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SEDIMENT-ASSOCIATED PESTICIDES IN AN URBAN STREAM IN GUANGZHOU, CHINA: IMPLICATION OF A SHIFT IN PESTICIDE USE PATTERNS

Huizhen Li,†‡ Baoquan Sun,†‡ Michael J. Lydy,§ and Jing You*†
†State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China
‡University of the Chinese Academy of Sciences, Beijing, China
§Fisheries and Illinois Aquaculture Center and Department of Zoology, Southern Illinois University, Carbondale, Illinois, USA

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Abstract—Pesticide use patterns in China have changed in recent years; however, the study of the environmental fate of current-use pesticides (CUPs) and their ecotoxicological significance in aquatic ecosystems is limited. In the present study, sediments were collected from an urban stream in the Chinese city of Guangzhou. Sediment-associated legacy organochlorine pesticides and CUPs—including organophosphates, pyrethroids, fipronil, and abamectin—were analyzed. Additionally, the relative toxicity of the sediments was evaluated with 10-d bioassays using *Chironomus dilutus*. Fifteen of 16 sediments collected from the stream were acutely toxic to *C. dilutus*, with 81% of the samples causing 100% mortality. Abamectin, fipronil, and pyrethroids (mainly cypermethrin) were identified as the principal contributors to the noted toxicity in the midges, with median predicted toxic units of 1.63, 1.63, and 1.03, respectively. Sediments taken from downstream sites, where residential and industrial regions were located, had elevated CUP concentrations and sediment toxicity compared with upstream sites. The present study is the first of its kind to link sediment CUPs, fipronil, and abamectin concentrations with toxicity in urban streams in China with a focus on shifting pesticide usage patterns. Environ. Toxicol. Chem. 2013;32:1040–1047. © 2013 SETAC

Keywords—Fipronil Abamectin Pyrethroids Urban stream Sediment toxicity

INTRODUCTION

China ranks as the largest producer of pesticides in the world, yet studies on environmental occurrence and ecotoxicological effects of pesticides have lagged far behind in the country. Most studies still focus on the legacy organochlorine pesticides (OCPs), even though most OCPs have been banned for use in China since the 1980s [1]. Conversely, studies on current-use pesticides (CUPs) in the environment in China are scarce [2–6] (Q. Huang, 2008, Master's thesis, Xiamen University, Xiamen, Fujian, China [in Chinese]).

Organophosphate insecticides (OPs) dominated Chinese markets until 2007, when pesticides with lower mammalian toxicity (e.g., pyrethroids, fipronil, and abamectin) were promoted as alternatives to the more toxic OPs (e.g., methamidophos). The production of pyrethroids has increased by more than 15% per year since 2007, and pyrethroids have been applied to one-third of the areas where pesticide application occurs in China (Q. Huang, 2008, Master's thesis, Xiamen University, Xiamen, Fujian, China [in Chinese]). At the same time, the demands for other CUPs, including chlorpyrifos and abamectin, have also increased, and the use of CUPs is expected to continue increasing in future years (http://market.chinabaogao.com/huagong/0120101S42011.html). The shift in pesticide application patterns has affected the occurrence of pesticide residues in the environment. A recent study of the composition of OP residues in the Jiulong River in Southeast China, for example, showed a different pattern than what was found in a previous study conducted seven years ago [2] (Q. Huang, 2008, Master's thesis, Xiamen University, Xiamen, Fujian, China [in Chinese]), and the restriction of the more toxic OPs helped explain much of the difference. The change in pesticide use patterns was also evident in a recent survey of pesticide residues in fruits and vegetables in Southeast China, in which cypermethrin was detected at the highest frequency and contributed the most to the total daily human intake of pesticide residues in that region [7].

Pesticide contamination in urban areas has drawn worldwide attention, and studies in the United States have suggested a link between pyrethroid occurrence and aquatic toxicity to nontarget species [8–14]. Urbanization in China has continued at an unprecedented pace and scale, and nearly half of the Chinese population lived in urban areas in 2010 (http://www.stats.gov.cn/english/newsandcomingevents/t20110428_402722237.htm); however, little attention has been paid to urban pesticide contamination in China. Guangzhou, China, the largest city in the Pearl River Delta (PRD), is one of the most rapidly growing areas in the world. It has a population of 12.7 million, an 83.8% urbanization rate (i.e., the proportion of the population that lives in urban areas compared with the total population), and a regional gross domestic product of 1,075 billion Renminbis in 2010 (i.e., 2.67% of the total gross domestic product of China) (http://www.gzstats.gov.cn/gzsq/201112/t20111229_27422.htm). Located in Southern China, Guangzhou possesses abundant water resources, with the Pearl River transecting the city and 231 urban streams scattered throughout the downtown area [15]. Because of the humid and warm weather, outbreaks of termites and other pests are frequent in Guangzhou, and thus pesticide usage for landscape maintenance and sanitary home use are high. In addition, during the rapid urbanization process, unbalanced development has occurred in some areas, and many so-called "urban villages" have appeared in this modernized city. Inhabited by poor and transient populations, urban villages

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All Supplemental Data may be found in the online version of this article.

[*] To whom correspondence may be addressed (youjing@gig.ac.cn).

are associated with overcrowding and squalid conditions. An investigation conducted in 2005 showed 139 urban villages in Guangzhou, accounting for 20.9% of the total area of Guangzhou, and the population in urban villages totaled 1.2 million (Z. Yang, 2011, Master's thesis, Ball State University, Muncie, IN, USA). As a unique phenomenon that formed during the process of China's urbanization efforts, urban villages are surrounded by modern urban infrastructure, and planting vegetable gardens is common practice in these villages. Thus, it was worthwhile to investigate the occurrence, toxicity, and potential sources of pesticide runoff in Guangzhou.

Recently, water quality issues in the PRD have caught the public's attention, and the input of untreated industrial and domestic sewage and pesticide runoff were considered the predominant reasons for the deterioration of the aquatic ecosystems in Guangzhou [16]. Since 2003, 38 new sewage treatment plants have been built, and water treatment capability has increased from 29 to 85% (http://special.gznews.gov.cn/2010/node_910/node_1015/2010/12/14/12923142657882.shtml). Thus, the contribution from point source pollution in aquatic ecosystems has been reduced. On the other hand, the increased areas of impervious surfaces prevents rain from infiltrating into the ground and has increased surface runoff. Consequently, the adverse impact of urban runoff has actually increased [17]. Urban streams in Guangzhou, which directly receive nonpoint source runoff, were ideal target sites to investigate the influence of urban runoff on aquatic ecosystems [15,18,19].

