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Identifying Organic Toxicants in Sediment Using Effect-Directed Analysis: A Combination of Bioaccessibility-Based Extraction and High-Throughput Midge Toxicity Testing

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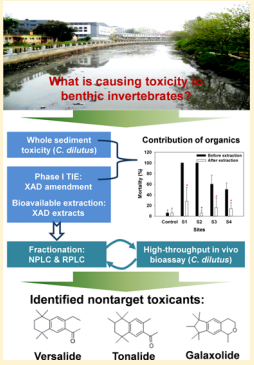
Supporting Information

ABSTRACT: Toxicity identification evaluation (TIE) and effect-directed analysis (EDA) were integrated to diagnose toxicity drivers in a complex system, such as sediment. In TIE manipulation, XAD resin was utilized as an amending agent for characterizing organic toxicants, which also facilitate a large-volume bioaccessibility-based extraction for EDA purposes. Both raw sediments in TIE and extract fractions in EDA were tested with *Chironomus dilutus* for toxicity using whole-sediment testing and a high-throughput microplate assay. This allowed for a direct link between whole-sediment TIE and EDA, which strongly strengthened the characterization and identification of toxicants. Sediments amended with XAD resin, as part of the TIE, significantly reduced midge mortality compared with unamended sediments, suggesting that organics were one class of main toxicants. On the basis of bioaccessible concentrations in sediment measured by XAD extraction, a group of previously unidentified contaminants, synthetic polycyclic musks (versalide, tonalide, and galaxolide), were found to explain 32−73% of the observed toxicity in test sediments. Meanwhile, three pyrethroids contributed to an additional 17−35% of toxicity. Surprisingly, the toxicity contribution of musks and pyrethroids reached 58−442 and 56−1625%, respectively, based on total sediment concentrations measured by exhaustive extraction. This suggested that total sediment concentrations significantly overestimated toxicity and that bioavailability should be considered in toxicity identification. Identifying nontarget toxicants sheds a light on application of the integrated TIE and EDA method in defining causality in a complex environment.



INTRODUCTION

While sediment provides important ecological functions and services for aquatic species, it is also a major sink for hydrophobic contaminants, potentially jeopardizing sediment-dwelling organisms. Urban waterways are one area with serious risk, which are subjected to a complex mixture of chemicals in many cases.[1,2] Before abating sediment-related risk, it is necessary to unravel causative agents in the complex system. A major challenge is that traditional assessment methods, which are mainly based on target chemical analysis, are unable to prioritize key drivers for ecological risk in a complex system.[3−5] For this reason, toxicity identification evaluation (TIE) and effect-directed analysis (EDA) have been proposed.[6−8]

Though TIE and EDA procedures are based upon the same principle of tracing key toxicants by sequentially reducing the complexity of environmental mixtures, they use different approaches to achieve this goal, with each having strengths and limitations.[9,10] Whole-sediment TIE incorporates the bioavailability of contaminants, as it generally utilizes in vivo sediment bioassays, but it is limited in its ability to pinpoint toxicants that are not commonly monitored or are of unknown identity.[9−11] Alternatively, EDA has the ability to identify nontarget organic toxicants in complex mixtures by using sophisticated fractionation and instrumental analysis techniques.[3,7,9] Traditional EDAs, however, may result in the introduction of errors in identifying key toxicants in sediment, because the bioavailability of contaminants was ignored.[12−14] Integrating TIE and EDA methods has been recommended to more accurately identify toxicants in sediment.[9,10]

To integrate TIE and EDA techniques, appropriate endpoints that complement one another are critical. While TIEs typically utilize in vivo endpoints such as mortality, growth, and reproduction of whole organisms,[6,9−11] most EDAs rely on in vitro endpoints using cell-based bioassays.[15−18] In vitro bioassays have many positive attributes, including high throughput, specificity, and sensitivity, yet they lack environmental relevance, as they generally do not consider

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bioavailability or are unable to account for toxicokinetic processes of pollutants. Furthermore, it is not practical to run conventional whole-organism bioassays for a large number of fractionated samples as is required by EDA.[7,9] Recently, in vivo toxicity testing has been successfully used in EDA, although it is still limited by its throughput.[19−21] It is imperative to develop high-throughput in vivo bioassay methods, which would promote the integration of TIE and EDA approaches.

