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The Use of Bioassays and Toxicity Identification Evaluation (TIE) Procedures to Assess Recovery and Effectiveness of Remedial Activities in a Mine Drainage-Impacted Stream System

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Abstract. Effluents from Walker Mine and its tailings pile have resulted in toxic concentrations of metals in Dolly and Little Grizzly Creeks. Recent remedial structures have greatly reduced metal loading, however the need to assess recovery of the receiving aquatic ecosystem exists so that future remediation priorities can be established. The objective was to contribute to this assessment using Toxicity Identification Evaluation procedures. Water samples were collected at several sites in Dolly and Little Grizzly Creeks. Untreated samples and samples passed through ion exchange columns, which remove cationic metals, were compared in side-by-side bioassays using *Pimephales promelas, Ceriodaphnia dubia,* and *Selenastrum capricornutum.* Samples were analyzed for total and dissolved copper, cadmium, zinc, and iron. Copper was the element responsible for toxicity. Toxicity was detected in the mine discharge and immediately downstream from the tailings where dissolved copper concentrations were 250 µg/L and 415 µg/L, respectively. Toxicity decreased at downstream sites but extended at least 6.4 km downstream. Improvement in bioassay performance by the treated waters verified metal toxicity. The results indicate that the mine effluent and tailings pile currently have the heaviest impact on Dolly and Little Grizzly Creeks and should be given the highest priority in future remedial programs.

Walker Mine, located on the western slope of the Sierra Nevada mountains (CA), is one of the numerous abandoned mines throughout the western United States. Like other mines, leachate from the Walker Mine workings, waste rock piles, and tailings have impacted the biotic communities in the receiving aquatic system. Since the opening of the mine, copper and other heavy metals have been drained into both Dolly Creek and Little Grizzly Creek. Sheehan (1980) reported total copper concentrations as high as 512 µg/L in Dolly Creek downstream of the mine effluent input (Figure 1). The elevated concentrations of copper had eliminated the fish community and severely impacted the benthic macroinvertebrate community in this section of the stream. Although other heavy metals also were present in the creeks, copper was responsible for the observed impacts (Sheehan 1980; Sheehan and Knight 1985). In July 1987, the U.S. Department of Agriculture and Forest Services (USDAFS) recorded copper concentrations as high as 1,050 µg/L in Dolly Creek below the mine discharge and 730 µg/L in Dolly Creek downstream of the tailings pile (USDAFS 1988).

Increasing awareness and concern of water quality degradation resulting from mine drainage and tailings leachate stimulated regulatory agencies to initiate remedial measures. Three structures have been repaired or constructed including (1) a retention dam immediately below the tailings to prevent further downstream erosion of the pile; (2) a levee to prevent lateral erosion of the tailings into Little Grizzly Creek; and (3) a seal in the mine portal to prevent acid mine drainage from entering into Dolly Creek. The mine seal, installed in November 1987, immediately reduced the total copper load by 98%, resulting in significantly reduced copper concentrations downstream (Croyle and Rosenbaum 1996).

Once remediation programs were initiated, the need to assess the biological recovery of the receiving waters existed to evaluate remediation effectiveness and plan for future remediation strategies. Often this evaluation requires extensive laboratory and field research. The use of the EPA early life stage three species bioassays has been acknowledged as a cost and time effective means of evaluating the toxicity of effluents and mine discharges (Nimmo *et al.* 1990; Fucik *et al.* 1991). These bioassays can be used as indicators of potential in-stream

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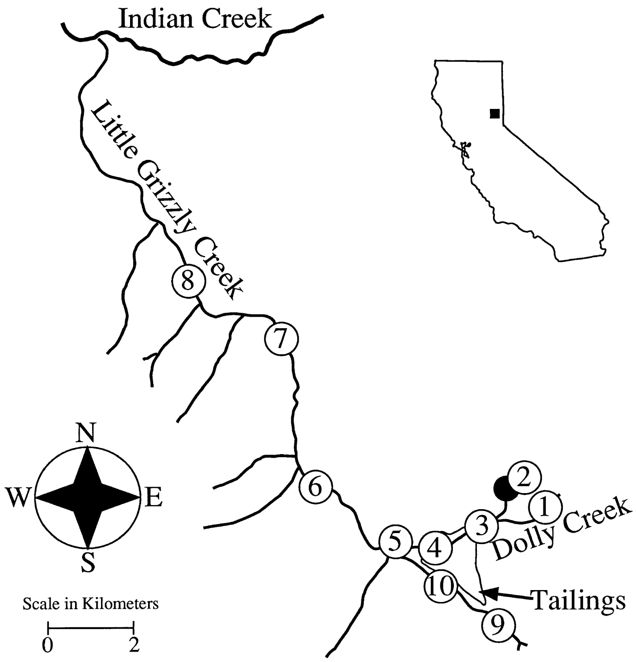

