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EFFLUENT BIOASSAYS AND STREAM INVERTEBRATE COMMUNITIES

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USE OF EFFLUENT TOXICITY TESTS IN PREDICTING THE EFFECT OF METALS ON RECEIVING STREAM INVERTEBRATE COMMUNITIES

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ABSTRACT

Intensive biological surveys were conducted during 1980 and 1981 on Prickly Pear Creek, Montana, a stream receiving metal-rich runoff from abandoned mines. The surveys characterized the macroinvertebrate benthic community and its physical/chemical environment. During 1981, static and flow-through bioassays were conducted using whole effluent and reconstituted freshwater. Total number of individuals and number of benthic taxa were more sensitive indicators of community change than other biological indices such as Shannon's diversity, evenness, and dominance. Stepwise multiple regression and principal components analyses showed changes in macroinvertebrate species richness and relative abundance to be linear negative functions of ambient metal concentratiions. Effluent bioassays showed potential for use in predicting in-stream biological effects from metal discharges. However, results of bioassays with brook trout showed a large discrepancy in tolerance between fish previously exposed to metals (acclimated) and those not exposed (nonacclimated). Avoidance behavior to sublethal concentrations may determine the presence of sensitive members of the ecosystem. Therefore, safe effluent criteria for toxicity should be based on presence of sensitive or desirable species in the receiving system, rather than LC50s alone. The concept of water effect (effluent: recovery zone) ratios merits further development.

Key words: onsite bioassays; mining; macroinvertebrates; water effect ratio; pollution impact.

INTRODUCTION

During 1980, the U.S. Environmental Protection Agency's (EPA) Office of Water Regulations and Standards issued a directive requiring documentation of water and biological quality in selected streams receiving mining, industrial, or municipal sewage treatment plant discharges. In response to this directive, a toxic metals study was designed to quantify four main objectives: 1) to document the concentration and distribution of toxic metals in selected streams receiving discharges from publicly owned treatment works (POTWs), mining activities, or industrial wastes; 2) to determine the biological state of receiving waters where the aquatic life criteria for toxic metals are exceeded, including sampling and analyzing fish, benthic invertebrates, and periphyton communities; 3) to report the extent to which criteria levels were observed to be exceeded; and 4) to develop explanatory hypotheses when healthy biota existed where criteria were exceeded.

Fifteen streams were originally sampled to provide a broad geographical representation and range of watershed types and uses, pollution sources, water quality characteristics, biota, and habitats. Results from the 1980 study indicate that, in some cases, known sensitive species of fish and invertebrates exist where EPA's acute and chronic criteria are exceeded. Analysis of these data led us to propose two hypotheses. First, metals can be chelated by organic and inorganic compounds in effluents and receiving streams, and are thus rendered biologically nonavailable. Second, fish are able to acclimate to sublethal metal concentrations which allow them to tolerate potentially toxic ambient levels.

To test these hypotheses, we conducted an intensive survey during 1980 and 1981 on Prickly Pear Creek, Montana. By the early 1860's, gold mining had begun near the upper reaches of Prickly Pear Creek, in the Corbin and Spring Creek drainages. Tailings and settling ponds from these long abandoned mines are a prominent feature of these drainages; the Montana Water Quality Bureau [1] reports that over 75% of Prickly Pear Creek was subject to streamback modifications and dredging during the mining process. Drainage containing high metal concentrations, including copper, zinc, silver, arsenic, and cadmium, is released from a mine adit and oxidized tailings, and then carried into Prickly Pear by Spring Creek. This paper discusses the importance of metal partitioning and biological acclimation in ameliorating metal toxicity.

METHODS

Study Area

Prickly Pear Creek forms its headwaters in the Elkhorn Mountains approximately 32 km southeast of Helena, Montana (Figure 1). The stream flows north for 64 km before entering Lake Helena and the Missouri River. Annual discharge of Prickly Pear Creek downstream from the confluence of Spring Creek during the 1979-80 water year ranged from 0.2 m^3/s to 1.2 m^3/s, with a mean of 0.5 m^3/s [1]. The study reach is generally characterized by continuous riffle flow interspersed with a few distinct pools. The substrate is primarily cobble and gravel throughout.

Five stations with similar riffle substrate and flow conditions were established in Prickly Pear Creek (Figure 1). One station was located in the control zone (011), and two stations (013 and 014) were located in the impacted zone 0.2 km and 1.6 km downstream from Spring Creek. One site (017) was located in the recovery zone approximately 8.5 km downstream from station 014. Station 018, located 2.4 km upstream from Montana City, was sampled only during 1981 (Figure 1).

Biological and Chemical Parameters

During 1980, five replicate box samples [2] were taken at each station (0.1 m^2 areal coverage) in Prickly Pear Creek. Collected invertebrate species were keyed to the lowest level possible, usually species. Oligochaetes were keyed only to class; chironomids were keyed to the family level during 1980 and to subfamily during 1981. Native fish were collected from each station using a seine and by electroshocking a 100 m reach at each station. Fish used in the bioassays were only those collected by seining.

Nutrients and metals sampled included all those listed in Table 1, although of primary importance to this study were those metals in excess of recommended criteria. Temporal variation was measured by integrating samples (ISCO model 1680) over a three-hour period continuously for 24 hours. Hence, at each site, there were eight 3-hour composite samples. In addition, one 24-hour composite was taken at each site. All nutrients were analyzed with a Technicon Auto Analyzer; the metals were analyzed using inductively coupled argon plasma emission spectroscopy (ICAP).

Static 24-hour and 96-hour flow-through bioassays were conducted at Prickly Pear Creek inside a mobile laboratory using a modified Mount and Brungs [3] diluter. The bioassays were run using a) Prickly Pear Creek water collected upstream from Spring Creek, b) Spring Creek water as a "whole" effluent, and c) laboratory reconstituted fresh water. All test chambers were placed in a water bath through which stream water at ambient temperature was circulated.

