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Identification of the Sediment-Associated Contaminants in the Illinois River Complex using Toxicity Identification Evaluation (TIE)

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Forward

The final report for this project "Identification of the Sediment-Associated Contaminants in the Illinois River Complex using a Toxicity Identification Evaluation (TIE)", conducted by Dr. Michael Lydy and W. Tyler Mehler, is comprised of the thesis of W. Tyler Mehler submitted to the Department of Zoology, Southern Illinois University – Carbondale in December 2009.

In addition, two papers have been published which are based on this project:

Mehler, W. Tyler, Jonathan D. Maul, Jing You, and Michael J. Lydy. 2009. Identifying the causes of sediment-associated contamination in the Illinois River (USA) using whole-sediment toxicity identification evaluation. *Environmental Toxicology and Chemistry* 29(1): 158-167.

Mehler, W. Tyler, Jing You, Jonathan D. Maul, and Michael J. Lydy. 2010. Comparative analysis of whole sediment and porewater toxicity identification evaluation techniques for ammonia and non-polar organic contaminants. *Chemosphere* 78: 814-821.

The thesis and research papers have been subjected to external scientific peer review and may not necessarily reflect the views of the Illinois Sustainable Technology Center.

IDENTIFICATION OF THE SEDIMENT-ASSOCIATED CONTAMINANTS IN THE ILLINOIS RIVER COMPLEX USING A TOXICITY IDENTIFICATION EVALUATION (TIE)

by

W. Tyler Mehler

B.S., Southern Illinois University Carbondale, 2005

A Thesis
Submitted in Partial Fulfillment of the Requirements for the
Master of Science Degree

Department of Zoology
In the Graduate School
Southern Illinois University Carbondale
December 2009

THESIS APPROVAL

IDENTIFICATION OF THE SEDIMENT-ASSOCIATED CONTAMINANTS IN THE ILLINOIS RIVER COMPLEX USING A TOXICITY IDENTIFICATION EVALUATION (TIE)

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for the Degree of

Master of Science

in the field of Zoology

Approved by:

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AN ABSTRACT OF THE THESIS OF

W. TYLER MEHLER, for the Masters of Science degree in ZOOLOGY, presented on JUNE 25, 2008, at Southern Illinois University Carbondale.

TITLE: IDENTIFICATION OF THE SEDIMENT-ASSOCIATED CONTAMINANTS IN THE ILLINOIS RIVER COMPLEX USING A TOXICITY IDENTFICATION EVALUATION (TIE)

MAJOR PROFESSOR: Michael J. Lydy

The difficulty of assessing risk of sediment-associated contaminant mixtures to benthic ecosystems is often attributed to understanding the bioavailable fraction of each contaminant. These issues have led to the development of the toxicity identification evaluation (TIE). Past pore water TIE testing on Illinois River sediments has indicated that ammonia was the primary contaminant. The current study, however, suggests that ammonia is no longer the primary contaminant of concern, but rather non-polar organics, including polycyclic aromatic hydrocarbons, are the primary cause for toxicity in the Illinois River Complex (IRC).

Summer of 2007 testing showed that six out of the seven sites that proceeded to Phase I testing exhibited a significant increase in survival with the addition of the non-polar organic amendment powdered coconut charcoal (PCC), while zeolite (ammonia amendment) and Resin Tech SIR 300 (cationic metals amendment) did not significantly increase survival suggesting that non-polar organics are the source of toxicity. In addition, Phase II testing suggested that concentrations of PAHs were high enough to cause the observed toxicity, which confirmed the results of Phase I testing. Additional seasonal-based sampling (i.e., fall, winter, spring, and summer 2008) supported the summer findings, with little variation between toxicity and concentrations, with 46% of the sites being improved with the addition of PCC in Phase I testing.

The results of Phase I and Phase II contradicted past pore water TIE studies as non-polar organics were suggested as the source of toxicity rather than ammonia. Thus, both pore water and whole sediment TIE methodologies were used on two selected sites. The results of this study suggested that discordance between the past pore water TIEs and the current whole sediment TIE were attributed to the methodologies and on a lesser note the test organisms used.

The present study provides data that could be used in combination with previous work to more accurately characterize the sources and spatial trends of toxicity in Illinois River sediments for future risk assessment and mitigation. Furthermore, the present study showed that while TIE methodologies are a valuable tool in assessing risk associated with contaminants in aquatic system, further research in understanding the role that each TIE method may serve in risk assessment is also important.

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CHAPTER 1

OVERVIEW

Two methods are widely used in aquatic risk assessment, the conventional contaminant-based approach and the toxicity-based approach. The conventional contaminant-based approach relies on the quantification of contaminants in sediment by exhaustive extraction, with relative toxicity being estimated by comparing those concentrations to published toxicity values. The downside of this approach is that toxicity is based on individual toxicant concentrations, and does not take into account the bioavailability or interactions of the constituents in sediment. In addition, with thousands of potential contaminants in a sediment sample, analytical measures of every chemical is impractical on a time and cost basis (Norberg-King *et al.* 2005).

The toxicity-based approach involves exposing organisms directly to a potentially toxic media and evaluates the risk of sediment contamination based on organism response (U.S. EPA 1991). The toxicity identification evaluation (TIE) method falls under this classification. In a TIE, toxicity is evaluated before and after a manipulation of the toxic media, which can substantially alter toxicity if a certain contaminant class is present. The degree of difference in toxicity between pre- and post-manipulation determines whether a particular contaminant class is a contributing factor to toxicity. If a difference in toxicity is observed, chemical quantification can be conducted to determine the specific compound(s) that may be the source of toxicity. Both pore water and whole sediment have been used in TIE procedures for sediment risk evaluation. A pore water TIE involves exposing test organisms to pore water extracted from the sediment, while a whole sediment TIE exposes organisms directly to the bulk sediment.

A TIE is conducted in two-phases: Phase I (characterization) and Phase II (identification) (U.S. EPA 1991). Phase I establishes the physical and chemical characteristics of the sediment using various manipulations to alter the bioavailability of a particular class of contaminants, such as cationic metals, non-polar organics, or ammonia (Ankley and Schubauer-Berigan 1995). Phase II depends heavily on the results of Phase I and uses various analytical techniques to identify the contaminants that may have contributed to the toxicity (U.S. EPA 1993a). The purpose of each phase is not to validate the previous phase, but to collectively validate the source of the toxicity using a weight of evidence approach (U.S. EPA 2007).

The Illinois River Complex (IRC), linking Chicago and Lake Michigan to the Mississippi River (Figure 2.1), has received considerable attention due to its ecological and economical importance. A comparison between studies of the 1920's (Richardson 1921, Richardson 1928) and studies of the late 1960's and early 1970's showed a dramatic decline of fingernail clams and snails in the IRC (Anderson 1977, Sparks *et al.* 1981). The conservation and protection of these benthic species is of the utmost importance due to their role in the dietary needs of bottom feeding fishes (Starrett 1972), as well as many diving ducks (Sparks 1977, Bellrose *et al.* 1979, Sparks and Ross 1992), as these populations have also seen dramatic declines (Sparks and Ross 1992). Addressing the cause of these reductions is needed to discern what type of remedial or pollution control should be employed on the IRC.

Thus, research in the IRC has been conducted to identify the cause of these reductions in the benthic biota, and have shown that IRC sediments were toxic to various benthic organisms (Sparks *et al.* 1981, Schubauer-Berigan and Ankley 1991, Sparks and

Ross 1992, Burton 1995). Furthermore, ammonia was commonly found at elevated concentrations corresponding to acute lethality of much of the benthic community including various snails, amphipods, mayflies, isopods, caddisflies, mollusks, and crayfish (Sparks and Ross 1992, Burton 1995). In addition, sediments in closer proximity to Chicago have been reported to have greater toxicity and higher pore water ammonia concentrations than downstream (Sparks and Ross 1992). Other studies have indicated potential contamination from heavy metals (Darmody *et al.* 2004), organochlorine pesticides (OCPs), polycyclic hydrocarbons (PAHs), and polychlorinated biphenyls (PCBs) (Schubauer-Berigan and Ankley 1991, Burton 1995, Groschen *et al.* 2000).

The present study used a whole sediment TIE to investigate the sources of toxicity on the IRC and examined spatial trends. Using a whole sediment TIE complements and strengthens previous pore water TIE work (Schubauer-Berigan and Ankley 1991, Sparks and Ross 1992, Burton 1995), while using a more environmentally realistic media to analyze toxicity.

In addition to evaluating spatial patterns, temporal trends were also evaluated to understand how these trends may influence the toxicological dynamics of sediments in the IRC. Temperature fluctuations and other water quality parameters that change by season have been shown to directly or indirectly influence toxicity in aquatic systems. Factors that can be affected include species sensitivity (Anderson and Koivusaari 1985, Smolarek *et al.* 1988, Kater *et al.* 2000), the efficiency of municipal wastewater treatment plants, toxic nature of contaminants (Emerson *et al.* 1975, Ankley *et al.* 1990, Frazier *et al.* 1996), and the amount of contaminant entering aquatic systems (i.e., seasonal agricultural run-off) (Goodfellow *et al.* 2005). Therefore, the whole sediment TIE study

was repeated in the fall, winter, spring, and subsequent summer to evaluate potential temporal effects.

A Sediment Quality Triad (SQT) approach was used on the IRC in the 1980-90's (Sparks 1984, Sparks and Ross 1992). The SQT approach involves the integration of chemical analysis, toxicity tests, and infaunal benthic structure analysis (Carr *et al.* 2003). An integral part of the study used a pore water TIE as a method for chemical analysis and toxicity testing in an attempt to identify the source(s) of toxicity (Sparks and Ross 1992). This study in addition to other work on the IRC have suggested that previous widespread losses of benthic organisms, such as fingernail clams, in the Illinois River have changed to localized or episodic conditions, and that general recovery of benthic fauna in the river was beginning in 1992 (Sparks and Ross 1992).

The objective of the present study was to provide the basis for a current Sediment Quality Triad (SQT) approach on the IRC. While the present study will not evaluate the infaunal benthic structure of the IRC, it will initiate much of the work that is needed for such analysis by characterizing potential sources of contamination, elucidating spatial and temporal patterns, and more accurately identifying the areas that need future risk assessment and mitigation.

In addition, a separate study was conducted to compare whole sediment to pore water TIE methodologies as well as differences in the sensitivity of the test organisms being used in past pore water TIEs and the present whole sediment TIE study (*Hyalella azteca* and *Ceriodaphnia dubia*, respectively). Thus, this study allows for direct comparisons with previous TIE work, while providing further information on the variability between pore water and whole sediment TIEs. Therefore, the present study

examined both the overall toxicological patterns on the IRC and also whether TIEs can effectively provide the basis for SQT approaches in other aquatic systems.

CHAPTER 2

EVALUATING THE ILLINOIS RIVER COMPLEX USING A WHOLE SEDIMENT TOXICITY IDENTIFICAITON EVALUATION (TIE)

ABSTRACT

Whole sediment toxicity identification evaluation (TIE) techniques were employed on the Illinois River Complex (IRC) to identify the sources of sediment toxicity that may have contributed to the decline in benthic invertebrate populations. The TIE focused on three classes of contaminants: ammonia, metals, and organics. Sediment toxicity was assessed using the amphipod *Hyalella azteca*, and the focus of the TIE was on assessing spatial and temporal patterns of contamination. Past studies suggested that ammonia was the major source of contamination in IRC sediments. However, the present study suggests that polycyclic aromatic hydrocarbons (PAHs) were the primary contributor of sediment toxicity. Phase I testing showed 46% of the site trials (12 of 26) exhibited increased *H. azteca* survival ($p < 0.05$) with the addition to powdered coconut charcoal (organic amendment), while zeolite (ammonia amendment) and Resin Tech SIR 300 (cationic metals amendment) did not increase *H. azteca* survival. Phase II testing revealed PAH concentrations were high enough to cause the observed toxicity, confirming Phase I results. Spatially, sediment toxicity as well as pore water ammonia concentrations declined with distance downstream from suspected contaminant sources, indicating a potential dilution or remedial effect. Temporally, pore water ammonia, metals, and PAH concentrations varied among sampling periods over an annual cycle for some sites near urbanized areas, while remaining temporally consistent at others. The results of the present study provide new information on the sources of toxicity within the IRC, and demonstrates the importance of evaluating spatial and temporal aspects in

whole sediment TIEs. This is particularly important for evaluations in riverine systems in which hydrologic processes can result in large variations in sediment toxicity on temporal and spatial scales.

INTRODUCTION

During the past 100 years, there has been an increase in the use of the Illinois River Complex (IRC) attributable to three major activities: (1) creation of the Chicago Sanitary and Ship Canal to move Chicago's waste water into the IRC and ultimately into the Mississippi River; (2) construction of levees, locks, and dams along the IRC; and, (3) a shift in land use in Illinois from forests and prairie to a mix of agricultural and urban landscapes (Darmondy *et al.* 2004). The dynamics of the river have changed due to these activities, which have caused increased erosion and sedimentation (Burton 1995, Darmody and Marlin 2002). Moreover, these activities may be responsible for the increases in sediment-associated contamination and noted declines in fingernail clams and benthic aquatic resources (Sparks *et al.* 1981, Sparks 1984, Burton 1995, Darmody and Marlin 2002, Darmody *et al.* 2004). Various classes of contaminants including ammonia (Schubauer-Berigan and Ankley 1991, Sparks and Ross 1992, Burton 1995, Groschen *et al.* 2000), metals (Schubauer-Berigan and Ankley 1991, Groschen *et al.* 2000), and non-polar organics, such as organophosphate (OP) and organochlorine pesticides (OCP) (Groschen *et al.* 2000), polychlorinated biphenyls (PCBs) (Groschen *et al.* 2000), and polycyclic hydrocarbons (PAHs) (Schubauer-Berigan and Ankley 1991, Sparks and Ross 1992, Burton 1995, Groschen *et al.* 2000), have been reported as potential sources of sediment toxicity in the IRC. The need to determine the sediment-

associated toxicity that each contaminant class may contribute for regulatory and research reasons has led to the development of the toxicity identification evaluation (TIE) procedures, which identifies the contaminant class causing toxicity in sediments by altering toxicity of those compounds by chemical or physical means (Mount and Anderson-Carnahan 1998).

Both pore water and whole sediment TIEs have been developed for sediment toxicity identification (Waller *et al.* 2005, U.S. EPA 2007). Three pore water TIEs have been conducted in the past on the IRC to identify toxic sites, and the sources of that toxicity (Schubauer-Berigan and Ankley 1991, Sparks and Ross 1992, Burton 1995). Two of these studies suggested that ammonia was the major source of toxicity, and sediments in close proximity to Chicago had greater toxicity and higher ammonia concentrations compared to downstream sediments. In addition to ammonia, PAHs were also responsible for the toxicity at some sites; however, no distinct spatial patterns were observed within the river (Sparks and Ross 1992, Burton 1995). The third study indicated that ammonia, non-polar organics, and metals all play a role in the toxicity in the Calumet Sag Channel, a major tributary of the IRC (Schubauer-Berigan and Ankley 1991).

Recent studies on other systems, however, have shown that toxicity attributed to pore water were inconsistent and did not always correlate with sediment toxicity (Waller *et al.* 2005). The pore water methodology calls for removal of the water from the sediment, and these extraction procedures alter the equilibrium dynamics of sediment and change contaminant bioavailability. Furthermore, pore water TIEs do not account for all potential uptake pathways, such as sediment ingestion. In contrast, the whole sediment

TIEs incorporate many biological uptake pathways and better represent *in situ* conditions (Adams *et al.* 2001, Ho *et al.* 2004, Waller *et al.* 2005).

The objectives of the present study were to evaluate sediment toxicity in the IRC using a whole sediment TIE, to identify the major source(s) of toxicity in the IRC, and to evaluate spatial and temporal patterns that may exist over the two-year span of the study with regard to toxicity and contaminant concentrations.

