Pergamon

Environmental Toxicology and Chemistry, Vol 13, No 12, pp. 1985-1997, 1994
Printed in the USA
0730-7268/94 $6.00 + .00

0730-7268(94)00147-2

Annual Review

TOXICITY OF METAL-CONTAMINATED SEDIMENTS FROM THE UPPER CLARK FORK RIVER, MONTANA, TO AQUATIC INVERTEBRATES AND FISH IN LABORATORY EXPOSURES

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(*Received* 31 *March* 1994; *Accepted* 17 *June* 1994)

Abstract—Sediments of the upper Clark Fork River, from the Butte and Anaconda area to Milltown Reservoir (230 km downstream), are contaminated with As, Cd, Cu, Pb, Mn, and Zn primarily from mining activities. The toxicity of pore water from these sediments was determined using *Daphnia magna* (48-h exposure), rainbow trout (96-h exposure), and Microtox®. However, pore-water data from these exposures were questionable because of changes in the toxicity of pore-water samples after 5 to 7 d of storage. Whole-sediment tests were conducted with *Hyalella azteca* (28-d exposure), *Chironomus riparius* (14-d exposure), rainbow trout (*Oncorhynchus mykiss*) 21- to 28-d exposure and *Daphnia magna* (2- to 22-d exposure). Sediment samples from Milltown Reservoir and the Clark Fork River were not generally lethal to test organisms. However, both reduced growth and delayed sexual maturation of amphipods were associated with exposure to elevated concentrations of metals in sediments from the reservoir and river. Relative sensitivity (most sensitive to least sensitive) of organisms in whole-sediment toxicity tests was: *Hyalella azteca* > *Chironomus riparius* > rainbow trout > *Daphnia magna*. Relative sensitivity (most sensitive to least sensitive) of the three end points evaluated with *Hyalella azteca* was: length > sexual maturation > survival. The lack of lethal effects on organisms may be related to temporal differences in sediment, acid-volatile sulfide, or organic carbon.

Keywords—Milltown Reservoir Clark Fork River Sediment Toxicity Invertebrates

INTRODUCTION

The Clark Fork River, located in southwestern Montana, is the largest tributary of the Columbia River in the northwestern United States. The upper Clark Fork River from the Butte and Anaconda area to at least 230 km downstream at Milltown Reservoir is contaminated with As, Cu, Cd, Pb, Mn, and Zn primarily from mining activities (see Fig. 1 in Canfield et al. [1]). Silver Bow Creek, the upper Clark Fork River, and Milltown Reservoir have been designated Superfund sites by the U.S. Environmental Protection Agency (EPA) because of metal-contaminated bottom sediments and arsenic (As) in drinking water [2].

The toxicity of chemicals in sediments is strongly influenced by the extent to which the chemical binds to sediment. As a consequence, different sediment types will exhibit different degrees of toxicity for the same total quantity of chemical [3]. For example, the toxicity of non-ionic organic chemicals in sediment is controlled primarily by the organic carbon content of the sediment [4]. The toxicity of many divalent metals may be controlled by the acid-volatile sulfide (AVS) phase of sediment [5]. Additionally, oxides of iron and manganese may regulate the bioavailability of metals in oxidized sediments [6,7].

This report is one in a series of reports assessing the nature and extent of sediment contamination at selected stations in Milltown Reservoir and the upper Clark Fork River. This assessment was accomplished by completing the following tasks: (a) ecological characterization and biological assessment for a soil contamination evaluation [8]; (b) preliminary field surveys and food-chain contamination evaluations for small mammals; (c) physical and chemical characterization of the sediment [9]; (d) whole-sediment toxicity tests; (e) benthic-community structure analysis [1]; (f) whole-sediment bioaccumulation tests [10]; (g) physiological changes and tissue metal accumulation in rainbow trout (*Oncorhynchus mykiss*) exposed to foodborne and waterborne metals [11]; and (h) development of an ecological risk assessment [12].

The objective of the present study was to assess the toxicity of metal-contaminated sediments and pore waters collected from Milltown Reservoir and the upper Clark Fork River to fish and aquatic invertebrates in laboratory toxicity tests and to evaluate how other factors in addition to metals influence metal toxicity. Pore-water toxicity tests were conducted with *Daphnia magna* (48-h exposure), rainbow trout (*Oncorhynchus mykiss*) (96-h exposure), and Microtox®. Whole-sediment toxicity tests were conducted with *Hyalella azteca* (28-d exposure), *Chironomus riparius* (14-d exposure), *D. magna* (2- to 22-d exposures), and rainbow trout (21- or 28-d exposure). Daphnids and trout were relatively insensitive in the whole-sediment toxicity tests [13];

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References to trade names or manufacturers do not imply government endorsements of commercial products.

Presented at the Symposium on Integrated Ecological Assessment of a Superfund Site: Milltown Reservoir, Clark Fork River, Montana, 13th Annual Meeting, Society of Environmental Toxicology and Chemistry, Cincinnati, Ohio, November 8-12, 1992.

therefore, results of whole-sediment toxicity tests are described for only amphipods and midges.

MATERIALS AND METHODS

Summary of sampling stations

Stations were selected to represent a gradient of sediment contamination that included relatively uncontaminated reference stations. Surface sediments were collected at six stations along the Clark Fork River from September 16–19, 1991: CF-01, Silver Bow Creek 1 km above a lime shack; CF-02, Clark Fork River near Warm Springs Pond; CF-03, Clark Fork River near Deer Lodge; CF-04, Clark Fork River at Gold Creek Bridge; CF-05, Clark Fork River at Turah Bridge; and CF-06, a reference station on Rock Creek within 5 km of its confluence with the Clark Fork River (see Fig. 1 in citation [1]). Sediments from seven stations in or near Milltown Reservoir were sampled from July 22–25, 1991: MR-01, a reference station on the Blackfoot arm of the Reservoir, and MR-02, MR-07, MR-11, MR-17, MR-19, and MR-25, all located on the Clark Fork arm of the Reservoir (see Fig. 2 in citation [1]).

Sample collection, handling, and storage

Sediment samples were collected from depositional zones with a petite Ponar grab (225-cm^2 area) or polypropylene scoop. Each sample was a composite of a minimum of 10 grabs/station taken from an area of about 100 m^2 in the reservoir and from an area about 3 × 6 m for the river samples. Polypropylene scoops were used in shallow backwater areas where bottom type or depth prevented use of a Ponar grab. Samples consisted of the upper 6 cm of the sediment surface. A total of 20 L of sediment from each station was placed in two 10-L high-density polyethylene (HDPE) containers. Samples were shipped to the laboratory by overnight courier for toxicity testing, bioaccumulation testing [10], benthic community assessment [1], and physical and chemical analyses [9]. The control sediment was a fine silt–clay agricultural soil collected near St. Louis, Missouri, and has been used in previous studies [4,14]. Sediments were held less than 3 weeks before the start of a test.

Culturing of test organisms

Amphipods were mass-cultured according to procedures outlined in Kemble et al. [13] using 80-L glass aquaria containing 50 L of well water (hardness 283 mg/L as $CaCO_3$, alkalinity 255 as $CaCO_3$, pH 7.8) and either maple leaves or "coiled-web material" (3M Co., Saint Paul, MN) as substrate. Amphipods were isolated by placing the substrate on a 5-mm-mesh sieve in a pan containing about 2 cm of well water. Well water was sprinkled over the substrate, flushing mixed-age amphipods into the pan below. Immature amphipods (<2 mm total length) used for testing were then rinsed into a no. 40 sieve (U.S. standard-sieve size 425 μm).

Midges were mass-cultured in polyethylene chambers (30 × 30 × 30 cm) containing 3 L of well water according to procedures of Ingersoll et al. [15]. To obtain first instar larvae (<24 h old) for testing, egg cases were placed in individual 100-ml glass chambers containing 50 ml of test water at 20°C for about 2 d before hatching occurred.

Daphnids were mass-cultured according to procedures described in Ingersoll et al. [15]. Eyed rainbow trout eggs were obtained from the Ennis Fish Hatchery, Ennis, Montana. Toxicity tests with trout were started about 5 d after hatching (except for trout in the toxicity test with Milltown Reservoir sediments, which hatched 1 week earlier than anticipated). Fish were not fed during holding, acclimation, or testing [13].

Toxicity tests

Pore-water toxicity tests were conducted with *Daphnia magna* (48-h exposure), rainbow trout (96-h exposure) and Microtox (15-min exposure). A minimum of 2 L of pore water was isolated from each homogenized-sediment sample by centrifugation at 4°C for 15 min at 5,200 rpm (7,000 *g*). After centrifugation, the supernatant was poured through a no. 60 (U.S. standard-sieve size 250 μm) polyester sieve into a 1-L amber glass bottle to remove any large floating particles. Pore-water samples were held at 4°C in the dark until testing. Aliquots of pore water were used for (a) metals analysis, (b) water-quality characterization, and (c) toxicity testing. The test water was reconstituted to be representative of the low hardness conditions in the Clark Fork River (CFR; hardness and alkalinity 100 mg/L as $CaCO_3$, pH 8.0, conductivity 200 μS/cm [13]).