The objectives of the present study were to: (1) analyze legacy OCPs and CUPs in 16 sediments collected from Chebei Creek, the longest stream in the heavily populated Tianhe District in Guangzhou, China; (2) assess sediment toxicity to *Chironomus dilutus*; (3) analyze the contribution of each class of pesticides to the noted toxicity; and (4) evaluate the usage patterns and potential sources of CUPs.

MATERIALS AND METHODS

Sample collection

Tianhe is the most populous district in Guangzhou, with a population of 1.43 million. The district was mainly farmlands before 1985, but it now is the economic and commercial center of Guangzhou (http://www.gzstats.gov.cn/gzsq/201112/t20111229_ 27422.htm). Chebei Creek, the longest stream in Tianhe, was selected as the representative stream for the present study. Chebei Creek originates at Longdong Reservoir and empties into the Pearl River, with a total length of 25.4 km and a drainage area of 80 km^2. As shown in Figure 1 and in the Supplemental Data, Figure S1, sediments were collected from 16 sites (S1–S16) chosen from different land uses along Chebei Creek, including a reservoir, a botanical garden, and industrial and residential areas. The top 5 cm of sediment was sampled using a stainless steel spade shovel, sieved through a 2,000-μm sieve to remove rocks and debris, and immediately transported to the laboratory. After passing through a 500-μm sieve, the sediment was homogenized and subsampled for chemical analysis and toxicity testing, and samples were stored at –20 and 4°C, respectively. Table S1 in the Supplemental Data provides descriptions of the sampling sites and sediment characteristics.

Chemicals and reagents

Sediment samples were analyzed for 27 OCPs, five OPs, nine pyrethroid insecticides, the phenylpyrazole insecticide fipronil and its two metabolites (referred to as fipronils), and the avermectin insecticide abamectin (Supplemental Data, Table S2). Fipronil and its metabolites, abamectin, and OCPs were obtained from AccuStandard. The pyrethroids and OPs were from ChemService, with the exception of tefluthrin and chlorpyrifos, which were from Sigma-Aldrich and Ultra, respectively. All of the solid standards had purities greater than 97% as indicated by the manufacturers. Additionally, 4,4'-dibromooctafluorobiphenyl and decachlorobiphenyl (Supelco) were used as surrogates, which were added into the samples before extraction, and d10-parathion (Cambridge) and d6-*trans*-cypermethrin (Dr. Ehrenstorfer GmbH) were used as internal standards for gas chromatography–mass spectrometry (GC/MS) quantification.

High-performance liquid chromatography–grade hexane was purchased from Burdick and Jackson. Analytical grade dichloromethane and acetone were purchased from Tianjin Chemical Reagent Factory and were redistilled before use. Copper powder was activated using concentrated HCl and sequentially washed with distilled water, methanol and dichloromethane. Silica gel and alumina adsorbents were sonicated with acetone, dried, and baked at 180 and 250°C, respectively, and anhydrous Na_2SO_4 was baked at 450°C for 4 h before use.

Sediment extraction and cleanup

The samples were extracted using a CW-2000 ultrasound-assisted microwave extractor (Xintuo) and followed a previously developed method [20]. Briefly, 20 g freeze-dried sediment, 2 g activated Cu powder, 100 ml extraction solution (hexane: acetone 1:1, v/v), and the surrogates were added into a 250-ml extraction flask. The ultrasound and microwave power were set at 50 and 100 W, respectively. The extraction was conducted for 6 min and was repeated with an additional 50 ml fresh extraction solution. The extracts were combined, filtered, and concentrated, and the solvent was exchanged to hexane using a Turbovap (Xintuo).

The extracts for pesticide analysis were cleaned using solid-phase extraction cartridges packed with primary/secondary amine and granular black carbon (Supelco). The dual-layer cartridge was prewashed with hexane before loading the extract. After the extract was loaded, 7 ml of a mixture of hexane and dichloromethane (7:3, v/v) was used to elute the target compounds from the cartridge. The eluent was collected, concentrated, and solvent exchanged to hexane and subsequently analyzed on GC/MS for OPs, pyrethroids, and OCPs after adding the internal standards. An additional 7 ml hexane and acetone solution (1:1, v/v) was used to elute the target analytes from the cartridge. After the completion of the OP, pyrethroid, and OCP analyses, the two effluents were combined, and fipronil and its metabolites were analyzed using GC/MS. The cleaned extracts were evaporated to near dryness, and two derivatization reagents—300 μl trifluoroacetic anhydride in acetonitrile (1:2, v/v) and 200 μl 1-methylimidazole in acetonitrile (1:1, v/v)—were added to the dry extract residues. After 10 min of derivatization in a refrigerator, the extracts were analyzed for abamectin using high-performance liquid chromatography following a previously developed method [21].

Instrumental analysis

The target contaminants—including OPs, pyrethroids, and fipronil and its metabolites— were analyzed on a Shimadzu QP-2010 Plus series GC/MS in negative chemical ionization mode. The OCPs were analyzed on the GC/MS in electron

