EFFECTS OF CADMIUM-SPIKED SEDIMENT ON CADMIUM ACCUMULATION AND BIOTURBATION BY NYMPHS OF THE BURROWING MAYFLY *HEXAGENIA BILINEATA*

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Abstract. We assessed accumulation of cadmium (Cd) and bioturbation by nymphs of the burrowing mayfly *Hexagenia bilineata* as indicators of exposure to Cd-spiked sediment in a 21-d test. Surficial sediments (top 5 cm) from Pool 7 of the Upper Mississippi River were spiked with Cd to concentrations of 3, 7, and 15 μg Cd g^{-1} dry weight. The experimental design was completely randomized, with three Cd-spiked sediment treatments plus an unspiked sediment control (1 μg Cd g^{-1} dry weight), and 10 nymphs in each of six replicates per treatment. Nymphs accumulated Cd during the 21-d exposure; mean concentrations varied from 0.22 to 6.24 μg g^{-1} dry weight, and tissue concentrations were correlated with Cd concentration in unfiltered test water (r = 0.93, P <0.01) and test sediment (r = 0.93, P <0.01). The effect of Cd on bioturbation by nymphs, as indicated by turbidity, differed significantly among treatments (P = 0.045) and over time within treatments (P = 0.01). Turbidity progressively decreased as Cd concentration in the sediment increased, up to 7 μg g^{-1}; however, turbidity in the 15 μg g^{-1} treatment (our greatest exposure concentration) did not differ significantly from the control. Concentrations of Cd in unfiltered, overlying test water increased significantly within treatments during the test, indicating that nymphs mobilized sediment-associated Cd into the overlying water, presumably through burrowing and respiratory activities.

Key words: accumulation, bioturbation, cadmium, *Hexagenia*, mayfly, spiked sediment

1. Introduction

Burrowing mayflies spend the majority of their life cycle in fine-grained sediment (Fremling and Mauck, 1980), thereby contributing to bioturbation (Malueg *et al.*, 1983; Odin *et al.*, 1995). They are also a significant benthic component in the food web of many aquatic ecosystems (Fremling, 1960) and may contribute to the trophic transfer of sediment-associated contaminants (Finley, 1985). Consequently, mayflies have been used as test organisms to evaluate sediment toxicity (Malueg *et al.*, 1983, 1984; Nebeker *et al.*, 1984; Giesy *et al.*, 1990; Bedard *et al.*,

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1992; Krantzberg and Boyd, 1992) and as sentinels of environmental contamination (Dukerschein *et al.*, 1992; Beauvais *et al.*, 1995).

Growth, gill beat frequency, swimming, crawling, grooming, feeding, and molting frequency have been examined as sublethal indicators of contaminant exposure in mayflies (Henry *et al.*, 1986; Diamond *et al.*, 1992). However, these measures require extensive handling of the organisms or are relatively insensitive, making them unsuitable for short-term exposures.

Many benthic invertebrates cause bioturbation, including the resuspension of sediment into the water column, through their burrowing, respiratory, feeding, and locomotor activities. Bioturbation is an important factor that influences physical and chemical (McCall and Fisher, 1980; Krantzberg, 1985; Matisoff *et al.*, 1985; Davis, 1993) processes at the sediment-water interface, including the availability and partitioning of sediment-associated metals (Krantzberg and Stokes, 1985; Thomann *et al.*, 1993). The mobility of sediment-associated cadmium (Cd), for example, may be increased during resuspension of sediments by a shift from reducing to oxidizing conditions and by altered pH (Khalid *et al.*, 1981; Förstner, 1987; Peterson *et al.*, 1996).

Fine-grained sediments of many lakes and rivers are anthropogenically enriched with Cd (Mathis and Cummings, 1973; Förstner, 1980; Bailey and Rada, 1984; Malueg *et al.*, 1984). *Hexagenia* nymphs mainly inhabit fine-grained sediment and can accumulate significant quantities of Cd after exposure to contaminated sediment (Burrows and Whitton, 1983; Suzuki *et al.*, 1988; Hare *et al.*, 1991a, 1991b; Odin *et al.*, 1995). In addition, both emergent adults (Dukerschein *et al.*, 1992) and nymphs (Beauvais *et al.*, 1995) of *H. bilineata* inhabiting contaminated sediments of the Upper Mississippi River have elevated concentrations of Cd.

Our primary objectives were to assess the sensitivity of accumulation of Cd, and bioturbation (turbidity generation) by nymphs of the burrowing mayfly *H. bilineata* as indicators of exposure to Cd-spiked sediment in laboratory microcosms for 21 d. Our secondary objective was to assess the mobilization of sediment-associated Cd into the water column by mayfly-mediated bioturbation. We hypothesized that (1) mayflies would accumulate Cd in response to elevated Cd concentrations in the sediment, (2) bioturbation by mayflies would decrease with increasing Cd concentration in sediment due to effects on burrowing and respiratory activities, and (3) bioturbation by mayflies would increase the concentration of sediment-associated Cd in the water column over the duration of the study. If bioturbation by mayflies proved to be a relatively sensitive measure in short-term laboratory exposures, it may provide an additional sublethal endpoint for assessing the toxicity of contaminated sediments on benthic invertebrates.

