Environmental Pollution 178 (2013) 135–141

Contents lists available at SciVerse ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Addition of contaminant bioavailability and species susceptibility to a sediment toxicity assessment: Application in an urban stream in China

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ARTICLE INFO

Article history:
Received 14 January 2013
Received in revised form
4 March 2013
Accepted 5 March 2013

Keywords:
Bioavailable toxic unit
Sediment toxicity
Tenax extraction
Matrix-solid phase microextraction
Cypermethrin

ABSTRACT

Sediments collected from an urban creek in China exhibited high acute toxicity to *Hyalella azteca* with 81.3% of sediments being toxic. A toxic unit (TU) estimation demonstrated that the pyrethroid, cypermethrin, was the major contributor to toxicity. The traditional TU approach, however, overestimated the toxicity. Reduced bioavailability of sediment-associated cypermethrin due to sequestration explained the overestimation. Additionally, antagonism among multiple contaminants and species susceptibility to various contaminants also contributed to the unexpectedly low toxicity to *H. azteca*. Bioavailable TUs derived from the bioavailability-based approaches, Tenax extraction and matrix-solid phase microextraction (matrix-SPME), showed better correlations with the noted toxicity compared to traditional TUs. As the first successful attempt to use matrix-SPME for estimating toxicity caused by emerging insecticides in field sediment, the present study found freely dissolved cypermethrin concentrations significantly improved the prediction of sediment toxicity to *H. azteca* compared to organic carbon normalized and Tenax extractable concentrations.

1. Introduction

The toxicological significance of current-use pesticides (CUPs) in aquatic ecosystems has drawn attention to several CUPs, including pyrethroids and fipronil, that have been identified as the principal contributors of sediment toxicity to the benthos (Amweg et al., 2006; Gan et al., 2012; Kuivila et al., 2012; Mehler et al., 2011a; Weston et al., 2004, 2005, 2008a,b). In those studies, organic carbon (OC) normalized sediment concentrations were used as a dose metric of toxicity. Because OC is an important factor controlling the partitioning of hydrophobic contaminants between sediment and porewater, OC-normalized concentrations have been proposed as a substitute for bulk sediment concentrations as the dose metric to estimate adverse effects of sediment-associated contaminants (Di Toro et al., 1991). Generally, OC-normalized CUP concentrations determined by exhaustive extraction were used in calculating toxic units (TU), without further considering the bioavailability of the contaminants. Consequently, the traditional TU approach may overpredict sediment toxicity. For example, over-predication of toxicity has been reported for the amphipods *Hyalella azteca* and *Eohaustorius estuarius* using pyrethroid-derived TUs at some sites (Amweg et al., 2006; Kuivila et al., 2012; Lao et al., 2012).

Similarly, other studies have shown OC-normalized concentrations may overestimate bioavailability of contaminants (Cornelissen et al., 2005; Reichenberg and Mayer, 2006). One of the reasons for this discrepancy is that contaminants have distinct sorption capacities to different types of OC, but the understanding of OC classification and the partitioning processes of contaminants within OC is still quite limited (Maruya et al., 2012). In addition, other factors may also affect bioavailability and toxicity of sediment-associated contaminants, including sediment grain size (Mehler et al., 2011b), sediment aromaticity and planarity (Lyytikainen et al., 2003), niches of the test organisms (Wang et al., 2004), and chemical properties (You et al., 2007). Thus, it is beneficial to incorporate bioavailability measurements into risk assessments to improve the accuracy of sediment toxicity estimates (Maruya et al., 2012).

Chemical extraction techniques, such as Tenax extraction and matrix-solid phase microextraction (matrix-SPME), have been developed to measure the bioavailability of sediment-associated contaminants (Cornelissen et al., 1997; Mayer et al., 2000; Reichenberg and Mayer, 2006). Bioavailable concentrations

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http://dx.doi.org/10.1016/j.envpol.2013.03.022

measured by desorption-based Tenax extraction better explained sediment toxicity of pyrethroid-contaminated sediments in the field compared to OC-normalized concentrations (You et al., 2008). Additionally, the freely dissolved concentrations of contaminants measured by matrix-SPME have also successfully assessed bioaccumulation potential and toxicity of pesticides (Ding et al., 2013; Harwood et al., 2012; Xu et al., 2007). However, most research to date has still focused on measuring the bioavailability of legacy contaminants, while CUPs are often the most important contributors to toxicity to benthic invertebrates (Maruya et al., 2012).

The objectives of the present study were to: 1) assess toxicity of CUPs in field-collected sediments to *H. azteca* using the traditional TU approach; 2) evaluate if sediment toxicity was overestimated with CUP-derived TUs; 3) investigate the reasons for the overestimation of toxicity; and, 4) check to see if bioavailability-based measurements, including Tenax extraction and matrix-SPME, could improve the accuracy in toxicity prediction of sediment-associated CUPs to *H. azteca*. An urban stream in Guangzhou, China, where CUPs were identified as major contributors to sediment toxicity, was chosen as the site for the present study.

2. Materials and methods

2.1. Sediment collection and analysis

Guangzhou is the third largest city in China and its urban waterways are heavily polluted (Mehler et al., 2011a). Chebei Creek is the longest stream in Tianhe, which is the most populous district in Guangzhou. The stream flows through industrial, residential and agricultural areas, receiving contaminants from various sources. Our previous study noted that CUPs in Chebei Creek sediment were extremely toxic to the midge *Chironomus dilutus* (Li et al., 2013). The severe pollution status, wide range of sediment characteristics, and multiple sources of contamination along the stream made Chebei Creek a good site to investigate the influence of bioavailability on sediment toxicity.

