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TOXICITY OF CONTAMINATED SEDIMENTS IN DILUTION SERIES WITH CONTROL SEDIMENTS

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ABSTRACT

The use of dilutions has been the foundation of our approach for assessing contaminated water, and accordingly, it may be important to establish similar or parallel approaches for sediment dilutions. Test organism responses to dilution gradients can identify the degree of necessary sediment alteration to reduce the toxicity. Using whole sediment dilutions to represent the complex interactions of in situ sediments can identify the toxicity, but the selection of the appropriate diluent for the contaminated sediment may affect the results and conclusions drawn. Contaminated whole sediments were examined to evaluate the toxicity of dilutions of sediments with a diversity of test organisms. Dilutions of the contaminated sediments were prepared with differing diluents that varied in organic carbon content, particle size distribution, and volatile solids. Studies were conducted using four macroinvertebrates and a vascular, rooted plant. Responses by some test organisms followed a sigmoidal dose-response curve, but others followed a U-shaped curve. Initial dilutions reduced toxicity as expected, but further dilution resulted in an increase in toxicity. The type of diluent used was an important factor in assessing the sediment toxicity, because the control soil reduced toxicity more effectively than sand as a diluent of the same sediment. Using sediment chemical and physical characteristics as an indicator of sediment dilution may not be as useful as chemical analysis of contaminants, but warrants further investigation.

Key Words: sediment, dilution, toxicity test, dose response

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INTRODUCTION

Sediments in many areas are contaminated with a wide range of chemicals from a variety of sources (IJC, 1987, 1988; NOAA, 1987). The hazards of these contaminants must be assessed prior to site specific remedial action. Such assessment needs to provide a measure of relative ecosystem degradation to establish priorities, and should include chemical and biological measures. Toxicity tests with interstitial water, elutriates, and whole sediments are used for assessing sediment contamination (Nebeker et al., 1984; Giesy and Hoke, 1989; Ingersoll and Nelson, 1990; Burton et al., 1989; Giesy et al. 1990). When the aqueous extraction (i.e., interstitial water and elutriates) is isolated, routine test procedures can be applied. However, kinetic studies revealed that, because of chemical or kinetic limitations, tests of aqueous extracts may not adequately represent the exposure of benthic invertebrates in the whole sediment matrix (Landrum, 1989; Landrum and Robbins, 1990; Boese et al., 1990).

Studies of contaminant dilutions have been used extensively in research, regulation, and monitoring to determine the relative degree of toxicity of aqueous materials (eg., effluents, receiving waters, pure chemicals) (Macek et al., 1978). Sediments with varying degrees of contamination can be evaluated in studies of whole sediment dilutions with a diversity of test organisms. These studies are based on principles similar to those for aqueous samples (U.S. Environmental Protection Agency, 1989). Studies of whole sediment dilutions have been conducted with sediments from Puget Sound (Chapman, 1988), Commencement and Elliott Bay (Pastorok and Becker, 1989; DeWitt et al., 1989), and the Detroit River (Giesy et al., 1990) comparing many toxicity test endpoints and species specific sensitivities.

Ideally, tests with whole sediment dilutions should produce a gradient of contamination that yields quantal responses of the test organisms. Responses to dilution gradients can identify the degree of necessary sediment alteration to reduce the toxicity. Studies of sediment dilution may provide details such as the volume of sediment exceeding a toxicity threshold, the selection of sites for remedial action, the priority of sites for remedial action, the amount of dilution to reduce toxicity to an acceptable limit, the toxicity of resuspended material, (6) the necessary time to eliminate toxicity at a specific rate of degradation, and the toxicity to a range of species (Giesy and Hoke, 1989). Furthermore, the dose response data from tests of sediment dilution contributes to the understanding of the effect on biota from complex chemical mixtures in sediments, especially where mortality is high. Studies of whole sediment dilutions would represent the complex interactions of in situ sediments more closely than tests of aqueous extracts (Chapman, 1988). However, selection of the appropriate diluent for the contaminated sediment may influence the results of the study and conclusions.

To assess the degree of sediment contamination with complex mixtures of contaminants, whole sediment matrices were used with test organisms exposed to collected sediments, but diluted with different control sediments. Studies were designed to investigate the influences that various diluents may have on sediment toxicity, and to evaluate the toxicity of dilutions of sediments from different stations. Dilutions of the contaminated sediments were prepared with different diluents, that varied in sediment chemical and physical characteristics, such as, organic carbon content, particle size distribution, and volatile solids. Several test organisms were used to assess the degree of toxicity, using both animals and plants, under varying conditions and test methods.

MATERIALS AND METHODS

Sediments

Sediment Collection

Contaminated sediments for tests with Hyalella azteca (Amphipoda), Chironomus riparius (Diptera), Diporeia sp. (Amphipoda), and Hydrilla verticillata (Angiosperma) were collected with a Ponar grab at sites from the Indiana Harbor of Lake Michigan, the Saginaw River, and the Buffalo River. Sediments tested with these test organisms were from stations 03 and 07 in the Indiana Harbor (Figure 1); at station 06 in the Saginaw River, MI (Figure 2); and at station 01 in the Buffalo River, NY (Figure 3). These sediments were collected for the Assessment and Remediation of Contaminated Sediments (ARCS), with the Great Lakes National Program Office (GLNPO), of the U.S. Environmental Protection Agency (Nelson et al., 1993). The sediment sampling stations were presumed or actual depositional areas representing point and non-point sources of contaminants. All of the ARCS sediments were mixed in a cement mixer, placed in high density polyethylene containers, and kept on ice before shipment to the participating laboratories. The sediments were stored in the laboratories at 4°C and placed into test systems within two weeks of receipt.

Contaminated sediments for tests with Ceriodaphnia dubia (Cladocera) were collected from the Little Scioto River (LSR) (Figure 4) in September and November 1989. The Little Scioto River is affected by several sources of contamination: an abandoned railroad yard, a municipal landfill and a wastewater treatment plant, a combined sewer overflow, and several industrial discharges (Sasson-Brickson and Burton, 1991). The contaminated sediment (River Mile 5.8 at State Hwy. 95) and the reference sediment (River Mile 8.1) were collected with an Ekman dredge. The contaminated sediment was observed to be contaminated with petroleum hydrocarbons that produced a surface film. Sediment was placed into 1-L high density polyethylene bottles, overlain with equal quantities of site water, and transported to the laboratory on ice for processing (Burton et al., 1989). Sediments were stored at 4°C and tests were started within 48 hours of collection.

Figure 1. Indiana Harbor, IN, sediment stations.