Daphnids were exposed in static toxicity tests for 48 h to 100, 50, 25, 12.5, and 6.25% pore water and to a negative control of CFR water. Before the test started, daphnids (<24 h old) were acclimated to CFR water by sequentially placing animals at 2-h intervals into 50:50 and 25:75 mixtures of well water to CFR water and then into 100% CFR water. Twenty daphnids, five at a time, were counted into each 250-ml test beaker containing 200 ml of water at 20°C. The photoperiod was 16.8 h light:dark at an intensity of about 500 lux. Aeration (about 2 bubbles/s) was used throughout the test because of low initial dissolved oxygen concentrations. Mortality was recorded in all treatments at 24 and 48 h. Death was defined as a lack of a response to prodding with a blunt probe during a 5-s observation period.

Exposures of rainbow trout to pore-water samples were similar to the exposure of daphnids except (a) test temperature was 10°C, (b) study duration was 96 h, and (c) 1-L test beakers containing 500 ml of water were used.

Microtox exposures were conducted at 15°C with luminescent bacteria (*Photobacterium phosphoreum*) and a model 500 Microtox instrument [16]. Full-strength (100%) pore water was tested using the "100%" test method [13]. Sodium chloride was substituted for the Microbics® osmotic adjusting solution (MOAS) to osmotically adjust the sample. Toxicity was measured as the 15-min EC50 value (the percent dilution of the pore waters that produced a 50% reduction in light output of the luminescent bacteria).

Whole-sediment toxicity tests were conducted with *Hyalella azteca* (28-d exposure) and *Chironomus riparius* (14-d exposure). Overlying water used in exposures was reconstituted CFR water. Biological observations included survival, total body length, weight, sexual maturation, or reproduction of test species. Amphipods were acclimated to CFR water by sequentially transferring animals at 24-h intervals into 50:50 and 25:75 mixtures of well water to CFR water and

then into 100% CFR water. Midge larvae were hatched in 100% CFR water.

Sediment samples were homogenized and placed into beakers 4 d before animals were added (day −4) using procedures described in Kemble et al. [13]. Midges and amphipods were exposed to 200 ml of sediment with 800 ml of overlying water in 1-L flow-through test beakers (4 replicates/treatment) at 20°C. Each beaker received 1.25-volume additions/d of overlying water starting on day −3. Aeration (about 2 bubble/s) was used in the whole-sediment Clark Fork River tests because of low dissolved oxygen in overlying water. The photoperiod was 16:8 h light:dark at an intensity of about 500 lux.

Tests were started on day 0 by counting 20 amphipods or 50 midges, five at a time, into the test beakers containing sediment and overlying CFR water. Amphipods were fed 6 mg of Purina® rabbit pellets in a water suspension three times a week for the first 7 d of the study and 12 mg three times a week for the last 21 d. Midges were fed 10 mg of Cerophyl® for the first 6 d of the test and 10 mg Hartz® Dog Treats and 6×10^7 algae cells (*Selenastrum capricornutum*) daily throughout the test. If excessive mold was observed on the sediment surface of any of the beakers, midges were fed only algae for that day [14].

Amphipods and midges were retrieved from test beakers at the end of exposures by wet-sieving the sediment. Overlying water (about 600 ml) was poured through a no. 50 U.S. Standard sieve and was swirled with enough action to suspend the upper 1 cm of the sediment. This slurry was then poured through the no. 50 sieve, and the contents were washed into an examination pan. The coarser sediment remaining in the beaker was rinsed through a no. 40 sieve, and the contents of this sieve were then washed into a second examination pan. Surviving organisms were removed from the examination pans and preserved in 8% sugar formalin until their length and sexual maturation were determined [14].

Amphipod body length was measured from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface with a Zeiss® interactive digital analysis system in combination with a Zeiss SV8 stereomicroscope at a magnification of 25×. Amphipods were classified as either "mature male" or "female or immature male" based on the presence of an enlarged second gnathopod. An enlarged second gnathopod of male amphipods was a consistent measure of sexual maturation (it is difficult to distinguish sexual maturation of females at this age). No attempt was made to quantify production of young amphipods. Body lengths of up to 20 midges/replicate were measured from 20- × 30-cm glossy black-and-white photographs (1-cm gridded background) at a magnification of 3.5× using a Houston Instrument True Grid® 1017 digitizing board. Midge lengths were measured from the anterior of the labrum to the posterior of the last abdominal segment [17].

Water quality

The following water-quality characteristics were measured in the pore-water samples: dissolved oxygen, chloride, temperature, conductivity, pH, alkalinity, total hardness, turbidity, total ammonia, and total sulfide [13]. Un-ionized ammonia concentrations (mg/L, as NH_3) were calculated using pH and temperature in the formula presented in Thurston et al. [18]. Hydrogen sulfide concentrations (mg/L) were calculated by adjusting the total dissolved sulfide concentrations to pH and temperature using the relationship presented in Broderius and Smith [19].

Dissolved oxygen, pH, conductivity, and temperature were measured at the beginning and end of the daphnid and trout pore-water exposures in the 100% pore-water, 25% dilutions, and negative control of dilution water (CFR water). Dissolved oxygen was measured at 48 h for the trout test in the 100% pore-water, 25% dilutions, and negative control.

In whole-sediment tests, the following water-quality characteristics were measured on day −1 (the day before animals are introduced to the test chamber) and at the end of each test: dissolved oxygen, chloride, temperature, conductivity, pH, alkalinity, total hardness, turbidity, total ammonia, and total sulfide, using procedures described in Kemble et al. [13]. Dissolved oxygen, pH, and conductivity concentrations in the overlying water were measured weekly.

Chemical characterization of pore water

Total and filterable concentrations of Al, As, Cd, Cr, Cu, Fe, Hg, Mn, Ni, Pb, Se, and Zn were measured in pore-water samples [9]. Fifty milliliters of each sample were vacuum-filtered through a 0.4-μm polycarbonate membrane, transferred to a precleaned 60-ml polyethylene bottle, and acidified with 0.5 ml high-purity HNO_3 before analysis of filterable metals. The remaining 50 ml of sample was acidified with 0.5 ml HNO_3, and aliquots were digested for "total recoverable metals" by one of two methods, depending on the analyte. For Hg and Se, samples were heated with potassium persulfate ($K_2S_2O_8$) and HCl [20]. For remaining analytes, digestion was accomplished by heating with HNO_3 and H_2O_2 [21].

Characterizations of sediment

Sediment subsamples from each station were characterized for (a) percentage water, (b) particle size, (c) cation exchange capacity, (d) organic carbon, (e) ash-free dry weight, and (f) metals and acid-volatile sulfide (AVS) [13]. Determination of AVS was patterned after EPA draft method 376.3 utilizing a sulfide electrode for detection of trapped sulfide ion generated after treatment with 3 N HCl [9]. Sediment characterization also included measurements of hydrogen sulfide, select pesticides, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) [9]. Whole sediment sampled the day that pore waters were prepared and the day whole-sediment toxicity tests were started (about 8 d later) exhibited very little change in AVS, As, Cd, Cu, Pb, or Zn [13]. Results of quality-control samples analyzed for physical characterization and water quality are summarized in Kemble et al. [13]. Results of quality-control samples analyzed for chemical characterization are summarized in Brumbaugh et al. [9].

Concentrations of metals in subsamples of whole sediments were determined by a total-acid digestion, a dilute hydrochloric acid (3 N HCl) extraction (SEM) at room temperature for 1 h simultaneously with acid-volatile sulfide

(AVS) determination, and a four-step sequential extraction (bound to oxides, organic matter, sulfides, and residual) [9,22]. Concentrations of Al, As, Cd, Cr, Fe, Hg, Mn, Ni, Pb, Se, and Zn were measured in extracts from the total acid digestion and the dilute HCl extraction of samples. Selected metals (As, Cd, Cu, Pb, and Zn) were also measured in the four-step sequential extraction.

Data analysis and statistics

Pore-water toxicity tests. Where mortality of daphnids or trout exceeded 50%, the EC50 was estimated by the probit method [23]. If data were incomplete (one or no partial mortality), a range bracketing the EC50 was determined (e.g., highest concentrations with 0% mortality and the lowest concentrations with 100% mortality), which is a conservative estimate of the EC50 confidence interval [23]. The EC50 for the Microtox test was estimated using linear regression equations that plotted log concentrations vs. log gamma (normalized ratio of light lost during the test to light remaining at time of measurement) [16].

Whole-sediment toxicity tests. Data for percentage survival and sexual maturation were arcsin transformed before analysis. Comparisons of mean survival and percentage sexual maturation (amphipod exposures) were made using a one-way analysis of variance (ANOVA) with mean separation by Fisher's protected least-significant difference test at $\alpha = 0.05$ [24]. Variance among treatment means for body length of amphipods and midges was heterogenous. Therefore, a rank analysis of variance was performed and mean differences determined using a t test on ranked means (at $\alpha = 0.05$). All statistical analyses were performed with Statistical Analyses System programs [25].

No-effect concentrations (NEC). No-effect concentrations, which are analogous to apparent-effects thresholds (AETs, [26]), were used to identify sediment characteristics that may have been associated with a toxic response. An NEC is calculated as the maximum concentration of a chemical (or material) in a sediment that did not significantly alter the particular indicator compared with the control. Exceeding an NEC for a particular chemical does not mean the chemical caused the effect. It is the concentration of a chemical associated with a response. However, if a sediment sample is toxic and the concentration of the chemical of interest is below the NEC, then the toxicity cannot be attributed solely to that chemical.