One fish species (Salvelinus fontinalis), and one invertebrate species, Ephemerella grandis (Insecta), were used in the bioasay tests. The E. grandis were captured upstream from the confluence of Prickly Pear and Spring Creeks where they were readily available near shore. Stream brook trout weighing 7 to 12 g were collected from the control station (011). Although this station served as a control, trace amounts of metals were present. Hatchery trout weighing 5 to 8 g were collected from the Bozeman National Fishery Research Laboratory. Nonacclimated (nonmetal-exposed) hatchery trout were placed in Prickly Pear Creek control water in the presence of equimolar concentrations of EDTA for two days prior to testing. The hatchery trout were acclimated to ambient Prickly Pear Creek metal concentrations by placing them into cages at the control site for ten days prior to testing.

Bioassays were conducted to determine the response variability between the two unrelated taxa, each with a known sensitivity to aquatic pollution. Specific tests included whole effluent (e.g., Spring Creek water) flow-through bioassays, using both stream and hatchery brook trout, E. grandis, and hatchery brook trout exposed to ambient metal concentrations in Prickly Pear Creek upstream from Spring Creek. The static bioassays used E. grandis individuals in Spring Creek water diluted with upstream Prickly Pear water, Spring Creek water diluted with reconstituted laboratory water, and reconstituted water spiked with copper.

RESULTS AND DISCUSSION

Chemistry

During 1980, mean concentrations of total copper, zinc, arsenic, silver, and cadmium in the impact zone (Table 2) were 3 to 5 times above recommended EPA acute criteria [4]. One-way analyses of variance (ANOVA) and Student-Newman-Keuls (SNK) multiple range tests [5] were used to determine patterns of difference between stations. Sample variance homogeneity was determined using Bartlett's test for homogeneous variances [5]. Concentrations of the five key metals examined, with the exception of arsenic, were significantly (α=0.05) greater in the impact zone than in either the control or recovery zones.

During 1981, mean total copper, silver, and zinc were 2 to 4 times in excess of the recommended criteria. Arsenic and cadmium levels did not exceed established national criteria levels. In general, metal concentrations during 1981 were considerably lower than those measured the previous year (Table 2).

Macroinvertebrates

During 1980 there were a total of 47 distinct macroinvertebrate taxa collected in five replicate box samples at four sites; only 8 of these were in concentrations greater than 5% of the total population (Table 3). The control zone site contained significantly higher (α=0.05) species richness and greater total numbers than did the impact zone stations. Total invertebrate numbers dropped an order of magnitude immediately downstream from the confluence of

Prickly Pear and Spring Creeks, and total number of taxa decreased to a third the upstream population (Figure 2). No significant differences among stations were observed with respect to Shannon's diversity, evenness, and dominance. As metal concentrations decreased further downstream, species richness and total numbers increased. However, stations in the recovery zone were not statistically different from those in the impact zone. Two possible reasons for this may be: 1) sample variances are sufficiently great to preclude parametric distinctions between the two sites; and 2) samples may not have been collected sufficiently far downstream (near Station 017) to reflect a true state of recovery. Total counts and number of taxa in the recovery zone never regained levels comparable to the control. This may be partly due to elevated zinc concentrations which at Station 017 were still in excess of recommended criteria and seven times greater than concentrations in the control zone.

During 1981, there were 61 distinct taxa collected at the four Prickly Pear sites (Table 3); of these only 9 were in concentrations greater than 5 percent of the total population. Species richness and mean counts per replicate at the impact site (014) were one-half those found in the control zone. Species richness at Station 011 was significantly ($\alpha = 0.05$) higher than at the other three sites; however, there were no statistical differences in relative abundances among stations. Species diversity and dominance were significantly lower at 014 than at any other site. Impact to the benthic community downstream from the confluence with Spring Creek was, in general, substantially less than that observed during 1980 (Figure 2), probably as a result of lower metal concentrations in 1981.

Forward stepwise multiple regression and factor analyses [6, 7, 8] were used to determine patterns of relationships between metal concentrations and macroinvertebrate populations in Prickly Pear Creek. Biological variables entering into the analyses included total number of individuals, total number of species, Shannon-Wiener diversity, species evenness, and Simpson's dominance. The five metals (both dissolved and total) reported in excess of recommended criteria during 1980 were used to develop statistically significant linear combinations which could account for the observed variance in measures of community structure.

Statistically significant regression equations emerged only for two community structural parameters: total number of individuals per sample (Totlind) and species richness (Nspecie) (Table 4). In the resulting regression equations, zinc and arsenic (both dissolved and total) were consistently the most important metals accounting for a major proportion of the variance in both total numbers and in species richness. Using dissolved metals only, zinc, arsenic, and copper combined to consistently explain over 90% of the observed variance in the two community parameters. The regression analyses were unable to explain at the $\alpha=0.05$ level the observed variance for diversity, evenness, and dominance.

Principal components analysis supports the results of the stepwise regressions and aids in determining how the benthic community responds to ambient metal concentrations. Both the dissolved and total forms of cadmium, copper, silver, and zinc load positively on the first principal component (Table 5). Both species richness and diversity (Hprime) load negatively on the first component, which alone accounts for 60.2% of the observed variance in the variables. The stronger, negative, loading of diversity and the high, positive, metal loadings implies a possible reduction in the species intolerant of the range of metals present. Further sampling, particularly behavioral drift in response to metal input, is necessary to verify this hypothesis.

The only metal loading strongly on the second principal component (Table 5) is arsenic, both the total and dissolved form. Total arsenic has a particularly high positive loading; the strong, negative, loadings for total number of individuals and species richness is understood in terms of the highly toxic nature of arsenic [9]. The positive loading for species' evenness (Even) indicates a "selective inhibition of crucial enzymes" [10], perhaps reducing several species' populations to low levels. The second principal component explains a further 31.9% of the observed variance among the variables; hence, a total of 92.1% of the observed variance is explained by both principal components.

