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IDENTIFYING THE CAUSES OF SEDIMENT-ASSOCIATED TOXICITY IN URBAN WATERWAYS IN SOUTH CHINA: INCORPORATING BIOAVAILABILLITY-BASED MEASUREMENTS INTO WHOLE-SEDIMENT TOXICITY IDENTIFICATION EVALUATION

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Abstract: Sediments in urban waterways of Guangzhou, China, were contaminated by a variety of chemicals and showed prevalent toxicity to benthic organisms. A combination of whole-sediment toxicity identification evaluation (TIE) and bioavailability-based extraction was used to identify the causes of sediment toxicity. Of the 6 sediment samples collected, 4 caused 100% mortality to *Chironomus dilutus* in 10-d bioassays, and the potential toxicants were assessed using TIE in these sediments after dilution. The results of phase I characterization showed that organic contaminants were the principal contributors to the mortality of the midges in 2 sediments and that metals and organics jointly caused the mortality in the other 2 sediments. Ammonia played no role in the mortality for any samples. Conventional toxic unit analysis in phase II testing identified Cr, Cu, Ni, Pb, and Zn as the toxic metals, with cypermethrin, lambda-cyhalothrin, deltamethrin, and fipronils being the toxic organics. To improve the accuracy of identifying the toxicants, 4-step sequential extraction and Tenax extraction were conducted to analyze the bioavailability of the metals and organics, respectively. Bioavailable toxic unit analysis narrowed the list of toxic contributors, and the putative toxicants included 3 metals (Zn, Ni, and Pb) and 3 pesticides (cypermethrin, lambda-cyhalothrin, and fipronils). Metals contributed to the mortality in all sediments, but sediment dilution reduced the toxicity and confounded the characterization of toxicity contribution from metals in 2 sediments in phase I. Incorporating bioavailability-based measurements into whole-sediment TIE improved the accuracy of identifying the causative toxicants in urban waterways where multiple stressors occurred and contributed to sediment toxicity jointly. *Environ Toxicol Chem* 2015;34:1744–1750. © 2015 SETAC

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INTRODUCTION

China has experienced rapid economic growth at an unprecedented pace in the past few decades. As a result of industrialization and urbanization, severe degradation of aquatic ecosystems has occurred. As a major sink of a vast number of chemicals, contaminated sediment became a great concern, particularly in urban waterways in China, as in many developing countries [1–3]. For example, more than 70% of urban sediment samples in the Pearl River Delta of South China were acutely toxic to *Chironomus dilutus* [3]. Sediments collected from an urban stream in Guangzhou, the largest city in the Pearl River Delta, showed tremendous toxicity to benthic invertebrates, with 94% and 81% samples being acutely toxic to *C. dilutus* and *Hyalella azteca*, respectively [4,5].

To set regulatory initiatives and remediation schemes, it is imperative to identify the causative toxicants. The complexity of sediment, however, made identifying the sources of toxicity rather challenging. A wide variety of contaminants were detected at high concentrations in the Pearl River Delta sediments, including organochlorine pesticides, polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), polybrominated diphenyl ethers (PBDEs), metals, and ammonia [1,2]. Recently, current-use pesticides, such as organophosphate, pyrethroid, and phenylpyrazole insecticides, were also found in this region and considered as plausible contributors to sediment

toxicity [3–6]. Nevertheless, the roles of other contaminants in toxicity were still unclear. Thus, more research is needed to better elucidate the contributions of various contaminants to sediment toxicity in such a complex system in which multiple stressors occurred and contributed to sediment toxicity jointly.

Toxicity identification and evaluation (TIE) has proven to be a valuable tool to identify the main toxicants in environmental samples by integrating bioassays and physical and chemical manipulations of samples [7]. During the development of TIE procedures, a variety of manipulations were used to characterize ammonia, metals, and organic pollutants; and their effectiveness and weakness were previously reviewed [7,8]. Most sediment TIEs have been conducted in North America and Europe, where the level of contamination was relatively low and sediment toxicity was mainly caused by a single class of contaminants [8]. The capability of using TIE to probe the major causes of sediment toxicity in heavily polluted environments in developing countries, like China, still needs to be validated.

