Toxicity of Metals in Water and Sediment to Aquatic Biota

By John M. Besser and Kenneth J. Leib

Chapter E19 of

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Abstract

We evaluated the toxicity of metals to aquatic biota of the upper Animas River and the lower reaches of Cement and Mineral Creeks based on toxicity tests with field-collected stream water and sediment, laboratory tests with selected metals of concern, and site-specific models of seasonal metal concentrations and metal toxicity thresholds. Stream water and sediment from several sites were toxic to amphipods, *Hyalella azteca*. However, stream water was less toxic than sediment, and the toxicity of stream water was reduced in the presence of sediment, presumably due to sorption of dissolved metals. The toxicity of stream water to amphipods and fathead minnows, *Pimephales promelas*, differed seasonally: toxicity was greater during late winter and early spring (before snowmelt) than in late spring or summer.

Toxicity of stream water was associated with elevated concentrations of several dissolved metals, especially zinc and copper. Laboratory tests demonstrated that dissolved zinc was highly toxic to amphipods, less toxic to fathead minnows, and least toxic to brook trout, *Salvelinus fontinalis*, the predominant fish species in the Animas River watershed study area. Significant toxic effects of zinc on early life stages of brook trout occurred at concentrations of 960 µg/L or greater. In contrast, both brook trout and fathead minnows were highly sensitive to copper; significant reductions in growth occurred at concentrations less than 10 µg/L. Models of the seasonal toxicity of zinc and copper in stream water were developed for three gauging stations near Silverton, Colorado. These models predicted extended periods of zinc toxicity to amphipods at all three sites and seasonal toxicity of copper to fathead minnows and brook trout in lower Mineral Creek (year-round) and in the Animas River downstream of Silverton (in winter). Predictions of these models were consistent with results of toxicity tests with stream water and with surveys of stream biotic communities, which suggests that dissolved copper concentrations in the study area are a significant limiting factor for brook trout and that dissolved zinc concentrations may limit populations of sensitive invertebrate taxa.

Introduction

Contamination of aquatic environments by acidic drainage and associated toxic metals is a widespread problem in watersheds affected by hard-rock mining (von Guerard and others, this volume, Chapter B). Erosion and weathering can mobilize high concentrations of toxic metals from exposed mineral deposits and altered rock, such as those occurring in the headwaters of Mineral Creek, Cement Creek, and the upper Animas River, but this 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 and others, 2000). Adverse effects of acidity and associated toxic metals on aquatic ecosystems may extend far downstream from sources of acidic drainage. Fish are absent and invertebrate fauna is limited in highly acidic streams (pH <4.5), and adverse effects on aquatic communities are also severe in mixing zones of acidic and neutral-pH waters: examples are the reach of Mineral Creek downstream of the confluence with South Fork Mineral Creek, and the reach of the Animas River downstream of the confluence of Cement Creek (Besser and Brumbaugh, this volume, Chapter E18). Colloidal precipitates of iron and aluminum oxyhydroxides formed upon neutralization of acidic water are typically enriched with high concentrations of potentially toxic metals, and these precipitates may be transported substantial distances downstream (Kimball and others, 1995; Church and others, 1997; Kimball and others, this volume, Chapter E9). Metals may remain in bioavailable forms in water and sediment well downstream of mixing zones, leading to metal bioaccumulation and toxic effects on fish and invertebrates (Kemble and others, 1994; Besser, Brumbaugh, and others, 2001; Besser and Brumbaugh, this volume).

Stream biota of the Animas River watershed study area have been adversely affected by more than a century of mining activities, which came to an end in the early 1990s (Jones, this volume, Chapter C). Recent surveys of aquatic communities in the watershed have documented severe impairment in headwater reaches most affected by acidic drainage, absence of fish in reaches below mixing zones of acidic tributaries, and reduced abundance of fish (principally brook trout) and reduced diversity and abundance of invertebrates in the downstream reach of the Animas River (Besser and Brumbaugh, this volume). In contrast, diverse and productive invertebrate communities and self-sustaining populations of brook trout occur in tributaries that are less impaired by acidic drainage.

Several recent studies have characterized the patterns of metal contamination associated with mining in the Animas River watershed study area. Church and others (1997) and Church, Fey, and Unruh (this volume, Chapter E12) have documented the distribution of elevated metal concentrations in fine bed sediment, relative to premining baseline concentrations. Leib and others (2003) and Wright, Simon, and others (this volume, Chapter E10) have reported that concentrations and loadings of metals varied in response to seasonal patterns of stream discharge (von Guerard and others, this volume, fig. 3). 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 increased, starting in late summer (August–September), reaching annual maxima during low flow in late winter (March–April). Nimmo and others (1998) reported that stream water from Cement Creek was highly toxic to three test organisms (rainbow trout, *Oncorhynchus mykiss*; fathead minnow; and a daphnid, *Ceriodaphnia dubia*) in acute (48 hr) on-site toxicity tests conducted during August 1997 (low flow) and June 1998 (high flow). Patterns of metal bioaccumulation by fish and other components of stream food webs suggested that elevated concentrations of copper and zinc corresponded more closely than concentrations of cadmium and lead to documented effects on invertebrate and fish communities (Besser, Brumbaugh, and others, 2001; Besser and Brumbaugh, this volume).

Purpose and Scope

This chapter describes a series of studies conducted to better define the underlying mechanisms of observed toxicity to aquatic biota of the Animas River watershed and to establish reliable thresholds for recovery. The objectives of these studies were:

- To determine the relative toxicity of stream water and fine bed sediment at sites in the upper Animas River and major tributaries
- To determine the seasonal range of toxicity in stream water
- To establish site-specific thresholds for toxicity of zinc and copper in stream water, and
- To develop models of the contributions of copper and zinc to toxicity of stream water.

