Biomonitoring in the Boulder River Watershed, Montana: Metal Concentrations in Biofilm and Macroinvertebrates, and Relations with Macroinvertebrate Assemblage

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Darren T. Rhea[1], David D. Harper[1], William G. Brumbaugh[2], and Aïda M. Farag[1]

[1]U.S. Geological Survey, Columbia Environmental Research Center, Jackson Field Research Station, P.O. Box 1089, Jackson, WY 83001

[2]U.S. Geological Survey, Columbia Environmental Research Center, 4200 New Haven Road, Columbia, Missouri 65201

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

Abstract

Portions of the Boulder River watershed, Montana, have contained elevated concentrations of arsenic, copper, lead, and zinc in water, sediment, and biology. We measured concentrations of As, Cd, Cu, Pb, and Zn in biofilm and macroinvertebrates, and assessed macroinvertebrate assemblage and aquatic habitat to monitor metals impacts in aquatic biology and compare with previous investigations. Concentrations of metals were generally larger in downstream sites compared with upstream or reference sites and two sites contained metal concentrations in macroinvertebrates greater than values reported to compromise health and survival of resident trout. Macroinvertebrate assemblage was affected by metal concentrations in biofilm and macroinvertebrates. However, macroinvertebrate metrics were significantly correlated with a greater number of biofilm metals (8) than metals in invertebrates (4). Lead concentrations in biofilm appeared to have the most significant impact on macroinvertebrate metrics. Trace metals continue to affect the aquatic health in the Boulder River watershed. Continued monitoring of metal concentrations in biofilm and macroinvertebrates along with assessments of aquatic habitat and macroinvertebrate assemblage will assist remediation efforts.

Background

Streams in the Boulder River watershed, Montana, are subjected to elevated concentrations of trace metals from abandoned mine adits and tailing piles. The result of these past mining activities is degraded water quality and aquatic habitat, and the potential for impaired aquatic life. Numerous reports and published works have identified the adverse effects of mining on the aquatic health in the Boulder River watershed. Initial investigations identified reduced survival of fish eggs (Nelson 1976), and reductions in invertebrate diversity (Gardner 1977) due to larger concentrations of metals below tributary confluences. In the 1990's, a broad, watershed-level assessment of the aquatic health was conducted throughout the Boulder River watershed to identify the influence of elevated metal concentrations on trout survival, biomass, and individual fish health. Farag et al. (2003) identified reduced trout survival in six tributaries devoid of resident fish, along with reduced fish health and biomass in one tributary capable of supporting trout. Further, Farag et al. (2004) also reported elevated concentrations of metals in biofilm and macroinvertebrates at sites throughout the watershed and that these components presented a pathway of exposure of metals to fish.

Biofilm (or aufwuchs), consisting of attached algae, bacteria, and associated detrital material that adheres to substrates in water (Horne and Goldman 1994), along with benthic macroinvertebrates have been used extensively as bioindicators of water quality and metal exposure in higher trophic levels (Poulton et al. 1995; Farag et al. 1998, 1999, 2000; Woodward et al. 1994, 1995). Similarly, a number of macroinvertebrate metrics have been developed to evaluate assemblage structure and associated processes resulting from habitat degradation or perturbation (e.g. Barbour et al. 1996). Though numerous metrics exist, many have likely been developed for specific geographies, requiring some initial investigation into their usefulness and relationships with metal contamination.

Remediation efforts have begun or are planned at several locations in the Boulder River watershed, dictating a need for monitoring data to evaluate progress in mitigation efforts and identify any adverse consequences of remediation. The purpose of this project was to measure trace element concentrations in biofilm and macroinvertebrates and conduct measures of aquatic habitat and macroinvertebrate assemblages at several sites with planned remediation. This information will serve as a baseline for future monitoring, evaluating future monitoring protocol and helping target areas where remediation might be more suited.