The bioavailability of sediment-bound contaminants should be considered when applying whole-sediment TIE [9–11]. Ignoring the difference in bioavailability when manipulating sediment in phase I of TIE (characterization) may exaggerate the toxicity of polar chemicals [12]. On the other hand, exhaustive extraction, which was conventionally used in phase II of TIE (identification), might produce a bias toward highly toxic but poorly bioavailable compounds, which actually posed limited effects on biota. Alternatively, bioavailability-based extractions represent more realistic exposure scenarios, yet their use in TIE is still limited [9,13].

The objectives of the present study were to 1) use a whole-sediment TIE to identify major toxicants causing sediment

toxicity in urban waterways of Guangzhou, China, and 2) integrate bioavailability-based extraction approaches into TIE testing to improve the accuracy in identifying the causative toxicant.

MATERIALS AND METHODS

Sampling sites and sediment collection

Guangzhou is the largest city in the Pearl River Delta, with a population of 12.9 million in 2013. Previous studies reported that urban waterways in Guangzhou were significantly deteriorated and exhibited prevalent toxicity to *C. dilutus* [4,5]. As shown in Supplemental Data, Figure S1, 6 surface sediments were collected in Tianhe and Panyu Districts of Guangzhou in April 2013. Before urbanization in the 1980s, the 2 districts were mainly farmland, but now they are highly urbanized areas with patches of agricultural, industrial, and residential activities. More details of sampling sites are provided in Supplemental Data, Table S1.

The top 5 cm of sediments were collected using a stainless steel spade by wading into the streams. The sediments were then passed through a 2-mm sieve to remove rocks and debris, stored in 4-L glass jars, and transported to the laboratory at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences. Sediments for toxicity testing and chemical analysis were stored at 4 °C and –20 °C, respectively. A control sediment was collected from a drinking water reservoir near Guangzhou, and the sediment showed no acute toxicity to *C. dilutus* [4,5].

Screening toxicity testing

The benthic invertebrate *C. dilutus* was used in the toxicity tests because Chironomidae are common species in the study area and the recommended organism for sediment TIE by the US Environmental Protection Agency (USEPA) [7]. The midges were cultured at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, following the protocols of the USEPA [14]. Ten-day sediment toxicity tests were performed with mortality as the endpoint, and the experimental design of the TIE is depicted in Supplemental Data, Figure S2.

As test sediment samples caused strong lethality to the midges, the sediments were tested in a dilution series to determine appropriate concentrations for TIE. The test sediments were diluted with control sediment on a wet weight basis (6.25–100% of the original sediment being used), and median lethal concentrations (LC50) of the test sediments were estimated. After an appropriate amount of control sediment was added, the diluted sediments were thoroughly mixed for 3 h before toxicity testing.

For acute toxicity testing, 60 g of wet sediment was distributed into a 400-mL beaker, and 300 mL of reconstituted water was added as overlying water. After settling for 24 h, 10 third-instar midge larvae were randomly introduced into each beaker. Overlying water (~150 mL) was renewed twice per day using an automatic water exchange system. The tests were performed in triplicate at 23 ± 1 °C with a 16:8-h light:dark photocycle, and the organisms were fed daily. Temperature, conductivity, pH, and dissolved oxygen in overlying water were monitored every day; and ammonia concentrations were measured at the beginning and the end of toxicity tests. At the end of 10-d exposure, the mortality of *C. dilutus* was numerated by sieving the organisms from the sediment with a 500-μm sieve.

TIE phase I: Characterization

Because the mortality of *C. dilutus* in sediments S5 and S6 was not significantly different from that in the control, the 2 sediments were excluded from TIE. Conversely, the other 4 sediments showed 100% mortality to the midges. According to the results of screening toxicity testing, the 4 sediments were diluted to suitable concentrations (12.5% of its original concentration for S1 and 50% for S2, S3, and S4) prior to TIE.

In phase I testing, 3 additives—zeolite (Foshan pet product factory), D401 cation resin (Tianjin Guangfu Fine Chemical Research Institute), and coconut charcoal (Guangzhou Jietan Trade)—were employed to differentiate the toxicity caused by ammonia, cationic metals, and nonpolar organics, respectively. The selection of the additives was according to previous research [7], and the procedures to prepare the additives are detailed in the Supplemental Data. Each sediment sample underwent the manipulations of mixing with 10% of zeolite, cation resin, or coconut charcoal. An unmanipulated treatment was also included, and 10% sand was added to compensate for physical dilution by the additives. Meanwhile, the control sediment was manipulated as the test sediments and included as the treated and untreated controls. All manipulated sediment samples were thoroughly mixed and equilibrated for 24 h before adding the midges.