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Methods

Toxicity studies focused on the conditions in the Animas River and the lower reaches of Cement and Mineral Creeks, near Silverton, Colo. (fig. 1). Samples for most toxicity tests were collected at four USGS streamflow gauging stations, three of which were located at the downstream end of the three major subbasins (A68, Animas River at Silverton; C48, Cement Creek at Silverton; and M34, Mineral Creek at Silverton); the fourth was located in the Animas River downstream of the confluences of these three streams (A72, Animas River below Silverton). During late summer 1998, additional samples were collected farther downstream on the Animas River at Elk Park (A73; about 7 km downstream of A72). A reference site was selected on the South Fork Mineral Creek (SMC; located downstream of the Forest Service Campground), which supports a self-sustaining population of brook trout and diverse and productive fish and invertebrate communities.

Toxicity Tests with Stream Water and Sediment

Samples of stream water and fine bed sediment for toxicity tests were collected during several sampling periods between fall 1997 and spring 2002. Samples of sediment were collected from all six study sites during fall 1997. Samples of sediment and stream water were collected from five sites (excluding C48) during late summer 1998. Samples of stream water were collected from four sites (excluding C48 and A73) during late winter 1999 (Besser, Allert, and others, 2001). Additional samples of stream water were collected at site A72 in late winter through late spring (March–May), 2002, to characterize toxicity before, during, and after spring snowmelt (Fey and others, 2002).

Figure 1. Location of sampling sites for toxicity studies in Animas River watershed study area.

Toxicity tests were conducted in general accordance with methods published by the U.S. Environmental Protection Agency (USEPA) (1994, 2000). Control and dilution water for these tests was a reconstituted water (hardness 113 mg/L; Besser, Allert, and others, 2001), with an ionic composition representative of late-summer conditions in the Animas River at Silverton (A68). Tests with fathead minnows (*Pimephales promelas*) were stocked with newly hatched larvae (less than 48 hr post-hatch) and lasted 7 days. Exposure chambers for fathead minnow tests were 1,000 mL beakers containing 250 mL of water. Tests with the amphipod, *Hyalella azteca,* were started with 7- to 14-day old individuals and lasted 7 days. Test chambers for amphipod tests were 300 mL beakers: beakers for sediment tests contained 100 mL of sediment and 150 mL of water; those for water-only tests contained 150 mL of water and a layer of fine sand. All toxicity tests were conducted under static-renewal conditions, with one replacement volume of test water added daily.

In 1997, amphipods were exposed to sediment with diluted well water (hardness approx. 140 mg/L as $CaCO_3$) in the laboratory. In 1998, amphipods were exposed to three treatments for each study site: stream water only (no sediment); sediment only (with control water); and sediment plus stream water. Survival of amphipods exposed to field-collected water and sediment were compared to survival in control water and control sediment (CTL, fig. 2). Control sediment was a formulated artificial sediment (Kemble and others, 1999; Besser, Allert, and others, 2001). Additional toxicity tests with stream water (minnows in summer 1998; minnows and amphipods in winter 1999 and winter/spring 2002) were conducted with stream water in a series of dilutions (100 percent, 50 percent, and 25 percent) prepared with reconstituted water.

Toxicity Tests with Zinc and Copper

Toxicity thresholds for zinc and copper were determined in laboratory toxicity tests with amphipods, minnows, and brook trout (*Salvelinus fontinalis*). Tests with amphipods and fathead minnows were conducted following methods similar to those previously described. Tests with both species were conducted for 7 days with endpoints of survival, for both species, and growth (average dry weight per survivor) and biomass (total dry weight of survivors), for fathead minnows. Early-life-stage toxicity tests with brook trout were conducted in general accordance with guidelines published by ASTM (1997), using a hard reconstituted water, designed to reflect ambient water quality during the late fall spawning period for this species (Besser, Allert, and others, 2001). This test water was similar to the reconstituted water used for toxicity tests with stream water, except for greater hardness (180 mg/L as $CaCO_3$), to better match late fall water quality in the Animas River at Silverton. Brook trout tests spanned the eyed egg, sac fry, and swim-up fry life stages (71 days exposure), with endpoints of survival, growth (average dry weight), and biomass.

Modeling Toxicity of Metals in Stream Water

We modeled seasonal variation in toxicity of zinc and copper in stream water for three gauging stations near Silverton (A68, A72, and M34; fig. 1) by comparing modeled dissolved metal concentrations with site-specific toxicity thresholds. Seasonal variation in dissolved metal concentrations in stream water was modeled by multiple regression (Leib and others, 2002). Toxicity thresholds for zinc and copper, determined from laboratory tests, were adjusted to account for the influence of seasonal differences in water hardness on toxicity (USEPA, 1999; Besser, Allert, and others, 2001).

Chemical Analysis

Concentrations of metals in water and sediment were determined by inductively coupled plasma–mass spectroscopy (May and others, 1997; Besser, Allert, and others, 2001). Samples of stream water were analyzed for “total recoverable” metals (unfiltered samples; microwave digestion with nitric acid) and dissolved metals (filtered through 0.45 µm membrane). Pore water was collected for chemical characterization in 1997 and 1998, by centrifugation or with passive diffusion samplers (Bufflap and Allen, 1995). Filtered stream water, unfiltered water samples from laboratory toxicity tests, and pore water were analyzed without digestion. Sediment samples collected in 1997 were subjected to a partial extraction (dilute HCl and hydrogen peroxide; Church, Fey, and Unruh, this volume). Samples collected in 1998 were analyzed for total recoverable metals. Water samples were analyzed for iron, zinc, copper, cadmium, and lead; sediment samples were analyzed for these metals and aluminum.