Bioassays

Onsite bioassay tests during 1981 emphasized whole effluent (Spring Creek water) toxicities at each site. Consequently, in this study, most tests were conducted using a serial dilution of Spring Creek water with either Prickly Pear Creek water or reconstituted fresh water. Results of static bioassays using E. grandis and serial dilutions of Spring Creek water with either Prickly Pear control zone water or reconstituted fresh water are shown in Figure 3. In both tests, the LC50 was at concentrations slightly less than

100 percent effluent. The test using reconstituted fresh water as the diluent demonstrated slightly greater toxicity although it was not statistically (p=0.05) different. A flow-through bioassay (Figure 4) using E. grandis resulted in a very similar LC50 value to the static tests; the static LC50 to flow-through LC50 ratio was 1.0.

The final test using E. grandis was conducted with copper spiked in reconstituted fresh water (Figure 5). The calculated LC50 was similar to that when copper was spiked in the effluent tests. This provides some evidence that Prickly Pear Creek water contains no factors capable of reducing metal toxicity. Total organic carbon and suspended solids were at low concentrations (TOC 2.8 to 7.8 mg/l; total residues 26 to 299 mg/l; total nonfiltrable residues 11 to 67 mg/l). Therefore, we would further expect that Prickly Pear Creek should not have toxicity-reducing characteristics [Cf. 11].

The initial test using brook trout was conducted with hatchery fish acclimated to Prickly Pear water for two days. EDTA was added to the medium to prevent exposure to background metal concentrations. Simultaneously, resident fingerling trout were collected from, and isolated in, control zone water without EDTA. Bioassay results (Table 7) indicate hatchery fish are more sensitive to metals than resident fish; even 100 percent concentrations of Spring Creek water did not produce mortality in resident stream fish.

A second test was conducted after hatchery fish were allowed to acclimate for ten days in Prickly Pear Creek control zone water. Subsequent tests were conducted with whole "effluent" spiked with copper and zinc. No mortality was observed in either group of fish even at the highest concentrations. It appears short term acclimation results in at least a 3-fold increase in metal tolerance. This phenomenon provides some insight into the apparent anomaly of observing fish where water quality acute criteria are exceeded. For example, substantial numbers of brook trout were observed throughout the control, impact, and recovery zones of Prickly Pear Creek. Ultimately, bioavailability of toxic metals to the stream biota appears to be a function of the effluent metal chelating capacity or that of the receiving stream water, as well as the extent of previous exposure to metals, and traditionally acknowledged water chemistry parameters such as hardness, alkalinity, and pH.

S. fontinalis and E. grandis are common in Prickly Pear Creek and represent sensitive species within the resident fauna. Furthermore, other members of their respective families are among the most sensitive species listed in EPA's criteria documents. Assuming metal concentrations under which these species live can be directly compared to national criteria, unacclimated hatchery trout demonstrate an LC50 value notably greater than those published for national criteria values. For example, the copper concentration was twice the national acute criterion, and the zinc concentration was three times greater than the national criterion. This discrepancy may be due to population differences, or perhaps some acclimation was occurring despite the addition of EDTA. After acclimation, brook trout LC50 values for copper and zinc were at least four times in excess of their national criteria values.

Mayfly LC50 values were similar to nonacclimated brook trout values, even though the potential for acclimation existed. Consequently, E. grandis may be much more sensitive to metal pollution than trout. Ultimately, this information strongly indicates that species selected for bioassays and their history of exposure to sublethal metal concentrations will critically influence bioassay results. This phenomenon must be considered when evaluating EPA's proposed protocols for criteria modification.

Relationship of Instream Biological Data to Bioassay Data

Benthic community structure will ultimately reflect changes at both the population and individual level. For this Prickly Pear Creek analysis, species richness and the total number of individuals per sample were found to be inversely related to concentrations of five metals found to be in excess of criteria levels. Stepwise regression and principal components analyses indicate arsenic and zinc to be particularly important in explaining the observed variance in the number of species found and in the total number of individuals present.

The importance of understanding yearly (and seasonal) variation in community structure prior to establishing site specific criteria (EPA draft protocol, 1982) is demonstrated by studying the responses in species richness and total counts to 1980 and 1981 metal concentrations. Although trends were similar both years, the relationship between biological parameters and metal concentrations in 1981 was weaker than in 1980. It will be necessary to define how seasonal variation in flow, temperature, rainfall (and snowfall) affect the interrelationships between the physical/chemical milieu and the resident biota. Once these are known for a site, "critical seasons" can be established during which it may be reasonable to stop (or increase) effluent release. For example, in the case of Spring Creek and Prickly Pear, there may be low flow periods when the relative importance of metals in Spring Creek, as they influence benthic macroinvertebrates, increases. When Prickly Pear Creek is subject to greater volumes of water (e.g., summer storm events, spring snowmelt), metals may be flushed from the Spring Creek sources and ultimately ambient levels would decrease, although the short-term episodic consequence would be just reverse. These chemical considerations must also be considered in light of scouring impact to the benthic community during episodic events. Further seasonal testing will more clearly define these relationships.

Flow-through and static bioassays on Prickly Pear Creek using Spring Creek water as the effluent indicate strongly that acclimation can occur in resident vertebrate species, even after only five to ten days. It demonstrates that species used in bioassays should be chosen with care to determine site-specific metal criteria and further, that resident biota may reflect changes in ambient metal levels with greater sensitivity than top level carnivores in the system.

Theoretically, a site-specific application factor relating LC50 concentrations to recovery zone metal concentrations can be developed for potentially toxic metals. An example of how this might be applied is demonstrated in the following manner:

Table 7 lists the measured LC50 concentrations for two key metals in the whole effluent flow-through test with *E. grandis* (Figure 4). Each value is divided by its respective ambient total metal concentrations in the recovery zone, i.e., at station 018 (Table 2). This ratio yields a site specific application factor. To obtain ambient impact zone levels which cause no significant reduction of the Prickly Pear Creek invertebrate community, the concentration of each metal would have to be reduced by the appropriate application factor. For example, zinc concentrations in the impact zone would be reduced by a factor of 5.4.