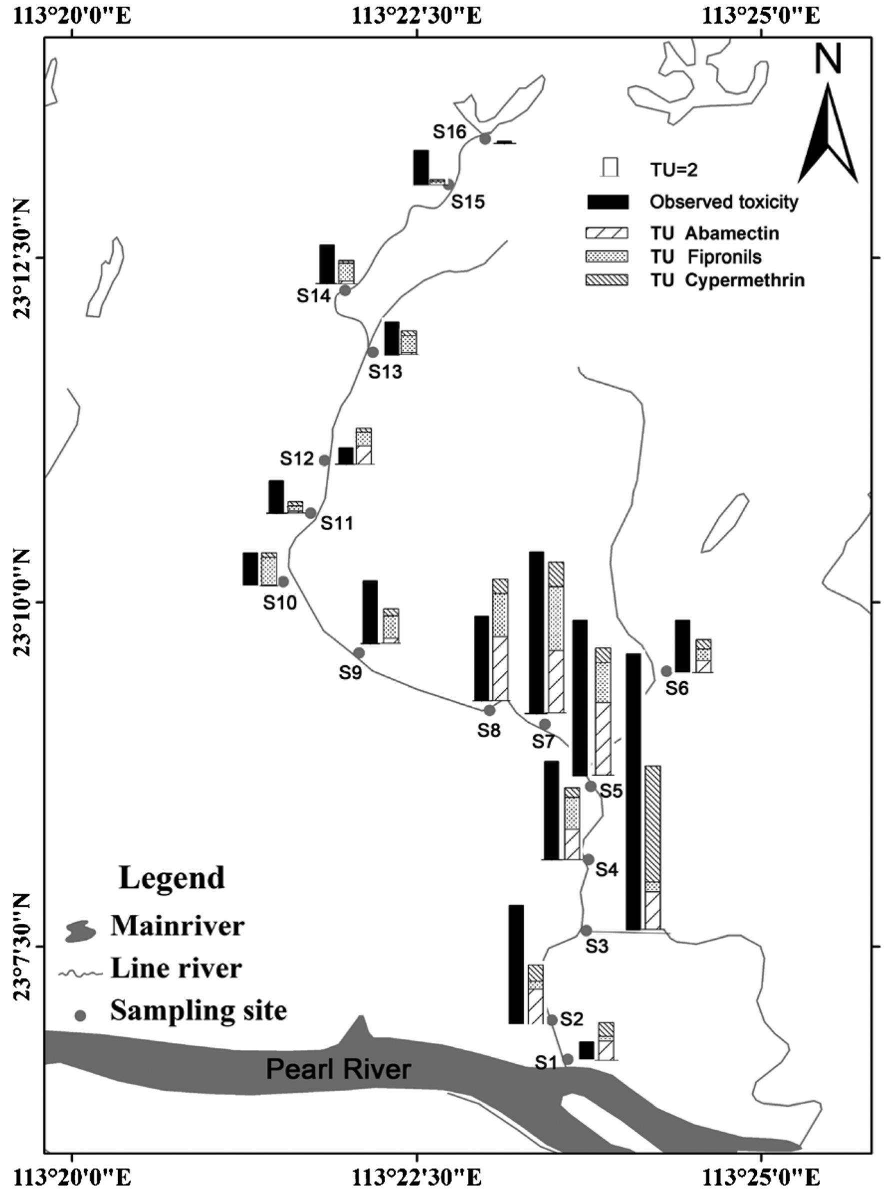

Fig. 1. Toxic units (TUs) derived from sediment concentrations of abamectin, fipronil and its metabolites (fipronils), and cypermethrin as well as the observed toxicity to *Chironomus dilutus* at each site along Chebei Creek in Guangzhou, China. The height of the first legend (TU = 2) was equal to a value of 2 for the observed toxicity or TU.

impact mode, and abamectin was analyzed on an Agilent 1200 high-performance liquid chromatography system equipped with a fluorescence detector [21]. More details on instrumental analysis are shown in the Supplemental Data.

A comprehensive set of quality control parameters included a solvent blank, a matrix blank, a matrix spike and its duplicate, and surrogate recovery. Moreover, the instruments were calibrated by analyzing calibration standards every 10 samples, and the variations of the calibration check standards were less than 20% for all analytes. No target compounds were detected in the blanks, and the recoveries of pesticides were from 79.3 to 152%. Before extraction, 4,4'-dibromooctafluorobiphenyl and decachlorobiphenyl were added to each sample as surrogates to evaluate performance of sample preparation processes, and recoveries were 69.6 ± 19.0% and 94.9 ± 25.0%, respectively. Reporting limits were used in the present study to define the lowest concentrations of the analytes that could be accurately quantified. The reporting limit was calculated by multiplying the lowest calibration standard concentration by the volume of the cleaned extract and then dividing that number by the dry weight of the sediment used for extraction.

Total organic carbon (TOC) and grain size of the sediments were also analyzed. After removing inorganic carbon with 1 mol/L HCl, the TOC of the sediment samples were analyzed using an elemental analyzer (ElementarVavio EL III). The size fraction distribution of the sediment was determined by wet sieving the sediment through 830-, 180-, and 58-μm sieves.

Toxicity testing

The benthic invertebrate *C. dilutus* was chosen as the test organism for the bioassays, because *Chironomus* species are native to the PRD, and this species is recommended by the U.S. Environmental Protection Agency (U.S. EPA) for toxicity testing [22]. Moreover, the midges are particularly sensitive to several target CUPs, including fipronil and abamectin. Midges were cultured at the Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, in accordance with U.S. EPA protocols, and third instar midge larvae were selected for the bioassays, with instar status determined using length and head capsule estimates [22].

Sediments collected from the PRD exhibited significant acute lethality to the midges [5]; therefore, 10-d sediment toxicity tests were used in the present study. Five replicates were used for all of the toxicity tests, and the bioassays followed standard U.S. EPA protocols [22]. Wet sediment (80 g) was transferred into a 400-ml beaker with 250 ml reconstituted water. After allowing the sediment to settle overnight, we added 10 midge larvae to each beaker and conducted bioassays at 23 ± 1°C under a 16:8 light:dark photocycle in an automated water delivery system. The overlying water was changed twice daily. Organisms were fed 1 ml ground fish food at 6 g/L per beaker per day, and water parameters (dissolved oxygen, conductivity, temperature, and pH) were monitored daily. At the end of the exposure, organism mortality was assessed by sieving the sediment and organisms with a 500-μm sieve.

Sediment collected from a drinking water reservoir near Guangzhou (Conghua) was used as control sediment; it showed no toxicity, and no target analytes were detected. For sediments with 100% mortality, dilutions were performed using the Conghua control sediment based on TOC content. Three separate dilutions of the original field sediments were performed and included 50%, 12.5%, and 6.25% dilutions. All of the toxicity tests were completed within two months of collection as recommended by the U.S. EPA protocols [22]. Given this time constraint, no further dilution testing was conducted, although mortality was still greater than 70% for some sediments even after being diluted to 6.25% of the original field sediments. In addition to using Conghua sediment as a control for diluting the field sediments, sediment collected from Longdong reservoir (S16) served as a reference sediment and was processed along with all of the diluted sediment samples. The Longdong reservoir sediment was from the origin of the target creek and showed no acute toxicity to the midges and therefore was a good choice as a reference sediment.