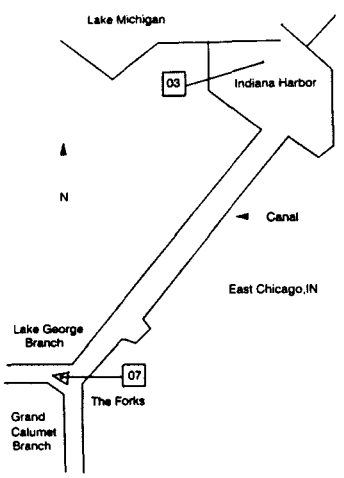


Figure 2. Saginaw River, MI, sediment stations.

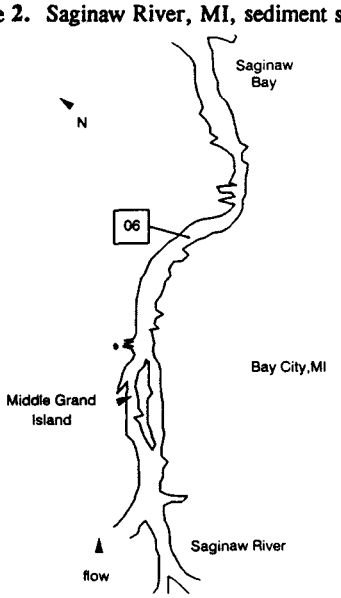


Figure 3. Buffalo River, NY, sediment stations.

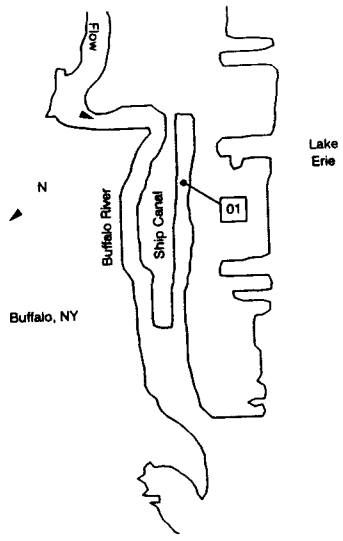
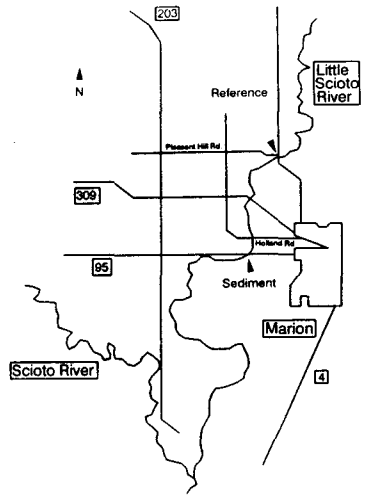


Figure 4. Little Scioto River, OH, sediment stations.



Physical and Chemical Characterization

ARCS

Characterization and chemistry of sediment from the ARCS stations were determined at the Battelle Marine Sciences Laboratory, Sequim, WA. Organic contaminants were quantified after solvent extraction and column cleanup with U.S. Environmental Protection Agency methods. Polynuclear aromatic hydrocarbons (PAHs) were detected by gas chromatography-mass spectrometry (GC-MS) with a selective ion monitoring method 8270 (U.S. Environmental Protection Agency, 1986), polychlorinated biphenyls (PCBs) and chlorinated pesticides by gas chromatography-electron capture detector method 8080 (U.S. Environmental Protection Agency, 1986). Chlorinated dioxins and furans were quantified by isotope dilution GC-MS method 8290 (U.S. Environmental Protection Agency, 1986).

X-ray fluorescence of freeze dried and ground sediments (Nielson and Sanders, 1983) was used to quantify As, Cr, Cu, Fe, Mn, Ni, Pb, and Zn, and atomic absorption (AA) was used to detect Ag, Cd, Hg, and Se (Nelson et al., 1993). For AA analysis, sediment was totally dissolved by a combination of nitric, perchloric, and hydrofluoric acids at elevated temperature and pressure. A graphite furnace with Zeeman background correction was used to quantify Ag, Cd, and Se; mercury was quantified by cold vapor.

Acid volatile sulfides (AVS) in the ARCS sediments were determined with the method of Cutter and Oattes (1987). In this method, hydrogen sulfide is selectively generated, cryogenically trapped, separated by gas chromatography, and detected by photoionization. The simultaneously extracted metals (SEM) are determined by analyzing the 1N HCl solution produced during the AVS determination (Nelson et al., 1993).

Total organic carbon was determined by combusting acid treated sediment at 800°C in an oxygen atmosphere. Sediment grain size was operationally classified by wet sieving through a 63 μm sieve. Percentage volatile solids or organic matter were determined by ashing dry sediments at 500°C for 2 h, measuring the ash weight, and calculating the percentage volatile solids as the fraction of weight loss.

Little Scioto River Sediments

The same organics (PAHs) and metals were quantified from sediments of the Little Scioto River as the ARCS sediments, with the addition of Ba, but without Hg (Ohio Environmental Protection Agency, 1988). Sediment dry weight of each sample was determined in triplicate after drying at 105°C for 24 h (Burton et al., 1989), and particle size was measured with a hydrometer (Day, 1956). Percentage volatile solids were determined using the same method as described for the ARCS sediments.

In exposures of Diporeia sp., the amount of sand and silt in each sediment was determined by sieving a known weight of wet sediment (about 1 g) with distilled water, through a 63-μm screen into a beaker. The remaining sediment sand on the screen was rinsed into a petri-dish and placed into a 60°C drying oven. The silt fraction (<63 μm) was allowed to settle for 24 h, the overlying water was removed, and the sediment was oven dried. The sand to silt ratio was calculated from both fractions. Mass balance was calculated as the sum of the two fractions divided by the dry weight that was calculated from the dry to wet weight ratio.

Sediment organic carbon was determined by placing a 1-2 g sample into scintillation vials and by adding 2 mL of 1N HCl to remove any carbonates. The vials were shaken for 24 h and then dried at 60°C. When dry, 20 mg samples were analyzed using a Perkin-Elmer 2400 C, H, N analyzer.

Sediment Dilutions

For the ARCS studies with Diporeia sp., H. azteca, and C. riparius, contaminated sediments were diluted with either a silica sand from Flat Rock Bagging, Flat Rock, MI (referred to as NOAA sand) or with a fine silt-clay soil collected at Florissant, MO (referred to as control soil) (Ingersoll and Nelson, 1990). Dilutions of sediment from the Indiana Harbor were made by using the control soil for the first experiment and sand as a diluent for the second experiment. Dilutions of sediment from the Saginaw River were made with NOAA sand. These control diluents were chosen because of their physical and chemical characteristics. Sand provides minimal contaminant sorptive properties, whereas a fine grained soil with organic matter will provide more.

Diporeia sp.

