Environmental Pollution 198 (2015) 15–24

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Tissue-specific bioaccumulation of human and veterinary antibiotics in bile, plasma, liver and muscle tissues of wild fish from a highly urbanized region

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ARTICLE INFO

Article history:
Received 17 July 2014
Received in revised form
5 December 2014
Accepted 15 December 2014
Available online

Keywords:
Antibiotics
Rivers
Fish tissues
Bioaccumulation
Human health risks

ABSTRACT

We investigated the bioaccumulation of antibiotics in bile, plasma, liver and muscle tissues of wild fish from four rivers in the Pearl River Delta region. In total, 12 antibiotics were present in at least one type of fish tissues from nine wild fish species in the four rivers. The mean values of log bioaccumulation factors (log BAFs) for the detected antibiotics in fish bile, plasma, liver, and muscle tissues were at the range of 2.06–4.08, 1.85–3.47, 1.41–3.51, and 0.48–2.70, respectively. As the digestion tissues, fish bile, plasma, and liver showed strong bioaccumulation ability for some antibiotics, indicating a different bioaccumulation pattern from hydrophobic organic contaminants. Human health risk assessment based on potential fish consumption indicates that these antibiotics do not appear to pose an appreciable risk to human health. To the best of our knowledge, this is first report of bioaccumulation patterns of antibiotics in wild fish bile and plasma.

1. Introduction

Pharmaceuticals in the environment have attracted great public attention during the past two decades due to their wide environmental occurrence and potential adverse effects to wild organisms (Daughton and Ternes, 1999; Kolpin et al., 2002; Fent et al., 2006). Among pharmaceuticals, antibiotics are one of the more commonly used pharmaceutical classes, and they are not only used for treatment of human diseases but also are used as veterinary drugs for growth promotion and disease prevention (Kümmerer, 2009). The annual consumption of antibiotics in the world was estimated to be more than 100,000 tons (Kümmerer, 2009). In the United States (FDA, 2009) and Europe (EMA, 2011), the annual usage of antibiotics was in the range of tens of thousands of tons. In China, the annual usage of antibiotics was estimated to be in the range of hundreds of thousands of tons, approximately 10 times higher than in the United States and Europe (Zhou et al., 2011).

After administration, antibiotics are excreted from human and animals, and subsequently released to municipal wastewater treatment plants (WWTPs) or farm wastewater treatment facilities (Sarmah et al., 2006; Sapkota et al., 2008; Schultz et al., 2010). Due to incomplete removal in wastewater treatment facilities, antibiotics were regularly detected in municipal WWTPs effluents (Hirsch et al., 1999; Zhou et al., 2013a), swine farm effluents (Zhou et al., 2013b), receiving river water and sediments (Kolpin et al., 2002; Yang et al., 2010; Liang et al., 2013), and soils (Ho et al., 2012).

Antibiotics may exert adverse effects on wild organisms after they enter into the receiving environment (Fent et al., 2006). Bacterial resistance to antibiotics is believed to be a big health concern (Cabello, 2006; Tao et al., 2010). It is also reported that use of antibiotics in human and animals accelerated development and transfer of antibiotic resistance genes in the environment (Su et al., 2012; Chen et al., 2013; Luo et al., 2010). Meanwhile, antibiotics such as tetracycline, chlortetracycline, norfloxacin, and sulfamethoxazole have been shown to have toxic effects on some aquatic organisms (Richards et al., 2004; Robinson et al., 2005; Yang et al., 2008).

The adverse effects of a pollutant on organisms may be correlated to internal chemical concentrations in tissues due to specific

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http://dx.doi.org/10.1016/j.envpol.2014.12.026
0269-7491/

bioaccumulation (Stadnicka et al., 2012). To date, there are few studies on the presence of antibiotics and their metabolites in aquatic biota outside of laboratory studies. Gao et al. (2012) reported that norfloxacin, ciprofloxacin and sulfamethazine were the most frequently detected antibiotics in fish muscle from the Hai River in China. Because of the use of antibiotics in aquaculture, some antibiotics can also be detected in target cultured fish from aquaculture ponds (Cabello, 2006; Gao et al., 2012). Laboratory experiments that documented uptake of ciprofloxacin by crucian carp showed bioaccumulation in visceral and muscle tissues (Nie et al., 2008). Blue mussels were shown to bioconcentrate oxolinic acid and oxytetracycline (Le Bris and Pouliquen, 2004). However, two studies in the United States and Germany showed no detection of antibiotics in fish liver and muscle samples from effluent-impacted rivers during screening of pharmaceuticals and personal care products (PPCPs) in fish muscle and liver tissues (Ramirez et al., 2009; Subedi et al., 2012). Recent studies demonstrate that pharmaceuticals, such as non-steroidal anti-inflammatory drugs and antidepressant drugs could accumulate in fish bile, brain and plasma tissues (Schultz et al., 2010; Brozinski et al., 2013; Mehinto et al., 2010), although some of these pharmaceuticals were less frequently detected in fish muscle (Ramirez et al., 2009; Subedi et al., 2012). Hence, pharmaceuticals like antibiotics may also display differential uptake and bioaccumulation in different biota tissues.

The objective of this study was to investigate the bioaccumulation characteristics of various antibiotics in the bile, plasma, liver and muscle tissues of wild fish. Fish samples were collected from four rivers: the Dongjiang, Shima, Danshui and Xizhijiang Rivers in the highly urbanized Pearl River Delta region, South China. The occurrence of 26 antibiotics was assessed in river water, sediments and fish tissues. The tissue-specific bioaccumulation profiles were then evaluated in different fish tissues from the four rivers by calculating the bioaccumulation factors. The results from this study expand our understanding of the bioaccumulation potential of polar contaminants such as antibiotics and their potential human and ecological risks.