Statistical Analysis

Data were transformed to improve normality and homogeneity of variance. Analysis of variance (ANOVA) was performed with SAS/STAT software (version 8.2; SAS Institute, Cary, N.C.). Concentration-response curves from toxicity tests were analyzed with ToxStat software (version 3.5; Lincoln Research Associates, Bisbee, Ariz.). Median lethal concentrations (LC50; for survival endpoint) or median effect concentrations (EC50; for other endpoints) were determined by the trimmed Spearman-Karber method (Hamilton and others, 1977). For toxicity tests with zinc and copper, lowest-observed-adverse-effect concentrations (LOECs; the lowest exposure concentration causing statistically significant adverse effect) were determined based on ANOVA, and concentrations associated with 25 percent inhibition of survival or growth (IC25s) were estimated by a linear interpolation technique (USEPA, 1994).

Results and Discussion

Toxicity of Sediment

Stream sediments from the Animas River watershed study area were not highly toxic to the amphipod, *Hyalella azteca*. Sediment toxicity tests conducted in late summer 1997 showed little evidence of toxicity to amphipods, except for slight reductions in growth of amphipods exposed to sediment from site C48 in lower Cement Creek (Besser, Allert, and others, 2001). In tests conducted in summer 1998, survival of amphipods in the artificial control sediment was low (50 percent), but amphipod survival was high (>80 percent) in four of the five field-collected sediments (fig. 2). Survival of amphipods exposed to all field-collected sediments was greater than that in the control, but survival of amphipods in sediment from A72 was significantly less than in sediment from the reference site, SMC.

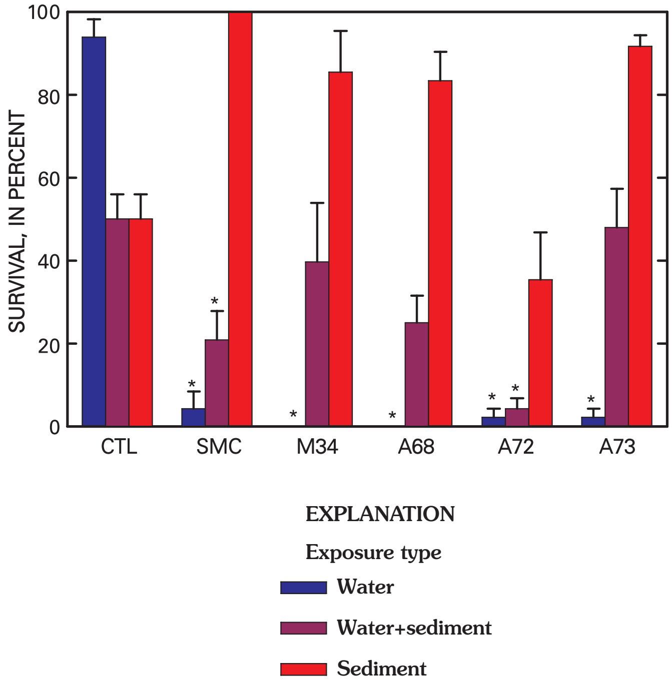

Figure 2. Survival of the amphipod, *Hyalella azteca*, during exposure to stream water and fine sediment, separately and in combination, August–September 1998. Means, with standard errors, n=6 per treatment. Asterisks indicate significant reduction in survival relative to corresponding control group (ANOVA/Dunnett's test).

Concentrations of most metals in stream sediment and pore water did not correspond closely to observed toxic effects on amphipods. Sediments from C48 and A72, both of which were toxic in one of the two years, contained greatest concentrations of sediment iron, but relatively low concentrations of other metals (table 1). Pore water from C48 sediment was acidic and contained highest concentrations of all metals analyzed in 1997 (Besser, Allert, and others, 2001), but greatest concentrations of iron and other metals in pore water were in sediments from A68 or M34, which were not toxic (tables 1 and 2).

Toxicity of Stream Water

Toxicity of stream water to amphipods and fathead minnows followed different spatial and temporal patterns. Stream water was highly toxic to amphipods in all seasons tested. In summer 1998, survival of amphipods in stream water from all five sites was significantly reduced relative to survival in the control water (fig. 2). Mortality of amphipods in site water ranged from 94 percent to 100 percent. Survival of amphipods was generally greater in tests with both stream water and sediment than in those with stream water only, suggesting that stream sediment reduced the toxicity of stream water by sorption of dissolved metals. Undiluted stream water from A68, A72, and M34 also caused 100 percent mortality of amphipods during late-winter low-flow conditions in April 1999 (Besser, Allert, and others, 2001), and water from

Table 1. Concentrations of metals in stream sediment, 1997 and 1998.

[For each site and metal, first value is result of partial extraction (1N HCl, 1 percent H_2O_2), September 1997, and second value is result of total-recoverable extraction, August 1998; n=1; -- indicates no sample]

Site	Aluminum (percent dry weight)	Iron (percent dry weight)	Cadmium (micrograms per gram dry weight)	Copper (micrograms per gram dry weight)	Lead (micrograms per gram dry weight)	Zinc (micrograms per gram dry weight)
A68	0.44 / 1.0	1.4 / 2.6	11 / 13	265 / 372	1,740 / 1,790	2,030 / 2,640
A72	0.47 / 1.9	2.5 / 8.4	3.7 / 7.0	175 / 447	782 / 782	676 / 1,010
A73	0.44 / 1.2	2.2 / 4.9	4.1 / 6.8	174 / 480	725 / 725	830 / 815
SMC	0.39 / 1.0	0.87 / 2.6	2.1 / 1.1	21 / 19	246 / 246	531 / 94
M34	0.53 / 1.5	2.5 / 4.7	1.2 / 2.6	148 / 156	221 / 221	349 / 196
C48	0.36 / --	3.8 / --	0.27 / --	42 / --	278 / --	94 / --

Table 2. Concentrations of metals in sediment pore water, August 1998.

