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Comparative analysis of whole sediment and porewater toxicity identification evaluation techniques for ammonia and non-polar organic contaminants

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ABSTRACT

Porewater and whole sediment toxicity identification evaluations (TIEs) were performed on contaminated Illinois River sediment and compared using two standardized toxicity-testing organisms (*Ceriodaphnia dubia* and *Hyalella azteca*). Results suggested that the choice of testing matrix (porewater versus whole sediment) significantly influenced characterization of toxicity. The porewater TIE suggested that ammonia was the major source of toxicity, while the whole sediment TIE indicated that non-polar organics, specifically polycyclic aromatic hydrocarbons, were the primary contributor to toxicity, with ammonia being a secondary contributor to toxicity. While the choice of test organism may have played a smaller role in the discordance between the TIEs, the data suggest that this factor alone could play a prevalent role in characterizing toxicity in other TIE assessments. Because porewater and whole sediment TIEs examine sediment toxicity differently, using both TIE approaches as part of a risk assessment may provide a more accurate risk estimate of sediment toxicity.

1. Introduction

A porewater toxicity identification evaluation (TIE) uses porewater to identify the contaminant class and/or specific chemical causing sediment toxicity in conjunction with analytical measurements to provide further evidence of the source of toxicity (Doe et al., 2001). An important advantage of using porewater as the testing media in a TIE over whole sediment was that porewater TIE guidelines were available in early 1990s and have been used frequently in risk assessment (Doe et al., 2001). The recent introduction of whole sediment TIE guidelines (US EPA, 2007) has stimulated debate toward which TIE methodology is a better approach to characterize sediment toxicity to benthic invertebrates. Studies have compared porewater and whole sediment toxicity testing in the past, and while often using different endpoints, these studies have shown that the sensitivity of the two approaches in addressing toxicity varies depending on the organism and contaminant class being examined (Bay et al., 2001; US EPA, 2007). However, evaluating different matrices in the TIE process (porewater versus whole sediment) to accurately characterize the source of toxicity has not been clearly addressed.

Previous porewater 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 metals as minor sources of toxicity (Sparks and Ross, 1992; Burton, 1995). However, a whole sediment TIE using *Hyalella azteca* was used to assess the same sites, and identified polycyclic aromatic hydrocarbons (PAHs), a non-polar organic, as a primary source of toxicity on the IRC (Mehler et al., in press). Since similar ammonia concentrations were observed between the studies, the contradiction between the outcome of the porewater TIE and the whole sediment TIE was surprising and stimulated further investigation on the two methodologies.

The objective of the present study was to compare the two TIE methodologies by conducting both porewater and whole sediment TIEs on two sediments collected from the IRC. An epibenthic and planktonic species (i.e., *H. azteca* and *C. dubia*, respectively) were used for both methodologies to examine species sensitivity and susceptibility and identify this effect on conclusions derived from each TIE methodology. Results of this research can be useful in TIE method and test organism selection in future TIEs and ultimately help improve risk assessments for contaminated sediment.

2. Materials and methods

2.1. Sediment and organisms

Two sediment samples (SS315 and SS308) were collected from the Chicago Sanitary and Ship Canal, a major tributary of the IRC

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(river-miles: 315 and 308, respectively). Approximately 20 L of sediment was collected from each site using a petite ponar (Wildco, Columbus, OH), and stored on ice (4 °C) prior to being received at Southern Illinois University-Carbondale (SIUC). The two sediments (SS315 and SS308) had total organic carbon (OC) content of 10.2% and 5.75%, respectively. These sediment sampling locations were selected, since these locations previously had exhibited acute toxicity to *H. azteca* (≈40–75% survival) (Mehler et al., in press). The OC in the porewater for SS315 and SS308 (372 and 657 mg L^{-1}, respectively) was determined using a Formacs Combustion TOC Analyzer (Skalar Analytical B.V., Breda, the Netherlands). Control sediment was prepared from a hydrated soil collected from Touch of Nature (TON) in Carbondale, IL, USA (Mehler et al., in press).

Mixed-aged cultures of *H. azteca* and *C. dubia* were originally obtained from the US EPA Duluth Mid-Continental Ecology Division and Texas Tech University (Lubbock, TX, USA), respectively, and have been cultured at Southern Illinois University-Carbondale (SIUC) in accordance with US EPA Protocols (US EPA, 2000). The two test organisms, juvenile *H. azteca* (14–21 d) and *C. dubia* (approximately 24 h) were used separately in each type of TIE. The *H. azteca* used for toxicity testing passed through a 1000 μm mesh sieve and were retained by a 500 μm mesh sieve (Schuler et al., 2006), while *C. dubia* less than 24 h old were obtained using standardized methods (US EPA, 2002).