For the tests with Diporeia sp., the ratio of contaminated sediments and diluent was determined on a dry weight basis. The dry to wet weight ratio of each sediment was measured and the appropriate amount of wet material was added. Control soil or NOAA sand equivalent to 1 kg dry weight, and an appropriate quantity of the contaminated sediment, were placed in a 4-L beaker with 300 mL of Lake Michigan water. The sediments were homogenized by stirring with an electric hand mixer to visual homogeneity. The contaminated sediments were then press sieved through a 1-mm screen into a 1-L beaker to eliminate large indigenous benthos. Dilutions of 50%, 33%, 20%, 10%, 4%, 2%, and 1% were made with NOAA sand or control soil and sediments from the Indiana Harbor; and 50%, 33%, 17%, 9%, and 4% with NOAA sand and sediment from the Saginaw River.

Sediment dilutions with NOAA sand or control soil, for tests with Diporeia sp., were examined to ensure that the nominal dilutions reflected the changes in the sediment properties for both the Indiana Harbor and Saginaw River sediments. Estimations of sediment physical characteristics were calculated for the percentage volatile solids, organic carbon and fines in the diluted sediments. The dilution proportion could be calculated by solving

for the dilution of the sediment physical characteristics. Simultaneous solution of the equation for two unknowns was performed with data from the percentage volatile solids, organic carbon, and fines. The equations for estimating contaminated sediment dilution with the control soil or NOAA sand are as follows:

$$OC_{DS} = OC_{CTRL}X + OC_{CS}Y$$

$$VS_{DS} = VS_{CTRL}X + VS_{CS}Y$$

OC Organic Carbon; VS Volatile Solids

$_{CTRL}$ Control Soil or NOAA Sand; $_{DS}$ Diluted Sediment; $_{CS}$ Contaminated Sediment

X Fraction of Control Soil or NOAA Sand; Y Fraction of Contaminated Soil

Hyalella azteca* and *Chironomus riparius

ARCS contaminated sediments for tests with H. azteca and C. riparius were diluted with either NOAA sand or control soil on a volume to volume basis, for a total volume of 200 mL. Test sediments were then stirred with a glass rod in each test chamber for one minute, and 800 mL reconstituted water (Ingersoll and Nelson, 1990) was added. Sediments were allowed to settle overnight under gentle aeration before the flow-through tests were started (Ingersoll and Nelson, 1990). Dilutions of 100%, 40%, 16%, 6%, 3%, and 1% were made with NOAA sand or control soil and sediments from the Indiana Harbor; and 100%, 75%, 50%, 25%, 13%, 6%, 3% and 2% with NOAA sand and sediment from the Saginaw River.

Hydrilla verticillata

The dilutions of sediments from the Buffalo and Saginaw River for tests with H. verticillata were made on a wet weight basis (g sediment to g Memphis sand or control soil). Memphis sand, obtained as sand box sand from a local supplier, was autoclaved and cleaned with nitric acid. The dilutions were as follows: for the experiments to determine peroxidase (POD) activity, the sediments were diluted with control soil to concentrations of 100%, 75%, 50%, and 25%. For the remaining endpoints measured, the sediments were diluted with Memphis sand to concentrations of 100%, 70%, and 40%.

Ceriodaphnia dubia

For the tests with C. dubia, contaminated sediment from the Little Scioto River was diluted with upstream reference sediment and mixed on a wet weight basis. The wet weight per mL of sediment (5 replicates) was determined and sediments were combined for a total of 500 mL in a 1-L high density polypropylene bottle and then shaken for 30 min on an Eberbach shaker table at 160 rpm. The concentrations of contaminated sediment, diluted with reference LSR sediment, were 100%, 75%, 50%, 25%, 13%, 6%, and 3%.

Test Organisms and Exposure Procedures

Diporeia sp.

Diporeia sp. were collected with a Ponar grab sampler at 24-29 m from Lake Michigan about 3 mi southwest of Grand Haven, MI. The organisms were gently sieved (1-mm) from the sediment, placed into plastic bags with

lake water, and transported on ice to the laboratory. At the laboratory, the animals were placed in plexiglass aquaria with Lake Michigan sediment and water, and held at 4°C.

A 28 d toxicity test with Diporeia sp. was modeled after the 10 d toxicity test with Rhepoxynius abronius (Swartz et al., 1985). Two centimeters of sediment and 700 mL of Lake Michigan water were carefully added to three replicate, 1-L beakers. The overlying water was gently aerated at 4°C for 24 h before the start of the test. After 24 h of aeration, 20 Diporeia sp. were added to each test chamber and illuminated with red light to encourage burrowing into the sediment. During the first two days occasional dead amphipods were replaced and amphipods found floating at the air-water surface were submerged. The test chambers were monitored for water loss throughout the study; Lake Michigan water was added if required. At the end of the study, the sediments were sieved through a screen (1-mm) and the number of surviving animals were recorded.

Hyalella azteca and Chironomus riparius

The amphipod, H. azteca, and the midge, C. riparius, were laboratory cultured at 20°C (±2°C) on a 16 to 8, light to darkness photoperiod, at a light intensity of about 25-50 foot candles. Cultures were maintained in a moderately hard water (hardness 283 mg/L as $CaCO_3$, alkalinity 255 mg/L as $CaCO_3$, pH 7.8) with gentle aeration (Ingersoll and Nelson, 1990).

The 14 d flow-through sediment tests were conducted as described by Ingersoll and Nelson (1990), with less than one day old C. riparius larvae, and about two week old immature H. azteca. The organisms were placed in a 1-L beaker with 200 mL sediment and 800 mL overlying water at 20°C. Each test chamber was stocked with 20 amphipods or 50 midges. Overlying water delivery rates were about 1 volume-addition $beaker^{-1}$ d^{-1}. Modified Mount and Brungs (1967) proportional flow-through diluters were used to deliver the overlying reconstituted water. Flow-splitting chambers (Ingersoll et al., 1990) delivered overlying water to each test chamber. Test organisms were sieved from sediments at the end of test and removed for counting.

During the tests, amphipods were fed Purina[R] Rabbit Pellets in suspension three times weekly, 6 mg for the first week and 12 mg for the second week. Midges were daily fed suspensions of Cerophyl[R], Selenastrum capricornutum, and Hartz[R] Dog Treats at a rate of 10 mg of Cerophyl (Day 1 to 6), 10 mg Dog Treats (Day 0 to end), and $6x10^7$ algal cells (Day 0 to end).

Total hardness and alkalinity as $CaCO_3$, pH, conductivity, and dissolved oxygen were measured in the overlying water (APHA, 1989; Ingersoll and Nelson, 1990). Water quality conditions were monitored on Day 0, Day 7, and Day 13 and included total water hardness as $CaCO_3$, pH, total alkalinity as $CaCO_3$, unionized ammonia, conductivity, dissolved oxygen, and turbidity.