MATERIALS AND METHODS

Study Site and Sample Collection

The IRC extends 525 km from the Des Plains River to the Mississippi River (Machesky *et al.* 2005). This major tributary of the Mississippi River drains 44% of the land area or approximately 75,000 km^2 of Illinois and portions of Wisconsin and Indiana (Machesky *et al.* 2005). Twenty-one surface sediments were collected from the IRC between river-miles 76 and 320 in the summer of 2007, with toxic sites being analyzed in the subsequent seasons (Figure 2.1). Site locations were selected based on previous studies, primarily those sampled by Sparks and Ross in 1992. Sites were chosen over a large portion of the IRC to identify spatial patterns in toxicity and contaminant concentration. Sediment samples were taken from the top five cm of the matrix using a petite ponar (Wildco, Columbus, OH, USA), and stored in 2 L glass jars in a cooler on ice (4°C) until the samples were received at Southern Illinois University-Carbondale. Fall 2007 toxic sediments were analyzed for total organic carbon (TOC) and soil texture by Midwest Laboratories, Inc. (Omaha, NE, USA).

Along with the sediment characteristics, water chemistry was evaluated at each site by retrieving water from the sediment-water interface using a Van Dorn sampler (Ben Meadows Co., Janesville, WI, USA). Temperature (°C), dissolved oxygen (DO) (mg/L), conductivity (µS/cm), pH, alkalinity (mg $CaCO_3$/L), and hardness (mg $CaCO_3$/L) were measured for each water sample. Temperature and DO were measured using a Yellow Springs Instrument Model 55 water quality meter (YSI Incorporated, Yellow Springs, OH, USA), pH was analyzed using an Acorn 6 pH meter (Oakton Instruments, Vernon Hills, IL, USA), and conductivity was measured using an Oakton WD-35607-10 meter (Oakton Instruments). Alkalinity and hardness were measured using titrations with a Model FF-2 Hach test kit (Hach Company, Loveland, CO, USA).

Organisms

The freshwater amphipod *Hyalella azteca* were used in all toxicity tests. Mixed-age cultures of *H. azteca* were originally obtained from the U.S. Environmental Protection Agency Environmental Research Laboratory in Duluth, MN, and have been cultured at the Fisheries and Illinois Aquaculture Center in accordance with standard protocols (U.S. EPA 2000). Juvenile *H. azteca* (14-21 d old) were used in the toxicity tests and were removed from the cultures by passing individuals through 500 and 1000 µm mesh sieves (Schuler *et al.* 2006).

Screening Toxicity Bioassays

A 10-d screening toxicity test was performed using standardized methods, and toxicity at each site was compared to the control sediment (U.S. EPA 2000). Control

sediment was prepared from a hydrated soil collected from Touch of Nature (TON) in Carbondale, IL, USA. The TON control sediment was used for all toxicity comparison purposes, as this sediment has been shown in past studies not to exhibit toxicity and with limited contamination. The TON soil was composed of 14% sand, 70% silt and 16% clay, and had a TOC content of 0.69%. Beside TON control sediment, reference sediment was also collected from Lower Peoria Lake (LPL) in the IRC. This site possessed similar physical characteristics as the contaminated sediments in the river and exhibited no toxicity to *H. azteca*, thus providing an environmentally realistic reference control. This reference sediment was used to monitor the influence of site characteristics on *H. azteca* survival, in addition it was also used in Phase I toxicity tests to ensure that the amendments alone did not cause toxicity. The LPL sediment was composed of 24% sand, 56% silt and 20% clay, and had a TOC content of 1.13%.

After homogenization, 60 g of wet sediment was placed into each jar, with six replicates per site to initiate the toxicity test. Water hardness can affect the toxicity of both organic and inorganic contaminants, thus the hardness of the test water was adjusted to simulate site water (U.S. EPA 2000). Two hundred and seventy-five ml of very hard-reconstituted water (270-300 mg $CaCO_3$/L) was used in the screening and Phase I toxicity tests and 10 juvenile *H. azteca* were distributed to each jar (U.S. EPA 1985). Exposures were conducted in a flow-through system, which renewed overlying water three times a day at 80-100 ml per renewal. The 350 ml jars, were placed into a temperature controlled water bath (23 ± 1°C), and the room set with a 16:8h light:dark photoperiod. The DO, conductivity, temperature, and pH were monitored daily in random beakers during the bioassays. *H. azteca* were fed daily during toxicity testing

with 1 ml of a yeast-cerophyll-trout chow (YCT) mixture. At the end of each test, mortality was assessed for each replicate by repeatedly gently swirling and pouring overlying water into a 500 µm mesh sieve. Organisms were then gently prodded to determine survival percentages for each replicate.

Phase I Testing: Characterization

In the screening toxicity tests, sites exhibiting significant differences in *H. azteca* survival from TON control sediment were indicative of sediment toxicity, and subjected to a suite of tests to identify the potential class(es) of contaminants responsible for the toxicity. Three main classes of contaminants were evaluated in Phase I testing, including ammonia, cationic metals and non-polar organics (specific methods are detailed later). Toxicity testing was conducted using six replicates for the un-amended sediment and for each amendment including Zeolite (ammonia), Resin Tech SIR-300 (metals) and powdered coconut charcoal (non-polar organics). Sand was added in proportion to amendment additions to sediments not receiving amending materials to compensate for potential dilution effects. Sediments were allowed to equilibrate for 24-36 h prior to the addition of test organisms (Burgess *et al.* 2000, Ho *et al.* 2004, U.S. EPA 2007). Phase I testing, excluding ammonia Phase I testing, was conducted using the same 10-d bioassay methodology used in the screening experiments.

Ammonia Characterization. Ammonia concentrations in sediment pore water were analyzed prior to toxicity screening due to the volatile nature of ammonia (see ammonia identification). Chemical quantification, prior to Phase I testing, allowed for

sediments with high pore water ammonia concentrations to be the sole focus of the Phase I zeolite additions.

Zeolite particles (Aquatic Eco-Systems, Apopka, FL, USA) were ground using two grinding stones, passed through a 1.4 mm sieve, and then hydrated with test water. Previous studies have showed that preferential binding to ammonia makes zeolite an ideal choice as an amending agent for this contaminant (Besser *et al.* 1998). The mixture was allowed to settle for 1-d before being decanted. A 20% (wet wt) amendment was used (12 g of zeolite slurry into 60 g of wet sediment) for each of the amended site sediments (Besser *et al.* 1998, U.S. EPA 2007). A four-day static test was conducted comparing the un-amended sediment to the zeolite-amended sediment, and total ammonia, DO, temperature and pH were measured daily in the overlying water.

Metal Characterization. Phase I metal analysis was conducted using a high purity-chelating agent, Resin Tech SIR-300 (ResinTech Inc, Cherry Hill, NJ, USA). The resin was composed of ~1 mm cross linked styrene-divinyl benzene beads with iminodiacetate functionality (R - CH_2 –N $(COOH)_2$), which has a strong affinity for cations (Burgess et al. 2000). The resin affinity for the metals of interest was $Cu^{2+} > Ni^{2+} > Cr^{6+} > Pb^{2+} > Zn^{2+} > Cd^{2+}$ (Burgess et al. 2000). The resin was rinsed five times with de-ionized water, and then stored at 4 °C in de-ionized water at a ratio of one part resin to three parts water. This methodology resolves pH fluctuations that might occur in the overlying water caused by the resin, while still having a high affinity for metal cations. The sediment and resin beads were combined at a ratio of 1:4 (wet wt) (15 g resin: 60 g wet sediment).

Non-polar Organic Characterization. Powdered coconut charcoal (PCC, Calgon Carbon, Pittsburgh, PA, USA) selectively removes organic contaminants, while having little influence on the toxicity of ammonia or metals or affecting aquatic organism health (Ho *et al.* 2004, U.S. EPA 2007). The PCC was produced from coconut husks after being pyrolyzed and ground into a powder (coarse: 105-595 μm). The PCC was hydrated (290 g PCC into 1000 ml of de-ionized water) in a vacuum flask and placed under a vacuum for at least 18 h. In the vacuum flask, air was removed under vacuum and water was able to effectively permeate into the PCC (Ho *et al.* 2004, U.S. EPA 2007). Next, the mixture was centrifuged using a 5702 R Eppendorf centrifuge (Eppendorf AG, Hamburg, Germany) at 2500 × g for 30 min at 4 °C. The resulting mixture was added to the sediment at 20% of the sediment weight (12 g of PCC for 60 g of wet sediment) (Ho *et al.* 2004, U.S. EPA 2007).

Phase II Testing: Identification

Phase II testing involved the analytical identification of contaminants in sediments. These concentrations were used to assess the risk of each contaminant class by using the toxic unit (TU) approach.

Ammonia Identification. Pore water was extracted from sediments immediately after collection (within 24 h) by centrifuging a 200 g aliquot of sediment at 2500 × g for 45 min at 4 °C. Total pore water ammonia concentrations were measured with an external calibration method using a Fisher Accumet AR20 meter coupled with a pH and

ion selective ammonia electrode (Fisher Scientific, Pittsburgh, PA, USA) which measures the total ammonia concentration.

Metal Identification. Metal (Cu^{2+}, Ni^{2+}, Cr^{6+}, Pb^{2+}, Zn^{2+} and Cd^{2+}) concentrations in sediments were quantified using a flame atomic absorption spectroscopy (FAAS 220, Varian Inc., Palo Alto, CA, USA) after acid digestion with concentrated nitric acid on a heating pad (95 °C) (U.S. EPA 1996). Standards for metal identification were purchased from Ultra Scientific (North Kingstown, RI, USA).

Non-polar Organic Identification. A suite of 20 OCPs, chlorpyrifos (an OP), seven pyrethroids, 44 PCB congeners, and 16 PAHs (PAHs taken from the U.S. EPA priority pollutant list) were analyzed in sediments for Phase II testing (see Table 2.1 for specific compounds). Pesticide standards were purchased from Supelco (Bellefonte, PA, USA). Standards for the PAHs and PCBs were purchased from Restek Corporation (Bellefonte, PA, USA) and Accustandard (New Haven, CT, USA), respectively. Sediment (10 and 2 g wet wt for OCP/OP/pyrethroid/PCB and PAH quantification, respectively) was thawed, thoroughly mixed with 5 g of diatomaceous earth, 1 g of silica gel, and 2 g of copper powder, and extracted at 100 ºC and 1500 pounds per square inch (psi) with a mixture of acetone and methylene chloride (1:1, v/v) on a Dionex 200 Accelerated Solvent Extractor (ASE) (Dionex, Sunnyvale, CA, USA). Two surrogates, 4,4'-dibromooctafluoro-biphenyl (DBOFB) and decachlorobiphenyl (DCBP) which were purchased from Supelco, were added to sediment prior to extraction to verify sample preparation efficiency for OCP/OP/pyrethroid/PCB quantification. For PAH analysis, 6-

methylchrysene was used as the surrogate. Extracts were solvent exchanged to hexane, dried by adding 12 g of anhydrous Na_2SO_4, and concentrated to 1 ml under a gentle stream of nitrogen gas.

Solid-phase extraction (SPE) with Envi-Carb II/primary secondary amines (PSA) dual-layer cartridges (Supelco) was used to clean sediment extracts for OCP/OP/pyrethroid analysis, and 7 ml of 30% methylene chloride in hexane was used as elution solvents. The eluents were condensed and solvent exchanged to 1 ml of acidified hexane (You *et al.* 2008).

Analysis of pesticides and PCBs was performed using an Agilent 6890 series gas chromatography (GC) equipped with an Agilent 7683 autosampler and an electron capture detector (ECD) (Agilent, Palo Alto, CA, USA). Two columns (a HP-5MS [30 m × 0.25 mm, film thickness: 0.25 µm] and a DB-608 [30 m × 0.25 mm, film thickness: 0.25 µm]) were used to confirm the analytical results. Qualitative identification was conducted using a retention window of 0.5%, while quantification was based on external standard calibration. After analyzing the pesticides, the extracts were further cleaned with concentrated sulfuric acid for PCB congener analysis. The methods from You *et al.* (2008) were used for pesticide analysis, while PCB analyses followed the methods outlined in Koch *et al.* (2006).

Sediment extracts for PAHs were cleaned by passing the extracts through a 20 ml column which was wet-packed with 10 g of 10% deactivated alumina, 3 g of 3% deactivated silica gel, and 3-5 g of anhydrous Na_2SO_4 from bottom to top. The column was eluted with 50 ml of hexane, followed by 50 ml of 10% (v/v) diethyl ether in hexane. Eluents were concentrated and solvent exchanged to 1ml of acetonitrile. Samples were

then analyzed on an Agilent 1100 High Performance Liquid Chromatograph (HPLC, Agilent Technologies, Palo Alto, CA, USA) equipped with a fluorescence detector (HPLC-FLD). The HPLC used an elution gradient of acetonitrile: water using a C18 (150 × 4.6 mm) column with a 1 ml/min flow rate. The elution profile was: 0-20 min of 60% acetonitrile in water, and 20-33 min of 100% acetonitrile.

Oil/Grease Identification. In addition to analyzing the standard contaminants, the U.S. EPA whole sediment TIE guidelines suggest that in sediments contaminated with PAHs, that the oils and grease that are often associated with PAHs should also be evaluated (2007). Oils and greases in combination with the PAHs are commonly referred to as the unresolved complex mixture (UCM) (Jonker *et al.* 2003, Jonker and Barendregt 2006, Jonker *et al.* 2006). Sediments were weighed, freeze-dried (24 h) and sonicated using a Bransonic Ultrasonic Cleaner 3510 (Bransonic Ultrasonic Corp., Danbury, CT, USA) with a mixture of methylene chloride: hexane (1:4, v/v) for 15 min. The extract was filtered and reduced in volume using a gentle stream of nitrogen until a constant weight was achieved. The remaining fraction was weighed and percentage of UCM was calculated by dividing the weight of UCM by the weight of dry sediment.

Temporal Patterns

Sites exhibiting toxicity in the summer of 2007 were evaluated and re-sampled in the fall and winter of 2007, and the spring and summer of 2008 to evaluate potential seasonal trends in the data. Seasonal testing was conducted using the same methods as described for the summer of 2007 samples. In addition to evaluating toxicity seasonally,

chemical concentrations of the three-contaminant classes were also examined to determine whether concentrations varied by season.

Data Analysis

Survival responses (% survival was done using an arcsine transformation) among site sediments and TON control sediment were compared using an analysis of variance (ANOVA) ($\alpha = 0.05$) and Dunnett's Multiple Comparison Test using SAS 9.1 (SAS Institute, Cary, NC, USA). Toxicity of site sediment was indicated by significant differences from the TON control sediment. Likewise comparisons were made between un-amended sediment and the amended sediment using the same statistical procedures during Phase I testing. Potential toxicity of amendments was evaluated by comparing toxicity of un-amended reference sediment (LPL) with the amended reference sediment using the same statistical analysis.

Toxic units (TU) were used to indicate the contribution of each contaminant to sediment toxicity, and were calculated by dividing the contaminant concentration at the site by the concentration of that contaminant causing 50% morality in the test population (i.e., LC_{50} value). For the organic contaminants, the concentrations were TOC normalized.

The LC_{50} values were taken from published literature values for *H. azteca* (PAHs: U.S. EPA 2004; OCPs, chlorpyrifos and pyrethroids: Weston *et al.* 2004; and ammonia: Ankley *et al.* 1995) with the exception of the PCB congeners. No LC_{50} values were found for individual PCB congeners or total PCBs for freshwater amphipods, thus the LC_{50} values of Aroclor 1254 with the marine amphipod, *Rhepoxynius abronius* were used

(Swartz *et al.* 1988). Metal toxicity was compared to threshold effects level (TEL) values for coastal and marine waters (MacDonald *et al.* 1996). The predominant exposure route of ammonia, for epi-benthic organisms, such as *H. azteca,* is the overlying water rather than pore water (Chapman *et al.* 2002, U.S. EPA 2007). Thus, for site SS315 which had the highest pore water ammonia concentration, ammonia TUs were calculated using overlying water concentrations. The remaining sites in which Phase I zeolite testing was not conducted, estimations of overlying water ammonia concentrations were extrapolated for these sites by multiplying the pore water concentration by the ratio of the overlying water and pore water at site SS315. The extrapolated overlying water concentrations were used to determine TU values for these sites in which Phase I testing was not conducted.