We chose to use the term NEC instead of AET because (a) we calculated NECs for both whole-sediment and pore-water concentrations, and AETs are classically calculated for only whole-sediment concentrations; (b) a minimum of 25 to 50 samples is recommended for calculating an AET, and only 15 samples from the reservoir and river were evaluated in the present study; and (c) we calculated effects relative to the control sediment, and AETs are typically calculated relative to reference sediments. We chose to develop NECs relative to control sediment because the reference sediment for Milltown Reservoir (MR-01) was contaminated with organic compounds (e.g., 730 ng/g total PCBs) [9] and was toxic to *Hyalella azteca* (e.g., reduced body length). Long and Morgan [27] reported an effect range-median (ER-M) of 400 ng/g for total PCBs in sediment. The ER-M is a concentration of a chemical in sediment above which effects are frequently or always observed or predicted among most species.

RESULTS

Physical characteristics

Physical characteristics of sediment samples are listed in Table 1. Sediment organic carbon content ranged from 0.76% for samples from station MR-02 to 6.9% for station CF-01. Mean organic carbon content in the control sediment was 1.2%. Percentage water ranged from 32% (mean of 4 replicates) for the control sediment to 67% for sediment from station CF-01. Sediment samples were predominantly sandy-loam-sized particles. The percentage of sand-sized particles (>50 μm) was about 10 to 15% greater for the river samples than for the reservoir samples (Table 1). Ash-free dry weights ranged from about 2.4% for station CF-05 sediment to 14.9% for station CF-01 sediment. Cation exchange capacity (CEC) of sediments ranged from 8.5 meq/kg for station CF-05 to 17.1 meq/kg for station MR-07 (Table 1).

Chemical sediment characteristics

Data are presented for filtered pore-water metals and SEM metals in whole sediment because these forms are most representative of bioavailable metals [9]. Analyses of total metals in pore-water and whole-sediment samples are described in Brumbaugh et al. [9].

In pore waters isolated from Milltown Reservoir sediments, concentrations of filterable Cr and Ni were similar to those in pore water from control sediment [9]. There was insufficient pore water available for determination of filterable Hg and Se. Results of total-recoverable element analyses indicated that very low concentrations of these elements were present in sediment samples from Milltown Reservoir [9]. The pore-water sample from station MR-19 was highest for As and Cu and among the highest concentrations for Pb, Cd, and Zn. Pore water from MR-01 was lowest for Cu and Zn but highest for Fe, Mn, and Ni. Concentrations of Al and Zn were highest in pore water prepared from station MR-11 sediment. For As, Cd, Cu, Pb, and Zn, proportions of filtered to total concentrations were highest for As and Zn and intermediate for Cd and Cu, while filterable Pb concentrations were low relative to total Pb [9]. Relatively low percentages of Al and Fe were filterable, but a very high percentage of Mn was filterable [9].

In pore water prepared from Clark Fork River sediment, all elements except Al were elevated in samples from at least one station compared with the control sample [9]. Pore-water concentrations of Cd, Cu, Fe, Mn, Ni, and Zn were highest in the pore water prepared from station CF-01 sediment. Pore water from station CF-06 had the highest concentration of Al and the lowest concentrations of As, Cu, Mn, and Zn. With the exception of As and Zn, dissolved metal concentrations were not markedly different among pore-water samples from CF-04, CF-05, CF-06, and control sediment. The highest concentration of As was in CF-03 pore water. The trends for proportions of dissolved to total metals in the Clark Fork River pore-water samples were similar to those observed for the reservoir [9].

Table 1. Physical and chemical characteristics of sediments from Milltown Reservoir and the Clark Fork River

Station	Total organic carbon (%)	Ash-free dry wgt. (%)	Cation exchange capacity (meg/kg)	Percent water	Particle size (%)			Sediment/soil class
					Sand	Silt	Clay	
Milltown Reservoir								
Control (rep. 1)	1.06	3.96	12.8	29	21	57	22	Silt loam
Control (rep. 2)	1.06	NM	14.8	29	10	68	22	Silt loam
MR-01 (ref.)	1.00	3.93	12.5	37	73	17	11	Sandy loam
MR-02	0.76	4.27	13.2	39	54	33	13	Sandy loam
MR-07	2.43	8.16	17.1	54	53	30	17	Sandy loam
MR-11	2.67	8.82	16.9	54	40	40	20	Loam
MR-17 (rep. 1)	1.94	8.63	15.5	56	51	32	18	Loam
MR-17 (rep. 2)	NM	NM	NM	56	47	36	17	Loam
MR-19	1.30	4.23	11.3	35	62	25	13	Sandy loam
MR-25 (rep. 1)	3.19	6.44	14.3	45	56	30	13	Sandy loam
MR-25 (rep. 2)	NM	NM	NM	45	56	28	16	Sandy loam
Clark Fork River								
Control (rep. 1)	1.30	3.70	11.6	35	9	58	33	Silt clay loam
Control (rep. 2)	1.18	NM	12.7	35	9	62	29	Silt clay loam
CF-01	6.90	14.93	15.6	67	52	28	20	Loam
CF-02	1.70	6.04	13.2	44	75	12	13	Sandy loam
CF-03	1.41	2.99	12.7	36	74	12	14	Sandy loam
CF-04	3.08	6.35	15.2	42	60	27	13	Sandy loam
CF-05	1.21	2.35	8.5	33	82	6	12	Sandy loam
CF-06 (ref.)	6.67	6.26	12.8	48	70	17	13	Sandy loam

NM = Not measured.

Concentrations of AVS and 12 simultaneously extracted elements in sediments were determined for samples on the day pore waters were prepared (Table 2). Mercury and selenium were at or below detection limits in all samples. Among the reservoir stations, concentrations of Cr and Ni were relatively constant among the samples and were similar to the control and reference (MR-01) sediments. Sample MR-11 was the highest for extractable Al, As, Cd, Cr, Cu, Fe, Pb, Mn, Ni, and Zn. The lowest concentrations of AVS and all analytes were in the control and reference sediments (MR-01). In the reservoir samples, AVS ranged from 48 to 745 μg/g dry sediment, compared with 19.1 μg/g in the reference sediment and <2 μg/g in the control sediment. There was no correlation between AVS and absolute amounts of any of the simultaneously extracted metals.

Among the river samples, the highest concentrations of extractable Al, As, Cd, Cr, Cu, Fe, Hg, Pb, Ni, and Zn were in sediment from station CF-01. The lowest concentrations of extractable As, Cd, Cu, Pb, Mn, and Zn were measured in sediment from station CF-06. Station CF-05 had the lowest concentrations of Al and Fe, whereas Cr and Ni were lowest in sediment from station CF-03.

Table 2. Concentrations of acid-volatile sulfide and simultaneously extracted metals in sediment samples

Station	AVS	Al	As	Cd	Cr	Cu	Fe	Hg	Pb	Mn	Ni	Se	Zn
Control	<2.0	1,495	2.1	0.184	1.96	5.7	3,300	<0.024	11.9	456	5.23	<0.025	<20
MR-01	19.1	635	1.2	0.035	0.99	10.6	2,523	<0.024	6.06	205	1.72	<0.025	<20
MR-02	500.0	1,480	5.3	1.59	2.58	141.0	3,948	<0.024	32.0	279	3.79	<0.025	538
MR-07	315.0	2,030	9.1	2.04	2.90	233.0	4,558	0.039	45.7	540	4.06	<0.025	680
MR-11	78.6	2,782	43.4	7.49	3.01	607.0	11,650	0.036	56.3	1,367	7.01	<0.025	3,244
MR-17	745.0	1,878	7.4	2.46	2.62	185.0	5,194	0.039	46.3	484	4.33	<0.025	658
MR-19	48.0	1,352	29.2	2.35	2.21	354.0	3,550	0.048	48.6	652	3.53	<0.025	767
MR-25	402.0	1,708	8.3	3.87	2.80	178.0	5,844	0.028	36.7	540	4.09	<0.025	1,064
Control	<2.0	1,408	1.5	0.167	1.65	<12.0	2,735	0.030	11.7	340	4.17	<0.025	<15
CF-01	8.3	4,562	202.0	31.30	6.18	6,971.0	18,470	0.840	569	2,962	8.16	<0.025	8,873
CF-02	612.0	1,777	23.8	3.12	3.05	325.0	7,313	0.030	62.4	4,348	4.15	<0.025	700
CF-03	165.0	1,148	24.8	1.69	1.72	287.0	3,665	0.020	55.0	468	2.34	<0.025	408
CF-04	415.0	2,124	10.8	1.93	3.86	251.0	6,093	0.040	50.2	638	4.19	<0.025	562
CF-05	251.0	990	2.7	0.762	4.45	77.0	2,262	<0.020	19.4	94	3.29	<0.025	294
CF-06	213.0	1,532	<0.5	0.074	2.37	<12.0	2,306	<0.020	3.5	16	2.46	<0.025	<15

Samples were collected for chemical analyses the day pore waters were prepared. Results expressed as μg/g dry wgt.

