Relations between Benthic Community Structure and Metals Concentrations in Aquatic Macroinvertebrates: Clark Fork River, Montana

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ABSTRACT

We sampled macroinvertebrate communities at six sites on the upper Clark Fork River, Montana, to determine relations between macroinvertebrate community structure and metals in invertebrates and the best benthic community metrics to use for ranking sites based on the relative severity of the effects of metals. Concentrations (μg/g) of six metals in invertebrates were determined: Al (range = 591-4193), As (2.7-34.1), Cd (0.13-8.38), Cu (26-1382), Pb (0.54-67.1), and Zn (212- 1665). Concentrations of As, Cd, Cu, Pb, and total metals were significantly correlated with at least one benthic metric. Copper (r = 0.88-0.94) and total metals (r = 0.90-0.97) provided the most highly significant correlations. Based on longitudinal site comparisons of metals in invertebrates, benthic community structure, and differences between proportionally scaled ranks, five benthic metrics provided the best indicators of relative impact: taxa richness, Ephemeroptera-Plecoptera-Trichoptera (EPT) richness, chironomid richness, percentage of the most dominant taxon, and density. The two sites with the highest accumulations of invertebrate metals also demonstrated the greatest relative degree of impact based on these parameters. The most meaningful combinations of metrics indicate that the benthic community at the most upstream site is being severely impacted by metals. Two sites demonstrated little or no negative impact, and three sites demonstrated low or moderate levels of negative impacts, which may be due to a combination of metals and other factors such as organic enrichment. We recommend that benthic community structure and metals in invertebrates collected from riffle habitats be used to determine relative impacts in metals-contaminated river systems, owing to their close relation to metal availability and transfer to higher trophic levels.

INTRODUCTION

The Clark Fork River basin in western Montana has received intensive mining activity since the late 1800's (Knudson 1984, Johnson and Schmidt 1988). Mine tailings containing high concentrations of copper, cadmium, and lead were discharged directly into the river before 1960 and have been incorporated into river sediments and floodplain topsoils (Rice and Ray 1985, Moore and Luoma 1990, Axtmann and Luoma 1991). Surface water quality has improved in recent years because of improved mine wastewater treatment, but high concentrations of metals remain in sediments, macrophytes, and benthic macroinvertebrates (Moore and Luoma 1990, Axtmann and Luoma 1991, Cain et al. 1992). Elevated levels of heavy metals in Clark Fork River invertebrates have been documented up to 381 km from its source near Butte, Montana (Cain et al. 1992). The river is a significant sport fishery for rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). However, age-0 fish of both species are absent or greatly reduced in many mainstem river reaches, and populations are well below carrying capacity (Knudson 1984, Chapman 1993). High concentrations of metals and associated pH depressions due to snowmelt and rainfall events have caused periodic fish kills in the Clark Fork River (Johnson and Schmidt 1988).

Benthic invertebrates can be used to monitor heavy metal contamination in streams (Nehring 1976, Winner et al. 1980, Burrows and Whitton 1983, Clements et al. 1988, Hoiland and Rabe 1992, Hoiland et al. 1994). Because of their importance in nutrient cycling and as a food source for fishes, benthic macroinvertebrates can provide a means of metals transfer to higher trophic levels (Dallinger et al. 1987, ASTM 1991). Aquatic invertebrates are the main food source for age-0 brown trout and rainbow trout (Carlander 1969, Hubert and Rhodes 1992), and in aquatic systems contaminated with metals, invertebrates are known to accumulate high metals concentrations (Spehar et al. 1978, Dixit and Witcomb 1983, Luoma 1983, Cain et al. 1992, Hare 1992, Timmermans et al. 1992, Verrengia and Kesten 1993).

Several studies have documented different effects of metals contamination on benthic communities, including increases in drift rates (Hynes 1960, Eyres and Pugh-Thomas 1978), decreases in benthic density (Waters 1972, Winner et al. 1980), reductions in sensitive taxa (La Point et al. 1984), and changes in distribution patterns (Clements et al. 1988, Roline 1988, Becker et al. 1990, Clements et al. 1992). In the upper Clark Fork River, longitudinal differences in benthic community structure have been documented from previous studies (Chadwick et al. 1986, McGuire 1989a, 1989b), and concentrations of metals in sediments appear to be correlated with metals in invertebrates (Cain et al. 1992). Because of severe metals contamination, no aquatic invertebrates had been found in the upper reaches of Silver Bow Creek (Fig. 1) until 1981, nine years after initiation of improved mine wastewater treatment (Chadwick et al. 1986). However, specific relationships between metals in riffle invertebrates and benthic community structure have not been well established. The objectives of this study were to (1) examine relations between benthic community structure and metals in invertebrates in the Clark Fork River, (2) identify ecologically relevant community parameters that are best suited as markers for evaluating metals-related impacts in this system, and (3) provide an overall ranking of relative impacts for sites on the upper Clark Fork River based on metals in invertebrates and benthic community structure.