RESULTS

Study Site Water

Mean (± standard deviation) water quality parameters for the summer of 2007 were DO 9.73 ± 3.00 mg/L, pH 8.12 ± 0.76, conductivity 820 ± 137 µS/cm, temperature 27.8 ± 1.9°C, hardness 254 ± 62.3 mg $CaCO_3$/L and alkalinity 148.2 ± 26.0 mg $CaCO_3$/L. Seasonal differences in some water quality parameters were evident, namely temperature, which was expected. Some differences were noted in DO, pH, conductivity and water hardness, between summer of 2007 and summer of 2008, suggesting that while statistically significant, the biological significance may be low. Of special note, was that DO, conductivity, and hardness values for winter were substantially greater compared to the other seasons (Table 2.2).

Tier I: Screening Toxicity Bioassays – Summer 2007

Twenty-one sampling sites were screened for toxicity in the summer of 2007, and the results are presented in Figure 2.2. Water-quality parameters (± standard deviation) for both the screening and Phase I toxicity tests, including temperature (22.9 ± 0.9 °C), DO (6.89 ± 0.67 mg/L) and pH (7.66 ± 0.35), were within U.S. EPA tolerance levels (U.S. EPA 2000). Water hardness was adjusted to simulate site waters, and conductivity and water hardness were 890 ± 164 μS/cm and 282.8 ± 53.5 mg/$CaCO_3$, respectively.

Of the 21 sites sampled, seven were chosen for further analyses based on their toxicity, using a whole sediment TIE approach as well as for analyzing seasonal patterns. Site toxicity only became significantly greater than controls above river-mile 277 (Number 14: Figure 2.1), with no significant toxicity being observed below this river-mile. The LPL sediment did not cause toxicity to *H. azteca*, thus it was chosen as the amendment reference sediment. Sediment TOC for the toxic sites and LPL sediment varied among sites (1.13-10.2%) (Table 2.3).

Tier II: Phase I Testing – Summer 2007

During Phase I testing only five of the seven toxic sites which previously were toxic in the screening toxicity tests had significantly greater toxicity from the control ($F_{7,39} = 20.13$, $p = 0.001$), and this variability between the screening and Phase I tests was in part due to different testing times and the low toxicity that occurred at these sites. Of those five sediments showing a significant effect, four showed significantly reduced

toxicity with the addition of PCC in the summer of 2007, suggesting that the source of the toxicity observed at these sites was non-polar organics (Figure 2.3).

Addition of Resin Tech SIR-300 to the sediments did not significantly reduce toxicity at any of the test sites, suggesting that metals were not the cause of the noted toxicity. Since Phase II testing of ammonia was conducted prior to Phase I testing, the site with the highest ammonia concentration (SS315) was solely tested using Phase I procedures. Site SS315 had approximately a 10-fold higher ammonia concentration than any other site. Zeolite did not significantly remove toxicity at SS315 (data not shown), but did reduce overlying total ammonia concentration by approximately 50%, from 0.4 TUs to <0.2 TUs. These results collectively suggested that non-polar organics were the source of toxicity at these sites, and that ammonia and metals were not suspected of contributing significantly to the noted toxicity.

Two sites (CS305 and SRCALRR) did not show a significant difference in toxicity from the control in Phase I testing even though there was a trend of increased toxicity. The addition of PCC and Resin Tech SIR-300 did not significantly reduce toxicity of CS305, but the PCC amendment significantly improved survivorship of *H. azteca* for the statistically non-toxic SRCALRR sediment ($p = 0.0017$). The ability of PCC to significantly reduce toxicity of statistically non-toxic sediments is an artifact in the statistical testing procedures that rarely occurs (2 of 26 trials).

Tier III: Phase II Testing – Summer 2007

Although Phase I testing suggested that non-polar organics were responsible for the noted toxicity, all three contaminant classes (metals, pore water ammonia, and non-

polar organics) were chemically analyzed to further strengthen the Phase I results. Chemical concentrations and the TUs associated with each class of contaminants for the summer of 2007 data are presented in Table 2.3. Total metal concentrations (µg/kg dry wt) at the sites were far below TEL, thus the TU values were < 0.1 (MacDonald *et al.* 1996). Ammonia TU values were below 0.1 at all sites, except for SS315 (TU=0.4). While overlying water concentrations for the sites were based on extrapolations from site SS315, Ankley *et al.* (1995) reported that at least 15-20 mg of total ammonia/L would be needed, in soft water, to cause appreciable mortality. All sites, excluding SS315, in very-hard water, exhibited pore water concentrations comparable to those reports. No pyrethroid pesticides were detected at any toxic site, and the sum pesticide and sum PCB TUs were all <0.2. The sum TU for the PAHs ranged from 0.74 to 4.6, and the TUs were high enough alone to cause the noted toxicity. In addition to the PAHs, the UCM was also analyzed. The UCM associated with the toxic spring 2008 sites were approximately four to eight times higher than that of the LPL reference site (Table 2.3).

Spatial and Temporal Assessment

Seven sites exhibiting toxicity in the summer of 2007 were re-tested for toxicity in the fall and winter of 2007, and spring and summer of 2008. The screening toxicity data was used to compare seasonal trends of toxicity at those sites. Results suggested that no seasonal trend in toxicity was evident and that toxicity remained fairly constant at most sites (Table 2.4). Spatially, as a whole, toxicity and pore water ammonia concentrations increased with closer proximity to Chicago (Figures 2.2 and 2.4, respectively).

Figure 2.5 shows the total concentration of metals, PAHs, and pore water ammonia in the toxic sites during the five seasons tested. Concentrations of the contaminant classes were, with a few exceptions, comparable regardless of season. Therefore, to determine whether spatial trends existed, pore water ammonia, total metals, and PAHs concentrations at each site were compared by averaging the concentrations among seasons. Contaminant concentrations in most cases were lower on the Calumet Sag Channel (Sites: Halstead, SRCALRR, Stony Creek and CS305) than those of the Chicago Sanitary and Ship Canal (Sites: SS315 and SS308), which was surprising, since the toxicity (average of the five seasons) was not dramatically different between the two branches of the river (Figure 2.5). The average concentration of total PCBs at most toxic sites, throughout the five seasons tested, was significantly greater than the LPL reference site (5-15 times higher). However, no significant differences were evident among the toxic sites. The presence of pesticides in sediments was patchy with no trend being evident seasonally or among sites (see Tables 2.5 and 2.6 for chemical concentrations of pesticides and PCBs, respectively).

DISCUSSION

Whole sediment TIE: Sources of Toxicity

The whole sediment TIE conducted in the present study suggested that non-polar organics, namely PAHs were the primary source of toxicity at these sites. Phase II testing was conducted to identify the potential contaminants by chemical analysis, after Phase I testing classified the source of toxicity as potentially non-polar organics. The TU data obtained from Phase II testing further supported Phase I findings, and suggested that

non-polar organics, such as PAHs, were at high enough concentrations to alone cause the noted toxicity at the toxic sites.

After the initial summer sampling period, toxic sites were sampled and analyzed in the four subsequent seasons. The addition of PCC, to characterize non-polar organic toxicity, significantly reduced toxicity in 12 of 26 trials (≈ 46%), not including the two statistically non-toxic sediments where PCC significantly improved survivorship. However, the percentage of sediments in which survivorship was significantly improved by the addition of PCC was surprisingly variable among seasons. Data collected in the summers of 2007 and 2008 illustrated the extent of the variability associated with the number of sites demonstrating improved survivorship with addition of PCC. For example, 80% of the summer 2007 samples showed significant improvement in *H. azteca* survivorship with the addition of PCC, while only 25% of the samples collected in summer 2008 showed significant improvement. In general, sediment PAH concentrations were fairly consistent throughout all of the seasonal sampling and consistently high enough to cause toxicity (Figure 2.5).

The variability of PCC to successfully remove toxicity may be a product of the matrix usually associated with PAHs. Though little research has been conducted on the UCM or 'oils' associated with PAHs, regarding toxicity or mode of action, it is believed that the oils associated with the UCM can cause toxicity (U.S. EPA 2007). A further understanding of the role UCM might play at toxic sites in the IRC, which have considerable higher UCM percentages than the reference site, is critical (Table 2.3). It is important to understand the role UCM plays in both toxicity at these sites and in determining effectiveness of PCC for whole sediment TIEs when working with PAH

contaminated sediments. In addition to the oils potentially causing toxicity, the role they play in disrupting the adsorption capability of activated carbon and how they affect bioavailability of other organic contaminants are issues that deserve more attention. Recent studies have shown that the oils associated with the UCM and other dissolved natural organic matter inhibited the adsorption capacity of activated charcoal by blocking sorption sites (Newcombe *et al.* 1997, Kwon and Pignatello 2005). Evaluating the oils that are present at each of the sites and how each interacts with PAHs is needed to better understand bioavailability of PAHs and the effectiveness of the PCC (Jonker *et al.* 2003). To address this issue, a study was conducted to determine whether the active sites on the PCC were saturated by the presence of the UCM, and varying amounts of coarse PCC (20, 30 and 40%) were added to two toxic sediments (Halstead and SS315). Results showed that the reduction of toxicity was similar regardless of the percentage of PCC added for both sediments (all additions for a site were non-significant when compared to one another), suggesting that the available active sites were not saturated by the UCM. It is possible that other contaminant(s) or the UCM itself may play a role in the toxicity, perhaps that the PAHs have a higher affinity to the UCM than the PCC (Jonker *et al.* 2006), and/or that the dynamics of the active sites of PCC are altered by the UCM in some other manner. Future studies investigating adsorption of organics with PCC, bioavailability, and the toxic effects of the UCM are needed to better explain the variation of PCC impact on toxicity reduction.

Furthermore, when investigating sites contaminated with PAHs, two factors associated with determining PAH TUs should be considered for accurate identification of toxicity. First, PAH TU values were based on LC_{50}s obtained from the equilibrium

sediment benchmarks (ESBs). Hawthorne *et al.* (2007) showed that TU values for individual sites might be overestimated due to differential bioavailability among PAHs in sediment, and that the organic carbon normalized partitioning coefficients (e.g., K_{oc} values) used by ESBs might not account for the heterogeneous distribution of organic contaminants in sediment. The exhaustive sediment extraction technique used in the present study may overestimate bioavailability. One approach that can reduce this source of variation for PAH toxicity would be to use freely dissolved pore water concentrations measured by solid phase microextraction methods (Hawthorne *et al.* 2006a, Hawthorne *et al.* 2006b, You *et al.* 2006, Hawthorne *et al.* 2007, You *et al.* 2007). Secondly, knowing concentrations of alkyl PAHs can provide additional information for sediment TIEs. Hawthorne *et al.* (2007) reported that up to 81% of the predicted toxicity noted in one of their study sites was caused by alkyl PAHs. These two factors show that using generalized TUs may over- or under-estimate toxicity. Despite these points, the evidence from the present sediment TIE suggested that PAHs and associated grease/oil in the sediments of the IRC was the major contributor of the toxicity at these toxic sites.

Spatial and Short-term Temporal Patterns

Each phase to the whole sediment TIE procedure provided insight into the toxicological dynamics associated with the IRC. Screening toxicity testing of summer 2007 samples suggested that sediments in closer proximity to Chicago had a greater likelihood of exhibiting toxicity, as no site below the confluence of the DuPage and Illinois Rivers (river-mile 277) was acutely toxic. In addition to site toxicity increasing as proximity to Chicago increases, a similar trend was observed for pore water ammonia

concentration (Figure 2.4). Both of these trends were similar to previous studies, and suggested that a dilution or remedial effect may be occurring down river of Chicago (Sparks and Ross 1992, Burton 1995).

While short-term temporal patterns (i.e., among seasons) were not observed in the present study, it is important to understand that differences among seasons were evident. For example, percent survival for site SS315 ranged from (42.0 to 75.0%) over the five sampling time points. It is also important to note that the IRC, and namely the Chicago area watershed, is a complex system with various inputs and uses and in conjunction with the hydrologic and flocculation/re-suspension dynamics of the sediment-water interface may, in part, have attributed to the differences among the seasons.

While in the present study seasonal trends in site toxicity and contaminant concentration were not apparent, it should be clarified that site water characteristics changed dramatically by season, which in many cases cannot be represented in the laboratory. For example, the difference in temperature from winter (≈ 7 °C) to summer (≈ 25 °C) can dramatically change species sensitivity, the relative state of various contaminants (e.g., ionized versus unionized ammonia), as well many other physical and chemical factors that can influence toxicity. Evaluating the water quality factors in conjunction with site toxicity and chemical concentration is critical in understanding what is actually occurring at the sediment-water interface of the studied site.

Evaluating spatial and short-term temporal patterns can allow for a more accurate focus for future risk assessment and mitigation. One example of the importance of spatial and short-temporal pattern analysis for future risk assessment is evident in the present study. The analyses of pore water ammonia concentrations along a large portion

of the IRC as well as additional sampling around site SS315 (Figure 2.6) further defined the source of the high concentrations of pore water ammonia at this site. Pore water ammonia concentrations increased with increasing proximity to site SS315 and then concentrations decreased afterwards. Site SS315 is located at the outfall of the Stickney Water Reclamation Plant (WRP), the largest municipal waste plant in the world (www.mwrd.org). Understanding the relationship between Stickney WRP and ammonia inputs to the IRC is currently unknown, but one that warrants further attention.

Long-term Temporal Patterns

The overall conclusion of the Sparks and Ross study of 1992 was that the IRC was improving, such that benthic fingernail clam (*Musculium tranversum*) and largemouth bass (*Micropterus salmoides*) were beginning to reappear in locations once abandoned. Based on the data generated from the present study, it is evident that the river has improved from previous widespread conditions of the 1970's and has changed to a system affected by localized or episodic problems, as mentioned in previous studies (Sparks and Ross 1992, Burton 1995), as many sites were characterized with little or no toxicity. However, determining whether the IRC has improved, is in a similar state, or has been further degraded since 1992 cannot be concluded, as more thorough investigations on the benthic populations would be needed. Sparks and Ross's study evaluated the biota, namely fingernail clam populations, and thus could be the reason that the authors felt they could provide an evaluation of the IRC's status as a whole (Sparks *et al.* 1981, Sparks 1984, Sparks and Ross 1992).

The present study suggested PAHs and the associated oil/grease were the major contributors of the noted sediment toxicity on the IRC, however ammonia, metals and other classes of organic contaminants were also detected at elevated concentrations. This finding was similar to a pore water TIE conducted in the Calumet Sag Channel in 1989 by Schubauer-Berigan and Ankley (1991). This study suggested that all three contaminant classes were responsible for the noted toxicity, while toxicity observed at approximately half of the sites was attributed to the non-polar organic contaminants, with PAHs and the associated oil/grease being the primary contributor (Schubauer-Berigan and Ankley 1991). In addition to the work by Schubauer-Berigan and Ankley (1991), two other pore water TIE studies have been conducted on the IRC, and these have suggested that ammonia was the main contributor to the toxicity at the sites, though at some sites the source of toxicity was believed to be PAHs (Sparks and Ross 1992, Burton 1995). Collectively, these four studies provide insight into the potential sources of contamination on the IRC, long-term temporal patterning of toxicity and contaminant concentration, and overall foundation for future risk mitigation.

Future Direction and Uncertainties

The variation associated with the ability of PCC removing toxicity in field sediments contaminated with PAHs, suggests that the application of whole sediment TIEs may be limited until the mechanisms on reduced sorption of hydrophobic organic contaminants by PCC are explored. It should be noted, however, that the use of PCC has been shown to effectively reduce toxicity for almost all non-polar organics including PCBs, various pesticides and in many cases PAHs (U.S. EPA 2007). Thus, this problem

should not discourage PCC usage in whole sediment TIEs, but rather encourage further research into understanding its capability as an amending agent. It should be mentioned, however, that PAHs were not the only contaminants whose TU values have uncertainty associated with them. Black carbon, acid volatile sulfides, and other sediment characteristics that could alter TU values for contaminants were not accounted for in the present study (U.S. EPA 2007). In addition, metals and PCB TU values were based on lab studies with a marine amphipod, due to limited published work with *H. azteca*. This should only be viewed as a minor issue for TIEs, as the conventional-contaminant based approach, as described in Chapter 1, which is still widely used, assesses toxicity based solely on TU values.