2.2. Phase I testing: sediment characterization

Phase I testing was conducted to characterize toxicity using porewater and whole sediment as test media with both *H. azteca* and *C. dubia*. Metals did not contribute to toxicity in previous TIE studies on the IRC (Sparks and Ross, 1992; Burton, 1995; Mehler et al., in press), thus only non-polar organics and ammonia were addressed in the present study. Powdered coconut charcoal (PCC) and zeolite were the non-polar organic and ammonia amendment in the whole sediment TIE, respectively. For the porewater TIE, C18 solid phase extraction (SPE) cartridges were used to remove toxicity from non-polar organics and zeolite was used to remove ammonia.

In Phase I toxicity testing, water hardness was adjusted to resemble site water (i.e., very hard water) (Mehler et al., in press). Preparation techniques for the amendments used were detailed in Mehler et al. (in press) as modified from past TIE studies (US 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 (US EPA, 2007). Dissolved oxygen (DO), conductivity, temperature, and pH were monitored daily in random beakers during toxicity testing.

2.2.1. Sediment toxicity tests with Hyalella azteca

Ten-d 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 (US 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-through 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 tests were performed at 23 ± 1 °C with a 16:8 h 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-d 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 assay to avoid the loss of ammonia during the daily water renewals, which would occur in a static-renewal flow-through 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 incubator with temperature and photoperiod controlled (23 ± 1 °C and 16:8 h light:dark, respectively).

2.2.2. Sediment toxicity tests with Ceriodaphnia dubia

Two-d 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 (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 incubator under similar conditions as *H. azteca* for 2 d without feeding.

2.3. Phase I testing: porewater characterization

Very hard water, which closely resembled IRC water, was prepared as a control using standard EPA protocols (US EPA, 2000). Site porewater samples were prepared by centrifuging sediment for 45 min × 2500g, and stored at 4 °C for no longer than one week prior to testing. Porewater was diluted at a 50:50 ratio with very hard water to alleviate initial low DO concentrations (3.5–4.5 mg L^{-1}) 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. Porewater was processed for non-polar organics and ammonia characterization by amending porewater 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, SPE-amended, and zeolite-amended treatments. 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 assays.

The procedures to amend site porewater with SPE included passing approximately 100–150 mL of porewater 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 porewater being passed through the SPE cartridge was collected and stored at 4 °C with a total of 200–250 mL being collected for analysis.

The zeolite-amended treatment reduced ammonia concentrations by shaking 200 mL of porewater 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, porewater was centrifuged again for 10 min × 2500*g* and stored at 4 °C.

2.3.1. Porewater toxicity tests with Hyalella azteca

Two-d static toxicity tests were conducted using 20 mL of diluted site porewater (50:50) in 25 mL disposable vials with

approximately 1 g of sand in each vial. Five organisms were placed into each of the eight replicates to initiate toxicity tests. Scintillation vials were placed into an incubator at 23 ± 1 °C and 16:8 h light:dark photoperiod (US EPA, 2007).

2.3.2. Porewater toxicity tests with Ceriodaphnia dubia

Two-d static tests were initially planned for porewater toxicity tests with *C. dubia*, however the testing was ended within 24 h due to early lethality (e.g., 100% lethality occurred). Ten millilitres of 50:50 diluted porewater was used, with 10 *C. dubia* being used per replicate. Eight replicates were used per treatment, and testing was conducted in the same incubator as the *H. azteca* porewater toxicity test.

2.4. Phase II testing: identification

Chemical concentrations of both ammonia and non-polar organics were determined in porewater and whole sediment. For a complete list of these contaminants refer to *Table* S1 in the supplemental material. Procedures for porewater and sediment preparation are detailed in the Phase I testing characterization methods above.

2.4.1. Ammonia identification

Porewater ammonia was assessed immediately after centrifugation and within 24 h of arrival at 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 porewater for each replicate. Concentrations of porewater ammonia were also assessed after zeolite manipulation to determine how much ammonia was reduced with the amendment. Total ammonia concentrations were assessed rather than unionized ammonia so that comparisons between sites and methodologies could be made (i.e., differences in pH among samples may confound comparisons of unionized ammonia).