For sediments treated with zeolites, 4-d static toxicity tests with 10 *C. dilutus* larvae were conducted in 4 replicates, as suggested by Besser et al. [15]. To avoid ammonia loss, overlying water was not renewed throughout the testing. The sediments manipulated with cation resin and coconut charcoal were run for 10-d toxicity tests in 5 replicates. The conditions for the 10-d bioassays in phase I TIE testing were similar to those in the screening toxicity testing discussed above in *Screening toxicity testing*.

TIE phase II: Identification

In addition to toxicity characterization, chemicals of concern were analyzed to identify the contaminants responsible for observed toxicity, including ammonia, metals, and organic pollutants (Supplemental Data, Table S2). To take the bioavailability of contaminants into consideration, bioavailability-based extraction approaches were used for assessing metals and organics in sediment in addition to the exhaustive extraction. The respective analytical methods for ammonia, metals, and organics are presented below.

Ammonia. Concentrations of ammonia in sediment pore-water were analyzed immediately on sediment collection. Wet sediment was thoroughly mixed, and 300 g of sediment was subsampled and centrifuged at 4000 g for 1 h to obtain porewater. After filtration, the total ammonia concentration in porewater was analyzed spectrophotometrically using salicylic acid as a color development reagent following a standard method proposed by the Chinese Environmental Protection Agency [16]. Analytical methods are detailed in the Supplemental Data.

Metals. The total and bioavailable concentrations were analyzed for 6 metals in sediment—Cd, Cr, Cu, Ni, Pb, and Zn—using microwave digestion and Bureau Commune de Reference of the European Commission sequential extraction, respectively. The Bureau Commune de Reference procedure speciated metals into 4 chemical mineralogical forms, which stood for various metal mobility and bioavailability—namely, acid-soluble, reducible, oxidizable, and residual fractions [17]. Metal concentrations were quantified on an inductively coupled

plasma–mass spectrometer with external calibration. More details on sample preparation and metal quantification are presented in the Supplemental Data.

Organics. A suite of organic contaminants that were previously found in the study area were analyzed, including 20 organochlorine pesticides, 7 organophosphates, 9 pyrethroids, fipronil and its degradation products, 15 PAHs, 24 PCBs, and 8 PBDEs. Ultrasound-assisted microwave extraction was used to analyze the total concentrations of contaminants in sediment [18]. In addition, a single-point 24-h Tenax extraction was used to measure bioavailable concentrations of sediment-bound organics [5]. Extracts were purified by solid-phase extraction cartridges or self-packed columns, and analytes were quantified by gas chromatography-mass spectrometry with internal calibration. More details on sample preparation and chemical analysis are shown in the Supplemental Data.

The contents of organic carbon in sediment were also analyzed. Sediment samples were freeze-dried, ground, and homogenized; and 1.5 g of sediment was soaked in 1 mol/L HCl solution for 3 h to remove inorganic carbon. The sediment was then washed with distilled water until neutral. Sediment organic carbon was determined with a Vario Elemental Analyzer after the sediment was dried at 60 °C for 12 h.

Data analysis

Sediment toxicity was defined as mortality significantly different from the mortality observed in control sediment. The comparison was conducted using a *t* test ($\alpha = 0.05$) with SAS Ver 9.1. The LC50 of the toxic sediment was calculated in screening toxicity testing by log-probit analysis with SAS and expressed as the percentage of original sediment in diluted sediment. Moreover, the effectiveness of sediment manipulations in phase I of TIE was evaluated by comparing the mortality of the midges in the amended and unamended sediments using a *t* test ($\alpha = 0.05$).