2. Materials and Methods

2.1. SEDIMENT COLLECTION

Sediments were obtained from Pool 7 of the Upper Mississippi River (Lake Onalaska, river mile 704.5). Surficial sediment (top 5 cm) was collected with a stainless steel van Veen dredge, placed into 3.8-L Nalgene® containers, and held on ice in the dark until taken to the laboratory. In the laboratory, sediment was passed through a 1.0-mm Nalgene® sieve to remove coarse particulate matter and large indigenous biota (Day *et al.*, 1995). Sediment was then homogenized, placed into four tared 3.8-L Nalgene® containers (one per treatment), and stored at 4 °C for 48 h. Three subsamples of homogenized sediment (each 20 to 25 g wet weight) were taken for analysis of wet to dry weight ratio and determination of volatile matter content.

2.2. SEDIMENT SPIKING

Sediment was spiked with Cd (added as $CdCl_2$; Baker Analyzed® , J.T. Baker Chemical Co.) to achieve target concentrations of 3, 8, and 16 μg g^{-1} dry weight. These concentrations were chosen because they are within the range found in contaminated sediments from many lakes and rivers (Förstner, 1980). The Cd concentration in our test sediments before spiking, was 1.0 μg Cd g^{-1} dry weight. Pore water (interstitial water) from each container of sediment was decanted and used as diluent to make the Cd stock solution for spiking a particular treatment. The sediment was spiked by dissolving the appropriate mass of $CdCl_2$ (calculation based on the wet weight to dry weight ratio of 3.553 for the sediment) in the pore water. The Cd solution was then placed back into the respective sediment treatment so the water content of the test sediment was not altered. The sediment-Cd mixture was then homogenized with a stainless steel blender (Waring Products, Model CB6, New Hartford, CT, U.S.A.) for 15 min at low speed (10 500 rpm) by alternating on-off intervals of 5 min. The homogenization procedure was conducted in a standard refrigerator (4 °C) to minimize heating of the sediment during mixing (ASTM, 1993).

The sediment (∼3.8 L for each treatment) was stored at 4 °C for 14 d to facilitate equilibrium between the Cd, sediment, and the pore water (ASTM, 1993). Samples (100 g wet weight) of sediment from the unspiked control treatment were taken before and after homogenization to assess the effects of homogenization on sediment particle size. In addition, a sample (minimum 5 g wet weight) of spiked sediment from each treatment was taken and stored frozen (–20 °C) in a Ziploc® bag until analysis for total Cd.

2.3. EXPOSURE SYSTEM

Nymphs of the mayfly *H. bilineata* were obtained from Pool 8 of the Upper Mississippi River (river mile 693.5), which contained a high density (476 m^{-2}) of nymphs. Mayflies were present at the sediment collection site in Pool 7, however, densities were lesser (213 m^{-2}) than at Pool 8. Therefore, to obtain a suitable number of organisms in the most efficient manner, nymphs were collected at the more easily accessible site in Pool 8. Nymphs were obtained with a standard ponar grab and were retained on a 1.0-mm mesh sieve that was washed with river water. Nymphs were retrieved from the sieve and measured to the nearest 1.0 mm from the tip of frontal process to the base of the caudal filaments. Only organisms within the 10–20 mm length interval were retained for testing.

The experimental design for the 21-d test was completely randomized, with four treatments, including three Cd-spiked sediment treatments and an unspiked sediment control, with six replicates per treatment. The experimental unit was a 4-L Pyrex® beaker that contained 750 g wet weight of Cd-spiked sediment (3±0.5 cm deep) and 3 L of overlying well water to obtain a water:sediment ratio of 4:1 (v/v) (Nebeker *et al.*, 1984).

Well water was slowly added to each beaker through Tygon® tubing connected to a 3.78-L Nalgene® carboy to minimize turbidity. Each beaker was then covered with a watch glass (20-cm diam.) to minimize evaporation and placed into a water bath held at 22±1 °C (ASTM, 1993). Water within the water bath was circulated with a thermostatically controlled, liquid circulation pump (Remcor Products Co., Model CFF-501, Franklin Park, IL, U.S.A.). To ensure low turbidity (≤5 NTU) in each beaker before the test, the water and spiked-sediment were allowed to settle for 4 d before test organisms were introduced. Dissolved oxygen concentrations in each beaker were maintained at ≥60% of saturation (ASTM, 1993) by aerating through 0.32-cm i.d. aquarium tubing placed ∼2.5 cm below the air-water interface. The photoperiod was 16 h light and 8 h dark.