Studies have shown that the predominant exposure route of water soluble contaminant (such as ammonia) to species which reside in the water column (e.g., *H. azteca* and *C. dubia*) is the overlying water (Chapman, 2002; US EPA, 2007). Thus, in whole sediment TIE testing, ammonia concentrations in overlying water were monitored every 2 d in both un-amended and zeolite-amended sediment. Three replicates in the overlying water were examined with 10 mL of overlying water per measurement.

2.4.2. Non-polar organics identification

Porewater was assessed for a suite of non-polar organics, including 20 organochlorine pesticides (OCPs), the organophosphate (OP) chlorpyrifos, and seven pyrethroid pesticides, as well as 16 PAHs (PAHs were taken from the US EPA priority pollutant list; US EPA, 2004) (*Table* S1) using liquid–liquid extraction techniques (LLE) (Wang et al., 2009). Briefly, 25 mL porewater was mixed with 50 mL dichloromethane in a separatory funnel, and was shaken for approximately 5 min. After separation, dichloromethane was collected, and the porewater was extracted twice more with dichloromethane. The extract was combined, concentrated, cleaned and solvent exchanged to acidified hexane 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). Pesticides were analyzed using an Agilent 6890 series gas chromatograph equipped with a micro-electron capture detector (Agilent, Palo Alto, CA, USA), using methods from You et al. (2008). Analysis of PAHs was performed using an Agilent 1100 High Performance Liquid Chromatograph equipped with a fluorescence detector (Mehler et al., in press). 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 detailed in Mehler et al. (in press). 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 dual layer cartridges (You et al., 2008) and a 20 mL alumina-silica column was used for the PAH cleanup. Instrumental analyses of extracts were the same as for porewater quantification.

2.5. Toxic unit determination

Predicted toxic units (TUs), which indicate the contribution of each contaminant to sediment toxicity, were calculated using the followingequation:

$$\text{Predicted TU}_i = \frac{C_i}{\text{LC50}_i} \quad (1)$$

where C was the contaminant concentration in the testing media, the LC_{50} was the concentration of a contaminant that would result in 50% mortality in a test population, and i was the individual contaminant being examined. Observed TUs were calculated by using the following equation:

$$\text{Observed TU} = \text{OPM} \times \frac{1}{50} \times \text{DF} \quad (2)$$

where OPM was the observed percent mortality at the site and DF was the dilution factor used in testing (which was one and two for whole sediment and porewater, respectively).

2.5.1. Non-polar organics

To determine TUs in the whole sediment TIE, non-polar organic pesticide LC50s based on sediment concentrations were taken from published literature values for *H. azteca* (OCPs, chlorpyrifos and pyrethroids: Weston et al., 2004). *C. dubia* is not commonly used in acute sediment toxicity testing, and LC50s based on sediment concentrations for non-polar organics could not be found. Using methods from Di Toro and McGrath (2000) and Di Toro et al. (2000) PAH TU values for both *H. azteca* and *C. dubia* were calculated based on Log K_{ow} for similar species (*Leptocheirus plumulosus* and *Daphnia pulex*, respectively).

In porewater testing, pesticides were below detection limits, and thus TU values were not determined for either species. The PAH TU values for the porewater TIE was initially based on published freely dissolved concentrations (Hawthorne et al., 2005), but this can often lead to erroneously high values. Thus, TU values were normalized for OC in the porewater, with the same methods as in sediment testing (Di Toro and McGrath, 2000; Di Toro et al., 2000).

2.5.2. Ammonia

The predominant exposure route of ammonia, for benthic and pelagic organisms (*H. azteca* and *C. dubia*, respectively) is the overlying water rather than porewater in whole sediment testing (Chapman, 2002; US EPA, 2007). Thus, ammonia TUs for both organisms in the whole sediment TIE were calculated based on overlying water concentrations. Conversely, in the porewater TIEs; TUs were based on porewater concentrations, since the organisms were directly exposed to the porewater matrix. Total ammonia TUs

for both whole sediment and porewater TIEs were based on extrapolations using unionized ammonia published literature values for *H. azteca* (Ankley et al., 1995) and *C. dubia* (Bailey et al., 2001).

