



Bioaccumulation of sediment-bound dichlorodiphenyltrichloroethane and heavy metals in benthic polychaete, *Nereis succinea* from a typical mariculture zone in South China



Huizhen Li^{a,b}, Fei Wang^{a,c}, Jing You^{b,*}

^a State Key Laboratory of Organic Geochemistry, Guangzhou Institute of Geochemistry, Chinese Academy of Sciences, Guangzhou 510640, China

^b School of Environment, Guangzhou Key Laboratory of Environmental Exposure and Health, and Guangdong Key Laboratory of Environmental Pollution and Health, Jinan University, Guangzhou 510632, China

^c Shenzhen Urban Planning and Land Resource Research Center, Shenzhen 518034, China

ARTICLE INFO

Article history:

Received 10 August 2016

Received in revised form 3 November 2016

Accepted 19 November 2016

Available online 26 November 2016

Keywords:

Sediment

DDT

Heavy metals

Bioaccumulation

Mariculture

Ecological risk

ABSTRACT

Bioaccumulation potential and associated ecological risk of sediment-bound DDT and its metabolites (DDXs) and heavy metals in Hailing Bay, a typical mariculture zone along the southern coast of China, were evaluated. The estuarine sediments were co-polluted by DDXs (120–4882 ng/g dry wt.) and heavy metals (292–409 mg/kg dry wt.). Both DDXs and metals in sediment were bioavailable to *Nereis succinea*, although the biota-sediment accumulation factors were less than 1 except for DDE. Significant transformation of DDT in organism was observed and DDE was the main metabolite. Ecological risk assessment showed that sediment-bound *p,p'*-DDT and *p,p'*-DDD frequently exhibited adverse effects on benthic community in the aquaculture zone, and heavy metals would cause moderate to considerable ecological risk, with Cd dominating the risk. The highest risk occurred in the shipyard area, indicating severe pollution and urgent need to control the source of DDT and heavy metals from aquafarming activities.

Capsule: Estuarine sediments in South China was co-polluted by DDT and its metabolites and heavy metals due to aquafarming activities, which resulted in bioaccumulation of the contaminants in benthic invertebrates and posed potential risk to species at higher trophic levels.

© 2016 Elsevier Ltd. All rights reserved.

1. Introduction

Sediments are known as a major sink for a variety of contaminants discharging into the aquatic system, and also act as a source of the contaminants to aquatic organisms and overlying water (Doong et al., 2002; Dural et al., 2007). Persistent toxicants, such as hydrophobic organic contaminants and heavy metals in sediments not only adversely affect benthic organisms (Mehler et al., 2011; Zhao et al., 2009), but also pose potential risk to species at higher trophic levels, including human via food chain transfer or direct consumption (Jones et al., 2014; Mearns et al., 2015; Yu et al., 2011a; Zhang et al., 2012).

Mariculture has rapidly developed over the past three decades worldwide due to the increasing demands for seafood. Fish farming activities, including feeding and boat operation and maintenance have caused the accumulation of persistent contaminants in estuarine sediment. For example, trash fish and fish meals used for feeding fish raised environmental loading of dichlorodiphenyltrichloroethane (DDT) and

heavy metals (Yu et al., 2011b; Zhang et al., 2012), and anti-fouling paints on boats partially explained fresh inputs of DDT and copper (Lin et al., 2009; Wu et al., 2016; Yu et al., 2011b). Consequently, deterioration of sediment quality in mariculture areas has raised more and more public attention. Previous studies measured the occurrence of DDT and heavy metals in sediment, water and fish samples from mariculture zones, providing evidence that those contaminants were bioaccumulative in fish and posed potential risk to human health (Hu et al., 2016; Qiu et al., 2011; Zhang et al., 2012).

Benthic invertebrates play a critical role in transferring sediment-bound contaminants to species at higher trophic levels, like fish. On one hand, benthic invertebrates act as a good indicator to sediment quality due to their direct contact with sediments (Ruus et al., 2005). On the other hand, some benthic invertebrates are important prey items to fish, so contaminants accumulated in these organisms may possibly transfer to fish via food chain (Mearns et al., 2015; Pan and Wang, 2012). However, little information is known on the bioaccumulation of sediment-bound contaminants to benthic polychaetes which are widely distributed in estuarine areas and are important prey items to fish. Research on the bioaccumulation of contaminants in the

* Corresponding author.

E-mail address: youjing@jnu.edu.cn (J. You).

polychaetes would provide better understanding on the bioavailability, toxicity and risk of sediment-bound contaminants and their transfer through food chain.

The objectives of the current study was to investigate the bioaccumulation potential of sediment-bound DDT and heavy metals in a benthic polychaete *Nereis succinea* from a typical mariculture zone in Hailing Bay, Guangdong, South China. The study site was chosen as Guangdong is one of the most important fishery culture bases in China, providing seafood to local and abroad people. Three specific aims were included: (1) to study the occurrence and composition of DDT and its metabolites (refers to DDXs, Table S1, “S” represents figures and tables in the Supplementary material thereafter) and heavy metals in the sediments; (2) to evaluate the bioaccumulation potential of DDXs and heavy metals in the polychaete by calculating the biota-sediment accumulation factors (BSAF); (3) to assess the potential ecological risk of DDXs and heavy metals.

2. Materials and methods

2.1. Chemicals and reagents

Neat compounds of DDT (*p,p'*- and *o,p'*-DDT) and its metabolites (*p,p'*-DDD, *o,p'*-DDD, *p,p'*-DDE, *o,p'*-DDE, *p,p'*-DDMU, *p,p'*-DBP, *p,p'*-DDNU and *p,p'*-DDM), surrogate standard (polychlorinated biphenyl (PCB)-67) and internal standard (*p,p'*-DDT-d8) were purchased from AccuStandard (New Haven, CT, USA). A multi-element stock solution of metal standards at the concentration of 100 µg/mL was purchased from General Research Institute for Nonferrous Metals (Beijing, China).

High performance liquid chromatography grade hexane was purchased from Burdick and Jackson (Ulsan, Korea). Prior to use, analytical grade acetone (Tianjin Chemical, China) was redistilled, anhydrous Na₂SO₄ was baked at 450 °C for 4 h, and copper powder was activated using concentrated HCl and then washed using de-ionized water and acetone in sequence. High purity concentrated sulfuric acid, nitric acid and hydrofluoric acid were obtained from Guangzhou Chemical Factory. The glassware used in the current study was soaked in a surface active cleaning agent (2% Decon 90, East Sussex, UK) for 24 h, rinsed using de-ionized water, dried at 120 °C and then baked at 450 °C prior to use. The digestion vessels and centrifuge tubes used for heavy metal analysis were soaked in diluted nitric acid for 24 h, rinsed using de-ionized water and dried at room temperature. Sea salt (Guangzhou Aqua-Pro Aquarium Factory) was dissolved in de-ionized water to prepare artificial seawater with a salinity of 28‰. The artificial seawater was aerated for at least 24 h prior to use.

