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Joint toxicity of sediment-associated DDT and copper to a polychaete, *Nereis succinea*

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Abstract As major components in antifouling paints, both dichlorodiphenyltrichloroethane (DDT) and copper are ubiquitous in estuarine sediment and have been detected at high concentrations in the harbors in South China. In the present study joint toxicity between DDT and copper to an estuarine polychaete, *Nereis succinea,* was examined using bioaccumulation potential, growth impairment and change in lipid peroxidation contents as sub-lethal endpoints. In general, the toxicity of DDXs (DDT and its metabolites) and copper acted independently and copper was more toxic to the lugworms at environmentally relevant concentrations. Nevertheless, co-exposure to copper led to a significant reduction in the bioaccumulation of DDXs when the concentrations of DDXs in sediment were high. The inhibition of DDX bioaccumulation by copper may be partially explained by the decrease in the bioavailability of sediment-associated DDXs which were estimated by biomimetic gut fluid extraction. The saturation of the solubilization agents or the inhibition of protease activity in gut fluid of *N. succinea* by copper limited the DDX bioavailability and the subsequent bioaccumulation.

Keywords Joint toxicity · DDT · Copper · Bioaccumulation · Bioavailability · *Nereis succinea*

Introduction

Enormous amounts of dichlorodiphenyltrichloroethane (DDT), a legacy organochlorine pesticide, have been historically applied worldwide. Consequently, DDT and its degradation products (designated as DDXs), e.g. dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE), are ubiquitous in the environment. Due to increasing awareness of its high persistence and bioaccumulation potential the use of DDT has been restricted for decades, resulting in the decreases in DDT concentrations in various environmental media (Li et al. 2014). Nevertheless, concentrations of DDT have hardly changed in some areas and DDT pollution is still a major concern in coastal regions in Asian countries (Monirith et al. 2003; Li et al. 2014). Antifouling paints were considered as one of input sources of fresh DDT in harbors (Lin et al. 2009).

Since the production in 1950s, DDT-containing antifouling paints have been extensively employed for boat maintenance (Cao and Zhang 2009). In China, approximately 11,000 tons of DDT was used to produce the antifouling paints from 1950 to 2005 (Global Environment Facility 2007). Many boats coated with DDT-containing paints are still in service up to now and pose a continuing threat to the estuarine ecosystem by releasing DDXs into the environment. Elevated levels of DDXs were detected in sediment samples collected from the harbors in Yangjiang and Hong Kong in South China and the highest concentrations reached 4,800 and 7,400 ng/g dry weight (dw), respectively (Lin et al. 2009; Yu et al. 2011), and

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antifouling paints on the boats which were berthed in the harbors were one of the important sources of sediment-associated DDXs. In addition, DDXs in fish collected in the harbor showed a positive relationship to DDXs in sediment, implying the transfer of DDXs from sediment to fish (Yu et al. 2011).

Beside DDT, copper is also a major component in the antifouling paints. For example, Singh and Turner (2009) reported that paint residues from the docks of a boat facility in Plymouth, UK contained approximately 300 mg/g copper in average. Further estimation of copper bioavailability using a protein digestion method suggested that approximately 4 % copper in paint residues were bioavailable and significant accumulation of copper in a marine macroalga, *Ulva lactuca* from the discarded paint particles confirmed this estimation (Turner et al. 2008, 2009). Antifouling paints were also found as a source of sediment-bound copper in the harbors in South China (Lin et al. 2009; Zhang et al. 2012). Since DDT and copper have the same input source of the paints for boat maintenance, the two contaminants frequently co-occurred in sediment. As a result, estuarine organisms may be simultaneously exposed to DDXs and copper. Little information, however, is available on the joint toxicity of these two contaminants to estuarine organisms.

The objectives of the present study were to evaluate the joint toxicity of sediment-associated DDT and copper to a marine benthic polychaete, *Nereis succinea* and further analyze the impact of the bioavailability of sediment-bound DDT and copper on their joint toxicity using gut fluid extraction (GFE). The benthic marine detritivore lugworm, *N. succinea*, was selected as the test organism because of its wide distribution in coastal muddy bed, its being an important prey for bottom-feeding fish and crustaceans, and a model organism in estimating the bioaccumulation of sediment-bound pollutants (Tian and Zhu 2011). In addition to bioaccumulation potential and growth impairment, change in lipid peroxidation (LPO) contents was also selected as a sublethal endpoint for assessing the toxicity of DDXs and copper. The LPO expressed the damage of organism lipids due to oxidative stress, which occurred when excess reactive oxygen species (ROS) were produced. In an organism ROS are generally maintained at a certain level and play an important role in normal cellular activities, but its production might increase when the organism was exposed to contaminants (Circu and Aw 2010).