2. Materials and methods

2.1. Study area

The Pearl River Delta region is a highly urbanized area in South China. Rivers in this region receive large quantities of treated and untreated domestic sewage. Sampling sites were located in the mainstream of the Dongjiang River and the 3 tributaries, the Shima, Danshui and Xizhijiang Rivers (Fig. 1). Sites M1–M8 are located in the Shima River, sites of S1–S7 are in the Danshui River, sites S8–10

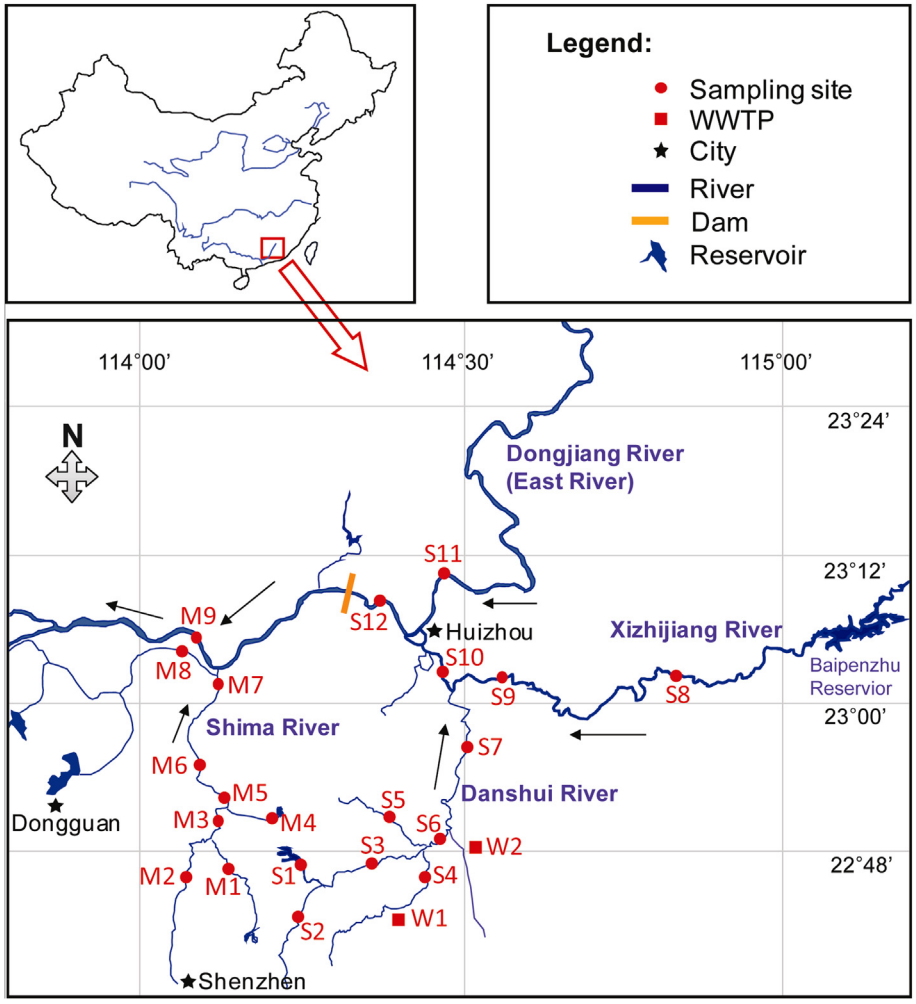

Fig. 1. Map of study area in the Dongjiang River system showing sites of water, sediments, and fish samples collected. The sampling campaigns were carried out on July 2012 (wet season) and December 2012 (dry season).

are located in the Xizhijiang River, and sites M9, S11 and S12 are located in the mainstream of the Dongjiang River. The effluents from two WWTPs (W1 and W2) in the basin of the Danshui River were also sampled. Detailed information describing the sampling sites and the quality parameters of water and sediments can be found in Tables S1–S3 and the text of Supplementary Information.

2.2. Sample collection

Sampling campaigns were carried out twice, in July 2012 (wet season) and December 2012 (dry season). One-liter surface water samples (grab samples with three replicates for each site) were collected and stored in amber glass bottles, and 50 mL of methanol and 400 μL of 4 M H_2SO_4 (adjust pH to 3.0) were added immediately to suppress bacterial activity as described in our previous studies (Zhou et al., 2012). Surface sediments were obtained by a stainless steel grab and stored in glass bottles, with 1 g of sodium azide added immediately to inhibit biodegradation. Water samples were transported to the laboratory on ice and stored at 4 °C in a cold room before further processing within 24 h. Sediment samples were freeze-dried immediately and kept at 4 °C for later extraction.

Fish were sampled by electroshocking with a pulsed direct current or by fish netting from 11 monitoring sites (Table S4). All the captured fish samples were kept alive in iced water aerated by portable air pumps and immediately transported to the laboratory after collection. Upon arrival in the laboratory, fish were anesthetized by immersion in tricaine methanesulfonate and sacrificed by rapid dissection. Blood was obtained from the fish caudal vein by a sodium heparin pre-rinsed syringe and stored at 4 °C for 8 h in 2 mL plastic vials. The vials were then centrifuged at 10,000 *g* for 10 min, and the supernatant (plasma) collected. Bile was obtained from fish gall bladder by a syringe needle and transferred to a cryogenic vial. Liver was separated from viscera and kept in a cryogenic tube. After removal of fish skin, muscle was obtained and cut to small pieces. All tissues collected were immediately stored at −20 °C until extraction.

The collected fish species in this study included tilapia (*Tilapia aurea*), crucian carp (*Carassius auratus*), common carp (*Cyprinus carpio*), leather catfish (*Clarias fuscus*), snakehead (*Ophicephalus argus*), grass carp (*Ctenopharynodon idellus*), chub (*hypophthalmichthys molitrix*), mud carp (*Cirrhinus molitorella*), and bream (*Parabramis pekinensis*). Fish lengths and weights are listed in Table S4. In total, 128 fish were collected in the two sampling campaigns. Due to the difficulty of dissection and complexity of wild fish, liver, plasma and bile samples could not be obtained for each fish. Hence, the numbers for bile, plasma, liver and muscle tissue samples were 105, 48, 91 and 128, respectively.

2.3. Sample extraction and instrumental analysis

The target antibiotics were selected based on the information released from the local health agency and detection frequencies of antibiotics reported in the literature. In total, 26 antibiotics, including 9 sulfonamides (SAs), 8 fluoroquinolones (FQs), 2 macrolides (MLs), 4 tetracyclines (TCs), and 3 others (lincomycin (LIN), ormetoprim (OMP), and trimethoprim (TMP)) were included in this study. The 9 SAs are sulfisoxazole (SSA), sulfadimethoxine (SDM), sulfadoxine (SDO), sulfameter (SM), sulfamonomethoxine (SMM), sulfamerazine (SMR), sulfamethoxazole (SMX), sulfamethazine (SMZ) and sulfapyridine (SPD); the 8 FQs are carbadox (CAR), ciprofloxacin (CFX), enrofloxacin (EFX), fleroxacin (FL), lomefloxacin (LFX), marbofloxacin (MAR), norfloxacin (NAR) and ofloxacin (OFX); the 2 MLs are dehydrated erythromycin (ETM-H_2O) and leucomycin (LCM); and the 4 TCs are chlortetracycline (CTC), doxycycline (DC), oxytetracycline (OTC) and tetracycline (TC).