2.2. Study area and sediment sampling

Hailing Bay in Yangjiang, Guangdong locates in the Pearl River Estuary which is one of the most important estuaries along the southern coast of China. Hailing Bay is famous for fish farming and tourism. The total area of aquaculture in Yangjiang has reached 39,600 ha, and Hailing Bay Shallow Sea Base is one of the largest bases in Yangjiang (http://www.yangjiang.gov.cn/english/Investment/Investment_Environment/Marine_Fishery/201203/t20120315_65843.html). Within Hailing Bay, Zhapo fishing harbor is a relatively large base with many aquaculture farms and fishing boats being set up. In total, seven surface sediments (0–10 cm) were collected from the mariculture zone in Hailing Bay using a stainless steel grab sampler. The sediment samples were stored in pre-cleaned plastic box, transferred back to the laboratory and stored at 4 and –20 °C for bioaccumulation testing and chemical analysis, respectively. Detailed information on the sampling sites is presented in Fig. S1 and Table S2. A reference sediment was collected from Zhanjiang harbor where few human activity was noted nearby.

2.3. Bioaccumulation testing

Twenty-eight day bioaccumulation testing was conducted using the polychaete *N. succinea* according to the protocols proposed by American Society for Testing and Material International (ASTM, 2005). The lugworms were purchased from an aquaculture farm in Zhanjiang, China and acclimatized for over 30 d in the laboratory before bioaccumulation testing. Artificial seawater was added into the container with sediment as overlying water (~10 cm), and the sediment was settled for 12 h before the tests. Ten lugworms of similar length (~15 cm) were selected and introduced in each testing chamber. The testing was conducted at 23 ± 1 °C with a 16:8 of light:dark photocycle. The overlying water was gently aerated during the testing, and water quality parameters, including salinity, dissolved oxygen, pH, and temperature were monitored every day. The overlying water was manually renewed every three days and the organisms were not fed during the testing. At the end of the bioaccumulation period, the organisms were sieved from the sediment, transferred to clean artificial seawater for 24-h gut purge, weighted and then stored at –20 °C for further analysis.

2.4. Chemical analysis

2.4.1. DDXs

The sediments and lugworms were freeze-dried, thoroughly homogenized, extracted using CW-2000 ultrasound-assisted microwave extractor (Xintuo, China), cleaned using self-packed column or gel permeation chromatography (GPC, Biotech, China), and analyzed for DDXs using an Agilent 7890-5975C gas chromatography-mass spectrometer (GC-MS, Agilent, USA). In brief, 5 g of dry sediment or 1 g of dry lugworm sample, 100 mL of a mixture of hexane and acetone (1:1, v/v), 1 g of copper powder (sediment sample only) and 50 ng of surrogate (PCB-67) were added into a 250-mL extraction flask. The extraction was conducted for 6 min with ultrasound and microwave power being set at 50 and 100 W, respectively, and the extraction was repeated with additional 50 mL of the extraction solvents. The extracts were combined, filtered, concentrated and solvent exchanged to 1 mL of hexane using a Turbovap (Xintuo). The sediment extract was cleaned using a wet-packed column (a diameter of 1 cm) with 6, 12 and 2 cm of alumina, silica gel and anhydrous sodium sulfate being packed from the bottom to the top. After loading the sample, 70 mL of a mixture of hexane and dichloromethane (7:3, v/v) was used to elute the target compounds. On the other hand, the organism extract was cleaned using GPC with dichloromethane being used as the mobile phase. The clean extracts were concentrated, solvent exchanged to hexane and then analyzed for DDT and its metabolites on GC-MS in electron impact ionization mode after adding 50 ng of *p,p'*-DDT-d8 as the internal standard.

The analytes were separated on a HP-5MS column (30 m × 0.25 mm × 0.25 µm) and helium was used as the carrier gas with a flow rate of 1.2 mL/min. The sample (1 µL) was injected into column in splitless mode with the temperature of injector at 260 °C. The temperature of the transfer line, ion source, and quadrupole was set at 260, 230 and 150 °C, respectively. The initial temperature of oven was set at 80 °C, and it ramped to 240 °C at a rate of 20 °C/min, held for 6 min, and ramped to 280 °C at 30 °C/min. Chemical qualification was achieved by simultaneous detection of target and qualifier ions within the retention time windows and chemical quantification was performed using internal standard calibration.

2.4.2. Heavy metals

Freeze-dried sediment (~0.2 g), 9 mL of concentrated nitric acid and 3 mL of 40% hydrofluoric acid (v/v) were added into a digestion vessel and digested at 180 °C for 20 min in a Xintuo XT-9900 microwave digestion system. After the system was cooled down, the digested solution was transferred into a 50-mL polyethylene terephthalate centrifuge tube. The digestion vessel was then rinsed with de-ionized water for three times and the solutions were combined. The solution was heated

at 80 °C using an Xintuo XT-9800 electric hot plate until near dryness, then 2 mL of concentrated nitric acid was added. The solution was finally diluted to 100 mL with deionized water for analysis. The freeze-dried organism tissue was grounded and thoroughly homogenized. Accurately weighted organism tissue (~0.3 g) was digested using concentrated nitric acid at room temperature for 2 d, and then the samples were heated to 120 °C until the tissues were completely digested.

The concentrations of the target heavy metals in sediment and organism tissue samples were analyzed by an Agilent 7700x inductively coupled plasma-MS. Five metals, i.e. Sc, Ge, Rh, In and Bi (1 mg/L) were used as internal standards in the calibration curves. The instrumental detection limits of the target heavy metals, i.e. As, Cd, Cr, Cu, Ni, Pb and Zn were 0.02, 0.005, 0.0037, 0.49, 0.056, 0.57 and 0.009 µg/L, respectively.

2.5. Quality assurance and quality control

A calibration standard of DDXs was analyzed on GC–MS every 10 samples and the relative percent variations between the calibration curve and the calibration standard were within 20%. Another calibration standard only with DDT was also analyzed on the instrument every 10 samples to ensure the possible degradation of DDT on the inlet was less than 20%. In addition, a method blank (solvent), a matrix blank (reference sediment or organism tissue), a matrix spike, and a matrix spike duplicate were analyzed along with the sample process. The concentrations of target compounds in the blanks were all lower than the detection limits. Recoveries of the target compounds in the matrix spikes ranged from 67% to 118%, and recoveries of the surrogate (PCB-67) in all the samples were 61%–90%.

The accuracy in quantifying heavy metals in sediment and organism samples was checked by concurrent analyses of the State Oceanographic Administration of China reference materials, marine sediment (GBW-07334) and shrimp tissue (GBW-10050). Recoveries of the target metals were 65%–117% and 77%–117% for reference marine sediment and shrimp tissue, respectively.

