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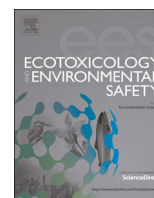
Integrated sediment quality assessment through biomarker responses and bioavailability measurements: Application in Tai Lake, China

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ABSTRACT

A weight of evidence (WoE) framework has been applied to assess sediment quality of a typical fresh-water lake, Tai Lake in China, where the sediments were contaminated by various chemicals but showed no acute lethality to the benthic invertebrate, *Chironomus dilutus*. A quantitative scoring method was employed to integrate three lines of evidence (LoE), including adverse effects in life cycle bioassays, biomarker responses, and bioavailability-based chemical analysis. Six biomarkers were determined in *C. dilutus* after the exposure to the sediments from Tai Lake and provided sensitive indication of sublethal effects at the molecular level. The biomarkers included cytochrome P450, glutathione S-transferase, carboxylesterase, acetylcholinesterase, catalase, and lipid peroxidation. The changes of the biomarkers were summarized for individual sampling sites by computing the integrated biomarker response (IBR) indices. Complementary information was also confirmed by the interrelationship of the LoEs. The IBR indices gained before pupation correlated well with the impairments of emergence of the midges, and altered acetylcholinesterase was corroborated by the detection of chlorpyrifos, an organophosphate pesticide. The relationship between bioavailable toxic units estimated by Tenax extractable concentrations of chemicals in sediment and the observed toxicity in the midges helped to identify the putative toxicity contributors to *C. dilutus*. Overall, the WoE method clearly distinguished the contaminated sites and ranked them by the level of contamination. Sediment-associated pesticides, particularly γ-hexa-chlorocyclohexane and chlorpyrifos, were the possible contributors to chronic toxicity to the midges.

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1. Introduction

There is a growing awareness of the risk of contaminated sediment in freshwater lakes (Johnson et al., 2001). A weight of evidence (WoE) framework has been developed to assess sediment quality by the integration of relevant lines of evidence (LoE), e.g., chemical characterization, toxicity testing, and biological surveys (Chapman, 1990; Burton et al., 2002). In addition, the responses of biomarkers in vivo were also used as LoEs for early warning signals of environmental stresses, particularly when test sediments did not cause mortality in the organisms. Biomarkers provided direct evidence for exposure and effects at the molecular level, including physiological, biochemical, and behavioral variations (Dickerson et al., 1994). Individual biomarkers, however, frequently failed to explain sediment quality data under field conditions due to the seasonal, physiological and biochemical variations, as well as the reliability of control organisms (Lagadic

et al., 1994). Rather, evaluating a battery of complementary bio-markers was preferred to assessing the risk of field sediments (Moore et al., 2004). To achieve this goal, Beliaeff and Burgeot (2002) applied integrated biomarker response (IBR) index to integrate the responses of multiple biomarkers into a single value for more intuitionistic assessment of the adverse effects of contaminants to organisms.

In addition to toxicological information, e.g., direct effects in the organisms and the IBR, chemicals of concern in sediment were a valuable LoE in a WoE investigation as well. Bulk sediment concentrations of sediment-bound contaminants were typically used as dose metrics for toxicity prediction, but they may over-estimate the toxicity due to ignoring the role of bioavailability (Luthy et al., 1997). Instead, biomimetic techniques have shown their potential of accurately predicting the bioavailability and toxicity of sediment-bound contaminants (You et al., 2011). Des-orption-based Tenax extraction measured the rapidly desorbing fractions of contaminants in sediment, and recent studies de-monstrated Tenax extractable concentrations better explained the bioaccumulation and toxicity of contaminants (Landrum et al.,

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2007; You et al., 2008; Lydy et al., 2015). It is advantageous to use IBR and bioavailability-measurements as quantifiable LoEs in assessing the risk of lake sediments which show low acute toxicity.

Tai Lake is the third largest freshwater lake in China. Previous studies reported that sediments in this lake contained a variety of contaminants, such as polycyclic aromatic hydrocarbons (PAHs) (Zhang et al., 2012), organochlorine pesticides (OCPs) (Wang et al., 2012), and metals (Fu et al., 2013). Meanwhile, genotoxicity of algae (Li et al., 2014) and the change in biomarker responses in goldfish (Wang et al., 2011; Yan et al., 2014) were also detected in Tai Lake. Therefore, this lake was selected as a representative freshwater lake for evaluating the effectiveness of incorporating IBR and bioavailability measurements into a WoE approach for sediment quality assessment.

The objectives of the current study were to measure the alteration of biomarker responses in the benthic invertebrate, *Chironomus dilutus* after being exposed to the sediments from Tai Lake, to analyze the bioavailability of organic contaminants in sediment using 24-h Tenax extraction, and finally to assess sediment quality by integrating three LoEs, including chronic toxic effects in the midges, biomarker responses, and bioavailability-based chemical analysis.

