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ENVIRONMENTAL RESEARCH BRIEF

Instream Tests for Toxicity Persistence from Heavy Metals

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Abstract

Instream toxicity tests used the larval fathead minnow *Pimephales promelas* and the cladoceran *Ceriodaphnia* in Prickly Pear Creek and Spring Creek, Montana, waters to examine toxicity persistence in a stream receiving metal inputs. The toxicity source was Spring Creek, a tributary of Prickly Pear Creek. Tailing and settling ponds, related to gold mining in the Spring Creek drainage, release zinc, copper, and cadmium to Prickly Pear Creek. Stream surveys characterized flow regimes, water quality, and biotic conditions in conjunction with toxicity testing. The study objectives were to: (1) develop a data base for validation of a toxicity persistence model; (2) assess the applicability of data from the Prickly Pear Creek study relative to model assumptions; and (3) assess field techniques for acquiring model input data.

Toxicity to test organisms was primarily due to zinc and copper in Spring Creek waters. Changes in Prickly Pear Creek toxicity downstream from the Spring Creek confluence were primarily due to dilution and hence were consistent with model assumptions. However, Spring Creek was not the sole source of toxicity in Prickly Pear Creek waters as unidentified toxicants were present in other tributaries. *Ceriodaphnia* was highly sensitive to toxicity in Spring Creek waters and provided useful model input data. *P. promelas* had a higher tolerance, and bioassay data from these organisms could not be used for model input. In the field, nutritional problems were encountered with test organisms using procedures described in bioassay protocols for each, suggesting either a quantitative food regime should be developed or a nonfeeding test be used in the future.

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Introduction

The U.S. Environmental Protection Agency's (EPA) Office of Water Regulations and Standards, Monitoring and Data Support Division (MDSD), is examining persistence and degradation rates of toxic wastes in streams. MDSD is seeking to identify methods most suitable for assessing instream persistence of whole effluent toxicity in receiving waters. Specifically, methods are required for site-specific assessment of effluent toxicities, both acute and chronic, prior to discharge, at the discharge point, and at points downstream where dilution, degradation, and partitioning to other compartments result in reduced toxicant concentrations. Particular interest centers on validation of toxicity models designed to predict instream toxicity persistence and validation of methods for acquiring input data for these models.

Instream toxicity testing has recently been conducted at several sites by EPA's Environmental Monitoring Systems Laboratory in Las Vegas, Nevada (EMSL-LV), and by the Environmental Research Laboratory in Duluth, Minnesota (ERL-D). Validation of a stream dilution model will be based on results from these investigations. Assumptions for the model presently being assessed are: (1) toxic chemicals and toxicity itself follow a conservative (not enhanced or degraded) mixing behavior; (2) physical, chemical, and biological interactions do not substantially alter toxicity at the point of complete mixing; and (3) variations in effluent toxicity are reflected in the varying toxicity of receiving waters and can be described by mass balance relationships.

To provide instream toxicity persistence data to MDSD, EMSL-LV conducted a stream toxicity study in the fall of 1983 at Prickly Pear Creek, Montana. The objectives of this study were to: (1) develop a data base to be used for model validation; (2) assess the applicability of data from Prickly Pear Creek relative to model assumptions; and (3) assess

field techniques for acquiring model input data. The study consisted of short-term acute and chronic toxicity tests using two test organisms and stream survey characterization of flow regimes, water quality, and biotic conditions.

Prickly Pear Creek flows north from its headwaters in the Elkhorn Mountains for approximately 64 km before entering Lake Helena and the Missouri River (Figure 1). Gold mining in the Corbin and Spring Creek drainage basin (draining into Prickly Pear Creek) began in the early 1860's. Tailing and settling ponds remain as prominent features within these drainages and release high concentrations of zinc, copper, and cadmium which are carried into Prickly Pear Creek via Spring Creek. Areas along Prickly Pear Creek were also subjected to extensive mining operations in the early 1900's. Over 75 percent of Prickly Pear Creek was subjected to stream bed modifications and dredging during the mining process.