[Metal concentrations in micrograms per liter; means (n=4), with standard error in parentheses. For each metal, means with the same superscript letter are not significantly different (analysis of variance, with Duncan's multiple-range test)]

Site	Aluminum	Iron	Cadmium	Copper	Lead	Zinc
A68	871 (688)^{a}	1,610 (1,253)^{b}	9.3 (0.9)^{a}	60 (34)^{a}	165 (111)^{a}	946 (261)^{a}
A72	204 (70)^{a}	8,180 (315)^{a}	0.09 (0.04)^{d}	5.3 (2.5)^{b}	7.6 (3.7)^{b}	42 (5.2)^{c}
A73	121 (40)^{a}	298 (99)^{b}	3.5 (0.1)^{b}	13 (1.6)^{ab}	4.0 (1.1)^{b}	167 (5.6)^{b}
SMC	218 (54)^{a}	154 (61)^{b}	0.86 (0.24)^{c}	9.1 (5.7)^{b}	5.5 (1.6)^{b}	63 (45)^{c}
M34	137 (56)^{a}	34,200 (675)^{a}	0.07 (0.02)^{d}	4.5 (1.1)^{b}	2.6 (0.9)^{b}	56 (3.0)^{c}

A72 caused 98–100 percent mortality of amphipods in three tests conducted before, during, and after spring snowmelt in 2002 (Fey and others, 2002). In contrast, stream water from most sites tested showed seasonal differences in toxicity to fathead minnows. In summer 1998, survival of minnows exposed to stream water was significantly reduced, relative to survival in control water, by undiluted stream water from only one site (SMC) of the five tested. In contrast, undiluted stream water from three of the four sites tested in late winter 1999 (M34, A72, and SMC) caused significant reductions in survival (fig. 3). Survival of minnows was unaffected by water from A68 during both seasons.

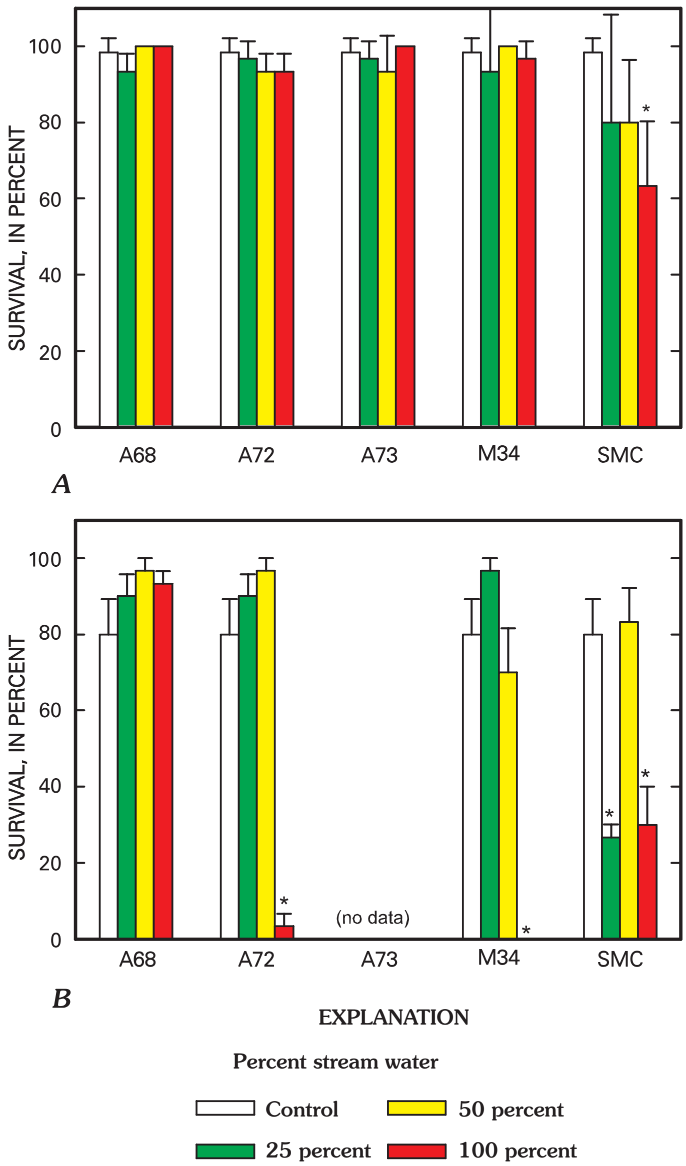

Figure 3. Survival of the fathead minnow, *Pimephales promelas*, during exposures to stream water and dilutions. *A*, August–September 1998; *B*, April 1999. Means, with standard errors; *n*=3 per treatment. Asterisks indicate significant reduction in survival relative to corresponding control group (ANOVA/Dunnett's test).

Differences in the toxicity of stream water among sites, test organisms, and sampling periods reflected overall trends in dissolved and total metal concentrations in stream water. Stream water from the most toxic site, M34, contained greatest concentrations of dissolved and total aluminum, iron, and copper (table 3). Water from A72, which was also toxic to both minnows and amphipods, contained a similar mixture of metals at somewhat lower concentrations. Attenuation of metal concentrations is evident at the downstream site A73, which was toxic to amphipods but not to fathead minnows in summer 1998. In addition, the relative toxicity of stream water to amphipods and minnows differed among sites. Water from A68, which was highly toxic to amphipods but not to minnows, had consistently high concentrations of dissolved and total zinc and cadmium, and lesser concentrations of copper, aluminum, and iron (table 3). In contrast, sites that were most toxic to minnows (A72 and M34) had consistently high concentrations of copper and iron and lower concentrations of zinc. The greater toxicity of stream water at A72 and M34 to fathead minnows in spring 1999, relative to summer 1998, was also associated with greater concentrations of iron and copper. The toxicity of water from SMC to minnows and amphipods during summer 1998 was associated with transient elevated concentrations of several metals, notably aluminum, apparently reflecting the influence of a localized thunderstorm during this test (Besser, Allert, and others, 2001). Although the South Fork Mineral Creek watershed has had a low level of mining activity compared to the rest of the study area, there is at least one large inactive mine upstream from this study site that could cause periodic impairment of water quality (Bandora mine, site # 332; Church, Mast, and others, this volume, Chapter E5).

