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CONTRIBUTION OF PYRETHROIDS IN LARGE URBAN RIVERS TO SEDIMENT TOXICITY ASSESSED WITH BENTHIC INVERTEBRATES *CHIRONOMUS DILUTUS*: A CASE STUDY IN SOUTH CHINA

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Abstract: The importance of pyrethroids as potential stressors to benthic organisms has gradually become evident in urban creeks; however, the occurrence and toxicity of sediment-associated pyrethroids are rarely studied in large rivers. In this context, 10 sediments from a large urban river (Guangzhou reach of the Pearl River in China) were assessed for pyrethroid occurrence and sediment toxicity to the benthic invertebrate *Chironomus dilutus*. One half of the sediments exhibited lethality to *C. dilutus* in a 10-d exposure and all surviving midges showed significant change of enzymatic activity. Moreover, mortality occurred during a 20-d exposure for all the sediments, in accordance with the high hazard quotients to benthic species estimated from pyrethroid residues in sediment. Pyrethroids were detectable in all sediments with the concentrations ranging from 2.43 to 61.2 ng/g dry weight, and permethrin and cypermethrin dominated pyrethroid composition. Acute toxic units for pyrethroids ranged from 0.03 to 0.56 (cypermethrin accounted for 13–81%) and showed a direct relationship with sediment mortality among the midges. This is consistent with the studies on small creeks in Guangzhou in which sediment-bound cypermethrin was found as a main stressor to benthic invertebrates. Comparatively, sediment toxicity and pyrethroid residues in large rivers were significantly lower than those in nearby creeks (urban tributaries). The difference may be partially explained by differing flow rates and water-carrying capacity among waterbodies at different scales; further validation is required. Overall, extensive use of pyrethroids has caused a threat to benthic species not only in small creeks but also in large rivers. *Environ Toxicol Chem* 2017;36:3367–3375. © 2017 SETAC

Keywords: Sediment toxicity contribution *Chironomus dilutus* Pyrethroids Pearl River Waterbodies at different scales

INTRODUCTION

To reduce environmental persistence of insecticides, minimize unwanted toxicity to nontarget organisms, and combat increasing resistance by pests, new generations of insecticides have been developed as replacements of legacy insecticides [1,2]. Recent studies showed that current-use pesticides were prevailing in aquatic environments and posed a high risk to nontarget aquatic species [3–5]. Stehle and Schulz [5] reviewed the occurrence of pesticides in aquatic environments at a global scale and found that current-use pesticides may cause greater threats to aquatic species than legacy pesticides. In particular, pyrethroids in sediments were considered to pose the greatest risk to benthic invertebrates; however, this conclusion was based only on limited data available thus far. Accordingly, more studies on the occurrence and risk of pyrethroids in different types of waterways are required to validate the statement [5].

The toxicity of pyrethroids to sediment-dwelling invertebrates has been noted globally [5,6]; nevertheless, the degree of toxicity to benthic invertebrates and the composition of pyrethroids were region-specific. Bifenthrin was considered as the key toxicant to sediment-dwelling invertebrates in developed countries (e.g., the United States and Australia [7,8]), whereas cypermethrin dominated the toxicity contribution in developing countries (e.g., China and Argentina [9,10]). In addition, most of the investigations on the occurrence and toxicity of pyrethroids were focused on sediments in small creeks. Potential risk of sediment-bound pyrethroids in large rivers was less studied, although the ecological functions of large rivers deserve more attention. Waterbodies at different scales (i.e., small creeks and large rivers) had distinct water-carrying capacity and in turn may contain different contaminants. Thus the claim that sediment-bound pyrethroids showed the greatest risk to benthic organisms at a global scale was questionable because of lack of representative sediment sites in large rivers [5].

The Pearl River is the second largest river in stream flow in China with a drainage area of $4.42 \times 10^5\,km^2$ [11]. Before flowing into the South China Sea, the river passes through Guangzhou, which is the largest city in South China and is experiencing an economic boom. The Pearl River Delta is one of the most pesticide-polluted areas in China [12,13]. Pyrethroids were frequently detected in sediments from urban creeks [10,14], and were considered as main toxicants in sediment [15,16]. However, similar to the cases in other regions of the world, the occurrence and toxicity contribution of pyrethroids in sediments were merely studied in small creeks in the Pearl River Delta, whereas the main channel, the Pearl River, was poorly investigated. Therefore, the Guangzhou reach of the Pearl River can serve as an ideal representative of large

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urban rivers in developing countries for better understanding sediment risk of pyrethroid residues to benthic species.

The aims of the present study were to: 1) evaluate acute and chronic toxicity of sediments collected from the Guangzhou reach of the Pearl River to the benthic invertebrate *Chironomus dilutus*, 2) analyze the occurrence and distribution of sediment-bound pyrethroids, 3) assess the contribution of pyrethroids to sediment toxicity to *C. dilutus* and their potential risk to the benthic community in the river, and 4) compare pyrethroid-related sediment toxicity in different types of waterbodies (e.g., large rivers and small creeks) using data in the present study and previous studies.

MATERIALS AND METHODS

Sediment sampling

As shown in Supplemental Data, Figure S1, 10 sediment samples were collected along the Guangzhou reach of the Pearl River, and detailed descriptions of the sampling sites are shown in Supplemental Data, Table S1. The sampling sites were classified into 4 categories: residential areas, commercial centers, rural areas, and public parks. Given that the study area was in a city with a large population, more sites were chosen from residential areas than from other areas. Meanwhile, a reference sediment collected from a reservoir in Conghua in northern Guangzhou was used as a control sediment.

