tested differed widely in their sensitivity to toxic effects of Cu and Zn under conditions in the upper Animas River. Amphipods were the most sensitive of the three taxa to Zn toxicity, with effects on survival at concentrations less than 100 µg/L in Animas Soft water, and brook trout were the least sensitive taxa to Zn toxicity, with effects on growth of early life stages at concentrations of 1,000 µg/L and greater and no significant effects on survival at concentrations up to 2,000 µg/L in Animas Hard water. Both brook trout and fathead minnows were highly sensitive to Cu, with significant reductions on growth in hard water at concentrations less than 10 µg/L and significant reductions in survival at concentrations between 20 and 30 µg/L. Amphipods were less sensitive to Cu, with significant reductions in survival occurring between 50 and 100 µg/L. These results indicate that the fathead minnows used for on-site toxicity tests were adequate surrogates for the responses of brook trout to Zn and Cu in stream water. The results of brook trout ELS tests suggest that Zn may not be a major contributor to the toxicity of stream water to brook trout in the Animas River and lower Mineral Creek, despite consistently high dissolved Zn concentrations (200-1,000 µg/L) at study sites in these reaches.

We modeled seasonal variation in toxicity of dissolved Zn and Cu, based on site-specific toxicity thresholds and seasonal variation in concentrations of these metals at three USGS sites near Silverton. Toxicity thresholds determined in ‘Animas’ reconstituted waters were adjusted for seasonal variation in hardness at these sites, based on exponential regressions of relationships between hardness and metal toxicity. The resulting site-specific thresholds were compared to concentrations of dissolved Zn and Cu predicted for each site by multiple regression models based on season (Julian date) and discharge. Modeled dissolved Zn concentrations at all three locations frequently exceeded thresholds for mortality of amphipods, but did not approach thresholds for reduced growth or survival of brook trout. In contrast, modeled Cu concentrations frequently exceeded thresholds for reduced growth and survival of brook trout and fathead minnows at two of the three sites. Model predictions were consistent with results of toxicity tests with amphipods and minnows in stream water and with the distribution of brook trout in recent surveys. These findings indicate that dissolved Cu concentrations in stream water are a significant limiting factor for brook trout in the upper Animas River watershed. However, the recovery of brook trout populations at some study sites may also be limited by toxicity of other metals, such as Fe and Al, and trout populations at all three sites near Silverton are probably affected by the reduced productivity of benthic invertebrates, resulting from toxicity of dissolved Zn and from degradation of physical habitats.

Introduction

Pollution of aquatic environments by acid drainage and associated toxic metals is a widespread problem in watersheds affected by historic and ongoing hard-rock mining. Hard-rock mining typically targets ore bodies containing sulfide minerals of both target metal(s) and other co-occurring metals. Some sulfide minerals, especially the abundant iron (Fe) sulfide, pyrite, can oxidize in the presence of oxygen to form sulfuric acid. The resulting acid drainage, which can mobilize high concentrations of toxic metals, occurs naturally through erosion and weathering of mineral deposits, but the process can be greatly accelerated by mining activities, which expose large amounts of sulfide minerals in mine tunnels, waste rock piles, and deposits of mine and mill tailings (Bove et al. 2000).

Both acidity and associated toxic metals generated by acid drainage can have adverse impacts on aquatic ecosystems which may extend far downstream from sources of acid drainage. The most severe impacts occur in highly acid streams and lakes, which can be nearly devoid of aquatic life except acid tolerant algae and microbes (Campbell and Stokes 1985). Severe impacts also occur on communities in and downstream of mixing zones of acid and neutral-pH waters. Despite neutral pH, high concentrations of dissolved and colloidal metals typically make the water column of mixing zones highly toxic to fish and invertebrates (Verbost et al. 1995, Henry et al. 1999). Al and Fe rapidly precipitate out of solution at neutral pH, forming colloidal hydrous oxides, which can be toxic (Smith and Sykora 1976) and, when deposited on stream substrata, cause severe degradation of benthic habitats. Some metals persist in bioavailable forms downstream of mixing zones, leading to metal bioaccumulation and hazards of chronic metal toxicity (Besser et al. in press). Metals that drop out of solution may become associated with periphyton, leading to contamination of stream food webs and dietary metal exposure to higher order consumers (Woodward et al. 1994, Farag et al. 1999). Metals associated with Fe/Al oxides can be transported substantial distances downstream (Kimball et al. 1995) and deposited as fine stream-bed sediments, which can be toxic to benthic invertebrates (Kemble et al. 1994).

The USGS Abandoned Mine Lands Initiative (AMLI) is a five-year project that uses a watershed approach to provide scientific information to help Federal land management agencies and local stakeholders plan remediation efforts in watersheds affected by historic mining activities (Nimick and von Guerard 1998). The AMLI focused on two study watersheds, the upper Animas River watershed in Colorado and the Boulder River watershed in Montana. Site-specific studies of the severity and mechanisms of impacts of acid drainage on stream biota are essential for development of appropriate and effective remediation plans. The general approach for biological studies in AMLI watersheds includes: (1) surveys of the status of fish and invertebrate communities, to document the cumulative impacts of mining, combined with other natural and anthropogenic influences on stream biota throughout the watershed, and to establish a baseline for monitoring effects of remediation; (2) studies of metal contamination in water, sediment, and stream food webs, to document associations between metal exposure associated with acid drainage and observed impacts on biotic communities; and (3) site-specific toxicity studies to document the critical routes of metal exposure that result in toxicity, to determine spatial and temporal variation in toxic effects, and to establish reliable goals for recovery of stream biota.

Study Objectives

This report describes a series of studies that characterized spatial and temporal variation in toxicity to stream biota of the upper Animas River watershed (Fig. 1). Previous studies have provided some useful information on the toxicity of stream water and sediments for a limited number of sites and sample dates, but provide little or no guidance regarding the underlying causes of observed toxicity and or thresholds for recovery of stream biota. Between 1998 and 2000, we conducted a series of studies to characterize toxicity and water quality at several sites in the upper Animas River and major tributaries near Silverton, Colorado. The objectives of these studies were: (1) to determine the relative toxicity of stream water and fine stream-bed sediments at sites in the upper Animas River and major tributaries; (2) to determine the seasonal range of toxicity in stream water; (3) to establish site-specific thresholds for toxicity of Zn and Cu in stream water; and (4) to develop models of the contributions of Cu and Zn to toxicity of stream water.

The Upper Animas River Watershed

The upper Animas River watershed (Fig. 1) is one of two watersheds selected for the Abandoned Minelands Initiative. The headwaters of the Animas River are in the mountainous terrain above the town of Silverton, in southwestern Colorado. Elevations in the upper watershed range from 9,320 ft. (2850 m) at Silverton to over 13,000 ft (3,900 m). The headwaters of the upper Animas River watershed above Silverton is drained by the Animas River, Cement Creek, and Mineral Creek. Downstream from Silverton, the Animas River flows generally south, passing through the rugged Animas Canyon and eventually draining into the San Juan River in northern New Mexico. The headwater streams of the upper Animas River watershed drain an area of Tertiary volcanic rocks. The predominant geologic feature in the area is the Silverton caldera, formed by the collapse of a volcano approximately 8 miles (13 km) in diameter. The boundaries of the caldera are approximated by the current courses of the upper Animas River and

Mineral Creek. The igneous intrusive and volcanic rocks, which filled the caldera, were later subjected to fracturing and hydrothermal alteration, leading to development of extensive deposits of ore minerals (Bove et al. 2000). Uplift, glaciation, and erosion of the area resulted in the rugged relief in the headwaters and led to exposure of mineral deposits near the surface. Weathering of these deposits, especially those in the area drained by Mineral and Cement Creeks, produce acidity and release metals to the surface waters and groundwater (Mast et al. 2000a).

Between 1,000 and 1,500 mining claims were staked in the upper Animas River watershed, upstream of Silverton, since placer gold deposits were discovered in 1871 (USGS 2000). Mining in the watershed peaked in the 1920s and 1930s and continued until 1991 when the Sunnyside Mine was closed. This historical mining activity produced miles of underground workings and large volumes of mine waste rock that have been pulverized to remove ore metals. Increased surface area and exposure to atmospheric oxygen has led to oxidation of large amounts of pyrite and other sulfide minerals, resulting in large anthropogenic sources of acidic drainage that have adversely affected water quality and aquatic and riparian habitats in the watershed.

Stream biota of the upper Animas River watershed have been dramatically affected by over a century of mining activities. Recent surveys of aquatic communities in the watershed have documented severe impacts in headwater reaches most affected by acid drainage, absence of fish in reaches below mixing zones of acid tributaries, and reduced abundance of fish (principally brook trout) and reduced diversity and abundance of invertebrates in the downstream reach of the Animas River (Butler et al, 2001; Table 1). In contrast, diverse and productive invertebrate communities and self-sustaining populations of brook trout occur in tributaries that are less impacted by acid drainage. Results of these surveys suggest that incremental recovery of brook trout populations occurred in some reaches of the Animas River during the 1990s, following mine closings and associated remediation activities, suggesting that additional remediation efforts may lead to further recovery of aquatic communities.

Studies conducted as part of the AMLI have characterized the patterns of metal contamination associated with mining in the upper Animas River watershed. Church and others (1997) documented the distribution of elevated metal concentrations in fine stream-bed sediments, relative to historic background concentration. Greatest metal concentrations in sediments occurred in stream reaches affected by large-scale ore milling activities, notably the upper Animas River upstream of Silverton, but metal concentrations above background levels also occur in sediments far downstream in the Animas River. This study also documented elevated concentrations of dissolved and colloidal metals in stream water. Loadings of metals from Cement and Mineral Creeks, which enter the Animas River at Silverton, produced elevated concentrations of dissolved Zn and colloidal Fe, Al, and Cu in the downstream reach of the Animas River. Investigations by Leib and others (in press) characterized the concentrations and loadings of metals in these two tributaries, and in the headwaters and downstream reaches of the Animas River, and found that concentrations and loadings of most metals varied in response to seasonal patterns of stream discharge. Concentrations of dissolved metals were generally lowest during the period of peak discharge in spring and early summer, due to dilution by melting snow, then gradually increase, starting in late summer (August-September) and reach annual maxima in late winter (March-April). Besser and others (2001) documented patterns of metal bioaccumulation in stream biota in the upper Animas River watershed that were consistent with elevated metal concentrations in stream water and sediments. Concentrations of Cu, Zn, Cd, and Pb were significantly elevated in periphyton, benthic invertebrates and liver tissue of brook trout from one or more sites in mining-impacted reaches of the upper Animas River relative to reference sites. Differences in metal bioaccumulation among invertebrate taxa were related to differences in feeding habitats, suggesting that dietary transfer contributed to bioaccumulation of metals in stream food webs. Concentrations of Cu in benthic invertebrates and trout corresponded most closely to documented impacts on invertebrate and fish communities.

Recent studies suggest that stream water, stream-bed sediments, and pore water (interstitial water in bed sediments) from the upper Animas River and tributaries are toxic to fish and invertebrates. Nimmo and others (1998) reported that stream waters from Cement Creek were highly toxic to three test organisms (rainbow trout, *Onchorhynchus mykiss*; fathead minnow, *Pimephales promelas*; and a daphnid, *Ceriodaphnia dubia*) in acute (48-hr) on-site toxicity tests conducted during August 1997 (low-flow) and June 1998 (high flow). Stream water from three other sites on the Animas River and one site in lower Mineral Creek were toxic to *C. dubia*, but not other taxa, during both testing periods. Similar toxic effects were observed in tests conducted with water from seepage pits in gravel bars, which were assumed to represent characteristics of pore waters in contact with contaminated sediments. Another series of studies examined the role of deposition of Fe/Al oxides and other metals on the suitability of stream cobble substrata by invertebrates (W. Clements, Colorado State University, written commun.). Results of these studies suggest that both substrata and stream water from the Animas River reduced colonization by benthic invertebrates. Toxicity studies with a metal-sensitive species of mayfly (*Baetis tricaudatus*) found that suspensions of colloids from the Animas River were not acutely toxic, but that ingestion of metal-contaminated periphyton from the Animas River was associated with reduced growth of *B. tricaudatus.*

The studies reported here focused on the conditions in the Animas River and the lower reaches of Cement and Mineral Creeks, near Silverton, Colorado (Fig. 1). Sample sites and site IDs used for this study are those established by the State of Colorado, Department of Public

Health and Environment, except where exceptions are noted. Most samples were collected at four USGS stream-flow gaging stations, one on each stream upstream of the confluence (A68, Animas River at Silverton; C48, Cement Creek at Silverton; and MC, Mineral Creek at Silverton), and one a short distance downstream on the Animas (A72, Animas River below Silverton). Two additional sites were sampled for some toxicity tests: A45, Animas River below Minnie Gulch (upstream of Howardsville); and A73, Animas at Elk Park (5 km downstream of A72). Biological communities at these sites exhibit a wide range of impacts of acid drainage (Table 1). A site on the South Fork of Mineral Creek (SMC), located approximately halfway between the Forest Service Campground and the confluence with the main stem of Mineral Creek, was selected as a reference site for toxicity tests. Although water quality of South Mineral Creek has been affected by mining, this site supports a self-sustaining population of brook trout and diverse and productive fish and invertebrate communities.

Methods

Sediment and water sampling

Samples of stream water and fine stream-bed sediments were collected from the Upper Animas River and tributaries during low-flow periods, in the late summers of 1997 (September 5-9) and 1998 (August 25-September 6) and in late winter of 1999 (April 6-8). Samples of fine sediment were collected from primary study sites during the 1997 and 1998 sampling periods. Stream gravels and associated fine sediments were collected from depositional areas, typically at the downstream or inshore portions of gravel bars, with acid-washed scoops constructed from PVC pipe (7.5 and 10 cm diameter). Samples were sieved with stream water through a polyethylene wash bucket (Wildco, Saginaw MI) with a bottom of stainless steel mesh (0.5 mm diameter mesh) and fine sediment passing through the sieve was allowed to settle in acid-washed polypropylene pans, then transferred to acid-washed polyethylene buckets. Sediments collected in 1997 were shipped to the USGS Columbia Environmental Research Center (CERC), Columbia Missouri, and refrigerated at 4°C for two weeks before testing and analysis. Sediments collected in 1998 were stored at ambient temperature and testing began within 48 hr of collection.

Stream water was sampled during late summer 1998 and late winter 1999. Samples collected during 1998 were used for on-site toxicity testing. Stream water was collected in acid-washed polyethylene carboys (20 L) by a series of sub-surface grabs across the stream cross-section. Samples were collected at two-day intervals, from the five primary study sites (A68, A72, A73, SMC, M34; seven samples each) and one secondary study site (A45; four samples each). Additional water samples were collected in late winter 1999, before significant snowmelt occurred in the upper Animas River watershed. Two samples were collected, three days apart, from primary sample sites, except A73, which was inaccessible during this period. Composite 20-L samples from each site were shipped by overnight courier for toxicity testing at CERC.

Toxicity tests with sediment and stream water

Toxicity tests with sediments – 1997. Laboratory toxicity tests were conducted during summer 1997 with fine stream-bed sediments at CERC with two species of benthic invertebrates: the midge, *Chironomus tentans*, and the amphipod, *Hyalella azteca*. Tests were conducted at 23 °C in water baths equipped with water-replacement system described by Zumwalt et al. (1994). Test chambers were 300 mL beakers with 'windows' of stainless steel screen to allow water to overflow, producing an exposure volume of approximately 250 mL. Homogenized sediment from each site (100 mL) and diluted CERC well water (Table 2) were added to eight replicate test chambers for each species one day before the start of the tests. Each replicate chamber was stocked with 10 test organisms. During the studies, overlying water in each beaker was replaced by four daily additions of diluted well water (100 mL per addition).