2. Materials and methods

2.1. Chemicals and reagents

Acetylthiocholine iodide, 5,5′-dithiobis(2-nitro-benzoic acid), α-naphthyl acetate, and ammonium molybdate were purchased from Aladdin Industrial Corporation (Shanghai, China). Glutathione, 7-ethoxycoumarin, 7-hydroxycoumarin, and 1-chloro-2,4-dinitrobenzene were obtained from J&K Scientific Limited (Guangdong, China). Thiobarbituric acid, tetramethoxypropane, and trichloroacetic acid were bought from Sinopharm Chemical Reagent Company Limited (Shanghai, China). Tenax TA beads (60–80 mesh) were purchased from Scientific Instrument Service Incorporation (Ringoes, NJ, USA). A mixture standard solution of 16 PAHs was purchased from Spex Certiprep Incorporation (Metu, NJ, USA), and standard solutions of 20 OCPs and eight organophosphate pesticides (OPs) were from Accustandard (New Haven, CT, USA) (Table S1 of the Supplementary data; "S" represents figures and in the Supplementary data thereafter). Two surrogates, decachlorobiphenyl (CB-209) and 4,4′-dibromooctafluorobiphenyl (DBOFB), were obtained from Supelco (Bellefonte, PA, USA). In addition, ^{13}C-CB-141, ^{13}C-CB-209, d10-parathion (Cambridge, Andover, MA, USA), 2-fluoro-1,1′-biphenyl, d14-*p*-terphenyl, and d14-dibenzo[a,h]anthracene (Dr. Ehrenstorfer, Germany) were used as internal standards for gas chromatograph–mass spectrometer (GC–MS) quantification. Hexane (HPLC-grade) was bought from Honeywell Company Limited (Korea). Dichloromethane and acetone (analytical grade) were obtained from Tianjin Chemical Reagent Factory (Tianjin, China) and were redistilled before use.

2.2. Study area and sediment collection

As shown in Fig. S1, five sediment samples were collected in the North of Tai Lake where significant degradation of water quality was noted (Wang et al., 2011). The five sites were numbered from T1 to T5, indicating the adverse effects from severe to slight, respectively, which were observed in chronic toxicity tests (Qi et al., 2015). Meanwhile, a control sediment was collected from a drinking water reservoir in Conghua, China. Surface sediment were collected using a stainless steel grab, passed through a 0.5 mm sieve, transported to the laboratory, and stored at 4 and −20 °C for toxicity testing and chemical analysis, respectively.

2.3. Bioassays and biomarker measurements

Life cycle toxicity tests were performed using *C. dilutus* in three replicates following a previously developed method (Du et al., 2013). Toxic endpoints included reduced survival and the impairments of growth, emergence and reproduction and detailed description of the testing can be found elsewhere (Qi et al. 2015). In brief, 20 newly hatched midge larvae (< 24 h) were gently transferred into a beaker which contained 60 g of wet sediment and 250 ml of reconstituted overlying water. The reconstituted water was prepared following the protocol suggested by the United States Environmental Protection Agency (USEPA, 2000) and aerated for at least 24 h before use. The tests were conducted using a 16:8 h light: dark photo-cycle and at 23 ± 1 °C and approximately 60% of overlying water was renewed twice every day. The larvae were fed once daily with 1 ml of fish food at varying concentrations considering their physiological difference at different life stages (at the first day: no feeding, from 2 to 7 d: 0.6 g/L, 8–12 d: 3 g/L, and 13 d to the end of testing: 6 g/L) (Du et al., 2013). Water quality parameters, including dissolved oxygen, temperature, pH, and conductivity were monitored daily and ammonia in overlying water was analyzed every 3 days.

After 20-d exposure to the sediments, the midge larvae were sieved from the sediment, enumerated, and rinsed three times with reconstituted water. Activities of six enzymes were determined in the midges, including the cytochrome P450 *O*-deethylase (P450), glutathione *S*-transferase (GST), carboxylesterase (CarE), acetylcholinesterase (AChE), catalase (CAT), and lipid peroxidation (LPO). A living larva was randomly selected to measure P450 level which was expressed 7-ethoxycoumarin *O*-deethylase activity using a microfluorimetric method (Desousa et al., 1995). Subsequently, the remaining organisms were homogenized in 100 mmol/L chilled phosphate buffer saline (PBS, pH 7.4) using a Bullet Blender Blue-24 homogenizer (Next Advance Incorporation, Averill Park, NY, USA) and centrifuged at 8000g for 20 min at 4 °C. The supernatant of the homogenate was decanted and used for determining enzymatic activities. Activity of GST was determined using 1-chloro-2,4-dinitrobenzene as a substrate through a kinetic approach (Habig et al., 1974) and CarE activity was determined spectrophotometrically using α-naphthyl acetate as a substrate (Vanasperen, 1962). The AChE activity was measured using a kinetic method with acetylthiocholine iodide as a substrate (Ellman et al., 1961). The CAT activity was determined by the method of ammonium molybdate (Goth, 1991) and LPO level was determined using thiobarbituric acid reaction (Ohkawa et al., 1979). In addition, the content of proteins in the midges was also quantified using the Bradford method with bovine serum albumin as a protein standard (Bradford, 1976). More details on enzymatic analysis are presented in the Supplementary data.