In addition to those uncertainties, the idea that the main contaminants causing the toxicity at these sites were different from those of the past studies was surprising due to the similarities in the pore water ammonia concentrations between the present and past studies (Sparks and Ross 1992, Burton 1995). This suggests that the differences found among these studies might be due to differences in the methodologies used and sensitivities of the test organisms employed and not the stressors at the sites. Other reports have suggested similar inconsistencies among sediment and pore water TIE methods (Winger *et al.* 2001, Chapman 2002, U.S. EPA 2007). To understand the difference between the past and present studies and to further strengthen the weight of evidence, which suggests that PAHs are the principle source of toxicity in the IRC, further work was conducted. This study, which is discussed in Chapter 3, addresses the differences between the past and present research by conducting both TIE methodologies on two selected sites using both test organisms from the previous pore water TIEs and the

present whole sediment TIE (*Ceriodaphnia dubia* and *Hyalella azteca*, respectively). This study allows for a direct comparison with past pore water TIE studies and elucidate the differences in whole sediment and pore water TIE testing as well as organism sensitivity.

CONCLUSION

By addressing toxicity as a function of response rather than the environmental concentrations of various toxicants provided better insight into the integrity and overall condition of the IRC. The addition of PCC in Phase I sediment TIE testing significantly reduced toxicity of a large portion of the toxic sediments in the IRC, which indicated organic contaminants were the major source of the observed toxicity. At the same time, the estimated TU values in Phase II TIE testing concluded that PAHs, a group of non-polar organic contaminants, were at high enough concentrations alone to cause the noted toxicity of all toxic sites. Similar to previous studies, pore water ammonia concentrations and toxicity increased with increasing proximity to Chicago, and suggested that the IRC in the Chicago area should be the focal point when attempting to address acute toxicity and future risk assessment and mitigation. Overall, the present study showed that whole sediment TIEs can be used to identify the source of sediment-associated toxicity and in assessing temporal as well as spatial concerns in riverine systems.

Table 2.1. List of specific compounds analyzed in Phase II testing of toxic sites on the Illinois River Complex.

	Class	Compounds
Organic Toxicants	PAHs	acenaphthene, acenapthylene, anthracene, chrysene, fluoranthene, fluorene, naphthalene, phenanthrene, pyrene, benzo[a]anthracene, benzo[b]fluoranthrene, benzo[k]fluoranthene, benzo[a]pyrene, benzo[g,h,i]perylene, dibenzo[a,h]anthracene and Indeno[1,2,3-cd]pyrene
	PCBs	Congeners: 8, 18, 28, 31, 43, 44, 48, 49, 52, 66, 70, 77, 86, 87, 95, 97, 99, 101, 105, 110, 114, 118, 123, 126, 128, 138, 153, 156, 157, 167, 169, 170, 174, 180, 183, 187, 189, 194, 195, 200, 201, 203 and 206
	Pesticides	OP: chlorpyrifos OCPs: *alpha*-BHC, *beta*-BHC, *gamma*-BHC, *delta*-BHC, *p,p'*-DDE, *p,p'*-DDD, *p,p'*-DDT, aldrin, *gamma*-chlordane, *alpha*-chlordane, diedrin, endrin, endrin aldehyde, endrin ketone, endosulfan I, endosulfan II, endosulfan sulfate, heptachlor, heptachlor epoxide and methoxychlor Pyrethroids: permethrin, lambda-cyhalothrin, cypermethrin, esfenvalerate, deltamethrin, cyfluthrin, bifenthrin
Heavy Metals		Cu^{2+}, Ni^{2+}, Cr^{6+}, Pb^{2+}, Zn^{2+}, Cd^{2+}
Ammonia		Total pore water ammonia (NH_4^+, NH_3)

Table 2.2. The average of water quality parameters (± standard deviation) for the seven toxic sites (n = 7) including dissolved oxygen (DO) (mg/L), pH, water hardness (mg $CaCO_3$/L), temperature (°C), conductivity and alkalinity (mg $CaCO_3$/L) for the five seasons tested.

	Summer 07'	Fall 07'	Winter 07'	Spring 08'	Summer 08'
DO (mg/L)	7.33 (0.98)	8.1 (0.78)	10.8 (1.29)	8.88 (1.38)	9.80 (2.26)
pH	7.24 (0.20)	7.21 (0.14)	7.27 (0.14)	7.48 (0.26)	8.01 (0.57)
Hardness (mg $CaCO_3$/L)	197 (61.3)	236 (20.2)	329 (48.8)	289 (20.8)	258 (19.9)
Temperature (°C)	25.9 (1.35)	16.3 (2.44)	7.33 (1.61)	17.2 (2.40)	25.4 (2.36)
Conductivity ($\mu S/cm^{-1}$)	739 (132)	1050 (91.2)	1740 (101)	1238 (67.1)	1053 (189)
Alkalinity (mg $CaCO_3$/L)	129 (16.3)	133 (14.6)	140 (20.9)	153 (15.4)	140 (20.9)

Table 2.3. Sediment total organic carbon content and contaminant concentrations of sum metals, total pore water ammonia, sum polychlorinated biphenyls (PCBs), sum organophosphate (OP) and organochlorine pesticides (OCP), sum polycyclic hydrocarbons (PAHs) and their respective sum toxic units (TUs) for the toxic sites in summer of 2007. The concentrations for organic contaminants were normalized to organic carbon (oc). The percent unresolved complex mixture (% UCM) was based on spring 2008 samples. BRL: below reporting limit (0.177 µg/g oc).

		LPL	SS308	Halstead	CS305	Stony Creek	SRCALRR	SS315
% total organic carbon		1.13	5.75	5.04	4.15	3.76	4.32	10.2
∑Metals (µg/g dry)		6.39	37.7	26.5	27.6	50.4	23.8	17.9
	∑TUs	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
Pore water ammonia (mg N/L)		6.48	36.6	26.2	13.7	19.4	21.7	541
	TUs	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	0.4
∑PCBs (µg/g oc)		4.45	11.5	15.7	21.1	37.2	34.5	7.6
	∑TUs	<0.1	<0.1	<0.1	<0.1	0.16	0.17	<0.1
∑OPs and OCPs (µg/g oc)		<RL	0.45	0.21	0.41	2.14	1.13	0.41
	∑TUs	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1	<0.1
∑PAHs (µg/g oc)		587	1934	1328	1198	1021	1267	4112
	∑TUs	0.74	2.8	1.9	1.8	1.4	1.6	4.6
% UCM		0.14	0.88	0.87	0.69	0.83	0.56	1.1

Table 2.4. Toxicity (± standard deviation) of toxic sites throughout the five seasons tested. Differing superscript letters show significant differences ($p < 0.05$) among the seasons for an individual site, as comparisons are not made between sites.

Toxicity (% survival)	CS305	SS315	SRCALRR	SS308	Stony Creek	Halstead	LPL
Summer 07'	74.0 (±5.48)^{a}	75.0 (±5.48)^{a}	61.7 (±11.7)^{ab}	58.3 (±17.2)^{a}	43.3 (±8.16)^{bc}	5.00 (±5.48)^{c}	93.3 (±8.16)^{a}
Fall 07'	16.7 (±15.1)^{b}	42.0 (±8.37)^{b}	48.3 (±14.7)^{b}	35.0 (±13.8)^{ab}	35.0 (±10.5)^{c}	38.3 (±11.7)^{a}	90.0 (±8.94)^{a}
Winter 07-08'	55.0 (±10.5)^{a}	58.3 (±11.7)^{ab}	70.0 (±8.94)^{ab}	46.7 (±12.1)^{ab}	55.0 (±8.37)^{ab}	48.3 (±13.3)^{a}	90.0 (±6.32)^{a}
Spring 08'	71.7 (±14.7)^{a}	53.3 (±19.7)^{b}	75.0 (±12.2)^{a}	41.7 (±11.7)^{ab}	53.3 (±15.1)^{ab}	30.0 (±17.9)^{ab}	91.7 (±9.83)^{a}
Summer 08'	68.3 (±7.53)^{a}	40.0 (±14.1)^{b}	70.0 (±16.7)^{ab}	25.0 (±21.7)^{b}	70.0 (±10.9)^{a}	13.3 (±8.16)^{bc}	91.7 (±7.52)^{a}

Table 2.5. Phase II organophosphate, organochlorine and pyrethroid insecticide analysis. Seasonal averages (µg/g organic carbon (oc)), standard deviation, the number of seasons detected (out of five), and the highest concentration of that individual compound detected throughout the seasons (highest conc. (µg/g oc)). The only non-polar organics that are reported are those above the reporting limit. The LPL winter sample was not taken, thus values for LPL are based on the remaining four seasons.

Site name	Non-polar organic	Average (µg/g oc)	Standard deviation	# of seasons detected	Highest conc. (µg/g oc)
CS305	Chlorpyrifos	0.090	0.049	5	0.161
	DDE	0.630	0.417	5	1.31
	DDD	0.519	0.195	4	0.718
	Aldrin	0.054	--	1	0.054
	Dieldrin	0.357	0.192	2	0.492
SS315	Chlorpyrifos	0.081	0.040	4	0.122
	DDE	0.347	0.110	4	0.500
	DDT	0.262	0.109	3	0.387
	DDD	0.373	0.183	3	0.552
	Aldrin	0.055	--	1	0.055
	Endosulfan I	0.089	0.036	3	0.110
SRCALRR	Chlorpyrifos	0.122	0.093	5	0.223
	DDE	0.549	0.209	4	0.843
	DDT	0.446	0.223	2	0.604
	DDD	0.347	0.072	3	0.412
	Aldrin	0.025	--	1	0.025
	Gamma-chlordane	0.190	--	1	0.190
	Dieldrin	0.309	0.094	3	0.400
	Alpha-chlordane	0.242	--	1	0.242
SS308	Chlorpyrifos	0.070	0.050	3	0.127
	DDE	0.288	0.211	4	0.590
	DDT	0.300	--	1	0.300
	DDD	0.351	0.193	4	0.619
	Aldrin	0.109	--	1	0.109
	Endosulfan sulfate	0.040	--	1	0.040
	Gamma-chlordane	0.166	--	1	0.166
	Dieldrin	0.161	0.085	2	0.221
Stony Creek	Chlorpyrifos	0.150	0.090	4	0.227
	DDE	0.767	0.232	5	1.01
	DDD	0.434	0.144	3	0.599
	Aldrin	0.090	--	1	0.090
	Gamma-chlordane	0.145	--	1	0.145
	Dieldrin	0.341	0.079	2	0.397
	Alpha-chlordane	0.281	--	1	0.281
Halstead	Chlorpyrifos	0.078	0.035	4	0.113
	DDE	0.331	0.156	4	0.521
	DDD	0.165	0.102	4	0.274
	Dieldrin	0.109	0.052	2	0.146
LPL	DDE	0.112	0.012	2	0.120
	Esfenvalerate	0.324	--	1	0.324

Table 2.6. Total ∑PCB congener concentrations (µg/g oc) for the five seasons tested. Superscript letters above the average concentration (Avg. Conc.) indicate significant differences ($p < 0.05$) amongst the sites using the average concentration of the five seasons tested (± standard deviation). Due to weather conditions LPL was not sampled in the winter (NA – Not Available).

∑PCB Congener (µg/g oc)	CS305	SS315	SRCALRR	SS308	Stony Creek	Halstead	LPL
Summer 07'	21.1	7.60	34.5	11.5	37.2	15.7	4.45
Fall 07'	29.4	15.6	55.3	19.8	23.8	25.6	0.948
Winter 07-08'	17.4	3.28	43.1	14.2	31.0	14.5	NA
Spring 08'	11.2	19.9	17.2	14.1	21.9	15.6	1.04
Summer 08'	12.9	7.79	10.5	22.5	9.03	31.6	0.759
Avg. Conc. (± st.dev)	18.4 (±7.27)^{abc}	10.8 (±6.74)^{bc}	32.1 (±18.4)^{a}	16.4 (±4.55)^{abc}	24.6 (±10.6)^{ab}	20.6 (±7.62)^{abc}	1.80 (± 1.77)^{c}

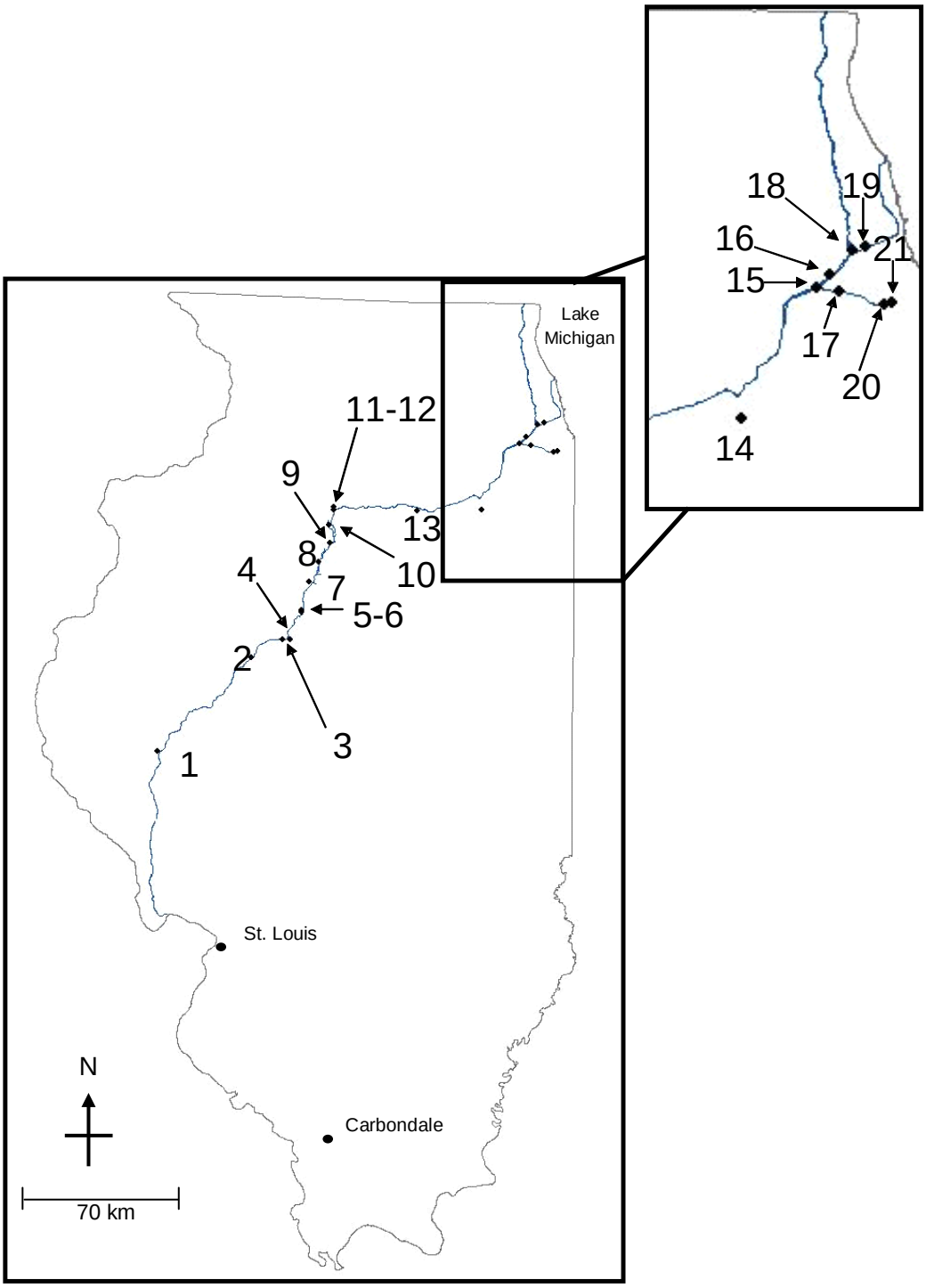

Figure 2.1. Sampling locations for Illinois River Complex (Illinois, USA) whole sediment toxicity identification evaluation sites. Numbering of sites refers to sampling location names, which are linked in Figure 2.2. Sites 15, 16, 18, and 19 lie on the Chicago Sanitary and Ship Canal and those numbered 17, 20, and 21 lie on the Calumet Sag Channel.

