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Toxicity Assessment of Sediments from the Grand Calumet River and Indiana Harbor Canal in Northwestern Indiana, USA

C. G. Ingersoll,[1] D. D. MacDonald,[2] W. G. Brumbaugh,[1] B. T. Johnson,[1] N. E. Kemble,[1] J. L. Kunz,[1] T. W. May,[1] N. Wang,[3] J. R. Smith,[4] D. W. Sparks,[5] D. S. Ireland[6]

[1] Columbia Environmental Research Center, U.S. Geological Survey, 4200 New Haven Road, Columbia, Missouri 65201, USA
[2] MacDonald Environmental Sciences Limited, #24-4800 Island Highway North, Nanaimo, British Columbia V9T 1W6, Canada
[3] Fisheries and Wildlife Sciences, 302 ABRN Building, University of Missouri, Columbia, Missouri 65211, USA
[4] Indiana Department of Environmental Management, 100 North Senate Ave., Indianapolis, Indiana 46206, USA
[5] U.S. Fish and Wildlife Service, 620 S. Walker St., Bloomington, Indiana 47403, USA
[6] U.S. Environmental Protection Agency, 401 M St. SW, Washington, DC 20460, USA

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Abstract. The objective of this study was to evaluate the toxicity of sediments from the Grand Calumet River and Indiana Harbor Canal located in northwestern Indiana, USA. Toxicity tests used in this assessment included 10-day sediment exposures with the amphipod *Hyalella azteca*, 31-day sediment exposures with the oligochaete *Lumbriculus variegatus*, and the Microtox® Solid-Phase Sediment Toxicity Test. A total of 30 sampling stations were selected in locations that had limited historic matching toxicity and chemistry data. Toxic effects on amphipod survival were observed in 60% of the samples from the assessment area. Results of a toxicity test with oligochaetes indicated that sediments from the assessment area were too toxic to be used in proposed bioaccumulation testing. Measurement of amphipod length after the 10-day exposures did not provide useful information beyond that provided by the survival endpoint. Seven of the 15 samples that were identified as toxic in the amphipod tests were not identified as toxic in the Microtox test, indicating that the 10-day *H. azteca* test was more sensitive than the Microtox test. Samples that were toxic tended to have the highest concentrations of metals, polycyclic aromatic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs). The toxic samples often had an excess of simultaneously extracted metals (SEM) relative to acid volatile sulfide (AVS) and had multiple exceedances of probable effect concentrations (PECs). Metals may have contributed to the toxicity of samples that had both an excess molar concentration of SEM relative to AVS and elevated concentrations of metals in pore water. However, of the samples that had an excess of SEM relative to AVS, only 38% of these samples had elevated concentration of metals in pore water. The lack of correspondence between SEM-AVS and pore water metals indicates that there are variables in addition to AVS controlling the concentrations of metals in pore water. A mean PEC quotient of 3.4 (based on concentrations of metals, PAHs, and PCBs) was exceeded in 33% of the sediment samples and a mean quotient of 0.63 was exceeded in 70% of the thirty sediment samples from the assessment area. A 50% incidence of toxicity has been previously reported in a database for sediment tests with *H. azteca* at a mean quotient of 3.4 in 10-day exposures and at a mean quotient of 0.63 in 28-day exposures. Among the Indiana Harbor samples, most of the samples with a mean PEC quotient above 0.63 (*i.e.*, 15 of 21; 71%) and above 3.4 (*i.e.*, 10 of 10; 100%) were toxic to amphipods. Results of this study and previous studies demonstrate that sediments from this assessment area are among the most contaminated and toxic that have ever been reported.

Correspondence to: C. Ingersoll; *email:* chris_ingersoll@usgs.gov

The Grand Calumet River and Indiana Harbor Canal Area of Concern (AOC) located in northwestern Indiana, has been subject to intensive industrial development throughout much of this century and is currently one of the most highly industrialized areas in the United States (Figure 1; MacDonald *et al.* 2002a, 2002b). Discharges of both point and nonpoint sources have released a variety of toxic, bioaccumulative, and other substances into the river system, including total organic carbon (TOC), nutrients, oil and grease, metals, phenolics, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), phthalates, and certain pesticides (MacDonald *et al.* 2002a, 2002b). Previous characterizations of sediments in the study area have reported a wide array of contaminants in sediment including metals and organic chemicals including PAHs, PCBs, and organochlorine pesticides (Hoke *et al.* 1993; Dorkin 1994; US EPA 1996a, 1996b). Sediment toxicity tests, benthic invertebrate community assessments, and fish community surveys have been conducted with samples from various locations throughout the assessment area (Lucas and Steinfeld 1972; Hoke *et al.* 1993; Polls *et al.* 1993; Burton 1994; Jop and Putt 1994; Canfield *et al.* 1996; Ingersoll *et al.* 1996). Results of these studies have documented that the sediments in the