Hydrilla verticillata

Hydrilla verticillata (Hydrilla) was cultured at 25°C under continuous illumination (about 238 fc) (Byl and Klaine, 1991). Algae-free cultures were obtained as described by Klaine and Ward (1984) and grown in sterilized Mason jars with a 10% Hoagland's nutrient medium (Hoagland and Snyder, 1933), supplemented with 200mg $NaHCO_3$/L. Stock cultures were transferred monthly to fresh medium.

Test sediment (100 g wet weight) was placed in a sterile 1-L Mason jar. This was gently covered with 400 mL of the nutrient medium. Three plants (three weeks old and measured for root and shoot length) were placed in each chamber and the roots were gently introduced into the sediments. The chambers were covered with inverted petri dishes.

After a 14 d exposure to the contaminated sediment, the hydrilla root and shoot length, dehydrogenase activity (DA), chlorophyll a concentration (Chl a), and POD activity were measured. Change in shoot length was determined by aseptically measuring the length of the shoot (mm) at the beginning and end of the test, and normalizing the value to a multiple of the original plant length. New root length (mm) was determined at the end of the exposure period, differentiated by the coloration of the tissue that was lighter than the older roots. Chlorophyll a was extracted from the shoot (Method 1004.3) and quantified (Method 1002G.1) in accordance with Standard Methods (APHA, 1989). The pigment concentration was reported as mg chl a/g dry plant weight. Peroxidase activity was determined using the method of Maehly and Chance (1954) and reported previously for use with hydrilla (Byl and Klaine, 1991).

Dehydrogenase activity in the whole plant was measured by a modified form of the triphenyl tetrazolium reduction method of Davies and Seaman (1980). The plant was incubated in the dark for 48 h in a solution of 0.05% (w/v) 2,3,5-triphenyl-2H-tetrazolium chloride (TTC) in aerated, dechlorinated tapwater at 37$\pm$1°C. The reduction product of TTC, triphenyl formazan (TPF), was batch extracted from the macerated plant for 24 h with spectrophotometric grade methanol in the dark at 37$\pm$1°C (Davies and Seaman, 1980). Triphenyl formazan was quantified spectrophotometrically at 515 nm with a chlorophyll blank, and the effect of turbidity was removed by subtracting the optical density at 750 nm. The TPF concentration was calculated from a standard curve and reported as mg TPF/g dry plant weight. The chlorophyll blank was prepared by diluting (1:5, v/v) the methanol extract of a non-TTC treated control plant.

Ceriodaphnia dubia

Ceriodaphnia dubia were cultured at Wright State University, Dayton, OH, with methods described by Knight and Waller (1987). Cultures were maintained at 25 $\pm$1°C, under a 16 to 8, light to dark photoperiod, in reconstituted hard water (U.S. Environmental Protection Agency, 1987). Dark gray plexiglass covered the culture beakers to reduce evaporation and to reduce light intensity to about 13 fc (Stemmer et al., 1990). Survival and reproduction were assessed in a seven day static test (U.S. Environmental Protection Agency, 1989), where the overlying water was renewed daily. The test was conducted by placing the organisms in ten 30-mL beakers to which 5 mL sediment and 20 mL overlying water were added. One C. dubia neonate, < 12 h old from the third brood, was added to each test chamber, which were exposed under the same conditions as the culture. In the tests with C. dubia, dissolved oxygen, temperature, and pH (APHA, 1989) in the overlying water were monitored daily. Alkalinity and hardness were measured at the beginning and end of the test.

Data Analysis

The percentage of contaminated sediment estimated to kill 50% of the test organisms was calculated by the Probit method. Computer programs (IBM-PC) for LC_{50} data analysis, the LC50 program, were obtained from

the Environmental Monitoring Support Laboratory of the U.S. Environmental Protection Agency at Cincinnati, OH. For tests with Diporeia sp., the percentage dilutions for the LC_{50} calculation were based on measured sediment characteristics, which were volatile solids, organic carbon and fine particles less than 63μm.

The percentage mortality and number of young per female of C. dubia per dilution were calculated. All young by each adult female, until death of the adult, or the end of the experiment, were included in the calculations. Mortality of some test organisms was related to handling stress and not replaced in the test system. These organisms were not included in survival estimates, and the offspring produced before a female's death were also not included in estimates of reproduction. Effect levels for responses of C. dubia were calculated by the Probit method.

RESULTS

Water Chemistry and Sediment Analyses

The softest water (about 60 mg $CaCO_3$/L) was used for the study with C. dubia, the hardness for the other overlying waters ranged from about 135 mg $CaCO_3$/L to 180 mg $CaCO_3$/L (Table 1). For studies with sediments from the Indiana Harbor with H. azteca, and tests with sediments from the Saginaw River, with H. azteca and C. riparius, the characteristics of the overlying water quality were similar to the in-flowing water; conductivity (μmhos) and hardness (mg $CaCO_3$/L) increased slightly (Table 2). Characteristics of the overlying water quality measured in the tests with C. dubia remained similar to the overlying water.

Table 1. Characteristics of overlying water used in sediment tests.

Overlying Water	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	pH	Conductivity (umhos/cm)	Unionized Ammonia (mg/L)	Sulfate (mg/L)	Chloride (mg/L)
NFCRC Reconstituted[1]	136	76	8.0	245	<0.004[2]	68	6.12
NOAA/Lake Michigan[3]	140	107	8.0	ND[4]	0.10	ND[4]	ND[4]
WSU Reconstituted[5]	160-180	110-120	7.6-8.0	ND[4]	ND[4]	ND[4]	ND[4]
MSU[6]	40-60	120-140	8.5	ND[4]	ND[4]	ND[4]	ND[4]

[1] National Fisheries Contaminant Research Center, water used for the Hyalella azteca and Chironomus riparius studies.

[2] Calculated unionized ammonia detection limits (Thurston et al., 1977).

[3] National Oceanic and Atmospheric Administration, Ann Arbor, MI, water used in the Diporeia sp. studies.

[4] Not determined.

[5] Wright State University, water used for the Ceriodaphnia dubia studies (USEPA, 1989).

[6] Memphis State University, water used for the Hydrilla verticillata studies.

Table 2. Characteristics of overlying water quality at test end for the Indiana Harbor, IN, study with Hyalella azteca, and the Saginaw River, MI, study with Hyalella azteca and Chironomus riparius.