2.6. Data analysis

Biota-sediment accumulation factor (BSAF) was applied to express the bioaccumulation potential of DDXs and metals from marine sediment to the benthic polychaete (Eq. 1).

$$BSAF = C_b / C_s \quad (1)$$

where C_b and C_s are the concentrations in the organism and sediment, respectively. For DDXs, C_b and C_s were normalized by the contents of lipid and organic carbon, respectively, while they were both on dry weight basis for heavy metals.

Potential risk of sediment-associated DDXs to benthic community was evaluated using risk quotient (RQ) and it was calculated using Eq. (2).

$$RQ = C_s / TOX_h \quad (2)$$

where TOX_h is the reference benchmark for ecological risk assessment. Three reference benchmarks recommended by the U.S. Environmental Protection Agency (USEPA) and National Oceanic and Atmospheric Administration (NOAA) were used in the current study to screen the ecological risk of sediment-bound DDXs (Burton, 2002). The description and threshold values for the benchmarks are presented in Table S3.

Potential risk of heavy metals in Hailing Bay sediments to marine ecosystem was assessed using ecological risk index (E_{RI}) based on the following equations (Hakanson, 1980).

$$E_r^i = T_r^i \times \frac{C^i}{C_o^i} \quad (3)$$

$$E_{RI} = \sum_{i=1}^7 E_r^i \quad (4)$$

where E_r^i is monomial potential risk factor. C^i and C_o^i are the measured sediment concentration of a specific heavy metal and its reference value, respectively. No background data of heavy metals for uncontaminated marine sediment in the study area is available, so the national soil background values in China (9.2, 0.07, 53.9, 20.0, 25.0, 23.6 and 67.7 mg/kg dry wt. for As, Cd, Cr, Cu, Ni, Pb and Zn, respectively) were used for risk assessment in the current study (Li, 1995; Zhang et al., 2012). T_r^i is the toxicity factor for the metals, with the values being 10, 30, 2, 5, 5, 5 and 1 for As, Cd, Cr, Cu, Ni, Pb and Zn, respectively (Wang et al., 2011). E_{RI} is the overall potential ecological risk of various metals based on the sensitivity of biological community in a study region. The potential ecological risk to benthic communities caused by heavy metals were then classified into the following five categories (Gan et al., 2000).

Low risk: $E_r^i < 30, E_{RI} < 100$.

Moderate risk: $30 \leq E_r^i < 50, 100 \leq E_{RI} < 150$.

Considerable risk: $50 \leq E_r^i < 100, 150 \leq E_{RI} < 200$.

Very high risk: $100 \leq E_r^i < 150, 200 \leq E_{RI} < 300$.

Disastrous risk: $E_r^i \geq 150, E_{RI} \geq 300$.

The comparison of bioaccumulation potential among treatments was performed by one-way ANOVA analysis followed by Tukey's post hoc tests ($\alpha = 0.05$). Principal component analysis (PCA) was used to evaluate the possible correlations among the occurrence of target contaminants and sedimentary factors, and it was performed using SPSS (Chicago, IL, USA).

3. Results and discussion

3.1. Co-occurrence of DDXs and heavy metals in sediment

As shown in Fig. 1 and Table S4, DDXs were detected in all the sediments from the mariculture zone in Hailing Bay, with concentrations ranging from 58.4 to 4882 ng/g dry wt. Two exceptionally high levels (1585 and 4882 ng/g dry wt.) were found in the sediments collected in Zhapo fishing harbor, where a shipyard, seawall and a beacon located nearby (Fig. 1). Previously, high DDX levels have also been reported in sediments sampled from Zhapo fishing harbor in 2010 (1200 and 4800 ng/g dry wt., (Yu et al., 2011b)), Hong Kong fishing harbor (up to 7400 ng/g dry wt., (Lin et al., 2009)), and Macau Harbor (1600 ng/g dry wt., (Mai et al., 2002)). Comparably, relatively low concentrations of sediment-bound DDXs were detected in the mariculture zone (S1, S2 and S3) and DDX concentrations decreased with increasing distances from the coast (Fig. 1). One of the reasons for the phenomenon was a possible source of DDXs from berth of fishing boats in the harbor and along the coast.

As shown in the composition profile of DDXs (Fig. S2), p,p' - and o,p -DDT were the dominant components in Hailing Bay sediments, accounting for 59% (47% and 12% for p,p' - and o,p -DDT, respectively) of the sum concentrations of DDXs on average. In addition, a batch of metabolites, including DDD (15% and 6% for p,p' - and o,p -DDD, respectively), DDE (7% and 0.2% for p,p' - and o,p -DDE, respectively), p,p' -DDMU (3%), p,p' -DBP (6%), p,p' -DDNU (3%) and p,p' -DDM (0.4%) were detected, as a result of significant degradation of DDTs in sediment. The ratios of (DDD + DDE)/(DDT + DDD + DDE) were all smaller than 0.5 in all the sediments except for the ratio of (o,p -DDD + o,p -DDE)/(o,p -DDT + o,p -DDD + o,p -DDE) in S3 sediment (0.73), indicating fresh input of DDT in this area. The result was consistent with previous studies that DDT-containing antifouling paints on boats has become a major source for severe DDT pollution in fishing harbors after the prohibition of DDT use in 1980s (Lin et al., 2009; Yu et al., 2011b).

Sum and individual concentrations of the seven metals (As, Cd, Cr, Cu, Ni, Pb and Zn) in the sediments collected from Hailing Bay were