Table 1
Concentrations (ng/L) of antibiotics in surface water samples from the Shima, Danshui, Xizhijiang, and Dongjiang Rivers.

Compound	Shima River			Danshui River			Xizhijiang River			Dongjiang River			All of the 4 rivers			
	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Frequency
SM	0–2.25	0.67	0.57	0–10.2	<1.13	0	0–1.49	0.18	0	0–6.09	1.55	0.28	0–10.2	0.74	0	48%
SMM	0–19.1	5.09	4.33	0–62.4	7.94	1.27	0.78–14.7	4.53	3.17	0–202	24.6	13.0	0–202	8.74	3.31	78%
SMX	0–150	45.5	26.5	0–98.1	29.4	16.2	0–59.0	15.9	9.29	0–19.3	5.95	4.82	0–150	30.3	15.7	79%
SMZ	0–31.0	10.3	6.92	0–147	20.7	8.03	0–39.4	13	11.2	0–86.7	23.8	13.0	0–147	16.1	8.27	76%
SPD	0–30.1	7.23	3.69	0–5.63	1.64	1.45	0–<0.98	<0.98	0	0–<0.98	<0.98	0	0–30.1	3.33	<0.98	62%
CFX	0–23.1	4.16	<3.63	0–5.48	<3.63	0	ND			ND			0–23.1	<3.63	0	44%
EFX	0–<1.29	<1.29	0	0–1.41	<1.29	0	0–15.1	1.65	0	0–5.11	<1.29	0	0–15.1	<1.29	0	17%
NFX	0–97.1	11.7	3.95	0–45.1	7.61	3.24	0–<2.93	<2.93	<2.93	0–5.34	<2.93	0	0–97.1	7.21	<2.93	67%
OFX	0–59.5	9.95	3.36	0–41.2	7.41	3.56	0–7.16	<0.96	0	0–4.39	<0.96	0	0–59.5	6.47	1.64	74%
ETM-H_2O	2.83–1578	672	610	0–906	255	185	0–166	58.1	30.1	0–609	82.9	27.9	0–1578	361	185	83%
CTC	0–452	37.0	0	0–338	38.9	8.06	0–36.6	4.72	0	ND			0–452	27.7	0	33%
DC	0–88.6	17.1	<4.43	0–81.7	5.04	0	ND			ND			0–88.6	8.19	0	29%
OTC	0–451	87.6	36	0–179	31.3	12.9	0–9.5	<2.08	0	0–61.9	7.52	0	0–451	45.1	9.48	52%
TC	0–178	25.2	7.28	0–85.5	13.5	7.76	ND			ND			0–178	14.1	0	38%
LIN	0–320	123	117	0–421	118	67.3	0–101	29.2	10.6	0–801	75.9	31.3	0–801	101	74.3	79%
TPM	0–50.1	23.7	26.9	0–47.1	19.3	20.3	0–15.8	4.3	2.1	0–15	3.15	2.54	0–50.1	16.5	14.4	83%

ND: not detected.
SM: Sulfameter; SMM: sulfamonomethoxine; SMX: sulfamethoxazole; SMZ: sulfamethazine; SPD: Sulfapyridine; CFX: ciprofloxacin; EFX: enrofloxacin; NFX: norfloxacin; OFX: ofloxacin; ETM-H_2O: dehydrated erythromycin; CTC: chlortetracycline; DC: doxycycline; OTC: oxytetracycline; TC: tetracycline; LIN: lincomycin; TMP: trimethoprim.

Table 2
Concentrations (μg/kg) of antibiotics in sediment samples from the Shima, Danshui, Xizhijiang, and Dongjiang Rivers.

Compound	Shima River			Danshui River			Xizhijiang River			Dongjiang River			All of the 4 rivers			
	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Range	Mean	Median	Frequency
SMM	0–<1.57	<1.57	0.79	0–3.59	<1.57	<1.57	0–<1.57	<1.57	<1.57	<1.57–4.29	1.73	<1.57	0–4.29	<1.57	<1.57	73%
SMX	0–<1.00	<1.00	0	0–3.07	<1.00	0	0–<1.00	<1.00	0	0–<1.00	<1.00	0	0–3.07	<1.00	0	28%
SMZ	0–6.68	<1.03	<1.03	0–5.14	<1.03	<1.03	0–1.97	<1.03	<1.03	<1.03–6.77	2.81	1.23	0–6.77	1.21	<1.03	85%
SPD	ND			0–2.84	<0.67	0	0–<0.67	<0.67	0	0–<0.67	<0.67	0	0–2.84	<0.67	0	15%
CFX	5.08–83.9	21.7	13.9	<4.03–139	19.0	6.77	0–8.37	4.51	4.57	<4.03–15.8	6.94	5.51	0–139	15.9	7.84	98%
EFX	<1.92–10.5	5.24	4.48	<1.92–47.4	9.14	4.17	<1.92–91.1	16.1	3.90	2.14–38.1	11.2	4.19	<1.92–91.1	9.12	4.28	100%
LFX	<1.35–8.00	3.09	2.46	<1.35–32.6	4.50	1.65	0–2.59	1.46	1.65	0–1.68	<1.35	<1.35	0–32.6	3.01	1.67	95%
NFX	6.54–277	61.7	28.7	5.67–120	37.5	17.2	0–37.1	14.8	13.4	0–16.7	9.14	10.2	0–277	38.3	16.5	95%
OFX	12.0–277	68.6	38.9	3.02–149	39.0	15.1	0–37.3	18.3	20.4	0–20.1	9.25	9.9	0–278	41.8	18.9	95%
ETM-H_2O	<1.59–48.9	15.1	13.3	<1.59–19.6	5.91	3.22	0–4.06	2.17	1.91	1.9–13.7	5.56	5.66	0–48.9	8.52	5.62	99%
CTC	0–147	21.1	5.05	0–40.4	7.87	4.98	0–15.6	6.92	7.4	0–93.8	23.1	8.91	0–147	14.7	5.18	80%
DC	0–43.0	11.2	<3.07	0–36.8	4.29	0	0–66.4	11.5	0	0–44.4	11.1	<3.07	0–66.4	8.81	<3.07	50%
OTC	0–663	137	46.5	0–2521	335	51.5	0–277	96.9	32.3	0–921	178	56.23	0–2521	206	49.4	75%
TC	0–269	52.0	18.3	0–1131	163	12.8	0–86.1	24.6	5.42	0–117	23.1	3.63	0–1131	82.3	12.9	75%
LIN	0–<4.43	<4.43	0	0–80.9	6.75	<4.43	0–5.62	<4.43	<4.43	0–22.3	<4.43	0	0–80.9	<4.43	0	35%
TPM	0–2.19	0.73	0.69	0–12.0	1.21	<0.64	0–0.95	<0.64	<0.64	<0.64–1.06	<0.64	<0.64	0–12.0	0.82	<0.64	91%