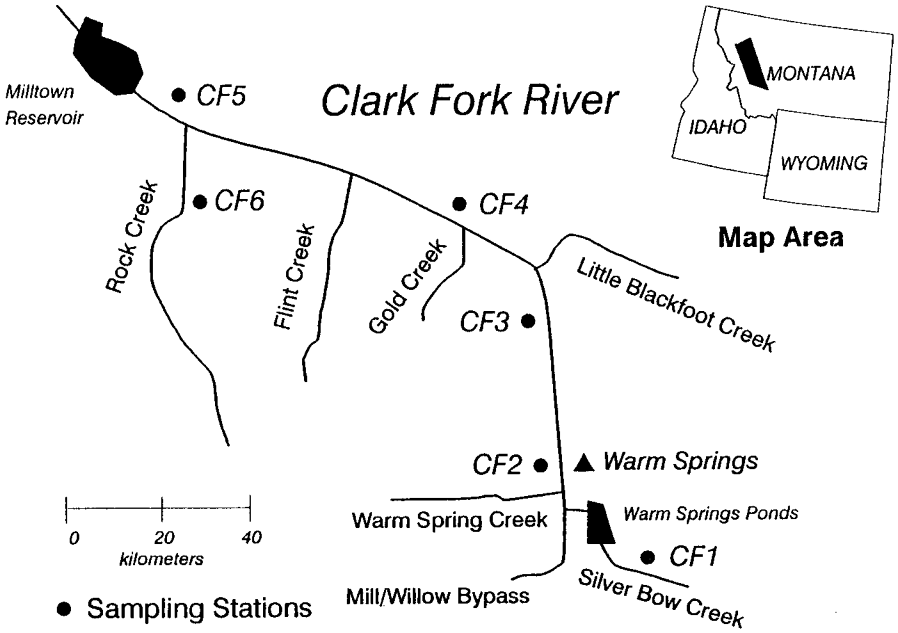

Figure 1. Location of sampling stations, upper Clark Fork River basin, Montana.

METHODS AND MATERIALS

Sample Collection

Benthic macroinvertebrates were sampled at six sites within the upper Clark Fork River drainage during the week of September 16-20, 1991: Site 1 (CF1), 1 km above the liming shack (at Silver Bow Creek, nearest to original source of metals); Site 2 (CF2), 2.7 km below Warm Springs Creek (at Warm Springs); Site 3 (CF3), 46 km below Warm Springs (at Deer Lodge); Site 4 (CF4), 98 km below Warm Springs (at Gold Creek); Site 5 (CF5), 198 km below Warm Springs (at Turah Bridge); and Site 6 (CF6), Rock Creek, 5 km above its confluence with the Clark Fork River (Fig. 1). Sites CF1-CF5 were equivalent to those used for collection of metals-contaminated sediments in other studies (Brumbaugh et al. 1994, Canfield et al. 1994, Ingersoll et al. 1994, Woodward et al. 1994, 1995). Rock Creek (CF6) is a tributary of the Clark Fork River and was chosen as a relatively non-contaminated reference stream. Based on visual examination of substrates and riparian vegetation of stream systems within the upper Clark Fork drainage, Rock Creek appeared to have habitats that were the most comparable to those at other Clark Fork River sites and was expected to contain a benthic fauna typical of the region.

Macroinvertebrate samples for analysis of community structure and determination of metals in invertebrates were taken from the same location within a riffle-run at each site. Five quantitative samples were taken at each site using a modified Hess sampler with an area of 0.1 m^2 and fitted with a removable bag of 320 μm mesh nylon netting. A current velocity meter, a 1 m measuring rod, and visual observations of substrate size were used to identify replicate locations that conformed to the following microhabitat characteristics: current velocity, 0.4-0.7

m/sec; depth, 10-30 cm; and visible substrate size of approximately 50% each of gravel/pebble (2-64 mm) and cobble (64-256 mm) (Wentworth 1922). Macroinvertebrate samples were preserved in the field with 10% buffered formalin and transferred to 80% ethanol before laboratory sorting. Sorted macroinvertebrates were identified to genus, and species when possible. The Oligochaeta (Tubificidae and Naididae) were identified to family. All species of *Hydropsyche* (Trichoptera: Hydropsychidae) were placed together because of the large number of small instars that could not be keyed to the species level. One riffle sample from site CF1 was lost during transport; therefore only four replicates for this site were included in the analysis.

