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Ecological Effects of Sediment-Associated Contaminants in Inner Burlington Harbor, Lake Champlain

Lake Champlain Basin Program

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Lake Champlain Basin Program Technical Reports

1. *A Research and Monitoring Agenda for Lake Champlain.* Proceedings of a Workshop, December 17-19, 1991, Burlington, VT. Lake Champlain Research Consortium. May, 1992.

2. *Design and Initial Implementation of a Comprehensive Agricultural Monitoring and Evaluation Network for the Lake Champlain Basin.* NY-VT Strategic Core Group. February, 1993.

3. (A) *GIS Management Plan for the Lake Champlain Basin Program.* Vermont Center for Geographic Information, Inc., and Associates in Rural Development. March, 1993.

   (B) *Handbook of GIS Standards and Procedures for the Lake Champlain Basin Program.* Vermont Center for Geographic Information, Inc. March, 1993.

   (C) *GIS Data Inventory for the Lake Champlain Basin Program.* Vermont Center for Geographic Information, Inc. March, 1993.

4. (A) *Lake Champlain Economic Database Project. Executive Summary.* Holmes & Associates. March 1993.

   (B) *Socio-Economic Profile, Database, and Description of the Tourism Economy for the Lake Champlain Basin.* Holmes & Associates. March 1993

   B) *Socio-Economic Profile, Database, and Description of the Tourism Economy for the Lake Champlain Basin. Appendices.* Holmes & Associates. March 1993

   (C) *Potential Applications of Economic Instruments for Environmental Protection in the Lake Champlain Basin.* Anthony Artuso. March 1993.

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5. *Lake Champlain Sediment Toxics Assessment Program. An Assessment of Sediment - Associated Contaminants in Lake Champlain - Phase 1.* Alan McIntosh, Editor, UVM School of Natural Resources. February 1994.

   Lake Champlain Sediment Toxics Assessment Program. An Assessment of Sediment - Associated Contaminants in Lake Champlain - Phase 1. Executive Summary. Alan McIntosh, Editor, UVM School of Natural Resources. February 1994.

6. *(A) Lake Champlain Nonpoint Source Pollution Assessment.* Lenore Budd, Associates in Rural Development Inc. and Donald Meals, UVM School of Natural Resources. February 1994.

   (B) Lake Champlain Nonpoint Source Pollution Assessment. Appendices A-J. Lenore Budd, Associates in Rural Development Inc. and Donald Meals, UVM School of Natural Resources. February 1994.

7. *Internal Phosphorus Loading Studies of St. Albans Bay. Executive Summary.* VT Dept of Environmental Conservation. March 1994.

   (A) *Dynamic Mass Balance Model of Internal Phosphorus Loading in St. Albans Bay, Lake Champlain.* Eric Smeltzer, Neil Kamman, Karen Hyde and John C. Drake. March 1994.

   (B) *History of Phosphorus Loading to St. Albans Bay, 1850 - 1990.* Karen Hyde, Neil Kamman and Eric Smeltzer. March 1994.

   (C) *Assessment of Sediment Phosphorus Distribution and Long-Term Recycling in St. Albans Bay, Lake Champlain.* Scott Martin, Youngstown State University. March 1994.

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11. (A) *Report on Institutional Arrangements for Watershed Management of the Lake Champlain Basin. Executive Summary.* Yellow Wood Associates, Inc. January 1995.

    (B) *Report on Institutional Arrangements for Watershed Management of the Lake Champlain Basin.* Yellow Wood Associates, Inc. January 1995.

    (C) *Report on Institutional Arrangements for Watershed Management of the Lake Champlain Basin. Appendices.* Yellow Wood Associates, Inc. January 1995.

12. (A) *Preliminary Economic Analysis of the Draft Plan for the Lake Champlain Basin Program. Executive Summary.* Holmes & Associates and Anthony Artuso. March 1995

    (B) *Preliminary Economic Analysis of the Draft Plan for the Lake Champlain Basin Program.* Holmes & Associates and Anthony Artuso. March 1995

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18. *Catalog of Digital Spatial Data for the Lake Champlain Basin .* Vermont Center for Geographic Information, Inc. September 1996.

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23. (A) *Lake Champlain Sediment Toxics Assessment Program. An Assessment of Sediment - Associated Contaminants in Lake Champlain - Phase 11. Executive Summary.* Alan McIntosh, Mary Watzin and Erik Brown, UVM School of Natural Resources. October 1997

    (B) Lake Champlain Sediment Toxics Assessment Program. An Assessment of Sediment - Associated Contaminants in Lake Champlain - Phase 11. Alan McIntosh, Mary Watzin and Erik Brown, UVM School of Natural Resources. October 1997

24. *Development of Land Cover/Land Use Geographic Information System Data Layer for the Lake Champlain Basin and Vermont Northern Forest Lands Project Areas.* Dr. Thomas Millette. October 1997

25. *Urban Nonpoint Pollution Source Assessment of the Greater Burlington Area.* Urban Stormwater Characterization Project. James Pease, VT Dept. of Environmental Conservation. December 1997

26. *Long-Term Water Quality and Biological Monitoring project for Lake Champlain.* Cumulative Report for Project Years 1992- 1996. VT Dept of Environmental Conservation and NYS Dept of Environmental Conservation. March 1998.

27. *Cumberland Bay PCB Study.* Clifford W Callinan, NY State Dept. of Environmental Conservation; Lyn McIlroy, Ph.D., SUNY Plattsburgh; and Robert D. Fuller, PhD., SUNY Plattsburgh. October 1998.

28. *Lake Champlain Underwater Cultural Resources Survey. Volume 1: Lake Survey Background and 1996 Results.* Scott A. McLaughlin and Anne W. Lessman, under the direction of Arthur B. Cohn, Lake Champlain Maritime Museum. December 1998.

29. *Evaluation of Soil Factors Controlling Phosphorus Concentration in Runoff from Agricultural Soils in the Lake Champlain Basin.* Frederick R. Magdoff, William E. Jokela, and Robert P. Durieux, UVM Department of Plant and Soil Sciences. June 1997.

30. *Lower Trophic Level Interactions in the Pelagic Foodweb of Lake Champlain.* Dr. Suzanne N. Levine, Dr. Mark Borchardt, Dr. Moshe Braner, Angela Shambaugh, and Susan Spencer of UVM School of Natural Resources and Marshfield Medical Research Foundation. July 1997.

31. Estimation of Lake Champlain Basinwide Nonpoint Source Phosphorus Export, William Hegman, Associates in Rural Development, Inc., Deane Wang and Catherine Borer, UVM Water Resources & Lake Study Center, September 1999.

32. *The Freshwater Mussels of the Lower Missisquoi Rivers: Current Status and the Potential for a Refugium from Zebra Mussel Impacts.* Paul Marangelo, VT Agency of Natural Resources, Dept of Environmental Conservation. August 1999.

33. *Ecological Effects of Sediment-Associated Contaminants in Inner Burlington Harbor, Lake Champlain.* Tetra Tech, Inc. September 1999.

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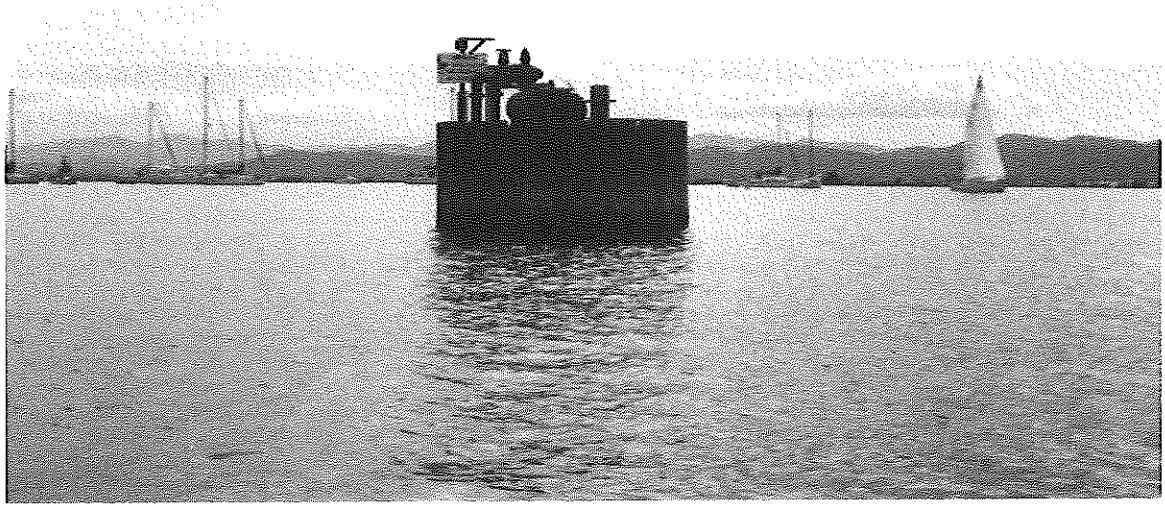
ECOLOGICAL EFFECTS OF SEDIMENT-ASSOCIATED CONTAMINANTS IN INNER BURLINGTON HARBOR, LAKE CHAMPLAIN



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EXECUTIVE SUMMARY

This project analyzed and compared current sediment and benthic ecological conditions in Burlington Harbor, Vermont with data collected previously. Twenty sites (10 relatively clean and 10 possibly impaired) were sampled in summer 1997 for whole sediment toxicity; sediment chemical parameters, including polycyclic aromatic hydrocarbons (PAHs), select metals, and several physicochemical parameters; vertical water column profile characteristics; organism tissue PAHs, lead, and protein expression (biomarkers); and benthic macroinvertebrate community integrity.

Highest concentrations of PAHs were observed near the old wastewater treatment plant outfall, the public boathouse and docks, and near the breakwater in the southern part of the harbor. Sediment PAH concentrations were considerably higher if based on the fine particle size fraction only. Metals were highest at the two different "reference" sites and at two sites near the breakwater. These results were consistent with previous observations from 1993. The ratio or difference of simultaneous extracted metals (SEM) to acid volatile sulfide (AVS) generally indicated low metal concentrations in interstial (pore) water at most sites. However, AVS was especially low ($\leq$ 1 μmole/g) at the two reference sites, suggesting that AVS may be an inappropriate parameter with which to evaluate potential metal toxicity at those sites. The spatial pattern of contaminants was consistent with earlier results; however, there were a significantly lower concentration of several metals and PAHs in surficial sediments compared to 3-4 years

ago. The decrease in sediment contaminants is coincident with the relocation of the Burlington municipal sewage outfall.

Hyalella survival was more sensitive to sediment characteristics than the fish endpoints and generally predicted greater impacts. Forward stepwise multiple regression analyses indicated that larval fish weight was related to SEM/AVS and lead concentration ($R^2 = 0.60$, $p < 0.02$). *Hyalella* survival was related to lead and log PAH sediment concentrations ($R^2 = 0.57$, $p < 0.01$). Follow-up acute and chronic (28 d) sediment toxicity tests with *Hyalella* on samples collected in spring 1998 confirmed sediment toxicity at several sites in the southern end of the harbor, while the "reference" site, in the northern part of the harbor, exhibited little or no toxicity.

Bioaccumulation tests using the worm *Lumbriculus* indicated that PAHs were not accumulated over a 28-day exposure period; however, lipid content of the worms was low; thus PAH bioaccumulation results were questionable. Lead showed very low accumulation factors based on sediment chemistry data collected in summer 1997 (BAFs <1.0), however, these data are suspect given the temporal and spatial heterogeneity observed in harbor sediment characteristics. Protein expression analyses indicated that several proteins were induced or suppressed in fish exposed to more contaminated sediments in the harbor. These results were consistent with growth effects observed in chronic fish toxicity testing.

A major difference between this and earlier biological studies of the harbor was a substantial increase in the number of zebra mussels, particularly in areas < 7 meters in depth. Forward stepwise multiple regression analyses indicated that benthic assemblage characteristics were

related to several physicochemical factors, including ammonia, organic nitrogen, organic carbon, and lead. PAH and SEM/AVS were often significant factors in regression models, but these were usually positively associated with benthic metrics, contrary to our expectations. However, two benthic metrics, percent dominance within the Chironomidae family and percent Oligochaetes, were directly related to sediment PAH concentration, consistent with the hypothesis that PAHs were affecting some components of the benthic invertebrate community in the harbor. Using sediment physicochemical measures to classify sites in the harbor, we observed two groups of sites in this study: one group consisted of breakwater and reference sites and the second group consisted of sites near either the old wastewater plant outfall, the oil dolphin, or the public boat docks. We observed that percent dominance within the Chironomidae family, and possibly percent Oligochaetes, were significantly different between these two site classes. Both of these fauna are typically indicative of sediment contamination.

A weight of evidence approach suggested that PAHs are likely to have long-term effects on biota and that fauna at sites nearest the old WWTP outfall or near the southern end of the breakwater are most at risk. Lead, zinc, copper, and silver exceeded NOAA ERL values and other chronic toxicity benchmarks at several sites in the harbor, however, lead was the only metal that was statistically related to biological effects. Given the fact that SEM/AVS ratios were generally < 1.0 in this study, metal concentrations in interstitial water were probably very low and theoretically not toxic. Thus, metal toxicity effects observed in this study were probably due to ingestion of contaminated sediments as opposed to interstitial water exposure. These results suggest that SEM/AVS may not be a reliable indicator of metal toxicity in harbor sediments.

Our results suggested that ingestion of metal-contaminated particles may be a source of toxicity to benthic biota in the harbor.

A qualitative examination of available data collected in this study, using the sediment quality triad approach, suggested that aquatic life was most at risk at sites closest to the old wastewater outfall and the public docks. Fauna at sites near the breakwater or in the northern part of the harbor appear to be less at risk due to sediment contaminants.

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1.0 INTRODUCTION

The Inner Burlington Harbor of Lake Champlain has received numerous toxicants from point and nonpoint sources in its watershed. Previous sediment sampling and analyses (McIntosh et al. 1996) demonstrated relatively high concentrations of silver, lead, and PAHs in the harbor, especially in the southern end, compared to sites outside the breakwater. This area of the harbor is near the old sewage outfall and oil dolphins but could also receive contaminants from the old rail yard and nonpoint sources in and around Burlington. That the surficial sediment (top 2-3 cm) at most sites had lower pollutant concentrations than sediments at greater depths suggests reduced inputs of these pollutants in recent history (past 30 years). However, these studies also indicated substantial temporal and spatial heterogeneity with respect to sediment contaminant concentrations and toxicity.

The case for toxic effects within Burlington Harbor was unclear based on previous work (McIntosh et al. 1996). Therefore, ecological and biological stress components, in addition to bioavailability and toxicity tests, were incorporated into the present study to establish a weight of evidence for the actual effects of the toxic contaminants in Inner Harbor. The overall objective of this project was to assess the hazard due to toxic contaminants in the sediments of Inner Burlington Harbor using a sediment quality triad approach (Chapman et al. 1992). Because certain potentially toxic contaminants were known to occur in Burlington Harbor, the objective of this project was divided into three major component questions.

- Have toxic sediments altered benthic communities of Burlington Harbor?
- Could such changes affect other ecological components of Lake Champlain?

- Do the toxic contaminants in Burlington Harbor sediments accumulate up the food chain and cause risks to higher terrestrial and aquatic trophic levels and human health?

2.0 METHODS

2.1 Sampling Design

Earlier work (McIntosh et al. 1996) indicated that most of the sites with high silver, lead, and PAH concentrations (the chief pollutants recognized) were located in the southern end of the harbor, from site BH20 south (Figure 2-1). Sampling locations in the present study were identified by reanalyzing the 1993-94 data from the harbor with a spatial statistical model known as kriging to estimate contaminant concentrations and uncertainties throughout the harbor (Appendix A).

Kriging is a geostatistical estimation method which incorporates a model of the spatial variability of data directly. The technique used here was ordinary kriging, which produces minimum-variance estimates by taking into account the variogram generated. A variogram is a plot of the average squared differences between data values as a function of separation distance and shows the general pattern of variability in a spatial framework using a geographic information system (GIS). There are four standard variogram models that could be generated: spherical, exponential, Gaussian and power models. For each chemical, the variogram was calculated and fitted with each of the four models by a non-linear least-squared procedure provided by SAS (SAS Institute 1997). Due to the small number of data points provided in the Phase 2 study for the inner harbor area (McIntosh et al. 1996), only 8 variograms were successfully modeled: copper, aluminum, iron, manganese, nickel, silver, nitrogen, and phosphorus. Because silver was highly correlated with PAH in the Phase 2 study (Pearson Correlation coefficient $r = .761$, $p = .0001$, $N = 20$; McIntosh et al. 1996), we could infer PAH spatial variability from that derived for silver.

