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Polyhalogenated Carbazoles in Surface Sediment from Sanmen Bay, East China Sea: Spatial Distribution and Congener Profile

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Abstract

Polyhalogenated carbazoles (PHCZs) have recently emerged as a group of halogenated pollutants with broad occurrences and bioaccumulation potential in aquatic systems. However, investigations on their occurrences in coastal waters remain very limited. In the present study we investigated PHCZs in surface sediment collected from 29 sites in Sanmen Bay, East China Sea. The results demonstrated a universal presence of PHCZs in sediment, with concentrations of ∑PHCZs (including all congeners) ranging from 7.7 to 17.5 ng/g dry weight (median: 11.3 ng/g dw). The PHCZ congener composition profile revealed a dominance of 3,6-dichlorocarbazole (36-CCZ) with comparable concentration with that of carbazole. Given that PHCZs are widely distributed in Sanmen Bay sediment and their concentrations rivaled other well-known persistent organic pollutants in the same area, this group of halogenated pollutants merits additional investigations of their potential risks to the studied aquatic system, as well as other important watersheds.

Keywords Polyhalogenated carbazoles · Spatial distribution · Congener profile · Sanmen Bay · Sediment

Polyhalogenated carbazoles (PHCZs) represent a group of emerging organic contaminants that contain the base structure of carbazole ($C_{12}H_9N$, CAS no. 86-74-8) and a complexity of halogen substitutions [i.e., chlorinated, brominated, iodinated, and mixed halogenated (Cl, Br, I)] (Parette et al. 2015). Carbazole is a *N*-containing heterocyclic compound that is naturally present in coal tar creosote, shale oil, crude oil and petroleum distillates (Clegg et al. 1997; Li and Suzuki 2010). This compound is used for the chemical synthesis of dyestuff, medicines, insecticides, polymers and plastics (Castorena et al. 2006; Zhang et al. 2018). Sources of PHCZs are composed of natural and anthropogenic emissions. While some chloro- and bromo-carbazoles are likely originated from natural processes, such as bacteria secretion (Mumbo et al. 2013) and volcanic eruptions (Pereira et al. 1980), the main anthropogenic sources of some PHCZs include chloroaniline herbicides (Chen et al. 2017), polyhalogenated indigo dyes (Parette et al. 2015), and photo-and semiconductors (Chen et al. 2017; Morin et al. 2005). As a result of their widespread use, PHCZs have been detected in soil (Mumbo et al. 2014, 2016; Tröbs et al. 2011), indoor dust and air (Fromme et al. 2018), organisms (Wu et al. 2017, 2018), and aquatic sediments from the United States (U.S.), Canada, Germany, Greece, and China (Chen et al. 2016; Grigoriadou and Schwarzbauer, 2011; Guo et al. 2014; Heim et al. 2004; Kronimus et al. 2004; Pena-Abaurrea et al. 2016; Peng et al. 2015, 2016; Wu et al. 2016a, b; Wu et al. 2017; Zhu and Hites 2005). Some congeners have been demonstrated to be bioaccumulative in aquatic ecosystems (Wu et al. 2017). Toxicity studies also revealed dioxin-like activities of PHCZs (Fang et al. 2016; Ji et al. 2019; Mumbo et al. 2016; Riddell et al. 2015). These charactersitics raise concerns on the environmental occurrences of PHCZs and potential risks to aquatic systems.

