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Distribution and bioconcentration of endocrine disrupting chemicals in surface water and fish bile of the Pearl River Delta, South China

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HIGHLIGHTS

- The bioconcentration and potential effect of EDCs in the PRD were investigated.
- The E2 activity equivalents suggest high risks in the investigated water.
- Occurrence of EDCs in fish bile can reflect that in ambient water.
- The investigated wild carp and algae can bioaccumulate phenolic EDCs.

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ABSTRACT

The distribution and bioconcentration of endocrine disrupting chemicals (EDCs) in water, algae, and wild carp bile of the Pearl River Delta (PRD), South China were investigated. 4-*tert* octylphenol (OP), 4-nonylphenol (NP), and bisphenol A (BPA) (unit, ng L^{-1}) in water were in the ranges of 1–14, 117–865, and 4–377, those (ng g^{-1} dry weight) in algae were in the ranges of 2–13, 53–282, and 16–94, and those (ng g^{-1}) in carp bile were in the ranges of 14–39, 950–4648, 70–1020, respectively. Estrone (E1) and 17α-ethinylestradiol (EE2) in water ranged from <LOQ to 1.58 ng L^{-1} and from <LOQ to 3.43 ng L^{-1}, respectively. In bile and algae, E1 ranged from nd to 30 ng g^{-1}, but EE2 was not detected. The E2 activity equivalents (EEQs) ranged from 1.20 to 10.97 ng g^{-1} in carp bile and from 0.07 to 8.06 ng L^{-1} in water. The EEQs in carp bile were significantly related to those in water, illustrating that occurrence of EDCs in carp bile can reflect that in ambient water in the PRD region. The bioconcentration factors (BCF, L kg^{-1}) of OP, NP, BPA, and E1 in algae were in the ranges of 482–7251, 131–740, 2846–12979, and undetectable, respectively, and those in carp bile were in the ranges of 1500–12960, 1648–11137, 3583–14178, and 13208–39623, respectively. The phenolic EDCs can be accumulated by wild carp bile and algae in the investigated aquatic ecosystems, which is also affected by the degree of the eutrophication. This study for the first time reported EDCs in carp bile and algae collected from the PRD, China.

1. Introduction

Endocrine disrupting chemicals (EDCs) in the aquatic environment have been of world wide concern due to their potential adverse effects in human and wildlife (Gross-Sorokin et al., 2006; Hotchkiss et al., 2008). EDCs encompass a wide range of natural and synthetic chemicals, including steroids, alkylphenols, phytoestrogens, dioxins, and pesticides, etc. (Sonnenschein and Soto, 1998; Metzler and Pfeiffer, 2001). Among these classes of chemicals, steroid estrogens, e.g., estrone (E1), 17β-estradiol (E2), and 17α-ethynylestradiol (EE2), and phenolic xenoestrogens, e.g., alkylphenols and bisphenol A, deserve particular attention as they possess high estrogenicity and moderate estrogenic potency, respectively.

Occurrence of EDCs in aquatic systems (Supporting information, SI, Fig. S1) has been extensively monitored, with spot sampling followed by laboratory analysis as the most widely used monitoring approach (Ternes et al., 1999; Jeannot et al., 2002; Richardson and Ternes, 2005). This approach, however, yields only instantaneous snap shots of pollutant levels and suffers from measurement uncertainties due to short- and long-term concentration variations. On the other hand, combined chemical analysis and bioassay can fully reveal water pollution level. Recent studies showed that

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numerous chemical substances, including estrogens and alkylphenols, tend to concentrate as glucuronide and sulfate conjugates in fish bile (Larsson et al., 1999; Ferreira-Leach and Hill, 2001; Legler et al., 2002; Pedersen and Hill, 2002). Moreover, analysis of fish bile, representative of recent exposure to bioavailable contaminants, was employed to identify and quantify polycyclic aromatic hydrocarbons (Ruddock et al., 2003; Johnson-Restrepo et al., 2008), chlorinated phenols (Brumley et al., 1998), resin, and fatty acids from pulp and paper mill effluents (Leppänen et al., 1998). Finally, a good correlation ($r = 0.81$ and $p = 0.0002$) was found between the bile E2 activity equivalents (EEQs) concentration and plasma vitellogenin induction in male bream, demonstrating that occurrence of biliary xenoestrogens can be an indication of internal exposure to these compounds (Legler et al., 2002). Therefore, analysis of fish bile may act as one important way in field surveys for EDCs.

To test the above-mentioned hypothesis, the present study used the Pearl River Delta (PRD) of South China as a case study to relate the concentrations of EDCs in water of reservoirs, lakes, and fish ponds to those in wild carp (*Cyprinuscarpio Linnaeus*) and in particulate algae. The PRD is one of the most developed and populated regions in China with deteriorated water quality due to continuing inputs of a variety of contaminants via domestic and industrial wastewater discharge (Cheung et al., 2003; Fu et al., 2003). On the other hand, few investigations on EDCs in surface waters of the PRD have been conducted (Chen et al., 2006; Peng et al., 2008; Gong et al., 2009), and none in fish samples. This study was the first time to report the levels of EDCs in bile of carp fish and particulate algae from the PRD, China.

