Distribution patterns and ecological risk of endocrine-disrupting chemicals at Qingduizi Bay (China): A preliminary survey in a developing maricultured bay

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Abstract

The occurrence and estrogenic activities of seven phenolic endocrine-disrupting chemical (EDC) compounds (nonylphenol (NP), octylphenol (4-OP), 2,4-dichlorophenol (2,4-DCP), 4-tertbutylphenol (4-*t*-BP), 4-tert-octylphenol (4-*t*-OP), tetrabromobisphenol A (TBBPA), and bisphenol A (BPA)) in the sediments of Qingduizi Bay (NorthernYellow Sea, China) in superficial sediments were investigated to evaluate their potential ecological impacts on the health of aquaculture organisms. All compounds, except 4-OP and 4-*t*-BP, were recorded in most sampling sites (1.06 - 28.07 ng g^{-1} dw in maricultural ponds (MPs), 1.98 - 8.22 ng g^{-1} dw in outer bay (OB)). BPA and 4-*t*-OP were the predominant EDC compounds in MPs and OB, respectively. Correlation between BPA and 4-*t*-OP indicated these compounds may share a similar source or pathway. Analyzed estrogenic activity revealed a low risk of total EDCs. The ranking of risk quotient showed 4-*t*-OP posed a median risk and TBBPA posed a high risk to the aquatic ecosystem.

Keywords: Endocrine-disrupting chemicals, Sediment, Pond mariculture, Risk quotient, Estrogenic activity, Qingduizi Bay.

Endocrine-disrupting chemicals (EDCs) are listed as priority substances in the Directive (Directive, 2013) due to evidence of significant estrogenic activity and teratogenicity to aquatic species, wildlife, and humans (Choi et al., 2004; Roepke et al., 2005; Yang et al., 2006). Alkylphenols (APs), tetrabromobisphenol A (TBBPA), and bisphenol A (BPA) mimic hormones and thus impair the reproductive systems of organisms (Fowler et al., 2012). They are considered to be xenoestrogens leading to metabolic disorders and imbalance of homeostasis in aquatic life (Maqbool et al., 2016).

APs are widely applied as a raw material in the manufacture of alkylphenol ethoxylates (APEOs), as well as in the preparation of curing agents, phenolic resins, heat stabilizers, polymers, and antioxidants (Ying et al., 2002; Esteban et al., 2014; Liao and Kannan, 2014). Nonylphenols (NPs) and octylphenols (OPs), present in the water column, sediments and living organisms, are the most prevalent APs (Lye et al., 1999; David et al., 2009). Similarly, BPA is also an important industrial material used for food cans, powder paints, engineering plastics, and dental fillings (Staples et al., 1998).

Xenoestrogenic phenols in the marine environment have been extensively investigated due to their ubiquitous occurrence and potential negative ecological effects (Tiwari et al., 2016; Diao et al., 2017; Hu et al., 2019). Domestic and industrial sewage discharges are the primary pathways for EDC release into the environment, including coasts and estuarine regions (Tiwari et al., 2016).

Organic pollutants, such as EDCs and polycyclic aromatic hydrocarbons, are adsorbed onto particulate matters and accumulated into sediments due to their hydrophobicity (Ying and Kookana, 2003; Keshavarzifard et al., 2018). Surface

63 sediments are the main reservoir for organic pollutants and are efficient proxies for
64 monitoring and evaluating benthic environmental quality (Yuan et al., 2015; Yang et al.,
65 2018). Resuspended sediments can release organic pollutants into the seawater via
66 physicochemical and biological processes, leading to secondary pollution in the
67 ecosystem (Gui et al., 2014). Investigating the levels of EDCs in mariculture areas using
68 sediment cores provides historical trends of organic pollutants, offering critical
69 information to support management strategies. However, analysis of surface sediments
70 to determine current concentrations of xenobiotic pollutants may offer adequate
71 baseline information for public healt risk management (Sugni et al., 2007).

72 Qingduizi Bay (39°41′59″–39°49′31″N, 123°11′41″–123°26′06″E) is an
73 important estuarine semi-enclosed bay in the northern Yellow Sea of China, covering a
74 tidal flat area of 130 km^2 (Compilation committee for survey of China bays, 1991) and
75 exposed to sewage discharge (e.g. organic pollutants: aquaculture food residues and
76 pesticides) from pond culture, dockyards, and sea farming along the coastline (Wu et
77 al., 2011). This bay is considered the most developed mariculture area in China, with
78 sea cucumber, jellyfish, and razor clam as the main productive species. Here, farming
79 areas increased from 5958.74 to 8356.55 hm^2 between 1990 and 2013, with pond
80 aquaculture covering approximately 5384 hm^2 (Liu et al., 2018).

