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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 freshwater 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-hexachlorocyclohexane and chlorpyrifos, were the possible contributors to chronic toxicity to the midges.

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 biomarkers 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 overestimate 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). Desorption-based Tenax extraction measured the rapidly desorbing fractions of contaminants in sediment, and recent studies demonstrated 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 8000*g* 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