Sanmen Bay, located in the middle part of Zhejiang Province, is a semi-enclosed bay as well as an important aquaculture region that opens to the East China Sea. The Bay covers about 775 km^2, and the average water depth is 5–10 m. The

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drainage basin of Sanmen Bay is approximately 3160 km^2, and there are more than 30 streams flowing into the Bay, among which Zhuyou River, Baixi River and Qingxi River are the largest (Liu et al. 2006; Xia 2011). The annual average runoff is 26.8×10^8 m^3, accounting for only 0.2% of in and out of the tidal volume of the bay; thus, the annual sediment transport volume is negligible (Xia 2011). As one of the most important aquiculture bases of Zhejiang Province, the Sanmen Bay have experienced large biogeochemical alterations due to the increasing anthropogenic activities, including industrial, aquaculture and municipal activities (Xu et al. 2018). Great amounts of pollutants, such as heavy metals (Liu et al. 2018), organochlorine pesticides (OCPs) (Yang et al. 2010) and polychlorinated biphenyls (PCBs) (Yang et al. 2011), have been discharged into the East China Sea and adjacent coastal areas. However, to date there has been no report on the occurrence and distribution of PHCZs in Sanmen Bay. Thus, our study aimed to understand the contamination status of PHCZs in the Bay by investigating the levels and distribution of a variety of PHCZ congeners in surface sediment.

Materials and Methods

Surface sediment (0–5 cm) was collected with a pre-cleaned stainless steel scoop in 29 sites across the Sanmen Bay during the period of 2016–2017 (Fig. 1). Samples were stored in pre-cleaned sampling bags, transported to the laboratory and then kept at − 20°C until chemical analysis.

The sediment samples were freeze-dried, ground into powder and passed through a 100-μm mesh stainless cloth sieve (Hogentogler & Co. Inc., Columbia, MD). The dried sediment (5 g) was ground with diatomaceous earth and spiked with surrogate standards (2,2′,3,4,4′,5,6,6′-octachlorobiphenyl (PCB-204) and 4′-fluoro-2,3,3′,4,5,6-hexabromodiphenyl ether (F-BDE-160)); 50 ng each), and then subjected to accelerated solvent extraction (Dionex