ND: not detected.
SMM: sulfamonomethoxine; SMX: sulfamethoxazole; SMZ: sulfamethazine; SPD: Sulfapyridine; CFX: ciprofloxacin; EFX: enrofloxacin; LFX: lomefloxacin; NFX: norfloxacin; OFX: ofloxacin; ETM-H_2O: dehydrated erythromycin; CTC: chlortetracycline; DC: doxycycline; OTC: oxytetracycline; TC: tetracycline; LIN: lincomycin; TMP: trimethoprim.

Details of standards, internal standards and reagents are described in Tables S5–S6 and in the text of Supplementary Information.

Simultaneous extraction of antibiotics in surface water and sediment has been developed in our previous study (Zhou et al., 2012). For fish tissue samples, due to the presence of bile acid in fish bile and lipid in liver and muscle tissues (Simpson and Wynne, 2000), it was essential to remove the bile acid and lipid to reduce the high matrix interference before instrumental analysis. This purification was carried out by using a two-layer SAX/PSA cartridge (SAX = strong anion exchange; PSA = primary secondary amine) to remove the matrix in bile, liver and muscle samples (Steinbach and Schwack, 2014), and the target antibiotics enriched by a subsequent HLB solid phase extraction (SPE) cartridge. Bile samples were adjusted pH to 3.0, and purified and enriched by SAX/PSA–HLB tandem cartridges. Plasma samples were adjusted pH to 3.0, and enriched by HLB cartridges. Liver and muscle samples were extracted using ultrasonic-assisted extraction modified from the method described by Ramirez et al. (2007); the raw extracts were then further purified and enriched by SAX/PSA–HLB tandem cartridges. Detailed extraction procedures for surface water, sediments and fish tissues are described in the Supplementary Information.

The target antibiotics were analyzed using UPLC–MS/MS (Agilent Ultra Performance Liquid Chromatography 1200 series UPLC system coupled to an Agilent 6460 triple quadrupole MS equipped with an electrospray ionization (ESI) source (Agilent, Palo Alto, CA, USA)) in multiple-reaction monitoring (MRM) mode. The instrumental analysis was performed in the positive mode. The method detection limits (MDLs) and method quantification limits (MQLs) for the target antibiotics were calculated based on the signal-to-noise values of 3 and 10 by analyzing spiked samples. Details of the instrumental conditions for the target antibiotics, recoveries, matrix effects, MDLs, and MQLs in fish tissues are provided in Tables S7–S11 and the text of the Supplementary Information.

2.4. Data analysis

The concentration range, mean value, median value and detection frequency were used to describe the contamination profiles for antibiotics in surface water, sediment and tissue samples. When calculating the range, median and mean values, data with the value less than MQL, then a half of MQL was substituted for the value, and for the data of ND (not detected), a zero was substituted. When calculating the detection frequency, data below MQL also were counted; ND results were not counted. Descriptive statistics and linear regression fitting were performed with the Microsoft Excel 2010 and Sigma Plot 10.0 software.

The bioaccumulation factors (BAFs) for an antibiotic in bile, plasma, liver and muscle tissues were calculated based on each fish and the corresponding concentration in surface water of that site. Due to the continued discharge of antibiotics into the receiving environments, the antibiotic bioaccumulation by wild fish is assumed to be pseudo steady state when calculating the BAFs. The equations for calculating BAFs in fish bile and plasma are described in the text of the Supplementary Information. Modeled log BAFs in fish muscle were used for comparison with the determined log BAFs. The modeling was based on the Arnot and Gobas model and conducted by the BCFBAF program of the software Epi Suite 4.1 released from U.S. Environmental Protection Agency (Costanza et al., 2012; USEPA, 2014). Chemical name and SMILES (simplified molecular-input line-entry system) structure were inputted as the basic parameters.

Potential intake of antibiotics by human consumers was estimated based on the average intake from eating fish muscle with the observed antibiotics concentrations (Dunnivant and Anders, 2006). The equation and corresponding parameters are described in

Table 3
Levels of antibiotics in fish bile, plasma, liver and muscle samples from the Shima, Danshui, Xizhijiang, and Dongjiang Rivers.

Compound	Bile (μg/L)				Plasma (μg/L)				Liver (μg/kg wet weight)				Muscle (μg/kg wet weight)			
	Frequency	Range	Mean	Median	Frequency	Range	Mean	Median	Frequency	Range	Mean	Median	Frequency	Range	Mean	Median
SMM	10%	0–20.4	<3.70	0	ND				ND				ND			
SMX	ND				2%	0–5.50	<4.54	0	ND				4%	0–<0.91	<0.91	0
SMZ	31%	0–49.4	2.41	0	83%	0–144	6.47	2.98	64%	0–3.12	0.88	<0.88	63%	0–8.00	<0.27	<0.27
CFX	12%	0–51.3	3.54	0	10%	0–<4.55	<4.55	0	8%	0–2.44	<1.04	0	5%	0–1.26	<0.46	0
EFX	10%	0–334	4.60	0	63%	0–34.7	<2.70	<2.70	18%	0–56.7	1.51	0	46%	0–6.96	<0.27	0
LFX	ND				8%	0–23.0	<5.30	0	ND				ND			
NFX	47%	0–83.9	6.42	0	ND				9%	0–7.67	<1.72	0	ND			
OFX	45%	0–37.8	5.02	0	21%	0–11.9	<2.16	0	86%	0–9.85	2.52	2.24	99%	0–16.8	1.34	0.66
ETM-H_2O	79%	0–285	26.1	12.54	92%	0–545	29.44	12.2	90%	0–2390	68.7	4.43	49%	0–2.36	<0.49	0
CTC	ND				ND				2%	0–326	<13.7	0	ND			
LIN	92%	0–58.6	8.80	6.18	25%	0–567	24.69	0	9%	0–1384	22.4	0	5%	0–26.2	<0.88	0
TMP	37%	0–4.01	0.50	0	21%	0–6.13	<4.34	0	25%	0–2.13	0.26	0	63%	0–0.39	<0.13	<0.13