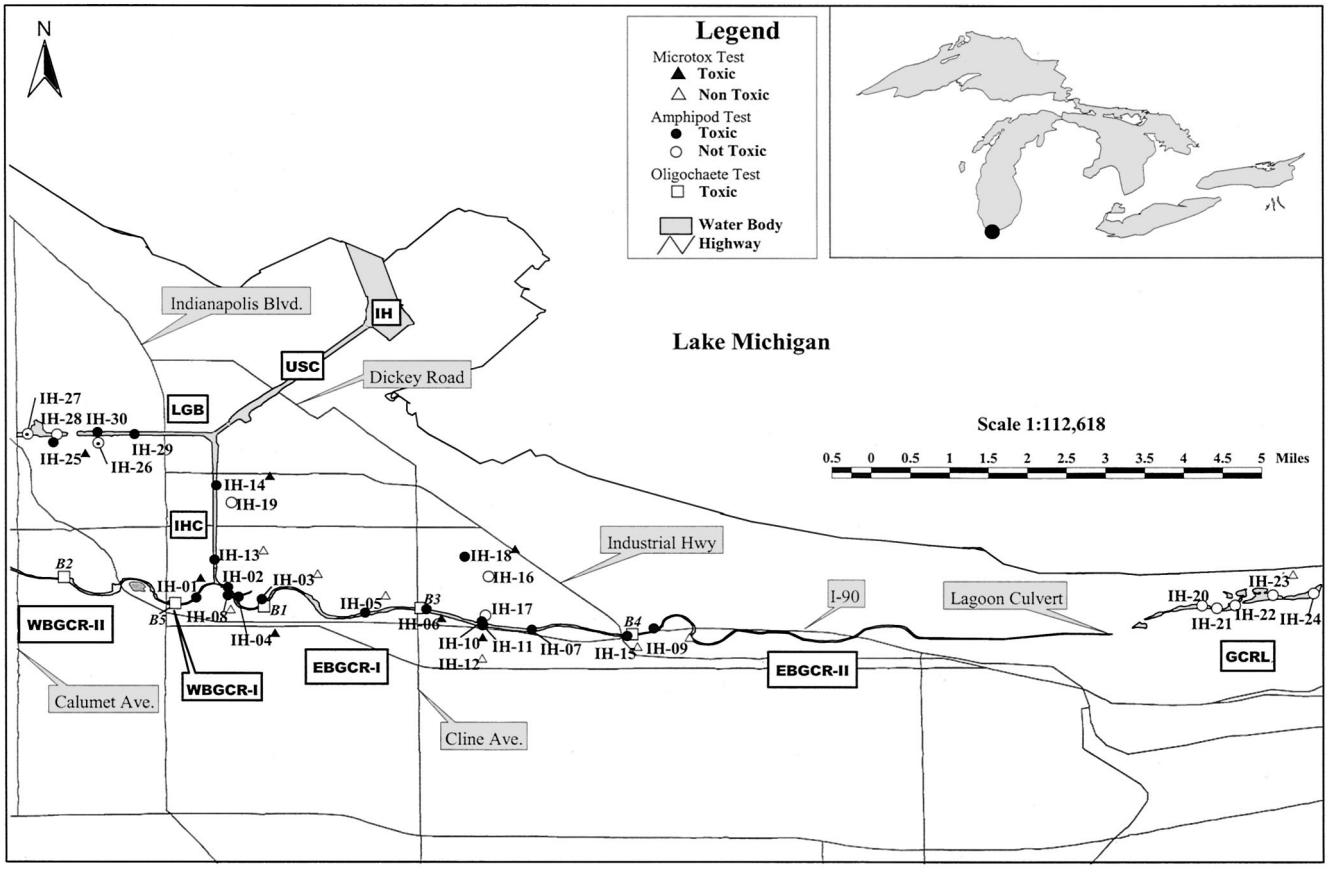

Fig. 1. Map showing locations of sampling stations for amphipod, oligochaete, and Microtox testing

assessment area are among the most contaminated and toxic ever evaluated (US EPA 1997; MacDonald and Ingersoll 2000).

The objective of the present study was to evaluate an additional 30 sampling stations in the assessment area where historic data on matching sediment toxicity and chemistry were limited (Figure 1; MacDonald and Ingersoll 2000). Toxicity tests used in this assessment included a 10-day sediment exposure with the amphipod *Hyalella azteca* (ASTM 2001a; US EPA 2000a), a 31-day sediment exposure with the oligochaete *Lumbriculus variegatus* (US EPA 2000a; ASTM 2001b), and the Microtox® Solid-Phase Toxicity (SPT) test (Johnson and Long 1998). MacDonald *et al.* (2002a, 2002b) describe the results of an integrated injury assessment for assessment area using data generated from the present study along with the results of historic studies in the assessment area dealing with sediment toxicity, chemistry, bioaccumulation, benthic invertebrate evaluations, and fish community status.

Materials and Methods

Sediment Collection

MacDonald *et al.* (2002a) provides a description of the sampling area illustrated in Figure 1. Grab samples of surficial sediment used in toxicity tests with *H. azteca* were collected at 30 stations from January 26 to March 20, 1999, using an Eckman grab (to a depth of about 10 cm; Figure 1). Samples from transect cores were also collected for chemical analyses at these same locations and additional locations along the assessment area (a total of 15 locations; MacDonald and Ingersoll 2000). Each surficial grab sample was made as a composite of at least five discrete grab samples (collected from within a 2-m radius of a transect core sample). Sediments were homogenized and subsamples were obtained for chemical analyses and for toxicity testing. Subsamples of each grab sample for toxicity testing were placed in two 1-L brown amber bottles and shipped on ice to the Columbia Environmental Research Center in Columbia, MO. Sediment samples were held at 4°C in the dark for up to 6 weeks before the start of the toxicity tests (ASTM 2001a).

Physical and Chemical Characterization of Sediment Samples

Chemical analyses of sediment samples included PAHs, total PCBs, organochlorine pesticides, total metals, acid volatile sulfides and simultaneously extracted metals (AVS and SEM), and pore water metals (total and dissolved; Table 1). Concentrations of PAHs were determined by gas chromatography and mass spectrometry (US EPA 1981, 1984). Concentrations of total PCBs (based on Aroclors) and organochlorine pesticides were determined by gas chromatography and electron capture (US EPA 1982). Concentrations of SEMs and AVSs were determined using procedures outlined in US EPA (1991). Total and filterable concentrations of metals (Ni, Cu, Zn, Cd, and Pb) in pore

Table 1. Whole-sediment physical and chemical characteristics of samples from the Grand Calumet River and Indiana Harbor Canal

Samples	Grain Size (% sand DW)	Total Organic Carbon (% DW)	SEM-AVS (μmole/g DW)	Toxic Units of Metals[a]	Total PCBs (μg/g DW)	Total PAHs (μg/g DW)	Mean PEC Quotient
Control-FS	13	1.2	−0.29	ND	ND	ND	ND
Control-WB	74	9.6	−25	ND	<0.07	0.50	0.14
IH-01	95	12	−11	1.4	<4.1	3,300	77
IH-02	ND	7.3	11	0.22	2.1	93	2.7
IH-03	ND	10	27	0.10	3.1	64	2.9
IH-04	ND	14	39	0.33	8.2	93	6.9
IH-05	ND	10	12	0.060	1.7	43	2.0
IH-06	54	8.8	−38	63	5.6	64	4.3
IH-07	44	10	5.3	0.16	18	140	12
IH-08	5.0	9.9	7.8	2.2	0.38	24	1.4
IH-09	51	14	13	1.3	<0.68	2.2	0.45
IH-10	29	13	27	ND	56	500	36
IH-11	94	0.45	0.10	ND	0.23	1.2	0.24
IH-12	36	14	18	0.99	2.7	67	3.2
IH-13	93	1.1	4.0	0.010	0.48	9.6	0.49
IH-14	14	4.9	4.8	0.0040	5.6	140	5.3
IH-15	96	1.1	−7.9	0.09	4.9	1,500	23
IH-16	82	4.8	−120	0.010	<0.32	<5.3	0.21
IH-17	95	0.17	−4.8	0.050	<0.15	<1.9	0.066
IH-18	14	7.2	465	11	0.22	<5.6	16
IH-19	66	13	12.3	1.7	<0.40	1.5	0.72
IH-20	42	13	−66	0.47	0.44	14	0.66
IH-21	58	11	−89	0.25	0.31	14	0.54
IH-22	53	13	−34	0.21	0.36	7.1	0.49
IH-23	60	9.9	−51	0.65	0.46	5.1	0.50
IH-24	75	13	−4.9	0.57	0.25	43	0.94
IH-25	43	2.0	−15	0.38	0.57	3.6	0.87
IH-26	99	3.6	−32	0.47	<0.77	<4.0	0.079
IH-27	65	17	−140	1.3	2.5	2.3	1.7
IH-28	56	6.7	−87	0.53	<2.7	4.6	0.72
IH-29	93	1.8	2.4	2.2	1.3	62	4.3
IH-30	58	16	−71	0.31	13	14	7.6

DW = Dry weight; ND = not determined.

[a] Sum toxic units for dissolved cadmium, copper, nickel, or zinc in pore water based on 10-day LC_{50} concentrations for *Hyalella azteca* in water-only exposures (US EPA 2000a; MacDonald and Ingersoll 2000).

water samples were determined by inductively coupled plasma mass spectrometry (May *et al.* 1997). Physical characterization of sediment samples included grain size and total organic carbon (TOC; Table 1) and were determined using procedures outlined in Kemble *et al.* (1994). Detailed results of these analyses are reported in Ingersoll *et al.* (1999) and MacDonald and Ingersoll (2000).

Toxicity test with L. variegatus

In August 1998, a toxicity test was conducted with sediments collected from five locations in the assessment area that corresponded to areas where the U.S. Fish and Wildlife Service had previously conducted bioaccumulation studies with barn swallows. The objective of this toxicity test was to determine if the oligochaete *L. variegatus* could be used to assess bioaccumulation of contaminants from sediments in the assessment area. Preliminary laboratory testing is recommended with sediments to determine if oligochaetes will survive and burrow into samples (US EPA 2000a).