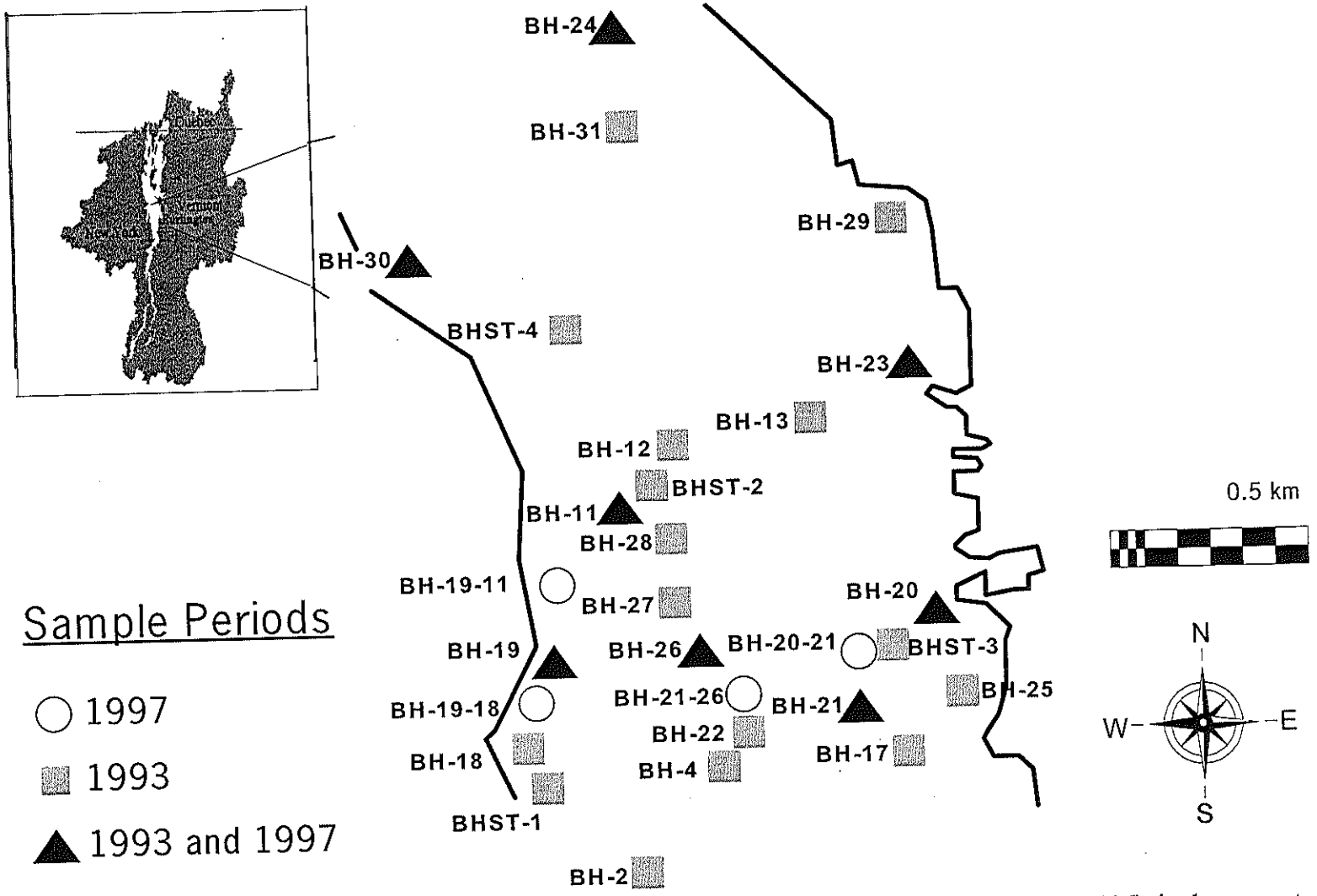

Figure 2-1. Map showing sites sampled in inner Burlington Harbor in 1993 only (McIntosh et al. 1996), in the present study only, and those sampled in both studies.

The sampling sites selected for the present study were those with the greatest uncertainty (using existing data), and the highest likelihood of contamination (Figure 2-1). Tetra Tech sampled 10 sites in the harbor and collected 10 samples from two different sites with historically low contaminant concentrations or toxicity ("reference sites") to help assess sediment quality in the harbor. Five reference samples were collected from site BH30 inside the harbor to take into account the background disturbance regime (due to ferry and other boat traffic). The remaining 5 reference samples were collected from site BH24 outside the harbor but nearby geographically (Figure 2-1) in an attempt to assess relatively undisturbed lake sediment conditions. Two sites (BH11 and BH23) were replicated once to obtain a quantifiable measure of the variability or uncertainty surrounding physicochemical measures obtained in this study. Replication was accomplished by compositing the first set of grabs for one sample, washing all equipment in lake water, repositioning the boat using differential global positioning, and collecting and compositing a second set of grabs for the same site. Eight of the sites sampled in this study were also sampled in the previous study (Figure 2-1).

Volunteers assisted in field sampling, sample processing, *in-situ* water column profile analyses (temperature, pH, dissolved oxygen, conductivity, Hydrolab field analyzer), and sample preservation. To ensure sample integrity and accurate data, several quality control steps were taken regarding volunteer participation. First, standard operating procedures (SOPs) concerning sample processing, measurement of water column parameters, and data recording were prepared by Tetra Tech and reviewed with the volunteers prior to sampling. Second, each sampling team was provided with SOPs and directed by a Tetra Tech staff member. Third, Tetra Tech conducted all sampling and supervised all water quality measurements and field notes made by volunteers. Fourth, Tetra

Tech staff supervised all benthic organism sorting conducted by volunteers on site and inspected and re-picked all samples sorted by volunteers.

2.2 Sediment Sampling and Types of Analyses Performed

Table 2-1 summarizes the types of analyses and timing of those analyses, as well as general location, for each site in this study. Benthic fauna and sediments were sampled in August 1997 similar to the timing of some of the previous studies. Sites were identified using differential global positioning and checked frequently during sampling to ensure proper location. Each site was sampled using a composite of 5-7 petite Ponar grabs as was previously done for toxicity, benthos, and surficial sediment chemical analyses (ASTM 1995a, McIntosh et al. 1996). Acceptability of sediment samples was judged using several criteria outlined in the approved Quality Assurance Project Plan (Tetra Tech 1997). If these criteria were not met, the sample was discarded and the site was resampled. Contents of three petite Ponar samples from each site were composited for benthic invertebrate analyses. The remaining 2-4 petite Ponar samples were composited and homogenized in the field using Teflon® or high density plastic equipment to obtain a representative sample from each site for chemical and toxicological analyses (ASTM 1995b). At each site, depth and sediment characteristics were recorded on field log sheets (Appendix B). Samples were handled and stored following standard operating procedures (Tetra Tech 1997), and samples were recorded on sample chain-of-custody forms for shipping off-site for analysis.

Table 2-1. Sampling site locations and analyses conducted. B = breakwater, O = near old outfall and oil dolphin, BH = boathouse, SCH = south, central harbor, REF = reference

Site	General Location	August 1997						May 1998			
			Sediment Chemistry		Toxicity Testing				Toxicity Testing		
		Tissue Analysis (Mussels)	Organics/ PAH/ Metals	PAH Fine Fraction	*P. promelas* (*protein expression analyses)	*H. azteca* 10 day	Benthic invertebrate analyses	Bioaccumulation *L. variegatus*	TIE	*H. azteca* 10 day	*H. azteca* 28 day
Control					✓	✓		✓	✓		✓
BH11	B		✓		✓*	✓	✓				
BH11B	B		✓		✓	✓					
BH19	B		✓		✓*	✓	✓				
BH19-11	B		✓		✓	✓	✓				
BH19-18	B		✓		✓	✓	✓			✓	✓
BH20	O	✓	✓		✓*	✓	✓				
BH20-21	O	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
BH21	O	✓	✓	✓	✓*	✓	sample lost	✓		✓	✓
BH21-26	SCH		✓		✓	✓	✓		✓	✓	✓
BH23	BH	✓	✓		✓	✓	✓	✓		✓	✓
BH23B	BH		✓	✓	✓	✓					
BH24 composite	REF	✓	✓		*						
BH24-1	REF				✓	✓	✓				
BH24-2	REF				✓	✓	✓				

Table 2-1. Sampling sites and analyses conducted (continued)

Site	General Location	August 1997						May 1998			
			Sediment Chemistry		Toxicity Testing				Toxicity Testing		
		Tissue Analysis (Mussels)	Organics/ PAH/ Metals	PAH Fine Fraction	*P. promelas* (*protein expression analyses)	*H. azteca* 10 day	Benthic invertebrate analyses	Bioaccumulation *L. variegatus*	TIE	*H. azteca* 10 day	*H. azteca* 28 day
BH24-3	REF				✓	✓	✓				
BH24-4	REF				✓	✓	✓				
BH24-5	REF				✓	✓	✓				
BH26	SCH		✓		✓*	✓	✓				
BH30 composite	REF	✓	✓							✓	✓
BH30-1	REF				✓	✓	✓				
BH30-2	REF				✓	✓	✓				
BH30-3	REF				✓	✓	✓				
BH30-4	REF				✓	✓	✓				
BH30-5	REF				✓	✓	✓				

Table 2-2 summarizes the analyses performed in this study, the methods used, and detection limits. Sediment chemical analyses included PAHs, simultaneously extracted metals (SEM), total organic carbon (% TOC), acid volatile sulfides (AVS), total organic nitrogen (TON), ammonia, particle size, and pH. Five metals (those showing the highest levels previously and those which are also used in the simultaneous extraction method) (Ankley et al 1996: silver, nickel, copper, lead, and zinc) were measured.

Table 2-2. Summary of parameters analyzed, methods used, and detection limits.

Parameter	Matrix	Method	Detection Limit
particle size	sediment	ASTM D4882 (1992)	1%
total organic carbon	sediment	USEPA - 9060	0.1%
total organic nitrogen	sediment	USEPA - 350.2 and 351.3	100 µg/Kg
ammonium	sediment	USEPA - 350.1	100 µg/Kg
pH	sediment	APHA 423	0.2 units
AVS	sediment	FGS - 0036	1.33 mg/Kg
SEM: copper, nickel, lead, zinc, silver	sediment	FGS - 0036/USEPA 1638 or 1639	1 mg/Kg
PAHs	sediment	USEPA 8270, 8310	5 µg/Kg
10 d *Hyalella* test	sediment	USEPA (1994)	N/A
28 d *Hyalella* test	sediment	USEPA (1996 draft)	N/A
7 d fathead minnow test	sediment	USEPA (1991a)	N/A
28 d *Lumbriculus* test	sediment	USEPA (1994)	N/A
Benthic macroinvertebrate assessment	sediment	Tetra Tech (1997)	N/A
temperature	water	BRF 050, APHA 2550	0.1° C
dissolved oxygen	water	BRF 050, APHA 4500-0(G)	0.2 mg/L
conductivity	water	BRF 050, APHA 2510	20 µhos/cm
pH	water	BRF 050, APHA 4500-HA+	0.2 units
organism protein expression patterns	tissue	Bradley et al. (1996)	0.1 µg/Kg
PAHs	tissue	USEPA 8270/8310	20 µg/Kg
lead	tissue	USEPA 6010/200.7	250 µg/Kg

Portions of the samples from three inner harbor sites were sieved to isolate the fine fraction less than 63μm and were also analyzed for PAHs, TOC, and TON because recent research has suggested that PAH bioavailability is much more strongly correlated with the concentration present in the fine fraction than in the sediment as a whole, particularly if cellulose fibers (such as from plant material) or gravel are present (Landrum pers. comm.).

Sediment grab samples are subject to chemical, biological, and physical changes as soon as they are collected. Therefore, sample handling, preservation, and storage techniques were designed to minimize any changes in composition of the sample by retarding chemical and/or biological activity and by avoiding contamination (ASTM 1995b). Collection methods, volume requirements, container specifications, preservation techniques, storage conditions, and holding times for sediment samples (from the time of sample collection) are summarized in Table 2-3.

Samples to be analyzed for trace metals did not come into contact with metal other than the stainless steel petite Ponar sampler, and samples to be analyzed for PAHs were stored in polyethylene containers. All sample containers were scrupulously cleaned (acid-rinsed for analysis of metals, solvent-rinsed for analysis of organic compounds) prior to use. All samples were placed in chilled storage (< 4°C) and shipped in insulated coolers overnight on ice to their final destination of analysis. The elapsed time between sample collection and analysis was as short as possible, never more than one week.

Table 2-3. Summary of procedures used for sediment sample collection, preservation, and storage.

Analyses	Sample Volume	Container	Preservation Technique	Storage Conditions	Holding Times
Sediment Chemical/Physical Analyses					
Simultaneous extracted metals (copper, nickel, lead, zinc, silver); acid volatile sulfide	100 g	Precleaned polyethylene jar	Refrigerate	≤ 4°C	7 days
Sediment: Polycyclic aromatic hydrocarbons	250 g	Solvent-rinsed polyethylene jar with Teflon® lid	Refrigerate	≤ 4°C/dark	14 days
tissue: PAHs and lead	250 g	Solvent-rinsed glass jar with Teflon® lid	Refrigerate	≤ 4°C/dark	14 days
Particle size	100 g	Whirl-pac bag	Refrigerate	< 4°C	28 days
Total organic carbon	50 g	Precleaned polyethylene container with Teflon®-lined lid	Refrigerate	≤ 4°C	14 days
Organic nitrogen Ammonia	≥ 50 g	Whirl-pac bag	Refrigerate	< 4°C	14 days
Sediment Toxicity Tests					
Reference and test sediments	1-2 L per site	Plastic bag or container	Completely fill and refrigerate	4°C/dark/ airtight	14 days
Benthic Macroinvertebrate Analyses					
Reference and test sediments	3-4 L per site	Plastic container	Ethanol	< 4°C	60 days

During the August 1997 sampling, sediment samples from each site were used in 10-day *Hyalella* acute (USEPA 1994) and in 7d fathead minnow (*Pimephales promelas*) survival and growth tests (USEPA 1991a). Each of the 10 potentially contaminated sites in the harbor was individually tested as were the 10 reference sites that were replicated for quality control. At test termination, a subsample of surviving fish from different samples was snap frozen (-80 C) in liquid nitrogen for later protein expression analysis (see section 2.8).

A second, abbreviated sampling was performed in May 1998, in accordance with the approved Work Plan, to collect sediment samples at 6 sites (a reference and 5 relatively contaminated sites) for chronic (28-d) *Hyalella azteca* sediment tests and ten-day *Hyalella* tests. *Lumbriculus variegatus* bioaccumulation tests (28d) and follow-up acute toxicity identification testing of pore water samples were conducted on a subset of the 6 sites.

2.3 Biological Analyses

Biological assessments, using benthic macroinvertebrates, were used in conjunction with other field and laboratory analyses to help determine the effects of sediment contamination and other stressors on the biota of Burlington Harbor. Bioassessments have been used successfully to evaluate sediment quality in the Great Lakes (Reynoldson et al. 1995), to assess overall biological quality of large reservoirs (TVA 1995), and to assess effects of non-point source pollution on lakes in Florida (FDEP 1994). Benthic macroinvertebrates live on or in the sediment for most or all of their life cycle; they have limited mobility; and they are longer-lived than microscopic organisms such as algae or plankton. This means that they cannot escape exposure, and they integrate stresses occurring throughout their life cycle. Benthic organisms are, therefore, widely considered the best indicators of aquatic pollution (e.g., Holland et al. 1989). Samples for macroinvertebrate analyses were sieved in the lab (600μm mesh) and preserved in 70% ethanol for later identification.

Tetra Tech used its Standard Operating Procedures for processing and subsampling benthic organisms (Tetra Tech 1997). Samples were mixed and placed into white enamel pans equipped

with a grid frame (30 individual cells). Cells were randomly chosen using a random number table, and all organisms within a cell were picked for identification. The original intention was that we would subsample each site until we obtained 200 ± 40 organisms. Due to relatively few individuals collected at some sites, we sorted each sample in its entirety. Organisms were enumerated and identified by macroinvertebrate specialists at Tetra Tech's Biological Research Facility. For this project, specimens were generally identified to genus (or lower) using the most current literature available. Worms were identified only to family because relatively few were encountered in this study.

Metrics were analyzed in two different ways. For the first type of analysis we separated sites into two groups: those adjacent to or very near known sources of human activity, including the oil dolphin, old wastewater outfall, or the public docks, and those sites further removed from these sources, including those near the breakwater and the two reference sites. A cursory examination of previous data suggested that these two groups of sites were different with respect to several physicochemical factors and, therefore, this classification may be appropriate as a first attempt. Furthermore, this classification approach did not make any presumptions concerning sediment contamination or contaminant bioavailability, both of which are difficult to assess with certainty. We examined this classification by performing studentized t-tests ($p = 0.05$) on means of each physicochemical factor between the two site groups. Metric values were then compared between the two site groupings also using t-tests at a significance level of 0.05. For these analyses, we averaged each attribute (metric) across field replicates for sites BH30 and BH24 in order to obtain a single value for each metric for each of these sites. This yielded a total of 11 unique sites for analysis (site BH20 biological sample was lost).