On day 0, 10 nymphs were introduced into each beaker beneath the air-water interface. Any nymphs that touched dry surfaces, were dropped, or were injured during handling were discarded (ASTM, 1993). Nymphs were not fed during the 21-d test (Bedard *et al.*, 1992). A subsample of 25 nymphs was taken to characterize the initial size of test organisms. Each nymph was weighed to the nearest 0.1 g, measured to the nearest 1.0 mm, and analyzed for dry weight by drying at 105 °C for 24 h to estimate the wet to dry weight ratio.

Turbidity (Hach Ratio/XR Model 43900 turbidimeter), pH (Beckman Model Φ11 meter), and dissolved oxygen and temperature (Yellow Springs Instrument Model 58 oxygen meter) were measured daily in each beaker for 4 d before introduction of the nymphs and during the test (n = 600 measurements for each variable). Hardness, alkalinity, and conductivity of the test water were measured in each beaker on days 1 and 20 (n = 48 measurements for each variable). Means and standard deviations (in parentheses) for physicochemical characteristics of water

in all beakers during the test were as follows: dissolved oxygen 7.4 mg L^{-1} (0.3), temperature 22.2 °C (0.2), pH 8.21 (0.09), alkalinity 142 mg L^{-1} as $CaCO_3$ (16), hardness 186 mg L^{-1} as $CaCO_3$ (12), and conductivity 378 μS cm^{-1} (20).

2.4. SAMPLE ANALYSES

2.4.1. *Test Water*

Turbidity was measured as an indicator of exposure of mayfly nymphs to Cd during the 21-d test. To ensure a representative turbidity sample from each beaker, a 50-mL composite water sample (five 10-mL subsamples) was withdrawn with a sampling device placed over the beaker. The device consisted of five 10-gauge stainless steel needles (15-cm in length) mounted to a circular piece of plexiglass (18.5-cm diam.), which enabled the collection of water at five fixed points, each 7 cm above the sediment-water interface. The composite water sample was homogenized, and a 25-mL aliquot was removed for analysis of turbidity. Twenty percent of the daily composite samples for turbidity were analyzed in duplicate. The volume of water (50 mL) removed from each beaker in the daily turbidity sample was replaced with fresh well water held at 22±1 °C.

Total recoverable Cd was determined on samples of unfiltered test water collected weekly (days 2, 9, and 16; n = 72 samples) and acidified to a pH <2 (16 N HNO_3 InstraAnalyzed® , J.T. Baker Chemical Co.). The samples were digested with 16 N HNO_3 (InstraAnalyzed®) in aluminum heating blocks (Cope *et al.*, 1994). Total recoverable Cd was determined in filtered (0.45-μm Nucleopore® polycarbonate membrane, 47-mm diam.) digestates with graphite-furnace atomic absorption spectrophotometry (Perkin Elmer Model 4100 equipped with a deuterium lamp for background correction).

2.4.2. *Sediment*

At the end of the test, a sample of sediment from each beaker was collected by passing the test sediments through a 1.0-mm Nalgene® sieve (without addition of water) into a Ziploc® bag and stored at –20 °C. Each sample was dried at 30 °C for 48 h, ground to a fine powder with a mortar and pestle, and stored in a Ziploc® bag until analysis.

Total recoverable Cd was determined in samples of sediment digested with an acid solution (InstraAnalyzed®) of 16 N HNO_3, 18 N HCl, and deionized water in aluminum heating blocks (Beauvais *et al.*, 1995). Digestates were filtered through 0.45-μm Nucleopore® polycarbonate membranes, and Cd was quantified by flame atomic absorption spectrophotometry (Instrumentation Laboratory Model 551).

Subsamples of sediment, taken from the control treatment before and after the homogenization procedure, were analyzed for textural composition with the sieve-pipet method (Guy, 1969; Plumb, 1981) to determine the content of sand (>62 μm), silt (4–62 μm), and clay (<4 μm). This procedure allowed assessment of potential changes in textural composition of sediment due to homogenization.

Dry weight and volatile-matter content of test sediment (control treatment) were determined by analysis of three 25-g subsamples of sediment (APHA *et al.*, 1992).

2.4.3. *Mayfly Nymphs*

On day 21 of the test, nymphs were recovered from each beaker by passing the test sediments through a 1.0-mm Nalgene® sieve. All nymphs retained on the sieve were rinsed with deionized water, counted, removed from the sieve, placed into Ziploc® bags, and held at –20 °C for 15 min to reduce metabolic activity to facilitate length measurement. Because we were interested in the bioavailability and potential trophic transfer of Cd from mayflies to predators in our test, we did not eliminate the sediment from the digestive tract of nymphs prior to Cd analysis (Hare *et al.*, 1989). Consequently, we refer to accumulation of Cd by mayfly nymphs rather than to bioaccumulation.

One composite sample from each replicate, each containing 5 nymphs, was analyzed for whole-body Cd. Individual nymphs in each composite sample were measured. Composite samples of nymphs were lyophilized in Ziploc® bags at –30 °C, ground into a fine homogenate, and digested with 16 N HNO_3 (InstraAnalyzed®) in aluminum heating blocks (Beauvais *et al.*, 1995; Dukerschein *et al.*, 1992). Unfiltered digestates were analyzed with graphite-furnace atomic absorption spectrophotometry (Perkin Elmer Model 4100).