For analysis of invertebrate metals, qualitative samples were collected with a 1 x 2 m kick seine from riffles and placed on a sorting table. Based on examination of site differences in benthic taxa collected during previous studies (Chadwick et al. 1986, McGuire 1989a, 1989b), a single, widespread species could not be used for metals comparisons. Therefore, alternating large and small organisms were randomly hand-picked with plastic forceps and placed in 8 x 16 cm clear polyethylene bags. Each of five plastic sample bags was half filled with organisms (approximately 100 g wet weight) to ensure that all samples had enough invertebrate tissue mass for analysis. We attempted to collect enough tissue mass from five locations within the designated riffle at each site. Because of the apparent low densities at site CF1, only three samples could be obtained from the designated riffle at this site. In terms of biomass, taxa that appeared to make up the largest percentage of the sample volume were recorded in field notes. All tissue samples were rinsed with river water, sealed, frozen on dry ice, and transported to the laboratory. Even though gut contents are not necessarily incorporated into invertebrate tissue, we did not allow 24 hr for invertebrates to purge their gut material before analysis, as was done in other studies (Cain et al. 1992).

Metal Analysis

Each invertebrate sample was homogenized, freeze-dried, and digested with nitric acid/hydrogen peroxide and microwave heating. Concentrations (μg/g dry wt.) of Al, As, Cd, Cu, and Pb in invertebrates were determined by graphite furnace atomic absorption; Zn was determined by conventional flame atomic absorption. More detailed descriptions of methods used for determination of metals in invertebrates, including detection limits, precision, and instrument performance, are given in a related study (Ingersoll et al. 1994).

Benthic Community Analysis

Eight benthic metrics were selected for use in comparisons with tissue metals and to aid in the determination of relative impacts and site ranking: (1) mean taxa richness, (2) mean EPT richness (number of taxa in Ephemeroptera, Plecoptera, and Trichoptera), (3) mean chironomid richness, (4) percentage contribution (relative abundance) of the most dominant taxon, (5) mean density (number/m^2), (6) EPT/chironomid ratio, (7) scraper/filtering collector ratio, and (8) the Hilsenhoff Biotic Index (HBI)(Hilsenhoff 1982, 1987). These metrics were chosen as possible indicators of toxic effects and overall benthic community health, and are commonly reported in benthic assessment studies (Klemm et al. 1990, Plafkin et al. 1989). Metrics related to taxa richness were calculated using genus-level identifications. However, species identifications were confirmed for some taxa to enhance interpretation of available tolerance information. Mean relative abundances (%) of the major functional feeding groups--scrapers, shredders, predators, filtering collectors, and gathering collectors (Merrit and Cummins 1984) were used in calculation of some metrics and to aid in the interpretation of site differences in invertebrate metals and uptake.

Statistical Analysis

Analysis of variance procedures were used to determine significant differences in means between sites for benthic metrics (one-way ANOVA, $P < 0.05$) and metals in invertebrates (ANOVA with Duncans multiple range test, $P < 0.05$). To examine relations between metals in invertebrates and macroinvertebrate community structure, metals and benthic metrics were ranked across sites using a proportional scaling method (Kreis 1988), which transforms values to a scale of 1-100 (1 = best, 100 = worst). Because many invertebrates are known to ingest particulates, sediment, and organic matter such as algae which are known to contain high levels of metals in the Clark Fork River (Axtmann and Luoma 1991), we ranked sites based on total recoverable metals (μg/g). For this paper, use of this data manipulation is based on the assumption that each benthic metric is of equal importance in determination of the final site ranking. Even though this is a difficult assumption to address, this scaling method retains the proportionality of the original data and provides parallel information that is directly comparable with site ranking based on sediment toxicity and benthos in other related studies on the Clark Fork River (Canfield et al. 1994, Ingersoll et al. 1994, Kemble et al. 1994).

To determine which benthic metrics were the best indicators of metals-related impacts in the Clark Fork River, statistical correlations between metals in invertebrates and benthic metrics were generated using means at each site ($n=6$). Because moderately high levels of Al and Zn are not acutely toxic to aquatic invertebrates, we examined relationships between metals and individual benthic metrics using total metals including Al and Zn, and total metals not including Al and Zn. A stepwise regression procedure was also used to find multiple variable relationships, with a 0.15 significance level as the criterion for entry of each variable into the model. Because both sets of data (benthic metrics, and metals in invertebrates) contain intercorrelated variables, no multiple variable models generated from this procedure were used in the interpretation of the results. Because of the large difference in invertebrate metals between CF1 and other sites, we also calculated these correlations with five sites ($n=5$), considering CF1 as an outlier.

To further assess which benthic metrics were the best indicators of relative impacts, we utilized a rank sum procedure (Zar 1984) and calculated the sum of absolute differences between site rankings for combinations of sediment metals reported in other studies (Brumbaugh et al. 1994, Canfield et al. 1994), metals in invertebrates, and benthic metrics. Means of proportional ranks were calculated for invertebrate metals, benthic metrics, sediment metals, and sediment toxicity (Kemble et al. 1994) to provide a set of overall rankings of Clark Fork River sites based on these parameters.