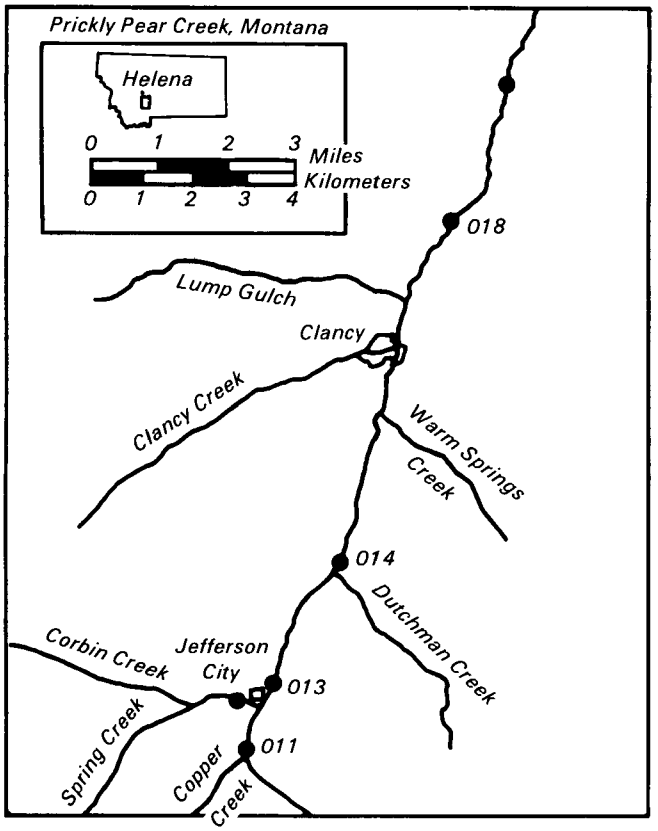

Figure 1. *Station locations on Prickly Pear Creek and Spring Creek, Montana.*

Procedures

Spring Creek toxicity and instream toxicity persistence in Prickly Pear Creek were determined using static renewal bioassays designed to measure both acute and chronic toxicity. Test organisms were the cladoceran *Ceriodaphnia*[1] and the larval fathead minnow *Pimephales promelas*. Toxicity tests were conducted with 24-hour composite stream and effluent water collected from September 30 through October 9, 1983. *Ceriodaphnia* toxicity testing actually began on October 1. Sampling stations are described in Table 1

Water quality and hydrological parameters were also measured as part of the study. For toxicity tests, grab samples of control waters were collected each day from the upstream Prickly Pear Creek station 011. These waters were diluted with Spring Creek water (the toxicity source) to obtain dilution test volumes with varying metals concentrations for comparison to ambient Prickly Pear Creek water toxicity.

Table 1. *Location of Stations on Prickly Pear Creek and Spring Creek, Montana, 1983*

Station No.	Description
011	Prickly Pear Creek, 1.1 km upstream from Spring Creek confluence
Spring Creek	Spring Creek, 100 m upstream from Spring Creek confluence
013	Prickly Pear Creek, 300 m downstream from Spring Creek confluence
014	Prickly Pear Creek, 3.8 km downstream from Spring Creek confluence, 100 m downstream from Dutchman Creek confluence
018	Prickly Pear Creek, 12 km downstream from Spring Creek confluence, 3 km downstream from Lump Gulch confluence

Results

Metal Concentrations

Spring Creek metal contributions caused significant increases in metals in Prickly Pear Creek water. However, approximately a two-fold decrease occurred between stations 013 and 018, due primarily to tributary dilution (Table 2). Dissolved metal concentrations were generally well below acute criteria in all downstream tributaries.

Total recoverable cadmium, zinc, and copper concentrations in Spring Creek and Prickly Pear Creek samples consistently exceeded U.S. EPA acute criteria for aquatic life during the toxicity testing period (Table 2). Concentrations of arsenic and lead were below the aquatic life criteria at all stations. Silver exceeded the acute criterion on October 6 at station 013, but was well below the acute criterion for all other dates and stations. Although cadmium exceeded the acute criterion, concentrations were below reported toxic levels for *Ceriodaphnia* and larval fathead minnows. Toxicity in test organisms was attributed to zinc and/or copper, but *Ceriodaphnia* bioassays indicated that another unidentified toxicant was present. Zinc and copper concentrations in Spring Creek were variable over the 9-day testing period with peak total recoverable concentrations on test days 1 and 5 (test numbers 1 through 9 refer to dates, October 1-9) (Figure 2).