Fig. 1. The Walker Mine study area

toxicity. An advancement of this laboratory assay involves the use of toxicity identification evaluation (TIE) procedures. A TIE is broadly defined as the characterization of the chemical group of a toxicant with the final goal of identifying the specific chemical responsible for toxicity. One TIE manipulation involves passing a water sample through ion exchange columns to remove heavy metals or oxyanions. Heavy metals and oxyanions can be removed from a sample and then compared to an untreated sample in a bioassay. When the organisms' performance in the sample with the metals removed significantly exceeds that of the untreated sample, the difference suggests a metal toxicity problem.

This study was designed to contribute to the assessment of the biological recovery at the Walker Mine while the remediation programs are being implemented by using these TIE procedures. The study had three objectives: (1) to examine the effectiveness of each remedial structure; (2) to determine the extent of downstream toxicity; and (3) to confirm metal toxicity by using ion exchange columns and metals analysis.

Materials and Methods

Mine Location and Sampling Area

The Walker Mine, an abandoned metals mine, is located in east central Plumas County, CA, on the western slope of the Sierra Nevada mountains. During the years of its operation (1904–1941), tailings from the mine mill were deposited in a natural basin at the confluence of Dolly and Little Grizzly Creeks. The tailings cover approximately 0.4 km^2 and range from 1 m to 14 m deep. Sampling sites were selected specifically to isolate remediation structures and to determine their effectiveness in reducing metal loading. The location of the mine and the tailings in relation to the sampling sites is illustrated in Figure 1. The rationale for selecting each site is explained below.

- Sampling Site 1, *Dolly Creek upstream from Walker Mine,* was selected to provide information on the quality of Dolly Creek upstream of any mine related impacts. A spring provides the primary source of flow to Dolly Creek.
- Sampling Site 2, *Walker Mine,* was chosen to provide information on the mine drainage before dilution. Samples were collected immediately below the pipe releasing mine drainage.
- Sampling Site 3, *Dolly Creek upstream from the tailings,* was selected to provide information on the quality of Dolly Creek downstream from the mine effluent but upstream of any tailings impact.
- Sampling Site 4, *Dolly Creek downstream from the tailings,* was selected to determine the impact of the tailings pile on the quality of Dolly Creek. Water samples were collected from Dolly Creek immediately below the tailings flash board dam.
- Sampling Site 5, *Little Grizzly Creek downstream of the confluence with Dolly Creek,* was selected to provide information on the quality of Little Grizzly Creek after the inflow of Dolly Creek.
- Sampling Sites 6, 7, and 8. Water samples were collected *6.4, 12.8, and 19.2 km downstream from the mine along Little Grizzly Creek.* This series of sites provided information on the extent of toxicity downstream from the tailings and mine.
- Sampling Site 9, *Little Grizzly Creek upstream from the tailings,* was chosen to provide information on the water quality of Little Grizzly Creek upstream of any mine related discharge.
- Sampling Site 10, *Little Grizzly Creek upstream from the confluence with Dolly Creek,* was chosen to provide information on the quality of Little Grizzly Creek before the entry of Dolly Creek. In particular, this site was selected to determine if seepage from the tailings pile was causing toxicity in this section of Little Grizzly Creek. Water samples were collected from Little Grizzly Creek approximately 50 m above its confluence with Dolly Creek.