After individual biomarkers were quantified, IBR index was calculated to integrate the six biomarkers into a single value (Beliaeff and Burgeot, 2002; Devin et al., 2014). Firstly, a mean (m) and a standard deviation (SD) were calculated for each biomarker at all sampling sites, and the level of a biomarker at individual sites (X) was standardized to the m and SD values of this certain biomarker to obtain a Y value (Eq. (1)). Secondly, a Z value was assigned as Y if the enzyme was activated at the site and $-Y$ for inhibition of the enzyme (Eq. (2)). Then, as shown in Eq. (3), an S value was computed as the sum of Z and |min|, and |min| was the absolute value of the minimum Z for the certain biomarker among the sites. Thirdly, the six biomarkers were put into an order of P450, GST, CarE, AChE, CAT, and LPO to arrange the biomarkers with similar functions to be adjacent on the star plot as suggested by Beliaeff and Burgeot (2002). Then an A value was calculated by multiplying two successive S values (S_i and S_{i+1}), and normalized by the sine function which took the number of biomarker (k) into

consideration (Eq. (4)). Finally, an IBR index was calculated for individual sampling site by summing up the A values of the k biomarkers (Eq. (5)) and then displayed using a star plot. The radius coordinate of the star plot represented the index at a given site.

$$Y = (X - m)/SD \tag{1}$$

$$Z = Y \text{ or } Z = -Y \tag{2}$$

$$S = Z + |\min| \tag{3}$$

$$A_i = \frac{S_i \times S_{i+1} \times \sin(2\pi/k)}{2} \tag{4}$$

$$IBR = \sum_{i=1}^{k} A_i \tag{5}$$

2.4. Bioavailability-based measurements

A variety of organic contaminants were analyzed in the sediment samples in a previous study including PAHs, PCBs, polybrominated diphenyl ethers (PBDEs), OCPs, OPs and pyrethroid pesticides and the toxic unit (TU)-based toxicity assessment suggested that contributions of PCBs, PBDEs, and pyrethroids to the chronic toxicity in the midges were relatively trivial, so chemical analysis in the present study only focused on PAHs, OCPs and OPs (Qi et al., 2015).

Bioavailable concentrations of the target contaminants were analyzed using a 24-h Tenax extraction following a method described in You et al. (2007). In brief, 5 g of wet sediment, 0.5 g Tenax, 0.5 g copper powder, 5 mg NaN_3 and 45 ml reconstituted water were placed in a 50- ml glass tube. The tubes were rotated at 20 revolutions/min. After 24 h, Tenax beads were separated from the sediment slurry with a stainless steel scoop and sonicated with 5 ml of a mixture of acetone: hexane (1:1, v/v) for 5 min. The extraction was repeated twice and the extracts were combined and solvent exchanged to 1 ml of hexane.

The procedures to purify the extracts and analyze the contaminants were detailed in a previous study (Qi et al., 2015). Briefly, a self-packed silica/alumina column was used to clean the extracts before analyzing PAHs and OCPs, and PSA/GCB solid phase extraction cartridges was used to clean the extracts for OP analysis. The PAHs and OCPs were analyzed using an Agilent 7890-5975 gas chromatograph–mass spectrometer (GC–MS) (Santa Clara, CA, USA) with electron impact ionization being used. A DB-5 MS column (30 m × 0.25 mm, film thickness 0.25 μm) was used for chemical separation and helium was used as carrier gas at a flow rate of 1.0 ml/min. Meanwhile, OPs were analyzed using a Shimadzu QP-2010-plus series GC–MS (Shimadzu Corporation, Kyoto, Japan) and negative chemical ionization was used. A DB-5 MS column (15 m × 0.25 mm, film thickness 0.10 μm) was used for chemical separation. Helium was used as the carrier gas with a flow rate being set at 1.0 ml/min, and methane was used as the NCI reaction gas. The performance of the instruments was checked by the injection of a calibration standard for every ten samples, and the relative differences between the checks were all within 20% for all analytes. A method blank, matrix blank, matrix spike, and matrix spike duplicate were processed every 20 samples. No target compounds were detected in the blanks, and the recoveries of target chemicals were in a range of 61–128%.

The contents of organic carbon (OC) in sediment were analyzed using an elemental analyzer (Elementar Vario EL III, Hanau, Germany) after removing inorganic carbon with 1 mol/L hydrochloric acid.