Test conditions were in general accordance with methods presented by USEPA (2000). Tests with *C. tentans* were started with second-instar larvae (10-12 days old) and test chambers were fed 0.5 mL (2 mg dry wt.) of a suspension of homogenized flake fish food (Tetra-Fin; Tetra-Werke) daily. Tests ended after 10 days, with endpoints of survival and growth (dry weight). Tests with *H. azteca* were started with 7- to 14-day old individuals. Amphipod test chambers were fed 1.0 mL (1.8 mg dry wt.) of a yeast-cereal leaves-Trout Chow© (Purina) suspension daily (USEPA 2000). Tests were ended after 14 days, with endpoints of survival and growth. Surviving amphipods were counted and preserved with osmotically-adjusted, buffered formalin. Total lengths were determined by digitizing total lengths along the dorsal margin of the carapace (ZIDAS image analysis system; Carl Zeiss).

On-site toxicity tests with sediment and stream water – 1998. Toxicity tests with stream water and bed sediment were conducted in late summer 1998 in a mobile laboratory at Silverton, Colorado. Stream water and sediment was added to toxicity tests within 48 hours of collection. Temperatures in exposure systems were maintained at 20-22 °C. Control water and dilution water for these tests was ‘Animas soft' reconstituted water (Table 2), prepared by dissolving reagent-grade salts ($NaHCO_3$, 15 mg/L; $CaSO_4$+2H_2O, 120 mg/L; $CaCl_2$+2H_2O, 33 mg/L; $MgSO_4$, 30 mg/L; and KCl, 4 mg/L) in ultrapure water produced by a cartridge-type purification system (resistance >18 megohms/cm; Barnstead). This salt mixture is a modification of the mixtures of salts recommended by ASTM (1997a) and USEPA (1994) to achieve a mixture of major ions more representative of conditions in the upper Animas River. The resulting reconstituted water is dominated by calcium and sulfate, with low alkalinity, approximating the proportions and seasonal ranges of major ions in the upper Animas River at A68 and A72 (Table 2). Only chloride, which does not form important complexes with either Zn or Cu in fresh waters (Stumm and Morgan 1996), occurred at consistently greater concentrations in the reconstituted waters than in stream water.

On-site toxicity tests were conducted with *H. azteca* in stream water and sediment following procedures similar to those described above. Amphipods were exposed to three treatments for each of the five primary study sites: stream water only (no sediment); sediment only (with Animas Soft water); and water plus sediment. Treatment groups consisted of six replicate exposure chambers, with eight animals per chamber. Test chambers in the water-only treatment contained a piece of coiled nylon mesh (3M) to provide a substratum for amphipods. Control sediment for sediment-only and water-plus-sediment treatments was a formulated artificial sediment consisting of 50% silt-sized and 50% sand-sized particles (Kemble et al. 1999). Exposures were conducted in a static test systems with a water-replacement system modified from Leppanen and Maier (1998), which used polypropylene syringe bodies (75 mL total volume) to deliver water additions to individual test chambers. During the studies, volumes of test water equal to approximately one-half of the water volume in the chambers (75 mL/chamber/day for chambers with sediment, 150 ml/chamber/day for chambers with water only) were added daily. Water for each treatment combination was added to a shallow plastic container, which split flow among six syringes draining into replicate test chambers. Excess water in test chambers overflowed into small plastic water baths, then into a common drain.

On-site toxicity tests with fathead minnows consisted of an abbreviated dilution series of stream water from the five primary sites. Tests were conducted in general accordance with the method of USEPA (1994). Stream water from each site was tested at full strength (100%) and diluted to 50% and 25% strength with Animas Soft reconstituted water, with three replicate test chambers per dilution. Control fish were exposed to 100% reconstituted water. Exposure chambers were 1,000 mL beakers containing 250 mL of water. Tests were started with ten newly-hatched larvae (less than 48-hr post-hatch) per beaker and lasted seven days. Minnows were fed newly hatched brine shrimp (*Artemia*) three times daily, and test water was replaced daily after water and detritus was siphoned from each beaker. Surviving minnows in each replicate group were counted, then dried at 60 degrees C. Total dry weight (biomass) of each replicate group and mean dry weight of surviving individuals (both) were determined for each replicate.

Toxicity tests with stream water – 1999. A second series of toxicity tests was conducted with stream water collected in April 1999. In these tests, both fathead minnows and amphipods were exposed to stream water diluted with Animas soft water to the same partial dilution series used for on-site fathead minnow tests (100%, 50%, 25%). Test conditions and apparatus were identical to those used for on-site tests, except that amphipod tests were conducted for seven days, with ten organisms per replicate and with survival as the only endpoint.

Toxic tests with zinc and copper in 'Animas' reconstituted water

Additional toxicity tests were conducted to determine toxicity thresholds for Zn and Cu to amphipods and minnows under the test conditions used for on-site tests, and to determine toxicity thresholds for these metals in chronic, early life stage exposures with brook trout. Tests with fathead minnows were conducted in the mobile laboratory concurrently with stream water toxicity tests during summer 1998. Test conditions were identical except that tests with Zn and Cu had two, rather than three, replicates per treatment. Tests with amphipods were conducted at CERC under the same conditions used for testing stream water in winter 1999. Exposure solutions were prepared daily from stock solutions of $ZnCl_2$+7 H_2O and $CuSO_4$+$5H_2O$, with a series of five concentrations of each metal prepared by 50% serial dilutions with Animas Soft reconstituted water.

Early life-stage toxicity tests with brook trout were conducted at CERC in general accordance with guidelines published by ASTM (1997b) Brook trout tests were conducted with 'Animas Hard' reconstituted water (average hardness 180 mg/L as CaCO3; Table 2), which contained greater calcium hardness to approximate levels occurring in early winter, the spawning period of brook trout. Reagent-grade salts ($NaHCO_3$, 15 mg/L; $CaSO_4$+$2H_2O$, 200 mg/L; $CaCl_2$+$2H_2O$, 33 mg/L; $MgSO_4$, 30 mg/L; and KCl, 4 mg/L) were dissolved in de-ionized water from the CERC reverse-osmosis/ion-exchange system (Culligan). Tests were conducted in proportional diluter systems, which delivered five dilutions of metal plus a control. Water with each concentration was fed to two duplicate aquaria, then split among four 6-L exposure chambers per concentration. The water replacement rate was three volume-additions per day. Water entering the diluter system and water in the water bath holding the exposure chambers was chilled to maintain a constant temperature of 10 °C. Water temperature was monitored daily throughout toxicity tests, and water-quality parameters (hardness, alkalinity, conductivity, pH, dissolved oxygen, and ammonia) were analyzed weekly. Water samples were colleted biweekly for metal analysis.

Tests were started with 15 eyed embryos, about 14 days before hatching, added to four replicate exposure chambers. Embryos were held in glass incubation cups (approx. 100 mL) with stainless steel screen bottoms, suspended in the exposure tanks under the water delivery tubing and incubated in darkness (under black plastic). Dead embryos were counted and discarded daily, the timing and success of hatching was recorded, and surviving fry were released to the test chamber and held under a 16-h light/8-hr dark photoperiod. Mortalities, defined as individuals that failed to respond to an external stimulus, were counted daily. Swim-up fry in both tests were fed three times daily (two times daily over weekends) with live brine shrimp nauplii and trout chow. Fish were not fed for the last 24-hr before the end of each test. Tests ended 30 days after the majority of trout reached the swim-up stage, resulting in a total exposure period of 71 d. At the end of the test, the number of surviving fish, the total dry biomass of surviving fish in each test chamber, and the average dry weight of surviving fish was determined for each replicate group.

Chemical analyses

Concentrations of metals in samples of water and sediment were determined by inductively-coupled plasma mass spectroscopy (ICP-MS; Perkin-Elmer/SCIEX Elan 6000; May et al 1997). Water samples were analyzed for iron (Fe), zinc (Zn), copper (Cu), cadmium (Cd), and lead (Pb); and sediment samples were digested and analyzed for these metals plus aluminum (Al). Samples for determination of total recoverable metals in water were acidified at the time of collection with ultra-pure HNO_3 (1% v/v) and analyzed without further digestion. Samples for determination of dissolved metals were filtered using a syringe-mounted filter with fluoropolymer (PVDF) membrane and polypropylene housing (Acrodisk LC-25 filter, Gelman; and 25-mL GasTite syringe, Hamilton Co.) before acidification. Water samples from toxicity tests were analyzed only for total recoverable metals, due to the expectation of low concentrations of suspended particulates in test chambers.

Samples of sediment pore water were collected for chemical characterization in 1997 and 1998, using two different methods. In 1997, two replicate test chambers were 'sacrificed' on day 0 of toxicity tests and sediments were transferred into polypropylene centrifuge bottles and centrifuged for 30 min at 3,000 rpm (2500 g) to separate sediment and pore water. Pore water was decanted, and filtered and acidified as described above for stream water. In 1998, pore water was collected from sediments during on-site toxicity tests, using passive diffusion samplers, or 'peepers', constructed from 12-mL polyethylene snap-top vials (Bufflap and Allen 1995). Samplers were prepared for use by the following steps: 1-cm diameter holes were cut in the lids of each vial; vials and lids were acid-washed, rinsed, and submerged in ultra-pure water; a polycarbonate filter membrane (>0.45 µm pore diameter; Nucleopore) was placed over the top of each vial; and the lid was sealed over the membrane. Samplers were stored in de-ionized water, then buried in sediments in toxicity test chambers one day before the start of the toxicity test. At the end of the test (15 d after samplers were added), samplers were retrieved, rinsed with ultrapure water, and the contents were retrieved by acid-washed stainless-steel syringe and acidified to 1% HNO_3 (v/v).

Filtered and acidified samples of stream water and pore water were analyzed without further preparation. Unfiltered water samples were digested with nitric acid in high pressure quartz reaction vessels before analysis of total recoverable metals. Samples of fine stream-bed sediment were digested by two different methods before metal analyses. Samples collected in September 1997 were subjected to a partial digestion with dilute acid and peroxide (2M HCl and 1% hydrogen peroxide at 50 °C), which was intended to dissolve metal oxide fractions, including amorphous Fe and Al hydrous oxides, but not metals associated with crystalline ore minerals (Church et al 1997). Samples collected during August-September 1998 were subjected to microwave digestion with concentrated nitric acid to extract total recoverable metals.

Quality assurance measures for analyses of metals in water samples included analysis of standard reference materials, duplicate analyses, sample spikes, ICP-MS interference checks, and analysis of field and reagent blanks. Recoveries of metals from reference solutions ranged from 76% to 100%, except for Fe. The generally higher recoveries for Fe (86 - 178%) suggest that matrix interferences may have produced a positive bias in sample results for Fe concentrations in water. Digestion and analysis of three aliquots of an unfiltered water sample produced relative standard deviations (RSDs) ranging from 1.1% to 6.0% for all analytes except Cu, which had an RSD of 63%, presumably due to contamination. Analytical precision, expressed as relative percent differences (RPD) for duplicate analyses ranged from 0.04% to 14.9%. Recoveries of analytes from aqueous matrix spikes for each element ranged from 91% to 124%, with average recoveries of 108% for 1998 samples and 101% for 1999 samples. Checks for interferences in ICP-MS analysis by five-fold dilutions of field-collected water samples indicated dilution percent differences of less than 15% for all elements except Fe, which ranged from 10% to 32%, consistent with a possible interference for this element. Analysis of a synthetic interference check solution for Cu, Zn, Cd, and Pb exhibited recoveries ranging from 94% to 117%. Blank contamination of porewater (peeper) samples was suggested by high concentrations of Zn in water from two samples (field blanks) from the 1998 studies (205 and 210 µg/L). However, peeper samples from control treatments contained low levels of Zn (15-22 µg/L), and most peeper samples equilibrated in field-collected sediments contained Zn concentrations less than 100 µg/L. Therefore, metal concentrations measured in peeper samples (Table 5) were not adjusted for blank contamination. There was no evidence of blank contamination of other metals in porewater samples, and no evidence of metal contamination in field blanks for surface water analyses. Digestion blanks analyzed with 1999 surface water samples indicated consistent contamination with Fe (180-210 µg/L), so Fe data reported for these samples were corrected for blank contamination.

Quality assurance results for sediment analyses were generally similar to those for water analyses. Recoveries from a sediment reference material were fair to good for all analytes except Al, which exhibited a low recovery of 21%. Low recoveries for a “total recoverable” digestion method are not unexpected, because NIST certified ranges for sediment are based on recovery of metals from a "total" digestion procedure, and these results are consistent with previous determinations of total recoverable metals in sediments at CERC. Triplicate digestion and analysis of sediment samples indicated RSD values less than 18% for all elements. Recoveries of elements from pre-digestion spikes of sediment samples ranged from 101% to

125%, with the exception of high recovery of Fe (159%) in one of the spikes, apparently due to the low spike/background ratio of 0.2 (i.e., five-fold greater Fe concentrations in the sample than in the spike). Blank equivalent concentrations (BEC) computed for procedural blanks for sediment digestion were less than the method detection limit (MDL) for all analytes except Cu, which exhibited a BEC slightly above the MDL. No blank correction was performed on sediment Cu concentrations.

Statistical Analysis

Data were transformed to improve normality and homogeneity of variance. Survival data were transformed to the arcsine of square roots of the ratio, survivors/number stocked. Metal concentrations were transformed to common logarithms, with values less than the MDL estimated as MDL/2 for statistical analysis. Analysis of variance (ANOVA) was performed with the SAS statistical software package (SAS Institute 1990). Two-way ANOVA was used to examine effects of site and exposure type (water, sediment, and water-plus-sediment) for on-site amphipod tests. One-way ANOVA was used to determine differences among sites or among serial dilutions. Differences among means were determined by Dunnett's test, for comparisons between treatments and controls, or Duncan's multiple-range test, for comparisons among sites.

Concentration-response curves from toxicity tests were analyzed with the ToxStat software program (WEST, Inc, Cheyenne WY). Following transformation, median lethal concentrations (LC50) or median effect concentrations (EC50; determined for endpoints other than mortality) were determined by the trimmed Spearman-Karber method (Hamilton et al. 1977).

For toxicity tests with Zn and Cu, data for survival, mean dry weight, and biomass (total dry weight per replicate group) were analyzed by ANOVA. Comparisons between controls and treatment groups were made by one-tailed Dunnett's test to determine lowest-observed adverse effect concentrations (LOECs) for Cu and Zn. LOEC is defined here as the lowest exposure concentration causing statistically significant adverse effect in a test endpoint, provided that all higher concentrations also cause significant effects. In addition, a linear interpolation technique was used to estimate concentrations of Cu and Zn associated with 25% percent inhibition of survival and growth (IC25; Norberg-King 1993, USEPA 1994).

Modeling toxicity of stream water

Toxicity of Zn and Cu in stream water at three USGS gages near Silverton (A68, A72, and M34) was modeled based on site-specific toxicity thresholds, determined as described above, and multiple regression models of seasonal variation in dissolved metal concentrations in stream water (Leib and others, in press). These models, which predict dissolved metal concentrations based on Julian date and average daily discharge, accounted for 76% to 91% of the variation in measured concentrations of Zn at AR68, A72, and M34 and Cu at A72 and M34. The models of dissolved Cu concentrations at A68, which did not follow the same seasonal pattern, accounted for 62% of observed variation. Similar models were also developed for seasonal variation in hardness, which modifies the toxicity of Zn and Cu, at all three sites. Average dissolved Zn and Cu concentrations for each Julian date were estimated using the regression models and average daily discharge (Leib et al., in press).