In the second type of analysis, we determined the physicochemical factors most related to each metric using Forward Stepwise Multiple Regressions. We controlled for Type 1 errors by limiting the analysis to < 4 independent factors because of the few number of sites available with concurrent biological and physicochemical data (N = 11). We also controlled for Type 1 errors by including in the model only those variables that had F statistics ≥ 1.0 and that improved the overall R^2 by more than 10%.

2.4 Laboratory Chemical and Tissue Analyses

Analytical methods for chemical constituents in sediments and tissues are summarized in Table 2-2. These analyses were performed by subcontract laboratories and reported to Tetra Tech as sample concentrations, along with the QA/QC information required. Methods for acid volatile sulfide (AVS), simultaneous extracted metals (SEM), and other analyses followed standard USEPA or APHA methods and were included in the QAPP for this study (Tetra Tech 1997). All chemical concentrations were calculated on a dry weight basis.

Tetra Tech made a special effort to use methods similar to those reported in the 1993 Phase 2 study (McIntosh et al. 1996) so that results from the two studies would be comparable. Detection limits and matrix spike recoveries in this study were very similar to those reported previously. Percent recoveries for PAHs in this study ranged between 42 and 94% and averaged 68.7% (Appendix C). Method blank measurements were always below the detection limits. Percent recoveries for metals (matrix spikes) in this study ranged between 76.4 and 125.3% and averaged 103.8% (Appendix C). Method blanks for metals were also below the detection limit.

Relative percent differences (RPD) for laboratory duplicates were < 15% for PAHs and < 10% for all metals, indicating high laboratory precision.

Zebra mussels (*Dreissena polymorpha*) were collected from several sites in the summer of 1997 and a composite sample of organisms collected at each site was analyzed for tissue PAHs and percent lipid content. Mussels were cleared and shelled prior to extraction and analysis. PAH concentrations were lipid normalized to more accurately express bioaccumulation of PAHs as a function of location in the harbor. PAH sediment concentrations were expressed on a dry weight basis and on the basis of organic carbon or organic nitrogen measured at each site to help interpret PAH toxicity and to compare observed concentrations with published criteria and threshold levels.

Pearson's Product Moment correlation analysis (using a significance level of 0.05) was used to infer relationships between pairs of physicochemical characteristics. For these analyses there was a total of 14 sites (10 harbor "test" sites, two of which were replicated, and a composite sample from each of the two "reference" sites). Principal components and factor analysis (Statistica, version 5.0) were also used to infer relationships among the physicochemical parameters measured and to help decipher differences among sites. A minimum eigenvalue of 1.00 was used in this analysis to minimize statistical artifacts. We also used a maximum of 10 iterations in the analysis to minimize spurious results.

2.5 Toxicological Analyses

Sediment toxicity test methods used in this project included: (1) *Hyalella azteca* 10d and 28d whole sediment toxicity test procedures; the 10d test measured lethality while the 28d test measured lethality as well as growth and fecundity; and (2) fathead minnow (*Pimephales promelas*) 7-day chronic whole sediment tests in which the endpoints were survival and growth (fish weight). Procedures for these tests are summarized in Tables 2-4 through 2-6.

All toxicity tests used standard randomization techniques and Good Laboratory Practices. Sediment samples were homogenized following check-in at the laboratory prior to testing to help ensure high test replicability (precision). At test initiation and during the tests, overlying water quality was characterized and recorded on standard bench sheets. Measurements included hardness, alkalinity, conductivity, pH, dissolved oxygen, temperature, and ammonia (APHA 1995). Growth (weight) data were collected using tared weigh boats, and the total and tare weights were recorded on standard bench sheets.

Tests were conducted using a manual renewal system in which overlying water in each chamber was replaced twice each day (ASTM 1995c; USEPA 1994). Differences in endpoint values among sites were determined using ANOVA and post-hoc Duncan multiple range means tests (p

Table 2-5. Summary of test conditions and test acceptability: *Hyalella azteca* 28 day whole sediment test

Parameter	Conditions
1. Test type	whole-sediment bioaccumulation test with renewal of overlying water
2. Temperature	23° C
3. Light quality	wide-spectrum fluorescent lights
4. Light intensity	500 to 1000 1x
5. Photoperiod	16L:8D
6. Test chamber size	400 ml beakers
7. Sediment volume	100 ml
8. Overlying water volume	175 ml
9. Renewal of overlying water	2 volume additions/day; one every 12 h
10. Age of test organisms	7-14d
11. Number of organisms per chamber	10
12. Number of replicate chambers/treatment	6
13. Number of organisms per concentration	60
14. Feeding	YCT food, 1.5- 2 ml daily
15. Aeration	None, unless dissolved oxygen in overlying water drops below 40% of saturation
16. Overlying water	surface water
17. Overlying water quality	Hardness alkalinity, conductivity, pH, and ammonia at the beginning and end of a test temperature and dissolved oxygen daily
18. Test duration	28 days
19. Endpoint	Adult survival, growth, reproductive, maturation
20. Test acceptability	Performance-based criteria specifications outlined in Table BRF043-2

Table 2-6. Summary of test conditions and test acceptability: *Pimephales promelas* 7 day survival and growth test

Parameter	Conditions
1. Test type	Static-renewal
2. Test duration	7 days
3. Temperature	25 ± 1°C
4. Light quality	Ambient laboratory illumination
5. Light intensity	500 - 1000 Lux
6. Photoperiod	16h light, 8h darkness
7. Test chamber size	300 ml
8. Test solution volume	175 ml overlying water and 100 ml sediment
9. Renewal of overlying water	Once every 24 hours
10. Age of test organisms	< 24 h
11. No. organisms per chamber	10
12. No. replicate chambers /treatment	4
13. No. organisms per treatment	40
14. Feeding	*Artemia nauplii* are made available twice daily
15. Test chamber cleaning	Siphon or pour off at least 70% of the water for test solution renewal
16. Aeration	None, unless DO concentration falls below 40% of saturation, at which time start gentle, single-bubble aeration
17. Overlying water	20% dilute mineral water
18. Endpoint	Survival and growth (weight)
19. Test acceptability	80% or greater survival in controls after 7 days; minimum dry weight of 0.25mg for control fish

< 0.05). Survival data were analyzed using an arcsine square root transformation, and fish weight data were log-transformed to satisfy variance homogeneity assumptions of analysis. Toxicity endpoints were related to physicochemical properties of the sediment using forward stepwise multiple regression analysis (Statistica, version 4.0). In an effort to control Type 1 errors due to the relatively small sample size ($N = 14$ for most regression analyses in this study), independent variables (physicochemical measures) were included in the model only if their F statistic exceeded 1.0, and they improved the overall R^2 of the model by more than 10%. To further guard against Type 1 errors, we included one factor (independent variable) for every 3 degrees of freedom to limit the model size and, therefore, potential spurious associations.

2.6 *In-Situ* Physicochemical Analyses

At each site, the depth profile (at 0.30 depth intervals) for temperature, dissolved oxygen, pH, and conductivity was constructed using a Hydrolab model H_20 multiprobe following standard procedures given by the manufacturer (Tetra Tech 1997). The Hydrolab equipment was calibrated at the beginning and mid-way through sampling. The equipment was rinsed and acclimated in site water at each new site prior to taking depth measurements. Measurements were recorded on pre-prepared log sheets (Appendix B).

2.7 Toxicity Identification Analysis

A screening-level toxicity identification evaluation (TIE) analysis was performed on interstial water samples from two sites (BH20-21, BH21-26), both of which exhibited acute toxicity to

Hyalella (10 d test) in samples collected in 1998. TIE procedures conformed to those recommended by USEPA (1991b) for Phase I acute toxicity identification of aqueous samples. Interstitial water was obtained by centrifuging sediment samples at 10,000g at 4° C for 30 minutes. This method of extracting interstitial water minimizes potential oxidative effects and yields fewer chemical artifacts than most other extraction procedures (Burton 1992). Organism survival was measured and compared among the various treatments.

Contaminants in this TIE included metals, PAHs, oxidants, ammonia, and certain polar organics and pesticides. A single interstitial water sample from each site was split into several subsamples, each one treated differently to selectively remove, or reduce, the bioavailability of one of the types of contaminant listed above. Metal bioavailability was reduced in one treatment using a series of EDTA concentrations. Ammonia and certain cationic metals were treated using clinoptylite, a resin that adsorbs these compounds. Nonpolar organics such as PAHs were removed using C_{18} resin columns, and oxidants were removed or reduced in bioavailability using thiosulfate addition. Polar organics, pesticides, and some metals were removed using activated carbon treatment. Each of these treatments conformed to methods in USEPA guidance (USEPA 1991b). Following treatments, each fraction was subjected to screening static acute (48 h) *Ceriodaphnia* testing (Table 2-7). Differences in survival among treatments were qualitatively compared at the end of the 48 h exposure period.

Table 2-7. Summary of test conditions for static acute *Ceriodaphnia dubia* pore water test.

PARAMETER	CONDITIONS
1. Test type:	Static
2. Test duration:	48h
3. Temperature:	20 ± 1°C
4. Light quality:	Ambient laboratory illumination
5. Light intensity:	10-20 uE/m^2/s (50-100 ft-c) (ambient laboratory levels)
6. Photoperiod:	16h light, 8h darkness
7. Test chamber size:	30 mL
8. Test solution volume:	20 mL
9. Renewal of test solutions	none
10. Age of test organisms:	less than 24h old
11. No. organisms per test chamber	5
12. No. replicate chambers per concentration	1
13. No. organisms per concentration or treatment:	5
14. Feeding regime:	YCT and *Selenastrum* while holding prior to the test; newly-released young have food available a minimum of 2 hours prior to use in a test
15. Test chamber cleaning	Cleaning not required
16. Test solution aeration	None
17. Dilution water:	pore water
18. Endpoint:	Mortality and/or immobility; LC50
19. Sampling and sample holding requirements	Samples are used within 36h of completion of the sampling period
20. Sample volume required:	100 ml
21. Test acceptability	90% or greater survival in controls

2.8 Protein Expression Analysis

Protein expression provides detailed information on an organisms' condition and on the environmental stressors affecting it. The effect of individual stressors, particularly chemicals, can be detected and measured, even in a complex environment (Bradley et al. 1996). The

information contained in the variability among the thousands of proteins in an organism provides the basis for assays of specific stressors (Bradley et al. 1996).

Proteome studies use two-dimensional gel electrophoresis (2-DGE) which makes it possible to quantify thousands of proteins simultaneously (Bradley 1990). Two-DGE is based upon a surface charge fractionation, during which proteins are separated in a pH gradient until they reach a stationary position where their net charge is zero. The pH at which a protein has zero net charge is called its isoelectric point (pI), which is followed by separation according to the molecular weight of the proteins. Molecular separation in the first dimension is by isoelectric focusing (IEF) and, in the second dimension, by Sodium Dodecyl Sulfate- Polyacrylamide Gel Electrophoresis (SDS-Page). The high resolution of 2-DGE results from the first and second dimension separations based on independent protein parameters. This allows precise tailoring of separation gradients in narrow or wide pI ranges, and separates small (mg) quantities of proteins without loss of resolution. The high resolution and reproducibility of 2D-gels make it possible to create "reference maps" of tissues or whole organisms.

Protein expression analyses were conducted on fathead minnow larvae exposed for 7 days to six different sediments representing a range of pollutant concentrations. Fish exposed in chronic sediment toxicity testing (August 1997) were collected at the end of the test, flash frozen in liquid nitrogen, and stored at -80 °C until analysis. Samples were homogenized in 30 µL phosphate-buffered saline (PBS), pH 7.4 (10mM NaPO4, 150 mM NaCl), 100mM phenylmethyl sulfonylfluoride (PMSF), and 100mM dithiothreitol (DTT), all obtained from Sigma (St. Louis). After homogenization, the samples were incubated for 15 minutes at room temperature (21 °C)

and then centrifuged in a microcentrifuge (Fisher Scientific, Pittsburgh, Pennsylvania) at 4°C for 5 minutes at 10,000 g. The supernatant was then removed and used for protein analyses.

Protein assays were performed in duplicate using a bovine serum albumin (BSA) standard following the Bradford method (Bradley 1989), as adapted by Bio-Rad (Richmond, CA). Protein samples were prepared for electrophoresis using Laemmli sample buffer. Equal amounts of the protein samples (30 μg protein) were separated according to their pI. The ID gels contained an acrylamide or monomer concentration of 4%, 20% Triton X-100, 9.2 M Urea, 1.6% Bio-Lyte 5/8 ampholyte and 0.4% Bio-Lyte 3/10 ampholyte; all were obtained from Sigma (St. Louis, MO). The tube gels, 7.5-cm x 1-mm, were obtained from Fisher Scientific.

Protein samples were electrophoresed for approximately 12 hours using a minigel apparatus (Bio-Rad, Mini-Protean II). For the first 10 minutes of the electrophoresis, the voltage was set at 500 V, after which the voltage was decreased to 400 V. After electrophoresis the gels were stored at -80°C for later use. Separation in the second dimension was performed using SDS-PAGE in a discontinuous buffer system. The acrylamide or monomer concentration of the stacking gel was 4%, and the resolving gel was 12% acrylamide. The samples were electrophoresed for approximately 1 hour at 140 V using the Mini-Protean II from Bio-Rad. Electrophoresis was continued until the same dye front reached the bottom of the gel. A cocktail of molecular weight standards from 97,400 daltons to 14,30 daltons (Promega, Madison, WI) was used along with each sample.

For silver staining, the gel was fixed in 30% ethanol and 10% acetic acid for 3 hours. Two 30 minute washes in 30% ethanol were followed by three 10 minute washes with distilled water. The gel was then incubated in 0.1% $AgNO_3$ for 30 minutes, rinsed, and developed in 2.5% $NaCO_3$ + 0.02% formaldehyde. After the first staining the gel was detained in Farmer's Reducing Solution, containing 0.23 M potassium ferricyanide and 0.1 M sodium thiosulfate, until the stain was no longer visible. After at least six 10-minute distilled water washes, the above described staining procedure was repeated. After the second developing cycle with 2.5% $NaCO_3$ + 0.02% formaldehyde, the reaction was stopped with 1% acetic acid after the desired resolution was achieved. The gels were then stored in plastic bags containing distilled water for later analyses.

Gels were scanned at high resolution using a Epson Scanner (Model:Expression 636) and Photoshop v.4.0 (Adobe) software. The two dimensional gel images were then compared using the Medical Electrophoresis Analysis Interactive Expert System, MELANIE (Bradley et al. 1996) to determine whether there were differences in the types of proteins expressed by larval fish exposed to sediments having different levels of contamination.

2.9 Bioaccumulation Testing

Sediment samples from three sites (BH23, BH21, BH20-21) and a control were collected in May 1998 and used in 28 day bioaccumulation testing using the worm *Lumbriculus variegatus* (ASTM 1995d; USEPA 1994). Procedures for this test followed those in USEPA's sediment testing guidance (USEPA 1994) and in ASTM guidance (ASTM 1995d) (Table 2-8). PAHs and

lead were measured in unexposed worms at test initiation and in exposed worms from each sediment sample at the end of the test. Worms were held in clean water for 24h prior to analysis to purge their intestines of sediment particles and ensure dry weight accuracy of the worms. A sample of the worms (approximately 0.4 g dry weight per sediment sample) was then digested and analyzed using gas chromatography and mass spectroscopy (USEPA 8270C) for PAHs and atomic absorption spectroscopy (USEPA 239.2) for lead (Table 2-2).

Table 2-8. Summary of test conditions for 28-day sediment bioaccumulation test with *Lumbriculus variegatus*

Parameter	Conditions
1. Test type	Whole-sediment bioaccumulation test with renewal of overlying water
2. Temperature	23° C
3. Light quality	Wide-spectrum fluorescent lights
4. Illuminance	500 to 1000 Lux
5. Photoperiod	16L:8D
6. Test chamber	4-L aquaria with stainless steel screens
7. Sediment volume	1 L
8. Overlying water volume	1 L
9. Renewal of overlying water	2 volume additions/day; once every 12 h
10. Age of test organisms	Adults
11. Loading of organisms in chamber	5 g/replicate
12. Number of replicate chambers/ treatment	3
13. Feeding	None
14. Aeration	None, unless dissolved oxygen in overlying water drops below 40% of saturation
15. Overlying water	Culture water, well water, surface water, site water, or reconstituted water
16. Test chamber cleaning	If screens become clogged during the test, gently brush the outside of the screen
17. Overlying water cleaning	Hardness alkalinity, conductivity, pH, and ammonia at the beginning and end; test temperature and dissolved oxygen daily
18. Test duration	28 days
19. Endpoint	Bioaccumulation of lead and PAHs
20. Test acceptability	Performance-based criteria specifications outlined in Table BRF037-2

3.0 RESULTS

3.1 Water Column Physicochemical Characteristics

Minor differences in pH, temperature, dissolved oxygen, and conductivity were observed between the surface and bottom at all locations (Appendix B). However, significant differences in some physicochemical parameters were observed at the lake bottom among sites. Linear regression analyses indicated that deeper sites had lower pH, temperature, and dissolved oxygen concentration (Figure 3-1). Conductivity was not related to depth ($p > 0.10$). Although pH and dissolved oxygen were lower at deeper sites, minimum dissolved oxygen concentrations were above 7.0 mg/L at all locations, and pH was generally between 7.5 and 8.5, indicating well-oxygenated and well-buffered conditions throughout the harbor.