Ultimately, a site-specific application can be related to "effluent" concentration by incorporating a factor which accounts for the approximate effluent LC50 dilution for the flow-through bioassay with E. grandis (Figure 4), in this example, 0.9. For example, the effluent concentration of zinc would have to be reduced by a factor of 4.86 (=5.4 * 0.9).

Part of the reduced metal toxicity in a receiving stream results from dilution. As evidenced by the Ephemerella flow-through bioassay, during low flows in late summer there is a 1:1 dilution of Spring Creek water with Prickly Pear water. Therefore, in our example, to compute a seasonal site-specific application factor, the effluent concentration for zinc would have to be reduced by a factor of 5.4 * 0.9 * 0.5 = 2.34 where: 0.9 = effluent LC50 percent dilution; 5.4 = LC50 concentration to recovery zone ambient concentration "application factor"; and 0.5 = level of effluent (Spring Creek) seasonal dilution.

If each metal concentration presently exceeding acute aquatic life criteria were to be appropriately adjusted, the recovery zone resident benthic community theoretically could be expected to shift upstream to the point of complete mixing of Spring Creek with Prickly Pear Creek. Use of this application factor concept would be further validated if we incorporate information on avoidance behavior as opposed to strictly mortality data [12] into our predictions concerning the presence and absence of sensitive species.

Some general conclusions, then, can be drawn from this study. Total numbers of individuals and total number of taxa in Prickly Pear Creek appear to be sensitive indicators of community change more so than other biological indices such as Shannon's diversity, evenness, or dominance. Changes in macroinvertebrate species richness and relative abundance in Prickly Pear were found to be linear functions of ambient metal concentrations.

Effluent bioassays may have potential for use in predicting in-stream biological impacts from metal discharges. Sensitivity of the test species, seasonality, and variation in community structural response all need to be taken into account. Finally, onsite effluent bioassays with brook trout indicate a large discrepancy between groups of fish previously exposed to metals (acclimated) and groups of fish not exposed to metals (nonacclimated). Variable or unknown histories of test organism exposure to metals can lead to misleadingly high LC50 results. However, using acclimated animals to develop criteria may not be appropriate for two reasons. First, intermittent discharges may not allow acclimation. Second, acute to chronic application factors may not be realistic, i.e., although short-term adult acclimation can occur, changes in metabolic functions may impair juvenile growth and subsequent reproduction.

RECOMMENDATIONS

1. Additional experimentation needs to be done on a site-specific basis to tighten statistical replicability with invertebrate field collections, if these are to be used in definition of control, impact, and recovery zones in receiving streams. This could be done by increasing the number of field replicates or by using alternative standardized samplers such as artificial substrates.

2. To evaluate the impact of a particular effluent on a receiving "control zone," organisms which demonstrate a sensitive reaction to the effluent should be used. Using a native organism will ensure acclimation to background water quality characteristics of each stream.

3. The validity of water effect ratios in site-specific criteria modification needs to be tested at additional locations using a variety of effluent types and quality of receiving waters (for example, with those having a higher TOC or suspended solid content). Further, this technique should be tested with other pollutants which are less stable or volatile (e.g., ammonia and organic compounds). The possibility of using this approach for all types of complex discharges should be evaluated.

4. Standard LC50 values do not predict "no effect" acute criteria values. Presence or absence of sensitive members of the aquatic ecosystem may stem from avoidance behavior to sublethal concentrations, since significant impact can occur at concentrations much less than the LC50 values.

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4. Standard LC50 values do not predict "no effect" acute criteria values. Presence or absence of sensitive members of the aquatic ecosystem may stem from avoidance behavior to sublethal concentrations, since significant impact can occur at concentrations much less than the LC50 values.

Thus, safe effluent criteria or standards for acute toxicity should be based on the presence or absence of sensitive or desirable species in the receiving system, rather than standard LC50s alone.

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Table 1. Chemical parameters collected in Prickly Pear Creek.

A. Technicon Auto Analyzer (mg/l)

- Total phosphate
- Orthophosphate
- Hydrolysable phosphate
- Kjeldahl nitrogen
- Total ammonia (NH_4)
- Nitrates + nitrites
- Total alkalinity

B. Additional Parameters (mg/l)

- Total Ca + Mg hardness
- Total organic carbon (carbon analyzer)
- Total residues
- Suspended residues
- Total sulfate

C. Metals - ICAP*

Cu, Cd, Zn, As, Ni, Ag, Cr, Se, Ca, Mg, Al, Pb (μg/l)

- Total recoverable
- Filtered through 0.45 μm
- Sediments

D. In-Situ Parameters

- Dissolved oxygen (mg/l)
- pH (SU)
- Conductivity (μmho)
- Temperature (°C)
- Turbidity (NTU)

* ICAP = Inductively Coupled Argon Plasma Emission Spectroscopy.

Table 2. Comparison of mean total concentrations (μg/l) of selected metals versus calculated acute and chronic water quality criteria (U.S. EPA 1980) for aquatic life, Prickly Pear Creek, Montana. Dashes indicate no chronic criterion has been established.

