

Aquatic Insects as Bioindicators of Trace Element Contamination in Cobble-Bottom Rivers and Streams

Daniel J. Cain, Samuel N. Luoma, James L. Carter, and Steven V. Fend

U.S. Geological Survey, Menlo Park, CA 94025, USA

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Trace element bioaccumulation was studied in immature benthic insects from two contaminated river systems to develop these animals as bioindicators. In one river, Cu, Cd, Pb, and Zn were analysed in insects and in fine bed sediments over a 381-km reach downstream of a large copper mining complex. In the other river, As contamination from a gold mine was assessed in insects and bed sediments over a 40-km reach. All insect taxa collected in contaminated river reaches had elevated whole-body trace element concentrations. However, direct comparisons of contamination using a single, common species among stations were limited because few species were distributed throughout the study reaches. Comparisons of contamination at taxonomic levels higher than species were complicated by element-specific differences in bioaccumulation among taxa. These differences appeared to be governed by biological and hydrogeochemical factors. The variation in element concentrations among species of the caddisfly *Hydropsyche* was slightly greater than within individual species. If this genus is representative of others, comparisons of contamination within genera may be a practical alternative for bio-monitoring studies when single species are not available.

On a étudié la bioaccumulation d'éléments traces chez des insectes benthiques immatures dans deux réseaux de cours d'eau pollués pour utiliser ces animaux comme bioindicateurs. Dans une rivière, on a mesuré Cu, Cd, Pb et Zn chez les insectes et dans les sédiments fins du lit sur un tronçon de 381 km en aval d'un grand complexe d'extraction du cuivre. Dans l'autre cours d'eau, on a mesuré la contamination par l'As provenant d'une mine d'or chez les insectes et dans les sédiments du lit sur un tronçon de 40 km. Tous les taxons d'insectes prélevés dans les tronçons pollués présentaient des concentrations élevées d'éléments traces à l'échelle corporelle. Toutefois, les comparaisons directes de la pollution sur une seule espèce commune aux diverses stations étaient limitées du fait qu'on retrouvait peu d'espèces disséminées tout au long des tronçons de l'étude. La comparaison de la pollution à des niveaux taxinomiques supérieurs à l'espèce était rendue difficile par des différences dans la bioaccumulation selon les éléments parmi les taxons. Ces différences semblaient régées par des facteurs biologiques et hydrogéochimiques. La variation des concentrations en éléments entre les espèces de la phrygane *Hydropsyche* était légèrement supérieure à celle qu'on observait au sein de chaque espèce. Si ce genre était représentatif des autres, la comparaison de la pollution entre les genres pourrait être une solution intéressante dans les études de surveillance biologique lorsqu'on ne peut étudier une espèce donnée.

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The extraction and processing of metal-rich ores in watersheds can result in extensive trace element contamination of streams and rivers by the downstream transport of waste material (Andrews 1987; Goddard 1988; Moore and Luoma 1990; Axtmann and Luoma 1991). Bioaccumulation data can be used to assess the occurrence and biological significance of such contaminants (Burrows and Whitton 1983; Hatakeyama et al. 1988; Lynch et al. 1988; Axtmann et al. 1991; Moore et al. 1991). The insect community is one of the most important faunal components in cobble-bottom streams, and immature benthic insects satisfy several important criteria established for biological indicators (Phillips 1980). Insects accumulate excessive amounts of trace elements when exposed to unnaturally high concentrations (Nehring 1976; Spehar et al. 1978; Burrows and Whitton 1983; Besser and Rabeni 1987). Some taxa tolerate high body burdens of trace elements (Burrows and Whitton 1983; Hatakeyama et al. 1988). Many taxa are sedentary; thus, body burdens reflect local conditions, although contaminant concentrations in taxa that tend to drift may also reflect conditions at upstream sites that were previously occupied (Gower and Darlington 1990). Time-integrated

measurements of exposure can be made with species whose immature aquatic life history extends for months or years. Lastly, benthic insects can be collected by simple, inexpensive methods.

There are, however, important, unresolved problems in using aquatic insects as bioindicators. Few studies have reported how bioaccumulation of trace elements is related to concentrations in the environment. The small size of most insects often requires many individuals to be pooled to ensure that each sample has sufficient biomass to meet analytical detection limits for trace elements. This method sacrifices information on individual variation. Also, in bioindicator studies, there is an implicit understanding that direct comparisons of contamination can only be made within species because of species-specific differences in contaminant uptake. However, populations of stream-dwelling insects vary considerably with time and location, and direct comparisons of contamination among locations may be limited by the absence of a single, common species. An alternative to studying a common species might be to group data from different taxa, e.g. genus, family, and order. This approach would allow comparisons of contamination among more locations, but

the effect of such pooling on the reliability of those comparisons has not been rigorously determined.

This paper considers three questions pertinent to using benthic insects as bioindicators of trace element contamination in rivers and streams: (1) which taxa are available for assessments of contamination over large spatial scales, (2) do chemical data from a suite of different taxa provide a body of evidence which reliably describes contamination gradients, and (3) in lieu of a single species, can strategies be developed for grouping taxa without a critical loss in sensitivity to changes in contaminant exposure?

The above questions are addressed with data from two separate, contaminated river systems in the United States. Spatial distributions of individual species and genera were examined in each river. The biological responses to contamination in these taxa were assessed by comparing bioaccumulation with contaminant concentrations in fine bed sediments. The influences of taxonomic relationship, feeding habit (Smock 1983a), and insect size (Smock 1983b; Krantzberg and Stokes 1988; Cain et al. 1989) on interspecies differences in bioaccumulation were examined.

Materials and Methods

Site Descriptions

The Clark Fork River in Montana and Whitewood Creek in South Dakota are similar in physical characteristics and origin of contamination. Both river systems have cobble-boulder and gravel-rubble substrata and have been extensively contaminated by large-scale mining in or near their headwaters. The river systems differ with respect to the primary contaminants and geochemical conditions affecting the chemical form and distribution of the contaminants and in the species composition of the benthic community, thus broadening the scope of our analysis of benthic insects as bioindicators.

A large copper mining and smelting complex was established in the headwaters of the Clark Fork River system in the 1860's. Mining declined in the 1970's, but between 1864 and 1972, more than 10^8 t of waste material enriched with heavy metals (e.g. Cu, Fe, Pb, Cd, and Zn) was released via Silver Bow Creek and Warm Springs Creek to the river (Fig. 1) and deposited in floodplains and bed sediments (Andrews 1987; Moore and Luoma 1990; Axtmann and Luoma 1991). Acid mine drainage is not common in this system. Nevertheless, contaminated bed sediments extend at least 550 km below the original site of mining activities (Johns and Moore 1985; Rice and Ray 1985; Brook and Moore 1988; Moore and Luoma 1990; Axtmann and Luoma 1991), presumably as a result of the transport of contaminated particulate materials. Trace metal contamination in benthic invertebrates has been detected 381 km downriver (Moore and Luoma 1990; Axtmann et al. 1991).

Whitewood Creek has been heavily contaminated by the discharge of tailings from a large gold mine located in the city of Lead (Fig. 2). From 1876 to 1977, mine tailings were discharged via a small tributary, Gold Run, into Whitewood Creek (Goddard 1988). The primary contaminant in Whitewood Creek is As which is derived from arsenopyrite, a common mineral in the gold-bearing deposits (Goddard 1988). Transport and deposition of the discharged tailings led to extensive downstream As contamination of bed and floodplain sediments and biota in Whitewood Creek and the Belle Fourche River (Cherry et al. 1986; Cain et al. 1989; Marron 1989; McKallip et al. 1989; Kuwabara et al. 1990). An important aspect of the

As contamination is remobilization of As and ferrous iron in acidic groundwater which flows from contaminated alluvial deposits into the creek (Fuller and Davis 1989). Arsenic is adsorbed to and coprecipitates with iron oxyhydroxides which are rapidly formed upon contact with the stream (Fuller and Davis 1989). The locations of some groundwater seeps are clearly visible by orange iron oxide precipitates covering the streambed.

Sample Collection

The immature stages (larvae, nymphs, and pupae) of benthic insects were collected with kick nets and by hand from riffle areas during low flow. Typically at each station, a single riffle was sampled repeatedly over a large area (tens of square metres) until an adequate number of individuals was collected. Aerial adults were collected with a sweep net (Whitewood Creek only). Specific taxa were targeted for collection based primarily on three considerations: wide distribution throughout the study reach, densities relatively high, and individuals relatively large. Additionally, taxa representing different trophic levels were selected.