2.5. QUALITY ASSURANCE

The accuracy of metal determinations for each batch of samples was determined by analyses of standard reference materials, spiked samples, replicate samples, procedural blanks, and calibration standards. All chemicals used in digestions and metal analyses were certified for trace metal analysis. All containers and non-metallic materials used in the test were acid-washed with 10% HNO_3 for 24 h and rinsed in deionized water before use.

Weekly analyses of U.S. Environmental Protection Agency (USEPA) quality control samples for turbidity (n = 27) yielded mean values within the certified range. Method precision, estimated from replicate analyses (n = 27) of quality control samples analyzed with weekly turbidity samples, ranged from 0.3 to 0.8% (relative standard deviation, RSD). Analysis of USEPA quality control samples for minerals and pH, done on days 1 and 20, yielded mean values for hardness, alkalinity, and pH within the certified ranges (n = 12).

During determinations of Cd in samples of unfiltered, overlying test water, we analyzed U.S. National Institute of Standards and Technology (NIST) Buffalo River sediment (SRM 2704) with each batch of samples. All 10 analyses of Buffalo River sediment yielded concentrations within the certified range. Mean recovery of Cd in overlying test water from three samples spiked after digestion was 92% (range 88 to 97%). Percent difference from analyses of Cd in five duplicate samples of overlying test water averaged 1.4% (range 0.1 to 4.8%).

Two certified standard reference materials were analyzed for Cd with each batch of sediment samples: NIST Buffalo River sediment and National Research Council of Canada (NRCC) PACS-1 sediment. Concentrations of Cd for each reference sediment sample analyzed (n = 6) were within the certified range. Recovery of Cd from seven triplicate samples of sediment spiked before digestion averaged 100% (range 86 to 110%). Method precision (RSD), estimated from analyses of seven triplicate samples of sediment averaged 2.9% (range 0.8 to 8.0%).

We analyzed NRCC dogfish muscle (DORM-1) with each batch of samples of whole mayfly nymphs. Concentrations of Cd in NRCC dogfish muscle (n = 3) were within the certified range. The percent difference from analyses of Cd in duplicate samples of mayfly tissue averaged 4.8% (range 1.6 to 8.0%).

2.6. Statistical analyses

Statistical analyses were performed with PC-SAS (SAS Institute Inc., 1987). All variables were examined for normality with the Shapiro Wilk's test and for homogeneity of variances with Hartley's F_{max} test (Sokal and Rohlf, 1981). The data for Cd concentration in nymphs and Cd in overlying test water had heterogenous variances and, therefore, were logarithmically transformed ($\log_{10}+1$) before analyses. Variation of treatment means for turbidity was evaluated with a one-factor, repeated measures analysis of variance (ANOVA). Repeated measures ANOVA allows the use of data from several dates to compare treatment effects without assuming that sampling dates are independent (Winer, 1971; Diggle, 1988). The main effect of the statistical tests was Cd concentration; the subplot contained the effects of time and interactions of Cd concentration and time on turbidity generated by mayfly nymphs. Variation in Cd concentration in whole nymphs and turbidity at the termination of the test (day 21) was evaluated with one-way ANOVA. Tukey's multiple comparison test was used to identify significant differences among treatment means. Pearson correlation coefficients between response variables and transformed Cd concentrations were calculated for nymphs and overlying test water. The interaction of nymph length, wet weight, and mortality with turbidity at the termination of the test was evaluated with the analysis of covariance (ANCOVA). A Type I error (α) of 0.05 was used to judge statistical significance.

3. Results

Mean measured concentrations of Cd in samples of sediment taken from the four treatments before and after the test were 1.0, 3.0, 7.0, and 15.0 μg g^{-1} dry weight, closely approximating our target concentrations of 1, 3, 8, and 16 μg g^{-1} dry weight (Table I). The homogenization procedure did not alter the textural composition of sediment samples. The percentages of sand, silt, and clay in samples of sediment from the control treatment were 3.4, 53, and 43%, respectively, be-

TABLE I

Mean Cd concentrations (± SD) in sediment, unfiltered test water, and mayfly nymphs, and mean turbidity during the 21-d test exposing nymphs of the mayfly *Hexagenia* to Cd-spiked sediment

Cd in sediment (μg g^{-1} dry wt)		Cd in unfiltered water (μg L^{-1})[b]				Mayfly nymphs		
Target	Measured[a]	Day 2	Day 9	Day 16	(NTU)[cd]	Turbidity (mm)[c]	SL (g)[c]	Wet wt Cd concn. (μg g^{-1} dry wt)[cd]
Unspiked control	1 ±0.1	0.06 ±0.01	0.14 ±0.05	0.17 ±0.08	236 a ±84	22 ±2	0.11 ±0.03	0.22 a ±0.04
3	3 ±0.1	0.20 ±0.04	0.47 ±0.13	0.57 ±0.14	177 b ±58	22 ±1	0.10 ±0.01	1.19 b ±0.22
8	7 ±0.1	0.58 ±0.25	0.99 ±0.38	1.18 ±0.41	124 c ±55	22 ±1	0.09 ±0.01	3.37 c ±0.47
16	15 ±0.6	1.78 ±0.37	2.55 ±0.52	3.19 ±0.58	212 ab ±49	23 ±1	0.11 ±0.01	6.24 d ±1.10

[a] Sediment samples taken on day 0 (n = 3 samples per treatment) and day 21 (n = 6 samples per treatment).