[1]Taxonomy uncertain; may be *C affinis* or *C reticulata* x *C affinis* From *Ceriodaphnia* Workshop (U S EPA Region VIII) in Fort Collins, Colorado, March 6-7, 1984, personal communication Dr Dorothy Berner, Museum of Comparative Zoology, Harvard University, Cambridge, Massachusetts

Table 2. *Total Recoverable Concentrations of Selected Metals in Spring Creek and Prickly Pear, and U.S. EPA Calculated Acute Criteria for Aquatic Life*
Mean Values are 10-day averages (September 30-October 9, 1983).
Number of days criteria were exceeded are given in parentheses.

Total Metals (µg/l)		Station 011	Spring Creek	013	014	018
Cadmium*	$\bar{x}$	2(6)	7.6(9)	5(10)	4(5)	3(6)
	Range	1-3	6-12	2-9	1-6	2-9
	Criterion Range	1.5-1.8	4.7-6.1	2.0-2.7	1.9-2.8	2.2-3.2
Lead	$\bar{x}$	13(0)	72(0)	30(0)	19(0)	15(0)
	Range	7-22	44-238	20-54	11-26	8-28
	Criterion Range	74-100	291-389	108-155	103-160	121-183
Zinc*	$\bar{x}$	100(10)	2119(10)	580(10)	236(10)	203(0)
	Range	49-183	1260-3625	481-656	261-372	169-232
	Criterion Range	180-224	464-562	238-303	230-308	255-338
Copper*	$\bar{x}$	12(2)	84(10)	28(9)	14(3)	12(0)
	Range	6-13	37-220	12-47	<6-22	7-15
	Criterion Range	12-15	33-41	16-20	15-21	17-23
Silver	$\bar{x}$	0.6(0)	1.6(0)	1.9(1)	0.2(0)	0.1(0)
	Range	<0.2-0.9	0.2-3.1	<0.2-11.2	<0.2-0.5	<0.2-4.3
	Criterion Range	1.2-1.9	8.5-12.8	2.1-3.5	2.0-3.6	2.5-4.4
Arsenic	$\bar{x}$	2(0)	27(0)	6(0)	4(0)	10(0)
	Range	<0.5-11	1.5-84	3-10	3-7	8-12
	Criterion Range	440	440	440	440	440

**Consistently exceeded recommended acute criteria for aquatic life.*

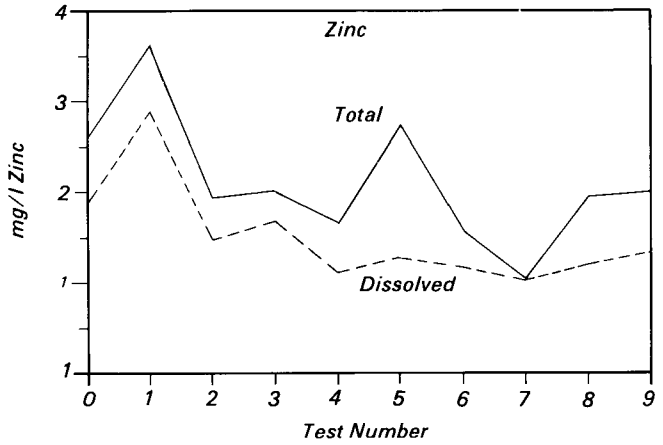

Figure 2. *Total recoverable and dissolved zinc concentrations, Spring Creek station, September 30 - October 9, 1983.*

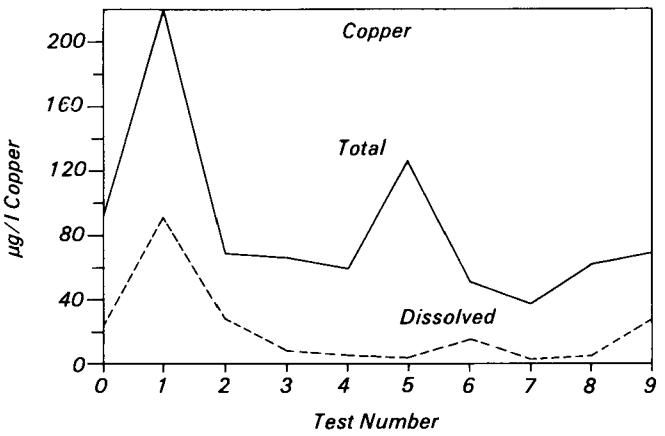

Figure 3. *Total recoverable and dissolved copper concentrations, Spring Creek station, September 30 - October 9, 1983.*