81 The ubiquity of EDCs in freshwater ecosystems is evident (Zhang et al., 2016).
82 However, the presence of these compounds in this area is limited. EDCs have been
83 studied in water and organisms of coastal systems worldwide (Salgueiro-González et
84 al., 2015; Chiu et al., 2018). However, few reports have addressed EDCs in developing

mariculture bays, which are considered sensitive and hazardous not only to the natural system but to human health. The main objectives of the present work are as follows: (i) perform a comparative study concerning the occurrence and distribution of EDCs in the surface sediments of mariculture ponds and outer bay (OB) of Qingduizi Bay; (ii) clarify the relationship among different phenolic EDC components and the correlation of these components with geochemical characteristics; and (iii) assess the potential risk of EDCs in sediments for benthic organisms. The results of this study will provide efficient baseline information for environmental monitoring and further policy decision-making on governance of aquaculture strategy and seafood safety.

The sediment samples were collected in August 2012 in two sites or aquatic systems in terms of presence or absence of aquaculture activity: (i) a maricultural pond (MP) occupied by numerous aquaculture ponds, and (ii) an OB without aquaculture activity. A total of 31 stations (15 in MPs and 16 in OB) perpendicular to the bay mouth were sampled (Fig. 1).

0–5 cm of sediments were sampled form the seabed surface using a van Veen grab (0.02 m^2). The samples were stored in a pre-cleaned glass jar in a freezer, and kept at −20 °C in the laboratory for further analysis. The wet samples were freeze-dried under conditions of –52 °C and <20 Pa with a vacuum refrigeration dryer (FD-1C-50, Beijing Biocool Instrument Co., Ltd., Beijing, China) for 36 h to a constant weight. Each dried sample was divided into two sub-samples: one for geochemical analysis and the other for EDC analysis. The sub-samples for EDC analysis were sieved through a 100 mesh sieve to remove large items (e.g., stones, sticks, and shells), homogenized, and stored

107 at 4 °C for further analysis.

108 Six geophysical indicators, including the percentage of total carbon (TC), total
109 nitrogen (TN), total organic carbon (TOC), organic matter (OM), mud fractions (silt–
110 clay), and ratios of TC and TN (C/N) of sediments, were estimated from the composite
111 sub-sample in each site. For TOC, samples were treated with 0.1 mol L^{-1} HCl solution
112 for 18 h to remove inorganic carbon and then freeze-dried before analysis. The
113 sedimental TN, TC, and TOC were determined using a Vario Macro CHN element
114 analyzer (Elementar Analysensysteme GmbH, Hanau, Germany). C/N values were
115 calculated using TN and TC ratios. OM was determined through loss on ignition (24 h
116 at 450 °C) according to the method described by Craft et al. (1991). The proportions of
117 mud (silt–clay fractions, <0.63 μm) were determined by sieving the dried samples
118 following Soares et al. (1999).

119 Seven phenolic EDC components, namely, BPA, TBBPA, nonylphenol (NP),
120 octylphenol (4-OP), 2,4-dichlorophenol (2,4-DCP), 4-tertbutylphenol (4-*t*-BP), and 4-
121 tert-octylphenol (4-*t*-OP), which are commonly detected in marine environments, were
122 selected for analysis in the present study. All the standards and chemicals applied in the
123 analysis were purchased from Chem Service (West Chester, USA).

124 Prior to machine analysis for EDCs, the sediment samples were pretreated
125 following the methodology described by Yuan et al. (2017). Briefly, the freeze-dried
126 sediments (approximately 5.0 g) were mixed with 1.0 g celite and ground into a powder
127 using a pre-cleaned pestle. Then, 100 μL of BPA-d_{16} (1 μg mL^{-1}) was added to the
128 samples to create a surrogate standard. The mixtures were extracted twice with

dichloromethane/acetonitrile (1:1, v/v) in the extract pond using an ASE350 apparatus (ASE350, Dionex, USA). The extraction conditions were: 2000 psi pressure, temperature 60 °C and 5 min, 60 s N_2 purge, static cycle three times. The crude extract was concentrated using a rotary evaporator and then diluted to 1 ml with water/methanol (20:80, v/v).