Materials and methods

Chemicals and sediment

The *p,p'*-DDT and $CuCl_2$ were used for spiking the sediments. Detailed information on the chemicals and reagents is presented in the Supplemental Data. A clean sediment was collected from an intertidal zone in Donghai Island, Zhanjiang, China and it was used as the control and to prepare the spiked sediments. After collection, the sediment was sieved through a 500-μm sieve to remove macrofauna, immediately transported to the laboratory and stored at 4 °C in the darkness. The density and water content of the sediment were 1.75 g/mL and 30.2 %, respectively. After removing inorganic carbon with 1 mol/L HCl, total organic carbon (TOC) of the sediment was determined using a vario EL III TOC analyzer (Elementar, Germany) and was 0.20 ± 0.02 %. Preliminary bioassay and analytical work indicated that the sediment had limited contamination and did not exhibit adverse effects to *N. succinea*.

Appropriate amounts of *p,p'*-DDT and $CuCl_2$ were spiked into sediment using acetone and water as the carrier, respectively. The sediment was thoroughly mixed for 3 h using a drill with a rotating stainless-steel blade. The volumes of the carrier for DDT spiking were equal (100 μL acetone/L sediment) across all sediments, including the controls which received acetone only. The $CuCl_2$ was spiked into sediment according to a method described by Hutchins et al. (2009) and the mass ratio of sediment to $CuCl_2$ solution was 4:1. After spiking, the sediments were aged at 4 °C for 30 days and homogenized again before use. The supernatant that developed from the sediment compaction was discharged at the end of the aging period.

Bioassays

Joint toxicity was evaluated using the sediments spiked with individual DDT and copper and their mixtures as shown in the experimental design in the Supplemental Data, Fig. S1. Nominal concentrations of DDT were 100 (A1), 5,000 (A2) and 10,000 (A3) ng/g dw, and they covered the range of sediment concentrations of DDXs in harbors in South China (Yu et al. 2011). Four nominal copper concentrations were used, including a concentration found in harbor sediment (50 μg/g dw, B1) (Zhang et al. 2012), two concentrations of sediment quality criteria for protection of aquatic life in China (100 and 250 μg/g dw, B2 and B3, respectively) (General Administration of Quality Supervision, Inspection and Quarantine 2002), and a high concentration (500 μg/g dw, B4). An additional DDT concentration (1,000 ng/g dw) was included in the original experimental plan of a partial 4 × 4 factorial design (Fig. S1a). However, spiking DDT at 1,000 ng/g dw in the sediments failed and were not included in the subsequent bioassays. Consequently, mixture toxicity testing was conducted using six treatments at different levels of DDT and copper (A1B1, A1B4, A2B2, A2B3, A3B1 and A3B4, Fig. S1b). In addition, the toxicity of individual

contaminants at the same concentrations as in the mixture testing was also simultaneously evaluated. Overall, DDT was spiked at three levels including A1 in treatments A1B0, A1B1 and A1B4, A2 in treatments A2B0, A2B2 and A2B3, and A3 in treatments A3B0, A3B1 and A3B4, while copper was at four concentrations of B1 (A0B1, A1B1 and A3B1), B2 (A0B2 and A2B2), B3 (A0B3 and A2B3) and B4 (A0B4, A1B4 and A3B4). Furthermore, the tests with solvent control were also simultaneously conducted (Fig. S1).

Mature lugworms were obtained from an aquaculture farm and acclimatized for a month in aquaria in the laboratory. The 28 day bioaccumulation tests with the lugworms were performed in triplicate following the standard protocol proposed by American Society for Testing and Materials (American Society for Testing and Materials 2000). The testing was conducted in a 2 L beaker containing 800 mL of sediment and 10 cm of overlying seawater. The sediment was settled for 12 h before eight lugworms being introduced into the beaker and the tests were carried out at 23 °C with a 16:8 h light:dark cycle. Individual lugworms of approximately 15 cm in length were sieved from the aquaria, transferred into clean seawater to purge the guts for 24 h and then added to test beakers. At the same time, eight lugworms were randomly selected, gut-purged for 24 h in clean seawater and analyzed for background concentrations of DDXs, copper, lipids, total proteins (TP), and LPO in organism. Each lugworm was weighed and the average wet weight per lugworm was 2.3 g.

Overlying water was renewed every 3 days and gently aerated and the organisms were not fed. Water quality parameters were monitored daily, including salinity, dissolved oxygen, pH and temperature. At the end of the 28-day testing, the lugworms were sieved from the sediments, washed with deionized water and transferred to clean seawater for 24-h gut purging process. After retrieved from the water, the lugworms were weighed and homogenized for LPO measurements or stored at −20 °C for chemical analysis.