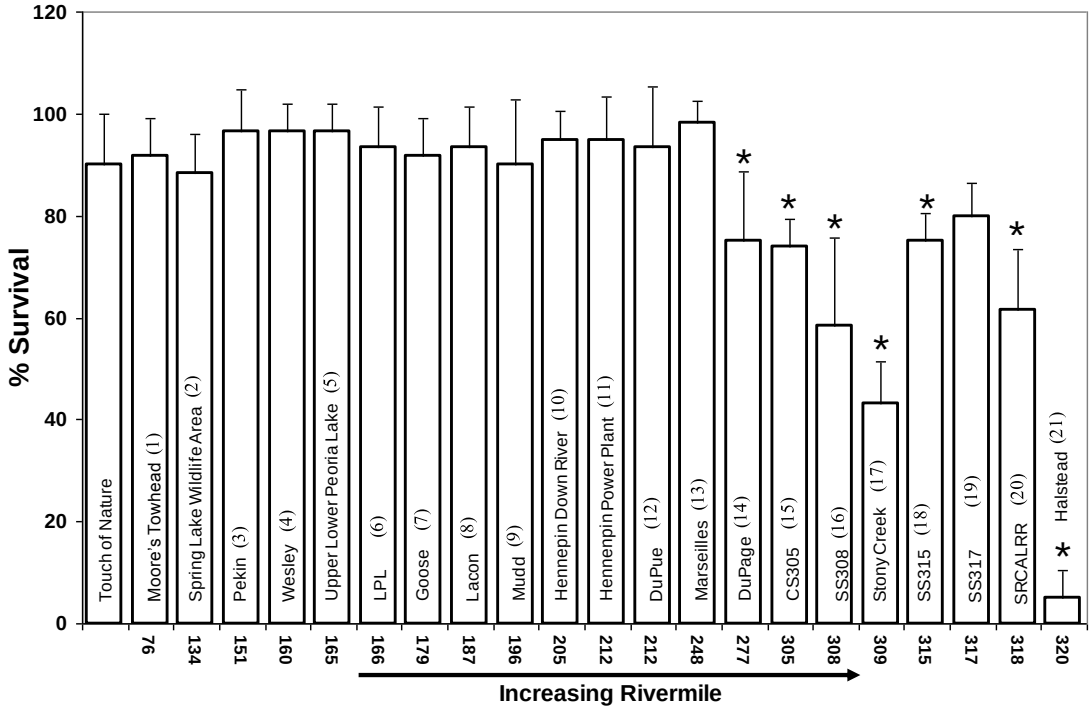

Figure 2.2. Summer 2007 site toxicity (percent survival for *Hyalella azteca*) with increasing river-mile. Asterisks (*) indicate significant differences ($p < 0.05$) in sediment toxicity between sites and Touch of Nature control.

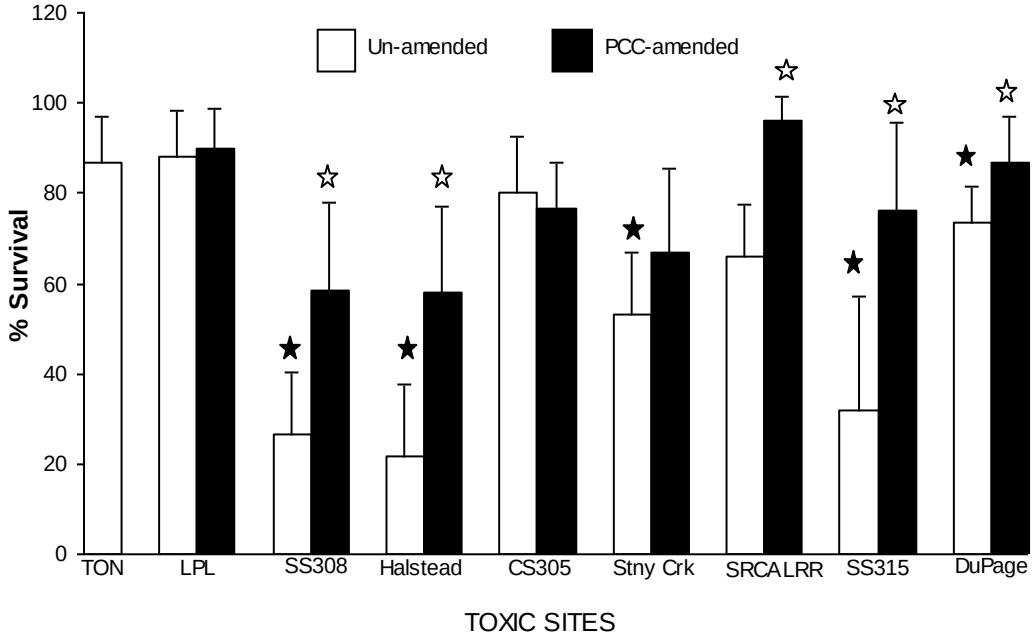

Figure 2.3. Summer of 2007 toxicity identification evaluation Phase I testing showing site toxicity (percent survival for *Hyalella azteca*) with and without amendments. Open and solid bars indicate mean percent survival in site sediment that was un-amended and those that were amended with powdered coconut charcoal (PCC), respectively. Error bars represent standard deviation. Solid and open stars indicate significant differences ($p < 0.05$) between un-amended site sediment and Touch of Nature control (TON) and between un-amended site sediment and PCC amended site sediment, respectively.

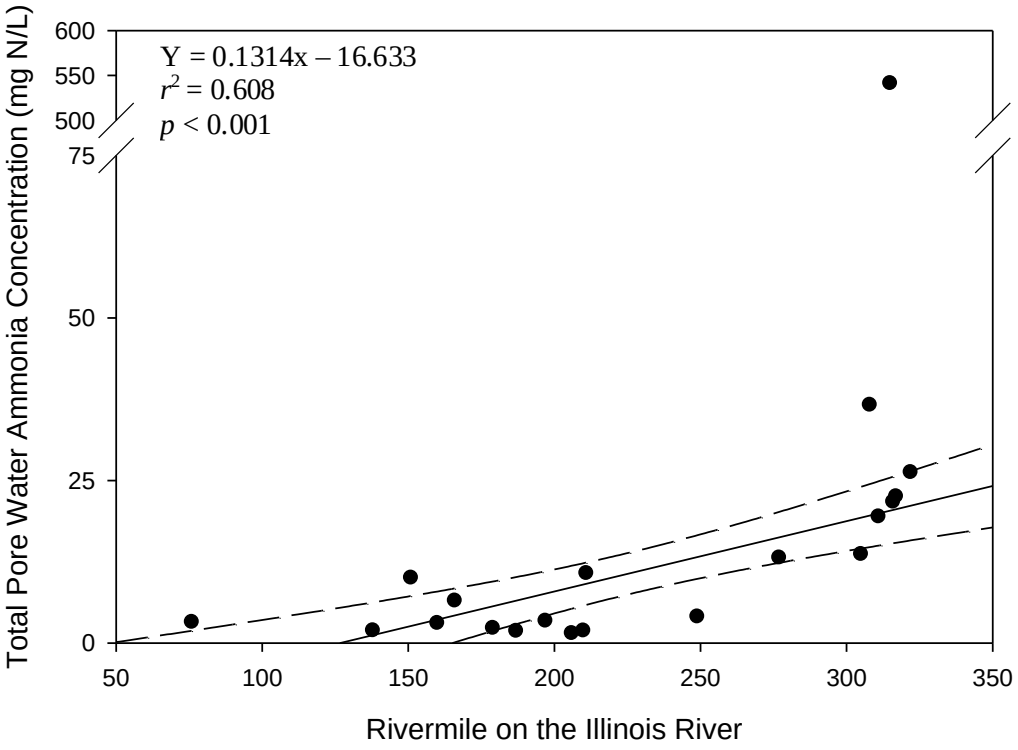

Figure 2.4. Linear regression of total pore water ammonia concentrations on river-mile of the Illinois River Complex in the summer of 2007. The 95% confidence intervals, modeled equation, and r^2 are provided. Each data point represents a sample.

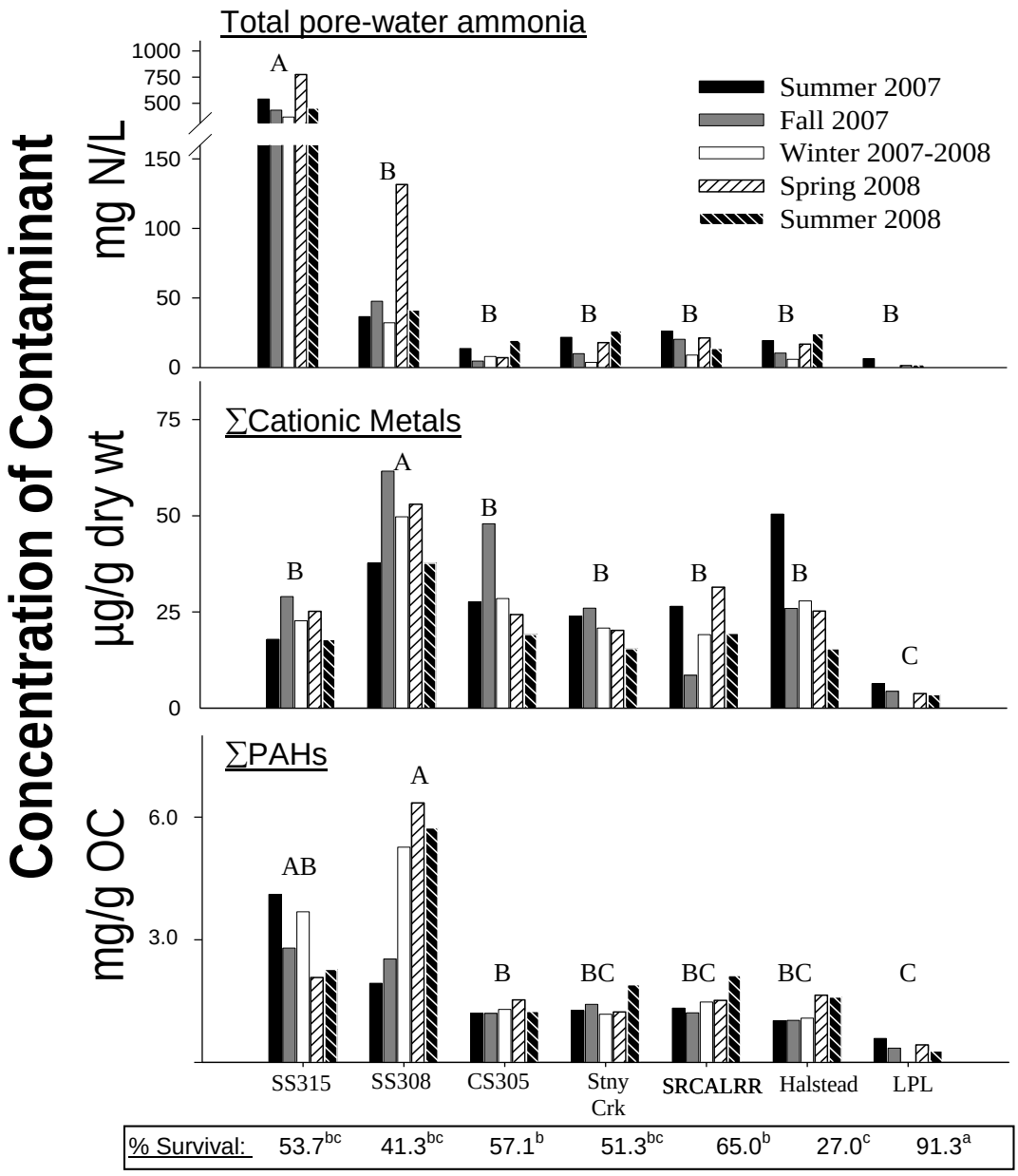

Figure 2.5. Results of the toxicity identification evaluation seasonal Phase II testing. Chemical concentrations of total pore water ammonia, sum metals, and sum polycyclic aromatic hydrocarbons (PAHs). The percentage values below each site name are the average percent survival of *Hyalella azteca* toxicity of each site for all seasons. Differing uppercase letters above figure bars and lowercase letters above percentages depict significant differences ($p < 0.05$) amongst site concentrations and site toxicity, respectively. For conversion of PAH concentrations to a dry wt basis, site-specific total organic carbon values can be found in Table 2.3.

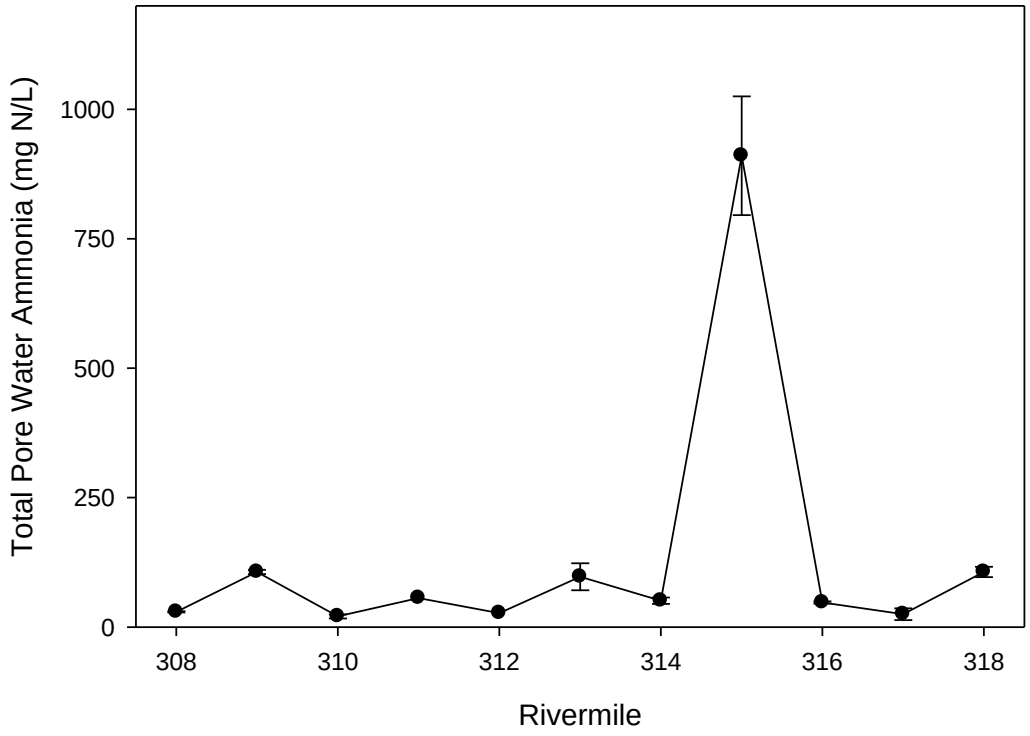

Figure 2.6. Total pore water ammonia concentrations taken each river-mile from 308 to 318 on the Chicago Sanitary and Ship Canal in the fall of 2008. Each point represents the mean of three measurements (± standard deviation).

CHAPTER 3

EXAMINING THE DIFFERENCES BETWEEN WHOLE SEDIMENT AND PORE WATER TOXICITY IDENTIFICATION EVALUATION (TIE) TECHINIQUES

ABSTRACT

In characterizing the sources of toxicity on the Illinois River Complex (IRC), discordance existed between previous pore water toxicity identification evaluations (TIEs), and the recent whole sediment TIE study (see Chapter 2). The present study was conducted to examine the effect of matrix type as the focus for the TIE (i.e., pore water versus whole sediment) and/or selected test organism (*Ceriodaphnia dubia* and *Hyalella azteca*) on the observed results of TIE patterns for the IRC. Both pore water and whole sediment TIEs were conducted focusing on two sites demonstrating historical toxicity. The pore water TIE suggested that ammonia was the major source of toxicity, which confirmed the past pore water TIE studies. However, the whole sediment TIE results indicated that non-polar organics, specifically polycyclic aromatic hydrocarbons (PAHs), were the primary contributor to toxicity, with ammonia contributing less to toxicity, confirming previous findings in the whole sediment TIE study. Results of the present study suggest that the choice of testing matrix, whether pore water or whole sediment, significantly influenced characterization of toxicity. While the choice of test organism may have played only a small role in the discordance between the TIEs, the data suggests that this factor alone could play a prevalent role in characterizing toxicity in other TIE assessments. The present study demonstrates that understanding the differences between pore water and whole sediment TIE methodologies and the differences in test organism sensitivity is critical to accurately identify the source(s) of toxicity in aquatic systems. In

addition, with pore water and whole sediment TIEs characterizing different aspects of the sediment, using both TIE procedures as part of a risk assessment provides a more accurate estimate of contaminant risk at a site.