The determination of EDCs was performed on a high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) comprising a Finnigan Surveyor MS Pump and Finnigan Surveyor autosampler (Thermo Electron Corporation, San Jose, CA, USA) with a Thermo Finnigan TSQ Quantum Discovery Max triple quadrupole mass spectrometer. A Waters Symmetry C18 column (150 mm × 2.1 mm i.d., 3.5 μm) connected to a Javelin guard column (Betasil C18, 2.1 × 20 mm, 5 μm) was used for separation. The flow rate of the mobile phase comprising methanol and 2% ammonia was 0.20 mL/min, and 10 μL of the extract was injected. The proportion of methanol was linearly increased from 50% to 80% within 3 min, held for 1 min, increased to 100% within 1 min, held for 5 min, reverted to 50%, and then held for 2 min. The negative ion multiple reaction monitoring mode was used, and the transitions of monitored ions are listed as follows: BPA: 227.10/212.30; TBBPA: 543.00/81.03; OP: 205.00/106.06; NP: 219.10/106.03; 2,4-DCP: 161.30/125.30; 4-*t*-BP: 149.00/133.30; 4-*t*-OP: 205.00/134.10. Nitrogen was used as the curtain and collision gas.

Strict quality control procedures were followed to ensure reliable results during analysis. The procedural blank and the spiked sample with standards were applied in

every twenty sediment samples to circumvent cross-contamination and interference. Extracted blank samples, calibration curves, and verification standards were analyzed at the beginning, middle, and end of each sample batch, respectively. The coefficient of determination (r^2) for each calibration curve was satisfactory (>0.99). 10μL, 50μL, and 100μL of BPA-d_{16} (1μg mL^{-1}) were spiked in blank control samples before extraction and analysis in recovery tests. Meanwhile, three parallel experiments were conducted in each blank and sediment samples, and the mean recovery of surrogate standards ranged from 72.3% to 81.2%. The limits of detection, defined as the concentration that yields an S/N equal to three, were 0.27 ng g^{-1} for BPA and 4-*t*-BP, 0.13 ng g^{-1} for TBBPA, 0.28 ng g^{-1} for NP, 0.26 ng g^{-1} for 4-OP, and 0.24 ng g^{-1} for 2,4-DCP, 4-*t*-OP. The final concentrations were corrected based on the recoveries. The results showed remarkable repeatability and reproducibility of the measurement.

Pearson correlation analysis was executed in R (R Core Team, 2017) with the package "ggcorrplot" to test the relationship between phenolic EDC compounds and geochemical factors. Concentrations below detection limits were assigned a value equal to half of the detection limit (Arditsoglou and Voutsa, 2012).

The estrogen equivalent concentrations (EEQs) of EDCs and their risk quotient were used to evaluate the potential ecological risk. The calculation of EEQs is based on the estradiol equivalency factor (EEF), which is defined as the relative potencies of individual EDC compounds with respect to β-estradiol (Gutendorf and Westendorf, 2001), as shown in equation (1):

$$EEQ_i = EEF_i \times C_i, \qquad (1)$$

where *i* refers to the compound *i* with concentration C. The EEFs were obtained from Gutendorf and Westendorf (2001) and Sun et al. (2013). To measure the level of estrogenic potential in sediment the total EEQ (EEQ_t) based on the EEQ of single estrogenic EDC was calculated by using Equation (2). EEQ_t was obtained according to the total EEQ concentrations of 4-*t*-OP, BPA, and NP. The EEF values of TBBPA and 2,4-DCP were not available. The equation is given as follows:

$$EEQ_t = \sum EEQ_i. \quad (2)$$

The ranking of risk quotient (RQ) is defined as the ratio of measured environmental concentration (MEC) of the target compound to the predicted no-effect concentration (PNEC), as follows:

$$RQ = MEC / PNEC. \quad (3)$$

The PNEC values were obtained by dividing the lowest observed toxicity value with the most sensitive species by an assessment factor based on eco-toxicity data (Yu et al., 2013). The data are normally derived from the chronic (i.e., no observed effect concentration) or acute toxicity data (EC_{50}) when chronic data are unavailable according to experimental situations (Garay et al., 2000). Risk ranking criteria for RQ were widely used to evaluate the potential risk of certain pollutants as follows: minimal risk if RQ was below 0.1, median risk if RQ was between 0.1 and 1, and high risk if RQ was larger than 1 (Blair et al., 2013). The PNEC values for 2,4-DCP, TBBPA, NP, 4-*t*-OP, and BPA were obtained from Sweeney and Currie (2002), Britain (2005), Sverdrup et al. (2006) , Liu (2012), Zhou et al. (2017), respectively.