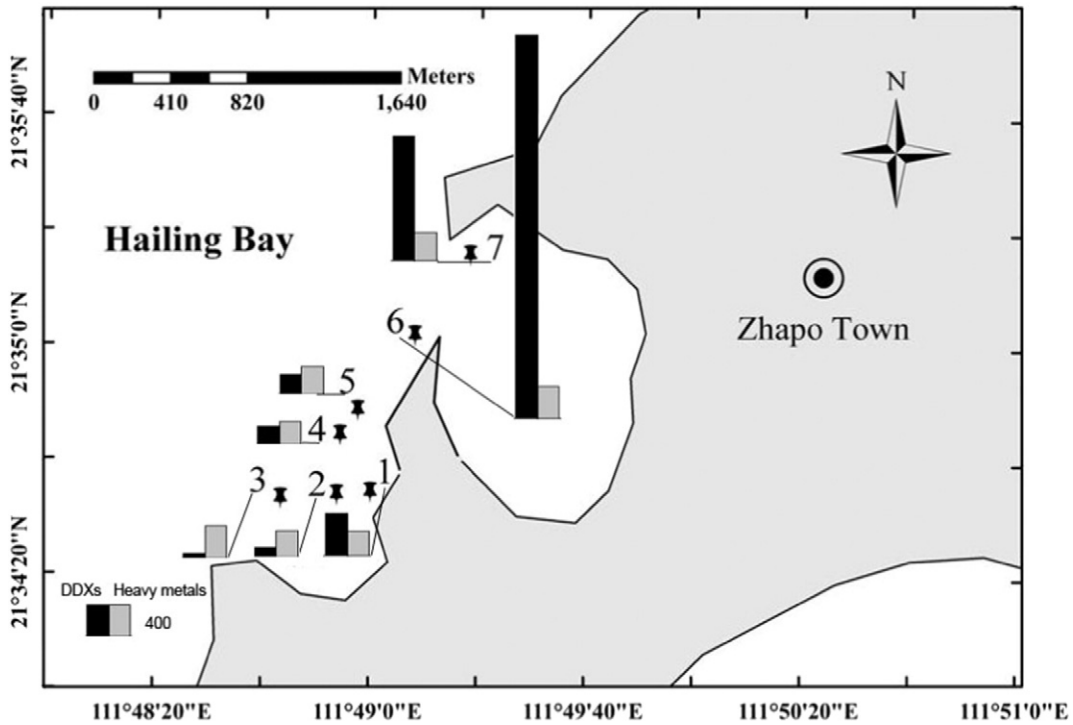


Fig. 1. Sampling map and the sum concentrations of DDT and its metabolites (DDXs, ng/g dry wt.) and heavy metals (mg/kg dry wt.) in the sediments collected from Hailing Bay, a typical mariculture zone in South China.

similar among sites (Fig. 1 and Table S5), except for copper whose concentrations in the sediments from the shipyard area (S6 and S7) were approximately two times higher than those in other sites (Table S5). In general, the concentrations of heavy metals in the current study were comparable to those previously reported in this area (Qiu et al., 2011; Zhang et al., 2012). The levels of sediment-bound metals in mariculture area in Hailing Bay were lower than those in the sediments collected from other polluted estuaries, such as Jinzhou Bay (Fan et al., 2014), Quanzhou Bay (Yu et al., 2008), and Kaohsiung Harbor (Lin et al., 2013) in China and Tinto Estuary in Spain (Vicente-Martorell et al., 2009), but were higher than those from non-aquaculture zones in Hailing Bay (Zhang et al., 2012) and some relatively clean estuaries, such as Changhua River Estuary in Hainan, China (Hu et al., 2013). The greater metal concentrations in mariculture area compared with non-aquaculture zone suggested that there was likely source of heavy metals related to aquaculture activities in Hailing Bay.

Overall, DDXs and heavy metals were co-occurred in the sediments in aquaculture zone and they possibly shared similar sources. Principal component analysis was performed to distinguish the sources of contaminants and the results are shown in Table 1. Sedimentary factors (total organic carbon (TOC), moisture content, density and grain size), major elements (Fe and Mn) and three heavy metal elements (As, Cr and Ni) shared PC 2, suggesting As, Cr and Ni in the sediments may mainly originate from natural source. Instead, DDXs, Cu and Pb shared PC 1, suggesting they may have similar anthropogenic source. Previous studies demonstrated that fresh DDT input in the study area mostly came from antifouling paints used for boats (Lin et al., 2009; Yu et al., 2011b). Copper is another widely used active component in antifouling paints (Lin et al., 2009). A significant correlation between sediment concentrations of Cu and DDXs was observed (Yu et al., 2011b), which supported the conclusion that DDXs and Cu shared the same source. In addition, tetraethyl lead was an antidetonant used in fuel oil, and application of leaded petrol might cause high levels of Pb residues in the environment (Cheng, 2003). Boat operation was frequent in aquaculture zone, which resulted in lead release and accumulation in the sediment. Therefore, PC1 may stand for a source of boat operation, maintenance

and repair relating to the use of oil and antifouling paints (Jessop and Turner, 2011; Yu et al., 2011b; Zhang et al., 2012). The conclusion was consistent with the spatial distribution of DDXs and Cu that sediments from the shipyard area in Zhapo Harbor (sediments S6 and S7) contained higher concentrations of DDXs and Cu than other sites (Tables S4 and S5). Furthermore, Zn and Cd shared PC4, suggesting these two elements may come from additional anthropogenic source relating to aquaculture activities (Basaran et al., 2010).

Table 1

The principle component analysis of sedimentary factors and target contaminants detected in the sediments collected from Hailing Bay, South China.

Component	PC				
	1	2	3	4	
Sedimentary factors	Total organic carbon	0.76			
	Moisture	0.94			
	Density	0.85			
	Grain size	0.82			
Heavy metals	Fe	0.70			
	Mn	0.85			
	Al			0.61	
	As		0.83		
	Cd				0.79
	Cr		0.87		
	Cu	0.841			
	Pb	0.77			
	Ni		0.85		
	Zn				0.72
DDXs	<i>p,p'</i> -DDT	0.97			
	<i>o,p'</i> -DDT	0.96			
	<i>p,p'</i> -DDD	0.87			
	<i>o,p'</i> -DDD	0.85			
	<i>p,p'</i> -DDE	0.99			
	<i>o,p'</i> -DDE	0.99			
	<i>p,p'</i> -DDMU	0.90			
	<i>p,p'</i> -DBP	0.99			
	<i>p,p'</i> -DDNU	0.69			
	<i>p,p'</i> -DDM	0.99			
	DDXs	0.95			

3.2. Bioaccumulation of DDXs and heavy metals in the polychaete

Quality of the overlying water was within the requirements by ASTM (2005) throughout the 28-d bioaccumulation testing, including temperature ($21.7 \pm 0.5 \text{ }^\circ\text{C}$), salinity ($26.8 \pm 0.5\text{‰}$), dissolved oxygen ($>70\%$ of saturation) and pH (7.5). No avoidance of lugworms to the sediments was observed. Variation in biomass of the lugworms at the beginning and the end of the testing was less than 20%. Moreover, lipid contents of the lugworms exposed to Hailing Bay sediments were comparable to that exposed to reference sediment (Table 2), suggesting no significant influence of sediment exposure on the behavior and growth of the organisms.

Significant bioaccumulation of DDT and its metabolites were observed in the lugworms after 28-d exposure to contaminated sediments, with sum concentrations of DDXs ranging from 3903 to 20,290 ng/g lipid (Fig. 2 and Table S4). Spatial distribution of sum DDXs in the lugworms were consistent with those in sediment in Hailing Bay, i.e. the sediments and exposed organisms from sites S6 and S7 contained the highest concentrations of DDXs (Fig. 2). Yet, the composition of individual DDXs in lugworms were quite different from those in sediment (Fig. S2). Instead of DDT being the dominant component in sediment, *p,p'*- and *o,p*-DDT only accounted for small portion (14% and 4%, respectively) of the sum concentrations of DDXs in the lugworms. Rather, the percentages of metabolites, including DDD (*p,p'*- and *o,p*-), DDE (*p,p'*- and *o,p*-) and *p,p'*-DDMU in the lugworms were significantly increased relative to the composition in the sediments (Fig. S2), indicating significant biotransformation of DDT occurred in the lugworms.