2.5. Data analysis

Significant differences of individual biomarkers between field sediments and the control were determined by a one-way analysis of variance and set at $p < 0.05$. In addition, the correlation between the IBR index and the impairment of emergence in chronic toxicity testing was evaluated with linear regression analysis using SPSS 16.0.

To assess the contribution of individual contaminants to the observed chronic toxicity in C. dilutus, bioavailable toxic units ($TU_{bioavailable}$) of the contaminants in sediment were calculated. As shown in Eq. (6), $TU_{bioavailable}$ is the ratio of OC-normalized Tenax extractable concentration of a contaminant (C_{24-h}) and its chronic median effective concentration (EC50). The C_{24-h} was calculated using the amount of contaminant extracted by Tenax resins divided by the amount of OC in the sediment used for extraction. The sum of TUs of contaminants in the same group was used to describe mixture toxicity assuming that the joint toxicity was concentration addition (Belden et al., 2007).

$$TU_{bioavailable} = \frac{C_{24-h}}{EC50(OC \text{ normalized})} \tag{6}$$

3. Results and discussion

3.1. Sediment toxicity and biomarker responses

Whole life cycle toxicity testing was conducted with the sediments collected from Tai Lake, and chronic toxicity to C. dilutus were observed at most sites, such as attenuated survivorship, reduced growth, impaired emergence, and low fecundity. Detailed results on chronic toxicity were presented elsewhere (Qi et al., 2015). In addition, a series of biomarkers were also measured in the midges after 20-d exposure to the sediments. As shown in Fig. 1, at least one biomarker was impacted in four of the five sediments. At T1 site, activities of GST, CarE, and AChE were reduced significantly compared with the control while LPO content was enhanced. After being exposed to T2 sediment, P450 level in the midges was considerably enhanced and GST activity was reduced. For the midges in T3 sediment, suppression of GST activity was companied with elevated LPO activity. The CAT activity was enhanced in the midges in T4 sediment, and none of the target biomarkers of the midges in sediment T5 were significantly different from the control.

The P450 and GST play important roles in the biotransformation of organic contaminants in the organisms during phases I and II metabolisms, respectively (Bucheli and Fent, 1995; Sheehan et al., 2001). As shown in Fig. 1A, significant enhancement of P450 level occurred at T2 site while GST activity was significantly inhibited at three sites (T1–T3). These results indicated that organic contaminants, such as PAHs, OCPs and OPs, might play a role in chronic toxicity to C. dilutus at sites T1, T2 and T3. The CarE is also associated with metabolic processes and protects the organisms against the damage by exogenous ester-containing compounds, e.g., OPs and pyrethroids (Newcomb et al., 1997). Meanwhile, AChE activity has been proposed as an effective tool for monitoring exposure of the organisms to cholinergic poisons, such as OPs and carbamate pesticides. The exposure of an organism to OPs and carbamates would lead to an accumulation of acetylcholine in the synapse of neuromuscular junction, and subsequently disrupt the function of the nervous system (Guilhermino et al., 1998). As shown in Fig. 1B, AChE activity at T1 site was remarkably suppressed with 41.4% inhibition. At the same time, CarE activity of
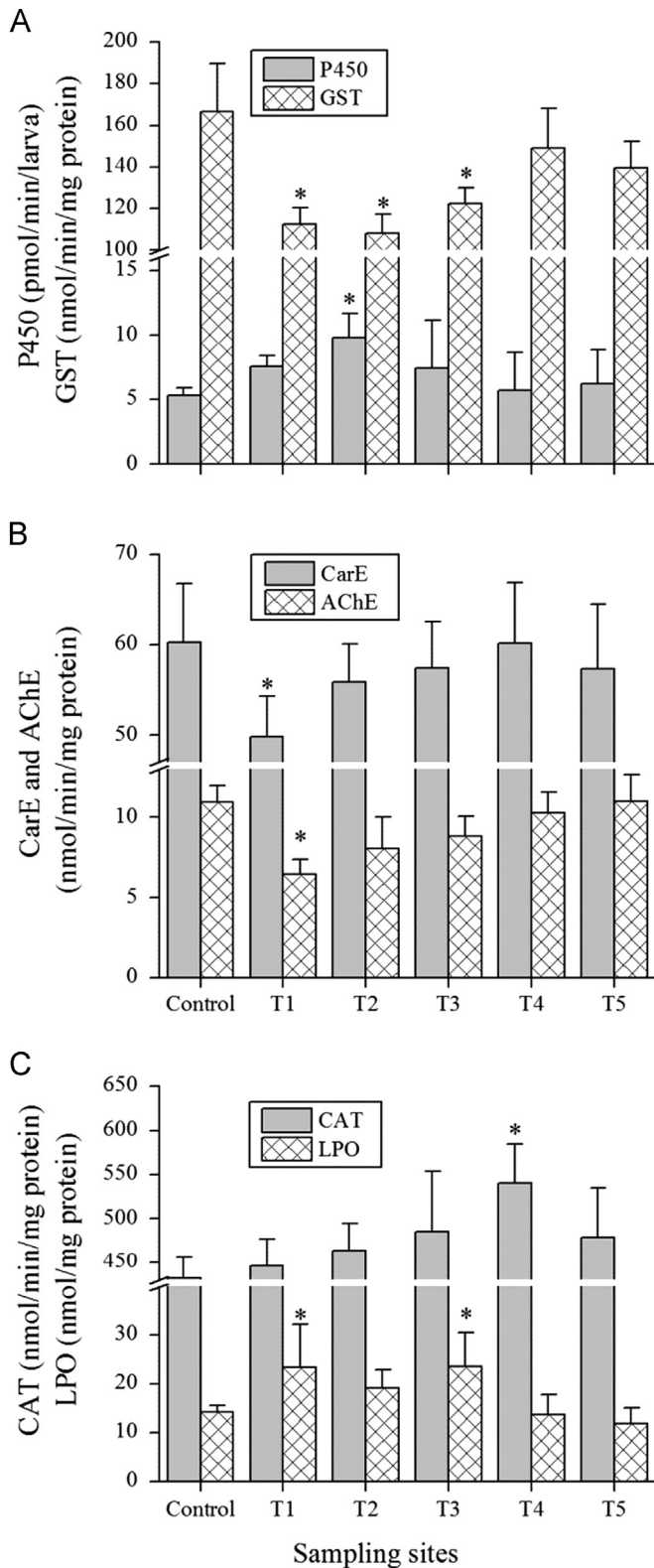
Fig. 1. The responses of biomarkers in *Chironomus dilutus* after 20-d exposure to the sediments in Tai Lake. The sampling sites T1–T5 are mapped in Fig. S1. Error bars denote the standard deviations ($n=3$) and significant differences between field sediments and the control were indicated by an asterisk ($p < 0.05$). (A) Cytochrome P450 *O*-deethylase (P450) and glutathione *S*-transferase (GST); (B) carboxylesterase (CarE) and acetylcholinesterase (AChE); (C) catalase (CAT) and lipid peroxidation (LPO).