Table 3. Water-quality measurements of pore water the day pore water was isolated from whole sediment

Station	pH	Alkalinity (mg/L)	Hardness (mg/L)	DO (mg/L)	Conductivity (μmho/cm @ 25°C)	Hydrogen sulfide (mg/L)	Total sulfide (mg/L)	Un-ionized ammonia (mg/L)	Total ammonia (mg/L)	Turbidity (NTU)	Chloride (mg/L)
Milltown Reservoir											
Control	6.96	48	180	7.1	451	<0.051	<0.10	0.003	0.89	249.0	18.5
MR-01	6.89	840	812	3.9	1,510	<0.056	<0.10	0.028	9.88	455.0	3.24
MR-02	7.36	336	288	3.9	532	0.085	0.28	0.030	3.60	56.0	ND
MR-07	6.91	792	612	2.3	1,387	0.102	0.28	0.081	29.60	191.0	9.45
MR-11	7.42	216	212	4.4	398	<0.029	<0.10	0.002	0.19	43.0	5.13
MR-17	6.96	572	496	5.0	1,048	0.084	0.15	0.031	11.40	94.5	15.5
MR-19	7.48	248	280	4.7	489	<0.027	<0.10	0.014	1.63	39.0	5.59
MR-25	7.03	272	248	2.8	519	0.060	0.12	0.009	3.17	93.5	11.3
Clark Fork River											
Control	6.73	52	124	8.4	294	<0.068	<0.10	0.001	0.67	220.0	14.2
CF-01	7.05	1,048	1,500	2.5	2,160	<0.050	<0.10	0.217	67.60	630.0	21.4
CF-02	7.87	304	288	5.1	619	0.053	0.42	0.138	6.32	51.0	11.7
CF-03	7.61	356	348	7.2	711	0.025	0.12	0.008	0.69	45.0	11.1
CF-04	7.31	548	500	4.9	987	0.048	0.14	0.030	4.72	81.0	12.9
CF-05	7.61	288	276	6.5	549	0.022	0.11	0.015	0.95	26.0	7.05
CF-06	7.40	132	124	5.9	234	0.095	0.30	0.003	0.51	67.0	1.88

ND = Not determined.

Concentrations of 23 organochlorine pesticides in whole-sediment samples were below 10 ng/g (the method lower limit of quantitation; MLLQ; [13]). Concentrations of total polychlorinated biphenyls in these samples were below the MLLQ of 50 ng/g except for MR-01 (730 ng/g), MR-07 (50 ng/g), and MR-11 (150 ng/g). Concentrations of polycyclic aromatic hydrocarbons (PAHs) in sediment samples were generally below the MLLQ (30 ng/g). Samples from MR-11 (≤145 ng/g), MR-19 (≤68 ng/g), and CF-01 (≤144 ng/g) exceeded the MLLQ for some compounds but were still well below reported sediment effect concentrations for PAHs [27].

Pore-water toxicity tests

Water-quality characteristics of pore water are listed in Table 3. After aeration, dissolved oxygen concentrations remained above 40% of saturation for the duration of the trout and daphnid exposures.

Pore waters prepared from MR-01, MR-02, MR-07, and MR-17 sediment samples were toxic to rainbow trout, with 96-h EC50s ranging between 50 and 100% pore water. The EC50 values for the control, and for MR-11, MR-19, and MR-25 treatments were greater than 100% pore water (Table 4). Pore-water samples prepared from Milltown Reservoir sediments were not acutely toxic in either the daphnid or Microtox exposures (EC50 >100% pore water; Table 4).

Among Clark Fork River sites only CF-01 pore water was toxic in the rainbow trout (EC50: 12 to 25% pore water), daphnid (EC50: 17% pore water), and Microtox (EC50: 19% pore water) exposures (Table 4). The influence of storage time on isolated pore-water samples was evaluated using Microtox. Pore-water was typically stored 5 to 7 d at 4°C before toxicity tests were started. The toxicity of pore-water samples prepared from CF-02 and CF-06 was dramatically reduced with 5 to 7 d of storage relative to 1 d of storage (Table 4).

Whole-sediment toxicity tests

Overlying water pH, alkalinity, total hardness, conductivity, and chloride measurements were similar among all stations, the control, and the inflowing CFR [13]. Un-ionized ammonia concentrations in CF-01 and CF-02 were elevated compared with other treatments and the control. By the end of the exposures, total-ammonia concentrations in all treatments except Station CF-01 were below the detection limit (<0.1 mg/L). Total-sulfide concentrations were below the detection limit (<0.1 mg/L) in overlying water for all stations, the control, and the inflowing CFR water. Dissolved oxygen measurements were at acceptable levels (>40% of saturation [28]) in all of the treatments throughout the exposures [13].

Milltown Reservoir. Survival of amphipods was not significantly reduced among any of the treatments relative to the control or reference (MR-01) sediments (Table 5). However, survival of amphipods in treatments MR-17 and MR-19 was significantly higher than in the control and reference sediment. Body length of amphipods was significantly reduced in all treatments relative to the control except for MR-25 and was significantly reduced in treatment MR-11 relative to the reference sediment (MR-01). Sexual maturation of amphipods was significantly reduced in treatments MR-07 and MR-11 relative to both the control and reference sediments (Table 5). Both body length and survival of midges in treatment MR-11 were significantly reduced relative to the control and reference sediments (Table 5). Indigenous organisms recovered at the end of whole-sediment exposures with reservoir sediments included oligochaetes, ostracods, a mussel, planaria, and copepods. Caddis fly cases were retrieved from several treatments; however, no caddis flies were retrieved. No pattern was evidenced between the toxic responses of amphipods or midges and the presence of these indigenous organisms.

Clark Fork River. Amphipod survival was significantly

Table 4. Pore-water toxicity results: The EC50 (95% confidence intervals in parentheses) for rainbow trout (*Oncorhynchus mykiss*; 96 h), *Daphnia magna* (48 h), and Microtox (15 min) exposures are calculated as a percentage of the pore-water sample

Station	*O. mykiss*[a]	*D. magna*[a]	Microtox[a]	Microtox[b]
Milltown				
Control	>100	>100	>100	ND
MR-01 (ref.)	59 (49–74)	>100	>100	ND
MR-02	50–100	>100	>100	ND
MR-07	50–100	>100	>100	ND
MR-11	>100	>100	>100	ND
MR-17	50–100	>100	>100	ND
MR-19	>100	>100	>100	ND
MR-25	>100	>100	>100	ND
Clark Fork River				
Control	>100	>100	>100	>100
CF-01	12–25	17 (10–26)	19 (18–20)	11 (11–11)
CF-02	>100	>100	>100	16 (8–29)
CF-03	>100	>100	>100	>100
CF-04	>100	>100	>100	>100
CF-05	>100	>100	>100	97
CF-06 (ref.)	>100	>100	>100	6

ND = Not determined.
[a]Pore water stored for 5–7 d before the start of the test.
[b]Pore water tested within 24 h of preparation.

reduced relative to the control and reference (CF-06) sediments in only treatment CF-01 (Table 5). Survival of amphipods in treatments CF-03 and CF-04 was significantly greater than in the control sediment but not the reference sediment. Amphipod body length was significantly reduced in treatments CF-01 and CF-03 relative to the control and reference sediments. Percentage maturation was significantly reduced in only the CF-01 sediment relative to the control and reference sediments. Midge survival and body length were not significantly reduced in any of the Clark Fork River treatments relative to the control or reference (CF-06) sediments. Midge body lengths in all treatments (except CF-03) were significantly greater than in the control sediment but not in the reference sediment (Table 5). Midge pupae were recovered from the CF-01 and CF-05 sediment samples. When amphipods were sieved from the sediment on day 28, indigenous animals were isolated, including oligochaetes, midges, and a snail. Indigenous organisms isolated on day 14 of the midge exposures included oligochaetes, a leech, and mussels. Again, there was no pattern between the toxic responses of amphipods or midges and the presence of these indigenous organisms.

DISCUSSION

Pore-water toxicity tests

Pore-water samples were stored at 4°C for up to 7 d before the start of the toxicity tests. The decision to store isolated pore water for this long after extraction was based on our experience with elutriate samples prepared from Great Lakes sediment [29]. However, the toxicity of isolated pore water in the Microtox assay changed with storage (Table 4). Similar changes were observed in toxicity of pore water to daphnids and trout (unpublished data). A flocculation of material occurred within the first 24 h of the pore-water exposures, which indicates metals may have precipitated with Fe and Mn oxyhydroxides. In the future, investigators should conduct toxicity tests immediately after isolating pore-water samples, and test solutions should be renewed frequently during exposures (e.g., every 12 or 24 h) to prevent changes in pore-water toxicity after storage. Given the problem associated with the changes in toxicity with storage of pore-water samples, the remainder of the discussion will focus on the whole-sediment toxicity data. However, pore-water chemistry was measured immediately after isolation from sediment and presumed to be valid [9], and therefore it was used in our evaluation of whole-sediment toxicity.

Whole-sediment toxicity tests

Whole-sediment samples from Milltown Reservoir and the Clark Fork River were toxic to amphipods, midges, and rainbow trout but not to daphnids (Table 5, [13]). The amphipod test identified sediment from six of the seven reservoir stations as toxic (e.g., significant reduction in length or sexual maturation relative to the control for MR-01, MR-02, MR-07, MR-11, MR-17, MR-19 samples). The whole-sediment midge test identified only one of the seven reservoir stations as toxic (MR-11), and the whole-sediment trout and daphnid tests [13] did not consistently identify any of the reservoir stations as toxic. The amphipod test identified two of six river stations as toxic (CF-01, CF-03), the trout and daphnid [13] tests identified one of six river stations as toxic (CF-01), and the midge test did not consistently identify any of the six river stations as toxic.