Toxicity Tests

Ceriodaphnia

Acute and Chronic Toxicity in Dilution Tests

Dilution tests with Spring Creek water produced acute effects (LC-50s) to *Ceriodaphnia* at dilution volumes varying from approximately 5 to 20 percent (Figure 3). There were no significant differences in Spring Creek acute toxicity in tests 2 through 5, 8, and 9, but toxicity was significantly higher in tests 1, 6, and 7. Higher toxicity in test 1 corresponded to high concentrations of zinc and copper in Spring Creek but this was not the case in tests 6 and 7.

Chronic toxicity, resulting in reduced neonate production, was only evident in tests 5 through 8 and occurred at dilution volumes of 5 to 10 percent Spring Creek water (Table 3). Reduced neonate production in tests 1 through 4 and in test 9 was in part or totally due to mortality, and chronic effects were not evident. Spring Creek chronic toxicity was greatest in tests 5 through 7 with significantly lower neonate production at dilution volumes of 5 percent Spring Creek water. Greater chronic toxicity in tests 6 and 7 was associated with greater acute toxicity due to the unidentified toxicant. Increased toxicity in test 5 was due to either the initial occurrence of the unidentified toxicant or to increased levels of zinc and copper. Overall, the

Table 3. *Mean Number of Neonates Produced and 95-Percent Confidence Limits,* Ceriodaphnia—*Tests 1 Through 9*
Chronic effect concentrations are noted for individual tests.
Comparisons were not made between tests.

Dilution Treatment Spring Creek (%)	Test 1	2	3	4	5	6	7	8	9
Control $\bar{x}$ (95% C. L.)	13.0 (11.7-14.4)	4.2 (1.9-6.5)	17.6 (15.5-19.7)	27.3 (21.8-32.7)	28.5 (26.2-30.8)	33.7 (31.8-35.9)	33.8 (22.8-28.7)	25.7 (11.7-14.4)	28.0 (15.5-19.7)
1% $\bar{x}$ (95% C. L.)	10.6 (8.4-12.8)	3.7 (2.5-4.9)	20.9 (15.1-26.5)	24.1 (21.2-27.0)	22.5 (16.7-28.4)	29 6 (26.5-32.9)	No Data	25.6 (23.2-28.1)	18.8 (14.0-23.9)
2.5% $\bar{x}$ (95% C. L.)	10.3 (8.6-12.0)	6.0 (2.5-9.4)	25.9 (23.4-28.4)	27.4 (26.6-28.2)	25.6 (22.6-28.6)	34.2 (33.2-35.2)	28.7 (27.0-30.3)	22.8 (17.5-28.2)	23.8 (20.9-26.6)
5% $\bar{x}$ (95% C L.)	10.1 (7.3-13.0)	7.2 (5.6-8.8)	25.3 (22 0-28.5)	21.8 (18.3-25.4)	9.9* (5.2-14.4)	21.2* (14.4-27.7)	22.9* (20.1-25.9)	18.4 (12.2-24.3)	18.9 (17.4-20.4)
10% $\bar{x}$ (95% C. L.)	1.0* (-0.5-2.5)	7.0 (4.3-9.5)	17.7 (15.6-19.8)	3.8* --	13.8 (9 6-18.0)	0	10 --	15.0* (12.6-17.4)	13.5 (10.4-16.6)
20% $\bar{x}$ (95% C. L.)	0	0*	0*	0	0	0	0	0	0*
Culture $\bar{x}$	23.3	26.5	28.6	38.5	37.9	35.8	30.9	25.3	25.2
Water (95% C. L.)	(20.1-26.8)	(22.4-30.6)	(26.8-30.5)	(32.6-44.3)	(35.1-40.7)	(34 0-37.6)	(28.7-33.0)	(22.6-28.1)	(21.8-28.6)

**Significantly different from control treatment, based on 95 percent confidence limits, indicating chronic effect level.*

relationship between toxicity and metal concentrations was poor, due primarily to the occurrence of the unidentified toxicant.