the midges at T1 site was also reduced, suggesting the possible presence of OPs and carbamates in Meiliang Bay of Tai Lake where T1 sediment was collected.
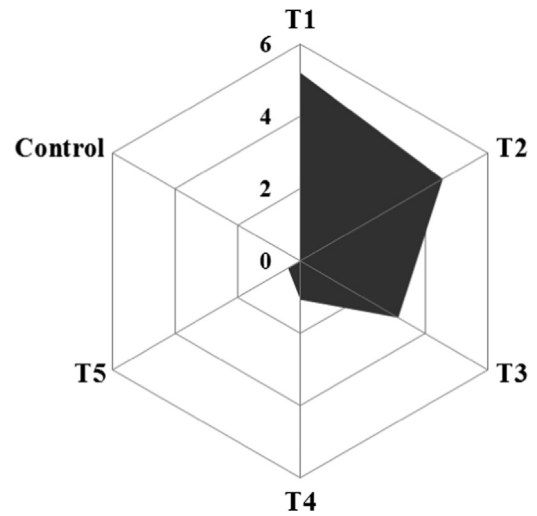

Fig. 2. Star plot of integrated biomarker response (IBR) indices of *Chironomus dilutus* after 20-d exposure to the sediments in Tai Lake. The sampling sites T1–T5 are mapped in Fig. S1.
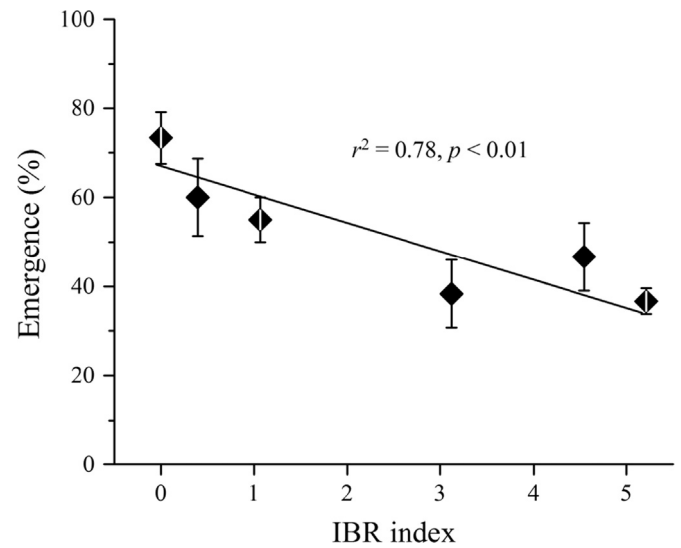

Fig. 3. Relationship between integrated biomarker response (IBR) indices determined at 20 d and percentages of emergence at 47 d for *Chironomus dilutus* exposed to the sediments in Tai Lake. Error bars denote the standard deviations ($n=3$).