Data analysis

Adverse effects in the bioassays were expressed as the observed toxicity and calculated from mean mortality of the organisms. For sediments that caused less than 100% mortality to the midges, observed toxicity was calculated using Equation 1

$$\text{Observed toxicity} = \frac{\text{Percent mortality}}{50} \quad (1)$$

When 100% mortality occurred, test sediment was diluted with control sediment, and Equation 2 was used

$$\text{Observed toxicity} = 2 + \frac{\text{Percent mortality}}{50} \times (n - 1) \quad (2)$$

The number 2 in the represented 100% mortality observed for the undiluted sediment, and the remaining portion of the equation calculated the toxicity contribution after sediment dilution and included a dilution factor n, which indicated how much the sediment was diluted and was the reciprocal of the percentage of the amount of original sediment used. Thus, n equaled 2, 8, and 16 for the three sediment dilutions, where 50%, 12.5%, and 6.25% of the original sediment was used for dilution, respectively (Supplemental Data, Fig. S2).

Traditionally, observed toxic unit was used to express toxicity noted in the bioassays, and it was calculated as 100 divided by the percentage of original sediment causing 50% mortality in a series of dilution tests. The traditional observed toxic unit calculation was not used in the present study for two main reasons. First, sediments from Chebei Creek showed extremely high toxicity to the midges, with two sediments still causing 100% midge mortality even when those sediments were diluted to 6.25% of their original value. To find the dose that would produce 50% mortality required further dilution of the test sediment to extremely low levels, which might significantly increase uncertainty in the toxicity evaluation because of the difficulty in homogenizing the sediment. Second, 13 of the 16 sediments in Chebei Creek exhibited 100% mortality to the midges in their original form and required extensive dilution. To establish a dose–response curve to find the percentage of original sediment causing 50% mortality required sediment being diluted to various doses showing a wide span of toxic responses. The requirement of conducting toxicity testing within two months after sediment collection [22] made it impractical to run more cycles of toxicity testing after three cycles of sediment dilution. As a consequence, the traditional observed toxic unit calculation was not applied in the present study; rather, the dilution factor (Eqn. 2) was introduced as a more practical way to compare the trend of mortality among the test sediments. To conduct an observed toxicity calculation using the dilution factor, the shapes of the dose–response curves were assumed to be similar for all of the sediment samples. The calculated observed toxicity values may not be the same as those obtained using the traditional method, but it is an appropriate calculation to compare toxicity among the sediments from the same creek.

In addition to the observed toxicity derived from the bioassays, a toxic unit (TU) estimated from the chemical analysis was used to evaluate the contribution of each contaminant to the noted toxicity and was calculated from measured sediment concentrations. The TU was the ratio of contaminant concentration in the sediment to the 50% lethal concentration (LC50) of the contaminant (Supplemental Data, Table S3)

$$\text{TU} = \frac{\text{Sediment concentration(TOC normalized)}}{\text{LC50(TOC normalized)}} \quad (3)$$

Although this may be slightly conservative, concentration addition was recommended as the most broadly applicable model for predicting mixture toxicity of pesticides [23]. Consequently, concentration addition (summation of TUs of individual contaminant) was used to assess sediment toxicity caused by a mixture.

Sediment toxicity was compared with the control and reference sediments using *t* tests, and a significant difference ($p < 0.05$) indicated sediments that were toxic to the midges. The comparisons and regressions were processed using SAS.

RESULTS AND DISCUSSION

Occurrence of pesticides

Mehler et al.'s recent study [5] analyzed a wide range of contaminants—including ammonia, metals, and hydrophobic organic contaminants in PRD urban waterways—and found that pyrethroids were the main cause of sediment toxicity to *C. dilutus*, a local benthic organism that has been widely used in sediment toxicity testing. Hence, the present study emphasized insecticides. Sediment concentrations of legacy OCPs in Chebei Creek sediment were relatively low (4.4 to 89.3 ng/g dry wt for sum OCPs; Supplemental Data, Table S4), and this was consistent with a previous study conducted on Guangzhou streams [15].

Sediment-associated CUP concentrations in Chebei Creek are shown in the Supplemental Data, Table S5. Because of their relatively high water solubility and shorter half-lives [24,25], the OPs had the lowest concentrations among the target CUPs, although they were widely used in China (Q. Huang, 2008, Master's thesis, Xiamen University, Xiamen, Fujian, China [in Chinese]). Chlorpyrifos was the only OP detected in sediment above the reporting limits, and concentrations ranged from less than the reporting limit to 17.8 ng/g dry weight. Compared with the OPs, pyrethroids were found at greater frequencies and concentrations. Pyrethroids were detected in all sediments except S16, which was located at the origin of the stream. Sum concentrations of pyrethroids at the other 15 sites ranged from 18 to 468 ng/g dry weight, with a median of 110 ng/g dry weight. Pyrethroids were also frequently detected in sediments from urban areas in California, USA [8–11], but the composition of the detected pyrethroid residues had different patterns in the two countries. Bifenthrin was the predominant pyrethroid detected in sediments collected in the United States [8–14]. In contrast, cypermethrin was the predominant pyrethroid in Chebei Creek sediments and accounted for 48% of sum pyrethroid concentrations, followed by permethrin, *lambda*-cyhalothrin, and fenpropathrin, with contributions of 24%, 11%, and 8.6%, respectively. These results were similar to our previous study in the PRD [5], and the high detection frequency and concentrations of sediment-associated cypermethrin corresponded to cypermethrin being the most commonly used pyrethroid in China [26].

Fipronil and abamectin were also detected in Chebei Creek sediments. Because fipronil sulfide and sulfone have similar toxic effects to midges as their parent compound [27], they were also included in the analyses. Fipronils were detected in all of the sediments at concentrations ranging from less than reporting limit to 54.2 ng/g dry weight. On average, fipronil had lower concentrations (1.9 ng/g dry wt) than fipronil sulfide (3.2 ng/g dry wt) and fipronil sulfone (7.8 ng/g dry wt), suggesting degradation of fipronil in sediments. Fipronil has been shown to transform to fipronil sulfone under both aerobic and anaerobic conditions, whereas fipronil sulfide is only formed under anaerobic conditions [28]. Both degradation products were detected in sediment in this stream, suggesting the presence of both aerobic and anaerobic conditions. To date, only two studies have reported occurrence of sediment-associated fipronil in China. Huang (Q. Huang, 2008, Master's thesis, Xiamen University, Xiamen, Fujian, China [in Chinese]) detected up to 2.6 ng/g dry weight fipronil in Jiulong River sediments, whereas fipronil sediment concentrations as high as 35 ng/g dry weight were reported in a drainage channel of a wastewater treatment plant [29]. Fipronil concentrations in the present study (less than the reporting limit to 9.1 ng/g dry wt) were in agreement with these previous studies [28] (Q. Huang,2008,Master's thesis, Xiamen University, Xiamen, Fujian, China [in Chinese]). Although no other study on the occurrence of fipronil metabolites in China was found, the sum concentrations of fipronil and its metabolites in Chebei Creek (less than the reporting limit to 54.2 ng/g dry wt) were similar to those in urban areas in California, USA (0.18–16 ng/g dry wt) [11], but lower than that in Texas, USA (<reporting limit to 560 ng/g dry wt) [12].