The top 5 cm of surface sediment were collected using a stainless bottom-grab dredge. Sediments were passed through a 2-mm sieve onsite to remove rocks and large debris, transported to the laboratory in the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, and then stored at 4 and –20 °C for toxicity testing and chemical analysis, respectively.

The contents of total organic carbon in the sediments were analyzed by an elemental analyzer (Elementar Vario El III) after removing inorganic carbon using 1 mol/L HCl.

Toxicity testing

The benthic invertebrate *C. dilutus* was chosen as the test organism in toxicity testing and cultured at the Guangzhou Institute of Geochemistry in accordance with the protocols suggested by the US Environmental Protection Agency (USEPA) [17]. The Chironomidae is widely distributed in the study area and *C. dilutus* is a standard species for sediment toxicity testing [17].

The toxicity of sediment samples from the Pearl River to the midges was evaluated using 10-d acute and 20-d chronic toxicity tests in an automated water delivery system. The tests were performed according to the methods recommended by USEPA [17] with minor modifications, and detailed descriptions of the methods were reported previously [15,18,19]. In brief, 80 g of wet sediment and 250 mL of moderately hard reconstituted water were transferred to each test beaker. After the sediments were settled overnight, 10 third instar midges or 10 first instar midges were randomly introduced into each beaker to initiate the 10-d acute or 20-d chronic toxicity tests, respectively. The toxicity tests were conducted in 5 replicates at 23 ± 1 °C and a 16:8-h light:dark cycle. Water change (~ 150 mL of overlying water) was performed twice every day. Water quality parameters including pH, dissolved oxygen, conductivity, and temperature were monitored daily and the concentration of ammonia in overlying water was measured at 0, 10, and 20 d. The midges were fed daily with 1 mL of 6 g/L fish food per beaker in acute bioassays. Various amounts of food were used in chronic toxicity testing with the midges (i.e., 0, 0.6, 3, and 6 g/L for the durations of 0–2, 3–7, 8–12, and 12–20 d, respectively) because of different food requirements for the midges at different life stages [20]. At the termination of the tests (10 or 20 d), surviving midges were collected from the sediment, counted, rinsed 3 times using distilled water, and weighed.

In addition to lethality, sublethal effects of the surviving midges after 10-d exposure to the sediments from the Pearl River were assessed by analyzing the activities of 5 enzymes including glutathione S-transferase (GST), carboxylesterase (CarE), acetylcholine esterase (AChE), catalase (CAT) and lipid peroxide, following a method described by Qi et al. [19]. Briefly, the thawed midges were homogenized in 1 mL of precooled phosphate buffer saline (0.1 mol/L) at 4 °C using a Bullet Blender Blue-24 homogenizer (Next Advance). Homogenate was centrifuged at 10 000 *g* for 10 min at 4 °C and the supernatant was decanted for analyzing enzymatic activity. The activities of AChE and GST were measured using kinetic methods with acetylthiocholine iodide [21] and 1-chloro-2,4-dinitrobenzene as the substrates, respectively [22], and CarE activity was determined by a photocolorimetric method [23,24]. The activities of CAT and lipid peroxide were measured using the ammonium molybdate [25] and thiobarbituric acid reactions [26], respectively. The Bradford method [27] was used to quantify protein contents in the midges and bovine serum albumin was used as a standard. More details on the procedures of enzymatic analysis can be found in the Supplemental Data.

Chemical analysis

Based on previous studies conducted in small creeks in the study area, pesticides and heavy metals were possible toxicity contributors to sediment toxicity [16]. Therefore, apart from pyrethroids, fipronil and its degradation products (fiproles), organophosphate pesticides, and heavy metals were also analyzed according to methods described by Yi et al. [16]. Supplemental Data, Table S2 lists the target contaminants analyzed in the present study.

Sediment was freeze-dried, thoroughly homogenized, extracted using an ultrasound-assisted microwave extractor (Xintuo), and analyzed for pesticides [28]. In brief, 10 g of dry sediment, 100 mL of a mixture of hexane and acetone (1:1, v/v), 50 ng of surrogates, and 2 g of activated copper powder were added into a 250-mL flask and the sample was extracted for 6 min with ultrasound and microwave power set at 50 and 100 W, respectively. After extraction, the supernatant was decanted and an additional extraction was conducted using 50 mL of fresh extraction solution. The 2 extracts were combined, filtered, and concentrated and the solvent was exchanged to 1 mL of hexane using a Turbovap (Xintuo).

The extracts were then cleaned using dual-layer solid phase extraction (SPE) cartridges packed with 300 mg of graphitized carbon black and 600 mg of primary secondary amine (Supelco). The SPE cartridge was conditioned with 6 mL of hexane before the extract was loaded onto the cartridge. Seven mL of a mixture of hexane and dichloromethane (7:3, v/v) were used to elute pyrethroids and organophosphate pesticides, and then 7 mL of acetone were added to elute fiproles. The cleaned extracts were concentrated, solvent exchanged to hexane and analyzed on the instrument after adding the internal standards of d6-*trans*-cypermethrin (AccuStandard) and d10-parathion (Dr. Ehrenstorfer).