Methods

Samples were collected from 10 locations throughout the Boulder River watershed (Table 1; Figure 1) during September 16-18, 2003. Sites were paired with other sites on the same stream or at intersecting tributaries for comparison purposes. Because of the extensive mining history in this watershed, many of the upstream sites cannot be considered "traditional" reference sites, therefore, they are not entirely void of mining impacts. However, because they are located above sites selected for remediation, they can be used for comparisons to assist with determinations about the success of remediation activities. Sites selected and comparisons included: Jack Creek above

Bullion Mine (JCA) compared with Jack Creek at mouth (JCM), Cataract Creek above Uncle Sam Gulch (UCC) compared with Lower Cataract Creek (LCC), Basin Creek at Basin (BCB) compared with Basin Creek below Buckeye Mine (BBB), and Upper Boulder River (BRRC) compared with Lower Boulder River (BRLG). Two additional sites Bullion Mine Tributary (BMT; paired with JCA), and Uncle Sam Gulch (USG; paired with CCA) were also included. Benthic macroinvertebrates could not be located in BBB, BMT, or USG, and were not collected from these sites for trace element analyses.

Benthic macroinvertebrates were collected for trace element analyses with a 30 x 30 cm kick net at 4 randomly selected riffles within a 100 m reach. Biofilm samples were collected by gently scraping the surface of rocks collected from the near-shore streambed. Biofilm and macroinvertebrate samples for trace element analyses were stored at 0^oC, and shipped under ice to the Columbia Environmental Research Center (CERC) for analyses. Arsenic, cadmium, copper, lead, and zinc were measured in four replicate biofilm and benthic macroinvertebrate samples from each site. All samples were lyophilized to a constant dry weight. Approximately 100 mg of each sample were digested in 1 mL of nitric acid and 1 mL of 30% hydrogen peroxide. Concentrations of metals were determined by inductively coupled plasma-mass spectroscopy (PE/SCIEX Elan 6000 ICP-MS). Quality control included measurements of predigestion spikes, postdigestion spikes, digestion replicates, and reference tissue samples.

Aquatic habitat and macroinvertebrate assemblage data were collected following methods described by the U.S. EPA Rapid Bioassessment Protocols for Use in Wadeable Streams and Rivers (Barbour et al. 1999). Briefly, habitat was quantified using a ten point visual assessment procedure based on parameters representative of reach scale and stream scale habitats (e.g. embeddedness, riffle frequency, bank stability). Macroinvertebrates were collected from riffle habitats at all sites to avoid variability in invertebrate diversity due to macrohabitat conditions (i.e. pools, runs, riffles). Samples were collected with a 30 x 30 cm kick net, and stored in 70% ethanol. Macroinvertebrate assemblage was quantified by subsampling 4 of 30 grids of the composite sample or until 200 (± 20%) individuals had been selected. Individuals were identified to Family using identification keys provided by Merritt and Cummins (1999). Barbour et al. (1999), define the predicted responses of multiple invertebrate metrics to increased perturbation. These predicted responses are indicated by arrows next to the parameters we utilized. Parameters included: total number of families (↓), number of Ephemeroptera-Plecoptera-Trichoptera (EPT) families (↓), percent EPT (↓), percent Plecoptera (↓), percent Diptera (↑), and proportion of Trichoptera as Hydropsychidae (↑).

Trace element concentrations in biofilm and benthic macroinvertebrates were statistically analyzed with one-way analysis of variance (ANOVA) including all sites, following tests for normality and homogeneity. If significant differences were detected, then the data were further analyzed using pair-wise comparisons of the least-squared means between corresponding locations (i.e. H_0: $LSMean_i = LSMean_j$). Invertebrate assemblage data were analyzed using Pearson's Correlation analysis to determine if metrics were positively or negatively related to metal concentrations in biofilm and invertebrates. Proportional data were square-root(x) transformed to adequately meet model assumptions. All statistical analyses were conducted with Statistical Analysis

System (SAS) software using the GLM, MEANS, CORR, and UNIVARIATE procedures (SAS Institute 1999).