Abamectin is extensively used in agricultural applications, is applied for fire ant control in homes, is used as an anthelmintic control agent for veterinary purposes, and is one of the most widely used pesticides in Southwest China [26]. To date, the investigations of abamectin have mainly concentrated on its degradability and toxicity to aquatic organisms in the laboratory setting, whereas monitoring field residues has not yet been conducted [21,30]. In the present study, abamectin was detected in 14 of the 16 sediments at concentrations ranging from 1.8 to 45.5 ng/g dry weight and a median of 9.1 ng/g dry weight, which indicated widespread presence of abamectin in urban streams.

Overall, both legacy OCPs and CUPs were detected in Chebei Creek sediments (Fig. 2 and Supplemental Data, Tables S4 and S5). Pyrethroids were the most dominant components (with an average percentage of 64.6% of the total pesticides), followed by OCPs (18.6%), abamectin (7.15%), fipronils (6.82%), and the OP chlorpyrifos (2.85%).

Contribution of pesticides to sediment toxicity

In addition to chemically analyzing sediment-associated pesticides, sediment toxicity was also assessed using the benthic invertebrate *C. dilutus*. Throughout the 10-d bioassays, water quality was within the required limits [22], with dissolved oxygen of 4.7 ± 1.4 mg/L, conductivity of 338 ± 30 μS/cm, temperature of 23.5 ± 0.6 °C, pH of 7.3 ± 0.3, and ammonia of 0.7 ± 0.3 mg/L, respectively. Midge mortality in the control sediments was less than 15%. In contrast, significant mortality was observed in 15 of the 16 sediments, with sediment S16 being the only non-toxic sediment. Sediment S16, which was collected at the origin of Chebei Creek, was used as the reference sediment because it was not toxic to the midges (<15% midge mortality). Thirteen sediments exhibited 100% mortality to the midges. To more accurately determine toxicity, dilution of those sediments causing 100% mortality was conducted, and sediment toxicity values expressed as observed toxicity are shown in Table 1. The observed toxicity values for Chebei Creek sediments ranged from 0.24 to 29.6, with a median of 4.86, and six sediments had observed toxicity greater than 8. Similar toxicity was noted in a previous PRD study of the PRD city sediments in Shenzhen city [5], indicating serious deterioration of aquatic ecosystems in urban areas in China. Through a Water Environment Restoration

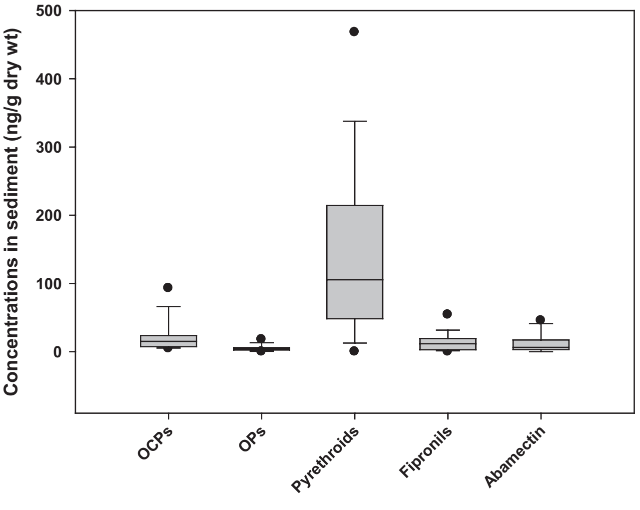

Fig. 2. Boxplot of sum concentrations (ng/g dry wt) of organochlorine pesticides (OCPs); organophosphate pesticides (OPs); pyrethroids; fipronil, fipronil sulfide, and fipronil sulfone (fipronils); and abamectin in the 16 sediments collected from Chebei Creek in Guangzhou, China. The two dots represent the highest and lowest concentrations. The three lines of the box represent 25%, 50% and 75% of the pesticide concentrations, and the bars represent the standard deviations.

Table 1. Observed toxicity and the toxic units (TU) of each class of contaminants to *Chironomus dilutus* exposed to the 16 sediments collected from Chebei Creek in Guangzhou, China

		TU						
Site	Observed toxicity	Pest[a]	Fip	Aba	Pyre	OPs	OCPs	PAHs[b]
S1	1.92	4.29	0.50	2.06	1.72	0.01	<RL[c]	0.05
S2	12.7	6.90	0.85	3.88	2.14	0.03	ND	0.09
S3	29.6	18.3	1.03	4.06	12.7	0.05	0.50	0.09
S4	10.5	8.32	3.41	3.23	1.62	0.03	0.03	0.06
S5	16.7	14.2	4.26	7.73	2.04	0.03	0.08	0.07
S6	5.62	3.70	1.24	1.27	1.17	0.02	ND	0.03
S7	17.3	16.9	6.79	6.67	3.28	0.05	0.05	0.12
S8	9.02	13.2	4.58	6.83	1.72	0.05	ND	0.05
S9	6.70	3.83	2.42	0.51	0.88	0.02	ND	0.02
S10	3.53	3.86	2.97	0.07	0.59	0.01	0.21	0.04
S11	3.46	1.42	0.57	0.19	0.57	0.01	0.07	0.04
S12	1.76	3.91	1.43	1.99	0.47	0.02	ND	0.09
S13	3.63	2.70	1.82	0.18	0.65	0.01	0.04	0.05
S14	4.10	2.52	1.89	0.32	0.30	0.01	ND	0.06
S15	3.81	0.59	0.32	ND	0.25	<0.01	0.01	0.02
S16	0.24	0.08	<RL[c]	ND	ND	<RL[c]	0.08	0.05
Median	4.86	3.88	1.63	1.63	1.03	0.02	0.07	0.05

[a] TU of all pesticides (Pest) including fipronil and its two metabolites, fipronil sulfide and fipronil sulfone (Fip); abamectin (Aba); pyrethroids (Pyre); organophosphate pesticides (OPs); and organochlorine pesticides (OCPs).