2. Materials and methods

2.1. Materials

Standards of 4-nonylphenol (NP) (mixture of compounds with branched side chains) (94%), 4-tert-octylphenol (4-t-OP) (93%), bisphenol A (BPA), estrone (E1), 17α-ethynylestradiol (EE2) and internal standard of bispentafluorobenzylbenzene (BPFBB) and derivatization reagent of pentafluorobenzoyl chloride (PFBOCl; purity >99%) were purchased from Sigma–Aldrich (St. Lois, MO). Deuterated bisphenol A (BPA-d_{16}) were purchased from Supelco. Estrone-2,4,16, 16-d_4 and 4-nonylphenol-d_5 (4-NP-d_5) were obtained from C/D/N Isotopes (Quebec, Canada). β-Glucuronidase and arylsulfatase extracted from Helix pomatia (glucuronidase, 100000 Sigma units; arylsulfatase, 7500 Sigma units) were purchased from Sigma–Aldrich.

2.2. Sampling sites and sample collection

Samples (36 in total) were collected from nine sites in the Zhujiang River, eight sites in the Dongjiang River, and nineteen sites in the park-lakes, fish ponds, reservoirs and other river streams of PRD, on August, 2011 (SI, Fig. S1). The Zhujiang River flows through the downtown of Guangzhou, which is highly urbanized with a population of 14 million and various industries. The Dongjiang River runs across the city of Dongguan, where manufacturing and processing industries are extremely developed and densely clustered. The two rivers join at the Shizhiyang waterway and flow into South China Sea. The other nineteen sites were selected due to the following two reasons. Firstly, the various sites were collected depending on their distances from the city center. The sites of YTQ, LWH, and DSH located in urban area, SK, LA, DWHG, and ZT were in the suburbs, while the sites of TTS and ZGJ were far away from the urban area. Secondly, the sites were collected according to their different industrialization levels. Some were located in the industry area (JM, JM-creek, and SWH).

Surface water samples at 0.5 m below the surface were collected with a stainless steel bucket, and stored in 10 L amber glass bottles. Upon transport to the laboratory, water samples were filtered through pre-combusted Whatman GF/F filter paper (0.7 μm) to remove suspended particles. An aliquot (50 mL) of each filtrate sample was analyzed for dissolved organic carbon (DOC) with a TOC-VCPH analyzer (Shimadzu). The rest of the filtrate was acidified with 6 M HCl to pH 2–3 to suppress microbial activities and stored at 4 °C prior to extraction. The samples for chlorophyll a (Chl a) were filtered through 0.45 μm cellulose acetate filters and then the membrane samples were extracted with 90% acetone for 24 h, and the Chl a concentrations were measured by using a UV–VIS spectrophotometer (See the Supporting Data for the detailed procedure). All pretreatment procedures including extraction were accomplished within 36 h after sampling.

The algae samples were collected by 200 and 300 mesh nylon nets and stored in glass bottles. Then algae samples were further separated from water by centrifugation and then freeze-dried.

Common carp, *C. linnaeus*, were captured with fishing nets from fish pond (FSBC), reservoir (ZT), and Zhujiang (ZJ) in August, 2011 and Dongjiang (DJ) in March, 2011, and at the same time, the water samples were collected. But in other sampling sites, no fish samples could be collected Fish samples were dissected immediately upon collection. Fish were stunned by a blow to the head and the spinal cord severed. Gall bladders were removed and bile fluid was obtained by puncturing the gall bladder with a needle and drawn into a syringe. Bile was stored in vials at −20 °C.

2.3. Sample extraction and derivatization

The procedures for sample extraction, purification, and derivation were published elsewhere (Peng et al., 2006; Gong et al., 2009, 2011). Briefly, EDCs in water samples were extracted with Oasis HLB cartridges, which were conditioned by passing 5 ml of ethyl acetate, 5 ml of methanol, and 15 ml of HCl (pH = 3) sequentially. Each filtered water sample (1 L each) was spiked with surrogate standards NP-d_5, BPA-d_{16}, and E1-d_4 and passed through a pre-conditioned cartridge at a flow-rate of 8–10 mL min^{-1}. The cartridge was washed with 10 mL of redistilled water/methanol (90:10, v/v) solution, and the cartridge was dried under vacuum for 30 min. After the cartridge was eluted with 10 mL of ethyl acetate at a flow rate of 1 mL min^{-1}, the extract was dehydrated with anhydrous sodium sulfate and concentrated to 1 mL by rotary evaporation prior to derivatization.