ND: not detected.
SMM: Sulfamonomethoxine; SMX: sulfamethoxazole; SMZ: sulfamethazine; CFX: ciprofloxacin; EFX: enrofloxacin; LFX: lomefloxacin; NFX: norfloxacin; OFX: ofloxacin; ETM-H_2O: dehydrated erythromycin; CTC: chlortetracycline; LIN: lincomycin; TMP: trimethoprim.

Supplementary Information. The risks posed by antibiotics to human were assessed by calculating a hazard quotient (HQ), which is derived from the ratio of the intake rate and the acceptable daily intake (ADIs) by adult (Boonsaner and Hawker, 2013). A HQ of 1.0 or above indicates a high risk of an adverse health effect, while conversely a hazard quotient below 1.0 indicates a low risk.

3. Results

3.1. Antibiotics in river water and sediments

In river water, 16 out of the 26 monitored antibiotics were detected during the two sampling campaigns (Table 1). The detected antibiotics included 5 SAs, 4 FQs, 1 ML, 4 TCs, and 2 other antibiotics. ETM-H_2O showed the maximum value among these detected antibiotics, up to 1578 ng/L. ETM-H_2O and TMP displayed the same highest detection frequency of 83%, followed by SMX, SMM, SMZ, OFX, and LIN with detection frequencies of higher than 70%. In sediments, the detected antibiotics were mostly the same as in surface water (Table 2), except that SM was only detected in surface water and LFX was only detected in sediments. OTC showed the maximum concentration among all of the detected antibiotics in the sediments, up to 2521 μg/kg, followed by TC at the maximum concentration of 1131 μg/kg. The detection frequencies of EFX, ETM-H_2O, CFX, NFX, OFX, LFX, and TPM were more than 90% in the sediments.

Mean values of the detected antibiotics in the Shima and Danshui Rivers were generally higher than those in the Xizhijiang and Dongjiang Rivers. The Shima and Danshui Rivers are the streams impacted by domestic sewage in this area. For most of the antibiotics, the sites S12 and M9 located downstream of Dongjiang River showed higher concentrations than the site S11 located upstream of Dongjiang River, due to the input of contributions from the Danshui, Xizhijiang and Shima Rivers. For example, ETM-H_2O had mass fluxes in sites S11, S12 and M9 of 0.96, 18.3, and 4.67 kg/d in the wet season, and 0, 0, and 3.2 kg/d in the dry season, respectively. The results suggest that the antibiotics from polluted tributaries and streams made significant contributions to the mass loading of antibiotics in the mainstream of the Dongjiang River.

A variety of antibiotics were also detected in the effluents from the two WWTPs in the Danshui River basin (Table S12). It can be seen that the mean values of SMX, SPD, NFX, OFX, ETM-H_2O, TC, and TMP in the effluents were 2 times higher than the mean values of these antibiotics in the Danshui River. Hence, for these antibiotics, the effluents were significant emission sources to the receiving rivers. But for SMZ and LIN, the mean values in the effluents were lower than those in the Danshui River, suggesting that other emission sources, such as untreated domestic wastewaters and wastewater from livestock farms, may also contribute to these rivers.

3.2. Antibiotics in fish bile, plasma, liver and muscle

The profiles of antibiotics in fish bile, plasma, liver, and muscle samples are displayed in Table 3, which includes the concentration range, mean and median concentrations, and detection frequencies. Twelve antibiotics were detected in at least one type of tissue, including 3 SAs (SMM, SMX and SMZ), 5 FQs (CFX, EFX, LFX, NFX and OFX), 1 ML (ETM-H_2O), 1 TC (CTC), and 2 others (LIN and TMP). SMZ, CFX, EFX, OFX, ETM-H_2O, LIN and TMP were detected in all four tissues, and these antibiotics also showed high detection frequencies in surface water or sediment samples (Tables 1 and 2), implying that the seven antibiotics were ubiquitous in all sampled environmental media.

Nine antibiotics were detected among 26 monitored antibiotics

in fish bile samples (Table 3). EFX showed a maximum concentration of 334 μg/L at site S12 in bile of crucian carp. ETM-H_2O, NFX, LIN and CFX also had maximum concentrations of more than 50 μg/L. LIN displayed the highest detection frequency at 92%, followed by ETM-H_2O at 79%. Meanwhile, LIN and ETM-H_2O also showed high mean and median concentrations, of 8.80 and 6.18 μg/L for LIN, and 26.1 and 12.54 μg/L for ETM-H_2O, indicating that these two antibiotics were the dominant antibiotics in bile.

Nine antibiotics were found in fish plasma samples, with the maximum concentrations for LIN, ETM-H_2O and SMZ up to 567, 545 and 144 μg/L, respectively (Table 3). The maximum occurrence for the three antibiotics was located in sites S6 (tilapia), S8 (mud carp) and S9 (grass carp), respectively. According to the detection frequencies and mean values (Table 3), ETM-H_2O, SMZ and EFX were the dominant antibiotics in plasma samples. SMX was only detected in one plasma sample of tilapia at site S7, and LFX was also only found in four plasma samples at sites S6, S7 and S10, but the two antibiotics were detected frequently in surface water or sediment samples.

Nine antibiotics were detected among the fish liver samples (Table 3). The ETM-H_2O and LIN displayed high maximum concentrations of 2390 μg/kg ww (crucian carp at site S9) and 1384 μg/kg ww (tilapia at site S7), respectively. ETM-H_2O, OFX and SMZ had high detection frequencies of 90%, 86%, and 64%, respectively. The three highly detected antibiotics in the liver samples also displayed high median and mean values (Table 3). CTC was only detected at 2 out of 91 liver samples with the concentrations of 326 μg/kg ww at site S8 and 57.4 μg/kg ww at site M8, respectively. The occasional detections were probably due to the exposure of wild fish in a highly polluted area.