Control water toxicity was evident in tests 1 through 3 with significantly lower neonate production in the control tests relative to the culture water tests (Table 3). Bioassays conducted on water collected from the tributary streams on October 16 revealed a potential source of control water toxicity due to Copper Creek inflow, located 100 m upstream from control station 011. Significant difference in neonate production (control vs. culture water) was also found in test 5, but this was probably due to nutritional differences in the two waters. The culture water supported high concentrations of algae (*Closterium*) and bacteria, and provided a greater food supply for *Ceriodaphnia* than was available in stream waters.

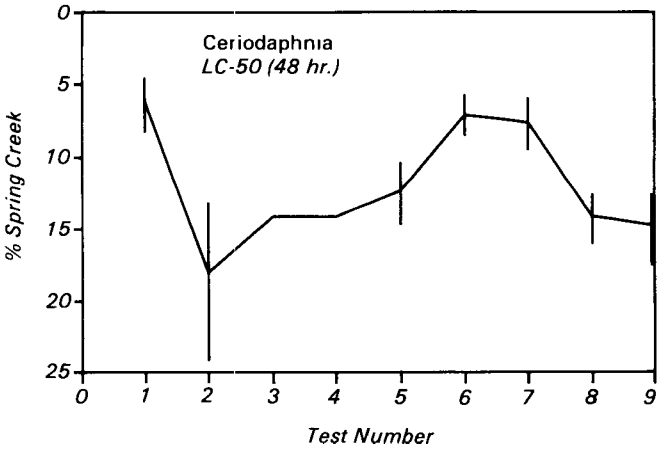

Figure 4. *Percentage Spring Creek water resulting in 48-hour LC-50s and 95-percent confidence limits,* Ceriodaphnia *tests. Confidence limits could not be determined for tests 3 and 4 because mortality was 100 percent in the 10 percent and 20 percent dilution treatments.*

Instream and Dilution Test Comparisons

Toxicity in Spring Creek dilution tests and instream Prickly Pear Creek tests was compared to determine if instream changes in toxicity were due strictly to inflow of Spring Creek water (Figures 4-7). Dilution volumes of Spring Creek water at instream stations 013, 014, and 018 were 17.3, 7.2, and 2.4 percent, respectively, and approximated dilution volumes of Spring Creek water used in the *Ceriodaphnia* dilution tests (20, 10, and 2.5 percent). Mortality in dilution and instream tests having comparable Spring Creek dilution volumes showed a high degree of similarity. However, higher mortality in some instream tests suggested an additional instream toxicant similar in nature to the unidentified toxicant in Spring Creek. Neonate production in dilution and instream test comparisons also showed no significant difference in a majority of the tests (Table 4).

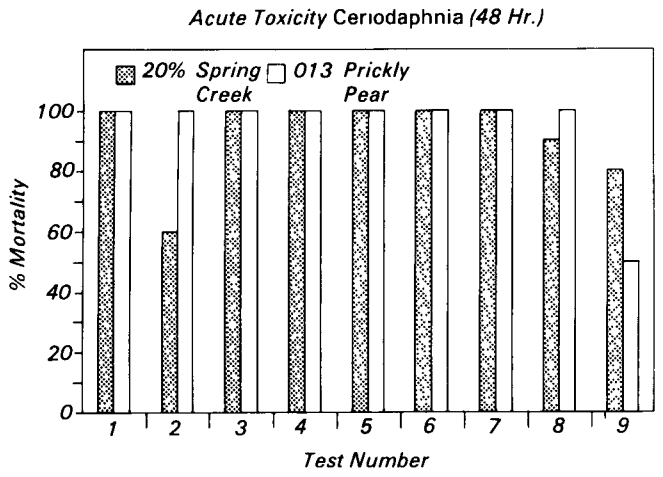

Figure 5. *Percent mortality in 20-percent Spring Creek water and Prickly Pear Creek station 013 treatments,* Ceriodaphnia *tests.*