For extraction of fish bile, a sample of 60–100 μL, with 1.5 mL of phosphate buffer (0.1 M, pH 6.0), 800 μL of Milli Q water, 10 μL of surrogate standards (100 mg L^{-1} NP-d_5; 5 mg L^{-1} BPA-d_{16} and 1 mg L^{-1} E1-d_4), and 10 μL of corresponding enzymes (100000 U mL^{-1} for β-glucuronidase and 7500 U mL^{-1} for sulphatase), was added to a 10-mL glass vial. Concentrations of enzymes were chosen according to Gibson et al. (2005). The glass vial was incubated overnight (17–18 h) in a 37 °C water bath with gentle shaking. After hydrolyzed bile, 300 μL of acetic acid and 2 mL of Milli-Q were added to the vial prior to SPE cleanup. The hydrolyzed bile sample was loaded onto a 200-mg Oasis HLB cartridge conditioned with 5 mL of methanol (MeOH) and 5 mL of 1% (v/v) acetic acid solution in Milli-Q water. The cartridge was rinsed with 2 mL of Milli-Q water and dried under vacuum for 10 min, and the target analytes were eluted with methanol (5 mL), ethyl acetate (3 mL), and hexane (3 mL). Finally, the combined eluents were evaporated to dryness under a gentle stream of nitrogen in a Zymark Turbovap LV Evaporator (Hopkiton, MA). The concentrated extract was redissolved in 200 μL of ethyl acetate. The derivatization method for EDCs was developed based on the information in the literature using pentafluorobenzoylation (Xiao and McCalley, 2000; Kuch and Ballschmiter, 2001; Xiao et al., 2001; Boitsov et al., 2004).

The dry algae samples were spiked with surrogate standards NP-d_5, BPA-d_{16}, and E1-d_4 and Soxhlet-extracted with 200 mL of acetone/DCM (1:1, v/v) for 24 h. Each extract was concentrated to 1 mL with a rotary evaporator and purified using a glass column. The column (200 mm × 10.5 mm i.d.) with a Teflon stopcock was dry-packed with 1.5 g of deactivated silica gel and topped with 1 g anhydrous sodium sulfate. The column was preconditioned with 10 mL of ethyl acetate/hexane (4:6, v/v). The 1 mL concentrated extract was transferred into the column and eluted with 20 mL of the mixture solvents of acetate/hexane (4:6, v/v). The elution was concentrated to 1 mL and blown to just dryness under a gentle flow of high purity nitrogen. The residual was dissolved in 1 mL MeOH and 100 mL redistilled water. The solution was extracted with ENVI-18 SPE cartridge conditioned sequentially with 5 mL MeOH and 5 mL redistilled water. After being dried under vacuum for 30 min, the cartridge was eluted with 8 mL of acetonitrile, and the extract was dehydrated with anhydrous sodium sulfate and concentrated to 1 mL by rotary evaporation prior to derivatization.

2.4. Instrumental analysis

Sample extracts were analyzed with a Shimadzu Model 2010 GC–MS equipped with an AOC-20i auto injector (Shimadzu, Japan) in the negative chemical ionization mode and mass spectra were scanned in the selected ion monitoring mode. Chromatographic separation was achieved with a J&W DB35-MS capillary column (30 m × 0.25 mm, 0.25 μm film thickness). Helium was used as the carrier gas and maintained at a constant flow rate of 1.0 mL min^{-1}. A sample volume of 2 μL was injected in the splitless mode with an inlet temperature of 300 °C. The column temperature was programmed from 80 °C (held for 1 min) to 220 °C at 10 °C min^{-1}, from 220 to 260 °C at 4 °C min^{-1}, from 260 to 300 °C (held for 8 min) at 5 °C min^{-1}, and from 300 °C to 310 °C (held for 15 min) at 20 °C min^{-1}. The MS interface temperature was maintained at 290 °C. Ion fragments of *m*/*z* 400, 414, 616, 464, and 490 were monitored for OP, NP, BPA, E1, and EE2, respectively. In the case of the surrogate and internal standards, *m*/*z* 466, 418, 630, and 468 were monitored for PBFBB, NP-d_5, BPA-d_{16}, and E1-d_4, respectively.

2.5. Quality assurance and quality control (QA/QC)

For every 10 water, bile, and particulate algae samples, a procedural blank and the spiked blank sample were processed. Surrogate standard recoveries (mean ± standard deviation) for QA/QC samples were 102 ± 4, 79 ± 9, and 110 ± 22% for NP-d_5, BPA-d_{16} and E1-d_4, respectively. Recoveries of the spiking standards (OP, NP, BPA, E1, and EE2) in the spiked sample were 94 ± 4%, 100 ± 7%, 100 ± 8%, 92 ± 9%, and 124 ± 10%, respectively (n = 4). On average, only NP at 10 ng L^{-1} and BPA at 2 ng L^{-1} were detected in the water blank samples (n = 4), and NP at 204 ng g^{-1}, OP at 3 ng g^{-1}, and BPA at 8 ng g^{-1}were detected in bile blank samples (n = 3), and 19.4 ng g^{-1} dry weight (dw) for NP and 3.4 ng g^{-1} dw for BPA were detected in particulate algae blanks (n = 3). In addition, surrogates (NP-d_5, BPA-d_{16}, E1-d_4) were added before extraction to quantify efficiency of sample preparation procedures, and the recoveries of the three chemicals ranged from 80 to 107% for water samples, 92–100% for bile samples, and 68–93% for particulate algae samples. The reported concentrations were not corrected by the surrogate recoveries, but the EDCs levels found in the method blanks were subtracted from the samples. Limit of quantification (LOQ) were set as 10:1 signal-to-noise ratio; for water samples, the LOQ of the OP, NP, BPA, E1 and EE2 were 0.2, 9, 0.9, 1 and 0.7 ng L^{-1}, for algae samples, the LOQ of these chemicals were 2.0, 6.0, 1.7, 1.3, and 2.0 ng g^{-1}, and for bile samples, the LOQ of these chemicals were 1.1, 9.5, 4.3, 2.0 and 3.0 ng g^{-1} bile, respectively.