Various biomimetic extraction methods have been developed to estimate the bioavailability of sediment-bound contaminants,[22,23] but most of these approaches only provide a limited amount of extracts, which is not enough for toxicity testing. Schwab et al.[24,25] amplified Tenax extraction (from 0.5 to 125 g of Tenax beads) and validated the application of large-volume Tenax extraction in EDA by retrieving large amounts of bioaccessible contaminants from sediment. Tenax extraction was also applied in identifying AhR-active contaminants in sediment contaminated by an oil spill.[26] Application of this method, however, was restricted by the high cost of Tenax beads. Additional polymeric materials, such as XAD resin, which are much less expensive compared with Tenax, have been used to sequestrate organic toxicants during whole-sediment TIEs as a replacement of commonly used charcoal.[27] Therefore, large-volume bioaccessibility-based extraction with appropriate polymers may serve as a link between TIE and EDA procedures, which can also take bioavailability into consideration.

The objective of the present study was to develop an integrated TIE and EDA method to more holistically diagnose major toxicants in sediment containing a complex mixture of organic contaminants. Large-volume XAD extraction was applied to characterize the toxicity contribution of organic contaminants in TIE as well as to gain sufficient bioaccessible contaminants for fractionation and bioassay in EDA. A high-throughput in vivo bioassay method with *Chironomus dilutus* as a model species was developed and used in EDA to match whole-sediment TIE, which also used midges as the testing species. Urban waterway sediment in Guangzhou, China has been reported as seriously contaminated with a mixture of organics and heavy metals based on the results of chemical analysis and bioassays with benthic invertebrates.[28−32] Nevertheless, the pollutants regarded as toxicants of concern only explained a small portion of effects observed in bioassay.[19,28] On the basis of the previous findings, sediments from Guangzhou were chosen as an example to evaluate the application of the integrated TIE and EDA method in uncovering additional toxicants, i.e., those not commonly monitored or those with unknown identity.

MATERIALS AND METHODS

Experimental Design. Large-volume XAD extraction and high-throughput midge toxicity testing methods were developed to link TIE and EDA techniques to identify major toxicants in sediment contaminated by a complex mixture of organics. Stepwise procedures of the newly developed method are shown in Figure S1. In short, sediment samples were first characterized using a phase I TIE for organic toxicants with XAD resin as the amending agent and third instar *C. dilutus* as the test organism. After the amending process, the XAD resin was collected and extracted, and the extract was cleaned using gel permeation chromatography (GPC) and fractionated using normal phase liquid chromatography (NPLC). The toxicity of GPC-cleaned XAD extracts and individual NPLC fractions was tested using the newly developed high-throughput midge bioassay method. The fractions showing significant mortality were further fractionated using reverse phase liquid chromatography (RPLC), and individual fractions were again tested with the midge bioassay. Possible toxicants in the toxic fractions were finally identified using GC−MS and GC−MS/MS, and toxicity contribution from the suspected toxicants was confirmed by constructing a dose−response relationship between midge mortality and chemical concentrations. Detailed information for each step of the experiment is presented below.

Sediment Collection. Four site sediments in Guangzhou urban waterways were sampled (Figure S2). Previous work reported that these sediments showed a high complexity of chemicals and unexplained toxicity.[28−32] Meanwhile, a control sediment was collected in a drinking water reservoir near Guangzhou, and this sediment showed no chronic toxicity to the midges.[33] More details on sediment sampling are presented in the SI.

Organism Culture. The benthic invertebrate, *C. dilutus*, was selected as the test organism in all bioassays, as it is a common test species for sediment toxicity and also as it has been used in previous TIE testing for samples collected in the study area.[28,30−32] Midges were cultured at Jinan University, Guangzhou, China according to the protocol by the United States Environmental Protection Agency.[34]

XAD Amendment and Extraction Confirmation. Large-volume XAD extraction played a critical role in linking TIE (as amending agent) and EDA (as extraction technique for fractionation) tests in the current study. Amberlite XAD-2 and XAD-4 resins (20−60 mesh) were obtained from Supelco (Bellefonte, PA, USA) with water contents of 40% and 55%, respectively. Prior to use, XAD resin was rinsed for three cycles by sonication with a mixture of hexane and acetone (1:1, v:v) and then rinsed with acetone once and deionized water three times. After washing, XAD resin was dried at 70 °C for 4 h. More information regarding chemicals and reagents can be found in the SI.

The efficiency of XAD extraction for organic compounds in water was evaluated using 17 compounds (100 ng/L) with a variety of chemical structures and a wide range of polarities (log K_{ow} ranging from 2.36 to 8.18), including three polychlorinated biphenyls (PCBs), three polybrominated diphenyl ethers (PBDEs), two polycyclic aromatic hydrocarbons (PAHs), and nine pesticides (Table S1). The extraction was conducted in three replicates using the procedures described below, and the recoveries of all test compounds ranged from 82% to 145%, indicating that the extraction method was valid.