The BSAF was calculated to evaluate the bioaccumulation potential of DDXs in the lugworms (Table 2). The BSAF values for *p,p'*- and *o,p*-DDT were lower than 0.33 across all sites, possibly due to significant biotransformation of DDTs in the organisms. Greater BSAF values for the metabolites than parent compound DDT supported the conclusion. On average, DDE had the highest BSAFs, i.e. 0.63 and 2.5 for *p,p'*-DDE and *o,p*-DDE, respectively, indicating that DDE was the main metabolite of DDT in the lugworms after exposure to Hailing Bay sediments. As shown in Table S4, concentrations of DDE, especially for *o,p*-DDE, were lower than the reporting limits in most of the sediments, yet *o,p*-DDE was detected at considerable concentrations in the lugworms, which indicated that DDE in *N. succinea* were formed in vivo. That DDE was the main metabolite of DDT was surprisingly different from a previous study on biotransformation of DDT in *N. succinea* which were exposed to laboratory spiked sediments, where DDD was the dominant metabolite (Wang et al., 2015).

Biotransformation process of DDT in benthic invertebrates showed species specific. For example, DDT was mainly transformed to DDD in a mussel *Perna viridis* (Kwong et al., 2009), a Mediterranean mussel *Mytilus galloprovincialis* and a Norway lobster *Nephrops norvegicus* (Perugini et al., 2004). DDT was also reported to be mostly metabolized to DDE in an amphipod *Hyaella azteca* (Lotufo et al., 2000) and five other invertebrates (Johnson et al., 1971). In addition, BSAF values for

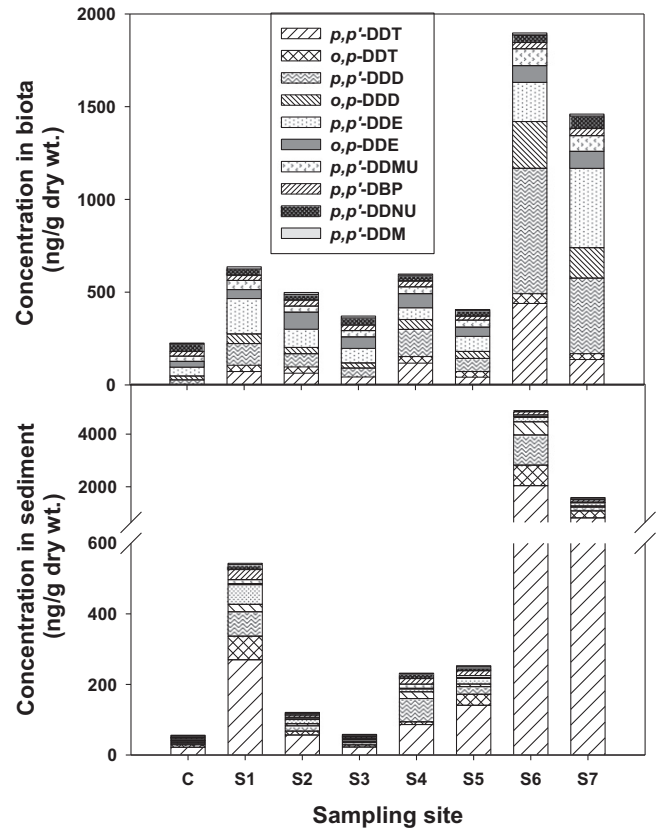


Fig. 2. Concentrations of individual DDXs in sediments collected from Hailing Bay and in the polychaete, *Nereis succinea* exposed to the sediments.

DDXs in S3 sediment was significantly higher than other sediments from Hailing Bay (Table 2), which suggested that sedimentary factors may influence the biotransformation process of DDXs in organisms.

Heavy metals were also detected in the lugworms after 28-d exposure to Hailing Bay sediments, suggesting they were bioavailable to the benthic invertebrates (Fig. 3 and Table S5). Different from metal distribution in sediment, metals in the lugworms showed obvious spatial variation. Lugworms exposed to sediments from the shipyard area in Zhapo Harbor (S6 and S7) had the highest body residues of metals, followed by sites S2 and S3 in the mariculture zone. Besides, the composition of individual metals in the sediments and lugworms were distinct (Fig. S3). On average, higher percentages of Zn and Cd in the total metals were detected in the lugworms (74% and 0.1%, respectively) compared with those in the sediments (31% and 0.06%, respectively). On the contrary, less percentages of Cr, Ni and Pb were found in the lugworms than those in the sediments, while percentages of As and Cu in the total metals were similar between the lugworms and sediments. The shifting

Table 2 Lipid content and biota-sediment accumulation factor (BSAF, g organic carbon/g lipid) of DDT and its metabolites for the polychaete, *Nereis succinea* exposed to the sediments collected from Hailing Bay, South China.

Site	Lipid content (%)	<i>p,p'</i> -DDT	<i>o,p</i> -DDT	<i>p,p'</i> -DDD	<i>o,p</i> -DDD	<i>p,p'</i> -DDE	<i>o,p</i> -DDE	<i>p,p'</i> -DDMU	<i>p,p'</i> -DBP	<i>p,p'</i> -DDNU	<i>p,p'</i> -DDM	<i>p,p'</i> -DDX	<i>o,p</i> -DDX
Reference	10.4	0	0	0.68	0.74	1.46	2.25	0.82	0.70	1.19	0.21	0.44	0.51
S1	8.6	0.02	0.04	0.14	0.20	0.28	1.31	0.35	0.08	0.20	0.26	0.09	0.12
S2	11.0	0.09	0.24	0.37	0.45	0.65	4.08	0.58	0.37	0.51	0.34	0.26	0.67
S3	9.5	0.24	0	0.99	0.90	2.25	5.25	1.22	0.89	1.24	0.58	0.76	0.98
S4	7.9	0.10	0.33	0.16	0.20	0.57	2.98	0.22	0.14	0.19	0.16	0.15	0.41
S5	9.0	0.03	0.09	0.30	0.45	0.46	2.13	0.57	0.13	0.30	0.10	0.12	0.26
S6	9.4	0.02	0.01	0.05	0.05	0.11	1.18	0.11	0.02	0.16	0.08	0.04	0.03
S7	10.5	0.01	0	0.10	0.13	0.12	0.57	0.16	0.01	0.04	0.04	0.03	0.03
Mean	9.6	0.07	0.10	0.30	0.34	0.63	2.50	0.46	0.23	0.38	0.22	0.21	0.36
Standard deviation	1.1	0.08	0.13	0.32	0.29	0.74	1.70	0.38	0.31	0.41	0.19	0.25	0.36