2.6. Data analysis

Hepatosomatic index (HSI) and condition factor (CF) were calculated as: HIS = $(W_L/W_B) \times 100$ and CF = $(W_B/L^3) \times 100$, where W_L is the weight of liver, W_B is the body weight and L is the length of the fish. Bioconcentration factor (BCF) was calculated by BCF = C_F/C_w, where C_F is the target contaminant concentration in fish bile and C_w is the water concentration (Mackay and Fraser, 2000). The estrogen equivalent concentrations (EEQs) of EDCs was calculated by using the equation (EEQs = $\sum EEF_{EDCs} \times C_{sample}$), where the $EEF_{(EDCs)}$ represents the estrogenic activity potencies of individual EDCs (OP (0.00093), NP (0.00063), BPA (0.00011), E1 (0.3), EE2 (2.2)) (Thorpe et al., 2006); and the C_{sample} represents the individual concentration of EDCs in water or bile sample.

3. Results and discussion

3.1. Aquatic physicochemical properties and fish biological parameters

Physiochemical parameters determined for the water samples are listed in SI Table S1. Values of water temperature, pH, and salinity varied in small ranges, but other parameters, particularly Chl a, changed widely among the sampling sites. The correlations between Chl a and other parameters (SI Fig. S2) were significantly positive for pH and Chl a (r = 0.43), Conductivity (COND) and Chl a (r = 0.38), UV_{254} and Chl a (r = 0.74), and Dissolved organic carbon (DOC) and Chl a (r = 0.82). As Chl a is determined by the quantity of algae in the water (Gosselain et al., 2000), higher pH values at these sites were related to the quantity of algae. The Chl a values (μg L^{-1}) were in the ranges of 14.8–91 (mean + standard deviation: 45 ± 35) in the reservoirs, 41.8–192 (108 ± 69) in the park lake, 42–270 (246 ± 111) in the fish pond, 8.58–24.6 (16.2 ± 6.7) in the rural river, 7.9–59 (30 ± 22) in DJ, and 68–133 (96 ± 23) in ZJ, which apparently followed the sequence: fish pond > park lake > ZJ > reservoir > DJ river > rural river (SI Fig. S3), suggesting different degrees of eutrophication in the investigated ecosystems. According to the level of Chl a, which at 10 μg L^{-1} is defined as the threshold of eutrophication in water, most of the sampling sites suffered from eutrophication except for two sites: Jiaomeng (JM) (8.58) and Shilong (D8) (7.88), where the EDCs levels were relatively high.

Biological parameters of the sampled carp are listed in Table S2. The carp samples were collected from fish ponds, reservoir, DJ, and ZJ River. They were different in weight, length, and condition factors, but most of the fish were about 1 or 2 years old, based on their weight and length. In additional, the hepatosomatic index (HSI) can provide an indication of the metabolic load in fish. In this study, the HSI values can be considered as background information of the fish samples.

3.2. Composition and distribution of EDCs in water samples

The concentrations of steroidal and phenolic EDCs in the water samples are listed in SI Table S1. The concentrations of 4-t-OP, NP, and BPA ranged from 1–14, 117–865, and 4–377 ng L^{-1}, respectively, whereas those of E1 and EE2 were <LOQ-1.58 and <LOQ-3.43 ng L^{-1}. The concentrations of NP and BPA were generally higher than those of 4-t-OP, E1, and EE2, and those of NP were the highest among the target EDCs. NP and 4-t-OP were detected at all samples, whereas BPA, E1, and EE2 were detected in 97%, 75%, and 86% of the samples.

Distribution of EDCs in Dongjiang and Zhujiang Rivers (SI Fig. S4) indicated that levels of NP at most sampling sites were

around 550 ng L^{-1}, and at a few sewage treatment plants outlet (e.g. D3, Z2, Z5, Z8 and Z9) and downstream site (Z1) the levels were higher than 600 ng L^{-1}. Lower levels of NP at sites of Z6 and Z7 were detected at approximately 200 ng L^{-1}, probably due to the two sites locating away from dense population area and pollutant source. Compared with previous NP data in Zhujiang River (Chen et al., 2006), it was found that the concentrations did not change obviously during the period from 2004 to 2011, suggesting that the NP contamination is still prevalent in some WWTWs, possibly due to their continuous use in car washes and other service industries (Pettersson et al., 2006). As to BPA, abnormal high concentrations were detected in D2, D3 and D4 (100, 377, 235 ng L^{-1}, respectively), which were more than three times higher than those in other sites ranging from 10 to 30 ng L^{-1}, indicating some major pollution sources around these areas. The distribution of OP was similar to that of NP, probably because they were of same origin. Estrogens (E1 and EE2) levels were higher near sewage treatment plants than in other sites.