Whole-Sediment Toxicity Identification Evaluation. Sediments (both contaminated and control sediments) for TIE characterization and subsequent EDA evaluation were amended in a 1-L conical flask containing 500 g of wet sediment, 500 mL of reconstituted water, and 20 g of XAD-2 and XAD-4 resins (1:1, w:w). This mixture was continuously mixed for 24 h at 23 °C in darkness using a magnetic stirrer at a rate of 800 rpm. At the end of extraction, the XAD resin was separated from the sediment slurry after 30 min of centrifugation at 4000 g. After the resin was removed, the amended sediment was settled for 24 h, and the overlying water was decanted before toxicity testing. Yi et al.[32] previously reported that these site sediments were highly toxic to the midges; therefore, a 2 day testing period instead of the standard 10 day

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testing period was performed following the US EPA protocol.[34] More details are presented in the SI.

Cleanup and Fractionation of XAD Extract. XAD resin recovered from the amended sediments in whole-sediment TIE testing were rinsed with deionized water and then sonicated with 50 mL of acetone once and 50 mL of a mixture of hexane and acetone (1:1, v:v) three times. After filtering and evaporation, the hexane layer was transferred to a clean flask, and the remaining aqueous solution was extracted with 20 mL of dichloromethane three times. The hexane and dichloromethane extracts were combined, concentrated, dried with anhydrous Na_2SO_4, solvent exchanged with 0.5 mL of dichloromethane, and filtered using a 0.22 μm filter before GPC cleanup.

A binary LC system equipped with an ultraviolet detector (Labtech, Beijing, China) was used to purify the extracts with a BioBeads S-X3 preparative column (20 × 300 mm, 38−75 μm, Labtech). Dichloromethane was used as the mobile phase at a flow rate of 5 mL/min. Humic acid and sulfur were used as representative macromolecule and small-molecule interferences, respectively, to determine the time window of fraction collection as detailed in the SI. The fraction between 10.5 and 18.0 min was collected for further testing.

Subsequent fractionations of the GPC-cleaned extracts were accomplished using the same LC system. The extract (0.3 mL in hexane) was first fractionated using NPLC with a cyanopropyl (CN) semipreparative column (10 × 250 mm, 10 μm, Waters, Ireland). A binary gradient of hexane and dichloromethane was used as the mobile phase at a flow rate of 4 mL/min. The elution program was set as 100% of hexane initially, reduced to 40% of hexane over 30 min, and then kept at 40% of hexane for 5 min. The previously mentioned 17 test compounds (5 μg/mL) were separated using this column with a retention time ranging from 4.7 to 33.6 min. No peak width was more than 1 min (Tables S1). As such, 35 fractions were collected at 1 min intervals. The collected fractions were subsequently solvent exchanged with 100 μL of DMSO for bioassays.

The fractions showing significant midge mortality were solvent exchanged to 0.3 mL of a mixture of acetonitrile and water (7:3, v:v) for RPLC fractionation using a C18 semipreparative column (10 × 150 mm, 10 μm, Agela Technologies, Tianjin, China). A mixture of acetonitrile and water was used as the mobile phase at a flow rate of 4 mL/min. The gradient elution program initiated at 70% of acetonitrile, and the percentage of acetonitrile gradually increased to 100% over 25 min and held for 10 min. Similar to the NPLC fraction, the retention time of the 17 test compounds was evaluated using this column, and retention time ranged from 3.3 to 32.2 min, with no peak width being more than 1 min (Tables S1). As a result, a total of 35 fractions at a time interval of 1 min were collected. After collection, water and acetonitrile were separated by adding 0.15 g of NaCl. Aqueous solution was then extracted with 2 mL of acetonitrile three times. All acetonitrile extracts were combined, dried with 0.2 g of Na_2SO_4, and solvent exchanged to 100 μL of DMSO for bioassay. Eventually, the fractions showing significant midge mortality were solvent exchanged to hexane for identifying suspected toxicants on GC−MS.

The 17 test compounds (50 ng) dissolved in dichloromethane were processed in triplicate following the entirety of the GPC purification and NPLC and RPLC fractionations, sequentially. Recoveries of these compounds at the end of RPLC fractionation were 44−71%. More details in developing NPLC and RPLC fractionation methods are provided in the SI.

High-Throughput Midge Toxicity Testing. Toxicity of the GPC-cleaned XAD extracts as well as the NPLC and RPLC fractions obtained from the extracts was tested using high-throughput midge toxicity testing as part of the EDA practice. The bioassays were performed in 12-well microplates with silanized glass inserts (2 cm i.d., 3 cm height), and 10 μL of test solution in DMSO was dosed into 3.99 mL of reconstituted water in each well. To avoid cannibalization, approximately 0.3 cm of clean sand was added as substrate at the bottom of the well. The tests were conducted in six replicates, and five third instar midge larvae were randomly introduced into each well to initiate the testing. The temperature and light/dark regime were set at 23 °C and 16:8, respectively. Neither water change nor feeding was conducted throughout testing. After 72 h of exposure, surviving midges were sieved, and survival was recorded.