The pesticides were analyzed on a QP–2010 plus series gas chromatography–mass spectrometry in negative chemical ionization mode (Shimadzu). A DB–5HT column (15 m × 0.25 mm, 0.1 μm) was used to separate the analytes. Helium was used as the carrier gas and the flow rate was set at 1.0 mL/min; methane was used as the negative chemical ionization reaction gas. Temperature of ion source and transfer line was set at 250 and 260 °C, respectively. Injection of 1 μL of extract was performed under high pressure and splitless mode. A programmable temperature vaporizing injection was used and initial temperature was set at 50 °C, held for 0.1 min, heated to 300 °C at 230 °C/min, and held for 5 min. The oven temperature was initiated at 60 °C, held for 1 min; heated to 180 °C at 10 °C/min, held for 1 min; then heated to 220 °C at 3 °C/min, held for 8 min; and lastly ramped to 300 °C at 20 °C/min, and held for 8 min. Pesticide quantification was conducted using internal standard calibration.

Six metals (Cd, Cr, Cu, Ni, Pb, and Zn) were analyzed in the sediments. After the sediment was freeze-dried, ground, homogenized, and sieved through a 150-μm sieve, 0.2 g of dry sediment were digested with 7 mL of HNO_3 and 3 mL of HF using a microwave digestion system (Xingtuo). The digested solution was evaporated to near dryness at 95 °C, 1 mL of HNO_3 was added, and the solution was then diluted using triple-distilled water. Metals were analyzed on an Agilent 7700 inductively coupled plasma–mass spectrometer.

Strict quality assurance and quality control were carried out, including a solvent blank, a matrix blank, a matrix spike and its duplicate for pesticide analysis. The instrument was checked by analyzing a calibration standard every 10 samples, and variation of each analyte was less than 20%. Two surrogates (4, 4'-dibromooctafluorobiphenyl and decachlorobiphenyl) were added to all samples before extraction to verify the performance of analytical procedures. Accuracy was validated by the recoveries of pesticides in matrix spike samples (58–126%) and the surrogates in all samples (71–108%). The reporting limits of pesticides in sediment samples were determined by multiplying the lowest concentration of the calibration standard and the volume of the final extract and then dividing by the mass of dry sediment being extracted. For quality assurance and quality control of heavy metals' analysis, a batch of blank, sample duplicate, and standard reference sediment was digested and analyzed following the same procedures. A Chinese standard reference sediment (GBW 07334) was used and the recoveries of the 6 heavy metals were from 66% to 131%.

Data analysis

A multiple analysis of variance based on the Tukey honest significant difference calculation was applied to compare the mortality and enzymatic activity of the midges exposed to the control and test sediments using R (Ver 3.2.5). A significant difference ($p < 0.05$) indicated that the test sediment was toxic to the midges. A *t* test was used to compare the concentrations of sediment-bound pyrethroids in different types of waterbodies using the package *Stat* in R.

Toxic unit was calculated to evaluate the contribution of contaminants to sediment toxicity to the midges (Equation 1). In addition, the hazard quotient was computed to evaluate the potential risk of sediment-associated contaminants to the benthic community in the river (Equation 2).

$$\text{Toxic unit} = C/\text{LC50} \quad (1)$$

$$\text{Hazard quotient} = \text{C}/\text{TEB or PEC} \quad (2)$$

where *C* is the sediment concentration of pesticides (organic carbon normalized) or heavy metals (dry wt basis) from the Guangzhou reach of the Pearl River, LC50 is the median lethal concentration of corresponding contaminant to *C. dilutus*, TEB is the organic carbon normalized threshold effect benchmark of the corresponding pyrethroids, and PEC is the probable effect concentration of the corresponding heavy metals. The LC50 values of the pyrethroids used for calculating toxic units were derived from sediment toxicity testing and have been widely used in previous assessments [29–31]. Limited sediment quality criteria for pyrethroids were available to date. Recently, Nowell et al. [31] developed likely effect benchmarks and organic carbon normalized threshold effect benchmarks for current-use pesticides based on spiked sediment bioassay data in the literature. Thus the organic carbon normalized threshold effect benchmarks based on *Hyalella azteca* toxicity data developed by Nowell et al. [31] were applied in the present study to assess the potential risk of sediment-associated pyrethroids to benthic invertebrates. To assess the contribution of pyrethroids to sediment toxicity, correlation analysis was performed between the probit of mortality and the toxic units of contaminants across sediments using R, and $p < 0.05$ indicated significance. Furthermore, a stepwise regression was performed using R to identify the main correlated variables for the observed sediment toxicity.

An integrated biomarker response (IBR) index was calculated to summarize biomarker expression by integrating quantifications of individual biomarkers into a single value [32]. First a *Y* value was calculated by the mean value of a biomarker (*X*) and mean (m) and standard deviation (SD) of the total biomarker values (Equation 3). Next a *Z* value was assigned to be *Y* or –*Y* according to the enzyme being activated or inhibited (Equation 4). Then an *S* value was calculated as the sum of *Z* and the absolute minimum value of *Z* for the certain enzyme in all sediment samples (Equation 5). As shown in Equation 6, A_i was then computed from multiplying 2 successive *S* values (S_i and S_{i+1}) and the sine function of the number of the enzymes (*k*) analyzed. Finally, an IBR index was calculated by summarizing the A_i values of individual enzymes (Equation 7).