The Clark Fork River was sampled along a 381-km reach downstream from Warm Springs Creek (Fig. 1). Discharge along this reach increases substantially due to tributary inflows. For example, mean discharge between October 1985 and September 1986 was $7.73 \text{ m}^3/\text{s}$ near station 34.6 and $208 \text{ m}^3/\text{s}$ near station 381.1 (Fig. 1). Based on preliminary sampling in 1984 and 1985, six taxa from two orders (Trichoptera and Plecoptera) were selected as bioindicators. These taxa were targeted for collection in August 1986 at 15 locations (Fig. 1). These samples were analysed for Cu, Cd, Pb, and Zn. In 1986, *Hydropsyche* (Trichoptera) was not separated to species. In August 1990, *Hydropsyche* spp. were collected and analysed separately. *Arctopsyche grandis*, another hydropsychid caddisfly, also was collected. Eight of the 15 stations sampled in 1990 were stations sampled in 1986 (Fig. 1). Metal concentrations in Clark Fork insects were referenced against the average metal concentrations in insects collected from two uncontaminated tributaries, Rock Creek and the Blackfoot River (Axtmann and Luoma 1991). Reference samples were collected in August 1986, April 1987, June 1987, August 1987, and August 1989 within 25 km of the confluences of these two tributaries with the Clark Fork (Fig. 1). Concentrations in the reference samples varied little among years. The largest year-to-year difference in concentration observed at either tributary for any taxon was $6 \mu\text{g Cu/g}$ (dry weight), $0.2 \mu\text{g Cd/g}$, $1.5 \mu\text{g Pb/g}$, and $45 \mu\text{g Zn/g}$.

Whitewood Creek changes from a relatively high-gradient, canyon-confined stream to a lower-gradient, more open, meandering stream with extensive overbank deposits as it flows northeast from the Black Hills to the prairie. Preliminary sampling in 1986 showed that the structure of the benthic community changed along the stream course. Consequently, few species were common to all stations within the study reach. In May-June 1987, 15 taxa from four orders (Ephemeroptera, Trichoptera, Plecoptera, and Hemiptera) were collected at stations 2-6, covering about a 40-km reach below the mine, and analysed for As (Fig. 2). Stations 5 and 6 were located within 200 m of one another to assess influences of localized input of As-laden groundwater on As concentrations in benthic insects. Reference concentrations were based on specimens collected in 1986 and 1987 from three stations: station (R1) located upstream of the mine, Spearfish Creek (SC), and the Belle

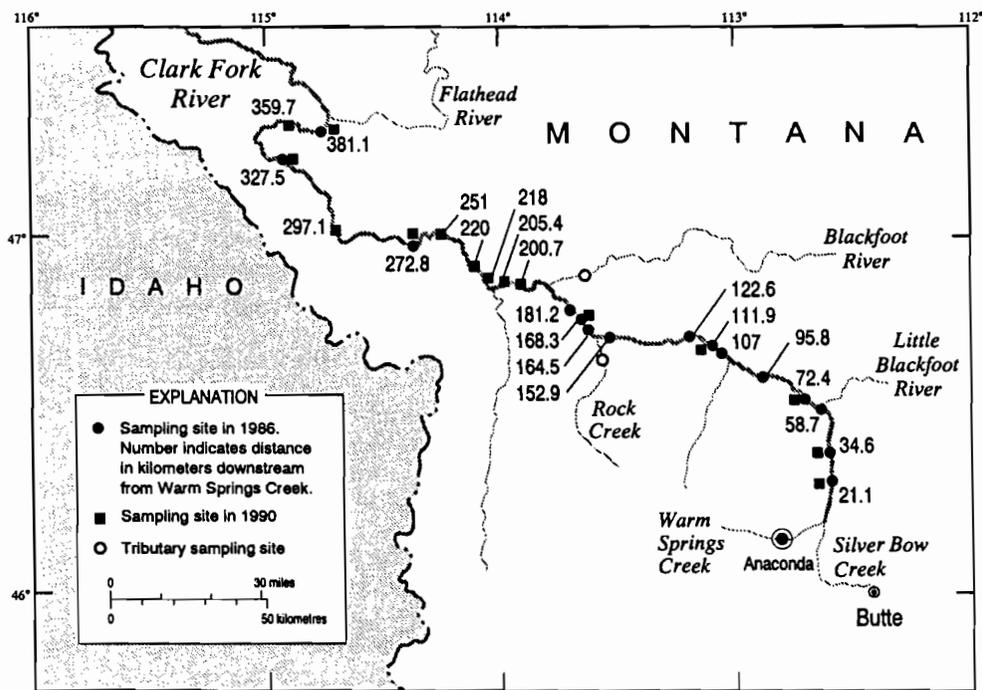


FIG. 1. Clark Fork River drainage basin in Montana, USA. Station locations are designated by distance in river kilometres downstream from Warm Springs Creek.

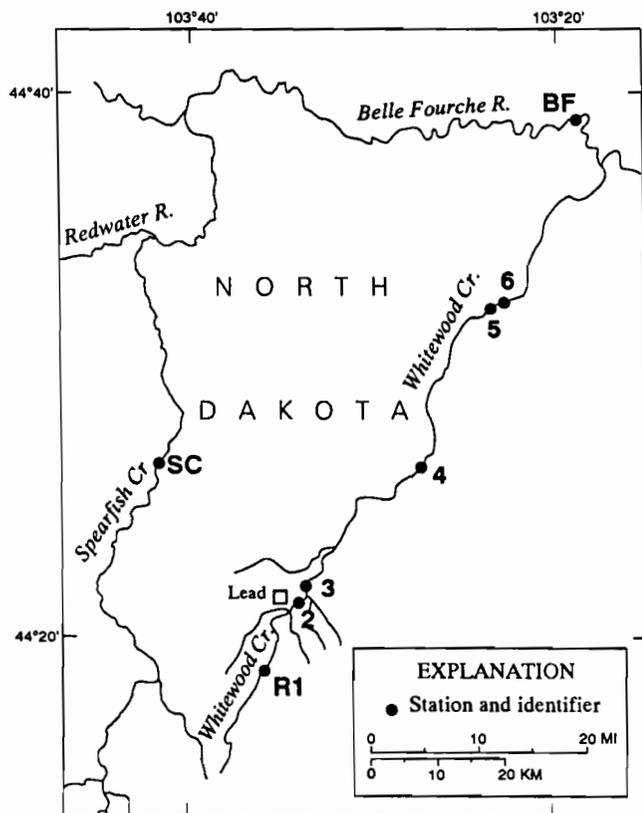


FIG. 2. Whitewood Creek, South Dakota, USA, showing stations.

Fourche River (BF) upstream of the confluence of Whitewood Creek (Fig. 2).

Insects collected for trace element analysis from the Clark Fork River and Whitewood Creek were initially sorted on site

by taxon and held for 4–6 h in plastic bags filled with ambient river water in a cooler to eliminate the contents of their guts. The river water was then decanted and the insects were frozen. Before freezing, some specimens were removed from the plastic bags and preserved in alcohol for taxonomic identification. The frozen samples were thawed in the laboratory and thoroughly rinsed with deionized water to remove particles from external surfaces. Individuals of the same species or genus were further sorted by size, and similar-sized individuals were pooled to make a sample with a total dry weight of at least 50 mg. Plecopterans were measured from the head to the last abdominal segment. The smaller taxa were sized by eye, and the average dry weight of individuals was determined from the total dry weight and the number of individuals in the composite sample.

Sample Analysis

Samples from the Clark Fork River were dried at 80°C, weighed, and then digested with hot 16 M HNO₃ reflux. The acid was evaporated after the sample solution turned clear, the residue reconstituted in 3 M HCl, and the solution analysed for Cu, Cd, Pb, and Zn by either flame atomic absorption spectrophotometry (AAS) or inductively coupled argon emission spectrophotometry.

Arsenic was determined in insects from Whitewood Creek. Samples were homogenized in a high-speed tissue homogenizer. The homogenate was freeze-dried, ashed in a muffle furnace at 450°C, reconstituted in 6 M HCl, and analysed by hydride-generation AAS.