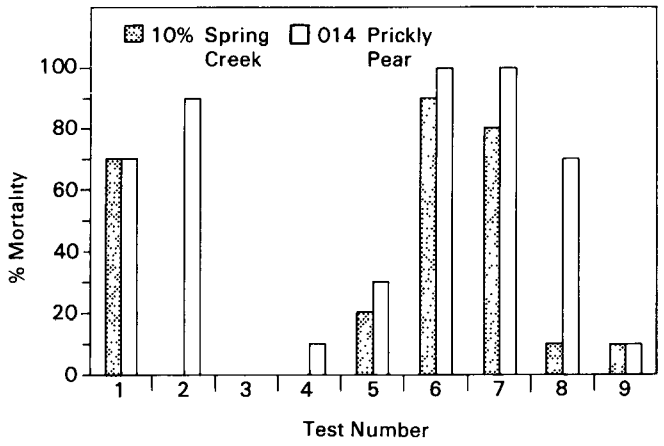

Figure 6. *Percent mortality in 10 percent Spring Creek water and Prickly Pear Creek station 014 treatments,* Ceriodaphnia *tests.*

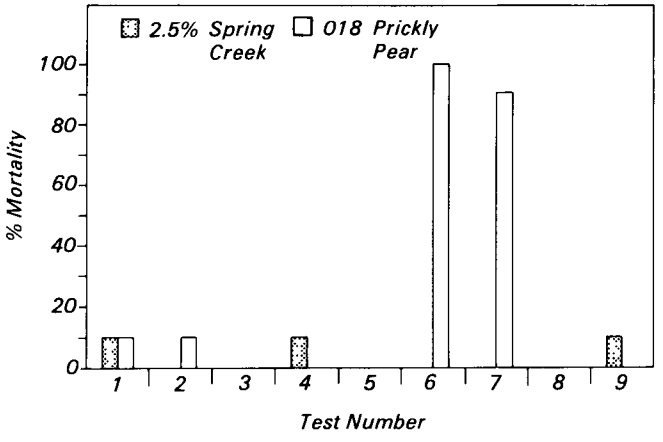

Figure 7. *Percent mortality in 2.5-percent Spring Creek water and Prickly Pear Creek station 018 treatments,* Ceriodaphnia *tests.*

Pimephales promelas

Larval fathead minnows were less sensitive to Spring Creek toxicity than were *Ceriodaphnia*. Estimated LC-50s for fathead minnows were at dilution volumes greater than 25-percent Spring Creek water, which was greater than dilution volumes found for instream Prickly Pear Creek stations (Figure 8). This lower sensitivity was also reflected in the instream station tests which showed little or no mortality.

Toxicity was highly variable for fathead minnows. Minimal mortality occurred in tests 2, 8, and 9. A significant decline in toxicity in tests 6 and 7 indicated that the unidentified substance toxic to *Ceriodaphnia* was not toxic to fathead minnows. Higher toxicity in tests 1 and 5 corresponded to elevated total recoverable concentrations of zinc and copper (Figures 2 and 7); however, a strong relationship for these metal concentrations and toxicity was not clearly evident in fathead minnow data.

High control mortality occurred after the third or fourth day and at test termination mortality was greater than 30 percent in six of the 10 tests (0, 1, 2, 4, 7, and 8). High control mortalities are usually indicative of procedural problems. However, mortality declined in the lower dilution treatments with little or no mortality at either 12.5 or 25 percent suggesting that Spring Creek water was ameliorating conditions in the control water. This mortality decline may have been due either to dilution of control water toxicity or to the addition of some factor enhancing survival.

Demonstration of chronic effects in fathead minnows was impossible due to highly variable growth rates. Growth was significantly increased with increased feeding in a separate feeding experiment, indicating test fish were probably underfed and that a quantitative food regime should be developed for future tests. Nevertheless, fathead minnows raised at EMSL-LV from identical egg batches showed variations in length approaching 400 percent after 30 days. This kind of growth variability highly influences test results and a nonfeeding lethality test may be more appropriate for field testing.

Stream Survey

Water Quality

Nonmetal stream water quality parameters revealed no additional sources of toxicity as ammonia, cyanide, and chlorine were below detection. All other water quality parameters were typical of fall conditions for good quality streams of the region.

Hydrology

Stream flow in Prickly Pear Creek increased from 11 cfs at station 011 to 37 cfs at station 018, with tributary inflows accounting for 62 percent of the increase. Flows increased between stations 013, 014, and 018 by approximately 5 and 8 cfs, respectively, as a result of ground water.

The percentage of Spring Creek water volume to the total water volume at downstream Prickly Pear Creek stations

Table 4. *Mean Number of Neonates Produced and 95-Percent Confidence Limits for Comparable Dilution and Station Treatments,* Ceriodaphnia—*Tests 1 Through 9*
Comparable dilution and station treatments were 20 percent and station 013; 10 percent and station 014; and 2.5 percent and station 018. Comparisons were not made between tests.