The sampling sites located in MPs showed higher levels and wider ranges of total

EDCs than those of the sites located in OB (Fig. 2). BPA, TBBPA, NP, 2,4-DCP, and 4-*t*-OP, were detected in most of the sites in Qingduizi Bay. 4-OP and 4-*t*-BP remained undetected (Table S1). Consistently, high concentrations of EDCs were found in the sites near the mouths of the Inna, Huli, and Diyin rivers.

4-*t*-OP, BPA, and TBBPA were the most dominant components, contributing 53.09%, 25.04%, and 8.04 % to the total concentration of ECDs (ΣEDCs), respectively. Overall, 4-*t*-OP was the predominant component detected in all sites, with concentrations ranging from not detected (n.d.) to 12.23 ng g^{-1} dw, and with the highest concentrations near the mouth of the Diyin River. BPA was the second most abundant phenolic EDC compound, with concentrations ranging from n.d. to 14.78 ng g^{-1} dw. Levels of BPA in MPs were significantly higher than those in OB, whereas other EDC compounds showed no significant statistical differences (Fig. 3). These results should be interpreted locally or should be compared with similar studies in other areas, since the study includes only one maricultural site and one non maricultural site. For a better estimation of the confidence intervals of the mean concentrations further global and transferable studies will require site replication for both MP and OB zones (Hurlbert, 1984).

BPA Levels in Qingduizi Bay were similar to those in the Yangtze River Estuary and its adjacent East China Sea (0.72–13.2 ng g^{-1} dw) (Bian et al., 2010). However the maximum concentrations observed were higher in the Shuangtaizi Estuary, China (n.d. −68.17 ng g^{-1} dw) (Yuan et al., 2017), the Beibu Gulf, China (3.07–22.80 ng g^{-1} dw) (Yang et al., 2013), and Masan Bay, Korea (2.70–50.30 ng g^{-1} dw) (Khim et al., 1999).

For other EDC compounds, such as TBBPA, NP, and 2,4-DCP, the levels were generally far below those of developed and industrial estuaries and bays around the world (Khim et al., 2001; Koh et al., 2006; Klosterhaus et al., 2013; Duan et al., 2014; Dong et al., 2015). NP and OP are more susceptible to biodegradation under aerobic conditions due to their straight side chain, and the half-lives of 2,4-DCP are relatively short, resulting in low concentrations of these compounds in the sediment of Qingduizi Bay (Ying and Kookana, 2003). The results also suggest old alkylphenols and 2,4-DCP inputs in the bay.

Organic contaminants are known to adsorb suspended and sediment particles due to their low solubility (Golding et al., 2005). The log octanol–water partition coefficient values (K_{ow}) of EDC compounds are above 4.0. Compounds with this values have a tendency to attach to particles and sediments containing high TOC (Khim et al., 2001). Contrary to the results of previous studies (Dong et al., 2015), correlation between EDCs and all measured sedimental geochemical factors was not significant (Table S2). These results confirm that proximity to sources (e.g., river mouths, aquaculture sewage discharge and channel excavation) may be the most important determinant for the dominance of EDCs (Khim et al., 2001; Yuan et al., 2017). Poor relationships among concentrations of TBBPA, NP, 2,4-DCP, and BPA suggest that their sources are independent of each other, while the significant correlation between BPA and 4-*t*-OP implies similar sources and transport pathways for both EDCs.

Phenolic EDCs have been proven to cause adverse effects on aquatic life due to their estrogenic activities (Campbell et al., 2006). These effects include feminizing,

malformation, and even death (Schwaiger et al., 2002; Bernet et al., 2008; McNair et al., 2015). Estrogenic activity is commonly used to describe interference effects of EDCs on the endocrine system of organisms. This activity was calculated in terms of the estradiol equivalent concentrations (EEQs). The screening of xenoestrogens will normally be conducted by representing its potency relative to a similar estrogen-like estradiol. To our knowledge, no systematic guideline refers to the harmful potential of EDCs in sediment.