	Water Quality Characteristic				
	pH	Alkalinity (mg $CaCO_3$/L	Hardness mg $CaCO_3$/L	Dissolved O_2 (mg/L)	Conductivity (μmhos/cm)
Indiana Harbor station 03 H. azteca					
100% (all Contaminated Sediment)	8.1	82	142	7.9	299
40%	8.1	66	175	8.0	270
6%	---	---	---	8.4	270
1%	---	---	---	8.9	270
0% (all Control Soil)	8.1	68	155	9.2	240
100% (all Contaminated Sediment)	8.1	82	142	7.9	299
40%	8.2	80	175	8.1	280
6%	---	---	---	8.4	275
1%	---	---	---	8.9	260
0% (all NOAA Sand)	8.2	76	155	9.6	270
Indiana Harbor station 07 H. azteca					
100% (all Contaminated Sediment)	8.0	80	156	7.8	317
40%	7.7	72	155	7.3	270
6%	---	---	---	8.0	260
1%	---	---	---	8.8	270
0% (all Control Soil)	8.2	60	145	9.1	250
100% (all Contaminated Sediment)	8.0	80	156	7.8	317
40%	7.9	64	165	7.6	265
6%	---	---	---	8.1	260
1%	---	---	---	9.8	270
0% (all NOAA Sand)	8.1	72	160	9.4	260
Saginaw River station 06 H. azteca					
100% (all Contaminated Sediment)	7.9	84	164	6.7	305
75%	8.0	---	168	9.6	290
13%	8.0	---	164	10.8	295
2%	7.9	---	164	10.0	260
0% (all NOAA Sand)	8.2	80	164	10.8	300
Saginaw River station 06 C. riparius					
100% (all Contaminated Sediment)	7.5	80	160	5.4	270
75%	7.6	---	---	5.6	300
13%	7.6	---	---	5.6	300
2%	7.7	---	---	6.9	300
0% (all NOAA Sand)	7.9	80	144	6.8	293

Percentage fines in ARCS sediments varied by 32% (Table 3). The NOAA sand was over 99% fines (Table 3) compared to the control soil which had a sand content of 8%, a silt content of 66%, and a clay content of 26%, constituting a silty loam soil (Ingersoll and Nelson, 1990). The range of percentage particle size for the LSR

sample was sand 68.7% to 87.5% (x=77.1, SD ±5.01, C.V.=6.59%), silt 12.5 to 31.3% (x=22.9 SD ±5.02, C.V.=21.9%), and clay was 0%. The LSR streambed is primarily composed of sand; silt fractions increase slightly downstream. The organic carbon content ranged from 0.26% in the NOAA sand to 14.3% in the LSR reference sediment. The sediment from the four ARCS stations contained concentrations of acid volatile sulfides which ranged from about 16 to 161 μM/g, with all SEM/AVS ratios less than one (Table 3).

The concentrations of total PAHs (sum of naphthalene to benzo(ghi)perylene) ranged from 0.02 μM/g to 3.8 μM/g in the ARCS sediments, and as high as 3.9 μM/g in the LSR sediment (Table 3). PAH concentrations in sediments from the Indiana Harbor station 07 and LSR, compared to the ARCS sediments at stations Indiana Harbor 03 and Buffalo River 01, varied almost an order of magnitude lower; the sediment from Saginaw River station 06 was two orders of magnitude lower. The concentrations for most of the metals in the ARCS sediments were significantly higher than background concentrations (Nelson et al., 1993), or concentrations in the control soil (Table 3). The highest concentrations of metals were in the sediment at Indiana Harbor station 07, and were almost two orders of magnitude greater than the control soil concentrations. Some metals in ARCS sediments at Saginaw River station 06, and Buffalo River station 01, were elevated by an order of magnitude greater than the metals in the control soil. The Indiana Harbor sediments were contaminated with Fe concentrations as high as 28.8% (Table 3).

Sediment Dilutions

For the control soil dilution of sediment from Indiana Harbor station 07, percentage fines did not show appreciable dilution, whereas percentage volatile solids and organic carbon were serially diluted (Table 4). For the NOAA sand dilution of sediment from Indiana Harbor station 07, all physical characteristics measured were diluted (Table 4). When comparing the measured sediment characteristics (Table 4) with the estimations (Table 5), fine sediment particles less than 63μm tracked the sediment dilution more closely in the sediment from the Indiana Harbor station 07 diluted with control soil, but did not follow the dilution with NOAA sand; a difference as high as 43.5%. In addition, volatile solids and organic carbon estimations did not follow the measured values, with differences as great as 53.2% for volatile solids and 14.2% for organic carbon (Table 5).

Table 3. Sediment chemical and physical characterization.

Contaminated Sediment	PAHs	Metals	Total SEM[1]	PCBs	TCDD	Organic Carbon (%)	Fe (%)	Volatile Solids (%)	Acid Volatile Sulfides (μM/g)	Sum SEM/AVS	Fines <63μm (%)
	(μM/g)				(nM/g)						
Indiana Harbor, station 03	0.5	112.9	3.40	0.0105	0.00059	7.7	19.7	---	33.5	0.010	77
Indiana Harbor, station 07	3.8	245.3	11.25	0.18	0.0006	8.8	28.8	---	71.4	0.158	76
Saginaw River, station 06	0.02	24.2	0.92	0.156	0.00071	2.1	1.5	---	15.5	0.059	45
Buffalo River, station 01	0.5	50.2	1.08	0.0003	0.00003	8.9	5.5	---	161.0	0.007	49
Little Scioto River	3.9	6.2	---	---	---	---	---	12.9	---	---	58[2]
Control Soil	.00072	1.7	---	0.03	---	1.3	---	4.0	---	---	75
NOAA Sand	---	---	---	---	---	0.26	---	0.1	---	---	1.1
Memphis Sand	---	---	---	---	---	---	---	---	---	---	0.0
Little Scioto River Reference	---	---	---	---	---	14.3	---	---	---	---	58[2]

[1] Simultaneously extracted metals, which includes Cd, Cr, Cu, Ni, Pb, and Zn.

[2] <50μm.

Table 4. Physical characteristics measured for dilutions of sediments from the Indiana Harbor and Saginaw River with control soil and NOAA sand, for studies with Diporeia sp. (standard deviations in parentheses).

Contaminated Sediment	Diluent	Nominal Sediment Dilution (%)	Volatile Solids (%)	Fines <63μm (%)	Organic Carbon (%)
Indiana Harbor station 07	Control Soil				
		33	8.9(0.02)	88.6(0.26)	3.9(0.26)
		17	6.4(0.03)	90.8(0.11)	2.7(0.12)
		9	5.1(0.03)	91.7(0.28)	1.9(0.31)
		4	4.2(0.03)	92.9(0.07)	1.3(0.15)
		2	3.6(0.05)	93.3(0.35)	1.2(0.06)
Indiana Harbor station 07	NOAA Sand				
		17	2.6(0.43)	12.3(0.27)	2.3(0.35)
		9	1.2(0.15)	6.2(0.42)	0.9(0.25)
		4	0.5(0.07)	2.9(0.37)	0.3(0.05)
		2	0.3(0.02)	1.8(0.07)	0.1(0.01)
		1	0.1(0.04)	1.1(0.09)	0.06(0.01)
Saginaw River station 06	NOAA Sand				
		50	2.25(0.03)	13.38(0.14)	0.97(0.09)
		33	1.53(0.03)	9.28(0.12)	0.52(0.09)
		17	0.79(0.02)	5.29(0.03)	0.29(0.04)
		9	0.49(0.02)	3.81(0.09)	0.17(0.02)
		4	0.27(0.02)	2.11(0.03)	0.09(0.02)

Table 5. Estimations of percentage sediment dilutions from physical characteristics for control soil and NOAA sand, in the studies with Diporeia sp., using sediments from the Indiana Harbor and Saginaw River.