Table 1. Mean relative abundance (%), and available tolerance information, for the five numerically dominant benthic taxa collected from riffles at six sites on the upper Clark Fork River, Montana, on September 16-20, 1991.

Ephemeroptera						
Baetis spp.			10.0	16.5	4.6	
*Ephemerella inermis/ infrequens				7.0	18.9	
*Epeorus spp.						12.7
*Paraleptophlebia spp.						10.7
Heptageniidae						9.1
Trichoptera						
Hydropsyche spp.	51.9	22.1	21.2			
Helicopsyche borealis				4.8		
Lepidostoma spp.						9.2
Hydroptila spp.			6.0			
Coleoptera						
**Optioservus spp.		20.6	6.2			
Diptera						
***Cardiocladius spp.	14.2					
**Thienemannimyia gp.	4.6	17.3	6.5			
**Cricotopus spp.	3.8	6.6			16.6	
**Microtendipes spp.		6.8				5.6
Orthocladius spp.						13.8
**Tipula spp.				2.9		
Oligochaeta						
Tubificidae				39.4		
Naididae					9.9	
Nematoda	8.9					

* At least one species is listed as intolerant to acidic conditions and/or heavy metals (Klemm et al. 1990)

** At least one species is listed as tolerant to acidic conditions and/or heavy metals (Klemm et al. 1990)

*** All species are listed as tolerant to acidic conditions and/or heavy metals (Klemm et al. 1990)

RESULTS

We identified 126 benthic taxa from riffle samples. The genus *Hydropsyche* was numerically the most dominant at sites CF1-CF3, while mayflies and orthoclad chironomids dominated at sites CF5 and CF6 (Table 1). Mayflies and stoneflies were completely absent at site CF1. Even though species identification was not possible within many groups, sites CF1-CF3 were dominated by some genera that include acid tolerant/metal tolerant species (Table 1, Klemm et al. 1990). Sites CF5 and CF6 were dominated by some genera that contain acid intolerant/metals intolerant species (Klemm et al. 1990). The riffle community at site CF4 was dominated by oligochaetes of the family Tubificidae and by *Baetis* spp. (Ephemeroptera: Baetidae) mayflies, and had a larger number of gathering collectors than other sites (Fig. 2). In samples analyzed for metals in invertebrates, substantial portions of the biomass collected from sites CF3-CF6 consisted of large stoneflies (Plecoptera: Perlodidae, *Isogenoides* spp., Pteronarcyidae, *Pteronarcys* spp.) and large cranefly larvae (Diptera: Tipulidae, *Tipula* spp.).

Total taxa richness, chironomid richness, and density were significantly lower at site CF1 and significantly higher at site CF5. The reference site (CF6) had the most evenly distributed benthic dominance, and at sites CF1 and CF4, percent relative abundance of the most dominant taxon was significantly higher than at other sites (Table 2). The Hilsenhoff Biotic Index (HBI), which is an indicator of the degree of organic pollution (Hilsenhoff 1982, 1987), was significantly lower at CF6 (mean = 3.48, S.D. = 0.31), and significantly higher at CF4 (mean = 7.21, S.D. = 0.99) than at other sites (Table 2). Values for all eight benthic metrics and proportionally scaled ranks for all sites are given in Table 2.

Levels of metals in invertebrates were highest at site CF1 and generally decreased longitudinally downstream. However, invertebrates collected at site CF4 contained the second highest levels of As, Cd, Cu, Pb, and Zn and the highest levels of Al (Table 4). Based on individual correlations between benthic metrics and metals in invertebrates, each of the six metals was significantly correlated with at least one benthic metric (Table 3). Results of the stepwise regression procedure matched that of individual correlations and identified the most highly significant relationships ($r > 0.91$, $P < .01$, Table 3) as those that would provide the most important model components. Copper and total metals