Sixteen sediments were collected along the stream as shown in Fig. 1. The top 5 cm of sediment was collected using a stainless steel spade shovel. Sediments were then passed through a 2 mm sieve to remove rocks and debris, transported to the laboratory, stored at 4 and −20 °C for toxicity testing and chemical analysis, respectively.

The CUPs analyzed included five organophosphate insecticides (OPs), eight pyrethroids, fipronil and its metabolites and abamectin and the sediment analysis using exhaustive extraction was detailed in Li et al. (2013). In brief, freeze-dried sediment was extracted for 6 min using an ultrasound-assisted microwave extraction (UAME) with 100 ml of a mixture of acetone and hexane (1:1, v/v) and ultrasound and microwave power being set at 50 and 100 W, respectively. After filtration, the extracts were concentrated using a Turbovap (Xintuo, Shanghai, China) and solvent exchanged to hexane. The dual-layer cartridges with primary secondary amine and granular black carbon (Supelco, Bellefonte, PA, USA) were used to clean the extracts and 7 ml of 30% dichloromethane in hexane (v/v) being used as elution solution. The cleaned extracts were concentrated, solvent exchanged to hexane and subsequently analyzed on the instruments.

Target CUPs, including OPs, pyrethroids and fipronil and its metabolites were analyzed on a QP-2010-plus series gas chromatography–negative chemical ionization–mass spectrometer (GC–NCI–MS) (Shimadzu Corporation, Kyoto, Japan). A DB-5HT column (15 m × 0.25 mm, 0.1 μm film thickness) was used to separate the analytes on GC–NCI–MS. Helium at a flow rate of 1.5 ml/min and methane were used as the carrier gas and NCI reaction gas, respectively. The temperature of the ion source and transfer line was set at 250 and 280 °C, respectively. The initial oven temperature was set at 60 °C, held for 1 min, heated to 200 °C at 10 °C/min, to 220 °C at 3 °C/min, held for 8 min, then to 300 °C at 50 °C/min, and held for 15 min. A programmable temperature vaporizing injection process was performed and the initial injector temperature was set at 60 °C, held for 0.1 min, and then jumped to 280 °C at 300 °C/min, and held for 17 min. Internal standard calibrations were used to quantify the analytes and d10-parathion was used as internal standard for OPs and fipronil and its metabolites, while d6-*trans*-cypermethrin was used for pyrethroids.

Abamectin was analyzed using an Agilent 1200 high performance liquid chromatography (Santa Clara, CA, USA) with fluorescence detection after derivatization and the separation was achieved using an Agilent Stablebond-C18 column (4.6 mm × 150 mm × 5 μm). Prior to analysis, the cleaned extracts were evaporated to near dryness, 300 μl of trifluoroacetic anhydride in acetonitrile (1:2, v/v) and 200 μl of 1-methylimidazole in acetonitrile (1:1, v/v) were added as derivatization reagents, and kept in a refrigerator for 10 min. A mixture of acetonitrile, methanol and water in a ratio of 47.5:47.5:5 (v/v/v) was used as the mobile phase and the flow rate of the mobile phase was set at 1.1 ml/min. Quantification was based on external calibration with six standards at the concentrations ranging from 10 to 500 ng/ml.

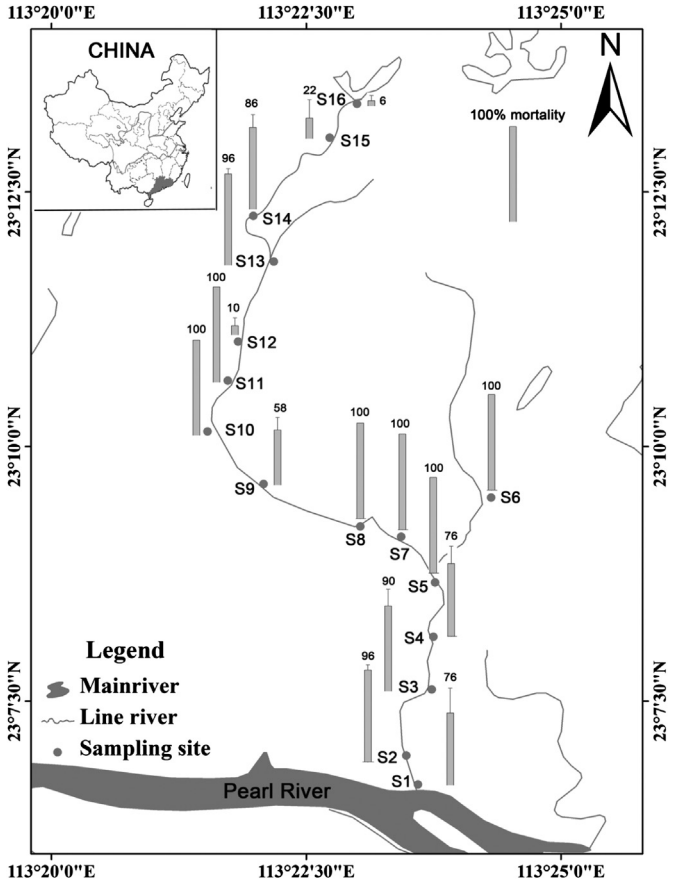

Fig. 1. The map of the sampling sites with mortality of *Hyalella azteca* being presented at each site along Chebei Creek in Guangzhou, China. The numbers above the bars are the average percent mortality of each site.