Seasonal variation in toxicity of Zn and Cu at the gaging stations was evaluated by comparing modeled dissolved metal concentrations with site-specific toxicity thresholds. Toxicity thresholds determined from tests with ‘Animas’ reconstituted waters were adjusted for differences in water hardness based on the relationships between chronic water quality criteria for metals and total hardness (mg/L as $CaCO_3$) established by USEPA (1999):

$$ZnWQC = e^{0.8473 \times \ln(hardness) + 0.884}$$

$$CuWQC = e^{0.8545 \times \ln(hardness) - 1.702}$$

Species-specific versions of these equations were derived by setting the equation equal to the appropriate threshold concentration from the toxicity test, entering the water hardness, and solving for the intercept value. The resulting equations were used to calculate toxicity thresholds for each site that accounted for seasonal differences in hardness, using hardness values predicted for each Julian date (Leib and others, in press). Site- and species-specific toxicity hazards for Zn and Cu for each site were compared by calculating toxic units, ratios of modeled metal concentrations in stream water to hardness-adjusted toxicity thresholds for each metal.

Results and Discussion

Toxicity of sediment and stream water

Summer 1997. Laboratory sediment toxicity tests conducted in late summer 1997 showed little evidence of sediment toxicity at most sites tested (Fig. 2). Survival of amphipods (*H. azteca*) did not differ significantly among sites. Growth of amphipods (expressed as total length) differed significantly among sites, but was significantly reduced, relative to the control group, only in sediments from Cement Creek (C48). Growth of amphipods was also reduced, relative to the reference site (SMC), by sediments from C48 and M34, and to a lesser extent by sediments from the other three sites (ANOVA/Duncan's test). Toxicity tests with midges (*C. tentans)* suggested greater toxic effects of sediments, although interpretation of these results was compromised by low survival (less than 50%) in the control group (Fig. 3). However, survival in sediments from A72, M34, and C48 were significantly reduced relative to the reference site, SMC (ANOVA/Duncan's test). Growth of midge larvae also differed significantly among sites, but there were no significant differences relative to the control sediment or to sediment from the reference site.

Summer 1998. On-site toxicity tests conducted in summer 1998 indicated that sediment was less toxic to amphipods than was stream water (Fig. 4). Water-only exposures with stream water from all five sites resulted in complete or nearly complete mortality of amphipods, and all stream water treatments were significantly different from the control group (Fig. 4). In contrast, sediment-only exposures (with reconstituted Animas soft water) with sediments from four of five sites resulted in high (80-100%) survival of amphipods. Survival of amphipods in sediment-only and sediment-plus-water treatments was greater than in water-only treatments. However, interpretation of data from these treatments was complicated by poor control performance, as survival in the controls (with formulated sediments) averaged only 50%, compared 100% in sediments from the reference site, SMC. When all data from the sediment-only tests were analyzed, survival did not differ significantly from controls. However, when controls were excluded from the analysis, survival in sediment from A72 (35%) was significantly less than in sediments from SMC (ANOVA/Duncan's test). Survival in water-plus-sediment treatments was generally intermediate between water-only and sediment-only treatments. Despite the low control survival, survival was significantly reduced in water-plus-sediment treatments from A68 and SMC (ANOVA/Dunnett's test). Site A72, which was most toxic in the sediment-only treatment, also had the lowest survival (4.2%) in the water-plus-sediment treatment. Growth of surviving amphipods did not differ significantly among sites in the sediment-only treatment (Fig. 4). In the water-plus-sediment treatment, growth was significantly reduced relative to controls by A73 and M34, which did not have significant reductions in survival, but not by the two other sites where survival was significantly reduced. Similarly, in the water-only treatment, growth of amphipods was not significantly reduced (and sometimes increased) relative to controls in groups with very low survival. These trends suggest that growth of surviving amphipods was more strongly influenced by differences in amphipod densities in test chamber (due to differential survival) than by toxic effects of water or sediment.

On-site toxicity tests indicated that stream water in summer 1998 was much less toxic to fathead minnows than to amphipods. The only significant toxic effects observed in minnows were a small, but significant reduction in survival in undiluted water from SMC (Fig. 5a), which corresponded to more substantial decreases in growth and biomass (Table 3). Two additional sites, A72 and M34, showed non-significant trends for decreased growth and biomass in greater concentrations of stream water.

Winter 1999. Toxicity tests with fathead minnows and amphipods demonstrated seasonal differences in toxicity of stream water from the upper Animas River watershed. In contrast to the minimal toxicity of stream water to fathead minnows in summer 1998, undiluted stream water from three of the four sites tested in winter 1999 (M34, A72, and SMC) caused significant reductions in survival of minnows (Fig. 5b). Survival of minnows was unaffected in undiluted water from A68. Although there were no significant reductions in survival in the 50% dilutions, survival of minnows in water from the three toxic sites followed the same trend for both 100% and 50% dilutions, with greatest toxicity in M34, followed by A72 and SMC. Although there was significant mortality of minnows in the 25% dilution of water from SMC, the absence of a consistent concentration-response trend suggests that this result was not due to toxic components of stream water. Trends in growth and biomass of minnows supported the rankings of toxicity of stream water among sites (Table 4). Biomass of surviving minnows followed the same statistical trends seen in the survival data, except for an additional significant decrease in biomass in the 50% dilution of water from M34. Mean growth of surviving minnows did not differ from controls at any of the dilution levels.

Toxicity tests with amphipods in winter 1999, conducted at four of the five sites tested in summer 1998. Undiluted stream water from A68, A72, and M34 caused 100% mortality of amphipods (Fig. 6). Water from A68 was most toxic to amphipods, as indicated by significant toxicity remaining through the 25% dilution. The toxicity of water from A72 and M34 was intermediate, with reduced but significant toxicity in the 50% dilutions and no significant toxicity in the 25% dilution. In contrast, stream water from the SMC reference site did not cause toxicity except in the undiluted (100%) treatment.

Association of toxicity with metal concentrations

The relatively minor toxicity of sediment from the upper Animas River watershed to midges and amphipods did not always reflect differences in metal concentrations in sediment among sites (Table 5). Sediments from Cement Creek (C48), the most toxic sediment tested in 1997, contained greatest concentrations of Fe, but relatively low concentrations other metals. Sediments from A72 and M34, which showed evidence of toxicity in both years, also contained relatively high concentrations of several metals, but greatest concentrations of total and acid-extractable Zn, Cd, and Pb, and of acid-extractable Cu occurred in sediments from A68, which showed no evidence of toxicity in either year. These data, although collected in two different years, suggest that a substantial fraction of all metals analyzed were associated with acid-soluble sediment phases, presumably hydrous oxides of Fe and Al.

Metal concentrations in pore waters differed widely among sites. Pore-water metal concentrations measured in centrifuged samples in 1997 (Table 6) were consistently less than those measured in peeper samples in 1998 (Table 7). These differences may reflect changes in sediment characteristics during storage (7-10 days) of the 1997 samples before they were centrifuged and analyzed -- for example, freshly-precipitated Fe oxides was evident on the walls of sample containers at the end of the storage period. Pore-water metal concentrations were most consistent between years for sediments from SMC and A73, which had much lower pore-water Fe concentrations in 1998 peeper samples. This suggests that dissolved pore-water metals were lost by sorption to Fe colloids. The toxicity of sediments from C48 in 1977 reflects the highly acidic pH (3.75) and high metal concentrations of C48 pore waters, relative to other 1997 samples. Metal concentrations in the 1998 pore-water (peeper) samples did not differ significantly between sediment-only and sediment-plus-water treatments, so data from these two groups were merged to allow statistical comparisons among sites (Table 7). Pore waters from sites A72 and M34, which showed the most consistent evidence for sediment toxicity (besides C48), contained high concentrations of Fe, but not other metals. In contrast, pore waters from A68, which contained greatest concentrations of Al, Cu, Zn, Cd, and Pb, showed no evidence of toxicity either year.

The toxicity observed in sediments from the upper Animas River watershed was associated with concentrations of labile Fe and Al oxides in sediments and to concentrations of aqueous Fe in sediment pore waters. Although sediments rich in Fe/Al oxides on benthic invertebrates had some toxic effects on amphipods in sediment-only toxicity tests, the presence of sediments in test chambers in water-plus-sediment test was associated with substantial reductions in toxicity, relative to stream water without sediments. However, this observation does not address the loss of habitat for benthic invertebrates due to embeddedness of stream gravels by Fe/Al precipitates.

Differences in the toxicity of stream water among sites and between sampling periods reflected overall trends in dissolved and total metal concentrations in stream water (Table 9). Stream water from M34, which had greatest overall toxicity overall in both seasons, contained greatest concentrations of dissolved and total Al, Fe, and Cu. Although Al was not analyzed in samples collected in winter 1999, other samples from M34 have been found to contain greater concentrations of dissolved Al during winter low-flow conditions, when pH at this site becomes more acidic (Mast et al. 2000b). Water from A72, which was also toxic to both species, contained a similar profile of metals at somewhat lower concentrations. Attenuation of metal concentrations is evident at the downstream site A73, which was toxic to amphipods but not minnows in summer 1998.

Differences in the toxicity of stream water to amphipods and fathead minnows among sites with different mixtures of aqueous metals suggests that these species differ in their sensitivity to individual metals. Water from the Animas River at A68, upstream of Cement and Mineral Creeks, which was highly toxic to amphipods and non-toxic to minnows during both seasons, had consistently high concentrations of dissolved and total Zn and Cd, and lesser concentrations of Al, Fe, and Cu, than other sites tested (Table 8). Water from further upstream at A45, which had greatest concentrations of dissolved Zn and Cd during summer 1998, was also not toxic to minnows. In contrast, sites that were most toxic to fathead minnows (A72 and M34) had consistently high concentrations of Cu and Fe but lower concentrations of Zn. The greater toxicity of stream water to fathead minnows at sites A72 and M34 in winter 1999, relative to summer 1998, was also associated with greater concentrations of Fe and Cu. Zn concentrations at these sites were also greater during winter, but remained less than those measured in water from A68, which was not toxic to minnows. The lack of surface runoff and predominance of ground-water inputs during winter snow cover was also reflected in greater ionic content (conductivity), hardness, and acidity, and reduced alkalinity, especially at A72 and M34 (Table 9).

Water from the reference site, SMC, which was toxic to both fathead minnows and amphipods, did not have elevated average concentrations of any of the metals analyzed. The unexpected toxicity of stream water from SMC to minnows during summer 1998 apparently reflects the influence of a localized thunderstorm in this drainage, which caused high discharge levels and high turbidity during collection of the first water sample from this site (August 25, 1998). Concentrations of all six metals analyzed in this unfiltered sample were two-fold to ten-fold greater than those in the four subsequent samples. Total Al in this sample (1330 µg/L) was greater than the average value for all other sites except M34.

Dissolved and/or total concentrations of several metals frequently exceeded national chronic water quality criteria (WQC) at the sites studied (USEPA 1999; Table 9). Hazards of toxicity of Zn and Cu were strongly suggested by numerous measured concentrations greater than WQC values. Differences in the concentrations of these two metals between sites (e.g. between A68 and A72) and between seasons were associated with differences in the responses of the two test organisms, suggesting that these metals contributed significantly to the observed of toxicity of stream water. Because the toxicity of both Cu and Zn can be modified by ambient water-quality characteristics, we conducted additional toxicity tests with these metals with amphipods, fathead minnows, and brook trout (described below) to develop toxicity thresholds for these metals under water-quality conditions that corresponded closely to ambient conditions in stream waters of the upper Animas River watershed.

Both Al and Fe also exceeded water quality criteria at all sites, except A68, during one or both sampling periods. However, the WQC for Al (87 µg/L as total Al) appears to be highly conservative for waters of circumneutral pH and does not consider the ameliorative effect of hardness (Ca) on Al toxicity (Gensemer and Playle 1999). At circumneutral pH, acute toxicity to fathead minnows and daphnids has been reported in the range 1,900 µg/L to 38,200 µg/L and chronic toxicity to these taxa has been reported in the range 742 µg/L to 3299 µg/L (USEPA 1988). Under acidic, low-Ca conditions (pH ≤5.5, Ca ≤3 mg/L), toxic effects of Al on brook trout (reduced survival and growth, and reduced swimming movements) have been reported in the range, 300-400 µg/l (Cleveland et al. 1986, Mount et al. 1988), but these toxic effects were reduced by small increases in Ca concentrations (Mount et al. 1988, Ingersoll et al. 1990). The influence of the much greater Ca concentrations that commonly occur in the upper Animas River watershed (20-120 mg/L) on Al toxicity have not been studied. Under acidic (pH 4.5-5.5), high-

Ca conditions, Al toxicity to fish is caused by respiratory stress resulting from formation of precipitates of Al hydrous oxides ($Al(OH)_3$) on gill surfaces (Gensemer and Playle 1999). These Al hydrous oxides (as well as Al sulfate-hydrous oxides) are formed in mixing zones in the upper Animas River watershed and may affect fish in downstream sites such as M34 and A72, especially during winter. However, toxic effects of Al in mixing zones tend to be localized, as toxic Al species are formed within seconds after neutralization, but toxicity decreases within minutes after mixing (Verbost et al. 1995).

Hazards of Fe to aquatic biota are also poorly predicted by the Fe WQC (1,000 µg/L, expressed as total Fe) which has not been updated since 1976 (USEPA 1999). Toxicity data for Fe are limited, although acute lethality of Fe (expressed as 96-hr median lethal concentration or LC50) has been reported to the crustacean, *Daphnia magna.* at 9,600 µg/L (Biesinger and Christenson 1972) and to fathead minnows at 1,300 to 7,000 µg/L (Pickering and Henderson 1966). Toxic effects of aqueous Fe on brook trout have been reported to vary widely under different test conditions, with 96-hr LC50s ranging from 410 µg/L to 4450 µg/L as total Fe (Decker and Menendez 1974). The toxicity of particulate Fe to brook trout is low, as indicated by no effects on egg hatchability, survival, or growth in a 90-d exposure to a suspension of 10,500 µg/L of Fe hydrous oxides (Smith and Sykora 1976). Standard methods for separation of dissolved and total metals (filtration with 0.45 µm pore diameter) typically overestimate dissolved Fe concentrations, because they do not remove Fe colloids which can be less than 1 µm diameter (Church et al. 1997). The toxicity of Fe in stream water may be best represented by concentrations of dissolved ferrous ions (Fe^{+2}), which have been found to be an average of 30% of total Fe in slightly acid waters (pH 5-7) and 3% of total Fe in slightly alkaline waters (pH 7-8; Loeffelman et al.1985). Ferrous iron is the predominant dissolved species in highly acidic streams, such as Cement and upper Mineral Creeks, where dissolved ferrous species and particulate Fe hydrous oxides undergo photo-reduction during daylight hours (McKnight et al. 1988), but the importance of this process in circumneutral and slightly acidic waters is not known. The available data suggest that hazards of Fe toxicity in stream waters of the upper Animas River watershed are greatest when elevated concentrations of dissolved Fe coincide with slightly acid conditions, as occurred in winter 1999 samples from sites A72 and M34 (Table 9). Although these conditions coincided with the greatest observed toxicity of stream water to fathead minnows, the additional testing necessary to characterize site-specific thresholds for Fe toxicity under these conditions was beyond the scope of this project.