A toxic unit approach was used to evaluate the toxicity contribution of individual contaminants, and 2 types of toxic unit were used in the present study, conventional and bioavailable toxic units (Equation 1 and Equation 2). The conventional toxic unit was calculated by dividing the total concentration of a contaminant (the content obtained by microwave digestion was used for metals and the concentration obtained by exhaustive extraction was used for the organics) by its LC50 value (Equation 1). Bioavailable concentrations estimated by bioavailability-based extraction were used for calculating the bioavailable toxic unit (Equation 2). The bioavailable concentrations were acid-soluble and Tenax-extractable concentrations for metals and organics, respectively. The concentrations of organic contaminants were organic carbon–normalized before toxic unit calculations. Mixture toxicity was estimated as the sum of toxic units (TU) of individual chemicals assuming that the joint toxicity was based on concentration addition.

$$\text{Conventional TU} = \frac{\text{Total concentration of a contaminant}}{\text{LC50 of the contaminant}} \quad (1)$$

$$\text{Bioavailable TU} = \frac{\text{Bioavailable concentration of a contaminant}}{\text{LC50 of the contaminant}} \quad (2)$$

The LC50 value of ammonia was obtained from Besser et al. [15], and LC50 values for pesticides were from several studies [3,4,19]. Not all LC50 values were available for metals,

PAHs, PCBs, and PBDEs, so the probable effect concentrations for freshwater sediment were used for metals and PCBs [20], equilibrium partitioning sediment benchmarks for PAHs [21], and the estimated-no-effect values for aquatic organisms for PBDEs [22].

RESULTS AND DISCUSSION

Screening toxicity testing

The quality of overlying water was monitored during the screening and phase I toxicity tests, and the parameters were all within the requirements of the USEPA [14], with temperature, conductivity, pH, dissolved oxygen, and ammonia of 23.1 ± 0.4 °C, $381 \pm 47\,\mu$S/cm, 6.9 ± 0.2, 5.8 ± 1.1 mg/L, and 0.4 ± 0.3 mg N/L, respectively.

The mortality to the midges by sediments S5 and S6 was not significantly different from the controls ($p > 0.05$), being 22% and 10%, respectively; thus, the 2 sediments were not further included in TIE. In contrast, the other 4 samples caused 100% mortality to *C. dilutus* and LC50 values were 10%, 24%, 28%, and 46% of original sediment for S1, S2, S3, and S4, respectively. As a consequence, these sediments were diluted with control sediment before processing phase I TIE. The most toxic sediment (S1) was diluted to 12.5% of its original concentration, and sediments S2, S3, and S4 were diluted to 50% of their original concentrations.

The high toxicity of the sediments in this area to benthic organisms has also been noted in previous studies [3–5]. Li et al. [4,5] reported that 81% and 38% of sediment samples collected from an urban stream in Guangzhou caused 100% mortality to *C. dilutus* and *H. azteca*, respectively. In the Pearl River Delta, 71% of sediment samples exhibited acute toxicity to *C. dilutus*, with one-third causing 100% mortality [3].

Phase I testing

To elucidate the causality of the observed mortality to the midges, the 4 sediments underwent phase I of toxicity characterization. The 3 materials used to manipulate sediment—zeolite, cation resin, and coconut charcoal—showed no effect on the midges in the controls (Figure 1). The addition of zeolite hardly changed the mortality of the sediment samples, suggesting that ammonia contributed little to the mortality (Figure 1A). As shown in Figure 1B, the lethality of sediments S2 and S3 was significantly reduced with the addition of cation resins, although the toxicity was not altered for samples S1 and S4. The present results suggested that metals might contribute to the mortality of S2 and S3. Finally, the addition of coconut charcoal significantly increased the survival of the midges in all of the sediments (Figure 1B), implying an important role of organic toxicants in the observed mortality in these sediments.

In short, ammonia was not a significant toxicant in the sediment samples. For samples S2 and S3, both metals and organics contributed to midge mortality; and organics were the main toxicants in sediments S1 and S4. The results were supported by previous research in the Pearl River Delta [3] and Guangzhou [4,5], where organic pesticides were found to be plausible contributors to sediment toxicity and the toxic contribution by ammonia was limited.