		Diluent			
		NOAA Sand		Control Soil	
Contaminated Sediment	Sediment Nominal Dilution (%)	Volatile Solids (%)	Fines <63μm (%)	Volatile Solids (%)	Organic Carbon (%)
Indiana Harbor station 07					
	50	---	---	19.0	---
	33	---	---	12.1	---
	17	12.8	16.3	5.9	18.1
	9	5.9	8.2	1.0	7.1
	4	2.5	3.8	2.0	2.4
	2	1.5	2.4	---	0.8
	1	0.5	1.5	---	0.5
Saginaw River station 06					
	50	55.5	56.9	---	---
	33	38.4	39.4	---	---
	17	19.9	22.5	---	---
	9	12.3	16.2	---	---
	4	6.8	9.0	---	---

Toxicity Tests

Indiana Harbor

For both diluents, NOAA sand and control soil, the toxicity of the diluted sediment from Indiana Harbor station 07, for studies with Diporeia sp., decreased monotonically as a function of greater dilution (Table 6). The two diluents resulted in LC_{50} values that differed almost a factor of two, although the 95% confidence intervals overlapped (Table 6). The control soil only slightly reduced toxicity of the Indiana Harbor station 07 sediment over that diluted with NOAA sand.

Table 6. Percentage mortality of Diporeia sp. exposed to Indiana Harbor sediment diluted with control soil or NOAA sand, and Saginaw River sediment with NOAA sand. Standard deviations are in parentheses.

Nominal Sediment Concentration (%)	Mortality (%)		
	Indiana Harbor station 07 and Control Soil	Indiana Harbor station 07 and NOAA Sand	Saginaw River station 06 and NOAA Sand
100	90(8.7)	---	55(4.1)
50	---	---	35(5.0)
33	93(5.7)	---	25(5.0)
17	73(7.6)	92(2.9)	36(2.9)
9	47(5.8)	83(7.6)	50(5.0)
4	28(7.6)	43(16.1)	63(2.9)
2	23(2.9)	40(5.0)	---
1	---	27(15.3)	---
Control	2(2.9)	1(2.9)	6(2.9)
LC_{50}[1]	4.5 (±1.23-10.95)	2.4 (±0.35-6.64)	NC[2]

[1] LC_{50} concentration resulting in 50% mortality, ±95% Confidence Limits, calculated using the Probit method.

[2] Not Calculated. The usefulness of any LC_{50} calculated from this data set is questionable because a concentration-effect relationship has not been demonstrated over a reasonable range.

The sediments from the Indiana Harbor that were diluted with control soil, and NOAA sand, were toxic to H. azteca, and remained toxic across the dilution series (Table 7). The control soil was more effective at reducing toxicity than the NOAA sand diluent for the H. azteca exposures. Diluting sediment from the Indiana Harbor station 07 with NOAA sand, to a 1% contaminated sediment, did not reduce toxicity, but diluting with control soil reduced the toxicity from 100% to 30% (Table 7). Control soil effectively reduced the toxicity in the H. azteca exposure with sediment from the Indiana Harbor station 03, a difference of about two percent (Table 7). The LC_{50} for Diporeia sp., and for H. azteca, exposed to sediment from Indiana Harbor station 07 diluted with control soil, differed about three percent.

Table 7. Percentage mortality of Hyalella azteca and Chironomus riparius exposed to sediment from Indiana Harbor and Saginaw River diluted with control soil and NOAA sand.

Nominal Sediment Concentration (%)	Mortality (%)					
	Indiana Harbor 03		Indiana Harbor 07		Saginaw River 06	
	H. azteca		H. azteca		H. azteca	C. riparius
	Control Soil	NOAA Sand	Control Soil	NOAA Sand	NOAA Sand	NOAA Sand
100	100	100	100	100	90	85
75	---	---	---	---	90	58
50	---	---	---	---	80	46
40	100	100	100	100	---	---
25	---	---	---	---	85	62
16	45	95	100	100	---	---
13	---	---	---	---	95	70
6	60	100	100	100	90	80
3	40	80	80	100	70	80
2	---	---	---	---	80	86
1	60	70	30	100	---	---
0	10	5	10	5	5	5
LC_{50}[1]	2.3 (±0.0-+∞)	.43 (±0.02-1.0)	1.4 (±1.0-1.9)	NC[2]	.00007 (±0.0-0.35)	NC[2]

[1] LC_{50} concentration resulting in 50% mortality, ±95% Confidence Limits.

[2] Not Calculated. The usefulness of any LC_{50} calculated from this data set is questionable because a concentration-effect relationship has not been demonstrated over a reasonable range.

Saginaw River

When the Saginaw River sediment from station 06 was diluted with NOAA sand, the Diporeia sp. response, and that of C. riparius, was not sigmoidal when plotted, but instead was U-shaped (Figure 5). Initial dilutions reduced toxicity as expected, but further dilution of the sediment resulted in an increase in toxicity. The LC_{50} obtained from exposure to Saginaw River sediment diluted with NOAA sand, for H. azteca, was less than one percent, but was not calculated for responses of Diporeia sp., and C. riparius, because a concentration-effect relationship was not demonstrated over a reasonable range (Tables 6 and 7).

For the H. verticillata exposure to Saginaw River sediment, no LC_{50}s were calculated for these responses because, again, a lack of effect over a reasonable range. The plant responses, irrespective of diluent (Memphis sand or control soil), were at times stimulatory. Dilution with Memphis sand inhibited the stimulatory response for root and shoot length, but further dilution of the Saginaw River sediment stimulated growth (Table 8). Dehydrogenase activity was the greatest at the 100% dilution with Memphis sand, but decreased to half the activity of the control at the 40% dilution. Chlorophyll a concentration increased two fold over that of control in the 40% dilution with Memphis sand, but remained at the control level for the other sediment dilutions (Table 8). An increase in POD activity was seen in the control soil dilution exposure, beginning with the initial dilution and continuing throughout the dilution series (Table 8).