2.6. Data analysis

Survival was compared among treatments using analysis of variance (ANOVA) ($\alpha = 0.05$) and Dunnett's multiple comparison test (SAS Institute, Cary, NC, USA). Comparisons were made between un-amended and the amended sediment toxicity. Potential toxicity of amendments was also evaluated by comparing toxicity of un-amended control with the amended control sediment.

3. Results

3.1. Phase I: characterization

In the whole sediment TIE, *H. azteca* survival for SS315 sediment was significantly improved by adding either zeolite or PCC, while no amendment significantly improved *H. azteca* survival for SS308 sediment (Fig. 1a). Survival of *C. dubia* was not significantly increased with the addition of either amendment for both sediments (Fig. 1b). In the porewater TIE, survival of both organisms was significantly increased by the zeolite manipulation for both sediments; however, the SPE amendments did not significantly reduce toxicity for either organism in either sediment (Fig. 1a and b). In all testing, the amendments for ammonia (zeolite) and non-polar organics (PCC and SPE) did not exhibit toxicity significantly different from controls for either species.

3.2. Phase II: identification

The zeolite amendment dramatically reduced ammonia concentrations from 359 and 111 mg N L^{-1} in the undiluted porewater to 22.5 and 12.0 mg N L^{-1}, for sediments SS315 and SS308, respectively (Table 1). In the whole sediment TIE, overlying water ammonia concentrations were approximately 10 times lower than porewater concentrations (Table 1). Zeolite additions reduced concentrations of overlying water ammonia by over half in the 4 d tests with *H. azteca* and in the 2 d tests with *C. dubia* (Table 1).

Pesticide concentrations in undiluted porewater were below reporting limits (i.e., 0.2 μg L^{-1}) in both un-manipulated porewaters, while elevated PAH concentrations were detected (Table 1). The SPE amendment reduced PAH concentrations for both porewater samples. Calculating the sum PAH TUs for *H. azteca* using freely dissolved LC50 values resulted in inflated TU values (30.4 and 664 for sediments SS315 and SS308, respectively). Thus, OC in porewater was measured and PAHs in porewater were normalized for OC, which resulted in lower TU values (Table 1).

Only a few pesticides were detected in sediment above the reporting limits of 0.035 and 0.020 μg g^{-1} OC for SS308 and SS315, respectively, (SS308 – DDT: 0.052 μg g^{-1} OC; SS315 – dieldrin, DDD, DDE, and DDT: 0.049, 0.37, 0.21 and 0.20 μg g^{-1} OC, respectively) resulting in low toxic units for *H. azteca* (<0.1 TU).