	Stations						
	Control		Impact			Recovery	
	011		013		014	017	018
	1980	1981	1980	1981	1980	1980	1981
Hardness (mg/l)	57	65	160	124	124	117	133
Total Arsenic							
actual ($\overline{x}$)	400.2	90.3	631.0	61.2	654.3	728.3	60.4
acute criterion	440	440	440	440	440	440	440
chronic criterion	-	-	-	-	-	-	-
Total Cadmium							
actual ($\overline{x}$)	4.5	3.2	24.5	3.4	15.5	3.5	3.4
acute criterion	2	2	5	4	4	4	4
chronic criterion	0.01	0.02	0.04	0.03	0.03	0.03	0.02
Total Copper							
actual ($\overline{x}$)	13.1	15.7*	109.1	112.0	34.1	18.7	27.2
acute criterion	13	15	35	27	27	26	29
chronic criterion	5.6	5.6	5.6	5.6	5.6	5.6	5.6
Total Silver							
actual ($\overline{x}$)	23.1	6.7	43.4	10.4	25.0	5.2	15.8
acute criterion	2	2	9	6	6	5	7
chronic criterion	-	-	-	-	-	-	-
Total Zinc							
actual ($\overline{x}$)	65.9	13.2	1963.8	1000.4	840.1	464.7	348.3
acute criterion	202	224	475	385	385	365	408
chronic criterion	47	47	47	47	47	47	47

* Dissolved copper: total measurements not available.

Table 3. Percent composition of common (>5%) invertebrate taxa collected in box samples in Prickly Pear Creek, Montana, 1980-81. Blanks indicate organism was not collected that station and year in greater than 5% relative abundance. Extended species list is available from the authors.

	Percent Composition							
	1980				1981			
	Control	Impact		Recovery	Control	Impact		Recovery
	011	013	014	017	011	013	014	017
Ephemeroptera								
Baetis tricaudatus	25	26	7	8	36	45	67	26
Baetis sp.					18			
Plecoptera								
Pteronarcella badia				7				
Trichoptera								
Arctopsyche grandis					6	14		
Brachycentrus sp.			21	24				32
Stactobiella sp.		6	26					
Diptera								
Chironomidae	39	44						
Orthocladiinae					9	10	7	
Diamesinae						15		
Simuliium sp.						8		12
Atherix variegata			30	16			7	11
Coleoptera								
Optioservus quadrimaculatus	7			17				6
Hydracarina								
Sperchon sp.			6	6				

Table 4. Stepwise multiple regression equations and results for Prickly Pear Creek ambient metal concentrations and benthic community structural parameters.

Equation	r^2	Overall F
All metals		
Totlind = 456.0 - 0.342 Astotl - 1.448 Zndiss -0.071 Asdiss	0.962	0.003
Nspecie = 27.6 - 0.150 Zndiss - 0.013 Astotl + 0.813 Cutotl	0.945	0.006
Dissolved metals only		
Totlind = 292.3 - 4.764 Zndiss - 0.226 Asdiss + 10.866 Cudiss	0.952	0.004
Nspecie = 23.0 - 0.220 Zndiss - 0.826 Asdiss +0.263 Cudiss + 0.150 Agdiss	0.983	0.006
Total metals only		
Totlind = 466.6 - 0.447 Astotl - 3.992 Agtotl	0.906	0.003
Nspecie = 26.1 - 0.550 Cdtotl - 0.012 Astotl	0.783	0.022

Table 5. Principal components analysis using varimax rotation of biological and metal (total and dissolved) data from Prickly Pear Creek, Montana. Factor loadings less than 0.5 are not shown.

Variable Name	Factor Loadings 1	2	3	Communality
Totlind		-0.934		0.999
NSpecie	-0.593	-0.776		0.954
Hprime	-0.863			0.825
Even		0.616	-0.784	1.002
Domin			0.943	0.970
AsDiss		0.655		0.684
AsTotl		0.906		0.928
CdDiss	0.944			0.966
CdTotl	0.930			0.978
CuDiss	0.815			0.795
CuTotl	0.987			0.990
AgDiss	0.690			0.497
AgTotl	0.906			0.831
ZnDiss	0.925			0.976
ZnTotl	0.924			0.939

Factor	1	2	3
Eigenvalue	8.025	4.256	1.054
% of Variance	60.2	31.9	7.9
Cumulative %	60.2	92.1	100.0

Table 6. Bioassay results using acclimated and non-acclimated brook trout, Prickly Pear Creek, Montana.

Test	Metal Concentrations (mg/l) Zn	Cu	LC50
Hatchery Trout			
Nonacclimated	1.45	0.088	75% of effluent*
Acclimated	2.13	0.188	>100% of effluent
Native Stream Trout			
"Nonacclimated"	1.88	0.155	>100% of effluent
"Acclimated"	2.13	0.188	>100% of effluent

*Effluent = Spring Creek water.

Table 7. Copper and zinc effluent LC50 concentration: recovery zone ambient concentration ratios for Prickly Pear Creek, Montana. LC50 concentrations are found on Fig. 4; ambient metal recovery zone concentrations are reported in Table 2.

	LC50/Recovery zone concentrations (mg/l)	Application factor
Copper	0.111/0.027	4.1
Zinc	1.890/0.348	5.4