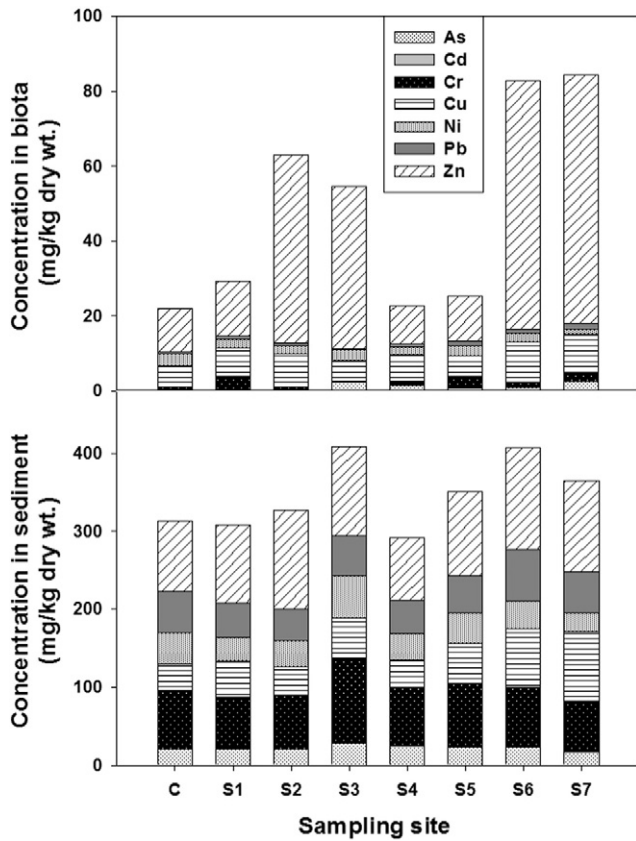


Fig. 3. Concentrations of individual heavy metals in sediments collected from Hailing Bay and in the polychaete, *Nereis succinea* exposed in the sediment.

composition of metals in organism and sediment was a result of different bioavailability of individual metals which was shown by their different BSAF values. The BSAFs of the seven metals in Hailing Bay sediments to benthic invertebrates were all less than 1, suggesting relatively low bioavailability of metals in the sediments. In comparison, Zn had the highest BSAFs (0.87 ± 0.14), followed by Cd (0.60 ± 0.27), As (0.32 ± 0.07), Cu (0.29 ± 0.09), Ni (0.11 ± 0.01), Cr (0.05 ± 0.02) and Pb (0.05 ± 0.01) (Table 3).

Sedimentary factors, chemical speciation and habitat and uptake routes of organisms are important parameters influencing the bioavailability and toxicity of metals in sediment (Zhang et al., 2014), and speciation analysis provided an effective approach to evaluate the biological and chemical availability of metals (Das et al., 1995; Zhang et al., 2012). Zhang et al. (2012) measured chemical speciation of the metals in Hailing Bay sediments and found that Cd appeared to be the most mobile, with over 50% on average in acid soluble fraction. The remaining metals showed less mobility with over 50% in residual fraction.

Table 3
The biota-sediment accumulation factors (BSAF, g dry wt. of sediment/g dry wt. of tissue) of heavy metals for the polychaete, *Nereis succinea* exposed to the sediments collected from Hailing Bay, South China.

Site	As	Cd	Cr	Cu	Ni	Pb	Zn
Reference	0.25	0.64	0.04	0.37	0.11	0.04	0.78
S1	0.30	0.58	0.08	0.32	0.12	0.05	0.74
S2	0.30	0.33	0.04	0.43	0.11	0.05	0.87
S3	0.29	0.29	0.02	0.24	0.08	0.03	0.90
S4	0.31	0.54	0.04	0.38	0.11	0.05	0.86
S5	0.28	0.68	0.07	0.24	0.10	0.05	0.66
S6	0.28	0.67	0.05	0.23	0.11	0.03	0.96
S7	0.48	1.09	0.07	0.20	0.11	0.05	1.08
Mean	0.32	0.60	0.05	0.29	0.11	0.05	0.87
Standard deviation	0.07	0.27	0.02	0.09	0.01	0.01	0.14

Low bioavailability of metals in Hailing Bay sediments in the speciation analysis by Zhang et al. (2012) was consistent with the low BSAFs in the current study. The high BASF values of Cd in the lugworms relative to other metals was also in accordance with its high mobility measured by speciation analysis, but greater bioaccumulation potential of Zn than other metals was not supported by speciation analysis in which most sediment-bound Zn was considered non-bioavailable (Zhang et al., 2012). This discrepancy showed that the bioavailability of sediment-bound metals was not only affected by sedimentary characteristics, other factors, such as organism behaviors, should also be considered. Previous studies have shown that different benthic organisms accumulated variable amounts of metals from the same sediment due to their difference in physiological and biochemical functions (e.g. Pempkowiak et al., 1999). Moreover, element regulation in organism is an important factor influencing their tissue concentrations of metals, particularly for essential metals like Zn (White and Rainbow, 1984; Yap et al., 2002). Yap et al. (2002) measured the total/speciation concentrations of metals in sediment and their body residues in benthic mussels, and found good correlations for Cd, Cu and Pb, but the correlation for Zn was not significant. The authors pointed out that Zn regulation in the organisms was a likely reason and it may also explain the greater bioaccumulation potential of Zn in the lugworms in the current study than the expectation from speciation analysis.

3.3. Ecological risk assessment

The HQ values were only calculated for *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE due to the lack of threshold values for *o,p'*-DDT and other metabolites (Table S6). In addition, *p,p'*-DDT, *p,p'*-DDD and *p,p'*-DDE were the dominant components in the sediments, accounting for 69% of sum DDXs on average (Table S4), so potential risk of the three compounds was calculated to estimate the overall risk of sum DDXs in Hailing Bay. Three threshold values, i.e. threshold effect level (TEL), probable effect level (PEL) and severe effect level (SEL) were applied to assess potential risk. The HQ-TEL values of DDXs were larger than 1 for all of the sediments from Hailing Bay, suggesting potential ecological risk. The levels of *p,p'*-DDT and *p,p'*-DDD were larger than PEL (HQ-PEL > 1) for 100% and 86% of the sediments, respectively, suggesting that most sediments contained *p,p'*-DDT and *p,p'*-DDD at concentrations high enough to cause frequent adverse effects on benthic community in the aquaculture zone. Furthermore, HQ-SEL values of *p,p'*-DDT and *p,p'*-DDD in respective 29% and 57% of the sediments were larger than 1, indicating severe ecological effects in the sediments from the shipyard area (sites S6 and S7).