$$Y = (X - \text{m})/\text{SD} \quad (3)$$

$$Z = Y \text{ or } Z = -Y \quad (4)$$

$$S = Z + |\text{absolute minimum}| \quad (5)$$

$$A_i = \frac{S_i \times S_{i+1} \times \sin(2\pi/k)}{2} \quad (6)$$

$$\text{IBR} = \sum_{i=1}^{k} A_i \quad (7)$$

RESULTS

Sediment toxicity to the midges

Mortality of *C. dilutus* after 10-d sediment exposure is shown in Figure 1 and Supplemental Data, Figure S2. Five of the 10 sediments from the Guangzhou reach of the Pearl River exhibited acute lethality to midges compared with the controls, with mortality of all sediments ranging from 10 ± 3% to 100%. A sediment collected from a residential area (S2) caused 100% mortality of the midges after 10-d exposure. Chronic toxicity of

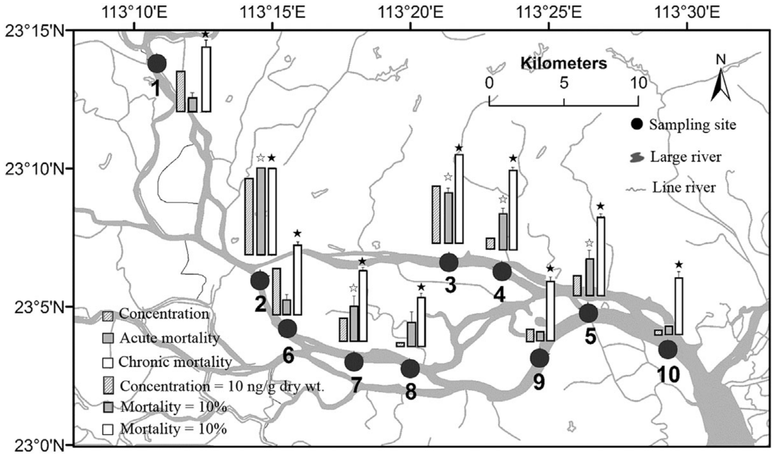

Figure 1. Spatial distribution of pyrethroid residues in sediment as well as acute and chronic mortality of *Chironomus dilutus* after exposure to sediments from the Guangzhou reach of the Pearl River in South China. Concentration of pyrethroids or mortality of *C. dilutus* is denoted by the length of the bar. Significant differences in acute and chronic mortality between test sites and control were determined by one-way analysis of variance and are indicated by asterisks (★ and ☆, respectively; $p < 0.05$).

the sediments was evaluated using first instar midges (Supplemental Data, Figure S3). All of the sediments from the Guangzhou reach of the Pearl River were chronically toxic to the midges, with mortality ranging from 65 ± 5% to 100%. Two sediments from residential areas (S2 and S3) caused complete mortality to the midges after 20-d exposure. As expected, the sediments showed greater effects to first instar midges than to third instar midges because of higher susceptibility of younger midges and longer exposure period of the chronic toxicity testing.

The responses of biomarkers in *C. dilutus* after 10-d exposure to sediments from the Pearl River are shown in Table 1. As a result of complete mortality of the midges exposed to S2 sediment, there were no biomarker data for S2. In general, 90% of the sediments from the Pearl River induced significant change of at least one enzyme, and 67% of the sediments impacted at least 2 enzymes. Compared with the controls, GST activity in the midges after exposure to sediments at S3 and S9 significantly increased. Elevated levels of lipid peroxide were observed in the midges exposed to sediments at S6, S7, S8, and S10, but no midges showed significant changes of CAT activity. Activity of CarE in the midges was inhibited by exposure to 3 sediments (i.e., at S4 and S5 from a commercial center and at S8 from a park). Finally, AChE activity in the midges exposed to one-half of the sediments (at S3, S4, S5, S6, and S7) was significantly inhibited.

The IBR indices were calculated for all site sediments to more comprehensively evaluate biomarker responses in the midges (Table 1). The IBR indices of all the sediments from the Pearl River were more than 10 times higher than that of the control sediment, suggesting that these sediments strongly affected biomarker expression (enzymatic activity) in the midges. The highest IBR index was found in sediment at S7 from a residential area, followed by at S4 from a commercial area.

Toxicity contribution from pyrethroids

As shown in Supplemental Data, Table S3 and Figure S4, bifenthrin, λ-cyhalothrin, cypermethrin, deltamethrin, fenpropathrin, and permethrin were detected with concentrations higher than the reporting limit in at least one sediment from the Guangzhou reach of the Pearl River. Esfenvalerate was detected in 5 sediments, but the concentrations were all lower than the reporting limit. No cyfluthrin was detected in any sediment. The

Table 1. Individual enzymatic responses and integrated biomarker response index in *Chironomus dilutus* after 10-d exposure to sediments collected from the Guangzhou reach of the Pearl River, South China[a]