Total OC, black carbon (BC), nitrogen and hydrogen were analyzed in the sediments using an elemental analyzer (Elementar Vario EL III, Hanau, Germany). After removing the inorganic carbon with 1 mol/L HCl, the sediments were heated at 60 °C until dry or at 450 °C for 4 h, and used for the OC or BC analysis, respectively. Sediment aromaticity and planarity were calculated using the ratios of hydrogen/carbon and carbon/nitrogen, respectively (Lyytikainen et al., 2003). Finally, the sediment grain size distribution was determined by wet sieving the samples sequentially through three sieves (830 μm, 180 μm and 58 μm).

2.2. Toxicity testing

Ten-day bioassays were performed with *H. azteca* in an automated water delivery system. Each beaker contained 80 g wet sediment and 250 ml reconstituted water. After sediment was allowed to settle overnight, 10 *H. azteca* (1–2 weeks old) were introduced into each beaker. Bioassays were conducted under a 16:8 light: dark cycle at 23 ± 1 °C using five replicates. Two 150 ml water changes, feeding with 6 mg of ground fish food per beaker, and water parameter monitoring including dissolved oxygen, conductivity, temperature and pH, were performed daily. Ammonia was monitored at the beginning and end of the testing. At the end of exposure, *H. azteca* mortality was determined by sieving the sediment and organisms with a 500 μm sieve. A sediment collected from the Conghua drinking water reservoir, which was not acutely toxic to *H. azteca* (mortality <14%), was used as control sediment for the bioassays.

2.3. Bioavailability measurements

Two bioavailability-based techniques were applied to incorporate bioavailability measurements into sediment toxicity assessments. As a desorption-based measurement, a single point 24-h Tenax extraction has been conducted to estimate bioavailability of sediment-associated CUPs (Cornelissen et al., 1997; Cui et al., 2013). About 3 g (dry weight, dw) of sediment, 3 mg of NaN_3 used to prevent microbial degradation, 40 ml of reconstituted water, several copper sheets

used to remove sulfur and 1 g of Tenax beads were added into a screw-capped glass tube. The tubes were rotated using a tube rotator (QB-228, Kylin-Bell, Haimen, China) at 20 revolutions per minute for 24 h. The tests were conducted in triplicate. At the end of the test, the beads were removed and sonicated with 5 ml of acetone for 5 min, followed by two additional 5-min sonication steps with 5 ml of acetone-hexane (1:1, v/v). The extracts were combined and solvent exchanged to hexane after removing water using anhydrous Na_2SO_4. Next, the extracts were cleaned by shaking vigorously with 30 mg of primary secondary amine and 100 mg of Florisil for 2 min. The supernatants were decanted, concentrated, and analyzed on GC–NCI–MS.

As an activity-based measurement, matrix-SPME measured the freely-dissolved CUP concentrations (Mayer et al., 2000; Cui et al., 2013). Ten cm of disposable SPME fibers coated with 10 μm polydimethylsiloxane were protected in a stainless envelope with 110 μm openings. Prior to use, the envelopes containing the fibers were cleaned by sonication with methanol and distilled water. After being dried at room temperature, the fibers were inserted into 15 g of wet sediment with several copper sheets in 25 ml vials. Testing was conducted using three replicates by gently shaking the vials on a HY-4 shaker (Fuhua Instrument, Jintan, China). After a 28-d exposure, the fibers were retrieved from the sediments, washed with distilled water, dried with a paper towel, and sonicated three times using 1 ml of hexane. The extracts were combined, concentrated, and analyzed for the target CUPs using GC–NCI–MS.

2.4. Quality assurance and quality control

Sediment analysis included a comprehensive set of quality control parameters including a solvent blank, a matrix blank, a matrix spike and its duplicate, and surrogate recovery. To minimize the matrix-induced chromatographic response enhancement, matrix-matched calibration standards with isotopic internal standards (d10-parathion and d6-*trans*-cypermethrin) were applied. A calibration standard solution was analyzed every 10 samples and the variations of target CUPs in the standards were all less than 20%. No target compounds were detected in the blanks and the CUP recoveries were from 83 to 152%. Dibromooctafluorobiphenyl and decachlorobiphenyl were added to each sample before extraction as the surrogates, with recoveries being 70 ± 19% and 95 ± 25%, respectively.

2.5. Data analysis

Sediment toxicity was compared to the controls using a *t*-test and a significant difference ($p > 0.05$) indicated that the sediment was toxic to *H. azteca*. The comparison and regression were processed using SAS (Version 8.02, SAS Institute).