Fine-grained bed sediments were collected simultaneously with the insect samples in the Clark Fork River in 1986 and in Whitewood Creek in 1987 following procedures described by Axtmann and Luoma (1991) and Cain et al. (1989). A polypropylene scoop was used to scrape sediment from the surface (0–1 cm) of several deposits in shallow, still water along the water's edge. The sediment was wet-sieved on site with ambient

river water through a 60- μm nylon mesh screen. Sediment collected from the Clark Fork River was transported on ice to the laboratory and dried at 80°C. Sediment from Whitewood Creek was allowed to settle for 24 h after collection. The river water was decanted, and the sediment was frozen and later freeze-dried. Dried sediments from both the Clark Fork River and Whitewood Creek were ground to homogeneous powders. Sub-samples were extracted with 0.6 M HCl to estimate the biologically available metal fraction (Langston 1980; Luoma and Bryan 1978, 1981, 1982). Clark Fork River sediments were analysed for Cd, Cu, Pb, Zn, and Fe by flame AAS. Whitewood Creek sediments were analysed for As by hydride-generation AAS.

Quality assurance of the analytical methods was checked with NIST standards. Bovine liver, oyster tissue, and river sediment (NIST standard reference materials 1577a, 1566a, and 1645) were analysed routinely to check the recoveries of Cd, Cu, Pb, Zn, and Fe. Albacore tuna (NIST reference material 50) was run with each group of samples analysed for As. Concentrations for most elements were usually within the 95% confidence limits of the certified mean concentrations. Zinc recoveries from 1577a and 1566a were typically 85–90% of the certified concentrations.

Statistical Analysis

The relationship between bioaccumulation and environmental contamination in the Clark Fork River was assessed by correlating trace metal concentrations in individual taxa with sediment metal concentrations (Table 1). Concentration data were log-transformed for the analysis.

The coefficient of variation was used to characterize the effect of pooling data from related species, as might be employed in a field sampling strategy. Several stations from each river were selected to provide a consistent suite and maximum number of taxa for this analysis (Table 1). Data from these stations were combined to increase the sample size. Stations selected in the Clark Fork River were located between 72.4 and 181.2 km (Table 1). Data from 1986 characterized the variance within hydropsychid caddisflies and stoneflies. Data from 1990 characterized the variance only among three species of hydropsychid caddisflies. Three Whitewood Creek stations were selected because the two major taxa collected in the stream in 1987, Trichoptera and Ephemeroptera, were well represented. Within orders, the median of the coefficients of variation for replicate

samples of all species collected at the selected stations was used to characterize the variation in trace element concentration at the species level. For higher taxonomic levels, the mean and coefficient of variation of trace element concentrations for all species within the same genus, family, or order were calculated at each station. The median of the coefficients of variation for all stations characterized the variation within each taxonomic group.

The effects of feeding habit and size on insect element concentrations were evaluated by analysis of covariance (ANCOVA). Although food preferences vary greatly among taxa and may change over the life history of a species, each taxon was assigned to one of four broad feeding categories based on descriptions of trophic relationships in Merritt and Cummins (1984): herbivores, detritivores, omnivores, or predators. The herbivore category included mayflies only. Detritivores comprised the caddisflies *Limnephilus* and *Hesperophylax* and the stonefly *Pteronarcys californica*. The omnivore category comprised several species of hydropsychid caddisflies. The predators included *Rhyacophila*, *Ambrysus*, and several perlid stoneflies. The ANCOVA included data from several stations (Table 1). Feeding habit was treated as the main factor, and the mean dry weight (size) of individuals in each sample was included as a covariate. The concentration of metals extracted in 0.6 M HCl from fine bed sediments (<60 μm) also was added as a covariate to adjust insect concentrations for differences in metal contamination among stations. Element concentrations and insect dry weights were log-transformed for the analyses. This analysis was limited to stations where at least three different feeding categories were represented in the collections (Table 1). In Whitewood Creek, four feeding categories were represented, but all four were not collected at any station. Therefore, the ANCOVA was run for two separate data sets (Table 1).

Results

Distribution of Taxa

The availability of species for trace element analysis differed among stations in both the Clark Fork River and Whitewood Creek. Of the six taxa targeted for collection in the Clark Fork River, only *Hydropsyche* spp. and *Isogenoides* sp. were collected at stations upstream of 60 km (Table 2). All of the targeted taxa were found between 61 and 191 km, although

TABLE 1. Summary of the data used in the different analysis of factors affecting bioaccumulation in insects. Feeding categories for ANCOVA: H = herbivore; D = detritivore; O = omnivore; P = predator.

Analysis	River	Year	Stations	Approach
Bioaccumulation vs. sediment contamination	Clark Fork	1986	15-stn. transect (21–381 km)	Correlation
Variation within taxa	Clark Fork	1986	72.4, 107, 164.5, 181.2	Coefficient of variation
	Clark Fork	1990	72.4, 111.9, 168.3	
	Whitewood Cr.	1987	2–4	
Effect of feeding habit and size	Clark Fork	1986	72.4, 107, 164.5, 181.2	ANCOVA: D, O, P
	Whitewood Cr.	1987	R1–3	ANCOVA: H, D, P
	Whitewood Cr.	1987	2–6	ANCOVA: H, O, P

TABLE 2. Distributions of benthic insects collected (+) for trace element analysis in the Clark Fork River, Montana, in August 1986, Whitewood Creek, South Dakota, in May–June 1987, and reference sites. “–” means the taxon was not collected because it was either rare or absent. All locations given in river kilometres except reference sites.

Taxon	Reference	Clark Fork River reaches (km)					Whitewood Creek station ID and location (km)				
		0–60	61–106	107–164	165–191	192–381	2 0	3 1	4 20	5 38	6 38.2
Trichoptera											
<i>Hydropsyche</i> spp.	+	+	+	+	+	+					
<i>Arctopsyche grandis</i>	+	–	+	+	+	+					
Plecoptera											
<i>Claassenia sabulosa</i>	+	–	+	+	+	+					
<i>Hesperoperla pacifica</i>	+	–	+	+	+	–					
<i>Isogenoides</i> sp.	+	+	+	+	+	–					
<i>Pteronarcys californica</i>	+	–	+	+	+	–					
Ephemeroptera											
<i>Baetis tricaudatus</i>	+						+	+	+	–	–
<i>Choroterpes</i> sp.	–						–	–	–	+	+
<i>Ephemerella inermis</i>	+						+	+	–	–	–
<i>Tricorythodes</i> sp.	–						–	–	–	+	+
Trichoptera											
<i>Hesperophylax occidentalis</i>	+						–	–	–	–	–
<i>Limnephilus</i> sp. and <i>Hesperophylax</i> sp.	–						+	+	–	–	–
<i>Cheumatopsyche</i> spp.	–						–	–	+	+	–
<i>Hydropsyche bronja</i>	–						–	+	+	+	–
<i>Hydropsyche oslari</i>	+						–	–	–	–	–
<i>Hydropsyche slossonae</i> and <i>oslari</i>	–						–	–	–	+	+
<i>Hydropsyche</i> spp.	+						+	+	+	–	–
<i>Rhyacophila acropedes</i> group	+						+	+	–	–	–
Plecoptera											
<i>Isoperla quinquepunctata</i>	–						–	–	+	–	–
<i>Hesperoperla pacifica</i>	+						+	–	–	–	–
Hemiptera											
<i>Ambrysus</i> sp.	–						–	–	–	+	+

some species were rare at some stations within this reach (Table 2). Below 191 km the densities of several stoneflies were insufficient for a trace metal sample. Specimens of all targeted taxa were collected in the tributaries. Taxa collected in Whitewood Creek appeared to reflect habitat differences between the upper and lower segments of the stream. Many of the taxa collected in the upper 30-km reach were replaced downstream by other taxa (Table 2). Seven of the 15 taxa were collected from at least one of the reference stations. Eight taxa were not available at any of the reference stations.

Bioaccumulation Related to Sediment Contamination

Trace element concentrations in all taxa in the Clark Fork River were elevated relative to reference samples and thus reflected contamination of the river (Table 3). Contamination in sediments and in most taxa progressively declined downstream (Table 3). Elevated Cu and Cd concentrations were still present in all taxa 191–381 km downstream of the origin of contamination. Metal-specific differences in bioaccumulation among taxa in the Clark Fork were apparent from differences in concentrations at the same stations, contrasting patterns in the downstream distributions of contaminants, and differences in correlations with metals in the sediments. Normalizing sediment metal concentrations to Fe concentrations in the sediments (e.g. Luoma and Bryan 1978; Langston 1980) had little

affect on correlations between sediment metal concentrations and metal bioaccumulation.