On average, EEQ_t value in MPs was higher than that in OB, resulting, potentially, in higher ecological and health risk for aquaculture populations. These results may be underestimated since 2,4-DCP, TBBPA, and other measured EDCs, such as estrone, 17β-estradiol, and synthetic estrogens, were not considered for the total EEQs.

The RQ values of 2,4-DCP, BPA, and NP at all sites (<0.1) suggest low risks for NP (Table 2). The RQ values of 4-*t*-OP reveal median risks. Site (P12) near the mouth of the Diyin River with an RQ>1 indicated a particularly high risk for the organisms. Regarding TBBPA, RQ values indicate high ecological risk for the system.

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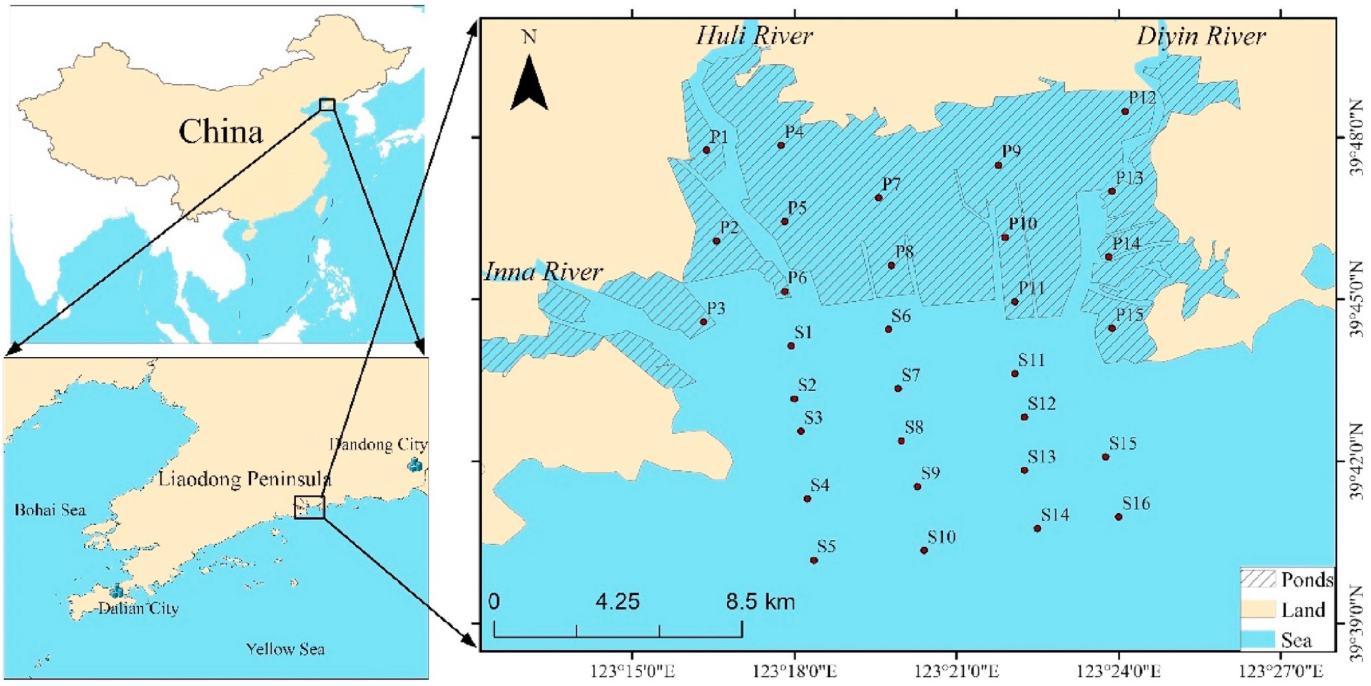
Figure legends

Fig. 1 Study area and locations of sampling sites in Qingduizi Bay

Fig. 2 Spatial distribution of ECDs in the surface sediments of Qingduizi Bay

Fig. 3 Box plots for EDC components in surface sediments of maricultural ponds (MPs) and outer bays (OB). The Shuangtaizi Estuary. The lower and upper hinges and the line in the box indicate the 25th, 50th, and 75th percentile EDC values, respectively. Circles represent outliers

Fig. 4 Pearson correlations of phenolic EDC compounds and physicochemical characteristics of sediments in Qingduizi Bay