Surface sediments were collected in 1998 from the following locations: Kennedy Avenue (B1), Columbia Avenue Bridge (B2), east of Cline Avenue Bridge (B3), east of Industrial Highway (B4), and Indianapolis Boulevard Bridge (B5; Figure 1). The day before the start of the exposures, 75 ml of sediment from each location was placed in a 300-ml beaker, and about 200 ml of overlying water was then gently poured into each beaker. Test conditions were similar to the conditions used to conduct the toxicity test with amphipods described below except that no food was added to the beakers, no replicates were tested, 20 adult oligochaetes were added to each beaker, and the test was conducted for 31 days. Oligochaetes used in the test were cultured using procedures described in Brunson *et al.* (1998).

Toxicity test with H. azteca

Mixed-age amphipods were mass cultured in 80-L glass aquaria containing 50 L of water that received about six volume additions/day of well water (hardness 280 mg/L as $CaCO_3$, alkalinity 250 mg/L as $CaCO_3$, and pH 7.80; Ingersoll *et al.* 1998). The amphipods were cultured at a temperature of 23°C and a light intensity of about 500 lux. Amphipods in the cultures were fed Tetramin® fish food (Ram Fab Aquarium Products, Oak Ridge, TN) and presoaked maple leaves *ad libitum*. Each aquarium contained six nylon substrates (20-cm-diameter sections of "coiled-web material"; 3M, St. Paul, MN). Amphipods

used to start the tests were obtained by collecting organisms from the mixed-aged cultures that passed through a #35 (500-μm opening) U.S. standard size sieve mesh and were stopped by a #40 (425-μm opening) sieve placed under water (ASTM 2001a). Amphipods were held in a 2-L beaker for 24 h before the start of a test. Use of this sieving technique resulted in an average length of amphipods of 1.23 mm (0.03 SE) at the start of the first set of sediment exposures and 1.32 mm (0.02) at the start of the second set of sediment exposures. The sizes of amphipods were comparable with the size of the known-age 7- to 8-day-old amphipods previously used to start sediment tests (Ingersoll *et al.* 1998; 1.2–1.6 mm). The first set of exposures (IH-01 to IH-15 samples) were started on March 5, 1999, and the second set of exposures (IH-16 to IH-30 samples) were started on March 30, 1999.

To test a range of TOC and grain size in the sediments, a negative control of West Bearskin sediment (Control-WB; 9.6% TOC, primarily sand particles; Ingersoll *et al.* 1998; Table 1) and a formulated sediment (Control-FS; 1.2% TOC, primarily silt-clay particles; Ingersoll *et al.* 1998; Kemble *et al.* 1999) were tested with each set of sediment samples. The formulated sediment was prepared from a dry mixture of materials and hydrated with a 50:50 volume of well water 3 days before the start of the sediment exposures. The formulated sediment was resuspended in this well water each of the 3 days before the start of the sediment exposures in order to better equilibrate the water quality characteristics of the pore water with the well water that was used as a source of overlying water in the sediment tests (Kemble *et al.* 1999).

Amphipods were exposed in 300-ml beakers containing 100 ml sediment and 175 ml of overlying water. Each sediment sample was thoroughly mixed using a stainless steel spoon and bowl and visually inspected to judge homogeneity; subsamples were then added to the exposure beakers the day before start of the sediment test (day −1). The spoons and bowls were rinsed with acetone, well water, and deionized water between samples. Plant fibers were removed by hand from samples IH-09, IH-16, IH-19, and IH-23 before the samples of sediment were placed in the beakers.

Exposures were conducted for 10 days at 23°C on a 16 light:8 dark photoperiod at a light intensity of about 200 lux. The source of overlying water was well water and there were two volume additions/day of water to each beaker using an automated system (Zumwalt *et al.* 1994). At the start of the exposure, about 20 amphipods were archived in a solution of 8% sugar formalin for measurement of length. A total of 10 amphipods were exposed in each beaker. Four replicates were tested for each sediment sample. Amphipods were fed 1.0 ml of YCT (yeast-Cerophyl®-Trout Chow®, 1,800 mg/L stock solution; US EPA 2000a) every day.

Hardness, alkalinity, conductivity, dissolved oxygen, pH, and ammonia were measured in the pore water samples before the start of the exposures (day −1) using methods outlined in Kemble *et al.* (1994; Table 2). Pore water samples were isolated by centrifugation at 5,200 rpm (7,000 *g*) for 15 min at 4°C (Kemble *et al.* 1994). About 20–50 ml of pore water was used to measure ammonia and a similar volume of pore water was used to measure the other water quality characteristics. A separate 20-ml subsample of pore water was used to prepare the filtered and nonfiltered samples for metal analyses (Ingersoll *et al.* 1999). A wide range in the water quality characteristics of the pore water was observed for hardness (256–12,000 mg/L as $CaCO_3$), alkalinity (70–3,860 mg/L as $CaCO_3$), conductivity (432–7,200 μmhos/cm), dissolved oxygen (1.1–9.0 mg/L), pH (6.38–9.58), total ammonia (0.859–54.2 mg/L), and unionized ammonia (<0.01–6.61 mg/L; Table 2). Hardness, alkalinity, conductivity, dissolved oxygen, pH, and ammonia were measured in overlying water on days 0 and 10 of the exposures. Conductivity and dissolved oxygen in overlying water were also measured on day 4 of the exposures. Overlying water quality characteristics were generally similar among treatments (Ingersoll *et al.* 1999). Dissolved oxygen in overlying water was at or above acceptable levels of 2.5 mg/L in all treatments throughout the study (ASTM 2001a; US EPA 2000a).

On day 10 of the exposures, sediments in each beaker were sieved through a #50 sieve (300-μm opening). The debris and amphipods remaining in the sieve were rinsed into a glass tray and searched for up to 20 min for organisms. Surviving amphipods were counted and preserved in 8% sugar formalin for later length measurements. Length of amphipods was measured along the dorsal surface from the base of the first antenna to the tip of the third uropod along the curve of the dorsal surface using a microscope and digitizing system (Kemble *et al.* 1994).

Microtox SPT Test

After completion of the amphipod exposures, 15 samples designated as toxic to *H. azteca* were tested with the Microtox SPT test. The Microtox SPT test determines the toxicity of chemicals in sediment by exposing bioluminescent bacteria (*Vibrio fisheri*; B-NRL 1117, Azur Environmental, Carlsbad, CA) directly to sediment suspended in solution (Microbics 1992; Johnson 1998; Johnson and Long 1998). A 300-mg (wet weight) sediment sample was placed in an SPT exposure tube with 3 ml of 2% NaCl diluent, stirred with a vortex mixer, and used to prepare a 12-tube 1:2 dilution series with three controls. Bacteria freshly obtained from a freeze-dried vial were introduced into each tube directly exposing the bacteria to the water-sediment matrix. The sample was briefly stirred with a vortex mixer and then incubated for 20 min at 15°C in a temperature controlled waterbath. After incubation, a SPT filtering device was inserted into each tube to facilitate the separation of solid and liquid materials. The supernatant containing exposed bacteria was transferred to a cuvette and placed in a luminometer for a 5-min stabilization period at 15°C where light emission was determined. A log-linear model was used to calculate EC_{50} values (expressed as percentage of sediment wet weight/ml; Johnson and Long 1998). All exposures were replicated with variation between samples of <10%.