Table 8. Hydrilla verticillata responses to exposures to Buffalo River and Saginaw River sediments diluted with control soil and Memphis sand. Standard deviations are in parentheses.[1]

Site	Nominal Sediment Concentration (%)	Dehydrogenase Activity (TPF/g plant dry weight)	Root Length (cm)	Shoot Length (cm)	Chl a (mg/g plant dry weight)	Peroxidase (POD) Activity
Buffalo River station 01						
Memphis Sand						
	100	0.34(.28)	9.5(7.1)	2.8(0.8)	6.0(4.5)	
	70	0.75(.13)	10.8(10.9)	3.5(1.1)	8.4(3.0)	
	40	0.11(.04)	16.5(7.4)	3.4(1.0)	7.5(3.2)	
	0	0.72(.19)	47.7(11.8)	4.2(0.6)	5.1(1.0)	
Control Soil						
	100					4.3(.2)
	75					4.2(.4)
	50					3.8(.2)
	25					3.1(.5)
	0					1.8(.6)
Saginaw River station 06						
Memphis Sand						
	100	3.0(.7)	21.6(24.7)	3.5(2.1)	4.7(1.2)	
	70	2.3(.4)	13.0(13.0)	2.7(1.2)	4.1(2.0)	
	40	1.0(.6)	23.4(30.5)	3.0(1.6)	8.5(2.0)	
	0	2.2(.6)	20.9(10.3)	3.6(1.2)	4.2(0.4)	
Control Soil						
	100					4.8(.3)
	75					4.9(.2)
	50					4.8(.4)
	25					4.1(.2)
	0					2.7(.4)

[1] The usefulness of any LC_{50} calculated from this data set is questionable because a concentration-effect relationship was not demonstrated over a reasonable range.

Buffalo River

Dilution with Memphis sand inhibited root and shoot length over that of control (Table 8), unlike the stimulation response seen with the Saginaw River sediment exposure. Dehydrogenase activity was the greatest with the control and the 70% dilution with Memphis sand, but decreased to almost half the activity of the control at the 100% dilution, and to 15% of the control activity at the 40% dilution (Table 8). Chlorophyll a concentration was greater than control for all of the dilutions with Memphis sand (Table 8). Continued increase in POD activity was seen in the control soil dilution exposure for the entire dilution series (Table 8). For the H. verticillata exposure to Buffalo River sediment, no LC_{50}s were calculated for these responses because of a lack of effect over a reasonable range.

Little Scioto River

For the C. dubia exposure, the toxicity of the diluted LSR sediment, decreased monotonically as a function of greater dilution (Table 9). The C. dubia responses were greatest in the most toxic sediment dilutions and decreased as the toxic sediment became more dilute, regardless of sampling date. Studies of LSR sediment dilutions with C. dubia provided endpoint comparisons, which indicated that reproduction was the most sensitive C. dubia response for this sediment dilution exposure. The mortality LC_{50} and the reproduction EC_{50} differed about 51%, and between sampling dates, reproduction differed less than 2% (Table 9).

Table 9. Response of Ceriodaphnia dubia to exposure to Little Scioto River sediment diluted with upstream reference sediment.

Nominal Sediment Concentration (%)	Mortality (%)	Surviving Young (#)
September 1989		
100	100	0.0
75	90	2.4
50	10	28.3
25	0	31.7
13	0	54.9
6	0	104.8
3	0	97.3
0	0	100.0
	LC_{50}[1] 61.2 (58.9-63.5)	EC_{50}[2] 10.17 (11.4-31.6)
		NOEL[3] 6.25
		LOEL[4] 12.5

Nominal Sediment Concentration (%)	Mortality (%)	Surviving Young (#)
November 1989		
100	100	0.0
75	100	0.0
50	90	0.7
25	70	7.5
13	60	25.4
6	0	83.4
0	0	96.4
	LC_{50}[1] 15.4 (±7.8-25.6)	EC_{50}[2] 11.5 (±5.7-22.0)
		NOEL[3] 6.25
		LOEL[4] 12.5

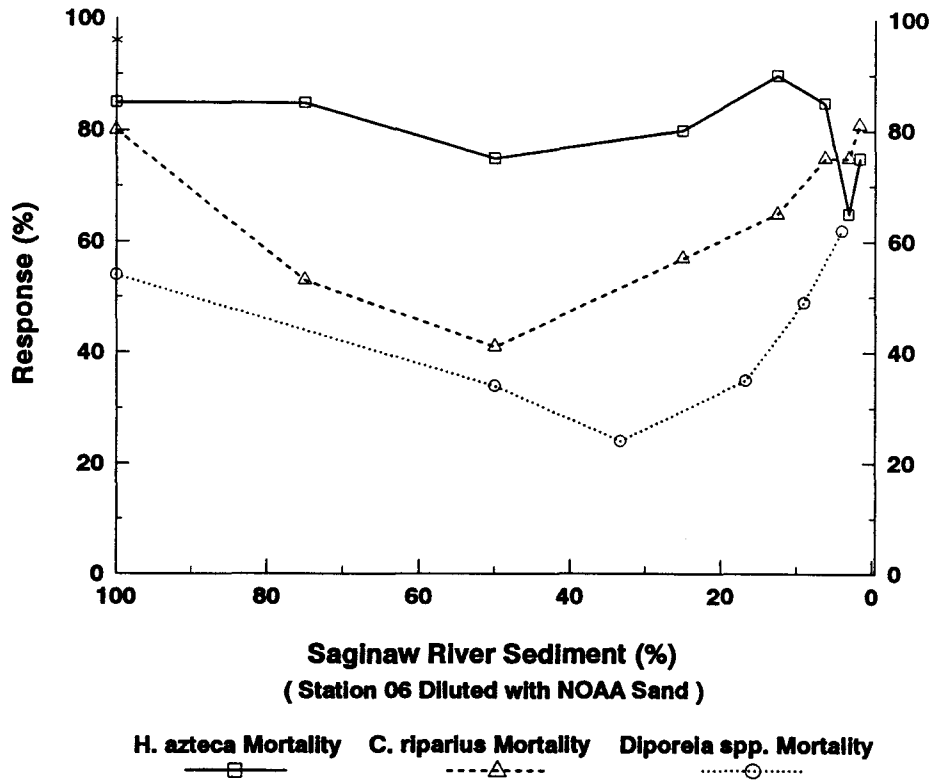
[1] ±95% confidence limits in parentheses.

[2] ±95% confidence limits in parentheses.

[3] NOEL = No Observable Effect Level.

[4] LOEL = Lowest Observable Effect Level.

Figure 5. Mortality (%) of Hyalella azteca, Chironomus riparius, and Diporeia sp. exposed to Saginaw River sediment from station 06 (%), diluted with NOAA sand.