Eight antibiotics were detected at least one time in the fish muscle samples (Table 3). The maximum concentrations for LIN and OFX were 26.2 μg/kg ww (tilapia at site M8) and 16.8 μg/kg ww (chub at site S10), respectively, followed by SMZ, EFX, CFX and ETM-H_2O at several μg/kg ww. The highest detection frequency for OFX was found to be 99% in fish muscle samples. Both SMZ and TMP showed the detection frequencies of 63%. But SMX, LIN and CFX displayed low detection frequencies (<5%) in fish muscle samples, despite that LIN was occasionally detected at a quite high concentration in the fish from site M8.

In general, antibiotics with different properties displayed different profiles in the four types of fish tissues. For example, OFX was detected in 4 types of tissues, with the detection frequencies increasing gradually from plasma (21%), bile (45%), liver (86%), and muscle (99%), suggesting that OFX was more likely to accumulate to lipid-rich tissues. In contrast, detection frequencies for LIN were almost the opposite, increasing gradually from muscle (5%), liver (9%), to plasma (25%) and bile (92%), suggesting that LIN was more likely to accumulate in hydrophilic tissues. This phenomenon was similar to the partitioning of these same antibiotics between surface water and sediment phases, e.g. OFX was more often detected in sediments than in surface water, while for LIN the detection frequencies were the reverse.

3.3. Bioaccumulation factors

Fig. 2 shows the profiles of log BAFs of antibiotics in fish bile, plasma, liver and muscles, and Table S13 lists detailed statistical information of the log BAFs for each tissue type.

In bile, the log BAFs for 9 detected antibiotics ranged between 0.04 and 4.55, with the maximum value for NFX at site S9 of the Xizhijiang River. The maximum log BAFs for most antibiotics were found in crucian carp and tilapia fish. The mean and median log BAFs for most of the antibiotics were higher than 2, except for TMP at 1.92. The median log BAFs for the detected antibiotics had the following order: CFX > EFX > NFX > OFX > SMM > SMZ > ETM-H_2O > LIN > TMP.

In plasma, the range of log BAFs for the 8 detected antibiotics was 0.37–4.73, with the maximum log BAF being found for EFX at site S6 of Danshui River. The maximum log BAFs for most of the

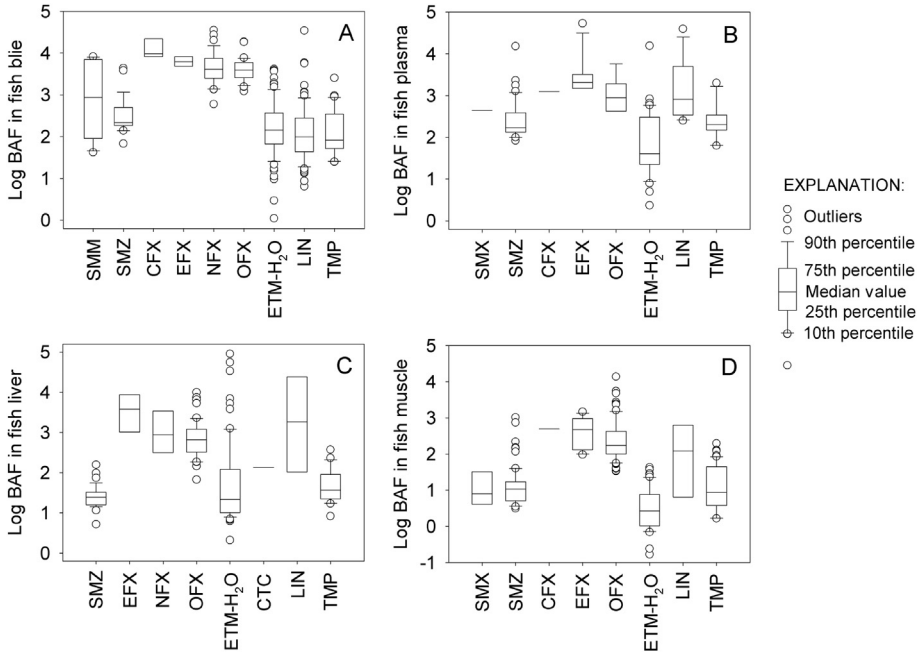

Fig. 2. The log bioaccumulation factors (log BAFs) of antibiotics in different fish tissues. A: bile; B: plasma; C: liver; D: muscle. The box plot graphically depicts the distribution of these results as five-number summaries (the smallest observation, lower quartile, median, upper quartile, and largest observation). SMM: sulfamonomethoxine; SMX: sulfamethoxazole; SMZ: sulfamethazine; CFX: ciprofloxacin; EFX: enrofloxacin; NFX: norfloxacin; OFX: ofloxacin; ETM-H_2O: dehydrated erythromycin; CTC: chlortetracycline; LIN: lincomycin; TMP: trimethoprim.

antibiotics were found to be in tilapia fish. The order of the median log BAFs was: EFX > CFX > OFX > LIN > SMX > TPM > SMZ > ETM-H_2O.

In liver, the range of log BAFs for the 8 detected antibiotics was 0.32–4.96. The maximum BAF was found for ETM-H_2O at site S9 of the Xizhijiang River. Most of the antibiotics with maximum log BAFs were found in tilapia and grass carp. The median log BAFs showed the following order: EFX > LIN > NFX > OFX > CTC > TMP > SMZ > ETM-H_2O.

In muscle, the range of log BAFs for the 8 detected antibiotics was −0.78 to 4.14. The maximum log BAF was found for OFX at site S10 of the Xizhijiang River. Most of the detected maximum BAFs were found in common carp, tilapia and grass carp. The median values for those antibiotics followed the order: CFX > EFX > OFX > LIN > SMZ > TMP > SMX > ETM-H_2O. In general, the FQs (CFX, EFX, NFX and OFX) showed higher mean and median log BAFs than those of other antibiotics in various fish tissues, while ML of ETM-H_2O showed lower mean and median values.

The determined log BAFs were compared with modeled values for fish muscle tissue. The results are shown in Table S14. The modeled log BAFs for most of the antibiotics were ranged from −0.05 to 0.174, and from −0.44 to 0.354 with and without biotransformation, respectively, when modeled using fish at the upper trophic level. The modeled log BAFs were clearly 1 to 2 orders less than those observed in fish muscle for most of antibiotics. While for the ML of ETM, the modeled log BAF was 0.97 (with biotransformation) or 2.154 (without biotransformation), which was higher than the determined mean and median values in fish muscle (Table 3). These results suggest that the modeled results could underestimate or overestimate the bioaccumulation for most of the ionizable antibiotics in wild fish tissues from highly impacted environments.