[b] TU of polycyclic aromatic hydrocarbons (PAHs).

[c] Lower than the reporting limit (RL). The RL was calculated by multiplying the lowest concentration of the calibration standards by the volume of the cleaned extract, and then dividing it by the dry weight of sediment extracted.

ND = not detected.

Project, 486 billion Renminbis were invested to restore water quality of urban streams in Guangzhou, including Chebei Creek from November 2008 to June 2010, and water quality was greatly improved (http://special.gznews.gov.cn/2010/node_910/node_1015/2010/12/14/12923142657882.shtml). However, after the completion of this project, several streams became degraded again over time, and contaminants released from the sediment might be one of the reasons [31].

Sediment TOC is known to affect bioavailability and toxicity of HOCs; thus, to better compare toxicity among sites, TOC-normalized sediment concentrations were used for TU calculations (Table 1 and Supplemental Data, Fig. S3 and Table S3). The sum TUs estimated from all pesticides was well correlated with observed toxicity for *C. dilutus* (observed toxicity = 1.18 TU + 0.48, $r^2 = 0.81$, $p < 0.05$, Fig. 3). As shown in Table 1, TUs for the legacy OCPs were all less than 0.01, with the exception of three OCPs (endosulfan, endrin, and nonachlor), which are still in use and contributed minimally to toxicity in sediments S3 and S10 with OCP TUs of 0.50 and 0.22, respectively. Chlorpyrifos was the only OP detected in sediment with TUs being less than 0.1 at all sites. Pyrethroids had a significant contribution to sediment toxicity to the midges in our previous PRD study [5], and their TUs in the present study were elevated as well. The sum pyrethroid TUs ranged from not detected to 12.7, with a median of 1.03 (Table 1). Similar to a previous study [5], cypermethrin contributed the most to pyrethroid toxicity to *C. dilutus* (82%) with a median TU of 0.88 (range from not detected to 12.7). Cypermethrin alone had greater than 1 TU in half of the 16 sediments, and greater than 2 TUs in sediments S3 and S7. Furthermore, a significant correlation was found between observed toxicity and pyrethroid TU (observed toxicity = 2.25 TU + 3.80, $r^2 = 0.78$, $p < 0.0001$), which supported the conclusion that pyrethroids (particularly cypermethrin) were one of the major contributors to the noted toxicity in *C. dilutus*.

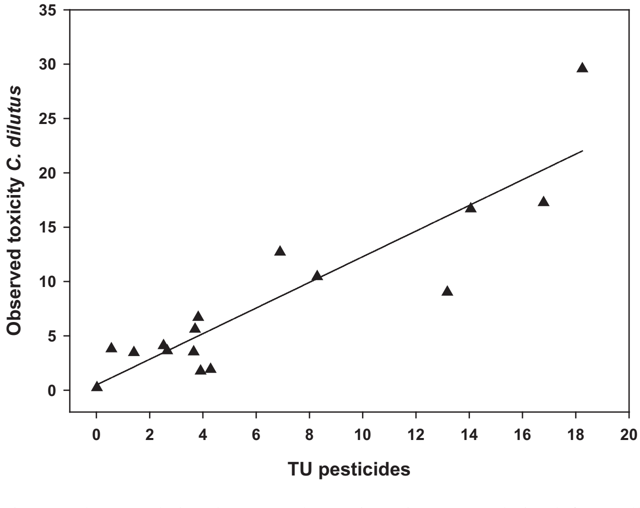

Fig. 3. The correlation between the toxic units (TUs) derived from all detected pesticide residues in sediments of Chebei Creek and the observed toxicity to *Chironomus dilutus*. The equation of the regression line was the observed toxicity = 1.18 TU + 0.48 ($r^2 = 0.81$, $p < 0.0001$).

As a γ-amino butyric acid–gated channel disruptor, fipronil was about 10 times more toxic than cypermethrin to *C. dilutus*, and LC50 values were 0.13, 0.16, and 0.12 μg/g OC for fipronil, fipronil sulfide, and fipronil sulfone, respectively [27]. To our knowledge, no study has been conducted in China that has examined fipronil toxicity to nontarget aquatic organisms; however, a recent report in California, USA, suggested that fipronil was a widespread contaminant with ecotoxicological significance [32]. In addition to its extensive use in agriculture as a replacement for OPs, fipronil was also a popular insecticide for termite control in Guangzhou before the restriction of its use in 2009 because of its high toxicity to shellfish and bees, but it is still being illegally produced and used under alternative names after its restriction (http://www.cnchemicals.com/PressRoom/PressRoomDetail.aspx?newsTypeId=r&prNewsId=61). In

Chebei Creek, fipronils were detected in all sediments tested, and TUs of the three compounds ranged from 0.02 to 6.79, with a median of 1.63. A significant correlation was found between the predicted TU of fipronils and observed toxicity (observed toxicity $= 2.14\,\text{TU} + 2.0, r^2 = 0.58, p < 0.05$) if the S3 sample was removed from regression analysis, as this sediment had extremely high toxicity but low concentrations of fipronils.

Abamectin, another γ-amino butyric acid–gated channel disruptor, has comparable toxicity to *C. dilutus* as fipronil, with an LC50 of 0.18 μg/g OC [21]. Fourteen of the 16 sediments (88%) contained detectable amounts of abamectin, and its TUs ranged from not detected to 7.73, with a median of 1.63. Similarly, regression analysis showed a strong correlation between abamectin-TUs and observed toxicity (observed toxicity $= 1.66$ TU + 2.86, $r^2 = 0.72$, $p < 0.001$), with the exception of S3. To our knowledge, no previous study has related abamectin residues in the environment with sediment toxicity, although its usage has surged worldwide in recent years. Mehler et al. [5] suggested the possibility of abamectin being the cause of sediment toxicity in the PRD according to toxicity identification and evaluation methods by addition of piperonyl butoxide, a synergistic agent for pyrethroids and abamectin. The present study identified abamectin as a major cause of sediment toxicity in Chinese urban streams.

In summary, Chebei Creek sediments were toxic to the midges. Abamectin, fipronils, and pyrethroids (mainly cypermethrin) contributed the most to the toxicity, with median TUs of 1.63, 1.63, and 1.03 (1.01 for cypermethrin alone), respectively (Fig. 1). Interestingly, sediment S3, which was the most toxic sediment sampled (observed toxicity value of 29.6), contained relatively low concentrations of abamectin (4.06 TUs) and fipronils (1.03 TUs) and high concentrations of pyrethroids (12.7 TUs, in which 12.4 TU was from cypermethrin), indicating that pyrethroids were the major contributor of toxicity for this sediment.