Quality assurance/quality control

Elemental recoveries of two reference solutions used as laboratory control samples ranged from 93 to 100%. Recoveries of elements from biological control material (CERC striped bass, NIST 1566b oyster, NIES 9 sargasso, and IRMM 414 plankton) ranged from 92 to 102%. Recoveries of elements from sediment control material (NIST 2704 and NRCC PACS-1) ranged from 70 to 100% with the exception of one poorer recovery for As (55%). Method precision from the triplicate digestion and analysis of invertebrate and biofilm samples exhibited percent relative standard deviations (%RSDs) of ≤ 26 %, with the exception of Pb for one biofilm replicate (51%). Instrumental precision from the duplicate analysis of invertebrate and biofilm digestates resulted in RPDs < 10%. Where spike/unspiked ratios were at least 10, method spikes of invertebrate and biofilm samples exhibited elemental recoveries ranging from 84 to 112%. Post-digestion spikes of invertebrate and biofilm digestates exhibited recoveries ranging from 77 to 99%. Finally, instrumental detection limits, method detection limits, and method quantification limits for the elements were within acceptable limits as specified by CERC guidelines. Measured concentrations of metals exceeded method detection limits in all field samples.

Results

Metal concentrations

Concentrations of As, Cd, Cu, Pb, and Zn in biofilm samples were significantly different among locations (**As**: $F_{9,30} = 18.12$, $P < 0.0001$; **Cd**: $F_{9,30} = 55.75$, $P < 0.0001$; **Cu**: $F_{9,30} = 153.5$, $P < 0.0001$; **Pb**: $F_{9,30}$ 26.50, $P < 0.0001$; **Zn**: $F_{9,30} = 91.42$, $P <$ 0.0001). Concentrations of metals in biofilm samples were generally larger in the downstream or non-reference sites than the upstream or reference sites (Table 2). Concentrations were somewhat variable across sites, however, all downstream sites had significantly greater concentrations of at least two metals compared with their respective reference. Basin Creek below Buckeye Mine had the greatest concentration of arsenic in biofilm (4357 μg/g dry wt.), whereas the largest concentrations of cadmium, copper, lead and zinc were obtained in biofilm from Uncle Sam Gulch (75.1, 4480, 1540, and 12380 μg/g dry wt, respectively).

Concentrations of As, Cd, Cu, Pb, and Zn in macroinvertebrate samples were significantly different among locations (**As**: $F_{6,21} = 40.85$, $P < 0.0001$; **Cd**: $F_{6,21} = 39.37$, $P < 0.0001$; **Cu**: $F_{6,21} = 58.76$, $P < 0.0001$; **Pb**: $F_{6,21} = 12.07$, $P < 0.0001$; **Zn**: $F_{6,21} =$ 24.16, $P < 0.0001$). Concentrations of metals in benthic macroinvertebrate samples followed a pattern similar to biofilm, although concentrations were less than those observed in biofilm (Table 3). All downstream sites had significantly greater concentrations of at least four metals than their corresponding reference. The greatest concentration of arsenic and copper in macroinvertebrates were obtained from JCM (56.0 and 342.5 μg/g dry wt, respectively), whereas, macroinvertebrates from LCC had the greatest concentrations of cadmium, lead, and zinc (24.4, 29.2, and 1327 μg/g dry wt, respectively).

Macroinvertebrate assemblage

Habitat was generally consistent across all sites, comprised mainly of riffle-pool habitat and dominated by gravel and cobble substrates (Table 4). Rapid Bioassessment scores were variable across study sites and generally reflected the amount of physical disturbance that had taken place, ranging from 95 at BRRC to 158 at UCC. Macroinvertebrates were readily available for identification at most locations, however, a total of only three specimens were recovered from the entire sample taken from USG, and only one individual was recovered from the entire sample collected at BMT (Table 4). There were several significant correlations between assemblage metrics and concentrations of metals in biofilm and invertebrates (Table 5). Total number of families had the highest number of significant correlations, and was negatively correlated with biofilm copper and lead, and invertebrate arsenic and copper. The proportion of Trichoptera comprised of Hydropsychidae was also significantly correlated with concentration of two metals in macroinvertebrates: cadmium and lead. Zinc was the only metal with no significant correlations with metals in biofilm or invertebrates. There were no significant correlations between physical habitat and any of the macroinvertebrate metrics.