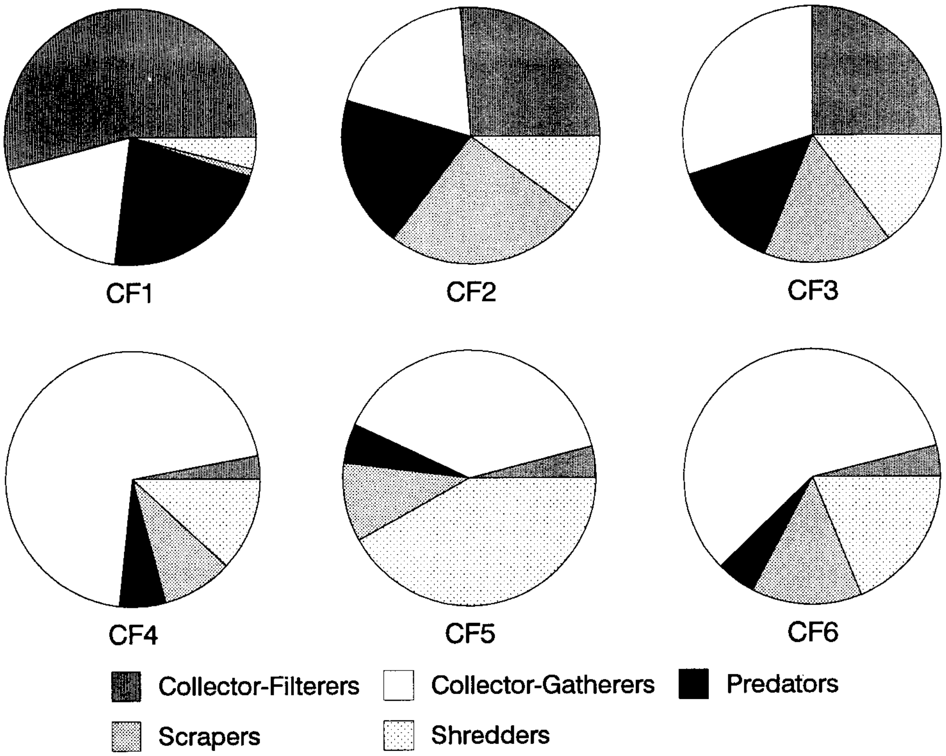

Figure 2. Percent relative abundance for five functional feeding groups at each sampling site on the Clark Fork River, Montana.

(μg/g, without Al, Zn) provided the strongest relationships (r = 0.92-0.94) with all the species richness metrics. Density (number/m^2) was significantly correlated with five individual metals and both combinations of total metals (Table 3). Taxa richness was highly correlated with Cd, Cu, Zn, and total metals (without Al, Zn). However, when only five sites were analyzed (excluding CF1), many of these correlations were not statistically significant (Table 3). Prior to this study, we did not anticipate the large disparity in levels of metals between CF1 and

Table 2. Mean and standard deviation of eight benthic metrics for riffle benthos collected on September 16-20, 1991 from six sites on the Clark Fork River, Montana. Numbers in parentheses indicate site ranking based on proportional scaling from 1-100 across sites (Kreis 1988). Means with the same letter are not significantly different (one-way ANOVA, p = 0.05).

Metric \ Site	**CF1** **n=4**	**CF2** **n=5**	**CF3** **n=5**	**CF4** **n=5**	**CF5** **n=5**	**CF6** **n=5**
Taxa Richness	14 **a**	44 **b**	53 **b**	49 **b**	62 **b**	52 **b**
S.D.	3	2	3	7	2	1
Ranking	(100)	(38)	(20)	(28)	(1)	(22)
EPT Richness*	5	18	23	21	28	24
S.D.	1	2	1	6	2	2
Ranking	(100)	(44)	(23)	(31)	(1)	(18)
Chir. Richness	5 **a**	13 **b**	14 **b**	14 **b**	19 **b**	17 **b**
S.D.	1	3	2	3	5	2
Ranking	(100)	(43)	(36)	(36)	(1)	(5)
% Dom. Taxon**	52 **a**	22 **b**	21 **b**	39 **a**	19 **b**	14 **b**
S.D.	12.7	1.5	0.7	8.2	3.6	4.9
Ranking	(100)	(23)	(20)	(68)	(14)	(1)
Density (#/m^2)***	909 **a**	15532 **b**	15023 **b**	11003 **b**	24248 **b**	18882 **b**
S.D.	460	5153	6695	6707	3496	3777
Ranking	(100)	(38)	(40)	(57)	(1)	(24)
EPT/Chir. Ratio**	2.05 **a**	1.04 **a**	2.61 **a**	2.99 **a**	1.32 **a**	2.73 **a**
S.D.	1.3	0.15	1.05	2.37	0.32	0.6
Ranking	(49)	(100)	(20)	(1)	(86)	(14)
Scraper/FC Ratio*	0.2	0.98	0.66	0.7	2.72	4.72
S.D.	0.01	0.13	0.11	0.07	0.39	0.91
Ranking	(100)	(80)	(87)	(86)	(43)	(1)
HBI**	4.68 **a**	5.45 **a**	5.17 **a**	7.21 **b**	5.04 **a**	3.48 **c**
S.D.	0.24	0.19	0.11	0.99	0.25	0.31
Ranking	(33)	(53)	(46)	(100)	(42)	(1)

* Analyzed with non-parametric ANOVA due to unequal variances. For both of these metrics, mean Wilcoxon Scores were lower for site CF1 and significant differences were found ($p > 0.05$).