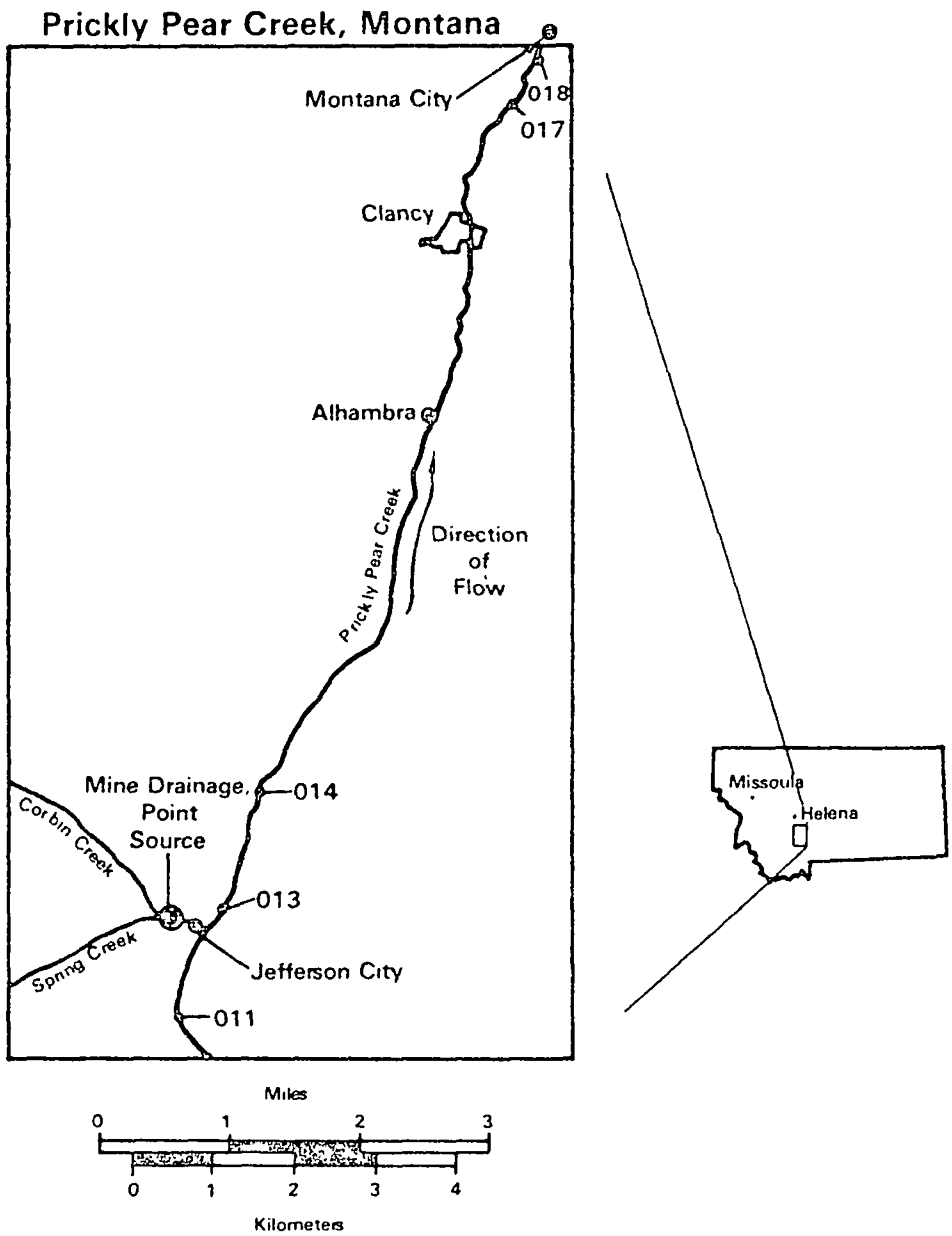
FIG. 1. Station locations on Prickly Pear Creek, Montana.

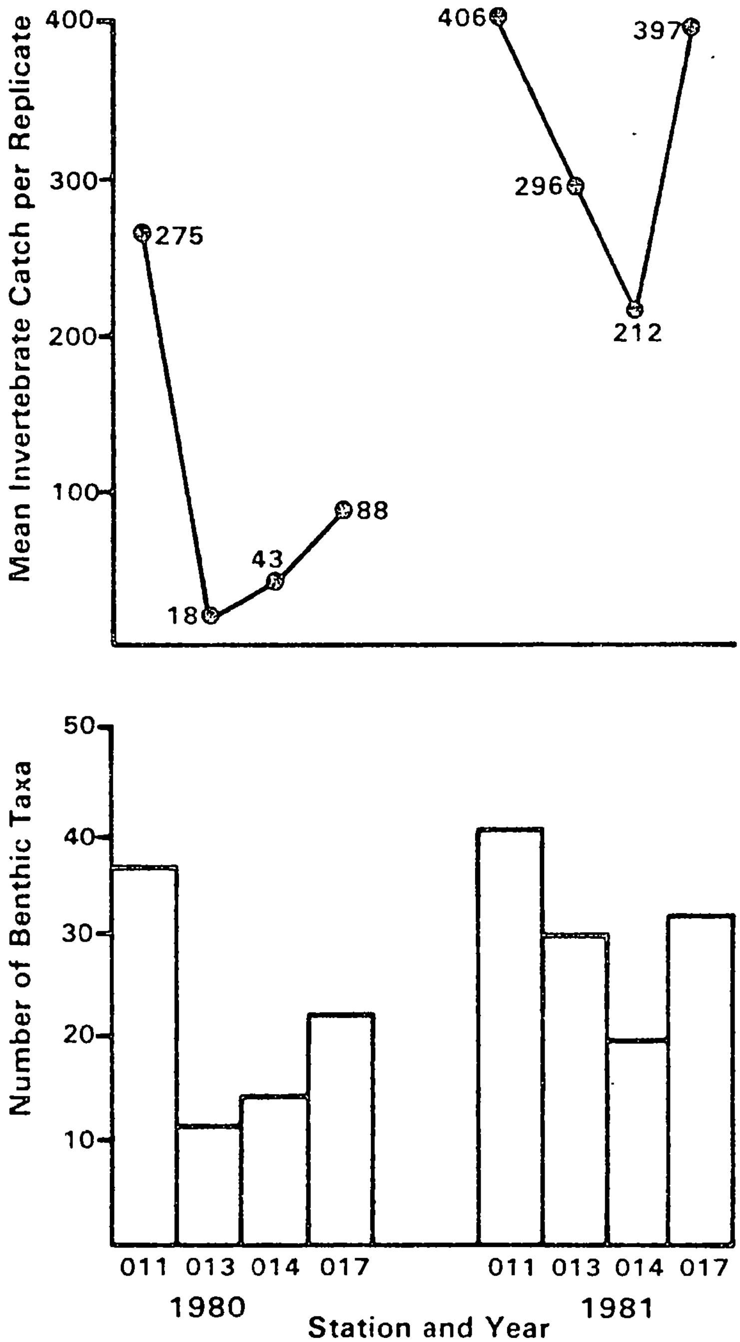
FIG. 2. Total number of macroinvertebrate taxa and mean count per replicate at control (011), impact (013, 014), and recovery (017) zone sites, Prickly Pear Creek, Montana. For clarity, error bars are ommitted.