Collection Methods

Sampling was conducted in April 1992 during the snow melt period. Bioassay water samples were collected as subsurface grab samples and immediately placed on ice for transport back to a centralized location for filtering. Samples for determining total metal concentrations also were collected as subsurface grabs using 1-L polyethylene bottles containing nitric acid. All bioassay and dissolved metal samples were filtered through a 0.45-µm in-line filter within 2 h of collection. Filtration of the bioassay waters prevents the columns from becoming clogged during application. Samples for dissolved metal analysis were filtered directly into 500-ml polyethylene bottles containing 1.25 ml 1:2 nitric acid. Bioassay and TIE waters were kept on ice until arrival at the laboratory where they were stored at 4°C.

Analytical Chemistry and Water Quality

Temperature, pH, and electrical conductivity (EC) were measured for each site at the time of sample collection. Total suspended solids were determined in the laboratory. Hardness, alkalinity, pH, and EC were measured in the laboratory for both treated and untreated samples. Metal concentrations were analyzed by California Department of Fish and Game at the Mussel Watch Laboratory using ultraclean reagents and facilities (Goetzl and Stephenson 1993).

Table 1. Dissolved (Dis) and total (Tot) metals concentrations (in µg/L) and total suspended solids (TSS) (in mg/L) in Dolly and Little Grizzly Creeks

	Copper		Cadmium		Zinc		Iron		
Site	Dis	Tot	Dis	Tot	Dis	Tot	Dis	Tot	TSS
1	<1	<1	<0.05	<0.05	<5	<5	5.2	58.8	11.4
2	**250**	**254**	0.10	0.22	8.2	8.3	6.5	6.7	0.85
3	**98.2**	**120**	<0.05	0.11	11.1	12.6	525	630	0.93
4	**415**	**518**	0.33	0.38	23.6	27.2	735	1080	1.64
5	**31.5**	**39.9**	<0.05	<0.05	1.7	3.7	218	361	0.35
6	**15.4**	**19.0**	<0.05	<0.05	0.9	1.0	104	139	0.77
7	**9.9**	**12.8**	<0.05	<0.05	0.8	0.9	81	97	0.68
8	**7.0**	**8.9**	<0.05	<0.05	0.5	1.10	69.5	83.0	0.66
9	<1	<1	<0.05	<0.05	0.9	0.9	117	190	0.16
10	<1	<1	<0.05	<0.05	0.3	0.8	312	451	1.10

Bold numbers indicate concentrations exceeding the numerical objectives set forth by the EPA criteria

Table 2. Water quality (pH, electrical conductivity, hardness, and alkalinity) of the ambient and amended post-column waters from Dolly and Little Grizzly Creeks

Site	pH	Electrical Conductivity (µmhos)	Total Hardness (mg/L $CaCO_3$)	Calcium Hardness (mg/L $CaCO_3$)	Magnesium Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)
1 ambient	8.00	147	74	48	26	77
1 rinsate	—	—	—	—	—	—
2 ambient	7.89	117	56	38	18	59
2 rinsate	—	—	—	—	—	—
3 ambient	7.90	125	62	34	28	58
3 rinsate	8.01	334	60	34	26	56
4 ambient	7.93	142	70	46	24	62
4 rinsate	7.85	310	66	42	24	58
5 ambient	7.73	72	36	22	14	36
5 rinsate	7.87	206	38	24	14	33
6 ambient	7.79	83	32	22	10	39
6 rinsate	7.87	226	36	24	12	39
7 ambient	7.90	89	40	26	14	40
7 rinsate	7.88	231	40	28	12	37
8 ambient	7.87	82	40	26	14	42
8 rinsate	7.75	223	44	28	16	38
9 ambient	7.85	59	28	16	12	31
9 rinsate	7.83	194	32	18	14	32
10 ambient	7.83	67	34	18	16	32
10 rinsate	7.74	186	38	22	16	28
control	7.76	295	90	48	42	58
c rinsate	7.77	394	90	48	42	60

Metal Toxicity Identification Evaluation Methods

All TIEs began within 12 h of sample collection. The procedures were applied to water collected from eight of the 10 sites. Sites 1 and 2 were excluded due to equipment and time limitations. Chelex 100 resin (Biorad, sodium form) was used to remove multivalent cations and Ag2-x8 resin (Biorad, chloride form) to remove anionic forms of metal and oxyanions. The specific procedures for resin column preparation, sample application to the resin columns, and sample amendments for bioassays are detailed in Connor *et al.* (1991). The column-treated waters of each site were amended to bring the hardness, alkalinity, and pH back to that of the respective untreated sample to ensure that the major difference was the missing heavy metals and oxyanions. Toxicity was not observed in upstream control amended rinsate waters, indicating that changes in ionic composition were not a problem.