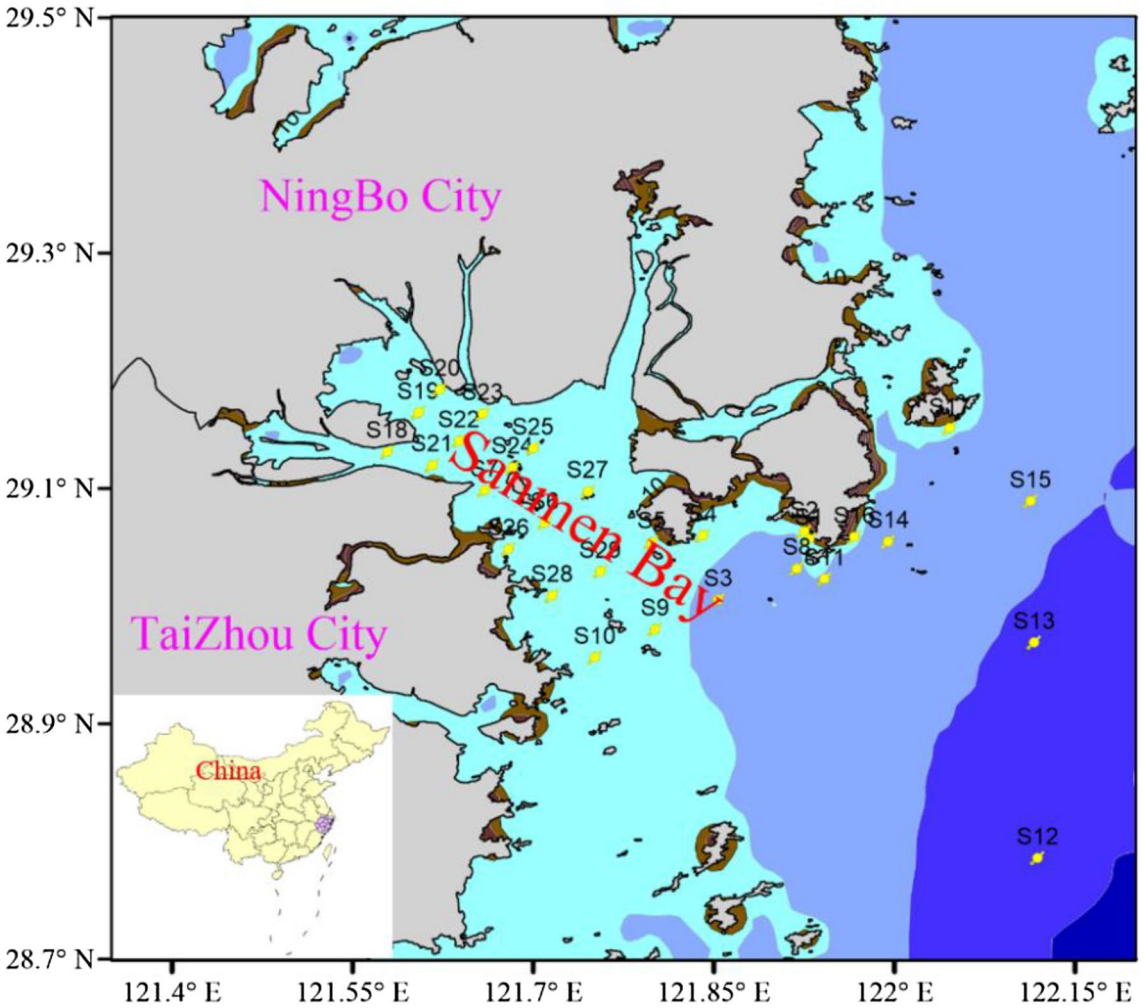

Fig. 1 Sampling sites of sediment samples

ASE 350, Sunnyvale, CA, USA) with two 5 min cycles with dichloromethane (DCM) at 100°C and 1500 psi. The extract was spiked with activated copper powder to remove sulfur element and then run through anhydrous sodium sulfate to remove moisture. Further purification was conducted by a Shimadzu Prominence Semi-Prep HPLC (Shimadzu America Inc., Columbia, MD) equipped with a Phenogel gel permeation chromatography column (300 × 21.2 mm, 5 µ, 100 Å; Phenomenex, Inc., Torrance, CA). Target compounds were collected in a fraction ranging from 16 to 45 min under a flow rate of 4 mL/min for the mobile solvent (DCM), which was further cleaned and separated on a 2-g Isolute silica solid phase extraction (SPE) cartridge. After the cartridge was prewashed with 10 mL of hexane (HEX), the extract was loaded and washed with 3 mL of HEX (discarded). Target PHCZs were eluted out with 11 mL of a mixture of HEX/DCM (40:60, v/v), which was concentrated and transferred to a gas chromatography (GC) vial. Internal standards (100 ng each of 3′-fluoro-2,2′,4,4′,5,6′-hexabromodiphenyl ether (F-BDE154) and Decachlorodiphenyl ether (DCDE)) were added prior to instrumental analysis. Reference standards of PCB-204, F-BDE-160, F-BDE154 and DCDE (purity > 99%) were purchased from AccuStandard (New Haven, CT). Sodium sulfate (10–60 mesh), diatomaceous earth, and high performance liquid chromatography (HPLC) grade solvents were purchased from Fisher Scientific (Hanover Park, IL).