The traditional TU approach was applied to evaluate the contribution of each contaminant to the observed toxicity. The TU was calculated by dividing the OC-normalized concentration of a contaminant by the 50% lethal concentration (LC50) to the test organism of that contaminant (Eq. (1)). The LC50 values were taken from the literature (Amweg et al., 2006; Ding et al., 2010, 2011; Hintzen et al., 2009; Maul et al., 2008; Maund et al., 2002). Concentration addition was recommended for predicting mixture toxicity of pesticides (Belden et al., 2007), thus summation of TUs of individual contaminants was used to assess the mixture toxicity in sediment.

$$\mathrm{TU} = \frac{\text{Sediment concentration(OC normalized)}}{\text{LC50(OC normalized)}} \quad (1)$$

Moreover, as shown in Eq. (2), bioavailability of the contaminants was taken into consideration in the toxicity evaluation using a bioavailable TU ($TU_{bioavailable}$), which was calculated by replacing the OC-normalized sediment concentration in Eq. (1) with the bioavailable concentration measured by Tenax extraction (OC-normalized Tenax extractable concentration, $C_{24\text{-h}}$) or matrix-SPME (OC-converted SPME measured concentration, C_{SPME}). The $C_{24\text{-h}}$ was the amount of chemical extracted by Tenax resin in 24 h divided by the amount of OC in sediment used for the extraction. The calculation of C_{SPME} from the freely-dissolved chemical concentration (C_{free}) was presented in Eq. (3).

$$\mathrm{TU_{bioavailable}} = \frac{C_{24-\mathrm{h}} \text{ or } C_{\mathrm{SPME}}}{\text{LC50(OC normalized)}} \quad (2)$$

$$C_{\mathrm{SPME}} = C_{\mathrm{free}} \times K_{\mathrm{OC}} = \frac{C_{\mathrm{fiber}} \times K_{\mathrm{OC}}}{K_{\mathrm{fw}}} \quad (3)$$

where, C_{free} and C_{fiber} were the freely-dissolved chemical concentration and SPME fiber concentration, respectively, and K_{OC} and K_{fw} were the partition coefficients between OC or fiber and water, respectively, and their values were taken from the literature (Laskowski, 2002; You et al., 2007). The OC normalized LC50 was used to calculate $TU_{bioavailable}$ due to the lack of Tenax- and SPME-based LC50 values. Although dividing C_{free} by the water-only LC50 values allows a direct estimation of $TU_{bioavailable}$ by SPME measurements (Ding et al., 2013; Xu et al., 2007), the calculation was not used in the current study, because 10-d water-only LC50 values for the target CUPs were not available. Instead, C_{free} values were converted to C_{SPME} before calculating $TU_{bioavailable}$ using the 10-d OC-normalized LC50 values as shown in Eqns. (2) and (3). Additionally, converting Tenax and SPME measurements to $C_{24\text{-h}}$ and C_{SPME} and using OC-based LC50 values for the $TU_{bioavailable}$ calculation also helped the comparison between bioavailable and traditional TUs.

3. Results and discussion

3.1. Toxicity estimation using the traditional TU approach

3.1.1. Observed toxicity to H. azteca

Throughout the bioassays, overlying water quality parameters were 4.8 ± 1.4 mg/L, 336 ± 38 μS/cm, 23.2 ± 0.6 °C, 7.2 ± 0.3 and 0.8 ± 0.3 mg/L for dissolved oxygen, conductivity, temperature, pH and ammonia, respectively. Mortality of the amphipods in control sediment was <14%, whereas mortality of test sediments ranged from 6 to 100% with a median of 93% (Table 1 and Fig. 1). In general, 13 of the 16 sediments (81%) had significant toxicity to *H. azteca*, and six of them (38%) caused 100% mortality of the organisms. Three sediments (S12, S15, and S16) had no acute toxicity to *H. azteca* and were located in a botanical garden, a tree nursery, and the origin of the stream (Longdong reservoir), respectively. Conversely, sediments with high toxicity were mainly located in the lower and middle sections of Chebei Creek, which received runoff from residential and industrial areas.

3.1.2. TU-predicted toxicity

Various types of CUPs including chlorpyrifos, pyrethroids, fipronil and its metabolites, and abamectin were detected in sediments in Chebei Creek and caused significant toxicity to *C. dilutus* (Li et al., 2013). To identify causality of the observed sediment toxicity to *H. azteca*, TUs of the detected CUPs were calculated (Table 1).

Chlorpyrifos was the only OP detected with concentrations higher than the reporting limits (RL) and its TUs ranged from non-detectable (ND) to 0.12. Fipronil and its metabolites, fipronil sulfide and fipronil sulfone, were detected with the sum TUs ranging from ND to 0.14. Abamectin was also detected in most sediments, but its TUs were all <0.1. Pyrethroids, however, accounted for 99% (96–100%, except for S16) of the sum TUs of all CUPs, with values ranging from ND to 46.3. Individually, six pyrethroids, namely cypermethrin, bifenthrin, *lambda*-cyhalothrin, esfenvalerate, fenpropathrin and permethrin, had >0.1 TU in at least one sediment, with median TUs being 3.1, 0.93, 0.27, 0.10, 0.05, and 0.05, respectively (Table 1). Cypermethrin was the major contributor to toxicity, which was consistent with previous studies in China (Mehler et al., 2011a; Wang et al., 2012). Moreover, toxicity to *H. azteca* was directly related with the sum TUs from all pyrethroids (Σpyr-TU) as shown in Fig. S1 in supplementary data (probit = 2.56 log TU + 4.70, $r^2 = 0.36$, $p < 0.05$).

Overall, the traditional TU analysis suggested that pyrethroids, especially cypermethrin, were the principal cause of sediment toxicity to *H. azteca* in Chebei Creek. Hence, the latter discussion is focused on pyrethroids.

3.2. Overestimation of toxicity by the traditional TU approach

As shown in Table 1, Σpyr-TU of the six sediments showing 100% mortality to *H. azteca* ranged from 3.08 to 19.5 with a median value of 5.90. The other seven toxic sediments also had high Σpyr-TUs ranging from 2.02 to 46.3. Two extreme cases were noted for sediments S3 and S12. The S3 site caused 90% mortality with a Σpyr-TU of 46.3, while the S12 site was not toxic to *H. azteca* despite having a Σpyr-TU of 2.02 (Table 1). These results implied that the TU approach considerably overestimated toxicity to *H. azteca* for most of Chebei Creek sediments.