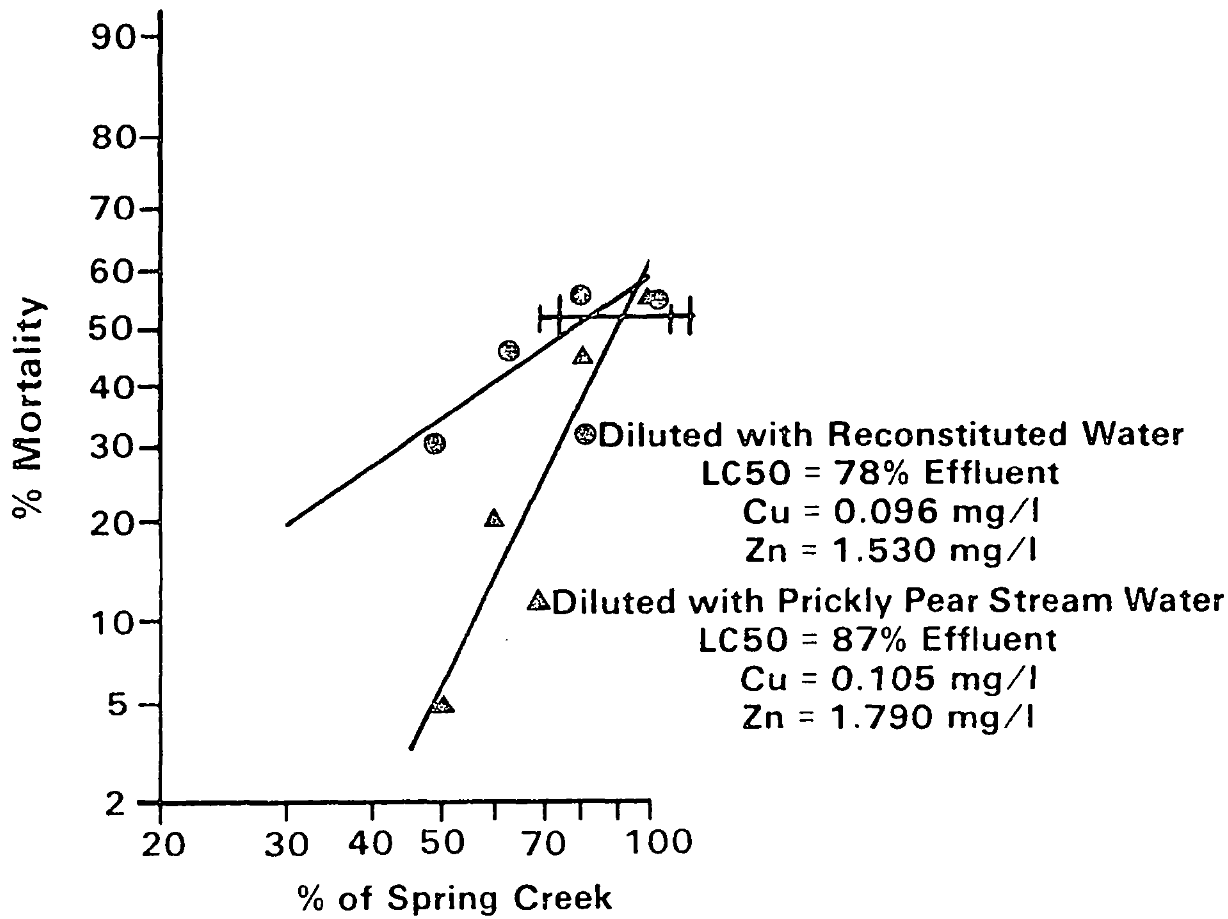
FIG. 3. Results of static bioassays with Ephemerella grandis in Prickly Pear Creek, Montana. Tests using reconstituted fresh water and stream (control zone) water are both shown. The horizontal bar is one standard deviation around the mean.

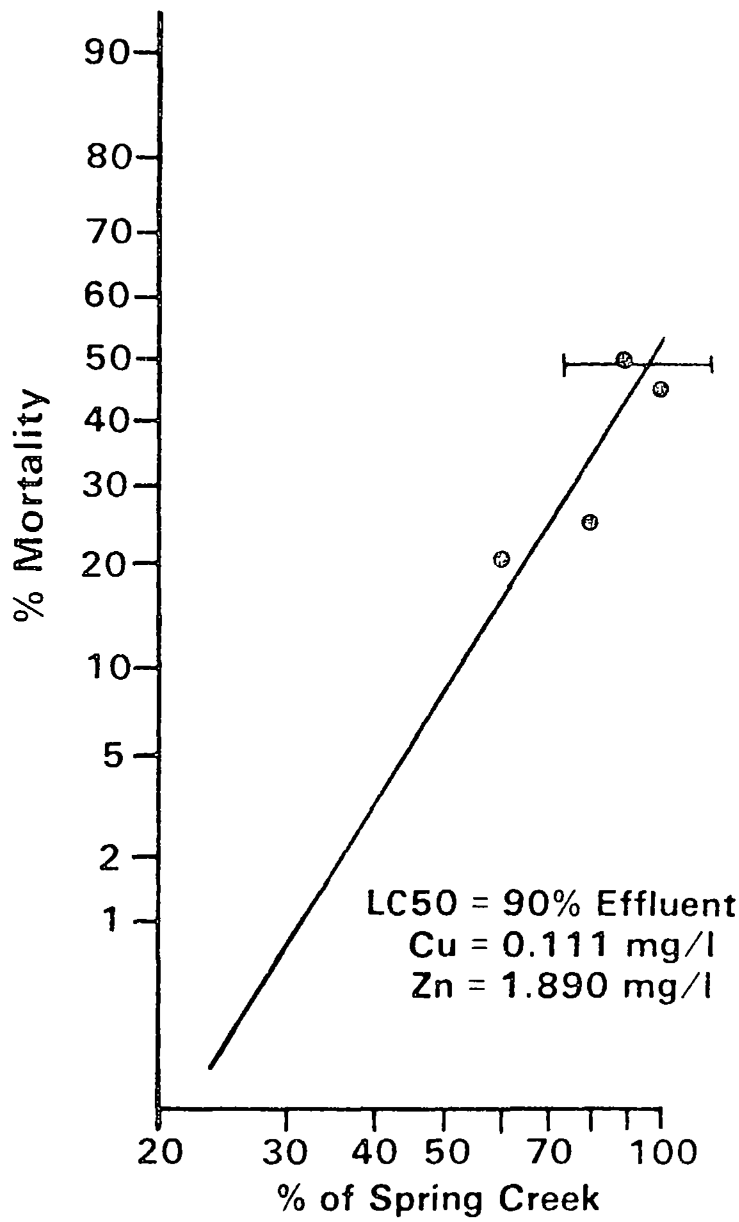
FIG. 4. Results of a flow-through bioassay with Ephemerella grandis in Prickly Pear Creek, Montana. The horizontal bar is one standard deviation around the mean.