After exposure and gut purging, fresh lugworms were homogenized in a glass homogenizer with a solution of 30 mmol/L Tris-NaCl buffer (pH 8.0; 0.15 mol/L NaCl, 5 mmol/L freshly prepared antiprotease, 2-mercaptoethanol, 0.1 mmol/L phenylmethylsulfonyl fluoride) being used as the homogenization buffer. The homogenized samples were centrifuged under 10,000×*g* at 4 °C for 30 min and the supernatant were decanted for analyzing the contents of TP and LPO. The TP was quantified by the Bradford Protein Assay using bovine serum albumin (BSA) as the standard (Bradford 1976) while LPO reaction was determined using a spectrophotometric method with thiobarbituric acid reagent suggested by Ohkawa et al. (1979). In brief, the homogenate was mixed with 10 % trichloroacetic acid solution which contained 1 mmol/L $FeSO_4$ and 0.67 % thiobarbituric acid. The mixture was incubated at 70 °C for 10 min and then the absorbance was measured at 532 nm on a Varioskan plate reader (Thermo Scientific, USA). Concentrations of the product were determined using a calibration curve with tetramethoxypropane as the standard and LPO levels were expressed as nmol of thiobarbituric acid reactive substances (TBARS) per mg TP. Additionally, lipid content in *N. succinea* was analyzed using a spectrophotometric method after acid digestion (van Handel 1985).

Target contaminants, including DDT and its degradation products and copper in the sediments were analyzed in three replicates before and after the bioassays. Chemical residues in the lugworms were also analyzed after the testing. The analytical procedures for sample preparation (solvent extraction and acid digestion for DDXs and copper, respectively) and instrumental analyses (gas chromatography/mass spectrometry (GC/MS) for DDXs and inductively coupled plasma atomic emission spectroscopy (ICP/AES) and atomic absorption spectrophotometry for copper) are detailed in the Supplemental Data. Information on the quality assurance and quality control for the analyses is also presented in the Supplemental Data.

Gut fluid extraction

The bioavailability of sediment-associated DDXs and copper to *N. succinea* was estimated using GFE following a method proposed by Voparil and Mayer (2004). A solution prepared by dissolving 5.0 g/L of BSA and 15.6 mmol/L of sodium taurocholate (ST) in the artificial seawater (BSA-ST solution) was used as a surrogate of gut fluid. The extraction was initiated by incubating 0.5 g dry sediment in 2 mL of the BSA-ST solution, which corresponded to a solid–fluid ratio of 0.25 g dry sediment/mL solution (Voparil and Mayer 2000, 2004). The sediment-solution slurries were shaken at 200 rpm for 4 h in the darkness and then centrifuged at 5,500×*g* for 30 min. The supernatants were decanted and filtered through 0.45 μm polytetrafluoroethene filters. Two types of controls were included in the GFE. One was a solvent blank of BSA-ST extraction solution without sediment being extracted and the other was sediment extracts using seawater as the extraction solution.

The DDXs in GFE supernatants were quantified with GC/MS after liquid–liquid extraction with dichloromethane and purified using concentrated sulfuric acid. To check the performance of analytical procedure and quantify DDXs on GC/MS, a surrogate (polychlorinated biphenyl-67 (PCB-67)) and internal standards (PCB-82 and *p,p'*-DDT-d8) were added to the samples before liquid–liquid extraction

and before GC/MS quantification, respectively. To analyze copper, the filtered GFE supernatants were dried at 80 °C and digested with 1 mL of 67 % HNO_3 at 85 °C. The digested solution was then diluted with appropriate amounts of deionized water before ICP/AES analysis.

Data analysis

Bioaccumulation potential of sediment-associated DDXs and copper to *N. succinea* was expressed as biota-sediment accumulation factors (BSAFs) (Eq. 1).

$$\mathrm{BSAF} = \frac{C_\mathrm{b}}{C_\mathrm{s}} \quad (1)$$

Where, C_b and C_s are chemical concentrations in tissue and sediment, respectively. To calculate BSAF for DDXs, C_b and C_s were normalized to tissue lipid and sediment OC, respectively, while copper concentrations on a dry weight basis were used.

In addition, the bioavailability of target compounds was estimated as gut fluid extractable fraction of a chemical in sediment using GFE and it was calculated by dividing the concentration of a chemical extracted by BSA-ST solution by its total concentration in sediment.

The bioavailability, bioaccumulation potential and sub-lethal toxicity of target contaminants to the lugworms among the treatments were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc tests ($\alpha = 0.05$).