Site	GST	CarE	AChE	CAT	LPO	IBR
	(nmol/min/mg protein)				(nmol/mg protein)	
S1	183 ± 27.5	51.2 ± 6.06	7.83 ± 1.47	415 ± 103	14.7 ± 3.47	3.59
S3	233 ± 81.5*	62.9 ± 14.1	6.32 ± 1.45*	497 ± 125	13.5 ± 0.58	3.97
S4	150 ± 22.6	43.5 ± 4.68*	5.09 ± 1.81*	372 ± 100	11.3 ± 9.81	4.17
S5	139 ± 2.21	43.4 ± 3.76*	5.82 ± 2.23*	328 ± 66.4	18.5 ± 1.01	3.06
S6	168 ± 46.1	60.1 ± 13.6	5.52 ± 0.72*	311 ± 42.3	26.7 ± 8.01*	2.49
S7	153 ± 28.7	46.3 ± 8.96	4.46 ± 1.38*	418 ± 54.1	27.3 ± 6.11*	6.50
S8	135 ± 33.6	44.2 ± 6.76*	7.74 ± 1.89	359 ± 72.5	30.9 ± 5.51*	3.07
S9	233 ± 37.5*	71.2 ± 8.06	7.05 ± 1.47	515 ± 203	17.7 ± 4.49	3.06
S10	143 ± 3.52	45.8 ± 2.58	7.06 ± 4.18	303 ± 51.4	26.9 ± 5.56*	2.08
Control	175 ± 17.7	61.7 ± 14.8	12.3 ± 0.72	444 ± 37.6	12.1 ± 1.76	0.15

[a]Significant difference between test sediment and the control was determined by a one-way analysis of variance ($n = 5$).
*$p < 0.05$.
GST = glutathione S-transferase; CarE = carboxylesterase; AChE = acetylcholine esterase; CAT = catalase; LPO = lipid peroxide; IBR = integrated biomarker response.

total concentrations of pyrethroids in the sediments ranged from 4.07 to 61.2 ng/g dry weight, with the highest concentration in S2 sediment. Permethrin was the dominant pyrethroid in sediments (0.88–35.4 ng/g dry wt), accounting for 62% of the total concentration of pyrethroids on average, followed by cypermethrin (22%) and bifenthrin (7.5%).

Toxic units were computed from sediment concentrations to predict the contribution of pyrethroids to acute sediment toxicity to *C. dilutus* (Figure 2 and Supplemental Data, Table S4). The total acute toxic units of pyrethroids ranged from 0.03 to 0.56, with a mean value of 0.22. Cypermethrin contributed the most to the toxic units, accounting for 51% on average (13–86%). A significant relationship was found for toxic units of pyrethroids and sediment toxicity (probit = 0.47 toxic unit + 3.56, $r^2 = 0.46$, $p < 0.05$; Figure 3), implying possible contribution of pyrethroids to sediment toxicity to the midges. However, the total acute toxic units of pyrethroids in sediment to the midges were usually smaller than 0.5 (except for at S2 [0.56]), suggesting that contribution of pyrethroids to the observed acute toxicity was limited and toxicity contribution from other pollutants in sediments should also be considered.

Chronic toxic units were calculated to evaluate the contribution of sediment-associated pyrethroids to the observed chronic toxicity to the midges. Because few chronic LC50 values of pyrethroids to *C. dilutus* are available, chronic toxic units were estimated from acute toxicity data. An acute-to-chronic ratio of 12.4 has been proposed to estimate chronic toxicity from acute LC50 values for pyrethroids [33]. The value of acute-to-chronic ratio was validated by experimental value for permethrin (13.4), whose acute and chronic LC50 values to *C. dilutus* were 24.5 and 1.83 μg/g organic carbon, respectively [20,34]. Accordingly, the total chronic toxic units of pyrethroids for these sediments were estimated by multiplying the acute toxic units by 12.4, resulting in chronic toxic units ranging from 0.37 to 6.94. This showed that sediment-bound pyrethroids in the Guangzhou reach of the Pearl River may be related to the chronic effects in the midges.

Potential risk to benthic community

The hazard quotient values were calculated to assess the potential risk of sediment-associated pyrethroids to the benthic community in the Guangzhou reach of the Pearl River (Table 2). In general, a hazard quotient value higher than 1.0 indicates

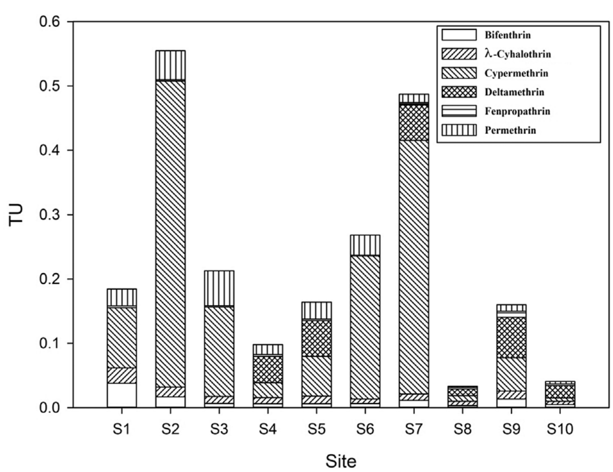

Figure 2. The toxic units (TUs) of individual pyrethroids to *Chironomus dilutus* in sediments from the Guangzhou reach of the Pearl River, South China.