Hyalella azteca are typically more sensitive than are midges to contaminated sediments [14,30,31]. In the present study, relative sensitivity (most sensitive to least sensitive) of test organisms was: *Hyalella azteca* > *Chironomus riparius* > rainbow trout > *Daphnia magna*. Relative sensitivity (most sensitive to least sensitive) of the three end points evaluated

Table 5. Whole-sediment toxicity results for *Hyalella azteca* (28 d) and *Chironomus riparius* (14 d) exposures

Station	Survival (%)	Length (mm)[a]	Mature males (%)
Hyalella azteca			
Control	86 (3.15) BC	4.45 (0.02) A	41 (3.35) AB
MR-01 (ref.)	93 (1.44) ABC	3.54 (0.10) C	28 (1.65) AB
MR-02	94 (4.73) AB	4.11 (0.08) B	39 (7.11) AB
MR-07	79 (6.25) C	4.06 (0.10) B	13 (5.45) C
MR-11	78 (8.51) C	2.62 (0.17) D	10 (3.03) C
MR-17	99 (1.25) A	4.10 (0.13) B	27 (4.25) B
MR-19	98 (2.50) A	3.99 (0.11) B	26 (1.66) B
MR-25	94 (2.04) AB	4.24 (0.09) AB	50 (10.22) A
Control	74 (2.39) C	3.89 (0.12) A	31 (6.79) A
CF-01	48 (9.68) D	2.85 (0.25) C	8 (5.00) B
CF-02	76 (6.88) BC	3.69 (0.11) AB	15 (1.58) A
CF-03	91 (5.54) AB	3.22 (0.13) BC	29 (7.03) A
CF-04	95 (2.04) A	4.03 (0.32) A	25 (6.84) A
CF-05	85 (6.45) ABC	3.83 (0.07) A	24 (3.40) A
CF-06 (ref.)	89 (3.75) ABC	4.01 (0.22) A	28 (2.17) A
Chironomus riparius			
Control	67 (2.38) B	10.7 (0.19) D	–
MR-01 (ref.)	79 (4.79) AB	12.1 (0.54) B	–
MR-02	76 (3.46) AB	11.8 (0.55) BC	–
MR-07	78 (2.22) AB	12.1 (0.14) B	–
MR-11	47 (12.84) C	8.1 (0.69) E	–
MR-17	82 (6.27) AB	13.8 (0.53) A	–
MR-19	75 (4.50) AB	10.7 (0.31) CD	–
MR-25	93 (3.11) A	11.8 (0.33) BCD	–
Control	81 (4.50) ABC	12.0 (0.33) C	–
CF-01	77 (7.19) ABC	16.0 (0.20) A	–
CF-02	64 (10.07) C	13.5 (0.54) B	–
CF-03	68 (5.12) BC	12.3 (0.43) C	–
CF-04	89 (1.71) A	13.3 (0.27) B	–
CF-05	78 (10.72) ABC	13.6 (0.15) B	–
CF-06 (ref.)	87 (2.38) AB	13.3 (0.17) B	–

Means (standard error of the mean in parentheses) sharing a common letter within a column at a site for a species are not significantly different ($p > 0.05$; $n = 4$).

[a]Starting body length of amphipods was 1.43 mm (0.06 SEM) for the Milltown Reservoir exposure and 1.31 mm (0.04 SEM) for the Clark Fork River exposure; midges were less than 24 h old at the start of the exposures.

in the 28-d whole-sediment toxicity test with *Hyalella azteca* was: length > sexual maturation > survival. Daphnids and trout were probably exposed to lower concentrations of metals in the water column than were amphipods and midges, which were in direct contact with the sediment [13]. The remaining discussion on the effects of metals associated with sediments will focus on *Hyalella azteca* responses, because this species was the most sensitive in the whole-sediment toxicity tests.

No-effect concentrations

Effects of sediment characteristics on toxicity were initially evaluated using regression analyses; however, analysis of sediment characteristics with toxicity was limited because one or two stations influenced the regression. Therefore, we chose to focus our evaluations of sediment toxicity on (a) no-effect concentrations (NECs) and (b) Sediment Quality Triad [1] approaches.

The sediment chemical and physical data listed in Tables 1, 2, and 6 were used to calculate each NEC. For example, for SEM Cu, significant reductions in body length were determined for each treatment relative to the control (e.g., Table 5). Treatments significantly different from the control were plotted vs. SEM Cu as circles, and treatments not significantly different from the control were plotted as triangles (Fig. 1). The NEC for SEM Cu is the treatment with the highest concentration of Cu in whole sediment that did not significantly reduce amphipod body length (e.g., 325 μg SEM Cu/g for sediment from station CF-02). Three sediment samples exceeded the NEC for SEM Cu: MR-19 (354 μg/g), MR-11 (607 μg/g), and CF-01 (6,971 μg/g).

Reported effect concentrations for sediment (e.g., ER-M [27] or water quality criteria [32]) are also plotted in Figure 1. For example, the ER-M for total Cu is 390 μg/g, similar to the NEC for SEM Cu of 325 μg/g for amphipod length (Fig. 1). Exceeding an NEC (or an ER-M) for a particular chemical does not mean the chemical caused the effect. It is the concentration of a chemical associated with a response. However, if a sediment sample is toxic and the concentration of the chemical of interest is below the NEC, then the toxicity cannot be attributed solely to that chemical. Table 7 lists NECs for *Hyalella azteca* body length and sexual maturation for (a) simultaneously extracted metals, (b) SEM metals normalized to AVS, (c) filtered pore-water metals, (d) pore-water ammonia and hydrogen sulfide, and (e) physical characteristics of the sediment samples. Samples exceeding each NEC and reported effect concentrations for NECs [27] are also listed in Table 7. Toxic units were calculated for (a) concentrations of pore-water metals normalized to water-only toxicity data [13] or (b) concentrations of SEM metals normalized to NECs [13]. A toxic unit is the concentration of metal in sediment or water divided by the threshold concentration in sediment or water. If the number is greater than one, a toxic effect would be predicted [33].

The NECs for (a) midge survival and length, (b) daphnid survival and reproduction, and (c) trout length and weight were greater than the highest concentration of chemicals in CF-01 sediment; hence, the NECs for these end points are not listed in Table 7. The NECs for survival of trout or amphipods were set most often by exposure to MR-11 sediment because CF-01 sediment was the only sample that reduced survival of amphipods or trout relative to the control. For this reason, the NECs for amphipod and trout survival are not cited in Table 7.

Reductions in amphipod length or maturation could not be attributed solely to concentrations of hydrogen sulfide measured in the pore-water samples, because concentrations were at or below the NEC in all of the samples. Pore water sampled from CF-02 sediment had the highest concentration of hydrogen sulfide, but amphipod length or maturation was not reduced with exposure to CF-02 sediment relative to the control (Table 7). Therefore, the NEC for hydrogen sulfide was ≥0.42 mg/L.

The un-ionized-ammonia NEC for amphipod length and maturation was 0.14 mg/L (Table 7). Only one sample (CF-01, 0.22 mg/L) exceeded the NEC for un-ionized ammonia measured in the pore-water samples. Hence, reduction in

Table 6. Dissolved metals measured in filtered pore-water samples

Station	Al	As	Cd	Cu	Cr	Fe	Mn	Ni	Pb	Zn
Milltown Reservoir										
Control	177.	<2.6	0.07	5.4	<0.4	0.16	0.2	6.1	0.4	<9.8
MR-01	12.0	15.7	<0.02	1.9	<0.4	3.47	17.5	5.1	0.3	<9.8
MR-02	<6.2	20.7	<0.02	3.7	0.4	<0.08	2.7	<1.8	<0.3	10.6
MR-07	<6.2	6.5	0.11	9.5	<0.4	<0.08	11.2	2.0	0.4	29.2
MR-11	16.5	25.0	3.78	89.9	<0.4	<0.08	3.1	2.2	0.9	187.
MR-17	<6.2	7.1	0.15	14.8	<0.4	<0.08	10.9	<1.8	<0.3	50.7
MR-19	8.6	168.	1.59	102.	<0.4	<0.08	7.3	2.3	0.9	183.
MR-25	9.7	10.1	0.32	15.1	<0.4	<0.08	4.4	1.9	<0.3	135.
Blank	<6.2	<2.6	<0.02	<1.4	<0.4	<0.08	<0.1	<1.8	<0.3	<9.8
Clark Fork River										
Control	186.	<1.1	0.08	4.68	0.67	0.24	<0.13	4.7	<0.21	3.9
CF-01	10.2	57.2	2.35	79.4	<0.46	3.49	77.4	20.3	<0.21	2,630.
CF-02	8.8	53.7	0.36	35.6	0.60	<0.04	12.7	4.1	1.22	166.
CF-03	2.7	72.2	0.26	16.4	<0.46	0.05	2.36	2.0	0.25	40.4
CF-04	<1.5	29.1	0.06	8.75	0.50	<0.04	11.6	4.5	0.29	28.0
CF-05	3.7	22.8	<0.05	8.67	0.69	0.04	0.50	3.9	<0.21	19.9
CF-06	39.4	2.9	<0.05	1.52	0.55	0.05	<0.13	2.4	0.39	2.0

Concentration units are μg/L except for Fe and Mn, which are mg/L.

amphipod length or maturation could be attributed solely to un-ionized ammonia in only the CF-01 sample. However, toxicity of ammonia to *Hyalella azteca* may not be pH dependent (G.T. Ankley, personal communication). Therefore, we also calculated an NEC for total-ammonia concentrations measured in the pore-water samples. The NEC for total ammonia was 6.32 mg/L for amphipod length and 9.88 mg/L for amphipod maturation (Table 7). Sediments from MR-01, MR-07, MR-17, and CF-01 exceeded the NEC for total ammonia in pore water. However, the toxicity of sediments from MR-02, MR-11, MR-19, and CF-03 to *Hyalella azteca* could not be attributed solely to ammonia or hydrogen sulfide because the concentrations were less than the NECs.