Reference standards of carbazole and target PHCZs, including 3-chlorocarbazole (3-CCZ), 3,6-dichlorocarbazole (36-CCZ), 1,3,6,8-tetrachlorocarbazole (1368-CCZ), 2,3,6,7- tetrachlorocarbazole (2367-CCZ), 3-bromocarbazole (3-BCZ), 2,7-dibromocarbazole (27-BCZ), 3,6-dibromocarbazole (36-BCZ), 1,3,6-tribromocarbazole (136-BCZ), 1,3,6,8-tetrabromocarbazole(1368-BCZ), 1-bromo-3,6-dichlorocarbazole (1-B-36-CCZ) and 1,8-dibromo-3,6-dichlorocarbazole (18-B-36-CCZ), were purchased from Sigma Aldrich (St. Louis, Missouri), Wellington Laboratories (Guelph, ON, Canada), or the Florida Center for Heterocyclic Compounds of the University of Florida (Gainesville, FL). Their purities were all over 98%. They were determined on an Agilent 7890B GC (Agilent Technologies, Palo Alto, CA) coupled to a single quadrupole mass analyzer (Agilent 5977A MS) in either electron impact (EI) mode (for carbazole, 3-CCZ, 36-CCZ, or 3-BCZ) or electron-capture negative ionization (ECNI) mode (for other PHCZs) (Wu et al. 2016a). A HP-5MS column (30 m × 0.25 mm × 0.25 µm, J&W Scientific, Agilent Technologies, USA) was used for the separation. The GC oven program and the MS parameters were introduced in Wu et al. 2016a). The GC injector was operated in pulsed-splitless mode with the injector temperature maintained at 260°C. The initial oven temperature was held at 50°C for 3 min, increased to 150°C at 10°C/min, and then to 300°C at 5°C/min (held for 10 min).

One procedural blank was run with every five samples. No target compounds were detected in any blank. The recoveries of surrogate standards were 86.2% ± 9.3% (mean ± standard deviation) and 96.1% ± 5.3% for PCB-204 and F-BDE-160, respectively. Reported concentrations were adjusted with the recoveries of PCB-204 (under EI mode) or F-BDE-160 (under ECNI mode). The method limits of quantification (MLOQs) were determined from replicate analyses (n = 8) of reference sediment spiked with target PHCZs (5 ng each congener). The MLOQ of individual congener was quantified by multiplying the standard deviation of levels retrieved from replicate analyses with a Student's t-value appropriate for a 99% confidence level (Wu et al. 2016a). Measurements below the instrumental detection limits (IDLs), defined as a concentration yielding a signal-to-noise ratio of five from GC–MS determination, were considered non-detectable (nd). Final concentrations of PHCZs were expressed on a dry weight (dw) basis. Figures 1 and 3 were processed with Surfer 13.0 (Golden Software, Inc., Denver, CO, USA).

Results and Discussion

Concentrations of carbazole and PHCZs in surface sediment are summarized in Table 1. PHCZs were detected in sediment from all of the 29 sites, with the detection frequency ranging from 0% for 2367-CCZ to 97% for 36-BCZ.

Table 1 Concentrations of PHCZ congeners (ng/g dw) in sediment from 29 sites across the Sanmen Bay, East China Sea

Congeners	Range	Median	DF[a] (%)
3-CCZ	< MLOQ[b]-2.7	0.6	72
36-CCZ	6.1–13.2	9.2	100
1368-CCZ	< MLOQ to < MLOQ	< MLOQ	0
2367-CCZ	nd	nd	0
3-BCZ	nd[c]-2.8	< MLOQ	41
27-BCZ	< MLOQ-0.8	< MLOQ	38
36-BCZ	< MLOQ-0.9	0.6	97
136-BCZ	< MLOQ-0.4	< MLOQ	3
1368-BCZ	< MLOQ-0.4	< MLOQ	7
1-B-36-CCZ	nd	nd	0
18-B-36-CCZ	nd	nd	0
Σ_{11}PHCZs[d]	7.7–17.5	10.9	–
Carbazole	1.3–6.2	2.9	100

[a]Detection frequency

[b]Below method limit of quantification (MLOQ)

[c]Non-detectable (S/N < 5)