The estimated ecological risk for heavy metals in sediments from Hailing Bay was presented in Table S7. Cadmium exhibited the highest ecological risk due to its high toxicity factor. The E_i^t values for Cd ranged from 65.2 to 133 (89.2 ± 23.1), with S2 sediment at very high risk ($100 \leq E_i^t < 150$) and the remaining sediments at considerable risk ($50 \leq E_i^t < 100$). The E_i^t values for the remaining six metals in Hailing Bay sediments were lower than 30, suggesting low risk, except for As in S3 sediment (30.4) at moderate risk. Based on the E_{Ri} values, three sediments (S2, S3 and S6) from Hailing Bay exhibited considerable risk and the remaining four sediments at moderate risk. Overall, moderate to considerable ecological risk for Hailing Bay sediments estimated from heavy metals were obvious, and was dominated by Cd. In general, risk from metals, i.e. Cd in Hailing Bay sediments in the current study was higher than that reported in the previous study (Zhang et al., 2012).

In addition to their potential risk to benthic community, DDXs and heavy metals in sediments from Hailing Bay were accumulated in the benthic polychaete, *N. succinea*. As sediment dwelling organisms, lugworms are important prey items to bottom dwelling fish species, suggesting possible transfer of contaminants to species at higher trophic levels in the food chain (Ruus et al., 2005). Yu et al. (2011a, 2011b) reported that bioaccumulation from food and overlying water were important loadings of DDXs in fish. Except for the two routes, transfer of

sediment-bound DDXs from sediment dwelling lugworms to fish, particularly for bottom dwelling fish may be another important loading (Mearns et al., 2015). Regarding non-metabolized metals, biomagnification of heavy metals, e.g. Hg was observed in fish feeding on benthic invertebrates near fish cages (Jones et al., 2014; Mearns et al., 2015). Furthermore, potential health risk of DDXs and heavy metals via consuming fish from Hailing Bay was also reported in previous studies (Qiu et al., 2011; Yu et al., 2011b). Therefore, DDXs and heavy metals in the sediments from mariculture zone in Hailing Bay not only exposed adverse effects on benthic community, but also caused potential risk to animals at higher trophic levels and even human health via food chain transport.

4. Conclusion and perspective

Pollution of DDXs and heavy metals in aquaculture zone in Hailing Bay were partially resulted from aquafarming activities, e.g. operation and maintenance of fishing boats, which was supported by the exceptional high residues of DDXs in sediments from shipyard area in Zhao Harbor. Sediment-bound DDXs and heavy metals were accumulated in the benthic polychaete, *N. succinea*, although bioavailability of the contaminants were low with BSAFs lower than 1 except for DDE. Significant biotransformation of DDT was observed and DDE was the main metabolites in the lugworms. Results of ecological risk assessment indicated that *p,p'*-DDT and *p,p'*-DDD in most of the sediments from Hailing Bay were estimated to cause frequent adverse effects on benthic community. In addition, moderate and considerable risk to the benthic community was estimated from sediment-bound heavy metals and Cd dominated the risk.

It should be noted that DDXs and heavy metals which were accumulated in sediment dwelling polychaete were possibly transferred to fish via food chain due to their persistence and biomagnification. Hailing Bay is an important aquafarming base in South China, providing seafood to local and abroad people. Thus, inputs of DDT and heavy metals from aquafarming activities caused deterioration of sediment quality and coastal ecosystem, and at the same time, posed potential health risk to whom consuming the polluted seafood. Therefore, continuous monitoring and further study on food chain transfer of DDXs and heavy metals from the benthic invertebrates should be conducted to comprehensive assess ecological and human health risks.

Acknowledgement

We thank Baoquan Sun, Xiaoyi Yi, Xin Chen, Hongxue Qi, Yuanquan Pei and Ping Ma for sample collection. This work was supported by the Natural Science Foundation of China (41273120, 41473106 and 41503091) and Natural Science Foundation of Guangdong Province, China (2015A030310219 and 2016A030312009). This is contribution No. IS-2315 from GIGCAS.

Appendix A. Supplementary data

Supplementary data to this article can be found online at doi:10.1016/j.marpolbul.2016.11.048.