Oxidative damage in the organisms was reflected by the changes in CAT and LPO. The cellular redox hemeostasis is generally maintained a balance between oxidants and antioxidants systems, and the reactive oxygen species (ROS), including superoxide anions, hydroxyl radicals (OH·), and hydrogen peroxide (H_2O_2), play a critical role in oxidative defense mechanisms. Environmental stressors, however, may cause excess ROS and trigger severe damage to biological macromolecules, e.g., proteins, DNA and lipids (Martindale and Holbrook, 2002). A number of defense systems are involved in combating the accumulation of ROS and CAT is a major antioxidant defense component which catalyzes the decomposition of H_2O_2 to water (Livingstone, 2001). The midges exposed to sediment T4 exhibited a CAT level significantly greater than the control, implying oxidative damage (Fig. 1C). Since OH· is an initiator of LPO (Cheng and Li, 2007), elevated LPO level demonstrates constant threats to membrane integrity and homeostasis of the organisms. The LPO level was enhanced significantly at sites T1 and T3, and it might be the consequence of the damage

of cell membranes by oxidative stress. At site T3, elevated levels of GST were also detected and it was reasonable because GST was involved in removing the products of LPO (Yang et al., 2001).

Overall, most sediment samples were chronically toxic to the midges, and chronic toxicity was consistent with the changes in enzymatic biomarkers. To use the information of biomarkers more comprehensively, IBR indices at all sampling sites were calculated and presented as a star plot. As shown in Fig. 2, the highest IBR indices were found in Meiliang Bay (T1 and T2), followed by Zhushan Bay (T3) and Gong Bay (T4), and the least impacted area was T5 from the conveying water district. Similarly, previous studies with goldfish also found relatively higher IBR indices in Meiliang Bay and Gong Bay than other areas in Tai Lake (Wang et al., 2011; Lu et al., 2013). Although the two species resided in different niches, IBR evaluation with the midges (the current study) and the goldfish (Wang et al., 2011; Lu et al., 2013) found similar contaminated areas in Tai Lake.

Moreover, IBR index were directly correlated with midges' emergence in chronic toxicity testing. Declined emergence of *C. dilutus* resulted in reduced reproduction and subsequent influence on the population of the organisms (Pascoe et al., 1989). Therefore, the emergence has been commonly used as a sensitive endpoint in the life cycle tests, but the determination of this endpoint took a long time after the pupation of midges (Benoit et al., 1997). As shown in Fig. 3, IBR index at 20 d correlated well with the emergence at 47 d ($r^2 = 0.78$, $p < 0.01$), which suggested that the determination of biomarkers before pupation could serve as a meaningful prediction regarding impaired emergence in *C. dilutus*.

3.2. Chemical analysis and bioavailability measurement

A suite of organic contaminants in the sediment samples were analyzed and PAHs, hexachlorocyclohexanes (HCHs), endosulfans and chlorpyrifos were considered as the putative contributors to sublethal toxicity in *C. dilutus* (Qi et al., 2015). Bulk sediment concentrations may overestimate sediment toxicity and cause misidentification of the causative agents of the observed toxicity due to different bioavailability of sediment-bound chemicals (Cornelissen et al., 2001). Recent studies demonstrated that sediment toxicity was better explained by bioavailability-based dose metrics than traditional TUs estimated from bulk sediment concentrations (You et al., 2008; Li et al., 2013; Lydy et al., 2015). To improve the accuracy in identifying putative toxicants in sediment, the bioavailability of PAHs, OCPs and OPs were measured using a 24-h Tenax extraction.

As shown in Table S2, the sum C_{24-h} of the 15 detected PAHs in sediments from different sites varied little, and the highest was at site T2 (31.2 μg/g OC) and the lowest at T5 (8.81 μg/g OC). On average, Tenax extractable PAHs accounted for 34% of bulk sediment concentrations. Nevertheless, this value was very low for 5- to 6-ring PAHs and only 3% could be extracted by Tenax within 24 h (Table S3). On the other hand, 5- to 6-ring PAHs are generally more potent than the 2- to 4-ring PAHs as demonstrated by their greater toxic equivalency factors (TEF) (Table S2) (USEPA, 1993). So the contribution of the total PAHs to sediment toxicity may be overestimated without considering the relatively low bioavailability of 5- to 6-ring PAHs.

The northern portion of Tai Lake was surrounded by farmland and agricultural runoff is a primary source of contaminants in this area (Wang et al., 2011). This explained the frequent detection of various pesticides in the sediments. The C_{24-h} values of OCPs are presented in Table S4 in the supplementary data. The site in Zhushan Bay (T3) had the highest OCP concentration at 0.53 μg/g OC. The OCP concentrations in Meiliang Bay (T1 and T2) were slightly lower, being 0.36 and 0.44 μg/g OC, respectively, and T5 contained the lowest OCPs at a concentration of 0.08 μg/g OC. In

Table 1

The Bioavailable toxic units (TU_bioavailable) of organochlorine pesticides (OCPs), organophosphate pesticides (OPs), and polycyclic aromatic hydrocarbons (PAHs) in sediments from Tai Lake. The sampling sites T1–T5 are mapped in Fig. S1.