Data Analyses

Statistical analyses for the amphipod exposures were conducted using one-way analysis of variance (ANOVA) at $\alpha = 0.05$ for all endpoints except length which was analyzed using a one-way nested ANOVA at $\alpha = 0.05$ (amphipods nested within a beaker). If the results of the ANOVA were significant, mean separation was performed relative to each of the control sediments by Fisher's protected least-significant difference test at $\alpha = 0.05$. Percent survival data were arcsine-transformed and length data were log-transformed before analysis. Spearman rank correlation procedures were used to evaluate relationships between responses in the toxicity tests to the physical or chemical characteristics of sediments. Statistical significance for the rank correlations was established at 0.005 for all comparisons to minimize experimental-wise error (Bonferroni method; Snedecor and Cochran 1982). All statistical analyses were performed with SAS programs (SAS 1998).

For the Microtox SPT test, EC_{50} values and 95% confidence intervals were calculated for each sediment tested. A toxicity reference index (TRI) was used to determine when a mixture of contaminants in the field-collected sediments adversely affected the bacteria in the Microtox SPT test (Johnson and Long 1998). A TRI was developed by spiking a control sediment (Control-FS) with pentachlorophenol (PCP) at 1 μg/mg (wet weight; Johnson and Long 1998). PCP was selected as a model reference toxicant because of its ubiquity as a problem chemical in sediment, its high K_{ow} value, and its high toxicity to aquatic biota. The control sample with an EC_{50} value of 0.5 (0.03–1.0 as percentage of wet weight sediment/ml) was assigned a TRI value of 1.0. A field-collected sample with an EC_{50} value < 0.5 indicated the

Table 2. Pore water quality characteristics measured at the start of the amphipod toxicity tests with samples from the Grand Calumet River and Indiana Harbor Canal

	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Conductivity (μmhos/cm)	Dissolved Oxygen (mg/L)	pH	Ammonia (total/unionized; mg/L)
First set of exposures						
Control-FS1	164	160	1,275	9.0	6.84	1.26/<0.01
Control-WB1	ND	70	432	2.7	6.75	1.95/<0.01
IH-01	1200	1,280	3,370	1.5	7.44	50.1/0.07
IH-02	300	1,325	2,480	5.7	7.88	27.5/0.10
IH-03	ND	1,250	1,933	2.2	7.28	54.2/0.05
IH-04	400	560	1,402	3.2	7.32	54.0/0.06
IH-05	384	420	1,120	4.1	7.41	12.0/0.02
IH-06	ND	3,860	7,200	1.1	9.58	32.6/6.61
IH-07	280	290	661	2.9	7.72	15.3/0.04
IH-08	448	640	1,119	2.5	7.09	22.7/0.01
IH-09	ND	420	1,556	2.6	7.50	2.06/<0.01
IH-10	440	422	964	2.0	7.45	26.8/0.04
IH-11	ND	ND	ND	ND	ND	ND
IH-12	384	486	1081	2.6	7.36	8.21/0.01
IH-13	ND	ND	ND	ND	ND	ND
IH-14	520	400	1097	4.6	7.38	4.49/0.01
IH-15	ND	ND	ND	ND	ND	ND
Second set of exposures						
Control-FS2	432	168	1,595	8.5	6.79	1.78/<0.01
Control-WB2	ND	58	481	2.5	6.61	2.24/<0.01
IH-16	500	510	1,069	2.7	7.30	5.05/0.01
IH-17	ND	ND	1,493	4.0	7.78	9.88/0.03
IH-18	340	100	2,520	7.2	7.57	0.859/<0.01
IH-19	650	340	1,507	3.9	7.20	1.54/<0.01
IH-20	430	528	1,168	2.8	7.17	ND
IH-21	316	320	690	2.7	7.36	3.68/0.01
IH-22	256	270	669	3.1	7.28	8.57/0.01
IH-23	624	698	1,958	1.8	6.94	11.4/<0.01
IH-24	422	390	1,217	1.6	7.27	7.17/0.01
IH-25	676	160	1,783	3.9	6.38	1.06/<0.01
IH-26	324	320	654	5.4	7.79	3.35/0.01
IH-27	388	454	1,434	2.3	7.11	13.3/0.01
IH-28	660	736	1,825	2.1	7.26	18.3/0.02
IH-29	526	876	1,655	1.4	7.05	52.6/0.03
IH-30	320	280	1,011	5.3	7.55	3.39/0.01

ND = Not determined (due to either insufficient sample volume or difficulty in making the measurement).

sample was more toxic than the reference toxicant. For example, a field sample with an EC_{50} value of 0.25 had a TRI of 2.0 (*i.e.*, spiked sample EC_{50} value/test sample EC_{50} value = 0.5/0.25) which indicated that the field sample was about twofold more toxic than the PCP reference sample.

Probable effect concentrations (PECs) were used to assess the relationship between sediment chemistry and toxicity. The PECs are effect-based sediment quality guidelines that were established as concentrations of individual chemicals above which adverse effects in sediments are expected to frequently occur in field-collected sediments (MacDonald *et al.* 2000). Mean quotients based on PECs were calculated to provide an overall measure of chemical contamination and to support an evaluation of the combined effects of multiple contaminants in sediments (MacDonald *et al.* 2000; US EPA 2000b; Ingersoll *et al.* 2001). A PEC quotient (PEC-Q) was calculated for each chemical in each sediment sample by dividing the dry-weight concentration of a chemical by the PEC for that chemical. In calculating concentrations of total PCBs or total PAHs, half the detection limit was used for compounds reported below the detection limit (Ingersoll *et al.* 2001). If the concentration was below the detection limit but above the PEC, this value was excluded from the calculation of the PEC-Q (and for evaluation by Spearman rank correlation).

We were interested in equally weighting the contribution of metals, PAHs, and PCBs in the evaluation of sediment chemistry and toxicity (assuming these three diverse groups of chemicals exert some form of collective toxic action). For this reason, we first calculated an average PEC-Q for up to seven metals in a sample based on dry weight concentrations of arsenic, cadmium, chromium, copper, lead, nickel, and zinc. A mean quotient was then calculated for each sample by summing the average quotient for metals, the quotient for total PAHs, and the quotient for total PCBs, and then dividing this sum by three (n = 3 quotients/sample; see Ingersoll *et al.* 2001 for additional details on the procedure used to calculate mean PEC-Q). Use of this approach for calculating the quotients was selected to avoid overweighting the influence of an individual chemical (*i.e.*, a single metal) on the combined mean quotient (US EPA 2000b).

Results and Discussion

Physical and Chemical Characteristics of Sediment Samples

A broad range in grain size and TOC was observed in the sediment samples collected from the assessment area (range of 5.0–99% sand and 0.17–17% TOC; Table 1). Therefore, two control sediments were evaluated in the toxicity tests with amphipods (Control-FS with low sand [13%] and low TOC [1.2%] and Control-WB with high sand [74%] and high TOC [9.6%]; Table 1). Results of the SEM and AVS analysis indicated that there was a high percentage of the samples with an excess of molar SEM concentrations relative to molar AVS concentrations (50% of the samples from the assessment area). A lower percentage of the samples (32%) had a sum toxic unit above 1.0 for dissolved pore water concentrations of cadmium, copper, nickel, or zinc (based on the 10-day water-only LC_{50} concentration for *H. azteca*; Table 1). Concentrations of AVS has been demonstrated to influence pore water concentrations and bioavailability of divalent metals in sediment toxicity and bioaccumulation tests (Ankley *et al.* 1996). An excess molar concentration of SEM relative to AVS and elevated concentrations of metals in pore water indicate that metals may contribute to the toxicity of a sediment sample (Table 1; Ankley *et al.* 1996).