3.2 Sediment Nitrogen, Organic Carbon, and Physical Characteristics

Table 3-1 summarizes physical characteristics, organic nitrogen, ammonia, and total organic carbon measured for each sediment sample. Highest organic nitrogen and ammonia concentrations were observed at the non-reference sites BH19, BH20-21, BH23, BH19-18 and BH11 (Table 3-1). Sites near the breakwater had higher ammonia concentrations and lower pH than other sites in the harbor (t-test, $p < 0.05$). Relatively high nitrogen was also observed at the reference site BH24 outside the harbor. Highest organic carbon was observed at BH20-21, BH19-18, BH19, and BH23. The public

boathouse and docks might be a source of nitrogen and carbon at BH23. It is not clear what the sources of nitrogen and carbon are for the remaining sites, as they are widely scattered in the harbor.

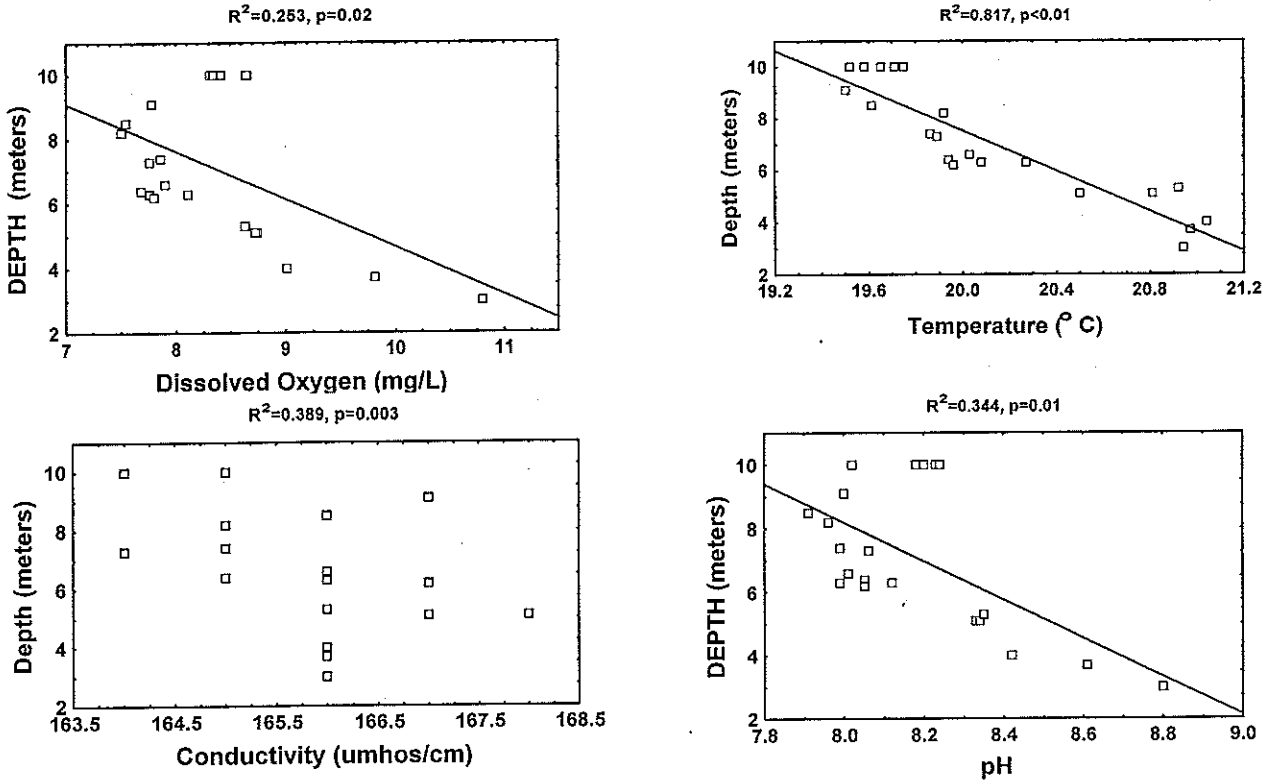

Figure 3-1. Bottom water temperature, dissolved oxygen, pH, and conductivity as a function of depth for each site sampled in inner Burlington Harbor, August 1997.

The two "reference" sites, BH24 and BH30, had the highest percent fines (Table 3-1), although sediment at BH24 contained more sand than most other sites. Coarser sediment was more common in the southern part of the harbor (BH20, BH20-21, BH21, and BH21-26) and at BH19 along the breakwater. Percent solids was relatively low at sites along the breakwater (BH11, BH19, BH19-11, BH19-18) compared to other sites in the harbor, indicating less compaction of sediment in that area.

Table 3-1. Summary of results for organic nitrogen (mg/kg), ammonia nitrogen (mg/kg), total organic carbon (TOC) (mg/kg), percent fines, and percent solids measured in sediment samples collected from Burlington Harbor sites in August 1997.

Station	Organic Nitrogen	Ammonia Nitrogen	Total Organic Carbon	Percent Fines	Percent Solids
BH24 reference (composite)	2500	400	290	95	42
BH30 reference (composite)	1600	360	470	81	36
BH11	2800	610	540	79	28
BH11B	3900	460	610	71	28
BH19	4300	670	790	31	24
BH19-11	2500	750	610	75	28
BH19-18	2800	650	1500	66	26
BH20	1300	170	78	35	60
BH20-21	3500	400	1600	50	40
BH21	1600	110	100	41	58
BH21-26	1500	280	260	40	53
BH23	3100	470	350	78	34
BH23B	3400	490	970	45	35
BH26	2300	290	290	70	42

Sediment ammonia concentration was directly correlated ($p < 0.05$) with organic nitrogen and total organic carbon, but organic nitrogen and organic carbon were not significantly related (Table 3-1, Appendix C). Percent solids in sediments was inversely related to depth ($r = -0.62$, $p < 0.05$, Table 3-1, Appendix C), while sediment ammonia concentration increased with increasing depth ($r = 0.56$, $p < 0.05$). Sediment ammonia concentrations could have been toxic at many of the breakwater sites if pore water had similar ammonia concentrations (Ankley et al. 1990; USEPA 1994).

3.3 Sediment Polycyclic Aromatic Hydrocarbons (PAHs)

Higher concentrations of PAHs were observed primarily near the old sewage outfall and oil dolphins (sites BH20, BH20-21, and BH21-26) as compared to either the breakwater sites or the two reference sites (Table 3-2), similar to results from the previous Phase 2 study. Site BH23 (near the public boathouse and docks) also had a relatively high PAH concentration. Mean PAH concentration in the fine sediment fractions (particle size < 0.63 μm) for sites BH20-21, BH23, and BH21 (the three most PAH-contaminated sites) combined was significantly greater than in the total samples (Figure 3-2; t-test, $p < 0.05$). Given that many lentic benthic species, such as midges and worms, often prefer fine sediments (Suedel and Rodgers 1994; Landrum and Robbins 1990), these preliminary results suggest that whole sample PAH measurements may underestimate the potential toxic stress on benthic organisms in the harbor.

The most abundant sediment PAH compounds in August 1997 were (in decreasing order) fluoranthene, pyrene, phenanthrene, benzo(a)pyrene, and benzo(K)fluoranthene (Appendix C, Table 3-2), all of which are byproducts of oil or gasoline combustion or degradation of oil products. These compounds were also some of the most common PAHs reported previously in the harbor (Watzin et al. 1997). Sediment biological effect concentrations, derived from either field assessments or sediment toxicological data, suggest that all of the above compounds, and/or total PAHs, could cause toxicity to benthic species at sites BH20-21, BH21, BH19-18, BH21-26, and BH23 (see Discussion, Section 4.0). PAH concentration was directly correlated with TOC concentration ($r = 0.542$, $p < 0.05$) and inversely related to depth ($r = 0.563$, $p < 0.05$) (Table 3-1, Appendix C).

Table 3-2. Summary of results for selected PAH compounds and total PAHs (μg/g) measured in sediment samples collected from Burlington Harbor sites in August 1997.

Station	Total PAH	OC Norm. PAH[1]	ON Norm. PAH[2]	Phenanthrene	Pyrene	Fluoranthene	Chrysene	Benzo(b)-fluoranthene	Benzo(k)-fluoranthene	Benzo(a)-pyrene
Method blank	0.05	<0.0	<0.0	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01	<0.01
BH 24 reference composite	0.26	0.90	0.10	0.05	0.07	0.07	<0.02	<0.02	<0.02	<0.02
BH 30 reference composite	2.64	5.62	1.65	0.42	0.47	0.64	0.31	<0.03	<0.03	<0.03
BH11	0.96	1.79	0.34	0.18	0.25	0.32	<0.04	<0.04	<0.04	<0.04
BH11B	3.46	5.67	0.99	0.50	0.54	0.82	0.32	<0.04	<0.04	0.21
BH19	1.83	2.32	0.43	0.33	0.46	0.63	<0.04	<0.04	<0.04	<0.04
BH19-11	1.14	1.87	0.46	0.21	0.32	0.36	<0.04	<0.04	<0.04	<0.04
BH19-18	5.27	3.51	2.39	0.77	0.77	1.31	0.42	<0.04	<0.04	0.58
BH20	2.88	36.92	2.21	0.42	0.60	0.78	0.25	<0.02	<0.02	0.20
BH20-21	18.13	11.33	5.85	2.00	2.13	3.50	1.65	1.40	1.80	1.98
BH21	9.76	97.60	6.10	1.02	1.45	2.07	0.76	0.52	0.67	0.83
BH21-26	5.26	20.23	3.51	0.66	0.87	1.36	0.55	<0.02	<0.02	0.53
BH23	3.65	10.43	1.18	0.56	0.62	1.00	0.41	<0.03	<0.03	<0.03
BH23B	8.51	8.77	2.93	1.20	1.37	2.14	0.77	<0.03	<0.03	0.89
BH26	2.90	10.00	1.26	0.40	0.57	0.79	0.31	<0.02	<0.02	0.17

[1] Organic carbon normalized PAH concentrations
[2] Organic nitrogen normalized PAH concentrations

We calculated two normalized PAH values for each site: one on the basis of organic carbon and the other based on organic nitrogen. Both of these normalized PAH values were highest at sites near the old wastewater plant outfall (BH20, BH20-21, BH21, BH21-26) and the boathouse (BH23), similar to our results using non-normalized PAH values. Organic carbon normalized PAH concentrations at sites closest to the old wastewater outfall and oil dolphins were especially high and indicate potential chronic toxicity according to USEPA's equilibrium partitioning sediment quality guidelines (USEPA 1992).

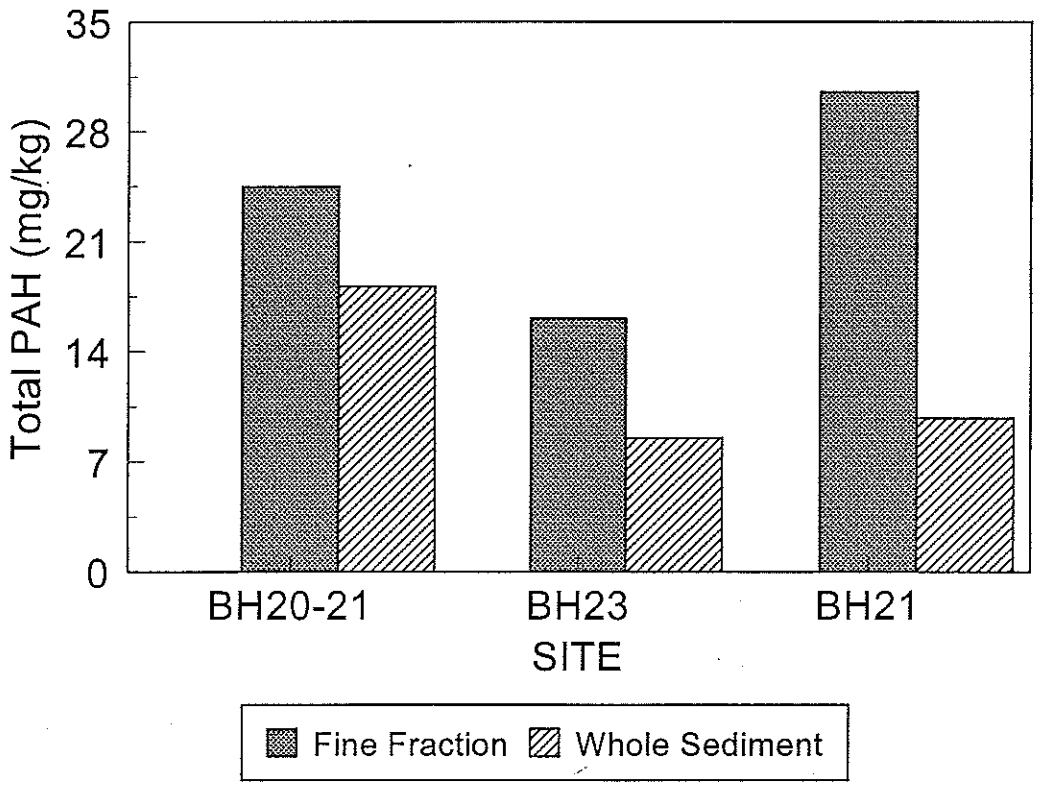

Figure 3-2. Total PAHs measured in the whole sediment versus the fine particle size fraction at three sites in Burlington Harbor, August 1997.

Tissue analyses of zebra mussels collected in August 1997 indicated that PAH concentrations were below the detection limit at all sites tested (Appendix C). Thus, PAHs were either not bioaccumulated

by these organisms or they were quickly metabolized or depurated. More extensive sediment and water column analyses would need to be conducted to determine if, and under what conditions, PAHs in sediments are mobilized into the water column and accumulated by filter feeders.

3.4 Sediment Metal Analyses

3.4.1 Silver

Table 3-3 summarizes results of metal and acid volatile sulfide analyses for each site. Silver exceeded NOAA's ERL value of 1.0 mg/kg at all sites except BH21 and BH26. No site exceeded NOAA's ERM of 3.7 mg/kg. Relatively high silver concentrations were observed at the two "reference" sites and at BH19. A significantly higher mean silver concentration was observed at the four breakwater sites (BH11, BH19, BH19-11, and BH19-18) than at the five sites sampled in the southern part of the harbor near the old wastewater discharge (BH20, BH20-21, BH21, BH21-26, and BH26; t-test, $N = 14$, $p = 0.02$). The 1993 Phase 2 study also observed relatively high silver concentrations near the breakwater (McIntosh et al. 1996).

3.4.2 Lead

Lead exceeded the ERL of 46.7 mg/kg at all sites but did not exceed the ERM of 218 mg/kg (Table 3-3). Highest lead concentrations were observed at the breakwater sites BH11, BH19, BH19-18, and BH24, the "reference" site outside the harbor. Similar to the pattern described above for silver, lead

was higher at the breakwater sites than at sites near the old wastewater outfall (t-test, N = 14, p = 0.013).

Table 3-3. Summary of results for metals (mg/kg), acid volatile sulfides (AVS) (*u*mole/g), simultaneously extracted metals (SEM) (*u*mole/g) SEM/AVS, and SEM-AVS observed in sediment samples collected from Burlington Harbor sites in August 1997.

Station	Ag	Pb	Ni	Cu	Zn	AVS	SEM	SEM/ AVS	SEM-AVS
BH24 reference composite	2.44	151.62	14.2	43.58	142.75	1.25	3.87	3.10	2.62
BH30 reference composite	2.04	111.38	11.09	35.77	103.61	0.94	2.89	3.07	1.95
BH11	1.98	188.97	20.55	43.91	167.56	25.75	4.53	0.18	-21.22
BH11B	1.66	151.63	18.51	32.90	136.09	21.93	3.66	0.17	-18.27
BH19	2.45	170.23	18.34	42.19	152.60	14.70	4.15	0.28	-10.55
BH19-11	1.59	121.08	13.83	32.15	114.04	19.86	3.08	0.16	-16.78
BH19-18	1.96	177.89	15.34	25.50	151.06	15.86	3.72	0.24	-12.14
BH20	1.62	51.30	5.62	9.65	58.03	4.59	1.40	0.30	-3.19
BH20-21	1.30	89.50	6.00	6.98	117.39	15.43	2.45	0.16	-12.98
BH21	0.62	85.78	4.60	11.68	91.84	1.64	2.09	1.27	0.45
BH21-26	1.64	141.80	7.74	35.68	152.39	2.14	3.72	1.74	1.58
BH23	1.73	123.69	12.53	28.02	137.44	13.72	3.37	0.25	-10.35
BH23B	1.02	91.37	9.95	16.39	102.67	12.89	2.45	0.19	-10.44
BH26	0.93	127.50	10.02	27.58	130.28	4.09	3.22	0.79	-0.87

3.4.3 Nickel

Nickel did not exceed the ERL of 20.9 mg/kg at any site in this study. However, one of the two field duplicate values at BH11 was 20.55 mg/kg, similar to the ERL (Table 3-3). Significantly lower nickel

concentrations were observed in the southern nearshore sites as compared to the breakwater sites (t-test, $N = 14$, $p < 0.001$), similar to the results observed with lead and silver above.