Toxicity thresholds for zinc and copper

Amphipods and fathead minnows. Survival of *H. azteca* in Animas Soft water decreased sharply with increasing concentrations of both Zn and Cu (Table 10). Treatments that established LOECs for both zinc (250 µg/L) and Cu (100 µg/L) were associated with 70% and 80% mortality, respectively. The narrow range of toxic effect of both metals were reflected in the similar values associated with 25% mortality (IC25) and 50% mortality (LC50). LOECs for survival of fathead minnows occurred at concentrations with relatively high survival (70-80%; Table 11), reflecting the more gradual slope of mortality curves and the lower within-treatment variation in the tests with fathead minnows. Survival LOECs for minnows corresponded quite closely to IC25s for both metals. For Cu, but not Zn, IC25s for growth and biomass (and EC50s, or median effect concentrations, for these endpoints) were lower than corresponding values for survival.

These results demonstrated substantial differences in the sensitivity of these two species to Zn and Cu, with amphipods more sensitive to Zn (LC50=220 µg/L, vs. 704 for minnows) and minnows more sensitive to Cu (LC50=35 µg/L, vs. 79 for amphipods). These differences are consistent with the responses of these species to stream water from different sites during 1998 and 1999. Site A68, which had consistently highest Zn concentrations and relatively low Cu concentrations, was highly toxic to amphipods, but was not toxic to minnows in summer or winter. The most toxic site to minnows was M34, which had greatest concentrations of Cu during both sampling periods. These results also suggest that seasonal differences in concentrations of these metals in stream waters affected the observed toxicity to these species. The effect of seasonal variation in metal concentration is evident for stream water from A72, which did not cause significant toxic effects on minnows during summer 1998, but caused nearly complete mortality and significant reductions in biomass during winter 1999, when total Cu concentrations approached the LC50 and exceeded the EC50 for biomass.

Brook trout. Early life-stages of brook trout were much more sensitive to Cu than to Zn in Animas Hard water (Table 12). Mortality of brook trout during Cu exposures occurred primarily in the period immediately after egg hatching. Although significant reductions in survival (LOEC) occurred at a greater Cu concentrations for brook trout than for minnows, the survival IC25 and LC50 of brook trout for Cu (24 and 29 µg/L, respectively) fell within the range of corresponding values for minnows. The narrow range between LC50 and IC25 for brook trout reflected the steep concentration-mortality curve. LOECs for reduced growth and biomass of brook trout occurred at lower concentrations than for fathead minnows, but IC25 and IC50 (50% inhibition concentrations) values for these responses were similar for the two species. Brook trout were less sensitive to Zn than either amphipods or fathead minnows. The most sensitive response of brook trout to Zn was reduced growth, with a LOEC of 1,000 µg/L (Table

12). However, the reduction in growth, relative to controls, was less than 25% at the highest Zn concentration tested (2,000 µg/L). Survival and biomass of ELS brook trout were not significantly reduced, and showed no clear trend toward decrease, at the highest concentration tested.

Comparisons of the results of our ELS tests with other studies of Zn and Cu toxicity to brook trout suggest that differences in water hardness, such as those occurring seasonally in streams of the upper Animas River watershed, will have a greater effect on thresholds for toxicity of Zn than those for Cu. In June 1999, on-site acute toxicity tests with Zn and Cu were conducted with juvenile brook trout using Animas River water as dilution water (written communication: Patrick Davies and Stephen Brinkman, Colorado Division of Wildlife, Fort Collins CO). Stream water was treated with an ion-exchange column to remove ambient metals before Zn or Cu was added for toxicity testing. The acute toxicity of Zn in these tests (96-hr LC50= 476 µg/L) was greater than that observed in our ELS test with Animas Hard water, which found no significant effect on survival at up to 2,000 µg/L). This difference in sensitivity of Zn between the on-site tests and our laboratory tests is consistent with the differences in hardness between the two test waters (42-45 mg/L as $CaCO_3$ for the Animas River water in June 1999 vs. 180 mg/L in the Animas Hard reconstituted water). Similarly, a published study of chronic Zn toxicity to brook trout in soft water (hardness = 45 mg/L) also found greater sensitivity of brook trout to Zn (significant reduction in survival of embryos and larvae at 1368 µg/L; Holcombe et al. 1979), than was observed in the ELS test with Animas Hard water (no significant effect on survival or embryos or larvae at 2,000 µg/L; Table 12). In contrast, thresholds for toxicity of Cu were remarkably similar between tests with different test waters. The acute toxicity of Cu to brook trout in the 1999 on-site studies (96-hr LC50 = 30 µg/L) was similar to that observed in our ELS test (29 µg/l). A previous study of Cu toxicity to ELS brook trout in soft water (hardness = 45 mg/L) found a LOEC of 17.5 µg Cu/L for both survival and growth (McKim and Benoit 1971), which was similar to the LOECs for growth and survival (6.3 and 25 µg/L, respectively) in our ELS test.

Additional short-term (7-d) toxicity tests in our laboratory provided more evidence of the role of water quality in modifying the toxicity of Zn and Cu to brook trout. These tests found that the toxicity of Zn was greater in the ASTM hard reconstituted water (ASTM 1997a) than in the Animas Hard water. Although these two test water have similar total hardness (180 mg/L), the lesser toxicity of Zn in the Animas Hard water is consistent with the greater concentrations of Ca in this hard water, compared to the ASTM water (Table 2). In contrast, the toxicity of Cu was greater in the Animas Hard water (Table 12). The greater toxicity of Cu in the Animas Hard water may be explained by the greater concentrations of carbonate in the ASTM water. Both formation of Cu-carbonate complexes and competition with Ca for gill binding sites can result in reduced toxicity of Cu (Meyer 1999), and the carbonate concentrations differ more widely between these two test waters (six-fold) than do Ca concentrations (two-fold) (Table 2). These results suggest that hardness (especially Ca concentrations) is a greater influence on the toxicity of Zn, than on the toxicity of Cu in streams of the upper Animas River watershed.

Toxicity thresholds established in the laboratory tests indicate that the organisms used for testing of seasonal toxicity of stream water were appropriate surrogates for identifying conditions that can be tolerated by resident brook trout. Thresholds for effects of Cu on growth and biomass of fathead minnows (both IC25s ≈9 µg/L) and brook trout (IC25s = 8-16 µg/L) were similar and thresholds for both species were at least as sensitive as WQCs for Cu (11 µg/L at a hardness of 120 mg/L, used in the fathead minnow test, and 15 µg/L at a hardness of 180 mg/L, used for the brook trout test; USEPA 1999). Since WQCs are intended to protect 95% of species for which appropriate toxicity data are available (USEPA 1986), these site specific thresholds can be expected to be protective of most sensitive aquatic species in the upper Animas River watershed. Similarly, the threshold for toxic effects of Zn on of fathead minnows should provide a substantial margin of safety for brook trout. Although the low toxicity threshold for Zn to amphipods (IC25 for survival = 183 µg/L in Animas soft water) was greater than the WQC for Zn (140 µg/L at a hardness of 120 mg/L), this threshold should protect most aquatic taxa. After adjusting for differences in hardness, the threshold for Zn toxicity to *H. azteca* would be protective for 89% (32 of 36) of the genera used to derive the WQC for Zn (USEPA 1999).

Modeled toxicity of zinc and copper in stream water

Seasonal variation in toxicity of Zn and Cu in stream water of the upper Animas River watershed reflects seasonal variation in concentrations of dissolved Zn and Cu in stream water, relative to site-specific thresholds for toxicity of these metals. Concentrations of Zn and Cu at the three gaging stations near Silverton, determined by multiple regression models based on discharge and season (Leib and others, in press), varied by approximately a factor of four over the course of an average seasonal cycle, with lowest concentrations in early summer and greatest concentration late winter (Figs. 8 and 9). Lower concentrations during summer reflect the effect of dilution by surface runoff of snow-melt and rainfall, whereas stream flow in winter is dominated by ground-water inflows, which typically contain much greater concentrations of metals (and other dissolved constituents). After adjustment for seasonal variation in hardness at all three sites, toxicity thresholds for Zn and Cu followed similar seasonal trends (Figs. 8 and 9). For Zn, the sensitivity of the three species, based on survival IC25s, varied in the order (from least sensitive to most sensitive): brook trout (no effect on survival at 2,000 µg/L) < fathead minnow < amphipod. For Cu, amphipods were less sensitive than fathead minnows and brook trout, which had similar sensitivity. For the two fish species, thresholds for effects of Cu on growth (IC25s = 8-9 µg/L) were lower than thresholds for survival (IC25s = 17-24 µg/L).

Greatest toxicity of Zn in stream water was predicted for site A68, where dissolved Zn concentrations exceeded hardness-adjusted toxicity thresholds for all three species during late winter and early spring (Fig. 7). Stream water from all three sites greatly exceeded the chronic water quality criteria for Zn and the threshold for reduced survival of amphipods year-round, consistent with our findings of significant mortality of this species at all three sites sampled during both summer and winter. Thresholds for Zn toxicity to fathead minnows were exceeded at A68 by a large margin during late winter and spring, and by a narrow margin at M34 during late winter. Only stream water at A68 approached the threshold (growth LOEC) for effects of Zn on brook trout. These predictions are consistent with the lack of toxicity of water from A68 and A72 to minnows during summer, but not with the absence of toxic effects on minnows in water from A68 during winter 1999. The lower Zn concentrations and lesser Zn toxicity predicted for site M34 suggests that the reduced survival of fathead minnows in stream water from M34 during both summer and winter cannot be attributed to Zn toxicity.

Modeled toxicity of Cu in stream water followed different patterns among the three sites (Fig. 9). Concentrations of dissolved Cu at site A68 were low year-round, well below toxicity thresholds for all three species tested and below chronic water quality criteria, but dissolved Cu concentrations were greater and more variable at both M34 and A72. Modeled Cu concentrations in stream water were greatest at site M34, where dissolved Cu exceeded thresholds for reduced growth and survival of fathead minnows and brook trout year-round. Dissolved Cu concentrations at site A72 were lower, but exceeded thresholds for reduced growth of minnows and trout from December through July. The accuracy of modeled Cu toxicity at A72 and M34 could be affected by uncertainty about the effect of seasonal variation in hardness on toxicity of Cu. If it is assumed that Cu toxicity is independent of hardness, toxicity at these sites would be underestimated during winter months (when thresholds determined at 180 mg/L hardness are adjusted upwards to reflect greater toxicity during winter) and overestimated during low-hardness period in summer. However, the brook trout toxicity thresholds exceeded at each site (survival and growth at M34, growth at A72) would not change.

Toxic units, ratios of modeled metal concentrations to hardness-adjusted toxicity thresholds, reflected differences in sensitivity among taxa, with greater hazards of Zn toxicity to amphipods and greater hazards of Cu toxicity to both fish species (Fig. 10). As a result, hazards to amphipods were greatest at A68, corresponding to greatest Zn concentrations, and hazards to fish were greatest at M34, reflecting greatest Cu concentrations. Seasonal variation in toxic units was reduced, relative to variation in dissolved Zn and Cu concentrations, because toxicity thresholds were adjusted for seasonal variation in hardness, which followed trends similar to those for Zn and Cu. Coefficients of variation (standard deviation as percent of mean) for toxic units over the annual cycle ranged from 8 to 41% for toxic units, compared to 36% to 65% for dissolved metals. However, cumulative toxic units (Zn+Cu) were greater at all three sites during the late winter sampling period than during the late summer sampling period, consistent with the generally greater toxicity of stream water during winter (Table 14). Significant reductions in survival of amphipods and fathead minnows occurred in all samples of stream water with 2.0 or greater cumulative toxic units. In some cases, observed toxicity was greater than or less than that expected from toxic unit calculations, such as the 100% mortality of amphipods at M34 during summer (1.0 toxic units) and the 90% survival of fathead minnows in the same sample (1.84 toxic units).

Both on-site toxicity tests and site-specific toxic unit models indicate that toxicity of Zn and Cu contribute to observed impacts on stream communities at study sites in the upper Animas River watershed. At the three Silverton stream-flow gaging stations, toxic effects of stream water on amphipods and fathead minnows were associated with concentrations of Zn and Cu, separately and in combination, that exceeded site-specific toxicity thresholds. Overall, toxic effects of stream water on these two species corresponded closely to observed impacts on stream biotic communities. Stream water was most toxic at sites with greatest impacts (M34 and A72), intermediate at sites with moderate community impacts (A68, A73), and least at sites with relatively diverse and abundant biotic communities (A45, SMC).

Toxicity thresholds established in laboratory tests indicated that populations of brook trout in streams of the upper Animas River watershed are probably subjected to toxic effects of Cu, but not Zn. Stream water exceeded Cu toxicity thresholds for brook trout at the two Silverton gaging stations where brook trout are absent (A72 and M34). For substantial portions of the years, water from A72 exceeded toxicity thresholds for reduced growth of brook trout, and water from M34 exceeded thresholds for effects on both growth and survival (Fig. 8). At A68, where brook trout have occurred in recent years, Cu concentrations in stream water did not exceed thresholds for Cu toxicity, and Zn concentrations only narrowly exceeded threshold for reduced growth during late winter (Fig. 7).

Conclusions

1. Metals in stream water caused more severe toxic effects on benthic invertebrates than did metals in bed sediments.

2. Observed toxicity of fine stream-bed sediments was associated with elevated concentrations of Fe and Al, but not Cd, Cu, Pb, or Zn, in both sediment and pore water.

3. The toxicity of stream water was reduced when sediments in toxicity test chambers, suggesting that sorption of dissolved metals to Fe/Al oxides or other sediment components may reduce the toxicity of stream water.

4. Stream water was highly toxic to amphipods (*Hyalella azteca*), with significant reductions in amphipod survival occurring at all sites tested in both 1998 and 1999.

5. Stream water was more toxic to fathead minnows (*Pimephales promelas*) during late winter 1999 (pre-snow melt) than in late summer 1998, consistent with greater concentrations of dissolved metals in stream water during winter.

6. Toxicity tests with Zn and Cu in reconstituted 'Animas' water indicated that amphipods were much more sensitive to Zn than minnows; conversely, fathead minnows were more sensitive to Cu than amphipods.

7. Early life stages of brook trout (*Salvelinus fontinalis*), the most widely occurring fish species in the upper Animas River watershed, were also much more sensitive to Cu than to Zn in exposures with reconstituted 'Animas' water.

8. Models of seasonal toxicity of Zn and Cu in stream water were consistent with observed toxicity of stream water to amphipods and minnows, and with patterns of distribution and abundance of brook trout in recent surveys.

9. Toxicity models indicated that dissolved Cu concentrations in the Mineral Creek at Silverton and, to a lesser extent, in the Animas River below Silverton were high enough to cause significant toxic effects on brook trout.

10. Toxicity models indicated that concentrations of dissolved Zn occurring in streams of the upper Animas River watershed are toxic to sensitive taxa of benthic invertebrates, but are not directly toxic to brook trout.

11. The toxicity of Fe and Al may also be a significant influence on populations of brook trout and benthic invertebrates of the upper Animas River watershed, especially in mixing zones of acid and neutral water and for short distances downstream, but additional studies are needed to adequately characterize these effects.

References

-ASTM, 1997a, Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphibians, E729-96, Annual Book of ASTM Standards, Volume 11.05: Philadelphia, PA, American Society for Testing and Materials, pp. 220-240.

ASTM, 1997b, Standard guide for conducting early life-stage toxicity tests with fishes, E1241-92, Annual Book of ASTM Standards, Volume 11.05: Philadelphia, PA, American Society for Testing and Materials, p. 550-577.

Besser, J.M., Brumbaugh, W.G., May, T.W., and others, 2001, Bioavailability of metals in stream food webs and hazards to brook trout (*Salvelinus fontinalis*) in the upper Animas River watershed, Colorado: Archives of Environmental Contamination and Toxicology 40, p. 48-59.