Effects on amphipod length and maturation could not be attributed solely to percent sand, silt, or clay in the whole-sediment samples. Sediment from CF-05 had the highest percent sand (82%), but amphipod length and maturation were not reduced with exposure to CF-05 sediment relative to the control (Table 7). Therefore, the NEC for percent sand was ≥82%. Similarly, the control sediment had the highest percent silt and clay relative to the other samples. Particle size did not affect the response of *Hyalella azteca* or *Chironomus riparius* exposures up to 28 d [13,14,34]. Hence, effects on amphipod length or maturation could not be attributed solely to particle size.

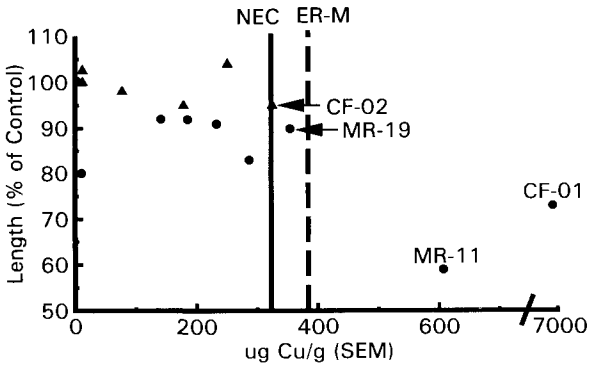

Fig. 1. Body length of *Hyalella azteca* (% of control) vs. SEM Cu (μg/g) for animals exposed to whole-sediment samples from Milltown Reservoir or the Clark Fork River. Circles represent lengths significantly less than their respective control; triangles represent lengths not significantly less than their respective control. NEC = no-effect concentration; ER-M = effect range-median (total Cu).

The total organic carbon (TOC) NEC for amphipod length or maturation was 6.67% (Table 7). The only sample that exceeded this NEC was CF-01, but this sample only exceeded the NEC by 0.23% TOC, which is less than the analytical variance associated with TOC (Table 1). Samples that exceeded the amphipod NECs for percent water in sediment were MR-07, MR-11, MR-17, and CF-01. Perhaps a sample with a larger volume of water would have a greater pool of dissolved contaminants available to the amphipods, or amphipod behavior may have been different with increased burrowing in sediments with a higher volume of water.

Concentrations of individual metals in sediments from up to five stations (MR-07, MR-11, MR-19, CF-01, and CF-03) exceeded metal NECs for amphipod length. Two stations (MR-11 and CF-01) exceeded metal NECs for amphipod maturation (Table 7). The amphipod length and maturation NECs for SEM Cr (4.45 μg/g) were well below the effect range-low (ER-L) of 80 μg/g for total Cr, and the NECs for SEM Ni (5.23 μg/g) were well below the ER-L of 30 μg/g for total Ni (Table 7 [27]). An ER-L is a concentration of a chemical in sediment above which adverse effects may begin or are predicted among sensitive species [27]. The NECs for Cr and Ni might have been higher had a broader range of sediment Cr or Ni been tested. Therefore, Cr and Ni may have been associated with an effect, but probably did not independently cause an effect.

Table 7. No-effect concentrations (NEC) for *Hyalella azteca* exposed to Milltown Reservoir or Clark Fork River sediments

Variable	NEC for length	NEC for maturation	Stations exceeding NEC for length	Stations exceeding NEC for maturation	Reported effect concentrations[a]
Simultaneously extracted metals (SEM; μg/g)					
As	23.8	24.8	MR-11, MR-19, CF-01, CF-03	MR-11, CF-01	33/85
Cd	3.87	3.87	MR-11, CF-01	MR-11, CF-01	5/9
Cu	325	354	MR-11, MR-19, CF-01	MR-11, CF-01	70/390
Cr	4.45	4.45	CF-01	CF-01	80/145
Ni	5.23	5.23	MR-11, CF-01	MR-11, CF-01	30/50
Pb	62.4	62.4	CF-01	CF-01	35/110
Zn	1,064	1,064	MR-11, CF-01	MR-11, CF-01	120/270
NEC-TU[b]	4.5	4.3	MR-11, CF-01	MR-11, CF-01	NA
NEC-TU As + Cu[c]	2.0	2.2	MR-11, MR-19, CF-01	MR-11, CF-01	NA
Sum SEM/AVS	1.54	11.8	MR-11, MR-19, CF-01, CF-03	MR-11, CF-01	1.0
Cu SEM/AVS	0.30	3.72	MR-07, MR-11, MR-19, CF-01, CF-03	MR-11, CF-01	1.0
Zn SEM/AVS	1.30	7.82	MR-11, MR-19, CF-01	MR-11, CF-01	1.0
Pore water (μg/L)					
H_2S (mg/L)	≥0.42	≥0.42	No stations	No stations	NA
Un-NH_3 (mg/L)[d]	0.14	0.14	CF-01	CF-01	NA
To-NH_3 (mg/L)[e]	6.32	9.88	MR-01, MR-07, MR-17, CF-01	MR-07, MR-17, CF-01	10.0[f]
As	53.7	168	MR-19, CF-01, CF-03	No stations	190
Cd	0.36	1.59	MR-11, MR-19, CF-01	MR-11, CF-01	1.1
Cu	35.6	102	MR-11, MR-19, CF-01	No stations	12
Ni	6.1	6.1	CF-01	CF-01	160
Zn	166	183	MR-11, MR-19, CF-01	MR-11, CF-01	110
Pore-water toxic units (normalized to water-quality criterion)					
As-TU	0.28	0.88	MR-11, CF-01, CF-03	No stations	NA
Cd-TU	0.33	1.45	MR-11, MR-19, CF-01	MR-11, CF-01	NA
Cu-TU	2.97	8.50	MR-11, MR-19, CF-01	No stations	NA
Ni-TU	0.04	0.04	CF-01	CF-01	NA
Zn-TU	1.51	1.66	MR-11, MR-19, CF-01	MR-11, CF-01	NA
Sum-TU	5.11	12.5	MR-11, MR-19, CF-01	MR-11, CF-01	NA
Pore-water toxic units (normalized to *Hyalella azteca* LC50[f])					
Cd-TU	0.13	0.57	MR-11, MR-19, CF-01	MR-11, CF-01	NA
Cu-TU	1.15	3.29	MR-11, MR-19, CF-01	No stations	NA
Ni-TU	0.008	0.008	CF-01	CF-01	NA
Zn-TU	2.26	2.49	MR-11, MR-19, CF-01	MR-11, CF-01	NA
Sum-TU	3.54	6.35	MR-11, MR-19, CF-01	MR-11, CF-01	NA
Physical characteristics					
TOC	6.67	6.67	CF-01	CF-01	NA
Sand (%)	≥82	≥82	No stations	No stations	NA
Silt (%)	≥63	≥63	No stations	No stations	NA
Clay (%)	≥31	≥31	No stations	No stations	NA
Water (%)	48	56	MR-07, MR-11, MR-17, CF-01	CF-01	NA

[a]Effects range low and Effects range median [24]; SEM/AVS [4]; pore-water metals: [33].
[b]Sum of SEM As, Cd, Cu, Pb, and Zn concentrations normalized to their respective NEC (e.g., NEC toxic units).
[c]Sum of SEM As and Cu concentrations normalized to their respective NEC (e.g., NEC toxic units for As and Cu combined).
[d]Un-ionized ammonia.
[e]Total ammonia.
[f]Approximate 10-d LC50 for *Hyalella azteca* (G. T. Ankley, EPA, Duluth, MN, personal communication).
NA = not applicable.

The SEM Zn NECs for amphipod length and maturation (1,064 μg/g) were well above the ER-M of 270 μg/g total Zn (Table 7). The NECs for SEM As, Cd, Cu, and Pb were similar to the ER-M or ER-L concentrations (Table 7). The toxicity of samples MR-11, MR-19, CF-01, or CF-03 may have been related to the NEC level being exceeded for at least one of these metals.

Concentrations of metals from filtered pore water were normalized to EPA water quality criteria [13,32] or to water-only LC50s for *Hyalella azteca* [13,30]. Concentrations of Cu or Zn in pore water were elevated compared with As, Cd, Pb, or Ni in samples prepared from MR-11, MR-19, CF-01, and CF-03 sediments. This indicates the toxicity of these whole-sediment samples may have been related to Cu or Zn. Moreover, stations MR-11, MR-19, and CF-01 exceed the Cu or Zn NECs for pore water.

Concentrations of filtered metals in pore water and SEM metals in sediment identified similar stations in excess of NECs (e.g., MR-11, MR-19, CF-01, CF-03; Table 7). This correspondence between sediment phases would be expected,

given that concentrations of individual metals in pore-water samples were correlated to concentrations of individual metals in whole-sediment samples [13]. Moreover, summing toxic units for pore-water chemistry (Table 7) or for whole-sediment chemistry [13] did not identify additional stations in excess of NECs. This correspondence would also be expected, given that concentrations of metals were correlated to each other in either pore-water or whole-sediment samples [13].