4. Discussion

4.1. Source and occurrence of antibiotics in receiving environments

The Pearl River Delta region has gone through rapid economic development in the past three decades. The population also has increased rapidly with the demand for labor in various industrial sectors. However, the rates of treatment of domestic sewage are still lower than 70% in Dongguan and Huizhou regions (Zhao et al., 2013). Discharge of untreated wastewaters and treated effluents from WWTPs with incomplete removal of antibiotics contributed to the presence of antibiotics in the Dongjiang River (Zhou et al., 2013a). Previous studies in this region had also demonstrated the ubiquitous presence of PPCPs such as triclosan and triclocarban and antifungal pharmaceuticals in the rivers (Zhao et al., 2013; Chen et al., 2014). Thus, treated and untreated domestic sewage could be a major source for antibiotics in the receiving environment. In addition, some small scatter-breeding farms are still present in Dongguan and Huizhou regions, despite large concentrated animal operations having been closed by the local government since 2005. Hence, incompletely treated wastewater from those small animal farms could also be an input source for antibiotics in the receiving environment.

In this study, 17 out of 26 antibiotics were found in surface water and sediment samples from Shima River, Danshui River, Xizhijiang River and Dongjiang River (Tables 1 and 2). Ten and 13 antibiotics showed the detection frequencies of higher than 50% in surface water and sediments, respectively. The maximum concentrations for the detected antibiotics could reach up to one thousand ng/L in surface water and several thousands μg/kg in sediments. The detected concentrations of antibiotics in surface water were comparable with those from our previous study in the Pearl River Guangzhou section (Yang et al., 2011), and previous studies in rivers from the United States (Kolpin et al., 2002), Switzerland (Giger et al., 2003), Germany (Hirsch et al., 1999), Spain (Ginebreda et al., 2010), and Japan (Managaki et al., 2007). In sediments, the measured antibiotics concentrations were substantially higher than those reported for river sediments from the United States (Kim and Carlson, 2007) and Spain (Martin et al., 2010), but comparable to our previous study in the Pearl River–Guangzhou section (Yang et al., 2010) and Liao River (Zhou et al., 2011).

4.2. Bioaccumulation of antibiotics in wild fish tissues

Although much work monitoring antibiotics in wastewater-receiving environments have been carried out, published studies on the bioaccumulation of antibiotics in wild aquatic organisms are very limited. This study shows that 10 antibiotics were detected in wild fish muscle and liver tissues, with maximum concentrations up to tens of μg/kg ww in muscle and several thousands of μg/kg ww in liver. Gao et al. (2012) reported that NFX, CFX and SMZ were the most frequently detected antibiotics in fish muscles from the Hai River. The reported highest concentrations for the antibiotics in Gao et al. (2012) are of the same order of magnitude as found in this study, while some reported values in fish muscles from the Pearl River Delta, China (NFX: 3.38–101 μg/kg dry weight, CFX: 4.60–33.3 μg/kg dry weight) (Nie et al., 2008) are higher than those found in this study.

In contrast, TMP and SMX were not detected in bream (*Abramis brama*) fish muscle samples collected at sites located downstream of WWTPs along the Rhine, Danube, and Elbe rivers in Germany (Subedi et al., 2012). Five selected antibiotics ETM, LIN, SMX, TMP and tylosin (TYL) were either not detected in fish muscle or liver samples in effluent-dominated rivers in the United States (Ramirez et al., 2009), although four of these antibiotics were detected in liver or muscle samples in this study. This difference may result from much higher antibiotics usage in China than in the United States (Zhou et al., 2012; Kümmerer, 2009).

For most antibiotics in fish muscle tissue, the modeled log BAFs were lower than the measured log BAFs values (Table S14). The bioaccumulation of hydrophobic organic pollutants is usually based on partitioning mediated by lipid content in fish muscle (Guo et al., 2008; Shi et al., 2009). The Arnot and Gobas model in the BCFBAF program of Epi Suite 4.1 software is also based on lipid partitioning for estimating bioaccumulation of hydrophobic organic contaminants (Costanza et al., 2012). However, many of the antibiotics in this study are ionizable compounds with functional groups, including amides, anilines, phenols, and carboxylic acids. In the BCFBAF program of Epi Suite 4.1 software, estimating the BAFs for ionizable compounds is also based on the lipid partitioning properties for the neutral form of the chemical only (Costanza et al., 2012). While in the field environment, antibiotics are likely present in the ionized form or as coexisting ionized and neutral forms. The uptake rate for ionized chemicals by fish gill is usually pH dependent (Erickson et al., 2006). The ionized form of a chemical also transfers across cell membranes, which may increase its bioavailability (Erickson et al., 2006). In comparison, the conceptual approach in the Arnot and Gobas model is thought to conservatively estimate uptake for potentially ionizable chemicals, as it only considers the neutral form (Costanza et al., 2012). This may explain why the modeled log BAFs values are lower than those determined from field data. Based on the criteria for the bioaccumulative properties of substances, a chemical with a BAF equal to or greater than 5000 are considered to be bioaccumulative (Government of Canada, 1995). In this study, only the log BAFs of OFX in 3 fish muscles (3.74, 3.74, and 4.14) are higher than the BAF of 5000 (log BAF = 3.70), suggesting that OFX may require further study to

determine if regulatory control should be considered.