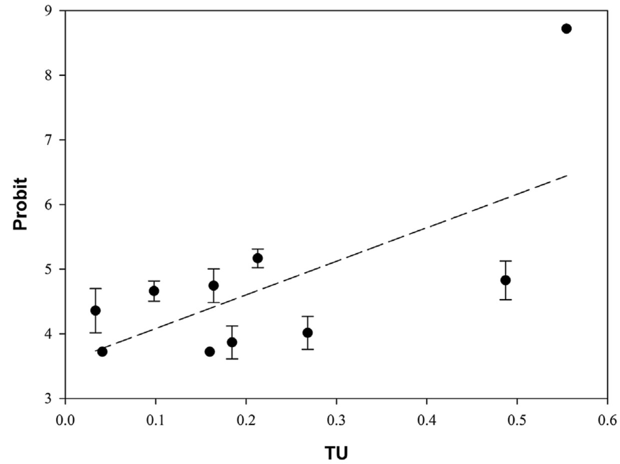

Figure 3. The correlation between acute sediment toxicity to *Chironomus dilutus* (probit of mortality) and the toxic units (TUs) of pyrethroids (probit = 0.47 toxic unit + 3.56, $r^2 = 0.46$, $p < 0.05$).

potential risk, and higher than 10 indicates significant risk. The total hazard quotient values of pyrethroids in sediments were in a range of 2.52 to 18.3 with a mean value of 8.87, suggesting that pyrethroids exhibited potential risk to the benthic community in the Guangzhou reach of the Pearl River. Special attention should be paid to sediments at S2 and S7, which had pyrethroid hazard quotient values of 18.3 and 16.8, respectively, indicating significant risk. Overall, 42% and 3.3% of the hazard quotients for individual pyrethroids were higher than 1.0 and 10, respectively. Individually, cypermethrin showed the greatest risk to benthic invertebrates in the sediments, with a mean hazard quotient value of 4.03 (0.1–13.0), followed by deltamethrin, λ-cyhalothrin, and permethrin.

DISCUSSION

Spatial distribution of sediment toxicity and pyrethroid residues

The midge *C. dilutus* was chosen as the representative species to investigate the toxicity of sediments from the Guangzhou reach of the Pearl River to benthic invertebrates via sediment bioassays. Acute and chronic lethality to the midges was observed for 50% and 100% of the sediments, respectively, suggesting that the sediments were threatening the survival of benthic invertebrates at an individual level in the studied river. Furthermore, all the sediments caused changes of enzyme activities in surviving midges and IBR indices of individual sediments were all higher than 10. Qi et al. [19] showed that emergence of midges was negatively related to IBR index. Decline of emergence would reduce the reproduction of the midges, which in turn would pose a risk on midge population [35,36]. Therefore, lethal and sublethal effects on the midges in the Guangzhou reach of the Pearl River at an individual level likely caused adverse effects on the midge population and further on the benthic community [37]. This was in accordance with the high hazard quotient values of pyrethroids in the sediment.

Insecticides were considered to be responsible for decreasing biodiversity in aquatic environment at a global scale [5]. Previous studies have investigated acute toxicity of sediments from urban waterways in the Pearl River Delta; however, the samples were merely collected from small creeks [10,15]. Mehler et al. [15] and Yi et al. [16] applied whole-sediment

Table 2. Hazard quotient of pyrethroids in sediments from the Guangzhou reach of the Pearl River, South China

Compound	TEB (μg/g OC)[a]	Site									
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
Bifenthrin	0.17	1.39	0.62	0.25	0.23	0.23	0.24	0.42	0.12	0.50	0.19
λ-cyhalothrin	0.023	2.94	1.85	1.30	1.17	1.44	0.88	1.21	0.82	1.50	0.61
Cypermethrin	0.049	2.55	13.0	3.80	0.63	1.68	6.08	10.8	0.24	1.41	0.14
Deltamethrin	0.02	0	0	0	2.44	3.33	0	3.28	0.62	3.77	1.15
Fenpropathrin	0.11	0.24	0.17	0.16	0.24	0.21	0.09	0.31	0.18	0.79	0.20
Permethrin	0.42	1.52	2.63	3.17	0.90	1.53	1.81	0.77	0.11	0.56	0.23
ΣPYR[b]		8.63	18.3	8.68	5.61	8.43	9.09	16.8	2.09	8.53	2.52

[a]The values of threshold effect benchmark were obtained from Nowell et al. [31].
[b]ΣPYR is the sum hazard quotient of pyrethroids.
TEB = threshold effect benchmark; OC = organic carbon.

toxicity identification and evaluation to identify the causes of sediment toxicity and analyzed a variety of possible toxicants including ammonia, metals, and hydrophobic organic contaminants in the creeks. Current-use pesticides (pyrethroids and fiproles) and heavy metals (Zn, Ni, and Pb) were identified as main toxicity contributors to the midges [16]. Consequently, the present study further evaluated the toxicity contribution of sediment-bound pyrethroids in this area from the large river (the Guangzhou reach of the Pearl River) using a toxic unit method and a significant relationship between sediment toxicity and pyrethroid toxic units was noted ($r^2 = 0.46$, $p < 0.05$).

Spatial distribution of sediment toxicity to the midges (acute and chronic toxicity) and pyrethroid concentrations in sediments along the river are mapped in Figure 1. Sediments containing elevated pyrethroid toxic units exhibited high acute toxicity to the midges, indicating possible contribution of pyrethroids to sediment toxicity to the midges (Figure 1). The Guangzhou reach of the Pearl River flows through countryside, residential, and commercial areas and, in general, sediments collected from residential areas (S2, S3, and S7) and commercial centers (S4 and S5) that had high population density had significant acute lethality to the midges. The effect of human activity on sediment toxicity has also been reported in previous studies in small creeks in the Pearl River Delta [10,15,38].