Discussion

Trace element concentrations in biofilm and macroinvertebrates provide a good indicator of metal transfer through the food web (Farag et al. 2004), and have been linked to health in other aquatic species (Farag et al. 1994, Woodward et al. 1995). Large concentrations of trace elements exist in the biofilm at many sites in the Boulder River watershed. Concentrations of trace metals in biofilm were within the range of concentrations measured during July 1997 in the Boulder River watershed (Farag et al. 2004), and in one case, arsenic measured in biofilm collected at BBB (4357 μg/g) was greater than any sample taken in 1997. Four locations sampled during 1997 were also sampled during this investigation, and provide some comparison. Metal concentrations in biofilm from BCB and BRLG were less than concentrations measured in 1997, with the exception of one element. Concentrations of arsenic, cadmium, copper, lead and zinc, in biofilm from BCB were 66, 83, 40, 143, and 85 % of concentrations measured in 1997, while at BRLG, concentrations for the same metals were 27, 106, 74, 58, and 84 % of concentrations taken in 1997. Concentrations of metals in biofilm from UCC and LCC did not follow any pattern when compared with samples collected in 1997. Concentrations of arsenic, cadmium, copper, lead, and zinc were 101, 77, 92, 98, and 121 % of 1997 values at UCC, and 58, 111, 62, 109, and 116 % of 1997 values at LCC.

Concentrations of trace metals in aquatic macroinvertebrates were similarly large throughout the Boulder River watershed and were comparable to concentrations measured in 1997. Concentrations of arsenic, cadmium, copper, lead and zinc in invertebrates were less than 1997 values in BCB (38, 38, 91, 57, and 56 %, respectively) and LCC (40, 41, 72, 86, and 55%, respectively). However, concentrations of metals in invertebrates from UCC and BRLG were neither consistently greater nor smaller. Concentrations of arsenic, cadmium, copper, lead, and zinc in invertebrates were 95, 29, 109, 65, and 50 % of 1997 values at UCC, and 44, 47, 174, 37, and 127 % of 1997 values at BRLG. Regardless of variability in measured concentrations of metals between 1997 and 2003, all concentrations during both years remain elevated.

The variability in metal concentrations between the 1997 and 2003 sampling events may be influenced by time of year in which samples were collected. Species of aquatic macroinvertebrates have greatly varying life history strategies and will emerge from the aquatic environment during different times of the year, creating a dynamic species assemblage that may vary (Merritt and Cummins 1999). Other research has indicated that concentrations of metals can vary among invertebrate species, particularly due to differences in functional feeding strategies (Farag et al. 1998). Therefore, differences in species assemblage may have contributed, at least in part, to the variability in metal concentrations in macroinvertebrates and possibly biofilm. Despite this, others have noted the importance of composite samples of macroinvertebrates for trace metals analysis, because they represent the food resources available for other aquatic organisms (e.g. Woodward et al. 1995). Future monitoring efforts should recognize the potential variability due to invertebrate assemblage, but continue to collect composite samples at precise locations and time to assist in long-term assessments.

Metal concentrations in biofilm and invertebrates in the Boulder River watershed remain at high levels, and may threaten overall aquatic health. Farag et al. (2003) identified reduced health of resident fish at lower Cataract Creek with invertebrate concentrations of arsenic, cadmium, copper, lead, and zinc of 63, 59, 340, 34, and 2410 μg/g respectively. These concentrations are larger than any observed during this current investigation, however, Farag et al. (1994) and Woodward et al. (1995) identified reduced survival, growth, and health of cutthroat trout fed invertebrate diets of 19, 2.3, 174, 15, and 648 μg/g of arsenic, cadmium, copper, lead and zinc, respectively. Concentrations of metals in invertebrates from LCC and JCM exceeded these levels.

Macroinvertebrate assemblage data provides a rapid and simple method to aid in assessments of aquatic health. All but one of the various metrics used during this investigation were correlated with metal concentrations in biofilm and macroinvertebrates. Additionally, all of the significant correlations followed the expected response of a particular metric to increased perturbation, lending further evidence to their usefulness. The total number of families in a sample provided the most effective approach to identifying differences in metal concentrations in macroinvertebrates, relating negatively to increasing arsenic and copper. Percent Trichoptera as Hydropsychidae was also positively related to cadmium and lead. Poulton et al. (1995) conducted an assessment of invertebrate community structure and metal concentrations in the Clark Fork River, Montana. Taxa number was identified as one of the best indicators of trace metal impacts. Although the authors used species-level comparisons of taxa richness, family-level comparisons may be just as useful. Others have identified the usefulness of invertebrate metrics based on families to evaluate environmental contamination (Hilsenhoff 1988; Feldman and Connor 1992; Lowell and Culp 2002). Family-level indices are more rapid, require less skill, and are therefore more repeatable than generic- and species-level indices (Hilsenhoff 1988). Future monitoring efforts in the Boulder River watershed should continue to utilize these metrics as an indicator of relative impact, particularly the number of taxa and the proportion of Trichoptera as Hydropsychidae, which are more related to concentrations of metals in invertebrates.