In contrast, when compared with muscle and liver tissues, there are no reported results for the detection of antibiotics in wild fish bile and plasma. Fish liver and gallbladder are the important digestion tissues with bile excreted to improve food digestion. Pollutants are transferred by blood to muscle, brain, liver and other digestion tissues after the uptake of pollutants by gill and gut. In this study, the profiles of antibiotics in fish bile and plasma showed that 11 antibiotics were detected in wild fish bile and plasma, with maximum concentrations up to several hundreds of μg/L. Especially in fish bile, the mean and median log BAFs for most of the detected antibiotics were higher than 2, with the median log BAFs range of 1.92–3.98 (Table S13). This suggests that wild fish bile and plasma tissues displayed higher bioaccumulation ability for ionizable antibiotics when exposed to antibiotics-contaminated environments. Laboratory studies also demonstrated the bioconcentration of some other pharmaceuticals in fish bile. Anti-inflammatory drugs diclofenac accumulated in the bile of rainbow trout with the log BCFs (bioaccumulation factors) between 2.51 and 2.98 (Mehinto et al., 2010; Kallio et al., 2010), which were comparable with the measured median log BAFs of antibiotics in wild fish bile in this study. Some psychoactive drugs and anti-inflammatory drugs also bioaccumulated in fish bile after 8-day laboratory exposures; among them, gemfibrozil can accumulate, with a maximum log BCF of 3.43 (Togunde et al., 2012). These pharmaceuticals were also found in bile of wild fish or caged fish immediately downstream of a wastewater treatment plant effluent outfall (Brozinski et al., 2013; Togunde et al., 2012).

In the field environment, the bioaccumulation of emerging pollutants such as polar pharmaceuticals to fish tissues is complicated by different exposure levels, different fish diets and unknown metabolism processes (Lahti et al., 2011). The uptake of antibiotics into fish can be either bioconcentration via gill uptake or bioaccumulation via food (Boonsaner and Hawker, 2013). Foreign chemicals will go through various phase I and phase II metabolisms in digestion tissues such as gill, kidney, bile, and liver after the chemicals enter into fish body (Lahti et al., 2011). However, in this study, the results obtained clearly indicate that antibiotics accumulated in wild fish tissues in antibiotics-contaminated environments, especially for fish bile and plasma tissues. Antibiotics in these tissues may cause adverse effects in fish when they are present in high internal concentrations (Stadnicka et al., 2012).

As shown in Tables 1–3, all of the detected antibiotics in fish tissues were also found to be present in surface water or sediment samples. The six antibiotics SMZ, EFX, OFX, ETM-H_2O, LIN and TMP also displayed high detection frequencies and high concentrations in fish tissues and in the environment, indicating that these antibiotics were accumulative in the wild organisms in the highly contaminated receiving environment. Some of the measured antibiotics, such as SMM, SMX, SPD, OTC, TC and NAR, also were detected at high concentrations and with high detection frequencies in surface water and sediments, but were either undetected or infrequently detected in fish tissues. Biotransformation of these antibiotics in fish tissues could be one potential cause. In human plasma, methyl, hydroxyl, and glucuronide metabolites of the antibiotic SMX have been reported (Vree et al., 1994). However, a recent laboratory study demonstrated that no metabolism of SMX was found in rainbow trout fish liver S9 fractions (Connors et al., 2013). Different fish species as well as other aquatic organisms may display different biotransformation ability of xenobiotics. Hence, further evaluation of the biotransformation ability for antibiotics in aquatic organisms is necessary.

4.3. Human health risk from fish consumption

Consumption of antibiotics contaminated fish may also pose potential risks to human consumers (Boonsaner and Hawker, 2013). The intake rates (mg/kg/d) of five frequently detected antibiotics (SMZ, EFX, OFX, ETM and TMP) from fish to human consumers were evaluated (Table S15). Since the majority of consumption was only through eating fish muscle, the maximum fish muscle concentrations of antibiotics were used assuming “a worst-case scenario”. On this basis, the intake rates of SMZ, EFX, OFX, ETM and TMP were 5.92×10^{-6}, 5.14×10^{-6}, 1.24×10^{-5}, 1.75×10^{-5} and 2.88×10^{-7} mg/kg/d for an adult with a body weight of 70 kg. The HQs were far below 1, ranging from 1.44×10^{-5} to 8.6×10^{-3}. The range of HQs so derived here suggests no direct detrimental effects from ingestion of antibiotics-contaminated fish. In other studies, risk evaluations for some other pharmaceuticals also demonstrated that no potential risks of adverse effects from combined fish consumption and drinking water (Bercu et al., 2008; Cunningham et al., 2009; Kumar and Xagoraraki, 2010).

The Ministry of Agriculture of the People's Republic of China (2002) also released a guideline, in which the Maximum Residue Limits (MRLs) were set for veterinary drugs in animal food for human consumption. In this guideline, the maximum MRLs for animal food (muscle) are set at the range of 50–200 μg/kg ww for SMZ, EFX, ETM and TMP (Table S15). The measured antibiotics in fish muscle in this study for the 4 antibiotics were all lower than the MRLs, indicating that the residual amounts of these antibiotics in fish muscle from this study met the criteria for safety for consumption. However, no MRL for OFX was set in the guideline. In this study, OFX was detected the maximum concentration of 16.8 μg/kg ww in fish muscle, which were only 2.5-fold lower than the lowest MRL of TMP (50 μg/kg ww). This suggests that OFX may need to be evaluated more thoroughly for human health risk and bioaccumulation ability as well. Further, the assessment conducted in this study did not consider the effects of antibiotic mixtures, although multiple antibiotics residues were shown to be commonly present in wild fish. In wild fish muscle, antibiotics were determined in mixtures with each other and with other types of pollutants (Pan et al., 2014). Hence, the intake of multiple antibiotics from fish consumption might pose a potential as-yet-unassessed health risk to humans.

5. Conclusions

A variety of antibiotics were found in surface water, sediments, and fish tissues from four rivers in the highly urbanized Pearl River Delta region. The maximum log BAFs for the detected antibiotics were more than 4 in fish bile (CFX, EFX, NFX, OFX, and LIN), plasma (SMZ, EFX, ETM-H_2O, and LIN, liver (EFX, OFX, ETM-H_2O, and LIN) and muscle (OFX) tissues, suggesting that many antibiotics accumulated in fish tissues in the contaminated rivers. Previous studies seldom focused on fish bile and plasma tissues, but these tissues clearly displayed strong bioaccumulation ability for some ionizable antibiotics, indicating a novel bioaccumulation pattern for antibiotics in the aquatic environment contaminated by these compounds. To the best of our knowledge, this is the first report on the detection of antibiotics in fish bile and plasma tissues.

Acknowledgments

The authors would like to acknowledge the financial support from National Natural Science Foundation of China (NSFC U1133005, 41101462 and 41121063) and the National Water Pollution Control Program (2014ZX07206-005). Thanks to E. T. Furlong (USGS) for editorial review of the revised manuscript and

other useful comments, and to two anonymous reviewers for their critical comments and suggestions. This is a Contribution No. 1999 from GIG CAS.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.envpol.2014.12.026.

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