Concentrations of total PCBs exceeded the PEC of 0.676 μg/g in 57% of the 23 samples with detectable concentrations of total PCBs. Similarly, the concentrations of total PAHs exceeded the PEC of 22.8 μg/g in 54% of the 26 samples from the assessment area with detectable concentrations of total PAHs. A mean PEC quotient of 3.4 was exceeded in 33% of the sediment samples and a mean quotient of 0.63 was exceeded in 70% of the 30 sediment samples from the assessment area. A 50% incidence of toxicity was reported in a database for sediment tests with *H. azteca* at a mean quotient of 3.4 in 10-day exposures and at a mean quotient of 0.63 in 28-day exposures (Ingersoll *et al.* 2001). Results of these chemical analyses of the mean PEC-Q indicate a high frequency of the samples from the assessment area would be expected to be toxic to sediment-dwelling organisms.

Toxicity Test with L. variegatus

At the start of the exposure (day 0), oligochaetes immediately burrowed into the B1 sediment. In contrast, oligochaetes did not burrow into the B2, B4, or B5 sediments (Figure 1). In addition, oligochaetes were observed making a whipping motion on the surface of the B5 sediment. The overlying water in the B3 sediment was too cloudy to observe the oligochaetes on day 0. On day 1, all of the oligochaetes in the B5 sediment appeared dead (as a "white ooze" on the sediment surface). Dead oligochaetes were also observed on the surface of the B3 sediment. Numerous oligochaetes were observed on the surface of the B2 and B4 sediments (oligochaetes on the surface of the B4 sediment were observed making a jerking motion). Throughout the remainder of the 31-day exposure, oligochaetes were observed on the surface of the B2, B3, and B4 sediments, and dead oligochaetes were periodically observed in these sediments. After 31 days, no oligochaetes were retrieved from the B2, B4, or B5 sediments, and only 4 of 20 oligochaetes were retrieved from the B1 sediment and 8 of 20 oligochaetes were retrieved from the B3 sediment.

Results of this toxicity test indicated that the samples from these five locations were too toxic to be used to assess bioaccumulation of contaminants from sediment using *L. variegatus*. This particular species is used for assessing bioaccumulation because it is relatively insensitive to many contaminants typically observed in sediment (US EPA 2000a). The decision was made to collect *Corbicula* spp. from the assessment area to evaluate bioaccumulation from sediment given the observation that this species was observed in the sediment samples evaluated in the toxicity test with *H. azteca* (MacDonald and Ingersoll 2000).

Toxicity Test with H. azteca

Survival of amphipods in both of the controls (Control-WB and Control-FS) was ≥90% at the end of both the first and second set of 10-day sediment exposures (Table 3). All but 1 of the 15 samples (IH-11) from the first set of exposures significantly reduced survival of amphipods relative to both the controls. In the second set of exposures, 4 of the 15 samples (IH-18, IH-25, IH-29, IH-30) significantly reduced survival of amphipods relative to the controls.

Mean length of amphipods was determined in treatments where survival was ≥40% (there were insufficient numbers of amphipods remaining in the treatments with survival <40% to measure length). Length of amphipods was not significantly reduced in any of the treatments relative to both of the controls (Table 3). Amphipods exposed in the formulated sediment (Control-FS) were significantly smaller than those exposed in the sediment from West Bearskin (Control-WB). Kemble *et al.* (1999) also observed lower growth of amphipods exposed for 10 days in formulated sediment compared to sediment from West Bearskin. However, after 28-day exposures, growth of amphipods was similar in formulated and West Bearskin sediments (Ingersoll *et al.* 1998; Kemble *et al.* 1999). This lower growth of amphipods after the 10-day exposures may have resulted from the limited nutritional quality of the materials used to prepare the formulated sediment. With longer exposures, there may have been increased bacterial build-up on the formulated sediment, which may serve as a source of nutrition for the amphipods.

If comparisons are made only to Control-WB, length of amphipods was significantly reduced in 6 of the 20 treatments where growth was measured (IH-03, IH-04, IH-05, IH-11, IH-13, IH-28). However, survival was also reduced in five of these six treatments. This supports the previous findings that growth of amphipods in 10-day sediment tests does not typically provide useful information beyond measuring survival alone; whereas growth of *H. azteca* in 28-day tests often provides unique information regarding sensitivity to contaminants (Ingersoll *et al.* 2001).

About half of the 30 samples had visible oily material in the pore water (IH-01 to IH-07, IH-10, IH-12, IH-27 to IH-30). Of these samples, only two (IH-27 and IH-28) were not toxic to amphipods. Six of the samples (IH-07 to IH-09,

Table 3. Response of *Hyalella azteca* in 10-day exposures and Microtox solid-phase sediment toxicity test to sediment samples from the Grand Calumet River and Indiana Harbor Canal[a]

	Hyalella azteca		Microtox SPT Test	
Samples	Survival (%)	Length (mm)	EC_{50} (Percentage Sediment Wet Weight/ml, 95% Confidence Intervals)	Toxicity Reference Index
First set of exposures				
Control-FS1	98 (2.50)	1.82 (0.05)	NM	NM
Control-WB1[b]	97 (3.33)	2.41 (0.07)	NM	NM
IH-01	0*	ND	0.020 (0.012–0.030)	25*
IH-02	8 (4.79)*	ND	NM	NM
IH-03	40 (10.8)*	1.81 (0.10)	2.5 (2.4–2.6)	0.20
IH-04	55 (15.5)*	1.92 (0.07)	0.26 (0.20–0.32)	1.9*
IH-05	65 (8.66)*	1.70 (0.07)	4.0 (3.3–4.8)	0.13
IH-06	3 (2.50)*	ND	0.40 (0.32–0.51)	1.3*
IH-07	5 (5.00)*	ND	NM	NM
IH-08	70 (4.08)*	2.13 (0.08)	0.77 (0.65–0.91)	0.65
IH-09	80 (4.08)*	2.64 (0.09)	2.9 (2.6–3.1)	0.17
IH-10	3 (2.50)*	ND	0.059 (0.026–0.13)	8.5*
IH-11	80 (7.07)	1.66 (0.07)	NM	NM
IH-12	15 (8.66)*	ND	1.5 (1.3–1.9)	0.33
IH-13	63 (11.8)*	1.72 (0.08)	1.2 (1.1–1.4)	0.41
IH-14	3 (2.50)*	ND	0.020 (0.016–0.022)	25*
IH-15	0*	ND	0.59 (0.53–0.65)	0.85
Second set of exposures				
Control-FS2	90 (4.08)	1.78 (0.05)	NM	NM
Control-WB2	95 (2.89)	2.62 (0.05)	NM	NM
IH-16	90 (6.45)	2.66 (0.08)	NM	NM
IH-17	98 (2.50)	2.88 (0.06)	NM	NM
IH-18	3 (2.50)*	ND	0.051 (0.049–0.053)	9.8*
IH-19	98 (2.50)	2.57 (0.06)	NM	NM
IH-20	90 (4.08)	2.63 (0.06)	NM	NM
IH-21	90 (4.08)	2.86 (0.06)	NM	NM
IH-22	88 (4.79)	2.80 (0.06)	NM	NM
IH-23	70 (12.3)	2.42 (0.05)	0.26 (0.19–0.36)	1.9*
IH-24	93 (4.79)	2.59 (0.06)	NM	NM
IH-25	0*	ND	0.14 (0.10–0.19)	3.6*
IH-26	95 (5.00)	2.87 (0.05)	NM	NM
IH-27	93 (4.79)	2.40 (0.06)	NM	NM
IH-28	80 (7.07)	2.26 (0.06)	NM	NM
IH-29	0*	ND	NM	NM
IH-30	0*	ND	NM	NM

ND = Not determined due to survival $<$ 40%; NM = not measured.