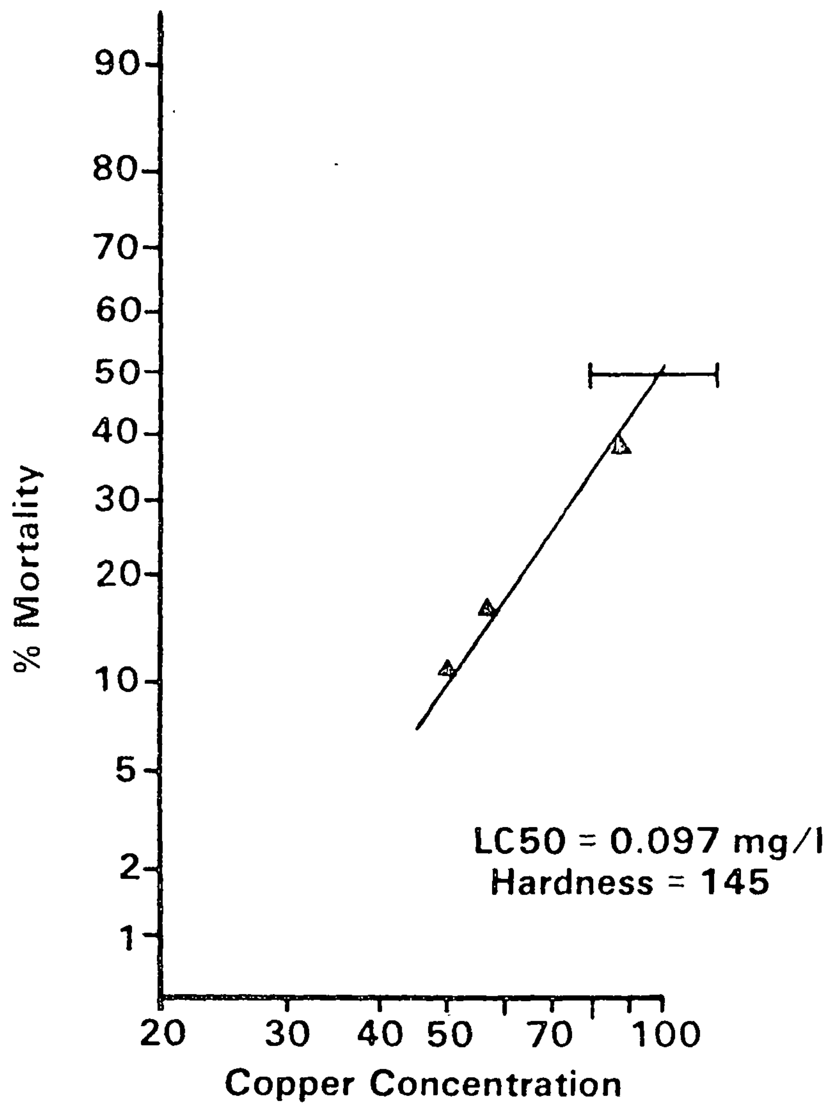
FIG. 5. Results of bioassays using Ephemerella grandis in reconstituted freshwater spiked with copper, Prickly Pear Creek, Montana. The horizontal bar is one standard deviation around the mean.


Prickly Pear Creek, Montana
Montana City
018
017
Clancy
Alhambra
Prickly Pear Creek
Direction of Flow
Mine Drainage, Point Source
014
Corbin Creek
013
Spring Creek
Jefferson City
011
Miles
0 1 2 3
0 1 2 3 4
Kilometers
Missoula
Helena


Mean Invertebrate Catch per Replicate
400
300
200
100
275
18
43
88
406
296
212
397
Number of Benthic Taxa
50
40
30
20
10
011 013 014 017
1980
011 013 014 017
1981
Station and Year


90
80
70
60
50
40
30
20
10
5
2
% Mortality
20
30
40
50
70
100
% of Spring Creek
Diluted with Reconstituted Water
LC50 = 78% Effluent
Cu = 0.096 mg/l
Zn = 1.530 mg/l
Diluted with Prickly Pear Stream Water
LC50 = 87% Effluent
Cu = 0.105 mg/l
Zn = 1.790 mg/l


90
80
70
60
50
40
30
20
10
5
2
1
% Mortality
LC50 = 90% Effluent
Cu = 0.111 mg/l
Zn = 1.890 mg/l
20
30
40
50
70
100
% of Spring Creek



90
80
70
60
50
40
30
20
10
5
2
1
% Mortality
LC50 = 0.097 mg/l
Hardness = 145
20
30
40
50
70
100
Copper Concentration