3.4.4 Copper

Copper exceeded the ERL of 34 mg/kg at both "reference" sites, BH11, BH19, and BH21-26; however, none of these sites exceeded the ERL by more than 30% (Table 3-3). Relatively low copper concentrations were observed at BH20 and BH20-21, both of which are slightly north of the old wastewater outfall. In contrast to the results observed with silver, lead, and nickel, breakwater sites had similar mean copper concentrations as the southern nearshore sites (t-test, $N = 14$, $p > 0.05$).

3.4.5 Zinc

Zinc exceeded the ERL of 150 mg/kg at BH11, BH19, BH19-18, and BH21-26; however, like copper, zinc rarely exceeded the ERL by more than 10% (Table 3-3). The "reference" site BH24, outside the harbor, had a zinc concentration approaching the ERL (142.75 mg/kg). Similar to results for copper, zinc concentrations were not significantly different between breakwater and southern near-shore sites (t-test, $N = 14$, $p > 0.10$).

3.4.6 SEM and AVS

Highest AVS concentrations were observed at the four breakwater sites, BH20-21, and BH23. AVS was approximately 1 μmole/kg or less at both "reference" sites and BH21 (Table 3-3), suggesting more

aerobic surficial sediment conditions at these sites as compared to other sites sampled. The 1993 Phase 2 study (McIntosh et al. 1993) also reported low AVS concentrations at our two "reference" sites. These results indicate that AVS is probably not a factor controlling metal bioavailability at these two sites and that other physicochemical characteristics such as organic carbon may be more important in this respect (Ankley et al. 1996).

Simultaneously extracted metal (SEM) concentration was highest at "reference" site BH24, the breakwater sites BH11, BH19, BH19-18, and BH21-26 (Table 3-3). The ratio of SEM/AVS was greater than 1.00 at the two "reference" sites (> 3.00 for both sites), BH21, and BH21-26 (Table 3-3), suggesting that the metals measured could be bioavailable (toxic) at these sites. However, as noted above, the very low AVS concentration at the two reference sites suggests that SEM/AVS comparisons are not an accurate indicator of metal bioavailability there.

We also examined the difference between SEM and AVS at each site because some researchers have suggested that the difference is a more accurate indicator of metal bioavailability than the ratio (Hansen et al. 1996). Difference values followed the same pattern among sites as those observed using the SEM/AVS ratio (Table 3-3). However, difference values did not exceed 2.62 at any site, indicating that SEM did not substantially exceed AVS in absolute concentration at any site in this study. Furthermore, most of the sites with SEM/AVS < 1.00 (with the exception of perhaps BH26) had ratios substantially less than 1.00, indicating that metals should not be bioavailable at those sites if interstitial (pore) water uptake is the primary exposure route of metals to benthic organisms (Ankley et al. 1996).

Copper, zinc, nickel, and lead concentrations in sediments among sites were highly correlated with each other and silver concentration was uncorrelated with zinc, suggesting either a different transport/fate mechanism or different sources for these two metals (Table C-1, Appendix C). Nickel, copper, and silver were each inversely related to PAH ($p < 0.05$, Table C-1). Principal Components and Factor Analysis of physicochemical data yielded a similar result. Factor 1 was correlated with metals, ammonia, and percent fines and Factor 2 was correlated with TOC, PAH, and percent solids (Figure 3-3). Thus, metal concentrations were generally inversely related to PAH and organic carbon concentrations in the harbor.

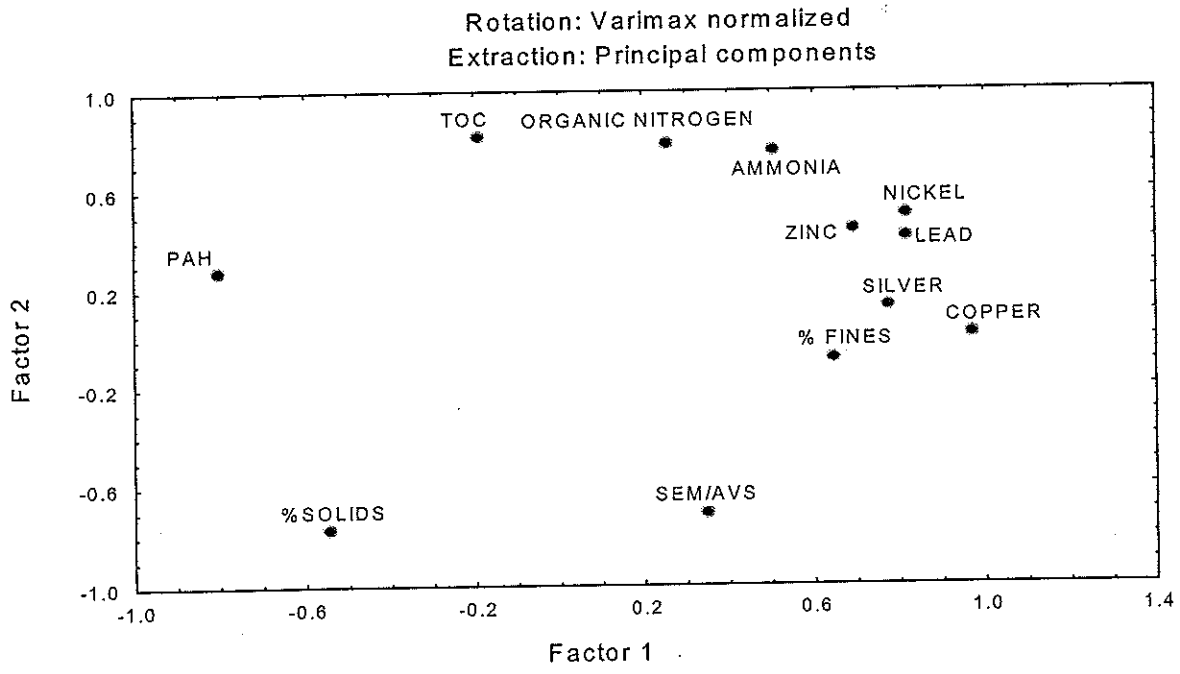

Figure 3-3. Summary of Principal Components Factor Analysis on physiochemical data collected for sediments in Burlington Harbor, August 1997.

3.5 Field Duplicate Analyses

Physicochemical measurements at the two sites with duplicate samples (BH23 and BH11) indicated reasonable agreement (precision) for most parameters as indicated by relative percent difference (RPD) values of generally < 40% (Table 3-4). Of the physicochemical parameters examined, PAH concentration had the highest RPD value, exceeding 30% at both sites. These results indicate that PAH concentration was more variable than other sediment parameters measured in this study.

Table 3-4. Summary of site replicate results for physicochemical and biological parameters measured on samples collected in August 1997 and relative percent difference (RPD) values between field replicates (units = mg/kg unless otherwise noted).

	Site 23			Site 11		
	A	B	RPD (%)	A	B	RPD (%)
TOC	350	970	16.1	540	610	6.7
Kjeldahl Nitrogen	3500	2900	9.4	3400	3500	1.4
Organic Nitrogen	3100	3400	4.6	2800	3900	16.4
Ammonia	470	490	2.1	610	460	14.0
% Solids	34	35	1.4	28	28	0
PAH	3.65	8.51	39.9	0.96	3.46	56.2
% Fines	78	45	26.8	79	71	5.3
AVS	440.1	413.4	3.1	825.8	703.3	8.0
SEM	303.4	221.4	15.6	422.9	340.8	10.7

3.6 Comparison Between 1997 and 1993 Sediment Contaminant Results

We compared contaminant concentrations from the 8 sites sampled both in this study and in the previous Phase 2 study to evaluate whether contaminant concentrations may have changed over time. T-tests of mean contaminant concentrations between years indicated that concentrations of some

metals and PAHs were lower in this survey than they were 4 years ago ($p < 0.05$; Figure 3-4). Mean SEM/AVS and lead and silver concentrations were not significantly different between years; however, some of these parameters (e.g., SEM/AVS) exhibited less variability among the eight sites in the present study than was observed in 1993 ($F = 76.8$, $p < 0.001$; Figure 3-4). Observed differences in contaminant concentration and/or variability in contaminant concentration between years are unlikely to be due to differences in field crews or laboratories. Both studies used a petite Ponar sampler, although the 1993 study used a winch to lower and raise the sampler rather than by hand as in the present study. Laboratory methods used in both studies, and the performance of those methods, were also very similar. As explained in section 2.4, we made a special effort to use similar analytical methods so that results could be compared between studies.

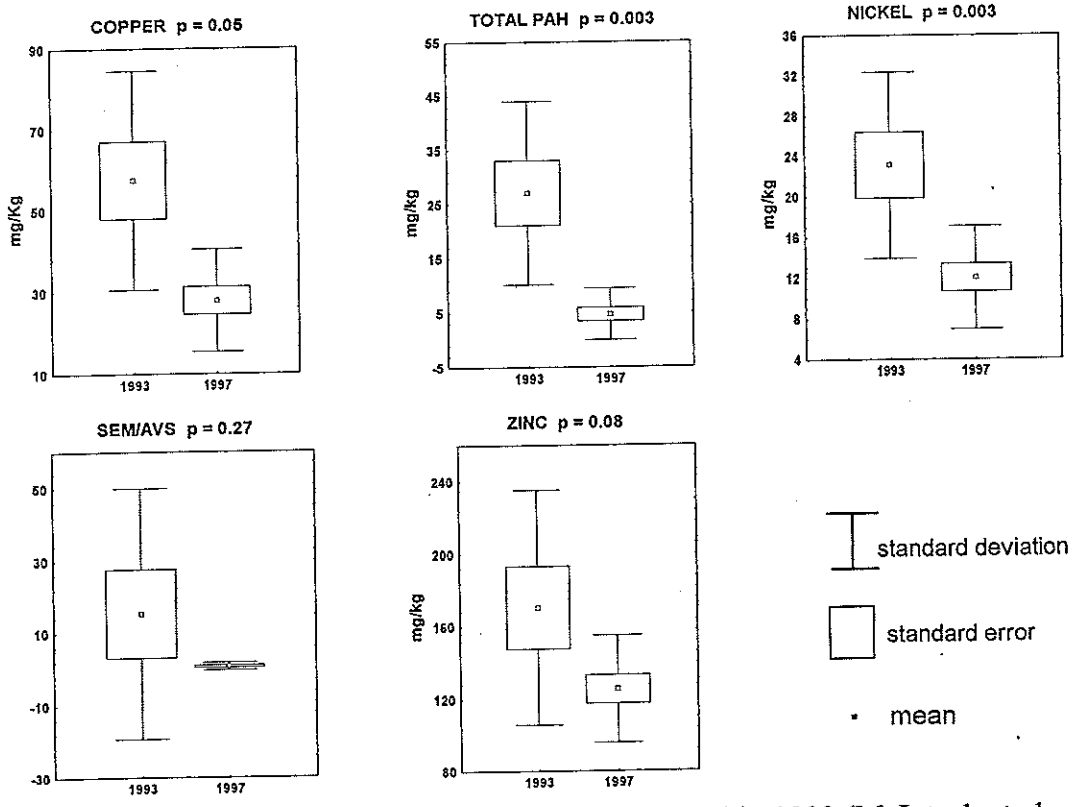

Figure 3-4. Comparison of sediment chemical concentrations reported in 1993 (McIntosh et al. 1996) and in the present study for the same locations in Burlington Harbor.

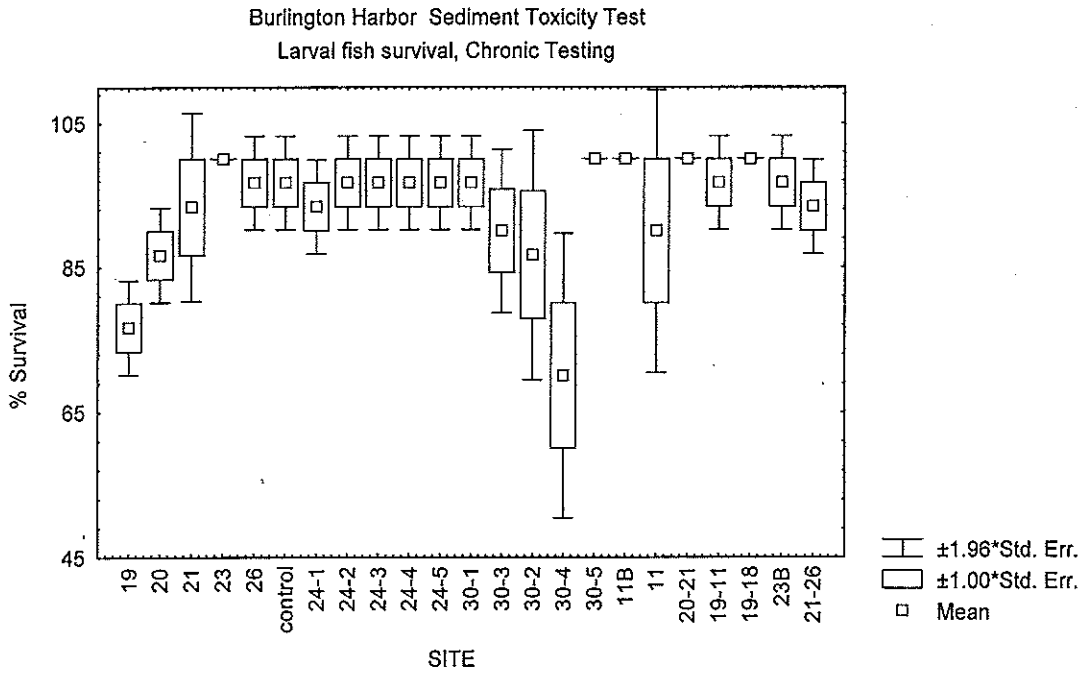

Figure 3-5a. Summary of survival results for the fathead minnow chronic sediment test on samples collected in August 1997.

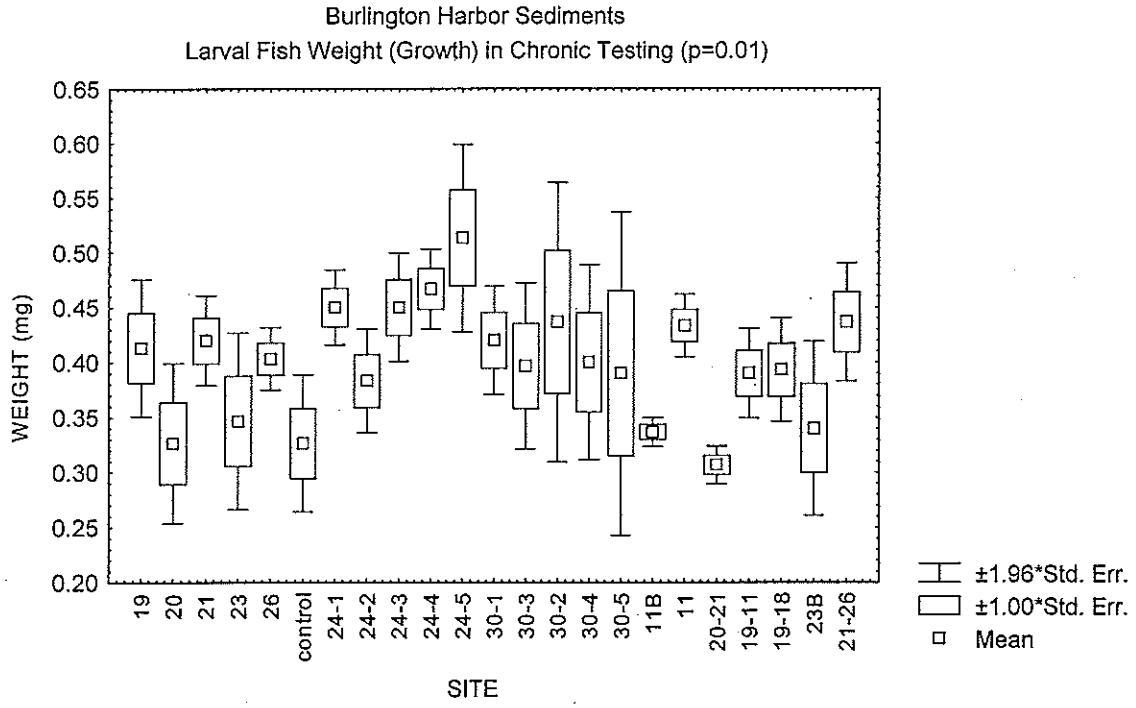

Figure 3-5b. Summary of fathead minnow weight (growth) results for chronic sediment test on samples collected in August 1997.