Fig. 1 shows the distribution of NP and BPA in other 19 water samples collected from reservoirs, park lakes, fish ponds, and other river channels. The sites of YTQ, LWH, and DSH located in urban area, SK, LA, DWHG, and ZT were in the suburb area, while TTS and ZGJ were far away from urban areas. Fig. 1a showed the concentrations of NP in urban, suburbs, and remote areas, which were obviously different between urban and non-urban sites. The sites (DSH, LWH, and YTQ) in urban areas were highly polluted, while the sites (ZGJ and TTS) located in non-urban areas were less impacted, demonstrating that the increased NP level was accompanied by the improved urbanization. For BPA (Fig. 1b), the water concentrations in industry areas such as the sites JM and SWH (Pangyu industry area) were about two to three times higher than in other non-industry area sites, illustrating that the sites with high level of BPA located in the vicinity of industry areas.

3.3. Concentrations of EDCs in carp bile

The concentrations of EDCs in the carp bile samples were shown in Table 1. OP, NP, and BPA were detected in all of the bile samples, and E1 was detected in 87.5% of the bile samples. The concentrations of OP, NP, BPA, and E1 varied in the ranges of 15–39 ng g^{-1} bile, 950–4648 ng g^{-1} bile, 70–1020 ng g^{-1} bile, and 7–30 ng g^{-1} bile with a mean (±SD, $n = 8$) of 27(±10), 2478(±1227), 347(±324) and 19(±7) ng g^{-1} bile respectively. The varied value of OP, NP and BPA were consistent with these differences in water; namely, the concentration gradient was in the sequence: NP > BPA > OP. When the concentrations among the four sampling sites were compared, it was found that the bile samples collected from the reservoir (ZT) were at relatively lower levels than those collected from Dongjiang River (DJ1, DJ2, DJ3) (Fig. 2). From the concentrations in water and bile, it can be found that higher concentration in the water led to higher concentration in the bile samples (Fig. 2). The concentrations of EDCs in the fish bile of the Pearl River Delta were compared with those reported in other countries (Table 2). The NP concentrations (950–4648 ng g^{-1} bile) in bile samples were slightly higher than those from Fenlon et al. (2010) who reported that the NP concentrations ranged from <blank to 2453 ng g^{-1} bile in thick-lip grey mullets collected from the Thames River, UK, but they were lower than the bile concentrations in the effluent-exposed fish in UK (5531–12 678 ng g^{-1} bile). They were also higher than that detected in sea bass (*Dicentrarchus labrax*) from aquaculture facilities in Spain, but lower than the concentration from Ebro river fish in Spain (Lavado et al., 2006; Fernandes et al., 2008; Vallejo et al., 2010). Moreover, BPA was detected with high concentrations after NP. The BPA values in this study were higher than those in the bile of English sole collected from United States coastal water (Da Silva et al., 2013), and was comparable to those in fish bile samples from UK Thames River, but lower than that in the effluent exposed fish bile samples (Fenlon et al., 2010). OP and E1 were in the similar level range, and the two chemicals were much lower than biliary levels from effluent-exposed fish, but were in the comparable level with those of the fish bile samples in Thames River, UK (Fenlon et al., 2010). In general, the levels of the EDCs in the fish bile samples of PRD are higher than those of the UK River (Fenlon et al., 2010), but much lower than those from the Effluent-exposed and river in Spanish ones (Lavado et al., 2006; Fernandes et al., 2008).

3.4. Relationship of EDCs levels in water and carp bile

Analysis of the fish bile can give the information of the EDCs levels in the water environment. As shown in Fig. 2, the bile sample collected from the reservoir (ZT) contained relatively lower chemical concentration than that from the DJ and ZJ Rivers, and also, the concentrations of EDCs in the water (ZT) were the lowest in the sample sites. In this investigation, it was found that the EEQs values in bile samples were significantly related to those in water