INTRODUCTION

The use of pore water in sediment toxicity testing began in the 1980's and has been used for an array of endpoints, test species, and testing methodologies. A pore water toxicity identification evaluation (TIE) is a method that uses pore water to identify the contaminant class and/or specific chemical causing sediment toxicity in conjunction with analytical measurements to provide further evidence into the source of toxicity (Doe *et al.* 2001). Until recently, an important advantage of using pore water as the testing media in a TIE over whole sediment was that pore water guidelines were available and have been used frequently in risk assessment (Doe *et al.* 2001). The recent introduction of whole sediment TIE guidelines (U.S. EPA 2007) has stimulated debate toward which TIE method is a better approach to characterize risk. Studies have compared pore water and whole sediment toxicity testing in the past, and while often using different endpoints, these studies have shown that the sensitivity of the two tests in addressing toxicity varies (Bay *et al.* 2001, U.S. EPA 2007). However, evaluating the different matrices in the TIE process (pore water or whole sediment) to accurately characterize the source of toxicity has not been clearly addressed.

Previous pore water TIE studies using *Ceriodaphnia dubia* investigated the sources of sediment toxicity on the Illinois River Complex (IRC), and identified ammonia as the major source of toxicity, with non-polar organics and cationic metals as

minor sources of toxicity (Sparks and Ross 1992, Burton 1995). However, our recent whole sediment TIE study using *Hyalella azteca*, which assessed the same site sediments (Mehler *et al.* 2009), identified polycyclic aromatic hydrocarbons (PAHs), a non-polar organic, as the major source of toxicity on the IRC, with little toxicity being attributed to either ammonia or cationic metals. The contradiction between the whole sediment and past pore water TIEs was surprising, since similar ammonia concentrations were noted between the studies.

The objective of the present study was to compare the two TIE methodologies by conducting both pore water and whole sediment TIEs on two sites shown previously to be contaminated with potential chemical mixtures. Although neither organism is commonly used in both TIE procedures, both test organisms from the past TIE research will be used using both TIE procedures (whole sediment: *Hyalella azteca*; pore water: *Ceriodaphnia dubia*) to examine species sensitivity. This research is critical for risk assessment as TIE methodology and organism choice may play a significant role in determining the source of contamination.

MATERIALS AND METHODS

Study Site and Sample Collection

Two sediment samples were collected from the Chicago Sanitary and Ship Canal (river-miles: 315 and 308), which is a major tributary of the IRC (Figure 2.1). The two sites (SS315 and SS308) exhibited acute toxicity in the previous whole sediment TIE study and were characterized using whole sediment Phase I and II procedures for five seasons (summer, fall, and winter of 2007, and the spring and summer of 2008) (Mehler

et al. 2009). The source of toxicity for both sites were characterized to be non-polar organics, and on average these sites contained the highest pore water total ammonia and polycyclic aromatic hydrocarbons (PAHs) concentrations, respectively (Mehler *et al.* 2009). Approximately 20 L of sediment from each site was taken using a petite ponar (Wildco, Columbus, OH), and then stored on ice (4°C) until the samples were received at Southern Illinois University-Carbondale. Sites SS315 and SS308 were analyzed in the previous whole sediment TIE and were composed of 10.2 and 5.75% total organic carbon (TOC), respectively (Mehler *et al.* 2009).

Organisms

Mixed aged cultures of *H. azteca* and *C. dubia* were originally obtained from the U.S. EPA Environmental Research Laboratory (Duluth, MN) and the Texas Tech Institute of Environmental Health and Safety (Lubbock, TX), respectively, and have been cultured at the Fisheries and Illinois Aquaculture Center in accordance with U.S. EPA Protocols (U.S. EPA 2000).

The two test organisms, juvenile *H. azteca* (14-21 d old) and *C. dubia* (≈ 24 h) were used separately in each type of TIE to compare and contrast organism sensitivity. The *H. azteca* that were used for toxicity testing were passed through a 1000 µm mesh sieve and retained by a 500 µm mesh sieve (Schuler *et al.* 2006), while *C. dubia* less than 24 h old were obtained using standardized methods (U.S. EPA 2002).

Phase I Testing: Whole Sediment Characterization

Control sediment was prepared from a hydrated soil collected from Touch of Nature (TON) in Carbondale, IL, USA. Phase I testing was conducted with both *H. azteca* and *C. dubia* and specific details for each species will be discussed shortly. Water hardness used in toxicity tests was adjusted to resemble site water (i.e. very hard water) (Mehler *et al.* 2009). Metals did not to play a role in toxicity in the previous TIE studies (Sparks and Ross 1992, Burton 1995, Mehler *et al.* 2009), thus non-polar organics and ammonia were the focus of the present study. Preparation techniques used for the non-polar organics and ammonia amendments (powdered coconut charcoal (PCC) and zeolite, respectively) were detailed in Mehler *et al.* 2009 as modified from past TIE studies (Besser *et al.* 1998, Ho *et al*. 2004, U.S. EPA 2007). Amendments were also added to control sediments to ensure that the amendments alone did not cause toxicity. Sand was added to sediments not receiving amending materials to discern any dilution effect, and sand additions were directly proportional to amendment additions. Sediments were allowed to equilibrate for 24-36 h prior to the addition of test organisms (Besser *et al.* 1998, Ho *et al.* 2004, U.S. EPA 2007). Dissolved oxygen (DO), conductivity, temperature, and pH were monitored daily in random beakers during bioassays.

Sediment Bioassays with Hyalella azteca. Ten-day toxicity tests were performed to compare survival in un-amended site sediment to survival in site sediment amended with PCC to characterize non-polar organic toxicity (U.S. EPA 2007). Eight replicates were used per treatment with 60 g wet sediment and 275 ml of very hard water per replicate, with 10 *H. azteca* per replicate. Toxicity tests were conducted in a flow-thru

system, with water renewals three times a day at 80-100 ml per renewal. *H. azteca* were fed daily during 10 d toxicity testing with 1 ml of yeast-cerophyll trout chow (YCT) and the tests were performed at 23 ± 1°C with a 16h:8h light:dark photoperiod.

Toxicity testing procedures for Phase I analysis for ammonia followed the same basic methods that were conducted for non-polar organics Phase I analysis with the following modifications. Four-day static tests were conducted to compare survival in un-amended site sediment to survival in site sediment amended with zeolite to characterize ammonia toxicity. The static method was chosen in this bioassay to avoid the loss of ammonia during the daily water renewals, which would occur in a static-renewal flow-thru test. These ammonia-zeolite tests were performed in the same manner as the non-polar organics-PCC testing, with the same number of organisms and replicates, without feeding. During the toxicity tests, total ammonia was assessed in the overlying water of both un-amended and amended treatments. Toxicity testing was performed in an Precision Scientific environmental chamber (Grand Rapids, MI, USA) with temperature and photoperiod controlled (23 ± 1°C and 16h:8h light:dark, respectively).

Sediment Bioassays with Ceriodaphnia dubia. Two-day static tests were performed with *C. dubia* for both non-polar organic and ammonia characterization using PCC and zeolite amendments, respectively, following the whole sediment testing procedures modified from Sasson-Brickson and Burton, Jr. (1991). Two hundred and fifty ml beakers were used with 100 ml of very hard water, and 30 g wet sediment per replicate. Eight replicates were used per treatment, with 10 *C. dubia* per replicate.

Toxicity tests were held in an environmental chamber under similar conditions as *H. azteca* for 2 d without feeding.

Phase I Testing: Pore Water Characterization

Very hard water, which closely resembled IRC water, was prepared as a control using standard EPA protocols (U.S. EPA 1985). Site pore water samples were prepared by centrifuging sediment for 45 min x 2500 g, and stored at 4 °C for no longer than one week prior to testing. Pore water was diluted at a 50:50 ratio with very hard water to alleviate initial low DO concentrations (3.5 – 4.5 mg/L) and abnormally high conductivities (>3000 mg $CaCO_3$), at the same time to reduce toxicity to levels that could be easily manipulated to ensure the performance of Phase I procedures. Pore water was processed for non-polar organics and ammonia characterization by amending pore water with a solid phase extraction (SPE) cartridge and zeolite, respectively, and the techniques for each will be discussed below. Eight replicates were used for each treatment, with those treatments being un-amended, solid phase extraction (SPE) amended, and zeolite amended treatments. As mentioned previously, past TIE studies showed that metals did not play a role in sediment toxicity on the IRC, and specifically at these two sites, thus metals were not evaluated in the present study. Control water was also manipulated with both SPE and zeolite methods to ensure that the amendments alone did not introduce toxicity. Conductivity, DO, temperature, and pH were monitored at the beginning and end of the bioassays.

The procedures to SPE amend site pore water included passing approximately 100-150 ml of pore water through a C18 SPE cartridge (1000 g bed wt, Grace Davison

Discovery Sciences, Deerfield, IL, USA) to retain non-polar organics. Before sample loading, SPE cartridges were conditioned with 5 ml of methanol and 5 ml of de-ionized water subsequently. The pore water being passed through the SPE cartridge was collected and stored at 4 °C with a total of 200-250 ml was collected for analysis.

The zeolite amended treatment reduced ammonia concentrations by shaking 200 ml of pore water with 20 g of zeolite for approximately 5 min, and zeolite was prepared using the same techniques as the whole sediment TIE experiment. After being amended, pore water was then centrifuged again for 10 min x 2500 g and stored at 4 °C.

Pore Water Bioassays with Hyalella azteca. Two-day static toxicity tests were conducted using 20 ml of diluted site pore water (50:50) in 25 ml scintillation vials with ~1 g of sand in each vial. Five organisms were placed into each of the eight replicates to initiate bioassays. Scintillation vials were placed into an environmental chamber at 23 ± 1°C and 16h:8h light:dark photoperiod (U.S. EPA 1993b)

Pore Water Bioassay with Ceriodaphnia dubia. Two-day static tests were initially planned for pore water bioassays with *C. dubia*, however the testing was ended within 24 h due to early lethality (e.g. 100% lethality occurred). Ten ml of 50:50 diluted pore water was used, with 10 *C. dubia* being used per replicate. Eight replicates were used per treatment, and testing was conducted in the same environmental chamber as the *H. azteca* pore water bioassay.

Phase II Testing: Ammonia Identification

Pore water ammonia was assessed immediately after centrifugation and within 24 h of arrival at Southern Illinois University-Carbondale (SIUC) using a Fisher Accumet AR20 meter coupled with a pH and ion selective ammonia electrode probe (Fisher Scientific, Pittsburgh, PA, USA) using a five-point external calibration. Three replicates were measured per site with 50 ml of site pore water for each replicate. Concentrations of pore water ammonia were also assessed after zeolite manipulation to determine how much ammonia was reduced with the amendment. In whole sediment TIE testing, ammonia concentrations in overlying water were also monitored every two days in both un-amended and zeolite amended sediment. Three replicates in the overlying water were examined with 10 ml of overlying water per measurement.

Phase II Testing: Non-polar Organics Identification

Pore water was assessed for a suite of non-polar organics, including 20 organochlorines (OCPs), the organophosphate (OP) chlorpyrifos, and seven pyrethroid pesticides, as well as 16 PAHs (PAHs were taken from the U.S. EPA priority pollutant list) (Table 2.1) using liquid-liquid extraction techniques (LLE) (Wang *et al.* 2009). Briefly, 25 ml of site pore water was mixed with 50 ml of methylene chloride in a 250 ml separatory funnel, and was shaken for approximately five minutes. After separation, the methylene chloride was collected, and the pore water was extracted twice more with methylene chloride. The solvent washes were combined, concentrated and solvent exchanged into acidified hexane (for accurate assessment of pyrethroid isomerization) and acetonitrile for pesticide and PAH analysis, respectively.

The chemical analysis was conducted in duplicate. The surrogates (4,4'-dibromooctafluoro-biphenyl (DBOFB) and decachlorobiphenyl (DCBP) for pesticides; and 6-methylchrysene for PAHs) and OCPs, OP and pyrethroids pesticide standards were purchased from Supelco (Bellefonte, PA, USA) and Chemservice (West Chester, PA, USA), while PAH standards were purchased from Accustandard (New Haven, CT, USA). Analysis of pesticides was performed in a similar manner as in the past whole sediment TIE study (Mehler *et al.* 2009). In short, pesticides were analyzed using an Agilent 6890 series gas chromatography (GC) equipped with an Agilent 7683 autosampler and electron capture detector (ECD) (Agilent, Palo Alto, CA, USA), using methods from You *et al.* (2008). Analysis of PAHs was performed using an Agilent 1100 High Performance Liquid Chromatography (HPLC) equipped with a fluorescence detector (HPLC-FLD). Qualitative identification was conducted using a retention window of 0.5%, while quantification was based on a five point external standard calibration.

Sediment extractions for pesticides and PAHs followed methods from recent whole sediment TIE work (Mehler *et al.* 2009). In short, sediments were extracted with an Accelerated Solvent Extractor (Dionex, Sunnyvale, CA, USA) in duplicate. The non-polar organic extracts were cleaned using two different techniques, pesticides were cleaned using SPE with Envi-Carb II/primary secondary amines (PSA) dual layer cartridges (You *et al.* 2008) and a 20 ml wet-packed alumina-silica column was used for the PAH cleanup. Further information regarding pesticide extraction and clean-up techniques can be found in You *et al.* (2008). The instrument analyses of extracts were the same as for pore water quantification.

Data Analysis

Survival responses were compared using an analysis of variance (ANOVA) (α = 0.05) and Dunnett's Multiple Comparison Test using SAS 9.1 (SAS Institute, Cary, NC, USA). Comparisons were made between un-amended and the amended sediment toxicity. Potential toxicity of amendments was evaluated by comparing toxicity of an un-amended control with the amended control sediment using the same statistical analysis.

Toxic units (TU) were used to indicate the contribution of each contaminant to sediment toxicity. In Phase II analysis, TUs were calculated by dividing the contaminant concentration at the site by the concentration of that contaminant causing 50% morality in the test population (i.e., LC_{50} value).

To determine TUs for Phase II in the whole sediment TIE, non-polar organic LC_{50} values were taken from published literature values for *H. azteca* (PAHs: U.S. EPA 2004; and OCPs, chlorpyrifos and pyrethroids: Weston *et al.* 2004), while *C. dubia* toxicity data could not be found in the literature as these organisms are not commonly used in whole sediment testing. Non-polar organic TU values for Phase II in the pore water TIE were initially based on published freely dissolved concentrations (Hawthorne *et al.* 2005).

The predominant exposure route of ammonia, for epi-benthic and pelagic organisms (such as *H. azteca* and *C. dubia*, respectively) is the overlying water rather than pore water in whole sediment testing (Chapman 2002, U.S. EPA 2007). Thus, ammonia TUs for both organisms in whole sediment testing were calculated based on overlying water concentrations rather than pore water concentrations. However, in pore water testing, TUs were based on pore water concentrations, since they are being

subjected to the pore water matrix. Total ammonia TUs for both whole sediment and pore water TIEs were based on extrapolations from published literature for *H. azteca* (Ankley *et al.* 1995) and *C. dubia* (Bailey *et al.* 2001).

RESULTS

Phase I Testing: Whole Sediment Characterization

H. azteca survival for SS315 site sediment was significantly improved by the addition of zeolite and PCC, while survival of *H. azteca* for SS308 sediment was not significantly improved by either amendment (Figure 3.1). *C. dubia* survival for both site sediments was not significantly increased with the addition of either amendment (Figure 3.2). Control tests showed both PCC and zeolite were not acutely toxic to either *H. azteca* or *C. dubia* (Figures 3.1 and 3.2).

Phase I Testing: Pore Water Characterization

Survival of both *H. azteca* and *C. dubia* (Figures 3.1 and 3.2) were significantly increased by the zeolite manipulation when compared to the un-manipulated sediment pore water for both SS315 and SS308 sites. On the other hand, the SPE cartridge did not significantly reduce toxicity at either site for either organism. Zeolite and SPE amendments were not acutely toxic to either *H. azteca* or *C. dubia* in control replicates (Figures 3.1 and 3.2).

Phase II: Ammonia Identification

Ammonia concentrations in the undiluted pore water were approximately 359 and 111 mg N/L for site SS315 and SS308, respectively. The zeolite amendment dramatically reduced ammonia concentrations to 22.5 and 12.0 mg N/L, for sites SS315 and SS308, respectively. The TU values for the undiluted pore water associated with both treatments can be viewed in Table 3.1.