Table 3-5. Results of Duncan multiple range test: Inter-site comparisons of fathead minnow survival in 7-day whole sediment tests, August 1997. + = $p < .05$, ++ = $p < .01$, +++ = $p < .001$.

Site	11	19	19-11	19-18	20	20-21	21	21-26	23	24	26	30
11	—	+										
19		—	+	++		++	+	+	++	+	+	
19-11			—									
19-18				—								
20					—							
20-21						—						
21							—					
21-26								—				
23									—			
24										—		
26											—	
30												—

BH20, BH20-21, and BH23 and the reference site BH24 (ANOVA, Duncan multiple range test, $p < 0.05$) (Table 3-6). Mean fish weight in the control was also lower than that in the reference site BH24 probably due to the relative lack of natural food available in our control sediment. Fish weight at all sites and the control was not significantly different from that observed at BH30, the harbor "reference" site.

Forward stepwise multiple regression analysis indicated that fish weight was related to SEM/AVS, organic carbon normalized PAH, and lead concentrations (Table 3-7). These three factors explained approximately 60% of the variance in fish growth observed among sites. A significant model could not be constructed for fish survival.

3.7.2 *Hyalella* 10-day survival test — August 1997

Figure 3-6 summarizes survival results for *Hyalella* in 10-day sediment tests. *Hyalella* survival appeared to be more sensitive than fish survival or growth. Except for the two "reference" sites sampled in this study, (sites BH24 and BH30), most sites exhibited significant reductions in *Hyalella* survival (Figure 3-6, Appendix E). *Hyalella* survival was higher in the pond sediment control than in all sites sampled in this study except BH20 (ANOVA, Duncan multiple range test, $p < 0.05$; Table 3-8).

Forward stepwise multiple regression analysis of *Hyalella* survival in 10-day tests yielded two significant factors, lead and log (PAH), which together accounted for 57% of the observed variance (Table 3-9).

Table 3-6. Results of Duncan multiple range test: Inter-site comparisons of fathead minnow dry weight in 7-day whole sediment tests, August 1997. + = p < .05, ++ = p < .01, +++ = p < .001

Site	11	19	19-11	19-18	20	20-21	21	21-26	23	24	26	30
11	—	+										
19		—				+						
19-11			—									
19-18				—								
20					—			+		+		
20-21						—	+	+		++		
21							—					
21-26								—				
23									—	+		
24										—		
26											—	
30												—

Table 3-7. Summary of forward stepwise multiple regression analysis of fathead minnow weight in 7-day whole sediment chronic tests.

Dependent Variable	Overall Model	Contributing Factors	Association	Partial Correlation	p-value
Fathead minnow weight	$R^2 = 0.604$	SEM/AVS	+	0.006	0.0134
	$p < 0.021$	Pb	+	0.283	0.0395
	$N = 14$	Carbon normalized PAH	+	0.286	0.207

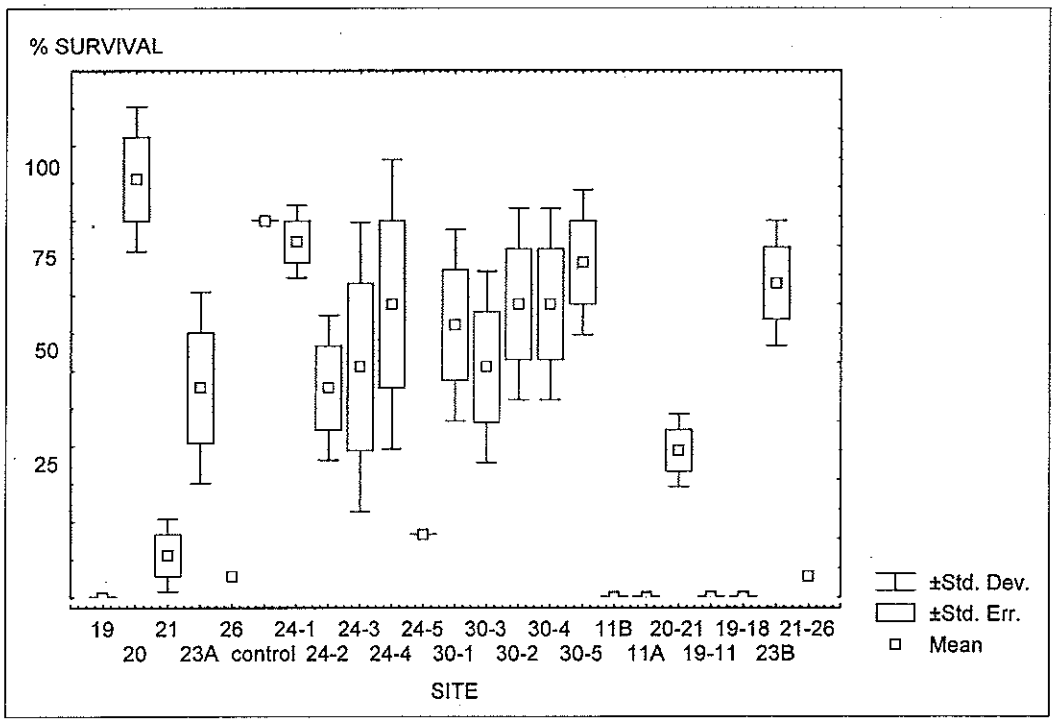

Figure 3-6. Summary of survival results for the 10-day *Hyalella azteca* whole sediment toxicity test on samples collected in August 1997.

Table 3-8. Results of Duncan multiple range test: Inter-site comparisons of *Hyalella azteca* survival in 10-day whole sediment tests, August 1997. + = p < .05, ++ = p < .01, +++ = p < .001

Site	11	19	19-11	19-18	20	20-21	21	21-26	23	24	26	30	Control
11	—				+++					+		++	+++
19		—			+++					+		++	+++
19-11			—						+	+		++	+++
19-18				—					+	+		++	+++
20					—		+++	+++	+++	+++	+++	++	
20-21						—							+++
21							—					++	+++
21-26								—		+		++	+++
23									—	+			++
24										—			+
26											—	++	++
30												—	+

Table 3-9. Summary of forward stepwise multiple regression analysis of *Hyalella* 10-day survival in whole sediment tests conducted in August, 1997.

Dependent Variable	Overall Model	Contributing Factors	Association	Partial R^2	p-value
Hyalella survival 10-day, August 1997	$R^2 = 0.573$	Pb	—	0.217	0.003
	$p < 0.009$	Log PAH	—	0.217	0.047
	N = 14				

Comparison of toxicity test results for the two replicated reference sites, (BH30 and BH24), indicated good agreement (high precision) for fish survival and growth but less for *Hyalella* survival. Coefficients of variation for the *Hyalella* 10d test were 12.9 and 62.7% for sites BH30 and BH24, respectively; 13.2 and 1.8% for fish survival; and 4.7 and 10.3% for fish weight (growth), respectively. Thus, *Hyalella* survival in 10d tests was a more variable endpoint than the fish endpoints examined in this study.

3.7.3 *Hyalella* 10-day Sediment Tests — May 1998

Follow-up 10-day sediment tests, using *Hyalella* and samples collected in May 1998, indicated toxicity at many of the same sites tested in August 1997 (Table 3-10; Appendix F). Ten-day survival was lowest at site BH20-21, followed by sites BH21-26 and BH23 (Duncan multiple range test, $p = 0.05$, Table 3-10). These sites had relatively high PAH concentrations in August 1997 and in earlier work (McIntosh et al. 1996). If sediment ammonia concentrations measured in 1997 at these sites were indicative of pore water ammonia concentration, then ammonia could also have been toxic to *Hyalella*.

Table 3-10. Summary of survival data observed in *Hyalella azteca* 10 day acute sediment toxicity testing on samples collected from select sites in May 1998. Means with the same superscript are not statistically different.

Site	% Survival	
	Mean	**SD**
Control	81.7 [1]	0.12
BH19-18	60.0 [1, 3]	0.24
BH20-21	16.7 [2]	0.05
BH21-26	43.3 [2, 3]	0.20
BH21	70.0 [1, 3]	0
BH23	46.7 [2, 3]	0.24
BH30	90.0 [1]	0.14

We observed considerable variability in *Hyalella* 10-day survival between August 1997 and May 1998 at five of the six sites examined (Figure 3-7). However, except for site BH19-18, the general pattern of toxicity among these sites was fairly similar in 1997 and 1998: site BH30 had the highest survival in both years and sites BH21, BH21-26, and BH23 exhibited intermediate survival in both years. The inter-annual variability exhibited in this test could be due either to uncertainties in sampling the exact same locations twice, changes in sediment contaminant concentration, or changes in contaminant bioavailability between seasons at these sites. These sources of variability were recognized in previous work (Watzin et al. 1997) and underscore the high degree of temporal and spatial heterogeneity in sediment characteristics in Burlington Harbor.

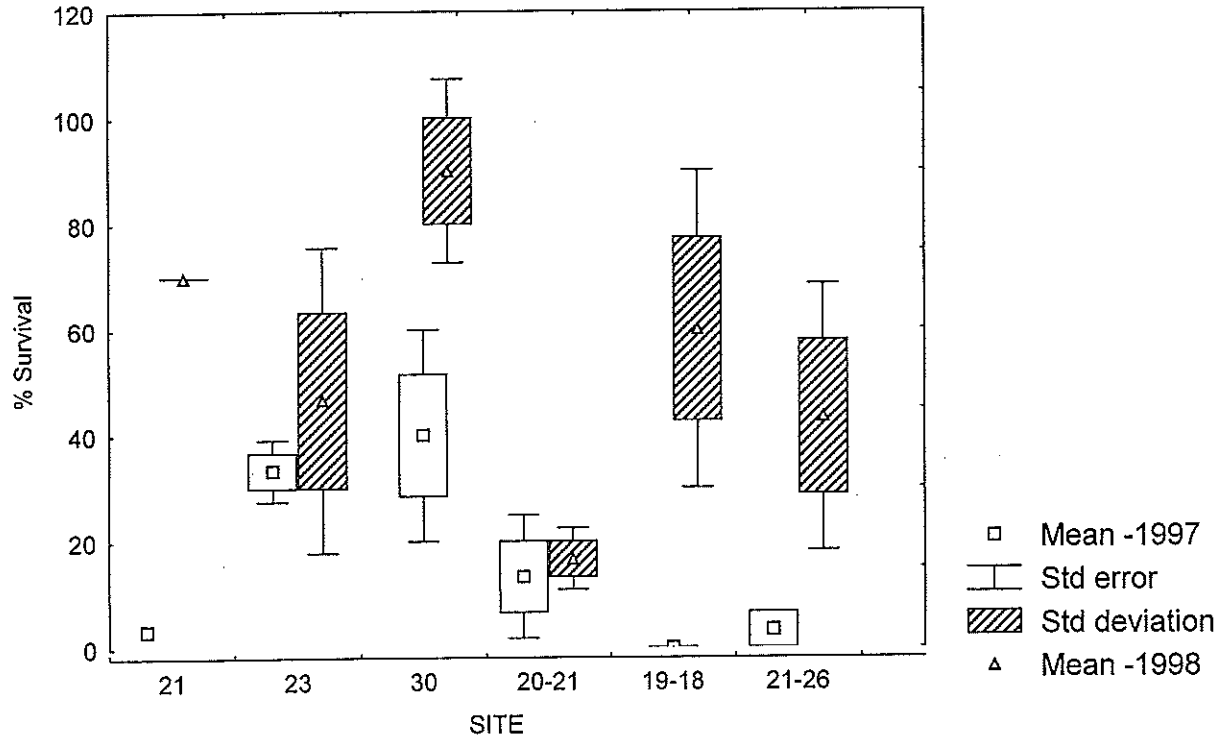

Figure 3-7. Comparison of *Hyalella* survival in 10-day whole sediment tests on samples collected in Burlington Harbor August 1997 and May 1998.

3.7.4 *Hyalella* 28-day Sediment Test — May 1998

Hyalella survival, growth, and fecundity in 28-day testing were poorest overall at site BH19-18 (Table 3-11; Appendix G). Although some of the six sites examined exhibited similar (within 10%) or lower survival in the 28-day *Hyalella* test than in the 10-day test, in two cases (BH20-21 and BH21-26), survival was unexpectedly greater in the chronic test. This may have been due to slightly different feeding regimes in the two test methods that, in turn, altered the bioavailability of contaminants. Alternatively, this may indicate method error for these tests. Sampling error was unlikely to be a factor because the same sediment samples from each site were split for acute and chronic tests and all samples were homogenized prior to partitioning into test chambers. Results of the chronic *Hyalella*

test suggest toxicity effects at site BH19-18 near the breakwater and perhaps at site BH21 (near old wastewater outfall) and at BH23 (boathouse).

Table 3-11. Summary of biological data measured in *Hyalella azteca* 28 day chronic sediment toxicity testing on samples from selected sites collected in May 1998. Sites with the same superscripts in common were not significantly different from each other (Duncan multiple range test, p >0 .05).

Site	% Survival	Length (mm)		Weight (mg)		Number of eggs/female	
		Mean	SD	Mean	SD	Mean	SD
Control	75.0[1]	3.44[3]	0.35	0.24[1]	0.04	0.82[1]	0.07
19-18	61.7[1,2]	2.87[1,2]	0.11	0.15[1,2]	0.06	0.12[2]	0.01
20-21	76.7[1]	3.32[1]	0.21	0.23[1]	0.07	0.95[1]	0.11
21-26	81.7[1]	3.01[1,2]	0.12	0.20[1,2]	0.04	0.72[1]	0.06
21	60.0[2]	3.45[3]	0.33	0.37[3]	0.08	0.65[1]	0.07
23	60.0[2]	3.79[3]	0.27	0.45[3]	0.06	0.87[1]	0.08
30	61.7[1,2]	3.08[1]	0.21	0.23[1]	0.06	0.52[1]	0.04

3.8 Toxicity Identification Evaluation of Interstitial Water

Acute (48 hour) toxicity tests were conducted on treated interstitial water from sediments collected at sites BH20-21 and BH21-26, both of which exhibited toxicity to *Hyalella* in the 1997 sampling. Results of these tests indicated no acute toxicity to *Ceriodaphnia dubia*, even in the untreated interstitial water (Appendix H). This result was not expected given the effects observed on *Hyalella* survival in whole sediment testing, however, the acute testing used a much shorter exposure period (2 vs 10 days). This result may also indicate that the contaminants are sediment-bound and that observed toxicity in *Hyalella* sediment tests resulted from organisms ingesting contaminated particles. PAHs or metals such as lead could conceivably cause such a result because they are often tightly

sorbed to sediment particles (Landrum 1989). This result was unlikely to be due to different species being used in the sediment and interstitial water tests because *Hyalella* and *Ceriodaphnia* have been shown to have very similar sensitivity to a range of contaminants in water-only testing (Burton 1992).

3.9 Bioaccumulation Analyses

Lumbriculus exposed for 28 days to sediments from three different sites in the harbor (BH20-21, BH21, and BH23, all of which were shown to have relatively high PAH concentrations and toxicity to *Hyalella*) did not accumulate PAHs (Table 3-12, Appendix I). All tissue PAHs were below detection at all three sites. This result suggests that if PAHs were accumulated by the worms during testing, they were metabolized and/or excreted prior to sampling at 28 days of exposure (Ingersoll et al. 1995).

Table 3-12. Initial and final tissue residues of lead and total PAHs in *Lumbriculus variegatus* exposed for 28 days to selected sediment samples from Burlington Harbor (collected in May 1998).

	Initial		Final		Final	
	Lead	PAH	Tissue Lead	Sediment* Lead	Tissue PAH+	Sediment* PAH
Treatment	(µg/kg)		(µg/kg)		(µg/kg)	
Control	< 590	< 35	<590	< 590	84	< 35
B23	---	---	8,800	123,690	< 4	6080**
B21	---	---	41,500	85,784	38	9760
B20-21	---	---	18,800	89,496	< 29	18,130

*** Sediment concentrations from August 1997 were used.**
+ Lipid normalized
**** Average of field duplicate results**

In contrast, lead was accumulated to some degree by worms exposed to the three harbor sediments (Table 3-12, Appendix I). Lead bioaccumulation factors (BAF) between 0.07 and 0.48 were observed

for these samples, assuming sediment lead concentrations measured at these same sites in August 1997. These BAFs are low and suggest a low potential for hazard due to lead bioaccumulation. All of these test results are uncertain because we were relying on chemical results from samples collected the previous August and harbor sediment physicochemical characteristics are spatially and temporally variable (Watzin et al. 1997). More extensive bioaccumulation testing would be necessary to adequately evaluate the risk from bioaccumulative contaminants in the harbor.