A similar finding has previously been reported with some sediments having high Σpyr-TUs, but unexpectedly low toxicity

Table 1
The mortality and toxic units (TU) of pesticides to *Hyalella azteca* in sediments collected from Chebei Creek in Guangzhou, China. The LC50 values (μg/g OC) used for calculating TUs, and the TU values for the contaminant with TUs greater than 0.1 for at least one sample were listed.

Site	Mortality (%)	TU									
		ΣPes	ΣPyr	Chl	ΣFip	Bif	Cyh	Cyp	Esf	Fen	Per
LC50				2.90[a]	4.10[b]	0.11[c]	0.45[d]	0.38[e]	0.89[d]	1.57[f]	10.8[d]
S1	76	7.47	7.42	0.03	0.01	0.92	0.91	5.17	0.05	0.32	0.05
S2	96	10.9	10.8	0.08	0.01	2.62	1.53	6.08	0.08	0.34	0.09
S3	90	46.5	46.3	0.11	0.02	0.98	1.42	43.6	0.03	0.21	0.03
S4	76	7.73	7.58	0.06	0.06	0.52	3.22	3.61	0.08	0.05	0.05
S5	100	10.6	10.3	0.06	0.10	2.35	2.18	5.61	0.05	0.05	0.08
S6	100	4.73	4.65	0.05	0.02	0.45	0.15	3.57	0.05	0.26	0.15
S7	100	19.8	19.5	0.11	0.14	6.95	3.09	9.09	0.07	0.17	0.10
S8	100	7.43	7.14	0.12	0.10	0.81	0.73	5.39	0.07	0.06	0.08
S9	58	6.08	5.98	0.06	0.04	2.94	0.25	2.62	0.05	0.07	0.04
S10	100	3.14	3.08	0.01	0.04	0.93	0.21	1.72	0.03	0.13	0.05
S11	100	3.14	3.11	0.01	0.01	1.09	0.20	1.64	0.02	0.08	0.08
S12	10	2.11	2.02	0.05	0.02	0.21	0.23	1.34	0.12	0.11	0.02
S13	96	3.46	3.42	0.01	0.03	1.05	0.29	1.80	0.06	0.14	0.05
S14	86	1.49	1.44	0.01	0.03	0.36	0.09	0.91	0.01	0.05	0.04
S15	22	1.31	1.30	0.01	ND	0.41	0.07	0.71	0.01	0.07	0.01
S16	6	ND[g]	ND	ND	ND	ND	ND	ND	ND	ND	ND
Median TU	93	5.41	5.32	0.05	0.03	0.93	0.27	3.09	0.05	0.1	0.05

The sum of pesticides (Σpes) included pyrethroids (pyr) (bifenthrin (Bif), *lambda*-cyhalothrin (Cyh), cypermethrin (Cyp), esfenvalerate (Esf), fenpropathrin (Fen), and permethrin (Per)), chlorpyrifos (Chl), and fipronil (Fip) and its metabolites fipronil sulfide and fipronil sulfone.

[a] LC50 value for chlorpyrifos from Ding et al. (2010).
[b] LC50 value for fipronil from Hintzen et al. (2009), and the LC50 values for fipronil sulfone and sulfone were 7.70 and 9.70 μg/g OC, respectively.
[c] LC50 value from Maul et al. (2008).
[d] LC50 value from Amweg et al. (2006).
[e] LC50 value from Maund et al. (2002).
[f] LC50 value from Ding et al. (2011).
[g] Non-detectable.

to *H. azteca* (Amweg et al., 2006; Kuivila et al., 2012; You et al., 2008). Due to the limited data on sediment toxicity to *H. azteca* in China, the present dataset was compared to the data reported from the United States (Amweg et al., 2006; Kuivila et al., 2012; Ng et al., 2008; Weston et al., 2004, 2005, 2008a,b) (Fig. 2). In the theoretical dose–response curve, one TU corresponds to 50% mortality and a sediment with TU greater than 1 should cause more than 50% mortality to the organisms. However, it is not the case we observed in Fig. 2. When calculated TU values were in the range of 0.5–5, sediment toxicity to *H. azteca* varied extensively; therefore, it was difficult to predict toxicity of the sediments with contaminants at concentrations within this range. This is consistent to previous finding by Lao et al. (2012) who suggested that the predicative ability of TUs to sediment mortality may be poor when TU values were between 1 and 5. Although Σpyr-TU > 5 usually corresponded to toxic sediment to the amphipods (Lao et al., 2012), there were some exceptions. For example, sediment S9 (the present study) and a sediment from Dallas, TX, US (Kuivila et al., 2012) had Σpyr-TUs of 5.98 and 13.3, respectively, but mortality to *H. azteca* was only 58 and 27%, respectively. Amweg et al. (2006) also noted a sediment was hardly toxic, while it had a TU > 5. The overestimation of sediment toxicity by Σpyr-TU may result from the errors in quantification and/or the reduced bioavailability and toxicity of pyrethroids in these sediments.