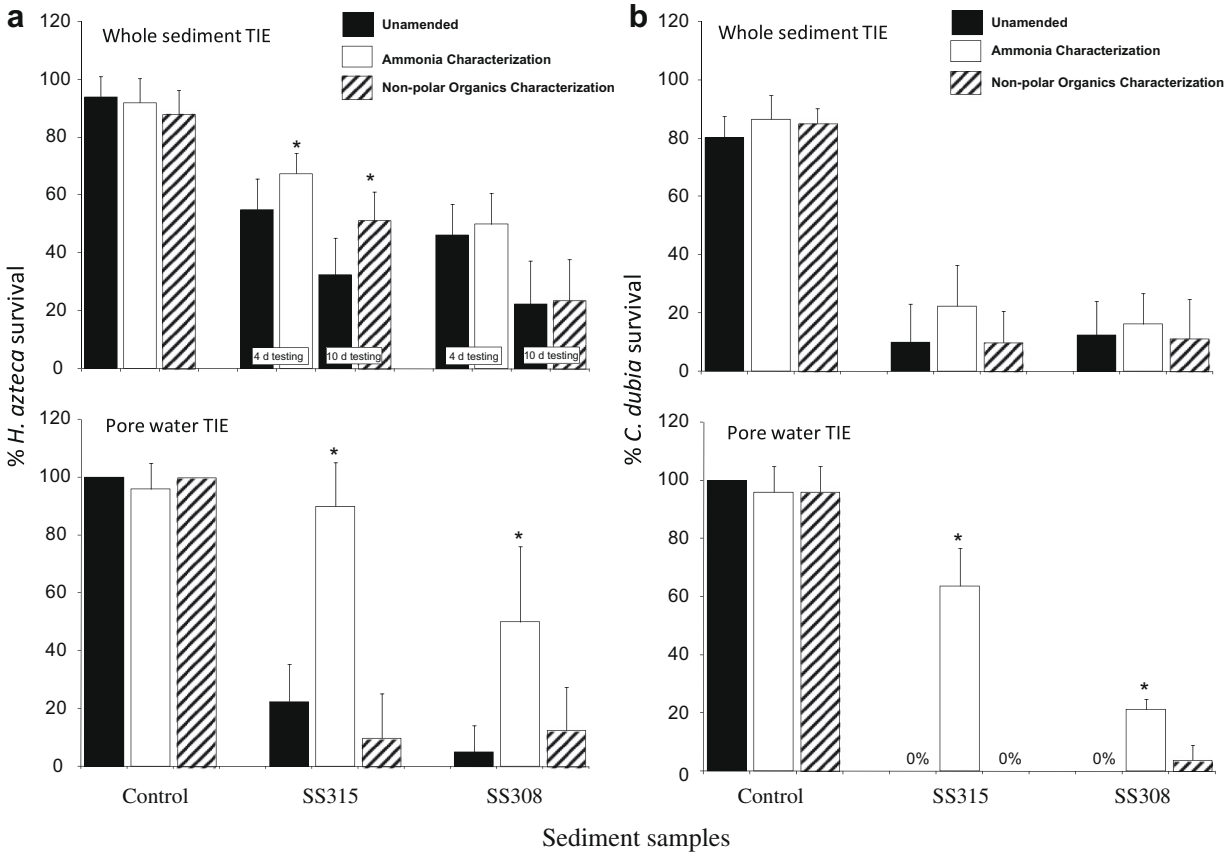

Fig. 1. Whole sediment and porewater toxicity identification evaluations (TIE) examining Phase I data for sites SS315 and SS308 for *Hyalella azteca* (a) and *Ceriodaphnia dubia* (b) showing site toxicity (percent survival) with and without amendments. Solid, open and striped bars indicate mean percent survival in site sediment that was un-amended, those characterized for ammonia (amended with zeolite) and those characterized for non-polar organics (amended in whole sediment and porewater with powder coconut charcoal (PCC) and solid phase extraction, respectively), respectively. Each bar represents eight replicates (±standard deviation). Stars indicate significant differences ($p < 0.05$) between un-amended 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.

Table 1
Observed (observed mortality/50 * dilution factor) and predicted (contaminant concentration (undiluted)/published LC50 contaminant concentration) toxic units (TUs) for whole sediment and porewater 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 porewater TIE testing were the mean concentrations of three separate replicates being taken every 2 d 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 *Fig.* S1 in the Supplemental material. 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 total organic carbon (oc).

			Phase II: total ammonia				Phase II: ∑PAHs			
			Obs. TUs	Conc. ($mg\,N\,L^{-1}$)	Predicted ammonia TUs	Post manipulation concentration ($mg\,N\,L^{-1}$)	Obs. TUs	Conc. ($\mu g\,g^{-1}$ oc)	Predicted ∑PAHs TUs[a]	Post manipulation concentration ($\mu g\,g^{-1}$ oc)
Whole sediment	*H. azteca* (2 d)[b]	SS315	0.90	37.9 (±4.21)	0.27	15.7 (±5.72)	1.35	641 (±39.6)	0.25	NA
		SS308	1.08	15.9 (±5.91)	0.11	8.58 (±4.54)	1.55	4405 (±629)	1.81	NA
	C. dubia (2 d)	SS315	1.80	40.3 (±0.85)	0.85	16.9 (±10.6)	1.80	641 (±39.6)	0.02	NA
		SS308	1.75	14.8 (±9.86)	0.31	2.07 (±0.23)	1.75	4405 (±629)	0.19	NA
				($mg\,N\,L^{-1}$)		($mg\,N\,L^{-1}$)		($\mu g\,L^{-1}$)		($\mu g\,L^{-1}$)
Porewater	*H. azteca* (2 d)	SS315	3.10	359 (±37.9)	2.56	22.5 (±0.69)	3.10	63.7 (±4.10)	0.03	21.9 (±8.23)
		SS308	3.80	111 (±13.8)	0.79	12.0 (±1.16)	3.80	1953 (±190)	0.52	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)	0.01	21.9 (±8.23)
		SS308	>4.0	111 (±13.8)	1.81	12.0 (±1.16)	>4.0	1953 (±190)	0.08	26.6 (±5.88)

NA = Not available.
[a] TUs for porewater PAHs were based on oc normalized concentrations for SS315 and SS308 (171.14 and 2973 $\mu g\,g^{-1}$ oc, respectively) and 2 d testing duration.
[b] Testing for ammonia and PAHs with whole sediment was conducted in 4 and 10 d tests, respectively.