[a] For the *H. azteca* exposures, means (n = 4; SE in parenthesis) with an asterisk within a column and within a set of exposures are significantly different from both of the control treatments (West Bearskin and formulated sediment). For the Microtox SPT test, samples with an asterisk are designated as toxic (TRI with PCP $>$ 1.0).

[b] One replicate in the control lost due to contamination (some sediment from IH-01 inadvertently dropped into one of the West Bearskin beakers on day 0 of the test).

IH-16, IH-19, IH-23) had substantial plant fibers and debris, which made isolation of amphipods at the end of the 10-day exposure difficult. Of these samples, survival of amphipods was marginally (yet significantly) reduced in the IH-08 (70%) and IH-09 (80%) treatments. Therefore, care should be taken in terms of classifying these two samples as toxic or nontoxic. Indigenous invertebrates were periodically observed at the end of the sediment exposures (oligochaetes, mollusks, nematodes, midges); however, the distribution of these organisms was not related to the toxicity of the samples (Ingersoll *et al.* 1999).

Figure 1 illustrates the pattern of toxicity observed in the samples across each of the major reaches in the assessment area. The incidence of toxicity was high across most of the reaches sampled in the assessment area. The exception to this pattern was that no toxicity was observed in the five samples collected from the easternmost portion of the assessment area (from GCRL: samples IH-20 to IH-24). No pattern in the severity of the toxicity of the samples was evident within the assessment area. For example in the LGB reach, none of the amphipods survived in the toxic samples (IH-25, IH-29, and IH-30), whereas survival in nearby nontoxic samples ranged from 80% to 95% (IH-26, IH-27, and IH-28; Figure 1 and Table 3). Similar variation in survival of amphipods was observed in the IHC and EBGCR-I reaches of the assessment area (Figure 1).

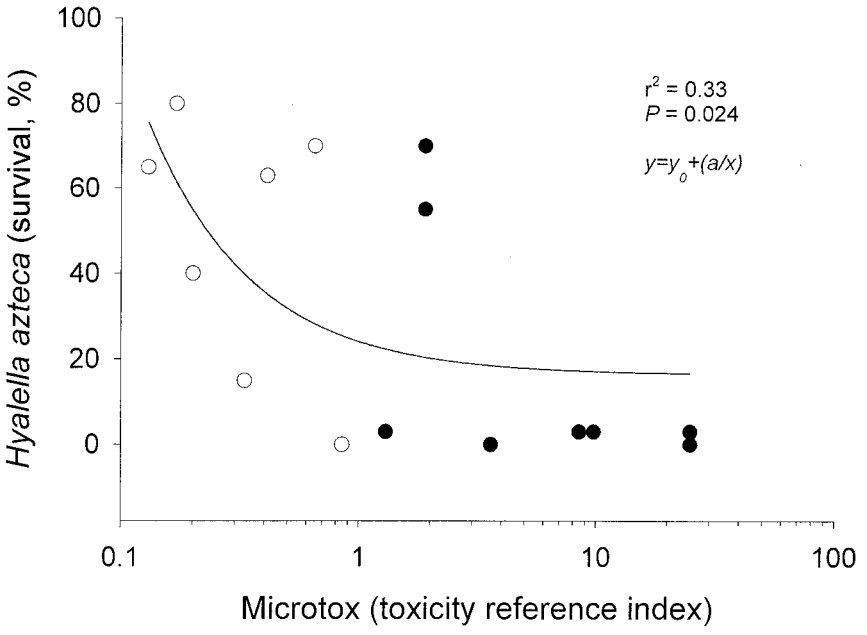

Fig. 2. Comparison of the response in the Microtox SPT test (EC_{50} expressed as TRI) to select toxic samples identified in the exposures with *Hyalella azteca* (percent survival). The closed symbols represent samples identified as toxic in the Microtox SPT test (Table 3)

Microtox SPT Test

A total of 15 samples that were identified as toxic in the amphipod exposures (Table 3) were also evaluated using the Microtox SPT test. Only 8 of these 15 samples were determined to be toxic in the Microtox SPT test (TRI $>$ 1.0; Table 3). Sediment identified as toxic in the Microtox SPT test had a TRI that ranged from 1.3 to 25 times greater than that of the PCP-spiked control sediment. Figure 2 illustrates the relationship between survival of amphipods and the Microtox TRI for these 15 samples (SigmaPlot 2000, line of best fit based on r^2). Of the seven samples in which survival of amphipods ranged from 0 to 5%, six were identified as toxic in the Microtox SPT test. However, in samples where survival of amphipods ranged from 15% to 80%, all were identified as nontoxic in the Microtox SPT test (Figure 2). Kemble *et al.* (2000) compared the response of the Microtox SPT test to the response of *H. azteca* in 28-day sediment tests and found that the Microtox SPT test generally identified all of the sediments that were lethal to amphipods. However, samples that reduced growth of amphipods in 28-day tests were often identified as not toxic in the Microtox SPT test. These data indicate that the response of *H. azteca* in either 10- or 28-day tests are more sensitive than the Microtox SPT test. A sample in the Microtox SPT test was identified as toxic only if it was more toxic than the control sampled spiked with PCP (TRI $>$ 1.0; Table 3). If toxicity were to be established as a TRI $>$ 0.2, there would be a 85% correspondence between the 10-day test with *H. azteca* and the Microtox SPT test (Figure 2). Additional research is needed to determine if toxicity in the Microtox SPT test should be established relative to reference conditions at a location of interest or if toxicity should be established relative to a mixture of chemicals or just PCP spiked into a control sediment (Johnson and Long 1998).

Comparisons of Sediment Characteristics to Toxicity Responses

No significant correlations were observed between grain size or TOC and either survival or length of amphipods or Microtox TRI (Spearman rank correlation $p > 0.005$; Table 4). Similarly, no significant correlations were observed between the chemistry of the pore water listed in Table 2 and either survival or growth of amphipods or Microtox TRI except for the significant negative correlation between total ammonia and amphipod length ($p < 0.005$; Table 4). Concentrations of total ammonia in pore water were elevated in many of the samples. However, samples with concentrations of total ammonia ranging from 7.17 to 18.3 mg/L (samples IH-17, IH-22, IH-23, IH-24, IH-27, and IH-28; Table 2) were not toxic to amphipods (Table 3). The concentrations of total ammonia in all of the samples were below the reported 10-day LC_{50} for *H. azteca* of 105 mg/L for pore water (Whiteman *et al.* 1996). This value was obtained in soft water and may represent a relatively low toxicity threshold for ammonia compared to the pore water in the present study (Ankley *et al.* 1995; Table 2). Additionally, Whiteman *et al.* (1996) reported that *H. azteca* avoided lethal concentrations of ammonia in sediment. Therefore, the toxicity of the sediments to *H. azteca* in the present study could not be attributed to the elevated concentrations of ammonia in pore water.