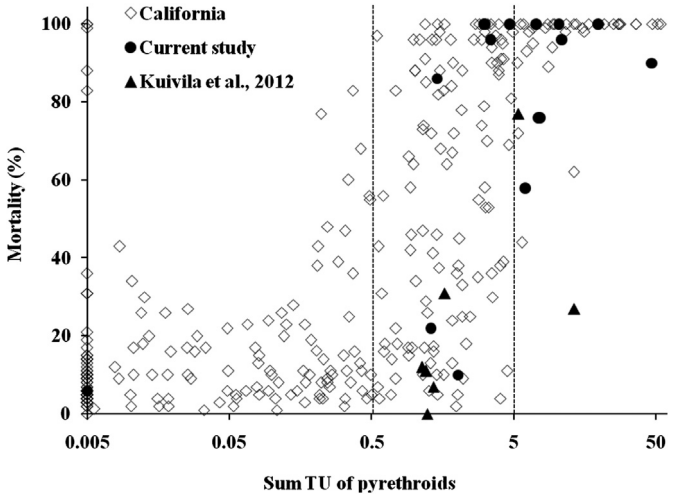

Fig. 2. The relationship between sediment toxicity to *Hyalella azteca* represented as mortality and toxic unit (TU) derived from organic carbon-normalized pyrethroid concentrations in sediment. Results of sediments collected from California, USA (open square) (Amweg et al., 2006; Ng et al., 2008; Weston et al., 2004, 2005, 2008a,b) and other sites in the USA (filled triangle) were from previous studies (Kuivila et al., 2012) and sediments from Chebei Creek in Guangzhou, China (filled circle) in current study were also included.

3.2.1. Confirmation of chemical quantification

Strict quality control plans were included in the present study for sediment analysis to ensure reliable quantification of CUPs in sediment. Provided cypermethrin was the most abundant CUP and the primary contributor to sediment toxicity to *H. azteca*, the accuracy of cypermethrin quantification was further confirmed with 10-d bioassays with *C. dilutus* using cypermethrin-spiked sediments. The OC-normalized LC50 (95% confidence interval) to *C. dilutus* determined in the present study was 1.31 (1.01–1.70) μg/g OC, which was comparable to that in the literature (1.34 μg/g OC) (Maund et al., 2002). The two lines of evidences (quality control in sediment analysis and the comparable LC50 values) collectively showed the quantification of CUP residues in sediments from Chebei Creek was accurate, and quantification error was excluded from the reasons for the toxicity overestimation.

3.2.2. Reduced bioavailability and toxicity

Reduced bioavailability of pyrethroids in sediment has been reported as one of the reasons for overestimation of toxicity using the traditional TU approach (You et al., 2008). Sequestration can significantly decrease bioavailability of sediment-associated contaminants and this sequestration is related to sediment characteristics, such as the amount and type of OC, particle size distribution, and aromaticity and polarity of the sediment (Cornelissen et al., 1997). Table S1 in the supplementary data details the characteristics of Chebei Creek sediments. Pyrethroid concentrations on a dry weight basis (C_s) were significantly correlated with OC and BC concentrations ($C_s = 16\ OC + 50$, $r^2 = 0.43$, $p < 0.01$ and $C_s = 371\ BC + 16$, $r^2 = 0.56$, $p < 0.01$, excluding S6), but were not related to sediment grain size distribution, aromaticity, or polarity. These significant relationships indicated pyrethroids tended to bind to OC, and OC-normalization would reduce the concentration variation among different sediments, while BC was also involved in the sorption process. However, in addition to total OC content, the types of OC should also be taken into consideration for estimating bioavailability of contaminants (Cornelissen et al., 2005; Kukkonen et al., 2005). Fleming et al. (1998) reported that different types of OC (peat or α-cellulose) significantly affected permethrin toxicity to *Chironomus riparius* at a constant OC content. In contrast, previous studies with spiked sediment showed BC significantly reduced the bioavailability of planar compounds, like benzo[a]pyrene, but had little effect on non-planar compounds, like permethrin (Pehkonen et al., 2010; Yang et al., 2009). The discrepancy among these studies may be due to the difference in OC types and sediment aging time. Therefore, additional research on sediment OC classification as well as the sorptive ability determination is required for more accurate prediction of contaminant bioavailability, especially for field sediments.

Furthermore, other contaminants in the sediment may also affect bioavailability and toxicity of pyrethroids. Elevated levels of heavy metals, especially lead, have been detected in sediment in Guangzhou urban waterways (Cheung et al., 2003). Mehler et al. (2011c) noted an antagonistic interaction between cypermethrin and lead to *C. dilutus*, and deemed that the co-existence of lead in sediment might reduce cypermethrin toxicity to *C. dilutus* in some field sediments. So, antagonistic interactions between cypermethrin and lead might also be one of the reasons for the unexpectedly low toxicity to *H. azteca* in Chebei Creek sediments.

In order to better understand the reasons for the overestimation of toxicity to *H. azteca*, toxicity to *H. azteca* was compared to data from our earlier study with *C. dilutus* (Li et al., 2013). Chebei Creek sediments exhibited greater toxicity to *C. dilutus,* with 88% of the sediments causing 100% mortality to the midges, and abamectin, fipronil and cypermethrin were the main contributors to toxicity to *C. dilutus*. The difference in toxicity between the two species may be explained by cypermethrin's antagonistic interaction with lead and the susceptibility of the two species to different CUPs. Although antagonistic interaction with lead may decrease cypermethrin toxicity to both species, the other two major contributors, abamectin and fipronils, can still cause high toxicity to the midges. Abamectin and fipronil were about 10 times more toxic than cypermethrin to *C. dilutus*, but much less toxic to *H. azteca* (Ding et al., 2011). To our knowledge, there are no studies on the joint toxicity of abamectin or fipronil and metals; therefore, it is difficult to determine if metals would also affect their toxicity. However, significant differences in sediment toxicity to the two species implied important roles of abamectin or fipronil in the toxicity to the midge.