Dissolved and (or) total concentrations of several metals in stream water frequently exceeded national chronic water-quality criteria (WQC) at the sites studied (USEPA, 1999; table 3). Hazards of toxicity due to dissolved zinc and copper were strongly suggested by numerous measured concentrations exceeding WQC values, and differences in toxicity among seasons and between species were consistent with variation in concentrations of these two metals. Concentrations of both aluminum and iron also frequently exceeded WQC values at all sites except A68. However, the potential toxic effects of waterborne aluminum and iron at these sites are uncertain. Relatively few studies have evaluated the toxicity of these metals, and most of these studies were conducted with dilute test water that differed substantially from the typical water quality of the study watershed. The greatest hazard of toxicity for these metals is associated with mixing zones of acidic and neutral water. The toxicity of aluminum to brook trout is generally thought to be greatest at pH 5.0–5.5, with toxicity thresholds in the range 300–400 µg/L (for example, Cleveland and others, 1986; Mount and others, 1988). However, toxicity of aluminum has been shown to be ameliorated by increased calcium concentrations, and most studies of aluminum toxicity have been conducted in water with calcium concentrations much lower than those typical of the Animas River watershed study area. The toxicity of aluminum in mixing zones seems to be associated with formation of oxyhydroxide colloids, but

Table 3. Dissolved and total metal concentrations in stream water during toxicity tests, August–September 1998 and April 1999.

[Metal concentrations in micrograms per liter; dissolved (filtered) samples are shown in italic bold; means, *n*=2–5 for 1998, *n*=2 for 1999. Water-quality criteria (WQC; USEPA, 1999) are listed for hardness of 100 mg/L for 1998 and 200 mg/L for 1999, except criteria for Al and Fe are independent of hardness; nd, not detected; --, no sample]

Site	Aluminum		Iron		Zinc		Copper		Lead		Cadmium	
	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999	1998	1999
A68	***43***		***94***	***nd***	***275***	***659***	***6.4***	***2.3***	***0.71***	***nd***	***1.1***	***2.1***
	77	--	637	201	290	613	8.3	8.4	3.7	3.0	1.1	2.1
A72	***42***		***402***	***2,150***	***175***	***625***	***5.9***	***8.3***	***<0.13***	***nd***	***0.73***	***2.3***
	1,129	--	1,622	4,805	240	574	19.2	27	5.0	9.2	0.90	2.1
A73	***61***		***151***		***178***		***4.7***		***<0.47***		***0.80***	
	1,062	--	1,420	--	265	--	18.4	--	6.5	--	0.96	--
SMC	***43***		***355***	***414***	***13***	***7***	***3.8***	***nd***	***0.42***	***nd***	***0.10***	***0.58***
	363	--	502	1,495	31	26	4.7	1.2	3.6	2.2	0.18	0.43
M34	***333***		***414***	***3,315***	***210***	***397***	***6.7***	23	***0.11***	***<0.3***	***0.73***	***1.9***
	1,382	--	2,020	5,605	180	347	24.8	37	5.4	9.6	0.75	1.4
WQC	87		1,000		120	216	9.3	16.9	3.2	7.7	2.5	4.2

these effects tend to be very localized as toxicity of newly formed colloids decreases rapidly (Verbost and others, 1995). Toxicity data for iron are also limited, and effects of dissolved iron on brook trout have been reported to vary widely under different test conditions. Toxicity of particulate iron to brook trout is low, and standard method for separation of dissolved metals (0.45 µm filtration) does not adequately separate iron colloids from truly dissolved metal iron (Church and others, 1997; Kimball and others, this volume). Measurements of filtered iron concentrations therefore tend to overestimate concentrations of dissolved iron. Toxicity of iron in stream water may be best represented by concentration of dissolved ferrous ions (Fe^{+2}), the predominant dissolved iron species in highly acidic streams, such as Cement Creek and upper Mineral Creek. Available data suggest that hazards of iron toxicity in the Animas River watershed study area are also associated with elevated concentrations of dissolved iron under slightly acidic conditions, such as winter conditions at A72 and M34 (table 3).

Toxicity Thresholds for Zinc and Copper

Toxicity thresholds determined from laboratory tests with zinc and copper are consistent with the observed differences in toxicity of stream water among species and seasons. Zinc was more toxic to amphipods than to fathead minnows, but copper was more toxic to minnows than to amphipods (table 4). These differences are consistent with the responses of these species to stream water, as water from A68 (highest zinc, low copper) was not toxic to minnows, whereas water from M34 (highest copper, low zinc) was highly toxic to minnows (figs. 2 and 3). The effect of seasonal variation in metal concentration is evident from repeated tests with fathead minnows exposed to stream water from A72, which was not toxic during late spring and late summer (1998 and 2002), but was highly toxic during late winter and early spring (1999 and 2002), when copper concentrations approached the LC50 for this species.

Toxicity tests with brook trout established chronic toxicity thresholds for the early life stages that are present in the Animas River watershed study area during winter low-flow conditions. Brook trout spawn in late fall, but hatching probably occurs during winter or early spring, the period of lowest stream flow and highest dissolved metal concentrations (Leib and others, 2003). Brook trout, like fathead minnows, were more sensitive to copper than to zinc (table 4). No effects on survival, and less than 25 percent reduction in growth, were observed at the highest zinc concentration tested (2,000 µg/L). The low sensitivity of brook trout to zinc in this test, which was conducted in hard water (180 mg/L), contrasts with tests conducted in softer water. A previous study found that early life stages of brook trout were more sensitive to zinc in soft water (LOEC for survival=1,368 µg/L at a hardness of 45 mg/L; Holcombe and others, 1979). Published thresholds for chronic toxicity of copper are more similar between tests with different test waters. A previous chronic study with brook trout in soft water found LOECs for survival and growth (17.5 µg Cu/L at hardness of 45 mg/L; McKim and Benoit, 1971) that were similar to those from our test with hard water (LOECs from 6 to 25 µg Cu/L; table 4). Additional short-term (7 day) toxicity tests conducted in our laboratory support the hypothesis that hardness, especially calcium, has a greater influence on the toxicity of zinc than on the toxicity of copper (Besser, Allert, and others, 2001).