Lead concentrations in biofilm had the most influence on the invertebrate metrics used. Lead concentrations in aquatic invertebrates have often been associated with uptake through digestion and linked to lead availability in food sources such as biofilm. For example, Hare et al. (1991) indicated that the primary source of lead in the mayfly nymph Hexagenia came from sediment consumed as food. Therefore, the number of correlations between biofilm lead concentrations and invertebrate assemblage metrics may be due to dietary lead sources altering the invertebrate community. Other metals in biofilm were also significantly correlated with macroinvertebrate metrics, and together, metal concentrations in biofilm provided twice as many significant correlations (8) as metal concentrations in macroinvertebrates (4). It may be deduced then, the metal concentrations in biofilm are having the most impact on the macroinvertebrate assemblage in the Boulder River watershed.

Trace metals exist in high concentrations in biofilm and macroinvertebrates at several locations in the Boulder River watershed, and these elevated levels have altered the macroinvertebrate community. Further, the concentrations of metals at two locations are greater than those reported in previous studies which have been linked with compromised health of other aquatic organisms. Efforts should continue to monitor the aquatic health and pathways of metal accumulation in the Boulder River watershed, to aid in restoration efforts and improve re-establishment of aquatic communities. Future monitoring efforts should continue at the precise location and time as previous efforts to facilitate long-term assessments of metals contaminated impacts.

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Table 1. Descriptions and locations of biomonitoring sites in the Boulder River watershed, Montana.

Site	Location	Latitude	Longitude
BCB	Basin Cr. Basin	46-16-16	112-15-42
BBB	Basin Cr. below Buckeye Mine	46-23-47	112-18-04
BRRC	Boulder R. above Kleinsmith Gulch	46-16-11	112-16-43
BRLG	Boulder R. below Little Galena Gulch	46-14-58	112-10-27
UCC	Upper Cataract Cr.	46-19-05	112-14-42
LCC	Cataract Cr. Below Uncle Sam Gulch	46-16-17	112-14-28
USG	Uncle Sam Gulch	46-19-04	112-14-44
JCA	Upper Jack Cr.	46-21-55	112-18-15
JCM	Jack Creak at confluence	46-20-47	112-20-19
BMT	Bullion Mine Tributary	46-21-53	112-18-17

Table 2. Trace metal concentrations (μg/g dry weight) in biofilm collected at sites in the Boulder River watershed, Montana (mean with standard error in parentheses). Data are presented as comparisons among sites with the least (denoted as †) and greatest relative amount of impact. Significant differences between lesser impacted and greater impacted sites, within a creek or on the mainstem, are indicated by asterisks ($\alpha = 0.05$).

Site	Arsenic	Cadmium	Copper	Lead	Zinc
BCB†	315 (132)	13.9 (1.1)	147.3 (14)	401.5 (158)	1962 (253)
BBB	4357 (1000)*	16.6 (3.8)	93.9 (16)	700.8 (160)*	1899 (400)
BRRC†	15.2 (3.7)	0.35 (0.09)	15.6 (3.0)	8.7 (2.8)	117.1 (30)
BRLG	69.8 (3.1)	18.4 (1.1)*	201.0 (28)	160.8 (18)	2695 (180)*
UCC†	131.8 (6.4)	17.8 (0.7)	238.0 (23)	250.3 (6.2)	2848 (60)
LCC	424.5 (28)	34.0 (2.5)*	525.3 (47)	422.5 (59)	4480 (390)*
USG	1778 (180)*	75.1 (4.7)*	4480 (140)*	1540 (136)*	12380 (398)*
JCA†	54.4 (17)	1.6 (0.4)	41.7 (12)	32.1 (15)	168.8 (47)
JCM	759.5 (58)	70.9 (9.3)*	1440 (314)*	343.8 (15)*	9148 (1100)*
BMT	2078 (80)*	3.1 (0.2)	398.5 (25)*	526.0 (23)*	273.8 (11)

Table 3. Trace metal concentrations (μg/g dry weight) in macroinvertebrates collected at sites in the Boulder River watershed, Montana (mean with standard error in parentheses). Data are presented as comparisons among sites with the least (denoted as †) and greatest relative amount of impact. Significant differences between lesser impacted and greater impacted sites, within a creek or on the mainstem, are indicated by asterisks ($\alpha = 0.05$).