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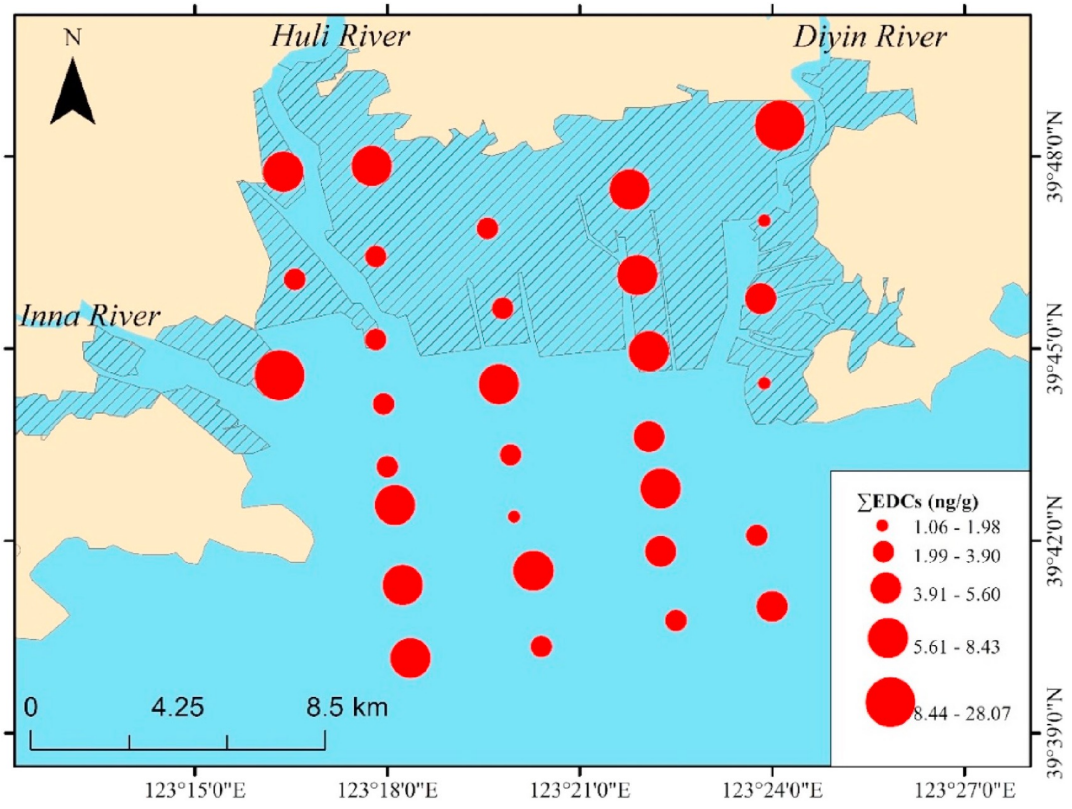
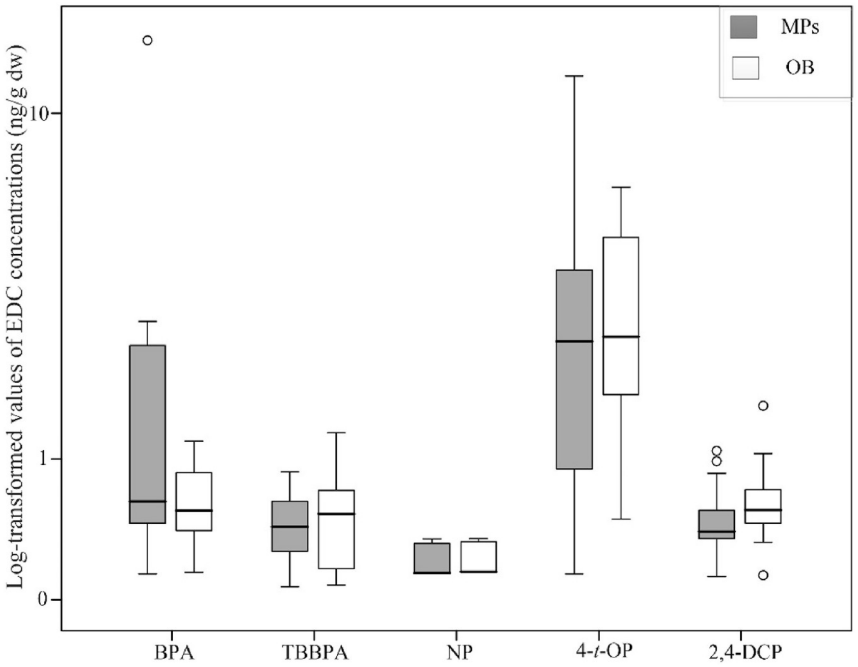


Fig. 2. Spatial distribution of ECDs in the surface sediments of Qingduizi Bay.