	Treatment		Treatment		Treatment	
Test	20%	013	10%	014	2.5%	018
1 $\bar{x}$	0	0	1.0*	6.6	10.3*	14.3
(95% C.L.)			(-0 5-2.5)	(3.8-9.3)	(8.6-12.0)	(12.5-16.0)
2 $\bar{x}$	0	0	7.0*	0	6.0*	11.8
(95% C.L.)			(4.3-9.5)		(2.5-9.4)	(9.9-13.7)
3 $\bar{x}$	0	0	17.7	19.2	25.9	23.4
(95% C.L.)			(15.6-19.8)	(18.1-20.3)	(23.4-28.4)	(18.8-26.8)
4 $\bar{x}$	0	0	3.8	1.0	27.4	20.3
(95% C.L.)			--		(26.6-28.2)	(13.9-26.6)
5 $\bar{x}$	0	0	13.8*	3.5	25.6	30.6
(95% C.L.)			(9.6-18.0)	(0.9-6.1)	(22.6-28.6)	(28.0-33.1
6 $\bar{x}$	0	0	0	0	34.2*	0
(95% C.L.)					(33.2-35.2)	
7 $\bar{x}$	0	0	10	0	28.7	14
(95% C.L.)			--		(27.0-30.3)	--
8 $\bar{x}$	0	0	15 0*	1.0	22.8	12.6
(95% C.L.)			(12.6-17.4)	(-0.6-2.8)	(17.5-28.2)	(2.9-22.3)
9 $\bar{x}$	0	0	13.5	5.8	23.8	23.0
(95% C.L.)			(10.4-16.6)	(-1.3-12.9)	(20.9-26.6)	(14.0-32.2)

**Significant difference in comparable dilution and station treatments based on 95-percent confidence limits.*

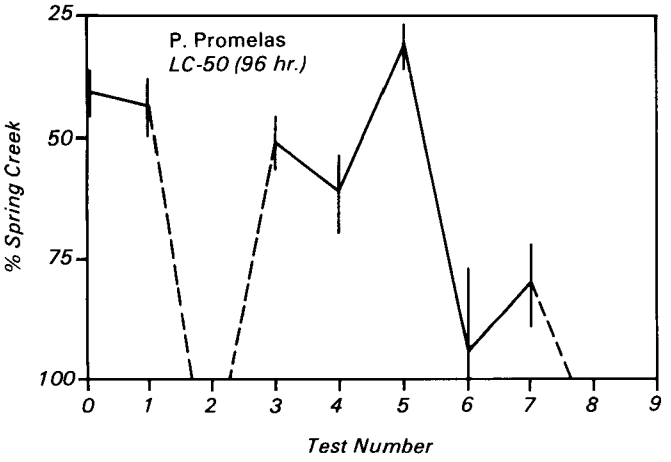

Figure 8. *Percentage Spring Creek water resulting in 96-hour LC-50s and 95-percent confidence limits,* Pimephales promelas *tests.*

013, 014, and 018 was 17.3, 7.2, and 2.4 percent, respectively, based on concentrations of Rhodamine WT injected into Spring Creek on September 23. Dye retention time from the Spring Creek confluence to station 018 was just over 11 hours.

Biota

Salmonid fishes were abundant at all Prickly Pear Creek stations but there was a downstream shift in species abundance from brook trout (*Salvelinus fontinalis*), to brook and rainbow trout (*Salmo gairdneri*) to brook, rainbow, and brown trout (*Salmo trutta*). The species shift in salmondis was probably due to increased pool habitats downstream.

Previous investigations conducted during summer have shown major reductions in both macroinvertebrate and periphyton numbers and diversity in the Prickly Pear Creek impact zone (station 013) and a gradual downstream recovery between stations 014 and 018. A superficial examination of macroinvertebrate communities indicated no evident reduction in either species types or species number in the impact zone. This lack of reduction may have been a physiological response to cooler water temperatures. Water temperatures during this investigation were approximately 7°C compared to summer temperatures of 16 to 20°C.