Results and discussion

Bioaccumulation potential of sediment-associated DDXs and copper

Control sediment (A0B0) contained trace amounts of DDT and DDD (25.9 ± 9.1 and 12.1 ± 3.9 ng/g dw, respectively), yet the concentrations of DDE and other degradation products of DDT as well as copper were all below the reporting limits in this sediment.

Significant degradation of DDT in sediments occurred during the aging period and this is consistent with previous reports in which extensive transformation of DDT to DDD and DDE in spiked sediments was observed (Lotufo et al. 2001; Ding et al. 2013). Concentrations of sediment-associated DDT, DDD and DDE before and after 28-day bioassays were presented in Table S1 in Supplemental Data. Sum concentrations of DDT, DDD and DDE ($\sum$DDX) at 28 days were less than those at 0 day and it could be explained by the relatively low analytical recoveries of DDXs in the sediments at 28 days. As shown in Table S1, recoveries of the surrogate PCB-67 were 83.1–115 % and 63.6–80.0 % for sediment samples at 0 and 28 days, respectively, indicating better analytical performance of samples at 0 day. Therefore, the measured concentrations of DDXs in sediment before bioaccumulation testing (0 day) were used for further calculations (Table 1). The measured $\sum$DDX in all spiked sediment ranged from 40.9 % to 136 % of the nominal concentrations. The low percentages of $\sum$DDX recovered in A2 and A3 groups were likely attributable to the losses of DDXs when discharging sediment supernatant at the end of aging period. The sediment had relatively low TOC content (0.20 ± 0.02 %), which was the main binding site for organic contaminants (Di Toro et al. 1991). Higher DDT concentrations in A2 and A3 groups than that in A1 group may require more OC for binding the chemicals, but TOC contents were uniform in all sediment. As a result, more DDXs lost to the supernatant in sediments of A2 and A3 groups.

Sediment concentrations of copper before and after the bioaccumulation testing were not significantly different (Table S2 in Supplemental Data), so average concentrations measured at 0 and 28 days were used for copper calculations (Table 2). Compared to DDXs, the measured copper concentrations were less deviated from the nominal values, ranging from 88.1 % to 105 % of the nominal concentrations. In addition to OC, other binding sites, such as iron and aluminum minerals may also play important roles in binding copper ions (Luoma and Bryan 1981), and iron and aluminum concentrations in the sediments used in the current study were abundant with mean levels at 1.92 % and 2.81 %, respectively.

Quality of overlying water was within the acceptable range throughout 28-day bioaccumulation testing, with temperature, salinity, concentrations of dissolved oxygen, and pH at 23 ± 1 °C, 27.5 ± 0.8 ‰, 7.9 ± 0.3 mg/L, and 7.9 ± 0.4, respectively. The concentrations of DDXs and copper in the lugworms after exposures were presented in Tables 1 and 2, respectively. In *N. succinea* DDD dominated the composition of DDXs. While DDD may be directly taken up from sediment as a form of DDD, extensive biotransformation of DDT to DDD in *N. succinea* was evident. With similar hydrophobicity, the ratios of the concentrations in biota and sediment of DDD were greatly larger than those of DDT (Table 1). Biotransformation of DDT to DDD also occurred in other marine invertebrates, for example, Kwong et al. (2009) reported DDT in mussels mainly transformed to DDD and the transformation to DDE was trace.

To evaluate the bioaccumulation potential of sediment-bound DDXs and copper to the lugworms, BSAFs were calculated (Fig. 1). The BSAF values of the total DDXs ranged from 0.25 to 0.60 g OC/g lipid, which were lower than those for other benthic polychaete (Mulsow and

Table 1 Concentrations of dichlorodiphenyltrichloroethane (DDT) and its degradation products dichlorodiphenyldichloroethane (DDD) and dichlorodiphenyldichloroethylene (DDE) in sediment and worm