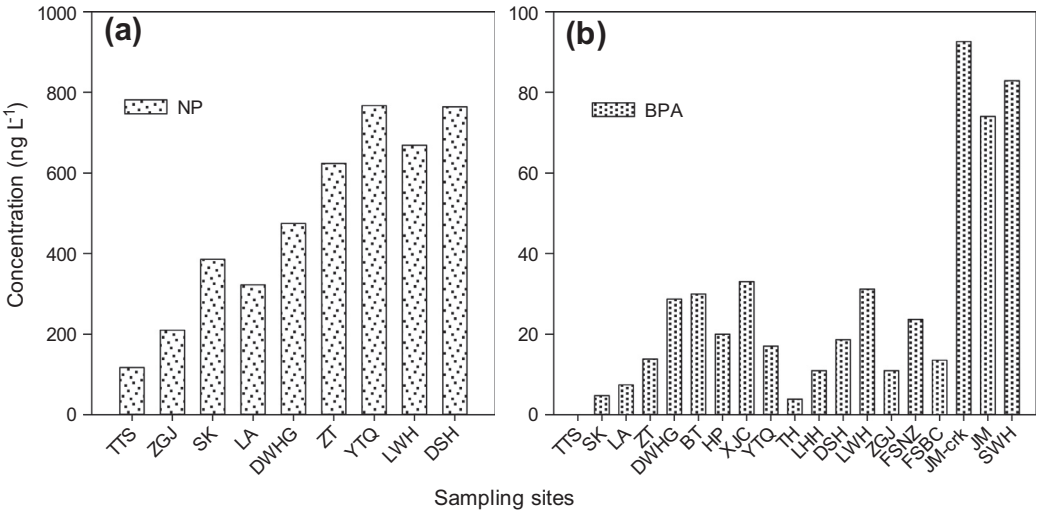

Fig. 1. Spatial distribution of 4-nonylphenol (NP) and bisphenol A (BPA) in the surface water samples from the reservoirs, city park-lakes, fish-ponds and rivers. For NP, YTQ, LWH, DSH sites represent urban areas, SK, LA, DWHG sites represent suburbs areas, and TTS, ZGJ sites represent remote areas.

Table 1
Concentrations and bioaccumulation factors (BCFs) of EDCs in carp bile (ng g^{-1} bile) and algae (ng g^{-1} dw) in different sampling sites.

	Sampling sites	OP		NP		BPA		E1	
		ng g^{-1}	BCF (L kg^{-1})	ng g^{-1}	BCF (L kg^{-1})	ng g^{-1}	BCF (L kg^{-1})	ng g^{-1}	BCF (L kg^{-1})
Carp bile	FSBC1(FB1)	15	2536	950	1648	70	6707	7	13208
	FSBC2(FB2)	24	4253	1416	2458	148	14178	15	28302
	FSBC3(FB3)	37	6393	2020	3505	78	7480	21	39623
	ZT	16	12960	1850	5681	179	12804	n.d.	–
	DJ1	39	3981	3775	9046	1020	9573	23	–
	DJ2	15	1500	2388	5721	382	3583	18	–
	DJ3	32	3303	4648	11137	300	7850	17	–
	ZJ	36	2921	2776	2165	597	10038	30	–
Algae	FSNZ	13	3419	267	690	72	4673	5	–
	XJC	12	7251	261	461	94	12979	30	–
	LA	12	1833	282	451	94	3310	nd	–
	ZT	2	482	53	131	16	4063	nd	–
	LHH	5	1858	166	166	26	1137	nd	–
	YTQ	2	2812	100	740	72	2846	nd	–

n.d. = Not detected.
"–" = On data.

samples ($r^2 = 0.76$, $p < 0.01$) (Fig. 3). This observation illustrated that analysis of fish bile could reflect the concentrations of EDCs in water. The concentrations of EDCs in bile proved that fish had absorbed these chemicals and reflected the internal exposure level to some extent. Moreover, as good relationship was observed between the bile concentrations of EDCs and the vitellogenin (VTG) concentrations in fish blood plasma, the high concentration in bile could imply some biological effect to the fish (Legler et al., 2002; Tyler et al., 2005). Legler et al. (2002) found a good correlation ($r = 0.81$, $p = 0.0002$) between bile EEQs and plasma vitellogenin induction in fish. As the effective concentration of E2 in water for inducing VTG in short term is between 8 and 10 ng E2/L (Thorpe et al., 2006), the EEQs in the carp bile and water, ranging from 1.20 to 10.97 ng g^{-1} and from 0.07 to 8.06 ng L^{-1} respectively, indicating some potential biological effect existing at some sampling sites. So the high EDCs levels in the investigated fish bile samples not only imply that the water of Pearl River Delta had been polluted by these chemicals but also suggest that the collected carp had assimilated these chemicals, which may cause some biological effect.

3.5. Bioaccumulation factors for EDCs in carp bile and algae

Based on the concentrations in water (SI Table S1) and in algae and carp bile (Table 1), the field bioconcentration factors (BCF) of OP, NP, and BPA, and E1 were calculated in algae and the carp samples. The BCF values of OP, NP, BPA, and E1 in algae were in the ranges of 482–7251, 131–740, 2846–12979, and no data, respectively, and in carp bile were in the ranges of 1500–12960, 1648–11137, and 3583–14178, and 13208–39623, with a mean of 4731, 5170, and 9027, and 27044, and a median of 3642, 4593 and 8712, and 28302, respectively. These in situ BCF values of OP and NP in this field study were consistent with those reported by other investigators. The BCF values had been reported for NP (34121) in roach bile and for *t*-OP in trout (56000) (Smith and Hill, 2004) and rud (20000) by Ferreira-Leach and Hill (2001) and Pedersen and Hill (2002). And these values were slightly lower than the EEQ BCF in the bile of fish exposed to sewage effluent reported by Tyler et al. (2005). BCFs in bile of fish exposed to effluents were 10 000–13 000 for E1 and E2 (Gibson et al., 2005). But, the field and laboratory BCF values of BPA in fish bile are unavailable for the comparison. When compared with the previous investigations on the bioaccumulation of NP in mussel and fish in laboratory experiments and field investigations (Ekelund et al., 1990; Ahel et al., 1993; Tsuda et al., 2000; Keith et al., 2001; Snyder et al., 2001), the BCF value of NP in carp bile in this investigation was consistent with that of mussel (Ekelund et al., 1990), while NP in the algae was consistent with those reported by Ahel et al. (1993). However, the higher BCF values for the carp bile and the algae in this investigation than in the previous investigations on other organism or other fish tissues were observed for the investigated EDCs (Table 1 and Table S3). The BCF values of E1 in carp bile were much higher than those (29–35) in crucian carp muscle and the laboratory BCF values (30) in fish muscle for E1 (Lai et al., 2002; Liu et al., 2012), and the BCF values of BPA were much higher than those (4–17) in crucian carp muscle and the laboratory BCF values (20–68) in medaka muscle for BPA (Staples et al., 1998; Liu et al., 2012). So we assume that the water-borne alkylphenols could be easily accumulated, but rapidly conjugated and eliminated via the liver/bile route in the carp. The other investigators pointed out that that biliary excretion is a major route for the elimination of alkylphenols (Smith and Hill, 2004).