In whole sediment TIE testing, overlying water ammonia concentrations were approximately 10 times lower than pore water concentrations (Table 3.1). Zeolite additions reduced concentrations of overlying water ammonia by over half in the 4-d tests with *H. azteca* as well as in the 2-d tests with *C. dubia* (Figure 3.3).

Phase II: Non-polar Organics Identification

Pesticide concentrations in undiluted pore water were below reporting limits (0.2 µg/L) in both un-manipulated site sediment pore waters, while elevated PAH concentrations were detected. The concentrations of PAHs in undiluted pore water were 30.7-fold lower in SS315 than those in SS308 (Table 3.1), with both concentrations being reduced with the SPE amendment. Calculating the sum PAH TUs for *H. azteca* using freely dissolved concentrations resulted in inflated TU values (SS315 and SS308—30.4 and 664, respectively), these TU values were inaccurate (see *Future Direction and Uncertainties* in the discussion*)* and thus were not reported in Table 3.1.

Few pesticides were detected above reporting limits (reporting limits for all pesticdes: 0.035 µg/g oc) in whole sediment. Those pesticides that were above reporting limits (SS308 – DDT: 0.052 µg/g oc; SS315 – dieldrin and DDD: 0.049 and 0.37, µg/g

oc, respectively) resulted in low toxic units (< 0.1 TU). Similar to pore water, site SS315 PAH sediment concentrations were lower (6.8-fold) than SS308 concentrations (Table 3.1). Concentrations of PAHs for whole sediment were comparable to those reported in the recent whole sediment TIE work (Mehler *et al.* 2009) and resulted in high TUs (Table 3.1).

DISCUSSION

TIE Methodology Differences

Phase I results of the pore water TIE strongly suggested that ammonia was the principle source of toxicity for both sites, with the zeolite addition significantly reducing toxicity at both sites for both organisms tested, while the SPE amendments (non-polar organic characterization) did not reduce toxicity. However, Phase I results of the whole sediment TIE for site SS315 showed that zeolite and PCC (ammonia and non-polar organic characterization, respectively) significantly reduced toxicity, and that neither amendment reduced toxicity in SS308. The differences between these two TIE outcomes can be understood more clearly by evaluating how the various differences in Phase I methodologies of pore water and whole sediment TIEs affects the characterization of both ammonia and non-polar organics, as well as examining the Phase II analytical results and the associated toxic units.

Ammonia. In *H. azteca* testing, predicted ammonia TUs during the pore water TIE were approximately 2.6 and 0.80 for SS315 and SS308, respectively, suggesting that ammonia was the source of toxicity for both sites, which supported the Phase I findings.

While in the whole sediment TIE, ammonia TUs that are based on overlying water concentrations (and use the same LC_{50} values as in pore water testing) were up to 10-fold lower than pore water ammonia TUs (0.27 and 0.11 for SS315 and SS308, respectively). Toxicity was significantly reduced in SS315 sediment suggesting that ammonia was a source of toxicity at this site; zeolite removed approximately 0.25 TU, which was close to the approximate predicted TUs associated with ammonia (Table 3.1). This was unexpected, as zeolite did not remove toxicity at site SS315 in previous Phase I testing in the whole sediment TIE in any of the five seasons tested (Mehler *et al.* 2009). This discrepancy may be due in part to the present study using two additional replicates, and even then the degree of significance was still low ($p = 0.15$), but this still suggests that ammonia plays a role in toxicity at site SS315. Alternatively, sites with considerably lower concentrations, such as site SS308, are most likely not impacted acutely by ammonia, which contradicts the pore water TIE findings.

In pore water TIE testing, the organism is subjected to the pore water matrix and in doing so a large portion of water soluble contaminants (such as ammonia). The direct interactions between the test organisms being used and the pore water itself, however, in sediment testing are negligible. Thus, water soluble contaminants may not be available at those concentrations when overlying water is added in whole sediment testing. Some authors have suggested that pore water testing may overestimate ammonia toxicity, especially for epi-benthic and pelagic organisms (such as *H. azteca* and *C. dubia*, respectively) who do not occupy niches which are commonly exposed to the pore water (Chapman 2002, U.S. EPA 2007). For these reasons the choice of test organism in pore water TIE testing is critical to accurately identify the source of toxicity. It should be

noted; however, that whole sediment TIEs are not entirely environmentally realistic, and that the amount of water being used in whole sediment TIEs would dramatically change the concentration and thus the results of the test. However, whole sediment tests may be the best choice available, since environmentally realistic conditions cannot be truly represented.

Non-polar Organics. As mentioned previously, pore water TIE testing suggested that ammonia was the source of toxicity, but PAH concentrations in phase II testing in the pore water at site SS308 were elevated, and concentrations of PAHs resulted in high TUs in extracted whole sediment for both sites. Two major issues arise; however, in trying to determine if non-polar organics, such as PAHs, do play a role in toxicity of these sites in both pore water as well as whole sediment TIE testing.

First, if PAHs are at high enough concentrations in the pore water TIE test to cause toxicity, then the SPE manipulation (which reduced PAH concentrations dramatically) should have reduced toxicity. One possible explanation why toxicity was not reduced could be attributed to ammonia toxicity. With the large degree of toxicity caused by ammonia in pore water TIE testing, the increase in survival with the SPE treatment maybe masked. To determine if this was the case, SS308 site sediment was reprocessed using the same Phase I pore water TIE procedures as stated earlier (un-amended, SPE amended, and zeolite amended) with an additional treatment using SPE and zeolite amendments simultaneously, which would remove both contaminant classes simultaneously. The results of this test suggested that non-polar organics were not the

source of toxicity in SS308 pore water, as toxicity in the zeolite and SPE treatment was not significantly higher than the zeolite treatment alone (data not shown).

The second issue was the hydrophobicity of PAHs and other non-polar organic contaminants. As in whole sediment testing, the unresolved complex mixture (UCM) or the oils and grease matrix that was associated with PAH contamination (Jonker *et al.* 2006) were still an issue, but other confounding factors in pore water testing include binding to glassware and dissolved organic carbon (DOC). As previously reported in the whole sediment TIE, the role that the UCM may play in causing toxicity, affecting the bioavailability of PAHs, as well as affecting the ability of PCC to bind non-polar organics is still unknown (Mehler *et al.* 2009) Interestingly, the UCM may serve as a more prevalent confounding factor in pore water than whole sediment TIE testing. The UCM (based on percent of dry wt) at site SS308 was examined before and after centrifugation with no significant differences in the percent composition of UCM in the sediment being observed, suggesting that the UCM was not represented in pore water TIE testing. If the UCM played a role in the toxicity or strongly bound non-polar organics, then pore water testing may grossly underestimate non-polar organic toxicity. If centrifuged sediment still possesses high concentrations of PAHs due to binding with the UCM, black carbon or other carbon sources, then adsorption or ingestion of the sediment, which may also contribute to toxicity, should also be evaluated. Additionally, in the pore water TIE bioassay, binding of the contaminants to glassware and dissolved organic carbon (DOC) may play a significant role between the contradicting results of the recent whole sediment TIE and past pore water TIE studies. Hawthorne *et al.* (2005) reported that up to 96% of the higher molecular weight compounds (with those being the more

toxic PAHs) can be bound to DOC, in some pore water samples, and thus may not be bioavailable to the test organism. Addressing DOC, UCM, as well as the other issues previously mentioned is important when trying to determine if the source of toxicity is non-polar organics when conducting pore water TIE testing.

Evaluating the TIE Methods

In determining which whole sediment and pore water TIE should be used in an aquatic risk assessment, it is important to understand that both TIEs have their strengths and limitations. Table 3.2 has been provided to addresses these concerns, by detailing the strengths and limitations of both types of TIEs.

While a bulk of the discussion has shown the drawbacks of pore water TIE testing, the benefits should not be overlooked as pore water TIE testing maybe the best suited choice for assessing toxicity in certain cases. Pore water testing in general is more efficient in terms of cost and time compared to whole sediment testing. A standard pore water test is typically conducted over a 2-d period of time and can be run in disposable scintillation vials, while whole sediment tests are usually 10 d in duration with substantially more space requirements. Another issue in whole sediment testing is the difficulty in using certain test organisms. Control recoveries of small organisms (such as *C. dubia* in the present study) and sediment avoidance issues (such as *H. azteca*) are both factors that increase variability and can confound whole sediment TIE results (Winger *et al.* 2001). Additionally, pore water TIEs are more sensitive in assessing toxicity than whole sediment bioassays. When comparing the sensitivity (based on an organism-toxicity response) of pore water to whole sediment with *H. azteca*, three times the amount

of toxicity was observed in pore water TIE testing, even though the test duration with pore water was five times shorter than whole sediment testing (Table 3.1). The increased sensitivity is caused by removing many of the bioavailability factors associated with the contaminant partitioning in various matrices as in whole sediment. While some authors would argue that the degree of toxicity in pore water testing is in many cases not environmentally relevant (Adams *et al.* 2001, Chapman *et al.* 2002, Ho 2002), pore water TIEs could provide a valuable assessment tool in determining toxicity in "worse" case scenarios or potentially characterizing sites in which sub-lethal effects have been observed.

With confounding factors being present in both whole sediment and pore water TIEs, the best option would be to conduct both TIE procedures (U.S. EPA 2007). In the present study, both TIE procedures were performed on two selected sites to evaluate the differences in matrix choice. The U.S. EPA (2007) suggests that conducting initial toxicity testing with both matrices to identify potential toxic sites in the beginning provides a larger scope to evaluate toxicity and to identify which TIE procedure needs to be used. In addition, it may facilitate more accurate decisions by risk assessors.

Organism Sensitivity and Susceptibility

The differences in methodologies played a large role in the outcome of both TIEs, but one consideration that occasionally is overlooked is test organism choice. The ramifications that test organism choice plays in characterizing the source of that toxicity in TIE testing have not been well documented. Previous pore water TIEs used *C. dubia* (Sparks and Ross 1992, Burton 1995), while the whole sediment TIE conducted recently

used *H. azteca* (Mehler *et al.* 2009). Comparing the differences between these organisms is critical to understand whether it plays a role in the discordance between past and present TIE research on the IRC.

The differences between the test organisms (*H. azteca* and *C. dubia*) in the present study are numerous. First, the organisms vary based on physiology, ecological niche, and functional feeding group. *H. azteca* are epi-benthic amphipods and feed by shredding (pair of cutting and tearing mandibles) plant and animal material; while *C. dubia* are pelagic cladocerans that feed by filter feeding (Smith 2001). With *H. azteca* having more direct interactions with the sediment one would believe it would be more susceptible to hydrophobic contaminants, such as non-polar organics, than a pelagic organism, such as *C. dubia.* The role that these differences play in the sensitivity between the two organisms is still somewhat unknown. Additionally, the difference in the size and age of the two species may play a large role in the sensitivity of the test organism. The critical body residues that are needed to cause a certain amount of toxicity may be the same for two different organisms. The time that may be needed for the two organisms to reach those critical body residues to cause that toxicity, however, maybe different (Rand *et al.* 1995). Studies have shown that *H. azteca* (Ankley *et al.* 1995) are less sensitive than *C. dubia* (Bailey *et al.* 2001) to many contaminants, such as ammonia with total ammonia LC_{50} values being approximately 140 and 47.3 mg N/L, respectively in water-only tests. The sensitivity differences between the two organisms can be observed in the present study, as site SS315 ammonia TU values for *H. azteca* were 2.3-fold lower than *C. dubia* (0.27 and 0.61, respectively) (Table 3.1).

In the present study, test organism choice may not have played a large role in the contradicting results between the whole sediment and pore water TIE studies. Sites with lower ammonia concentrations (as were reported by Sparks and Ross 1992, Burton 1995, Mehler *et al.* 2009) could inherently be toxic to *C. dubia*, but not to *H. azteca*, and thus be mischaracterized due to this reason alone. For these reasons, it is imperative that the objectives of a study justify why a particular organism was chosen. The location of sediments in a highly channelized riverine system (> 6 meters), such as the IRC, make choosing an environmentally relevant TIE test organism quite difficult. Habitats that *H. azteca* typically inhabit are limited, with neither of these organisms most likely having direct interactions with the sediments (Smith 2001). Studies on the benthic community structure would provide insight toward the most site- relevant organisms to select for TIE purposes. However, choosing more environmentally relevant organisms may be difficult, as organisms that would be found presently in these areas would represent the organisms that thrive in contaminated sediments. The best choice may be to use organisms that were found at these sites historically, such as the fingernail clam. Although these organisms might be quite difficult to rear and use, they would provide a more accurate surrogate to evaluate and understand the risk associated with these contaminants to the benthic ecosystem of the IRC. It should be noted, however that the test organisms used in the present whole sediment TIE study as well as the past pore water TIE studies are organisms that are commonly used in TIE studies and are easy to culture and use than other test organisms. Future studies should consider using both past test organisms for comparison purposes as well as investigating organisms that are critical in the ecosystem

being studied, which in this case is the fingernail clam (Sparks 1984, Sparks and Ross 1992).

Future Direction and Uncertainties

Determining accurate bioavailable concentrations in comparison to the observed toxicity is difficult in both pore water and whole sediment TIE testing. In the present study, target contaminant concentrations were determined by exhaustive analytical methods, which do not represent the freely dissolved concentrations that are available to the test organism. The extraction concentrations are useful in determining the total concentrations that are at the site and determining a general idea of what might be causing toxicity, but it tells us relatively little toward what is actually available and predicting the amount of toxicity that is observed from each contaminant class. Additionally, TU values for whole sediment were based on equilibrium sediment-benchmarks and may not represent what is bioavailable to the organism as K_{oc} values vary among sediments (Hawthorne *et al.* 2007). This general idea also exists in pore water testing as non-polar organics may bind to DOC and not be bioavailable to the test organism. By performing liquid-liquid extractions with pore water, and not accounting for DOC, this may lead to erroneous concentrations that have been reported (with PAHs) often exceeding solubility limits (Hawthorne *et al.* 2005). Additionally, Phase II procedures (chemical quantification) for both matrices can be difficult to accomplish even at concentrations that would cause toxicity. For example, the amount of pore water needed in the TIE study to determine contaminant concentrations was limited to 25 ml, due to the difficulty and time needed to extract large volumes of pore water. By using

only 25 ml, reporting limits (0.4 µg/L) were high and may have masked potential toxicity of highly toxic contaminants such as pyrethroids and certain PAHs (e.g. dibenz[ah]anthracene LC_{50}:0.28 ng/ml – Hawthorne *et al.* 2005). Other issues such as black carbon, mixture effects of contaminants, glassware binding, and water quality parameters can influence TUs, regardless of TIE chosen. With these confounding factors TUs should be seen as estimates which have limitations. While this is a confounding factor in the Phase II procedure, it is a significant and prevalent issue in the conventional contaminant-based method, as this method bases toxicity solely on chemical concentrations. For these reasons, the conventional contaminant-based method has reverted to use of solid-phase microextraction (SPME) fibers to resolve many of these confounding factors with high success. Use of SPME fibers, while not commonly used in TIEs, could enhance the TIE procedure greatly (Hawthorne *et al.* 2006a, Hawthorne *et al.* 2006b, You *et al.* 2006, Hawthorne *et al.* 2007, You *et al.* 2007).

CONCLUSIONS

The present study confirmed that differences between pore water and whole sediment TIE methodologies could characterize the source of toxicity differently, and the same is true for the use of different test organisms. Evaluating and understanding the differences between the two types of TIE methodologies is important in determining the circumstances in which a certain TIE technique should be employed. With TIEs becoming a common procedure in risk assessment in various aquatic systems, understanding the variability associated with the TIE methods is difficult, but if investigators can conduct both TIE methodologies much of this variability is addressed.

Perhaps, more importantly, each TIE method evaluates a different component of a toxic site-sediment, and by conducting both TIE procedures helps provide a stronger weight of evidence than using one TIE method alone.