Overall, a variety of factors may change bioavailability and toxicity of sediment-associated pyrethroids to *H. azteca*. Consequently, the ability of the traditional TU approach to evaluate sediment toxicity was impaired. However, it is impossible to theoretically correct the TU calculation unless a full understanding of the factors affecting bioavailability was achieved. Alternatively, bioavailability-based techniques have been proposed to provide a more accurate estimation of sediment toxicity (Reichenberg and Mayer, 2006; You et al., 2011; Cui et al., 2013), and a bioavailable TU approach was compared to the traditional TU approach for assessing toxicity to *H. azteca*.

3.3. The application of the bioavailable TU approach

Tenax extraction and matrix-SPME were applied to evaluate the potential of using bioavailability-based methods to assess sediment toxicity. Sediment samples were analyzed for pyrethroids using Tenax extraction and matrix-SPME in addition to exhaustive UAME (Tables S2-S4). Tenax extraction detected six pyrethroids including bifenthrin, *lambda*-cyhalothrin, cypermethrin, esfenvalerate, fenpropathrin and permethrin at concentrations > RLs in at least one sediment, and $C_{24\ h}$ for Σpyr and cypermethrin accounted for 34% (17–55%) and 34% (15–70%) of bulk sediment concentrations, respectively. For matrix-SPME, only *lambda*-cyhalothrin and cypermethrin were detected at concentrations > RLs. On average, C_{SPME} accounted for 3.5% (1.6–5.3%) of the bulk sediment concentration. Lee et al. (2003) suggested literature-reported K_{oc} values may be underestimated due to association of chemicals to dissolved organic matter, thus it should be noted that C_{SPME} may also be underestimated. However, the impact was similar among sediments for which cypermethrin was the major contributor to toxicity.

To determine if bioavailability-based measurements can improve the accuracy of sediment toxicity assessments, $TU_{bioavailable}$ measurements for cypermethrin derived from Tenax extraction and matrix-SPME were compared to the noted toxicity in the bioassays (probit mortality to *H. azteca*). The toxicity contribution of *lambda*-cyhalothrin, the other pyrethroid detected in matrix-SPME samples, was negligible compared to cypermethrin, so only cypermethrin was included in the assessment. A significant correlation was observed for Tenax measurements (probit = 1.85 log $TU_{24\text{-}h}$+ 6.70, $r^2 = 0.30$, $p = 0.04$, excluding S3) (Fig. 3B), and it was slightly improved compared to the traditional TUs from exhaustive UAME (probit = 2.29 log TU_{UAME} + 5.58, $r^2 = 0.26$, $p = 0.06$, excluding S3) (Fig. 3A). A previous study showed improved toxicity estimations using $TU_{bioavailable}$ measures from Tenax extraction for the selected sediments, which had extremely low toxicity compared to the prediction by Σpyr-TU (You et al., 2008). Additionally, compared to the other two methods, $TU_{bioavailable}$ derived from matrix-SPME measurements significantly improved toxicity prediction (probit = 3.10 log TU + 9.78, $r^2 = 0.52$, $p = 0.004$, excluding S3) (Fig. 3C). Previous studies with laboratory-spiked sediments also suggested the SPME-determined freely dissolved pyrethroid concentration had a good ability to predict sediment toxicity to benthic organisms (Xu et al., 2007; Ding et al., 2013). Additionally, studies with field sediments contaminated with legacy contaminants (e.g. polychlorinated biphenyls or polycyclic aromatic hydrocarbons) showed that direct measurement of contaminant porewater concentration was the most accurate method to predict body residues in *Lumbriculus variegatus* (Trimble et al., 2008) and toxicity to *H. azteca* (McDonough et al., 2010). Because sensitivity of detection using SPME for pyrethroid analysis was low, Harwood et al. (2013) suggested that it was difficult to measure freely-dissolved pyrethroid concentrations in field sediments using SPME fibers. Conversely, cypermethrin was detected at concentrations > RLs in 75% of Chebei Creek sediments, which made the present study the first successful attempt to use matrix-SPME for estimating toxicity caused by emerging contaminants