Bioassay Methods

Bioassays were conducted using standard EPA protocol (US EPA 1994). The test organisms used were the fathead minnow *Pimephales promelas,* the cladoceran *Ceriodaphnia dubia,* and the green alga *Selenastrum capricornutum.* All organisms were maintained at 25 ± 1°C. EPA moderately hard reconstituted water served as the laboratory control water.

Statistical Methods

All of the toxicological endpoints were analyzed using an ANOVA and Duncan's multiple range test (Geng and Hill 1989) except for the

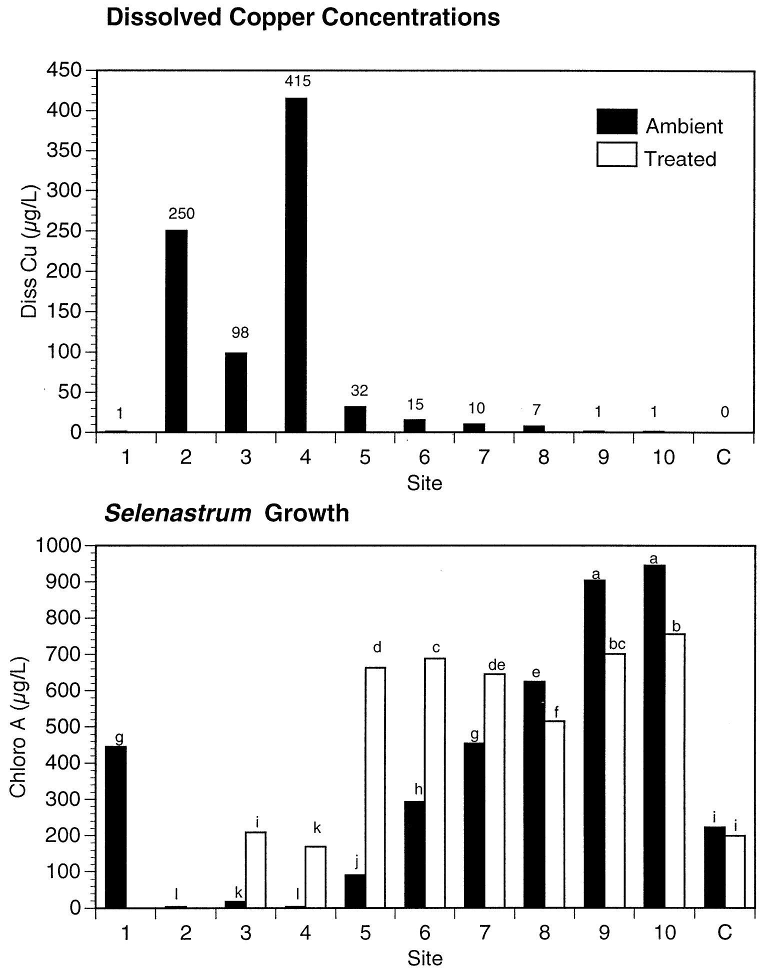

Fig. 2. Copper concentrations measured at each site and *Selenastrum* sp. growth as chlorophyll [A] (μg/L) for treated and untreated samples. For algal growth, significant differences are represented by dissimilar letters above the columns. Samples from Sites 1 and 2 were not treated with ion-exchange columns

Ceriodaphnia survival. The daphnid's survival was analyzed with Fisher's Exact Test ($p < 0.05$) where survival at each site and its respective treated water were compared to an upstream control (Zar 1996).

Results

Analytical Chemistry and Water Quality

Total and dissolved copper, cadmium, zinc, and iron concentrations are presented in Table 1. Total suspended solids also are included in Table 1 because of its relationship to the bioavailability of metals in natural waters (O'Donnel *et al.* 1985). The field temperatures ranged between 4°C and 10°C. Hardness, EC, pH, and alkalinity are presented for both ambient and amended column-treated waters in Table 2. The elevated electrical conductivity in the column-treated waters are due to sodium, chloride, and sulfate ions added during the application and amendment processes.