As suspected concentrations of PAHs in porewater, when OC normalized, were less than those in sediment samples (Table 1).

4. Discussion

4.1. TIE methodology differences

Phase I results of the porewater TIE strongly suggested that ammonia was the principle source of toxicity with the zeolite addition significantly reducing toxicity at both sites for both organisms tested. However, the SPE amendments did not reduce toxicity although PAH concentrations were dramatically reduced. However, Phase I results of the whole sediment TIE for SS315 showed that zeolite and PCC (ammonia and non-polar organic characterization, respectively) significantly reduced toxicity for *H. azteca*, and that neither amendment reduced toxicity in SS308 or in *C. dubia* testing at both sites. For a better understanding of the differences between the two TIE outcomes, ammonia and non-polar organics will be discussed separately in regards to methodology differences between porewater and whole sediment TIEs as well as the Phase II analytical results and the associated TUs.

4.1.1. Ammonia

For *H. azteca*, predicted ammonia TUs during the porewater TIE were approximately 2.6 and 0.80 for SS315 and SS308, respectively, suggesting that ammonia was a source of toxicity for both sediments, which supported the Phase I findings. However, in the whole sediment TIE, ammonia TUs were based on overlying water concentrations, which were up to 10-fold lower than porewater ammonia TUs (0.27 and 0.11 for SS315 and SS308, respectively). Toxicity was significantly reduced in SS315 sediment by zeolite amendment suggesting that ammonia was a source of toxicity for the sediment. Phase II analysis indicated that the ammonia concentration in the overlying water column was reduced with the addition of zeolite by approximately 22.2 mg N L^{-1} or 0.16 TU (see *Figure* S1, Supplemental material), which was close to the observed toxicity removed (TUs of approximately 0.27). This was consistent with Phase I results; suggesting ammonia played a role in sediment SS315 toxicity. Alternatively, sediment SS308 that had considerably lower ammonia concentration (3.2-fold) was most likely not acutely impacted by ammonia, which contradicts the porewater TIE findings.

In the porewater TIE, organisms were exposed to the porewater directly, in which concentrations of water-soluble contaminants (such as ammonia) were elevated. However, the direct exposure of the organisms to the porewater in sediment testing was negligible. Thus, porewater testing may overestimate ammonia toxicity, especially for epibenthic and pelagic organisms (such as *H. azteca* and *C. dubia*) that do not occupy microhabitats that solely involve porewater (Chapman, 2002; US EPA, 2007). Choosing test organisms that better represent exposure scenarios may alleviate this bias. It should be noted, however, that whole sediment TIEs might lack some environmental realism in that the amount of overlying water used in whole sediment testing does not represent the large volume of water present in field conditions, and thus may misrepresent toxicity of water-soluble contaminants such as ammonia.

4.1.2. Non-polar organics

In the whole sediment Phase I analysis, the non-polar organic amendment (PCC) characterized toxicity for only one of four Phase I trials (i.e., SS315 with *H. azteca*). Phase II analysis of the whole sediment TIE, however, demonstrated that PAH concentrations in the

porewater of SS308 sediment were elevated and PAH concentrations in sediment and corresponding TUs were high enough to cause toxicity (Table 1). Additionally, TU estimates in the present study were only for the 16-priority pollutant PAHs (Table 1), thus TU estimates may underestimate toxicity because substituted PAHs were not accounted. Hawthorne et al. (2007) reported that alkyl-substituted PAHs caused up to 81% of the predicted toxicity noted in one of their study sites. Alternatively, use of equilibrium partitioning sediment benchmarks (ESBs) to assess toxicity for those 16 PAHs yielded TU values of 0.90 and 6.44 for SS315 and SS308, respectively. Use of ESBs would provide a higher protective estimate for assessing risk, and further strengthens the conclusion that PAHs are a source of toxicity in whole sediment testing. Issues with PCC successfully reducing toxicity in sediments contaminated with PAHs have been previously documented (US EPA, 2007; Mehler et al., in press), and may account for the low characterization of non-polar organics as the source of toxicity in Phase I testing. The unresolved complex mixture (UCM), which is the oil and grease matrix associated with PAH contamination, may confound toxicity characterization in whole sediment Phase I testing by causing toxicity, affecting PAH bioavailability, and changing the ability of PCC to bind to non-polar organics (Mehler et al., in press). The inconsistency in Phase I and II results in the whole sediment TIE indicate further investigation is still needed to fully understand the contribution of non-polar organics, such as PAHs, to the observed toxicity. The high PAH concentrations in combination with our previously published whole sediment TIE (Mehler et al., in press) suggest that PAHs are a source of sediment toxicity at these sites.