Biesenger, K.E., and Christensen, G.M., 1972, The acute toxicity of some heavy metals to different species of aquatic insects: Journal of the Fisheries Research Board of Canada, v. 29, p. 1691-1700.

Bove, D.J., Mast, M.A., Wright, W.G., and others, 2000, Geological control on acidic and metal-rich waters in the southeast Red Mountains area, near Silverton, Colo., Proceedings of the 5th International Conference on Acid Rock Drainage, vol. 1, pp. 523-533.

Bufflap, S.E., and Allen, H. E., 1995, Sediment pore water collection methods for trace metal analyses: a review: Water Research, v. 29, p. 2051-2054.

Butler, P., Owen, R., Simon, W., and others 2001, Use Attainability Analysis for the Animas River Watershed: Durango, Colorado, Animas River Stakeholders Group.

Campbell, P. G. C., and Stokes, P. M., 1985, Acidification and toxicity of metals to aquatic biota: Canadian Journal of Fisheries and Aquatic Sciences, v. 42, p. 2034-2049.

Church, S.E., Kimball, B.A., Fey, D.L, and others, 1997, Source, Transport, and Partitioning of Metals between Water, Colloids, and Bed Sediments of the Animas River, Colorado, Open-file Report 97-151: Denver, Colorado, U.S. Geological Survey, 135 p.

Cleveland, L., Little, E.E., Hamilton, S.J., and others, 1986, Interactive toxicity of aluminum and acidity to early life stages of brook trout: Transactions of the American Fisheries Society, v. 115, p. 610-620.

Decker, C, and Menendez, R., 1974, Acute toxicity of iron and aluminum to brook trout. Proceedings West Virginia Academy of Sciences, v. 46, p. 159-167.

Farag, A.M., Woodward, D.F., Brumbaugh, W.G., and others, 1999, Dietary effects of metals-contaminated invertebrates form the Coeur d'Alene River, Idaho, on cutthroat trout: *Transactions of the American Fisheries Society*, v. 128, p. 578-592.

Gensemer, R.W., and Playle, R.C., 1999, The bioavailability and toxicity of aluminum in aquatic environments: Critical Reviews in Environmental Science and Technology, v. 29, p. 315-450.

Hamilton, M.A., Russo, R.C., and Thurston, R.V., 1977, Trimmed Spearman-Karber method for estimating median lethal concentrations in toxicity bioassays: *Environmental Science and Technology*, v. 11, p. 714-719.

Henry, T.B., Irwin, E.R., Grizzle, J.M., and others, 1999, Acute toxicity of an acid mine drainage mixing zone to juvenile bluegill and largemouth bass: *Transactions of the American Fisheries Society*, v. 128, p. 919-928.

Holcombe, G.W., Benoit, D.A., and Leonard, E.N., 1979, Long-term effects of zinc exposures on brook trout (*Salvelinus fontinalis*): *Transactions of the American Fisheries Society*, v. 108, p. 76-87.

Ingersoll, C.G., Mount, D.R., Gulley, D.D., and others, 1990, Effects of pH, aluminum, and calcium on survival and growth of eggs and fry of brook trout (*Salvelinus fontinalis*): Canadian Journal of Fisheries and Aquatic Sciences, v. 47, p. 1580-1592.

Kemble, N.E., Brumbaugh, W.G., Brunson, E.L., and others, 1994, Toxicity of metal-contaminated sediment from the upper Clark Fork River, Montana, to aquatic invertebrates and fish in laboratory exposures: *Environmental Toxicology and Chemistry*, v. 13, p. 1985-1997.

Kemble, N. E., Dwyer, F. J., Ingersoll, C. G., and others, 1999, Tolerance of freshwater test organisms to formulated sediments for use as control materials in whole-sediment toxicity tests: Environmental Toxicology and Chemistry, v. 18, p. 222-230.

Kimball, B.A., Callendar, E., and Axtmann, E.V., 1995, Effects of colloids on metal transport in a river receiving acid mine drainage, upper Arkansas River, Colorado, USA: *Applied Geochemistry*, v. 10, p. 285-306.

Leib, K.J., Mast, M.A., and Wright W.G., in press, Characterization of Selected Water-Quality

Constituents and Seasonal Loadings in the Upper Animas River Basin, Southwestern Colorado: U.S. Geological Survey, Water Resources Investigation Reports.

Leppanen, C.J., and Maier, K.J., 1998, An inexpensive and efficient modular water-renewal system for bulf sediment toxicity testing: *Environmental Toxicology and Chemistry*, v. 17, p. 969-971.

Loeffelman, P.H., Van Hassel, J.H., Arnold, T.E.., and others, 1985, A new approach for regulating iron in water quality standards, In: Bahner, R. C., and Hansen, D. J., Eds., Aquatic toxicology and hazard evaluation; eighth symposium, ASTM STP 891: Philadelphia, PA, American Society for Testing and Materials, p. 137-152.

Mast, M.A., Verplank, P.L. Yager Y.B., Wright, W.G., and others, 2000a, Natural sources of metals to surface waters in the upper Animas River watershed, Proceedings of the 5th International Conference on Acid Rock Drainage, vol. 1: Littleton, Colorado, Society for Mining, Metallurgy, and Exploration, pp. 513-522 .

Mast, M. A., Evans, J. B., Leib, K. J., and Wright, W.G., 2000b, Hydrologic and water-quality data at selected sites in the upper Animas River watershed, southwestern Colorado, 1997-99. Open-File Report 00-53: Denver, Colorado, U.S. Geological Survey.

May, T.W., Wiedmeyer, R.H., Brumbaugh, W.G., and others, 1997, The determination of metals in sediment pore waters and in 1N HC l-extracted sediments by ICP-MS: *Atomic Spectroscopy*, v. 18, p. 133-139.

McKim, J.M., and Benoit, D.A., 1971, Effects of long-term exposures to copper on survival, growth, and reproduction of brook trout (*Salvelinus fontinalis*): *Journal of the Fisheries Research Board of Canada*, v. 28, p. 655-662.

McKnight, D.M., Kimball, B.A., and Bencala, K.E., 1988, Iron photoreduction and oxidation in an acidic mountain stream: Science, v. 240, p. 637-640.

Meyer, J.S., 1999, A mechanistic explanation for the ln(LC50) vs. ln(hardness) adjustment equation for metals: Environmental Science and Technology, v. 33, p. 908-912.

Mount, D.R., Mount, D.R., Ingersoll, C.G., and others, 1988, Effect of long-term exposure to acid, aluminum, and low calcium on adult brook trout (*Salvelinus fontinalis*). 1. Survival, growth, fecundity and progeny survival: Canadian Journal of Fisheries and Aquatic Sciences, v. 45, p. 1623-1632.

Nimmo, D.R., Castle, C.J., and Besser, J.M., 1998, A toxicological reconnaissance of the upper

Animas River watershed near Silverton, Colorado (abstract), Nimick, D.A., and von Guerard, P., Science for Watershed Decisions on Abandoned Mine Lands: Review of Preliminary Results, Denver, Colorado, February 4-5, 1998, p. 19.

Norberg-King, T.J., 1993, A linear interpolation method for sublethal toxicity: the inhibition concentration (ICp) approach (Version 2.0): National Effluent Toxicity Assessment Center Technical Report 03-93, U.S. Environmental Protection Agency, Environmental Research Laboratory, Duluth, MN.

Pickering, Q.H., and Henderson, C., 1966, The acute toxicity of some pesticides to fish. Ohio Journal of Science, v . 66, p. 508-513.

SAS Institute, 1990, Statistical Analysis System: SAS/STAT User's Guide, Version 6, Fourth Ed.: Cary, NC, SAS Institute, Inc.

Stumm, W., and Morgan, J. J., 1996, Aquatic chemistry: chemical equilibria and rates in natural waters, 3rd edition: New York, John Wiley and Sons.

Smith, E.J., and Sykora, J.L., 1976, Early developmental effects of lime-neutralized iron hydroxide suspension on brook trout and coho salmon: *Transactions of the American Fisheries Society*, v. 105, p. 308-312.

USEPA, 1986, Quality Criteria for Water - 1986, EPA 440/5-86-110: U.S. Environmental Protection Agency, Office of Water Regulations and Standards, Washington, DC.

USEPA, 1988, Ambient Water Quality for Aluminum - 1988. EPA 440/5-88-008: Washington, DC, U.S. Environmental Protection Agency.

USEPA, 1994, Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, Third Edition, EPA/600/4-91/002: Washington, DC, U.S. Environmental Protection Agency.

USEPA, 1996, 1995 Updates: Water Quality Criteria for the Protection of Aquatic Life in Ambient Water, EPA-820-B-96-001: Duluth, Minnesota, U.S. Environmental Protection Agency.

USEPA, 1999, National recommended water quality criteria -- correction, EPA 822-Z-99-001: Washington, DC, U.S. Environmental Protection Agency.

USEPA, 2000, Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates: EPA 600/R-99/064: Washington DC, U.S. Environmental Protection Agency.

USGS, 2000, Interim report on the scientific investigations in the Animas River watershed, Colorado, to facilitate decisions by the U.S. Bureau of Land Management and the U.S. Forest Service, March 29, 2000 meeting, Denver, Colo. Open-File Report 00-245: Denver Colorado, U.S. Geological Survey.

Verbost, P.M., Berntssen, M.H.G., Kroglund, F., and others, 1995, The toxic mixing zone of neutral a nd acidic river water: Acute aluminum toxicity in brown trout (*Salmo trutta* L.): Water, Air, and Soil Pollution., v. 85, p. 341-346.

Nimick, D. A., and von Guerard, P., 1998, Science for Watershed Decisions on Abandoned Mine Lands: Review of Preliminary Results, Denver, Colorado, February 4-5, 1998: Open-File Report 98-297, U.S. Geological Survey, Denver Colorado

Woodward, D. F., Brumbaugh, W. G., DeLonay, A. J., and others, 1994, Effects on rainbow trout fry of a metals-contaminated diet of benthic invertebrates from the Clark Fork River, Montana: Transactions of the American Fisheries Society, v. 123, p. 51-62.

Zumwalt, D.C., Dwyer, F.J., Greer, I.E., and others, 1994, A water-renewal system that accurately delivers small volumes of water to exposure chambers: *Environmental Toxicology and Chemistry*, v. 13, p. 1311-1314.

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

	Benthic Invertebrates				Trout	
	Number of taxa		Number per m^2		Fish /300M	
Location (Site ID)	1992	1997	1992	1997	1992	1997-98
Upper Animas River						
Above Howardsville (A45)	12	11	163	575	134	327
Above Silverton (A68)*	14	3.3	171	98	23	2
Below Silverton (A72)	9	2.7	20	97	0	0
Elk Park (A73)	12	5.3	84	309	13	23
Tributaries						
Cement Cr. (C48)	1	0	3	0	0	--
Mineral Cr. (M34)	3	1.8	4	24	0	--
S. Mineral Cr. (SMC)	20	15	1090	2769	80	--

* Fish sampled 2 km upstream of A68.

Table 2. Chemical characteristics of stream water in the Animas River near Silverton, Colorado, and of control and dilution waters used for toxicity testing. Values are mean or range of measured values, except nominal concentrations for ionic constituents in reconstituted waters. Sources (for Animas River): Mast et al. 2000b, Butler et al. 2001.

	Animas River		Reconstituted Waters			Well water
	A68	A72	Animas Soft	Animas Hard	ASTM hard	(33% dilution)
Calcium	17-69	15-110	37	55	28	85
Magnesium	1.1-3.5	1.3-6.8	6.1	6.1	24	8.3
Sulfate	28 - 286	36 - 310	91	135	163	16
Sodium	0.9 - 2.9	0.9 - 4.1	4.1	4.1	51	7
Chloride	<1 - 3	<1 - 6	18	18	4	7
pH	6.1- 7.5	4.9-7.2	7.1-7.6	7.2-7.4	7.8 - 8.0	7.5 - 7.9
Total hardness	50-185	20-300	104-121	180-182	160-180	82 - 92
Total alkalinity	16-36	50-130	13-23	16-22	110 - 120	70 - 84
Conductivity	120-430	120-640	310	480	590 - 600	170 - 200

Table 3. Growth of fathead minnows in on-site toxicity tests with stream water, August 1999. Mean growth and total biomass (mg dry wt.) for each treatment group, with standard error (N=6 for controls, N=3 for treatments). Asterisk indicates means significantly less than controls (ANOVA/Dunnett's test).

Site		Growth (mg/fish)			Biomass (mg)		
	Dilution	25%	50%	100%	25%	50%	100%
Control			0.33 (0.03)			3.25 (0.33)	
A45				0.39			3.6
A68		0.44 (0.03)	0.33 (0.02)	0.36 (0.02)	4.1 (0.4)	3.3 (0.2)	3.6 (0.2)
A72		0.44 (0.04)	0.41 (0.03)	0.33 (0.04)	4.3 (0.5)	3.9 (0.4)	3.1 (0.4)
A73		0.38 (0.03)	0.34 (0.03)	0.39 (0.02)	3.7 (0.4)	3.2 (0.5)	3.9 (0.2)
M34		0.38 (0.01)	0.37 (0.03)	0.25 (0.02)	3.5 (0.4)	3.7 (0.3)	2.4 (0.2)
SMC		0.32 (0.02)	0.30 (0.07)	0.13 (0.05)*	3.1 (0.2)	2.6 (0.9)	0.9 (0.4)

Table 4. Growth of fathead minnows in laboratory toxicity tests with stream water, April 1999. Mean growth and biomass (mg dry wt.) for each treatment group, with standard error (N=6 for controls, N=3 for treatments). Asterisk indicates means significantly less than controls (ANOVA/Dunnett's test).

Site		Growth (mg/fish)			Biomass (mg)		
	Dilution	25%	50%	100%	25%	50%	100%
Control			1.03 (0.35)			6.8 (0.9)	
A68		0.68 (0.03)	0.62 (0.02)	0.62 (0.03)	6.1 (0.5)	5.9 (0.1)	5.7 (0.1)
A72		0.63 (0.06)	0.54 (0.04)	0.90 (--)	5.7 (0.6)	5.2 (0.4)	0.90 (--) *
M34		0.55 (0.03)	0.30 (0.05)	--	5.3 (0.4)	2.0 (0.1) *	--
SMC		0.44 (0.04)	0.63 (0.01)	0.32 (0.11)	1.2 (0.1)	5.3 (0.5)	1.2 (0.5) *

Table 5. Concentrations of metals in fine bed sediments from streams of the Upper Animas River watershed, Colorado, 1997 and 1998. Partial extraction (1N HCl, 1% H_2O_2) of samples from September 1997 and 'total recoverable' extraction of samples from late August 1998 (N=1).

Site	Al (%) (Partial / Total)	Fe (%) (Partial / Total)	Cu (µg/g) (Partial / Total)	Zn (µg/g) (Partial / Total)	Cd (µg/g) (Partial / Total)	Pb (µg/g) (Partial / Total)
A68	0.44 / 1.0	1.4 / 2.6	265 / 372	2030 / 2640	11 / 13	1740 / 1790
A72	0.47 / 1.9	2.5 / 8.4	175 / 447	676 / 1520	3.7 / 7.0	782 / 1010
A73	0.44 / 1.2	2.2 / 4.9	174 / 480	830 / 1760	4.1 / 6.8	725 / 815
SMC	0.39 / 1.0	0.87 / 2.6	21 / 19	531 / 297	2.1 / 1.1	246 / 94
M34	0.53 / 1.5	2.5 / 4.7	148 / 156	349 / 905	1.2 / 2.6	221 / 196
C48	0.36	3.8	42	94	0.27	278

Table 6. Concentrations of metals in sediment pore water from streams of the Upper Animas River watershed, Colorado, September 1997. Samples were obtained by centrifugation, followed by filtration (0.45 μm pore diameter).