Bioassay and Toxicity Identification Evaluation

The results of the three species toxicity tests are presented in Figures 2, 3, and 4. The toxicological endpoints for all three species exhibit similar graphic profiles with the greatest depression occurring in Dolly Creek just below the tailings pile.

All tests passed EPA criteria for test acceptability, except that the *Ceriodaphnia* reproduction in the laboratory control water was lower than the recommended 15 neonates per female. This criterion was not met because of insufficient test waters to continue the tests for the extra day that would have allowed adequate reproduction.

Table 3 summarizes the significant differences in test species' performance between the ambient site waters and their respective upstream controls (Site 1 or 9).

Discussion

Dolly Creek becomes toxic at the point where the mine portal discharge enters the creek. Toxicity increases as the water flows over the tailings pile, then decreases gradually downstream from the flash board dam. The data clearly demonstrate a correlation between copper concentration and bioassay performance. Copper concentrations exceeded the criteria set forth by the EPA to protect aquatic life from the mine portal to 19.2 km downstream (US EPA 1984). No other metals exceeded these water quality criteria. Laboratory studies have shown copper to be toxic below the concentrations found in samples collected from Dolly and Little Grizzly Creeks. Carlson *et al.*

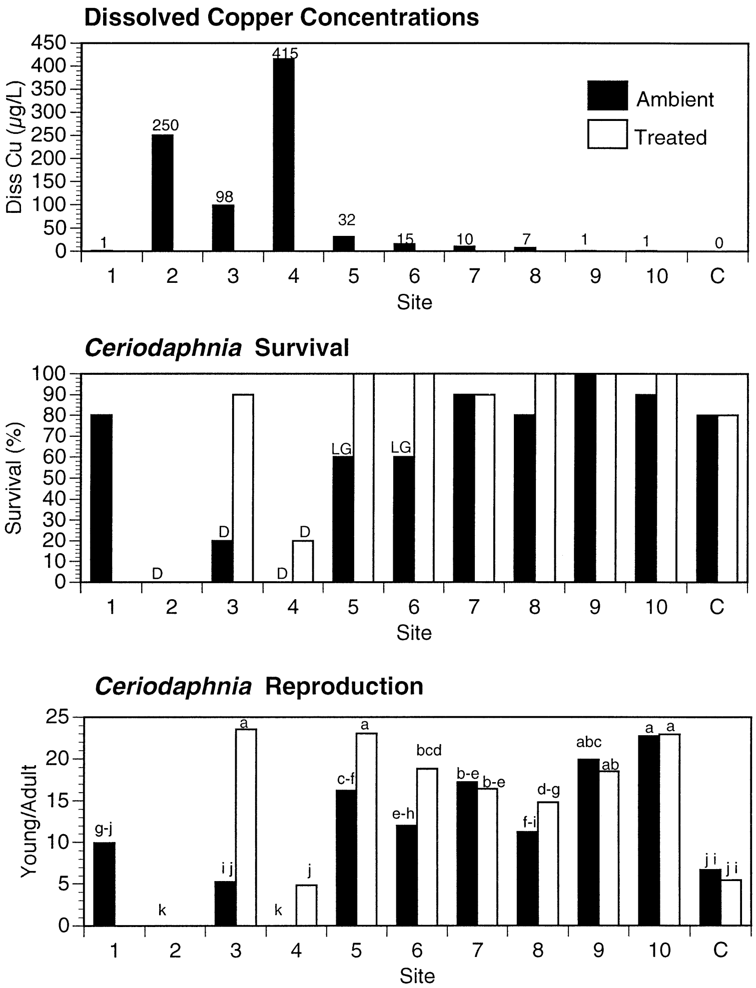

Fig. 3. Copper concentrations measured at each site and the two endpoints for *Ceriodaphnia* sp. For survival, columns labeled D or LG represent significant differences from Site 1 (Dolly Creek control) and from Site 9 (Little Grizzly Creek control), respectively. For reproduction, significant differences are represented by dissimilar letters above the columns. Samples from Sites 1 and 2 were not treated with ion-exchange columns