[d]Sum of 3-CCZ, 36-CCZ, 1368-CCZ, 2367-CCZ, 3-BCZ, 27-BCZ, 36-BCZ, 136-BCZ, 1368-BCZ,1-B-36-CCZ and 18-B-36-CCZ

Concentrations of ∑PHCZs (including all PHCZ congeners) ranged from 7.7 to 17.5 ng/g dw in the study sites. The median concentration (i.e., 10.9 ng/g dw) in Sanmen Bay was lower than what has been reported in surface sediment from the Great Lakes (median: 38.0 ng/g dw) (Guo et al. 2017) and the Saginaw River basin (Michigan, U.S.; median: 18.9 ng/g dw) (Wu et al. 2016a), but one order of magnitude greater than the levels reported in surface sediment collected from Lake Tai of China (median: 1.5 ng/g dw) (Wu et al. 2016b), San Francisco Bay (median: 9.3 ng/g dw) (Wu et al. 2017), and the rivers and coastal water of the North Sea estuary (Germany; median: 0.6 ng/g dw) (Chen et al. 2016). Concentrations of ∑PHCZs in Sanmen Bay also rivaled those of well-known persistent organic pollutants (POPs) in the same Bay area, including polychlorinated biphenyls (PCBs; 9.3–19.6 ng/g dw), organochlorine pesticides (OCPs; range 3.0–7.4 ng/g dw), dichlorodiphenyltrichloroethanes (DDTs; range 0.7–3.0 ng/g dw), and hexachlorocyclohexanes (HCHs; range: 0.4–1.0 ng/g dw) (Yang et al. 2010, 2011). The inter-regional and inter-chemical comparison demonstrated the relative abundance of PHCZs in Sanmen Bay and raised concerns on their potential ecological risks to benthos.

The sources of PHCZs remain controversial, but a combination of anthropogenic and natural sources is likely (Wu et al. 2018). Halogenated indigo dyes have been suggested a likely source of 1368-BCZ and some other PHCZs (Parette et al. 2015). A full array of chlorinated carbazoles were reported at sites approximate to a chlorine production facility (Takasuga et al. 2009). In addition, a significant linear relationship was found between carbazole and total PHCZ concentrations in the sediment ($F = 17.63$, $p < 0.001$). This might suggest that at least some PHCZ congeners could share similar sources with carbazole or even be synthesized from carbazole through anthropogenic or natural processes. Natural sources are also possible for some PHCZs. For example, enzymatic synthesis by chloroperoxidase from marine fungus (*Caldariomyces fumago*) can produce some bromo- and chloro-carbazoles (Mumbo et al. 2013). Future studies are critically needed to better elucidate congener-specific sources of PHCZs.

The composition profile of PHCZ congeners in surface sediment from the Sanmen Bay was dominated by 36-CCZ (83.1%), followed by 3-CCZ (5.6%) and 36-BCZ (5.1%) (Fig. 2). Bromine-containing carbazoles were much less abundant than chlorinated congeners, with 36-BCZ relatively more abundant than other brominated carbazoles. This composition pattern resembled what has been reported in sediment from other aquatic bodies, such as the Saginaw Bay River system, coastal North Sea waterbodies, and the San Francisco Bay, where 36-CCZ was generally the predominant congener (Chen et al. 2016; Wu et al. 2016a, b, 2017). For example, 36-CCZ accounted for 76.6% of the

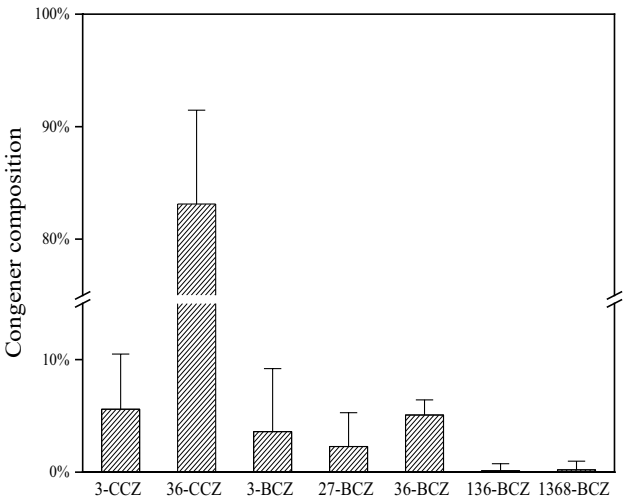

Fig. 2 Mean composition of PHCZ congeners in surface sediment. Error bars represent standard deviations