In addition, this observed higher BCF values in this investigation may depend on living habitat and trophic levels of fish, and environmental behaviors of EDCs. As other investigation demonstrated that the bioavailability, uptake, fate of PAHs by aquatic

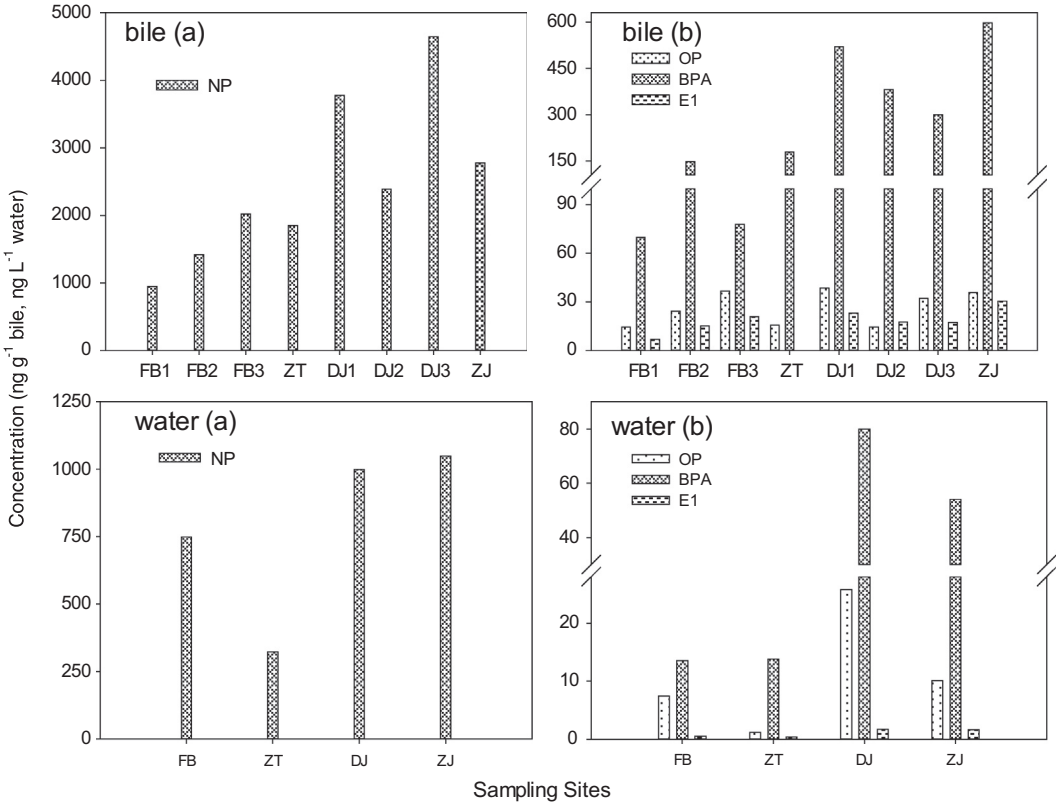

Fig. 2. The concentrations of endocrine disrupting chemicals (EDCs) (OP, NP, BPA and E1) in the carp bile and in the ambient water.

Table 2
Comparison of EDCs in the carp bile (ng g^{-1} bile) with other reports in the world.

Fish species	Sampling site	NP	OP	BPA	E1	Literature
Carp	Blank workup	204	3	8	n.d.	This study
	Blank workup	428 ± 149	n.d.	6 ± 0.6	3.7 ± 0.3	Fenlon et al. (2010)
	Fish ponds	950–2020	15–37	70–148	7–21	This study
	Reservoir	1849	16	179	n.d.	This study
	DJ	2388–4648	15–39	300–1020	17–23	This study
	ZJ	2776	36	597	30	This study
Roach	Thames-River, UK	195–2453	n.a.	<blank-68	3–97	Fenlon et al. (2010)
	Effluent-exposed, UK	5531–12678	n.a.	763–1951	565–1426	(Fenlon et al. (2010)
Rainbow trout	Effluent-exposed, UK	37953–50655	n.a.	n.a.	966–1739	(Gibson et al. (2005)
Juvenile rainbow trout	Effluent-exposed, Sweden	24000	n.a.	23000	4000	Pettersson et al. (2006)
Thick-lip grey mullets	Estuary of Urdaibai, Spain	2300–10660	n.a.	n.a.	n.a.	Vallejo et al. (2010)
Sea bass	Aquaculture facilities, Spain	360–898	4–10	n.a.	n.a.	Fernandes et al. (2008)
Carp	Ebro river, Spain	140–59830	10–1300	n.a.	n.a.	Lavado et al. (2006)
Barbel	Ebro river, Spain	170–340000	50–2240	n.a.	n.a.	(Lavado et al. (2006)
English sole	Puget sound, WA, USA	n.a.	n.a.	<6.3–52	n.a.	Da Silva et al. (2013)

n.a. = Not analyzed.
n.d. = Not detected.