[b] Twenty-four unfiltered, overlying test water samples analyzed each week.

[c] Mean turbidity (with length as a covariate), mean standard length (SL), wet weight, and concentrations of Cd in nymphs on day 21.

[d] Means with a different letter were judged to be significantly different (α = 0.05) with Tukey's *hsd* test.

fore homogenization and were 3.3, 53, and 44%, respectively, after homogenization. Volatile matter content of test sediment (control treatment only), analyzed on samples of sediment taken before homogenization, was 8.4% (SD 0.1%).

Mayfly nymphs accumulated Cd during the 21-d exposure to Cd-spiked sediment. On day 21, mean whole-body concentrations of Cd in nymphs ranged from 0.22 μg g^{-1} dry weight in the control to 6.24 μg g^{-1} dry weight in the 15 μg g^{-1} treatment, a 27-fold variation ($P < 0.01$, Table I). In contrast, concentrations of Cd in sediment treatments varied only 14 fold (1 to 15 μg g^{-1} dry weight, Table I). Concentrations of Cd in nymphs were correlated with Cd concentration in unfiltered test water (r = 0.93, $P < 0.01$) and test sediment (r = 0.93, $P < 0.01$). Overall, mortality of nymphs was minimal (mean 7%) during the test and was unrelated to Cd exposure (ANOVA, $P = 0.22$).

The effect of Cd on bioturbation (turbidity generation) of sediment by nymphs differed among treatments (repeated measures ANOVA, $P = 0.045$), over time within treatments ($P = 0.01$, Figure 1), and was concomitant with an increase in the concentration of Cd in overlying, unfiltered test water (Figure 2). Nymphs exposed to Cd-spiked sediment (except the 15 μg g^{-1} treatment during days 0 to 8) generated less turbidity than nymphs in the control treatment (Figure 3).

Length of nymphs had a significant effect on turbidity. On day 21, the concentration of Cd in test sediment explained only 35% of the variation in turbidity (one-way ANOVA, $P < 0.05$). However, when length was included as a covariate with concentration of Cd in sediment, 85% of the variation in turbidity was explained (ANCOVA, $P < 0.01$). With length as a covariate, turbidity on day 21 was significantly different among all treatments, except between the control and the 15 μg Cd g^{-1} treatments and between the 3 and 15 μg Cd g^{-1} treatments (Tukey's *hsd*). Nymphs grew significantly in all treatments during the test. Length of nymphs ranged from 10 to 20 mm on day 0 and from 15 to 28 mm on day 21. However, at day 21 length (ANOVA, $P = 0.65$) and wet weight (ANOVA, $P = 0.47$) of nymphs did not differ among treatments (Table I).

Mean concentrations of Cd in unfiltered test water increased weekly within each treatment and differed among treatments (repeated measures ANOVA, $P < 0.01$, Table I). As expected, the concentration of Cd in unfiltered test water was strongly correlated with that in test sediment (r = 0.97, $P < 0.01$).

4. Discussion

4.1. Accumulation of cadmium

The accumulation of Cd in mayfly nymphs was linearly related (r = 0.93, $P < 0.01$) to the concentration of Cd in sediment. Odin *et al.* (1995), who exposed nymphs of *Hexagenia rigida* to Cd-spiked sediment (range 0.6 to 10.0 μg g^{-1}) for 15 d, likewise found that the accumulation of Cd in *H. rigida* was correlated (r = 0.89)