References

- ASTM, 2005. Standard Test Method for Measuring the Toxicity of Sediment-associated Contaminants with Freshwater Invertebrates. ASTM E 1706-05. ASTM International.
- Basaran, A.K., Aksu, M., Egemem, O., 2010. Impacts of the fish farms on the water column nutrient concentrations and accumulation of heavy metals in the sediments in the eastern Aegean Sea (Turkey). *Environ. Monit. Assess.* 162, 439–451.
- Burton, G.A., 2002. Sediment quality criteria in use around the world. *Limnology* 3, 65–76.
- Cheng, S., 2003. Heavy metal pollution in China: origin, pattern and control. *Environ. Sci. Pollut. Res.* 10, 192–198.
- Das, A.K., Chakraborty, R., Cerver, M.L., Guardia, M.D.L., 1995. Metal speciation in solid matrices. *Talanta* 42, 1007–1030.
- Doong, R.A., Sun, Y.-C., Liao, P.L., Peng, C.K., Wu, S.C., 2002. Distribution and fate of organochlorine pesticide residues in sediments from the selected rivers in Taiwan. *Chemosphere* 48, 237–246.
- Dural, M., Göksu, M.Z.L., Özak, A.A., 2007. Investigation of heavy metal levels in economically important fish species captured from the Tuzla lagoon. *Food Chem.* 102, 415–421.
- Fan, W., Xu, Z., Wang, W.X., 2014. Metal pollution in a contaminated bay: relationship between metal geochemical fractionation in sediments and accumulation in a polychaete. *Environ. Pollut.* 191, 50–57.
- Gan, J., Jia, X., Lin, Q., Li, C., Wang, Z., Zhou, G., Wang, X., Cai, W., Lu, X., 2000. A primary study on ecological risk caused by the heavy metals in coastal sediments. *J. Fish. China* 24, 533–538 (in Chinese).
- Hakanson, L., 1980. An ecological risk index for aquatic pollution control: a sedimentological approach. *Water Res.* 14, 975–1001.
- Hu, B., Cui, R., Li, J., Wei, H., Zhao, J., Bai, F., Song, W., Ding, X., 2013. Occurrence and distribution of heavy metals in surface sediments of the Changhua River Estuary and adjacent shelf (Hainan Island). *Mar. Pollut. Bull.* 76, 400–405.
- Hu, S., Su, Z., Jiang, J., Huang, W., Liang, X., Hu, J., Chen, M., Cai, W., Wang, J., Zhang, X., 2016. Lead, cadmium pollution of seafood and human health risk assessment in the coastline of the southern China. *Stoch. Env. Res. Risk A.* 30, 1379–1386.
- Jessop, A., Turner, A., 2011. Leaching of Cu and Zn from discarded boat paint particles into tap water and rain water. *Chemosphere* 83, 1575–1580.
- Johnson, B.T., Saunders, C.R., Sanders, H.O., Campbell, R.S., 1971. Biological magnification and degradation of DDT and aldrin by freshwater invertebrates. *J. Fish. Res. Board Can.* 28, 705–709.
- Jones, H.J., Swadling, K.M., Butler, E.C.V., Barry, L.A., Macleod, C.K., 2014. Application of stable isotope mixing models for defining trophic biomagnification pathways of mercury and selenium. *Limnol. Oceanogr.* 59, 1181–1192.
- Kwong, R.W.M., Yu, P.K.N., Lam, P.K.S., Wang, W.X., 2009. Biokinetics and biotransformation of DDTs in the marine green mussels *Perna viridis*. *Aquat. Toxicol.* 93, 196–204.
- Li, T., 1995. Element abundances of China's continental crust and its sedimentary layer and upper continental crust. *Chin. J. Geochem.* 14, 26–32.
- Lin, T., Hu, Z., Zhang, G., Li, X., Xu, W., Tang, J., Li, J., 2009. Levels and mass burden of DDTs in sediments from fishing harbors: the importance of DDT-containing antifouling paint to the coastal environment of China. *Environ. Sci. Technol.* 43, 8033–8038.
- Lin, Y., Chang-Chien, G., Chiang, P., Chen, W., Lin, Y., 2013. Multivariate analysis of heavy metal contaminations in seawater and sediments from a heavily industrialized harbor in southern Taiwan. *Mar. Pollut. Bull.* 76, 266–275.
- Lotufo, G.R., Landrum, P.F., Gedeon, M.L., Tigue, E.A., Herche, L.R., 2000. Comparative toxicity and toxicokinetics of DDT and its major metabolites in freshwater amphipods. *Environ. Toxicol. Chem.* 19, 368–379.
- Mai, B., Fu, J., Sheng, G., Kang, Y., Lin, Z., Zhang, G., Min, Y., Zeng, E.Y., 2002. Chlorinated and polycyclic aromatic hydrocarbons in riverine and estuarine sediments from Pearl River Delta, China. *Environ. Pollut.* 117, 457–474.
- Mearns, A.J., Reish, D.J., Oshida, P.S., Ginn, T., Rempel-Hester, M.A., Arthur, C., Rutherford, N., Pryor, R., 2015. Effects of pollution on marine organisms. *Water Environment Research* 87, 1718–1816.
- Mehler, W.T., Li, H., Lydy, M.J., 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.
- Pan, K., Wang, W.X., 2012. Trace metal contamination in estuarine and coastal environments in China. *Sci. Total Environ.* 421–422, 3–16.
- Pempkowiak, J., Sikora, A., Biernacka, E., 1999. Speciation of heavy metals in marine sediments vs their bioaccumulation by mussels. *Chemosphere* 39, 313–321.
- Perugini, M., Cavaliere, M., Giammarino, A., Mazzone, P., Olivieri, V., Amorena, M., 2004. Levels of polychlorinated biphenyls and organochlorine pesticides in some edible marine organisms from the Central Adriatic Sea. *Chemosphere* 57, 391–400.
- Qiu, Y.-W., Lin, D., Liu, J., Zeng, E.Y., 2011. Bioaccumulation of trace metals in farmed fish from South China and potential risk assessment. *Ecotoxicol. Environ. Saf.* 74, 284–293.
- Ruus, A., Schaanning, M., Øxnevad, S., Hylland, K., 2005. Experimental results on bioaccumulation of metals and organic contaminants from marine sediments. *Aquat. Toxicol.* 72, 273–292.
- Vicente-Martorell, J.J., Galindo-Riño, M.D., García-Vargas, M., Granado-Castro, M.D., 2009. Bioavailability of heavy metals monitoring water, sediments and fish species from a polluted estuary. *J. Hazard. Mater.* 162, 823–836.
- Wang, Y., Yang, Z., Shen, Z., Tang, Z., Niu, J., Gao, F., 2011. Assessment of heavy metals in sediments from a typical catchment of the Yangtze River, China. *Environ. Monit. Assess.* 172, 407–417.
- Wang, F., Pei, Y., You, J., 2015. Biotransformation of dichlorodiphenyltrichloroethane in the benthic polychaete, *Nereis succinea*: quantitative estimation by analyzing the partitioning of chemicals between gut fluid and lipid. *Environ. Toxicol. Chem.* 34, 360–368.
- White, S.L., Rainbow, P.S., 1984. Regulation of zinc concentration by *Palaemon elegans* (Crustacea: Decapoda): zinc flux and effects of temperature, zinc concentration and moulting. *Marine Ecology-Progress Series* 16, pp. 135–147.
- Wu, C., Bao, L., Tao, S., Zeng, E.Y., 2016. Significance of antifouling paint flakes to the distribution of dichlorodiphenyltrichloroethanes (DDTs) in estuarine sediment. *Environ. Pollut.* 210, 253–260.
- Yap, C.K., Ismail, A., Tan, S.G., Omar, H., 2002. Correlations between speciation of Cd, Cu, Pb and Zn in sediment and their concentrations in total soft tissue of green-lipped mussel *Perna viridis* from the west coast of Peninsular Malaysia. *Environ. Int.* 28, 117–126.
- Yu, R., Yuan, X., Zhao, Y., Hu, G., Tu, X., 2008. Heavy metal pollution in intertidal sediments from Quanzhou Bay, China. *J. Environ. Sci.* 20, 664–669.
- Yu, H., Guo, Y., Bao, L., Qiu, Y., Zeng, E.Y., 2011a. Persistent halogenated compounds in two typical marine aquaculture zones of South China. *Mar. Pollut. Bull.* 63, 572–577.

- Yu, H., Shen, R., Liang, Y., Cheng, H., Zeng, E.Y., 2011b. Inputs of antifouling paint-derived dichlorodiphenyltrichloroethanes (DDTs) to a typical mariculture zone (South China): potential impact on aquafarming environment. *Environ. Pollut.* 159, 3700–3705.
- Zhang, W., Liu, X., Cheng, H., Zeng, E.Y., Hu, Y., 2012. Heavy metal pollution in sediments of a typical mariculture zone in South China. *Mar. Pollut. Bull.* 64, 712–720.
- Zhang, C., Yu, Z., Zeng, G., Jiang, M., Yang, Z., Cui, F., Zhu, M., Shen, L., Hu, L., 2014. Effects of sediment geochemical properties on heavy metal bioavailability. *Environ. Int.* 73, 270–281.
- Zhao, Z., Zhang, L., Wu, J., Fan, C., 2009. Distribution and bioaccumulation of organochlorine pesticides in surface sediments and benthic organisms from Taihu Lake, China. *Chemosphere* 77, 1191–1198.