Site	Arsenic	Cadmium	Copper	Lead	Zinc
BCB	8.1 (1.4)	6.8 (1.0)	83.8 (8.7)	7.1 (1.2)	520.8 (26)
BRRC†	5.5 (1.4)	0.1 (0.01)	33.2 (1.6)	1.1 (0.1)	239.8 (34)
BRLG	11.69 (1.8)	5.54 (0.3)*	170.5 (16)*	14.1 (2.2)*	848.8 (62)*
UCC†	7.1 (1.1)	4.6 (0.6)	84.2 (3.7)	7.3 (1.7)	527.3 (31)
LCC	25.0 (4.4)*	24.4 (3.2)*	246.0 (33)*	29.2 (6.1)*	1327 (179)*
JCA†	9.3 (2.3)	3.5 (0.3)	45.4 (2.3)	5.8 (1.2)	363.3 (23)
JCM	56.0 (4.8)*	14.9 (0.5)*	342.5 (12)*	20.2 (2.8)*	924.8 (44)*

Table 4. Summary of Rapid Bioassessment habitat scores and macroinvertebrate indices for sites in the Boulder River watershed, Montana. Upstream or reference sties are denoted by †.

	Habitat		Macroinvertebrates					
Site	Dominant substrate	Score	Taxa (families)	EPT Taxa	EPT (%)	Plec. (%)	Dip. (%)	Hyd/Tri (%)
BCB†	cobble	112	13	9	81.4	14.9	9.3	48.9
BBB	sand	141	14	9	32.3	14.3	67.1	44.4
BRRC†	cobble	95	12	8	72.3	4.76	16.2	28.1
BRLG	cobble	117	14	11	90.7	4.8	4.4	9.2
UCC†	cobble	158	16	13	60.8	28.6	19.0	20.5
LCC	cobble	119	9	6	68.2	22.5	31.8	89.4
USG	sand	133	3	0	0	0	100	N/A
JCA†	cobble gravel	145	15	12	73.6	42.1	21.9	16.1
JCM	cobble gravel	130	9	8	97.1	44.1	2.9	60.0
BMT	cobble gravel silt	98	1	0	0	0	100	N/A

Table 5. Pearson correlation coefficients (*P*-value) for relationships between metal concentrations in biofilm and macroinvertebrates and macroinvertebrate assemblage metrics (presented for square-root transformed data).

		Macroinvertebrate Metric					
Type	Metal	Families	EPT	%EPT	%Plec.	%Dip.	%Hyd.
Biofilm	Arsenic	-0.4851 (0.1553)	-0.5298 (0.1152)	**-0.6535** **(0.0404)**	-0.3400 (0.3364)	**0.7289** **(0.0168)**	0.4255 (0.2932)
	Cadmium	-0.2191 (0.5431)	-0.2399 (0.5043)	-0.1133 (0.7553)	0.0656 (0.8572)	0.0218 (0.9523)	0.5588 (0.1499)
	Copper	**-0.5997** **(0.0669)**	**-0.6561** **(0.0394)**	-0.5347 (0.1113)	-0.3206 (0.3665)	0.3789 (0.2801)	0.5426 (0.1647)
	Lead	**-0.5784** **(0.0798)**	**-0.6707** **(0.0338)**	**-0.6955** **(0.0255)**	-0.4372 (0.2064)	**0.6584** **(0.0385)**	0.5993 (0.1164)
	Zinc	-0.2170 (0.5470)	-0.2578 (0.4720)	-0.1364 (0.7071)	0.0102 (0.9777)	0.0320 (0.9301)	0.5301 (0.1766)
Invertebrates	Arsenic	**-0.7853** **(0.0364)**	-0.4922 (0.2619)	0.5676 (0.1838)	0.5238 (0.2275)	-0.3673 (0.4176)	0.5985 (0.1557)
	Cadmium	-0.6523 (0.1131)	-0.5041 (0.2486)	0.1847 (0.6917)	0.4409 (0.3221)	0.0531 (0.9100)	**0.7344** **(0.0601)**
	Copper	**-0.7171** **(0.0697)**	-0.4694 (0.2880)	0.5263 (0.2249)	0.3001 (0.5132)	-0.3704 (0.4134)	0.5597 (0.1914)
	Lead	-0.6149 (0.1417)	-0.4367 (0.3272)	0.2813 (0.5412)	0.3335 (0.4648)	-0.0528 (0.9104)	**0.7589** **(0.048)**
	Zinc	-0.6133 (0.1431)	-0.4715 (0.2855)	0.2635 (0.5680)	0.1839 (0.6931)	-0.0655 (0.8891)	0.5689 (0.1826)