Results of the SEM and AVS analysis of the whole-sediment samples indicated that 50% of the samples from the assessment area had an excess of molar SEM concentrations relative to AVS (Table 1, Figure 3). A lower percentage of the samples (32%) had a sum toxic unit (TU) above 1.0 for pore water concentrations of cadmium, copper, nickel, or zinc (based on 10-day LC_{50} concentrations for *H. azteca* in water-only exposures; Table 1). Elevated concentrations of metals in pore water and associated toxicity might be expected when the molar

Table 4. Spearman rank correlation for toxicity data and the physical or chemistry data presented in Tables 2 and 3

Variable	Toxicity Endpoint		
	H. azteca Survival	*H. azteca* Length	Microtox TRI
Pore water ammonia (total)	−0.26 (26)	−0.72 (15)*	−0.13 (13)
Pore water ammonia (unionized)	−0.37 (26)	−0.45 (15)	0.03 (13)
Grain size	0.36 (25)	0.14 (15)	0.15 (12)
TOC	−0.05 (29)	0.04 (18)	−0.23 (15)
SEM-AVS	−0.30 (29)	−0.43 (18)	−0.15 (15)
Toxic units metals	−0.11 (27)	−0.031 (17)	−0.16 (14)
Total PCBs	−0.66 (25)*	−0.57 (15)	0.16 (13)
Total PAHs	−0.64 (29)*	−0.38 (18)	0.30 (15)
Average PEC quotient for metals	−0.74 (29)*	−0.49 (18)	0.53 (15)
Mean PEC quotient	−0.77 (29)*	−0.57 (18)	0.60 (15)

Significant correlations are designated with an asterisk at $p < 0.005$ (sample number in parentheses)

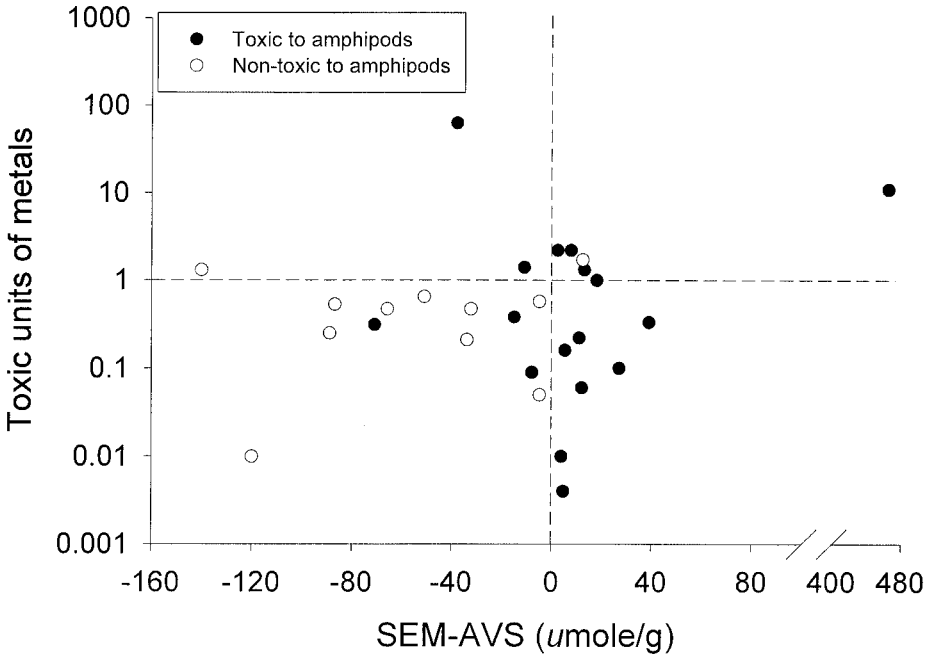

Fig. 3. The relation between the molar concentration of simultaneously extracted metals to acid volatile sulfide (SEM-AVS) and toxic units of metals in the sediment samples

concentration of SEM exceeds AVS (Ankley *et al.* 1996). In the present study, no significant correlation was observed between SEM-AVS and TU for metals ($p > 0.005$). Of the samples with SEM-AVS > 0, only 38% had pore water TU for metals > 1.0 (Figure 3). The lack of correspondence between SEM-AVS and pore water metals indicates that there are variables in addition to AVS controlling the concentrations of metals in pore water (Figure 3). For example, TOC has also been shown to play an important role in the partitioning of metals into pore water (Ankley *et al.* 1996). However, there was a higher correspondence between SEM-AVS and pore water metals at elevated concentrations of metals in pore water (of the nine samples with TU > 1.0 for metals, 67% of these samples had SEM-AVS > 0; Figure 3). U.S.EPA (1997) suggests SEM-AVS > 5 to be a better predictor of metal toxicity in sediments rather than SEM-AVS > 0. In the present study, there were several samples that were toxic with an excess of SEM-AVS between 0 and 5 (Figure 3). However, other chemicals may have been contributing to the toxicity of the samples. Furthermore, the one sample with SEM-AVS of 12.3 was not toxic to amphipods. Hence, the utility of a cut point of SEM-AVS > 5 in the present study could not be adequately evaluated.

The incidence of toxicity in the amphipod exposures was 87% at SEM-AVS > 0 and was 78% at porewater TU > 1.0 (Figure 3). In contrast, the incidence of toxicity was only 25% in samples with SEM-AVS < 0 and porewater TU < 1.0 (Figure 3). Results of these analyses indicate that metals likely caused or substantially contributed to toxicity observed in some of the sediment samples from the assessment area.

A significant negative correlation was observed between survival of amphipods and total PCBs, total PAHs, average PEC-Q for metals, or the mean PEC-Q measured in whole sediments (Spearman rank correlation $p < 0.005$; Table 4). A significant negative correlation was also observed between length of amphipods and the concentration of total ammonia

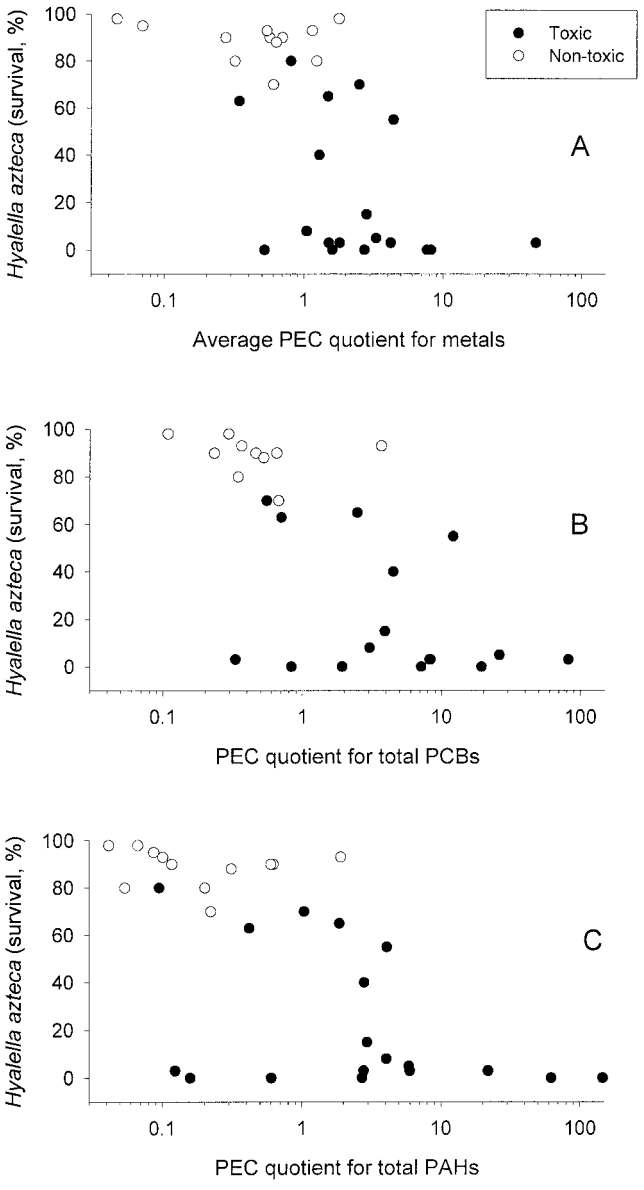

Fig. 4. The relation between the PEC quotient for metals, PCBs, or PAHs and the response of *H. azteca* in the 10-day tests (as percent survival)