A lack of an effect of metals in samples with toxic units greater than 1.0 in pore water or in whole sediment [13] could be related to several factors including (a) pore-water isolation techniques [13], (b) metal complexation by dissolved organic carbon (DOC), (c) binding of metals by AVS, (d) differences in water hardness of pore water, (e) toxicity of metals was less than additive [35], or (f) *Hyalella azteca* are less sensitive to some metals [13].

In summary, sediment samples form MR-01, MR-07, MR-17, and CF-01 exceeded the amphipod NECs for total ammonia in pore water or percent water in the whole sediment. In addition, sediment from CF-01 exceeded all of the NECs listed in Table 7, except the NECs for particle size and hydrogen sulfide. Toxicity of sediments from MR-02, MR-11, MR-19, and CF-03 to *Hyalella azteca* could not be attributed solely to ammonia or hydrogen sulfide. The toxicity of MR-01 sediment may have been related to organic contaminants.

Concentrations of metals in sediments from up to five stations (MR-07, MR-11, MR-19, CF-01, CF-03) exceeded metal NECs for amphipod length. Two stations (MR-11, CF-01) exceeded metal NECs for amphipod maturation. The toxicity of MR-02 to amphipods could not be attributed solely to metals. Perhaps this station received input of an unmeasured organic contaminant because of its location near the confluence of the Blackfoot River.

Factors controlling metal bioavailability

Divalent metals in sediments with molar SEM/AVS ratios ≤ 1.0 would not be predicted to be bioavailable [5,35,36]. In the present study, the sum SEM/AVS NEC for amphipod length (1.54, Table 7) was exceeded in samples from stations MR-11 (24.3), MR-19 (11.8), CF-01 (960), and CF-03 (2.15). A sum SEM/AVS NEC ≥ 1.0 for other end points or species indicates insensitivity or low metal bioavailability.

The Cu SEM/AVS NEC for amphipod length was 0.30 (Table 7). Sediment samples exceeding this NEC were MR-07 (0.37), MR-11 (3.89), MR-19 (3.72), CF-01 (423), and CF-03 (0.88). A ratio less than 1.0 would be predicted to be nontoxic. Hence, Cu SEM/AVS ratios of 0.37 for MR-07 sediment and 0.88 for CF-03 sediment indicate metals or factors other than Cu may have contributed to toxicity. Similar stations exceeded the sum SEM/AVS and Cu SEM/AVS NECs. This correspondence would be expected, given that SEM Cu and Zn were highly correlated ($r^2 = 0.73$) and molar concentrations of Cu and Zn accounted for $\geq 95\%$ of the sum SEM for Cu, Zn, Cd, Hg, Pb, and Ni in river and reservoir sediments [9]. Perhaps the Cu SEM/AVS NEC of 0.3 could be used to evaluate historic data for potential problem areas in Milltown Reservoir and Clark Fork River [13].

The influence of AVS on bioavailability of Cd or Ni in sediments has been investigated by Di Toro et al. [5], Ankley et al. [35], and Carlson et al. [36] in 10-d toxicity or bioaccumulation tests. However, the influence of AVS on other divalent metals in sediment, including Cu, Zn, Pb, and Hg, has not been thoroughly evaluated. Ankley et al. [37] and Bennett and Cubbage [38] reported Cu was not toxic to *Hyalella azteca* in 10-d tests at SEM/AVS ratios up to 59. However, their samples had very high TOC (7 to 14%). In addition, sediment SEM/AVS ratios increased during their 10-d tests due to decreasing AVS concentrations (26-fold increase over 10 d) [38]. In contrast, AVS concentrations remained relatively constant in the 28-d exposures in the present study [13]. Carlson et al. [36] and Ankley et al. [35] also reported relatively constant AVS concentrations during 10-d exposures.

Perhaps Cu bioavailability in sediment is reduced by organic carbon in addition to AVS [37]. In the present study, sediment TOC was generally between 1 and 3%. The exceptions were CF-06 (6.67%) and CF-01 (6.90%). The SEM/AVS ratio for the CF-01 sample was 960 and would be expected to be extremely toxic. Although the CF-01 sample was one of the most toxic samples tested, survival of amphipods (48%) was not severely affected.

The toxicity of the metals in pore water may be modified by DOC (e.g., [39]). Concentrations of DOC in pore water were not measured in the present study. Complexation of metals by DOC might account for the lack of metal toxicity in the CF-02 pore-water sample to *Hyalella azteca* [13]. Future studies evaluating the bioavailability of metals in sediment should include measurements of DOC in pore water in addition to TOC and AVS in whole sediment.

Each sediment sample contained a complex mixture of metals. Additional information is needed to determine specific contaminants that may have caused the observed toxicity. The cause of sediment toxicity or the interactive effects of sediment toxicants can be determined by spiking sediment with individual chemicals or with mixtures of chemicals [40]. Once the cause of sediment toxicity has been identified, better decisions can be made regarding remediation options.

Data from sediment spiking tests should be compared with field data on chemical concentrations in natural sediments and observed biological effects. Furthermore, a range in sediment TOC, DOC, and AVS should also be evaluated. Toxicity identification evaluations (TIEs) could also be used to assess the cause of acute toxicity of sediment pore-water samples (e.g., [35]). For example, sodium thiosulfate or EDTA could be used to chelate toxic metals, and pH adjustments could be used to evaluate ammonia toxicity. However, the TIE approach may be inappropriate for evaluating pore-water samples from Milltown Reservoir and the Clark Fork River because of (a) changes in the toxicity of pore water with storage and (b) the lack of acute toxicity in pore-water samples.

Brumbaugh et al. [9] reported high variability in sediment chemistry within stations in both the river and reservoir. Molar ratios of SEM (Zn + Cu)/AVS in the upper 6 cm of individual cores sampled from stations CF-02 and MR-11 varied almost 100-fold. Variation within each 6-cm layer and throughout the microenvironment in which burrowing inver-

tebrates inhabit may be even greater Understanding this vari ability in sediment chemistry is particularly important for interpreting benthic community data [1] The average concentrations of Cu, Zn, and AVS in the upper 6 cm of core samples from these stations were in reasonable agreement with the corresponding composite samples used to conduct the toxicity tests This indicates homogenization of sediment required for toxicity testing did not cause large changes in average sediment chemistry However, the potential for tox icity to sediment-dwelling organisms within each station may be highly localized

Sediment samples from Milltown Reservoir and the Clark Fork River were not generally lethal to test organisms exposed in the laboratory However, sublethal effects on test organisms were associated with exposure to elevated concentrations of metals in sediments from the reservoir and river Canfield et al [1] reported that total abundance of benthic organisms/m^2 did not follow a consistent pattern relative to concentrations of metals in the sediment samples However, the number of Chironomidae genera was higher at stations that were toxic in laboratory tests and had higher concentrations of metals in sediment (CF-01, CF-03, MR-11, MR-19) Therefore, lab tests and benthic community evaluations both provide evidence of metal-induced degradation to aquatic communities in the reservoir and the river

Acknowledgement—We thank L R Farley, H Fisher, E K Henry, L B King, J K Young, and D C Zumwalt for technical support in conducting the laboratory exposures, M R Ellersieck and L S Sappington for assistance analyzing data, I E Greer and K Srigley Werner for culturing test organisms, J B Hunn for assistance on quality control and quality assurance, J M Besser, T J Canfield, P R Heine, and R L Knowlton for collection of sediment samples, J Arms, J A Lebo, J C Meadows, M J Walther, R H Weidmeyer, and J L Zajicek for chemical analyses of samples, and D C Chapman, G A Pascoe, P V Winger, and two anonymous reviewers for their helpful review comments on the manuscript This work was supported in part through interagency agreement DW14935406 01-0 Although the information in this report was funded in part by the U S Environmental Protection Agency, it may not necessarily reflect the views of the agency, no official endorsement should be inferred

REFERENCES

1 **Canfield, T.J., N.E. Kemble, W.G. Brumbaugh, F.J. Dwyer, C.G. Ingersoll** and **J.F Fairchild.** 1994 Use of benthic invertebrate community structure and the Sediment Quality Triad to evaluate metal contaminated sediment in the upper Clark Fork River, Montana *Environ Toxicol Chem* **13** 1999–2012

2 **Pascoe, G.A.** and **J.A. DalSoglio.** 1994 Planning and implementation of a comprehensive ecological risk assessment at the Milltown Reservoir–Clark Fork River Superfund Site, Montana *Environ Toxicol Chem* **13** 1943–1956

3 **Di Toro, D.M., C.S. Zarba, D.J Hansen, W.J. Berry, R.C. Swartz, C.E. Cowan, S.P. Pavlou, H E. Allen, N.A. Thomas** and **P.R. Paquin.** 1991 Technical basis for establishing sediment quality criteria for non ionic organic chemicals by using equilibrium partitioning *Environ Toxicol Chem* **10** 1541–1583

4 **Adams, W.J., R.A. Kimerle** and **R.G. Mosher.** 1985 An approach for assessing the environmental safety of chemicals sorbed to sediments In R D Cardwell, R Purdy and R C Bahner, eds , *Aquatic Toxicology and Hazard Evaluation Seventh Symposium* STP 854 American Society for Testing and Materials, Philadelphia, PA, pp 429–453