Similar observations were also reported in other regions [8]. A recent review on whole-sediment TIE [8] showed that ammonia only contributed 7% of sediment toxicity in all samples tested. Conversely, 90% of sediment toxicity was caused by the organics, either singly or in combination with other toxicants. Metals were also important toxicants, and 17%
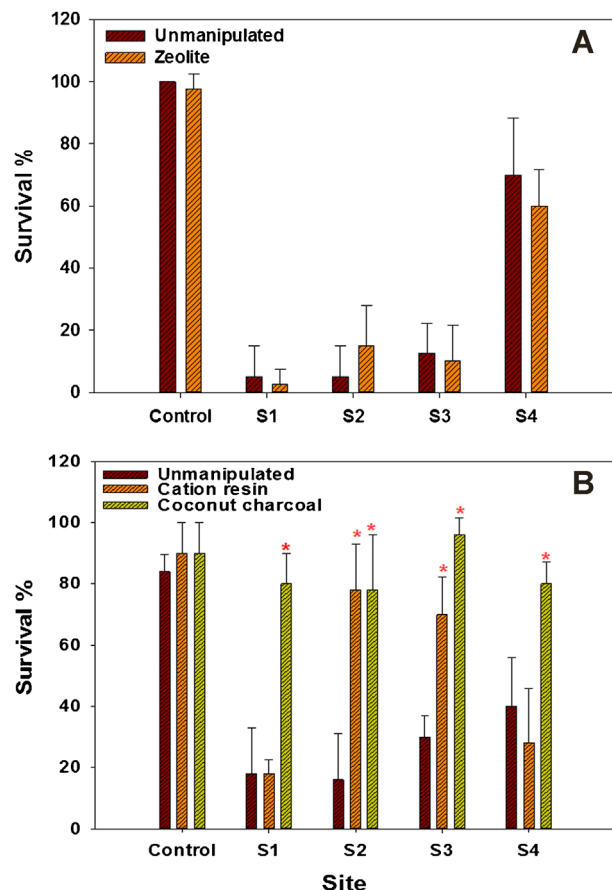
Figure 1. The change in the survival of *Chironomus dilutus* by the addition of (**A**) zeolite or (**B**) cation resin and powdered coconut charcoal to test sediments collected from urban waterways in Guangzhou, China. The control sediment was collected from a drinking water reservoir near Guangzhou. The unmanipulated treatments were mixed with 10% sand. Error bars represent mean survival with standard deviations ($n = 4$ and 5 for [**A**] and [**B**],, respectively). * Significant differences between the treatments and control ($p < 0.05$).

of toxicity of sediments was contributed by metals [8]. Different from previous studies in North America and Europe, in which sediment toxicity was mainly caused by a single class of contaminants [8], half of the sediments from urban waterways in Guangzhou caused lethality to the midges by joint toxicity of organics and metals, indicating the complexity of sediment contamination in this area.

Phase II testing

Concentrations of ammonia in porewater were measured as soon as the sediments were collected. As shown in Table 1, toxic units of ammonia to *C. dilutus* were <0.3 in all the sites, implying that the toxicity of ammonia to the midges was trivial. This supported the results in phase I testing.

As shown in Supplemental Data, Table S3, the respective concentrations of Cd, Cr, Cu, Ni, Pb, and Zn were 0.5 mg/kg to 1.4 mg/kg, 37.9 mg/kg to 88.2 mg/kg, 52.0 mg/kg to 175 mg/kg, 16.9 mg/kg to 53.9 mg/kg, 180 mg/kg to 360 mg/kg, and 154 mg/kg to 550 mg/kg dry weight, which were comparable to previous studies in Guangzhou [1,23]. Hu et al. [24] reported that Cd, Cu, Zn, and Pb in soils of Guangzhou were much higher than their background levels. Agricultural activities before urbanization, traffic activities, industrial effluents, and domestic sewage were considered as potential sources of metals in urban areas [1,24,25].

Because the LC50 values were not available for all target metals, the probable effect concentration values were used to derive toxic units as a conservative prediction (Table 1). The toxic units of metals were 7.37, 4.95, 3.29, and 3.91 for sediments S1, S2, S3, and S4, respectively (Table 1). The high toxic units of S2 and S3 showed that metals might be toxic enough to cause the mortality of *C. dilutus*, as shown in phase I results. The toxic units of S1 and S4 were also high, but the contribution of metals to the toxicity of these 2 sediments was not evident in phase I testing. The conflicting results required better understanding the bioavailability of the metals in these sediments.