Toxicity thresholds determined from our laboratory tests indicate that the organisms used for testing the seasonal toxicity of stream water were appropriate surrogates for determining conditions that can be tolerated by resident aquatic biota of the Animas River watershed (table 4). Thresholds for copper toxicity were similar for fathead minnows and brook trout. The threshold for toxic effects of zinc on fathead minnows provides a substantial margin of safety for brook trout. Amphipods are much more sensitive to zinc than brook trout, and the available toxicity literature suggests that the threshold for zinc toxicity to amphipods should protect a large proportion of aquatic invertebrate taxa (USEPA, 1999).

Table 4. Toxicity thresholds for copper and zinc from laboratory tests.

[Hardness of test water=113 µg/L. LOEC, lowest observed effect concentration; IC25, 25 percent inhibition concentration; EC50, median (50 percent) effect concentration. See text and Besser, Allert, and others (2001) for derivation of toxicity thresholds]

Species	Endpoint	Copper (µg/L)			Zinc (µg/L)		
		LOEC	IC25	EC50	LOEC	IC25	EC50
Amphipod (*H. azteca*)	Survival	100	64	79	250	183	220
Fathead minnow (*P. promelas*)	Survival	12.5	17	35	500	458	704
	Growth	100	9.2	55	>1,000	>1,000	>1,000
	Biomass	50	8.7	11.4	500	>1,000	>1,000
Brook trout (*S. fontinalis*)	Survival	25	24	29	>2,000	>2,000	>2,000
	Growth	6.3	8.1	17	1,000	>2,000	>2,000
	Biomass	6.3	9.8	17	>2,000	>2,000	>2,000

Modeled Toxicity of Zinc and Copper

Seasonal variation in toxicity of stream water of the Animas River watershed reflects seasonal variation in concentrations of dissolved zinc and copper, relative to site-specific toxicity thresholds for these metals. Concentrations of zinc and copper typically followed similar seasonal patterns at the three gauging stations near Silverton (fig. 1), with lowest concentrations in early summer and greatest concentrations in late winter (fig. 4; Leib and others, 2003). The only exception was dissolved copper at site A68, Animas River at Silverton, which remained at very low concentrations during the entire low-flow period (summer through early spring) but showed a moderate increase during high flows in late spring. Thresholds for toxicity of zinc and copper to brook trout and amphipods (table 4), adjusted for seasonal variation in hardness using the regressions published by USEPA (1999; Besser, Allert, and others, 2001), followed similar seasonal trends (figs. 4 and 5). Modeled thresholds for toxicity of copper and zinc at all three sites reached minima in late spring, corresponding to the annual minimum for water hardness.

Greatest toxicity of zinc in stream water was predicted for site A68, where dissolved zinc concentrations exceeded hardness-adjusted toxicity thresholds for all three species during late winter and early spring (fig. 4). Stream water from all three sites greatly exceeded both the chronic water-quality criteria for zinc and the threshold for toxic effects of zinc on survival of amphipods year-round (table 4; USEPA, 1999), consistent with the findings of our toxicity tests. Only stream water at A68 approached the threshold for chronic toxicity of zinc on brook trout. These predictions are consistent with the absence of toxic effects in fathead minnows exposed to water from A68 and A72 during late spring and summer. The lesser zinc toxicity predicted for site M34 suggests that reduced survival of fathead minnows in stream water from M34 during both summer and winter cannot be solely attributed to zinc toxicity.

Modeled toxicity of copper in stream water followed different patterns among the three sites (fig. 5). Dissolved copper concentrations were greater and had more seasonal variability at both M34 and A72. Concentrations of dissolved copper at A68 were below toxicity thresholds and water-quality criteria year-round, except for a brief period during early spring snowmelt. Modeled copper concentrations in stream water exceeded toxicity thresholds for brook trout year-round at M34, during winter and spring at A73, and during late spring at A68. Because the threshold for chronic toxicity of copper to brook trout may not be strongly influenced by hardness, the severity of the risks associated with exceeding toxicity thresholds for copper may differ among the three sites. Toxic effects were predicted for brook trout at A72 and M34 during periods of high water hardness (greater than that in toxicity tests), when hardness-adjusted toxicity thresholds may underestimate actual toxicity hazards. In contrast, dissolved copper concentrations at A68 exceeded toxicity thresholds for brook trout only during snowmelt, the annual minimum for hardness, when hardness-adjusted toxicity threshold may over-predict toxicity.

Toxic units, ratios of modeled metal concentrations to hardness-adjusted toxicity thresholds, indicated greater hazards of zinc toxicity to amphipods and greater hazards of copper toxicity to both fish species. Toxic units of 1.0 and greater indicate that dissolved metal concentrations exceed hardness adjusted toxicity thresholds (Besser, Allert, and others, 2001). The sum of toxic units for copper and zinc, an estimate of potential additive toxicity of these metals, was greater during late winter than during the late summer, consistent with observed seasonal differences in toxicity. Cumulative hazards to amphipods were greatest at A68, primarily due to high zinc concentrations, and hazards to trout were greatest at M34, primarily due to high copper concentrations. Hazards to trout, estimated from cumulative toxic units, were only slightly greater at A72, where trout are absent, than at A68, where both adult trout and juveniles have been collected in recent