5 **Di Toro, D.M., J.H. Mahony, D.J. Hansen, K.J. Scott, M.B. Hicks, S.M. Mayr** and **M. Redmond.** 1990 Toxicity of cadmium in sediments The role of acid volatile sulfides *Environ Toxicol Chem* **9** 1487–1502

6 **Chao, T.T.** and **P.K. Theobald.** 1976 The significance of secondary iron and manganese oxides in geochemical exploration *Econ Geol* **71** 1560–1569

7 **Brook, E.J.** and **J.N. Moore** 1988 Particle size and chemical control of As, Cd, Cu, Fe, Mn, Ni, Pb, and Zn in bed sediment from the Clark Fork River, Montana, USA *Sci Total Environ* **76** 247–266

8 **Linder, G., R Hazelwood, D. Palawski, M. Bollman, D. Wilborn, J. Malloy, K. DuBois, S. Ott, G. Pascoe** and **J.A. DalSoglio.** 1994 Ecological assessment for wetlands at Milltown Reservoir, Missoula, Montana Characterization of emergent and upland habitats *Environ Toxicol Chem* **13** 1957–1970

9 **Brumbaugh, W G., C.G. Ingersoll, N.E. Kemble, T.W. May** and **J.L. Zajicek.** 1994 Chemical characterization of sediments and pore water from the upper Clark Fork River and Milltown Reservoir, Montana *Environ Toxicol Chem* **13** 1971–1983

10 **Ingersoll C.G., W.G. Brumbaugh, F.J. Dwyer** and **N.E. Kemble.** 1994 Bioaccumulation of metals by *Hyalella azteca* exposed to contaminated sediments from the upper Clark Fork River, Montana *Environ Toxicol Chem* **13** 2013–2020

11 **Farag, A.M., C.J. Boese, D.F. Woodward** and **H.L. Bergman.** 1994 Physiological changes and tissue metal accumulation in rainbow trout exposed to food-borne and waterborne metals *Environ Toxicol Chem* **13** 2021–2029

12 **Pascoe, G.A., R.J. Blanchet, G. Linder, D. Palawski, W.G. Brumbaugh, T.J. Canfield, N.E. Kemble, C.G. Ingersoll, A. Farag** and **J.A. DalSoglio.** 1994 Characterization of ecological risks at the Milltown Reservoir–Clark Fork River Sediments Superfund Site, Montana *Environ Toxicol Chem* **13** 2043–2058

13 **Kemble, N.E. et al.** 1993 Sediment toxicology In C G Ingersoll, W G Brumbaugh, A M Farag, T W La Point, and D F Woodward, eds , Effects of metal contaminated sediment, water, and diet on aquatic organisms, May 10, 1993 U S Environmental Protection Agency, Helena, MT, pp 2 1–2 100 Available from NTIS, Springfield, VA PB93-215952

14 **Ingersoll, C.G.** and **M.K. Nelson.** 1990 Testing sediment toxicity with *Hyalella azteca* (Amphipoda) and *Chironomus riparius* (Diptera) In W G Landis and W H van der Schalie, eds , *Aquatic Toxicology and Risk Assessment 13th Volume* STP 1096 American Society for Testing and Materials, Philadelphia, PA, pp 93–109

15 **Ingersoll, C.G., F.J. Dwyer** and **T.W. May.** 1990 Toxicity of inorganic and organic selenium to *Daphnia magna* (Cladocera) and *Chironomus riparius* (Diptera) *Environ Toxicol Chem* **9** 1171–1181

16 **Microbics Corporation** 1989 *A Microtox® Manual, How to Run Toxicity Test Using the Microtox Model 500* Carlsbad, CA

17 **Smock, L.A.** 1980 Relationships between body size and biomass of aquatic insects *Freshwater Biol* **10** 375–383

18 **Thurston, R.V., R.C. Russo** and **K. Emerson.** 1974 Aqueous ammonia equilibrium calculations Technical Report 74-1 Montana State University, Bozeman, MT

19 **Broderius, S.J.** and **L.L. Smith** 1977 Direct determination and calculation of aqueous hydrogen sulfide *Anal Chem* **49** 424–428

20 **Presser, T.S.** and **I. Barnes.** 1985 Dissolved constituents including selenium in waters in the vicinity of Kesterson National Wildlife Refuge and the west grassland, Fresno and Merced Counties, California Water Resour Invest Rep 85 4220 U S Geological Survey, Menlo Park, CA

21 **U.S. Environmental Protection Agency.** 1983 Methods for chemical analysis of water and wastes EPA 600/4-79-020 Environmental Monitoring and Support Laboratory Cincinnati, OH

22 **Moore, J.N., W.H. Ficklin** and **C. Johns** 1988 Partitioning of arsenic and metals in reducing sulfidic sediments *Environ Sci Technol* **22** 432 437

23 **Stephan, C.E.** 1977 Methods for calculating an LC50 In F L

Mayer and J L Hamelink, eds *Aquatic Toxicology and Hazard Evaluation* STP 634 American Society for Testing Materials, Philadelphia PA, pp 65 84

24 **Snedecor, G.W.** and **W.G. Cochran.** 1982 *Statistical Methods*, 7th ed The Iowa State University Press, Ames, IA

25 **SAS Institute.** 1989 *SAS® User's Guide Statistics, Version 5 Edition* Cary, NC

26 **Barrick, R., S. Becker, L. Brown, H. Beller** and **R. Pastorok.** 1988 Sediment quality values refinement 1988 update and evaluation of Puget Sound AET, Vol 1 PTI Environmental Services, Bellevue, WA

27 **Long, E.R.** and **L.G. Morgan.** 1991 The potential for biological effects of sediment sorbed contaminants tested in the National Status and Trends Program NOS OMA 52 Technical Memorandum National Oceanic and Atmospheric Administration, Seattle, WA

28 **American Society for Testing and Materials.** 1993 Standard guide for conducting sediment toxicity tests with freshwater invertebrates E1383 93a In *Annual Book of ASTM Standards*, Vol 11 04, Philadelphia, PA, pp 1173–1199

29 **Coyle, J.J., E.A. Crecelius** and **C.G. Ingersoll.** 1993 Elutriate toxicity tests *Daphnia magna* and Microtox In C G Ingersoll, D R Buckler, E A Crecelius and T W La Point, eds , Biological Assessment of Contaminated Great Lakes Sediment Final Report U S Environmental Protection Agency, Chicago, IL, pp 2-1–2 24

30 **Ankley, G.T., M.K. Schubauer-Berigan, J.R. Dierkes** and **M.T. Lukasewycz.** 1991 Sediment toxicity identification evaluation Phase I (characterization), phase II (identification), and phase III (confirmation) modifications of effluent procedures EPA 600/6 91/007 U S Environmental Protection Agency, Duluth, MN

31 **Nelson, M.K., J.J. Coyle, L.B. King, N.E. Kemble, E.A. Crecelius** and **I.F. Greer.** 1993 Whole sediment toxicity tests In C G Ingersoll, D R Buckler, E A Crecelius and T W La Point, eds , *Biological Assessment of Contaminated Great Lakes Sediment* Final Report U S Environmental Protection Agency, Chicago, IL, pp 4 1-4-44

32 **U.S. Environmental Protection Agency.** 1986 Quality criteria for water 1986 EPA 440/5 86 001 Washington, DC

33 **Rand, G.M.** and **S.R. Petrocelli.** 1985 *Fundamentals of Aquatic Toxicology* Hemisphere, Washington, DC

34 **Ankley, G.T., D.A. Benoit, J.C. Balogh, T.B. Reynoldson, K.E. Day** and **R.A. Hoke.** 1994 Evaluation of potential confounding factors in sediment toxicity tests with three freshwater benthic invertebrates *Environ Toxicol Chem* **13** 621–626

35 **Ankley, G.T., G.I. Phipps, E.N. Leonard, D.A. Benoit, V.R. Mattson, P.A. Kosian, A.M. Cotter, J.R. Dierkes, D.J. Hansen** and **J.D. Mahony.** 1991 Acid volatile sulfide as a factor mediating cadmium and nickel bioavailability in contaminated sediments *Environ Toxicol Chem* **10** 1299–1307

36 **Carlson, A.R., G.L. Phipps, V.R. Mattson, P.A. Kosian** and **A.M. Cotter.** 1991 The role of acid volatile sulfide in determining cadmium bioavailability and toxicity in freshwater sediment *Environ Toxicol Chem* **10** 1309–1319

37 **Ankley, G.T., V.R. Mattson, E.N. Leonard, C.W. West** and **J.L. Bennett.** 1993 Predicting the acute toxicity of copper in freshwater sediments Evaluation of the role of acid volatile sulfide *Environ Toxicol Chem* **12** 315 320

38 **Bennett, J.** and **J. Cubbage.** 1992 Copper in sediments from Steilacoom Lake, Pierce County, Washington Report WA 12 9080 Washington State Department of Ecology, Olympia, WA

39 **Winner, R.W.** and **J.D. Gauss.** 1986 Relationship between chronic toxicity and bioaccumulation of copper, cadmium and zinc as affected by water hardness and humic acid *Aquat Toxicol* **8** 149–161

40 **Lamberson, J.O.** and **R.C. Swartz.** 1992 Spiked-sediment toxicity test approach In M Kravitz, ed , *Sediment Methods Classification Compendium* EPA 823 R-92 006, Washington, DC, pp 4-1–4 10