(1986) demonstrated chronic toxicity to *Ceriodaphnia* and fathead minnows to be around 17 µg/L and 85 µg/L, respectively. A study by Spehar and Fiandt (1986) reported a chronic EC_{50} of 56 µg/L to daphnids and 11 µg/L to the minnows. Belanger *et al.* (1988) have determined the 48-h LC_{50} to *Ceriodaphnia* to be 35 µg/L and found a 53% reduction in reproduction for a 7-day test at 9.9 µg/L. Baylock *et al.* (1985) have shown a 50% reduction of growth to *Selenastrum* in 40 µg/L Cu. As part of other studies, our laboratory has run several reference toxicant tests with Cu as $CuSO_4$. The 7-day LC_{50} values in soft water (hardness of 50 mg/L as $CaCO_3$) averaged 20 µg/L for the fathead and 30 µg/L for the daphnid. The 4-day EC_{50} for *Selenastrum* was 8 µg/L. Although these studies suggest differences in species sensitivity in relation to copper toxicity, these values can be used to establish a range of copper concentrations that might be expected to cause toxicity in natural waters. A number of factors, such as hardness, pH, alkalinity, dissolved organic carbon, and total suspended solids are known to affect the bioavailability and therefore, the toxicity of copper (US EPA 1984; Michnowicz and Weaks 1984; Nelson *et al.* 1986).

Zinc concentrations were lower than concentrations known to affect these species, with one exception. The sample collected immediately below the tailings contained zinc concentrations close to the *Selenastrum* EC_{50} generated by our laboratory (15 µg/L). Because our EC_{50} experiment was conducted at a hardness of 32 µg/L, a hardness less than half of Site 4, a definitive conclusion cannot be made about the role of zinc toxicity at this site. In-house assays also demonstrate that the *Ceriodaphnia* 7-day LOEC of 100 µg/L in soft reconstituted water, is more than three times the Zn concentration found at Site 4. Benoit and Holcombe (1978) demonstrated that one of the more sensitive life stages of the fathead minnow, egg adhesion, was effected at 145 µg Zn/L.

The deviation from EPA criteria for test acceptability is not believed to have qualitatively affected the results of this study. Keating *et al.* (1989) suggest that poor daphnid performance in reconstituted water may be due to the presence of an organic and/or a lack of trace nutrients. This theory may apply to natural and rinsate waters as well. Columns can emit trace organics and remove beneficial trace nutrients from the water.

As an objective of the study, we evaluated each of the remedial structures for its effectiveness on reducing the metal impacts. The levee successfully prevents lateral seepage, as indicated by the low copper concentrations and high organisms' performance at Site 10. Since its installation, the mine seal has reduced copper loading significantly, however the mine discharge remains acutely toxic to the daphnid and alga. Upon the