Site	Metal concentration (μg/L)					
	Al	Fe	Cu	Zn	Cd	Pb
A68	27	57	7.5	226	0.87	1.8
A72	16	nd	4.6	142	0.89	0.7
A73	19	nd	9.7	169	1.3	1.5
SMC	310	112	14	47	2.2	0.26
M34	17	nd	5.2	69	0.63	1.6
C48	3058	417	106	821	3.5	43

Table 7. Concentrations of metals in sediment pore water from streams of the Upper Animas River watershed, Colorado, August 1998. Samples were obtained from 'peeper' samplers (0.45 µm pore diameter). Data from 'sediment only' and 'sediment plus water' treatments was pooled for statistical analysis (see text). Means (N=4), with standard error in parentheses; means followed by the same letter are not significantly different (ANOVA/ Duncan's test).

Site	Metal concentration (µg/L)					
	Al	Fe	Cu)	Zn	Cd	Pb
A68	871 (688)	1608 (1253) b	60 (34) a	946 (261) a	9.3 (0.9) a	165 (111) a
A72	204 (70)	8184 (315) a	5.3 (2.5) b	42 (5.2) c	0.09 (0.04) d	7.6 (3.7) b
A73	121 (40)	298 (99) b	13 (1.6) ab	167 (5.6) b	3.5 (0.1) b	4.0 (1.1) b
SMC	218 (54)	154 (61) b	9.1 (5.7) b	63 (45) c	0.86 (0.24) c	5.5 (1.6) b
M34	137 (56)	34175 (675) a	4.5 (1.1) b	56 (3.0) c	0.07 (0.02) d	2.6 (0.9) b

Table 8. Dissolved and total metal concentrations in stream water of the Upper Animas River watershed, Colorado. Means of samples collected during toxicity tests in 1998 (August 25-September 6; N = 2-5) and 1999 (April 5-8; N=2). Water quality criteria (WQC; USEPA 1996, 1999) are listed for hardness of 100 (for summer 1998) and 200 mg/L (for winter 1999), except where criteria are hardness-independent; all WQC values expressed as total recoverable metals.

Site	Al		Fe		Zn		Cu		Pb		Cd	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
A45	57 108	–	nd 495	–	288 277	--	4.6 8.2	--	0.32 0.87	--	1.3 1.2	--
A68	43 77	–	94 637	nd 201	275 290	659 613	6.4 8.3	2.3 8.4	0.71 3.7	nd 3.0	1.1 1.1	2.1 2.1
A72	42 1129	–	402 1622	2150 4805	175 240	625 574	5.9 19.2	8.3 27	<0.13 5.0	nd 9.2	0.73 0.90	2.3 2.1
A73	61 1062	–	151 1420	--	178 265	--	4.7 18.4	--	<0.47 6.5	--	0.80 0.96	--
SMC	43 363	–	355 502	414 1495	13 31	7 26	3.8 4.7	nd 1.2	0.42 3.6	nd 2.2	0.10 0.18	0.58 0.43
M34	333 1382	–	414 2020	3315 5605	210 180	397 347	6.7 24.8	23 37	0.11 5.4	<0.3 9.6	0.73 0.75	1.9 1.4
WQC	87		1,000		120	216	9.3	16.9	3.2	7.7	2.5	4.2

Table 9. Water quality characteristics in undiluted stream water during toxicity tests. Means of samples collected August 25-September 6, 1998 (N = 4-7) and April 5-8, 1999 (N=2).

	Conductivity (µmhos/cm)		pH		Total Alkalinity (mg/L as $CaCO_3$)		Total Hardness (mg/L as $CaCO_3$)	
Site	1998	1999	1998	1999	1998	1999	1998	1999
A45	160	–	7.37	–	21	–	89	--
A68	203	334	7.64	7.55	31	21	113	152
A72	275	479	7.07	6.36	17	9	150	231
A73	263	–	7.27	–	17	–	145	--
SMC	109	302	7.55	7.06	21	55	59	146
M34	224	448	6.96	5.71	11	6	125	162

Table 10. Toxicity of copper and zinc to amphipods, *H. azteca*, in seven-day toxicity tests in 'Animas Soft' reconstituted water. Means for each treatment group (N=6 for controls, N=2 for treatments), and toxicity thresholds for each endpoint and metal (with 95% confidence intervals where indicated).

Metal and Threshold	Concentration (µg/L)	Survival (%)
Copper	0	80 (0)
	12.5	87 (3)
	25	73 (9)
	50	77 (9)
	100	20 (20)*
	200	7 (7)*
LC50		79 (61-102)
IC25		64 (52-78)
LOEC		100
Zinc	0	80 (0)
	62.5	70 (6)
	125	80 (6)
	250	30 (10)*
	500	0 (0)*
	1,000	0 (0)*
LC50		220 (175-279)
IC25		183 (163-225)
LOEC		250

Table 11. Toxicity of copper and zinc to fathead minnow, *Pimephales promelas*, in seven-day toxicity tests in 'Animas Soft' reconstituted water. Means for each treatment group (N=6 for controls, N=2 for treatments), and toxicity thresholds for each endpoint and metal (with 95% confidence intervals where indicated).

Metal and Threshold	Concentration (µg/L)	Survival (%)	Growth (mg)	Biomass (mg)
Copper	0	98 (2)	0.33 (0.03)	3.3 (0.3)
	6.3	100 (0)	0.32 (0.04)	3.2 (0.4)
	12.5	80 (0)*	0.16 (0.01)*	1.3 (0.1)*
	25	65 (15)*	0.15 (-)*	1.4 (-)
	50	35 (5)*	0.19 (0.04)	0.7 (0.1)*
	100	15 (5)*	0.15 (0.05)*	0.3 (0.2)*
LC50/IC50		35 (25-51)	55	11.4
IC25		17 (14-29)	9.2	8.7
LOEC		12.5	100	50
Zinc	0	98 (2)	0.33 (0.03)	3.3 (0.3)
	125	100 (0)	0.36 (0.08)	3.6 (0.8)
	250	95 (5)	0.38 (0.02)	3.7 (0.4)
	500	70 (0)*	0.48 (0.01)	3.4 (0.1)
	1,000	30 (10)*	0.36 (0.09)	1.0 (0.1)*
	2,000	0 (0)*	--	--
LC50/IC50		704 (542-916)	>1,000	>500
IC25		458 (434-483)	>1,000	>500
LOEC		500	>1,000	1,000

Table 12. Toxicity of copper and zinc to brook trout, *Salvelinus fontinalis,* in early life-stage toxicity tests in 'Animas Hard' reconstituted water. Means for each treatment group (N=4), and toxicity thresholds for each endpoint and metal (with 95% confidence intervals where indicated). Asterisks indicate means that are significantly different from controls (Dunnett's test, p≤0.05).

Metal and Threshold	Concentration (µg/L)	Survival (%)	Growth (mg)	Biomass (mg)
Copper	0	88.3	45.8	601
	6.3	93.3	37.3*	521*
	12.5	96.7	27.4*	396*
	20	68.4*	14.8	151*
	50	3.4*	--	--
	100	0*	--	--
LC50/IC50		29(26-32)	17 (14-19)	17 (16-18)
IC25		24 (21-29)	8.1 (6.4-9.7)	9.8 (7.9-11.2)
LOEC		25	6.3	6.3
Zinc	0	86.7	43.7	565
	125	95	40.5	576
	250	88.4	40.3	533
	500	90	41.0	553
	1,000	100	38.1*	572
	2,000	98.3	36.9*	544
LC50/IC50		>2,000	>2,000	>2,000
IC25		>2,000	>2,000	>2,000
LOEC		>2,000	1,000	>2,000

Table 13. Toxicity of copper and zinc to swim-up brook trout, *Salvelinus fontinalis,* in seven-day toxicity tests in two reconstituted test waters. Means for each treatment group (N=3, except N=6 for controls), and toxicity thresholds for each endpoint and metal (with 95% confidence intervals where indicated). Asterisks indicate means that are significantly different from controls (Dunnett's test, p≤0.05).

Metal and Threshold	Concentration (µg/L)	Survival (%) Animas Hard	Survival (%) ASTM Hard
Copper	0	100	98.3
	6.3	100	100
	12.5	100	96.7
	20	90	100
	50	20*	83.3*
	100	0*	3.3*
LC50		38 (33-43)	64 (58-72)
IC25		31 (24-34)	56 (47-60)
LOEC		50	50
Zinc	0	93.3	96.7
	500	100	100
	1,000	96.7	93.3
	2,000	100	40*
	4,000	60	6.7*
	8,000	3.3*	0*
LC50		5,400 (4,940-5,910)	1,900 (1,600-2,200)
IC25		3,320 (2,510-5,040)	1,350 (1,250-1,550)
LOEC		8,000	

Table 14. Seasonal variation in toxic units for Cu and Zn in stream water from gaging stations near Silverton, Colorado. Toxic units are ratios of modeled Cu and Zn concentrations in stream water on September 1 and April 1 (approximate dates of toxicity tests) to hardness-adjusted toxicity thresholds (IC25s for reduced survival). 'Total' toxic units are sums of Cu and Zn values; values associated with significant reduction in survival are underlined.

		Summer (Sept. 1)			Winter (Apr. 1)		
Species	Site	Cu	Zn	Total	Cu	Zn	Total
H. azteca							
	A68	0.05	2.06	<u>2.11</u>	0.04	3.82	<u>3.86</u>
	M34	0.35	0.65	<u>1.00</u>	0.35	1.46	<u>1.81</u>
	A72	0.05	1.38	<u>1.43</u>	1.14	2.09	<u>3.23</u>
P. promelas							
	A68	0.20	0.82	1.02	0.14	1.53	1.76
	M34	1.58	0.26	1.84	1.43	0.54	<u>1.97</u>
	A72	0.20	0.55	0.75	0.55	0.54	<u>1.09</u>

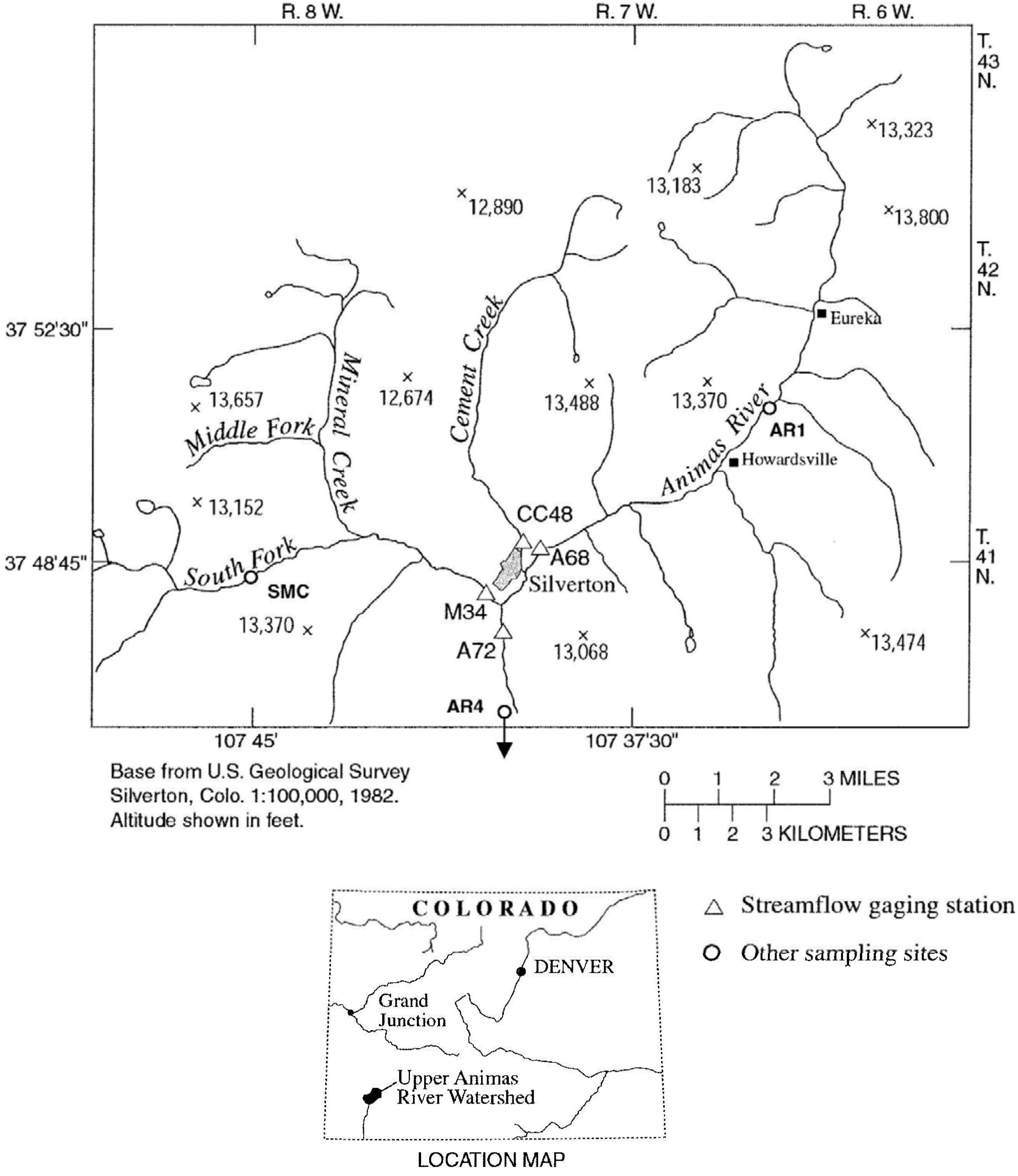

Figure 1. Map of the upper Animas River watershed, Colorado, showing sampling sites. Sites include three USGS gaging stations near Silverton: A68 (=AR2), Animas R. at Silverton; A72 (=AR3), Animas R. below Silverton; C48 (=CC), Cement Creek at Silverton (CC48); and M34 (=LMC) lower Mineral Creek at Silverton.