A suite of organic contaminants was quantified in the sediments. The sum concentrations of PAHs, PBDEs, and organochlorine pesticides were 1294 ng/g to 3826 ng/g, 97.5 ng/g to 2502 ng/g, and 5.20 ng/g to 8.59 ng/g dry weight, respectively; and no PCBs were detected above the reporting limits in all of the samples (Supplemental Data, Tables S4–S6). Meanwhile, high levels of current-use pesticides were detected (Supplemental Data, Table S7). Three organophosphates were detected, including chlorpyrifos, diazinon, and terbufos. Cypermethrin, lambda-cyhalothrin, and permethrin were the most dominant pyrethroids in the sediments, with their concentrations being 18.0 ng/g to 130 ng/g, 9.87 ng/g to 90.7 ng/g, and 20.4 ng/g to 132 ng/g dry weight, respectively. In addition, deltamethrin was detected at 227 ± 23 ng/g dry weight in S4. The detection of pyrethroids at high concentrations was previously reported in the Pearl River Delta with cypermethrin and permethrin in the ranges of 1.44 ng/g to 219 ng/g dry weight and not detected to 91.4 ng/g dry weight, respectively [6]. Other than pyrethroids, fipronil and its degradation products (fipronil sulfide and fipronil sulfone) were detected at concentrations from 0.49 ng/g to 15.4 ng/g dry weight, which was similar to the finding in the previous study in

Table 1. Conventional toxic units of ammonia, metals, current-use pesticides, organochlorine pesticides, polycyclic aromatic hydrocarbons, and polybrominated diphenyl ethers in sediment samples from Guangzhou, South China

Sediment	Toxic unit					
	Ammonia[a]	$\sum$Metal[b]	$\sum$CUP[c]	$\sum$OCP[d]	$\sum$PAH[e]	$\sum$PBDE[f]
S1	0.24	7.37	3.52	0.04	0.07	0.11
S2	0.18	4.95	4.37	0.14	0.07	0.08
S3	0.10	3.29	4.24	0.13	0.13	0.02
S4	0.19	3.91	3.52	0.03	0.05	0

[a]The 50% lethal concentration (LC50) value of ammonia was obtained from the literature [15], and the ammonia concentration is detailed in Supplemental Data, Table S1.
[b]The toxic units of metals were derived from the probable effect concentration [20], and the concentrations of individual metals are shown in Supplemental Data, Table S3.
[c]The CUPs included organophosphate insecticides, pyrethroids, and fipronil and its degradation products. The LC50 values of the CUPs were from the literature [3,4,19], and the concentrations of individual CUPs are shown in Supplemental Data, Table S7.
[d]The LC50 values of OCPs were from the literature [3], and the concentrations of individual OCPs are shown in Supplemental Data, Table S6.
[e]The toxic units of PAHs were derived from equilibrium partitioning sediment benchmarks [21], and the concentrations of individual PAHs are shown in Supplemental Data, Table S4.
[f]The toxic units of PBDEs were derived from sediment benchmarks [22], and the concentrations of individual PBDEs are shown in Supplemental Data, Table S5.
CUP = current-use pesticide; OCP = organochlorine pesticide; PAH = polycyclic aromatic hydrocarbon; PBDE = polybrominated diphenyl ether.

the Pearl River Delta (below reporting limit to 54.2 ng/g dry wt) [4]. Contamination by current-use pesticides in urban areas is also of great concern in the United States, and recent studies have demonstrated the relationship between current-use pesticide occurrence and sediment toxicity to benthic organisms [26–28].

Among the organics analyzed, persistent organic pollutants contributed little to the mortality of midges (Table 1). The toxic units of PAHs, organochlorine pesticides, and PBDEs were 0.05 to 0.13, 0.03 to 0.14, and 0 to 0.11, respectively. Relatively, current-use pesticides had high toxic units of 3.52 to 4.37. Because a toxic unit of 1 corresponded to 50% mortality, current-use pesticide concentrations were high enough to cause 100% mortality in all sediments.

As shown in Figure 2A, cypermethrin, lambda-cyhalothrin, deltamethrin, and fipronils were the major organic toxicants. Cypermethrin was a dominant toxicant in all sediments, with its toxic units being 1.29, 2.64, 0.96, and 1.08 for S1, S2, S3, and S4, respectively. Deltamethrin caused sediment toxicity in S4 with a toxic unit of 2.11, and lambda-cyhalothrin and fipronils contributed considerably to sediment toxicity in the other 3 sediments with toxic units of lambda-cyhalothrin being 0.46, 0.31, and 0.25 and toxic units of fipronils being 1.64, 1.25, and 2.93 for S1, S2, and S3, respectively. The results were consistent with previous studies in which pyrethroid and fipronil insecticides were regarded as the principal sediment-associated toxicants to benthic invertebrates in the Pearl River Delta [3–5].