∑PHCZ concentrations in the Saginaw Bay River sediment, followed by 3-CCZ (17.9%), 1368-CCZ (1.6%), and 1-B-36-CCZ (1.4%). This congener was also found to be dominant in bivalve (*Mytilus californiaus*), sport fish (e.g., striped bass (*Morone saxatilis*)), and harbor seal (*Phoca vitulina*) from the San Francisco Bay and lake trout (*Salvelinus namaycush*) from the Laurentian Great Lakes (Wu et al. 2017, 2018). These patterns may imply a relative abundance of 36-CCZ during the synthesis reactions or its resistance to degradation compared with other congeners. The dominance of 36-CCZ may be attributed to the enhanced stability of *para*-substituted products due to the high charge density at the *para* positions compared to the *ortho* positions (Bonesi and Erra-Balsells 1997). The pattern may also be due to an electrophilic aromatic substitution pattern favoring halogen substitution at the *ortho* and *para* positions relative to the nitrogen atom on the carbazole (Mumbo et al. 2013). For example, Mumbo et al. (2013) reported that enzymatic synthesis of PHCZs from a source material of carbazole could result in a dominance of products with halogen substitution at the 3,6 positions relative to nitrogen on the carbazole. However, in the San Francisco Bay study, the PHCZ congener profiles were also found to vary between different species, suggesting congener-specific biomagnification/biotransformation. For example, while 36-CCZ dominated over other congeners in most species, double-crested cormorant eggs (*Phalacrocorax auratus*) exhibited an elevated abundance of 136-BCZ compared with 36-BCZ and other congeners (Wu et al. 2017). Therefore, additional studies are needed to better elucidate congener-specific environmental behavior, food-web transfer, and fate in the ecosystems.

Concentrations of ∑PHCZ exhibited a clear spatial distribution in Sanmen Bay (Fig. 3). From the inner Bay (e.g.,