3.10 Protein Expression Analyses

Appendix J shows the two-dimensional protein patterns for fathead minnows exposed in chronic sediment testing. Image analysis using MELANIE showed that approximately 500 protein spots were present on the gels. Comparison of protein patterns observed in fish exposed to site BH24 sediment (considered a reference sediment for this analysis because it had the lowest PAH concentration) and site BH21 (the most contaminated site), revealed that at least two protein spots were new or induced in site BH21 fish and were not present on any other gel, including the control.

At least five protein spots were present only on the gel from fish exposed to reference site sediments (BH24) and not on gels representing more contaminated sites. These proteins may have been suppressed in fishes exposed to sediment from the more contaminated sites (BH11, BH19, BH20, BH21, and BH26).

Pair reports generated by MELANIE identified protein spots present on composite gels generated from at least three gels at two different sites. Features (protein spots) on the 2D image from site BH24, for

example, were compared to features on images from other sites. Features unique to site BH24 exposed fish can be interpreted as proteins that are suppressed in fish exposed to other sediments. This analysis indicated that six proteins were present in all protein patterns obtained from the different sites (BH11, BH19, BH20, BH21, BH26) except for site BH24 (reference).

Several proteins in fathead minnows were induced or suppressed as a result of exposure to contaminated sediments in the harbor. This may be due to interference of the contaminants with transcription factors that result in the activation of certain fish genes. Fish exposed to the reference sediment did not express these proteins. It is reasonable to assume that contaminants measured in this study (specifically PAHs) and/or those measured previously (PCBs, DDE) that are present in these sediments have influenced the protein patterns of the fish. Spiked sediment exposure experiments, using a range of PAH concentrations, would need to be carried out to confirm that the proteins expressed or inhibited match those observed in this study. However, these preliminary analyses, together with the toxicity test results presented earlier, indicate that the more contaminated harbor sediments produce biological effects that could have ecological consequences in the harbor.

3.11 Biological Assessment

Despite efforts to collect at least 200 organisms at each site, most samples had between 80 and 180 organisms per 0.07 sq. meters (3 petite Ponar samples) (Table 3-13, Appendix K). Sites outside the harbor, BH24-2, BH24-3, BH24-5, had the fewest invertebrates; this appeared to be related to the especially coarse sandy substrate observed there.

Table 3-13. Benthic macroinvertebrate metric values calculated for each site sampled in Burlington Harbor, August 1997.

Site Name	No. Of Organisms	Total Taxa	Chiro-nomid Taxa Richness	Chironomidae Percent Dominance	Percent Oligochaeta	Percent Crustacean/ Mollusc	Crustacean/ Mollusc Taxa Richness
BH11	154	22	11	37.11	22.73	9.74	5
BH19	60	17	9	25.93	3.33	30.0	5
BH19-11	60	22	7	41.67	11.67	40.0	10
BH19-18	80	15	6	28.13	0	25.0	5
BH20	233	17	2	61.54	0	31.48	11
BH20-21	172	10	1	100	0	52.32	8
BH21	Sample lost in laboratory accident						
BH21-26	142	21	11	46.39	1.41	23.94	4
BH23	521	20	3	72.73	0	78.50	9
BH24-1	157	16	9	31.11	0	58.60	3
BH24-2	241	7	0	0	0	83.33	3
BH24-3	95	8	0	0	0	82.11	3
BH24-4	Benthic data not included due to questionable sort data by volunteer (QC).						
BH24-5	47	8	4	50.00	8.51	4.26	2
BH26	81	17	7	67.16	4.94	6.17	3
BH30-1	154	19	9	27.45	1.30	53.90	5
BH30-2	80	13	5	28.57	3.75	83.75	6
BH30-3	131	22	8	35.00	1.53	78.63	9
BH30-4	153	21	10	39.39	0.65	57.51	7
BH30-5	159	23	13	45.71	0	69.81	6

Physicochemical data presented earlier in this report indicated some significant differences between sites near the breakwater or outside the harbor and those located in the vicinity of the old wastewater

outfall, the oil dolphin, or the public boathouse and docks. We were interested in determining which benthic invertebrate attributes measured in this study discriminated between these two types of sites.

We initially examined six metrics, each of which could be ecologically relevant to Burlington Harbor: number of taxa; number of chironomid genera; percent individual crustacean or molluscs; number of crustacean and mollusc species; percent Oligochaetes; and percent dominance within Chironomidae (Table 3-13). Only the metric percent dominance within the Chironomidae family was significantly different between site classes ($p < 0.01$, Figure 3-8a). Percent Oligochaetes was possibly different between site types as well ($p = 0.07$, Figure 3-8b). We concluded from this analysis that these two metrics could be useful in discriminating between near-shore contaminated sites and other sites in the harbor.

We used correlation analysis to determine whether either of these two metrics was related to a gradient of sediment physiochemical constituents. Percent dominance within Chironomidae was directly related to the logarithm of PAH concentration (Figure 3-9a) and inversely related to nickel, copper, silver, and lead concentrations ($p < 0.05$). Though preliminary, these results suggest that percent dominance within Chironomidae may be a useful indicator of sediment PAH stress on benthic invertebrates in the harbor.

Percent Oligochaetes was correlated with ammonia (Figure 3-9b), suggesting that percent Oligochaetes could be a useful indicator of ammonia stress in the harbor. However, this preliminary result needs further evaluation, because interstitial ammonia concentrations were not measured in our study and

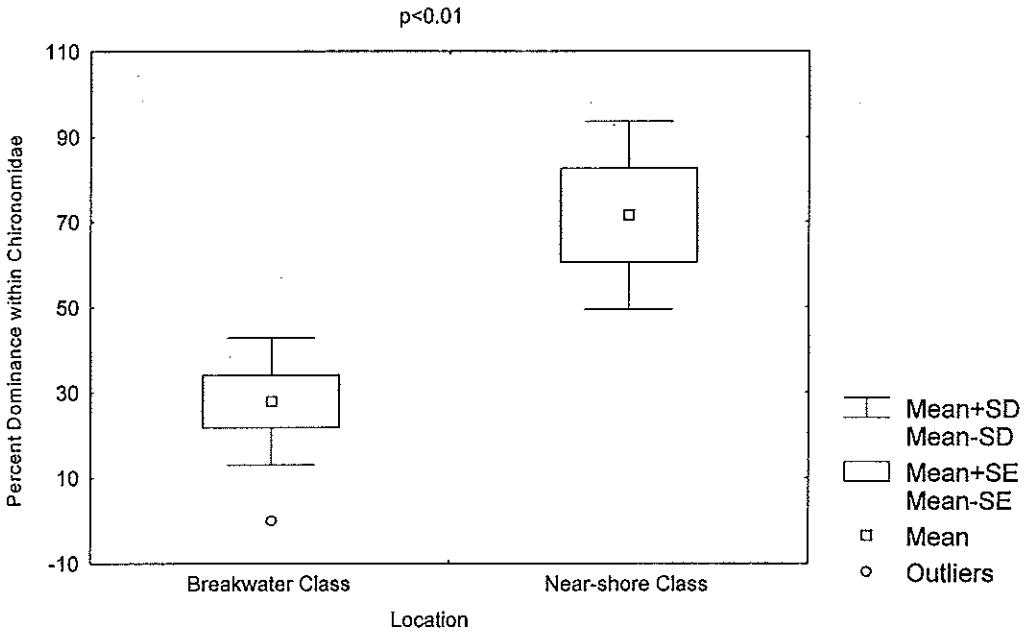

Figure 3-8a. Comparison of percent dominance within Chironomidae families between sites near the breakwater or "reference" areas in the northern part of the harbor (Breakwater Class) and sites near the old wastewater outfall, oil dolphin, and public docks (Near-Shore Class).

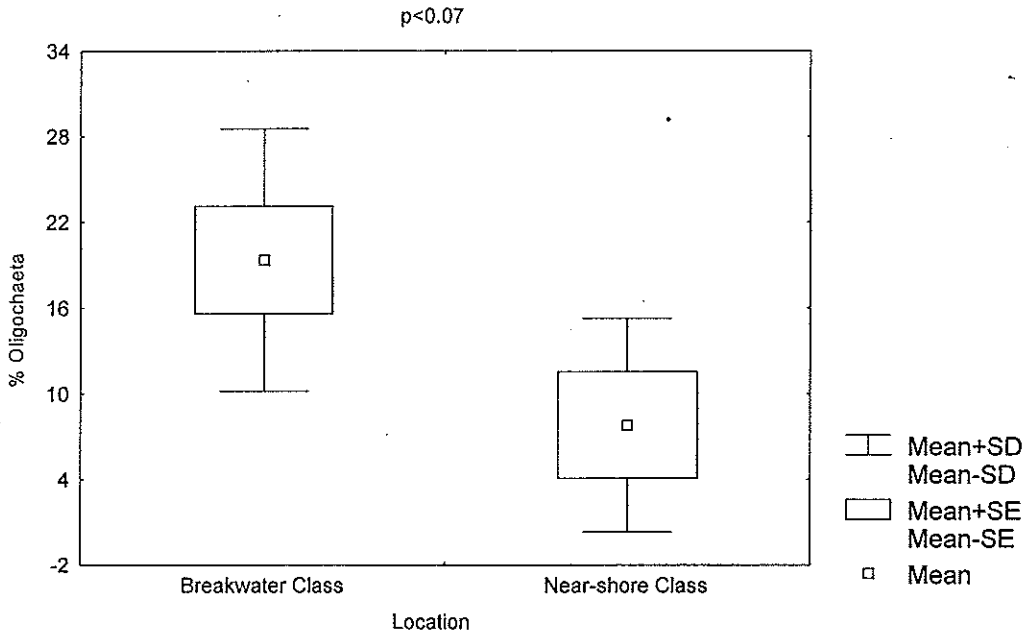

Figure 3-8b. Comparison of percent Oligochaetes between sites near the breakwater or "reference" areas in the northern part of the harbor (Breakwater Class) and sites near the old wastewater outfall, oil dolphin, and public docks (Near-Shore Class).

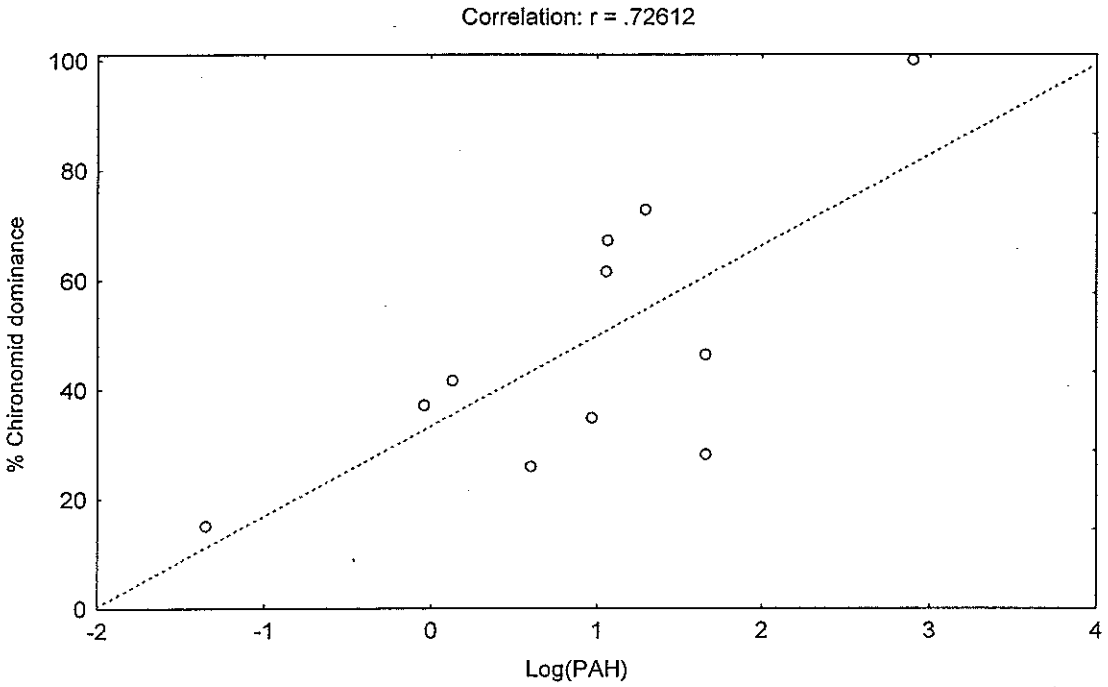

Figure 3-9a. Relationship between percent dominance within chironomidae and the logorithm of PAH concentration for samples collected in Burlington Harbor, August 1997.

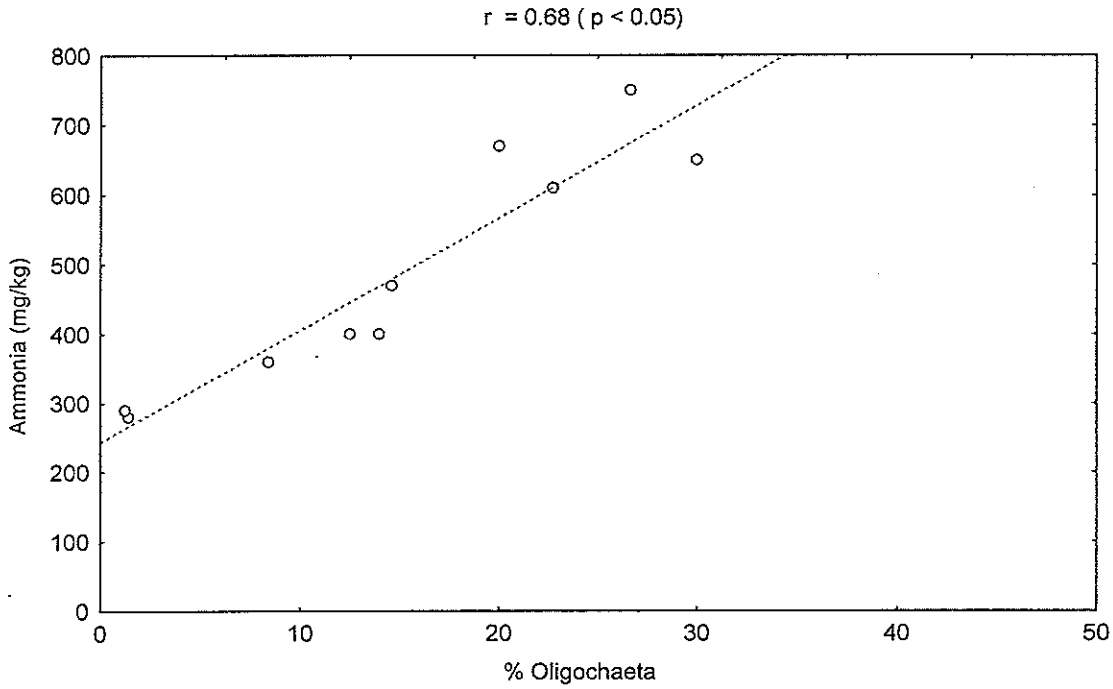

Figure 3-9b. Relationship between percent oligochaetes and ammonia concentration for samples collected in Burlington Harbor, August 1997.

recent reports have indicated that benthic invertebrates are more susceptible to interstitial, rather than sediment-bound, ammonia (Canfield et al. 1996; ASTM 1995c).

One major difference between biological results gathered in the present study and those obtained previously is the recent invasion, and growing abundance, of *Dreissena polymorpha* (zebra mussels) in the harbor. Zebra mussels were collected at most nearshore sites (e.g., sites BH23, BH21, BH20-21) and appeared to be limited to < 7m depth. Multiple regression analysis indicated that lead, ammonia, and organic carbon normalized PAH were most related to the number of molluscan and crustacean taxa (Table 3-14). Zebra mussels and isopods were often collected in association with macrophytes in the August 1997 sampling. In the May 1998 sampling, zebra mussels were collected in most areas of the harbor where there was hard substrate, even to a depth of 10 m.

Table 3-14. Summary of multiple regression analyses of benthic invertebrate assemblage metrics, summer 1997.

Dependent Variable	Overall Model	Contributing Factors	Association	Partial R^2	p-value
Taxa richness	$R^2 = 0.794$	TOC	—	0.242	.001
	$p < 0.008$	Ammonia	+	0.243	.003
	$N = 11$	log (PAH)	+	0.258	.017
Number of crustacean and mollusc genera	$R^2 = 0.880$	Pb	—	0.269	< 0.001
	$p < .001$	Ammonia	+	0.283	0.002
	$N = 11$	Organic carbon-normalized PAH	+	0.328	0.096
Percent crustacean and mollusc individuals	$R^2 = 0.504$	SEM/AVS	+	0.131	0.058
	$p = 0.156$	Pb	—	0.144	0.105
	$N = 11$	Organic nitrogen	+	0.227	0.121

Table 3-14. Summary of multiple regression analyses of benthic invertebrate assemblage metrics, summer 1997 (continued).