Bioavailability of metals and organics

Because sequestration would extensively reduce the bioavailability of sediment-bound contaminants and subsequent

Figure 2. (**A**) Conventional toxic units and (**B**) bioavailable toxic units of the potentially toxic metals and organics in the sediments collected from urban waterways in Guangzhou, China.

toxicity, it is desirable to integrate bioavailability-based extraction into whole-sediment TIE [10]. To improve the accuracy of identifying the causes of sediment toxicity to the midges, the bioavailability of metals and organic contaminants in sediment were quantified by Bureau Commune de Reference sequential extraction and Tenax extraction, respectively. Although the term "bioavailability" is used in the present study, it is the bioaccessibility of sediment-bound organics being quantified by Tenax extraction [29,30].

The toxicity of metals in sediment is strongly dependent on their chemical forms, and the total metal content in sediment was a poor indicator of metal toxicity [31]. The Bureau Commune de Reference sequential extraction was widely adopted to assess the bioavailability of sediment-bound metals [32]. Metals were separated into 4 fractions with Bureau Commune de Reference extraction, and the percentages of individual fractions in the total concentrations are shown in Supplemental Data, Table S3. The bioavailability of a metal in acid-soluble, reducible, and oxidizable fractions decreases successively; and the metal in residual fraction was considered not bioavailable [25]. Thus, the concentration of metal in the acid-soluble fraction was regarded as the bioavailable concentration.

As shown in Supplemental Data, Table S3, Zn was the most bioavailable metal, with acid-soluble fractions >50% (53–62%) in all sediments. The bioavailability of Ni followed, with acid-soluble fractions of 36% to 49%. Comparably, the bioavailability of Cu was relatively low, with acid-soluble fractions of 1% to 12%. The bioavailability of Cr was poor because its acid-soluble fractions were <2% and most Cr was in the residual fraction (58–72%). Although acid-soluble fractions of Pb were small (4–12%), its total concentrations were high in the sediments. As a result, Pb might still be toxic. As shown in Figure 2B, Zn, Ni, and Pb were the potentially toxic metals and Zn contributed the most to the toxicity in most sediments. The possible risk of Zn was also previously noted in the Pear River estuary as a dominant contaminant in sediment [33].

The measurements of bioavailability confirmed the toxicity of metals to *C. dilutus* in sediments S2 and S3, which was recognized in phase I testing. The considerable bioavailability of metals in sediments S1 and S4 also suggested that metals might cause lethality to the midges in these 2 sediments. Nevertheless, the mortality of the midges was not decreased when manipulating sediments S1 and S4 with cation resin. One possible reason for this discrepancy was sediment dilution. As discussed earlier, phase I testing was conducted using diluted sediments (12.5% for S1 and 50% for S2, S3, and S4). Therefore, one should be cautious when characterizing the contaminant class when sediment was diluted, especially when the toxicity was caused by multiple stressors. In addition, sediment organic carbon was an important factor controlling the bioavailability and toxicity of metals [7]. The organic carbon contents in S1 and S4 (7.1% and 9.0%, respectively) were much higher than those in S2 and S3 (3.0% and 1.4%, respectively); thus, the toxicity of metals in S1 and S4 was relatively lower, although the concentrations of metals on a dry weight basis were similar. The content of acid-volatile sulfide (AVS) in sediment significantly affected the bioavailability of the metals, and AVS normalization was proposed to estimate metal bioavailability [34,35]. Yet, AVS contents were not measured in the present study; thus, the influence of AVS on the metals in these sediments could not be evaluated.