Suspect Screening and Toxicant Quantification. Suspects in the toxic fractions were screened on a Shimadzu QP-2010 Plus gas chromatograph−mass spectrometer (GC−MS) based on a NIST05 library and a compound composer software containing a database with semiquantification methods for 942 analytes.[19] In brief, analytes was separated using a HP-5MS column (30 m × 0.25 mm, 0.25 μm, Agilent, USA), and compounds were identified in scan mode with electron impact ionization. Sample (1 μL) was injected in pulsed splitless mode, with helium used as a carrier gas at a flow rate of 1.2 mL/min. Temperatures of the injector, ion source, quadrupole, and transfer line were set at 250, 250, 150, and 260 °C, respectively. The oven temperature was initially set at 60 °C and held for 1 min, increased to 180 °C at a rate of 20 °C/min and held for 1 min, increased to 240 °C at 5 °C/min and held for 3 min, and finally increased to 300 °C at 10 °C/min and held for 6 min.

Suspected toxicants in bulk sediment, GPC-cleaned XAD extract, and laboratory spiked samples were quantified using a Shimadzu TQ8040 GC−MS/MS on electron impact ionization mode. Sediment samples were extracted with accelerated solvent extraction and cleaned with solid phase extraction. Detailed information on sediment sample preparation and toxicant quantification is presented in the SI.

Toxicity Confirmation. As discussed in greater detail in the results and discussion below, musks that were not regularly monitored in the environment were identified as potential toxicants based on the integrated TIE and EDA method. The toxicity of the suspected toxicants was confirmed by establishing dose−response relationships with neat compounds using the high-throughput midge bioassay method as described above.

Data Analysis. Midge mortality in individual test samples was compared with the control using a one-way analysis of variance followed by a Dunnett's test for multiple comparisons (SAS 9.1, SAS Institute Inc., Cary, NC, USA). Significance ($p < 0.05$) indicated significant toxicity of the test sample compared with the control.

Mortality was used as the endpoint in the bioassays, so the LC50 of the test sediment ($LC50_{test\ sediment}$) was calculated to describe sediment toxicity based on EDA bioassays. Relative enrichment factor (REF) is used as a dose metric in constructing a dose−response relationship and obtaining

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$LC50_{test\ sediment}$, which is dimensionless. Detailed calculations are presented in the SI.

The toxicity contribution of each suspect toxicant (i) in sediment is calculated based on bulk sediment toxicity testing and sediment concentrations using the following equations.

$$\text{observed sediment toxicity} = \frac{\text{percent mortality}}{50} \quad (1)$$

$$\text{TU}\ (i) = \frac{C\ (i)}{\text{LC50}\ (i)} \quad (2)$$

$$\text{toxicity contribution}\ (i) = \frac{\text{TU}\ (i)}{\text{observed sediment toxicity}} \quad (3)$$

Where TU is the toxic unit of each suspect toxicant, C is the organic carbon (OC) normalized sediment concentration, and LC50 is the OC normalized medium lethal concentration to *C. dilutus* in sediment toxicity testing. Because of the lack of 2-d sediment LC50 values of musks and pyrethroids, 10-d sediment LC50 values of pyrethroids were directly used in calculating TU, while sediment LC50 values of musks were obtained by extrapolating from their water LC50 values. Detailed information on the LC50 values is presented in Table S2.

RESULTS

Whole-Sediment TIE. All four sediments showed acute lethality to *C. dilutus*, with S1 and S2 sediments causing 100% mortality in the 48-h bioassay (Figure 1). Compared with

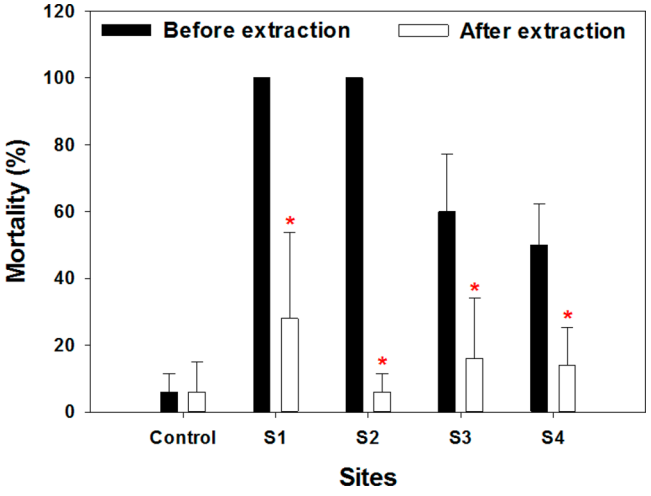

Figure 1. Sediment toxicity to *Chironomus dilutus* before and after sediment extraction with XAD resin. The asterisk indicates significant difference between sediment toxicity before and after XAD extraction ($p < 0.05$).