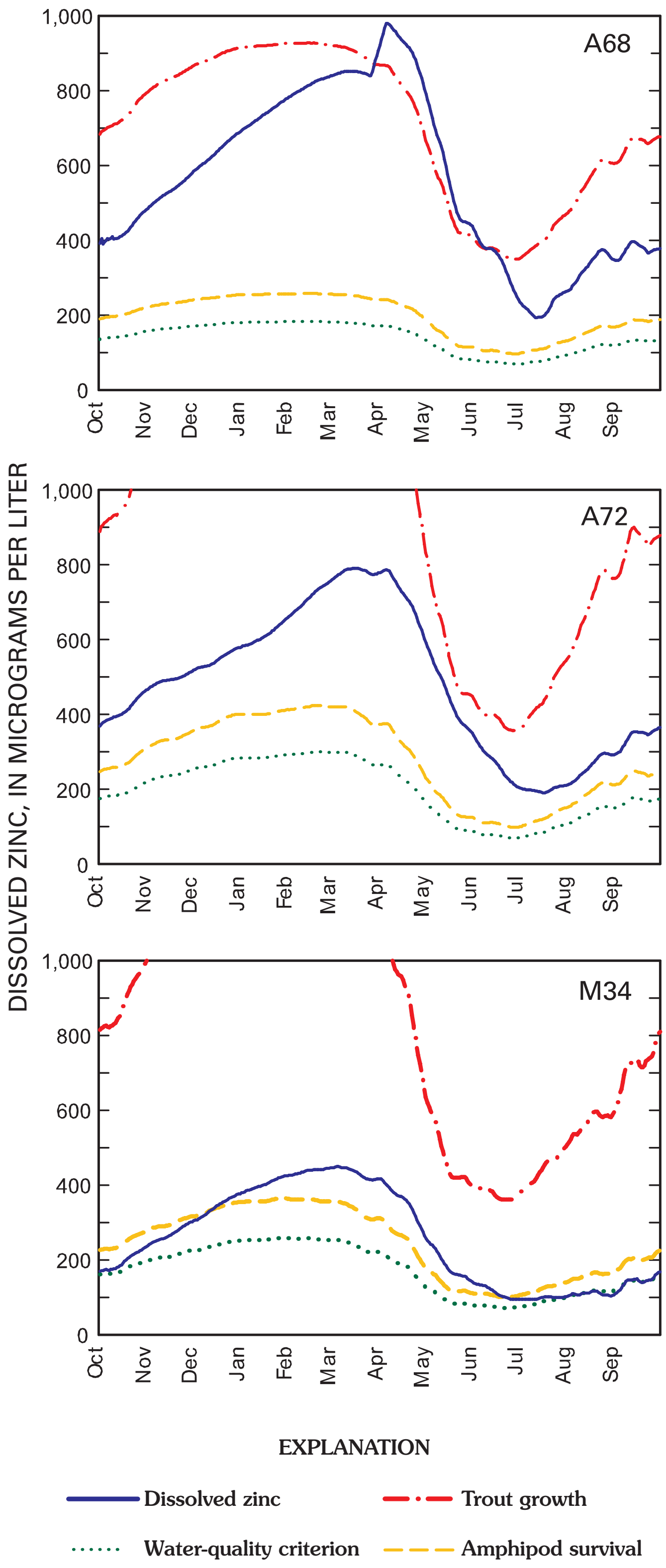

Figure 4. Seasonal variation in modeled dissolved zinc, hardness-adjusted toxicity thresholds, and chronic water-quality criteria. Dissolved zinc and hardness modeled as described by Leib and others (this volume). Toxicity thresholds for trout and amphipods adjusted for variation in hardness using the slope from the chronic water-quality criterion equation (USEPA, 2002).