Copper and Cadmium

Copper concentrations differed among taxa by a factor of about 2 at reference stations and by a factor of 3.5 within each reach in the Clark Fork (Table 3). Concentrations in hydropsychid caddisflies decreased downstream (Fig. 3a). Copper in *Hydropsyche* spp. and in *Arctopsyche grandis* correlated significantly with Cu in sediments (Table 4). Copper accumulation by *Hydropsyche cockerelli* and *Hydropsyche occidentalis* was virtually the same where these species were collected together in 1990 (Fig. 3a). Differences were evident between *H. occidentalis* and a third species at the two most upstream stations (Fig. 3a). However, Cu in these *Hydropsyche* spp. was typically twice that found in *A. grandis* in both 1986 and 1990 (Fig. 3). Differences in Cu concentrations and downstream patterns also occurred among stoneflies (Fig. 4). *Pteronarcys californica* was collected at only eight stations in the Clark Fork. At most of these stations, Cu concentrations in this species were higher than those in either of the other two species of stonefly or in *Hydropsyche* spp. Copper concentrations in *P. californica* decreased downriver, but tissue concentrations were not correlated significantly with Cu in sediments (Table 4). The insignificant correlation may be attributed to the species' relatively narrow distribution (samples were collected from only eight

TABLE 3. Trace metal concentrations (mean \pm 1 SD, $\mu\text{g/g}$ dry wt.) in insects and sediments from the Clark Fork River in August 1986 and from reference sites. Samples were collected at three stations within the specified reach except for *Hesperoperla* sp. ($n = 1$). “—” means the taxon was not collected because it was either rare or absent.

Element	Taxon	Reference	Reach			
			0-60	106-164	191-183	
Copper	Trichoptera					
	<i>Hydropsyche</i> spp.	18 \pm 4	204 \pm 18	72 \pm 8	27 \pm 5	
	<i>Arctopsyche grandis</i>	11 \pm 2	—	33 \pm 4	20 \pm 2	
	Plecoptera					
	<i>Pteronarcys californica</i>	32 \pm 3	—	115 \pm 6	—	
	<i>Claassenia sabulosa</i>	34 \pm 11	—	68 \pm 0.3	51 \pm 3	
	<i>Hesperoperla pacifica</i>	28 \pm 3	—	46	—	
	<i>Isogenoides</i> sp.	16	84 \pm 8	68 \pm 4	—	
	Sediment	18 \pm 5	779 \pm 228	408 \pm 103	129 \pm 53	
	Cadmium	<i>Hydropsyche</i> spp.	0.2	2.8 \pm 0.6	2.2 \pm 0.6	0.7 \pm 0.2
<i>Arctopsyche grandis</i>		0.2 \pm 0.1	—	1.4 \pm 0.3	0.6 \pm 0.1	
<i>Pteronarcys californica</i>		0.1 \pm 0.1	—	1.0 \pm 0.1	—	
<i>Claassenia sabulosa</i>		0.1	—	1.4 \pm 0.1	0.6 \pm 0.03	
<i>Hesperoperla pacifica</i>		0.2 \pm 0.1	—	1.0	—	
<i>Isogenoides</i> sp.		<0.4	1.8 \pm 0.2	1.4 \pm 0.3	—	
Sediment		<0.3	6.6 \pm 1.5	3.5 \pm 0.9	1.6 \pm 0.3	
Lead		<i>Hydropsyche</i> spp.	1.8 \pm 2.2	12.8 \pm 1.5	8.1 \pm 1.3	3.1 \pm 0.9
		<i>Arctopsyche grandis</i>	1.2 \pm 1.1	—	4.1 \pm 0.4	2.1 \pm 0.3
		<i>Pteronarcys californica</i>	0.6 \pm 0.4	—	7.4 \pm 1.3	—
	<i>Claassenia sabulosa</i>	<0.1	—	0.4 \pm 0.1	0.4 \pm 0.2	
	<i>Hesperoperla pacifica</i>	<0.2	—	1.0	—	
	<i>Isogenoides</i> sp.	<0.4	2.6 \pm 1.5	1.2 \pm 0.4	—	
	Sediment	11 \pm 1	125 \pm 40	90 \pm 22	40 \pm 3	
	Zinc	<i>Hydropsyche</i> spp.	112 \pm 2	270 \pm 36	233 \pm 20	122 \pm 22
		<i>Arctopsyche grandis</i>	136 \pm 8	—	191 \pm 9	134 \pm 1
		<i>Pteronarcys californica</i>	183 \pm 20	—	253 \pm 9	—
<i>Claassenia sabulosa</i>		177 \pm 19	—	238 \pm 14	200 \pm 5	
<i>Hesperoperla pacifica</i>		311 \pm 42	—	387	—	
<i>Isogenoides</i> sp.		288	328 \pm 8	357 \pm 10	—	
Sediment		54 \pm 1	1073 \pm 337	894 \pm 179	354 \pm 73	

stations) and the variability in the tissue concentrations among stations. Concentrations of Cu in *Isogenoides* sp. also showed a detectable decrease downstream which correlated with sediment Cu concentrations (Table 4). In contrast, concentrations of Cu in *Claassenia sabulosa* changed little over 320 km (Fig. 4), although concentrations were significantly higher throughout the Clark Fork than at reference stations. *Claassenia sabulosa* was not found in the upper 60 km. Downstream trends in other species were more obvious because of the high tissue concentrations in this reach.

Cadmium concentrations in most taxa in the upper 164 km of the Clark Fork exceeded concentrations in reference populations by an order of magnitude (Table 3). Differences in concentrations among taxa within a given reach were generally less than twofold. The highest concentrations occurred in *Hydropsyche* spp. (Table 3). Differences in Cd accumulation were evident between *Hydropsyche* spp. at the three most upstream stations where contamination was greatest, but not at the rest of the stations (Fig. 5a). Cadmium contamination in *Hydropsyche* spp. was also greater than in *A. grandis* at the most contaminated stations in the river in 1986 (Fig. 5b), but

differences between the two caddisflies were less under less contaminated conditions (e.g. in 1990 when concentrations were about half of those in 1986; Fig. 5). Concentrations of Cd in the caddisflies correlated significantly with sediment Cd concentrations (Table 4). In the plecopterans, Cd in *Isogenoides* sp. and in *C. sabulosa* showed a small downstream decrease (data not shown) which correlated significantly with sediment concentrations (Table 4). Concentrations of Cd decreased downstream in *P. californica*, but did not correlate significantly with sediment concentrations (Table 4) because of variability in the data similar to that observed with Cu.

Lead and Zinc

The differences in Pb concentrations among taxa from uncontaminated and contaminated stations exceeded an order of magnitude (Table 3). Concentrations of Pb in *Hydropsyche* spp. and *A. grandis* reflected the downstream decrease in Pb concentrations in sediments (Table 3). Lead in stoneflies was not significantly correlated with Pb in sediments (Table 4), although Pb in *P. californica* decreased from 40 $\mu\text{g/g}$ at 72 km to 4 $\mu\text{g/g}$ at 181 km.

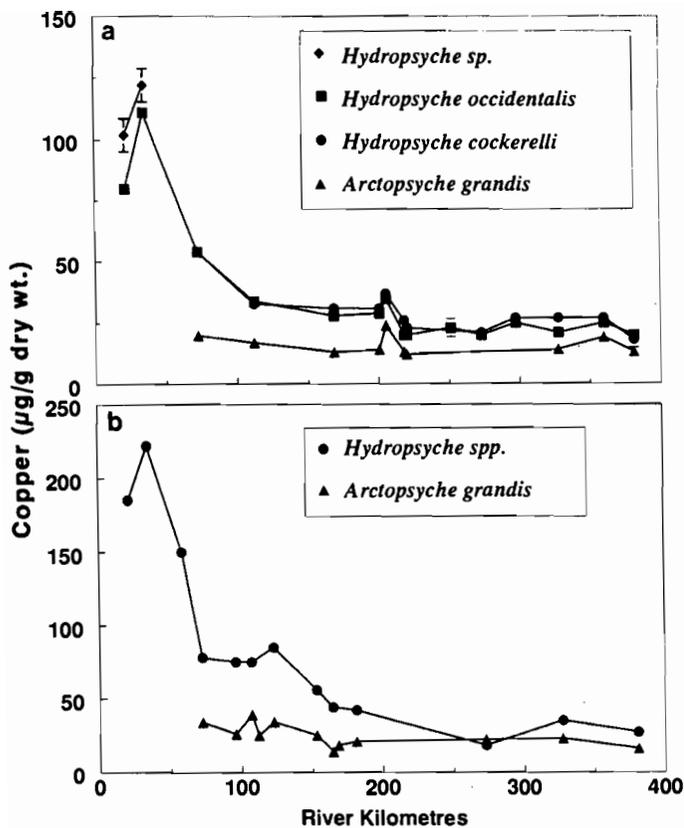


FIG. 3. Downstream distributions of Cu concentrations in hydropsychid caddisflies in the Clark Fork River in (a) 1990 and (b) 1986. Concentrations are for composite samples ($n = 1-7$). Error bars are ± 1 SE of the mean concentration.