Group	Treatment	Concentration in sediment (ng/g dry weight)				Concentration in worm (ng/g dry weight)				Bioavailability (%)			
		DDE	DDD	DDT	∑DDX	DDE	DDD	DDT	∑DDX	DDE	DDD	DDT	∑DDX
A1	A1B0	BRL	32.9 ± 1.8	51.8 ± 2.0	86.3 ± 3.8	ND	245 ± 113	ND	245 ± 113	ND	12.1 ± 0.0	7.73 ± 0.01	9.26 ± 0.01
	A1B1	BRL	39.7 ± 1.6	74.4 ± 4.9	116 ± 6	ND	345 ± 104	ND	345 ± 104	ND	10.1 ± 0.0	5.38 ± 0.00	6.92 ± 0.00
	A1B4	BRL	44.4 ± 2.6	88.2 ± 1.9	136 ± 5	ND	471 ± 141	ND	475 ± 142	ND	9.01 ± 0.01	4.53 ± 0.00	5.90 ± 0.00
A2	A2B0	48.9 ± 1.2	735 ± 35	1,341 ± 38	2,125 ± 24	191 ± 20	8,687 ± 207	1,794 ± 18	10,672 ± 169	15.5 ± 4.6	25.1 ± 4.0	42.0 ± 12.5	35.5 ± 9.4
	A2B2	49.2 ± 2.2	750 ± 100	1,528 ± 51	2,327 ± 145	153 ± 35	6,582 ± 1,541	1,644 ± 240	8,380 ± 1,787	14.9 ± 1.6	25.7 ± 1.8	36.4 ± 3.5	32.5 ± 2.8
	A2B3	48.5 ± 0.7	829 ± 28	1,702 ± 84	2,580 ± 99	205 ± 4	7,123 ± 3,123	1,657 ± 784	8,916 ± 4,015	15.3 ± 1.3	23.1 ± 3.4	34.9 ± 9.0	30.7 ± 6.9
A3	A3B0	100 ± 0	1,105 ± 14	2,890 ± 203	4,095 ± 217	211 ± 33	8,023 ± 2,402	1,360 ± 544	9,594 ± 2,678	17.4 ± 3.1	27.6 ± 1.7	39.6 ± 6.7	35.8 ± 5.2
	A3B1	99.3 ± 2.5	1,551 ± 307	4,305 ± 805	5,955 ± 1,107	123 ± 13	5,349 ± 551	1,153 ± 403	6,625 ± 893	15.8 ± 2.6	19.4 ± 3.4	26.2 ± 4.7	24.3 ± 4.1
	A3B4	102 ± 1	1,399 ± 207	3,834 ± 278	5,334 ± 464	194 ± 84	7,512 ± 2,362	1,461 ± 752	9,167 ± 3,154	17.0 ± 2.3	25.8 ± 2.0	35.4 ± 8.1	32.5 ± 6.4

DDT, DDD and DDE were designated as DDXs. The bioavailability of the chemicals measured by gut fluid extraction is also presented

BRL below reporting limit, *ND* not detected

Landrum 1995; Lotufo et al. 2000) and a freshwater amphipod (Lotufo et al. 2001). For copper, the BSAF were from 0.32 to 0.79 g dry sediment/g dry worm. Rapid biotransformation and limited uptake may contribute to the low bioavailability of a compound (Sijm et al. 2000). Although *N. succinea* was capable of transforming DDT, the calculated BSAF values of DDXs already took DDT and its major metabolites into consideration. Therefore, the low BSAF values for DDXs were not the result of the in vivo biotransformation of DDT. Instead, the reduction in sediment ingestion rates of the lugworms under the stress of the contaminants may by the reason for the low BSAF values. Lotufo et al. (2000) reported that the polychaete *Neanthes arenaceodentata* tended to avoid foods which were spiked with DDT at elevated concentrations, and consequently, feeding rates decreased when DDT concentrations in foods increased. In the current study the losses of wet weights of *N. succinea* in the spiked sediments were greater compared to the controls, suggesting that the lugworms were adversely affected by the contaminants (Supplemental Data, Fig. S2). Moreover, the BSAF values of DDXs in the current study were calculated from data at one time point of 28 days whereas the values reported by Lotufo et al. (2000) were kinetically derived steady-state BSAFs. Although 28 days was long enough for most chemicals to reach equilibrium in benthic organism and commonly used in bioaccumulation testing, kinetically obtained BSAFs may be significantly higher than those calculated at one time point in case that the steady states were not reached at that time (American Society for Testing and Materials 2000). Boese et al. (1997) reported that kinetically derived and 28-day BSAF for DDXs in marine deposit-feeding clam *Macoma nasuta* were different with values of 0.83 ± 0.40 and 0.23 ± 0.08, respectively. Similarly, this may contribute to the lower BSAF values for DDXs in the current study than those reported by Lotufo et al. (2000).

Evaluation of joint toxicity of DDXs and copper

Joint toxicity between DDXs and copper to *N. succinea* was evaluated using the change in bioaccumulation potential (BSAFs) as the endpoint (Fig. 1). The presence of copper in sediments spiked with DDT at low and middle concentrations (groups A1 and A2) did not obviously alter the BSAFs for DDXs. Alternatively, the BSAFs of DDXs dropped significantly with the addition of copper to the sediments with the highest concentration of DDT in group A3 (Fig. 1). Similar phenomena were also observed for bioaccumulating DDD in the lugworms because DDD was the dominant DDX (Supplemental Data, Fig. S3). The BSAF values of copper in all treatments were not significantly different (Fig. 1), implying that the bioaccumulation