Metals in Sediment and Tissue

Sediment metal concentrations in Spring Creek and at station 013 were approximately an order of magnitude higher than those found at the control station 011 (Table 5).

Table 5. *Mean Sediment Metal Concentrations in Spring Creek and Prickly Pear Creek, Montana, September 27-29, 1983*

	Sediment Metal Concentrations (mg/kg)				
Station	Cadmium	Lead	Zinc	Copper	Silver
011	3	135	502	133	1
Spring Creek	29	3612	4975	1142	36
013	30	3240	4937	967	34
014	14	1243	2765	372	12
018	9	668	1680	202	6

No arsenic analysis

Concentrations decreased downstream but at station 018 were still substantially higher than concentrations found at the control station.

Tissue metal concentrations were highest in periphyton followed by macroinvertebrates and fish (Table 6). Periphyton and macroinvertebrate tissue concentrations were highest at station 013 and decreased downstream. Fish tissue metal concentrations were not exceptionally high and there was no substantial difference in tissue concentrations between stations. Previous investigations have found significantly higher tissue metal concentrations in most organs (kidneys, gills, brains, heart, and gonads) from fish collected in the impact areas of Prickly Pear Creek. However, muscle tissue did not have elevated metal concentrations. In this investigation, whole fish were used for tissue analyses and the inclusion of muscle tissue probably masked metal concentrations in the organs.

Conclusions

Metal concentrations in Prickly Pear Creek were significantly increased downstream from Spring Creek, which contributed elevated levels due to gold mine tailing and settling ponds in the drainage basin. Concentrations of cadmium, zinc, and copper measured over a 10-day period exceeded U.S. EPA acute criteria for aquatic life at one or more of the downstream sampling stations in Prickly Pear Creek. Elevated metal concentrations were the only identifiable water quality problems observed in Prickly Pear Creek during this investigation.

Spring Creek toxicity to test organisms (*Ceriodaphnia* and *P. promelas*) was primarily due to zinc and copper. Other unidentified toxicants were present and Spring Creek was not the only source of toxicity for Prickly Pear Creek waters; however, changes in toxicity (persistence) in Prickly Pear Creek were primarily due to downstream dilution of Spring Creek water. Spring Creek toxicity exhibited a conservative behavior in its downstream distribution in Prickly Pear Creek and was consistent with toxicity model assumptions.

Sensitivity of the two test organisms to toxicity in Spring Creek and Prickly Pear Creek was very different. *Ceriodaphnia* was highly sensitive, and bioassay results were applicable in assessing toxicity persistence in Prickly Pear Creek. *P. promelas* had a higher tolerance and could not be used in assessing toxicity persistence. Although sensitivity of the two animals was different, both appeared to be highly representative of toxic effects in Prickly Pear Creek native fish and macroinvertebrate communities found in previous studies.

Problems were encountered in the field bioassay procedures used for both organisms. These problems were related to the food regimen used in each bioassay. Cerophyl proved to be a better food source than yeast in *Ceriodaphnia* tests. Chronic toxicity was not measured in *P. promelas*, apparently because of underfeeding, and either a quantitative food regime should be developed for this test or a nonfeeding test should be used in future field testing.

Table 6. *Tissue Metal Concentrations in Prickly Pear Creek, Montana, September 27-29, 1983*

		Tissue Metal Concentrations (mg/kg)					
Organism	*Station*	*Cadmium*	*Lead*	*Zinc*	*Copper*	*Silver*	*Arsenic*
Periphyton	011	1	35	285	46	1	6
	013	37	1588	4640	1190	19	343
	014	9	175	1615	135	2	--
Macrophyte	014	12	252	2630	330	4	61
Macroinvertebrates	011	1	18	326	37	1	2
	013	12	165	2038	276	2	32
	014	4	47	660	65	1	8
	018	2	26	444	37	<1	7
Fish							
Salvelinus fontinalis	011	<1	3	70	11	<1	<1
	011	1	7	230	20	<1	1
	013	1	10	92	10	<1	<1
	014	1	5	145	14	<1	<1
	014	1	8	225	8	<1	1
Salmo gairdneri	014	1	12	255	16	<1	<1
Salmo trutta	018	1	10	220	12	<1	<1
Cottus *spp.*	011	<1	8	135	10	<1	<1
	014	1	6	265	28	<1	<1
	018	<1	6	255	7	<1	<1

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