measured in pore water. No other significant correlations were observed between these variables and the length of amphipods or Microtox TRI ($p > 0.005$). Figure 4 illustrates the relationship between survival of amphipods and the average PEC-Q for metals (A), the PEC-Q for total PCBs (B), or the PEC-Q for total PAHs (C). With increasing quotients for metals, PCBs, or PAHs, there was an increasing degree of toxicity observed in the amphipod test. For metals, total PCBs, and total PAHs, samples with a PEC-Q at or above 1.0 to 2.0 were frequently toxic to amphipods. However, there were some samples with quotients ranging from 0.1 to 1.0 that were also toxic to amphipods (Figure 4). In these instances, multiple chemicals may be contributing to the observed toxicity. Therefore, the mean PEC-Q based on metals, total PCBs, and total PAHs was calculated for each sample to weight equally the contribution of metals, PCBs, and PAHs in the evaluation of sediment chemistry and toxicity.

Figure 5 illustrates the relationship between survival of amphipods and mean PEC-Q in the sediment samples from the assessment area. In the amphipod test, all of the samples with a mean quotient > 2.0 were toxic ($n = 13$), none of the samples with a mean quotient < 0.3 were toxic ($n = 4$), and 38% of the samples between a mean quotient of 0.3 to 2.0 were toxic ($n = 13$). Plotting the data based on mean PEC-Q (Figure 5) reduced the variability in toxicity observed at quotients < 1.0 based on metals, PAHs, or PCBs alone (Figure 4). A 50% reduction in survival was estimated at a mean quotient of 2.3 (see Figure 5 for description of the equation for this line; SigmaPlot 2000). Results of these analyses are similar to the findings reported in Ingersoll *et al.* (2001) where a 50% incidence in toxicity was observed in a database for sediment tests with *H. azteca* at a mean quotient of 3.4 in 10-day exposures ($n = 670$) and 0.63 in 28-day exposures ($n = 160$).

Toxicity in the Microtox SPT test also increased with increasing mean PEC-Q in the sediment samples (Figure 5). In the Microtox SPT test, 86% of the samples with a mean quotient > 3.0 were toxic ($n = 7$), whereas only 25% of the samples with a mean quotient between 0.45 and 3.0 were toxic ($n = 8$; no samples were tested at a mean quotient < 0.45). Kemble *et al.* (2000) reported a low incidence in toxicity in the Microtox SPT test at a mean quotient > 1.0 (50% toxicity was observed in 12 samples from Waukegan Harbor located in western Lake Michigan). It is not surprising to find a lower correspondence between PECs and the response of bacteria given that the PECs described in MacDonald *et al.* (2000) were derived using whole-sediment toxicity tests with benthic invertebrates.

In summary, the results of this study and previous studies demonstrate that sediments from the assessment area are among the most contaminated and toxic sites that have ever been reported (Lucas and Steinfeld 1972; Hoke *et al.* 1993; Burton 1994; Jop and Putt 1994; Canfield *et al.* 1996; Ingersoll *et al.* 1996; US EPA 1997; MacDonald and Ingersoll 2000). More samples were identified as toxic in the amphipod tests compared to the Microtox test, indicating that the 10-day *H. azteca* test was more sensitive than the Microtox test. A combination of PEC-Qs, SEM and AVS, and measures of pore water metals were used to determine that concentrations of metals, PAHs, and PCBs in sediments from the assessment area were sufficient to cause or substantially contribute to the observed sediment toxicity in the tests with amphipods and with Microtox (MacDonald *et al.* 2000). Companion papers by MacDonald *et al.* (2002a, 2002b) describe the findings from an integrated injury assessment for the assessment area using the results of the current study along with the results of historic studies on sediment toxicity, chemistry, bioaccumulation, benthic invertebrate evaluations, and fish community assessments.

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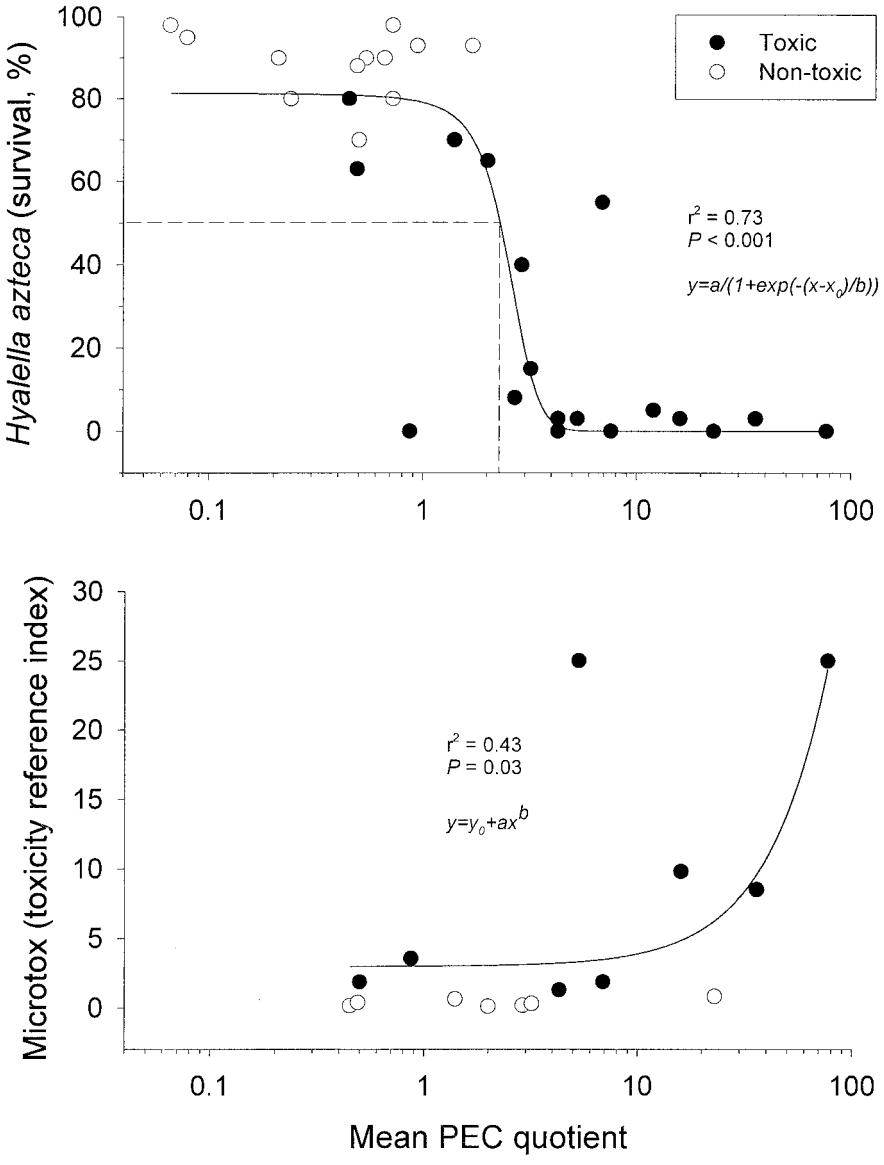

Fig. 5. The relation between the mean PEC quotient and the response of *H. azteca* in the 10-day tests (as percent survival) or the response in the Microtox SPT test (as the EC_{50} expressed as the TRI)

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