Bioavailable toxic units estimated by Tenax extraction better reflected sediment toxicity caused by organic contaminants
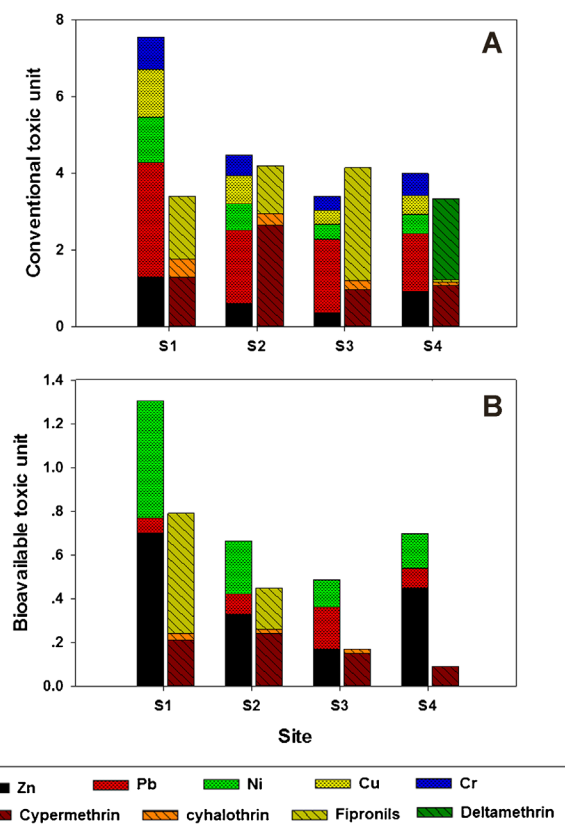
Table 2. The total and bioavailable concentrations of current-use pesticides, organochlorine pesticides, polycyclic aromatic hydrocarbons, and polybrominated diphenyl ethers in sediment samples from Guangzhou, South China[a]

		Concentration (ng/g dry wt)			
	Sediment	$\sum$CUP	$\sum$OCP	$\sum$PAH	$\sum$PBDE
Exhaustive extraction	S1	365	5.20	3826	502
	S2	223	8.59	2395	2502
	S3	63.1	7.78	1294	1724
	S4	587	7.41	3402	97.5
Tenax extraction	S1	77.4	2.56	674	ND
	S2	30.9	3.35	417	ND
	S3	10.9	1.69	307	ND
	S4	32.2	1.94	439	ND

[a]The total and bioavailable concentrations were measured by exhaustive extraction and Tenax extraction, respectively.
CUP = current-use pesticide; OCP = organochlorine pesticide; PAH = polycyclic aromatic hydrocarbon; PBDE = polybrominated diphenyl ether; ND = not detected.

[5,36,37]. A 24-h Tenax extraction was used because it directly approximated the rapid desorbing fraction and was an excellent indicator of the bioavailable concentration among chemical classes and sediments [38–41]. Table 2 lists the sum Tenax-extractable concentrations of individual groups of target contaminants in sediment, and the concentrations of individual compounds are presented in Supplemental Data, Tables S4–S7.

As shown in Table 2 and Supplemental Data, Tables S4–S7, bioavailable fractions of organic contaminants were much smaller than their bulk sediment concentrations, and percentages of Tenax-extractable concentrations in bulk concentrations were 13% to 24%, 22% to 49%, and 5% to 21% for PAHs, organochlorine pesticides, and current-use pesticides, respectively. These were similar to values in previous studies, which reported that Tenax-extractable concentrations accounted for 17% to 55% for pyrethroids [5] and 10% to 60% for PCBs [39]. Because only small portions of organic contaminants in sediment were bioavailable, the conventional toxic unit approach would overestimate the toxicity. Alternatively, a bioavailable toxic unit was calculated and cypermethrin, lambda-cyhalothrin, and fipronils were the putative organic toxicants (Figure 2B). Although deltamethrin was detected in S3 at a high concentration, its low bioavailability limited its toxicity.

Overall, sediment toxicity to midges in urban waterways of Guangzhou was mainly caused by metals (Zn, Ni, and Pb) and current-use pesticides (cypermethrin, lambda-cyhalothrin, and fipronils). Although the total sediment concentrations were effective for screening purposes, they may lead to bias in identification of causative toxicants. To improve the accuracy in TIE, it is important to take the bioavailability into consideration.

CONCLUSIONS

Sediments in the urban waterways of Guangzhou were severely polluted and highly toxic to benthic organisms, and the toxicity was caused by metals and organic contaminants jointly. To better elucidate the causality of sediment toxicity in the presence of multiple stressors, bioavailability-based measurements were applied and the accuracy of toxicity identification was improved.

SUPPLEMENTAL DATA

Tables S1–S7.
Figures S1–S2. (1.6 MB DOC).

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