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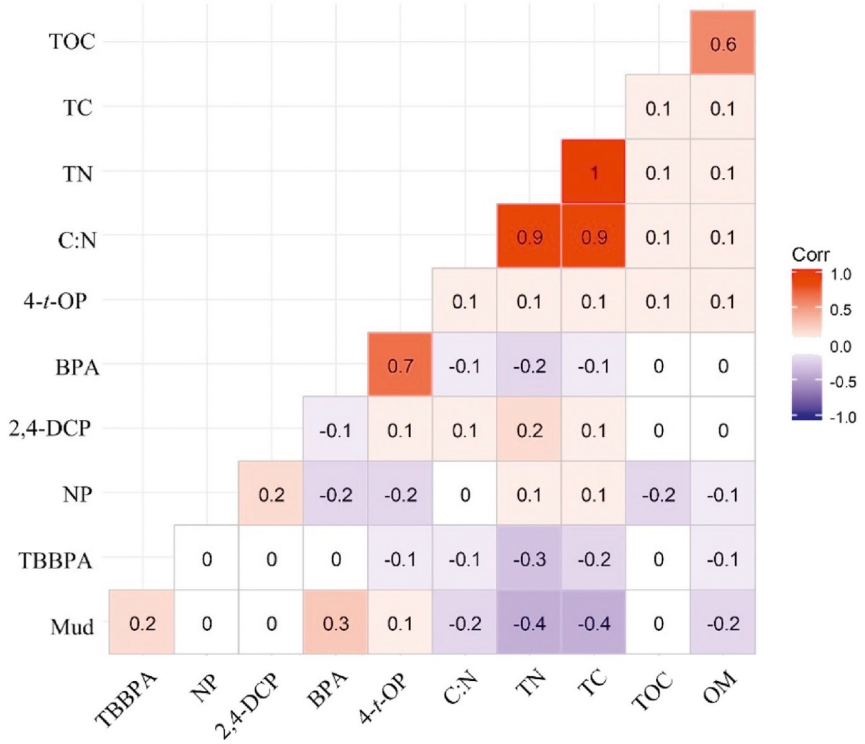

Fig. 4. Pearson correlations of phenolic EDC compounds and physicochemical characteristics of sediments in Qingduizi Bay.

Tables

Table 1 Estrogenic activity of sediments (ng g^{-1} dw) measured in the mariculture ponds and outer bay of Qingduizi Bay.

EDC compounds	EEF (10^{-3})	Mariculture Ponds (10^{-3})		Outer Bay (10^{-3})	
		Ranges	Average	Ranges	Average
4-*t*-OP	[a] 6.5	0.88–79.49	20.18	3.09–42.84	20.08
NP	[a] 2.3	0.32–0.80	0.47	0.32–0.79	0.51
BPA	[b] 1.0	0.14–14.78	2.37	0.14–1.17	0.61
EEQ_t	--	23.02		21.20	

Estradiol equivalent factor (EEF)

Total estrogen equivalent concentration (EEQ_t),

[a] Gutendorf and Westendorf (2001),

[b] Sun et al. (2013)

442 **Table 2** Variations in the risk quotient (RQ) of five phenolic EDC compounds in

443 Qingduizi Bay.

Compound	PNEC (ng g^{-1} dw)	Mariculture Ponds (%)			Outer Bay (%)		
		RQ < 0.1	0.1 < RQ < 1	RQ > 1	RQ < 0.1	0.1 < RQ < 1	RQ > 1
4-*t*-OP	7.4	6.7	86.6	6.7	12.5	87.5	0
TBBPA	0.3	0	40.0	60.0	0	37.5	62.5
NP	39.0	100	0	0	100	0	0
BPA	46.0	100	0	0	100	0	0
2,4-DCP	41.7	100	0	0	100	0	0

444 Predicted no-effect concentration (PNEC) for target compound.

445 Risk quotient (RQ) and the number of sites (in percent).

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