Although some OCPs and OPs are still in use in China today, toxic contributions to *C. dilutus* from these two classes of insecticides were negligible in the present study. In addition to the target pesticides, 16 polycyclic aromatic hydrocarbons (PAHs) and 15 polybrominated diphenyl ethers (PBDEs) were also analyzed in Chebei Creek sediment (H. Li, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou, China, unpublished data). Threshold effect concentrations were derived from sediment toxicity testing data and are commonly used for assessing sediment risk caused by PAH contamination [33]; therefore, threshold effect concentrations on a sum PAH basis were used for estimating TUs for the PAHs (0.02 to 0.12 for all sediments, Table 1). No TU calculation was conducted for PBDEs, because no benchmark values were available. However, a previous study showed that sediments from electronic waste recycling sites with much greater PBDE concentrations than the sediments in Chebei Creek were not acutely toxic to midges [5]; therefore, the contribution from PBDEs in the present study was considered minimal.

Metal contamination was also reported in urban streams in Guangzhou, and the predicted TU from metal concentrations reported in the literature ranged from 0.09 to 58.3 (mean value of 1.52) TUs, based on effect-range median values. This suggests that heavy metals in some of the Guangzhou urban streams might play a role in the noted sediment toxicity [15]. However, our previous study indicated that metals had relatively low contribution to acute toxicity to the midges exposed to sediment collected from the Pearl River Delta [5]. Another contaminant that could contribute to sediment toxicity to *C. dilutus* was ammonia. Fu et al. [34] reported that ammonia concentrations in sediment pore water of Guangzhou urban streams ranged from 19.9 to 152 mg/L. The highest ammonia concentration of 78.6 mg/L (0.54 TU) in the previous PRD study [5] was found in a sediment from Guangzhou, and it was within the range found in the Fu et al. study [34]. Therefore, ammonia might also contribute minimally to sediment toxicity in Chebei Creek at some sites, but its contribution was most likely lower than that of the CUPs.

Distribution and sources of pesticides

In general, the spatial trends of the four classes of CUPs were similar along the stream, whereas OCPs showed a different pattern (Fig. S3). Sediments with more CUP residues were mainly from the down- and middle-stream segments of the stream, where residential (sites 1–4 and 6) and industrial areas (sites 5, 7, and 8) dominated. Li et al. [4] suggested that population density correlated, to some extent, with CUP occurrence in sediment because of their extensive homeowner use. Conversely, OCPs were detected at higher concentrations in upstream sites. The legacy OCPs were widely used for agricultural purposes in the study areas, which formerly were agricultural lands before the formation of the Tianhe District in 1985. Thus, it was difficult to identify the historical sources of OCPs versus current inputs of other contaminants. Additionally, TOC-normalized OCP concentrations in Longdong Reservoir (S16) were relatively high because of the extremely low TOC content of this sediment. The legacy pesticide DDT and its metabolites dominated at S16, whereas no CUPs were detected at concentrations greater than reporting limits, and atmospheric deposition may be the source for the trace legacy OCPs found at this site.

Figure 1 mapped the distribution of observed toxicity and TU values of abamectin, fipronils, and cypermethrin along the creek, and it showed sediment toxicity followed a similar geographic distribution, with highly toxic sites being mainly located in the downstream sites of the stream. This correlation further supported the conclusion that CUPs were the main cause for the sediment toxicity in Chebei Creek.

Rice fields, vegetable gardens, and fruit fields accounted for 9.0, 19, and 8.7% of the total area of Guangzhou, respectively [18]. Chlorpyrifos, fipronil, and abamectin were recommended as the replacements for the more toxic OPs for pest control in rice and vegetable culturing (http://www.chinapesticide.gov.cn/doc07/07102301-1.html). Because of the unbalanced urbanization process, many urban villages have arisen in modernized areas in Guangzhou, where planting vegetable gardens is common. Therefore, urban villages scattered within residential areas might be an important source of the three classes of CUPs. In contrast, pyrethroids were mainly used in professional and home pest control, and their sediment concentrations showed a correlation with an urban fingerprint [4,6]. Trace concentrations of pyrethroids and fipronil were detected in the wastewater treatment plant effluent [29,35]. Additionally, poor sanitation conditions in the urban villages promote the breeding of mosquitoes, which would result in the extensive use of mosquito control agents (mainly pyrethroids); therefore, urban villages might also be a source for pyrethroids, similarly to the other three vegetation-use CUPs.

CONCLUSIONS

An urban stream in the highly urbanized Chinese city of Guangzhou was selected as the study area to evaluate ecotoxicological significance of CUPs. High levels of sediment-associated CUPs, including abamectin, fipronils, and pyrethroids, were detected in the stream and correlated well with the noted midge toxicity. The present study suggested that CUPs released by residential use, urban landscape maintenance, and vegetable gardens, in addition to the use of mosquito control agents in urban villages, have caused degradation of the aquatic ecosystem. Future risk assessments in China should pay special attention to the complicated land-use patterns created by the unbalanced development during rapid urbanization, such as urban villages in modernized cities.

SUPPLEMENTAL DATA

The Supplemental Data presents information on instrumental analysis.

Tables S1-S5.

Figs. S1-S3. (389 KB DOC).