original sediments, the mortality of *C. dilutus* was significantly reduced after XAD amendments in phase I TIE manipulation for all sediment samples (Figure 1). Results from whole-sediment TIE suggested that organic contaminants were the main class of toxicants in these sediments, and EDA would be beneficial in identifying suspect toxic organic contaminants.

Toxicity of XAD Extracts and Fractionation in EDA. Negative controls of the high-throughput bioassay showed no mortality to *C. dilutus*, and the XAD extracts from the control sediment showed minimal toxicity (<10% mortality). In addition, LC50 values of a standard chemical, imidacloprid, to midges determined by the standard toxicity testing and the high-throughput in vivo toxicity test methods were 3.21 (2.25−4.45) and 2.84 (2.17−3.70) μg/L, respectively, suggesting that this high-throughput in vivo bioassay method was valid. Conversely, GPC-cleaned XAD extracts of the four sediments caused pronounced mortality to *C. dilutus*, with median lethal concentrations (LC50) at 0.025, 0.041, 0.019, and 0.025 of the original sediment for samples S1, S2, S3, and S4, respectively (Figure S3). Significant dose−response relationships between REF values and midge mortality confirmed significant contribution of organic contaminants in the XAD extracts to sediment toxicity measured by whole-sediment TIE.

Results of the first NPLC fractionation and subsequent bioassays indicated that fraction 17 of all four samples caused significant mortality to midges (ranging from 73 ± 25% to 100%), and fraction 28 of S1, S2, and S3 showed mortality from 30 ± 17% to 100% (Figure 2). Mean midge mortality in the other fractions for the four samples was lower than 25%, except for fractions 14, 16, 18, and 34 for sediment S2. Similar patterns of bioactive fractions in the four samples suggested the presence of similar contaminants in these sediments. Considering toxicity significance and sample size for conducting bioassays for the fractions, only fractions 17 and 28 of sample S3 were chosen for further RPLC fractionation. Subsequent fractionation using RPLC with these two fractions of S3 resulted in a single toxic fraction for each NPLC fraction, i.e., S3-17-14 and S3-28-7 (site-NPLC fraction-RPLC fraction) (Figure 3). These two toxic fractions were then screened for potential toxicants on GC−MS.

Identification of Toxicants. The two toxic RPLC fractions (S3-17-14 and S3-28-7) had some peaks present in their respective GC−MS chromatograms, yet no suspect contaminants were identified through screening the Shimadzu compound composer database. Further, full scan chromatograms of the two toxic RPLC fractions were compared with the chromatograms of their respective NPLC toxic fractions S3-17 and S3-28. Chromatograms of the NPLC fraction and its respective RPLC fraction were compared. Peaks that occurred simultaneously in the two chromatograms were regarded as the peaks of suspected toxicants that caused adverse effects in both fractions. Only one peak overlapped in the chromatograms of fractions S3-17 and S3-17-14, and the peaks had similar mass spectra (Figure S4). Compared with standard mass spectra in the library, the peak showed match degrees over 80% for two synthetic polycyclic musks (versalide and tonalide), while similarities of mass spectra for other compounds were all below 1.5%. Galaxolide is another isomer with the same molecular weight (Table S3). Therefore, the three musks were considered as candidate toxicants in these samples. Then, bulk sediment samples and their respective XAD extracts (S1−S4) were analyzed for the three musks on GC−MS/MS with standard compounds. The three musks were detected in all four samples, with sum concentrations ranging from 500 to 4269 ng/g dry wt. in sediment (Table S4). Additional information, such as molecular structures and hydrophobicy for the three compounds are available in Table S3.