Contaminant[a]	TU_bioavailable[b]				
	T1	T2	T3	T4	T5
p,p′-DDE	< 0.01	< 0.01	BRL[c]	< 0.01	< 0.01
p,p′-DDT	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Endosulfan I	0.56	0.71	0.74	0.61	0.51
Endosulfan II	BRL	BRL	BRL	BRL	BRL
Endrin	0.13	0.18	0.25	BRL	0.10
γ-HCH	0.74	BRL	BRL	BRL	BRL
Methoxychlor	BRL	BRL	BRL	BRL	BRL
ΣTU_bioavailable−OCP	1.43	0.89	0.99	0.61	0.61
Chlorpyrifos (OP)	0.28	0.29	0.31	0.16	0.08
ΣTU_bioavailable−pesticide	1.71	1.18	1.30	0.77	0.69
ΣTU_bioavailable−PAH	0.026	0.033	0.032	0.021	0.011

[a] Only contaminants with at least one TU_bioavailable being greater than 0.01 are presented.
[b] The TU_bioavailable was calculated from Tenax extractable concentration of contaminants in sediment and its chronic median effect concentrations (EC50). Tenax extractable concentrations of individual contaminants and EC50 values of PAHs and pesticides are presented in Tables S2, S4 and S5.
[c] BRL: below reporting limits. The RL for individual contaminants are presented in Tables S2, S4 and S5.

all the sites, β-HCH was the dominant OCP with C_{24-h} ranging from 0.18 to 0.43 μg/g OC. In addition, the OP, chlorpyrifos, was also detected in all sediments with C_{24-h} being from 0.025 to 0.089 μg/g OC in all five sites (Table S5). The detection of chlorpyrifos was confirmed by the observation of AChE inhibition in *C. dilutus*. As a broad spectrum OP, chlorpyrifos is bioactivated to chlorpyrifos-oxon, which is a potent AChE inhibitor. The order of the levels of AChE inhibition in the five sediments coincided with C_{24-h} of chlorpyrifos.

To predict the causative contaminants to the chronic toxicity to the midges, TU_bioavailable of individual contaminants were computed from their respective C_{24-h} and chronic EC50 values. Assuming concentration addition for chemicals in the same class, joint toxicity was estimated as the sum of TU_bioavailable of individual contaminants. Before computing the TU_bioavailable of PAHs, chronic EC50 of individual PAHs were derived from the Tenax-based EC50 of benzo[a]pyrene (BaP) for the emergence of *C. dilutus* (14.2 μg/g OC) (Du et al., 2014). The TEF values suggested by the USEPA (1993) were used to convert toxicity data of individual PAHs to their BaP equivalents. Due to the lack of chronic toxicity data, EC50 values for individual pesticides were estimated by dividing its median lethal concentration (LC50) in acute toxicity testing by its acute to chronic ratio and the values are shown in Tables S4 and S5 in the Supplementary data.

As shown in Table 1, the sum TU_bioavailable of all pesticides in the sediments from Tai Lake ranged from 0.69 to 1.71. Compared to pesticides, ΣTU_bioavailable of PAHs were small at all sites, ranging from 0.011 at T5 to 0.033 at T2. This implied that PAHs contributed little to the observed toxicity. Conversely, the TU_bioavailable of OCPs and OPs suggested that both of them were responsible for the chronic toxicity in the midges. Among all OCPs, endosulfan I had the highest TU_bioavailable, being from 0.51 to 0.74 at all sites. At T1, γ-HCH also made a great contribution to the toxicity with a TU_bioavailable of 0.74, but this OCP was not found above its reporting limit in other sediments. The OP chlorpyrifos was considered as another toxicity contributor with TU_bioavailable from 0.08 to 0.31 at all sites.

Overall, several pesticides, including γ-HCH, endosulfan I and chlorpyrifos, were plausible stressors to *C. dilutus* in the sediments from Tai Lake. The relationship between TU_bioavailable and observed

Table 2
Ranking the sediment samples from Tai Lake by a weight of evidence (WoE) framework from scores of individual lines of evidence (LoE).

	LoE type	T1	T2	T3	T4	T5	
LoE1	Chronic bioassays (5^a)	4	3	2	2	0	Adverse effects at organism level
LoE2	Biomarkers (6^a)	4	2	2	1	0	Biomarker responses at the molecular level
LoE3	$TU_{bioavailable}$ [b]	1	1	1	0	0	Bioavailability-based chemical analysis
WoE [c]		0.75	0.5	0.42	0.25	0	

[a] The number of measured toxic endpoints and biomarkers. The scores of LoE1 and LoE2 were the number of measurements which were significantly different from the control. More information is presented in Tables S6 and S7.