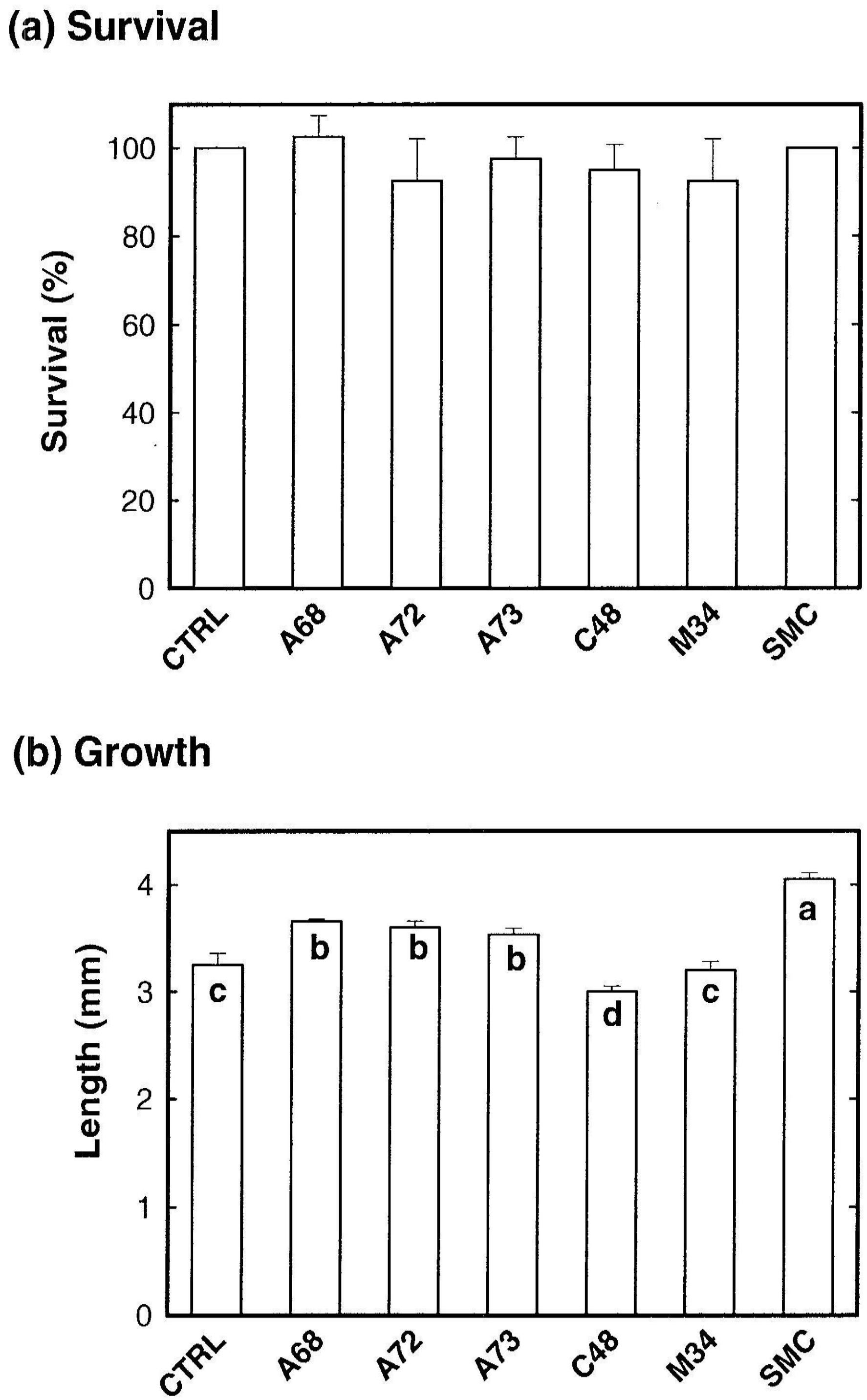

Figure 2. Toxicity of sediments of the Upper Animas River and tributaries to the amphipod, *Hyalella azteca*, September 1997. Means, with standard errors; means with same letter are not significantly different (ANOVA/Duncan's test).

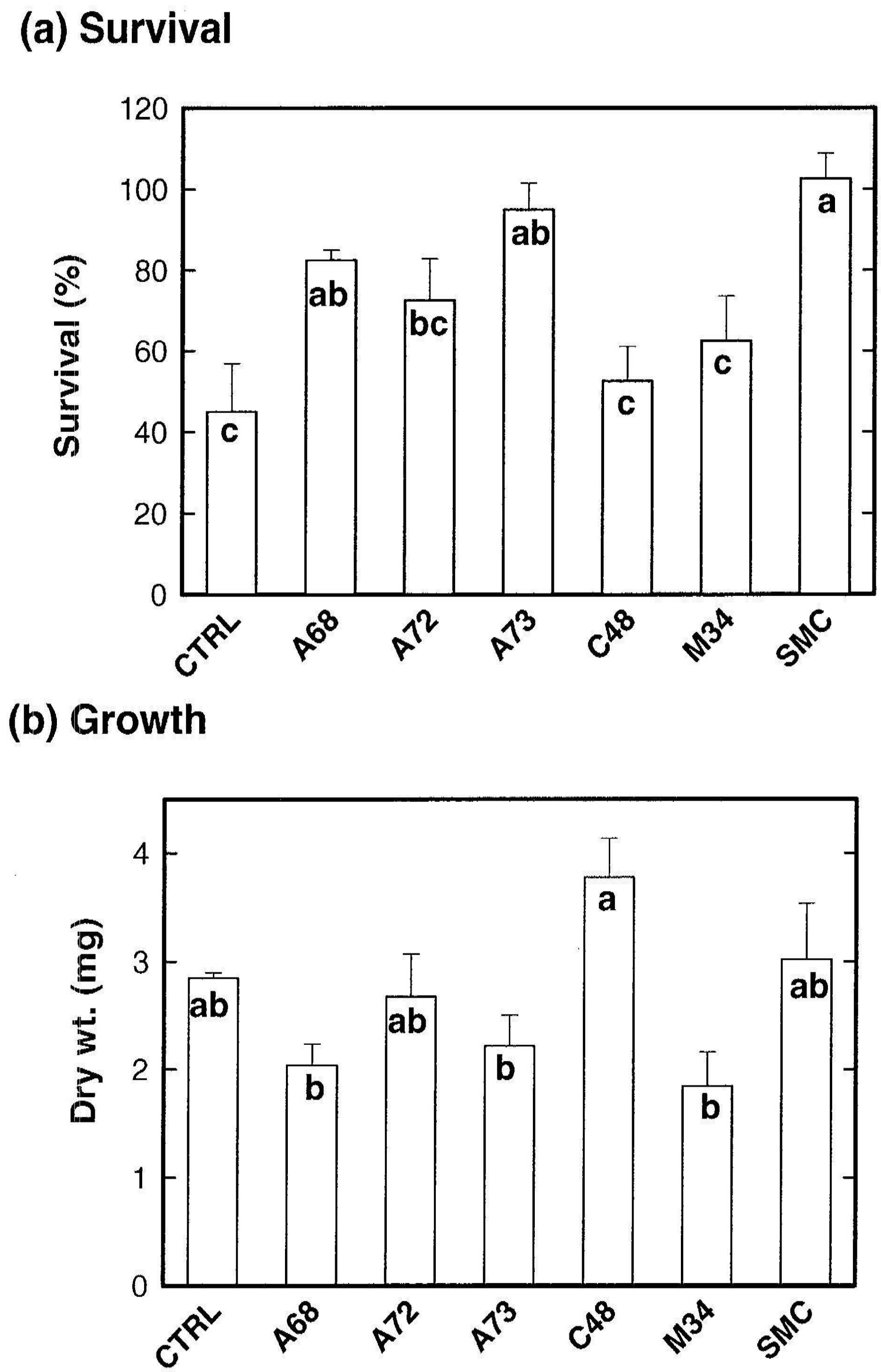

Figure 3. Toxicity of sediments of the Upper Animas River and tributaries to the midge, *Chironomus tentans*, September 1997. Means, with standard errors; means with same letter are not significantly different (ANOVA/Duncan's test).

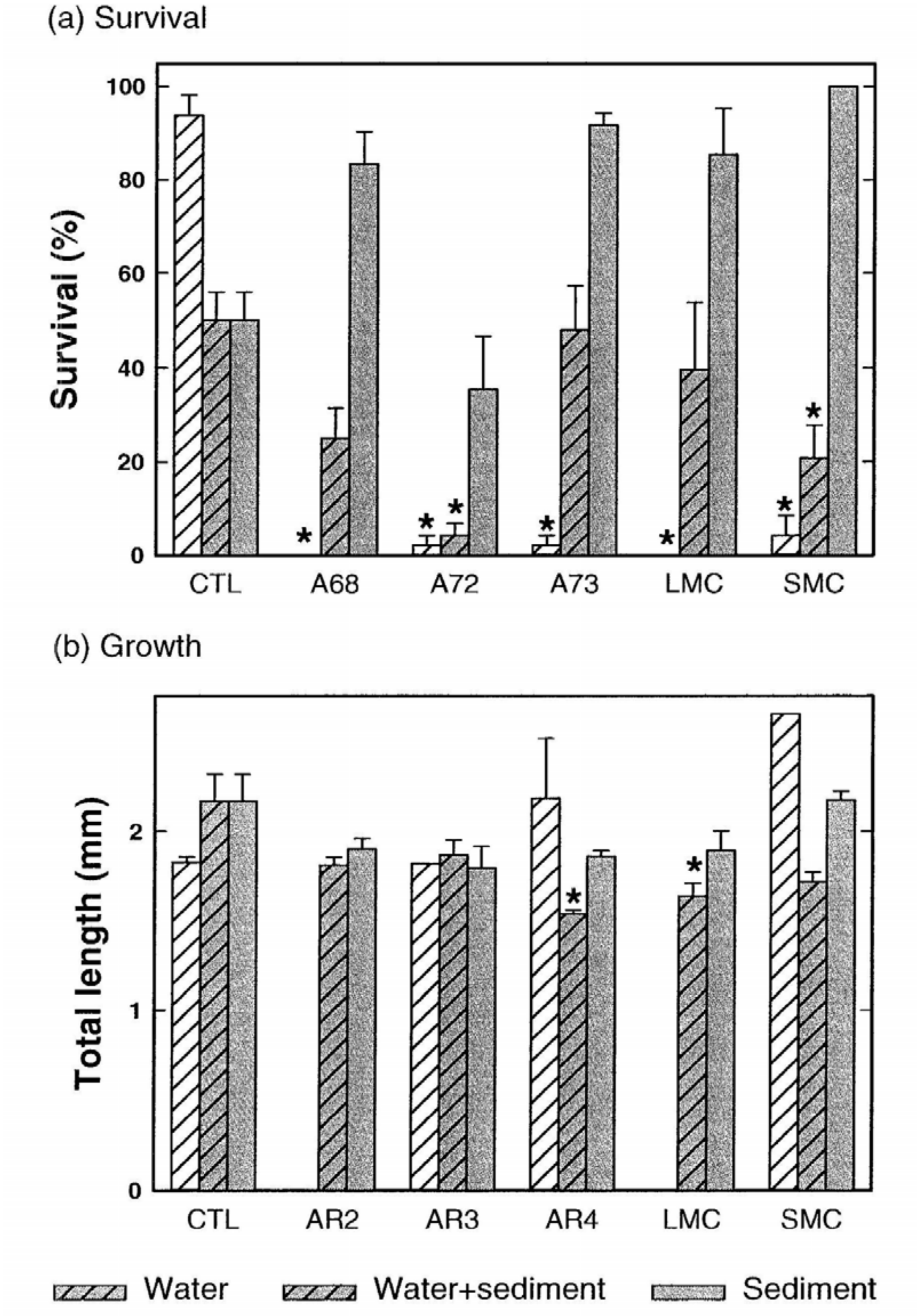

Figure 4. Toxicity of stream water and fine sediment, separately and in combination, to the amphipod, *Hyalella azteca*, August-September 1998. Means, with standard errors; bars with asterisks are significantly less than control (ANOVA/Dunnett's test).

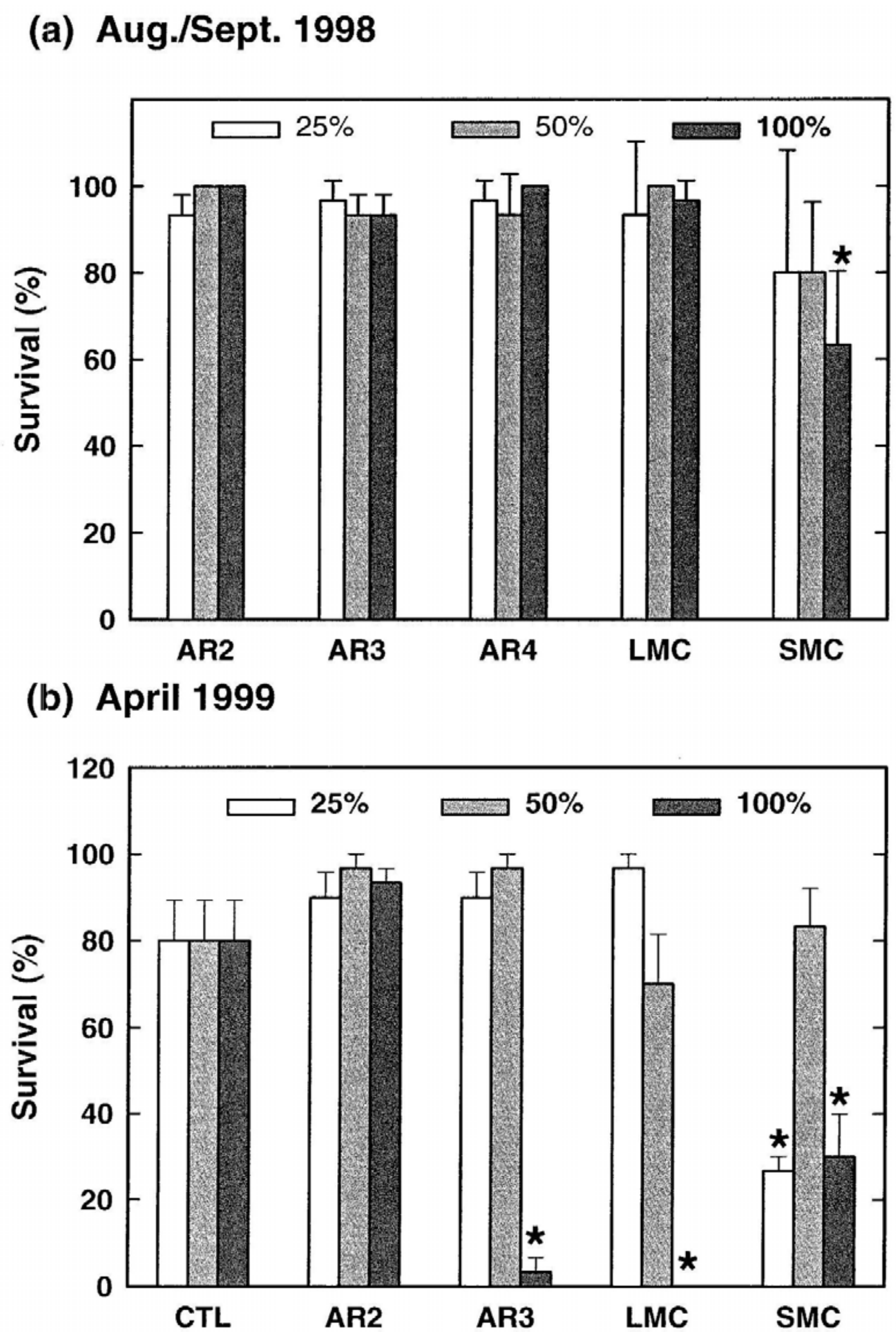

Figure 5. Toxicity of stream water, undiluted (100%) and serial dilutions, to the fathead minnow, *P. promelas*: (a) August-September 1998; (b) April 1999. Means, with standard errors; asterisk indicates significantly less than control (ANOVA/Dunnett's test).