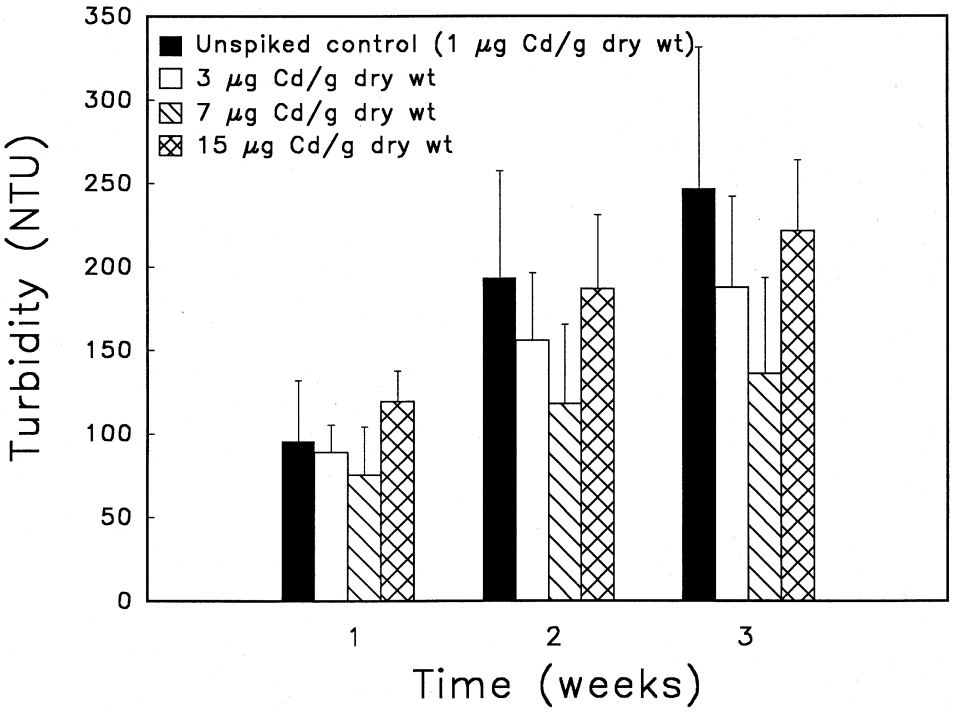

Figure 1. Weekly mean turbidity (with SD) during the 21-d test with nymphs of the mayfly *Hexagenia* exposed to Cd-spiked sediment.

with the concentration of Cd in spiked sediment. The concentration of Cd in our sediment treatments varied 14-fold, yet the mean whole-body concentrations of Cd in nymphs varied 27-fold. Beauvais *et al.* (1995), who quantified Cd in sediments and nymphs of *H. bilineata* from the Upper Mississippi River, similarly found that Cd concentrations varied less than three-fold (range 1.19 to 3.23 μg g^{-1} dry weight) in surficial sediments but varied almost 20-fold in *Hexagenia* (range 0.13 to 2.35 μg g^{-1} dry weight). The minimum and maximum concentrations of Cd in surficial sediments from the Upper Mississippi River in the study of Beauvais *et al.* (1995) were similar to the two lowest sediment concentrations (1.0 and 3.0 μg Cd g^{-1} dry weight) in our study. Nymphs exposed to 1 and 3 μg g^{-1} in sediment in our test accumulated concentrations of Cd (0.22 to 1.19 μg g^{-1} dry weight) similar to those (0.12 to 2.6 μg g^{-1} dry weight) measured by Beauvais *et al.* (1995), indicating that our 21-d laboratory test on Cd accumulation yielded environmentally realistic results.

Hexagenia nymphs are detritivores that spend the majority of their life cycle in the sediment (Fremling, 1960); therefore, accumulation of sediment-associated Cd through the diet is a significant route of uptake by these organisms. For example, laboratory studies with mayfly nymphs and sediments spiked with radiolabelled Cd have shown that the major site of Cd accumulation was in the gut of nymphs,

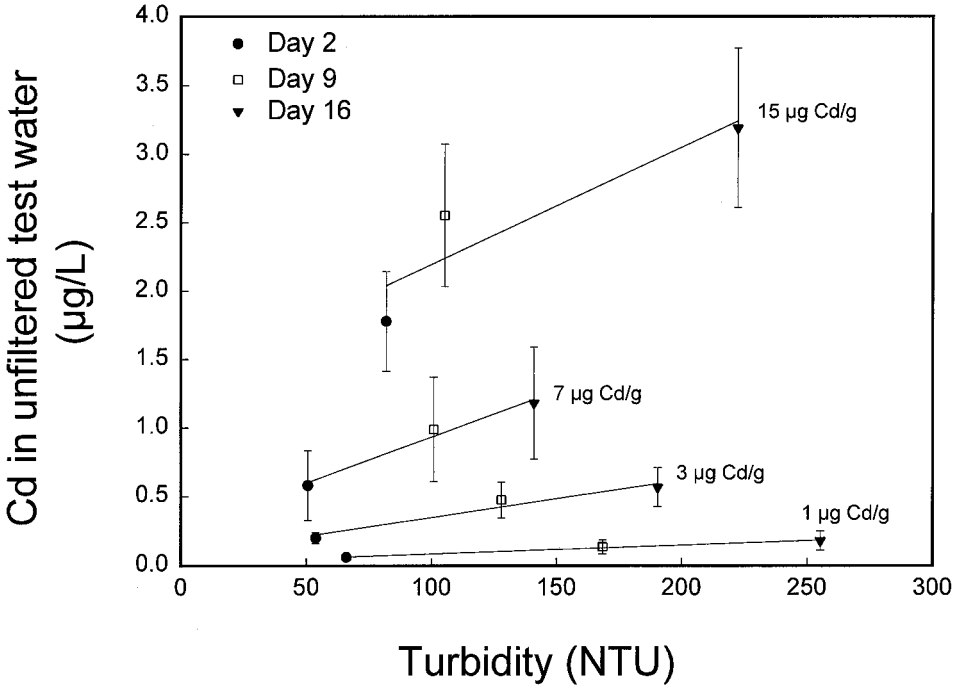

Figure 2. Relation between mean concentration of Cd (with SD) and turbidity in unfiltered test water during the 21-d test exposing nymphs of the mayfly *Hexagenia* to Cd-spiked sediment.