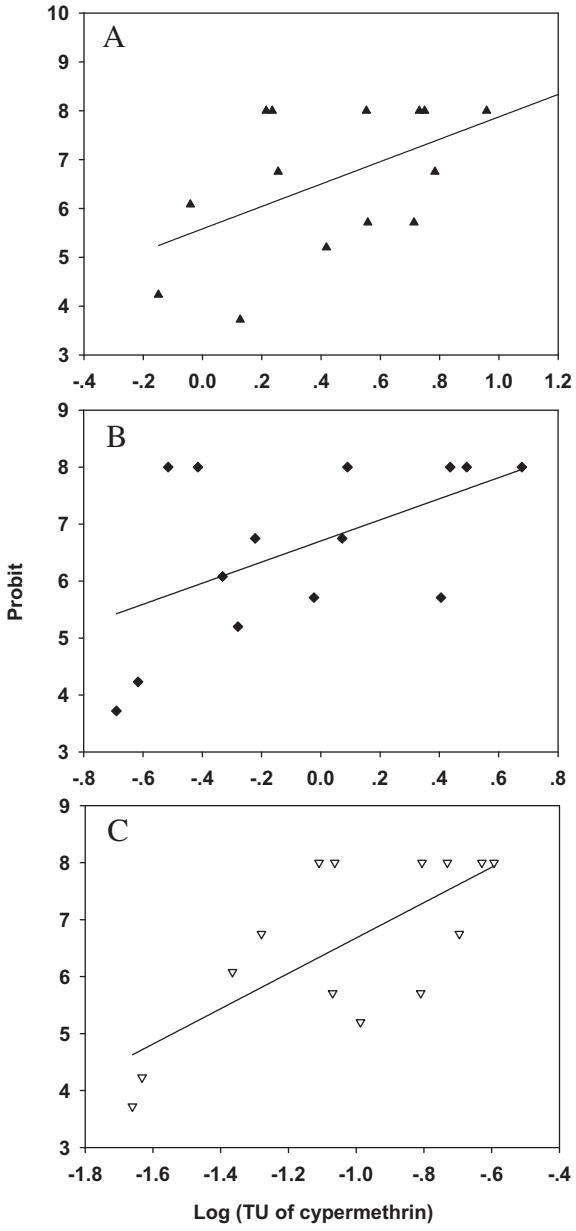

Fig. 3. The relationship between sediment toxicity to *Hyalella azteca* in probit form and the toxic units (TU) derived from cypermethrin in Chebei Creek sediments determined using three methods: organic carbon-normalized cypermethrin concentrations in sediment determined by exhaustive extraction (A, probit = 2.29 log TU + 5.58, $r^2 = 0.26$, $p = 0.06$); Tenax extractable concentrations of cypermethrin measured by Tenax extraction (B, probit = 1.85 log TU + 6.70, $r^2 = 0.30$, $p = 0.04$); freely dissolved concentrations of cypermethrin in sediment porewater predicted by matrix-solid phase microextraction (C, probit = 3.10 log TU + 9.78, $r^2 = 0.52$, $p = 0.004$).

acting as specific mode of actions (e.g., sodium channel disrupting) in field-collected sediment.

Although both approaches improved toxicity estimation, matrix-SPME showed a slightly better prediction of sediment toxicity than Tenax extraction, possibly because the two techniques measured different fractions of contaminants in sediment (Reichenberg and Mayer, 2006). Tenax extraction measured the rapidly desorbing fraction of contaminants in sediment, or that fraction potentially accessible to the organisms, but was not a direct measure of the partitioning of sediment-associated contaminants. Rather, C_{free} measured by matrix-SPME represented chemical activity at equilibrium and it controlled the partitioning process a contaminant among phases (Reichenberg and Mayer, 2006). The main exposure route for *H. azteca* to contaminants in sediment is partitioning from porewater and overlying water (Wang et al., 2004). The extremely low C_{free} found in the present study suggested that most of the detected pyrethroids in Chebei Creek sediments were not bioavailable via partitioning. Although a good correlation was noted for the fractions of contaminants available for desorption (Tenax extraction) and partitioning (matrix-SPME) (You et al., 2011), different desorption rates of cypermethrin from the sediment to the porewater may induce variability using the single time-point Tenax extraction as the bioavailability measurement. Therefore, C_{free} measured by SPME better predicted exposure of cypermethrin to *H. azteca*, and subsequently toxicity. Similarly, Trimble et al. (2008) reported that matrix-SPME served as a better chemical technique in bioavailability prediction across matrices than Tenax extraction, and the reason was that matrix-SPME directly measured the equilibrium concentrations of contaminants in sediment porewater, which was not affected by the sequestration or desorption processes among various sediments. These results suggested that it is beneficial to use $TU_{bioavailable}$ derived from bioavailability-based measurements in sediment toxicity assessments, especially in the case when toxicity was overestimated by traditional TUs. If the water-only LC50 values are available, $TU_{bioavailable}$ can be directly calculated from C_{free} (Ding et al., 2013; Xu et al., 2007) and avoid possible underestimation of C_{SPME} due to the inaccurate K_{oc} values (Lee et al., 2003).

4. Conclusions

Sediment toxicity evaluations suggested that pyrethroids, especially cypermethrin, were the major contributors to toxicity to *H. azteca* in urban streams in Guangzhou, China. The traditional TU approach considerably overestimated the toxicity of sediment-associated pyrethroids due to their low bioavailability. Chemical sequestration and possible antagonism of multiple contaminants might be the reasons for the low bioavailability and toxicity to *H. azteca* in sediments with elevated bulk sediment concentrations. The $TU_{bioavailable}$ value, derived from the bioavailability-based measurements, significantly improved the prediction of sediment toxicity to *H. azteca*. Compared to the desorption-based approach (Tenax extraction), the activity-based approach (matrix-SPME) provided a better estimation of sediment toxicity to *H. azteca*. Additionally, significant differences in susceptibility of *H. azteca* and *C. dilutus* to different CUPs suggested the limitations in using single species in bioassays. Hence, it is important to incorporate chemical bioavailability and multiple species tests in sediment risk assessments.

Acknowledgments

This research was financially supported by NSFC (41222024, 41273120, and 41121063) and the Scientific Research Foundation for the Returned Overseas Chinese Scholars, State Education Ministry. We thank J. Pang, Z. Lin and J. Du for assistance in sediment sampling and A. D. Harwood and the two anonymous reviewers for their constructive comments. This is contribution No. IS-1651 from GIGCAS.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2013.03.022.

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