Table 3.1. Observed and predicted toxic units for whole sediment and pore water toxicity identification evaluation (TIE) Phase II testing for sites SS315 and SS308 with *Hyallela azteca* and *Ceriodaphnia dubia*. Concentrations of ammonia (± 95% confidence intervals (CIs)) in whole sediment and pore water TIE testing were the mean concentrations of three separate replicates being taken every two days and the mean concentration of three replicates at the beginning of the test, respectively. For a more accurate depiction of ammonia concentrations in whole sediment testing over time see Figure 3.3. Concentrations of the sum polycyclic aromatic hydrocarbons (∑PAHs) (± 95% CIs) were the mean concentration of two replicates at the beginning of the test. The concentrations for organic contaminants were normalized to organic carbon (oc). ** - testing for ammonia and PAHs with whole sediment was conducted in 4- and 10-d tests, respectively. NA – Not Available.

			Phase II: Total Ammonia				Phase II: ∑PAHs			
Matrix	Organism (Test Duration)	Site	Obs. TUs	Conc. (mg N/L)	Predicted Ammonia TUs	Post-manipulation Concentration (mg N/L)	Obs. TUs	Conc. (µg/g oc)	Predicted ∑PAHs TUS	Post-manipulation Concentration (µg/g oc)
Whole sediment	*H. azteca* (**)	SS315	0.90	37.9 (± 4.21)	0.27	15.7 (± 5.72)	1.35	641 (± 39.6)	0.90	NA
		SS308	1.08	15.9 (± 5.91)	0.11	8.58 (± 4.54)	1.55	4405 (± 629)	6.44	NA
	C. dubia (2 d)	SS315	1.80	40.3 (± 0.85)	0.85	16.9 (± 10.6)	1.80	641 (± 39.6)	NA	NA
		SS308	1.75	14.8 (± 9.86)	0.31	2.07 (± 0.23)	1.75	4405 (± 629)	NA	NA
				(mg N/L)		(mg N/L)		(µg/L)		(µg/L)
Pore water	*H. azteca* (2 d)	SS315	3.10	359 (± 37.9)	2.56	22.5 (± 0.69)	3.10	63.7 (± 4.10)	NA	21.9 (± 8.23)
		SS308	3.80	111 (± 13.8)	0.79	12.0 (± 1.16)	3.80	1953 (± 190)	NA	26.6 (± 5.88)
	C. dubia (1 d)	SS315	> 4.0	359 (± 37.9)	5.84	22.5 (± 0.69)	> 4.0	63.7 (± 4.10)	NA	21.9 (± 8.23)
		SS308	> 4.0	111 (± 13.8)	1.81	12.0 (± 1.16)	> 4.0	1953 (± 190)	NA	26.6 (± 5.88)

Table 3.2. Strengths (+), limitations (-), and factors that are neither a strength nor limitation (+/-), for whole sediment and pore water toxicity identification evaluations (TIEs).

Whole sediment TIE	(+/-)	Issue in TIE procedure	(+/-)	Pore water TIE
10-d testing, initial glassware cost	-	Cost and time (sampling, setup, testing duration)	+	Quick setup and 2-d testing
Beakers/jars/flow-thru system/environmental chamber	-	Space and equipment requirements	+	Disposable scintillation vials/environmental chamber
Not a concern	+	Adsorption to test chambers	-	Problems w/ hydrophobic compounds
Bioavailability addressed	+	Issues regarding bioavailability	-	Bioavailability not addressed
Difficult, requires further sub-lethal analysis	-	Evaluating sub-lethal aspects	+	Can evaluate using acute data
Addressed	+	Dietary route of exposure	-	Not addressed
Effective	+	Test organism (benthic)	+	If pore water is route of exposure
Effective	+	Test organism (non-benthic)	-	Not environmentally relevant
Difficult	-	Use of small test organisms	+	Easy to score and use
Environmentally relevant	(+/-)	Sensitivity of testing procedure	(+/-)	More sensitive, could be used to address sub-lethal or "worse-case" scenarios
Avoidance issues with sediment	(-)	Variability of testing procedure	(+)	Homogenous matrix that can't be avoided
Not a concern (emulate field conditions)	(+)	Water quality parameters	(-)	Low dissolved oxygen, high conductivity, oxidation issues

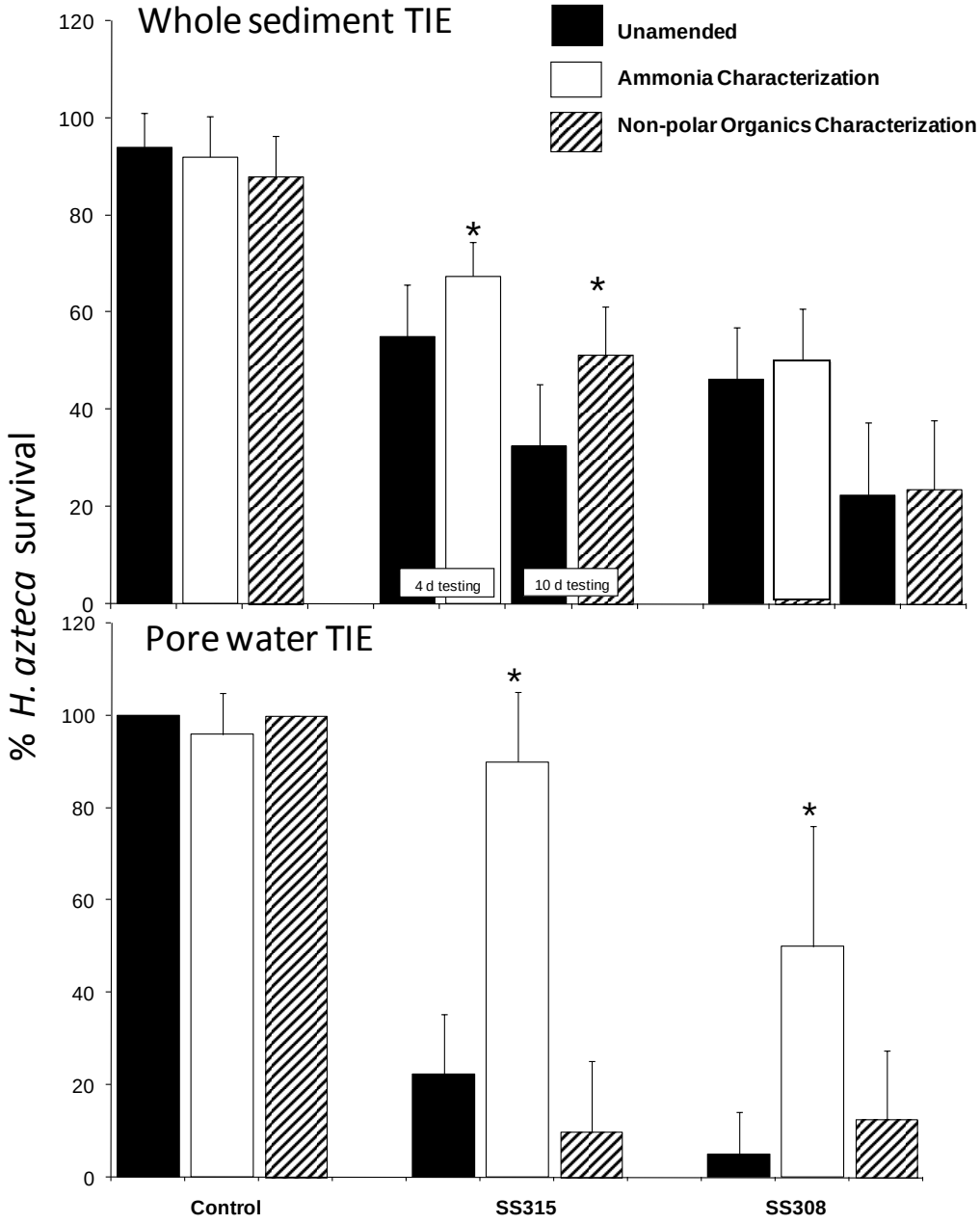

Figure 3.1. Whole sediment and pore water toxicity identification evaluations (TIE) examining Phase I data for sites SS315 and SS308 for *Hyalella azteca* showing site toxicity (percent survival) with and without amendments. Solid, striped and open bars indicate mean percent survival in site sediment that was un-amended, those characterized for non-polar organics (amended in whole sediment and pore water with powder coconut charcoal (PCC) and solid phase extraction (SPE), respectively), and those characterized for ammonia (amended with zeolite), respectively. Each bar represents eight replicates (± standard deviation). Stars indicate significant differences ($p < 0.05$) between un-amended site sediment and amended site sediment. In whole sediment TIE testing, zeolite and PCC were conducted over 4- and 10-d, respectively, thus the solid bar to the left of the amended treatment was conducted over the same duration.

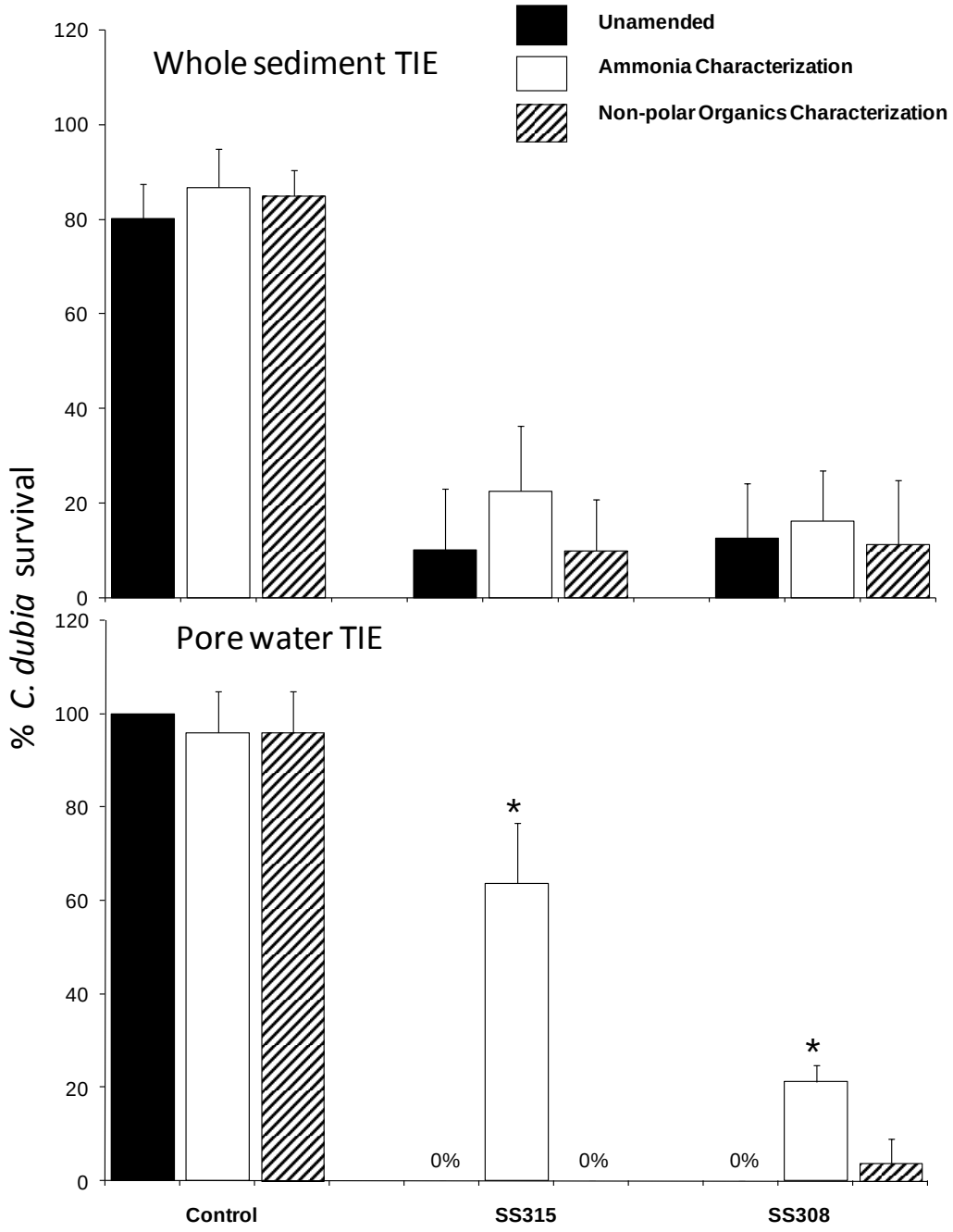

Figure 3.2. Whole sediment and pore water toxicity identification evaluations (TIE) examining Phase I data for sites SS315 and SS308 for *Ceriodaphnia dubia* showing site toxicity (percent survival) with and without amendments. Solid, striped, and open bars indicate mean percent survival in site sediment that was un-amended, those characterized for non-polar organics (amended in whole sediment and pore water with powder coconut charcoal (PCC) and solid phase extraction (SPE), respectively), and those characterized for ammonia (amended with zeolite), respectively. Each bar represents eight replicates (± standard deviation). Stars indicate significant differences ($p < 0.05$) between un-amended site sediment and amended site sediment.

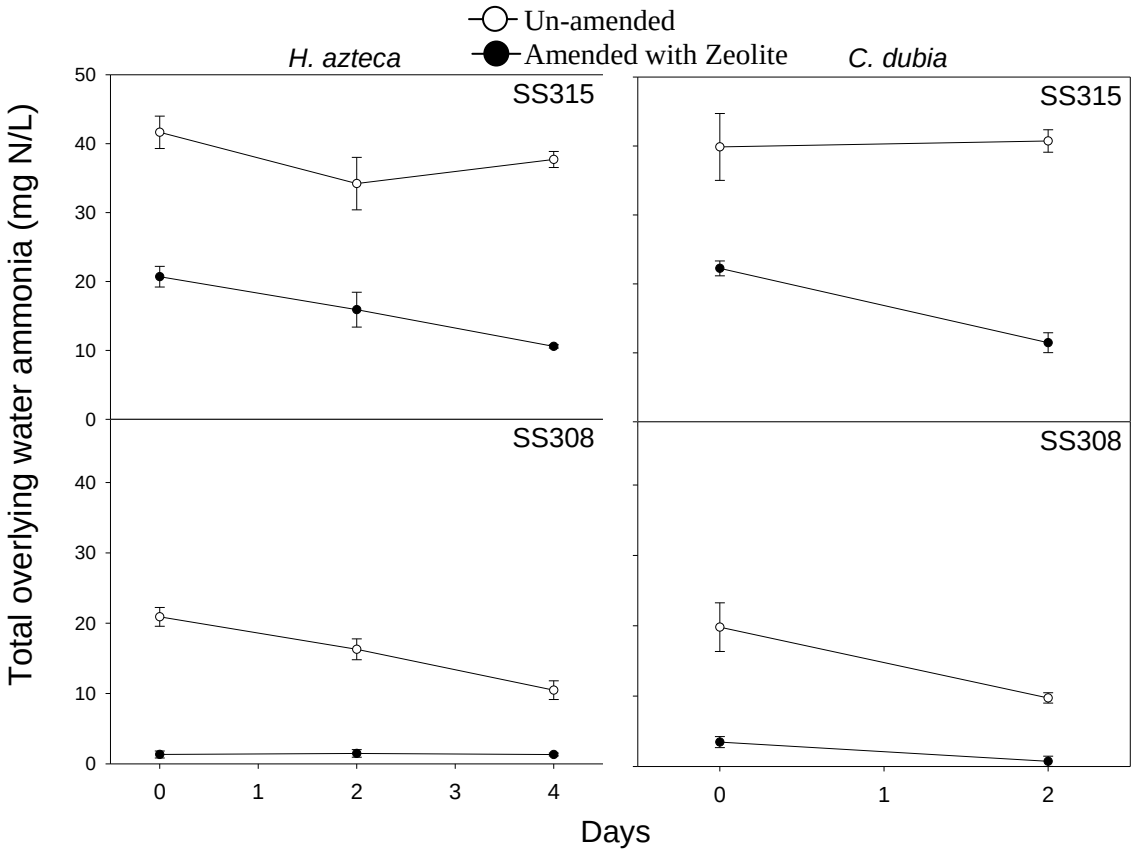

Figure 3.3. Whole sediment Phase II testing using zeolite with *Hyalella azteca* and *Ceriodaphnia dubia*, showing overlying water total ammonia concentrations over time. Lines with open circles and solid circles signify those un-amended and those amended with zeolite, respectively. Each time point is the average of three separate replicates (± standard deviation). Day 0 indicates the day that the test organisms were added to the sediment; zeolite had already been placed into amended samples for one day.

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