Dependent Variable	Overall Model	Contributing Factors	Association	Partial R^2	p-value
Number of chironomid genera	$R^2 = 0.572$	Lead	+	0.286	0.014
	$p < 0.03$	TOC	—	0.286	0.141
	N = 11				
Percent dominance within Chironomidae	$R^2 = 0.887$	PAH	+	0.372	< .001
	$p < .001$	TOC	—	0.405	0.004
	N = 11	SEM/AVS	—	0.109	0.016
Percent Oligochaetes	$R^2 = 0.699$	Ammonia	+	0.398	< 0.003
	$p = 0.015$ = 0.008	Organic carbon normalized PAH	+	0.398	.035
	N = 11				

For each of the other five metrics that were identified in Table 3-13, we performed forward stepwise multiple regression analyses using physicochemical measures as the independent factors. Factors considered in models included depth, percent fines, TOC, ammonia, lead, silver, SEM/AVS, organic nitrogen, and PAH (both organic carbon normalized, and non-normalized). Metals other than lead and silver were not included in these analyses because other metals were either relatively low in concentration (often < ERL) or they were highly correlated with either lead or silver.

Results of these analyses are summarized in Table 3-14. Ammonia, organic nitrogen, and/or organic carbon were significant explanatory factors for several biological metrics. Thus, sediment physical properties and nitrogen appeared to be major factors explaining benthic invertebrate assemblage characteristics in the harbor. The previous Phase 2 study (Watzin et al. 1997) reported a significant effect of particle size on several benthic assemblage attributes.

Two metrics, percent Oligochaetes and percent dominance within Chironomidae, were directly related to sediment PAH concentration, consistent with the possibility that PAHs are having toxic effects on certain benthic fauna in the harbor. However, other metrics were either unrelated to PAH concentration or related in a way that was not consistent with our knowledge of benthic ecology (e.g., taxa richness was positively associated with PAH concentration, Table 3-14).

4.0 DISCUSSION

Five separate types of analyses were conducted in this study (toxicity, chemical, biological, bioaccumulation, and protein expression) in an attempt to determine sediment quality at various places in Burlington Harbor using a weight of evidence approach. Bioaccumulation analyses (both laboratory *Lumbriculus* testing and zebra mussel tissue PAH analysis) indicated that PAHs were not accumulated and lead was accumulated only slightly. Given the preliminary nature of these analyses, bioaccumulation results are considered uncertain and should not be evaluated on an equal basis with toxicity, chemistry, and biological results. Protein expression analysis of sediment-exposed fish suggested changes in certain proteins at most harbor sites as compared to site BH24 outside the harbor. Thus, these results, though interesting as supporting information, are too preliminary and apparently not discriminating enough to be useful in a weight-of-evidence assessment approach. We, therefore, followed the standard sediment triad approach (Chapman et al. 1992; Chapman et al. 1997), using sediment contaminant data, toxicity test results, and benthic macroinvertebrate measures, to assess overall sediment quality at sites in Burlington Harbor.

Figure 4-1 summarizes results of the sediment quality triad analysis. Effects based on each type of data were classified as low, intermediate, or high, depending on the magnitude and/or consistency of the data for each data type. In general, we observed reasonable concordance among the three types of data for each site. Sites BH20-21, BH21-26, and BH23 all had relatively high PAH contamination, intermediate or high toxicity, and relatively poor benthic integrity. Sites BH20,

Site	Chemical		Toxicity		Benthic Invertebrates		
	PAH	SEM/ AVS	P. promelas	Hyalella 10 d or 28 d	Taxa Richness	% Chir Dominance	Percent Oligochaetes
11	○	○	◎	●	○	○	○
19	○	○	○	●	○	○	○
19-11	○	○	○	●	○	◎	○
19-18	◎	○	○	◎	◎	○	○
20	○	○	○	○	○	●	●
20-21	●	○	○	●	●	●	●
21	●	●	○	●	ND	—	—
21-26	◎	●	○	◎	○	◎	●
23	◎	○	○	◎	○	●	●
24	○	●*	○	◎	●	○	○
26	○	○	○	●	○	●	●
30	○	●*	◎	○	○	◎	◎

*Interpretation uncertain because AVS concentration was approximately 1 µmole/g

Figure 4-1. Qualitative summary of sediment quality triad analysis Burlington Harbor, 1997 - 1998; ○ = small concentration or effect, ◎ = intermediate, ● = high

BH30, and possibly BH24 had relatively low PAH contamination, low toxicity, and relatively intermediate or high benthic integrity measures, suggesting relatively unimpaired conditions at those sites. The breakwater sites exhibited some inconsistencies among the three types of data. PAHs and SEM/AVS measures indicated generally little contamination, but these sites also exhibited toxicity and/or low benthic integrity (Figure 4-1). These results suggest that there may have been some other contaminant present (e.g., ammonia) at those sites.

Results of all analyses conducted by Tetra Tech, and previously by the University of Vermont (Watzin et al. 1997), indicated that PAHs as a whole and, specifically, a few individual PAHs (fluoranthene, pyrene, phenanthrene), are present at potentially toxic levels in surficial sediments, particularly in the southern part of the harbor. Other potential chemical stressors measured in either our study or by the University of Vermont included silver, lead, and copper. Results of simultaneous extracted metals (SEM) and acid volatile sulfide (AVS) analyses suggested that the metals observed at most sites were unlikely to be bioavailable in the interstitial water because the excess sulfide present should result in metal sulfide precipitates or tightly bound complexes that are not easily taken up by aquatic organisms through water uptake (Ankley et al. 1996). Of the four sites at which SEM/AVS ratios were greater than 1 (signifying the potential for metal toxicity), two (sites BH30 and BH24) exhibited little toxicity in whole sediment tests, and both sites exhibited benthic community metrics indicative of relatively good sediment quality either in the present study or in previous work (Watzin et al. 1997). However, *Hyalella* toxicity and some benthic macroinvertebrate metrics were negatively correlated with certain metals, notably lead. Thus, lead and perhaps other metals could be toxic to benthic fauna via ingestion of metal-contaminated sediment as opposed to interstitial water exposure. Alternatively, *Hyalella* and benthic macroinvertebrates could have been

responding to some unknown chemical (e.g., PCBs?; McIntosh et al. 1996) that was spatially correlated with metals.

Table 4-1 summarizes available relevant criteria or ecological threshold values pertaining to PAH toxicity and freshwater organisms. Sources of information included USEPA water and sediment quality criteria documents, data from the ARCS Program (USEPA 1996), Long and Morgan's values (1991), Ontario Ministry of Environment sediment quality guidelines (Persaud et al. 1993), and other published data. The ARCS values, USEPA's sediment quality criteria, and Washington State's thresholds are normalized on the basis of organic carbon concentration. Other values listed are not computed using normalized PAH concentrations.

Table 4-1. Summary of available toxicological benchmarks or criteria and bioconcentration factors (BCFs) reported for PAHs common in Burlington Harbor and for total PAHs. * = µg/g organic carbon

Chemical	Source	Concentration (µg/g)	BCF
Fluoranthene	USEPA SQC	620*	200-1800 (fish)
	Washington State	100*	
	USEPA, chronic WQC	6.16 µg/L	
	Long and Morgan ERL	85.3	
	Florida DEP threshold level	46.9	
	ARCS ERL (*Hyalella*)	2.1*	
	ARCS ERM (*Hyalella*)	7.6*	
Pyrene	Washington State	1,000	970 (fish)
	Long and Morgan ERL	665	2,700 (invertebrates)
	Florida DEP threshold	153	
	ARCS ERL (*Hyalella*)	2.1*	
	ARCS ERM (*Hyalella*)	18.3*	
Phenanthrene	USEPA proposed chronic WQC	6.3 µg/L	325 (invertebrates)
	Long and Morgan ERL	240	
	Florida DEP threshold	86.7	

Table 4-1. Summary of available toxicological benchmarks or criteria and bioconcentration factors (BCFs) reported for PAHs common in Burlington Harbor and for total PAHs (continued).

Chemical	Source	Concentration (µg/g)	BCF
	ARCS ERL (*Hyalella*)	1.0*	
	ARCS ERM (*Hyalella*)	13.8*	
Total PAHs	Washington State	370 (for low mol. Wt. PAHs)	
	ARCS ERL (*Hyalella*)	15*	
	ARCS ERM (*Hyalella*)	105*	
	Long and Morgan ERL	4.0	
	Long and Morgan ERM	44.8	

Using organic carbon-normalized concentrations, the acute (lethality) values range between 7 and 347 mg/kg for individual PAHs that were found in harbor sediment. Chronic values range between 1 and 665 mg/kg, depending on the compound and the data source. Fluoranthene, which was the most abundant PAH observed in our study and in previous work, has acute and chronic values of approximately 8 and 2 mg/kg organic carbon, respectively. If we assume that the bulk sediment concentrations measured are in equilibrium with the interstitial water concentration (an assumption used by USEPA in their sediment-quality criteria development for nonionic organic pollutants such as PAHs), then pore water concentrations could conceivably exceed the proposed USEPA water quality criteria for select PAHs (~ 6 µg/L), resulting in chronic toxicity to aquatic life at several sites, particularly BH21, BH20, and BH21-26 near the old wastewater outfall and the oil dolphins.

Data derived from the ARCS Program (USEPA 1996), in which *Hyalella*'s sensitivity to sediment PAHs was investigated at a number of sites in the Great Lakes, indicated that a total PAH concentration of 15 mg/kg organic carbon was chronically toxic to *Hyalella*. A fluoranthene

concentration of 7.6 mg/kg organic carbon was acutely toxic as well. These PAH concentrations were exceeded at a number of sites, especially in the southeastern portion of the harbor.

The risks associated with sediment PAHs were further evident if PAH concentrations were based on the fine sediment fraction only. Site BH21, for example, had a normalized PAH concentration of 30.5 mg/kg in the fine sediment fraction (as opposed to 9.7 mg/kg based on the sediment as a whole), which far exceeds most of the acute and chronic threshold values proposed for sediment PAHs (Table 4-1). Because benthic fauna are most likely to come into contact with, or ingest fine sediment, these results suggest long-term effects due to PAHs in the southern part of the harbor.

The USEPA has developed a sediment quality criterion of 620 mg/kg organic carbon for fluoranthene based on an equilibrium partitioning model (DiToro et al. 1991). Washington State has a sediment quality criterion of 100 mg/kg organic carbon for fluoranthene. These criteria were generally not exceeded in the harbor, suggesting that acute risks to fauna from fluoranthene are unlikely.

Recent work has indicated that many PAHs are subject to photoinduction or greater toxicity when exposed to UV radiation (Ankley et al. 1994). In laboratory studies in which UV radiation approximated that observed at shallow depths (1-2 m) in clear water, acute effects were observed at lower levels of PAHs than without UV present. More significantly, delayed effects on survival were observed several days after UV radiation was removed. Thus, elevated sediment PAH concentrations in shallow areas of the harbor (such as observed at site BH23), may represent

Table 4-2 summarizes available toxicological data for the most abundant anthropogenic metals encountered in our study: copper, lead, zinc, and silver. These sediment benchmarks are not normalized in any way and are derived using an apparent effects threshold (AET) approach and whole sediment metal concentrations. If bulk sediment concentrations are assumed to be similar to interstitial water concentrations, all USEPA metal water quality criteria would have been exceeded. However, much of the metal measured in harbor sediments may not be present in interstial water because the SEM/AVS measurements rarely exceeded one. Only at sites BH30, BH24, BH21, and BH21-26 were the SEM/AVS ratios greater than 1.0, signifying the possibility of bioavailable metals in pore water at those sites. These sites were recognized in previous work (McIntosh et al. 1996). Sediments from sites BH30 and BH24 did not produce toxicity in our study, although sites BH21 and BH21-26 did. The latter sites were also characterized by high PAH concentrations.

Table 4-2. Summary of available toxicological benchmarks or criteria reported for metals common in inner Burlington Harbor.

		Metal Concentration (μg/L or kg)*			
Source of Information	**Nickel**	**Copper**	**Lead**	**Zinc**	**Silver**
Water					
USEPA Acute WQC	789.0	9.2	74	65	0.92
USEPA Chronic WQC	87.7	6.5	3.2	58.9	0.12
Sediment					
Ontario MOE LEL	16,000	16,000	31,000	120,000	---
Hazardous Substance Database	---	34,000	---	150,000	---
Long and Morgan ERL	20,900	34,000	46,700	150,000	1,000
Long and Morgan ERM	51,600	270,000	218,000	410,000	3,700
Ontario MOE SEL	75,000	110,000	250,000	820,000	---
ARCS ERL (*Hyalella*)	19,514	16,965	---	384,000	---
ARCS ERM (*Hyalella*)	47,500	209,000	---	544,000	---

* At 50 mg/L as $CaCO_3$ hardness

Sediment toxicity can occur over a wide range of metal concentrations, depending on the availability of complexing or oxidizing agents. In the ARCS Program (USEPA 1996), for example, the probable effect level (PEL) for copper was approximately 50 mg/kg based on 28-day *Hyalella* toxicity tests. Sediment copper concentrations in our study were rarely greater than 40 mg/kg. Silver, copper, lead, and zinc exceeded Long and Morgan's ERL values in the harbor, similar to results reported previously (McIntosh et al. 1996). However, Long and Morgan values are not normalized to account for metal bioavailability. Given the well oxygenated lake bottom, metals in surficial sediments are probably maintained in an oxidized state, such that sediment interstitial or pore water is not likely to contain toxic concentrations of metals (Ankley et al. 1996). However, lead was related to several toxicological and biological responses in this study suggesting that organisms may be responding to sediment-associated, and not pore water lead (and perhaps other metals). Therefore, SEM/AVS may not be a reliable indicator of metal toxicity in the harbor. The high lead concentrations observed at several breakwater sites in particular (e.g., BH11, BH19, and BH19-18) suggest that this metal may pose a long-term risk to aquatic life in the harbor.

5.0 CONCLUSIONS

Results of the present research support earlier work conducted between 1990 and 1993 in the harbor. Specifically, the southern end of the harbor and the breakwater area appear to have generally higher metal and PAH sediment concentrations, more sediment toxicity, and poorer benthic assemblage attributes than other sites. The present sampling at select new locations generally confirmed and lent more certainty to the spatial patterns of contaminants reported earlier (Watzin et al. 1997). Toxicity and biological data for multiple samples taken at reference sites inside and outside of the harbor in this study also confirmed the high degree of spatial heterogeneity reported in earlier studies. The fact that sediment samples from site BH24, outside the harbor, showed as much variability in toxicity and fewer invertebrate taxa than site BH30 inside the harbor suggests that sediment disturbance and contaminant accumulation are important factors immediately outside the harbor as well as inside the harbor.

Biological and toxicological results appeared to be related to sediment ammonia, organic nitrogen, PAH, and lead concentrations. A similar role for PAH was observed in the previous study (Watzin et al. 1997). However, it is not clear to what extent temporal variability affects this relationship. We observed lower sediment concentrations of PAHs, copper, nickel, and zinc in this study as compared to 1993. The relocation of the sewage outfall outside the harbor and implementation of some stormwater runoff controls in between these two studies may have been responsible for the observed decrease in these contaminants. Follow-up monitoring should be conducted to confirm this trend and to ascertain the spatial heterogeneity in chemical and toxicity levels in the harbor.

Perhaps one of the biggest biological differences observed between the current study and studies conducted earlier is the presence of zebra mussels in the harbor. In 1997, this species appeared to be confined to sites < 7m depth and was especially associated with aquatic macrophytes. In the spring sampling, zebra mussels were abundant in most areas of the harbor. This species was absent from the harbor in 1993. The long-term effect of this invasion is unclear, particularly with respect to PAHs and other contaminants in the harbor.

Chronic (28-d) *Hyalella* testing, using samples collected in the spring, further confirmed the presence of chronically and acutely toxic conditions for those sites at which PAH concentrations were highest. Protein expression analyses on fathead minnows exposed in chronic sediment tests suggested that there were several differences in specific proteins expressed in fish from PAH-contaminated sites relative to fish exposed to low PAH concentrations. These results further support the contention that PAHs are a source of stress to aquatic fauna at some sites in the harbor (especially southern part).

Zebra mussel tissue analyses indicated that PAHs were not accumulated. *Lumbriculus* bioaccumulation tests on selected sediment samples taken in the spring also indicated that PAHs were not bioaccumulated. These data suggest that the relatively low weight PAHs common in harbor sediments (e.g., phenanthrene, pyrene, and fluoroanthene) are metabolized or excreted by aquatic fauna, if they are ingested at all, and not accumulated to any appreciable extent. However, it is not known whether sediment PAHs are mobilized and potentially accumulated by benthic fauna under other lake conditions or seasons. Furthermore, the biological cost of metabolizing and/or excreting PAHs by aquatic fauna is unknown at present. Comparisons between our data and

available benchmark values indicated that PAHs and lead are likely sources of long-term stress in the harbor. Given that the harbor is relatively shallow and contained (by a breakwater), and that there are frequent episodes of turbulence and sediment resuspension, historic deposits of PAHs and lead may continue to affect aquatic life in the harbor. Follow-up monitoring may be warranted to identify whether lead poses significant ecological and human health risks in the harbor and whether other contaminants measured previously, such as PCBs, are also significant stressors to aquatic life in Burlington Harbor.

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