** Analysis with 1/square root data transformation.

*** Analysis with square root data transformation.

other sites. Correlations were calculated with all sites and only five sites, because data for CF1 had the most influence on the relationships. No significant relationship was found between the EPT/chironomid ratio and any of the metals. Based on longitudinal site comparisons, statistical correlations between invertebrate metals and benthic community structure, and sums of differences between ranks (Table 5), five metrics provided the best overall benthic indicators for determining relative metals-related impacts: taxa richness, EPT richness, chironomid richness, percent dominant taxon, and density. The two sites with the highest accumulation of invertebrate metals (CF1, CF4) also demonstrated the greatest relative degree of negative impact based on these benthic community indicators (Table 5). The most meaningful combinations of metrics (Table 6) indicate that the benthic community at CF1 is being severely impacted by metals. Sites CF5 and CF6 demonstrated little or no negative impact, and sites CF2, CF3, and CF4 demonstrated moderate levels of negative impacts which may be due to a combination of metals and other factors such as organic enrichment.

Table 3. Correlation coefficients (r) for eight benthic metrics vs. metals in invertebrates (μg/g) from six sites sampled on September 16–20, 1991, on the Clark Fork River, Montana. Values represent correlations using means for all six sites (6), and for five sites (5) excluding CF1 as an outlier.

		Metals in Invertebrates						Total	Total
Metric	**# of Sites**	**Al**	**As**	**Cd**	**Cu**	**Pb**	**Zn**	**w/Al,Zn**	**wo/Al,Zn**
Taxa Richness	(6)	−.05	−.81	−.89*	−.94**	−.90*	−.92**	−.64	−.95**
	(5)	−.42	−.57	−.13	−.42	−.41	−.03	−.41	−.43
EPT Richness	(6)	−.10	−.83*	−.88*	−.93**	−.90*	−.90*	−.65	−.93**
	(5)	−.45	−.65	−.01	−.51	−.47	−.07	−.48	−.51
Chironomid Richness	(6)	−.14	−.87*	−.87*	−.93**	−.91*	−.88*	−.68	−.93**
	(5)	−.51	−.75	−.18	−.71	−.56	−.15	−.58	−.64
% Dominant Taxon	(6)	+.56	+.88*	+.79	+.77	+.90*	+.79	+.91**	+.78
	(5)	+.89*	+.77	+.66	+.65	+.88*	+.86*	+.99**	+.97*
Density $(\#/m^2)$	(6)	−.37	−.94**	−.84*	−.90*	−.94**	−.84*	−.83*	−.90*
	(5)	−.81	−.89*	−.28	−.87	−.80	−.36	−.80	−.81
EPT/Chir. Ratio	(6)	+.52	+.20	+.05	+.03	+.14	+.10	+.04	+.05
	(5)	+.52	+.33	+.04	+.44	+.39	+.07	+.87	+.32
Scraper/FC Ratio	(6)	−.43	−.81*	−.59	−.56	−.67	−.52	−.68	−.57
	(5)	−.54	−.78	−.74	−.83	−.68	−.61	−.69	−.79
HBI	(6)	+.85*	+.48	+.01	+.04	+.22	+.06	+.61	+.02
	(5)	+.85	+.91*	+.89*	+.88*	+.93*	+.93*	+.83	+.96*

* Indicates statistical significance at the $P < .05$ level

** Indicates statistical significance at the $P < .01$ level

Table 4. Concentrations of total recoverable metals ($\mu g/g$ dry wt.) in invertebrates collected from riffles in the Clark Fork River on September 16-20, 1991. Means (standard error of the mean in brackets) sharing a common letter within a column are not significantly different (ANOVA w/Duncan's, $P < 0.05$). Numbers in parentheses represent site ranking based on proportional scaling from 1-100 across sites (Kreis 1988).

			Metals			
Site (n)	**Al**	**As**	**Cd**	**Cu**	**Pb**	**Zn**
CF1 (3)	b 1354 [74.9] (22)	a 34.1 [2.15] (100)	a 8.38 [0.66] (100)	a 1382 [156] (100)	a 67.1 [6.41] (100)	a 1665 [50.5] (100)
CF2 (5)	b 1368 [86.5] (22)	b 14.6 [1.46] (39)	d 1.20 [0.07] (14)	d 122 [5.44] (8)	b 10.7 [1.31] (16)	d 304 [9.86] (7)
CF3 (5)	b 1305 [264] (21)	b 13.1 [1.14] (34)	d 1.43 [0.10] (17)	c 181 [20.9] (12)	b 9.93 [1.19] (15)	d 293 [1.92] (7)
CF4 (5)	a 4193 [85.4] (100)	a 26.8 [1.61] (77)	b 2.20 [0.07] (26)	b 266 [11.1] (19)	a 32.2 [0.98] (48)	b 453 [7.99] (17)
CF5 (5)	c 591 [60.1] (1)	c 3.39 [0.27] (3)	c 1.76 [0.16] (21)	e 48 [3.61] (3)	c 3.83 [0.13] (6)	c 359 [22.4] (11)
CF6 (5)	c 830 [124] (8)	c 2.70 [0.24] (1)	e 0.13 [0.01] (1)	f 26 [0.93] (1)	d 0.54 [0.09] (1)	e 212 [7.22] (1)