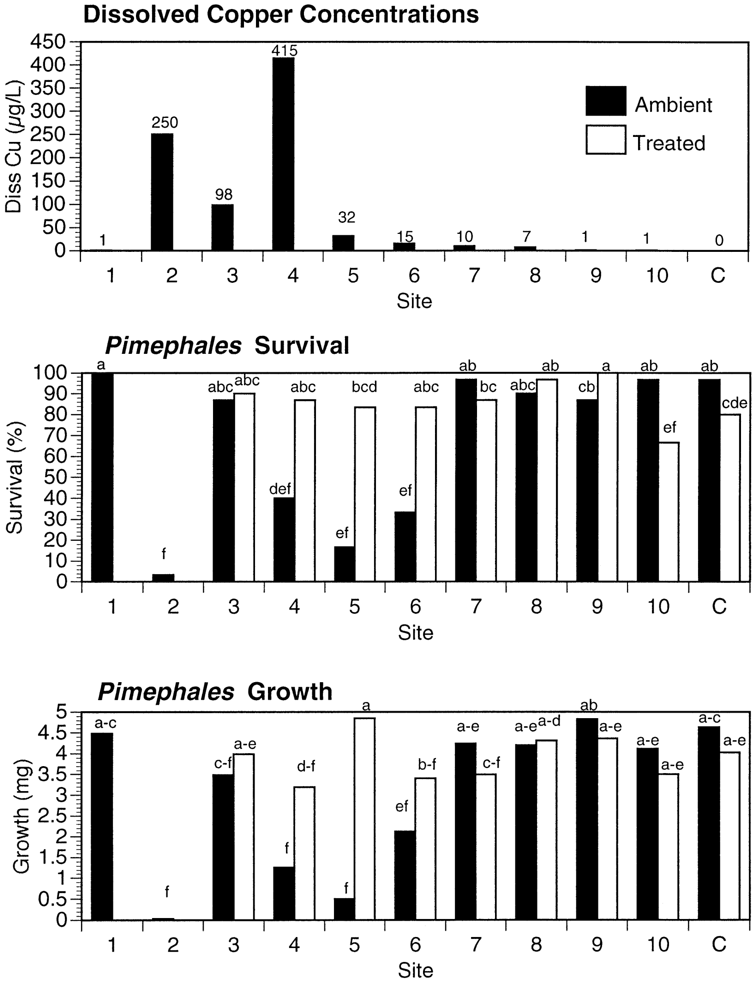

Fig. 4. Copper concentrations measured at each site and the two endpoints for *Pimephales* sp. For both survival and growth, significant differences are represented by dissimilar letters above the columns. Samples from Sites 1 and 2 were not treated with ion-exchange columns

effluent's dilution in Dolly Creek (Site 3), toxicity is reduced, but still present. Metal concentrations had no impact on minnow growth and survival at this site. The retention dam prevents downstream erosion of the tailings, but it does not prevent metal leaching from the tailings. Once Dolly Creek flows through the tailings, the water becomes increasingly toxic to the daphnid and alga and chronically toxic to the fish. Toxicity extended at least 6.4 km downstream to the daphnid and minnow and 19.2 km downstream to the alga. These conclusions are supported by unpublished data on benthic macroinvertebrate and fish community structure (Maier *et al.* unpublished data).

The leachate from the pile currently is the greater source of copper contamination to both Dolly and Little Grizzly Creeks. Most of the contamination arises from metals dissolving into the water as Dolly Creek passes over the tailings pile. Stabilizing the tailings is considered a high priority of future remediation programs. Regulatory agencies are considering economical methods to prevent the direct contact between the Dolly Creek and the tailings in future corrective programs. Interactions between precipitation, runoff, and the tailings should be given special attention. The mine seal has reduced copper loading into Dolly Creek. The current effluent continues to impact aquatic life, based on the laboratory bioassays, but to a lesser degree compared to before sealing. Additional remedial strategies should be considered here.

The use of ion exchange columns confirms the toxicity of metals to the organisms. This is evident with the improved organisms' performance in the treated waters where the corresponding ambient waters were toxic. Chelex resin has a higher binding affinity to heavy metals than to Ca and Mg. Recognition of metal removal became apparent with the complete loss of hardness in column-treated waters following sample application.

TIE procedures and biomonitoring are valuable tools in assessing the progress of remedial programs. The amount of metal contamination varies with precipitation and as remedial programs are implemented, laboratory experiments should be done periodically. The use of ion exchange columns works well to identify and confirm metals as a toxicant, especially downstream in aquatic systems where the origin of a pollutant may not be so apparent.

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Table 3. Summary of bioassay performance

	Dolly Creek Sites				Little Grizzly Creek Sites					
	1	2	3	4	5	6	7	8	9	10
Selenastrum growth	N*	I*	B	B	B	B	B	I	T	T
Ceriodaphnia survival	N*	I*	B	I	B	B	T	T	T	T
Ceriodaphnia reproduction	N*	I*	R	B	R	B	T	T	T	T
Pimephales survival	N*	I*	T	B	B	B	T	T	T	T
Pimephales growth	N*	I*	T	I	B	I	T	T	T	T

N = No significant difference in test species performance relative to the upstream control; I = significant inhibition in test species performance relative to the upstream control; R = significant improvement in test species performance in amended rinsate water relative to the corresponding ambient water; B = both significant inhibition in test species performance relative to the upstream control and improvement in test species performance in amended rinsate water relative to the corresponding ambient water; T = no significant difference in test species performance relative to the upstream control and in test species performance in amended rinsate water relative to the corresponding ambient water
* Ambient water was not applied to ion exchange columns

and no endorsement should be assumed. We would like to thank the following people for their support: Jon Goetzl, Mark Stephenson, Bill Croyle, Jerry Bruns, Louise Lampara, and Lisa Brattin.

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