suggesting the primary route of exposure was by ingestion of sediment (Hare *et al.*, 1991a). In our test, we did not eliminate the sediment from the digestive tract of nymphs prior to Cd analysis because we were interested in the bioavailability and potential trophic transfer of Cd from mayflies to predators (Hare *et al.*, 1989). Nymphs accumulated significant quantities of Cd during the 21-d exposure, indicating that the uptake of Cd by nymphs from the sediment may represent a significant pathway for the trophic transfer of Cd.

4.2. BIOTURBATION BY HEXAGENIA

Burrowing and respiratory activities by nymphs significantly increased turbidity during the 21-d test. Malueg *et al.* (1983), who exposed nymphs of *H. limbata* to various contaminated sediments for 5 d, likewise found that turbidity increased due to burrowing and respiratory activity of nymphs. Moreover, they found that turbidity of the reference control sediment was greater than that of test sediments and suggested that the differences were likely due to reduction of respiratory activity or to mortality of mayflies.

Mayfly nymphs in our study generated turbidity in all treatments. Turbidity progressively decreased as Cd concentration in the sediment increased up to 7 μg g^{-1}, but turbidity in the 15-μg g^{-1} treatment (our greatest exposure concentration)

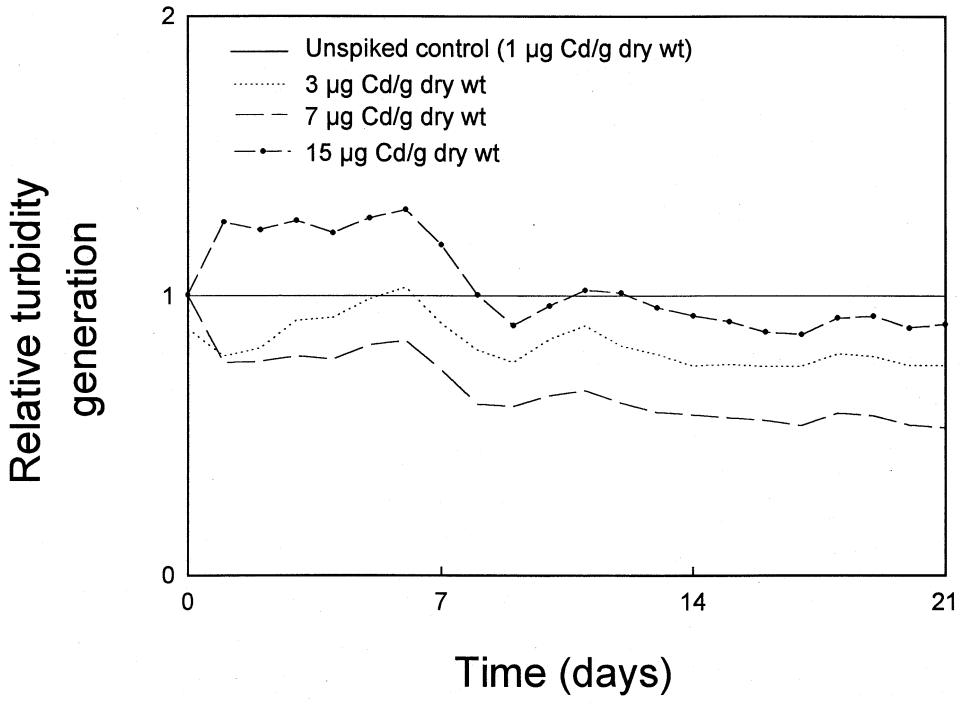

Figure 3. Relative turbidity generation by nymphs of the mayfly *Hexagenia* exposed to Cd-spiked sediment for 21 d (relative turbidity generation is the ratio of turbidity in a given Cd-spiked sediment treatment to the turbidity in the control treatment).

did not differ significantly from the control (Table 1, Figure 3), which was an unexpected result. In a similar study, Odin *et al.* (1995) exposed nymphs of *H. rigida* to Cd-spiked sediment (range 0.6 to 10.0 μg g^{-1}) for 15 d. In contrast to our results, they observed decreased turbidity throughout the entire range of exposure; however, their greatest exposure concentration (10 μg g^{-1}) was lesser than ours (15 μg g^{-1}). They suggested that the highest concentrations of Cd in their test sediment (up to 10 μg g^{-1}) may have inhibited the locomotor activity of *H. rigida*. In our test, sediment-associated Cd seemed to have a U-shaped effect on turbidity, i.e., turbidity decreased progressively to a Cd concentration of 7 μg g^{-1} in the sediment, above which, turbidity increased. The reason for the high turbidity in the 15 μg g^{-1} treatment relative to the other Cd treatments in our test is unknown.