DISCUSSION

The most comparable habitat available at all sites was sampled to reduce the influence of habitat differences on benthic community structure. By using a multimetric assessment approach (Plafkin et al. 1989), our goal was to provide discrimination between impacts related to metals and those due to other possible sources such as organic enrichment. We did not use diversity indices in this approach because they have been criticized (Washington 1984), and other studies have shown diversity indices to be of limited value in evaluating longitudinal recovery of benthic organisms in Silver Bow Creek (Chadwick et al. 1986). Also, community dissimilarity due to contaminants often cannot be readily distinguished from that due to other variables such as habitat-related differences; therefore, we did not use similarity indices in our evaluation. Sites CF1-CF5 had comparable substrate and riparian vegetation, however longitudinal differences in stream size (order) and visual signs of organic enrichment were observed. Rock Creek (CF6) contained different riparian vegetation, and stream substrates had a lower degree of embeddedness than sites in the Clark Fork River. Even though our choice of benthic metrics was somewhat arbitrary, we used those that are the least influenced by these factors, or those that would help identify specific differences due to impacts other than metals.

Our results indicate that the riffle invertebrate community at some Clark Fork River sites is negatively impacted, at least in part, by heavy metal contamination. At site CF1, significantly reduced species richness (Table 2), a lack of stoneflies and mayflies, the presence of acid tolerant/metals tolerant taxa such as *Cardiocladius* spp. (Diptera: Chironomidae), and high levels of metals in invertebrates indicate that this site is severely impacted by heavy metals. This result is consistent with the findings of Chadwick et al. (1986), who reported invertebrate recovery in Silver Bow Creek did not begin at most stations until several years after implementation of mine wastewater treatment. At our site CF1, they also reported low benthic densities similar to our estimate of 909/m^2, and a high dominance of Hydrophsychid caddisflies (*Hydropsyche* spp.), which first became established in 1981 at some sites. Our data for site CF1 indicates a continuation of this trend.

Comparisons of invertebrate metals with those at CF5 and CF6, and site rankings based on benthic community metrics also indicate an overall negative impact at sites CF2, CF3, and CF4. At these sites, however, benthic community effects due to metals appear to be more subtle and difficult to distinguish from other factors such as organic enrichment. The difficulty in separating the combined effects of metals and organic enrichment in streams has been documented elsewhere (La Point et al. 1984). In general, levels of metals in invertebrates drop sharply between CF1 and CF2 and gradually decline downstream (Table 4). The abrupt drop in metals between these two sites, which are only 1.5 km apart (Fig. 1), is in part due to the Warm Springs settling ponds that were built in the 1950's to precipitate metals (Chadwick et al. 1986).

Table 5. Rank comparisons between eight benthic metrics and six metals in invertebrates and sediments from the Clark Fork River, Montana. Numbers represent a total sum of absolute differences between site rankings determined by proportional scaling from 1-100 (Kreis 1988). Values for n equal the number of comparisons included in the sum total (six metals = with Al & Zn, four metals = excluding Al, Zn).

	Invertebrate Metals		***Sediment Metals**	
Metric / Metals	**6 metals (n = 36)**	**4 metals (n = 24)**	**6 metals (n = 36)**	**4 metals (n = 24)**
Taxa Richness**	567	300	464	336
EPT Richness**	587	312	506	368
Chir. Richness**	619	326	590	424
% Dominant Taxon**	671	402	786	558
Density (#/m^2)**	687	378	763	540
EPT/Chir. Ratio	1643	1086	1533	1015
Scraper/FC Ratio	1346	827	1591	1073
HBI	1255	874	1662	1118

* Levels of sediment metals (μg/g) from Canfield et al. (1994) were re-ranked excluding their data from Milltown Reservoir

** Based on lower sums of differences between ranks, these metrics were selected as the best overall benthic components to be used for site ranking (Table 6).

Table 6. Final rankings of six sites on the Clark Fork River, Montana, based on benthic metrics and levels of metals in invertebrates and sediments. Values represent means of proportionally scaled ranks from Table 2, Table 4, and other sources (1 = best, 100 = worst).