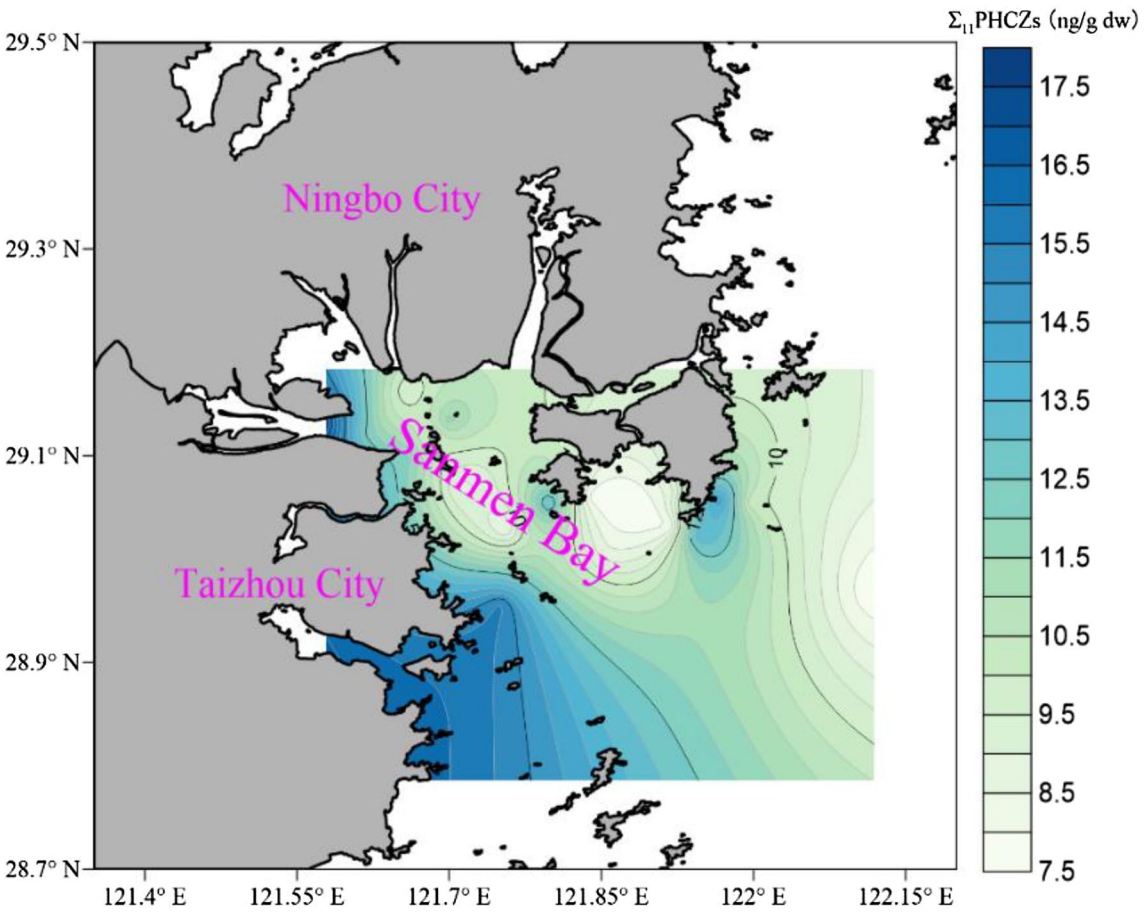

Fig. 3 Spatial distribution of PHCZs in surface sediment from Sanmen Bay

sites S18, S19 and S20) to outer Bay sites (e.g., sites S12, S13, and S15), a clear PHCZ concentration gradient was observed, similar to the pattern observed for other legacy contaminants such as PCBs, OCPs, DDTs and HCHs (Yang et al. 2010, 2011). This pattern demonstrates urban influences on the coastal sites. However, elevated concentrations were observed at sites S11 and S16 (i.e., 13.3 and 14.7 ng/g dw, respectively) near the outer Bay region. These two sites are located near the Xiangshan County of Ningbo city, where one of the largest sewage outfall districts in Sanmen Bay basin is based; Similarly, elevated concentrations of chemical oxygen demand (COD_{cr}), ammonia–nitrogen and total phosphorus (TP) were also reported at these locations (Yao and Huang 2015). This demonstrates an important influence of sewage discharge on the sediment contamination of PHCZs and other persistent organic pollutants. Among the shoreline sites, those from the southern Bay exhibited relatively greater PHCZ concentrations than those from northern Bay, despite that the norther Bay is located near a metropolitan area with a greater human population density than the southern Bay (Fig. 3). It is noted that intense electronic waste (e-waste) dismantling and recycling activites have been reported in the Taizhou region (Han et al. 2009; Zhou et al. 2017). Contaminants including PHCZs may be released from e-waste related activities and disharged to the Bay through surface runoff and air deposition (Wang et al. 2011; Yang et al. 2011). Therefore, urban runoff, sewage discharge, and e-waste activities together shape the spatial distributio pattern of PHCZs in Sanmen Bay. Other factors, such as coastal or tidal currents and atmospheric deposition, may also influence the distribution of contaminants in this area (Moon et al. 2007; Jiang et al. 2018), but they are beyond the scope of the present study.

Overall, in the present study we investigated the concentrations and composition profile of a suite of PHCZ congeners in Sanmen Bay of East China Sea. The results revealed broad distributions of PHCZs in the studied area with levels at the high end of the range of concentrations reported in other water bodies worldwide. Our data add to the knowledge of environmental occurrences of this group of halogenated pollutants and further support the assumption that they are globally distributed. Despite of an increasing number of reports on the environment occurrences of PHCZs, their origins or sources remain insufficiently elucidated. Further

laboratory and field studies are needed to provide a more comprehensive understanding of their sources, environmental behavior, fate, and toxicities of this group of chemicals.

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