Surprisingly, no overlapping peak was found in the GC−MS chromatograms of S3-28-7 and S3-28. Although the reason for this is unclear, it is expected to be a result of the relatively high polarity of the compounds in fraction 28. As such, further toxicity confirmation was focused on the three suspect

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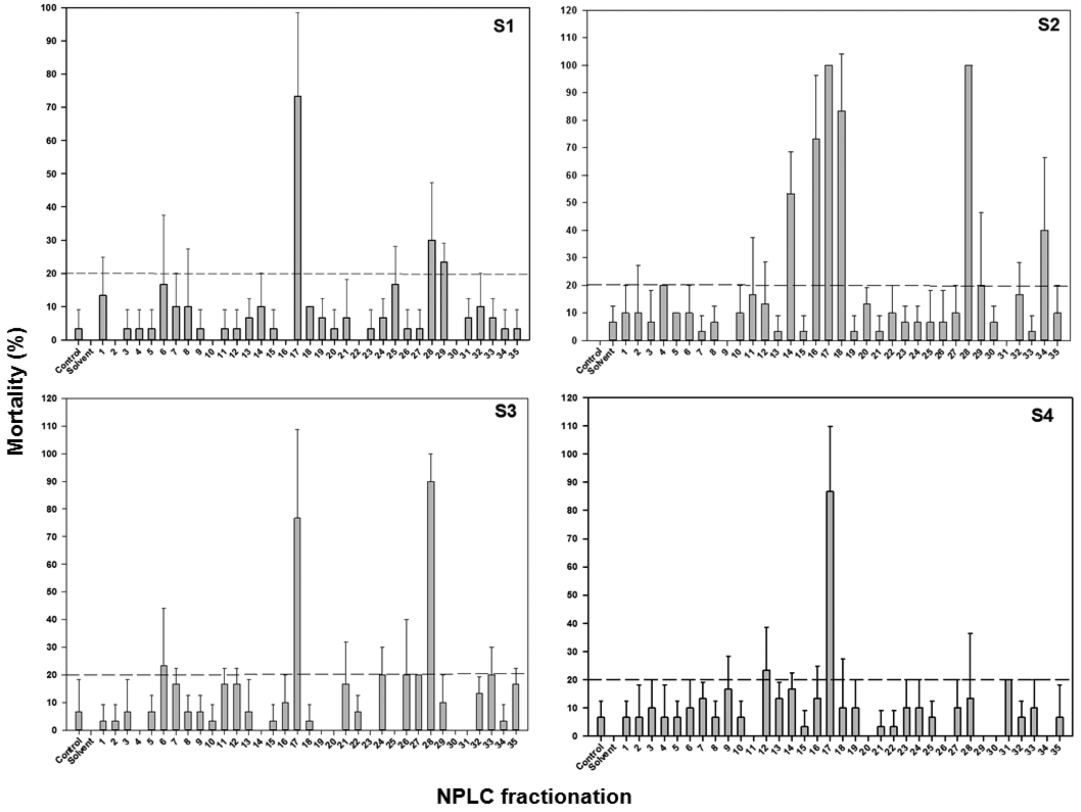

Figure 2. Toxicity of normal phase liquid chromatography (NPLC) fractionated extracts to *Chironomus dilutus*. S1, S2, S3, and S4 indicate the four site samples collected in Guangzhou, China.

toxicants in fraction 17. Toxicity confirmation of the musks in these sediment samples was achieved by establishing their dose−response curves to *C. dilutus* using their respective neat standards. The LC50 values for the musks in 72 h water-only toxicity testing were 6.27, 119, and 275 μg/L for versalide, tonalide, and galaxolide, respectively, suggesting that the musks might cause acute toxicity to midges, especially versalide.

Bioaccessible fractions of the three musks in sediment measured by XAD extraction explained 32%, 36%, 73%, and 63% of the noted mortality to the midges for S1, S2, S3, and S4, respectively (Table 1). Other than the three musks, the bulk sediments and their respective XAD extracts were analyzed for pyrethroids, which were regarded as sediment toxicants in the previous TIE test.[32] Three pyrethroids (cypermethrin, permethrin, and bifenthrin) were detected in the samples, and their toxicity contributions were 21%, 27%, 17%, and 35% to midges for S1, S2, S3, and S4, respectively, based on bioaccessible concentrations in sediments measured by XAD extraction (Table 1). Toxicity contributions based on total concentration in sediments measured by exhaustive ASE extraction were 59%, 58%, 442%, and 121% for musks and 91%, 56%, 1625%, and 422% for pyrethroids (Table 1).