Table 2 Concentrations of copper in sediment and worm and the bioavailability of sedimentary copper which were measured by gut fluid extraction

Group	Treatment	Sediment concentration (μg/g dry weight)	Worm concentration (μg/g dry weight)	Bioavailability (%)
B1	A0B1	52.2 ± 0.3	27.2 ± 13.9	26.2 ± 0.5
	A1B1	50.1 ± 1.0	39.6 ± 10.0	25.6 ± 0.6
	A3B1	45.5 ± 0.8	29.1 ± 12.2	29.0 ± 0.5
B2	A0B2	103 ± 3	65.6 ± 2.0	17.5 ± 0.7
	A2B2	92.9 ± 1.0	30.2 ± 17.3	15.7 ± 0.4
B3	A0B3	220 ± 2	99.7 ± 26.8	11.4 ± 0.7
	A2B3	235 ± 2	165 ± 50	11.0 ± 0.7
B4	A0B4	452 ± 5	336 ± 84	9.64 ± 0.26
	A1B4	479 ± 7	253 ± 93	10.5 ± 0.3
	A3B4	492 ± 12	334 ± 54	9.85 ± 0.06

of sediment-bound copper to *N. succinea* was not likely affected by the co-occurrence of DDXs.

Beside bioaccumulation potential, joint toxicity of DDXs and copper was also assessed for sublethal effects, including the impairment of growth and the alteration of LPO contents. As shown in Fig. 2a, c, neither weight reduction nor LPO contents of the lugworms were related to tissue concentrations of DDXs. Conversely, increasing copper concentrations in the lugworms caused significant losses of worm weights and LPO contents (Figs. 2b and 2d), demonstrating that the observed sublethal toxicity to *N. succinea* was likely attributed to sedimentary copper. This is similar to a previous study on joint toxicity of an organic contaminant and a metal by Tang et al. (2011) who concluded that copper induced greater toxicity to *Daphnia*

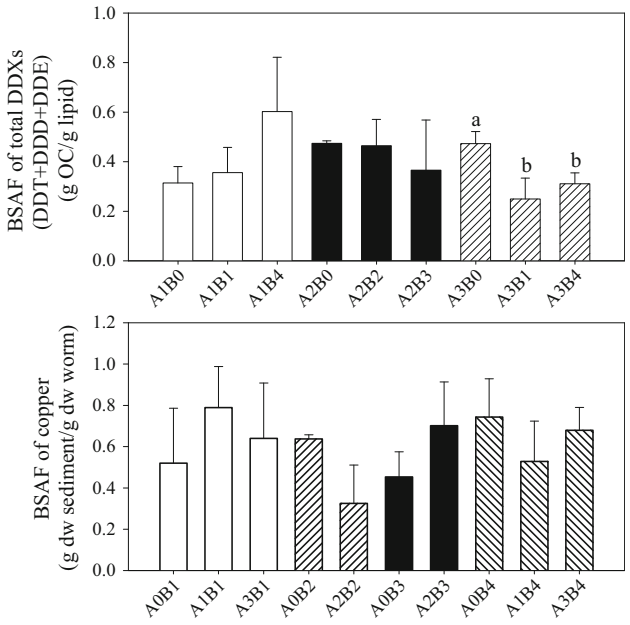

Fig. 1 The biota-sediment accumulation factor (BSAF) values of the total DDXs (*upper panel*) and copper (*lower panel*) in *N. succinea* after 28-d bioaccumulation testing. Different *lowercase letters* denote significant differences ($p < 0.05$). *OC* organic carbon

magna than pentabrominated diphenyl ethers at their respective environmentally relevant concentrations. As shown in Fig. S2, wet weights of the lugworms in control sediment were slightly dropped at the end of the bioassays and it was because TOC contents in the sediments were low and the organisms were not fed throughout the testing. Therefore, it was reasonable to note a positive weight loss due to the limited food resources even though no copper was detected in the lugworms (Fig. 2b). The LPO was related to ROS which kept at a certain level in organism in normal condition (Circu and Aw 2010), hence the dose–response curve of LPO and copper concentrations in tissue also had a positive intercept (Fig. 2d). Overall, the exposure to sedimentary copper at environmentally relevant concentrations induced growth impairment and oxidative stress in *N. succinea.*

While copper in sediment sublethally impaired *N. succinea*, co-existence of DDXs hardly affected the toxicity of copper. That is, DDXs and copper behaved independently to *N. succinea* at sublethal toxicity levels. Antagonistic interaction of a metal (lead) and a pesticide (cypermethrin) in *Chironomus dilutus* were previously noted and the competition of the contaminants to the receptor sites, such as calcium channel, was considered as a plausible explanation (Mehler et al. 2011). Similarly, a competition for target sites may happen for DDT and copper. As a neurotoxin, DDT may bind to axonic membranes of nerve fibers and damage the normal functioning of voltage-sensitive sodium channels (Bloomquist 1996). Meanwhile, copper may also act on the same target sites by binding to functional sulfhydryl (–SH) groups of membrane proteins, e.g. Na^+/K^+-ATPase (Li et al. 1996). Thus, antagonistic effect may occur if DDT and copper competed for the same receptor sites (i.e. the sodium channel), yet it was not observed in the current study. Rather, the toxicity of DDT and copper behaved independently.