The discordance characterizing non-polar organic toxicity between whole sediment and porewater TIEs could be due to two factors. First, if PAHs were at high enough concentrations to induce toxicity, then the SPE manipulation (which reduced PAH concentrations dramatically, Table 1) should have reduced toxicity. This was not the case in the porewater TIE. One possible explanation was that high ammonia toxicity in porewater TIE testing masked the increase in survival with the SPE treatment. To determine if this was the case, SS308 sediment was reprocessed using the same Phase I porewater TIE procedures (un-amended, SPE-amended, and zeolite-amended) with an additional treatment using SPE and zeolite amendments simultaneously for both contaminant classes. Results indicated no difference in toxicity reduction between the zeolite and SPE treatment compared to the zeolite treatment alone. This suggests that SPE manipulation did not significantly reduce toxicity of the SS308 porewater.

Another reason for the discordance could be the characteristics of the non-polar organic contaminants such as low water solubility and high hydrophobicity. Strong absorbents, such as black carbon, may reduce the amount of non-polar organics partitioning to porewater, especially planar PAHs. The UCM, as discussed earlier, may also interfere with the porewater TIE. The UCM in sediment SS308 was examined before and after centrifugation to extract porewater, and no significant difference in UCM contents in sediment was observed, suggesting minimal UCM in the porewater. If UCM played a role in the toxicity of strongly bound non-polar organics, then porewater testing may grossly underestimate non-polar organic toxicity. Secondly, binding of the non-polar contaminants in the porewater to the glassware and dissolved organic carbon (DOC) may cause significant variation between whole sediment and porewater TIEs. Hawthorne et al. (2005) reported that up to 96% of the higher molecular weight compounds (with those being the more toxic PAHs) may bind to DOC in porewater, and thus were not bioavailable. While OC was analyzed in the porewater and provides more realistic TUs than using freely dissolved concentrations, use of sediment K_{oc} values for porewater OC samples has its limitations (Brannon et al., 1995). Additionally, TUs for PAHs in the present study were estimated by using predictive narcosis models (Di Toro and McGrath, 2000; Di Toro et al., 2000) using similar test organisms, since published LC50 values are not widely available. Therefore, TUs only provided limited estimates of toxicity. In addition, Phase II chemical quantification can be difficult to accomplish even at concentrations that would cause toxicity. For example, the amount of porewater 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 porewater. By using only 25 mL, reporting limits (PAHs: 0.4 $\mu g\ L^{-1}$) were high and may mask potential toxicity of highly toxic contaminants such as pyrethroids and certain PAHs (e.g., dibenz[ah]anthracene LC50: 0.28 $\mu g\ L^{-1}$ – Hawthorne et al., 2005). Lastly, the porewater testing could not account for the exposure route of sediment ingestion, which may be significant for some hydrophobic contaminants (Lydy and Landrum, 1993; Leppanen and Kukkonen, 1998; Morrison et al., 1996.) In summary, porewater TIEs may underestimate toxicity caused by non-polar organics.

4.2. Evaluating the TIE methods

Both TIE techniques provide valuable information in characterizing the risk associated with various contamination sources, with each technique having strengths and limitations (Table 2). Porewater testing generally is more efficient in terms of cost and time requirements when compared to whole sediment testing. A standard porewater test is typically conducted over a 2 d period in disposable containers, while whole sediment tests are usually 10 d in duration with substantially more space requirements. Control recoveries of small organisms (e.g., *C. dubia*) and sediment avoidance issues (e.g., *H. azteca*) are both factors that increase variability and are confounding factors in this study as well as other whole sediment TIE results (Winger et al., 2001).

Additionally, porewater TIEs are more sensitive in assessing toxicity than whole sediment assays. For example, in the present study, *H. azteca* toxicity was three times greater for the porewater TIE testing, despite test duration five times shorter than whole sediment testing (Table 1). While some authors would argue that the degree of toxicity in porewater testing is in many cases not environmentally relevant (Adams et al., 2003; Chapman, 2002; Ho, 2002), porewater TIE could provide a valuable assessment tools in determining toxicity in "worse" case scenarios or potentially characterizing sites in which sub-lethal effects would be observed in a quick and cost-effective manner (US EPA, 2007).