Zinc concentrations varied by a factor of 2–3 among taxa at reference and contaminated stations (Table 3). Zinc concentrations in sediments correlated with Zn in trichopterans but not in plecopterans (Table 4). However, all taxa were less responsive to Zn contamination than other metals. For example, Zn in *Hydropsyche* spp. in the 0- to 60-km reach exceeded reference concentrations by only a factor of 2.4 (Table 3). In the same reach, Zn in *Isogenoides* sp. was only 1.1 times greater than reference concentrations.

Arsenic

In Whitewood Creek, As concentrations were elevated downstream of the mine at stations 2–4 in all taxa that could be compared with reference populations (Table 5). However,

the details of the downstream trends were difficult to assess because no single species was available at all stations. Differences in As between stations 5 and 6 appeared to reflect important localized processes. High As concentrations were found in sediments and in *Choroterpes*, *Tricorythodes*, and *Ambrysus* collected at station 6 within the plume of a groundwater seep which was marked by the iron oxide stain on the stream bottom. In contrast, concentrations were lower in these taxa and sediment collected approximately 200 m upstream at station 5. At station 6, *Hydropsyche* sp. were collected near midchannel and outside the boundary of the plume. This slight difference in location probably explains why concentrations in this taxon were similar between stations 5 and 6.

Arsenic concentrations differed among taxa from reference areas by an order of magnitude (Table 5). A similar level of variation was observed among taxa from contaminated stations, although concentrations were higher. In general, As concentrations in mayflies and caddisflies were higher than in stoneflies and *Ambrysus* sp. (Table 5). This was the only consistent difference in As concentrations that appeared related to taxonomy.

Effects of Aggregating Data by Taxonomic Level

The variance in trace element concentrations usually increased as data for species were systematically aggregated by genus, family, and order (Fig. 6). Combining data for *Hydropsyche cockerelli* and *H. occidentalis* from the Clark Fork River increased the median coefficient of variation at the genus level for Cd, Pb, and Zn, although the differences in concentrations of these metals between the species were small (e.g. see Fig. 3a, 5a). Including *A. grandis* and aggregating all data at the family level (Hydropsychidae) further increased the coefficient of variation for Pb and Cu. The variance in bioaccumulation data in Trichoptera in Whitewood Creek and in Plecoptera in the Clark Fork River also increased as species were grouped by family and order (Fig. 6). The median coefficient of variation for As in four mayfly species in Whitewood Creek increased slightly from 28 to 34% when the species were combined at the order level. The increase in variance from species to order was greater for Pb, Cu, and As than for Cd and Zn (Fig. 6). Lead in stoneflies in the Clark Fork and As in caddisflies in Whitewood Creek were especially variable.

Influence of Feeding Habit on Bioaccumulation

Feeding habit had a clear influence on concentrations of Pb in insects from the Clark Fork (ANCOVA, Table 6). At the four stations analysed, the average Pb concentrations were highest in the detritivore, intermediate in omnivores, and low-

TABLE 4. Correlation of whole-body insect metal concentrations and acid-extractable (0.6 M HCl) metal concentrations in bed sediment for samples collected from the Clark Fork River in August 1986. Correlation coefficients, r , and sample sizes (in parentheses) are shown. Significance levels: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Taxon	Copper	Cadmium	Lead	Zinc
Trichoptera				
<i>Hydropsyche</i> spp.	0.86 (13)***	0.71 (13)**	0.74 (13)**	0.74 (13)**
<i>Arctopsyche grandis</i>	0.63 (12)*	0.84 (12)***	0.61 (12)*	0.69 (12)*
Plecoptera				
<i>Claassenia sabulosa</i>	0.53 (12)	0.69 (12)*	0.26 (12)	0.53 (12)
<i>Hesperoperla pacifica</i>	0.00 (7)	-0.63 (7)	0.69 (5)	0.43 (7)
<i>Isogenoides</i> sp.	0.74 (12)**	0.66 (12)*	0.36 (11)	0.25 (12)
<i>Pteronarcys californica</i>	0.68 (8)	0.51 (8)	0.66 (8)	0.37 (8)

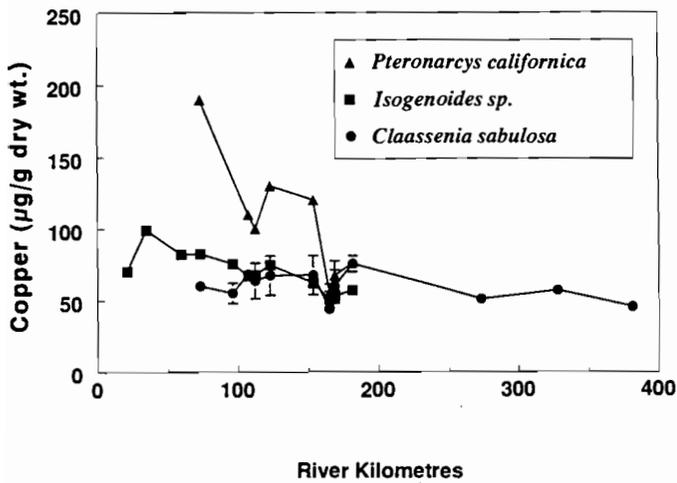


FIG. 4. Downstream distributions of Cu concentrations in stoneflies in the Clark Fork River in 1986. Concentrations are for composite samples ($n = 1-4$). Error bars are ± 1 SE of the mean concentration.

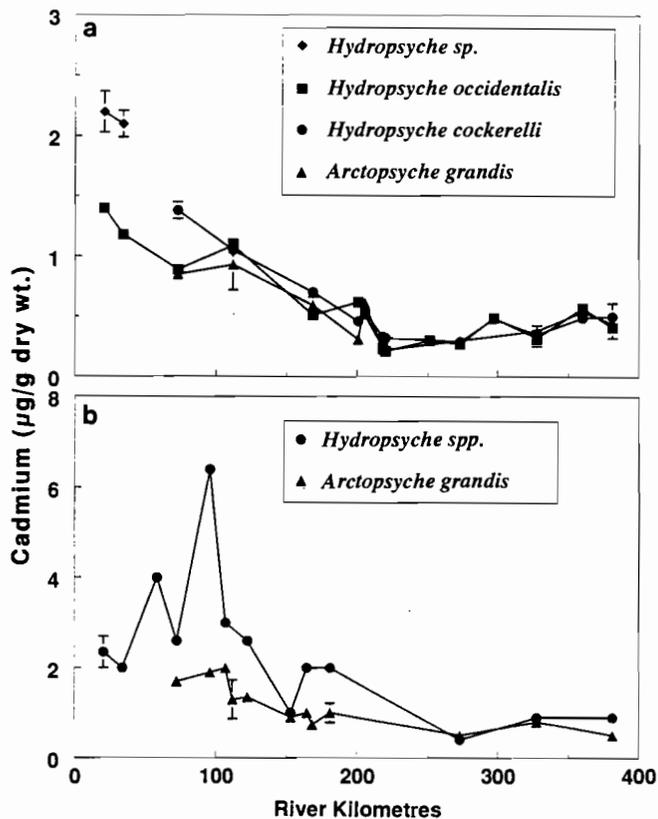


FIG. 5. Downstream distributions of Cd concentrations in hydropsychid caddisflies in the Clark Fork River in (a) 1990 and (b) 1986. Concentrations are for composite samples ($n = 1-7$). Error bars are ± 1 SE of the mean concentration.

est in predators (Table 7). Feeding habit also may have contributed to the high Cu concentrations observed in the detritivore *P. californica*. Other differences (e.g. Cd) between feeding categories in the Clark Fork were relatively small (Table 7), although some were statistically significant (Table 6). In some instances, differences in concentrations among taxa within a feeding category were as great as differ-

ences observed between feeding groups. For example, Zn concentrations in *C. sabulosa* were more similar to those in *P. californica* and *Hydropsyche* spp. than to those in the other two predaceous stoneflies (Table 7).

As with taxonomic level, differences among feeding categories in the Clark Fork were greatest where contamination was highest and diminished as contamination levels approached background. For example, Cu concentrations were $190 \mu\text{g/g}$ in the detritivore *P. californica* at 72 km (Table 7). At the same station, Cu in omnivores and predators averaged 56 and $61 \mu\text{g/g}$, respectively. At 164 km where contamination was lowest among the four stations, concentrations were $55 \mu\text{g/g}$ in the detritivore and averaged $29 \mu\text{g/g}$ in the omnivores and $43 \mu\text{g/g}$ in the predators (Table 7). Differences in Pb concentrations between *P. californica* and omnivores also were less at 164 km than at 72 km.