DISCUSSION

When sand was used as the diluting matrix, the response pattern of the sediment dilution tests were not consistent between sediments. The response curves within collection areas (Indiana Harbor, Saginaw River, Buffalo River) were not consistently sigmoidal or U-shaped, as observed in exposures of Diporeia sp., H. azteca, C. riparius, and H. verticillata (i.e., Chl a and dehydrogenase activity). U-shaped dose-response curves were reported for Eohaustorius estuarius and R. abronius exposed to dilutions of contaminated sediments from Commencement Bay and Elliott Bay (DeWitt et al., 1989). An overall non-linear relationship was shown between triphenyl formazan production (dehydrogenase activity) and dilution of Texas reservoir sediments, with monotonicity only occurring over a small range of sediment slurry dilutions (Burton and Lanza 1986). These distinct response patterns are not easily explained.

For Diporeia sp., the Indiana Harbor sediment probably contained sufficient PAHs (3.8 μM/g) (Table 3) to

produce mortality by 28 d, with an LC_{50} of 0.601 μM/g (Landrum et al., 1991). For the Saginaw River sediment, the PAH concentration at 0.02 μM/g (Table 3) was below the no effect level (Landrum et al., 1991). Before or after dilution, the resulting toxicity in Saginaw River sediment was apparently from more than PAH compounds. Also, differences in the toxicity of PAH mixtures is probable. In addition to PAH compounds, sediments from the Indiana Harbor and Saginaw River contained PCBs and tetrachlorodioxins at concentrations greater than 0.002 μM/g. In studies of mixtures of chlorinated hydrocarbons, concentrations less than 0.002 μM/g do not yield toxicity after nine day exposures for Diporeia sp. (Landrum et al., 1991). Since some of the sediments were toxic at or below the no effect level, for one or more of the contaminants, suggests that the observed toxicity resulted from additive or cumulative effects of the complex mixture of contaminants. For sediment which contained elevated contaminant concentrations, compared to controls, like sediment from the Indiana Harbor 07, and for concentrations of unidentified contaminants, this type of contaminant interaction probably occurred in both the undiluted sediment and the dilutions.

Control sediment of a similar composition to the test sediments is perhaps the most environmentally realistic diluent, particularly if it originates from the same ecosystem, e.g., reference sediments. Dilution with reference sediments may simulate existing contaminant gradients occurring at the test site, as seen in the C. dubia exposure to LSR sediment. In spite of sediment manipulation (and possible sediment chemistry alteration) for the studies with C. dubia, both mortality and reproduction produced sigmoidal dose-response curves.

The response of organisms to mixtures of contaminants in the sediment matrix is complex. The observed responses depend on a multitude of factors, such as particle size (DeWitt et al., 1989), feeding characteristics of the organisms (Boese et al., 1990), and chemical changes from sediment manipulation in oxygenated environments (ASTM E1391-90, Burton 1991). If the sediments are altered causing particle size distribution to be less favorable to the organisms, then the sediment may only appear to become more toxic when actually it may be added stress from habitat modification. This effect was observed in marine sediments when R. abronius was exposed to dilutions of sediment from Commencement Bay (DeWitt et al., 1989). These alterations of the sediment matrix may be factors contributing to the U-shaped dose-response curves seen in the Diporeia sp. and C. riparius exposures to Saginaw River sediment diluted with NOAA sand. The alteration of particle size distribution probably was not a factor for the test organisms used in any of these dilution studies since all survived well in the diluent controls, and have not previously shown sensitivity to particle size distributions (Landrum, 1989; Ingersoll and Nelson, 1990; Byl and Klaine, 1991).

Diluting sediments may reduce the amount of available food to the organisms requiring them to spend more energy foraging. If contaminants accumulated by an organism come primarily from foods, in general, as is projected from kinetics studies for strongly sorbed contaminants (Landrum 1989, Landrum and Robbins 1990), dilutions may not reduce the total exposure but increase energetic stress. The combination could result in increased toxicity of diluted sediments, assuming that the concentration of food remains the same. Furthermore, with the reductions of measured sediment organic carbon, test organisms that may feed on sediment organic matter with little or no associated contaminants, may be food limited because of the dilutions. It was shown with sediment from the Indiana Harbor and Saginaw River that the organic carbon from the contaminated sediment was

diluted with the addition of more diluent (Table 4), subsequently becoming increasingly scarce. This unpredictable effect may account for the U-shaped dose-response curves observed in the exposures with Saginaw River sediment from station 06 diluted with NOAA sand.

As estimated by physical characteristics, dilutions of sediments from the Indiana Harbor and Saginaw River for tests with Diporeia sp. were greater than were nominally planned. It is possible that the physical characteristics measured are not appropriate indicators of these sediment dilutions, but because of costly chemical analyses, one can not afford to have contaminants analyzed in each dilution; alternate measurements are desirable. The sediment characteristics were investigated to see if they tracked well with the sediment dilution series, and to give a more realistic view of the changes of the diluted toxic sediment. Undoubtedly, further research is needed to evaluate the utility of using sediment physical characteristics to predict the actual dilution of sediments. Characterization is needed for the actual processes (i.e, mixing) and influencing factors (i.e., oxygenation for volatile sulfides) that prevented the sediments from more closely matching the expected nominal proportions.

Diluting sediments can modify the chemical environment by oxygenating the sediment, thereby reducing AVS that in turn may modify the bioavailability of heavy metals (DiToro et al., 1991). The diluent may have more or less AVS (or other complexing agents) and thereby alter metal availability. Also, additions of uncontaminated sediment increases the number of binding sites of metal and organic contaminants, as shown in the H. azteca exposure to Indiana Harbor sediment diluted with both control soil and NOAA sand. Toxicity to H. azteca was reduced (mortality decreased) when control soil was used as the diluent. Furthermore, the diluent may alter sediment porosity, thus altering availability and desorption/adsorption kinetics. The toxicity of the sediments may be due to any or all of the constituents in the matrix. Although AVS may be oxidized during the manipulations involved in sediment dilutions, dilution with an uncontaminated sediment could reduce contaminant bioavailability by providing additional metal-binding components, including, but not limited to, sulfide, as well as binding sites for organic contaminants. However, this may not be the case for dilution with sand, which changes sediment volume and texture, but not binding capacity.

Toxicant effect levels are traditionally determined using monotonic response curves. Results of the present studies show sediment dilutions may not produce monotonic responses and therefore, violate statistical assumptions used in conventional procedures (e.g. Probit, binomial, and moving-average). The use of dilutions has been the foundation of our approach for assessing contaminated water, and accordingly, it may be important to establish similar or parallel approaches for sediment dilutions. Although, from these studies, it is clear that traditional methods applied to this complex matrix are inadequate. To explain changes in sediment chemistry and contaminant bioavailability from sediment dilution with varying diluents, and to advance our understanding of the appropriate approach for this type of assessment, further research is necessary.

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