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REFERENCES

1. Guo Y, Yu H, Zeng EY. 2009. Occurrence, source diagnosis, and biological effect assessment of DDT and its metabolites in various environmental compartments of the Pearl River Delta, South China: A review. *Environ Pollut* 157:1753–1763.
2. Zhang Z. 2001. Environmental behavior and risks of organic pesticides in the estuarine agricultural watershed. PhD dissertation. Xiamen University, Xiamen, Fujian, China (in Chinese).
3. Zhang Z, Dai M, Hong H, Zhou JL, Yu G. 2002. Dissolved insecticides and polychlorinated biphenyls in the Pearl River Estuary and South China Sea. *J Environ Monit* 4:922–928.
4. Li H, Mehler WT, Lydy MJ, You J. 2011. Occurrence and distribution of sediment-associated insecticides in urban waterways in the Pearl River Delta, China. *Chemosphere* 82:1373–1379.
5. Mehler WT, Li H, Lydy MJ, You J. 2011. Identifying the causes of sediment-associated toxicity in urban waterways of the Pearl River Delta, China. *Environ Sci Technol* 45:1812–1819.
6. Wang J, Li H, You J. 2012. Distribution and toxicity of current-use insecticides in sediment of a lake receiving waters from areas in transition to urbanization. *Environ Pollut* 161:128–133.
7. Chen C, Qian Y, Chen Q, Tao C, Li C, Li Y. 2011. Evaluation of pesticide residues in fruits and vegetables from Xiamen, China. *Food Control* 22:1114–1120.
8. Weston DP, Holmes RW, You J, Lydy MJ. 2005. Aquatic toxicity due to residential use of pyrethroid insecticides. *Environ Sci Technol* 39: 9778–9784.
9. Amweg EL, Weston DP, You J, Lydy MJ. 2006. Pyrethroids insecticides and sediment toxicity in urban creeks from California and Tennessee. *Environ Sci Technol* 40:1700–1706.
10. Holmes RW, Anderson BS, Phillips BM, Hunt JW, Crane DB, Mekebri A, Connor V. 2008. Statewide investigation of the role of pyrethroids pesticides in sediment toxicity in California's urban waterways. *Environ Sci Technol* 42:7003–7009.
11. Lao W, Tsukada D, Greenstein DJ, Bay SM, Maruya KA. 2010. Analysis, occurrence, and toxic potential of pyrethroids, and fipronil in sediments from an urban estuary. *Environ Toxicol Chem* 29:843–851.
12. Hintzen EP, Lydy MJ, Belden JB. 2009. Occurrence and potential toxicity of pyrethroids and other insecticides in bed sediments of urban streams in central Texas. *Environ Pollut* 157:110–116.
13. Ding Y, Harwood AD, Foslund HM, Lydy MJ. 2010. Distribution and toxicity of sediment-associated pesticides in urban and agriculture waterways from Illinois, USA. *Environ Toxicol Chem* 29:149–157.
14. Kuivila KM, Hladik ML, Ingersoll CG, Kemble NE, Moran PW, Calhoun DL, Nowell LH, Gilliom RJ. 2012. Occurrence and potential sources of pyrethroid insecticides in stream sediments from seven U.S. metropolitan areas. *Environ Sci Technol* 46:4297–4303.
15. Li M, Zhong J, Li S, Zheng W. 2005. Investigation and assessment of sediment pollutants in the brooklets in Guangzhou. *Guangzhou Environmental Sciences* 20:1–5 (in Chinese).
16. Zhu Z, Deng Q, Zhou H, Ouyang T, Kuang Y, Huang N, Qiao Y. 2002. Water pollution and degradation in Pearl River Delta, South China. *Ambio* 31:226–230.
17. Xia H, Lin F. 2010. Study on quality characterization of runoff in Guangzhou urban area. *Chin J Environ Sci Manag* 35:129–131 (in Chinese).
18. Wang C, Fang Z, Zheng S, Jiang Y. 2003. Concentrations and distribution of heavy metals in sediments and benthic organisms from sewage stream in Guangzhou city. *Chin J Saf Environ* 3:40–43 (in Chinese).
19. Wang X, Nie X, Huang Z, Zhu D, Li K. 2006. Toxicity of the lixivium of the sediment from streams of Guangzhou to aquatic organisms. *Asia J Ecotoxicol* 1:180–185 (in Chinese).
20. Li H, Wei Y, You J, Lydy MJ. 2010. Analysis of sediment-associated insecticides using ultrasound assisted microwave extraction and gas chromatography-mass spectrometry. *Talanta* 83:171–177.
21. Ding Y, Weston DP, You J, Rothert AK, Lydy MJ. 2011. Toxicity of sediment-associated pesticides to *Chironomus dilutus* and *Hyalella azteca*. *Arch Environ Contam Toxicol* 61:83–92.
22. U.S. Environmental Protection Agency. 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. EPA 600/R-99/064. Washington, DC.
23. Belden JB, Gilliom RJ, Lydy MJ. 2007. How well can we predict the toxicity of pesticide mixtures to aquatic life? *Integr Environ Assess Manag* 3:364–372.
24. Bondaenko S, Gan J. 2004. Degradation and sorption of selected organophosphate and carbamate insecticides in urban stream sediments. *Environ Toxicol Chem* 23:1809–1814.
25. Gan J, Lee SJ, Liu WP, Haver DL, Kabashima JN. 2005. Distribution and persistence of pyrethroids in runoff sediment. *J Environ Qual* 36:834–841.
26. Whittle B. 2010. Communities in peril: Asian regional report on community monitoring of highly hazardous pesticide use. Pesticide Action Network Asia and the Pacific, Penang, Malaysia.
27. Maul JD, Brennan AA, Harwood AD, Lydy MJ. 2008. Effect of sediment-associated pyrethroids, fipronil, and metabolites on *Chironomus tentans* growth rate, body mass, condition index, immobilization, and survival. *Environ Toxicol Chem* 27:2582–2590.
28. Lin K, Haver D, Oki L, Gan J. 2008. Transformation and sorption of fipronil in urban stream sediments. *J Agri Food Chem* 56:8594–8600.
29. Yang X, Ying G, Kookana RS. 2010. Rapid multiresidue determination for currently used pesticides in agricultural drainage waters and soils using gas chromatography-mass spectrometry. *J Environ Sci Health B* 45:152–161.
30. Tisler T, Erzen NK. 2006. Abamectin in the aquatic environment. *Ecotoxicol* 15:495–502.
31. Yu G, Lei H, Liu G, Bai T, Liu K, Chen R. 2007. Research on the characteristics of sediment release in a heavily polluted tidal river and control technologies. *Acta Sci Circumstant* 27:1476–1484 (in Chinese).
32. Gan J, Bondarenko S, Oki L, Haver D, Li JX. 2012. Occurrence of fipronil and its biologically active derivatives in urban residential runoff. *Environ Sci Technol* 46:1489–1495.
33. Swartz RC. 1999. Consensus sediment quality guidelines for polycyclic aromatic hydrocarbon mixtures. *Environ Toxicol Chem* 18:780–787.
34. Fu G, Yu Y, Ye H, Chen F. 2010. Nitrogen and phosphorus pollution of river in urban area of Guangzhou. *Water Resour Prot* 26:24–28 (in Chinese).
35. Chen M, Ren R, Wang Z, Lin X, Zhang S. 2007. Residues of pyrethroid pesticides in wastewater of the sewage treatment plant. *Environ Monit China* 23:27–30 (in Chinese).