Correspondingly, pyrethroid concentrations in sediments from residential areas (S2, S3, S6, and S7) were the highest, followed by agricultural areas (S1 and S9), commercial centers (S4 and S5), and parks (S8 and S10). Urban application of pyrethroids is mainly for mosquito control (household and public) and landscaping and structural maintenance (homeowners and professionals). The S2 and S3 sediments were collected from the old town of Guangzhou where most of the buildings were constructed over 50 yr ago. The needs of structural maintenance promoted the use of insecticides, resulting in high pyrethroid residues in the surrounding aquatic environment. The other 2 residential sites (S6 and S7) were located adjacent to urban villages that had a high density of transient population and poor hygienic conditions. It is reasonable to detect high levels of pyrethroids in the sediments as a result of massive application of pyrethroids in household mosquito controlling in urban villages [39]. Sediments at S1 and S9 were collected from vegetable planting areas, and pyrethroid concentrations in sediment at S1 were as high as those from residential areas but from different sources (agricultural use; Figure 1 and Supplemental Data, Table S3). Comparatively, pyrethroids in sediments from the parks were one order of magnitude lower than those from residential areas.

Overall, similar spatial distribution of sediment toxicity and pyrethroid residues showed human activity played an important role in pesticide application, which in turn affected pesticide occurrence and sediment toxicity to benthic organisms. Land-use pattern strongly impacted environmental distribution of pyrethroids and residential use of pyrethroids caused severe deterioration of urban waterways [8,40]. Apart from pesticide application and land-use patterns, conditions of a receiving waterbody (e.g., scale, stream flow, as well as amount, size, and structure of particle matters) would also significantly affect pyrethroid distribution in the aquatic environment [6,41].

Sediment-associated pyrethroids in waterbodies at different scale

Sediment concentrations of pyrethroids in the Guangzhou reach of the Pearl River were generally lower than those in the small creeks in the same city (e.g., 6.4 times lower than those in Chebei Creek) [10,14]. Chebei Creek is one of the 231 urban tributaries of the Guangzhou reach of the Pearl River, and some areas these tributaries flow through are utilized for similar land-use purposes. The Pearl River is the second largest river in China, with an approximate cross-sectional flow of 300 m^3/s, whereas Chebei Creek is 19.4 km with an approximate cross-sectional flow of 0.2 m^3/s. The scale of waterbody probably influenced pyrethroid residues in sediment, consequently the toxicity to benthic organisms. That is, 94% of the Chebei Creek sediments caused acute lethality to midges and 81% of them caused complete mortality [10]. Nevertheless, only 10% of the sediments from the Guangzhou reach of the Pearl River caused complete acute mortality to midges.

Flow rate, the contents of suspended particles, and dissolved organic carbon are important parameters influencing the fate and transport of pyrethroids in waterways [42,43]. Urban creeks directly received runoff and discharges containing pyrethroids from the surrounding landscaping and residential belts [10]. Usually, water flow in small creeks is slower than that in large rivers. For example, flow velocity in urban creeks in Guangzhou (0.039 ± 0.021 m/s) was approximately 7 times slower than that in the Guangzhou reach of the Pearl River (0.28 ± 0.22 m/s). Slow water flow in Chebei Creek enhanced the deposition of suspended particles containing pyrethroids to bottom sediment in the tributary instead of rushing into the Pearl River, which resulted in higher sediment concentrations of pyrethroids in urban creeks than that in the main channel of the river. Meanwhile, high stream flow velocity and cross-sectional flow in large rivers caused considerable dilution of contaminants and were other important reasons for the lower pyrethroid residues in sediment from large rivers [44].

Furthermore, pyrethroid residues in sediments from large rivers flowing through urban areas were compared with those in urban creeks to better understand the influence of the scale of waterbody on the occurrence of pyrethroids. Literature data with detailed concentrations were collected and summarized in Supplemental Data, Table S5. Most of the studies to date were conducted in small creeks, whereas very few measured pyrethroids in sediments from large rivers. Individually, the mean concentrations of bifenthrin, cypermethrin, and permethrin in sediments from urban creeks were obviously higher than those from large rivers (Supplemental Data, Figure S5). Conversely, sediment concentration of λ-cyfluthrin and esfenvalerate in large rivers was higher than that in creeks. The chemical-specific distributions of pyrethroids in waterbodies at different scale were likely related to their application patterns. Whereas bifenthrin, cypermethrin, and permethrin were heavily used in urban areas, λ-cyhalothrin and esfenvalerate were equally used in urban and agricultural activities [6,45]. As discussed in this section, pyrethroids originated from urban runoff may have a greater chance to be deposited in small creeks, resulting in higher residues for the 3 pyrethroids. In addition to urban runoff, however, there was additional agricultural input of λ-cyhalothrin and esfenvalerate into large rivers when the rivers flowed through agricultural areas. Nevertheless, it should be noted that application patterns (e.g., amount, rate, and composition) of pyrethroids directly influenced their regional distribution in waterways [45]. For example, sediment concentrations of pyrethroids in Chebei Creek, which flows through an urbanized and populous area in Guangzhou [10], were significantly higher than those in urban streams in the Guangzhou College City area, where urbanization and population density were lower than in the Chebei Creek location [38] (Supplemental Data, Table S5). High urbanization and population density probably promoted the use of pyrethroids and correspondingly increased their residues in aquatic systems.