Results from the ANCOVA suggested that feeding habit might differentiate bioaccumulation among some species within higher taxonomic groups. For example, characterizing Plecoptera by feeding category separated *P. californica* from the predaceous stoneflies (*C. sabulosa*, *Hesperoperla pacifica*, and *Isogenoides* sp.) and helped explain differences in Pb and Cu concentrations within that order. On the other hand, differences in metal accumulation between the filter-feeding omnivores *Hydropsyche* spp. and *A. grandis* were not explained by feeding category.

The influence of feeding habit on As concentrations was not clear. Feeding category was not a significant factor in differences in As concentrations among taxa representing herbivores, detritivores, and predators at stations R1, 2, and 3, but significant differences were detected among herbivores, omnivores, and predators at stations 2-6 (Table 6). Arsenic concentrations in the predators at these stations were low relative to herbivores and omnivores. However, the effect of feeding habit was difficult to separate from the effect of size (see below).

Effect of Size on Bioaccumulation

Most differences in metal concentrations among taxa from the Clark Fork River did not appear to result from differences in dry weight (size) (Table 6). In the subset of data analysed by ANCOVA, the mean dry weight of individual specimens for all taxa ranged over more than an order of magnitude; however, dry weight only had a significant effect on Cu. A significant interaction between feeding habit and dry weight was detected for Cu and Cd: concentrations in omnivores decreased as dry weight increased. This was a result of differences in metal concentrations between *Hydropsyche* spp. and *A. grandis*. Concentrations of all metals (including Pb and Zn, although these were not statistically significant) in the smaller *Hydropsyche* spp. were typically 2 times greater than in *A. grandis*.

Arsenic concentrations both within and among insect taxa from Whitewood Creek were negatively related to dry weight. For example, As concentrations in all taxa collected at stations R1, 3, and 6 correlated significantly ($p < 0.05$) with the average dry weight of insects composing the samples (Fig. 7) even though the species composition of the community changed from station R1 to station 6. Much of the variation in As concentration among taxonomic groups could be accounted for by differences in the insects' dry weights (sizes). Further, the relative sizes of taxa within different feeding categories may have influenced the statistically detectable effect of feeding habit on As concentrations. Predators were typically the largest specimens collected and had low As concentrations. Herbivores and omnivores were generally small and had high As concentrations.

TABLE 5. Arsenic concentrations (mean \pm 1 SD, $n = 1-9$ composite samples, $\mu\text{g/g}$ dry wt.) in insects and sediments from Whitewood Creek, South Dakota, in May-June 1987 and from reference sites. The caddisflies *Limnephilus* sp. and *Hesperophylax* sp. were not separated before As determination. “-” means the taxon was not collected because it was either rare or absent.

Taxon	Reference	Station				
		2	3	4	5	6
Ephemeroptera						
<i>Baetis tricaudatus</i>	10	65 \pm 15	80 \pm 15	24 \pm 2	-	-
<i>Choroterpes</i> sp.	-	-	-	-	156 \pm 28	278 \pm 86
<i>Ephemerella inermis</i>	16 \pm 0.2	40 \pm 1	59 \pm 28	-	-	-
<i>Tricorythodes</i> sp.	-	-	-	-	92 \pm 20	625 \pm 80
Trichoptera						
<i>Hesperophylax occidentalis</i>	6 \pm 3	-	-	-	-	-
<i>Limnephilus</i> sp. and <i>Hesperophylax</i> sp.	-	8	10 \pm 0.03	-	-	-
<i>Cheumatopsyche</i> spp. <i>Hydropsyche</i>	-	-	-	56 \pm 8	59	-
<i>H. slossonae oslari</i>	7 \pm 3	58 \pm 10	37 \pm 6	98 \pm 26	-	-
<i>H. bronta</i>	-	-	20	80 \pm 4	29	-
<i>H. sp.</i>	2 \pm 1	-	-	-	-	-
<i>H. sp.</i>	-	-	-	-	66 \pm 25	77 \pm 15
<i>Rhyacophila</i> sp.	1 \pm 1	4	64	-	-	-
Plecoptera						
<i>Isoperla quinquepunctata</i>	-	-	-	10 \pm 4	-	-
<i>Hesperoperla pacifica</i>	2 \pm 1	5 \pm 1	-	-	-	-
Hemiptera						
<i>Ambrysus</i> sp.	-	-	-	-	25 \pm 5	73 \pm 42
Sediment	18 \pm 4	84 \pm 1	145 \pm 7	440 \pm 18	660 \pm 4	764 \pm 74

Size differences within taxa could bias comparisons of metal contamination between stations at Whitewood Creek. For example, individual *Hesperophylax* sp. with an average dry weight of 7 mg at reference station R1, above the mine, had As concentrations only slightly lower than those in larger specimens of *Hesperophylax* sp. and *Limnephilus* sp. (average dry weight of 44 mg) at station 3 located below the mine (Fig. 7). From this comparison alone one might wrongly conclude that station 3 was not contaminated.

Arsenic Contamination of the Exoskeleton

The strong size effect on As concentrations in insects from Whitewood Creek suggested that As was associated with the exoskeleton of the larvae and nymphs. Arsenic concentrations in pupae and adult specimens from Whitewood Creek were only 15-50% of the As concentration in larvae and nymphs (data not shown). Cessation in feeding and/or the loss of the gut and its contents during metamorphosis could have affected the As concentrations in pupae. However, the most likely cause for these differences is that the exoskeleton of immature specimens, which is shed during ecdysis and which was intentionally not collected with the pupal samples, was enriched with As relative to internal tissues. Arsenic body burdens (micrograms) were calculated from the average insect dry weight and As concentrations for the different developmental stages. These results were used to estimate the total amount of As in the larvae or nymph exoskeleton (assuming that the differences in As among life stages were entirely due to As sorbed to the exoskeleton). Arsenic associated with the exoskeleton ranged from 32% of the total body burden in taxa collected above the mine to 56-99% at stations below the mine (Table 8).

Arsenic-bearing particles appeared to contribute to the surface contamination of Whitewood Creek insects. Scanning

electron microscopy - energy dispersive X-ray analysis (SEM-EDX) showed that the external surfaces of specimens were littered with inorganic particles (Fig. 8), despite repeated rinsing with deionized water prior to As analysis. The particles appeared to be simply trapped by setae. The particles on the insect surfaces were similar in character to As-enriched iron oxyhydroxides that precipitate when contaminated groundwater is discharged into Whitewood Creek (Fuller et al. 1988). In those particles on the insects in which As was detected, Fe was the major constituent (Fig. 9). The As/Fe molar ratio of iron oxyhydroxides from the stream (Fuller et al. 1988) was similar to the As/Fe molar ratio in insects (Cain et al. 1988). Iron particles also were observed in the gut cavity, but with much lower frequency than on the exoskeleton. In only one case (a specimen of the filter-feeding *Hydropsyche* sp.) did an Fe particle in the gut have detectable concentrations of As.

Discussion

The concentrations of trace contaminants in the bodies of all benthic insects in the Clark Fork River and Whitewood Creek were elevated where sediments were contaminated, indicating that bioaccumulation is a general, community-level response to contamination in these lotic environments. Thus, analysis of tissues from any member of the benthic insect community might be used to detect exposure to contaminants. However, taxa differ in their suitability as bioindicators because of differences in distribution within study reaches and differences in bioaccumulation.