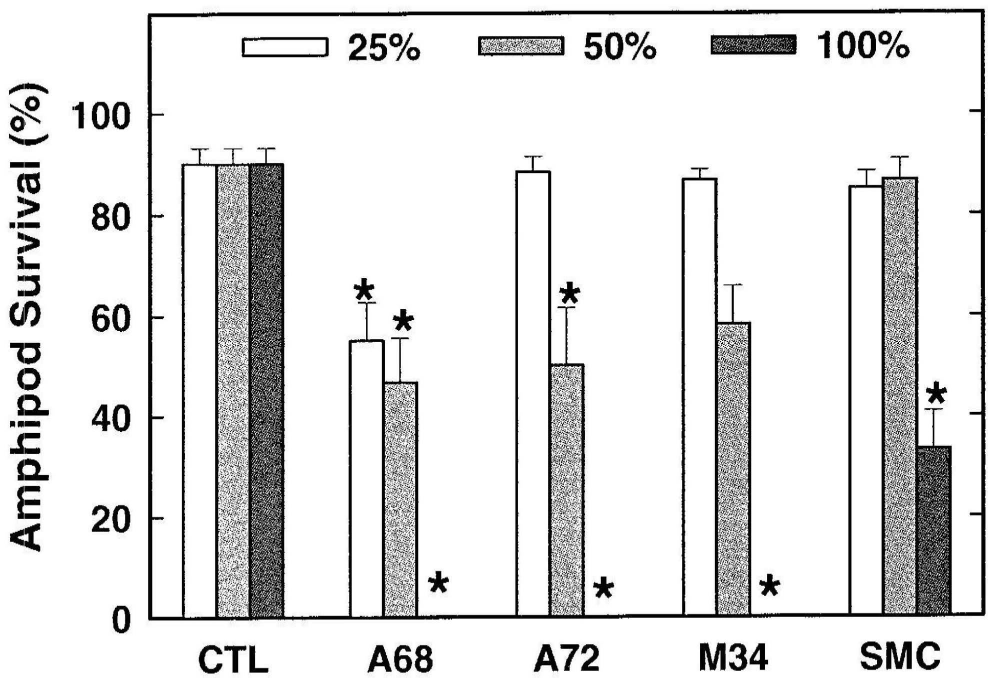

Figure 6. Toxicity of stream water, undiluted (100%) and serial dilutions, to the amphipod, *H. azteca*; April 1999. Means, with standard errors; asterisk indicates significantly less than control (ANOVA/Dunnett's test).

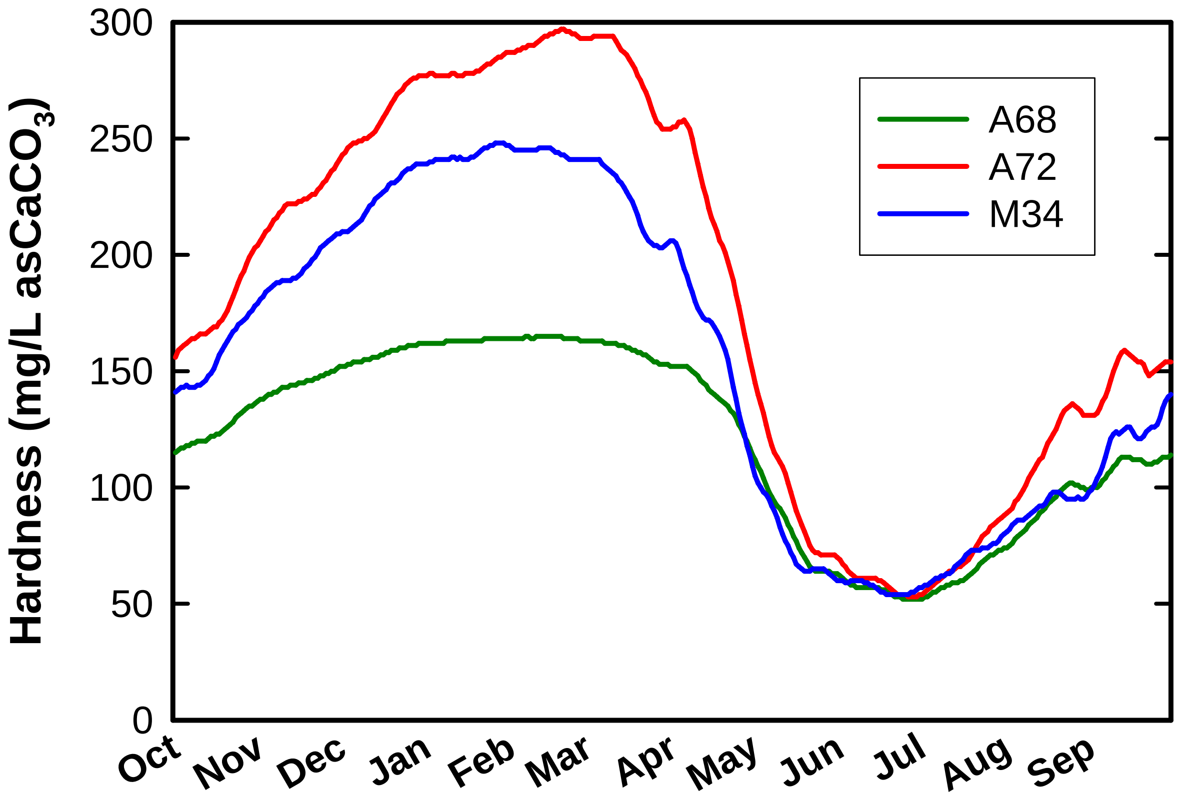

Figure 7. Seasonal variation in hardness at three USGS gaging stations near Silverton, Colorado. A68, Animas River at Silverton; A72, Animas River below Silverton; and M34, Mineral Creek at Silverton.

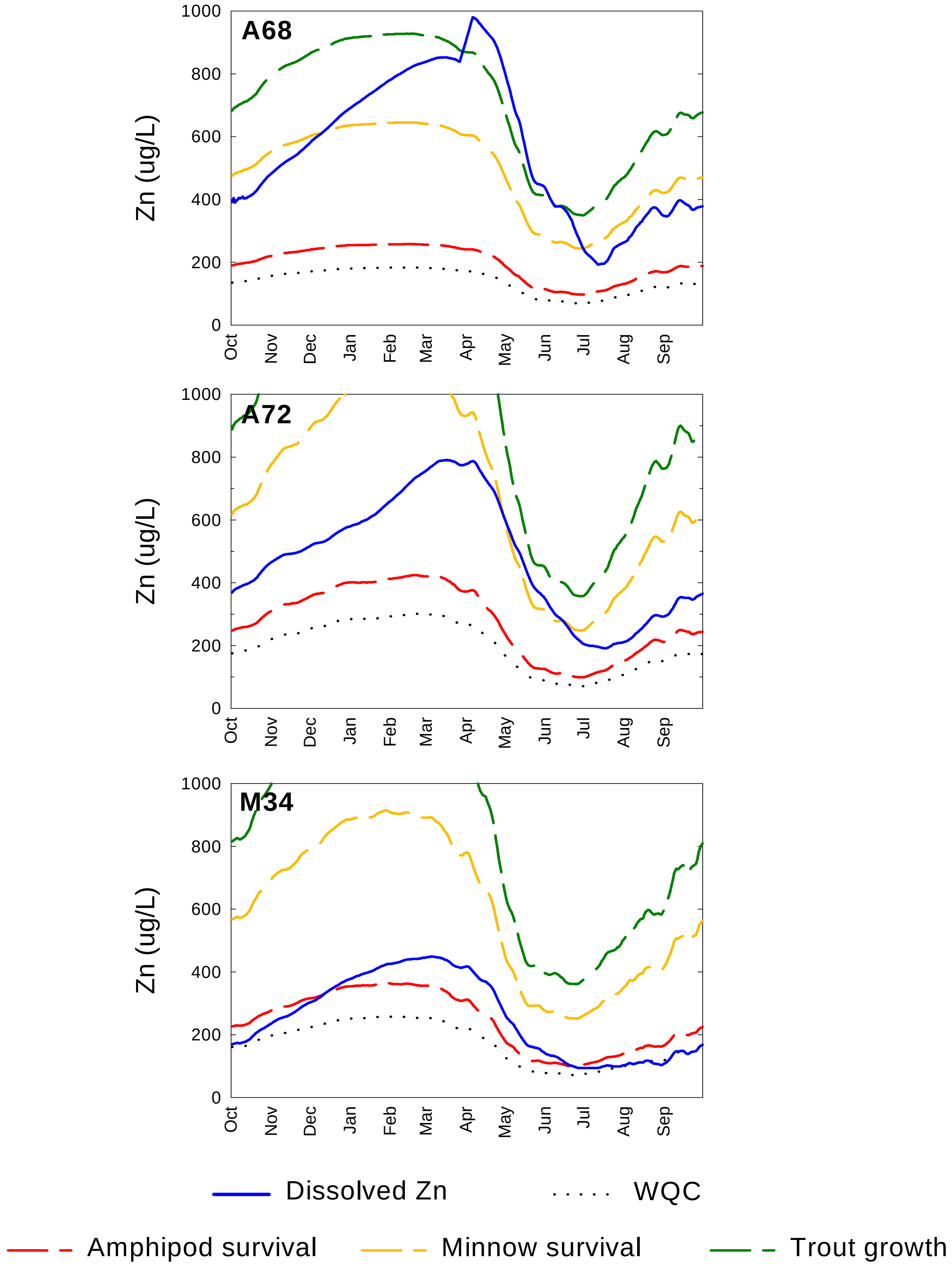

Figure 8. Modeled seasonal variation in dissolved Zn and hardness-adjusted thresholds for Zn toxicity at USGS gaging stations near Silverton, Colorado: A68, Animas River at Silverton; A72, Animas River below Silverton; and M34, Mineral Creek at Silverton.

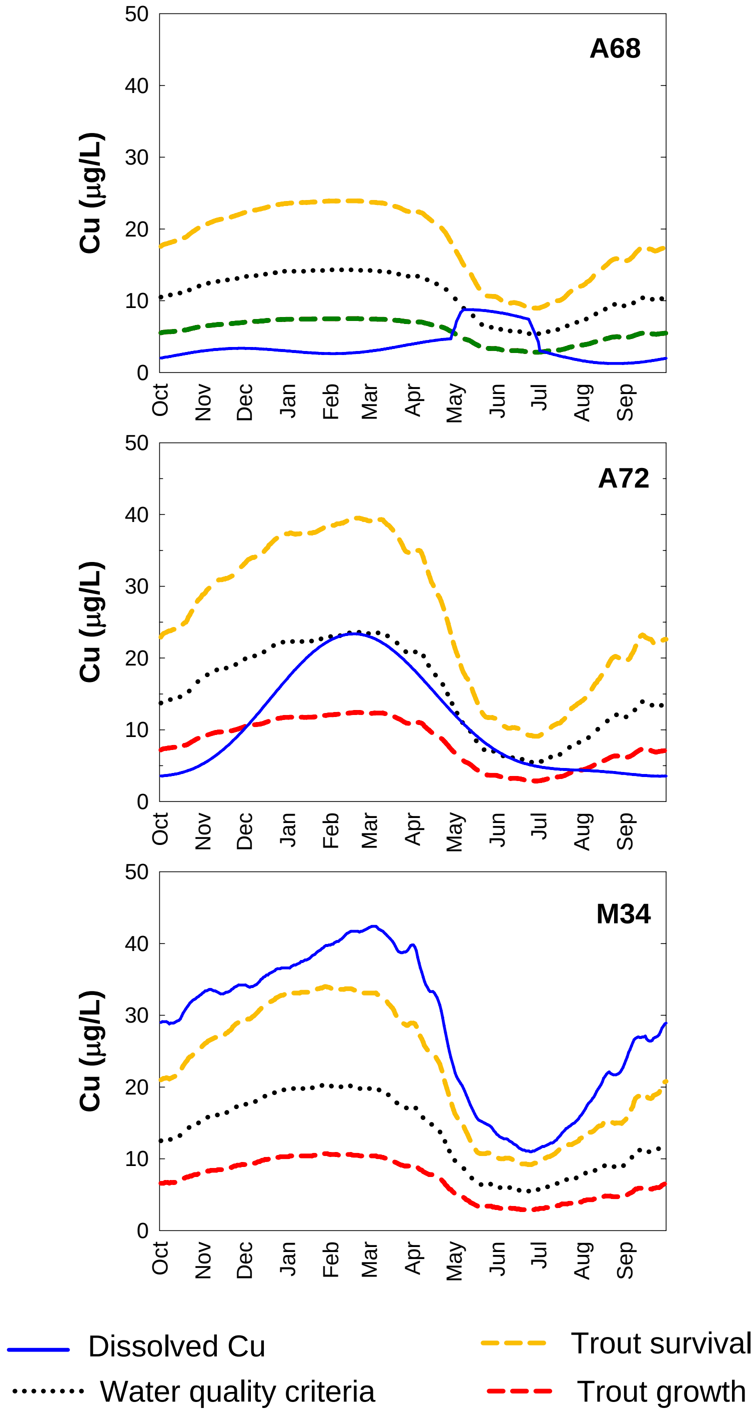

Figure 9. Modeled seasonal variation in dissolved Cu and hardness-adjusted thresholds for Cu toxicity at USGS gaging stations near Silverton, Colorado: !68, Animas River at Silverton; A72, Animas River below Silverton; and M34, Mineral Creek at Silverton.

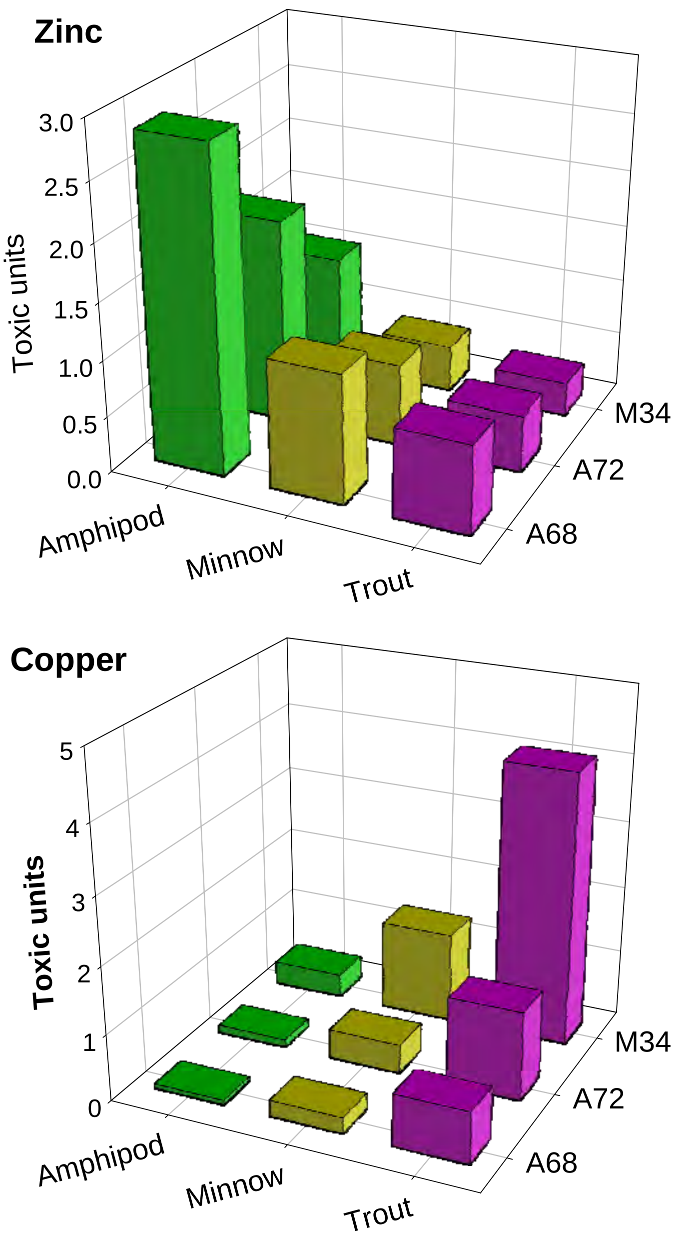

Figure 10. Modeled toxicity of Zn and Cu in stream water at three USGS gaging stations near Silverton, Colorado. Annual mean toxic units for each metal were calculated from ratios of dissolved metal to hardness-adjusted toxicity thresholds for growth of brook trout and survival of fathead minnow and amphipods (IC25s, except Zn LOEC used for brook trout).