Additional toxicity contributors

Similar to the findings in urban creeks in Guangzhou [16,39], pyrethroids may partially contribute to the observed sediment mortality to the midges in the Guangzhou reach of the Pearl River, as suggested by the multiple lines of evidence including toxic units, hazard quotients, and correlations of contaminant residues with toxicity. Nevertheless, the contribution of pyrethroids to acute mortality of *C. dilutus* was limited, with the maximum acute toxic unit of 0.56. This implied that contaminants other than pyrethroids existed in the large rivers causing the toxicity. Previous studies have detected various contaminants in sediment in the Guangzhou reach of the Pearl River: heavy metals, persistent organic pollutants (such as polycyclic aromatic hydrocarbons [PAHs], polychlorinated biphenyls [PCBs], polybrominated diphenyl ethers [PBDEs], and organochlorine pesticides), and ammonia [46–49]. Some of these compounds may have contributed to the toxicity as well.

In a toxicity identification and evaluation study on urban creeks in Guangzhou, Yi et al. [16] found that 2 pyrethroids (λ-cyhalothrin and cypermethrin), fiproles, and 3 metals (Zn, Ni, and Pb) were the main toxicants in sediments causing acute mortality of the midges. Accordingly, heavy metals, organophosphate insecticides, and fiproles were added to the list of target analytes—in addition to pyrethroids—and their contributions to the lethality to the midges were also evaluated. Because acute mortality of PAHs, PCBs, PBDEs, and organochlorine pesticides to the midges was extremely low, they were excluded from the analysis [15,16].

All of the 6 analyzed metals (Cd, Cr, Cu, Ni, Pb, and Zn) were detected in the sediments from the Guangzhou reach of the Pearl River (Supplemental Data, Table S6) and the concentrations were similar to previously reported data on the river [49]. The total toxic units of sediment-bound metals ranged from 0.15 to 0.45, with a mean of 0.26 (Supplemental Data, Table S7). Whereas the mean value was similar to the mean toxic unit of pyrethroids (0.22), the much lower relative standard deviation of metal toxic units (37%) across sediment samples compared with relative standard deviations of pyrethroid toxic units (80%) and acute mortality of the midges (77%) suggested that metals were more equally distributed along the river and less related to acute mortality than pyrethroids spatially. However, the risk from sediment-bound metals in the Guangzhou reach of the Pearl River should not be ignored, as indicated by high hazard quotient values calculated based on PECs (5.55–14.5; Supplemental Data, Table S8) that suggested significant long-term effects of metals on benthic invertebrates. Chlorpyrifos was the only organophosphate pesticide detected in sediments, with toxic units ranging from < 0.01 to 0.04 (0.01 ± 0.01), implying limited contribution of organophosphate pesticides to sediment toxicity (Supplemental Data, Table S8). Fiproles were detectable in sediments but their concentrations were all lower than the reporting limit.

Overall, the total toxic units of analytes (pyrethroids, chlorpyrifos, and metals) ranged from 0.19 to 1.05, and metals accounted for approximately one-half of the total toxic units on average (Supplemental Data, Table S8). Although the contribution of toxic units from pesticides (pyrethroids and chlorpyrifos) was only one-half of the total toxic units, pesticides' toxic units were significantly correlated to midge mortality (probit = 5.78 toxic unit + 3.42, $r^2 = 0.48$, $p < 0.05$). Specifically, pyrethroids alone had a significant correlation with sediment toxicity ($r^2 = 0.46$, $p < 0.05$; Figure 3), as did organophosphate pesticides ($r^2 = 0.46$, $p < 0.05$). On the contrary, metals were not significantly related to the mortality ($p > 0.05$). Furthermore, a stepwise regression was performed to identify the correlated variables. Sediment-associated pyrethroids were the correlated variable to the observed sediment toxicity (adjusted $r^2 = 0.58$, $p < 0.05$); however, heavy metals and organophosphate pesticides were excluded variables ($p > 0.05$). The results confirmed that pyrethroids were the major correlated variable to the observed toxicity in the list of analytes and partially explained sediment mortality to the midges in the Guangzhou reach of the Pearl River. Further nontargeted chemical analysis—which may be achieved by effect-directed analysis [50,51]—is required to more comprehensively identify toxicity contributors to the observed sediment toxicity.

CONCLUSIONS

The Guangzhou reach of the Pearl River was selected as the study area to evaluate the toxicity contribution of sediment-associated pyrethroids to benthic invertebrates in large urban rivers. One-half of the sediments exhibited acute lethality to *C. dilutus*, and all sediments were chronically lethal to the midges. Pyrethroids were detected in the sediments and permethrin was the dominant pyrethroid. Although toxic unit analysis showed that sediment-associated pyrethroids only partially contributed to the acute lethality of the midges, the total hazard quotients of pyrethroids were higher than 10, indicating their potential risk on the benthic community.

Comparatively, sediment toxicity and pyrethroid residues in sediments from the Guangzhou reach of the Pearl River were significantly lower than those in small creeks that flow into the Pearl River. Faster flow rate and dilution caused by higher cross-sectional flow in the large river may be the reasons for lower sediment toxicity and pyrethroid residues in the Pearl River than in its tributary Chebei Creek. Similar to the case in small creeks, pyrethroids were the possible contributors to the observed toxicity to benthic organisms in large rivers; however, they can only explain a portion of sediment toxicity. To more comprehensively identify key toxicity contributors in river sediments contaminated by a complex mixture of toxicants, nontargeted chemical analysis (e.g., effect-directed analysis) is required.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3919.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (lihuizhen@jnu.edu.cn).

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