Changes in the composition of the benthic community prevented direct comparisons of As contamination in any one species among all stations in Whitewood Creek or between contaminated and reference stations. Differences in community composition were probably caused by changes in stream con-

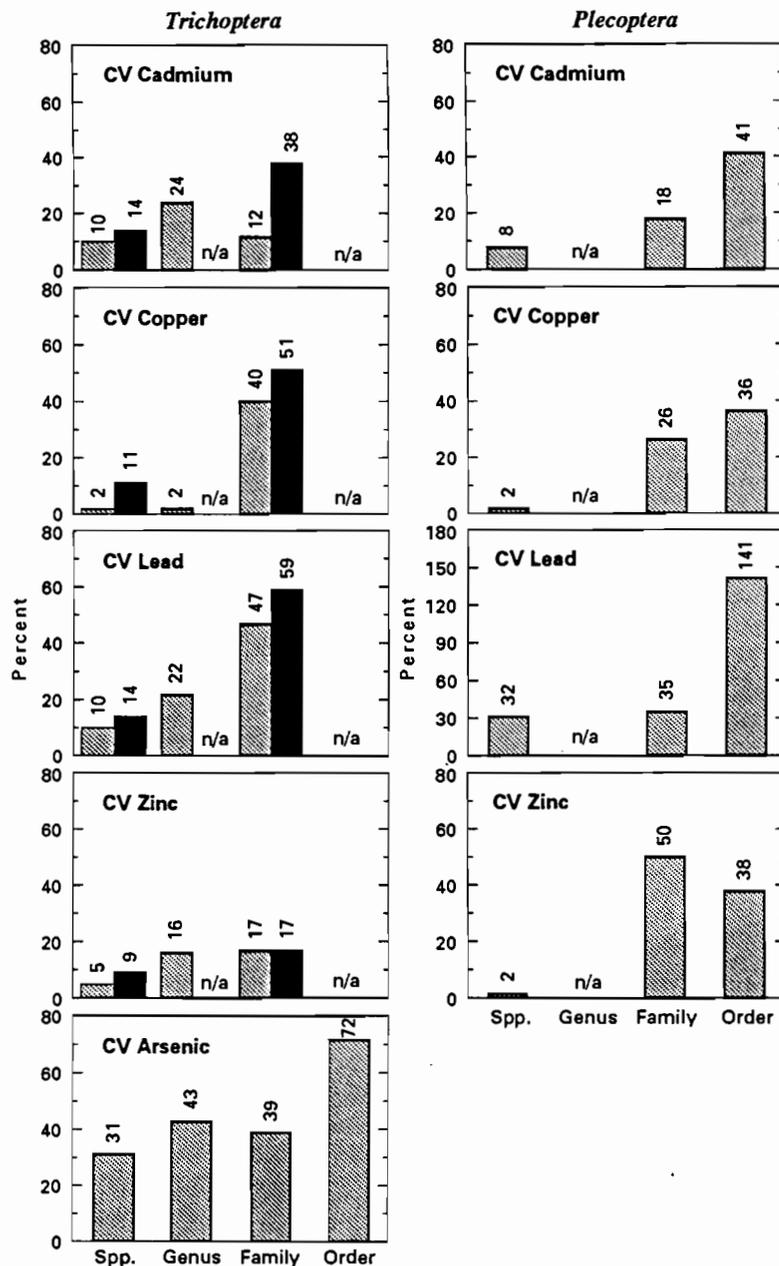


FIG. 6. Coefficients of variation (%) for trace element concentrations within different taxonomic groups. Each value is the median of the coefficients of variation for several stations in the Clark Fork River and in Whitewood Creek. Spp. is the median coefficient of variation for replicate ($n > 1$) composite samples of species. Values for higher taxonomic levels resulted from aggregating concentration data of all species within the specified taxon. Cadmium, Cu, Pb, and Zn data are from the Clark Fork River. Trichoptera are represented by Hydropsychidae, exclusively. Dark bars are for 1986 and light bars are for 1990. Arsenic data are from Whitewood Creek. "n/a" means taxa were not available for grouping at that level.

ditions and the character of the benthic habitat (Cain et al. 1988). In the Clark Fork River, changes in the structure of the benthic community appeared to be related to the effects of the pollution (Chadwick and Canton 1986; McGuire 1988, 1989). In the upper 60-km reach, densities of many taxa including mayflies and stoneflies are significantly lower than farther downstream, and some taxa (e.g. *C. sabulosa* and *P. californica*) are absent. Downstream, more species are represented in the assemblage, although population densities of species vary among stations (McGuire 1988, 1989; pers. obs.). Because of their wide distribution and relatively high densities,

Hydropsyche spp. were better than other taxa for assessing metal contamination over broad spatial scales.

Trace element bioaccumulation is typically species dependent, but similarities may exist among groups of related species. For example, concentrations of trace metals may be higher in mayflies than in other taxa (Nehring 1976; Burrows and Whitton 1983; Besser and Rabeni 1987; Hatakeyama et al. 1988). Differences (or similarities) in bioaccumulation have been explained by functional and morphological characteristics. Feeding habit was cited as a cause of differences in trace element accumulation among insect species (Elwood et al. 1976;

TABLE 6. ANCOVA probability levels for the effects of feeding habit, size (dry weight), and contamination (sediment metal concentration) on trace element concentrations in insects; ns means not significant at $p \leq 0.05$. Data for Cd, Cu, Pb, and Zn are from four stations in the Clark Fork River where taxa representing three feeding categories (detritivore, omnivore, and predator) were collected in August 1986. Data for As are from stations in Whitewood Creek sampled in May–June 1987.

	Cd	Cu	Pb	Zn	As ^a	As ^b
Factor						
Feeding habit	0.013	0.017	0.000	0.000	ns	0.018
Covariate						
Dry weight	ns	0.016	ns	ns	0.016	0.012
Contamination	ns	0.000	0.031	ns	0.000	0.000
Interaction						
Feeding \times weight	0.028	0.001	ns	ns	ns	ns
Feeding \times contamination	ns	0.009	ns	ns	ns	ns

^aIncludes detritivores, herbivores, and predators from stations R1–3.

^bIncludes herbivores, omnivores, and predators from stations 2–6.

TABLE 7. Trace metal concentrations in insects having different feeding habits. Values in bold are the averages of concentrations in composite samples collected at four stations in the Clark Fork River in 1986 where all feeding categories were present (Table 1). The minimum and maximum concentrations in individual taxa from the four stations are shown in parentheses.

Feeding category	Cu	Cd	Pb	Zn
Detritivore	102	1.1	14.1	234
<i>Pteronarcys californica</i>	(55–190)	(0.2–2.8)	(2.5–39.8)	(208–248)
Omnivore	43	1.9	4.5	198
<i>Hydropsyche</i> spp.	(44–78)	(2.0–2.6)	(1.8–8.0)	(221–234)
<i>Arctopsyche grandis</i>	(14–34)	(1.0–1.7)	(1.8–3.0)	(172–185)
Predator	54	1.2	0.8	338
<i>Claassenia sabulosa</i>	(44–60)	(1.6–1.7)	(0.1–2.1)	(209–248)
<i>Hesperoperla pacifica</i>	(36–42)	(1.2–2.1)	(0.2–0.6)	(471–483)
<i>Isogenoides</i> sp.	(50–82)	(0.8–1.1)	(0.3–1.7)	(335–342)

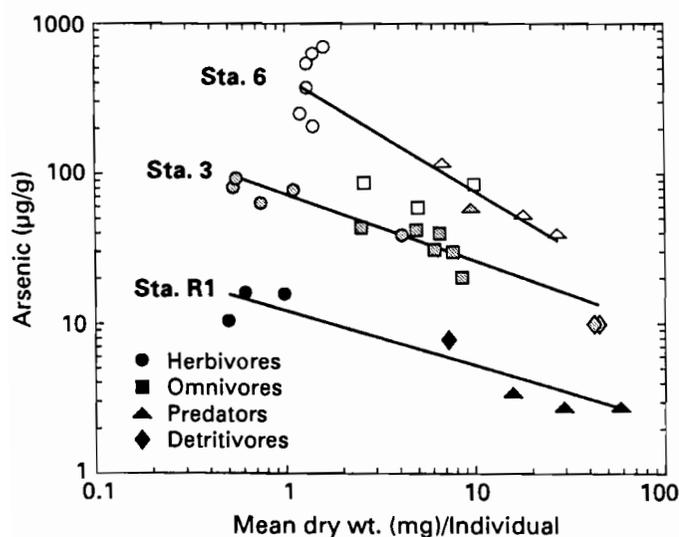


FIG. 7. Arsenic concentrations in taxa from stations R1, 3, and 6 in Whitewood Creek as a function of the insects' dry tissue weights. Each symbol is a single composite sample. Different symbol shading indicates different stations.

Smock 1983b). Some authors have suggested that the lowest trace element concentrations occur in predators (Nehring 1976; Burrows and Whitton 1983; Smock 1983b; Timmermans et al. 1989), and insects that ingest sediments may have exceptionally high concentrations of some elements (Smock 1983b). The

latter may result from concentrations of metals associated with undigested material in the digestive tract (Elwood et al. 1976; Smock 1983b; Hare et al. 1989). The external body surface also may be an important site of metal accumulation in insects (Elwood et al. 1976; Smock 1983b; Krantzberg and Stokes 1988; Cain et al. 1989; Timmermans and Walker 1989; Gower and Darlington 1990; Hare et al. 1991; van Hattum et al. 1991), thus creating a size dependence on concentrations. Bioaccumulation of essential and nonessential trace elements may differ. Chironomids appear to regulate Zn and Cu in contaminated lakes, but not Cd and Pb (Krantzberg and Stokes 1989).