[b] The scores of LoE3 were assigned as 0 or 1 in cases of the values of sum bioavailable toxic unit ($TU_{bioavailable}$) of all detected contaminants being < 1 or ≥ 1, respectively.

[c] Overall average score of individual LoEs.

toxicity was commonly used as additional evidence for toxicant identification (Li et al. 2013). The linear regression between the emergence of *C. dilutus* and $TU_{bioavailable}$ were analyzed for the putative toxicants, and the r^2 values of the regression was 0.83 for chlorpyrifos but only 0.27 for endosulfan I (Table S3). The different r^2 for the two pesticides confirmed that chlorpyrifos was one of the main causative agents in these sediments, but endosulfan I was not a major contributor to the observed chronic toxicity. A recent bioassay-directed analysis study (Hu et al., 2015) also found that chlorpyrifos was one of predominant pollutants in sediments from Tai Lake. The result in the current study was consistent with their findings.

3.3. Integrated assessment of sediment quality

Integrated assessment of sediment quality in Tai Lake was performed with a WoE framework which integrated three LoEs using a qualitative scoring method (Table 2). Various methods, such as listing evidence, best professional judgment, causal criteria, logic, indexing, scoring, and quantification, have been proposed to integrate the LoEs (Linkov et al., 2009). The scoring method was chosen in the current WoE framework because it provided a simple scheme that was understandable to the laymen and was successfully used in a great many studies (Semenzin et al., 2008; Hwang et al., 2013).

Two LoEs represented biological effects, including observed chronic toxicity and biomarkers. The effects in test sediments were scored 0 if they showed no significant difference from the control, otherwise a value of 1 was assigned. After chronic exposure to site sediments, remarkable toxicity to *C. dilutus* was noted at most sites (Qi et al., 2015) and the scores of chronic toxicity (LoE1) for each site are presented in Table S6 in the supplementary data. Biomarkers were evaluated as LoE2 and provided more sensitive indication of sublethal responses at the molecular level (Table S7). Meanwhile, $TU_{bioavailable}$ values were used as additional LoE (LoE3) for assessing chemical stress. Chemical stress at individual sites was scored 0 or 1 when the sum of $TU_{bioavailable}$ for all detected contaminants was < 1 or ≥ 1, respectively. Finally, WoE scores for bioassays, biomarkers and chemical stressors were averaged to gain an integrated score and each LoE was equally weighted.

The test sites were ranked qualitatively by scores and the overall evaluation of hazard for the five sites were in the order of $T1 > T2 > T3 > T4 > T5$ (Table 2). The most contaminated area was Meiliang Bay (T1 and T2), followed by Zhushan Bay (T3) and Gong Bay (T4). On the contrary, sediment quality the conveying water district (T5), which was the outlet to Wangyu River and was close

to Yangtze River, was not significantly different from the control site. The relatively low level of contamination at T5 was likely due to the dilution effect of water diversion from Yangtze River to Tai Lake. Two pesticides in sediment, γ-HCH and chlorpyrifos, were the plausible contributors of chronic toxicity to *C. dilutus* in Tai Lake, especially in Meiliang Bay.

None of the sediments collected from Tai Lake showed acute lethality to the midges, thus it is valuable to include the measurements of biomarkers as additional LoE to provide an efficient sentinel in early warning for contaminants in the field. As mentioned above, impaired emergence of *C. dilutus* (after pupation) was successfully predicted by the IBR indices (before pupation). Furthermore, alteration of specific biomarkers helped identifying principal toxicants at the sites, for instance, the inhibition of AChE confirmed the finding of elevated chlorpyrifos in Meiliang Bay (T1). Overall, the application of multiple LoEs approach distinguished and ranked contaminated sites successfully. The integration of chemical and biological information also provided information on identifying the causative chemicals for the observed chronic toxicity.

4. Conclusions

Sediment quality in a typical freshwater lake in China was assessed by integrating multiple LoEs, including chronic toxic effects in organism level, biomarker responses in molecular level, and bioavailability-based chemical analysis. Although no acute lethality was noted for *C. dilutus* after exposure to the sediments from Tai Lake, chronic toxicity in growth, emergence and reproduction were evident along with the change in biomarkers. The measurements of bioavailability of sediment-bound contaminants increased the accuracy in identifying the causative agents to the toxicity. Using a qualitative scoring method, the contaminated sites were clearly distinguished and ranked by the level of contamination. Pesticides in sediment, particularly γ-HCH and chlorpyrifos, were the possible contributors to chronic toxicity to the midges. In addition, complementary information of the study sites can be achieved by the interrelationship between individual LoEs. The alteration of AChE corroborated with the detection of OPs and the IBR correlated well with the impairment of emergence of *C. dilutus*.

Appendix A. Suplementary data

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.ecoenv.2015.05.007.

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