Metric / Site	CF1	CF2	CF3	CF4	CF5	CF6
5 Benthic Metrics	(100)	(35)	(25)	(42)	(1)	(11)
5 Benthic Metrics + HBI	(100)	(38)	(27)	(55)	(1)	(3)
All 8 Benthic Metrics	(100)	(56)	(34)	(53)	(17)	(1)
6 Invertebrate Metals	(100)	(19)	(19)	(54)	(7)	(1)
4 Invertebrate Metals	(100)	(19)	(19)	(42)	(8)	(1)
*6 Sediment Metals	(100)	(11)	(6)	(9)	(1)	(2)
*4 Sediment Metals	(100)	(10)	(9)	(7)	(3)	(1)
*_Hyallela_ Toxicity Rank	(100)	(47)	(31)	(6)	(19)	(1)

* Re-ranked from Canfield et al. (1994) excluding their data from Milltown Reservoir

In contrast with the findings of others who have reported metals concentrations in invertebrates and sediments collected from the Clark Fork River (Cain et al. 1992, Canfield et al. 1994), metals in invertebrates at the Gold Creek site (CF4) were elevated over those at Warm Springs (CF2) and Deer Lodge (CF3) and were more comparable with the findings of Woodward et al. (1994, 1995). This site also had a significantly higher concentration of Al in invertebrates (Table 4) and noticeably higher amounts of filamentous algae and periphyton in riffle areas than at other sites. Algae and periphyton are known to contain elevated levels of metals in the Clark Fork River (Axtmann and Luoma 1991). Invertebrate detritivores and those in proximity to fine substrate materials in the stream bed are known to concentrate some metals at higher levels than in sediments (Dixit and Witcomb 1983, Smock 1983a, 1983b, Timmermans et al. 1989). This finding has been reported in *Pteronarcys californica* (Cain et al. 1992), *Tipula* (Elwood et al. 1976), and tubificid oligochaetes (Chapman 1985), all of which dominated in numbers or biomass at site CF4 (Table 1, Fig. 2). Gathering collectors may ingest larger amounts of detritus and particulate-borne metals because of their feeding habits. Elevated Al concentrations in invertebrates from site CF4 indicate higher particulate loads were present in these organisms (Ingersoll et al. 1994). We measured total metals present because invertebrate gut contents are a potential source of uptake by higher trophic levels. This factor could be ecologically significant for large taxa such as *Tipula* spp., in which gut contents are known to contain as much as 50% of the total body burden of metals (Elwood et al. 1976).

Site CF4 also had a significantly higher mean HBI (Table 2), which indicates an elevated level of organic pollution (Hilsenhoff 1982, 1987). This result is also supported by the dominance of tubificid oligochaetes at this site (relative abundance = 39.4 %, Table 1), which is known to be an indicator of metals-polluted systems (La Point et al. 1984) and elevated organic pollution.

This finding is consistent with other studies that have reported high numbers of tubificid oligochaetes in riffle habitats with rock-rubble substrate in metals-polluted systems (Winner et al. 1980). Because primary productivity and detritus can be directly utilized by the functional feeding groups that dominate at site CF4 (Fig. 2), organic enrichment may be stimulating additional transfer of metals through the food chain. Based on our results, organic enrichment may be creating an additional negative impact on the benthic community at site CF4 and to a lesser extent at sites CF2 and CF3.

Other studies have found severe impacts on benthic community structure in Silver Bow Creek (Chadwick et al. 1986) and to a lesser extent on the upper 60 km of the Clark Fork River, which included our sites CF2 and CF3 (McGuire 1989a, 1989b). Our trends in relative metals concentrations of As, Cd, Cu, and Pb in invertebrates were also similar to those measured in other studies (Cain et al. 1992, Woodward et al. 1994, 1995). Our site rankings and relationships between metals in invertebrates and community structure also indicate that analysis of metals in riffle invertebrates may be an ecologically meaningful component in determining the relative risk of metal uptake and availability to higher trophic levels.

Benthic community structure is related to invertebrate metals in the Clark Fork River when relative comparisons between sites are being made. The overall site ranking reported here shows some degree of similarity with that based on community structure and invertebrate toxicity testing of sediments collected from backwater habitats at the same sites (Canfield et al. 1994), except Canfield et al. (1994) did not identify a significant metals-related impact at site CF4. More importantly, moderate to high densities of benthic organisms are present in the Clark Fork River, even at sites containing high levels of metals in invertebrates (Table 4), which indicates that, to some degree, the benthic community may be able to tolerate and adjust to metals contamination. Ecologically, this phenomenon may be more important than changes in community taxa richness and biomass because metals-contaminated invertebrates have been known to represent a major source of metal uptake and accumulation in salmonids (Dallinger et al. 1987). Clark Fork River trout are known to feed opportunistically on the same invertebrates we found to be dominant in riffles at sites CF2-CF5 (D. Reiser, unpublished report, EA engineering, Science, and Technology, Redmond, Washington). In other studies, diets of invertebrates collected from riffles at Warm Springs (CF2) and Gold Creek (CF4) reduced growth and survival in brown and rainbow trout, and produced histopathological changes in trout liver tissue (Woodward et al. 1994, 1995). Yet, in our study, the negative impacts on community structure we detected at these sites could not fully account for these effects on higher trophic levels. When metals contamination is present in river ecosystems, we recommend that ecological assessment studies include analysis of benthic community structure and metals in riffle invertebrates owing to their close relationship to food chain availability and trophic transfer.

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