Although previous reports on joint toxicity of DDXs and copper are limited, several studies on mixture toxicity of

DDXs demonstrated that DDXs seldom interact with other contaminants. For example, the toxicity of DDT and lindane was independent in several marine species including a dragonet (*Callionymus lyra*), a sole (*Solea solea*), a common prawn (*Palaemon serratus*) and an oyster (*Crassostrea gigas*) (Bocquené et al. 1995). Lydy and Austin (2004) also reported that pre-exposures with DDE at concentrations of 0.2, 2, and 20 μg/kg did not modify the susceptibility of *Chironomus tentans* to chlorpyrifos and diazinon.

On the other hand, the interactions of copper with other contaminants were more variable and the mixture effects were species- (Kraak et al. 1993; Zhu et al. 2011) and exposure-dependent (Banks et al. 2003). The interaction between metals and pesticides was also complicated. Different conclusions have been drawn when studying the joint toxicity between metals and pesticides, e.g. organophosphate and pyrethroid insecticides (Forget et al. 1999; Mahar and Watzin 2005; Kungolos et al. 2009; Mehler et al. 2011). Nevertheless, the majority of the studies suggested that there was no obvious interaction when organisms were co-exposed to metals and pesticides (Lister et al. 2011). So did in most treatments of co-exposures of DDXs and copper in the current study.

In general, no interaction occurred for exposing the lugworms to DDXs and copper simultaneously using bioaccumulation potential, growth impairment and LPO change as sublethal toxicity endpoints except that the co-existence of copper in sediment induced a significant drop of BSAFs of DDXs at the highest DDT concentration.

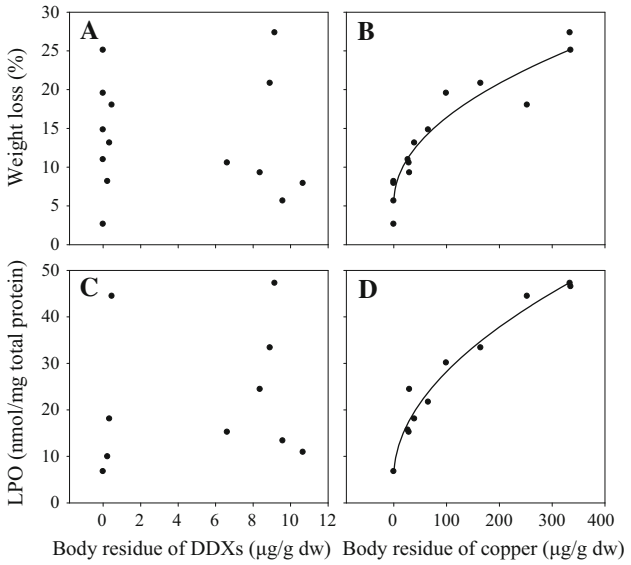

Fig. 2 The relationships of growth impairment and change in lipid peroxidation (LPO) of the worm with body residues of DDXs (**a**, **c**) and copper (**b**, **d**), respectively. Body residues of copper was significantly related with weight loss ($Y = 1.2\ X^{0.5} + 5.3$, $r^2 = 0.91$, $p < 0.01$) and LPO contents ($Y = 2.2\ X^{0.5} + 5.6$, $r^2 = 0.96$, $p < 0.01$), respectively

The impact of bioavailability on bioaccumulation

It has been reported that the interaction between chemicals might alter their bioavailability, and subsequently the toxicity (Mochida et al. 2006; Zhou et al. 2012). To gain a better understanding on the inhibition of the bioaccumulation of sediment-bound DDXs at elevated concentrations by the presence of copper, the bioavailability of DDXs and copper in sediment was estimated using GFE (Tables 1 and 2). As shown in Table 1, the bioavailability of DDXs, which was the fraction of DDXs being extracted from the sediment by the gut fluid, from the sediments at low DDX concentrations (A1 group) was much smaller than that in high-DDX concentration-spiked groups (A2 and A3). On the contrary, the bioavailability of copper decreased when the concentrations of copper in sediment increased. This is consistent with the result observed by Weston et al. (2002). They also found that when chemical concentrations in sediment increased, the fractions being extracted by the gut fluid for benzo[a]pyrene increased from 50 to 70 %, but the fractions for mercury reduced (Weston et al. 2002). However, the reason for the different trends between the organics and the metals was still unclear.