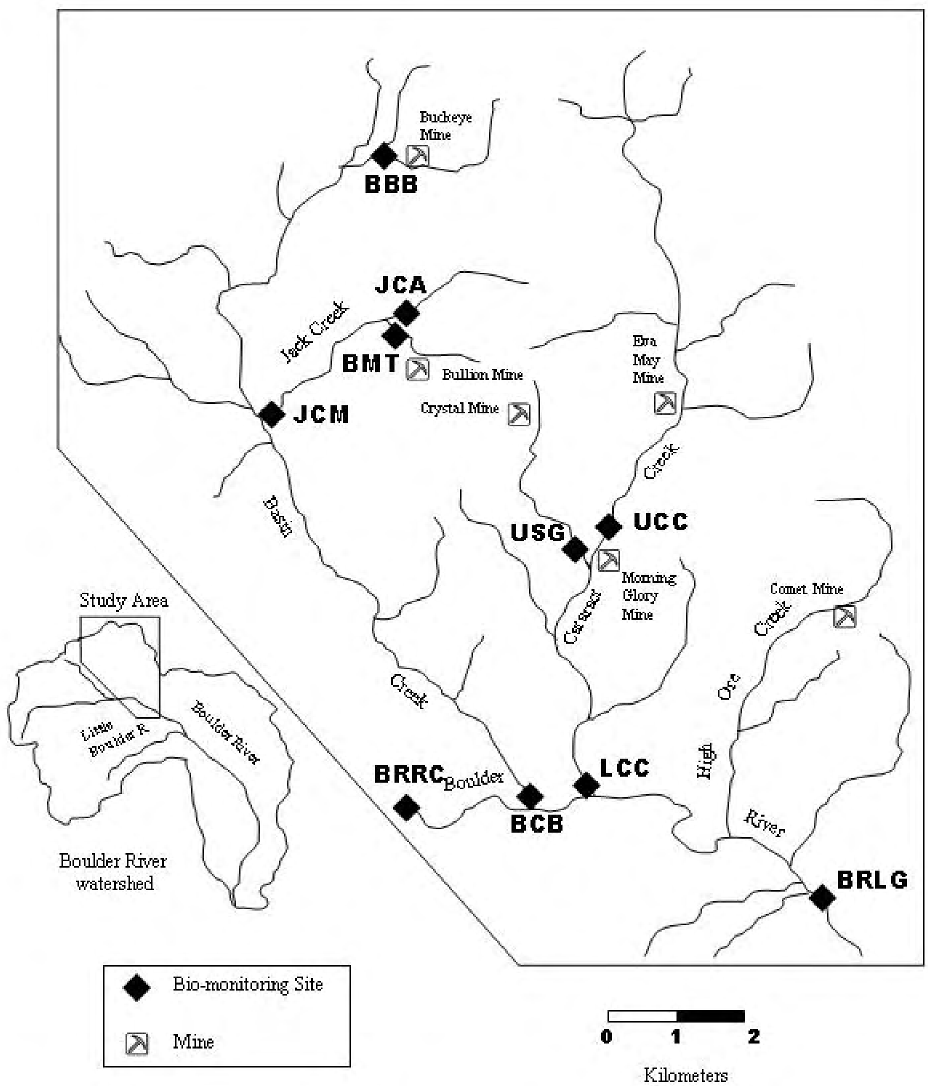

Figure 1. Map of the Boulder River watershed showing biomonitoring locations.