Although the length of nymphs did not differ significantly among treatments at the beginning or end of our test, nymphal length influenced turbidity. The resolution of the effects of sediment-associated Cd on turbidity was increased by 50% when length of nymphs was added as a covariate in statistical analysis (ANCOVA). This outcome may have implications for using turbidity as an indicator of Cd exposure with burrowing mayflies. We started the test with nymphs ranging from 10 to 20 mm in length. Perhaps, studies beginning with a narrower size-range

of nymphs would allow better resolution of sublethal effects among treatments. Growth (length and wet weight) and mortality of *Hexagenia* nymphs did not seem to be sensitive indicators of Cd exposure in our study. Odin *et al.* (1995) similarly found that mortality and growth of *H. rigida* were not affected by exposure to Cd-spiked sediment (0.6 to 10 μg g^{-1}) in a 15-d test.

4.3. MOBILIZATION OF CADMIUM

Our analyses of unfiltered water showed that nymphs mobilized sediment and associated Cd into the overlying water. Mean concentrations of Cd in unfiltered test water increased weekly within each treatment and differed among treatments (repeated measures ANOVA, $P < 0.01$, Table I). Among all treatments at day 16, mayflies had mobilized into the water column an average of 0.02% of the total mass of Cd initially spiked into the sediment. This proportion is similar to that of Odin *et al.*, (1995), who found that 0.04% of the Cd initially added to their 10 μg g^{-1} sediment treatment was mobilized into the overlying water by day 15 of their test with *H. rigida*.

We did not analyze samples of filtered test water for dissolved Cd in our study. However, Cope *et al.*, (1994), who studied Cd-contaminated sediments (1.3 to 21.4 μg g^{-1}) from the Mississippi River Basin, developed an empirical relation between concentrations of Cd in filtered and unfiltered test water. Their test was conducted under physicochemical conditions and duration (28 d) similar to ours (dissolved oxygen 7.3 mg L^{-1}, temperature 21.9 °C, pH 7.8, alkalinity 107 mg L^{-1} as $CaCO_3$, hardness 147 mg L^{-1} as $CaCO_3$, and conductivity 307 μS cm^{-1}). Based on the linear relation (Cd concentration in filtered water = 0.0078 + 0.0038 $\times$ Cd concentration in unfiltered water, r = 0.99) of Cope *et al.*, (1994), the predicted concentrations of Cd in filtered water (dissolved fraction) from our test would have ranged from 8–15 ng L^{-1} on day 2 to 8–20 ng L^{-1} on day 16. Although these concentrations are relatively small, they are directly attributable to mayfly-mediated bioturbation. This Cd previously unavailable in the water column represents an additional route of Cd exposure for mayflies, which circulate overlying water through their burrows during respiration (Fremling, 1960; Fremling and Mauck, 1980), and for other organisms living in the water column.

Bioturbation and subsequent suspension of sediment are important factors influencing availability and partitioning of sediment-associated Cd. Bioturbation, for example, has recently been shown to cause oxidation of acid volatile sulfide (AVS), thereby altering the partitioning of sediment-associated Cd and enhancing its bioavailability to sediment-dwelling organisms (Peterson *et al.*, 1996). Although AVS affects Cd toxicity, it forms only under anaerobic conditions (Leonard *et al.*, 1995). AVS was not measured in our study because respiring mayfly nymphs move water through burrows, which maintains aerobic conditions in the sediment (Fremling, 1960; Fremling and Mauck, 1980). Moreover, mayfly nymphs are intolerant of low dissolved oxygen in the sediment (Fremling and Johnson, 1990); therefore,

an overlapping distribution of nymphs and AVS in the sediment would not be expected.

5. Conclusions

Sediments from many lakes and rivers contain Cd concentrations within the range tested here (1.0 to 15.0 μg g^{-1} dry weight, Förstner, 1980). Mayfly nymphs exposed to sediments within this concentration range accumulated significant quantities of Cd during the 21-d exposure, suggesting that nymphs inhabiting areas contaminated by Cd may provide a substantial dietary source of Cd for fish and other predators. The turbidity generated by nymphs decreased progressively as the concentration of Cd in the sediment increased up to 7 μg g^{-1} dry weight. However, the reversal of this trend in our greatest exposure concentration (15 μg Cd g^{-1}) is unexplained. Given the uncertainties revealed in our laboratory test between Cd in sediment and turbidity generated by mayflies, extrapolation of our results to the measurement of turbidity as a substitute for bioturbation-mediated release of compounds from contaminated sediments seems unwarranted. Bioturbation is not a suitable sublethal endpoint of toxicity. However, turbidity generated through bioturbation (i.e., suspension of sediments) by nymphs may serve as an indirect source of Cd exposure for other aquatic organisms in the water column, by altering its partitioning and availability.

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