The bioavailability of DDXs and copper was not related to their bioaccumulation potential across all groups, but a significant relationship was noted for the bioavailability of DDXs to their BSAF values in group A3 in which the bioaccumulation of DDXs was inhibited by copper (Fig. 3). This implied that reduced bioavailability of

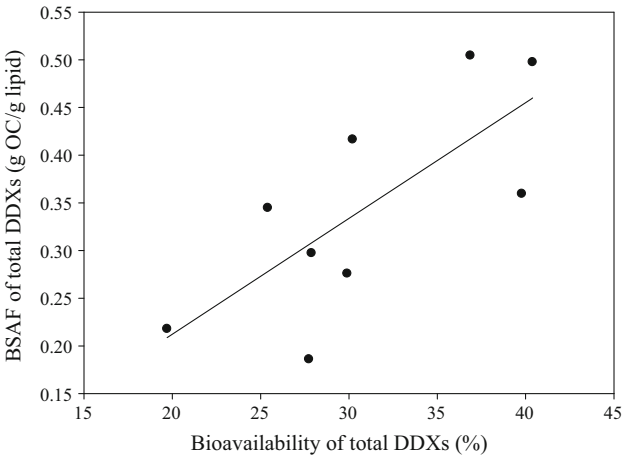

Fig. 3 The relationship ($Y = 0.01\ X - 0.03$, $r^2 = 0.55$, $p < 0.05$) between the biota-sediment accumulation factor (BSAF) of the total DDXs and the bioavailability of DDXs in sediment spiked with dichlorodiphenyltrichloroethane (DDT) at the highest concentration

sediment-associated DDXs due to the presence of copper may play a role in their decreased bioaccumulation potential.

Voparil and Mayer (2000) suggested that surfactant micelles and proteinaceous materials in gut fluid of marine deposit-feeders were the two important agents which were responsible for the solubilization of polycyclic aromatic hydrocarbons. Large globular proteins offered a hydrophobic interior environment to solubilize hydrophobic compounds and proteins in gut fluid were also important to sorb the metals by complexation (Weston et al. 2002). Hence, copper and DDXs may compete for the limited proteinaceous materials in the gut fluid, resulting in the drop of the bioavailability, particularly when chemical concentrations were high. The inhibition of bioaccumulation of DDXs by the presence of copper in A3 group may be a result of reduced bioavailability which was due to the saturation of solubilization agents in gut fluid of *N. succinea*. Similar result was also reported by Voparil and Mayer (2000) who found that the saturation of digestive agents limited the extraction of polycyclic aromatic hydrocarbons from sediment. However, the specific mechanism on the binding behaviors and whether DDT and copper share the same binding sites of proteinaceous materials in the gut fluid remain unknown. Furthermore, the reduction in DDX bioavailability may be partially explained by the inhibitory effects on digestive processes in lugworms induced by copper. The addition of copper in gut fluid of the lugworms or other deposit feeders at toxic levels would result in decreases in protease activity, which was critical in hydrolyzing sediment-bound organic materials (Chen and Mayer 1998). As a result, the fraction of extractable DDXs decreased because of the strong association between sediment-bound organic materials and DDXs.

Conclusions

Given the same input source of antifouling paints for boat maintenance, DDXs and copper are ubiquitous in estuarine sediment and simultaneously acted on benthic marine organisms. Joint toxicity between DDXs and copper to *N. succinea* was investigated using bioaccumulation potential, growth impairment and change in LPO contents as the endpoints. The two chemicals behaved independently in most treatments and copper showed greater sublethal toxicity to the lugworms at environmentally relevant concentrations. Exceptionally, co-exposure of copper reduced the bioaccumulation potential of DDXs when contaminant concentrations were extremely high. Reduced bioavailability of sediment-associated DDXs explained their decrease in bioaccumulation potential when copper co-existed and the decline in bioavailability might result from the saturation of solubilization agents and the inhibition of protease activity by copper in the gut fluid in *N. succinea*.

In case of the harbor sediment in which concentrations of DDXs up to 4,800 ng/g dw (Yu et al. 2011), co-occurrence of copper may reduce the bioaccumulation of DDXs in deposit-feeders and the subsequent risks on the higher trophic levels. More attention, however, should be paid to copper relative to DDXs, because the copper would produce greater sublethal toxicity to the lugworms at environmentally relevant concentrations.

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Conflict of interest The authors declare that they have no conflict of interest.

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