In this study, examples of all of the influences cited above were evident, but these effects were element specific, species specific, and influenced by the hydrogeochemical nature of the contamination. Bioaccumulation of Pb was clearly influenced by feeding habit, with concentrations highest in a detritivore and lowest in predators. However, this pattern of accumulation was not consistent with other elements. Copper concentrations were highest in a detritivore where sediments were highly contaminated in the Clark Fork, but where contamination was less severe, the concentrations were similar to those in other taxa. Feeding habit had no discernible influence on Cd and Zn bioaccumulation. The size of individuals clearly influenced As concentrations in Whitewood Creek because of the contamination of external surfaces of the body. Hare et al. (1991) reached a similar conclusion for As in a lake-dwelling insect. In Whitewood Creek the exoskeleton was contaminated with As-enriched iron oxides which are produced from acidic groundwater inflows. Localized areas of exceptional As contamination

TABLE 8. Arsenic content (average $\mu\text{g}/\text{individual} \pm 1 \text{ SD}$, $n = 1-5$) in larva, nymph, pupa, and adult specimens in Whitewood Creek. The percent As associated with the larva/nymph exoskeleton was estimated from the difference in As content between those life forms and the pupa and adult phases.

Station	Taxon	Life form			% As in exoskeleton
		Larva/nymph	Pupa	Adult	
R1	<i>Hesperophylax</i> sp.	56	38		32
2	<i>Hesperophylax</i> sp. and <i>Limnephilus</i> sp.	134	58		57
3	<i>Hesperophylax</i> sp. and <i>Limnephilus</i> sp. <i>Rhyacophila</i> sp.	436 \pm 11	194 \pm 41		56
		606	17		97
4	<i>Isoperla</i> sp. <i>Hydropsyche</i> spp.	55 \pm 11		2 \pm 1	96
		386 \pm 68		5 \pm 0.1	99

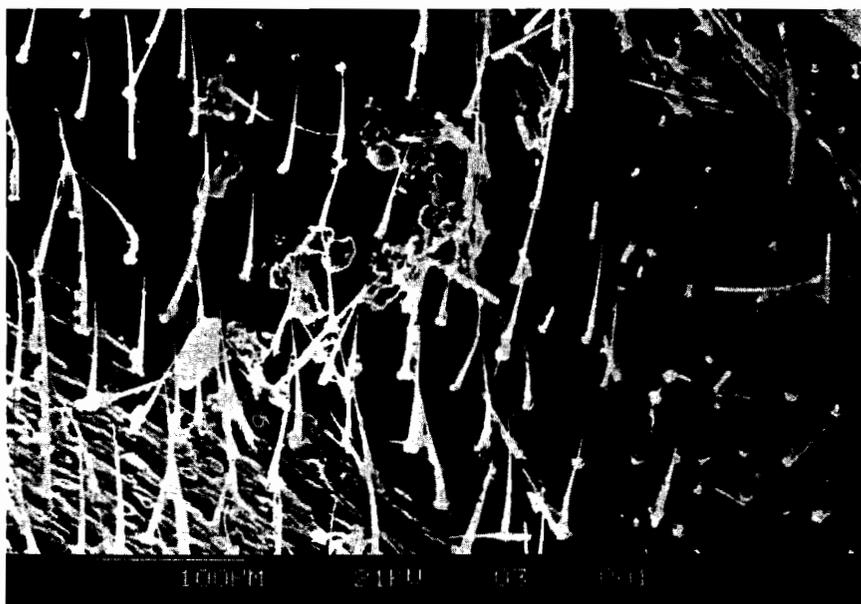


FIG. 8. Scanning electron micrograph of particles (oval and irregularly shaped objects near center of field) among setae of a specimen of *Hesperoperla pacifica* collected from station 2 in Whitewood Creek.

were common in this system where groundwater seeps contacted the stream channel. Size was not generally important in interpreting metal concentrations in insects from the Clark Fork River, where acid inflows are not common. Size appeared to be an important consideration for comparisons only between *Hydropsyche* spp. and *A. grandis*. However, *A. grandis* is more predaceous and ingests larger particles than *Hydropsyche* spp. (Wiggins 1977), and therefore, differences in food selection also could have influenced bioaccumulation of trace metals in these taxa. Insects accumulated Zn the least of all the elements in response to sediment contamination in the Clark Fork River. Physiological regulation of Zn may explain this observation. Copper regulation was not evident in any species, except perhaps *C. sabulosa*. In this species, Cu concentrations throughout the Clark Fork were higher than reference concentrations, but changed little over more than 300 km of the river despite a significant change in sediment Cu contamination.

These examples illustrate the complexity of contaminant accumulation in stream benthos. Bioaccumulation is the result of biotic and abiotic processes whose relative importance in

determining contaminant concentrations may differ from one situation to another.

Consideration of the processes that affect bioaccumulation may help direct strategies for effectively using benthic insects as biomonitors in lotic environments. Because the size, age, or developmental stage of individuals can affect concentrations, these factors should be considered. Where concentrations are size dependent, station comparisons are best made with data that account for differences in insect size. Concentrations of contaminants derived from whole-body analysis may also reflect contamination from sediments and food in the gut. The influence of undigested material would probably be greatest for taxa that ingest sediments (Smock 1983b) and in environments where differences in sediment contamination and tissue bioaccumulation are large. In this study, animals lost fecal material during the short depuration period, but complete evacuation of the gut could not be assured. Sediment in the gut may have contributed to the downstream patterns in metal concentrations in some taxa in the Clark Fork River and to differences in metal bioaccumulation among feeding groups. However, ANCOVA

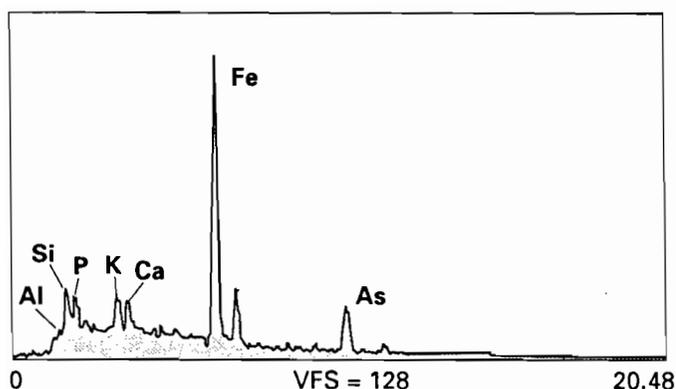


FIG. 9. Energy dispersive X-ray analysis of particle containing Fe and As found on the exoskeleton of a specimen (*Choroterpes* sp.) collected at station 6, Whitewood Creek.

implies that gut content does not affect tissue concentrations of all elements equally. This interpretation is consistent with the direct analysis of the metal concentrations in the gut contents of a burrowing mayfly (Hare et al. 1989) and in *P. californica* from the Clark Fork River (Axtmann et al. 1991). Quantifying the contribution of gut content will be important in determining tissue incorporation of trace elements and the influence of trophic level on bioaccumulation.

This study has demonstrated the difficulty in utilizing a single, cosmopolitan species for biomonitoring cobble-bottom river systems. One strategy for countering this problem would be to combine species for analysis. Our results indicate that this approach would sacrifice precision and sensitivity because of the inherent variability in trace element bioaccumulation among taxa. The problems with combining taxa are most severe at the most contaminated sites, since variability in contaminant concentrations among taxa appears to be greatest in such situations. Changes in species composition among stations also would lead to intractable errors.

Although the increases in variance and the potential biases that result from combining taxa are smaller for some elements than for others, samples should be separated to the lowest taxon practical in order to maximize sensitivity in multielement studies. Ideally, a single species should be compared throughout a study area. In lotic environments, this may be possible over short reaches of a river system, but improbable in studies covering long reaches of a river or an entire basin where a variety of habitats are encountered. Also, species identifications may be difficult to ascertain. In lieu of single species, comparisons of contamination at the genus level appear to be a practical alternative. Differences in trace element concentrations among *Hydropsyche* spp. were relatively small. This may be because the genus comprises ecologically and morphologically similar species (Wiggins and Mackay 1978). If *Hydropsyche* is typical, then grouping species of a genus may be an acceptable compromise to preserve sample precision while reducing analytical costs.

In many cases, it may be necessary to integrate information from several different taxa to assess contamination. Multispecies approaches can be effective in documenting the intensity and extent of contamination in lotic environments (e.g. Moore et al. 1991). When using this approach, taxa should be carefully

selected to allow the greatest possible opportunity to make relative comparisons of contamination over space and time.

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