DISCUSSION

Toxicant Identification. Three musks (versalide, tonalide, and galaxolide) were identified as one group of toxicants to midge mortality for sediments from urban waterways in Guangzhou, China. Synthetic polycyclic musks have been intensively used as additives in fragrances and a broad range of household products, such as perfumes, soaps, shampoos, lotions, and cleaning agents.[35−37] Although the production of versalide was discontinued in the 1980s as a result of likely neurotoxicity,[38] this musk remains in various environmental matrices, e.g., treated and untreated urban wastewater[39] and aquatic biota, e.g., mussels, oysters, and clams.[40] Tonalide and galaxolide are still in use currently. Frequent detections of high levels of musks in sediments from Guangzhou (Table S4) supported their ubiquitous existence in the aquatic system. Even with several reports on their occurrence in various environmental matrices and biota, synthetic polycyclic musks have long been ignored regarding their ecological risk. They were just recently regarded as emerging contaminants with potential risk and are not commonly included in risk assessments.[35,37,41,42] As the musks are not in the list of commonly monitored analytes and have limited aquatic toxicity data, they would not be identified as contaminants of concern in any of the past TIE studies conducted in this area.[31,32]

In addition to the musks, pyrethroid insecticides have been found to be the principal organic toxicants to *C. dilutus* in urban waterways of Guangzhou.[28,30−32] However, they were not identified as suspect toxicants in the present EDA practice. The use of water-only bioassays may reduce the toxicity of hydrophobic contaminants due to glassware binding. An alternative exposure method, such as passive dosing, may help to resolve the issue.[19] The toxicity contribution of pyrethroids to midge mortality in these sediment samples was comparable with musks based on bioaccessible concentrations in sediment (Table 1). By integrating TIE and EDA approaches, a more holistic picture of causality can be determined, and a better understanding of the system complexity is gained, which allows more accurate risk mitigation and remediation efforts to be undertaken. Qi et al.[19] suggested oxidative stress induced by sediment-bound

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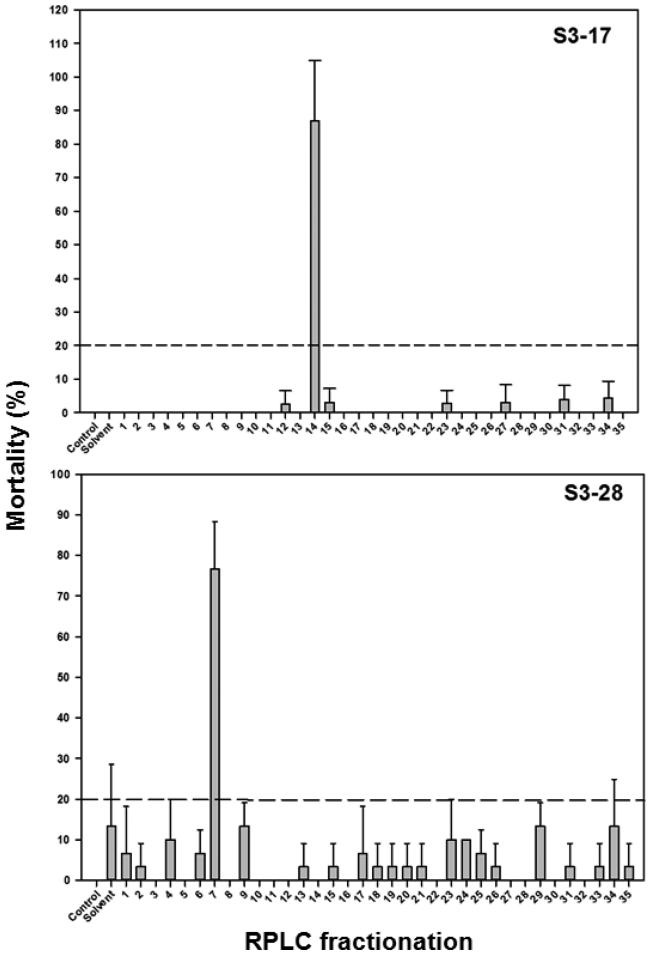

Figure 3. Toxicity of reversed phase liquid chromatography (RPLC) fractionated extracts to *Chironomus dilutus*. S3-17 and S3-28 indicate the normal phase liquid chromatography (NPLC) fractions 17 and 28, respectively, of the S3 sample collected in Guangzhou, China.

pesticides might be one of the main toxic pathways to the midges in the study area. The musks were also considered as neurotoxicants and caused oxidative damage to aquatic organisms at environmental concentrations.[43,44]

Integrating Whole-Sediment TIE and EDA. The XAD resin used in the current study is as effective as coconut charcoal that was used in previous whole-sediment TIE,[32] as both amendments significantly reduced sediment toxicity (Figure 1). The biggest strength of using XAD resin is that the organic contaminants adsorbed by XAD resin could be recovered as the bioaccessible fraction for further EDA analysis, which is not possible for charcoal or other carbon materials. Traditional phase II whole-sediment TIE (i.e., identification) generally focused on analyzing a limited number of target contaminants and defined causality by correlating sediment concentrations of the detected contaminants and their respective toxicity thresholds.[6,7,9,10] Although the whole-sediment toxicity testing considers bioavailability in phase I TIE characterizing contaminant class (traditionally ammonia, heavy metals, and nonpolar organics), phase II TIE often ignores the issue of bioavailability when identifying the contaminant(s) within that class.[9,10]

Recent studies started to address these Phase II TIE limitations by utilizing resin-assisted extraction and evaluating the toxicity of the extracts from these resins, e.g., XAD[27,45] and Tenax.[32] Unfortunately, direct linking the bioaccessible fraction of a specific contaminant to the toxicity is still challenging due to the presence of multiple stressors, particularly some unknown chemicals.[13,29] With the help of EDA, the toxicity of chemicals not routinely monitored or with unknown identity, e.g., musks in sediments from the Guangzhou urban area, could be unraveled.