With confounding factors present in both whole sediment and porewater TIEs; the best option would be to conduct both TIE procedures (US EPA, 2007). The US EPA (2007) suggests that conducting initial toxicity testing with both matrices to identify potentially toxic sites provides a larger scope to evaluate toxicity and to identify which TIE procedure would provide the best information for protection of biota at the site. Arguably, using both matrices through the entire TIE procedure provides more evidence and may facilitate more accurate decisions by risk assessors.

4.3. Organism sensitivity and susceptibility

The difference in methodologies played a large role in the TIE outcome. However, selection of the test organism must also be considered. The consequence of test organism choice in characterizing sources of toxicity in TIEs has not been well documented. Two test organisms (*H. azteca* and *C. dubia*) were compared in the present study and these organisms vary based on taxonomic group, physiology, ecological niche, and functional feeding group. *H. azteca* are benthic amphipods and feed by shredding plant and animal material, while *C. dubia* are pelagic filter feeders (Smith, 2001). Because *H. azteca* are exposed to the sediment more directly, this species may be more susceptible to hydrophobic organics than *C.*

Table 2
Strengths (+), limitations (−), and factors that are neutral (neither a strength nor limitation) (±) for whole sediment and porewater TIEs. A brief description for the rationale for strength and limitation is provided.

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

dubia. The role that these differences play in the sensitivity between the two organisms is unknown. Additionally, variation in size and age of the two species may influence the sensitivity of the test organism. The critical body residues that are needed to cause a certain level of toxicity may be the same for each organism. The time needed for the two organisms to reach critical body residues and cause toxicity, however, maybe different (Rand et al., 1995).

Studies have shown that *H. azteca* are less sensitive than *C. dubia* to many contaminants, such as ammonia with total ammonia LC50s approximately 140 and 47.3 mg N L^{-1}, respectively in water-only tests for *H. azteca* (Ankley et al., 1995) and *C. dubia* (Bailey et al., 2001). The present study demonstrated the variation in sensitivity between the two organisms with SS315 sediment predicted ammonia TU values for *H. azteca* 2.3-fold lower than *C. dubia* (0.27 and 0.61, respectively) (Table 1). Alternatively, studies have shown that *H. azteca* were more sensitive to fluoranthene, a PAH, than the cladoceran *D. magna*, with 10 d water-only LC50 values of 30.3 and 102.6 μg L^{-1}, respectively (Suedel and Rodgers, 1996). The differences in sensitivity between the two species depending on contaminant class is one that warrants further attention, especially in areas where mixtures of the contaminants occur, as is the case with the sites SS315 and SS308.

In the present study, test organism choice may not have played a primary role in variation of results between the whole sediment and porewater TIE studies. However, some IRC sediments with lower ammonia concentrations reported by Sparks and Ross (1992); Burton (1995), and Mehler et al. (in press) could inherently be toxic to *C. dubia*, but not *H. azteca*. For these reasons, it is imperative that the objectives of a TIE study justify and provide rationale for test organism selection. Studies on the benthic community structure would provide insight toward the most site-relevant organisms to select for TIE purposes. However, using native organisms that are found at the sites may be difficult, as organisms that would be currently found in these areas would represent the organisms that thrive in contaminated sediments. It should be noted however that the test organisms used in the present TIE study are organisms that are commonly used in TIE studies. Thus, the test organisms chosen for this study allow for comparisons to previous TIE research.

5. Conclusions

The present study confirmed that porewater and whole sediment TIE methodologies might characterize the source of toxicity differently. Furthermore, the use of different test organisms may also yield different conclusions among TIE methods. Evaluating and understanding the variation in outcomes between the two types of TIE methodologies is important for determining situations in which each of the TIE techniques would be most useful for identifying contaminant risk to aquatic organisms. With TIEs becoming a common procedure in risk assessment, understanding the variability associated with each method is critical. Each TIE methodology evaluates sediment toxicity at a site differently, with both having their own strengths and weaknesses. Therefore, conducting both whole sediment and porewater TIE procedures may assist in providing a stronger weight of evidence than a single TIE method.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.chemosphere.2009.11.052.

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