organisms from contaminated media (water, sediments, and food) were also affected by a variety of physical (e.g. lipophilicity, temperature, etc.) and biological parameters. As a general rule in fish, water is dominant pathway of exposure for organic compounds with log K_{ow} lower than 5, while suspended particles can be used for some fish species as food and can contribute substantially to bioaccumulation for PAHs with log K_{ow} higher than 5 (Landrum, 1989). The physicochemical properties of a given chemical, the physiological components of the uptake process, biotransformation and blood flow as well as fatty acid composition and lipid content in aquatic animals could all affect the uptake and accumulation of organic chemicals for fishes (Barron, 1990). All of those above may contribute to the unpredictability of the bioaccumulation of EDCs in fish.

Another interesting result (Fig. 4) is that the sequence of the field BCF among OP, NP, and BPA was different in the varied levels of the eutrophication. The BCF value of NP in the fish bile from the oligotrophic water environment (Chl a, 2–3 ng L^{-1}) was higher than that of BPA and 4-t-OP, which was presumed to be reasonable based on log K_{ow} of the target compounds (4-NP >4-t-OP, BPA). However, bile samples from the eutrophic water bodies (Chl a > 10), the BCF value of NP was lower than that of 4-t-OP and BPA. The varied eutrophication degree means different amount of algae in the water. As algae can also accumulate OP, NP, and BPA, and the BCF values of OP and BPA in the investigated algae were an order of magnitude higher than those of NP (Table 1), the large amount of algae in water might be one of the reasons for the different BCF sequence. Moreover, the abnormal BCF values of NP for the

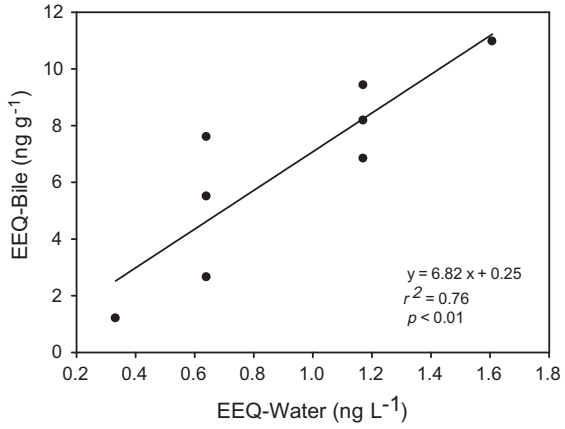

Fig. 3. Relationship between the E2 activity equivalents (EEQs) in the water and in the carp bile.

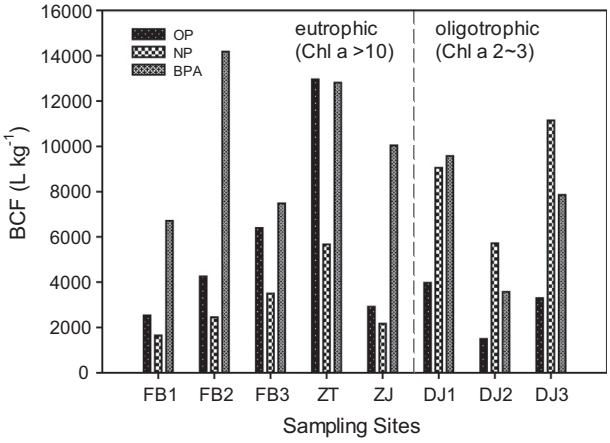

Fig. 4. The variation of the bioaccumulation factors (BCFs) of OP, NP and BPA in the carp bile sampled from the different water eutrophication environment.

investigated algal samples, which are different from the predicted values from its log K_{ow} value, suggest that NP could be degraded by some algal species in the field. It was indeed reported in the laboratory that NP can be degraded by some algal species (Writer et al., 2011; Jurgens et al., 2002). In a word, the exact mechanism for the lower in situ BCF values for NP in this field study is not clear and warrants further investigation.

4. Conclusion

This work investigated endocrine disrupting chemicals (EDCs) in water and wild carp bile of the Pearl River Delta (PRD), South China. The relatively high concentrations of NP and BPA in carp bile were detected. The EEQs in the bile samples were significantly related to those in the water samples, illustrating that occurrence of EDCs in fish bile can reflect that in ambient water in the PRD region. The BCF in carp bile were comparable to other reports and could be affected by water eutrophication level.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.chemosphere.2014.01.048.

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