Moving Forward with Bioaccessibility-Based and High-Throughput EDA Approaches. To date, a majority of EDA studies have relied on exhaustive extractions.[3,10,12] Exhaustive extractions may result in a high bias toward highly hydrophobic contaminants, which may actually not be bioavailable. Exhaustive extraction significantly overestimated the toxicity of the suspected toxicants in sediments from Guangzhou, especially for pyrethroids in S3 and S4, which had predicted high toxicity contributions up to 1625% and 422%, respectively (Table 1). Although toxicity contributions were overestimated by using 10-d sediment LC50 values instead of 2-d values in calculating TUs (Table S2), a 2 orders of magnitude difference between bioaccessible concentration-based TU and total concentration-based TU of pyrethroids in S3 indicated significant overestimation (Table 1). Similar overestimation of pyrethroid toxicity has been previously found in bulk sediment toxicity evaluation in the study area.[29] Bioavailable and bioaccessible fractions of pyrethroids measured by solid phase microextraction and Tenax extraction, respectively, improved correlations between toxic units of pyrethroid (i.e., cypermethrin) and mortality of test organisms.[29] XAD extraction, which is cost-effective and owns the same advantages as Tenax extraction, has been successfully applied in sediment toxicity identification in the present study and moved forward with bioaccessibility-based EDA.

The high-throughput in vivo bioassay is low cost and labor efficient and owns the advantage in assessing toxicity using the

Table 1. Observed Sediment Toxicity of the Four Test Sediments, Toxic Units (TU) of Three Musks and Three Pyrethroids, and Toxicity Contribution of the Musks and Pyrethroids in Sediment to *Chironumus dilutus*

parameters		S1	S2	S3	S4
observed sediment toxicity		2.0	2.0	1.2	1.0
based on bioaccessible concentration in sediment measured by XAD extraction	TU (musks)	0.64	0.71	0.87	0.63
	TU (pyrethroids)	0.42	0.55	0.21	0.35
	toxicity contribution (musks)	32%	36%	73%	63%
	toxicity contribution (pyrethroids)	21%	27%	17%	35%
based on total concentration in sediment measured by accelerated solvent extraction	TU (musks)	1.18	1.15	5.30	1.21
	TU (pyrethroids)	1.82	1.12	19.51	4.22
	toxicity contribution (musks)	59%	58%	442%	121%
	toxicity contribution (pyrethroids)	91%	56%	1625%	422%

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same organism (the midge) in whole-sediment TIE and EDA. To date, most TIE studies utilized an in vivo bioassay, yet EDA practices utilized an in vitro bioassay. Making direct links between in vivo and in vitro toxicity is challenging and requires interpretation for species extrapolation.[3,9,10] Simultaneous use of in vivo and in vitro bioassays in EDA would provide complementary evidence in defining causality and allow mechanistic integration for toxicity in TIE.[28−32]

Overall, an integrated TIE and EDA approach combining bioaccessibility-based XAD extraction and high-throughput in vivo midge bioassay was developed. The method identified nontarget contaminants, i.e., three musks (versalide, tonalide, and galaxolide) as one group of main toxicants to *C. dilutus* in sediments from Guangzhou, China. Other than musks, previously identified pyrethroids also contributed obvious toxicity to the midges. Significant toxicity overestimation of the suspect toxicants calls for considering bioavailability in sediment toxicity identification.

More studies are required not only to incorporate the integrated TIE and EDA methods into budget-constrained risk assessments but also, perhaps more importantly, to provide information on nontarget toxicants that are not commonly evaluated. This was the case in the present study that synthetic musks, a group of extensively used chemicals but not commonly considered as toxicants of concern, were identified as potential toxicants. These identified chemicals could be added in the list of routinely monitored chemicals in future sediment risk assessment.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.8b05633.

Chemicals and reagents; sediment sampling; whole-sediment toxicity testing; GPC cleanup; sediment extraction and cleanup; instrumental analysis; data analysis; tables and figures as described in the text (PDF)

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Notes

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