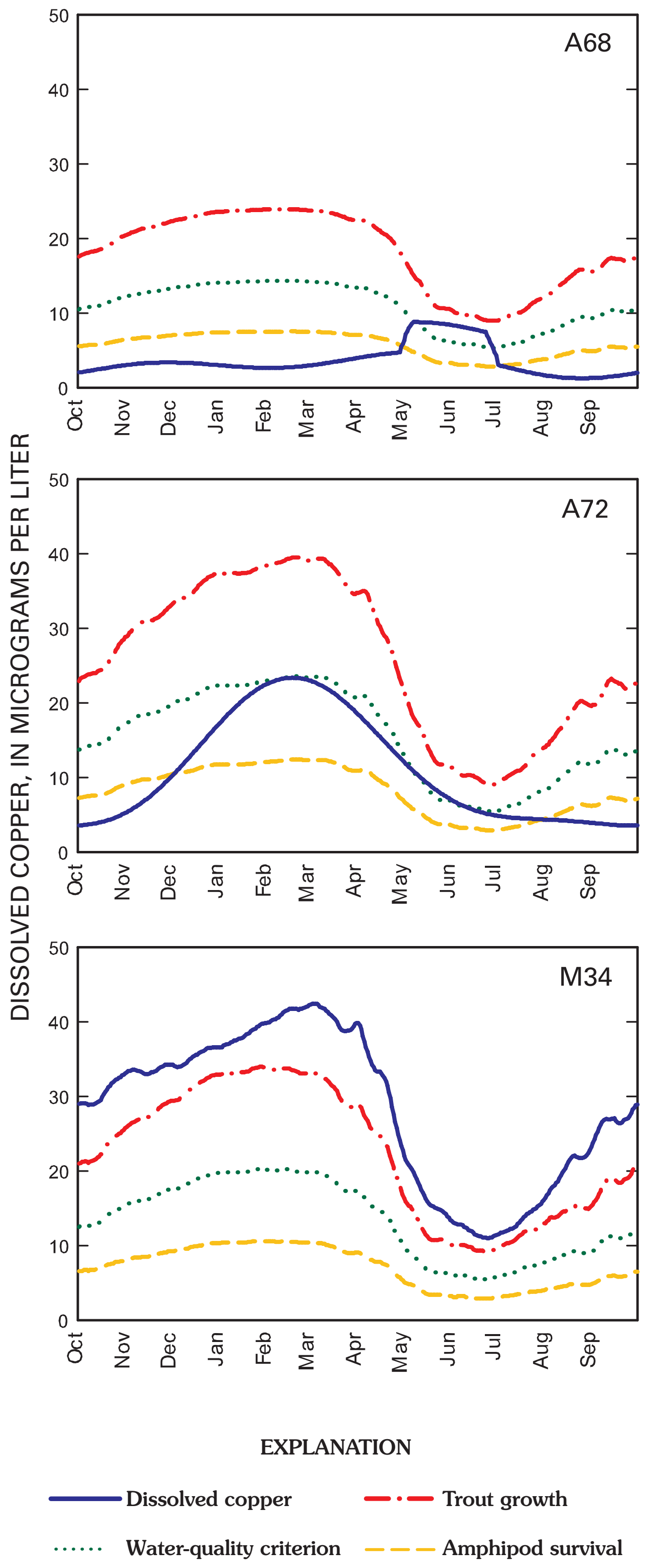

Figure 5. Seasonal variation in modeled dissolved copper, hardness-adjusted toxicity thresholds, and chronic water-quality criteria. Dissolved copper and hardness modeled as described by Leib and others (this volume). Toxicity thresholds for trout and amphipods adjusted for variation in hardness using the slope from the chronic water-quality criterion equation (USEPA, 2002).

years. However, hazards at A72 were predominantly due to copper, where toxic units for copper averaged greater than 1.0 on an annual basis, whereas both copper and zinc contributed equally to cumulative toxic units for trout at A68, and neither individual metal had toxic units greater than 1.0 on an annual basis.

Both toxicity tests with stream water and site-specific toxicity models indicated that toxicity of zinc and copper contributes to adverse effects on aquatic biota of the Animas River watershed study area. Observed and predicted toxic effects of stream water corresponded closely to impairment of stream biotic communities. Toxicity of stream water was greatest at sites where abundance and taxonomic richness of fish and invertebrate communities are most impaired (M34 and A72), intermediate at sites with moderate impairment (A68, A73), and lowest at sites with the most diverse and abundant biotic communities (SMC). Modeled toxicity, based on ambient metal concentrations and site-specific toxicity thresholds, suggests that populations of brook trout in streams of the Animas River watershed study area are probably affected by toxic effects of copper but not zinc.

References Cited

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

Besser, J.M., Allert, A.L., Hardesty, D., Ingersoll, C.G., May, T.W., Wang, N., and Leib, K.J., 2001, Evaluation of metal toxicity in streams of the upper Animas River watershed, Colorado: U.S. Geological Society Biological Science Report, 2001–001, 78 p.

Besser, J.M., Brumbaugh, W.G., May, T.W., Church, S.E., and Kimball, B.A., 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, v. 40, p. 48–59.

Bove, D.J., Mast, M.A., Wright, W.G., Verplanck, P.L., Meeker, G.P., and Yager, D.B., 2000, Geologic controls on acidic and metal-rich waters in the southeast Red Mountains area, near Silverton, Colo., *in* ICARD 2000; Proceedings of the Fifth International Conference on Acid Rock Drainage, Volume 1: Society for Mining, Metallurgy, and Exploration, Inc., p. 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.

Church, S.E., Kimball, B.A., Fey, D.L., Ferderer, D.A., Yager, T.J., and Vaughn, R.B., 1997, Source, transport, and partitioning of metals between water, colloids, and bed sediments of the Animas River, Colorado: U.S. Geological Survey Open-File Report 97–151, 135 p.

Cleveland, Laverne, Little, E.E., Hamilton, S.J., Buckler, D.R., and Hunn, J.B., 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.

Fey, D.L., Wirt, Laurie, Besser, J.M., and Wright, W.G., 2002, Water quality and aquatic toxicity data of 2002 spring thaw conditions in the upper Animas River watershed, Silverton, Colorado: U.S. Geological Survey Open-File Report 02–488, available online (Version 1.1) at URL http://pubs.usgs.gov/of/ofr-02-488/.

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.

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. 76–78.

Kemble, N.E., Brumbaugh, W.G., Brunson, E.L., Dwyer, F.J., Ingersoll, C.G., Monda, D.P., and Woodward, D.F., 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. 8, 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, U.S.A.: Applied Geochemistry, v. 10, p. 285–300.

Leib, K.J., Mast, M.A., and Wright, W.G., 2003, Using water-quality profiles to characterize seasonal water quality and loading in the upper Animas River basin, southwestern Colorado: U.S. Geological Survey Water-Resources Investigations Report 02–4230, 46 p. Available online at http://pubs.usgs.gov/of/wri/wri024230/.

May, T.W., Wiedmeyer, R.H., Brumbaugh, W.G., and Schmitt, C.J., 1997, The determination of metals in sediment pore waters and in 1N HCl-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.

Mount, D.R., Ingersoll, C.G., Gulley, D.D., Fernandez, J.D., La Point, T.W., and Bergman, H.L., 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 [abs.], *in* Nimick, D.A., and von Guerard, Paul, eds., Science for watershed decisions on abandoned mine lands; review of preliminary results, Denver, Colorado, February 4–5, 1998: U.S. Geological Survey Open-File Report 98–297, p. 19.

USEPA (U.S. Environmental Protection Agency), 1994, Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms, Third Edition: U.S. Environmental Protection Agency, EPA/600/4–91/002, 340 p.

USEPA, 1999, National recommended water quality criteria—Correction: U.S. Environmental Protection Agency, EPA 822–Z–99–001, 25 p.

USEPA, 2000, Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates: U.S. Environmental Protection Agency, EPA/600/R–94/064, 133 p.

Verbost, P.M., Berntssen, M.H.G., Kroglund, F., Lyderson, E., Witters, H.E., Rosseland, B.O., Salbu, B., and Wendelaar Bonga, S.E., 1995, The toxic mixing zone of neutral and acidic river water—Acute aluminum toxicity in brown trout (*Salmo trutta* L.): Water, Air, and Soil Pollution, v. 85, p. 341–346.